

Shaofan Li
Bohua Sun
Editors

Advances in Cell Mechanics

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Preface

During the past decade, molecular and cellular biophysics has emerged as one of the most active and exciting areas at the frontier of scientific research. It is a multi-disciplinary field that has many important applications in medicine, health care, life science and biology in general. In essence, the origins of many diseases and illness are rooted at the cellular level. Like molecular and cellular biology, molecular and cellular biophysics is also an indispensable part of the very foundation of contemporary medical and life sciences. In fact, cellular biophysics not only provides a theoretical foundation toward understanding biological processes such as the function of organs, tissues and their interactions, but also provides practical answers to medical treatment and pathology including molecular mechanism of diseases and infections. Because of rapid development of nano-science and nano-technologies, molecular and cellular biophysics not only offers a practical means but also the promise to treat and cure many diseases with which we as a human society are still struggling with. However, unlike molecular cellular biology, molecular and cellular biophysics, in particular molecular and cellular biomechanics, is still a field at its infancy. It is precisely because of this that makes cellular mechanics a promising and exciting research field to study and to work.

In this book, we have selected nine research works at the forefront of molecular and cellular biomechanics to be introduced to our readers. It is our opinion that these works represent the current trend and future directions of cellular biomechanics research. By compiling these different topics into one volume, a unique perspective is provided on the current state of cell mechanics research and what lies in the future.

Among these contributions, Romero and Arribas presented their groundbreaking work on three-dimensional cell model, cell growth dynamics algorithm, and associated large-scale finite element simulation through Chapter 1. In Chapter 2, Zeng, Li, and Kohles presented their work on multiscale simulations of soft contact and adhesion of stem cells. In this work, a soft matter model has been developed to model the mechanical mechanism of the mechanotransduction of stem cells. Focusing on molecular mechanics and genetic mechanism of cellular biology, Wu, Wang, and Cohen presented their molecular dynamics modeling of proteins in Chapter 3. More specifically, they employed a molecular dynamics and principal component analysis (MD-PCA) approach studying sickle hemoglobin-hemoglobin interaction, which is the main cause of sickle cell anemia. In Chapter 4, Qin, Chou, Kreplak, and Buehler presented their latest work on atomistic and coarse-grain modeling and simulations of cellular intermediate filaments. They not only presented their own work, but also provided a detailed tutorial on modeling and simulations of intermediate filament networks. Complementary to Qin et al's work, in Chapter 5, Hatami-Marbini and Mofrad give a tutorial overview of

cytoskeletal mechanics and rheology, and the topics that they have touched on are from mechanics of intermediate filament, rheology of cytoskeleton network, experimental measurements and techniques, to computation and simulation approach. To capture the complexity of cell's constitutive behaviors, in Chapter 6, Vernerey presented a multiphase mixture cell model and its application to cell-substrate interactions. In the proposed multiphase cell model, it combines the continuum description of the stress fiber, mass transport, cytosol fluid motion, and G-actin monomer motion, etc. Not only does the multiphysics model couple and combine several different aspects of cell biology, but the author has also applied the latest extended finite element method (X-FEM) and level-set method to simulate cell contact and adhesion with an extracellular substrate. This has demonstrated how applied and computational mechanics can solve complex problems in cell mechanics. To investigate mechanotransduction of cells through a purely thermodynamic approach, Sarvestani presented his analytical cell adhesion model in Chapter 7 that takes into account the effects of substrate stiffness and how it affects the growth of nascent adhesion areas. In Chapter 8, Kohles provided a detailed account on the experimental biomechanics of a single cell, in which he has discussed a state-of-the-art opto-hydrodynamics technique for measuring isolated cellular mechanical properties. Finally, in Chapter 9, Shen discussed his nonlocal shell model to simulate the buckling of microtubules inside a cell. This is a good example that clearly shows how non-classical continuum mechanics can contribute to the understanding of cellular biology.

Finally, it is our hope that this volume will disseminate much useful information in cellular biomechanics to a larger community outside the area of applied mechanics, while arousing public interest in cell mechanics research and applications. Ultimately, we envision that these works would promote more in-depth study and research in cell mechanics and cellular biophysics.

Shaofan Li and Bohua Sun
February 4, 2011

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Chapter 1 Modeling and Simulations of the Dynamics of Growing Cell Clusters

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Abstract: A phenomenological discrete model for the dynamics of growing cell clusters is presented. Each cell is modeled as a growing deformable solid which can interact mechanically with its neighbors by means of adhesion and repulsion forces. By defining simple behavior rules based on the age and the mechanical state of the cells, simple cluster dynamics can be reproduced. The framework is far from complete, but describes the essential features required for more complete mechanical simulations of cell ensembles.

Keywords: cell mechanics, finite elements, population dynamics, cellular automata

1.1 Introduction

The study of cell cluster dynamics is fundamental for understanding biological phenomena such as embryology, tissue repair, and most importantly, solid tumors. Up to now, biochemistry has been the main discipline employed to study such processes, with undeniable success. There is, however, growing awareness that mechanics also plays a crucial role in these dynamical processes and new avenues to research and analysis are now opened.

Since the classical work of Young^[1] and later Eaves^[2], the mechanical effects on tumors have been widely studied. For example, in the key work of Helmlinger et al.^[3] experimental evidence was provided to support the idea that the growth of multicellular tumor spheroids is controlled by pressure. More surprisingly, their findings are demonstrated “... *regardless of host species, tissue of origin, or differentiation state.*” These, and similar ones^[4,5] motivate the study of single cell mechanics, multiple cell mechanical interactions, and their effect on the global dynamics of growing ensembles.

Several approaches have been investigated to understand the mechanics of

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growing cell clusters, cancer in particular^[6]. The oldest models employ partial differential equations that treat tumors as continua with deterministic growth (see [7] and references therein). While these approaches allow of the study of complete tumors, they can not provide enough details of the mechanical effects at the micro scale, since cells are smeared out and are not represented.

The second type of approaches employed for studying cell population dynamics is based on cellular automata (see [8-10] among many others). These methods represent individual cells and their behavior, so they allow much richer resolution than smeared models. In an effort to keep the computational cost low, many of the geometrical/mechanical details are often ignored. Some cellular automata include sophisticated discrete evolution laws, and even some crude mechanical behavior.

The computational model that is presented in the current work advocates the use of individual entities to represent each cell in the cluster, just as cellular automata. However, in contrast with the latter, the proposed models have sufficient mechanical and geometrical details so as to replicate, at least qualitatively, the most important phenomena that seem to be at the heart of their mechanical behavior. The underlying motivation is to provide a test-bed for the mechanical response of cell clusters based on first principles and as few ad hoc evolution rules as possible. This environment will allow test hypothesis on the effect of mechanical variables on growth or elimination of cell colonies while providing very high definition pictures of the geometry and internal variables of the ensembles.

The basic mechanical features that are represented in the model are: deformation, material response, growth, adhesion, and repulsion. To replicate *in silico* all these effects, a nonlinear finite element model is employed. In it, every single cell is defined as a Deformable solid of ellipsoidal shape capable of following complex deformation modes. The material for each body is homogeneous and isotropic whose constants are chosen so as to qualitatively match experimental results. Growth, an attribute that most cellular automata do not incorporate, is described in a mechanically sound manner, whose rate is predetermined based on each cell's age. A key ingredient of the model is the numerical treatment of cell-to-cell interaction forces. The computational cost of a molecule-based, membrane-to-membrane interaction model is prohibitive. Thus, a macroscopic model is incorporated into the finite element computations using standard penalty constraints and surface-to-surface potentials. Finally, a decision tree is employed to capture the most basic events of the life cycle of each cell. At each instant, a cell might be divided or die depending on random decisions whose probability function depends on the cell's age and its mechanical stresses.

The model described in the current article still fails to become predictive.

In order to do so, larger number of cells need to be considered in each cluster, and additional effects should be incorporated into the model (among others, transport of nutrients and oxygen, pH, etc). Nevertheless, it provides a higher level of resolution than cellular automata and will be progressively improved and validated.

The outline of the rest of the article is as follows: In Section 1.2 the geometrical description of individual cells is discussed, both at the continuum and the computational levels. Then, in Section 1.3, their mechanical equilibrium is formulated. Section 1.4 describes the two mechanical interactions which are considered among cells, namely, interpenetration constraints and adhesion. The simplified logic for the life cycle of each cell is presented in Section 1.5 and details of the whole numerical implementation are provided in Section 1.6. A large-scale numerical simulation of hundreds of interacting cells is described in Section 1.7, using all the model features presented before. The article concludes with a summary of results in Section 1.8.

1.2 Single cell geometry and kinematics

In order to model the salient features of deformable growing cells, a deformable body is defined whose mechanical response resembles that of a cell. Sophisticated cell mechanical models can be found in the literature that are used to study various aspects of the cell deformation. They employ either tensegrities to reproduce the mechanical response of the cytoskeleton as in, for example, [11, 12], or continuum finite elements as in [13, 14]. In all cases, high accuracy in reproducing simple mechanical tests can only be obtained at the expense of complex equations and computational models, which, in practice, preclude their use for studying the joint behavior and interactions of hundreds of cells. In this work, rather than attempting to accurately model the mechanics of individual cells, we identify the *essential* mechanical features of their behavior and model them, at least, in a qualitatively correct fashion.

We propose next an isotropic, homogenized cell model that accounts, in an approximate manner, for the mechanical response of the cell, its membrane, its cytoskeleton, organelles, etc. The equations are the standard equilibrium partial differential equations of a deformable nonlinear solid with growth. In this section we focus on their geometric description and kinematics, including growth. Both the continuum and numerical models will be described, illustrating the generality and flexibility of the approach.

1.2.1 The continuum model

Each cell is assumed to be a set of particles that occupy, in the reference growth-free configuration, an open set $\mathcal{B}_o$ of $\mathbb{R}^3$ and particles labeled by $\mathbf{X}$. At time t , the cell occupies a region $\mathcal{B}_t \in \mathbb{R}^3$. Particles $\mathbf{X} \in \mathcal{B}_o$ are mapped onto points $\mathbf{x} \in \mathcal{B}_t$ by the deformation φ so that $\mathbf{x} = \varphi(\mathbf{X}, t)$.

The deformation gradient $\mathbf{F}_t = \mathbf{grad}[\varphi]$ is the function that maps neighborhoods of $\mathbf{X}$ onto their deformed counterpart at $\mathbf{x}$. By assumption, we consider motions so that the deformation gradient is of the form

$$\mathbf{F} = \mathbf{F}_d \mathbf{F}_g. \quad (1.1)$$

The term $\mathbf{F}_g$ accounts for the growth of the material and it is assumed, in our model, to be known a priori. The term $\mathbf{F}_d$ is the part of the deformation gradient that is associated with strains and stresses, and the only part that enters the formulation of the material model. Hence, we define the right Cauchy–Green deformation tensor as

$$\mathbf{C} = \mathbf{F}_d^T \mathbf{F}_d. \quad (1.2)$$

See Fig.1.1 for an illustration of this kinematic concept.

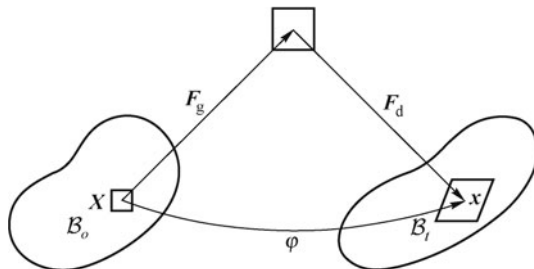


Fig. 1.1 Cell kinematics, including growth, described at the differential level.

In living organisms, growth is controlled by space, nutrients, biological signals, etc, and it is anisotropic in general. In the proposed framework, however, we will employ the simplest model of growth that will allow us to concentrate on the mechanical phenomena. It consists of an *ad hoc* rule for the growth part of the deformation gradient which, furthermore, is homogeneous, isotropic and of the form

$$\mathbf{F}_g(\mathbf{X}, t) = g(t) \mathbf{I}. \quad (1.3)$$

In the previous equation $g : [0, \infty) \rightarrow [1, G]$ is a continuous, monotonically increasing function from 1, the reference un-grown state, to G , a constant related to the volume of the adult cell.

1.2.2 The numerical model for the cell geometry

Every cell is discretized with a nonlinear finite element model. A special mesh generator has been defined to create cells of ellipsoidal shape with arbitrary dimensions and with selectable mesh density. Other types of cell geometry could be used, since this step is completely independent of the rest of the model but, for simplicity, results shown in this article are restricted to random ellipsoidal geometry (or spherical, as a special case).

Figure 1.2 shows three identical cells with three different mesh densities. For the dynamic simulations it is crucial that cells can be created/eliminated automatically, and that the end user only selects the desired resolution. This choice has the most dramatic impact on the overall CPU cost of all other assumptions.

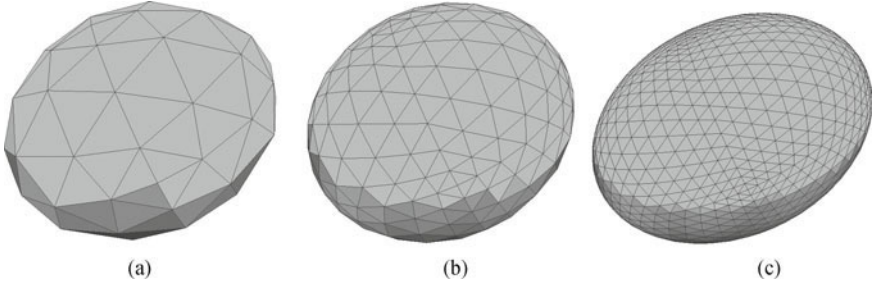


Fig. 1.2 Three cell meshes with different levels of mesh refinement.

1.3 Single cell equilibrium and material model

The equilibrium equations of mechanics are imposed over every volume element of the cell using a nonlinear finite element formulation. This solution step is exact, up to the finite element error, and accounts for every internal stress and strain component in the cell.

1.3.1 Cell equilibrium

The equilibrium equations of the cell are the standard balance of equations in continuum mechanics. If $\mathbf{S}$ is the second Piola–Kirchhoff stress tensor, then the balance of linear momentum can be stated as

$$\begin{aligned} \operatorname{div} [\mathbf{FS}] &= \mathbf{0} & \text{in } \mathcal{B}_g, \\ \mathbf{FSN} &= \mathbf{T} & \text{on } \partial\mathcal{B}_g. \end{aligned} \tag{1.4}$$

In the previous equations, operator $\text{div}[\cdot]$ is the material divergence and $\mathbf{T}$ is known forces on the cell boundary. Alternatively, the equilibrium can be formulated as the position that minimizes the potential energy

$$V(\varphi) = \int_{B_o} W(\mathbf{grad}[\varphi], \mathbf{F}_g) dV + V_{\text{ext}}(\varphi) , \quad (1.5)$$

where W is a stored energy function on the cell material and V_{ext} is the potential of the external forces.

In an abstract way, the solution of the mechanical cell problem consists in finding φ given a known growth law $\mathbf{F}_g$ and known surface tractions $\mathbf{T}$. Details of the formulation and solution of Eq.(1.4) in the context of the finite element method can be found in standard references. See, for example, Ref. [15].

To close the formulation of the boundary value problem of single cell mechanics suffices to define a constitutive law for the homogenized material.

1.3.2 The material model

The choice of constitutive law is completely independent of the rest of ingredients of our model. Hence, any sensible model could be employed, possibly incorporating finite strain elastic, viscous, or even plastic effects.

In our simulations we have employed hyperelastic material models, the simplest ones that might be used to approximate the most elementary mechanical behavior as reported throughout the literature regarding “traction” tests of the cells^[16,17]. The goal is to obtain cell-like deformable solids whose mechanical response resembles that of typical cells. To this end, a standard neo-Hookean model is employed as defined in [18]. The symmetric Piola-Kirchhoff stress tensor is thus defined by $\mathbf{S} = 2\partial_{\mathbf{C}}W$, where W is the stored energy function of the form

$$W = U(J) + \frac{\mu}{2}(I_1 - 3) - \mu \log J, \quad U(J) = \frac{\lambda}{2}(\log J)^2 , \quad (1.6)$$

with $I_1 = \text{tr}[\mathbf{C}]$ and $J = \sqrt{\det(\mathbf{C})}$. The two elastic constants λ, μ are obtained by fitting experimental data with the model.

1.3.3 Determination of material constants

In order to select representative elastic constants for Eq.(1.6) we use the experimental data provided in [19]. In this reference force-displacement curves are reported for compression tests performed over C2C12 mouse myoblasts.

It is not the goal of this modeling phase to find the value of the elastic constants of the material that these cells are made of. Rather, a phenomenological value is sought out so that when a cell of the type described in Section 1.2 is employed in a numerical simulation, its overall response matches the experimental results. This distinction is relevant because it allows us to employ relatively coarse finite element meshes without unacceptable levels of inaccuracy, at least in the dominant deformation modes. In turn, this results in material constants which are mesh-dependent. In the simplest possible model in this section, a single node is defined in the cell interior. This crude discretization is sufficient for a qualitative approximation.

In our simulations a spherical cell-like body of volume $2\,520\,\mu\text{m}^3$ is considered. The material model is neo-Hookean with stored energy function defined in Eq.(1.6). For each level of mesh refinement, the corresponding Young's moduli and Poisson's ratio are obtained to fit the experimental data of the compression tests. For the coarse mesh, the one employed in the rest of the simulations, the elastic constants found have values $\lambda = 40.3\,\text{kPa}$ and $\mu = 4.48\,\text{kPa}$.

To carry out the simulation, a fully grown cell is placed between two rigid surfaces which are brought close to each other, compressing the cell and deforming it. Figure 1.3 shows four stages during the compression test and Fig.1.4 depicts the compression force vs. the relative diameter change obtained with the numerical model described above, superimposed to the experimental results alluded to above. The agreement is good enough for qualitative purposes. The change of slope in the deformation curve is due to the discretization error in the coarse mesh employed.

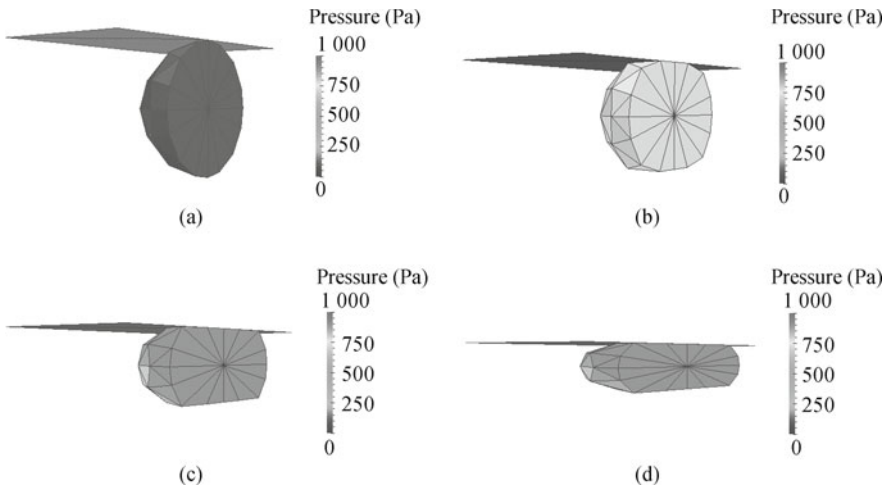


Fig. 1.3 Cell compression test. The cell is cut through a vertical plane to show the stress distribution in the inside (color plot in the book end).

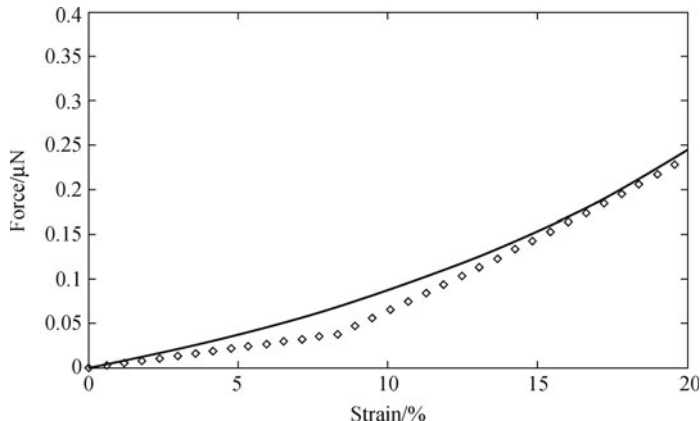


Fig. 1.4 Compression force vs. relative diameter change in the compression test of a cell-like spheroid. The continuous line shows a fit of the experimental results reported in [19]. The dots correspond to the numerical results obtained with the numerical model.

1.4 Modeling cell interactions

Having defined a model for individual cells, it remains to incorporate the rules that will serve to represent the interaction between cells, eventually governing the global system dynamics. As in the case of single cells, it is not trivial to determine which complex interactions should be considered, and which should be neglected. In any case, and as before, it is not possible to attempt modeling all interactions. Rather, one should aim to select the ones that most crucially affect the overall response.

The most fundamental effect of mechanical nature among cells is attraction/repulsion forces. The detailed picture of cell-to-cell forces is described in detail in the literature [20] and their accurate description might be crucial for the study of certain problems^[21]. In our analysis, having already made simplifications at the level of the cell mechanics, it becomes reasonable to attempt again to capture the most basic effects in a qualitatively correct way, but without excessive complexity that would preclude a full numerical solution.

With these considerations we aim to model cell repulsion first. The micromechanical mechanisms behind cell interpenetration are complex and would require a high resolution of the cell geometry^[22,23]. Using, for example, Lennard–Jones type potentials to model such effect would entail almost a molecular description of the cell membrane. Instead, we propose to coarse grain all the repulsive forces within the variational formulation of the prob-

lem (as required for the finite element implementation) using the simplest penalty method. Using this standard technique^[24], the penetration of the two external surfaces of the cell is “artificially” avoided. Of course, the repulsion forces no longer are physically based, but they nevertheless account for the right amount of resultant guaranteeing the constraint.

Cell adhesion is also represented in the model. As before, the micromechanical causes for such forces are complex and their detailed resolution is outside the goals of the current model. We propose to model them by defining a surface-to-surface attraction that accounts for these effects. As described below, it can be easily incorporated within the finite element formulation and adjusted so as to fit experimental data.

1.4.1 Cell-to-cell contact

Interpenetration forces guarantee that two cells, even when they get close to or pressed against each other, do not overlap. Ignoring their molecular explanation, their overall effect can be described in a purely geometrical manner and, in this sense, easily implemented. In fact, at the cell scale, these forces can be described as contact forces. This type of interactions has been thoroughly studied in the context of Solid Mechanics and its numerical implementation is well documented^[25,26].

The simplest numerical strategy to enforce interpenetration is based on penalization. Given two cells $\mathcal{B}_1, \mathcal{B}_2$, let Γ_1, Γ_2 denote, respectively, their boundaries. For each point $\mathbf{X}_1 \in \Gamma_1$, the signed distance to Γ_2 is denoted $\Delta(\mathbf{X}_1)$, and defined as being positive if $\mathbf{X}_1$ is outside $\mathcal{B}_2$ and negative otherwise. Then, the penetration of $\mathbf{X}_1$ is defined as

$$\pi(\mathbf{X}_1) = \langle -\Delta(\mathbf{X}_1) \rangle, \quad (1.7)$$

where $\langle \cdot \rangle$ denotes the Macaulay bracket. By symmetry, the same argument applies to the penetration of the $\mathcal{B}_2$ into $\mathcal{B}_1$. Then, to enforce the interpenetrability of either body into its neighbour it suffices to augment Eq.(1.5) by the penalization term

$$\int_{\Gamma_1} \frac{\kappa_p}{2} \pi(\mathbf{X})^2 \, dS + \int_{\Gamma_2} \frac{\kappa_p}{2} \pi(\mathbf{X})^2 \, dS. \quad (1.8)$$

The penalization constant κ_p must be positive and large in comparison with any characteristic stiffness of the cell. Recommendations for the choice of this contact constants are provided in standard texts on finite elements and we refer to the references alluded to before.

1.4.2 Cell-to-cell adhesion

When the surfaces of two neighboring cells become close, adhesion forces appear that try to bring them together and to keep them in this way. As in the case of the repulsion forces, we propose a phenomenological model that accounts for this type of interactions in such a way that it is qualitatively correct and matches closely experimental data. Following the results reported in [19], adhesion forces are incorporated between membrane surfaces closer than a cut-off distance δ_o . Based on the experimental data of this article, adhesion forces per unit of surface on one cell and unit of surface of the opposing cell have constant value κ_a as long as their relative distances are below δ_o , and zero otherwise.

Let $\gamma_1 = \varphi(\Gamma_1)$ and $\gamma_2 = \varphi(\Gamma_2)$ be the boundaries of the deformed cells. To include adhesion effects on the formulation, an additional force per unit area of the surface γ_1 must be added at each point $\mathbf{x}_1 \in \gamma_1$ of the form

$$\mathbf{F}_1(\mathbf{x}_1) = \int_{\gamma_2} \mathbf{f}(\mathbf{x}_1, \mathbf{x}_2) \, dS_2, \quad (1.9)$$

where the force $\mathbf{f}$ per unit of area squared has value

$$\mathbf{f}(\mathbf{x}_1, \mathbf{x}_2) = \kappa_a \mathcal{H}(\delta_o - |\mathbf{x}_2 - \mathbf{x}_1|) \frac{\mathbf{x}_2 - \mathbf{x}_1}{|\mathbf{x}_2 - \mathbf{x}_1|}, \quad (1.10)$$

and $\mathcal{H} : \mathbb{R} \rightarrow \{0, 1\}$ is the Heaviside step function. Similarly, points on the surface γ_2 are attracted to γ_1 with a force per unit area

$$\mathbf{F}_2(\mathbf{x}_2) = \int_{\gamma_1} \mathbf{f}(\mathbf{x}_2, \mathbf{x}_1) \, dS_1. \quad (1.11)$$

From the previous two equations it follows that

$$\int_{\gamma_1} \mathbf{F}_1(\mathbf{x}_1) \, dS_1 + \int_{\gamma_2} \mathbf{F}_2(\mathbf{x}_2) \, dS_2 = \mathbf{0}. \quad (1.12)$$

The Equation (1.10) models adhesion forces of constant moduli (per unit of surface squared) when the distance between the interacting surfaces is below a threshold δ_o . This represents bonds that are created when the cell membranes are close to each other and only break when separated beyond δ_o .

To implement the adhesion force on the surface γ_1 (respectively γ_2) the following integral must be evaluated

$$\int_{\gamma_1} \mathbf{F}_1 \, dS_1 = \int_{\gamma_1} \int_{\gamma_2} \mathbf{f}(\mathbf{x}_1, \mathbf{x}_2) \, dS_2 \, dS_1. \quad (1.13)$$

In the context of a finite element model, the previous integral is approximated with the finite sum

$$\int_{\gamma_1} \int_{\gamma_2} \mathbf{f}(\mathbf{x}_1, \mathbf{x}_2) \, dS_2 \, dS_1 \approx \sum_{T_i \in \gamma_1} \sum_{T_j \in \gamma_2} \mathbf{f}(C_i, C_j) A_i A_j, \quad (1.14)$$

where T_i, T_j are the triangles on the cell surfaces, C_i, C_j their centers, and A_i, A_j their corresponding areas.

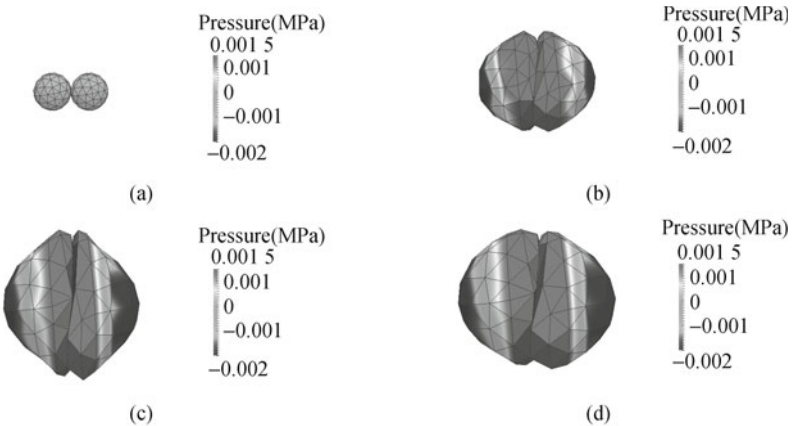
1.4.3 Cell-to-cell interaction test

To illustrate the effect of the two types of cell interactions defined in this section we show next some results of the virtual test of two cells interacting coming into contact with each other, and then pulled apart.

Two identical spherical cells of initial diameter $5.6 \, \mu\text{m}$ are placed with their centers fixed at a distance also of $5.6 \, \mu\text{m}$. Growth in the cells progressively increases their volume, eventually multiplying its original value by 3. Then, due to their proximity and the restriction on their centers, both cells come into contact, deforming while they press one against the other.

Once the two cells are fully grown and the contact forces are developed, they are slowly pulled apart. In this process, the contact forces are reduced. Eventually, the two previously contacting surfaces no longer try to interpenetrate and adhesion forces are then activated which attempt to keep them together.

Figure 1.5 describes a complete sequence of the contact/adhesion test. Pressure builds up when the two cells are pressed one another, especially close to the interacting surfaces. When the two bodies are separated, pressure decreases and adhesion pulls the two cells together. Figure 1.6 plots the values



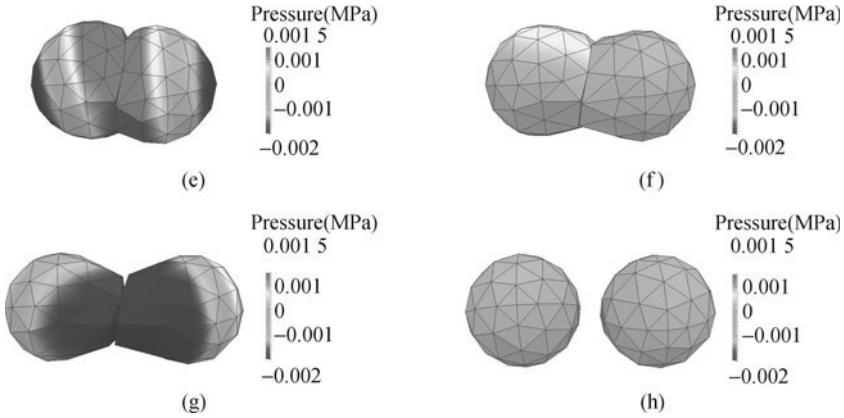


Fig. 1.5 Adhesion test. The centers of the two cells are initially fixed, and contact occurs due to cell growth. When the cell on the right is pulled apart from the one on the left the contact stresses are relaxed and adhesion helps to keep the interacting surfaces glued (color plot in the book end).

of the interaction forces during the whole process. The contact force peaks at approximately $8 \mu\text{N}$ and the latter only drops to $-0.5 \mu\text{N}$.

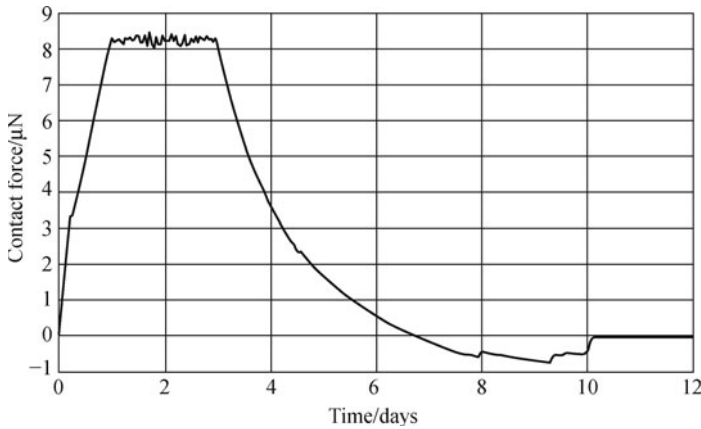


Fig. 1.6 Adhesion test. Contact/adhesion forces between the two interacting cells.

The repulsion force is a function of the initial distance between the cell centers, hence somewhat arbitrary. The adhesion force, however, only depends on the cell surface area and adhesion model, hence providing a good estimate of the magnitude of such forces in more complex simulations. The value of the adhesive constant employed for this test is $\kappa_a = 2 \times 10^{-3} \mu\text{N}/\mu\text{m}^3$, and δ_o is a random variable with uniform distribution in the interval $[2, 3.5] \mu\text{m}$. Both constants can be varied to match experimental results. The simula-

tion presented in this section verifies that the contact/adhesion formulation described in this section, being simple, suffices to capture the essential response in cell-to-cell mechanical interactions.

1.5 Modeling the cell life cycle

The last part of the simulation framework to be defined corresponds to the phenomenological description of the life cycle of each cell: birth, growth, duplication, and death. The algorithmic rules set up to mimic these aspects are extremely simplistic, but still go beyond what is usually employed in cellular automata because the internal pressure of the cell is used to modulate the random distributions that govern the critical changes: duplication and death. Figure 1.7 shows the basic logic governing the cell life cycle. There are

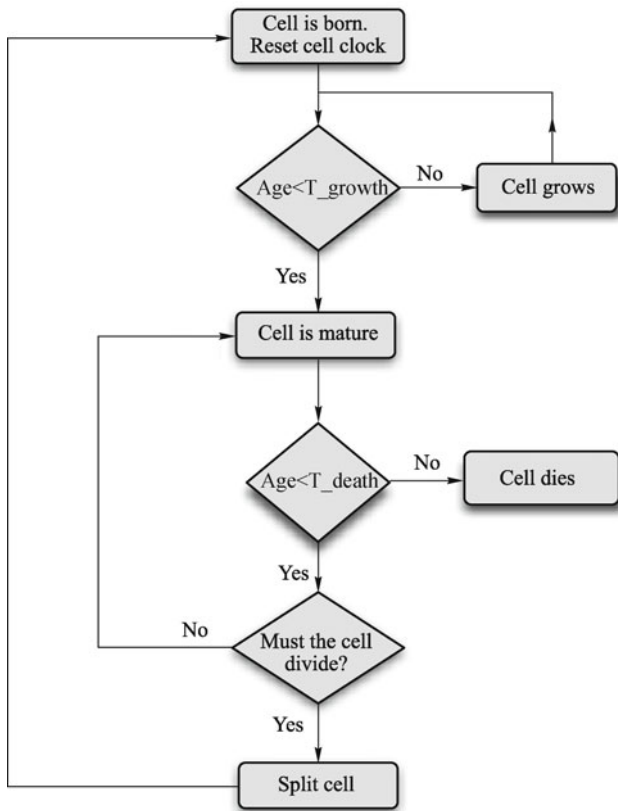


Fig. 1.7 Cell life cycle logic diagram. A key issue of the proposed model is that every decision depends on the mechanical state of the cell.

obvious similarities with the expected rules that might control the evolution of a standard cell automaton. The distinct feature of the proposed framework is that every decision is “mechanically informed”, using the average pressure in each cell to regulate each random variable sampled.

The life cycle of each cell can be summarized as follows: Each cell is created as an ellipsoidal solid with random orientation and volume (within a pre-determined interval). A finite element mesh is created as described in Section 1.2. Each of the cells holds an internal “clock” that measures the elapsed time since its creation. For a pre-determined period of time, the cell grows isotropically as described in Section 1.3 reaching at the end an “adult” volume which will remain unchanged (except for the possible deformation) until the eventual death or duplication of the cell.

Once a cell is considered to be adult it might divide itself. A random variable is sampled at birth determining a dividing pressure (in our simulations we have employed an exponential distribution). Approximately every hour (the precise interval is randomly selected for each cell), the average pressure in the cell is compared with the dividing pressure. If the former is smaller, the cell divides, and otherwise it remains quiescent. Due to this algorithm, cells in the cluster surface are more prone to divide those in the core.

When a cell divides, two small cells are generated close to the center of the parent cell with random orientation. For geometrical reasons, each of these child cells must have a rather small volume so that, in their creation, they do not overlap with neighboring cells but rather fit within the volume previously occupied by the parent cell. In a short period, each of the two children grows until its volume becomes close to half of the volume of its parent cell, with the differences related to the randomness in the cell original dimensions. These variations are small and, more importantly, have no effect on average upon the balance of mass. We note that this algorithmic growth is unrelated with the cell true biological growth which is described in Section 1.2.

At birth, every cell also samples a random life duration, which in our simulations followed a Weibull distribution. If a cell becomes too old, it simply dies and disappears. This often happens to cells in the core, whose average pressure is high and rarely divides, whereas in the cells closer to the surface such phenomenon is more unlikely, because the cells’ clocks reset every time they divide. The last two phenomena have been observed in experiments, as explained in Introduction, and are ultimately responsible for the saturated growth laws shown in Section 1.7.

1.6 Details of the numerical implementation

The computational model described in the previous sections accounts for the most significant mechanical effects within a cell cluster. Despite the simplifications made, the model is complex and very demanding in terms of CPU and memory. In this section we comment on the data structures and algorithmic strategies underlying the whole framework.

1.6.1 The finite element model

The robustness of the whole simulation code depends on the numerical treatment of the mechanical effects (kinematics, material modeling, contact, adhesion...) as well as an efficient treatment of the data structures, which are fairly complex.

The core of the simulation framework is a finite element code for dynamic, explicit, nonlinear, solid mechanics. The formulation employed for modeling the cell response is the simplest one for finite strain problems. Its discretization is based on pure displacement elements with isoparametric interpolation of the type described in [15].

Standard finite element codes are, in most cases, not well suited for handling frequent data creation and elimination. With this in mind, a hierarchical, object-oriented data structure had to be implemented from the outset. This type of data organization simplifies the definition of cells, to which their elements and nodes can be related and from which they extract information. In this structured database, cell creation and deletion imply the corresponding memory allocation/deallocation of the dependent entities (elements and nodes). While nothing prevents a simpler code from solving the kind of multicellular problems we are interested in, experience has shown that the book-keeping is so complex that unless the programming language takes care of it, the task is overwhelming.

1.6.2 Contact/adhesion interface detection

The most time-consuming phase of the computation is the numerical treatment of contact and adhesion. While the computation of the corresponding forces is fairly simple, there is a large number of possibly interacting surfaces which grows very rapidly with the increasing number of cells. It is hence mandatory to use efficient sorting methods that can quickly identify possible contact pairs and neglect those which are physically impossible.

The specific structure of the cell ensemble can be used in this respect. At a first level, pairs of cells which are relatively close are identified. Then, interface elements (for contact and adhesion) are created between the opposing faces of close pairs. At the same time, whenever two previously interacting cells become too distant, the interface elements are removed. These two processes entail a relatively complex dynamic allocation/deallocation of data structures. However, in order to handle large populations of cells it is absolutely mandatory.

1.6.3 Time integration

The evolution of the cell system is described by force equilibrium equations, growth, and behavior logic. The characteristic time of growth and duplication, the dominant forcing effects of the model, is much longer than the characteristic time for wave propagation within the deformable cells, suggesting that the problem is quasi-static. However, due to the complexity of the model, it is more convenient to employ an explicit time-integration scheme which does not require the linearization of the equilibrium equations.

The time-integration scheme selected for advancing the system equations is the classical central difference explicit method. The stability condition for this method imposes a severe restriction on the time step size which makes the computations simple, but is time consuming. We have adopted a technique known as mass-scaling, commonly used in other applications of solid mechanics where the use of explicit solutions for quasi-static problems is desired. This numerical trick consists in using a fake density for the cell material so that the time step size is increased, but without perturbing the response at the time scale of the main effects. Details of the specific scaling employed in the cell simulations are provided in Section 1.7.

Artificial damping is also employed for the dynamic simulation. Since the cell material model and the contact forces are elastic, there are no dissipative effects in the system. The frequent collisions detected by the contact algorithm produce elastic waves and oscillations which are unphysical. To eliminate them, artificial (Rayleigh) damping is introduced in the integrator. This is also a standard strategy in explicit dynamics.

This strategy is closely related to Dynamic Relaxation, a technique used to solve (complex) quasistatic problems by obtaining the long-term response of a dynamic problem in which both mass and damping have been artificially chosen so as to maximize the time step size and damp out the transient response. The implementation of this type of methods can be found, for example, in [27, 28], and depends on estimates of the largest and smallest

eigenvalues in the problem, which must be adaptively updated along the solution. Since in our simulations the mesh changes frequently, but otherwise is quite homogeneous, it is more efficient to precalculate the artificial density and mass and keep it constant throughout the simulation, saving the computational time associated with the eigenvalue estimation.

1.6.4 Parallelization

The computation burden of the described system is mainly due to the calculation of internal forces in every finite element in each cell, and the contact interactions. For large clusters of cells, the number of elements and contact surfaces quickly grows and the computations become fairly involved.

At the same time, most of the calculations are localized and the whole calculation can be effectively distributed over several CPUs without much difficulty. The internal forces in the finite elements can be independently computed, so do the contact/adhesion forces. Since no system of equations needs to be solved in an explicit solution, the overall flow of the program can be distributed over several CPUs. At this moment, our code runs in a multicore, shared memory computer, substantially reducing the computing time compared with a standard serial solution.

1.7 Numerical results

Using all the modeling assumptions described in the previous sections, we show some of the results obtained from a full simulation of the initial stages in the growth of a tumor.

The problem set up is very simple. A single cell, of the type described in Sections 1.2 and 1.3, is created and allowed to grow for one day. After this initial growth, and approximately every hour, the average pressure of the cell is compared with a reference pressure randomly sampled at the cell's birth. As explained in Section 1.5, if the average pressure is lower than the reference pressure, the cell splits. In our simulations, an exponential distribution of mean 120 Pa is chosen to sample the reference pressures.

Each of the newly created cells originally has a volume of approximately $1/125$ of the parent cell, but quickly grows so that the sum of the two volumes equals that of the parent. This initial growth is completely artificial and used for computational purposes only. After the volume is recovered, and hence the original mass, the children cells start to grow according to the model defined in Section 1.2. Figure 1.8 depicts the growth function employed in

our simulations. From time $t = -0.2$ day to time $t = 0$ day, the function g grows linearly from 1 to $125/2$. In this interval, the mass of the parent cell is recovered. The real cellular growth takes place when t is positive, where g is again defined as a linear function peaking at 125 when $t = 1$ day. From that instant, the cell is assumed to be adult, its volume remains constant, and the duplication logic applies to each of the children, as explained before.

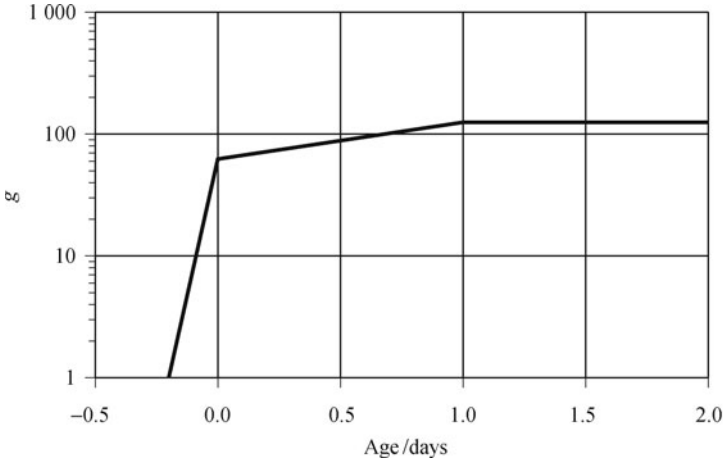


Fig. 1.8 Cell growth function. Negative ages are purely numerical. The value of the growth function remains constant when time $t = 1$ day.

Under this dynamics, the number of cells quickly reaches several hundred, and the tumor radius grows accordingly. In the tumor, exterior cells divide more frequently than interior cells because pressure increases in a cell when it is surrounded by others. In this way, the number of neighbors of a cell, and its relative position within the whole population control its metabolism.

The results shown below correspond to a simulation of 55 days in cell time. At the end of the simulation the total number of cells is 490, and 23 generations have taken place. The mechanical model is integrated for approximately 600 000 steps using the central differences method. In the final instant reported below, the finite element model has over 620 000 elements and 32 000 nodes.

As explained in Section 1.6, the dynamic equilibrium equations are advanced in time using the central differences explicit integrator. Given that the Young modulus of the cells' homogenized material is $E = 15$ kPa and the Poisson's ratio is $\nu = 0.45$, a fictitious cell density is chosen to be $\rho = 10^{20}$ kg/m³. As explained before, this abnormally high density is purely numerical, since the problem is quasistatic, and chosen so that the characteristic time step is of the order of 1 second. To damp out the elastic vibrations, an

artificial Raleigh damping of value 0.1 is employed. Since the quasistatic problem is transformed into a dynamical problem, boundary conditions become unnecessary.

The mechanical interactions among cells, i.e. contact and adhesion, are modeled as described in Section 1.4. Particularly, the contact constant κ_p is automatically selected by the code in such a way that it is as large as possible, but at the same time it does not reduce the critical time step resulting from the cell's elements. The adhesion constant is chosen as $\kappa_a = 2 \times 10^{-3} \mu\text{N}/\mu\text{m}^5$ and the cutoff distance δ_o is a random variable with uniform distribution in the interval $[2, 3.5] \mu\text{m}$.

Figures 1.9 to 1.11 show six snapshots of the tumor development. The coloring in the figures indicates the cell generation, showing higher division activity on the borders. The deformation of cell can be better appreciated in the cross section of Fig.1.12.

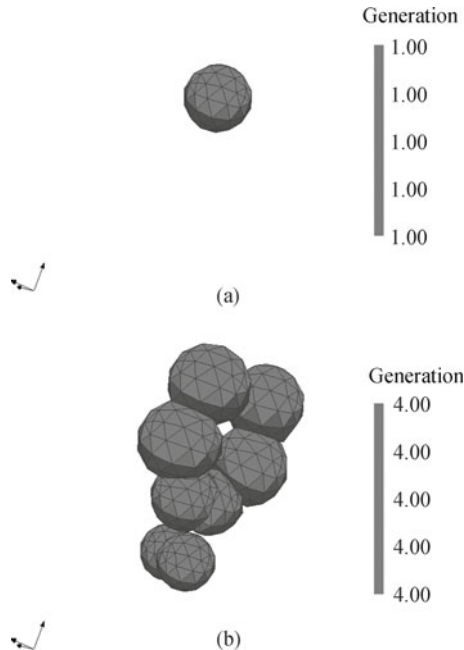


Fig. 1.9 Initial cell (a) and population on day 8 (b) (color plot in the book end).

Figure 1.13 depicts a histogram of the cell age at the moment of division. Since this is random variable, and it is modulated by the average pressure, the distribution is far from uniform. Most cells divide close to their second day of life, but eventually, and especially if their pressure is high, they can live up to 25 days without dividing.

Finally, Fig.1.14 shows an approximate value of the radius of the tumor,

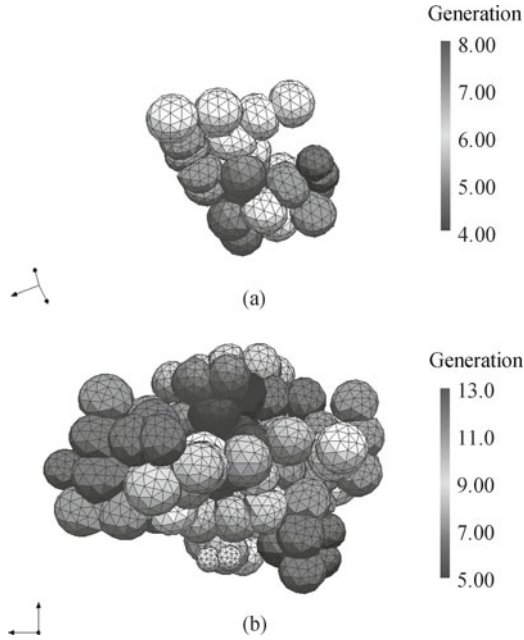
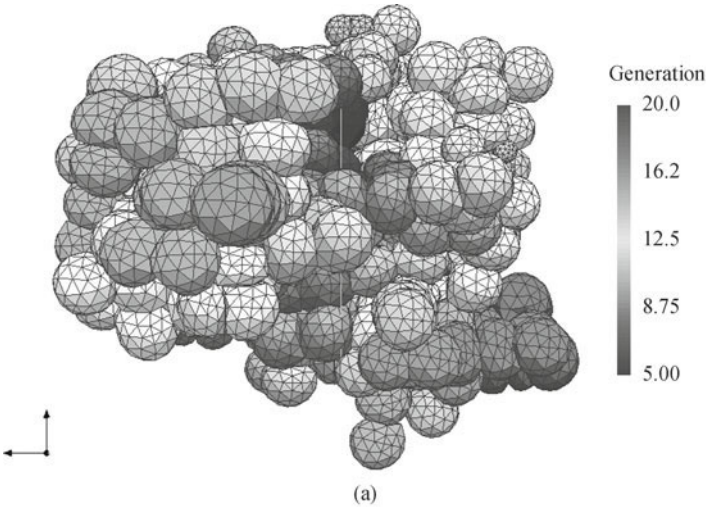


Fig. 1.10 Cell population on day 17 (a) and day 33 (b) (color plot in the book end).

defined as the radius of the smallest sphere enclosing all the cells. The results indicate that the initial radius growth is fast but then the growth becomes linear. This resembles Gompertz law for tumors, which indicates that the radii of solid tumors initially grow exponentially but only linearly at later stages.



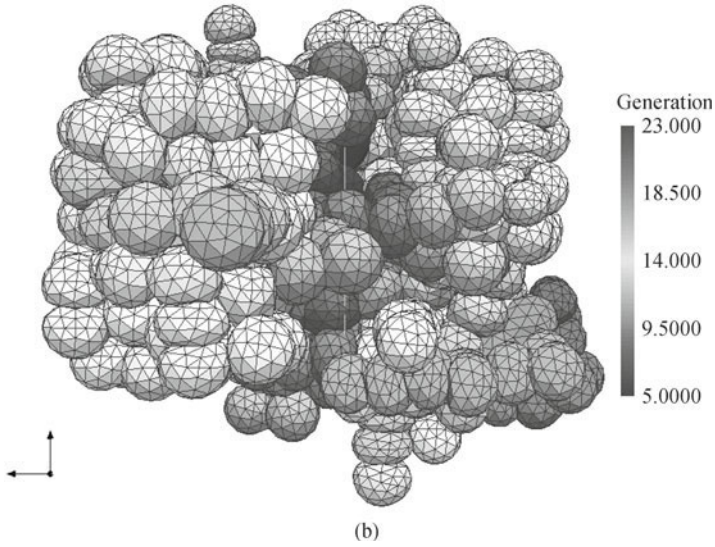


Fig. 1.11 Cell population on day 50 (a) and day 55 (b) (color plot in the book end).

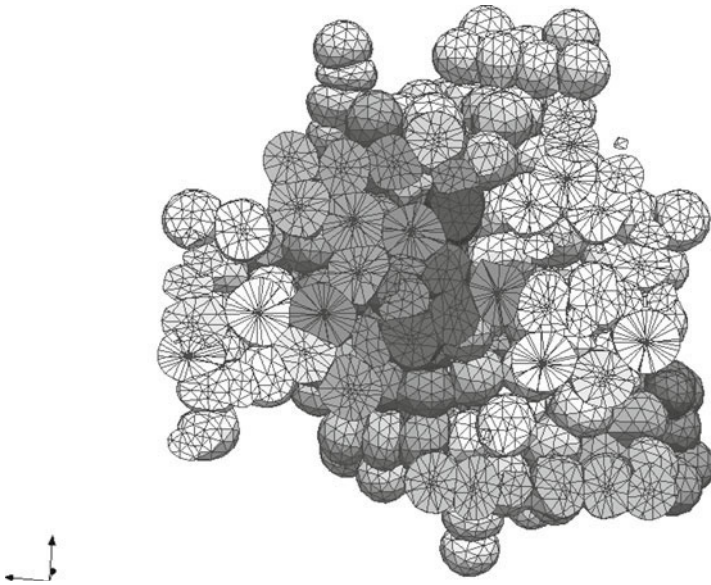


Fig. 1.12 Cluster section (color plot in the book end).

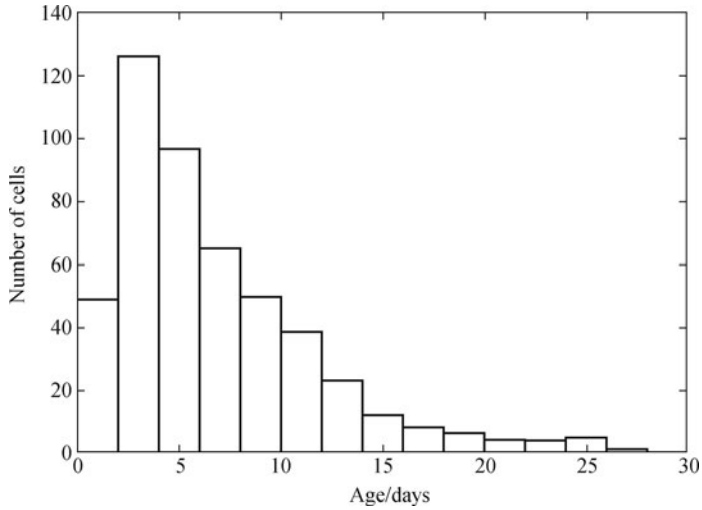


Fig. 1.13 Histogram of cell age at division.

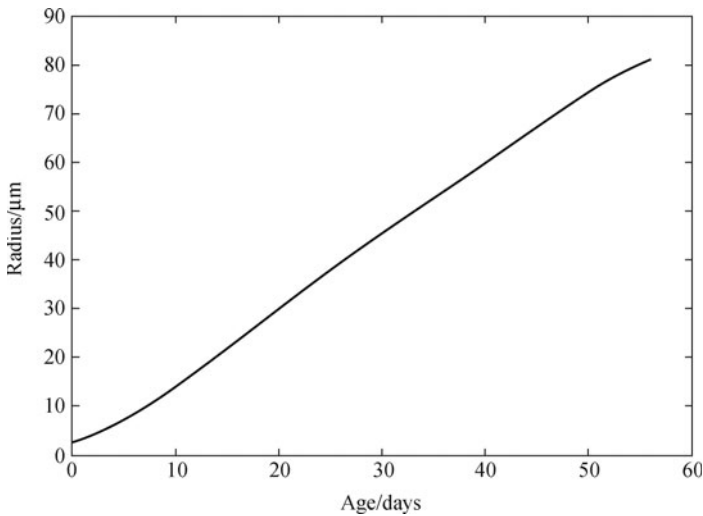


Fig. 1.14 Approximate radius vs age.

1.8 Summary and conclusions

The large scale simulation of growing cell clusters can be approached from the stand point of nonlinear Computational Mechanics. Despite the enormous

complexity of the mechanical behavior of cells and their interactions, simplified models can be formulated which account for the essential phenomena: deformation, growth, cell-to-cell contact/adhesion, and simplified life cycle, improving upon existing cellular automata.

In this work we have proposed a mechanical and computational model of cell ensembles. The simplifications alluded to above result in tractable equations which can be solved using techniques of nonlinear finite elements. The proposed framework includes nonlinear homogenized cell mechanics providing a simple model for deformation and growth. To describe cell interactions, a penalty formulation is used to account for cell-to-cell contact forces, and an effective adhesion model replaces membrane adherence. The involved changes that take place during the life time of a cell are accounted for with a simple logic decision tree which is modulated by the mechanical stimuli over each cell.

An important feature of the design of the presented simulation framework is that each of its essential components is independent of the rest, and can be improved if required for an enhanced resolution. For example, viscoelastic material models can be easily employed for the cells replacing the current hyperelastic ones. Similarly, the adhesion forces can be improved to account for the relative distance of the interacting surfaces. Also, mesh refinement will lead to increased spatial resolution of the stress and pressure fields. In this article, however, we have only employed the simplest models at each stage, leaving for future work possible improvements.

There is, to the authors' knowledge, no other simulation framework with similar capabilities to the one presented herein. Despite its obvious limitations, it offers higher resolution than standard cellular automata, and has enough flexibility to incorporate, if desired, additional features that will enhance its predictive capabilities (for example, transport of nutrients, pH, cell migration, etc.). The model, already as simple as presented herein, has a very high computational cost and all the improvements described above will substantially increase it. The use of distributed computing is the best alternative to alleviate this cost, and we are currently working in this direction.

In the long term, the goals of models such as the one presented in this work are to be able to reproduce the growth/pressure coupling described in [3]. Then, building also on the detailed surface maps of tumors that will be generated by the simulations identify areas of higher activity and model new scenarios resulting from different boundary conditions (both mechanical and chemical). The results presented herein are preliminary and work is in progress in many directions.

Acknowledgements

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Chapter 2 Multiscale Biomechanical Modeling of Stem Cell-Extracellular Matrix Interactions

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Abstract: A multiscale cell model has been developed to study the mechanotransduction of pluripotent stem cells in an attempt to explain the mechanical information exchange between the cells and their extracellular environment leading to a biologic response. In the model, the macroscale cell is modeled as liquid crystal with a hyperelastic nucleus. A nanoscale adhesive model was introduced to describe the interaction between receptors and ligands. We have developed and implemented a Lagrange type meshfree Galerkin formulation and related computational algorithms for the described cell and adhesive contact model. A comparison study with experimental data was conducted to validate the parameters of the cellular computational model. By using a soft matter physics modeling approach, we have simulated the adhesive contact process between cells and different extracellular matrix substrates. The simulation shows that the cell can sense substrate elasticity by responding via cell spreading, altered cell contact configurations, and altered molecular conformations.

Keywords: cell spreading, focal adhesion, hyperelasticity, liquid crystal, meshfree method, multiscale simulations, soft matter contact physics, stem cells

2.1 Introduction

A major recent advance in cellular and molecular biology is the discovery that cell behavior depends on the sensitivity toward both the rigidity and surface micro-structures of the surrounding extracellular matrix (ECM) environment. For example, Discher et al.^[1] and Engler et al.^[2] reported that

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matrix elasticity directs stem cell lineage specification. The ability of the cell to sense the environmental mechanical stimulus and subsequently to mediate its own coordinated responses is defined by the phenomenon of mechanotransduction. As a process of cellular signal transduction in response to mechanical stimuli, mechanotransduction plays an important role in the healthy physiologic processes of cell motility, angiogenesis, embryonic development, tissue regeneration, and wound healing. However, abnormal stimuli may guide mechanotransduction toward a sequelae of diseases in cardiology, orthopedics, neurology, and oncology. The exact molecular mechanism of mechanotransduction related to cellular focal adhesion is unknown and is under active investigation.

Pluripotent and multipotent stem cells are unspecific cells (adult or embryonic) that have two defining properties, they have the ability to: (a) differentiate into other functional cell types in the human body, and (b) self-regenerate. Even though it is generally believed that transcription regulation or genetic factors play an important role in this decision-making process, neither the topology nor the dynamics of the regulatory networks are fully known. If stem cells can sense and respond to physical signals during cell-cell or cell-ECM interactions, then they can integrate and analyze this information, consequently changing their morphology, dynamics, behavior, and eventually their fate^[3].

Understanding the influence of biomechanical factors on the fate of stem cells will eventually help the development of synthetic approaches that may cultivate or trigger stem cells to differentiate into desirable and functional phenotypes^[4]. Because of its scientific and clinical importance, a major focus of molecular cell biology is the study of mechanotransduction with particular focus on stem cells^[5-7]. While understanding the interplay between cellular contractile activity, the stiffness of the surrounding tissues and the resulting mechanical stresses-strains are crucial for establishing a biomechanical-based mechanotransduction model. The physical process of mechanotransduction is realized through the contact and adhesion between cells and their extracellular environment.

Cellular adhesion and spreading depend heavily on the interactions between the cell and the surrounding ECM substrates. When cultured onto artificial adhesive surfaces, cells first flatten and deform extensively as they spread^[8]. Not all cell types respond to substrate stiffness in the same way, but many including endothelia cells^[9], mammary epithelial cells^[10] and mesenchymal stem cells^[2] exhibit increased spreading and adhesion on stiffer substrates compared to compliant ones^[11].

Recently, several general cell contact and focal adhesion models have been proposed, notably Freund and Lin^[12], Ni and Chiang^[13], and Deshpande et al.^[14] Continuum models have also been developed recently to predict cell adhesion in the early stages of culture^[15,16] as well as to simulate cell motility^[17]. In order to understand the precise biomechanical sensing process during cell contact and adhesion and to explain possible mechanotransduction

mechanisms, we have developed the following multiscale cell model. In a recent paper^[18], the present authors have reported some early results of this effort.

This article is organized into six sections: In Section 2.2 we report the construction of our cell and ECM model; in Section 2.3 we discuss the cell adhesion and contact algorithm; Section 2.4 is focused on the meshfree implementation of the computational cell model; in Section 2.5, validation of the cell model and numerical simulations are presented; in Section 2.6 we discuss some important issues of the cell model.

2.2 Cell and ECM modeling

The main objective of this work is to advance stem cell modeling specifically and cell modeling in general. Therefore, we have systematically constructed a cellular biomechanical model by mathematically treating stem cells as soft matter.

2.2.1 Basic hypothesis and assumptions

The cell membrane is essentially a lipid bilayer. Presently, the most successful cell model is the fluid mosaic model, or the lipid bilayer model^[19]. This approach captures two essential features of the lipid bilayer: fluidity and diffusion. A well-established and very successful mechanics or mathematics model for the cell membrane is Helfrich's liquid crystal cell membrane model^[20], which is based on the fluid mosaic model. Because Helfrich's liquid crystal cell membrane model has successfully predicted the bi-concave shape of red blood cells, it has been regarded as the first triumph of soft matter physics.

From a structural viewpoint, a cell consists of the membrane wall, cytoplasm, microtubes, the cell nucleus, and the cytoskeleton or the cell's scaffold. The cell nucleus plays a key role in the cell's response to mechanical forces^[21]. According to Maniotis et al^[22], the intracellular nucleus is about nine times stiffer than the cell's cytoplasm. Based on these observations, we modeled the cell nucleus as a hyperelastic material, which has been used previously by Caille et al^[21] to model the nucleus of endothelia cells. To extend Helfrich's liquid crystal membrane model, we also use a bulk nematic liquid crystal material to model the outer layer of the cell membrane.

The rationale for such a soft matter physics cell model is that cell cytoplasm does not just consist of liquid, it contains cell organelles and many weakly cross-linked polymer networks, such as actin filaments or intermediate filaments. Depending on the phenotype, the content, i.e., microstructure and the concentration, of these filaments may be different. In this work, we

address the modeling of stem cells, where the cell cytoskeleton may be less developed than that of other functional cells. Therefore, the cytoplasm region of our stem cell model contains a lower cytoskeleton component with a considerable amount of liquid. Hence, the liquid crystal model becomes a suitable option for modeling our interpretation of cellular cytoplasm. For simplicity, in this preliminary two-layer cell model, we do not distinguish between the cell membrane and cell cytoplasm. A refined soft matter physics model that distinguishes between the cell membrane and cell cytoplasm will be reported in a follow-up paper.

Here, the ECM is modeled as a substrate consisting of a hyperelastic block, as used previously for both cell and gel models^[23,24] (Fig.2.1). In the following sections, we shall describe both the hyperelastic constitutive model and the liquid crystal model applied in our cell-ECM modeling approach.

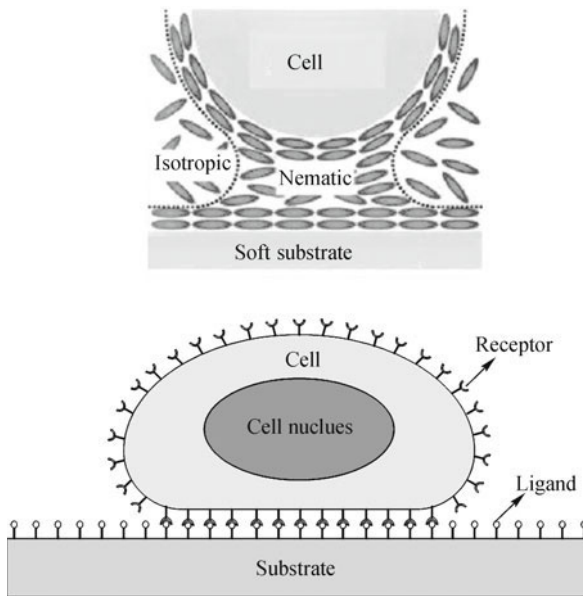


Fig. 2.1 Soft matter physics cell model and soft adhesive contact model.

2.2.2 Hyperelastic model

The hyperelastic constitutive model is used to represent the intracellular scaffold and plasma aggregates, which are assumed to be isotropic and nonlinear, exhibiting an elastic response with large strains. There are more than twenty hyperelastic models for describing rubber-like materials, a comparison of different hyperelastic models can be found in [25]. In our research, we adopted the modified Mooney-Rivlin material^[35] to model the cell nucleus and ECM.

The strain energy density function W for the modified Mooney–Rivlin material is given as

$$W = C_1(I_1 - 3I_3^{\frac{1}{3}}) + C_2(I_2 - 3I_3^{\frac{2}{3}}) + \frac{1}{2}\lambda(\ln I_3)^2 \quad (2.1)$$

where C_1, C_2 and λ are material constants and $\mathbf{C} = \mathbf{F}^T \cdot \mathbf{F}$ is the right Cauchy–Green deformation tensor. The three invariants of the right Cauchy–Green tensor are defined as

$$I_1 = \text{tr}(\mathbf{C}), \quad (2.2)$$

$$I_2 = \frac{1}{2}\{[\text{tr}(\mathbf{C})]^2 - \text{tr}(\mathbf{C}^2)\}, \quad (2.3)$$

$$I_3 = \det(\mathbf{C}). \quad (2.4)$$

The corresponding constitutive relations can be expressed in terms of the second Piola–Kirchhoff stress tensor $\mathbf{S}$, as well as the right Cauchy–Green tensor invariants,

$$\mathbf{S} = 2\{(C_1 + C_2 I_1)\mathbf{I} - C_2 \mathbf{C} - (C_1 I_3^{\frac{1}{3}} + 2C_2 I_3^{\frac{2}{3}} - \lambda \ln I_3)\mathbf{C}^{-1}\}. \quad (2.5)$$

After the second Piola–Kirchhoff stress is obtained, the first Piola–Kirchhoff stress tensor can be immediately computed as $\mathbf{P} = \mathbf{S} \cdot \mathbf{F}^T$, which can then be substituted into the developed meshfree Galerkin formulation to calculate the internal nodal force (shown later).

If the substrate is modeled as a Mooney–Rivlin hyperelastic medium, its elastic stiffness tensor is a fourth order tensor that can be evaluated as

$$\begin{aligned} \mathbb{C} = 4 \frac{\partial^2 W}{\partial \mathbf{C} \partial \mathbf{C}} &= 4C_2 \mathbf{I} \otimes \mathbf{I} + \frac{4}{3}(C_1 I_3^{\frac{1}{3}} + 4C_2 I_3^{\frac{2}{3}} - \lambda)\mathbf{C}^{-1} \otimes \mathbf{C}^{-1} \\ &\quad - 4(C_1 I_3^{\frac{1}{3}} + 2C_2 I_3^{\frac{2}{3}} - \lambda \ln I_3)\mathbf{C}^{-1} \odot \mathbf{C}^{-1} - 4C_2 \mathbb{I}. \end{aligned} \quad (2.6)$$

By making the elastic constants, C_1, C_2 and λ , dependent on spatial coordinates, one can model the substrate with heterogeneous stiffness.

2.2.3 Liquid crystal model

Liquid crystals have biphasic properties by combining liquid and solid characteristics. For instance, a liquid crystal may be fluidic similar to a liquid, while having a long range orientational order and therefore a solid elasticity associated with deformations of the same order. There are many different types of liquid crystal phases (e.g. smectic, nematic, isotropic), which can be distinguished by their different optical properties (e.g. birefringence). In this cell modeling approach, we adopt a simplified version of the Ericksen–Leslie theory^[26] as the governing equations for the nematic liquid crystal

component. The strong forms of the simplified Ericksen–Leslie theory are

$$\rho_0 \frac{D\mathbf{v}}{Dt} = \nabla \cdot \boldsymbol{\sigma} + \mathbf{b}, \quad \forall \mathbf{x} \in V(t), \quad (2.7)$$

$$\rho_0^d \frac{D\tilde{\mathbf{h}}}{Dt} = \gamma \{ \nabla \cdot \nabla \otimes \mathbf{h} - \mathbf{r}(\mathbf{h}) \}, \quad \forall \mathbf{x} \in V(t) \quad (2.8)$$

where $\mathbf{v}$ is the flow velocity field, $\mathbf{h}$ is the nematic liquid crystal director field, $\mathbf{b}$ is the body force in the current configuration, ρ_0 and ρ_0^d are the densities of the fluid and director fields in the reference configuration, respectively. Here, the differential gradient operator is acting in the spatial configuration, i.e., $\nabla := \frac{\partial}{\partial x_i} \mathbf{e}_i$. In addition, γ is the director elastic constant, $\mathbf{r}$ is a Landau–Ginzburg type potential that governs the evolution of the director field

$$\mathbf{r} = \frac{dR(\mathbf{h})}{d\mathbf{h}} = \frac{\mathbf{h}}{\epsilon^2} (|\mathbf{h}|^2 - 1), \quad \text{and} \quad R(\mathbf{h}) = \frac{1}{4\epsilon^2} (|\mathbf{h}|^2 - 1)^2 \quad (2.9)$$

and finally, the Cauchy stress is determined as

$$\boldsymbol{\sigma} = -p\mathbf{I} + 2\mu\mathbf{d} - \eta\nabla \cdot (\nabla \otimes \mathbf{h} \odot \nabla \otimes \mathbf{h}) - \mathcal{G}. \quad (2.10)$$

In Eq.(2.10), $p = \kappa(1 - J)$ is the hydrostatic pressure, κ the bulk modulus, $\mathbf{F}$ the deformation gradient, $J = \det(\mathbf{F})$ the Jacobian determinant, μ viscosity, η a positive constant, and $\mathcal{G}$ the contribution from the contact boundary condition, which can be expressed as

$$\mathcal{G} = \begin{cases} A(\mathbf{h} \cdot \nabla \varphi_s) \mathbf{h} \otimes \nabla \varphi_s & \text{planar anchoring} \\ A[(\mathbf{h} \cdot \mathbf{h}) \nabla \varphi_s - (\mathbf{h} \cdot \nabla \varphi_s) \mathbf{h}] \otimes \nabla \varphi_s & \text{homeotropic anchoring} \end{cases} \quad (2.11)$$

where A is a contact constant.

2.3 Contact and adhesion models for cell-substrate interactions

The interplay between a cell and its ECM is defined by complex interactions between ligands and receptors. In this research approach, we do not model the exact molecular mechanisms of the adhesion phenomenon or the detailed molecular motions during this complex process. Instead, we model an overall adhesion effect between cells and their substrates. The specific attractive adhesion force may be simulated by a cohesive potential, or an attractive potential force, while the steric interaction between the cell membrane and the substrate is treated as the repulsive force.

We have developed two different computational algorithms and modeling techniques to simulate cell adhesive contact: (a) the regular continuum mechanics contact plus an adhesive attraction force, and (b) the cohesive contact.

2.3.1 The adhesive body force with continuum mechanics contact

The main features of the developed computational contact algorithm are: (a) using the regular continuum contact mechanics to simulate a repulsive force, and (b) using a postulated adhesive potential to mimic an attraction force. The continuum contact mechanics algorithm is illustrated in Fig.2.2. In the proposed adhesive contact algorithm, the adhesive force is modeled by a special body force determined by the distance between a point on the cell membrane and its shortest distance to the substrate as described in Eq.(2.13). In this case, we do not model the repulsive force, but adopt the conventional finite element or meshfree contact algorithm to enforce the impenetrable condition between the cell and its substrate. By assuming that the density and

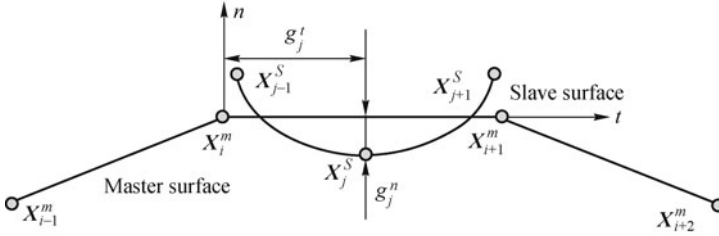


Fig. 2.2 A penetrated slave particle and corresponding master segment.

the size of the substrate are much greater than the density and the size of the cell, we may neglect the direct adhesive force from the cell to the substrate. The total virtual work contribution from the adhesive contact force may be written as

$$\delta\Pi_{AC} = \int_{\Omega_0} B(r) \frac{\mathbf{r}}{r} \cdot \delta \mathbf{u} d\Omega + \int_{\Gamma_c^{(1)}} \mathbf{f}^c \cdot \delta \mathbf{g} dS \quad (2.12)$$

where the adhesive attraction force is modeled by the body force

$$B(r) = Ge^{-\frac{r}{d_0}} \quad (2.13)$$

where G and d_0 are constants and $\mathbf{r}$ is the position vector between nodal particles on the cell and the corresponding surface element of the substrate. For the adhesive contact, the membrane may be in contact with the ECM. During this process, interpenetration of the cell membrane and the ECM surface is not permitted. The impenetrable condition is enforced to identify the repulsive force by using finite element-based continuum mechanics contact algorithm. Hence, the second integral in Eq.(2.12) is a surface integral, in which $\mathbf{g}$ is the gap vector, and $\mathbf{f}^c$ is being modeled as the repulsive normal force plus a contact frictional force.

The basic concept behind the classical contact procedure is that the two contacting bodies first penetrate into each other within a single explicit time integration step while additional nodal forces are introduced into the contacting nodal points so that the impenetrability conditions are strictly enforced.

We adopted the exact enforcement of the impenetrability condition in a single time step as previously described^[27].

In our contact simulations, both cell and ECM can be made deformable. Typically the contacting surfaces are designated as “master” and “slave”. Here, we treated the cell’s outer surface as the slave and the ECM substrate surface as the master.

The contact algorithm begins with prediction of the slave particles at time step n , the contact-detection algorithm is then used to search all the inter-penetration particles based on the determinant value of the meshfree moment matrix^[28]. When all penetration points are detected, the next step is to calculate the normal gap and relative tangential velocity between the intrusion slave particles and the closest master surface locations (Fig.2.2). The procedures are outlined as follows:

- (i) Calculating the normal (n) and tangential (t) gaps

$$g_j^n = (\mathbf{x}_j^s - \mathbf{x}_i^m) \cdot \mathbf{n}_i \quad (2.14)$$

$$g_j^t = (\mathbf{x}_j^s - \mathbf{x}_i^m) \cdot \mathbf{t}_i \quad (2.15)$$

with

$$\mathbf{t}_i = \frac{\mathbf{x}_{i+1}^m - \mathbf{x}_i^m}{\|\mathbf{x}_{i+1}^m - \mathbf{x}_i^m\|} \quad (2.16)$$

$$\mathbf{n}_i = \mathbf{e}_3 \times \mathbf{t}_i \quad (2.17)$$

where $\mathbf{n}_i$ is the outward normal vector of the i -th master segment matching with the j -th penetrated slave particle, $\mathbf{e}_3$ is the unit vector pointing outward from the plane.

- (ii) Calculating the normal and tangential forces

The contact force $\mathbf{f}^c$ has two components: the normal repulsive contact force and the tangential contact friction force. The normal direction repulsive force may be defined by

$$\mathbf{f}_j^n = \frac{2M_j^s g_j}{\Delta t^2} \mathbf{n}_i = f_j^n \mathbf{n}_i. \quad (2.18)$$

In the tangential direction, the classical Coulomb friction model is adopted in modeling the forces between the slave body and the master body. To enforce the stick or static condition, we have

$$\mathbf{f}_j^{\text{stick}} = -\frac{M_j^s}{\Delta t} \mathbf{v}_j^t. \quad (2.19)$$

The tangential force cannot exceed the force limit at which the interface can hold. After reaching the limit, a slip or dynamic condition should be applied

$$\mathbf{f}_j^{\text{slip}} = -|\mu_k f_j^n| \frac{\mathbf{v}_j^t}{\|\mathbf{v}_j^t\|}. \quad (2.20)$$

The tangential force shall be the minimum of these two forces or

$$\mathbf{f}_j^t = -\min(|\mu_k \mathbf{f}_j^n|, \|\mathbf{f}_j^{\text{stick}}\|) \frac{\mathbf{v}_j^t}{\|\mathbf{v}_j^t\|} \quad (2.21)$$

where μ_k is the friction coefficient which is dependent upon the two interacting surface materials, $\mathbf{v}_j^t$ is the relative tangential velocity between the j -th slave particle and the i -th master segment.

(iii) Updating the contact force for each master contact particle

During the simulation, the substrate is deformable. Therefore, the contact force needs to be added to the master nodal particles to make sure that the total force is balanced. We use linear interpolation to distribute the contact forces of the two nodal particles to the corresponding master segment,

$$\mathbf{f}_i^n = -(1 - \alpha) \mathbf{f}_j^n \quad (2.22)$$

$$\mathbf{f}_i^t = -(1 - \alpha) \mathbf{f}_j^t \quad (2.23)$$

$$\mathbf{f}_{i+1}^n = -\alpha \mathbf{f}_j^n \quad (2.24)$$

$$\mathbf{f}_{i+1}^t = -\alpha \mathbf{f}_j^t \quad (2.25)$$

where

$$\alpha = \frac{g_j^t}{\|\mathbf{x}_{i+1}^m - \mathbf{x}_i^m\|}, \quad (0 \leq \alpha < 1). \quad (2.26)$$

(iv) Redistributing the contact forces to neighboring particles within the substrate

The nodal force vectors calculated above are the exact vectors for each penetrating slave particle and the corresponding master nodal surface particles. In the meshfree approach, we redistribute each exact nodal force to its supporting nodal particle. After the force redistribution, the contact force at particle I becomes

$$\bar{\mathbf{f}}_I = \sum_{J=1}^{n_{\text{node}}} N_I(\mathbf{X}_J) \mathbf{f}_J. \quad (2.27)$$

In the finite element method (FEM) interpolation, $N_I(\mathbf{X}_J) = \delta_{IJ}$, we then recover the exact nodal force vector.

In living biologic cell contact situations, the spatial densities for both ligands and receptors change with time during the adhesive process. In fact, both ligands and receptors are capable of self-assembly, a complex mechano-diffusion process^[12].

We hypothesize that the density of the receptors is directly related to the spatial density and magnitude of the director field associated with the liquid crystal theory, so that the weak forms of Eqs.(2.39) and (2.40) are explicitly coupled through the spatial density and magnitude defined by the director field, i.e., $\beta_1(t) = c_1 \rho^d(t) \|\mathbf{h}\|$ and $\beta_2(t) = c_2 \rho^d(t) \|\mathbf{h}\|$, where c_1 and c_2 are

constants. A cellular model coupling mechano-diffusivity and the described contact/adhesion theory within a soft matter physics modeling approach will be presented in a separate paper. As a preliminary study presented in this paper, we consider that the densities of ligands and receptors within the contact zone remain fixed.

In this work, the interface between ligands and receptors is modeled as an interactive zone that separates the cell from the substrate, and the adhesive body force distribution varies with the magnitude of the gap distribution. The adhesive interaction is strong near the contact zone, and it decays as material points move away from the contact zone. The attractive component of the adhesive contact model is similar to a special version of the coarse-grain FEM adhesion contact algorithm as proposed for long-range van der Waals forces by Sauer and Li^[29].

2.3.2 The cohesive contact model

As mentioned above, the interfacial zone between ligands and receptors is modeled as a gap (< 100 nm) that separates the cell and its substrate, and the cohesive force distribution varies with the magnitude of the gap distribution.

In our meshfree simulation, we adopt a cohesive potential^[30]. A similar potential was used to model the interactions between cell and a rigid substrate when studying cell crawling^[17]

$$\Phi(r) = \sigma \left[\left(\frac{\varepsilon}{r} \right)^4 - 2 \left(\frac{\varepsilon}{r} \right)^2 \right] \quad (2.28)$$

where σ is the energy depth and r is the gap length at a specific location. The adhesive force vector can be calculated as

$$\mathbf{F}(r) = -\frac{\partial \Phi}{\partial \mathbf{r}} = \phi'(r) \frac{\mathbf{r}}{r} \quad (2.29)$$

where

$$\phi'(r) = \frac{4\sigma}{\varepsilon_0} \left\{ \left(\frac{\varepsilon}{r} \right)^5 - \left(\frac{\varepsilon}{r} \right)^3 \right\}. \quad (2.30)$$

Taking the ligand-receptor bond density into consideration, the adhesive force between the cell membrane and the substrate surface can be calculated as follows:

$$\mathbf{f}(r) = N_b \mathbf{F}(r) = N_b \frac{4\sigma}{\varepsilon_0} \left\{ \left(\frac{\varepsilon}{r} \right)^5 - \left(\frac{\varepsilon}{r} \right)^3 \right\} \frac{\mathbf{r}}{r} \quad (2.31)$$

where N_b is the bond density. Here we choose $N_b = 10\,000 \mu\text{m}^{-2}$, $\varepsilon_0 = 10$ nm, $\sigma = 0.024$ pN $\cdot \mu\text{m}$ from Roy and Qi^[17] as well as $\varepsilon = 100$ nm.

In the meshfree computation, the cohesive force between the cell and substrate is calculated based on the following point-interaction algorithm.

For the surface particle j associated with the cell membrane, we assign

$$\mathbf{f}_j^C = \mathbf{f}_{ij} \quad (2.32)$$

where $\mathbf{f}_{ij}$ is the force between the j -th surface particle of the cell and the corresponding i -th master surface segment of the substrate (Fig.2.3) and

$$\mathbf{f}_{ij} = N_b \phi'(r_{ij}) \frac{\mathbf{r}_{ij}}{r_{ij}}, \quad \mathbf{r}_{ij} = \mathbf{r}_i - \mathbf{r}_j, \quad \text{and} \quad r_{ij} = |\mathbf{r}_{ij}|. \quad (2.33)$$

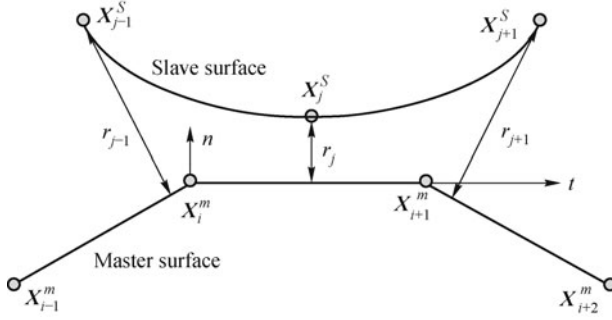


Fig. 2.3 Cell surface particles and corresponding substrate surface segments.

As noted in the protocol, the contact force needs to be added to the master nodal particles to make sure that the total force is balanced. A linear interpolation distributes the contact force to the two nodal particles of the corresponding substrate segment as follows:

$$\mathbf{f}_i^S = -(1 - \alpha) \mathbf{f}_j^C, \quad (2.34)$$

$$\mathbf{f}_{i+1}^S = -\alpha \mathbf{f}_j^C \quad (2.35)$$

where

$$\alpha = \frac{(\mathbf{x}_j^C - \mathbf{x}_i^S) \cdot \mathbf{t}_i}{\|\mathbf{x}_{i+1}^S - \mathbf{x}_i^S\|}, \quad (0 \leq \alpha < 1) \quad (2.36)$$

with

$$\mathbf{t}_i = \frac{(\mathbf{x}_{i+1}^S - \mathbf{x}_i^S)}{\|\mathbf{x}_{i+1}^S - \mathbf{x}_i^S\|}. \quad (2.37)$$

Again, the nodal force vectors here are the exact vectors for each penetrating slave particle and corresponding master nodal surface particle. In meshfree approach, Eq.(2.27) is then applied as described earlier along with the FEM interpolation.

$$\bar{\mathbf{f}}_I = \sum_{J=1}^{n_{\text{node}}} N_I(\mathbf{X}_J) \mathbf{f}_J. \quad (2.38)$$

2.4 Meshfree Galerkin formulation and the computational algorithm

In order to simulate cell spreading over the substrate under extremely large deformation, a Lagrangian formulation is developed for the soft matter physics cell model under finite deformation, and a Galerkin weak formulation is derived and applied in the follow-up numerical computation. The main advantage of adopting a meshfree Lagrangian formulation in simulation is its ability to avoid remeshing and easy tracking the cell surface and its interface. Consequently, the corresponding simulation results are insensitive to mesh-distortion during cell spreading, of which the mesh-based FEM has difficulties in handling such large deformations. Moreover, the ability to track a dynamic interface makes the calculation of surface curvature and hence surface tension much easier than that of the Eulerian formulation.

As noted previously, the numerical simulations are conducted by using meshfree methods^[31]. The meshfree method has advantages in computing large deformation problems compared with traditional FEM. In our meshfree simulation, both the cell and its substrate are discretized by a set of particles and then further represented by interpolation functions (Fig.2.4).

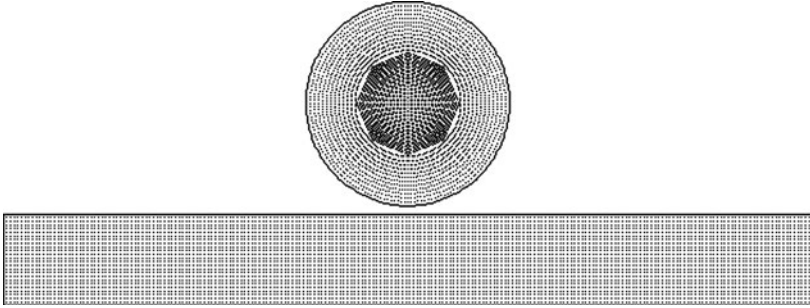


Fig. 2.4 Meshfree discretization of the cell and extracellular matrix model.

The weak form of balanced linear momentum under finite strain condition can be expressed as

$$\begin{aligned}
 & \sum_{i=1}^2 \int_{\Omega_0^{(i)}} \rho_0^{(i)} \ddot{\mathbf{u}}^{(i)} \cdot \delta \mathbf{u}^{(i)} d\Omega^{(i)} + \sum_{i=1}^2 \int_{\Omega_0^{(i)}} \mathbf{P}^{(i)} : \delta \mathbf{F}^{(i)} d\Omega^{(i)} \\
 &= \sum_{i=1}^2 \int_{\Omega_0^{(i)}} \rho_0^{(i)} \mathbf{B}^{(i)} \cdot \delta \mathbf{u}^{(i)} d\Omega^{(i)} + \sum_{i=1}^2 \int_{\Gamma_t^{(i)}} \bar{\mathbf{T}}^{(i)} \cdot \delta \mathbf{u}^{(i)} dS^{(i)} \\
 &+ \sum_{i=1}^2 \delta \Pi_{AC}^{(i)}
 \end{aligned} \tag{2.39}$$

where $\mathbf{B}$ is the body force, $\mathbf{P}$ is the first Piola–Kirchhoff stress, $\bar{\mathbf{T}}$ is the

prescribed traction on the traction boundary $\Gamma_t^{(i)}$, $i = 1$ corresponding to cell and $i = 2$ corresponding to the ECM substrate. Note the last terms in Eq.(2.39), $\delta\Pi_{AC}^{(i)}$, denote the virtual work contribution from adhesive contact, which will be discussed in detail in the next section.

The nematic director evolution is described for the cell only, due to simplicity. We do not introduce $i = 1$ in the formulation. Therefore, a weak form of the governing equation can be expressed as

$$\begin{aligned} \int_{\Omega_0} \rho_0^d J \frac{D\mathbf{h}}{Dt} \cdot \delta\mathbf{h} d\Omega &= - \int_{\Omega_0} \gamma \{ (\mathbf{F}^{-1} \cdot \mathbf{F}^{-T}) \cdot (\nabla_{\mathbf{x}} \otimes \mathbf{h}) \} : (\nabla_{\mathbf{x}} \otimes \delta\mathbf{h}) d\Omega \\ &+ \int_{\Gamma_t} \gamma \{ \mathbf{N} \cdot (\mathbf{F}^{-1} \cdot \mathbf{F}^{-T}) \cdot (\nabla_{\mathbf{x}} \otimes \mathbf{h}) \} \cdot \delta\mathbf{h} dS \\ &+ \int_{\Gamma_c} \gamma \{ \mathbf{N} \cdot (\mathbf{F}^{-1} \cdot \mathbf{F}^{-T}) \cdot (\nabla_{\mathbf{x}} \otimes \mathbf{h}) \} \cdot \delta\mathbf{h} dS \\ &- \int_{\Omega_0} \gamma J \mathbf{r}(h) \cdot \delta\mathbf{h} d\Omega \end{aligned} \quad (2.40)$$

where Γ_c denotes the contact boundary, assuming that

$$\mathbf{N} \cdot (\mathbf{F}^{-1} \cdot \mathbf{F}^{-T}) \cdot (\nabla_{\mathbf{x}} \otimes \mathbf{h}) = \mathbf{0}, \quad \forall \mathbf{x} \in \Gamma_t$$

as well as

$$\mathbf{h} = \bar{\mathbf{h}} \Rightarrow \delta\mathbf{h} = 0, \quad \forall \mathbf{x} \in \Gamma_c.$$

This considers the following meshfree interpolation

$$\mathbf{u}(\mathbf{X}, t) = \sum_{I=1}^{n_{\text{node}}} N_I(\mathbf{X}) \mathbf{d}_I(t), \quad (2.41)$$

$$\mathbf{h}(\mathbf{X}, t) = \sum_{I=1}^{n_{\text{node}}} N_I(\mathbf{X}) \mathbf{h}_I(t). \quad (2.42)$$

Note that even though the director field, $\mathbf{h}(\mathbf{X}, t)$, is essentially a part of the displacement gradient, the robustness of the meshfree computation allows us to use equal-order interpolation without severe numerical instability.

Following the standard meshfree discretization procedure^[31], we obtain the following discrete equations of motion with the understanding that those equations apply to both the cell and the substrate

$$\mathbf{M} \ddot{\mathbf{d}} = \mathbf{f}^{\text{ext}} - \mathbf{f}^{\text{int}}(\mathbf{d}) \quad (2.43)$$

where $\mathbf{M}$ is the lumped mass matrix, $\mathbf{f}^{\text{int}}$ is the internal force array arising from the current state of stress, $\mathbf{f}^{\text{ext}}$ is the external force array including

body forces and surface traction and contact forces, where

$$\mathbf{M}_{IJ} = \int_{\Omega_0} \rho_0 \mathbf{N}_I \mathbf{N}_J d\Omega, \quad (2.44)$$

$$\mathbf{f}_I^{\text{int}} = \int_{\Omega_0} P_{iJ} \mathbf{N}_{I,J} \mathbf{e}_i d\Omega, \quad (2.45)$$

$$\mathbf{f}_I^{\text{ext}} = \int_{\Omega_0} \rho_0 \mathbf{B}_i \mathbf{N}_I \mathbf{e}_i d\Omega + \int_{\Gamma_t} \bar{\mathbf{T}}_i \mathbf{N}_I \mathbf{e}_i dS + \int_{\Gamma_c} \bar{\mathbf{f}}_i \mathbf{N}_I \mathbf{e}_i dS. \quad (2.46)$$

At time $t_{n+1} = t_n + \Delta t$, the discrete equation of motion can be written as

$$\mathbf{M} \mathbf{a}_{n+1} = \mathbf{f}_{n+1}^{\text{ext}} - \mathbf{f}_{n+1}^{\text{int}}. \quad (2.47)$$

If the central difference scheme is used in the time integration, we have

$$\mathbf{d}_{n+1} = \mathbf{d}_n + \Delta t \mathbf{v}_n + \frac{1}{2} \Delta t^2 \mathbf{a}_n, \quad (2.48)$$

$$\mathbf{a}_{n+1} = \mathbf{M}^{-1} (\mathbf{f}_{n+1}^{\text{ext}} - \mathbf{f}_{n+1}^{\text{int}}), \quad (2.49)$$

$$\mathbf{v}_{n+1} = \mathbf{v}_n + \frac{1}{2} \Delta t (\mathbf{a}_n + \mathbf{a}_{n+1}) \quad (2.50)$$

where $\mathbf{d}$, $\mathbf{a}$, $\mathbf{v}$ denote the nodal displacement, acceleration and velocity arrays, respectively.

For the nematic liquid crystal theory, we can define the internal force for the director field as

$$\mathbf{f}_I^h = - \left(\int_{\Omega_0} \mathcal{P}_{i,J} N_{I,J}(\mathbf{X}) \mathbf{e}_i d\Omega + \int_{\Omega_0} \gamma J r_i N_I(\mathbf{X}) \mathbf{e}_i d\Omega \right) \quad (2.51)$$

where the generalized internal force for the director field is then defined as

$$\mathcal{P} = \gamma (\mathbf{F}^{-1} \cdot \mathbf{F}^{-\text{T}}) \cdot (\nabla_{\mathbf{x}} \otimes \mathbf{h}). \quad (2.52)$$

If the central difference scheme is again used in the time integration, we have

$$\mathbf{v}_{n+1}^h = \mathbf{M}_h^{-1} \mathbf{f}_{n+1}^h, \quad (2.53)$$

$$\mathbf{h}_{n+1} = \mathbf{h}_n + \Delta t \mathbf{v}_{n+1}^h. \quad (2.54)$$

2.5 Numerical simulations

As an application of soft matter physics and a multiscale contact-adhesion algorithm, we have uniquely simulated cell-ECM contact and adhesion mechanics. To ensure a meaningful simulation, we have first conducted a validation test of the proposed cell model. In doing so, we identified critical parameters of the soft matter model. We then applied the verified material model in order to simulate contact between a cell and various substrates with different stiffnesses. We have also simulated the interaction between a cell and a substrate with non-uniform stiffness. Finally, we have also compared simulation results obtained from the two defined contact-adhesion algorithms.

2.5.1 Validation of the material models

To validate the proposed cell model, we have simulated cell deformation under direct compression and compared those results with experiment measurements of endothelial cells^[21]. Here, a constant force is applied to the top and bottom rigid microplates, while the boundary nodes are in contact with the cell surface. The classical contact algorithm is then applied at the two contact surfaces. In the simulation, the cell deformation is defined as the relative reduction in height, i.e., $(H_0 - H)/H_0$. We first set the whole cell as a hyperelastic Mooney-Rivlin material and fit the force-deformation curve (Fig.2.5c) to get the material constants. Then we input the fitted mathematically-defined hyperelastic Mooney-Rivlin material within the cell nucleus. The material outside the nucleus region is modeled as a nematic liquid crystal.

Figure 2.5a and b demonstrates the cell shape before and after deformation. The force-deformation curve is plotted in Fig.2.5c. The applied compressive forces increase non-linearly as a function of the cell height reduction. From the simulation (Fig.2.5c and Fig.2.6), one can find that the force re-

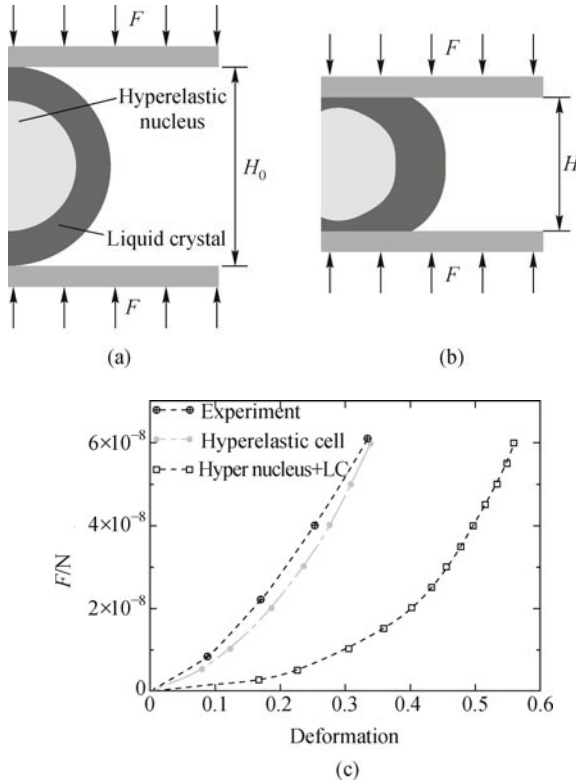


Fig. 2.5 Validation of the cell model: (a) before deformation, (b) after deformation, (c) comparative force-deformation curves.

quired to create the same deformation for endothelial cells is greater than that for stem cells, which is reasonable considering that stem cells are structurally underdeveloped.



Fig. 2.6 Deformation of the cell model under force $F = 3 \times 10^{-8}$ N: (a) hyperelastic cell, (b) liquid crystal cell with a hyperelastic nucleus.

Since the two phenotypes are dramatically different, it is inaccurate to fit the stem cell model with the experimental data obtained from endothelial cells. It is apparent that our stem cell model is much softer than previous characterizations of endothelial cells.

2.5.2 Cell response in four different stiffness substrates

The cell is modeled as a vertical circular plate in two-dimensions (2D) with a diameter of $D = 10 \mu\text{m}$. The substrate is modeled as a vertical 2D slice sectioned from a horizontal plate with a resulting dimension of $(L \times H = 39.78 \mu\text{m} \times 4.5 \mu\text{m})$. The exact problem arrangement is shown in Fig.2.7. Plain strain is assumed in the simulation. In the meshfree computation, a total of 4 455 particles are used in the discretization of the cell, and 5 525 particles are used to form the meshfree discretization of the substrate. The time step is chosen as $\Delta t = 5 \times 10^{-12}$ s. The dilation parameters are selected as $(\rho_x = 1.48\Delta x, \rho_y = 1.48\Delta y)$ and $(\Delta x = 0.174 \mu\text{m}, \Delta y = 0.2 \mu\text{m})$ for the cell, $(\Delta x = 0.18 \mu\text{m}, \Delta y = 0.18 \mu\text{m})$ for the substrate. The nucleus of the cell is modeled as a hyperelastic Mooney–Rivlin material, where the initial density is $\rho^0 = 1.0 \times 10^3 \text{ kg/m}^3$ and the material constants are $C_1^n = 2.126 \times 10^3 \text{ Pa}$, $C_2^n = 1.700 \times 10^2 \text{ Pa}$ and $\lambda^n = 1.700 \times 10^5 \text{ Pa}$. The region beyond the hyperelastic nucleus is modeled as a nematic liquid crystal, where the density of the liquid crystal is chosen as $\rho^0 = 1.0 \times 10^3 \text{ kg/m}^3$, the density of the director field is $\rho_d^0 = 1.0$ and the material properties and constants are $\kappa = 2.2 \times 10^9 \text{ Pa}$, $\mu = 1.0 \times 10^{-3} \text{ kg/(m} \cdot \text{s)}$, $\eta = 5.0 \times 10^{-8}$, $\varepsilon = 1.0 \times 10^{-6}$, $\gamma = 1.0 \times 10^{-4}$, as defined earlier. The friction coefficient for the contact algorithm is chosen as $\mu_k = 0.1$. Finally, the substrate is modeled as a hyperelastic Mooney–Rivlin material. Four different substrates

with different stiffness are considered in this analysis. The densities for the four substrates are the same as the cell nucleus. We set the material constants $C_1 = 1.265 \times 10^4$ Pa, $C_2 = 1.012 \times 10^3$ Pa and $\lambda = 1.012 \times 10^6$ Pa. The material properties for the four different substrates are chosen as

$$\begin{aligned} C_1^{S1} &= C_1, & C_1^{S2} &= 2C_1, & C_1^{S3} &= 5C_1, & C_1^{S4} &= 10C_1 \\ C_2^{S1} &= C_2, & C_2^{S2} &= 2C_2, & C_2^{S3} &= 5C_2, & C_2^{S4} &= 10C_2 \\ \lambda^{S1} &= \lambda, & \lambda^{S2} &= 2\lambda, & \lambda^{S3} &= 5\lambda, & \lambda^{S4} &= 10\lambda. \end{aligned}$$



Fig. 2.7 Computational model of cellular spreading on different substrates (the colors represent different modeled materials, color plot in the book end).

The classical continuum contact algorithm has been used in these simulations. The constants $G = 9.8 \times 10^6$ N/kg and $d_0 = 1.0 \times 10^{-5}$ m are used for the adhesive potential force (Eq.(2.13)). The cell is held static initially and the initial gap between the cell and substrate is held at 400 nm. The bottom surface of the substrate is fixed during the entire simulation time. The adhesive forces will draw the cell into contact with the substrate, and then the cell will spread under the adhesive and contact forces. Simulations have been carried out for cells in contact with four substrates of different elastic moduli (stiffnesses) as described above.

From these simulations, one may observe the cell spreading over time. In Fig.2.8 and Fig.2.10, we display the cell shapes and stress contours over four different substrates with different stiffnesses under the same contact conditions over the same time-frame. One may find that the contact between the cell and softest substrate (Substrate-I) (Fig.2.8a and Fig.2.10a) generates the least cell spreading, and the contact between the cell and Substrate-II (Fig.2.8b and Fig.2.10b) has the second least cell spreading, while the contact between the cell and Substrate-III (Fig.2.8c and Fig.2.10c) has the second greatest spreading. When the substrate stiffness continues to increase, the cell on the Substrate-IV has the greatest spreading (Fig.2.8d and Fig.2.10d).

It may be noted that although Case IV generates the greatest cell spreading, the difference between Case IV and Case III is very small, which means the spreading may not increase any more when the stiffness of the substrate reaches a specific value. However, within a certain range, cell spreading area is directly related to the stiffness of the substrate, and it is purely a phenomenon of a soft (lower) elasticity. Based on this model, the stem cell can

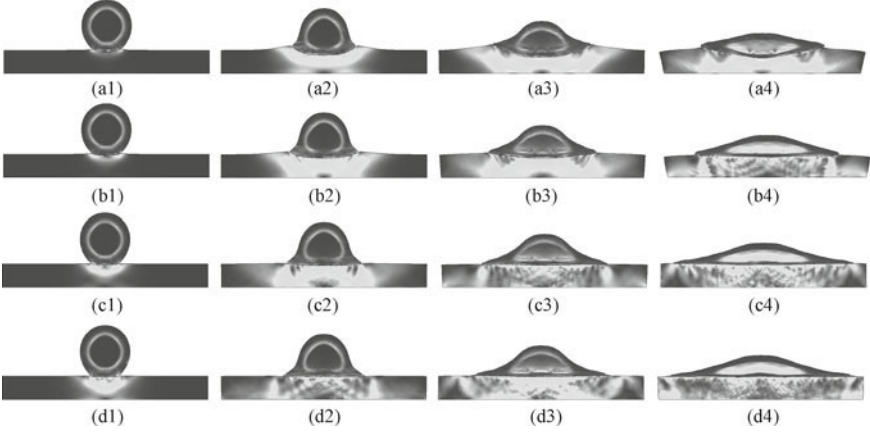


Fig. 2.8 Cell spreading over substrates with different stiffnesses (classical contact theory): (a) Substrate-I, (b) Substrate-II, (c) Substrate-III, (d) Substrate-IV.



Fig. 2.9 Cell nucleus configurations with different ECM stiffness(classical contact): (a)=b4, (b)=d4.

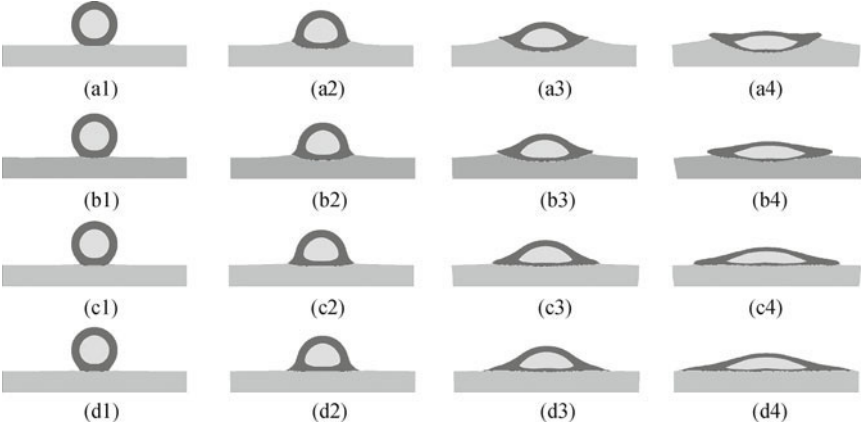


Fig. 2.10 Cell spreading over substrates with different stiffnesses (cohesive contact): (a) Substrate-I, (b) Substrate-II, (c) Substrate-III, (d) Substrate-IV.

be interpreted as a mechanical sensor, which can translate the mechanical information (material properties) of the substrate into its resulting shape, configuration, and size. As an overall perspective, this is the definition of mechanotransduction.

Zooming in to Fig.2.8, we juxtapose the two last sequences of cell contact

process in Fig.2.9. From Fig.2.9b, one finds that under the stiff substrate, the shape of the cell nucleus becomes concave in contact with the convex shape of the cell nucleus under softer substrates. This finding indicates that the deformed shape of the cell nucleus also depends on the stiffness of the ECM. We have identified this as strong evidence supporting cell mechanotransduction without incorporating the concept of “tensegrity” on the structure of cell scaffold^[5]. In fact, embryonic stem cells do not have well-developed cell-scaffold structures. To the best of authors’ knowledge, this type of cell responses has not been previously reported.

2.5.3 Cell response to a stiffness-varying substrate

It is well known that cells prefer to migrate towards stiffer regions on a substrate with a stiffness gradient. This phenomenon is known as durotaxis. In our simulation, the material properties of the substrate are dependent upon a function of position x to observe the cell spreading motion in various directions

$$\begin{aligned} C_1^S &= C_1(0.1 + 9.9r), \\ C_2^S &= C_2(0.1 + 9.9r), \\ \lambda^S &= \lambda(0.1 + 9.9r) \end{aligned}$$

where r is defined as: $r = (x + L/2)/L$ with the center of the substrate at $x = 0$. The exact problem arrangement is shown in Fig.2.11. In Fig.2.12 and Fig.2.13, we display a time sequence of a cell contacting a stiffness-varying deformable substrate from two different contact algorithms. Without varying the stiffness within the substrate, the cell should move equally in both left and right side directions. From the simulation results, one can see immediately that the cell moves towards the right side much faster than to the left side of the substrate due to the stiffness gradient. This indicates a preference by the

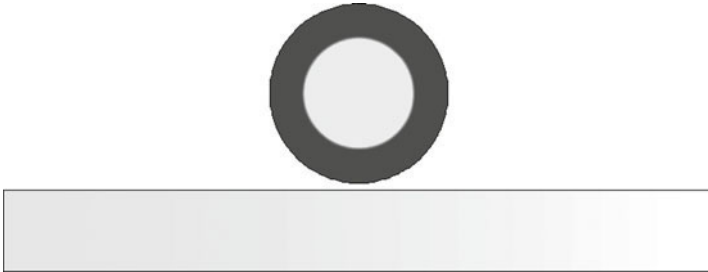


Fig. 2.11 Computational model of cell spreading on a stiffness-varying substrate, the color in the substrate stands for different stiffnesses, the yellow color at the right end indicates the highest stiffness (color plot in the book end).

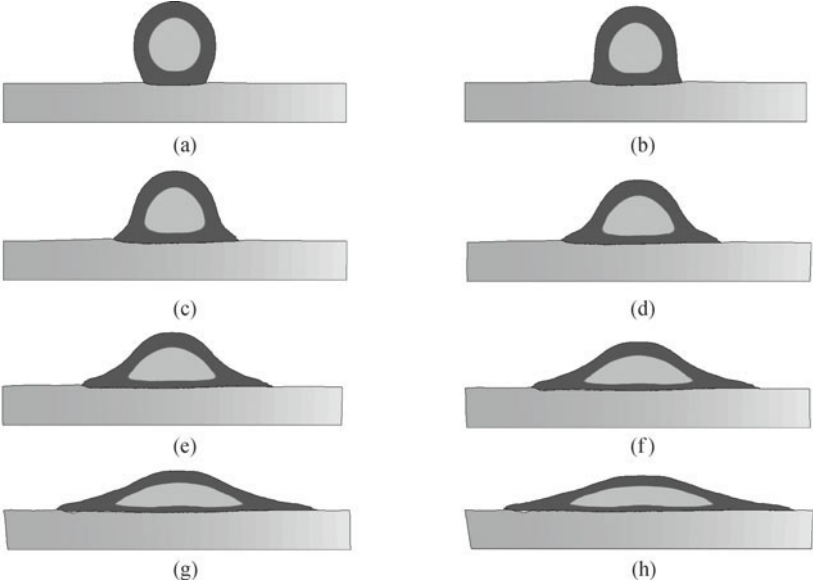


Fig. 2.12 Time sequence of cell contact with a stiffness-varying substrate (classical contact theory) (color plot in the book end).

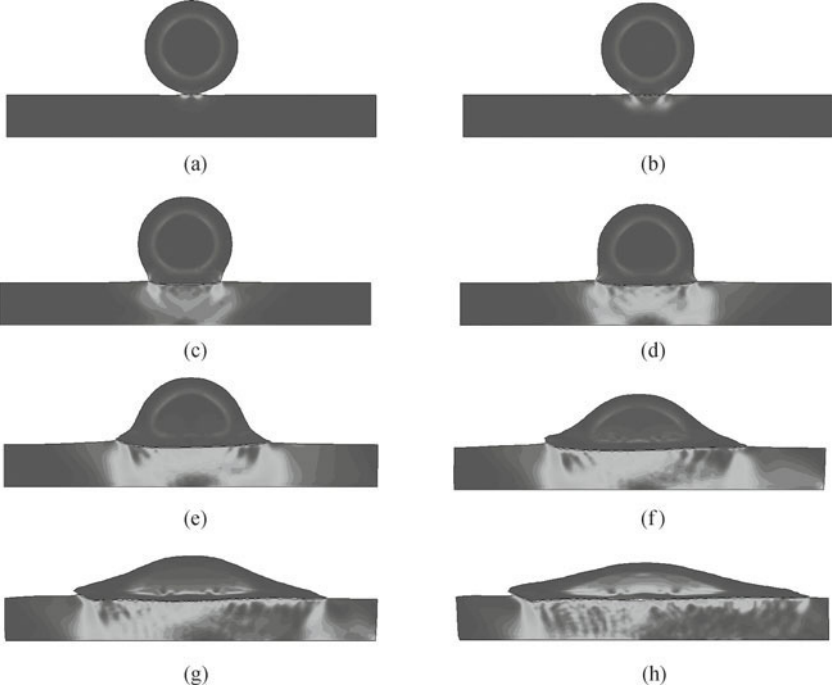


Fig. 2.13 Time sequence of cell contact with a stiffness-varying substrate (stress contour from the cohesive contact model, color plot in the book end).

cell in favor of a stiffer ECM substrate. These results generally agree with reported experimental measurements^[32,2] as well as numerical simulations^[13] of cell adhesion and migration.

2.5.4 Comparison of two different contact algorithms

We also compared two contact algorithms in this simulation. In both approaches, the cell is modeled as a nematic liquid crystal with a hyperelastic nucleus while the substrate is modeled as a hyperelastic material.

In the classical contact algorithm, the adhesion force is acting on every particle in the cell as a body force, while the repulsion force only acts on the surface particles. The interaction between the outer layer and the core of the cell quickly draws the whole body of the cell toward the substrate.

In the second contact algorithm, the colloidal adhesive force quickly decays when the distance between a point and the contact zone increases. Thus, the primary effect is on the cell membrane when the cell and substrate are in direct contact with each other.

Although the two contact algorithms give similar results, the mathematical physics behind each approach is different. For example, the second contact algorithm is more mechanically based. If one takes a close look at the simulation results (Fig.2.12 and Fig.2.13), one can identify that the results from the cohesive contact approach give a clearer indication that the cell is indeed in favor of a stiffer substrate (color plot in the book end).

2.5.5 Three-dimensional simulation of cell spreading

In the following simulations, the cell is modeled as a spherical ball in three-dimensions (3D), with a diameter of $D = 10 \mu\text{m}$ (Fig.2.14). The substrate is modeled as a 3D horizontal circular plate with a dimension of $(R \times H = 15 \mu\text{m} \times 5 \mu\text{m})$. The cell nucleus in the 3D model is smaller than in the 2D model. In the meshfree computation, a total of 4 341 particles are used in discretizing the cell and 16 640 particles are used to form the discretization of the substrate. The nucleus of the cell is modeled as a hyperelastic Mooney–Rivlin material. In this original presentation, we have developed a 3D computational algorithm and modeling techniques to simulate the cell adhesive contacts.

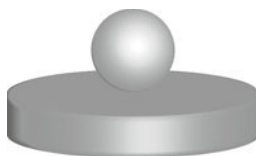


Fig. 2.14 The 3D computational model.

Previous results have indicated a similar 3D cell spreading. In Fig.2.15, we display the cell shapes and stress contours associated with three different substrate stiffnesses under the same contact conditions and time frames. As expected within a certain range, cell spreading area is directly proportional to the stiffness of the substrate.

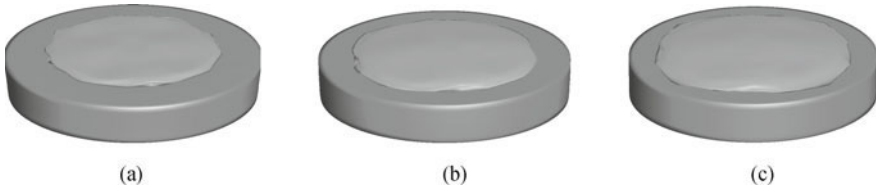


Fig. 2.15 Cell spreading over substrates with different stiffnesses: (a) Substrate-I, (b) Substrate-II, (c) Substrate-III (color plot in the book end).

Since this is a mesoscale continuum simulation, one can measure the stress level at every position of the cell. In Fig.2.16, we display computation results that have been brought about for cells in contact with three substrates of different elastic moduli (stiffness). We found that the stress distributions in both the cell and the substrate are different for different substrate elasticities, which show that the cell can sense the substrate elasticity and response accordingly. The signals between the cell and substrate are hypothetically transmitted through the adhesive contact interaction models. In addition, we have sampled the effective stress in the cell nucleus at the same time instance. We have then plotted the magnitude of the effective stress versus the different values of substrate stiffness. One can clearly observe that as the substrate stiffness increases, the magnitude of the effective stress increases (Fig.2.17). Similar correlations have been observed in experimental measurements of the contractile forces of stem cells^[1]. In Fig.2.18, we display the time sequence of a cell contacting on the soft Substrate-I. The color contour is the effective stress contour and the white arrow represents the director field. Comparing with the 2D results, 3D simulations provide a possible way to capture the cell morphology changes previously described^[13].

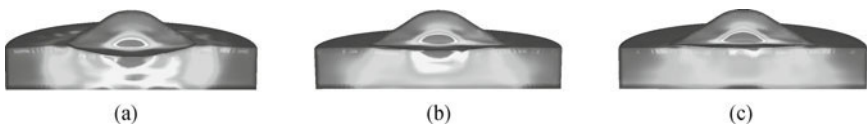


Fig. 2.16 Effective stress over substrates with different stiffnesses: (a) Substrate-I, (b) Substrate-II, (c) Substrate-III (color plot in the book end).

Recent experimental results have demonstrated that geometrical properties such as cell shape and configuration play an important role in regulating stem cell lineage^[33]. Particularly, experimental results^[34] have shown that the advancing adhesion front of a circular contact zone associated with a 3D

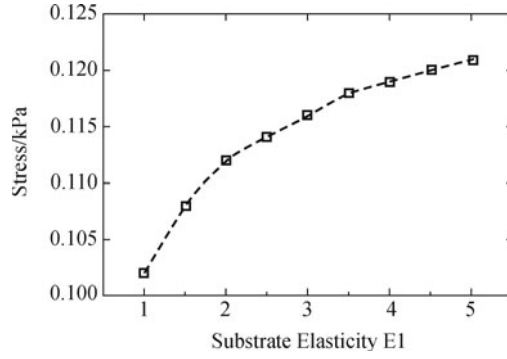


Fig. 2.17 Effective stress vs. elastic stiffness of the substrate.

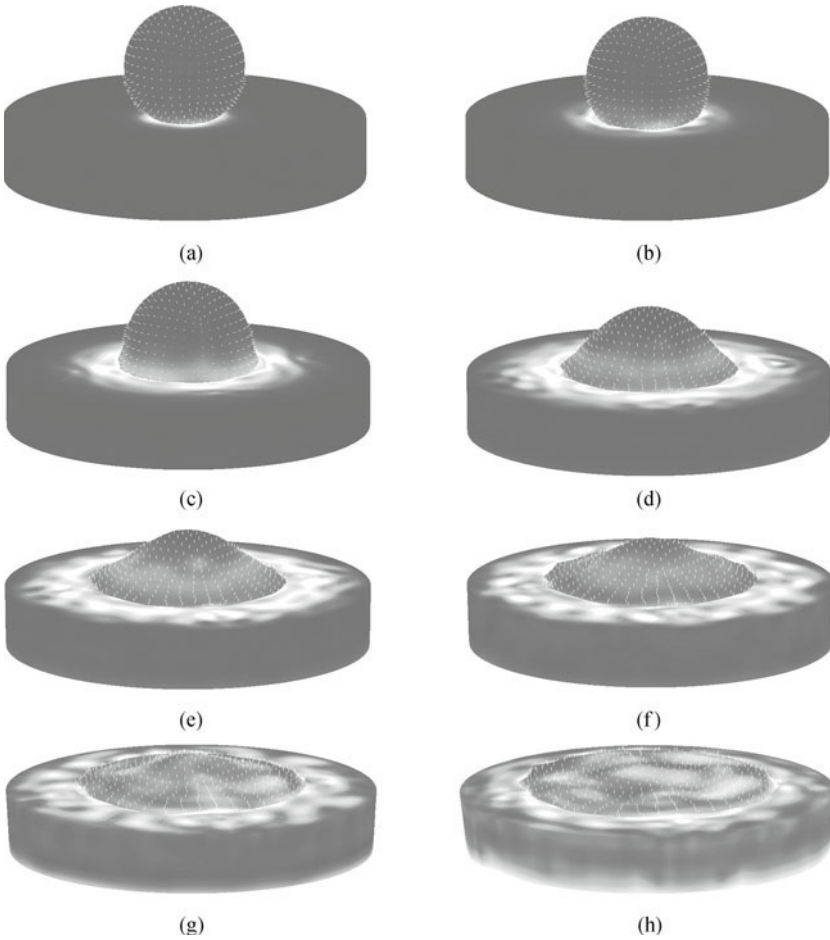


Fig. 2.18 Time sequence of cell contact with the soft Substrate-I (color plot in the book end).

vesicle is unstable under small perturbation. Once achieving a critical value, it easily becomes modulated or sinusoidal in nature. This particular feature of cell spreading can be captured by our soft matter physics cell model numerically. In Fig.2.19, a time sequence of cell spreading images on a soft substrate from a top-down view is displayed, which reveals a clear picture of the spreading modulation as the contact area increases.

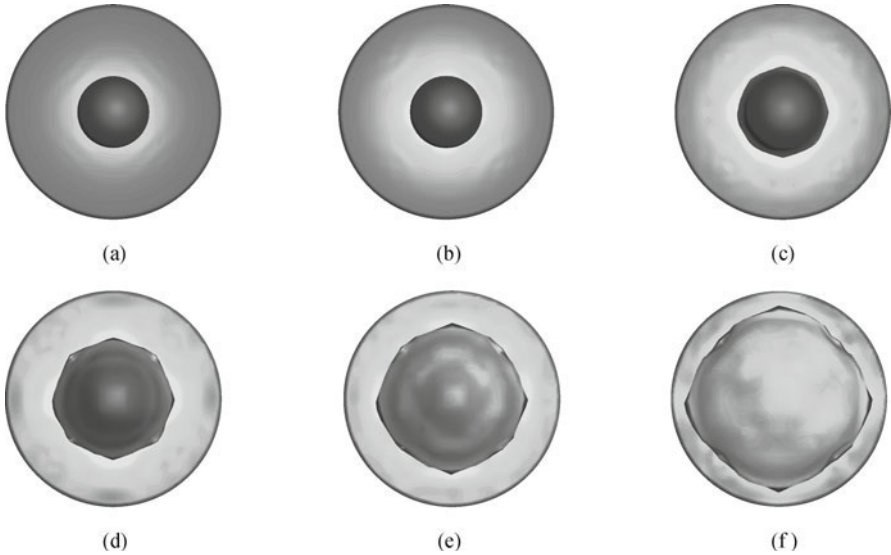


Fig. 2.19 Time sequence of cell contact with the soft Substrate-III (top view) (color plot in the book end).

2.6 Discussion and conclusions

In conclusion, the described soft matter physics cell model may be applicable to simulations of stem cell contact. The presented models provide possible explanations of cell mechanotransduction and other adaptive issues at the larger scale level.

Our simulations have shown that by using the proposed soft matter physics cell model, when a cell is in contact with a substrate, (a) its local effective stress measurement may depend on stiffness of the substrate, (Fig.2.17); (b) the area of cellular spreading depends on the stiffness of extracellular substrate (Fig.2.8, Fig.2.10, Fig.2.15); (c) during the soft contact iteration, the cell is in favor of stiffer substrate (Fig.2.12 and Fig.2.13); (d) the adhesion front of the cell will become non-uniform once the spreading radius reaches a certain value (Fig.2.19).

It should be noted that cell behavior, in particular stem cell behavior, is a complex biological phenomenon. The proposed soft matter physics cell model

is only intended to model the mechanical behavior of cells at a coarse level, which may not explain the molecular mechanisms of cell motion, evolution, and proliferation. An in-depth study requires an understanding of molecular cell biology including all relevant concepts in biochemical, biophysical, and biomechanical factors as well as their interactions at different scales.

Developing soft matter physics models for stem cells may help us understand the biomechanical and biochemical behavior of cells. It has been shown in this paper that the soft matter approach can offer explanations regarding the interaction between stem cells and its mechanical niche. In some cases, the soft matter physics model has even shown a predictive power. It is the authors' opinion that by combining the soft matter model with a molecular simulation, we may be able to achieve qualitative prediction of cell behavior while corroborating with experimental observation. The predictive stem cell model may provide both scientific insight and clinical guidance on a host of health care problems, such as regenerated medicine and drug design and delivery problems.

The soft matter physics cell model presented in this work is indeed primitive, but it may provide a useful approach for more realistic and more accurate modeling of cells, especially stem cells. Eventually more sophisticated models will simulate self-assembly of focal adhesions, cell division dynamics, cellular proliferation and so on.

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Chapter 3 Modeling of Proteins and Their Interactions with Solvent

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Abstract: Sick cell anemia is the first disease whose cause was pinpointed at a genetic level. Hydrophobic interaction is the main cause for sickle hemoglobin (hemoglobin S) sticking to itself. Interaction of water with hydrophobic objects plays a major role in molecular self-assembly processes^[1-3], as well in the process of aggregation of hemoglobin S. In this chapter, slow motion analysis and model reduction methods were employed for the study of hemoglobin-hemoglobin interaction. Through this study, a new computational framework was presented for calculating the normal modes and interactions of proteins, macromolecular assemblies, and surrounding solvents. The framework employs a combination of Molecular Dynamics (MD) simulation and Principal Component Analysis (PCA). It enables the capture and visualization of the molecules' normal modes and interactions over time scales that are computationally challenging, providing a starting point for experimental and further computational studies of protein conformational changes. We hope that the modeling protocols or procedures established for this known pathology will eventually shed some light on other complex biological systems that are not well known.

Keywords: protein, coarse-graining, multi-scale modeling, molecular dynamics, and hemoglobin

3.1 Introduction

Computational and mathematical models help biologists and medical researchers to understand the causes of disease, molecular level processes, and much more. These models uncover a multitude of biological facts, such as genome sequences and protein properties. Experimental and computational

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methods are integrated into biological research to better understand complex biological systems. Computational biology provides powerful methods to address critical scientific questions outside a laboratory.

Understanding a protein's mechanics is a prerequisite for getting an insight into its biological function, since most proteins perform their functions through structural deformation, also called conformational change. Such conformational change has been modeled by atomic level simulation such as molecular dynamics (MD) simulation.

It is difficult to theoretically explain and predict the conformational change in protein binding or interaction, though there are standard methods to experimentally probe this phenomenon. These methods include fluorescence resonance energy transfer, X-ray crystallography^[4], cryoelectron microscopy (cryo-EM)^[5], NMR^[6], etc. Conformational change is not accessible to MD simulation available today due to its computational cost. It happens on millisecond scales^[7,8] compared to MD simulation on nanosecond scale. Therefore other computational methods must be applied. One of these methods is normal mode analysis (NMA), which has been proven useful for studying collective motion of biological macromolecules. In addition, NMA with coarse-grained modeling of protein structures of large proteins has been a computational alternative to atomic level simulation. Studies^[9-11] show that some of the lowest frequency normal modes of several proteins, including hemoglobin, are strongly correlated with the conformational change.

Proteins and other biological macromolecules are large and chemically inhomogeneous. Their dynamic behavior or conformational changes are in time scales from femtosecond (high-frequency local bond vibration) to millisecond (protein folding)^[12]. This complex system is difficult to study theoretically. Although NMA provides information about slow protein motion^[10,11], its harmonic approximation limits its use to small amplitude motion in the potential energy surface associated with a local minimum^[13]. It is difficult, using NMA, to analyze such large-scale effects as the hydrophobic effect^[14], which plays an important role in protein-protein interaction. A combination of MD simulation and principal component analysis (PCA) provides a systematic way to study protein slow motion. This method overcomes some of the difficulties of NMA.

Protein-protein interaction such as macromolecular docking has been studied extensively. The ultimate goal is the prediction of the three-dimensional structure of the macromolecular complex as it would occur in a living organism. If the bond angles, bond lengths, and torsion angles of the components are not modified at any stage of formation of the complex, it is known as rigid body docking. In general, hemoglobin-hemoglobin interaction caused by hydrophobic interaction could be treated as rigid body docking. Docking processes that include conformational change, or flexible docking are much more complicated. Flexible docking could, in theory, be studied by quantum mechanics and other methods. Slow motion analysis is suitable for some rigid body docking.

Sickle cell anemia is the first disease whose cause was pinpointed at a genetic level. The switch of a single DNA base pair in the hemoglobin gene from A to T changes an amino acid in hemoglobin from glutamic acid to valine^[15]. Red blood cells (RBCs) are composed largely of hemoglobin. In normal RBCs, hemoglobin is globular. Normal hemoglobin tends to form a protective layer of surrounding water molecules. This protective water coating keeps hemoglobin molecules separating from each other. Normal RBCs have a flexible membrane and can easily squeeze through capillary vessels. In the mutated hemoglobin molecule, one normally hydrophilic spot becomes slightly hydrophobic and, in deoxygenated states, tends to lose its protective layer of water molecules. Consequently, the hemoglobin molecules tend to stick together and form a chain of hemoglobin beads. In a cascading event, such chains form bundles, eventually altering the red blood cell membrane from flexible to stiff. As a result, the affected RBCs switch from their normal dumbbell shape to a sickle shape. Stiff sickle cells tend to block capillary vessels and directly contribute to sickle cell anemia.

Hydrophobic interaction is the main cause for sickle hemoglobin (hemoglobin S) sticking to itself. Interaction of water with hydrophobic objects plays a major role in molecular self-assembly processes^[1-3] as well in the process of aggregation of hemoglobin S.

In this chapter, slow motion analysis and model reduction methods were employed for the study of hemoglobin-hemoglobin interaction. Through this study, a new computational framework was presented for calculating the normal modes and interactions of proteins, macromolecular assemblies and surrounding solvents. The framework employs a combination of MD simulation and PCA. It enables the capture and visualization of the molecules' normal modes and interactions over time scales that are computationally challenging, providing a starting point for experimental and further computational studies of protein conformational changes.

The new multiscale methodology based on Singular Value Decomposition (SVD) and Principal Component Analysis (PCA) was applied to a relatively small mutant T4 phage lysozyme. These results are compared with published data obtained by NMA and finite element methods. The eigenmodes captured are in quantitative agreement with previously published results.

With this success on a small protein, the same technique will be implemented in a much more complicated hemoglobin-hemoglobin molecule interaction model, in which thousands of atoms in hemoglobin molecules are coupled with hundreds of thousands of T3 water molecule models. In this model, complex inter-atomic and inter-molecular potentials are replaced by nonlinear springs. We employ the same method to get the most significant modes and their frequencies of this complex dynamical system. More complex physical phenomena can then be further studied using these coarse-grained models with SVD-based model reduction methods. We also hope that the modeling protocols or procedures established for this known pathology will eventually shed some light on other complex biological systems that are not

as well-known.

3.2 Classical molecular dynamics

In classical molecular dynamics (MD), atomic trajectories are computed by solving Newton's equation of motion Eq.(3.1), which is a system of second order ordinary differential equation (ODE),

$$\mathbf{M} \frac{d^2}{dt^2} \mathbf{q}(t) = -\nabla U(\mathbf{q}(t)), \quad (3.1)$$

where $\mathbf{q}$ is the position vector, $\mathbf{M}$ is the diagonal mass matrix, $U(\mathbf{q})$ is the potential energy, and $-\nabla U(\mathbf{q})$ represents the force vector.

In practice, the following system of first order ODEs are solved,

$$\dot{\mathbf{q}} = \mathbf{M}^{-1} \mathbf{p}, \quad \dot{\mathbf{p}} = -\nabla U(\mathbf{q}), \quad (3.2)$$

where $\mathbf{p}$ is the momentum vector.

The potential energy, U , is typically given by

$$U = U^{\text{bo}} + U^{\text{nbo}}, \quad (3.3)$$

$$U^{\text{bo}} = U^b + U^a + U^d + U^i, \quad (3.4)$$

$$U^{\text{nbo}} = U^{LJ} + U^e. \quad (3.5)$$

Note that U^{bo} terms are bonded potential, U^{nbo} terms are unbonded potential, U^b and U^a terms are harmonic (linear and angular spring) potentials that model covalent bond interaction, U^d terms are dihedral potential, U^i terms are improper potential, U^e terms are the coulomb potential, and U^{LJ} terms model a van der Waals attraction and hard core repulsion. The coefficients in these potentials are determined experimentally, often aided by theoretical approaches such as *ab initio* quantum mechanical calculation.

It is unrealistic to expect that accurate trajectories can be computed in long-time intervals in MD simulation because MD trajectories are chaotic. One can only expect that the trajectories will have the correct statistical properties, which can be verified by using the initial velocities randomly generated from a Maxwell distribution.

The computational difficulty of molecular dynamics simulation resides in the computation of the force $\mathbf{f}_i$ that is a gradient of an anharmonic potential field prescribed to all atoms. Further, the time step for integrating the equation of motion is typically in the order of femtoseconds, whereas protein function occurs at much larger time scale, from nanoseconds to seconds. The accessible time scale for molecular dynamics is commonly in the order of nanoseconds^[7,8]. However, Ref.[16] reported that millisecond-scale MD simulation has been performed on Anton, which is a recently completed special-purpose supercomputer designed for MD simulation of biomolecular systems. Even with this considerable improvement, MD simulation may be

computationally inhibited for large protein mechanics, where large spatial and temporal scales are required.

3.2.1 Coarse-grained model

Coarse-grained models have been widely used for large-scale protein analysis, which enormously reduces the degrees of freedom. Since the dominant motion of a protein structure is represented by a carbon backbone chain^[17], in coarse-grained models protein structure is represented by α -carbon atoms for the protein backbone chain. Moreover, the high computational costs usually arise from the complicated anharmonic potential field. Consequently, the simplification of such a potential field for a protein structure described by α -carbon atoms is the key issue for the coarse-grained modeling of proteins.

3.2.1.1 Normal mode analysis

Normal mode analysis (NMA) is a computational alternative to atomic simulation such as MD for understanding large protein mechanics^[9,18-20]. The principle of NMA is similar to that typically employed for structural mechanics. Once the stiffness matrix (Hessian matrix) for a structure is constructed, the modeling analysis provides the vibration information of such a structure. The stiffness matrix for a protein structure is usually established based on the computation of the second gradients of anharmonic potential field prescribed to all atoms. In general, calculation of a stiffness matrix is implemented at equilibrium position, which is obtained by minimization of anharmonic potential. This implies that for large proteins, the computation of a stiffness matrix along the minimization process is a computationally expensive process.

NMA is also referred to as quasi-harmonic analysis^[21], since the modal analysis is implemented with harmonic approximation to potential energy V for small displacement,

$$V \approx V_o + \frac{1}{2} \sum_{i,j} K_{ij} (r_{ij} - r_{ij}^o)^2, \quad (3.6)$$

where r_{ij} is the displacement between atom i and j with $\mathbf{r}_{ij} = \mathbf{r}_j - \mathbf{r}_i$, r_{ij}^o is the initial displacement, and K_{ij} is the Hessian matrix (stiffness matrix) for a protein structure given by $K_{ij} = \partial^2 V / \partial r_i \partial r_j$.

Quasi-harmonic analysis is used to solve the eigenvalue problem such as $K_{ij} v_j = \omega^2 m_i v_i$, where ω is the natural frequency, v_i is the normal mode corresponding to natural frequency ω , and m_i is the atomic mass for i^{th} atom. The cross-correlation matrix representing the thermal fluctuation motion can be computed from equilibrium statistical mechanics theory,

$$L_{ij} = \sum_{n=1}^{3N} \frac{k_B T}{m_i} \omega_n^2 (v_i \otimes v_j), \quad (3.7)$$

where k_B is the Boltzmann's constant, and T is the absolute temperature.

The subscript n for natural frequency and normal mode represents the mode number^[22]. It should be noted that summation goes from 7 to $3N$, where N is the total number of atoms, and rigid body modes correspond to six zero eigenmodes.

3.2.1.2 Gō model

Ref.[23] introduced a more simplified potential field for α -carbon atoms so that α -carbon atoms are prescribed by a potential field consisting of covalent bonds for consecutive α -carbon atoms and non-bonded interaction (i.e. van der Waal's interaction) for native contacts. The thermal fluctuation behavior of protein structures has been well described by Gō model.

Low-frequency normal modes relevant to protein dynamics are insensitive to details of potential field^[21]. Ref.[23] pointed out that short-range interaction may govern the protein dynamics. Furthermore, the motion of protein structure fits well with a backbone chain represented by α -carbon atoms. Gō potential can be addressed as follows:

$$V \approx \sum_i \left[\frac{k_1}{2} (r_{i,i+1} - r_{i,i+1}^o)^2 + \frac{k_2}{4} (r_{i,i+1} - r_{i,i+1}^o)^4 \right] + \sum_{i,j} 4\epsilon \left(\frac{1}{r_{i,j}^6} - \frac{1}{r_{i,j}^{12}} \right), \quad (3.8)$$

where r_{ij} is the distance between i^{th} and j^{th} α -carbon atoms.

Superscript zero indicates the equilibrium state. The first summation represents the nonlinear elastic energy for covalent bonds, while the last summation shows the non-bonded interaction for native contact. Native contact is defined so that α -carbon atoms i and j are in the native contact if r_{ij} is less than a specific cut-off distance, such as 10 Å.

3.2.1.3 Elastic network model

In an elastic network model (ENM), the system is represented by a network of beads connected by elastic springs. Generally one bead represents one amino acid as shown in Fig.3.1. However, elastic network models can be used with an all-atom description as well. The ENM is widely used for its simplicity. Although ENM only includes harmonic fluctuation, it correctly represents the topology of the system and yields the right pattern of the principal modes, which normally associate with protein functions.

ENM was first studied by Ref.[24]. The author assumed the harmonic approximation to potential field prescribed to α -carbon atoms. The harmonic potential field can be presented only for native contacts and covalent bonds with identical force constant,

$$V \approx \sum_{i,j} \frac{\gamma}{2} (r_{ij} - r_{ij}^o)^2 H(r_c - r_{ij}^o), \quad (3.9)$$

where γ is a force constant, r_{ij} is the distance between i^{th} and j^{th} α -carbon atoms, superscript o indicates the equilibrium state, r_c is the cut-off distance

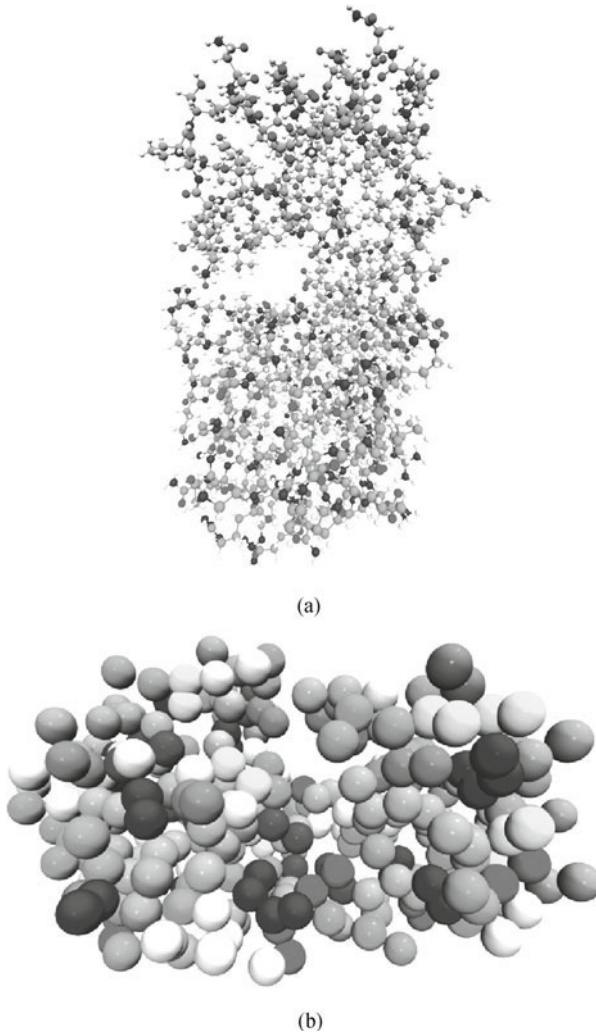


Fig. 3.1 Model T4 Lysozyme (PDB: 3lzm) with elastic network mode. Each bead stands for one residue and is connected with abstract springs within a cutoff distance. Colored by atom type: H white; O red; N blue; C cyan; S yellow; P tan: (a) molecular structure with CPK view, (b) elastic network model (color plot in the book end).

defining a native contact, and $H(x)$ is the Heaviside unit step function defined as $H(x) = 0$ if $x < 0$; otherwise $H(x) = 1$.

3.2.2 High performance computing

The implementation of Principal Component Analysis (PCA) and other analytic methods in this work involve massive-matrix manipulative operation and other computational work. They are computationally expensive. There are very few tools to do such analysis efficiently. A parallel computing package was developed and the details are explained in Section 3.5.1.

High-performance computing (HPC) uses supercomputers and computer clusters to solve advanced computation problems. In this research, Franklin, a massively parallel processing (MPP) system, was used to test the methods and produce the result.

Franklin belongs to National Energy Research Scientific Computing Center. It has 9 572 computer nodes and each node has quad processor core in October 2010 with a theoretical peak performance of 9.2 GFlop/sec per core (4 Flop/cycle if using *SSE128* instructions). More information about Franklin system is listed in Table 3.1.

Table 3.1 Franklin system specifications (<http://www.nersc.gov/nusers/systems/franklin/about.php>)

Franklin Specifications	
Number of compute nodes	9 572
Processor cores per node	4
Number of compute processor cores	38 288
Number of spare compute nodes	20
Processor core type	Opteron 2.3 GHz quad core
Processor core theoretical peak	9.2 GFlop/sec
System theoretical peak (compute nodes only)	352 TFlop/sec
Physical memory per compute node	8 GB
Memory usable by applications per node	7.38 GB
Number of login nodes	10
Switch interconnect	SeaStar2
Measured MPI point-to-point bandwidth	1.6 GB/sec
Measured MPI point-to-point latency	6.5 – 8.5 μ s
File system	Lustre
Usable disk space	436 TB
Theoretical IO bandwidth	32 GB/sec aggregate
Batch system	Torque/Moab

3.3 Principal component analysis

As a linear combination of all state variables, for instance, nodal unknowns in finite element analysis, the general coordinate $\xi(t)$ can be introduced as follows:

$$\xi(t) = \sum_{i=1}^m \phi_i x_i(t) = \boldsymbol{\phi}^T \mathbf{x}(t), \quad (3.10)$$

where m represents the total number of channels or state variables and $x_i(t)$ is the i^{th} time-dependent variable.

If the variance of $\xi(t)$ within the time interval $[t_0, t_1]$ is maximized, the data spreads mostly in this general direction. In consequence, this direction, represented as a linear combination of all state variables, will be called the principal direction, as illustrated in Fig.3.2. Of course, it is anticipated that the total number of principal direction r should be much smaller than the spatial dimension m . Denote that the variance of $\xi(t)$ can be expressed as,

$$\xi(t) = \int_{t_0}^{t_1} (\xi(t) - \bar{\xi})^2 dt, \quad (3.11)$$

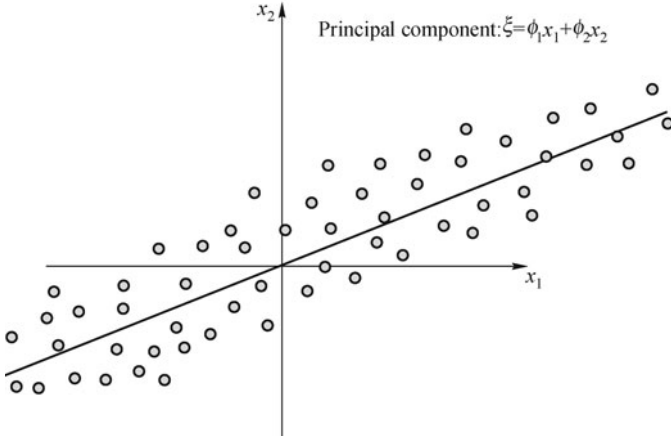


Fig. 3.2 Illustration of principal component.

with time averaged value,

$$\bar{\xi} = \int_{t_0}^{t_1} \xi(t) dt / (t_1 - t_0). \quad (3.12)$$

It is often more practical to use temporal discretization instead of dealing with infinite dimensional problems in the time domain. For example, there are n time snapshots within the time interval $[t_0, t_1]$. For a typical k^{th} channel

or variable, denoted as $\bar{x}_k = \sum_{j=1}^n x_{kj} / n$, the variance of this channel can be

expressed as $\sum_{j=1}^n (x_{kj} - \bar{x}_k)^2$. Consequently, the variance based on the general

coordinate ξ in Eq.(3.10) can be expressed in terms of $\sum_{j=1}^n (\xi_j - \bar{\xi})^2$, where

the mean value of ξ is defined as $\bar{\xi} = \sum_{j=1}^n \xi_j / n$.

The procedure to maximize the variance ξ in $\mathcal{R}^n$ leads to an optimization problem: find $\phi \in \mathcal{R}^n$ so that $\phi^T (x_k - \bar{x}_k)(x_k - \bar{x}_k)^T \phi$ is maximized subject to the constraint $\phi^T \phi = 1$.

It is then clear that the principal component ϕ is in fact the eigenvector of the covariance matrix,

$$\mathbf{A} = (x_k - \bar{x}_k)(x_k - \bar{x}_k)^T, \quad (3.13)$$

and the number of principal components r depends on the rank of the covariant matrix $\mathbf{A}$.

Principal component analysis involves diagonalization of the positional covariance matrix $\mathbf{A}$ to identify an orthogonal set of eigenvectors or “modes” describing the direction of maximum variation in the observed conformational distribution. Covariance matrix $\mathbf{A}$ is calculated with Eq.(3.13). PCA diagonalization of the covariance matrix involves the following eigenvalue problem:

$$\mathbf{A}\mu = \lambda\mu, \quad (3.14)$$

where μ is the eigenvectors and λ is the eigenvalues of covariance matrix $\mathbf{A}$.

One motivation for PCA is to reduce the dimensionality of the MD trajectory data and provide a concise way to visualize, analyze, and compare large-scale collective motion observed over the course of the simulation. Particularly eigenvectors with the largest eigenvalues provide the biggest contribution to the observed covariance^[25,26]. The so-called “essential” modes from a PCA are usually a selection of these eigenvectors and associated eigenvalues that collectively account for a large percentage of the total observed motion.

3.3.1 Three oscillators system analysis with PCA

In the following example, PCA will be used to analyze a simple dynamical system. This example illustrates the PCA procedure and its relationship with the original data. Consider the three body system shown in Fig.3.3, where the edge points are fixed and cannot move. According to the mathematical model^[27], there are three degrees of freedom in the system. Therefore, the dynamics equation of the system can be written as,

$$M\ddot{x} + Kx = R(t), \quad (3.15)$$

$$M = \begin{pmatrix} m & 0 & 0 \\ 0 & 2m & 0 \\ 0 & 0 & 3m \end{pmatrix}, \quad K = \begin{pmatrix} 2k & -k & 0 \\ -k & 2k & -k \\ 0 & -k & 2k \end{pmatrix}, \quad (3.16)$$

$$K\phi = \omega^2 M\phi,$$

with $\mathbf{x} = [x_1 \ x_2 \ x_3]^T$, m standing for mass and k for stiffness of the spring, ω for natural frequency of this system.

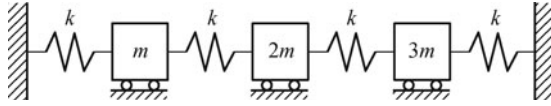


Fig. 3.3 Illustration of three oscillators system. Three bodies (not affected by gravity), each with mass m ; $2m$; and $3m$, attached to four springs, each with spring constant k .

With the standard procedure, the natural frequency can be calculated by solving the generalized eigenvalue problem. The eigenvalues equal ω^2 , where ω denotes natural frequency. Table 3.2 shows the eigenvalues and the corresponding eigenvectors for the calculation result.

Table 3.2 Eigenvalues and eigen vectors for the spring system

Number	Eigenvalue	Eigenvector
1	1.181 61	0.402 98, 0.468 37, 0.272 19
2	2.236 07	-0.408 25, 0.408 25, 0.408 25
3	3.455 02	0.065 39, -0.337 58, 0.871 35

To build a dynamical system based on these three bodies, Verlet integration is used to generate trajectories for each integration or timestep. A trajectory matrix $\mathbf{A}$ is built where each row holds a body's displacement data and each column records displacements at one time step. PCA analysis will be performed as introduced elsewhere.

Verlet integration is a numerical method used to integrate Newton's equation of motion. It is frequently used to calculate trajectories of particles in MD simulation. Newton's equation of motion for conservative physical systems is,

$$\mathbf{M}\ddot{\mathbf{x}}(t) = \mathbf{F}(\mathbf{x}(t)) = -\nabla V(\mathbf{x}(t)), \quad (3.17)$$

where t is the time, $\mathbf{x}(t)$ is the ensemble of the position vectors of N objects, V is the scalar potential function, $\mathbf{F}$ is the negative gradient of the potential giving the ensemble of forces on the particles.

Typically, an initial position and initial velocity are given. To discretize and numerically solve this initial value problem, a time step $\Delta t > 0$ is chosen and the sampling point sequence $t_n = n\Delta t$ is considered. The task is to construct a sequence of points that closely follow the points on the trajectory of the exact solution.

Verlet integration can be seen as follows using the central difference approximation to the second derivative,

$$\mathbf{a}_n = \frac{d^2\mathbf{x}(t_n)}{dt^2} \approx \frac{\mathbf{x}_{n+1} - 2\mathbf{x}_n + \mathbf{x}_{n-1}}{(\Delta t)^2}. \quad (3.18)$$

The Verlet algorithm uses Eq.(3.18) to obtain the next position vector from the previous two,

$$\mathbf{x}_{n+1} = 2\mathbf{x}_n - \mathbf{x}_{n-1} + \mathbf{a}_n(\Delta t)^2. \quad (3.19)$$

Two systems have been built. The first system is running in ideal condition; the second system is running with noise. To add noise to this system, random disturbing displacements are added at every integration. To stimulate the system, the initial position of every mass is set corresponding to one eigenvector of the natural frequencies, shown in Table 3.2 as k , with $k = 1, 2, 3$; the initial velocities are set to zero; every time step Δt is set with $\Delta t = 0.05$ and total integration or time steps $nstep = 200$.

Once the displacement matrix has been calculated, the covariance matrix is produced as Eq.(3.13). Eigenvectors for this covariance matrix are listed in Table 3.3. The new trajectory is produced by projecting data back corresponding to each of the eigenvector.

Table 3.3 Eigenvector and Rayleigh–Ritz results with Verlet algorithm

k	Eigenvector	Rayleigh–Ritz quotient
1	−0.508 70, −0.674 89, −0.534 55	1.539 42
2	−0.212 10, 0.710 23, 0.671 25	1.937 58
3	−0.013 60, 0.167 64, −0.985 75	3.367 64

The Rayleigh–Ritz method is used to find the approximate real resonant frequencies of systems with multiple degrees of freedom, such as spring mass systems^[28]. Figures 3.4, 3.5, and 3.6 show the frequency computed by Rayleigh–Ritz with initial position corresponding to natural frequency. Based on linear algebra, the Rayleigh–Ritz quotients must lie between the minimal

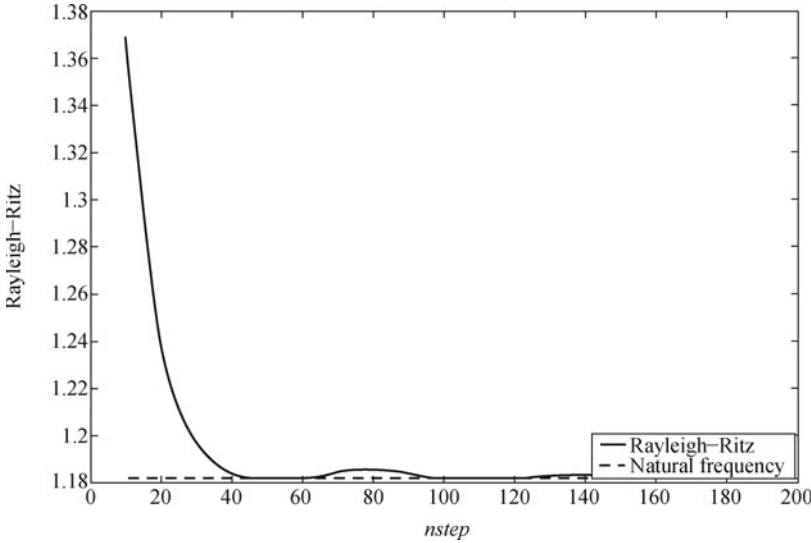


Fig. 3.4 Frequency computed by Rayleigh–Ritz with initial position corresponding to the first natural frequency. Dash line denotes the corresponding natural frequency.

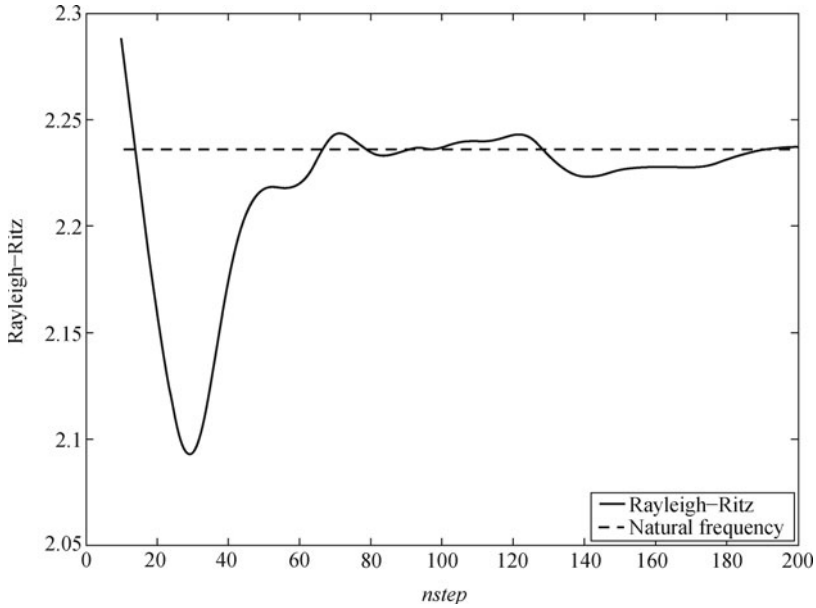


Fig. 3.5 Frequency computed by Rayleigh–Ritz with initial condition corresponding to the second natural frequency. Dash line denotes the corresponding natural frequency.

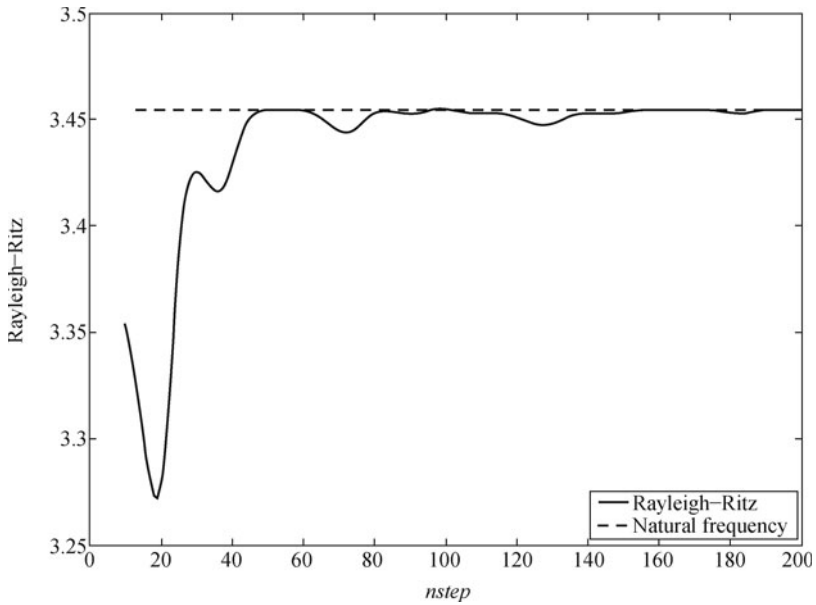


Fig. 3.6 Frequency computed by Rayleigh–Ritz with initial position corresponding to the third natural frequency. Dash line denotes the corresponding natural frequency.

and maximal eigenvalues. It can be easily observed that the dominant eigenmode looks like the corresponding eigenvectors and Rayleigh–Ritz quotients are approximately equal to the corresponding eigenvalues. Most importantly, if one imposes an excitation with frequency around a generic natural frequency on the system, the result is resonance. In addition, the Rayleigh–Ritz quotient will tend to converge with the natural frequency as the number of snapshots $nstep$ increases based on the computing result.

The projected trajectories present the motion of the original set. Eigenval-

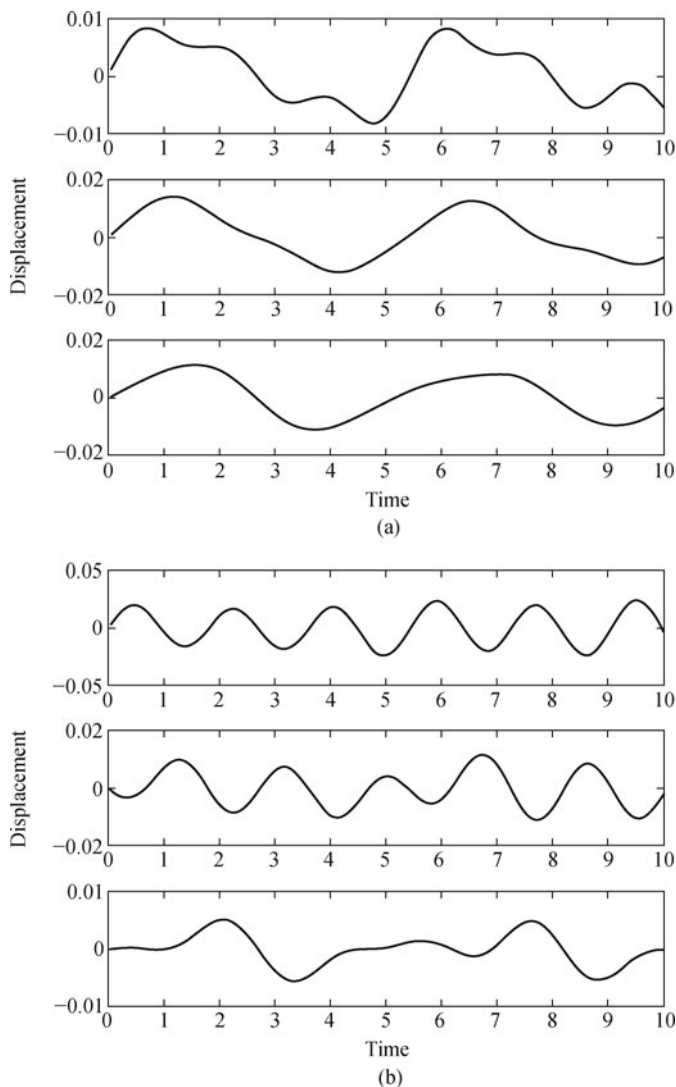


Fig. 3.7 Time vs. displacement without noise: (a) excited by initial displacement with $k = 1$, (b) excited by initial displacement with $k = 3$.

ues for the covariance matrix show how important each mode's contribution is to the whole motion. The percentages of the variance for each mode are: 97.196 9, 2.003 5, 0.799 6. In other words, one mode dominates the entire motions of the system. This result is true for any type of initial displacements proportional to the eigenmode.

The original displacements are shown in Fig.3.7. Projected displacements after PCA are shown in Fig.3.8. After the PCA projection, the modes of displacements are close to the original motion. Similar phenomena as shown in Figs.3.9 and 3.10 can be discovered after applying noise in the spring

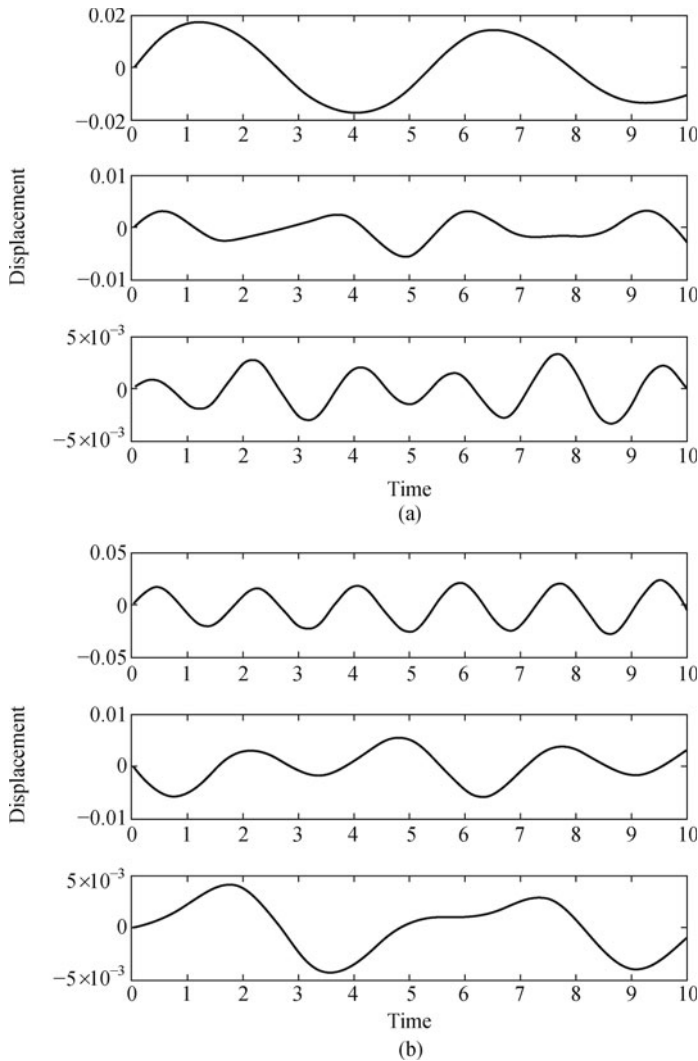


Fig. 3.8 Time vs. projected displacement without noise: (a) excited by initial displacement with $k = 1$, (b) excited by initial displacement with $k = 3$.

system. In the latter case, the motion reinstated using PCA is between the system eigenmode and the dominant mode.

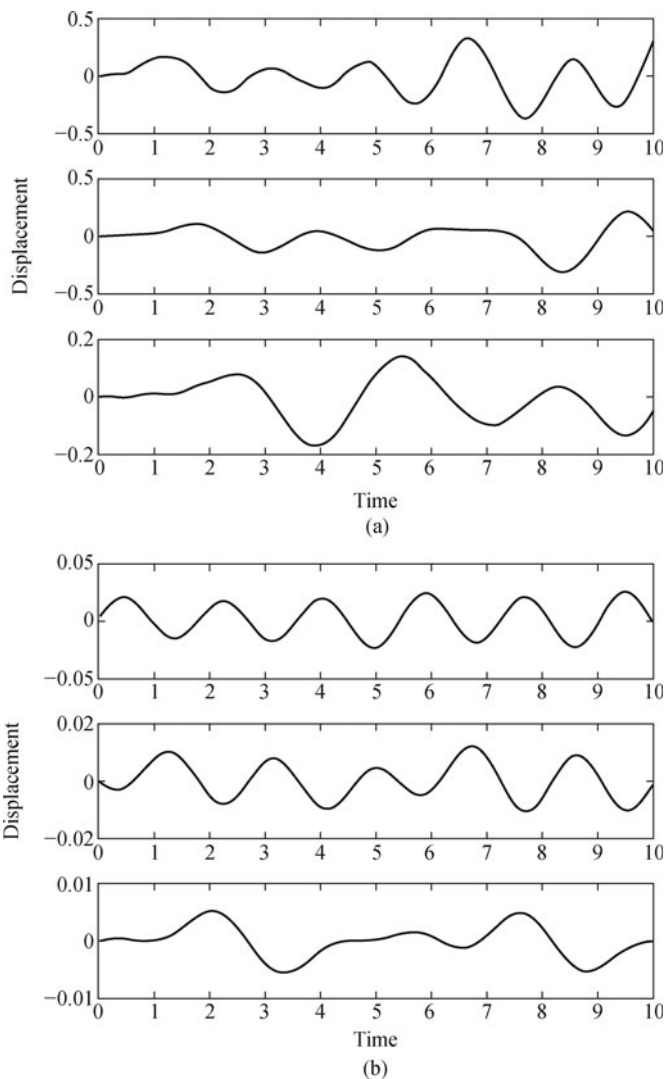


Fig. 3.9 Time vs. displacement with noise: (a) excited by initial displacement with $k = 1$, (b) excited by initial displacement with $k = 3$.

PCA is also applicable to the study of complex systems, for example, the conformation (slow motion) analysis of biological macromolecules. Biological macromolecules such as proteins are large and have thousands of degrees of freedom. It is theoretically difficult to explain and predict the conformational change in proteins. Slow motions such as protein folding can be dominant

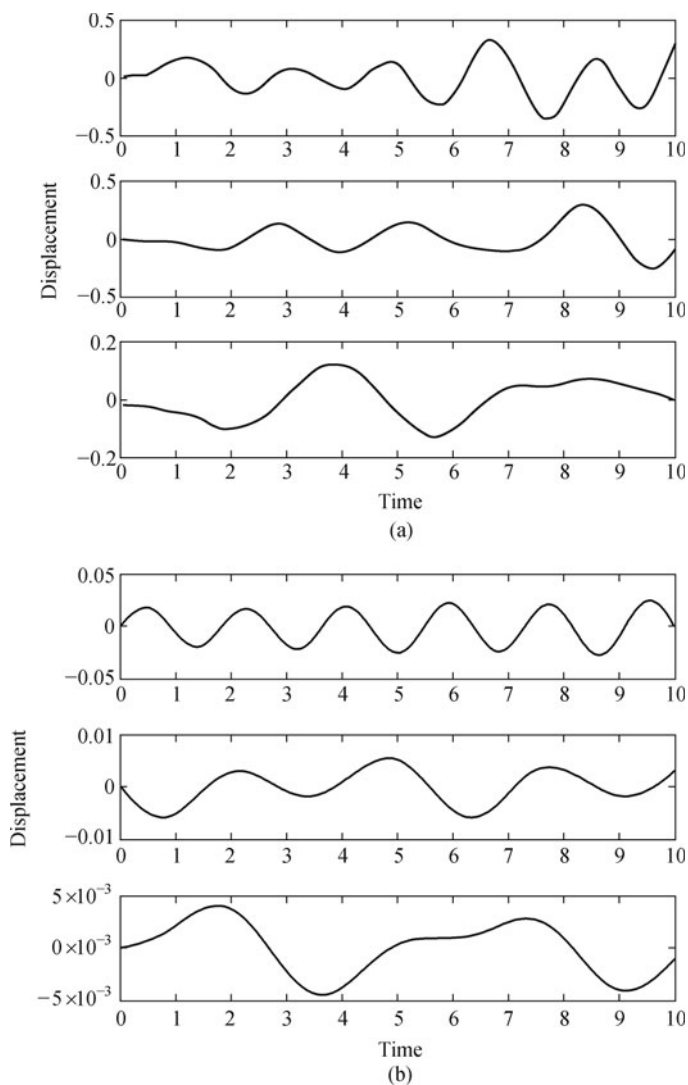


Fig. 3.10 Time vs. projected displacement with noise: (a) excited by initial displacement with $k = 1$, (b) excited by initial displacement with $k = 3$.

motions. The projection of dominant motion provides an effective way to study protein slow motion.

3.3.2 Quasi-harmonic analysis

Quasi-harmonic analysis (QHA)^[12,29] and related principal component analysis techniques, based on the eigenvalue decomposition of an ensemble of protein conformation, have provided a useful method for identifying motion, particularly at long-time scales^[30]. QHA captures the large-scale conformational fluctuation in a protein by diagonalizing the mass-weighted covariance matrix, known as the atomic fluctuation matrix ($F_{\alpha\beta}$). For a system with N atoms, $F_{\alpha\beta}$ is a $3N \times 3N$ symmetric matrix, defined as:

$$F_{\alpha\beta} = \langle m_{\alpha}^{\frac{1}{2}}(x_{\alpha} - \langle x_{\alpha} \rangle) m_{\beta}^{\frac{1}{2}}(x_{\beta} - \langle x_{\beta} \rangle) \rangle, \quad (3.20)$$

where α and β represent the $3N$ degrees of freedom in Cartesian space; m is the mass of the atom and the quantity within $\langle \rangle$ denotes an average over the ensemble of structures in MD simulation.

The inverse square roots of the eigenvalues determined by diagonalizing $F_{\alpha\beta}$ represent the frequencies associated with the protein eigenmode. The eigenvectors represent the displacement vectors of the individual atoms. The lowest frequencies correspond to large-scale cooperative motion in the protein; the higher frequencies represent localized motion. For a system with N atoms, there are $3N - 6$ internal modes. However, due to computational costs, typically only a limited number of slow modes (lowest frequencies) is computed.

QHA allows identification of protein motion at a variety of time scales that are related to the length of the MD simulation. The atomic fluctuation matrix can be computed from protein conformation sampled during a single MD simulation that is of limited duration. It also can be computed with a collection of MD trajectories that represent a long-time scale.

QHA is computationally expensive, especially with a large number of atoms and the long MD simulation. Various methods have been developed to minimize the computational cost, such as time-averaged normal coordinate analysis (TANCA)^[31]. A significant advantage of QHA over other methods is that it allows identification of slow conformational fluctuations that span distant areas of the protein energy landscape, such as the reaction pathway during enzyme catalyzing^[32,33]. Resisting some of the limitations of NMA-based approaches, QHA provides a methodology to explore protein motions with longer time scales.

The difference between QHA and PCA is that in computing the atomic fluctuation matrix ($F_{\alpha\beta}$) QHA takes mass to amplify the motion of heavy atoms (that is, atoms other than hydrogen). Hydrogen atoms move faster and are more likely to relate to high frequency motion. QHA and PCA also study protein conformational changes by exploring locations of each residue. These locations are usually represented by the location of the alpha-carbon of each residue. Motion of hydrogen atoms is related with localized motion. Therefore QHA is good at describing slow motion than PCA. The research

on these phenomena is presented in Section 3.5.3.

3.3.3 Equilibrium conformational analysis

Equilibrium conformational fluctuation plays an essential role in the biological function of proteins. These fluctuations range from subtle rearrangements of a few atoms to large-scale movement involving the entire protein. Protein motion time scales occupy a correspondingly large range, from femtoseconds to microseconds or even longer^[33,34]. The lower end of this range, or fast motion, is commonly known as vibration, which includes bond stretching and angle bending. The upper end of this range, or slow motion, involves considerable conformational changes^[4,5,7-9,18], such as rotation of side chains and movement of flexible loops. Slow fluctuations have been studied in relation to possible involvement with protein function^[32-42]. However, proteins are chemically inhomogeneous^[14]. The complexity of protein dynamic behavior makes it difficult to obtain a theoretical description of thermal fluctuation. In this research, a combination of classical molecular dynamics (MD) simulation and principal component analysis (PCA) is introduced to study equilibrium conformational fluctuation of proteins.

Protein motions and their possible role in protein function are studied with normal mode analysis (NMA) and its various extensions. Conformational fluctuation and individual protein structures are associated with protein structure and function. NMA is based on harmonic approximation of the potential energy minimum, which is computed by diagonalizing the Hessian matrix. However, recent research shows that slow conformational changes of proteins are largely anharmonic^[43]. Furthermore, NMA ignores solvents, but water plays an important role in protein conformation. In fact, NMA is not well suited for protein conformational change that covers widely separated areas of the conformational energy landscape, such as enzyme catalysis^[30].

The increase in available computing power provides a systematic way to study protein conformational changes with MD simulation. A combination of MD simulation with PCA and its extensions have given an insight into protein and DNA dynamics. These methods have been successfully applied to the thermodynamics of a number of nucleic acid interactions including sequence selective drug-DNA association^[44], large conformational transition in DNA^[45], and protein-DNA complexes^[46]. A generalized package of this methodology has been built in this research and is demonstrated in this chapter.

3.4 Methods and procedures

3.4.1 Framework

One motivation of this research is to build a generalized procedure for protein equilibrium conformational fluctuation analysis. This method may be applied to any protein with known atomic coordinates. The standard procedure is as follows:

(1) Select an appropriate PDB file from Protein Data Bank (PDB) and conduct an MD simulation. Save the trajectories matrix $\mathbf{A}_{3m \times n}$, where m is the number of atoms and n is the number of snapshots from simulation shown as follows:

$$\mathbf{A}^T = \begin{pmatrix} x_{11} & x_{21} & \dots & x_{m1} & y_{11} & \dots & y_{m1} & z_{11} & \dots & z_{m1} \\ x_{12} & x_{22} & \dots & x_{m2} & y_{12} & \dots & y_{m2} & z_{12} & \dots & z_{m2} \\ \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ x_{1n} & x_{2n} & \dots & x_{mn} & y_{1n} & \dots & y_{mn} & z_{1n} & \dots & z_{mn} \end{pmatrix}^T.$$

- (2) Clean the data to remove translation and rotation.
- (3) Compute the covariance matrix with Eqs.(3.13) and (3.20).
- (4) Solve the eigen problem with covariance matrix to get eigenvectors and eigenvalues.
- (5) Assess the number of modes required to accurately represent the conformational change by analyzing overlap coefficients and eigenvalues.
- (6) Calculate a new “mode matrix” by projecting selected eigenvectors or “modes” back to covariance.
- (7) Analyze conformational changes based on the results of previous steps.
- (8) Perform correlation analysis.

3.4.2 Overlap coefficients

The overlap coefficients (OCs) of the α -carbon atoms are calculated to measure how well each PCA mode represents all motions^[47]. PCA modes ($\mathbf{v}_i$) are compared with a vector ($\Delta\mathbf{x}$), that describes the displacement of α carbon atoms from the minimized starting simulation position. The structure is experimentally determined by X-ray crystallography. This vector is calculated following alignment of the minimized and crystal structures with the MD average structure to remove rotation and translation of the center of mass.

The weight factor ($\alpha^{(i)}$) for each PCA mode is calculated as follows:

$$\alpha^{(i)} = \frac{\mathbf{v}_i \cdot \Delta\mathbf{x}}{\|\mathbf{v}_i\|^2}. \quad (3.21)$$

It measures whether or not $\Delta\mathbf{x}$ can reasonably be represented in the vector space described by various subsets of PCA modes. A subset S of modes is calculated as a reconstructed vector ($\tilde{\mathbf{v}}$) as follows:

$$\tilde{\mathbf{v}} = \sum_{i \in S} \alpha^{(i)} \mathbf{v}_i. \quad (3.22)$$

Then the overlap coefficients which show the similarity between $\Delta\mathbf{x}$ and each PCA mode ($\mathbf{v}_i$ or $\tilde{\mathbf{v}}$) are calculated by the angle between the two vectors:

$$\cos \theta = \frac{\mathbf{v} \cdot \Delta\mathbf{x}}{\|\mathbf{v}\| \cdot \|\Delta\mathbf{x}\|}. \quad (3.23)$$

The relative error in the reconstructed vectors can be computed as:

$$\varepsilon = \frac{\|\Delta\mathbf{x} - \tilde{\mathbf{v}}\|}{\|\Delta\mathbf{x}\|}. \quad (3.24)$$

This error simply provides a measure of how well the displacement due to the recalculated vector recapitulates the observed crystallographic $\Delta\mathbf{x}$ and thus allows users to assess the number of modes required to accurately represent the conformational change.

3.4.3 Correlation analysis

Correlations between the fluctuations of residues, measured at α -carbon atoms, give additional insight into protein function^[48-50]. An unweighted pair group method with arithmetic means (UPGMA) clustering algorithm is used to define groups of residues with similar correlation patterns under equilibrium fluctuation. The correlation is measured by constructing correlation matrices. The details of correlation matrices can be found in Section 3.4.6.

A correlation analysis procedure similar to Ref.[47] is followed:

- (1) Construct a positional correlation matrix S_{ij} (Section 3.4.6).
- (2) Compute the effective “distance” d_{ij} , based on the correlation measure:

$$d_{ij} = \sqrt{1.0 - S_{ij}^2} \quad (3.25)$$

The distances between residues represent the strength of the relationship between them, with the closest residues having the highest correlation.

- (3) Generate a “treelike” representation of correlated residues by the UPGMA method, based on the calculated effective distances. The UPGMA method is presented in Section 3.4.7.

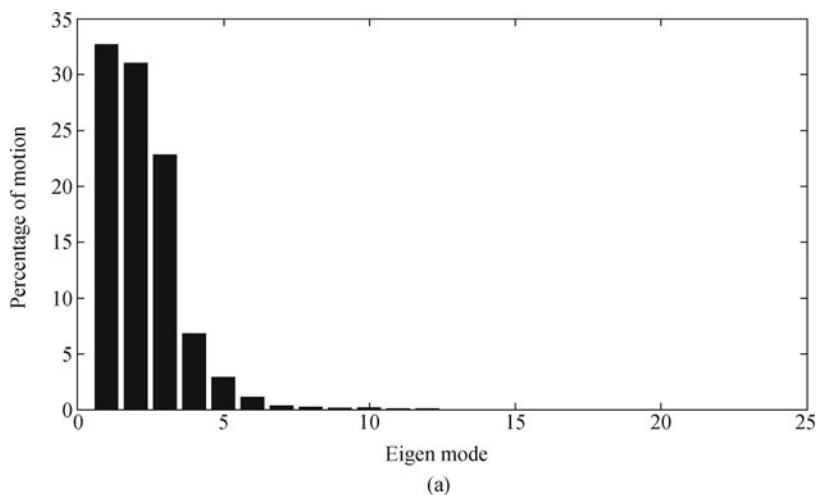
- (4) Conduct a depth-first search for choosing the smallest clusters that contain at least one residue from each of the groups defined by the selection criteria. Experimental information plays an important role in the definition of these clusters.

(5) Complete the cluster selection by figuring out other important residues of the selection criteria that were not found in the initial cluster selection and by finding the clusters that contain those identified residues but do not contain any residue selected in the previous depth first step.

Correlation matrices give insight into the pairwise correlation of residues. While residue clusters are likely to involve inter-domain communication, they do not show individual residue interaction that could be important for the protein functional mechanism. Although the correlations identified by clustering do not provide direct information about the series of events that generate the correlations, they do provide a starting point for experimental and further computational studies designed to model the protein conformational change.

3.4.4 PCA with MD simulation

Based on the eigenvalues from PCA analysis as illustrated in Fig.3.11, the dominant motions were the first three motions for each system. Each mode was projected back corresponding with its eigenvector. The motions are shown in Figs.3.12 and 3.14. Rotation is dominant in the third motion being evident from Fig.3.14. The other two motions are a combination of rotation and translation. This is generally true for any simulation of a protein system because translation and rotation are usually the dominant motions of the original data set. This is one evidence that PCA captures slow conformational change. However, further significant motions lie behind the dominant rotation and translation. To bring these motions to the foreground, alignments are used to remove rotation and translation before PCA.



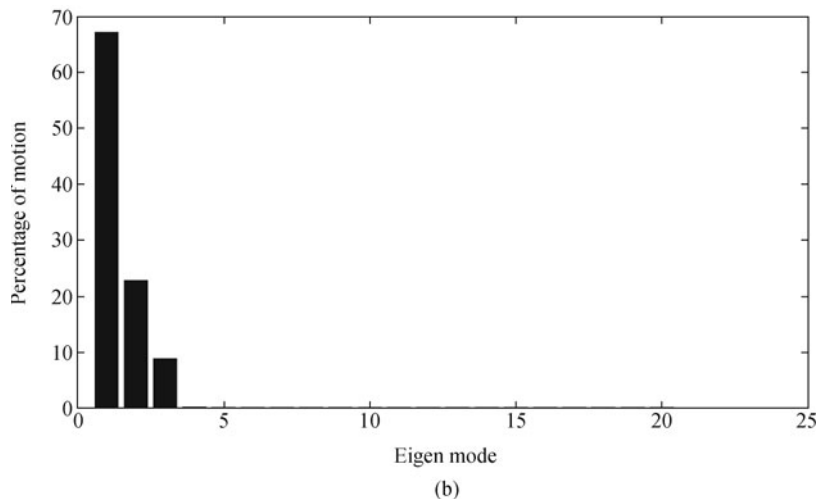


Fig. 3.11 PCA eigenvalues with original data for T4 lysozyme MD simulations: (a) vacuum, (b) solvent.

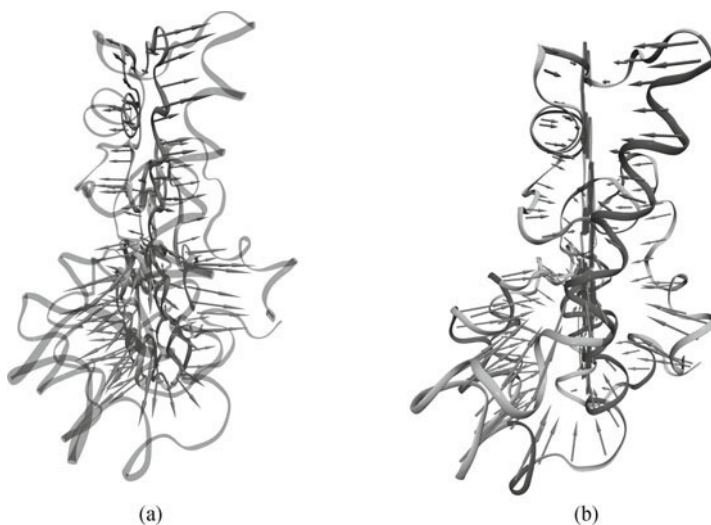


Fig. 3.12 Non-aligned PCA mode 1 displacement visualizations of MD simulation without solvent. The starting structure is shown in new-cartoon representation colored by structure. The destination structure is shown in blue ribbon. The red arrows indicate the direction of displacement based upon 1st PCA mode of MD simulation without solvent: (a) expansion, (b) contraction (color plot in the book end).

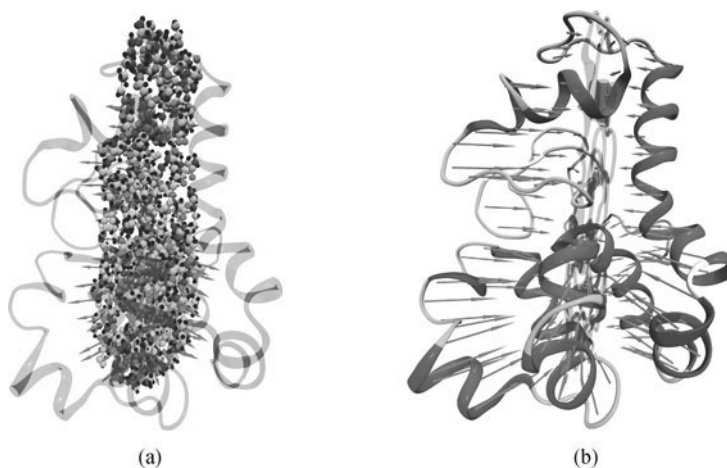


Fig. 3.13 Non-aligned PCA mode 2 displacement visualizations of MD simulation without solvent. In CPK representation colored by atom type: H black; O red; N blue; C cyan; S yellow; P tan. The starting structure is shown in new-cartoon representation colored by structure. The destination structure is shown in blue ribbon. The red arrows indicate the direction of displacement based upon 2nd PCA mode of MD simulation without solvent: (a) expansion, (b) contraction (color plot in the book end).

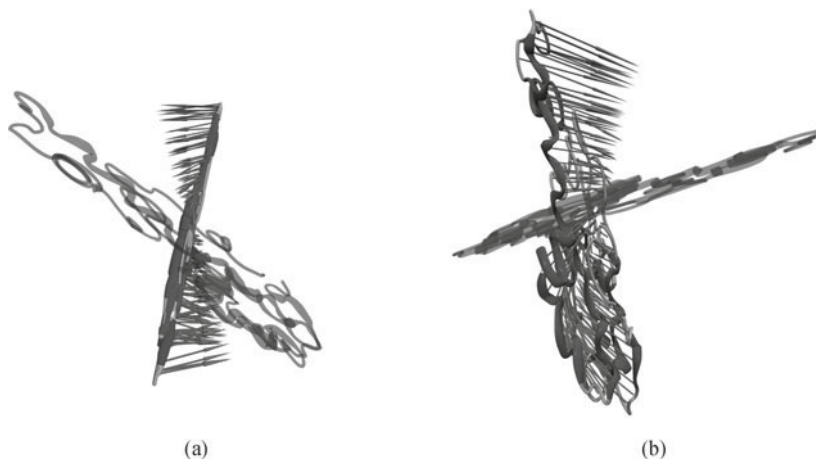


Fig. 3.14 PCA mode 3 displacement visualizations of MD simulation without solvent. The starting structure is shown in new-cartoon representation colored by structure. The destination structure is shown in blue ribbon. The red arrows indicate the direction of displacement based upon 3rd PCA mode of MD simulation without solvent: (a) expansion, (b) contraction (color plot in the book end).

A reference structure is defined for alignment. The coordinates of each snapshot is aligned with the reference structure. The reference structure is defined by the user and usually is the initial position or the averaged position of all snapshots. Translation alignment can easily be done by moving all coordinates by a vector $\mathbf{v}$ which equals $\mathbf{v}_o - \mathbf{v}_r$, where $\mathbf{v}_o$ and $\mathbf{v}_r$ hold the coordinates of the center of mass of the original and reference systems.

Alignment for rotation is calculated by the Kabsch algorithm, which is introduced in Section 3.4.5. The center of mass is traced by $(\bar{x}_1, \bar{x}_2, \bar{x}_3)$. At each time step, the center of mass can be evaluated as follows:

$$\bar{x}_k = \sum_{i=1}^N \frac{m_i x_k^i}{m}, \quad \text{for } k = 1, 2, 3, \quad (3.26)$$

where N is the total number of atoms, x_k^i represents the position of the i^{th} atom in the k direction, and m is the total mass expressed as $\sum_{i=1}^N m_i$.

3.4.5 Kabsch algorithm

The Kabsch algorithm^[51,52] is a method for calculating the optimal rotation matrix that minimizes the RMSD between two sets of points, namely, rotation alignment.

The algorithm calculates only the rotation matrix. Both sets of coordinates must be transformed to their centroid first. Note that in general, the “rotation matrix” represents a transformation to a different orthonormal basis rather than a rotation about a single axis.

The algorithm starts with two sets of paired points, P and Q . Each set of points can be represented as an $N \times 3$ matrix. The first row consists of the coordinates of the first point, the second row of the coordinates of the second point, the n^{th} row of the coordinates of the n^{th} point:

$$P \text{ or } Q = \begin{pmatrix} x_1 & y_1 & z_1 \\ x_2 & y_2 & z_2 \\ \vdots & \vdots & \vdots \\ x_n & y_n & z_n \end{pmatrix}.$$

The algorithm works by calculating a covariance matrix, $\mathbf{A}$,

$$\mathbf{A} = \mathbf{P}^T \mathbf{Q}. \quad (3.27)$$

The optimal rotation $\mathbf{R}$ is based on the matrix formula $\mathbf{R} = (\mathbf{A}^T \mathbf{A})^{\frac{1}{2}} \mathbf{A}^{-1}$. However, in the case that $\mathbf{A}$ does not have an inverse, another approach with singular value decomposition (SVD) was also introduced in Ref.[51, 52]. First, calculate the SVD of the covariance matrix $\mathbf{A}$,

$$\mathbf{A} = \mathbf{U} \sum \mathbf{V}^T. \quad (3.28)$$

Next, decide whether you need to correct the rotation matrix to insure a right-handed coordinate system

$$d = \text{sign}(\det(\mathbf{A})). \quad (3.29)$$

Finally, calculate the optimal rotation matrix $\mathbf{R}$,

$$\mathbf{R} = \mathbf{V} \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & d \end{pmatrix} \mathbf{U}^T. \quad (3.30)$$

3.4.6 Positional correlation matrix

A scalar correlation matrix was defined in Cartesian space by equation

$$S_{ij} = \frac{\langle (\mathbf{R}_i - \langle \mathbf{R}_i \rangle) (\mathbf{R}_j - \langle \mathbf{R}_j \rangle) \rangle}{\sqrt{\langle (\mathbf{R}_i - \langle \mathbf{R}_i \rangle) (\mathbf{R}_i - \langle \mathbf{R}_i \rangle) \rangle \langle (\mathbf{R}_j - \langle \mathbf{R}_j \rangle) (\mathbf{R}_j - \langle \mathbf{R}_j \rangle) \rangle}}, \quad (3.31)$$

to denote the correlation between atoms i and j ^[47].

The value of S_{ij} ranges from -1.0 to 1.0 , with positive and negative values indicating correlated and anti-correlated motions. $\mathbf{R}_i$ and $\mathbf{R}_j$ are the positions of atoms i and j and $\langle \rangle$ denotes the trajectory average over snapshots. The matrix is calculated across all α carbon atoms of the protein.

3.4.7 Cluster analysis

The unweighted pair group method with arithmetic mean (UPGMA)^[53] is a simple agglomerative or hierarchical clustering method. The algorithm examines a pairwise distance matrix (or similarity matrix) to construct a rooted tree. At each step, the nearest two clusters are combined into a higher-level cluster. The distance between any two clusters A and B is the average of all distances between pairs of objects x in A and y in B :

$$\frac{1}{|A| \cdot |B|} \sum_{x \in A} \sum_{y \in B} d(x, y). \quad (3.32)$$

UPGMA algorithm is illustrated in Fig.3.15.

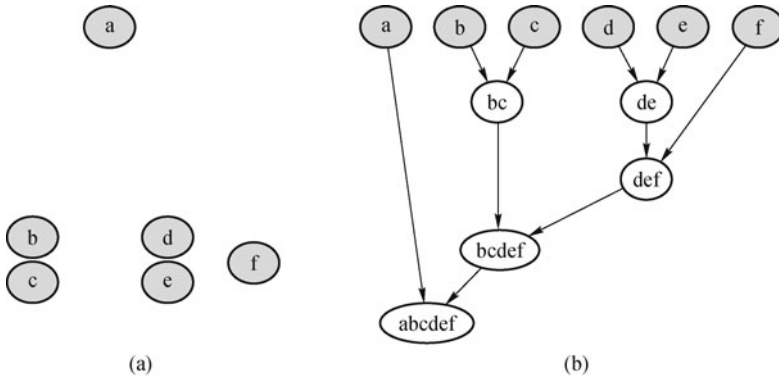


Fig. 3.15 Illustration of UPGMA algorithm: (a) raw data, (b) clustering with UPGMA. Source: Wikipedia.

3.5 MD simulation with T4 lysozyme

The T4 phage lysozyme molecular dynamics simulation utilizes the NAMD parallel molecular dynamics package version 2.6^[54] with the CHARMM force field^[48]. The initial structure was determined by X-ray crystallography^[55], retrieved from the Protein Data Bank (PDB ID 3LZM; residue numbers 1–164).

Two systems, one with solvent and the other in vacuum, were built for MD simulation. The PSF package in VMD^[56] is used to generate the structure file (PSF file) for MD simulation. First, the coordinates missing in the crystal structure are reconstructed. For the solvent system, the minimized structure is solvated in a periodic truncated cubic simulation box of 29 712 TIP3P water molecules, providing a minimum of 15 Å of water between the protein surface and any box edge. The water box is rotated to minimize the system size. Sodium and chloride ions are added to neutralize the total system and achieve a salt concentration of 0.05 mol/L. Ion placement is random; minimum distances between ions and molecules as well as between any two ions are set as 5 Å. The parameter setting is shown in Table 3.4.

Table 3.4 MD simulation parameter setting for T4 lysozyme

Item	Setting
Periodic boundary conditions	Yes
Cutoff distance	12 Å
Switch distance	10 Å
Pair list distance	13.5 Å
Time step	2.0
Rigid bonds	All
Non-bonded frequency	2

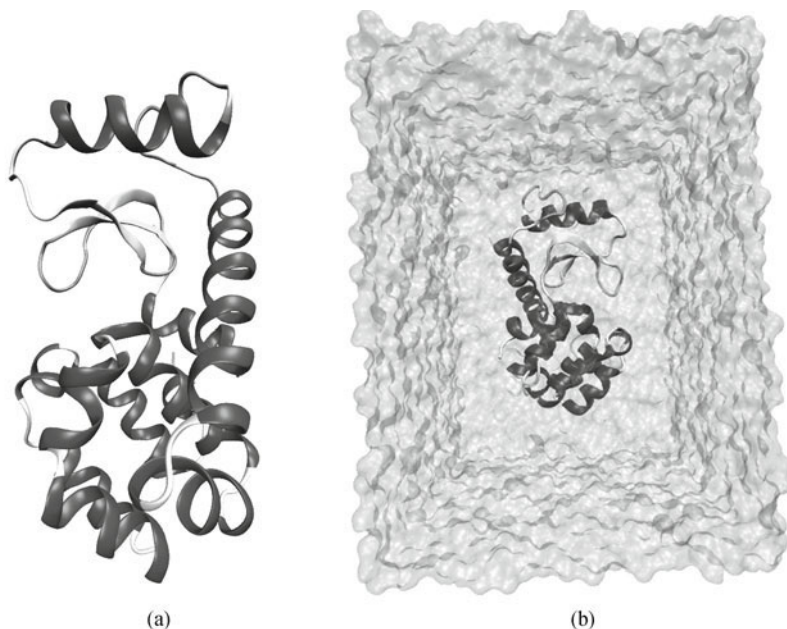


Fig. 3.16 Schematic representation of the energy-minimized molecular structure analyzed for T4 lysozyme with structure view, colored by structure type: Alpha-Helix purple, 3₁₀-Helix blue, Pi-Helix red, Extended-Beta yellow, Bridge-Beta tan, Turn cyan, Coil white: (a) molecular structure in vacuum, (b) molecular structure in water box (color plot in the book end).

Periodic boundary conditions are selected for simulation with solvent. The periodic boundary condition involves surrounding the system under study with identical virtual unit cells. The atoms in the surrounding virtual systems interact with atoms in the real system. These modeling conditions are effective in eliminating surface interaction of the water molecules and creating a more faithful representation of the *in vivo* environment than what a water sphere surrounded by vacuum provides^[57].

In the solvent simulation, energy minimization was performed in 20 000 steps. There were no energy changes during last 1 000 time-steps. Following minimization, the entire system was heated in 10 K increments up to 300 K with micro-canonical ensemble (NVE) equilibration per temperature step. The simulation was conducted at 300 K. Atomic trajectories were calculated in one-femtosecond (fs) time steps using SHAKE^[58] constraints on hydrogen-heavy atom bonds. The total production simulation length is 15 nanosecond (ns). The first 9 ns were discarded as an “equilibration period”. This extensive relaxation/equilibration period was necessary due to drift in the potential energy.

A similar procedure was devised in vacuum, except that during the heating stage each step is 50 K. The total simulation length is 40 ns. In both

simulations, snapshots were recorded every picosecond (ps) for analysis.

3.5.1 Equilibration measures

It is important to assess whether or not the simulation achieved equilibrium or steady-state sampling before analyzing conformational fluctuation. Analysis of the molecular dynamics of the T4 lysozyme was performed as described in Section 3.5. The root mean-squared deviation (RMSD) of atomic position relative to the starting structure was calculated to measure equilibration and simulation stability.

RMSD is a numerical measure of the difference between two structures defined as,

$$\text{RMSD} = \sqrt{\frac{\sum_{i=1}^{N_{\text{atoms}}} (r_i(t_1) - r_i(t_2))^2}{N_{\text{atoms}}}}, \quad (3.33)$$

where N_{atoms} is the number of atoms whose positions are being compared and $r_i(t)$ is the position of atom i at time t .

Figure 3.18 shows the results of the RMSD calculation. There is an initial rapid rise in RMSD that levels off around 10 ns in vacuum. By contrast, the RMSD in solvent was stable except during the first 0.2 ns. The solvent simulation was heated slowly to create a stable simulation result. The system was relaxed during the heating stage. The rapid rise reflects a significant conformational change during the simulation as demonstrated in Fig.3.17.

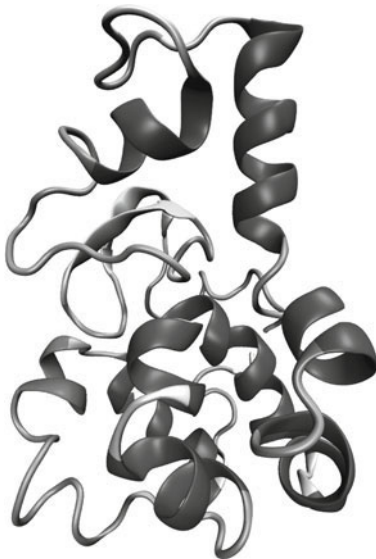


Fig. 3.17 Illustration of T4 lysozyme structure after 40 ns simulation in vacuum (color plot in the book end).

Without the “protection” of water molecules, the α -helix on the top bends toward the lower part. This is one of the natural modes^[59]. Based on these results, the last 5 ns simulation with solvent was mainly used for fluctuation analysis.

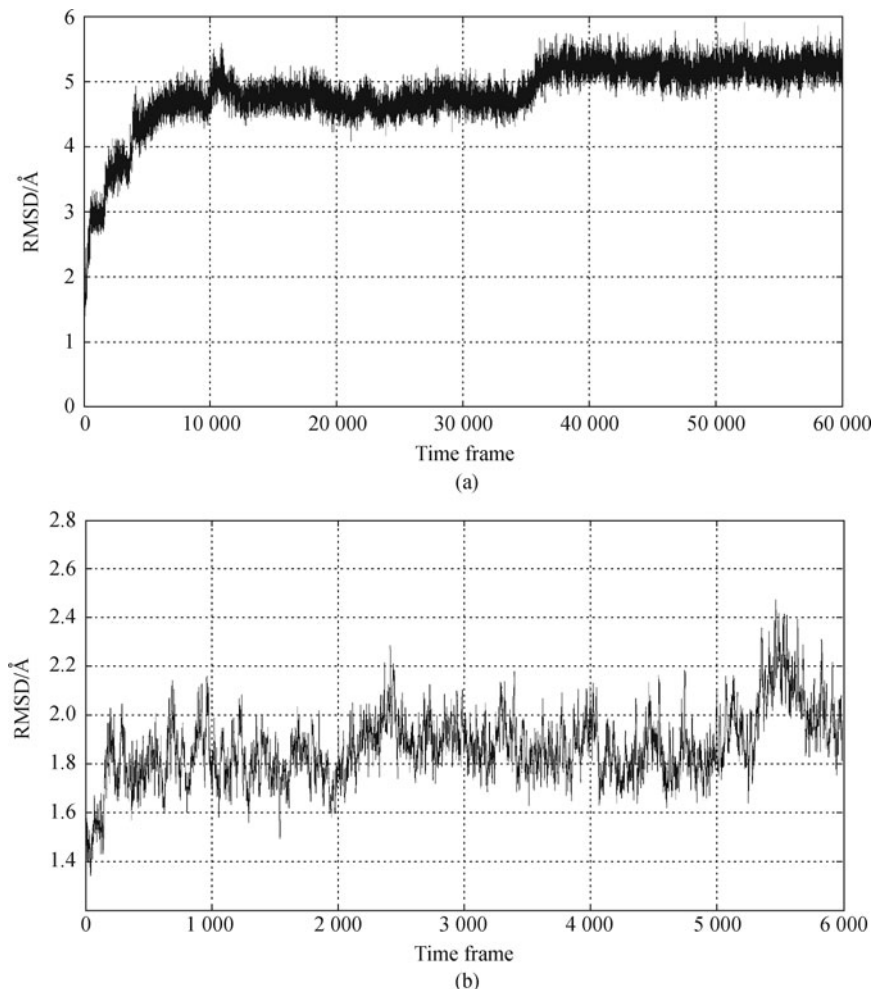


Fig. 3.18 Root mean-squared deviation (RMSD) of atomic position relative to the starting structure with T4 lysozyme simulation: (a) RMSD value with T4 lysozyme simulation in vacuum, (b) RMSD value with T4 lysozyme simulation with solvent.

3.5.2 Fluctuation analysis

Equilibrium thermal fluctuation of residues provides the first quantitative

evaluation of the proposed MD-PCA framework and helps to understand protein function by local conformational flexibility. The root mean square fluctuation (RMSF) of α -carbon atoms is plotted as a function of residue number to show local conformational change. Atomic α -carbon fluctuations are compared to published results such as the All Atom Method (ATM), Rotational-Translational Block (RTB), Gaussian Network Model (GNM), and Finite Element Method (FEM) (Fig.3.19).

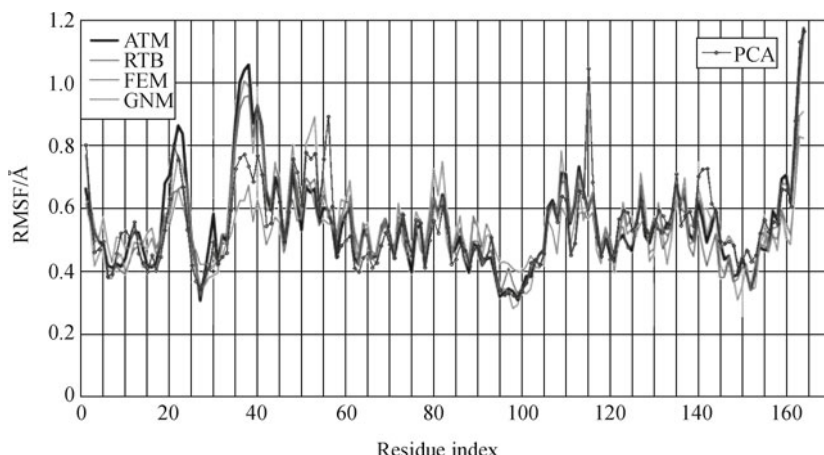


Fig. 3.19 RMSFs of the α -carbon atoms at 300 K for T4 lysozyme. Methods used are: All Atom Method (ATM), Rotational-Translational Block (RTB), Gaussian Network Model (GNM), Finite Element Method (FEM), Data generated from simulation (PCA) (color plot in the book end).

The T4 lysozyme shows flexibility at the solvent-exposed β -hairpin turn between residues 20 and 24, also at the short loop between residues 35 and 38. The solvent-exposed α -helix at residues 39–51 can be found in Fig.3.19. These RMSF results are in quantitative agreement with other methods except at residue 115. This suggests that the α -helix containing residue 115 tends to bend at that spot. In the simulation without water, that α -helix does bend at residue 115 without solvent-exposed “protection”.

3.5.3 Mode selection and evaluation

This research analyzes the motion of all atoms in the system, including the hydrogen atoms in the protein, which form a large part of the protein atoms and which have high frequency modes. In this section, eigen analysis and overlap analysis are used to evaluate the dominant modes of motion. Both PCA and QHA are used in the analysis.

Three models are tested with data taken from the same simulation. Model one is PCA with α -carbon atoms only; model two is PCA with all atoms;

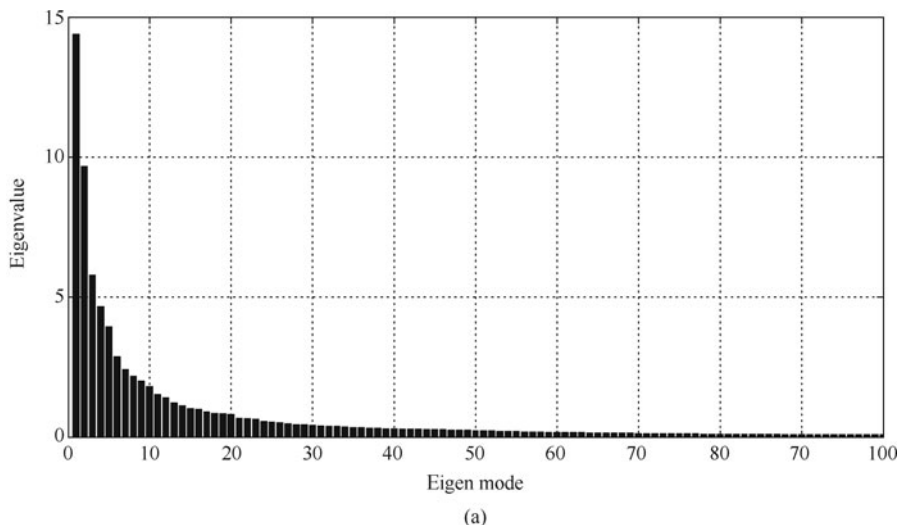
model three is QHA with all atoms. PCA with α -carbon model represents the benchmark solution.

PCA transforms a number of possibly correlated variables into a number of uncorrelated variables called principal components. Its operation can be thought of as revealing the internal structure of the data in a way that best explains the variance in the data. PCA is sensitive to the relative scaling of the original variables. In the following, the similarities and differences among these three models are presented.

3.5.4 Eigenvalue analysis

Eigenvalues and eigenvectors are calculated according to the standard procedure in the three models. The results are shown in Figs.3.20, 3.21, and 3.22. The screen test first proposed by Ref.[60] yields the best results in practice to decide how many eigenmodes should be taken. Cattell suggests finding the place where the smooth decrease of eigenvalues appears to level off to the right of the eigenvalue plot. Following this suggestion, the first 10 eigenmodes are selected in all three models.

Although all atom PCA discovers the same number of dominant modes (slow motion) as α -carbon PCA does, the slow motions are disturbed. The cumulative energy of the first 10 is 59.1% in α -carbon PCA, 42.6% in all atom PCA, 46.2% in QHA. Quasi-harmonic analysis magnifies each atom's motion by its mass. In essence, QHA is a mediated method between all atom PCA and α -carbon PCA.



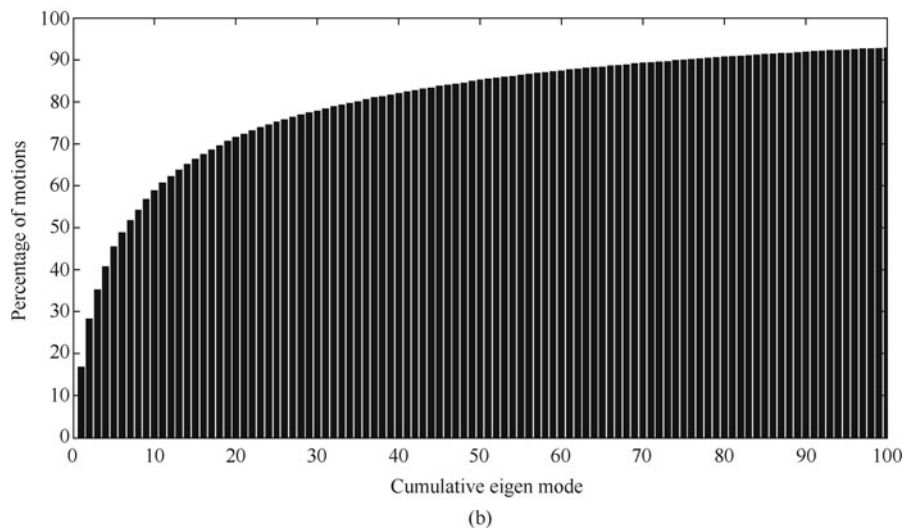
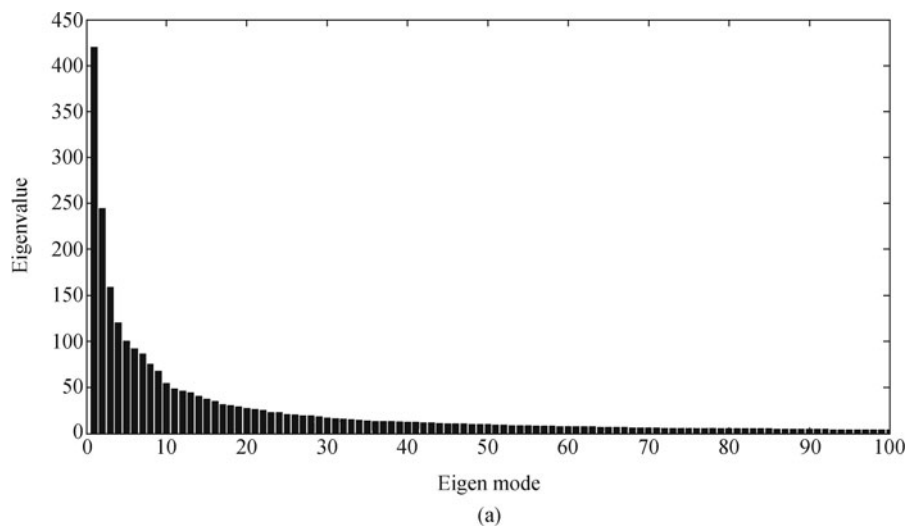


Fig. 3.20 PCA eigenvalues calculated with α -carbon for T4 lysozyme MD simulations: (a) eigenvalue, (b) cumulative percentage of eigen mode.



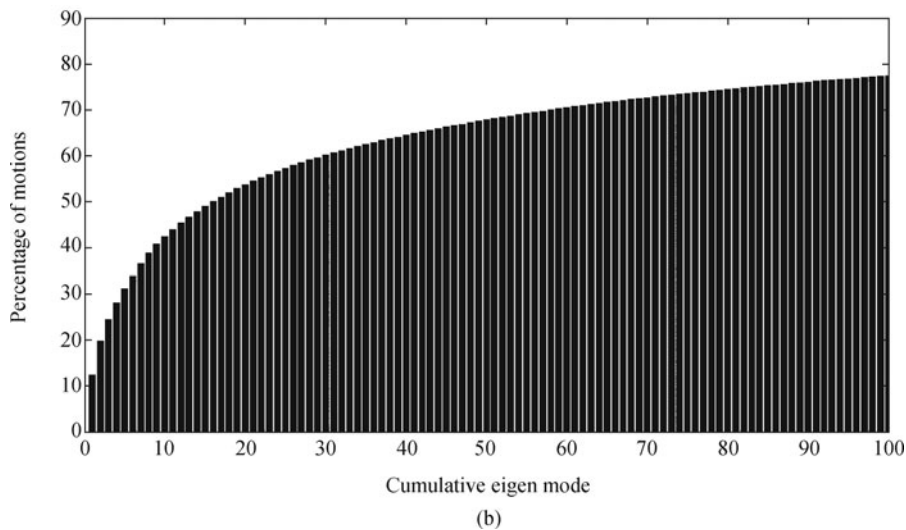
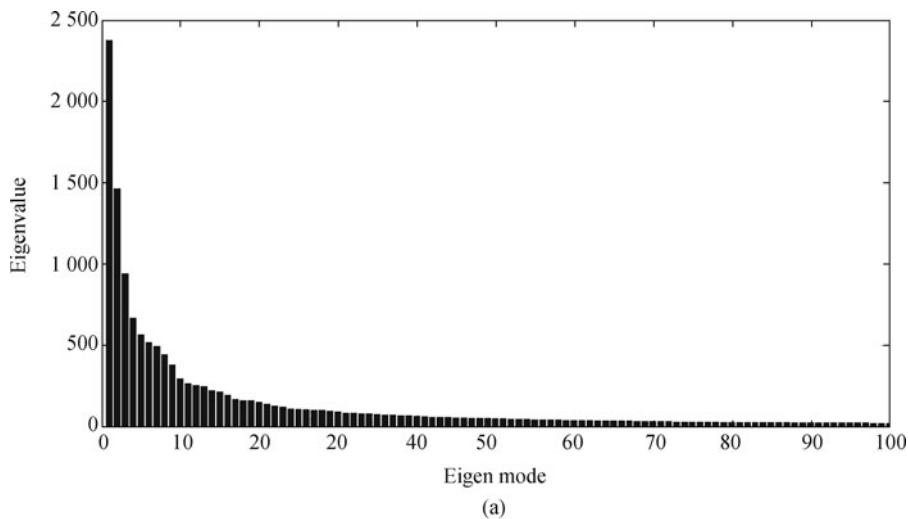


Fig. 3.21 PCA eigenvalues calculated with all atoms for T4 lysozyme MD simulations: (a) eigenvalue, (b) cumulative percentage of eigen mode.



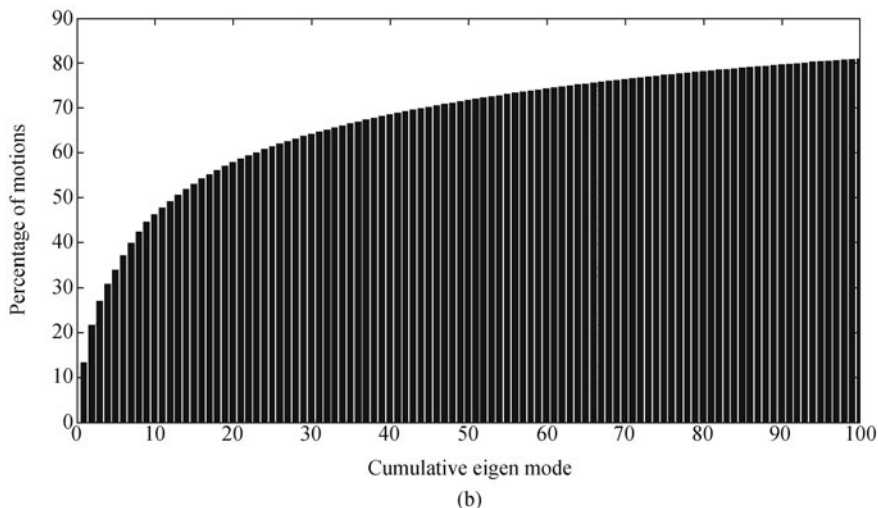


Fig. 3.22 QHA eigenvalues calculated with all atoms for T4 lysozyme MD simulations: (a) eigenvalue, (b) cumulative percentage of eigen mode.

3.5.5 Overlap evaluation

With the calculated PCA/QHA modes placed into experimentally determined structural rearrangements, the overlap coefficients (OCs) were calculated as shown in Section 3.4.2. The results are shown in Figs.3.23, 3.24 and 3.25. The full basis set of 492 α -carbon PCA modes gives a very high overlap ($\cos \theta = 1.000$), as done by all atom PCA/QHA, while carrying low error at 0.000 01 for α -carbon PCA, 0.000 06 for all atom PCA, and 0.000 6 for all atom QHA. From this PCA basis set, a minimal set of modes was identified that represented the observed displacement. The first 10 modes were selected based on eigenvalue analysis.

The first and largest atom PCA mode accounts for 12.6% of the total motion, but slightly overlaps ($\cos \theta = 0.17$) the changes observed in the X-ray structure. This largest mode represents an asymmetric “twisting-like” mode as shown in Fig.3.28. The second mode accounts for 7.3% of the total motion but has a greater overlap ($\cos \theta = -0.29$). This is a “flapping-like” motion as shown in Fig.3.29. A similar result is obtained with the all atom QHA method. Different distributions of the errors with the QHA method is also illustrated in Fig.3.26. It seems that errors tended to occur at the begin and end modes. This is evident that QHA can “separate” slow motion from high frequency motion.

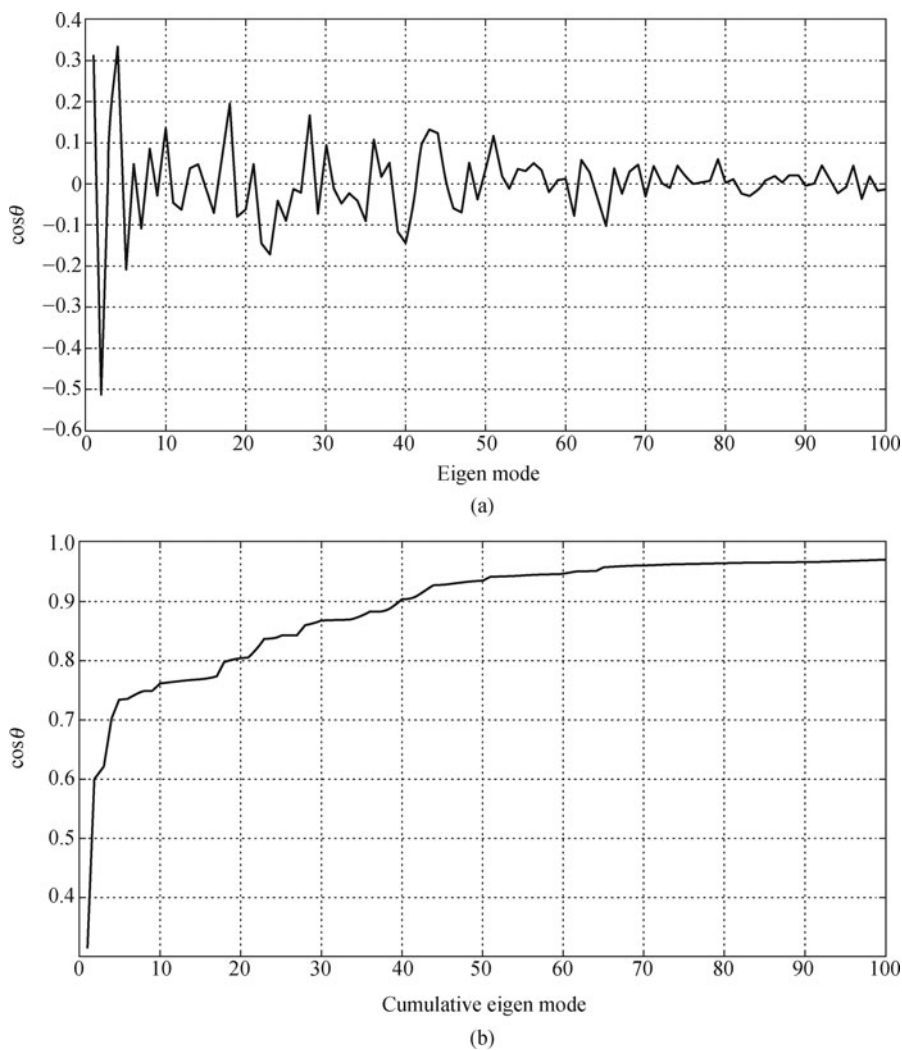


Fig. 3.23 PCA motions overlap measurement calculated with α -carbon for T4 lysozyme MD simulations: (a) overlap for individual mode, (b) overlap for cumulative modes.

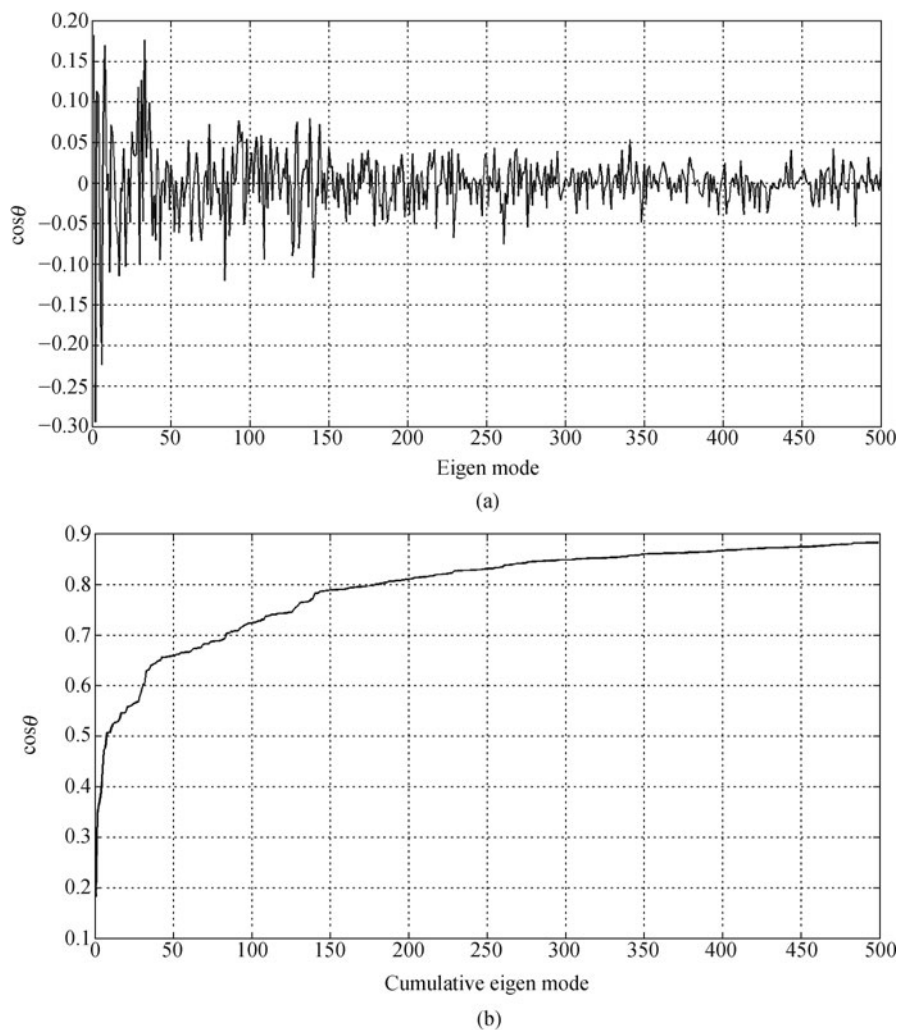


Fig. 3.24 PCA motions overlap measurement calculated with all atoms for T4 lysozyme MD simulations: (a) overlap for individual mode, (b) overlap for cumulative modes.

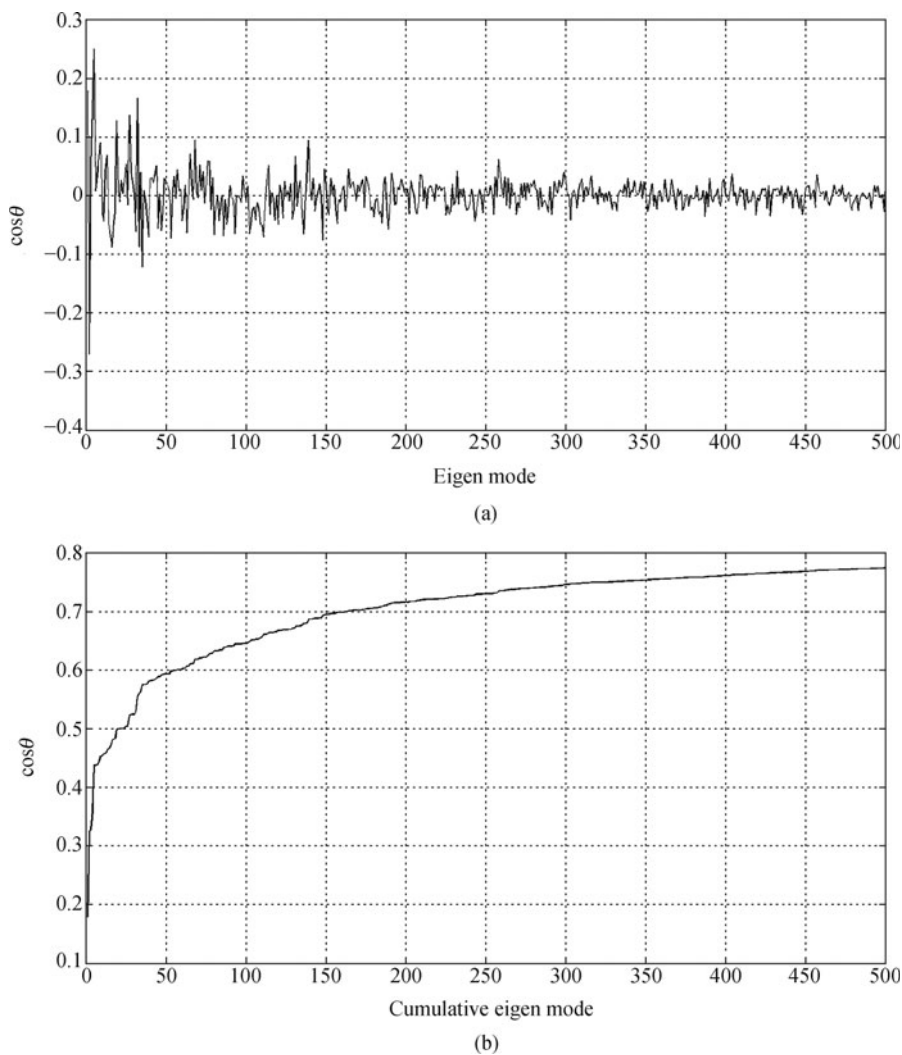
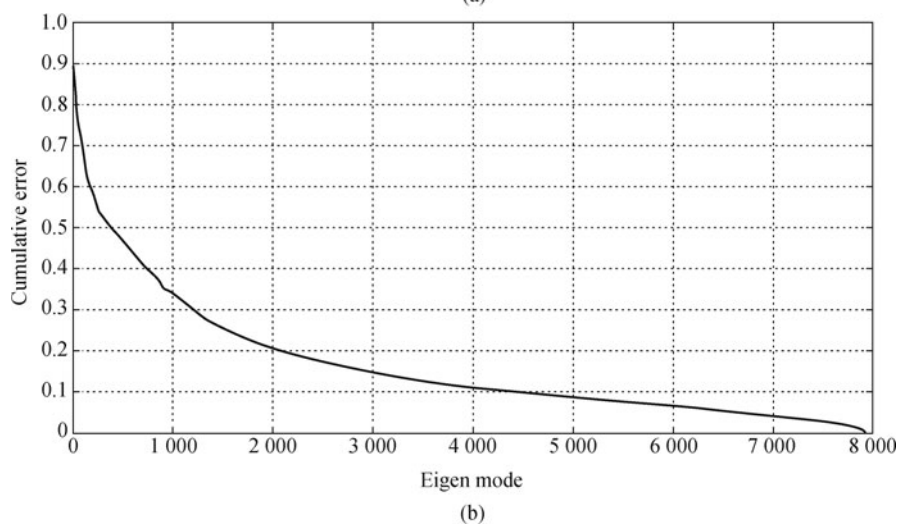
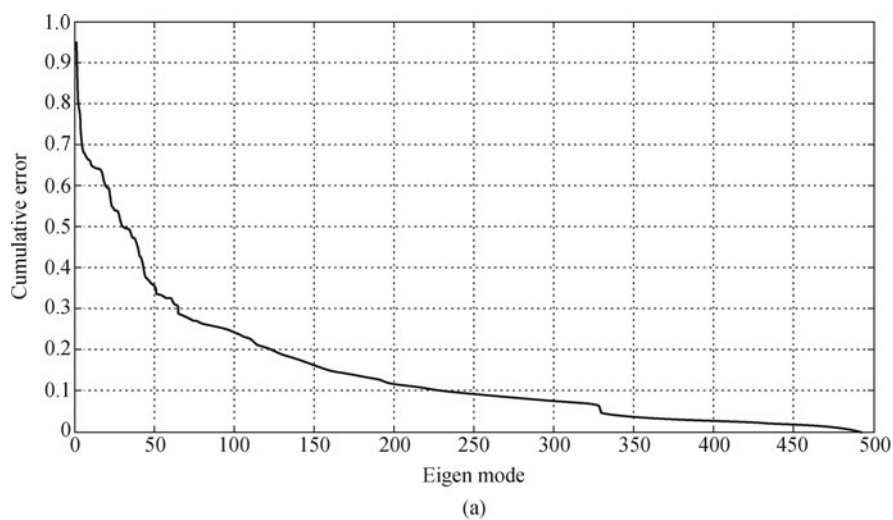


Fig. 3.25 QHA motions overlap measurement calculated with all atoms for T4 lysozyme MD simulations: (a) overlap for individual mode, (b) overlap for cumulative modes.



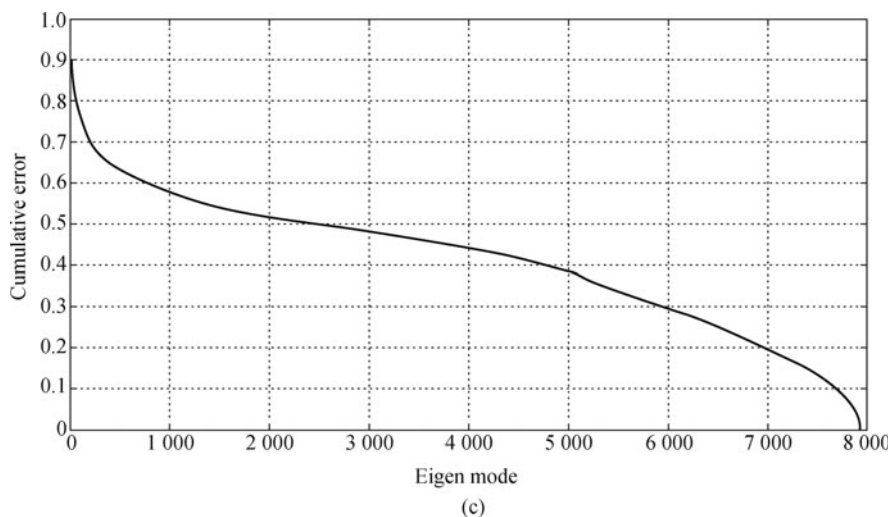


Fig. 3.26 PCA and QHA motions error measurement calculated with all atoms for T4 lysozyme MD simulations: (a) overlap for individual mode, (b) overlap for individual mode, (c) overlap for cumulative mode.

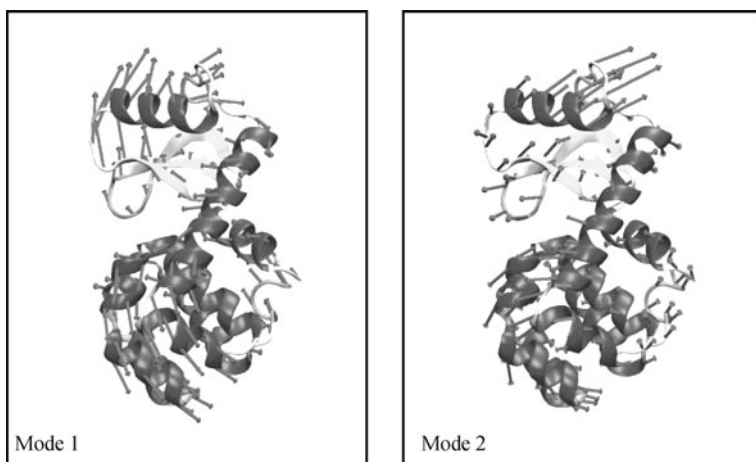


Fig. 3.27 Schematic representation of the two lowest eigenmodes of T4 lysozyme computed using the FEM (color plot in the book end).

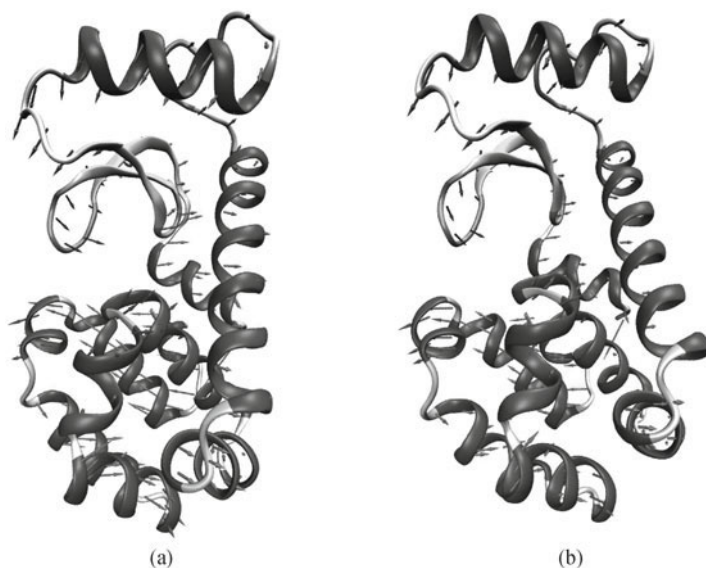


Fig. 3.28 The 1st eigenmode displacement visualization of T4 lysozyme MD simulation with solvent. The starting structure is shown in new-cartoon representation colored by structure. The red arrows indicate the direction of displacement based upon 1st eigenmode of MD simulation with solvent: (a) generated from PCA, (b) generated from QHA (color plot in the book end).

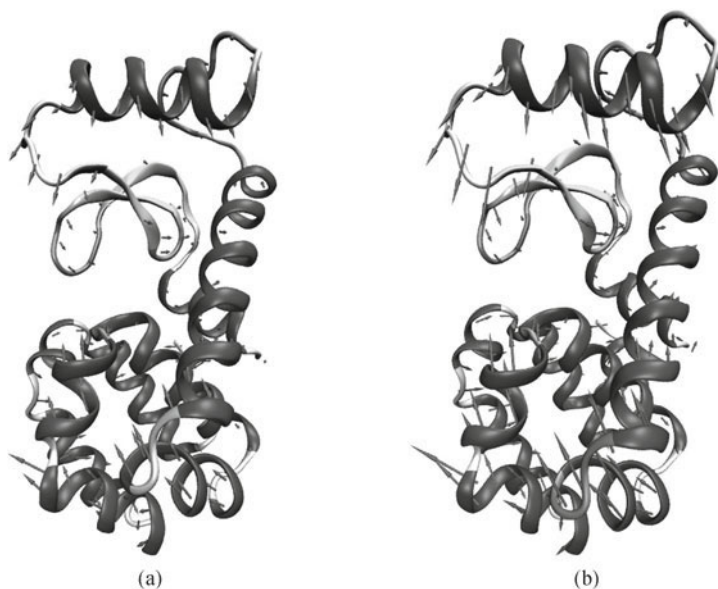


Fig. 3.29 The 2nd eigenmode displacement visualization of T4 lysozyme MD simulation with solvent. The starting structure is shown in new-cartoon representation colored by structure. The red arrows indicate the direction of displacement based upon 2nd eigenmode of MD simulation with solvent: (a) generated from PCA, (b) generated from QHA (color plot in the book end).

3.5.6 Identification of slow conformational flexibility

The lowest modes of proteins describe their most characteristic conformational change, which are often directly related to their function. The lowest mode of the T4 lysozyme is a hinge-bending mode similar to that of native hen egg lysozyme. Three lowest modes are presented in Figs.3.28, 3.29, and 3.30. They are compared with the results from Ref.[61]. The second mode shows the hinge-bending mode.

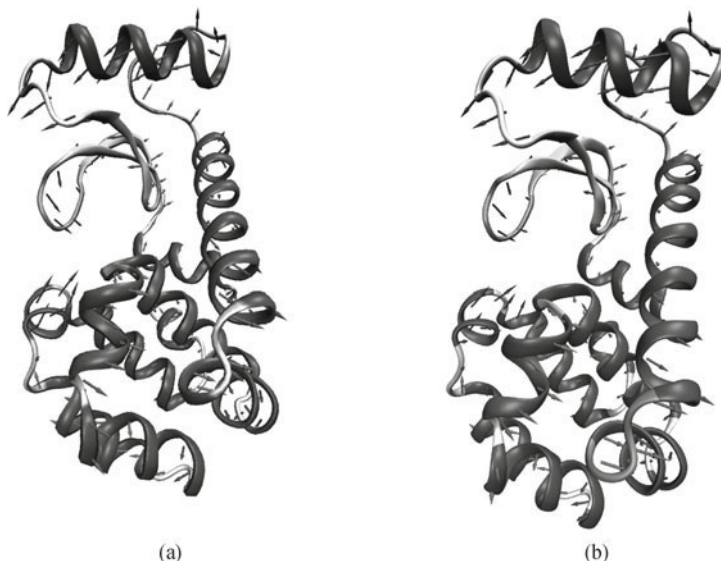


Fig. 3.30 The 3rd eigenmode displacement visualization of T4 lysozyme MD simulation with solvent. The starting structure is shown in new-cartoon representation colored by structure. The red arrows indicate the direction of displacement based upon 3rd eigenmode of MD simulation with solvent: (a) generated from PCA, (b) generated from QHA (color plot in the book end).

3.5.7 Correlation analysis of T4 lysozyme

A cluster analysis of residues was carried out from the correlation matrix and is shown in Section 3.4.3. It depicts possible interactions among groups of residues. This method provides a starting point for experimental and computational studies designed to determine more fine-grained conformational changes in the protein.

Figure 3.31 generated by Ref.[61] serves as a basis for evaluation of these results. In Fig.3.31, where results were calculated from the raw simulation data, the (anti-) correlation is weak. However, after projecting the first 10

eigenmodes to generate a new “cleaned” data set, the (anti-) correlation results were in closer agreement with Bathe’s. However, more correlations between residue groups are found in this map, suggesting that more interactions have been identified with the PCA-based method.

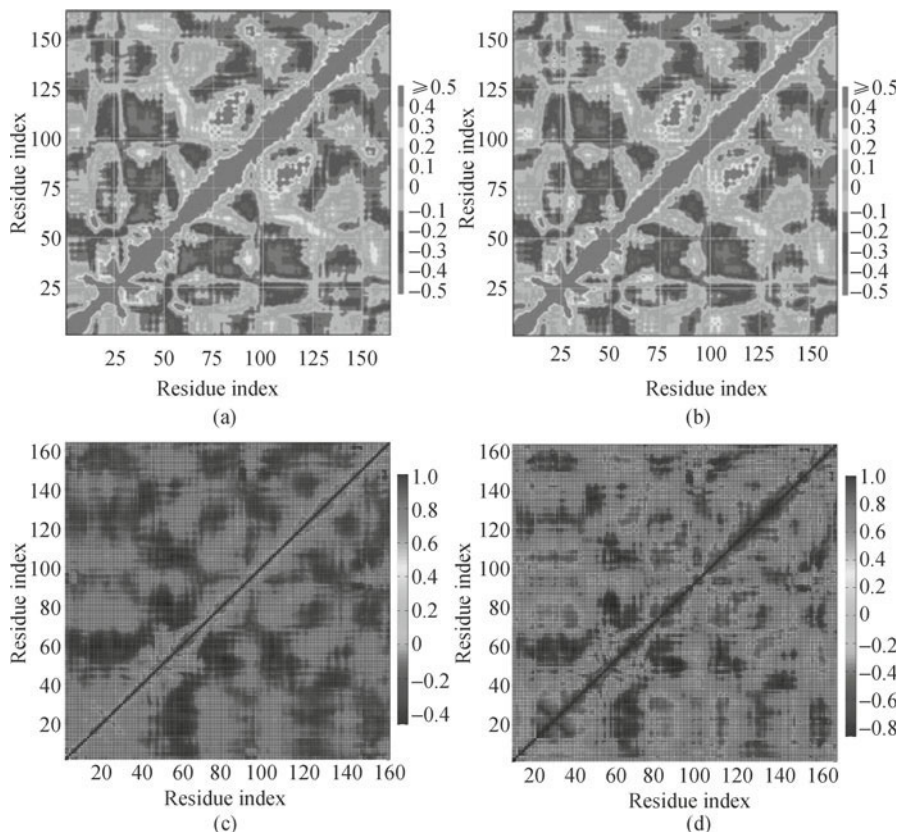


Fig. 3.31 Correlated fluctuation of α -carbon atoms computed for T4 lysozyme: (a) all Atom Method (ATM) vs. Normal Mode Analysis (NMA), (b) RTB procedure, (c) original simulation data, (d) PCA method (color plot in the book end).

Clustering was performed using the UPGMA algorithm. In Fig.3.32, the cluster shows only “contact-based” relationships. This might be caused by the conformational change in which a new harmonic balance has been reached. In Fig.3.33, the red α -helix group shows the contribution of the “hinge-bending” mode. Furthermore, interaction between the two lower α -helices are strong too.

This chapter presents the MD-PCA framework of analysis applied to the equilibrium conformational fluctuation of the T4 lysozyme. This T4 lysozyme case study indicates that the PCA/QHA analysis using all atoms is effective



Fig. 3.32 Residue clusters based on the correlation matrix for MD simulation without solvent. Clusters generated using the UPGMA algorithm were selected based on binding site residues, etc. These clusters are simply groups of residues with concerted conformational fluctuations that could be important for inter-domain communication. Different clusters are indicated by color (color plot in the book end).

in extracting slow motions of the protein, yielding results in agreement with previous studies, as well as some novel and potentially useful new results.

It is difficult to theoretically explain and predict the conformational change of proteins that occur during binding or interaction. Conformational change is not accessible to molecular dynamics simulations today due to their high computational costs. Therefore other computational methods must be applied. Previous studies^[9,18] show that some of the lowest-frequency normal modes of several proteins, including hemoglobin, are strongly correlated with the large amplitude conformational change of these proteins. In this chapter, the MD-based PCA method previously described is applied to large-scale (low-frequency) protein-protein interaction, ignoring local (high-frequency) motion. This method reveals that the dominant motion of aggregation of sickled hemoglobin molecules is caused by hydrophobic effects. This discovery demonstrates that it is feasible to model slow motions due to hydrophobic effects with MD-based PCA.

The MD-PCA framework is used to analyze hemoglobin-hemoglobin

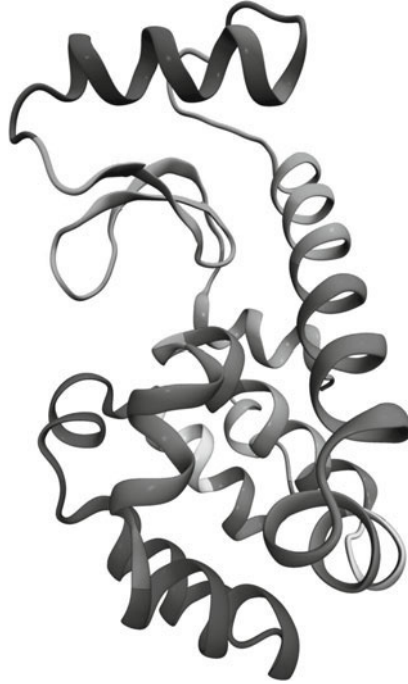


Fig. 3.33 Residue clusters based on the correlation matrix first 10 PCA modes. Clusters generated using the UPGMA algorithm were selected based on binding site residues, etc. These clusters imply groups of residues with concerted conformational fluctuations that could be important for inter-domain communication. Different clusters are indicated by color (color plot in the book end).

interaction in this chapter. An artificial environment resembling sickled hemoglobin interaction is built. The simulation produces a noticeable “aggregation” process. The dominant modes calculated using PCA in this simulation show a damping-approach process during hemoglobin-hemoglobin interaction. Furthermore, this interaction displays a “softball-like” shape change in each hemoglobin molecule. That is, the atomic motions have greater amplitude opposite the point of interaction. The amplitude diminishes along with the distance to the “interaction-spot”. This suggests that there is not a single “attractive force bond” that causes the aggregation of hemoglobin, which matches the results of [1, 2, 62, 63].

3.6 Hemoglobin and sickle cell anemia

Hemoglobin is the main ingredient of red blood cells (RBC) in vertebrate blood. Hemoglobin is an iron-containing oxygen-transport metallo-protein.

The primary structure of hemoglobin, like other protein molecules or polypeptides, is a long chain of many monomers or amino acids. The well-known secondary structures are α helices and β sheets. The α - and β -globins are derived from gene clusters. The α -globin genes are on chromosome 16 and the β -globin genes are on chromosome 11. Both gene clusters contain not only the major adult genes, α and β , but other expressed sequences that are utilized at different stages of development. The orientation of the genes in both clusters is in the 5' to 3' directions, with the earliest expressed genes at the 5' end. These basic structures eventually form tertiary and quaternary structures. In humans, the hemoglobin molecule is most often an assembly of four globular protein sub-units, as shown in Fig.3.34. Each subunit is composed of a protein chain tightly associated with a non-protein heme group. Each heme group contains one iron atom that is directly responsible for the transport of oxygen from the lungs to other tissues and transport of carbon dioxide from tissues to the lungs. The four polypeptide subunits are bound to each other by salt bridges, hydrogen bonds, and hydrophobic interaction. There are two kinds of contacts between the α and β chains: $\alpha_1\beta_1$ and $\alpha_2\beta_2$. The predominant hemoglobin in adulthood is HbA ($\pm 97\%$), which consists of two α and two β -globin chains ($\alpha_2\beta_2$). Other hemoglobin types are HbA₂ (2 to 3.5%; $\alpha_2\delta_2$) and HbF ($< 2\%$; $\alpha_2\gamma_2$).

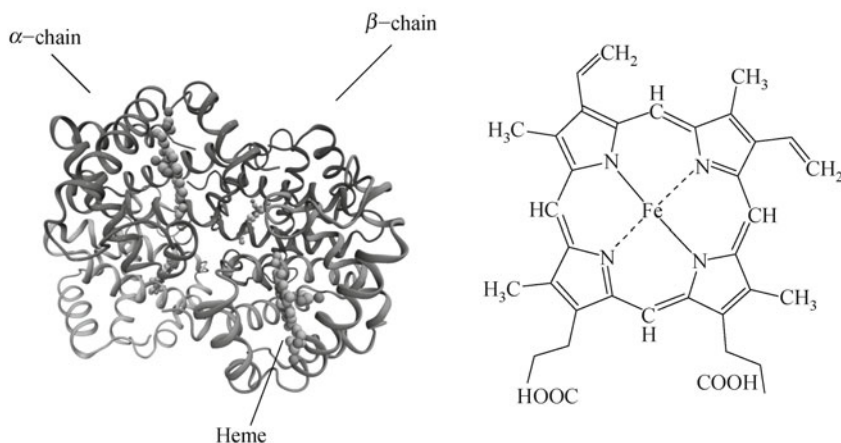


Fig. 3.34 Quaternary structure of hemoglobin and its oxygen carrier heme.

Although secondary and tertiary structures of various hemoglobin subunits are similar, reflecting extensive homology in amino acid composition, the variations in amino acid composition that do exist impart marked differences in hemoglobin's oxygen carrying properties. In addition, the quaternary structure of hemoglobin leads to physiologically important allosteric interaction between the subunits. One specific mutation in the genes for the hemoglobin protein results in a series of consequences, from the type

of amino acid to the conformation or shape and the function of the complete hemoglobin molecule, which eventually leads to sickle cell anemia.

This particular point mutation, β_6 , replaces glutamic acid with the less polar valine at the sixth position of the β chain, forming an abnormal globin: β^s . This results in the formation of “sickle hemoglobin” or HbS ($\alpha_2\beta_2^s$).

In Fig.3.35, hemoglobin tetramers are represented as circles. Each quarter corresponds to one protein subunit. The β_6 mutation is indicated as a protrusion from the circle in the β_2 subunit and the hydrophobic pocket is a kink in the β_1 subunit. The interaction of different HbS tetramers and the formation of the fiber or chain in the deoxygenated state is also illustrated in Fig.3.35. Upon deoxygenation, HbS has a certain probability of forming a hydrophobic interaction with an adjacent S globin, ultimately resulting in the polymerization of HbS. The hydrophobic residue provides a nucleating site for protein-protein interaction or “sticky patch”. Figure 3.36 provides an illustration of this docking phenomenon.

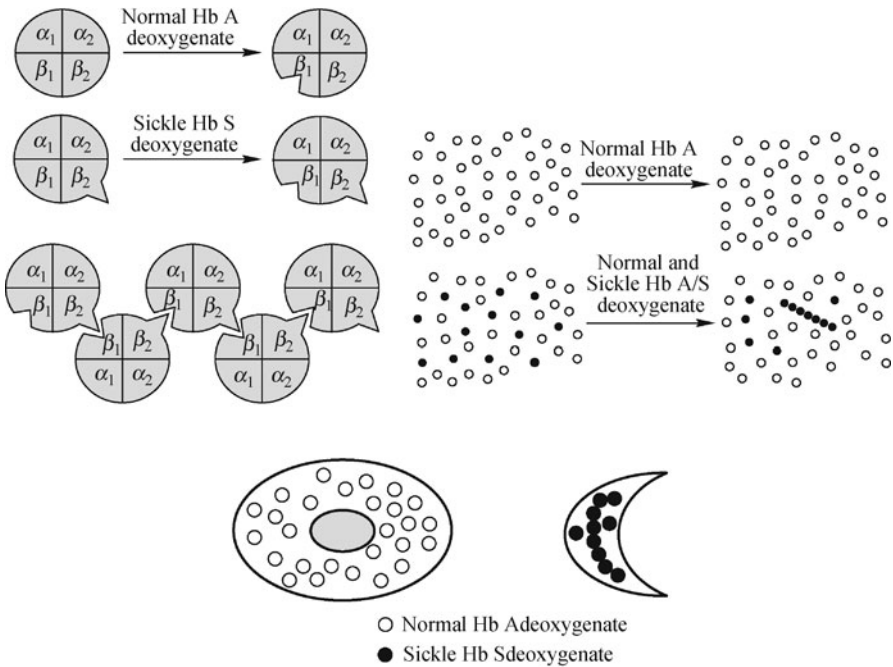


Fig. 3.35 An illustration of the genetic cause of macroscopic property changes of red blood cells.

In essence, the underlying problem in sickle cell anemia is that the valine for glutamine substitution results in hemoglobin tetramers that aggregate into arrays upon deoxygenation. This aggregation leads to deformation of the red blood cell, making it relatively inflexible and unable to traverse the capillary beds. Repeated cycles of oxygenation and deoxygenation lead to

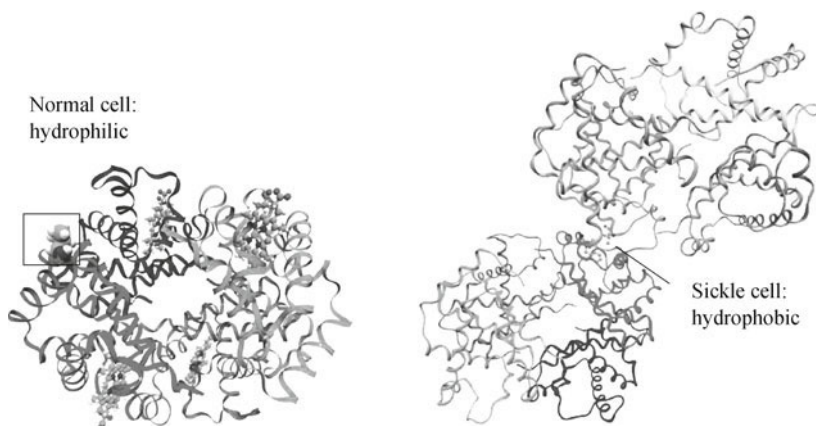


Fig. 3.36 Sickle mutation alters a hydrophilic β -globin site to a hydrophobic one.

irreversible sickling. The end result is clogging of the fine capillaries. Because bones are particularly affected by the reduced blood flow, frequent and severe bone pain results. This is the typical symptom of a sickle cell crisis. Long-term recurrent clogging of the capillary beds leads to damage to the internal organs, particularly the kidneys, heart and lungs. The continual destruction of the sickled red blood cells leads to chronic anemia.

Under anaerobic conditions, sickle cell hemoglobin (HbS) polymerizes into highly elongated cables. Such polymers distort the shape and suppleness of RBCs, resulting in a sickle-like appearance compared to the donut shape of normal RBCs. The rigid and distorted sickled RBC has difficulty in passing through the small capillary, thereby blocking blood flow. However, only deoxy-HbS, other than oxy-HbS, polymerizes. This is consistent with the fact that RBC sickling occurs in the capillaries where the O_2 concentration is relatively low and the deoxy-HbS concentration is relatively high. The sickle cell phenotype arises from a single mutation in the β -globin gene resulting in an amino acid substitution at the sixth residue of the β -chain with β -Val6 in HbS substituted for β -Glu6 in normal HbA. The hydrophobic β -Val6 side-chains are exposed on the surface of two β -chains of HbS and they can fit into hydrophobic pockets created by the side-chains of β -Phe85 and β -Leu88, and also on the β -chain surface^[64,65], as shown in Fig.3.37. The spacing between these residues is such that deoxy-HbS molecules associate with each other and polymerize. However, the geometrical spacing between these residues is different for the oxy-HbS conformer and it does not polymerize.

This red blood cell system (sickle or normal) was chosen as a test system for a multi-scale and multi-physics modeling protocol because there is a wealth of clinical data for sickle cell diseases and, more importantly, because the sole cause of this hereditary disease, a mutation of a base pair in the β -globin gene, is known. Hydrophobic effect is the molecular level cause and it is a slow motion during protein-protein interaction. The test sought

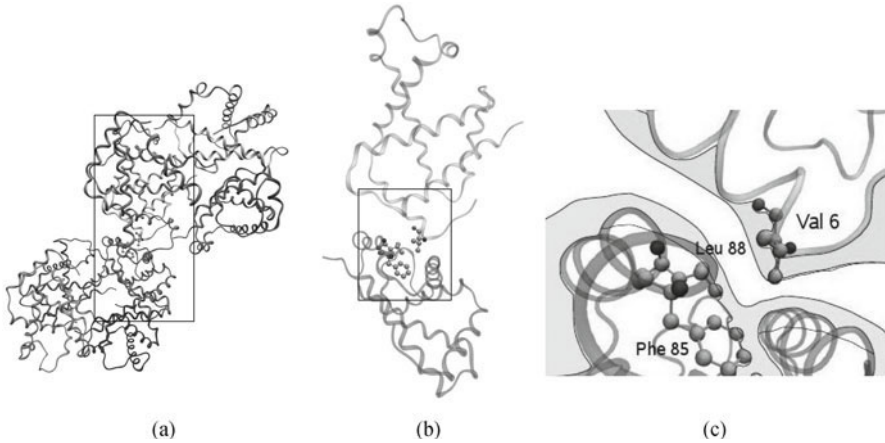


Fig. 3.37 Donor and acceptor during deoxygenated states: (a) global view, (b) enlarged view from (a), (c) donor and acceptor, enlarged view from (b).

for determining whether this MD-based PCA method aims to reveal this conformational change.

3.6.1 Molecular dynamic simulation with NAMD

The threshold for sickled hemoglobin aggregation is unknown, which makes it difficult to simulate this process. An artificial system was built by separating two docked mutated hemoglobin molecules from each other for a little distance (4 Å to 5 Å) along the axis of their center of mass. The initial docked configuration was obtained from Ref.[66] (PDB ID: 1BZ0). The initial system is illustrated in Fig.3.38.

The PSF package in VMD^[56] is used to generate a structure file (PSF file) for MD simulation. First, the coordinates missing in the crystal structure are reconstructed. The minimized structure is solvated in a periodic truncated cubic simulation box of 111 738 TIP3P water molecules, providing a minimum of 15 Å of water between the protein surface and any periodic box edge. The water box is large enough that the protein does not interact with its image in the next cell. The water box is rotated to minimize the system size.

Ions are placed in the water to represent a more typical biological environment. They are especially necessary because the protein being studied carries six excess negative charges. Six extra sodium ions are added to make the system neutral. The ions present shield the regions of the protein that carry the charge and make the entire system more stable. They are placed in regions of potential minima, since they will be forced into those regions during the simulation anyway. Sodium and chloride ions are added to neutralize the total system and achieve a salt concentration of 0.05 mol/L. Minimum

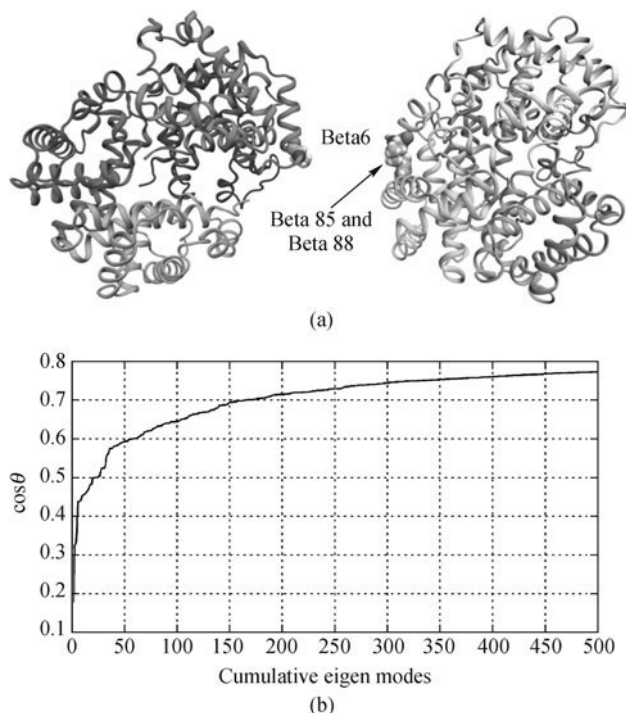


Fig. 3.38 Initial simulation position for HbS: (a) global view without water, (b) a closer look of the pocket.

distances between ions and the protein molecule as well as between any two ions are set as 5 Å.

A periodic boundary condition was selected for simulation with solvent. The use of a periodic boundary condition involves surrounding the system under study with identical virtual unit cells. The atoms in the surrounding virtual systems interact with atoms in the real system. These modeling conditions effectively eliminate surface interaction of the water molecules and create a more faithful representation of the *in vivo* environment than a water sphere surrounded by vacuum provided^[57].

Energy minimization involves searching the energy landscape of the molecule for a local minimum. MD minimization and equilibration simulation involve more than one minimization-equilibration cycle, often fixing and releasing molecules in the system.

Energy minimizes in 180 fs and equilibrates with the atoms in the protein fixed in space in 120 fs, minimizes the system again in 60 fs and equilibrates in 120 fs again, this time with the protein free to move. Fixing the protein allows the water, which typically responds much faster to forces than the protein, to do the relaxing in the first step. This saves computational effort and prevents the introduction of artifacts from an unstable starting structure.

A final 100 fs energy minimization reaches the “minimized” position. The parameter settings are shown in Table 3.5.

Table 3.5 MD simulation parameter setting for hemoglobin interaction

Item	Setting
Periodic boundary conditions	Yes
Cutoff distance	12 Å
Switch distance	10 Å
Pair list distance	13.5 Å
Timestep	2.0
Rigid bonds	All
Non-bonded frequency	2

It is important to check if this system has the possibility of docking. After energy minimization, a vacuum space forms between β -Val6 side-chains and the surface of another β -Phe85 and β -Leu88 (Fig.3.39). This leads to a docking process of the two hemoglobin molecules.

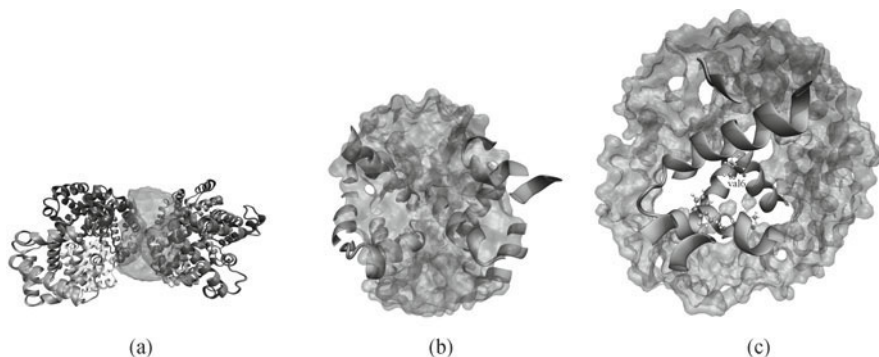


Fig. 3.39 Illustration for energy minimization position with HbS simulation: (a) global view, for illustration water was shown within 20 Å of residue $\beta 6$ only, (b) a closer look of the pocket, (c) rotation by 90° (color plot in the book end).

There were no energy changes during last 1 000 time steps in energy minimization. Following minimization, the entire system was heated at 5 K up to 310 K with micro-canonical ensemble (NVE) equilibration per temperature step. The production simulation was conducted at 310 K. The trajectory was calculated with one femtosecond (fs) time step using SHAKE^[58] constraints on hydrogen-heavy atom bonds.

In order to represent an environment more typical of the human body, a constant temperature of 310 K was set. The temperature is controlled by Langevin dynamics deploying a damping frequency of 5 ps⁻¹ to all non-hydrogen atoms. Langevin dynamics is a means of controlling the kinetic energy of the system, thus controlling the system temperature and/or pres-

sure. The method uses the Langevin equation for a single particle:

$$m_i \frac{d^2 x_i(t)}{dt^2} = F_i x_i(t) - \gamma_i \frac{dx_i(t)}{dt} m_i + R_i(t). \quad (3.34)$$

The second term on the right side represents a frictional damping that is applied to the particle with frictional coefficient $\gamma_i m_i$. The third term represents random forces that act on the particle (as a result of solvent interaction). These two terms are used to maintain particle kinetic energy to keep system temperature at a constant value.

The Particle Mesh Ewald method (PME)^[67,68] is used to compute the electrostatic forces. The grid spacing is kept below 1.0 Å. Multiple time stepping is carried out using the Verlet impulse/*r*-RESPA integration scheme^[69]. Van der Waals interaction is truncated at 13.5 Å using a switching function starting at 12.0 Å. Full periodic boundary conditions are imposed. The coordinates and energy are saved every 1 ps.

The whole process of hemoglobin-hemoglobin interaction could be long. MD simulation works at a nanosecond scale and cannot reveal this whole process. In addition, microscopic details missed by experiments, such as the behavior of hydrogen bonds and the role of water, could be studied and explored with slow motion analysis.

3.6.2 Conformational change analysis

The beginning approach between the two hemoglobin molecules occurs during the “heating” stage. Figure 3.40 shows the distance between the centers of mass of two HbS. The first 660 ps time steps are the heating stage, followed by 60 ps equilibrating steps. A rapid drop can be noticed during the heating stage. After their initial displacement, these two molecules were pushed toward each other, and then reached a local equilibration. The distance of the last 9 ns shows a “damping” stage between two molecules.

Figure 3.41 shows the results of RMSD during regular simulation after the heating and equilibrating stages. RMSD is a measure of equilibration and simulation stability. Although the distance doesn’t change during the last 9 ns, the RMSD changes indicate continued conformational changes.

Figure 3.42 illustrates the conformational changes for each residue. Blue areas indicate higher RMSF scores and red ones reflect lower RMSF scores. This shows that the most conformational fluctuation occurs on the surface at the far end of each hemoglobin. The insides of each hemoglobin are more rigid than the surface, except at the point of interaction. Therefore, the dynamic motion of each hemoglobin is not a rigid body motion, but more likely the motion of a soft ball.

This method enables the study of conformational changes by analysis of energy as well. Figure 3.43 shows the energy calculation. To separate the

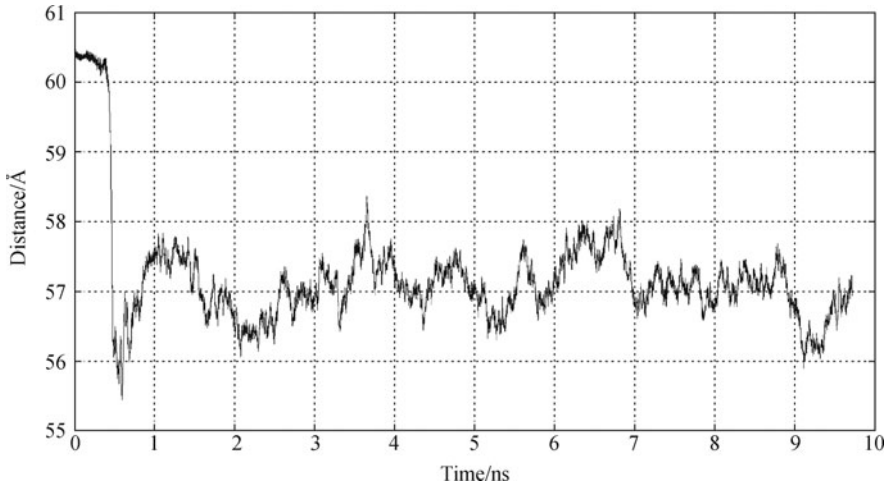


Fig. 3.40 Distance of center of mass of two hemoglobin molecules.

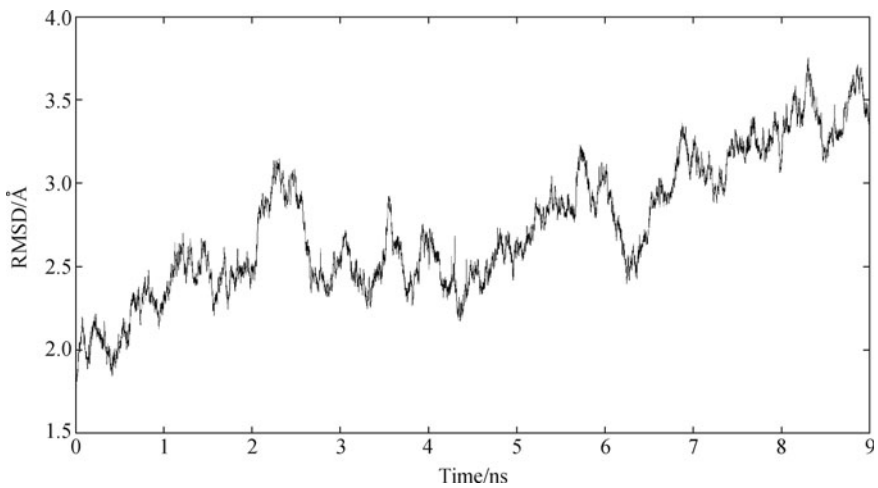


Fig. 3.41 RMSD of HbS simulation.

heating stage from regular simulation, the start of regular simulation is set as time zero. During the heating stage, both kinetic energy and potential energy increase. During the heating stage, the velocities are reassigned so the energy does not reflect the conformational change.

Van der Waals (VDW) forces are relatively weak compared to normal chemical bonds, but play a fundamental role. Figure 3.43 shows the calculation of VDW potential. As the two molecules approaching, VDW increases until the so-called strong repelling position is fixed. VDW then decreases while the distance increases. After equilibration, VDW and other poten-

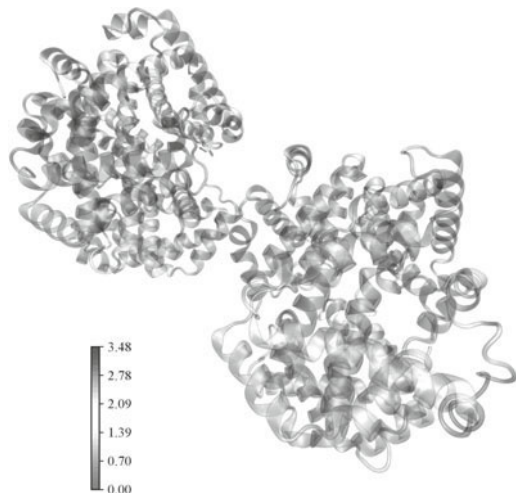
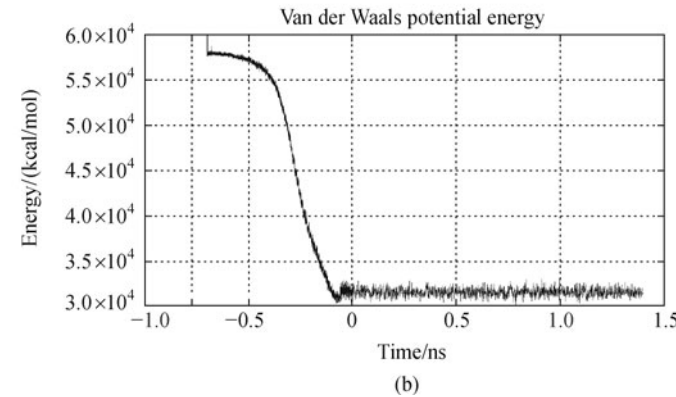
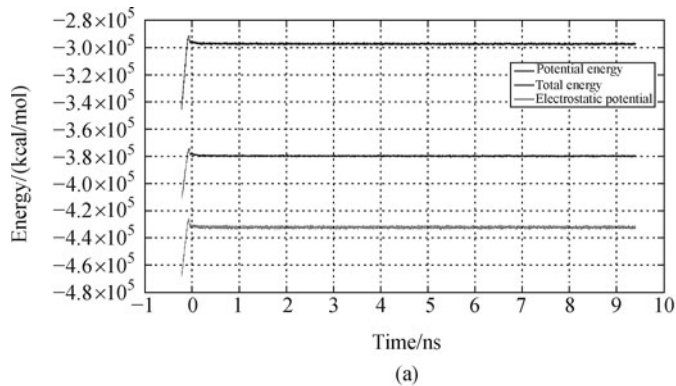


Fig. 3.42 Illustration of RMSFs for hemoglobin interaction simulation, colored by RMSF value for each residue (color plot in the book end).



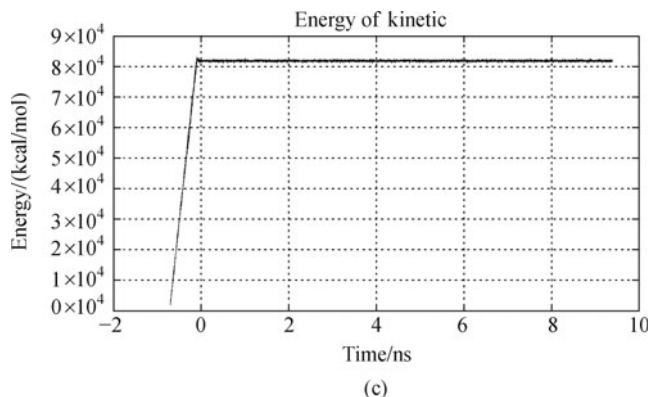


Fig. 3.43 Energy plotting, heating and equilibrating stages shown before time step 0: (a) potential energy, (b) van der waals energy, (c) kinetic energy.

tials remain stable within a local potential well. However, the conformational changes have not ended. These two molecules could reach another equilibration place and relative energy will continue to change as well.

3.6.3 PCA analysis

The principle components of the MD results are calculated accordingly. The eigenvalues are shown in Fig.3.44. In Fig.3.44, the “elbow” turn is at the 5th eigenvalue and the cumulative energy content of the first five eigenvalues is 63%. The first five eigenmodes are selected as the dominant modes for study.

The lowest mode shapes computed using PCA are projected onto the energy-minimized structures of hemoglobin in Fig.3.45 to visualize the directional behavior of their large length-scale collective motion. The lowest mode shapes of proteins describe their most typical conformational changes, which are often directly related to their function.

Based on the lowest mode shape, each hemoglobin molecule oscillates around the point of contact. The whole molecule is not rigid during this oscillation. The residues on the contact chain, namely, chains B and H, shown in Fig.3.45, move with smaller amplitude; the residues on the outer chains, namely, chains A, C, E and F, move with larger amplitude. Furthermore, the farther the distance from the point of contact, the larger the amplitude of the motion of each residue. This result shows that the driving force for hemoglobin-hemoglobin aggregation is not a bonding force. Rather, the water environment plays an important role in the motions, hydrophobic in nature, the effect of which involves the entire surface of the hemoglobin molecule.

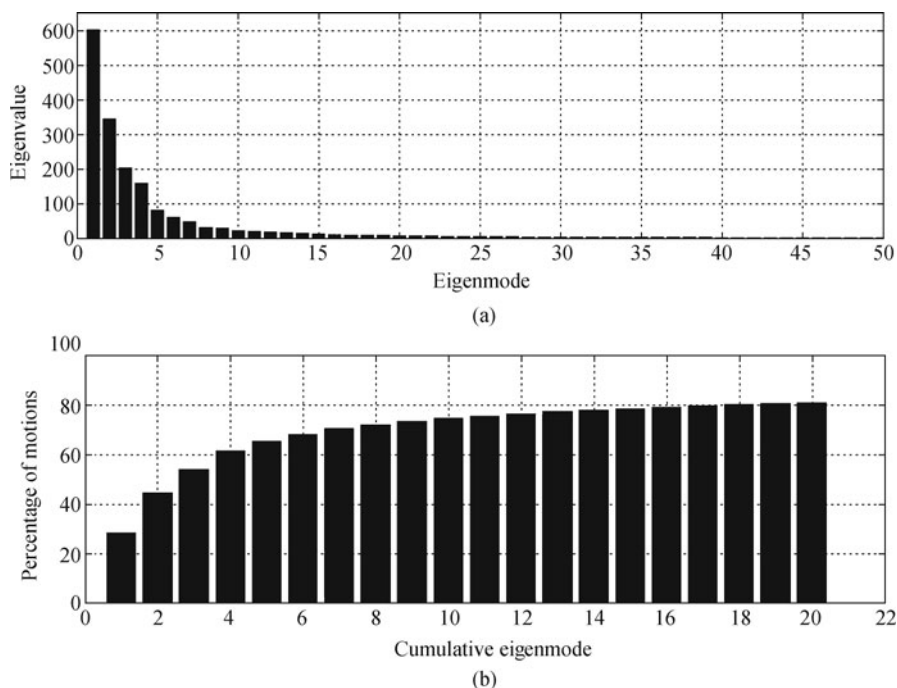


Fig. 3.44 PCA eigenvalues calculated with α -carbon for hemoglobin interaction: (a) eigenvalue, (b) cumulative energy content for each eigenvector.

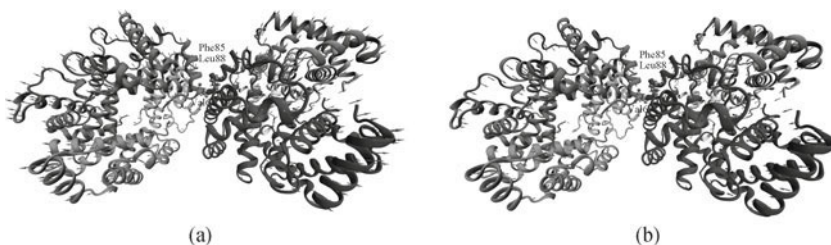


Fig. 3.45 Hydrophobic motion captured by PCA analysis, colored by chains: chain A blue, chain B gray, chain C orange, chain D yellow, chain E tan, chain F silver, chain G green, chain H red. The red arrows indicate the direction of displacement based on 1st eigenmode, the lengths of arrows indicate the amplitude: (a) eigenvalue, (b) cumulative energy content for each eigenvector (color plot in the book end).

3.6.4 Correlation analysis with HbS interaction

A correlation matrix showing the clustering of the motions of residues was produced, as shown in Section 3.4.3. This indicates a possible interaction between groups of residues. The correlation matrix is based on Eq.(3.31). The

clusters were identified by the UPGMA algorithm introduced in Section 3.4.3. Figure 3.46 shows the correlation map and Fig.3.47 illustrates the clusters, with different clusters indicated by color.

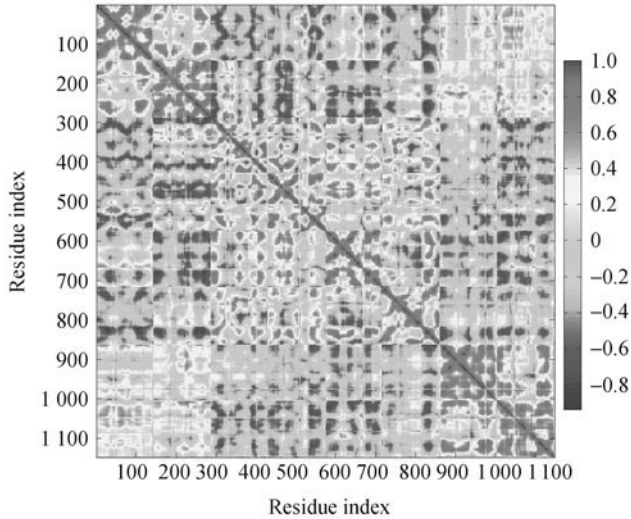


Fig. 3.46 Correlation of HbS result (color plot in the book end).

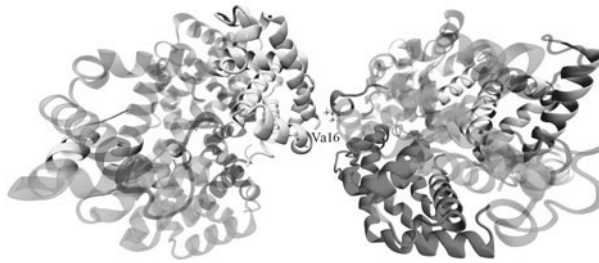


Fig. 3.47 Residue clusters based on the correlation matrix first 5 PCA modes. Clusters generated using the UPGMA algorithm were selected based on including binding site residues. These clusters imply groups of residues with concerted conformational fluctuation that could be important for inter-domain communication. Different clusters are indicated by color (color plot in the book end).

The hydrophobic “pocket”, formed by residue β -Val6 on chain H and residue β -Phe85 and β -Leu88 on chain B, has been studied. The correlated residues with β -Phe85 and β -Leu88, which are in the same cluster, are in yellow; whereas the correlated residues β -Val6 are in red. The yellow cluster indicates that the motion related to β -Phe85 and β -Leu88 comes from outside α -helices. The driving “forces” are horizontal, which tend to “push” the protein molecules closer. On the other hand, the red cluster has vertical motion

and contributes shear stress to the docking place. This stress may lead to the separation of these two molecules. The heme near the docking spot plays an important role. It may contribute resistance to the shear stress. The formation of this heme is important. Hemoglobin aggregation occurs only during a deoxygenated state. The possible reason is the heme during deoxygenated stage which supports the stability of the docking pocket. Of course, this hypothesis needs further validation by experiments or other methods such as quantum mechanical calculation.

3.7 Conclusion

In this work, the MD-PCA framework is used to study sickled hemoglobin-hemoglobin interaction. The fine details of protein motion are produced to predict the hemoglobin-hemoglobin interaction. A correlation matrix based on clustering of residue motion is produced. The clustering suggests a possible explanation for hemoglobin aggregation and points to the role of water during this intermediate state. These analyses provide a starting point for further study and for exploring protein conformational change using the micromechanical and elastic properties of protein.

Acknowledgements

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Chapter 4 Structural, Mechanical and Functional Properties of Intermediate Filaments from the Atomistic to the Cellular Scales

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Abstract: Living systems have developed effective strategies in adapting to the environment during the last 3.5 billion years. Here we review joint experimental, computational and theoretical multiscale studies of the mechanical properties of intermediate filament (IF) proteins, an important component in the cell's cytoskeleton and a key player in cell mechanics, migration and disease processes including cancer. We review the general field of intermediate filament mechanics, highlight recent advances, challenges, and explain the opportunities that now exist at the interface of engineering and biology. A general theme of our discussion is the consideration of how material properties change at different structural scales and how this relates to biological function, from a bottom-up perspective that link the nano- to the macroscale.

Keywords: intermediate filaments, hierarchical structure, biomechanics, multi-scale modeling, computational simulation, materiomics, nuclear lamina

4.1 Introduction

Living systems have developed effective strategies in adapting to the environment during the last 3.5 billion years. The structures of plants, fungi and

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bacteria cells are stabilized and protected mainly by their cell wall, which is essential for their survival. Animal cells lack such a cell wall structure, and other strategies are required to stabilize their cells and tissues^[1]. Moreover, the behavior of many animal cells can not be achieved by having a cell wall, such as cell migration and contraction. Migration refers to the movement of cells which is a central process in the development and maintenance of organs, while contraction refers to the shortening of a cell and the concomitant development of tension that is important in generating force in muscles for locomotion and wound healing by closing the extracellular matrix. The ability of cells to survive in varied and extreme conditions, and to generate movement requires appropriate means to achieve the integrity, as well as an effective ability to generate and withstand mechanical stress. Cytoskeleton systems in metazoan cells serve as a scaffold to achieve those mechanical requirements. It maintains the cell shape, protects the cell, enables cellular motion, is involved in cell division process, and plays other important roles in intracellular transport for both mass and signals. For eukaryotic cells, there are three major components of the cytoskeleton: microtubules (MTs), intermediate filaments (IFs) and microfilaments (MFs). They have different main functions in maintenance of the cell shape. IFs and MFs function as ropes to bear tension while MTs function as support beams to bear compression. The name of IFs was termed in 1968 because their diameters are 8–12 nm, appearing to be intermediate in size between those of the other cytoskeletal components, MTs (25 nm) and MFs (7–9 nm)^[2,3].

IFs are composed of transcellular networks of both high rigidity and flexibility that integrate individual cells both dynamically and functionally into large-scale tissues. Compared to MTs and MFs, IFs are rather flexible, and in contrast to MTs and MFs, which break at smaller strain when subjected to tensile stress, IFs become highly elastic and resist large deformation^[3]. Earlier studies focused on analyzing the mechanical signature of human vimentin, which is a specific type of IFs found in the cytoplasm of fibroblasts, leukocytes, and blood vessel endothelial cells, representing the most widely distributed type amongst all intermediate filaments^[1]. Studies focusing on analyzing its mechanical signature have suggested that vimentin IFs are highly sensitive to applied forces at low levels, while they can sustain extremely large deformation of up to 300% under tension^[1,4,5]. It has also been observed that due to severe stiffening, the tangent modulus of IFs increases manifold during deformation, a property that is believed to be crucial for providing mechanical stability to cells at large stretch. Due to its unique mechanical signature IFs have also been referred to as the “safety belts of cells” to reflect their capacity to prevent exceedingly large cell stretch^[6–8]. IFs are also found in the cell’s nucleus in the form of lamin, where they form a dense mesh-like network providing mechanical integrity and biochemical functions at the cytoskeleton-chromatin interface^[9–11].

In humans, IF proteins are encoded by at least 65 genes, giving rise to a large protein family with limited sequence identity, as summarized in

Table 4.1^[12]. This constitutes the greatest difference between the IF system and both the MT and MF systems. These two systems (MTs and MFs) are engaged in many basic cellular functions so that mutations in their subunit proteins, tubulin and actin, are much less tolerated than mutations in IF proteins. The difference may be explained by that as far as the key cell functions are concerned (cell adhesion and migration, organelle shaping and positioning), IFs seem to play the role of a mediator by providing cellular signposts through post-translational modifications^[13]. This constitutes an important functional difference between both the MT and MF systems, which are the main players in the above mentioned cellular functions, and is believed to cause the difference in their mutation tolerance. Nevertheless, recent work has revealed a multitude of disease mutations in various IF proteins, leading to complex diseases that directly reflect the intricate expression patterns of IF genes. As summarized in Table 4.2, the diseases manifest tissue, cell, or nuclear defect and fragility in the skin, cornea, or muscle and some of the laminopathies support the point that intermediate filaments are a scaffold to maintain cell and tissue integrity and functionality^[12]. Amongst all IF proteins, lamin A is the one with the largest number of identified mutations, 230 so far, causing a complex set of at least 13 different human diseases including lipodystrophies, muscular dystrophies and even progeria syndromes. Although the disease mechanism is not at all clear in any of these cases, several models involving stress, cell fate and gene expression have been proposed. In addition, disturbances in signalling pathways, caused by mutations in IF

Table 4.1 The type, characteristic and location of intermediate filaments^[12]

Name	Type	Size	Gene	Cell or tissue distribution
Cytoplasmic keratins	I	40–64	17	Epithelium, hair
	II	52–68	12	Epithelium, hair
Vimentin	III	55	10	Mesenchyme
Desmin	III	53	2	All muscle
Glial fibrillary acidic protein	III	52	17	Astrocytes
Peripherin	III	54	12	Peripheral neurons
Syncoilin	III	54	1	Muscle (mainly skeletal, cardiac)
Neurofilaments	IV	61–110	8–22	Central nervous system
α -Internexin	IV	61	10	Central nervous system
Nestin	IV	240	1	Neuroepithelial
Synemin nuclear	IV	180, 150	15	All muscle
Lamins A and C	V	62–78	1	Nuclear lamina
Lamins B1 and B2	V	62–78	5, 19	Nuclear lamina
Other				
Phakinin	Orphan	46	3	Lens
Filensin	Orphan	83	20	Lens

Table 4.2 Overview over diseases associated with mutations in intermediate filaments^[12].

Disease name	Main clinical features	Associated genes
Chronic pancreatitis	Malabsorption, pain, weight loss	Keratin 8
Cirrhosis and hepatitis	Variceal bleeding, ascites, confusion	Keratins 8, 18
Epidermolysis bullosa simplex	Fluid-filled bullous skin lesions, generalized or localized	Keratins 5, 14
Epidermolytic hyperkeratosis	Hyperkeratosis, skin fragility	Keratins 1, 10
Ichthyosis bullosa of Siemens	Bullous ichthyosis, erythema	Keratin 2e
Charcot–Marie–Tooth disease	Symmetrical muscle weakness, wasting, foot deformities, difficulty walking, reduced tendon reflexes	Lamins A and C
Dilated cardiomyopathy	Conduction defects in the heart are present	Lamins A and C
Familial partial lipodystrophy	Substantial redistribution of adipose tissue	Lamins A and C
Hutchinson–Gilford progeria	Premature aging, alopecia, absence of ear lobes, dystrophic nails	Lamins A and C
Muscular dystrophy	muscle weakness, cardiomyopathy with conduction blocks	Lamins A and C
Alexander disease	Early-onset megalencephaly, progressive spasticity, dementia	Glial fibrillary acidic protein
Amyotrophic lateral sclerosis, sporadic form	Rapid loss of motor function	Neurofilament, heavy chain
Desmin-related myopathy	Peripheral and distal muscle weakness, arrhythmias, restrictive heart failure	Desmin

proteins, may be responsible for some aspects of IF diseases in general.

Recent advances in applying a materiomics approach to study disease mechanisms^[14]. This advantage inspires us that for a better understanding of the IF system, we need to use the mechanistic insight to reveal relations between structural, mechanical, and functional properties in the biological context. Therefore, we focus on defining structural properties of IFs from a materials science perspective, and on their role in mechanical materials phenomena, specifically materials failure, in diseased or altered physiological conditions. We will summarize recent advances in understanding the structure (with an explicit consideration of different hierarchical levels), property (for example strength or elasticity) and function (for example disease and material failure character) of the IF system.

4.1.1 Hierarchical structure of vimentin intermediate filaments

Vimentin, as one of most prominent types of IFs, forms hierarchical structures as shown in Fig.4.1, ranging from H-bonds, clusters of H-bonds in alpha-helical turns, alpha helical proteins, dimers, tetramers, unit length filaments, full-length filaments to the cellular level at the scale of micrometers^[15].

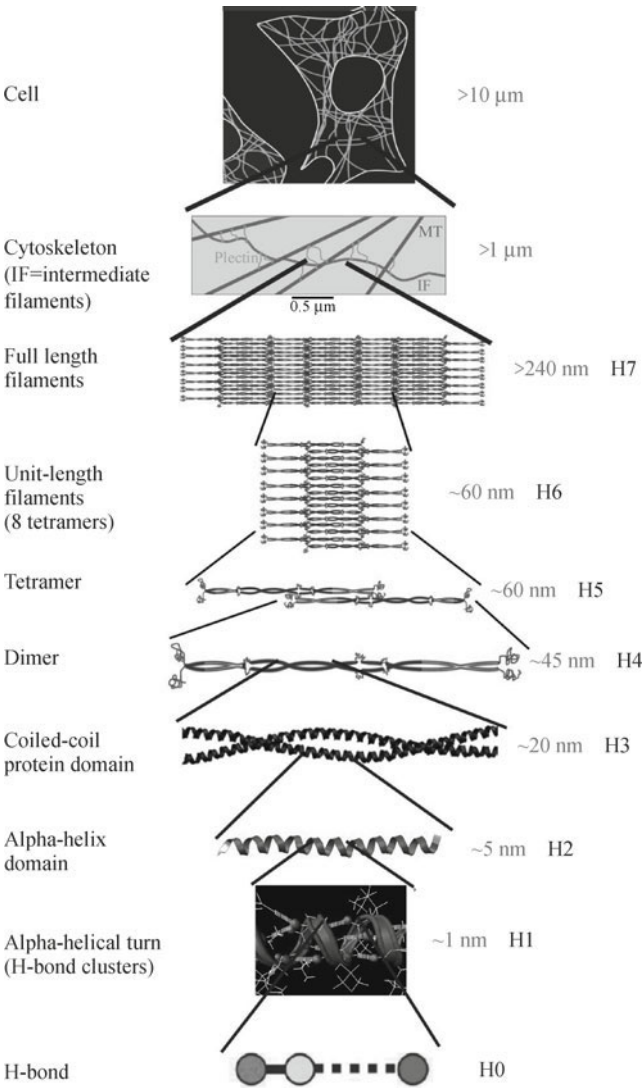


Fig. 4.1 The hierarchical structure of intermediate filaments, from atomic to cellular scales (for original reference see Ref.[15], color plot in the book end).

Each vimentin dimer, forming the fundamental building block of IFs, is

composed of two chains, with each containing 466 amino acids. Four rod-like structural segments are consisted in the dimer sequentially in the order of 1A, 1B, 2A and 2B, connected by linkers L1, L12 and L2 (Fig.4.2a). Outside the rod-like structure, there is an amorphous head and an amorphous tail domain. It has been suggested based on experimental work that the four structural segments are coiled coils, which are double-stranded protein motifs where each strand is an alpha-helix with heptad-repeated substrings. This seven-residue repeat generally has apolar residues at the first and fourth positions, forming a left-handed hydrophobic stripe. Following the orientation of this stripe, the two alpha helical strands wrap around each other. The coiled coil structure is believed to increase the stability compared with a single alpha helical structure. So far, the crystallized structure (available in the protein data bank) are limited to parts of the four segments that make up the entire dimer. For example, for vimentin, a section of the 1A and 2B domains has been crystallized and their atomic structures identified based on x-ray diffraction experiments^[16-20] (these structures are found in PDB entries 1gk4, 1gk6, 1gk7 and 3klt).

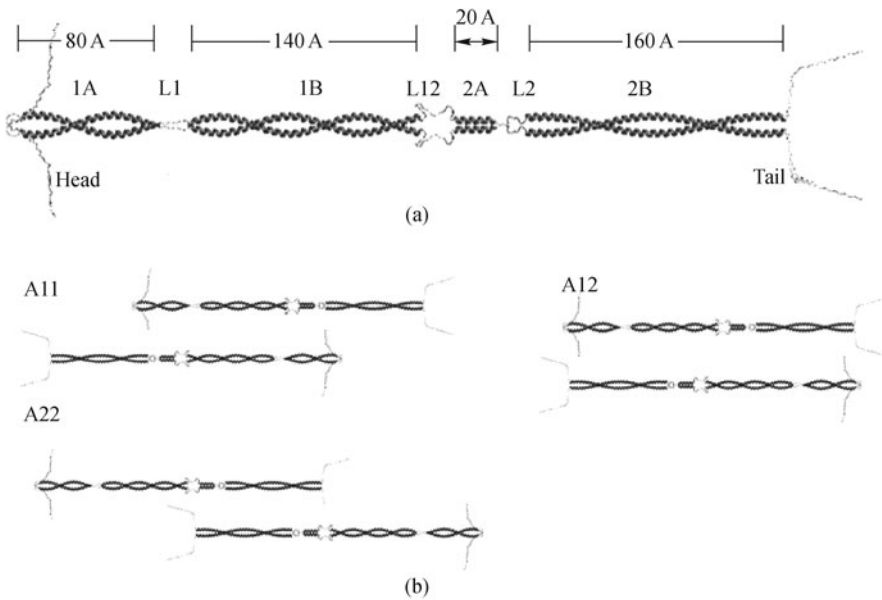


Fig. 4.2 Schematic representation of vimentin dimer and tetramer before molecular relaxation. Panel A: schematic geometry of the dimer geometry, including labels identifying the various segments and linker domains. Panel B: typical geometry of vimentin tetramer before relaxation observed in experiments: A11, A12 and A22.

There are, however, persistent experimental challenges in identifying the remaining parts of the vimentin IF structure. Since many parts of IFs are intrinsically disordered structures^[21], x-ray diffraction studies on naturally

occurring or recombinantly produced IF bundles do not provide sufficient data to produce a full atomistic model of entire IF dimers. Solid state nuclear magnetic resonance has been successfully utilized to derive atomistic models of amyloid fibrils, for example^[22]. However, amyloid peptides are typically much smaller than IF dimers, so that this approach may remain unsuccessful as well. Finally, cryo-electron tomography offers the promise of molecular-level imaging of single IFs, but the best tomograms are currently limited to a resolution around 5 nm^[23], whereas Angstrom resolution is required to directly reveal the atomic structure of proteins. Therefore, most of the atomic structure of vimentin dimer has not been determined by experiments yet. At the next hierarchical level two dimers come together and interact with each other to form a tetramer structure. Three distinct types of antiparallel arrangements are found in experiments for this interaction as shown in Fig.4.2b^[24]: A11, the coiled-coil domain of helix 1B of one dimer interacts with the same domain of a second dimer; A12, the coiled-coil domain of helix 2B of one dimer interacts with the 1A and 1B domains of a second dimer; A22, the coiled-coil domain of helix 2B of one dimer interacts with the 2B domains of a second dimer. Normally, A11 and A22 are found in the assembling process at the tetramer stage but A12 is only found in the higher assembling stage.

A higher level structure of IFs is the unit length filament, which includes a bundle of eight tetramers. It is observed in experimental studies that the head and tail domains play roles at assembling of structural levels higher than tetramer level. The headless IF proteins form only dimers and tetramers under conditions of ionic strength lower than and pH higher than standard buffer, but no higher-order structures (and particularly, no octamers are formed)^[24]. This has been shown independently for headtruncated desmin, cytokeratins, and vimentin^[24]. Therefore, there are two possible reasons: (a) the head domain is needed to allow additional dimers to assemble to the A11 and A22 tetramers; or (b) headless IF protein dimers associate in the A12 mode that does not allow further assembling. This finding indicates that the head domain is necessary for productive formation of octamers from tetramers. Another concern that has remained for quite some time is the role of the amorphous tail domain in assembling process or lateral interaction of individual IFs. By analyzing early assembly intermediates of tailless vimentins, it has been shown that they form IFs in a manner analogous to intact vimentin. However, tailless IFs exhibit no significant tendency for lateral aggregation. It is also shown that tailless vimentins lose control of lateral growth, ending with increasing number of dimers/tetramers at filament cross sections. This finding indicates that the tail domain is necessary for controlling the conformation and interaction of full length filaments^[25].

4.1.1.1 *In silico* modeling of intermediate filaments

Due to the lack of a structural model of IFs, relatively little is known of the physical material concepts of intermediate filaments. Specifically, our un-

derstanding of fundamental filament properties, such as the basis for their great extensibility, stiffening properties, and their exceptional mechanical resilience, remains limited. This has so far prevented us from answering fundamental structure-function relationship questions related to the mechanical role of intermediate filaments, which is crucial to link structure and function in the protein material's biological context. The availability of a structural model of IFs, especially dimers, as the most basic subunit, is also the key to understand the mechanisms of IF-related genetic diseases, where structural flaws cause major structural changes of biologically relevant properties. To address this issue a hierarchy of *in silico* techniques with different levels of accuracy, ranging from atomistic to coarse-grained representations, was recently applied in order to enable a bottom-up multi-scale analysis of vimentin IFs at different hierarchical levels and time scales^[15]. Atomistic level molecular dynamics simulations were carried out by using the CHARMM19 all-atom energy function with an effective Gaussian model for the water solvent^[26,27], as well as by using explicit solvent simulations with the CHARMM force field and TIP3 water^[28] implemented in NAMD^[29] for validation of the effective Gaussian simulations. In this chapter we compare the geometric and mechanical properties of intermediate filaments with the observation in experiments, reviewing some of the key results from earlier molecular modeling work.

4.1.1.2 Molecular structure of vimentin dimer and tetramer

We used a homology method to set up a structural model of intermediate filament dimer and tetramer structures (for details regarding the approach in this and following sections, see Ref.[15]). Each polypeptide chain that composes the dimer has the identical 466 amino acid sequence and reflects the exact sequence of human vimentin intermediate filaments^[18]. The amino acids are connected by angles of $\phi = -58^\circ$ and $\psi = -47^\circ$ to reflect the known conformation of alpha-helical segments^[30], and by $\phi = 180^\circ$ and $\psi = 180^\circ$ for the other unstructured parts of the chain. Note that the parameters ϕ and ψ are two characteristic dihedral angles that define the conformation of the protein backbone chain; these angles describe how two neighboring amino acids are connected to one another (for details see Ref.[31]). According to the sequence analysis, the 1A, 1B, 2A and 2B segments possess an initial alpha-helical structure, while the other parts of the chain are initially unstructured^[16-18].

Each amino acid within the polypeptide chain takes the topology and local coordinates according to the optimized geometry in the CHARMM 22 force field^[28]. Due to the polarities of the amino acids, the apolar amino acids in the 1A, 1B and 2B segments generate a hydrophobic stripe that winds around its helical axis with a left-handed heptad repeat. Via coordinate displacements and transformations, we combine two initially straight alpha-helical chains at a specific distance and coiling angle to form a coiled coil structure. This configuration is energetically favored since it shields apolar residues from the polar solvent environment^[32]. The coiling angle is 3.51 deg/Å for 1A, 3.85

deg/Å for the 1B and 2.46 deg/Å for the 2B segment, as shown in Fig.4.3. The coiling angles are different in different segments because the twist angle of the hydrophobic stripe on each of the segments differs. This twist angle is determined by linear fitting of the angular coordinate θ of the hydrophobic amino acids with respect to their axial coordinate z in the $R-\theta-z$ coordinate system of C_α atoms. In the 2A segment, the hydrophobic stripe is nearly parallel to the axis of the alpha-helix, resulting in a parallel alpha-helical bundle.

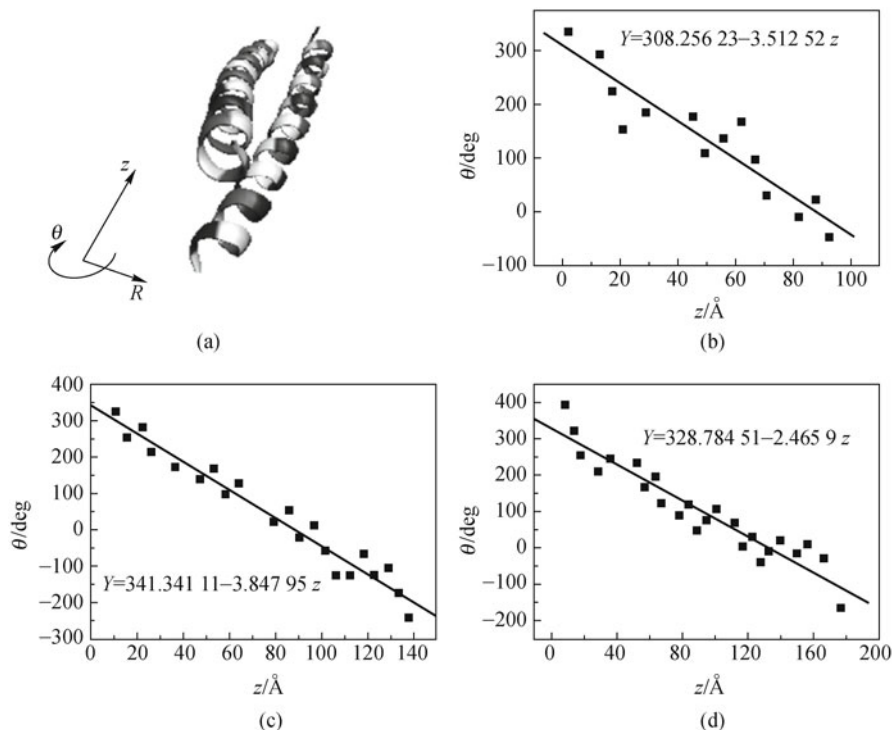


Fig. 4.3 The geometry character of each coiled-coil domain in a vimentin dimer. Panel A: the schematic representation of a coiled coil segment, and the relative coordinate used to form the model. The hydrophobic amino acids are white, while other amino acids are colored; Panel B: the coordinates of the hydrophobic stripe along a chain in 1A domain; Panel C: the coordinates of the hydrophobic stripe in 1B domain; Panel D: the coordinates of the hydrophobic stripe in 2B domain (data taken from Ref.[15], color plot in the book end).

Figure 4.4 shows a snapshot of the molecular model of the vimentin dimer and tetramer after sufficient equilibration using the CHARMM19 all-atom energy function with an effective Gaussian model for the water solvent^[26,27], displaying the characteristic segmented geometry with coiled coil regions connected through linker domains. The total length of the dimer without the

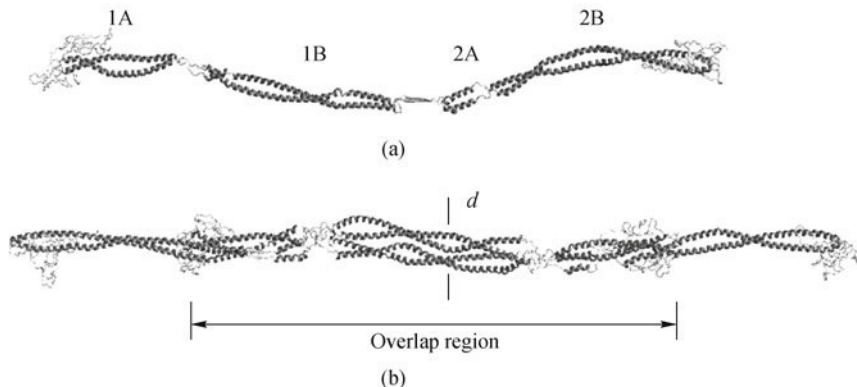


Fig. 4.4 Molecular structures of the vimentin dimer and tetramer. Panel A: snapshot of the atomistic model of the dimer. The blue part denotes the stutter part around the stutter (amino acids 345–351). Panel B: snapshot of the atomistic model of the tetramer (images taken from Ref.[15], color plot in the book end).

head and tail domain is ≈ 48.7 nm. From dynamical analyses of the molecular dynamics trajectory of the dimer at 300 K, we observe that the linker domains represent soft, hinge-like structures that connect much stiffer coiled coil segments. This heterogeneous distribution of the bending stiffness along the molecule's axis severely affects the filaments' motion by enhancing its mobility due to an effectively reduced persistence length, which might be significant for IF self-assembly. Thereby, the linker domains act as hinges around which the molecular structure can rather easily bend and rotate. For the tetramer, we consider the combination of two dimers in the A11 form^[17]. The total length of the tetramer is ≈ 61.3 nm, which agrees with the length of unit length filaments observed in experiment. The overlap part of the tetramer has a length of ≈ 36 nm. We find that the head segment of each dimer is coiled around the other dimer. From an analysis of the equilibrium fluctuations it can be seen that compared with the dimer structure, the tetramer structure is notably stiffer. This is likely due to its increased area moment of inertia, resulting in an effectively increased persistence length. We summarize a comparison between the simulation and experimental measurements as shown in Fig.4.5.

4.1.1.3 Structural validation against experimental results with multiple methods

The modeling process and the results reviewed in Section 4.1.1.2 are based on simulations in implicit solvent, and thus, an important question remains about the structural and dynamical behavior of the resulting proteins in explicit solvent^[36]. Several earlier comparisons between explicit and implicit solvents have been reported in the literature, focusing on exploring the effects of the solvents in conformation sampling and energy landscape in folding^[37,38].

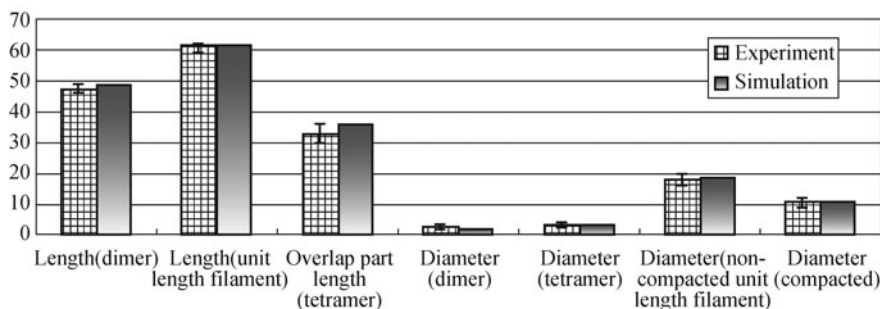
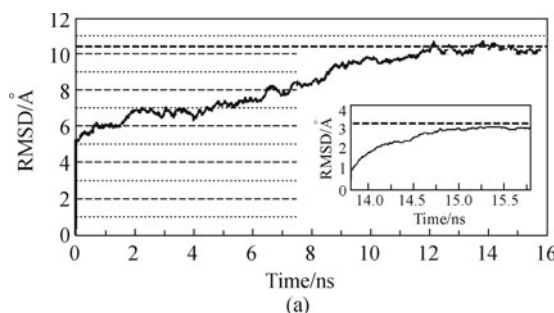


Fig. 4.5 Comparison of structural properties of the IF dimer and tetramer between experiment and simulation (data taken from Ref.[15], experimental results from [17, 33-35]).

We have carried out large-scale validation simulations and structural analyses of the atomistic model using structural equilibration in large-scale atomistic simulations in explicit solvent, with system sizes up to 500 000 atoms and simulations carried out at 20 ns time scales. The most important result is that our studies confirm the stability of the molecular model, at least at the time-scales accessible to our explicit simulations (for details, see original work reported in Ref.[36]).

Figure 4.6 shows the evolution of the root mean square deviation (RMSD) for the dimer (Fig.4.6a) and the tetramer (Fig.4.6b) in explicit solvent (compared with the initial structure obtained from implicit solvent equilibration). The results show that after 12 ns, the RMSD values for both the dimer and tetramer converge to a constant value, suggesting that both structures have reached a stable configuration in the explicit solvent model (the final structures are the structures shown in Figs.4.7a and b, respectively). Furthermore, the overall relatively small deviation of the structure provides evidence for the fact that the implicit model is a reasonable approximation for the dimer and tetramer structures, at least at the time scales considered here.

For the dimer shown in Fig.4.7a, it can be seen that the linker L12 forms a parallel beta-sheet in both explicit and implicit solvent models, as observed in experiments^[39]. By comparing the two structures, we also observe that the



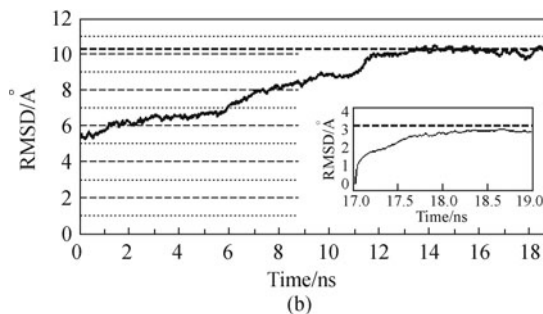


Fig. 4.6 Root mean square displacement (RMSD) analysis of explicit solvent, for the dimer (panel A) and the tetramer (panel B). The deviation is calculated with respect to the starting configuration, which is the result of equilibration in implicit solvent. The inserts in each panel depict the RMSD analysis during the last 2 ns of equilibration^[15,36].

2A segment (parallel helices) and L12 (beta-sheet) are only marginally stable. Same as in the dimer case, we also present a detailed analysis of the structural character of the equilibrated protein in Fig.4.7b. The analysis shows that the tetramer structure is more stable than the dimer, and most of the structural features are well conserved after explicit solvent equilibration. The

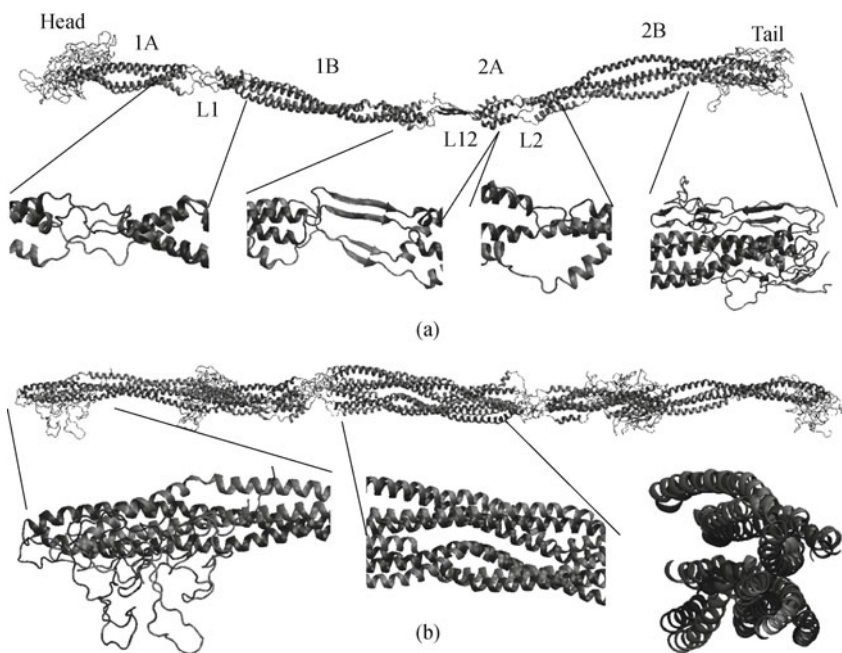


Fig. 4.7 Structure of the vimentin intermediate filament dimer (panel A) and tetramer (panel B), depicting a comparison between implicit and explicit solvent simulation results (color plot in the book end)^[36].

structure of the overlapped coiled coil domain, which is mainly composed of a pair of 1B segments in dimers arranged in an antiparallel form, does not change its molecular structure significantly during the long-time equilibration, indicating that this part is rather stable. This observation is also supported experimentally, since the headless or tailless dimers are still able to form tetramer structure in the A11 form, and the interaction is mainly caused by the 1B domain within the dimer.

Additional analysis of the dynamical properties of each amino-acid in the dimer and tetramer during the simulation is shown in Fig.4.8, where the RMSD value for each residue in the simulation is depicted. These results also reflect the relative flexibility of each domain during the equilibration process. We observe that the peaks shown in Fig.4.8a correspond to the linker, head and tail segments, providing the evidence that these domains are among the most flexible ones in the dimer structure and as such undergo the largest structural fluctuations. The peak RMSD values P corresponding to the link-

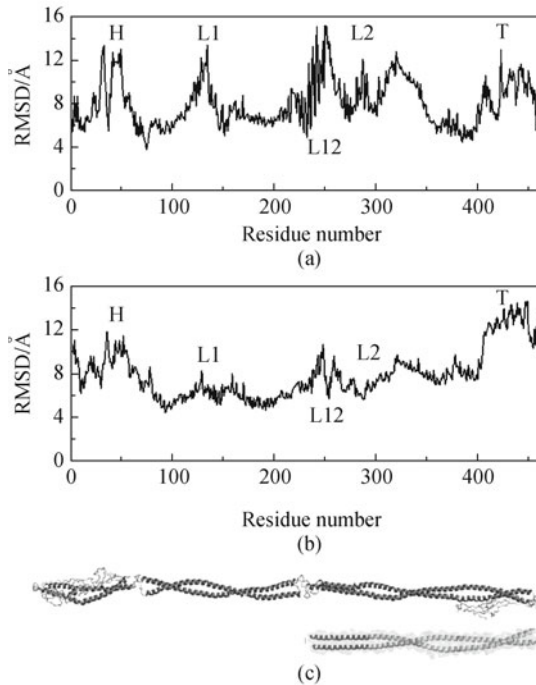


Fig. 4.8 Root mean square displacement (RMSD) analysis during the equilibrium process in explicit solvent, reflecting the local flexibility of each domain, for the dimer (panel A) and the tetramer (panel B). Panel C, the molecular structure of the vimentin with a continuous coil2 domain, the insert part is the coil2 segment (AA263 to AA405). (Reprinted from [20], Copyright © 2010, with permission from Elsevier)

ers have a relation as $P_{L12} > P_{L1} > P_{L2}$, indicating the relation of the stiffness of each linker $K_{\theta 2} < K_{\theta 1} < K_{\theta 3}$. It is also interesting to note that the right part (amino acid numbers 291 to 351) of the 2B segment is more stable than the left part (amino acid numbers 351 to 405), as shown by the peaks in these regions. Experiments have revealed that a stutter defect is located around amino acid number 351. This information provides evidence that the 2B segment is marginally stable from the stutter region on, and until the beginning of the tail domain, but more flexible before the stutter region.

The RMSD value of the tetramer (Fig.4.8b) shows that the interaction between two dimers significantly affects their flexibility and stability. As the two anti-parallel dimers combine, the overlapped part becomes more stable than the other domains. Moreover, we do not find a peak value at the L2 domain. There are two possible reasons to explain this interesting observation. The first one is that this linker tends to interact with the other dimer and its flexibility is significantly reduced. The second possibility is that this part tends to form some structure more stable than the amorphous region. Recent experimental studies have confirmed that the latter possibility is more likely the case, where the L2 domain in vimentin is in an alpha-helix conformation after crystallization (within structure with PDB ID 3klt^[20], as depicted in Fig.4.9), which integrates the 2A and 2B domains together as a single, homogeneous coil2 domain. This finding indicates that it is not necessary to introduce an amorphous L2 to increase flexibility in facilitating IF assembling. The RMSD analysis shown here provides some evidence to confirm this point as it shows that the L2 domain does not necessarily need to be flexible to keep the tetramer stable.

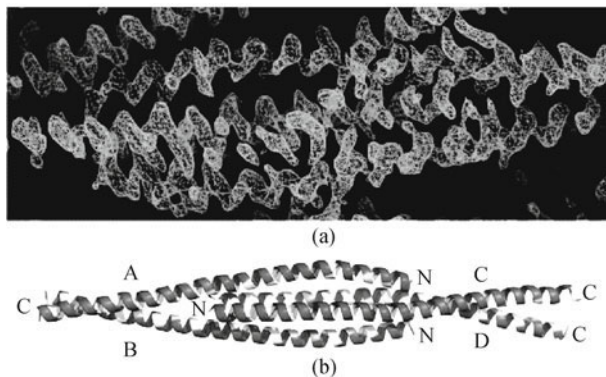


Fig. 4.9 The crystallized structure of the 2A, L2 and part of 2B domain close to the N terminal of vimentin dimer (AA263–AA334 with PDB ID 3klt) (color plot in the book end). (Reprinted from Ref.[20], Copyright © 2010, with permission from Elsevier)

We also verify the stability of our model by coarse-grained representation, where this model is generated from the full atomistic model (for de-

tails on this method, see Ref.[15]). We use the MARTINI force field^[26]. The model for the tetramer includes 43 801 particles (4 052 for the protein plus 39 749 for the water molecules), while the corresponding full atomistic system included 506 915 particles (29 924 for the protein plus 476 991 for the water molecules). Figure 4.10 shows the full view of the coarse-grained model as well as a comparison with the full atomistic one. Each amino-acid is coarse-grained into 1 to 5 particles. This coarse-grained method reduces the particle number and thereby increases our ability to simulate much longer time-scales. We carry out an equilibration for 300 ns = 0.3 μ s. The RMSD analysis shown in Fig.4.10 suggests that the tetramer structure has reached an equilibrated state after ≈ 150 ns. Most importantly, there is no conformational or structural change during the equilibration process. Further structural analysis included a radial distribution function (RDF) analysis, which provided evidence that the structural character of the coarse-grained model remains the same during equilibration.

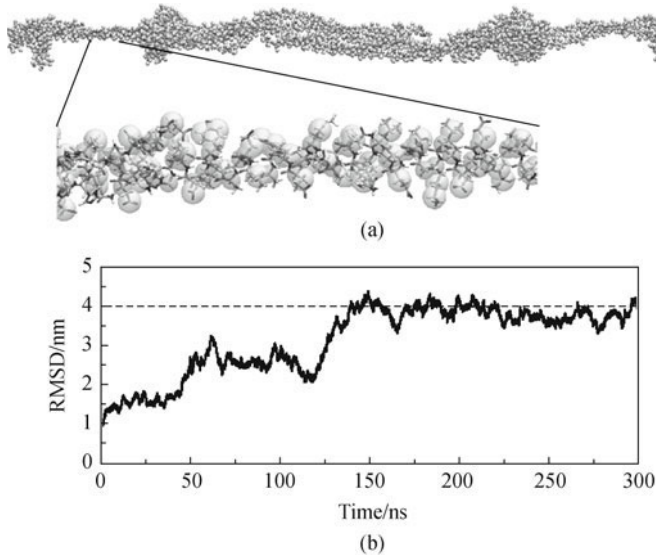


Fig. 4.10 Coarse-grained representation of the vimentin tetramer (panel A) and structural stability of the vimentin tetramer structure at time scales of hundreds of nanoseconds (RMSD analysis shown in panel B), here using the MARTINI force field^[15].

4.1.2 The structural and physiological character of keratin

Keratin, another kind of intermediate filament, is the key component of hair, nail and skin in vertebrates, including mammals; and is special due to the

relatively high density of stronger disulfide bonds. The human intermediate filament database has shown that at least 54 of 70 intermediate filament genes are keratins, making this a prominent form of IFs that are widely found^[40]. Keratin is generally divided into type I (KRT9–KRT24) and type II (KRT1–KRT8) proteins according to their physical and chemical properties. For example, keratin type I is neutral or basic and keratin type II is acidic. In contrast to other intermediate filaments, keratin homodimers are very unstable, and the basic structural unit of intermediate filaments is a heterodimer of one type I keratin where its partner is type II keratin, such as KRT5 and KRT14^[41,42]. Indeed, in Hatzfeld's and Weber's paper (see Ref.[42]), it is shown that keratin proteins do not assemble into intermediate filaments if the keratins are type I or type II homodimers. They also found that keratin heterodimers based intermediate filaments are more stable than homodimers. Like all intermediate filament proteins, the characteristic of keratin is the central alpha-helices domain which is composed of four helices (1A, 1B, 2A, and 2B), a number of linker domains (L1, L12, and L2) and the termination by globular head domains.

Inside the cell, keratins not only play a key role in maintaining the stability of the cell, but they also contribute the stability of adjacent cells. As shown in Fig.4.11a, an analysis of immunofluorescence staining images show that the nucleus is tightly embraced by keratin intermediate filaments. From the nucleus outwards, keratins spread towards the cell wall and attach to desmoplakin protein which is a component of desmosomes which forms intercellular junctions as depicted in Fig.4.11b (for the immunofluorescence image) and Fig.4.12.

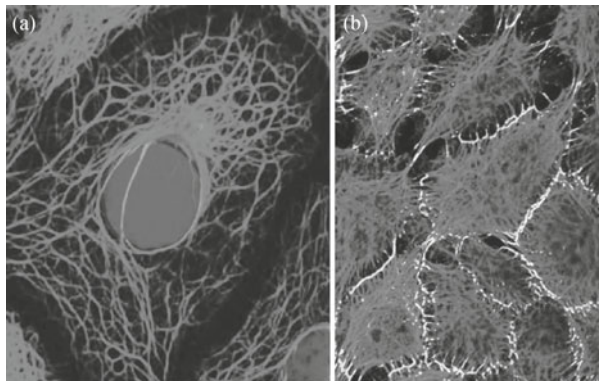


Fig. 4.11 Keratin intermediate filament in epithelial cells: (a) the keratin intermediate filament (K18) and the nucleus (liver carcinoma cell) are stained in red and blue respectively, (b) keratin filaments (in red) and the desmoplakin (in green), a component of desmosomes, are labeled in cultured keratinocytes of line HaCaT. The location of desmoplakin and desmosomes in a cell is shown in Fig.4.2. a and b are the immunofluorescence staining images (color plot in the book end). (Reprinted from Ref.[43], Copyright © 2008, with permission from Springer)

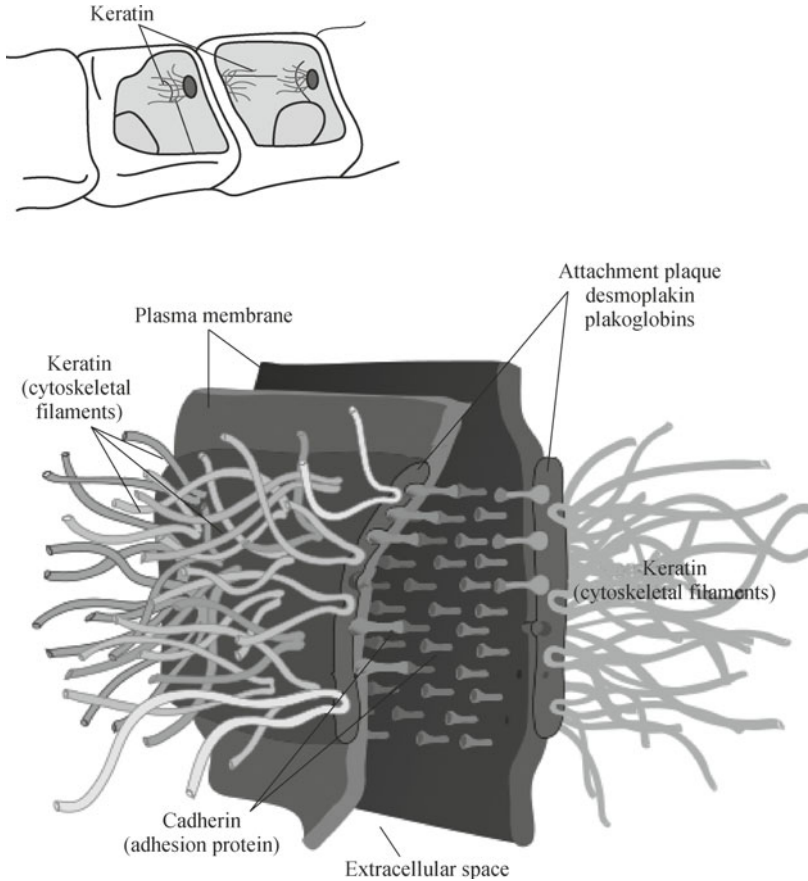
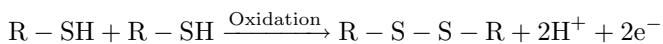


Fig. 4.12 Keratin intermediate filaments are attached to desmoplakin in the desmosome, which is known as the complex responsible for cell-to-cell adhesion in animal cells. (image taken from Ref.[44], color plot in the book end).

There are typically large amounts of the sulfur-containing amino acid cysteine in keratins. For example, human hair has approximately 14% cysteine which is shown in Fig.4.13a. In fact, when hair burns, the particular pungent odor stems from the sulfur compound in cysteine. Keratin proteins are important structural stabilizers of epithelial cells, and the disulfide bonds in cysteine residue play an important role in achieving the characteristic mechanical properties in keratins^[45,46]. The disulfide bond can be formed by two cysteine residues in oxidizing environments^[47,48] (see Fig.4.13b), as described in the following chemical reaction:



The geometry of a disulfide bond in a larger protein, the vascular cell adhesion molecule-1 (VCAM-1; Protein Data Bank number: 1vsc) is shown in

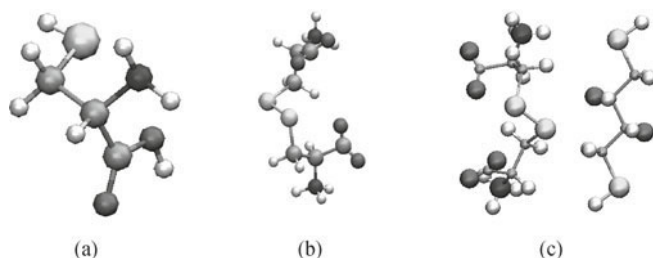


Fig. 4.13 Cysteine is an amino acid with the chemical formulation $C_3H_7NO_2S$. Panel A: image of one cysteine residue. Panel B: illustration of how a disulfide bond is formed between two cysteine residues. Panel C: model of a disulfide bond under a reducing agent, DTT, in explicit solvents water molecules are not shown in the figure. The carbon, hydrogen, nitride, oxygen and sulfur atoms are colored blue, white, dark blue, red and yellow respectively (color plot in the book end).

Fig. 4.14^[49]. In each immunoglobulin domain of VCAM-1, there is a disulfide bond buried in its core, serving as the stabilizer to retain the folded structure of this protein. Indeed, a major consideration of the source of the great stability of keratin IFs is the disulfide bonds that mediate cross-linking at different scales. For example, the process of hair perming uses the mechanism of cleaving the disulfide bonds first and then reforming the new disulfide bonds after the desired hair style has been shaped.

The structure and mechanisms associated with disulfide bonds have been a subject of several recent investigations. Recently, the reaction pathway of disulfide bond formation and breaking has been studied by Car-Parinello molecular dynamics which is based on quantum mechanics theory^[50-52]. They show that the disulfide bond breaks under the presence of a redox reagent, dithiothreitol (DTT). However, quantum-level simulation of the disulfide bond in explicit solvent is time-consuming and requires significant computational resources. Thus the ReaxFF force field^[53], capable of properly describing bond breaking in a classical molecular dynamics simulation can be effectively applied to studying disulfide bonds at larger scales. By using the ReaxFF force field in preliminary studies, we observed how molecules such as DTT affects the peak force of disulfide bond breaking in explicit solvent environment; effectively reducing the mechanical strength. In the future, such reactive studies could extend to the mechanics of disulfide bond in keratins which contain hundreds of thousands of atoms.

Finally we note that more than 16 diseases related to around 160 known keratin mutations have been discovered so far^[54]. However, there are still many open questions, about particularly what kind of mechanism the mutations affect the configurations and properties of keratins, and how to relate the mutation to the disease. Keratin intermediate filaments occupy a decisive position in maintaining epithelial cell integrity, thereby suggesting an importance of mechanical loading in the progression of this disease. Once it is revealed how mutations affect the occurrence of particular diseases, we may

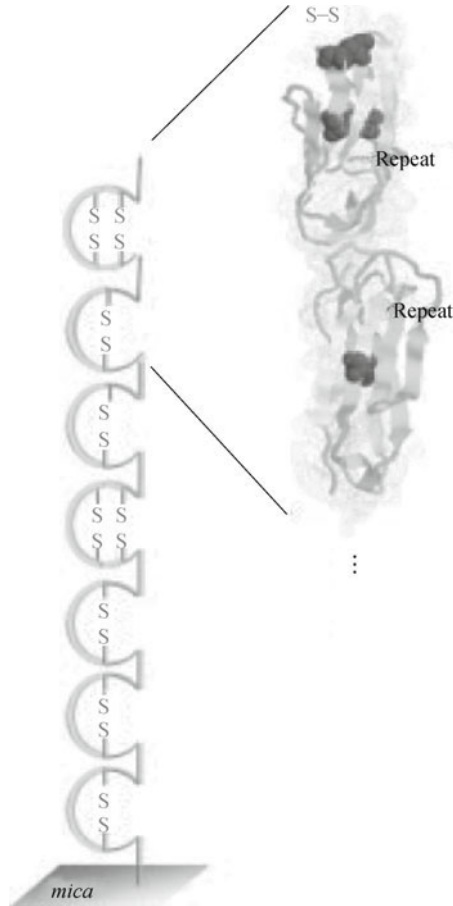


Fig. 4.14 The schematic diagram of force tension experiment by AFM. There are seven immunoglobulin domains of VCAM. The disulfide bonds, shown in red, stabilize the VCAM protein structure (color plot in the book end). (Reprinted from Ref.[49], Copyright © 2004, with permission from the American Society for Biochemistry and Molecular Biology)

be able to indentify targets that could result in novel therapeutic approaches.

4.2 Connecting filaments to cells level function and pathology

The mechanical properties of IFs have been studied for almost a century at the tissue level, especially in keratin-based appendages such as hair, wool and quills^[55]. With the development of experimental nanomechanical approaches

based on the atomic force microscope (AFM) and optical tweezers (OTs), it is now possible to measure the mechanical properties of single IFs^[56]. The new frontier is clearly the cell level where it is more difficult to measure mechanical properties of single filaments or networks. In the following sections, we will review our current understanding of IF mechanical and mechanotransduction properties in the cell context.

4.2.1 Bending and stretching properties of IFs in cells

Basically all the quantitative measurements of IFs mechanical properties have been performed *in vitro* on single filaments, networks and macroscopic bundles. The emerging picture is that IFs are flexible filaments with a persistence length in the 400–1 000 nm range^[55]. Obtained values depend on the method used^[57,58] and the IF tested^[58]. Stretching single IFs using an atomic force microscope (AFM) revealed a maximum extensibility of 250% associated with a 4-fold reduction in diameter^[56,59], in close agreement with the observations made in simulation (see Ref.[15]). Force versus extension data on single IFs showed force plateaus^[60] that were attributed to coiled coil unfolding events, and a nonlinear increase in force above 100% extension^[15,59]. Since all these experimental measurements have been obtained on *in vitro* reconstituted filaments made of recombinant proteins, they do not necessarily reflect the mechanical properties of single IFs *in vivo* in which remains an important challenge.

The only evidence for the flexibility of IFs *in vivo* comes from live cell imaging data on PtK2 epithelial cells transfected with fluorescently labeled K8 or K18^[61]. Yoon and coworkers observed K8/K18 tonofibrils exhibiting wave-like movements, with a wavelength around 1–2 μm , similar to the buckling waves propagating along individual microtubules (MTs)^[62]. For MTs, the buckling waves have been observed in cells when an MT tip pushes against the cell wall^[62]. For the keratin tonofibrils the waves have been observed within the keratin network and their propagation was inhibited by treatment with a phosphatase inhibitor, Calyculin A^[61]. Based on the comparison with MTs, the tonofibrils' waves are an indicator of compressive stresses being dissipated within the keratin network.

So far, three different groups have observed the extensibility of IFs *in vivo*. Chang and coworkers analyzed the interaction between the vimentin IF network and melanosomes, pigment granules, in *Xenopus laevis* melanophores^[63]. They observed that the vimentin network helps to regulate melanosomes movements. In one case, they followed a granule while it was bending and ultimately stretching a single vimentin IF to double its original length, i.e. 100% extension. Another example of single IF stretching comes from the work of Lewis and colleagues who studied the architecture of desmin IFs in the sonic muscle fibers of type I male midshipman fish^[64]. Fluorescence

imaging of these muscle fibers under tension revealed that desmin IFs are extensible reversibly to at least 60% in this model system^[64]. Finally, Fudge and coworkers stretched NEB-1 keratinocytes transfected with GFP-K14 cultured on silicon membranes up to 133%^[8]. The keratin network followed the extension of the membrane without breaking. However the deformation was not reversible. The keratin bundles of stretched and relaxed cells showed buckling waves with a wavelength around 2.8 μm similar to the one observed in PtK2 cells^[61]. Interestingly, the stretched keratin bundles did not show any clear reduction of diameter when observed by transmission electron microscopy^[8].

Overall, the few currently available measurements of IFs mechanical properties within cells confirm that IFs are much more flexible and extensible than other cytoskeletal elements. However the role played by the large family of intermediate filament binding proteins (IFAPs) remains elusive.

4.2.2 IFs responding differently to tensile and shear stresses

Recent studies have shown that cells are able to sense the stiffness of the substrate they are attached to^[65]. This sensing involves cytoskeletal remodeling^[66]. In the particular case of the IF network, several studies demonstrated a completely different response to tensile and shear stresses.

Cells in culture are very sensitive to continuous and cyclic stretching stresses below 20% extension. Stretching induces many different signal transduction pathways that generally lead to actin cytoskeleton remodeling^[67]. The most well-known effect is the change in orientation of actin stress-fibers in response to a low frequency, 1 Hz, cyclic stretch^[68]. In the case of the IF network, Yano and coworkers reported changes in the expression patterns of K6 and K10 in human epidermal keratinocytes stretched continuously by 20% for 24 h^[69]. However at that level of stretching the keratin network remains structurally unaffected^[70]. Above 20% extension, the keratin network starts to align in the stretch direction as expected for the passive mechanical response of a network^[8].

The situation is completely different when one looks at the response to shear stresses. Endothelial cells that line blood vessels exhibit well developed K8/K18 networks in culture. When exposed to a shear stress of 30 dyn/cm^2 similar to the one due to blood flow in a vessel, the keratin network is formed from thick and wavy tonofibrils instead of individual filaments^[71]. This effect is visible after 60 min exposure to shear stress and is an active remodeling of the keratin network through the phosphorylation of K18 by protein kinase C (PKC) ζ ^[71]. Mutating the specific K18 phosphorylation site at Ser33 or inhibiting PKC ζ by a peptide antagonist yielded a partial disassembly of the keratin network in response to shear stress^[71]. The phosphorylation of K18 at Ser33 allows binding of 14-3-3 ζ to the keratin network and this interaction is responsible for the observed remodeling^[71].

Even though the *in vitro* characterized mechanical properties of IFs point towards their involvement in cell mechanics at large deformations or large forces, the currently available *in vivo* data show that IFs are involved in the cell response to moderate physiological stresses.

4.2.3 Mechanotransduction through the intermediate filament network

Maniotis and coworkers demonstrated early that the IF network is involved in propagating mechanical stresses from the cell surface to the nucleus^[72]. However, it is shown only recently that the proteins responsible for the linkage between cytoskeletal IFs and the nuclear lamina have been identified, namely, Nesprin and SUN proteins^[73,74]. Similarly, the involvement of the IF network in cell migration and especially the direct and indirect interactions between IFs and integrins have only been unveiled in the past few years^[75-77]. The emerging picture is that the IF network is a major constituent of the cytoskeletal continuum. Still, its response to mechanical stresses remain barely studied (see the discussion in the previous section) and the main evidence for the involvement of IFs in mechanotransduction comes from genetic diseases where IF proteins are mutated^[78]. Mutations in keratins, desmin and lamin A yield a wide spectrum of pathologies some of which exhibit mechanical failure of the skin, skeletal muscles or heart muscles^[78]. In one case, *in vitro* measurements indicated that mutated desmin IFs have different mechanical properties than the wild-type ones, thus pointing towards impaired mechanotransduction as a molecular explanation for the observed pathologies^[79]. Unfortunately, there have been no *in vivo* or cell studies performed to confirm this hypothesis. Most of the cell level studies on mutant IFs have concentrated on their assembly properties^[80] and their involvement in signal transduction pathways^[81].

4.3 Experimental mechanics

Several experimental approaches have been developed to characterize the mechanical properties of IFs from the single filament level to networks and cells. In this review we will focus on three experimental methods, AFM, bulk rheology and particle tracking microrheology.

4.3.1 Single filament mechanics

Due to their assembly mechanism, single IFs are apolar structures^[82] that

tend to stick well to solid supports^[83]. For these reasons, optical tweezers approaches have been unsuccessful in determining the elastic properties of single IFs. Instead, the AFM has been used extensively to determine the mechanical properties of single IFs. Imaging in liquid of vimentin IFs deposited on different surfaces yielded a 1 micron estimate for their persistence length^[57]. This corresponds to an apparent Young's modulus of 8 MPa for a 10 nm wide filament. However IFs are bundles of two long stranded coiled coils, so their mechanical properties are anisotropic, which means that their elastic properties have to be described by a Young's modulus (E) and a shear modulus (G). To measure these quantities, Guzman and coworkers imaged vimentin IFs, unfixed and fixed with a chemical crosslinker, suspended above the 200 nm pores of an aluminate membrane^[84]. They measured the deflection of the vimentin filaments above the pores for different forces applied by the AFM cantilever on the surface. They obtained an estimate for E around 1 GPa and an estimate for G around 2–3 MPa. Another application of the AFM is the controlled manipulation of nanostructures on surfaces^[85]. During the manipulation, the AFM cantilever is submitted to a torque that provides an estimate of the force applied on the nanostructure during manipulation^[86]. Kreplak and coworkers took advantage of this fact to measure the extensibility of several IFs^[56] and the stress-strain curve of a single desmin IF^[59]. They observed a maximal extensibility of 250% associated with a nonlinear stress-strain curve above 100% extension. This method has been used successfully to detect nanomechanical differences in desmin filaments harboring point mutations associated with myofibrillar myopathies^[79].

4.3.2 Rheology of IF networks *in vitro*

The first demonstration that IFs have very different mechanical properties compared to actin filaments and microtubules came from bulk rheological measurements^[87]. The experiment revealed that a suspension of vimentin IFs can support shear strains of several 100% while hardening in the process^[87]. Since this early work, bulk rheology has been applied to several IF types, neurofilaments^[88], vimentin^[89], desmin^[58], keratins K5/K14^[90] and K8/K18^[91]. Most measurements are taken at low frequency, around 1 Hz, and low shear strains, 1%–5%, and provide an estimate of the elastic properties of the network as a function of protein concentration, for example^[89–91]. Experiments performed over a large frequency range, from 10 to 10^4 Hz, provide a direct measure of the persistence length of the filaments within a network. In the case of desmin, a value of 900 nm was obtained in comparison to 400 nm for vimentin^[58]. Since, IF networks have nonlinear rheological properties, varying the amount of stress or strain can provide additional information. Using a pre-stress method where a small oscillatory stress is applied in addition to a constant stress, Lin and coworkers characterized

the nonlinear rheology of a vimentin network in the presence of magnesium ions^[89]. Above a given pre-stress, the measured differential modulus varies as pre-stress exponent $3/2$ that can be explained as the straightening of non-extensible filaments^[89]. However at very large pre-stresses, vimentin networks in presence of magnesium ions deviate from this behavior due to the extensibility of the filaments^[92]. As a result, it is possible to estimate the apparent Young's modulus of a single filament within the network, which is 9 MPa^[93]. The next step is to understand the rheological properties of mixed network of, for example, IFs and actin^[94], or actin and microtubules^[95]. Furthermore, two groups recently demonstrated that composite networks of actin filaments and myosin filaments can have contractile behavior comparable to the ones of a cell, in the presence of ATP and cross-linkers^[96,97]. It would be interesting to know how IFs contribute to the rheological properties of these active networks.

4.3.3 IF networks rheology in cells

Another way to measure the local viscoelastic properties of a filamentous network is particle tracking microrheology^[98]. Microspheres of, for example, polystyrene are embedded within the network and followed optically by video microscopy. The trajectory of the spheres can be analyzed in terms of the Brownian motion of a particle in a viscoelastic medium. This technique allowed the measurement of both the elastic modulus of a vimentin network and its mesh size^[99]. Interestingly the same approach can be used to probe the local rheological properties of IF networks within cells. Alveolar epithelial cells were treated with detergent and high salt to prepare intact keratin networks suitable for particle tracking microrheology measurements^[100]. These experiments revealed different elastic moduli and mesh sizes at the cell periphery and at the perinuclear region^[100]. Furthermore the application of shear stress prior to cell treatment induced an homogeneous distribution of mesh sizes across the cells due to keratin network rearrangements^[100]. The next step is to perform active microrheology experiments where one particle is manipulated by a laser trap^[101] or a magnet^[102] while another one nearby in the cell is followed by video microscopy. Ideally, this should be done with organelles such as the melanosomes mentioned in Section 4.2.1 that are embedded in a dense vimentin network^[63].

4.4 Case studies

4.4.1 Single vimentin filament mechanics

As reviewed in the beginning of this chapter intermediate filaments form hierarchical structures, ranging from dimers, tetramers, unit length filaments, and full-length filaments to the cellular scale. The intermediate filament dimer and tetramer represent the most fundamental building block. Although there exists clear evidence that intermediate filaments play a key role in important physiological and pathological mechanisms, the mechanical and structural properties and their relation remain elusive for this protein material. Earlier molecular dynamics studies have focused solely on a small section of the 1A and 2B domains^[103,104], without considering the properties of the entire dimer structure. Furthermore, recent experimental studies using AFM analyses focused on the mechanical properties of entire filaments^[5,105]. Here we present a series of systematic molecular dynamics simulations to probe the response of entire intermediate filament subunits to mechanical tensile stretch.

4.4.1.1 Steered molecular dynamics—the *in silico* stretching simulation method

In experiments, optical tweezers and AFM are powerful tools that are usually applied to studying the elastic property and strength of protein nanostructures. As reviewed above those tools are able to stretch the protein and record the force-extension relationship during the entire stretching process. Such stretching experiment usually gives a characteristic sawtooth pattern of spaced peaks that result from the sequential unfolding owing to its particular microstructure^[106]. In computational simulations, the steered molecular dynamics (SMD) approach, which implements the basic concept of applying external forces to one or more atoms in the protein, are usually applied to studying the behavior of the protein structure under stretching, mimicking the process of experimental deformation with AFM. Figure 4.15 gives an example of the unfolding of the modular protein titin I27^[107], marking one of the earliest applications of this technique. By comparing the force-extension curve from AFM (Fig.4.15a) and the conformation change obtained in SMD simulation, it could be predicted that the mechanical resistance in this protein module arises from a patch of six hydrogen bonds bridging the A' and G strands, and the hump region is caused by the hydrogen bonds between A and B strands (Fig.4.15b). The authors further verified this by introducing a point mutation to break the interaction between strand A and B, and as a result a difference force-extension curve is obtained as expected by their hypothesis. These early studies provided clear evidence that the SMD method is a promising tool to find relationships between mechanical and microstruc-

tural properties of protein structures^[108]. This mechanistic insight, which is hard to obtain by AFM tests only, is important to reveal the deformation mechanism of protein structures, as well as to predict the properties after mutation. A similar methods has also been applied to study the deformation mechanisms of intermediate filaments as will be discussed in the following sections. The use of SMD provides important mechanistic insight into deformation behavior of protein filaments that complements experimental studies.

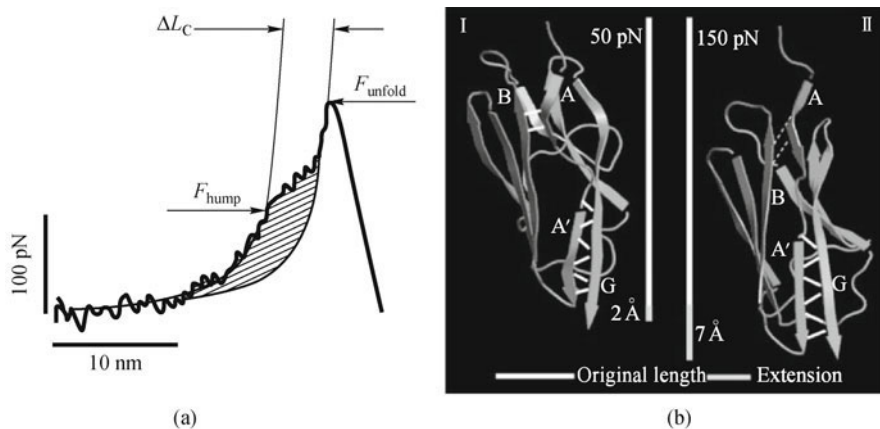


Fig. 4.15 Titin I27 and its elasticity. Panel A: the force extension curve of Titin I27 obtained via AFM stretching. Panel B: the cartoon representation of I27 model before and after stretching in SMD simulations. (Reprinted from [107], Copyright © 1999, with permission from Nature Publishing Group)

In order to investigate the mechanical properties of IF dimers and tetramers, we implement a uniaxial tensile test. The C^α atoms at the end of two 2B segments are pulled by using an SMD spring (harmonic, with a force constant of $10 \text{ kcal/mol/\AA}^2$), while the other end of the subunit is fixed, as shown in Fig.4.16a and b for a dimer and tetramer, respectively. As illustrated in Fig.4.16c, this simulation set up mimics the AFM experiment in applying force on the filament, the SMD spring is elastic to decrease the fluctuation of the stretching force as well as avoiding artificial impact force applied on the protein structure. The pulling force F is recorded versus the position. The simulations were carried out at different pulling velocities ranging from $0.0001 \text{ \AA/ps}$ to $1 \text{ \AA/ps}$. We record the force-extension curve and analyze the mechanical properties by computing the engineering strain defined as $\varepsilon = \Delta L/L_0$.

4.4.1.2 The mechanical property of dimer and tetramer^[15,109]

The dimer structure is stretched until all helical domains are fully extended. Figure 4.17 shows the force-strain curves of the dimer at a constant pulling rate of $0.01 \text{ \AA/ps}$, and the structural character of the dimer is given in

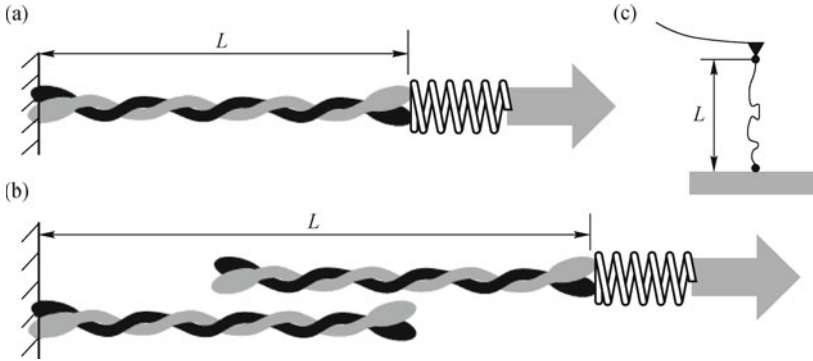


Fig. 4.16 Schematic of the pulling geometry and implementation of mechanical tensile test on dimer (panel A) and tetramer (panel B). This simulation set up is based on the AFM test on protein materials (panel C) (For details see original papers, Refs.[15,109]).

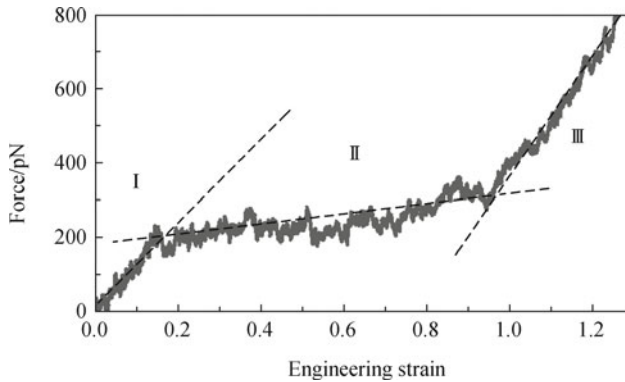


Fig. 4.17 The force-strain curve of the dimer under stretching for constant pulling rate ($0.01 \text{ \AA/ps}$) (For details see original papers, Refs.[15,109]).

Fig.4.18. The simulations reveal three distinct regimes of deformation. In the first regime (I), the pulling force increases linearly with strain until it reaches an angular point, where a dramatic change in the slope emerges (the angular point corresponds to the point where the first unfolding in the protein occurs). Within this region, no rupture or domain unfolding is observed until the applied strain is higher than 12%. In the second regime (II), the force-strain curve resembles a plateau regime where the pulling force remains almost constant. It is found that unfolding of all alpha-helical domains occurs in this regime. For this region, 2A segment begins to uncoil first, and other segments start to uncoil in sequence. We observe that the uncoiling of alpha-helical structures occurs simultaneously with the formation of beta-sheet protein domains, a phenomenon referred to as α - β transition. With this structural transition, the back bones of both strands start to align with

the pulling direction. In the third regime (III), the stretching force increases rapidly as the strain increases, indicating a significant stiffening of the local (tangent) elastic properties. This is caused by pulling the unfolded polypeptide backbone of the protein, where the stretching of covalent bonds leads to a much increased stiffness, as shown in Fig.4.18, of the strain beyond 95%. The overall characteristics of the force-strain curves of the dimer share similarities with stretching of individual alpha-helices^[103,110] as well as with myosin coiled-coil structures^[111]. Regime (II) represents the main unfolding regime, where the average force in this regime approximates the protein's unfolding force. The slight increase of the force in this regime (approximately 1.079 pN per % strain, as shown in Fig.4.17) is due to the fact that as more turns in the alpha-helical protein are unfolded, a larger rupture force is required (since the failure probability is decreased for a smaller number of remaining convolutions^[112]). It is noted that in experimental and simulation studies of the myosin coiled-coil protein, the strain-force curve shows a very similar behavior, and the same level of unfolding force^[113].

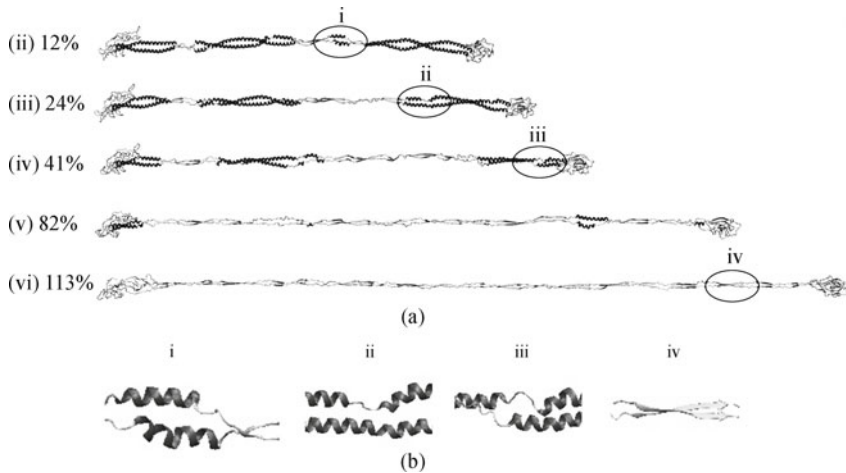


Fig. 4.18 Simulation snapshots during pulling of the vimentin dimer at different applied pull strains (panel A). Panel B shows detailed views of the molecular structure at various levels of strain^[109].

Calculation of the Young's modulus for small deformation (calculated in the pre-angular point regime (I) shown in Fig.4.17, where the dimer is deformed homogeneously before unfolding sets in) leads to values in the range of 380–540 MPa. This is in close agreement with experimental results from corresponding vimentin intermediate filament bending experiments, which show moduli in the range of 300–900 MPa^[114]. This agreement supports the notion that intermediate filaments should be considered as a continuous body in the very small deformation regime where no unfolding or sliding occurs between individual dimers.

Figure 4.19 summarizes the unfolding history of each domain in tension. We observe that the 2A segment ruptures first at 12% strain, and the reason may be that while the other three segments are coiled coils, the 2A segment is essentially a parallel alpha-helical bundle, which has a lower mechanical stability against tensile stretch^[16,115]. Once the 2A segment is fully uncoiled at $\approx 20\%$ strain, the segments 1B, 1A and 2B begin to uncoil sequentially. This unfolding sequence is highly repeatable. It has been suggested that this characteristic deformation pattern could play an important role in biomechanical signaling pathways, where specific levels of strain may correspond to particular changes in the molecular structure. Thereby the unfolding of specific domains might induce biochemical signaling, which may be part of the coupling between deformation state and biological processes.

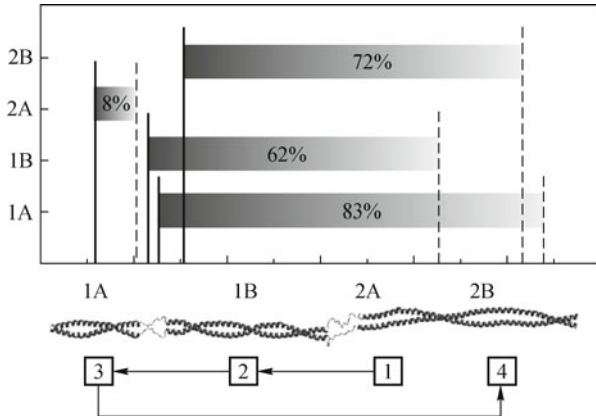


Fig. 4.19 Unfolding history maps for the dimer as it undergoes tensile deformation with increasing strain^[109].

We extend the mechanical analysis by performing stretching simulations with pulling rates ranging from $0.0001 \text{ \AA/ps}$ to $1 \text{ \AA/ps}$. The unfolding force at the angular point (denoting the onset of domain unfolding in the protein) is plotted as a function of the pulling speed in Fig.4.20. The results show a significant dependence on the unfolding force as a function of pulling velocity, reaching a plateau value for small pulling speeds on the order of $\approx 100 \text{ pN}$. Using those results to fit the Bell model whose details are given in [109, 110], the following is obtained:

$$f = \frac{k_B T}{x_b} \ln v - \frac{k_B T}{x_b} \ln v_0, \quad (4.1)$$

where f is the unfolding force, k_B is Boltzmann constant, T is the temperature, v is the pulling speed, and v_0 is the natural bond-breaking speed defined as

$$v_0 = \omega_0 x_b \exp\left(-\frac{E_b}{k_B T}\right). \quad (4.2)$$

Here $\omega_0 = 1 \times 10^{13} \text{ s}^{-1}$ is the natural vibration frequency. We obtain for the slow deformation regime, $E_b = 14.05 \text{ kcal/mol}$ and $x_b = 4.27 \text{ \AA}$. For the fast deformation regime, we obtain $E_b = 5.18 \text{ kcal/mol}$ and $x_b = 0.64 \text{ \AA}$, where E_b and x_b physically equal the energy barrier height and width to unfold the molecule. In earlier studies^[116] it has been suggested that the protein structure strength increases as a weak power of rate under slow loading. Based on this suggestion, we use an overall fitting function to the data defined as

$$f_{\text{fit}}(v) = A \exp\left(\frac{\ln v}{B}\right), \quad (4.3)$$

which empirically captures the asymptotic trend of the strength properties for a very wide variation of pulling speeds. The parameters $A = 222.77 \text{ pN}$ and $B = 6.634$ are obtained by fitting Eq. (4.3) to the overall simulation results as shown in Fig.4.20. The prediction of Eq. (4.3) for pulling velocities comparable to those used in experiment (on the order of $8\text{E-}12 \text{ \AA/ps}$) is $\approx 10 \text{ pN}$, being in agreement with experimental results^[35] that reported a strength of 15.7 pN in regime (II). For the coiled coil myosin structure, the unfolding force measured by atomic force microscopy (with a cantilever speed of $1 \text{ \mu m/s}$) is $\approx 33 \text{ pN}$ ^[113], while from our model the unfolding force for this pulling velocity is found to be 28 pN . This comparison suggests a rather good agreement with experimental results.

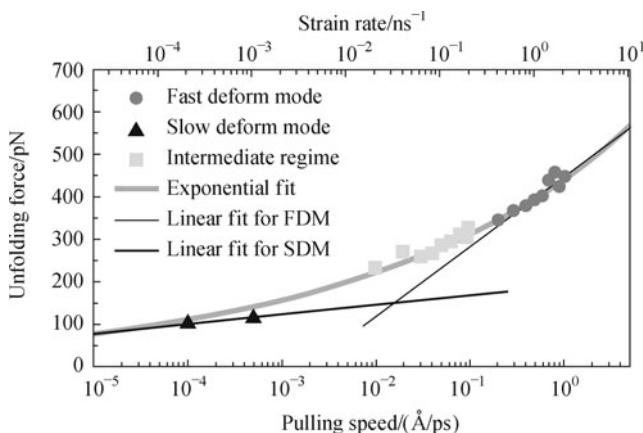


Fig. 4.20 Unfolding force as a function of the pulling speed and corresponding strain rate (data taken from Ref.[109]).

We continue with an analysis of the tetramer mechanical properties. Figure 4.21 shows the complete force-strain curves of a vimentin tetramer under stretching. The simulations reveal four distinct regimes. In the first regime (I), the pulling force increases linearly with the strain until it reaches the angular point, and we do not observe unfolding phenomenon before overall 10% strain. In the second regime (II), the force-strain curve forms a plateau,

where the pulling force keeps almost constant, which is similar to the case of the deformation properties of the dimer. From simulation snapshots in Fig.4.22a, we find that the 2B segments unfold first, starting from the stouter location, then expand throughout the entire segment. In the third regime (III), we observe a significant transition in the force-strain behavior. During this transition, the pulling force changes from the low-force plateau (<1 nN) to a peak value of 7.3 nN, marking the onset of the final regime (IV). Beyond the peak value, the force in regime (IV) is rather bumpy and its level begins to decrease with the increasing strain.

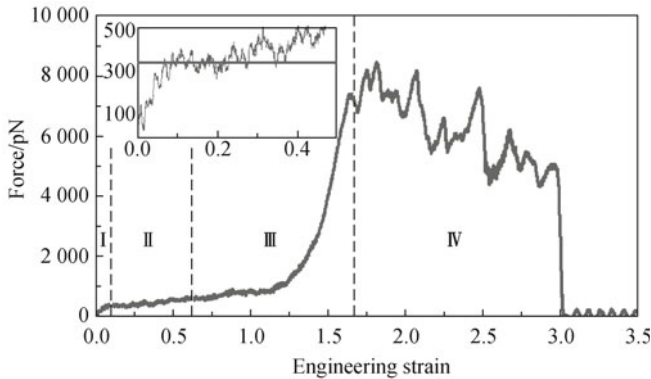


Fig. 4.21 The force-strain curve of the dimer under stretching for a constant pulling rate ($0.1 \text{ \AA/ps}$) (for details see Ref.[15,109]).

The force plateau (II) observed in Fig.4.21 relates to the stepwise unfolding of the coiled coil segments. We observe that at the end of the plateau regime, the tetramer behaves differently from the dimer. The overlapped part in center begins to uncoil (Fig.4.22a), and the pulling force approaches a much broader transition region, where the structure becomes significantly stiffer and eventual fails by interdimer sliding. The sliding process is composed of many rupture events of hydrogen bonds, that is why the stretching force is much bumpy. The two dimers are completely detached at $\approx 300\%$ strain (Fig.4.4b). The comparison between the dimer and tetramer reveals a significant difference in the mechanical behavior.

The stretching study of the tetramer indicates that the deformation of IFs involves the $\alpha - \beta$ transition and subsequent sliding^[117]. This conclusion suggests that significant filament thinning should occur during stretching. Indeed, this behavior has been observed experimentally in atomic force microscopy and electron microscopy studies of desmin IFs^[5,105] as shown in Fig.4.22c, being in qualitative agreement with our predictions. The experimental evidence for microstructure transition in intermediate filaments under stretching is based on a small angle X-ray diffraction study of stretched hard alpha-keratin fibers^[118].

To compare our result with that of AFM stretching of single filaments,

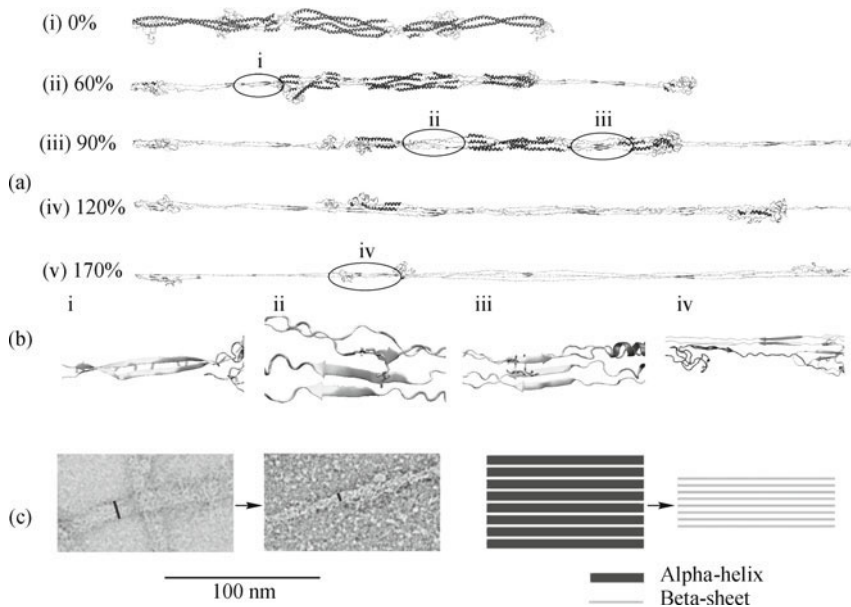


Fig. 4.22 Simulation snapshots during pulling of the dimer at different pull strain (a), (b) shows detailed views of the molecular structure at various levels of strain, (c) shows the experimental evidence that alpha-beta transition takes place during stretching^[15,105] (color plot in the book end).

considering the fact that unit length filaments fuse from the head to the tail to form full length filaments, we consider the force-strain curve from 60% strain onwards which corresponds to the beginning of the third region. The force is divided by the cross-sectional area of the tetramer, $A_0 = 32.9 \text{ nm}^2$ to obtain a measure of the stress. To account for rate effects that results in force estimates much larger than those seen in experiments^[108,110], we use a force scaling factor of 7.1. This value is based on earlier studies that for beta-sheet proteins the strength at $0.1 \text{ \AA/ps}$ pulling speed is 1700 pN ^[119]. For slow pulling on the order of $10^{-11} \text{ \AA/ps}$ the strength is found to be 240 pN ^[120]. This facilitates a direct comparison with experimental results using AFM^[5], as given in Fig.4.23.

A quantitative comparison with AFM experiments to summarize the mechanical property of single IF filaments^[5] is presented here as shown in Fig.4.24. It is shown that there is overall good agreement between simulation and experiment. The resulting maximum stress for the filament is predicted to be $\approx 97 \text{ MPa}$ (found at $\approx 205\%$ strain) from simulation, close to experimental measurements of 90 MPa at similar levels of strain^[5]. The transitions of the stress-strain behavior and the maximum strength in the two methods show overall good agreement. However, in the lower strain regime before the transition, the experimental stress is higher, possibly caused by effect of sliding of the filament on the surface during stretching. From the analysis of

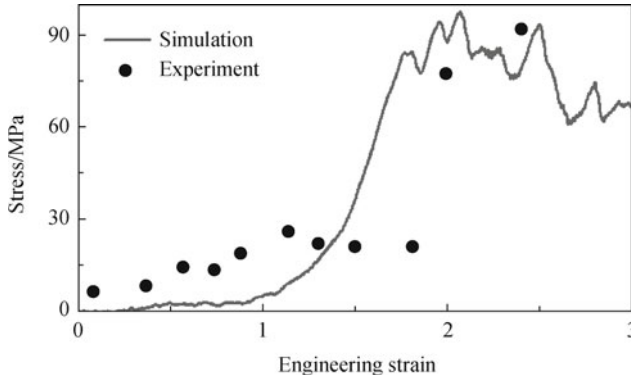


Fig. 4.23 Comparison of stress-strain curve of IF filaments between experiment and simulation as reported in Ref.[105].

the stress-strain behavior, we extract the tangent modulus for the full length filament to be $\approx 3\text{--}12$ MPa below 50% strain. This modulus is close to experimental results, where a range of $\approx 7\text{--}15$ MPa was reported^[5,35]. We note that the initial tangent Young's moduli found here are significantly smaller than typical values reported for α -keratins (1.8 GPa), microtubules (0.9 GPa)^[121] and F-actin (2.6 GPa)^[122], confirming the characteristic properties of IFs. In both simulation and experiment, the tangent modulus increases by a factor of ≈ 20 once 150% strain is reached, leading to severe stiffening. The tangent modulus in simulation approaches ≈ 185 MPa before failure, similar to experimental results. The reason to cause differences between simulation and experiment is not yet well understood. Possible explanations include the presence of defects, structural irregularities, and the fact that much longer filaments are considered in experiment, whereas the small system considered in our simulations is perfect without any structural flaws.

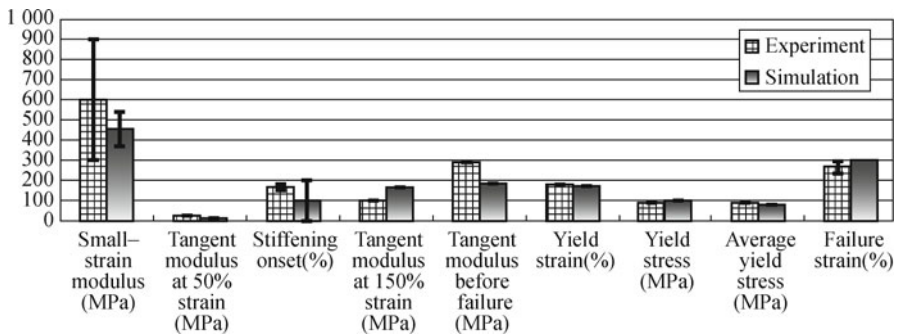


Fig. 4.24 Comparison of mechanical properties of IF filaments between experiment and simulation (experiment results from Refs.[5, 35, 105, 114], the comparison shows an overall good agreement).

4.4.1.3 Plasticity of intermediate filaments^[123]

In this section, we review a study of the conformation of vimentin subunits to gain further insights into the dynamic properties of vimentin, desmin and keratin K5/K14 subunits. Subunits embedded in vitreous ice appeared as thin straight rods but the use of a molybdate staining solution and/or glutaraldehyde favored more compact conformations and aggregation into meshworks. AFM imaging in air of filaments adsorbed on mica after one hour of assembly was surrounded by subunits displaying a wide range of conformations from dots to flexible rods as shown in Fig.4.25. The assembly in the presence of

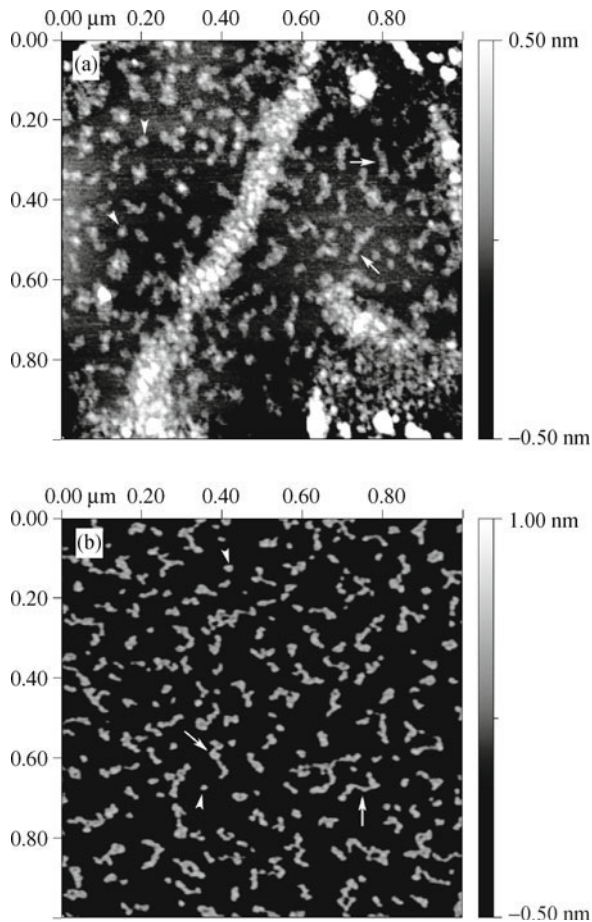


Fig. 4.25 AFM images in air of IFs assembled for 1 h at 37 °C and adsorbed to mica. (a) Vimentin at 0.1 mg/mL in 25 mM Tris-HCl (at pH 7.5), 125 mM NaCl. Flat filaments are visible as well as vimentin subunits. (b) Keratin K5/K14 at 0.1 mg/mL in 25 mM Tris-HCl (at pH 7.5), 100 mM NaCl. In this picture we only show the IF subunits. In both cases, notice the presence of dot-like structures (arrowheads) along side curly threads (arrows) (Figure adopted from Ref.[123]).

divalent ions such as calcium, or glutaraldehyde fixation biased the distribution of conformations towards the most compact ones. MD simulations of a full atomistic single vimentin dimer and tetramer in solution and adsorbed to a coarse-grained mica surface enabled us to map in detail the type of conformations accessible to an IF subunit.

In our AFM experiments, we assumed that the conformations of the subunits on mica are very similar to the ones they would have in solution. This hypothesis can be tested *in silico* through MD simulations. We started with the conformations of the vimentin dimer and tetramer presented in Fig.4.2. After an equilibration time of 50 ns, we observed different conformations of the subunits with and without adhesion energy (Fig.4.26). We find the main effect of the mica substrate is to keep the subunit conformation in a straighter form compared to its equilibrium state in solution. We measure the end-to-end length L_{ee} of the dimer and tetramer in wt/wo presence of the mica. The dimer has an L_{ee} of only (21.5 ± 0.5) nm in solution compared to (47.9 ± 0.1) nm on mica and the tetramer has an L_{ee} of (36.5 ± 2.7) nm in solution compared to (58.5 ± 0.3) nm on mica. The adhesion energy to the substrate seems to “freeze” the dynamic properties of the dimer and tetramer, because before adhesion, the conformation of the subunit is driven by entropy while after adhesion the adhesion energy starts to take effect. This means that during the absorption of an IF subunit to a mica surface in solution, the final conformation on the substrate should not be modified significantly. In other words, the difference in morphology between subunits imaged by AFM in air and cryo-negative staining can be attributed mainly to dehydration effects.

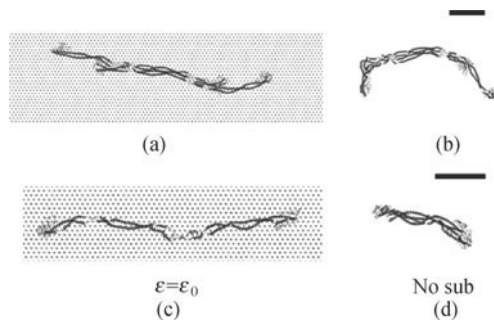


Fig. 4.26 The conformation of vimentin dimers and tetramers at equilibrium with and without substrate presence. The equilibrated conformation of the tetramer (a) and dimer (c) on the substrate with adhesion. The equilibrated tetramer (c) and dimer (d) without substrate adhesion. The scale bar is 10 nm for (a), (b) and (c), (d)^[123].

As for the equilibrium conformations in solution, they are in good agreement with experimental data. We already know from previous studies that the dimer is only soluble in at least 4 M urea^[82], so the collapse into a compact 20 nm long rod (Fig.4.26d) was expected. For the tetramer, the

equilibrium conformation shows a similar smoothly bent shape (Fig.4.26b) which agrees with the cryo-stained tetramers (Fig.4.27). Overall, these data indicate that IF subunits are indeed very “plastic” structures that can bring about dramatic shape changes depending on their environments.

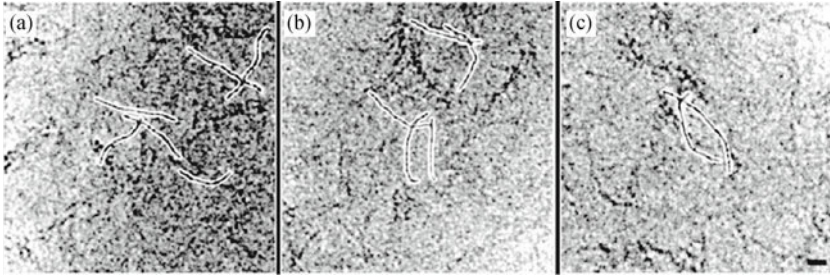


Fig. 4.27 Gallery of cryo-negative stained desmin tetramers. The rod shape of the tetramers is highlighted for clarity. Not all the depicted tetramers are single ones; they may be overlapping or cross-linked end to end by the staining agent. Nevertheless the flexible nature of the individual tetramers is clearly visible. Scale bar: 10 nm, figure was adopted from Ref.[123].

The flexibility of IF subunits is likely to stem from two different structural elements. All IF proteins form either homo- or hetero-dimers comprising two double stranded coiled coil segments interspersed by a flexible L_{12} linker^[124]. The coiled coil segments are around 20–25 nm long that is similar to the persistence length of a coiled coil measured by atomic force microscopy, i.e. 25 nm^[125]. Hence, the predicted average angle between the tangents at both ends of one segment is around 70° . The linker L_{12} is predicted to be formed of two poorly structured polypeptide chains^[126] and its extreme flexibility has been assessed experimentally for invertebrate IFs^[127]. EM of glycerol sprayed and rotary metal shadowed dimers revealed strong kinks at the L_{12} linkers with angles of 90° and smaller^[127]. Our MD simulation of the vimentin dimer (Fig.4.26d) is in perfect agreement with this idea.

4.4.2 Network mechanics

Having understood the basic structural and mechanical properties of IFs, we can now upscale our simulation to study the physical and physiological roles this filament system plays in cells. IFs are often neglected in cell mechanics models based on principles such as tensegrity^[128] or soft glassy materials^[129]. It is generally argued that IFs are too flexible compared to MFs and MTs to have any major impact on cell elasticity at small strains or even on cell adhesion and cell motion. However early studies on vimentin deficient cells and cells treated with acrylamide pointed towards a pivotal role of IFs in cell stiffness, stiffening and growth^[130,131]. Recently, Bhattacharya et al. demon-

strated that vimentin IFs mediate cell adhesion strength through their interactions with plectin and $\beta 3$ integrin^[132]. Along the same line, skin fibroblasts of plectin deficient mice generate less traction forces at focal adhesion sites compared to skin fibroblast of wild-type mice^[133]. Interestingly, the same cells are also deficient in long distance propagation of mechanical stresses in the absence of plectin^[133]. Still a similar disruption of mechanotransduction remains to be demonstrated in the absence of IFs instead of plectin.

So far the extensibility of IFs has already been demonstrated in sonic muscle fibers of the type I male midshipman fish^[64]. In this special skeletal muscle developed for high frequency contraction, the desmin IF network is better organized in three dimensions than any other vertebrate skeletal muscle^[134]. This particularity enabled Lewis et al. to follow by immunofluorescence the deformation of the desmin network in a muscle fiber stretched to 60% strain^[64]. Such strains did not induce breakage of the filaments and the deformation was reversible at the resolution of immunofluorescence^[64]. Based on this result and our nanomechanical data on single desmin IFs^[59,79], it is straightforward to propose that IF networks may behave as “security belts” in tissues exposed to large tensile strains (early presentation of this hypothesis in Ref.[135]).

4.4.2.1 Multiscale modeling of intermediate filaments and networks

A future direction to test key hypotheses and to elucidate structure-property links between molecular and microscopic length-scales is to build network level models based on the molecular-level bottom-up models of IF structure and mechanical behavior described above^[136], as shown in Fig.4.28. This blueprint illustrates the potential of using a hierarchical multi-scale approach, where a mesoscale model at the level of cellular protein networks is built based on the constitutive behavior of individual molecular-level protein building blocks^[137]. Such multi-scale models, integrated with multi-scale experimental studies, could be crucial to further our understanding of IF proteins in the context of extreme mechanical deformation of cells and mechanotransduction^[14].

4.4.2.2 The deformation and rupture of intermediate filament network

Figure 4.29 shows snapshots of deformation of a two-dimensional intermediate filament network, which features a small geometric irregularity (modeled as a crack-like defect) that could represent an inclusion in the network such as the cell nucleus^[138-140]. In this network model, a square lattice of interconnected full length filaments is considered, where each filament behaves with the mechanical loading in what we obtained via atomic simulation (Fig.4.23), mimicking the overall behavior shown in Fig.4.29. This simulation illustrates the great level of robustness of IF networks against catastrophic failure.

The failure of this network is another interesting process to investigate.

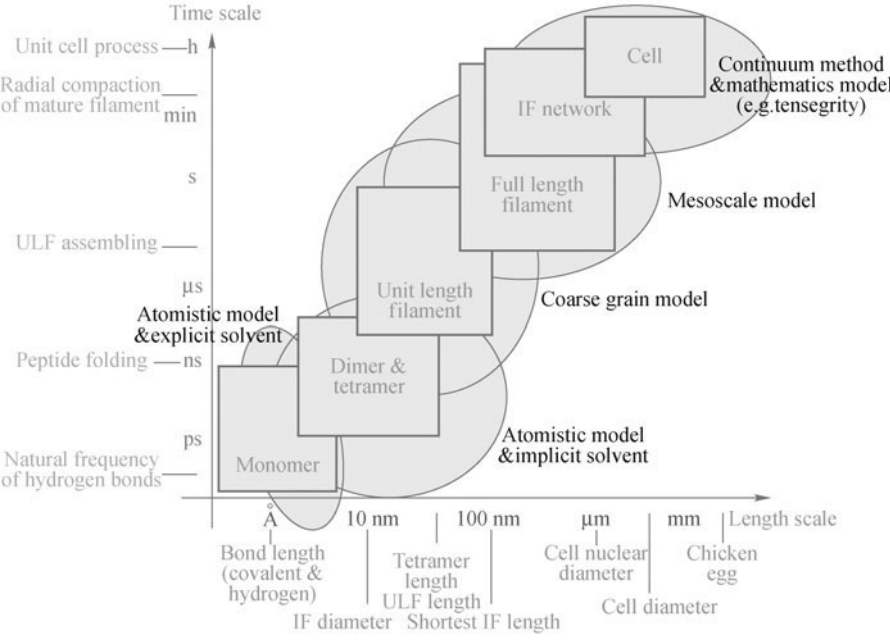
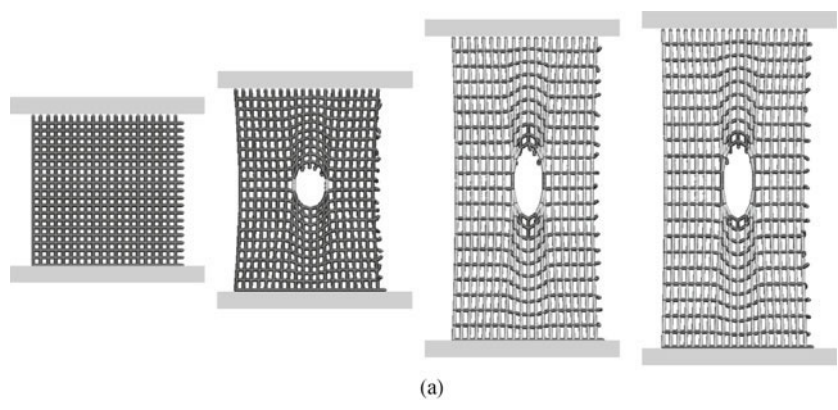


Fig. 4.28 The basic approach of the step-by-step procedure of studying the structural and mechanical properties of IF structures by using multi-scale experiment and simulation (further details see Ref.[14], color plot in the book end).

We have been able to track the crack propagation history by applying loading on a larger model as shown in Fig.4.30. Here we show that by applying multi-scale method, we are not restricted to predicting the structural property of materials near equilibrium state, but also able to reveal the real time dynamic process, especially for material failure, in biological systems.



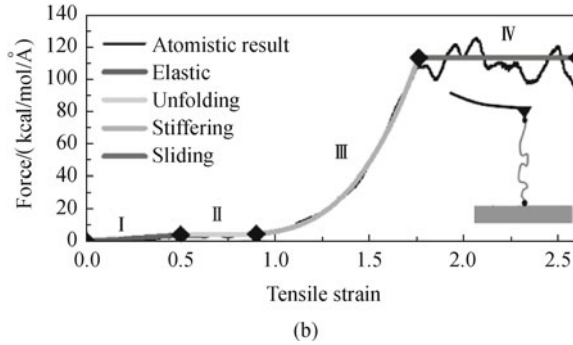


Fig. 4.29 Snapshots of a larger-scale intermediate filament network deformation (a). The mechanical response of each filament is fitted fully according to the result of full atomic modeling as given in Fig.4.23. Each filament under tension is colored according to the strain state, as shown in the force-strain curve (b)^[138,141] (color plot in the book end).

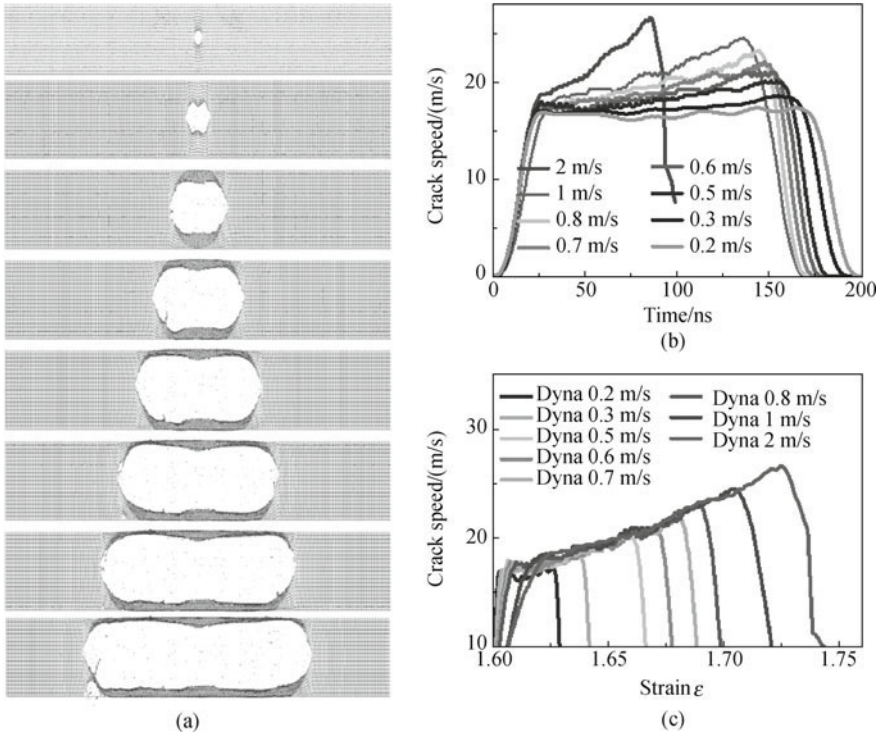


Fig. 4.30 Snapshots of the intermediate filament network under tension after the applied strain exceeds the strength limit as shown in Fig.4.30. We record the crack speed as a function of time with different loading rates and find that the crack speed is a function of applied strain rather than the loading rate^[141] (color plot in the book end).

4.4.3 The mechanical role of intermediate filament in cellular system

By utilizing a simple empirical coarse-grained computational model of the intermediate filament network in eukaryotic cells, we show that intermediate filaments play a significant role in the cell mechanical behavior at large deformation. Figure 4.31 depicts a view of experimentally stretched cells stretched uniaxially at about 75% strain as well as the mesoscopic cell model at 59% strain. We observe that as the cell is stretched, the intermediate filament network undergoes tensile deformation in the areas between the pulled extrem-

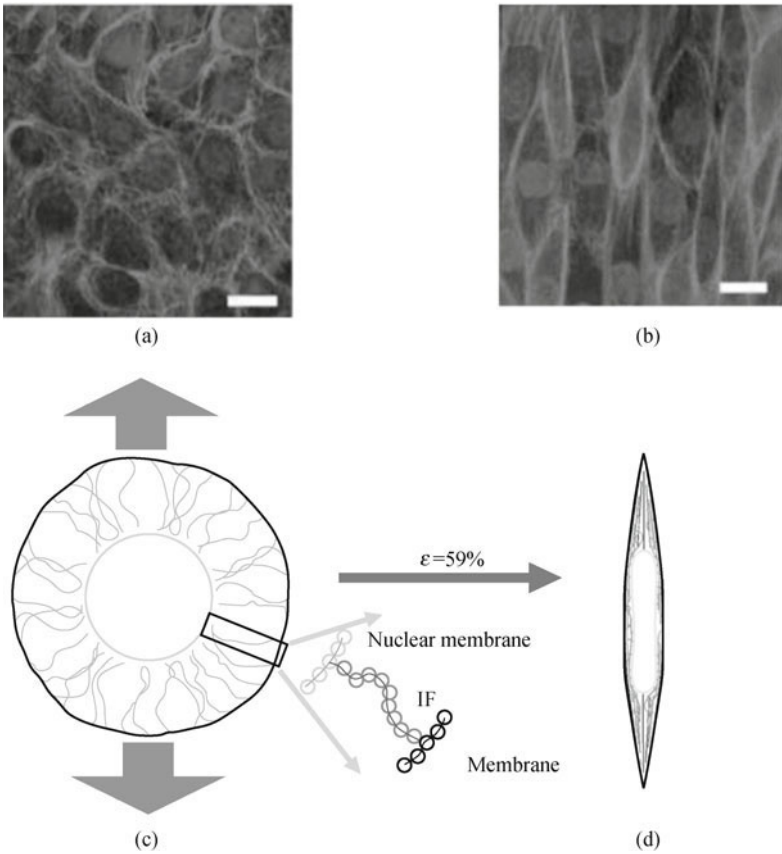


Fig. 4.31 Cell model geometry, boundary conditions and illustration of the role of intermediate filament network for maintaining cell integrity at large deformation. (a) and (b): intermediate filament network in MDCK cells (scale bar is approximately 25 μm) under static and strained conditions. (Experimental images were reprinted from Ref.[55], Copyright © 2007, with permission from John Wiley and Sons). (c) and (d): snapshots of the cell model deformation under mechanical load (at 0% and 59% strain, respectively) (color plot in the book end).

ities and the nucleus, and is being compressed and bent in the cell's lateral domain. This snapshot illustrates that intermediate filaments are stretched significantly, and are thus important for maintaining cell integrity at large deformations^[142].

Using this model, we investigated the mechanical behavior of a cell under tension and changed the filament number to study its effect on cells' properties as shown in Fig.4.32. As the intermediate filament density decreases, the cell mechanical properties exhibit greater softening and a reduced stiffness. The results show that intermediate filament deficient cell models display an altered mechanical behavior, featuring a softer mechanical response at large deformation while the mechanical properties remain largely unchanged at small deformation. The computational results are qualitatively consistent with the experimental measurements.

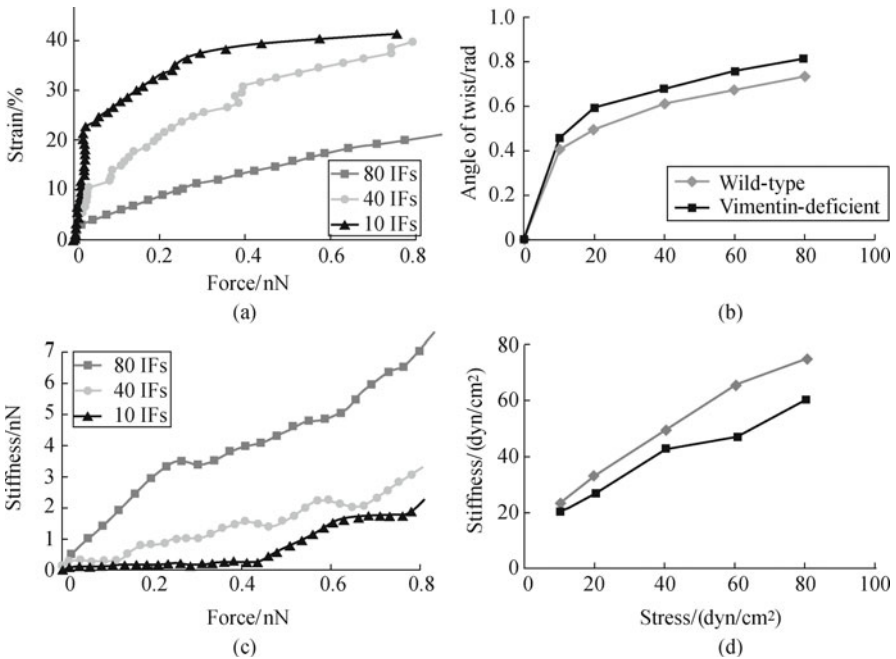


Fig. 4.32 Mechanical response of the cell under tension for several intermediate filament densities, as predicted by the mesoscale model ((a) and (c)), as well as a qualitative comparison with experimental results ((b) and (d)) were reprinted from [143], Copyright © 2000, with permission from the American Physiological Society). It is noted that $1 \text{ dyn/cm}^2 = 0.1 \text{ Pa} \approx 0.008 \text{ nN}$ on a cell cross section (assuming a diameter of $10 \text{ }\mu\text{m}$).

Our results suggest that intermediate filaments contribute to cell stiffness and deformation at large deformation, and thus play a significant role in maintaining cell structural integrity in response to applied stress and strain. Our model opens the door to future study disease states, the effects of amino

acid mutations, and how structural changes at different levels in the cell's structural makeup influence biomechanical properties. We realize the current 2D model is far from enough to simulate the complex behavior of cells, to cross this barrier, we are working a more complicate but also dedicate 3D model as shown in Fig.4.33. We hope to apply this model to capturing the character of cells in the future.

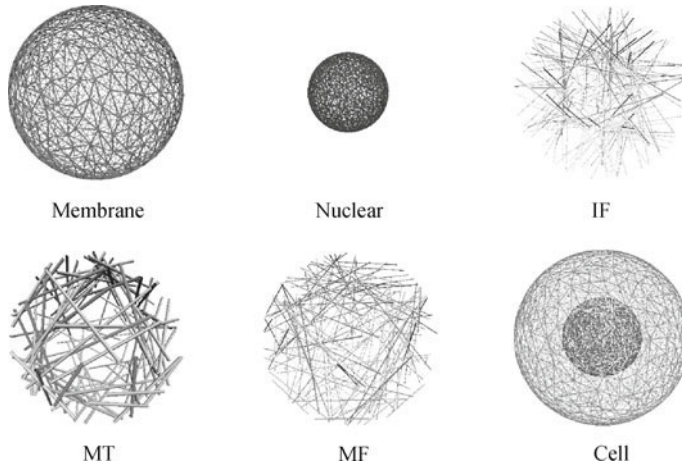


Fig. 4.33 An illustration of 3D structural models of cells, summarizing the main components of the cell's skeleton system.

4.5 Conclusion

In this chapter we reviewed the general field of intermediate filament mechanics, highlighted recent advances, challenges, and explain the opportunities existing at the interface of engineering and biology. A general theme of our discussion is the consideration of how material properties change at different structural scales and how this relates to biological function, and analyzed from a bottom-up perspective. Key results reviewed here, such as the identification of structural models of intermediate filaments, its associated mechanical properties, and upscaling the nanomechanical properties to larger scales (that reach several tens of micrometers), provide exciting opportunities for quantitative links between protein chemistry (genetics) and functional properties (physiology), which can effectively be studied by using the materiomics approach used here.

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Chapter 5 Cytoskeletal Mechanics and Rheology

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Abstract: Cells are basic functional units of life and control a wide range of intra- and extra-cellular activities. They are highly complex structures with unique biomechanical properties to withstand the physiological environment as well as mechanical stimuli. Studies related to the mechanics of single cells are aimed at describing the molecular mechanisms responsible for the physical integrity of the cells as well as their biological functions. These studies have significant implications for biotechnology and human health. Recent advanced and innovative experimental techniques for measuring forces at piconewton resolutions and displacements over nano-meter scales have greatly facilitated this area of research. Moreover, tremendous research efforts have been devoted to the development of multiscale multiphysics computational models for the mechanical properties and functions of cells. This chapter reviews recent numerical and experimental studies in the area of cytoskeletal mechanics and rheology. For this purpose, basic modeling techniques for the mechanics of semiflexible actin filaments as well as various experimental and computational methods for measuring the mechanical behavior of cells are discussed.

Keywords: living cells, cytoskeletal mechanics, semiflexible actin filaments, cell mechanobiology

5.1 Introduction

Cells are the basic functional units of life and control a wide range of intra- and extra-cellular activities. They are dynamic and ever changing systems

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composed of numerous components with distinct mechanical, chemical and biological properties. Cells alter their mechanical properties in response to external stimuli in order to perform various functions to which mechanics and biology are often intrinsically linked. Many cells migrate both during development as organisms grow and at maturity in health- and disease-related states, e.g. wound repair and atherosclerosis. Moreover, cells sense and respond to externally applied mechanical forces and transduce them into a cascade of biochemical events influencing their behavior. The cellular processes involved in sensing and responding to mechanical stimuli are studied in the fast growing field of mechanotransduction. The ability of a cell to perform the above functions depends on its integrity and its particular shape which, are maintained through the structural stiffness and rheology of the cytoskeleton. A detailed understanding of cytoskeletal mechanics and rheology is required to determine the molecular basis for cellular phenomena such as cell migration and cell adhesion. Cytoskeleton, the primary source of the cell structural integrity and stiffness, comprises of a system of highly entangled protein filaments that permeate the microfluidic space of the cytosol. It behaves elastically in response to quick deformations while behaving similar to a viscous fluid-like material in slow deformations. Actin filaments, intermediate filaments, microtubules, and their cross-linking proteins are the major components of the cytoskeletal network. Microfilaments and microtubules are made of a chain of globular proteins while intermediate filaments are composed of long fibrous subunits (Fig.5.1). These protein filaments are collectively responsible for cell structural properties and motilities.

Microtubules are polymers of tubulin heterodimer, α -tubulin and β -tubulin. The tubulin monomers organize themselves into a small hollow cylinder with an outer diameter of approximately 25 nm. Microtubules with half-lives of only a few minutes are highly dynamic and polarized structures undergoing constant polymerization and depolymerization. They radiate from the centrioles, located near nucleus, and interact with motor proteins to move intracellular organelles inside the cell.

There are different intermediate filaments in different cell types; however, they are all formed from the assembly of fibrous proteins such as Keratin. Intermediate filaments consist of a central α -helical domain with over 300 residues. These dimers assemble into a staggered array to form tetramers; the end-to-end connection of tetramers yields protofilaments. An intermediate filament has a ropelike structure and is composed of approximately eight protofilaments. Intermediate filaments are fairly stable and exhibit high resistance to solubility in salts.

The filamentous actin (F-actin) is constructed through polymerization of the globular monomeric actin, G-actin, into two twisted strands which are loosely wrapped around each other. Actin microfilaments are often concentrated beneath the cell membrane and reinforce the cell surface against external forces. F-actin has a diameter of about 8 nm and is polarized with a pointed (minus) and a barbed (plus) end. The polymerization of actin occurs

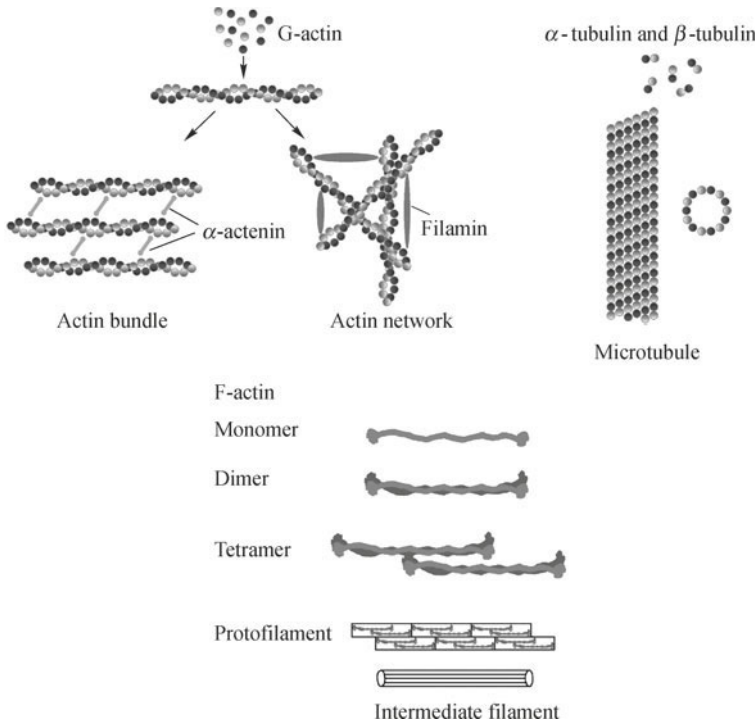


Fig. 5.1 Schematic plot for the structure of an actin filament, a microtubule and an intermediate filament (color plot in the book end).

though the hydrolysis of the Adenosine-5'-triphosphate (ATP) of actin subunit to adenosine diphosphate (ADP) plus an inorganic phosphate. Monomer addition and filament growth occur through ATP binding to the positive barbed end whereas depolymerization occurs preferentially at the pointed ends. Filament growth and organization are regulated by many factors, including ionic concentrations, actin binding proteins (ABPs), a variety of capping, binding, branching, and severing proteins. The assembly and disassembly of microfilaments allow a cell to change its shape and to accommodate cellular movement. For example, white blood cells are able to move through capillary walls and enter the damaged tissue through cytoskeletal remodeling.

A plethora of actin monomers along with a large number of actin-monomer-binding proteins are required for the rapid growth of actins in motile cells and also for sudden reorganization of actins in response to intra- and extra-cellular stimuli. These proteins connect actin to microtubules, actin to intermediate filaments or actin to both of these fibers. Actin binding proteins such as α -actinin and filamin organize actin filaments into tertiary structures such as fiber bundles (known as stress fibers) and three-dimensional lattice-like networks. Stress fibers form at focal adhesions, i.e. the points in the cell at which actin myosin contractility is resisted. At the focal adhesion,

the actin cytoskeleton is anchored to transmembrane protein integrins. For the stress fiber growth, the cytoskeletal microstructure is required to be regulated and organized by recruiting myosin motor molecules and actin cross-linkers. Molecular motor protein myosins belong to actin binding protein category and play an important role in the cytoskeleton dynamic behavior. Myosins are present in all muscle cells forming a well-defined structure, the sarcomere, and produce active contraction. In skeletal muscle cells and cardiac myocytes, the regularly arranged sarcomeres appear in a clearly observable striated pattern. In non-muscle cells these contractile machineries are mainly involved in functions such as maintaining a resting level of cell tension, changing cell shape, and cell migration.

Despite the importance of the cytoskeletal rheology, the relation between the microstructural details and the macroscopic rheological behavior of the cytoskeleton remains elusive. Several fundamentally different models have been proposed for the mechanics and rheology of the cytoskeleton; a thorough review of these models has recently been presented by the authors^[97]. Here, we briefly review some of the theoretic and experimental attempts to analyze and understand the mechanics, microrheology, and macrorheology of the cytoskeleton. The structure of this chapter is as follows. First, basic concepts for modeling semiflexible filaments are introduced. The chapter then discusses various experimental techniques used to measure macroscopic behavior of cells under external loads. A brief review of recent computational advancement in cytoskeletal modeling is given in the end.

5.2 Modelling semiflexible filament dynamics

This section discusses a recent methodology to study and characterize the behavior of the semiflexible filaments^[8,9]. The cytoskeleton is highly heterogeneous and has an intricate yet diverse structure. Its three broadly important filaments are F-actin, microtubules, and intermediate filaments. These filaments are classified as being semiflexible because of their long persistence length with the respect to their contour length. The persistence of actin, microtubules, and intermediate filaments are about 10 μm , 6 μm , and 1 μm , respectively. The persistence length is the length over which the thermal bending becomes appreciable and the contour angles become correlated^[1,2]. For modeling purposes, a semiflexible filament may be represented as a continuously isotropic Euler beam. For any conformation Ω of the semiflexible filament, the total free energy $H(\Omega)$ is given by

$$H(\Omega) = \frac{EI}{2} \int_L dl \left(\frac{\partial \theta(l)}{\partial l} \right)^2,$$

where L is the contour length, $\theta(l)$ is the filament angle at the distance l along the filament, and E and I denote the filament Young's modulus and cross-

sectional rigidity, respectively. The probability $p(\Omega)$ of finding the filament in a particular configuration Ω is given by Boltzmann distribution,

$$p(\Omega) = \frac{1}{Z} \exp\left(-\frac{H(\Omega)}{K_B T}\right) = \frac{1}{Z} \exp\left[-\frac{EI}{2K_B T} \int_L dl \left(\frac{\partial \theta(l)}{\partial l}\right)^2\right],$$

where K_B is the Boltzmann constant, T is the absolute temperature, and Z is a normalization constant. From the above equation, the persistence length can be defined as

$$L_p = \frac{EI}{K_B T}.$$

This expression captures the competition between flexural rigidity and thermal energy in determining the conformation or shape of the filament. From equipartition theory, $K_B T/2$ is the mean energy for each degree of freedom (here bending deformation). It can be shown that persistence length is also expressed as

$$\langle \mathbf{t}(l) \cdot \mathbf{t}(l+r) \rangle_l = \exp\left(\frac{-r}{2L_p}\right),$$

where $\mathbf{t}(s)$ is the tangent vector, r is the distance from initial position l along a filament and $\langle \rangle_l$ denote averaging over l . In semiflexible polymers, the entropic contribution to the free energy is small and the enthalpic or strain energy contribution plays a more significant role.

Many of artificial polymers can be categorized as being flexible, i.e. the polymer persistence length is much smaller than its contour length and therefore it turns many times in the solution and looks like a relatively compact disordered coil. The theory for the behavior of network formed by these flexible polymers, where the persistence length is small compared with both the length of the polymer and the entanglement distance in the solution, has been well developed^[1]. As the persistence length becomes larger and comparable to (or larger than) the contour length, the dynamic of the filaments changes. These filaments are called semiflexible filaments and the properties of the networks formed by these fibers are less developed.

Cytoskeleton is a highly heterogeneous, active and dynamic structure consisting of semiflexible filaments with interconnection lengths of the same order as fiber persistence length. The biophysical properties of the cytoskeleton have been studied and measured in living cells. For example, F-actin networks subjected to oscillatory shear deformation show negative normal stresses as strong as shear stresses^[3]. This phenomenon does not appear in the behavior of flexible polymer networks; their response is like most materials where the tendency to expand in the direction normal to the applied shear forces yields positive normal stresses. Another unique property of actin networks is the observation of both stiffening and softening. Unlike simple polymer gels, networks of semiflexible filaments nonlinearly stiffen in order to resist large deformations and maintain the network integrity^[4,5]. A reversible

stress-softening behavior in compression, essential in preventing catastrophic fracture of actin networks, has also been reported^[6]. Flexible and rigid polymers exhibit only monotonic stiffening in compression. The origins of these anomalies and unique macroscopic behavior are under study and there is no comprehensive theory yet. It seems that additional complexities due to nonhomogeneous protein microstructure are required to be considered for the proper description of the cytoskeletal behavior. The underlying filament deformations leading to such a network behavior are still unknown^[7].

A new approach has recently been suggested to dynamically simulate semiflexible filaments in a physically consistent but computationally efficient manner^[8,9]. The prevalent method for dynamically simulating semiflexible filaments is the string-of-beads idealization. In a weak force field F^P , a particle drifts with a velocity v proportional to the force. The proportionality constant is called the frictional coefficient ξ . Considering the random Brownian forces F^B generated by constant collision with solvent molecules,

$$\xi v(t) = F^P(t) - F^B(t).$$

For fluctuating semiflexible filaments, the conserved force F^P is the internal bending force of the worm model,

$$\xi v(t) = EI \frac{d^2\theta}{dt^2} - F^B(t).$$

In order to determine the points on the filament at which the fluctuation, dissipation, and elastic forces are applied and balanced, a filament is often discretized into a string of beads. The viscous and Brownian forces are then applied at each spherical bead center. The discretization of a filament into beads leads to an error in the hydrodynamics. The diffusion coefficient of a rigid representation of a string of beads approaches that of a rigid cylindrical rod only at infinite number of beads^[1]. Furthermore, this way of discretization yields errors in determining bending angles and contour length of a continuous filament. In order to tackle these problems and decrease the computational cost, an idealization of a semiflexible filament as a string of continuously flexible rods has been proposed. The Brownian forces due to solvent collision over flexible rods are considered as normal and parallel forces acting on the surface of the rods. Neglecting the hydrodynamic screening and end effects, it is assumed that Brownian force on a segment of a long filament is the same as the force acting on the segment when it is free in the solution. Moreover, the influence of segment bending capacity is neglected in the analysis. Therefore, the Brownian force on a segment that is a part of a flexible filament is obtained by considering a freely diffusing and rigid segment. The resulting force is projected into two perpendicular directions and its variance in each direction is determined by the effective translational and rotational diffusion coefficient of the segment^[8]. The Brownian fluctuation dynamics of a semiflexible filament is obtained from balancing simultaneously the Brownian and other forces acting on each segment of the filament. The Brownian

forces are determined from Gaussian distributions with variance determined by the diffusion coefficients. The variance for the net Brownian force F_i in a time interval Δt is given as

$$\langle F_i F_i \rangle = 4 \frac{(K_B T)^2}{D_i} \Delta t,$$

where the diffusion coefficients D_i is calculated from the segment configuration at the beginning of time interval Δt . The direct application of the well established diffusion coefficients of cylindrical rods as well as their bending mechanics to the analysis is the clear advantage of this methodology. The low computational cost of this methodology allows using larger and more realistic networks to model and investigate the mechanics of cytoskeletal networks with large filament aspect ratios. In the next section, recent experimental techniques and computational methods developed for studying the mechanical properties of the cytoskeleton are briefly reviewed.

5.3 Experimental measurements

Experimentally, the behavior of the cytoskeleton is determined either by monitoring the Browning movements of inherent or introduced particles (passive method) or by direct application of external forces (active measurements)^[10,11]. Passive techniques examine the motion of inherent or introduced particles due to thermal fluctuations. If a medium is soft enough, thermal fluctuations of a microscopic probe are measurable and represent the linear response of viscoelastic parameters of the medium surrounding the probe. For example, in passive microrheology, the displacements of micro-sized beads embedded into the cytoskeleton are monitored using either video recordings and particle tracking or laser beam interferometry^[12-17]. There are two versions of this technique; one-particle method and two-particle method. In the former, the positions of individual particles are recorded and the complex shear modulus of the environment is calculated from the mean square displacement of the Brownian motion using the Fluctuation Dissipation theory^[11]. In this method, active movement of the probe particles may influence the medium viscoelastic properties and cause errors in the measurements. Two-particle microrheology has been developed to avoid this problem by measuring the cross correlation of the displacement fluctuations of two particles located at a given distance from each other.

In active techniques, the mechanics is derived from probing the structure via applying a direct force using optical tweezers, atomic force microscopy, osmotic pressure, and hydrodynamics flow among others^[10,11,18,19]. Unlike the passive measurements, the active microrheology methods incorporate applying localized forces at the site of the interrogation. In the next subsections, a summary of these techniques is given.

Nevertheless, before discussing active microrheology methods, a note is in place about frequency-dependent viscoelasticity measurements. Cells show viscoelastic properties at macroscopic scales; experiments based on dynamic mechanical analysis are usually used to study their viscoelasticity. In these tests, a small amplitude oscillatory strain, $\varepsilon_0 \sin(\omega t)$, is applied to a material and the resulting stress, $\sigma_0 \sin(\omega t + \delta)$, is measured where δ is the phase shift between stress and strain, ω is frequency of strain oscillation, and t is time. The in-phase stress response is called the storage elastic modulus, $E'(\omega) = \sigma_0/\varepsilon_0 \cos(\delta(\omega))$, which is a measure of the stored mechanical energy. The out-of-phase response is called the loss elastic modulus, $E''(\omega) = \sigma_0/\varepsilon_0 \sin(\delta(\omega))$, and it measures the energy dissipated as heat in the material. Loss and storage moduli are frequency-dependent, i.e. depending on applied frequencies, a material can have solid-like or liquid-like behavior. Complex variables are used to express modulus as $E^*(\omega) = E'(\omega) + iE''(\omega)$ where i is the imaginary unit. A straightforward and robust approach to characterize the cell microrheology is to determine its complex modulus $E^*(\omega)$ from oscillatory measurements over a wide range of frequencies. The real and imaginary parts of $E^*(\omega)$ represent the elastic energy stored and the frictional energy dissipated within the cell at different oscillatory frequencies, respectively.

5.3.1 Glass microneedles

Glass needles are thin enough to apply small but meaningful forces without damaging the cell. They are first used to apply nanonewton or smaller forces on neurons and to initiate neurite extension^[20]. Cells are deformed by poking them with glass needles which are calibrated to determine their bending constant. The calibration process starts with a precalibrated large rod; this rod is used to calibrate a rod slightly smaller than itself. Each rod is then used to calibrate a rod smaller than itself sequentially until the thin microneedle rod is calibrated. In the experiment, two needles are often mounted in a micromanipulator; one needle is calibrated for its bending constant and used as the needle applied to the cell, while the other needle is used as an unloaded reference for bending of the calibrated needle and for possible drift of the micromanipulator system^[21,22].

5.3.2 Cell poking

The cell poker is a device to apply forces locally to the surface of live cells using an oscillating glass needle tip^[23,24]. The cell is suspended in fluid from a coverslip on top of a vertical glass needle which is attached to a wire needle at its opposite end. The wire needle is coupled to a piezoelectric actuator

which oscillates the glass needle up and down. The glass needle tip indents the cell surface; the applied force is determined by measuring the difference in displacement between the wire needle and the glass needle. The cell poker provides phenomenological information about the viscoelastic properties of cells, about mechanical changes induced by external stimuli, and about the function of the cytoskeleton in the cell mechanical response. Changes in cell shape and cytoskeletal organization associated with physiological processes and due to the physical forces are detectable with the cell poker^[25]. Although the cell cytoskeletal network is neither isotropic and homogenous nor much thicker than the amount of indentation, Hertz model may be used to estimate analytically the cell poking results. In this model, the force displacement curves are estimated in terms of Young's modulus and Poisson's ratio of the domain.

5.3.3 Atomic force microscopy

The atomic force microscope (AFM) is essentially a high-resolution and very sensitive cell poker. It was invented about twenty five years ago and is considered as a classical tool for imaging, measuring, and manipulating materials at nanoscale. AFM tip is used to exert controlled forces to the samples and record the corresponding displacements in order to probe the mechanical properties of the material surface. It is operated in both static and dynamic modes such as contact, tapping, and noncontact modes. The cell properties are probed by applying either pushing or pulling forces as long as the tip is strongly bonded to the cell surface. The spatial inhomogeneity of cells is a problem when indentation is done with conventional sharp tips (~ 10 nm)^[26]. In order to control the inhomogeneities and create a well-defined probe geometry, polystyrene beads of known radius can be attached to the AFM tips^[27,28]. The range of measured forces is from 10 pN to 100 nN with estimated Hertz model-based Young's modulus of 0.1–10 kPa. In the tapping mode, where cells are probed with a rapidly oscillating AFM tip, it is observed that cells stiffen^[29]. AFM has been widely used to probe oscillatory mechanics of cells as well as to estimate their apparent Young's moduli assuming that they are pure elastic materials^[30]. At high frequencies, the viscous drag acting on the cantilever should be corrected to obtain reliable measurements of the complex modulus^[31]. AFMs have also been used for measuring forces between receptors and ligands; quantification of these forces is essential to develop a better understanding for cell-cell interaction, e.g. rolling of flowing leukocytes on vascular surfaces^[32].

5.3.4 Micropipette aspiration

The viscosity and elastic response of cells floating in a solution or attached to a surface can be estimated by pulling on the cell membrane using a small diameter micropipette and measuring the length of aspiration. It is a technique based on the principle of hydrostatic pressure transmission. During the last sixty years, micropipette aspiration has been used to study the mechanical properties of a variety of cells such as monocytes, red blood cells, leukocytes, and erythrocytes^[33-40]. Because of the capability of this method to probe cells that are attached to a surface as well as those suspended in solution, it is a powerful method to probe the viscoelasticity properties of non-adherent blood cells; mechanics of suspended leukocytes determines their transportation and retention in the body. Mechanics of cells in suspension is required for better understanding of cancer cell properties; these cells grow in suspension and a canonical feature of them is the loss of anchorage dependence. Micropipette aspiration measures the cortical tension in the cell membrane, the cytoplasmic viscosity and the cell elasticity. Cortical tension is the tangential tension at the cell surface resisting the increase of surface area; it is conceptually similar to surface tension that pulls a water drop into a sphere. Micropipettes can be used to perform creep tests by applying a step pressure and measuring the projection length of the cell into the micropipette as a function of time.

5.3.5 Microplates

The microplate method has been developed to measure viscoelasticity of surface-adherent cells. In this method, the entire cell is probed by applying loads in physiological range. The deformation is accurately measurable and very large deformations can be applied. Basically, the cell is sandwiched between two glass plates; the cell is tightly attached to the bottom plate which is rigid and the top flexible plate is brought in contact with the top cell surface. The rigid plate is then moved to produce compression, extension, or shear which can be measured from the deflection of the flexible microplate^[41,42]. This technique is used to measure the creep response (the slow deformation under constant stress) or the stress relaxation (the stress relieve under constant strain)^[43]. It is noted that measurements for one single cell at a time can only be performed and the method does not allow regional differences in cell properties to be probed.

5.3.6 Parallel-plate flow chambers

In this method, the fluid flow through a chamber surface coated with a cell monolayer is used to study response of cells to fluid flow; a cellular probe is used to measure this response. Several cell types such as vascular endothelial cells and osteocytes are physiologically exposed to fluid flow and shear stress. Cells sense these external forces and react accordingly; this process is crucial for many regulatory processes. For example, endothelial surface layer has multifaceted physiological functions and behaves as a transport barrier, as a porous hydrodynamic interface in the motion of red and white cells in microvessels, and as a mechanotransducer of fluid shearing stresses to the actin cortical cytoskeleton of the endothelial cell^[44]. Endothelial cells adopt an elongated shape in the flow direction if they are subjected to a shear flow. A similar situation exists for osteocytes in bone where mechanosensing controls bone repair and adaptive restructuring processes^[45]. It is believed that strain-derived flow of interstitial fluid through lacuno-canalicular porosity mechanically activates the osteocytes. There are three candidates stimulating cells: wall shear stress, streaming potentials, and chemotransport^[46,47]. Controlling the wall shear stress and measuring its effect on fluid transport, bone cell nitric oxide, and prostaglandin production can be used to study the nature of the flow-derived cell stimuli^[47]. Fluid shear stress rate is also an important parameter for bone cell activation^[48].

5.3.7 Optical tweezers

In the optical tweezers technique, controlled manipulative forces are applied to the cells while leaving the cell wall intact^[49]. The mechanical properties of living cytoplasm can be studied with minimal damage through application of highly local internal forces. Optical tweezers method is based on trapping a bead with laser beam focalized by a microscope objective. In other words, a laser beam passing through a high-aperture objective lens spatially traps a particle as the scattering force pushing the particle away from the focus point balances the gradient force pulling the particle toward the focus point of the laser. The trapped microbead is partially embedded onto the cell surface and used to apply local forces on the cell. The limited power of the laser bound the applied forces within the range of 1 pN to 100 pN. This method has a wide range of applications ranging from manipulating cells and transporting foreign materials into single cells to delivering cells to specific locations and sorting cells in microfluidic systems. Principal strength of using tweezers for these characterizations includes non-contact force for cell manipulation, high force resolution, wide range of frequencies, and amiability to liquid medium environments. In this method, the microbead usually causes local unwanted remodeling events which alter the structure under study. For further discus-

sion, the reader is referred to a recent review on recent development of optical tweezers^[50].

5.3.8 Magnetic traps

In this technique, the ferromagnetic microbeads coated with a protein which binds to the intracellular proteins are used to apply large forces both on the surface of cells and in their intracellular environment. Several cell types such as white blood cells and fibroblasts exhibit elastic moduli of $\sim 1\text{--}10$ kPa, therefore, large forces on order of nanonewton are needed to study the deformation of these cells^[51]. A high magnetic field is first used to magnetize the beads and create parallel magnetic moments in order to apply a torque. Ferromagnetic particles are used to apply torques and paramagnetic particles to apply force only^[52–56]. Forces up to 10 nN may be generated using paramagnetic beads and forces of several piconewtons are generated using ferromagnetic beads^[57–59].

5.4 Computational models

The biomechanical models developed for the mechanics of a cell are either based on the continuum level and macroscopic observations (phenomenological models) or based on the cell nano-structure (micromechanics models). In the former, it is assumed that cell behaves as a continuum material for which a constitutive model based on the experimental observations can be chosen to describe its behavior. The cytoskeleton has been modeled as a simple viscoelastic continuum, porous gel and soft glassy material. These continuum-level models may only be used to describe the cell behavior if the length scale of interest is much larger than the dimensions of the cell microstructure. In micromechanics methods, the main microstructural features of the system are included in the model.

There are instances where a cell behaves at macroscopic length scales which are two to three orders of magnitude larger than its microstructural characteristic lengths. The behavior of erythrocytes/neutrophils in micropipette aspiration and magnetocytometry-induced deformations are among cases where continuum models successfully describe the overall mechanical properties^[38,39,57,60–62]. The cell environment consists of ionic water solution and charged macromolecules that are influencing its biomechanical and biochemical properties. For example, it is observed that the cell volume changes under osmotic loadings due to changes in ion concentrations. While many of continuum level models neglect the interactions of these different phases and consider the cell as a single-phase material, multi-phasic models have been developed to take into account the interactions between solid, fluid and

free ions in the cytoskeleton^[63]. Most of the work in this area as well as the first multi-phasic model have been developed in order to study the articular cartilage and chondrocyte cells. Few notes are in place about the range of applicability and accuracy of continuum-level modeling of the cell behavior. In these models, the small but important Brownian motions of cytoskeleton due to thermal fluctuations are neglected. Moreover, the infinite number of time scales, a characteristic feature of power-law rheology of the cell response, is replaced with a limited number of time constants^[43]. Finally, although we have the freedom to incorporate inhomogeneous and isotropic properties for the representative continuum domain through selecting proper constitutive laws (i.e. force displacement relation), these models often cannot be related to and derived from the cell microstructure. Therefore, the continuum-level methods are highly specific to the experimental observations and usually cannot be generalized^[64].

In continuum-level models, the fibrous and discrete structure of the cytoskeleton is missing. In discrete models, a network of randomly cross-linked fibers is used to represent the cytoskeleton microstructure. A complete review of these models is given in [65, 97]. Since cytoskeletal filaments are heavily cross-linked on the scale of their thermal persistence length, they store energy in bending, twisting and stretching modes of deformation. Cytoskeleton is a semiflexible network whose behavior is non-affine. The models for flexible networks^[1] are unable to provide a thorough description for the mechanical behavior of semiflexible networks. Over past decades, extensive efforts have been devoted to developing a unified and general model to describe the elasticity and unique features of semiflexible gels such as large shear moduli, nonlinear response, and power-law scaling of the cytoskeletal rheology,^[66-72]. The cytoskeletal filaments resist bending and exhibit thermally induced fluctuations due to Brownian forces. A filamentous protein is therefore considered as an elastic rod with bending and stretching rigidities resulting from both elastic and entropic response. The langevin equation describes the hydrodynamics drag force of the filaments through the solvent.

Affine network models are effective in modeling the behavior of networks whose individual filaments only rotate and uniaxially deform under uniform loadings, i.e. the macroscopic strain distributes uniformly (affine deformation). These models are not valid where the behavior is non-affine and bending deformations of filaments become important^[66,67,70,73]. As discussed in reference [65], boundary value problem on dense random fiber networks can be solved using Stochastic Finite Element Method^[72].

As stated earlier, cytoskeleton is an active biological system responding to external stimuli through actin-myosin interactions which lead to formation of stress fibers. The interplay of multiple phenomena including myosin contraction, actin network elasticity, the internal and external constraints imposed on the cytoskeleton creates the stress distribution inside the cell. The plethora of actin binding proteins controls the organization (e.g., local microstructure and connectivity) of actin filaments. These filaments are as-

sembled and disassembled in response to various kinds of signals resulting from cell locomotion, cell division and extension^[74-76]. The mechanical stability of the cell is due to dynamic arranging and rearranging of cytoskeletal filaments via crosslinking molecules. For instance, Arp2/3 complex forms 70° branching networks, fimbrin and α -actinin put F-actins in parallel bundles, and filamin and spectrin form three-dimensional actin networks. Filamin and spectrin are protein complexes consisting of multiple actin-binding domains which arrange actin filaments into dense meshworks. Other types of ABPs are those which are used as a physical support or scaffold and are less directly involved in regulating actin structure. Myosins are a member of this category of ABPs.

There are over fifteen different types of myosins using actin filaments to move their specific cargo inside the cell. Myosins are molecular motors which produce movement through ATP hydrolysis and can generate forces on order of pico-Newtons inside the actin network or bundles. The mechanical properties and structure of cytoskeleton are greatly influenced by the contribution of forces generated by myosins. Thus, their effect in cell mechanics cannot be overlooked. The effect of motor molecules may be included in the filamentous network models for the cytoskeleton. In these models, internal forces exerted by myosin motor molecules induce a state of pre-stress condition inside the system. These networks, whose elasticity is controlled by myosin, are often called active networks and their behavior is different than that of passive networks. It has been shown experimentally that these active myosin stresses stiffen the network by two orders of magnitude^[77]; however, a mathematical and theoretic model of active network has not yet been developed (Fig.5.2).

The stress field in the cytoskeletal network depends on the contractile machinery called stress fibers. They are formed by the actin-myosin interactions and are characterized by repeating units of myosin proteins. The movement of myosin motors in the stress fibers causes actin filaments to contract and slide past one another. The dynamics of stress fibers has not yet been well understood and is currently under study. It is known that their elasticity is a function of myosin spacings and changes over time. Cellular functions such as wound healing, proliferation, shape stability, and apoptosis depend heavily on how the force is acted upon the cell. Forces transmit into and out of the cell cytoskeleton through these bundles at the basal surface, where it interacts with the surrounding extracellular matrix, and where cytoskeletal contractility is resisted. At these interaction sites (focal adhesion), stress fibers often form^[78,79]. They also form along the direction of external forces, e.g. it is found that stress fibers orient along the direction of fluid flow in endothelial cells under shear stress^[80,81]. The mechanical properties of stress fibers can be non-uniform due to the variations in myosin spacings along stress fiber length. Moreover, the direction and strength of stress fibers depend on stress field within the cell. Forces are transferred and propagated directly and in a band-like structure from the cytoskeleton stress fibers to the discrete sites on the nuclear envelop^[82,83]. As stress fibers enable the cell

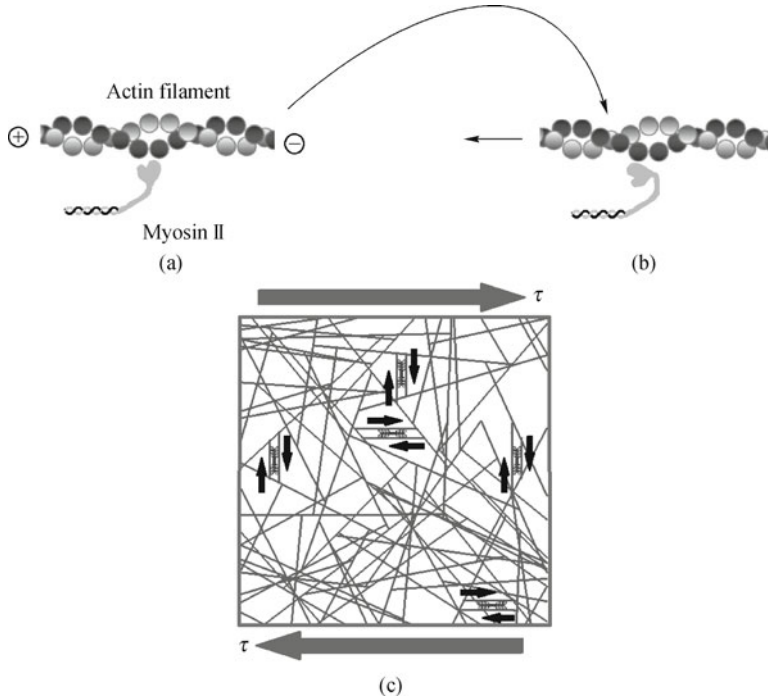


Fig. 5.2 Schematic of a bipolar myosin filament interacting with two actin filaments. The $\pm$ signs indicate the polarity of actin; myosin moves toward the plus end. (a) Myosin head attaches to the actin; high energy configuration. (b) The myosin head bends and pulls on the actin filament; power stroke. (c) Active networks subjected to external loadings. In active networks, myosin molecular motors control the network elasticity and induce a state of pre-stress inside the network (color plot in the book end).

to focus myosin contractility along a specific direction of resistance, the cell can stiffen directionally to protect itself against excess stress and strains in this particular direction. Moreover, the cell explores the matrix rigidity and migrates in the direction of increasing rigidity through stress fibers^[84]. Cell locomotion is believed to depend on and even be controlled by changes in substrate rigidity. Particularly, cell response on culture surfaces is dictated by substrate rigidity; actin stress fibers are oriented along the stiffest direction of the microfabricated substrates^[85].

In literature, stress fibers have often been simulated by continuum models. These models predict the diffusion of stress away from the points of matrix attachment, as opposed to the directed and focused stress propagation observed in experiments. A recent study, however, showed that in translating the discrete microscale actin-myosin interaction to the continuum scale, these models are missing the perpendicular component of the myosin force acting on the actin filaments^[83]. Inclusion of this correction in the model

recovers the directed and focused nature of stress fibers. Moreover, the stress fiber arrangements for simple cell-matrix attachments predicted by this model match those observed in experimental studies. It is noted that key aspects of the interactions are missed in coarse graining and in approximating discrete macromolecular interactions by a continuum model. Therefore, a multi-scale approach retaining the discrete nature of the macromolecular interactions and keeping material properties in the discrete macromolecular scale is required. Such a model requires taking into account the discrete nature of cytoskeleton as well as the effects of hydrodynamic interaction on the behavior of individual filaments^[8,9].

It has been shown that elastic modulus of stress fibers in living endothelial cells is approximately 10–15 kPa which remains constant over large strains up to 12%^[86]. This stiffness is a function of myosin II since disturbing its activity by adding myosin inhibitor blebbistatin causes a 30% loss of the modulus^[87]. The elastic modulus of fibroblast cells also decreases due to the application of this myosin inhibitor^[88]. This further confirms the importance of the tension generated by myosin to the overall cellular stiffness. Despite considerable experimental and computational efforts devoted to study basic physical principles of cell contractility due to myosin II motors, our understanding of this process is still limited. It is not yet clear how contractility and pattern formation changes with microscopic parameters such as number of myosin motors, number of cross-linkers and density of actin filaments. It is known that the motor activity inside the cell is a controlled process which results in the formation of stress fibers in cells on flat substrates and the contractile rings during cytokinesis^[89–91]. The contractile rings, which are composed of actin, myosin II, septins and GTP-binding hetero-oligomers, generate a furrow which partitions one cell into two. There have been some efforts to describe these rings using continuum-level hydrodynamics models whose accuracy has been confirmed by *in vitro* studies^[92,93]. In these models, the actin network is modeled as active viscoelastic polar gel which is forced out of its equilibrium state because of the ATP hydrolysis. These studies suggested that the assembly and disassembly of cytoskeletal structures can be tuned via varying the concentration of local myosin II. Furthermore, F-actin cross-linkers such as filamin A and α -actinin must be present for the contraction of F-actin networks induced by myosin II at physiological ATP concentrations^[94]. In other words, myosin cannot generate large forces if actin filaments are not cross-linked. The dependence of contractility on the number of cross-linkers and myosin motors per actin filament has been studied by constructing well-controlled model system of purified actin, myosin, and α -actinin^[95]. The results of recent theoretical studies show that the force generated by myosin is not sufficiently large for ring contraction during cytokinesis unless the actin filaments are heavily cross-linked^[96].

5.5 Conclusion

In this chapter, current experimental and computational methods are concisely reviewed to shed some light on the cytoskeletal rheology. Living cells exhibit physical properties similar to those of a liquid or a solid depending on properties of external stimuli; the transition between these two states is among unresolved problems of the field. Understanding and characterizing the underlying molecular events involved in the distinctive response of cells are among major goals of cell mechanics research. Despite tremendous progress in the cytoskeletal mechanics and rheology over the past decades, there is still a long way to go before developing a thorough understanding for the basis of cell individual and collective mechanical behavior. Recent advancement in nano-mechanics and imaging has brought a wealth of information about the molecular composition and properties of cytoskeletal constituents. Furthermore, the ever improving microrheometry-based experiments have expanded our understanding for the overall macroscopic behavior of cells. Nevertheless, the key question remaining to be answered is how these unique properties are emerged from cell generic microstructural features.

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Chapter 6 On the Application of Multiphasic Theories to the Problem of Cell-substrate Mechanical Interactions

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Abstract: Tissue functions and properties are determined by the interactions between cells and their extra-cellular matrix. Cells, which are the active component of tissues, can sense mechanical stimuli provided by their surrounding matrix and respond by generating mechanical forces. These forces may then influence the reorganization of the matrix and begin a sequence of cell/matrix crosstalks that feedback onto themselves. Our understanding of these interactions and their influence on the evolution of soft tissue is thus critical to understand many phenomena in biological tissues, such as wound healing, or tissue morphogenesis. Experimental observations tell us that cell shape and size are intrinsically linked to its function, properties and motion. For instance, cell spreading area is known to control the expression of mitogen agents, and thus cell division. Inversely, cell elongation and orientation are linked to the properties and deformation of the surrounding extracellular matrix. The origins of these behavior can be traced down to the multiphasic nature of cytoplasm and the rich spectrum of chemo-mechanical processes occurring within it, including stress-fiber polymerization, structural deformation and mass transport. Such multiphasic media have traditionally been very well described by mixture theory based on a continuum representation of interpenetrating phases and their interactions. The objective of this chapter is to introduce such a formulation of cell mechanics in order to capture the fundamental mechanisms of cell contractility and its interaction with an underlying elastic substrate. As such, the cell is considered as a mixture of four different phases including a passive cytoskeleton, a distribution of contractile stress-fibers, the cytosol and a population of globular actin monomers dissolved in the cytosol. After discussing the general formalism based on balance laws and constitutive relation, the chapter introduces a numerical strategy, based on the extended finite element, to capture the contraction of cells on compliant substrate. Model prediction and comparison with experiments are finally provided and discussed.

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Keywords: mechanotransduction, cell-matrix interaction, mixture theory, chemo-mechanical coupling, finite elements, levelset method

6.1 Introduction

Soft biological tissues are very particular types of materials that have the ability to change their structure, properties and chemistry in response to external cues. This fast response capability can be attributed to the out-of-equilibrium nature of the tissue structure, resulting from a constant crosstalk between a population of cells and their surrounding extra-cellular matrix (ECM). These interactions allow cells to sense stimuli conveyed by the ECM^[1] (such as force, deformation or flow) and the ECM to restructure due to the action of cells (characterized by traction forces^[2,3] or enzyme degradation^[4]). In other words, the properties and behavior of cells completely depend on their ECM, while the structure of ECM is the result of cell's activity. This type of feedback mechanism, commonly found in biological systems, is very likely to be at the origin of a lot of critical behavior in live tissue, including morphogenesis^[5-7], remodeling^[8] wound healing^[9] and angiogenesis and cancer dynamics^[10-12].

A critical aspect of cell mechanics is thus to understand how cells are able to sense their mechanical environment and react by deforming and applying forces to their surroundings. A number of studies have been performed to characterize cell contractility and force generation. Common strategies consist of using arrays of microneedles^[13] or microcontact printing^[14] in order to control the spatial distribution of adhesion islands. By constraining the regions where cells can attach to the substrate, these techniques effectively eliminate behavior such as spreading and motility in order to concentrate on the contractility and force generation. Using actin staining procedure, the structure of stress-fibers can be revealed and compared to the forces measured at focal adhesion. However, the complexity of phenomena involved in both passive and active mechanical behavior of cells makes it difficult to provide quantitative explanations based uniquely on experimental observations. The use of mathematical models is thus essential to validate experimental findings, make prediction that can guide experiments and more generally, provide a more complete understanding of certain observable phenomena.

Modeling of the passive response of the cytoskeleton has traditionally taken different routes according to the application in mind. One can generally find two types of models in the literature: structural models and continuum models. On the one hand, the strength of structural models resides in that they can accurately describe the forces in individual components of the cytoskeleton and their relation to the overall cell response. This feature has particularly been used with the tensegrity model^[15-18] to explain the relationship between cell rigidity and the level of pre-stress in the actin network. In other instances, continuum models may be powerful in explaining

cytoskeleton mechanics. On the other hand, continuum models have been used to describe the elastic and visco-elastic mechanical behavior of the cell under load. In this class of models, a promising approach consists of mixture (or multiphasic) models, which considers the cytoskeleton as a mixture of a solid skeleton, saturated in a solution of water and macromolecules. This approach has mainly been used to explain the change in cell volume due to osmotic loading and the cell's viscous behavior^[19,20].

Mathematical models of the cell's active behavior aim at understanding the evolution of cell substrate adhesion, cell contraction and the mechanisms of locomotion. In this context, cell contraction was coupled with adhesion and protrusion advance to describe the underlying mechanisms of locomotion^[21,22] and their dependence on substrate properties^[23]. More recent studies by Desphandes et al. introduced a bio-mechanical model that is able to describe cytoskeleton contraction by considering molecular mechanisms associated with stress-fiber formation and focal adhesion assembly. While not considering mass balance within the cytoskeleton and the combined effects of a fluid and solid phase, this approach provided a promising means of capturing the relationship between cell contractibility and stress architecture depending on substrate properties and applied forces.

The study of multiphase materials undergoing internal mass transport, mass exchange and deformation has been very well described by the theory of mixture^[24,25]. By considering the interactions between individual solid and fluid phases within the continuum theory, this approach provides an excellent platform onto which the various aspects of fibroblast contraction can be incorporated in a natural fashion. This chapter presents an approach that views the cell as a mixture of a solid cytoskeleton (in which stress-fibers may be formed from actin polymerization) and freely moving actin m-monomers dissolved in an interstitial fluid, the cytosol. This approach has the following attraction: it satisfies the basic balance equations of a continuum (mass, momentum, energy), it allows for mass transfer between constituents through kinetic equations, that display a dependency on mechanical stress and deformation, and it incorporates attractive features from both filopodia lamellipodia and cell contractibility models, which makes this model attractive for future studies of cell spreading involving both protrusion growth and stress-fiber formation.

The chapter is organized as follows. First, we provide a description of the chemo-mechanical processes occurring during cell spreading and the associated change in the cytoskeleton and force generation. The advantages of a continuum description is then discussed to characterize these processes. Section 6.3 introduces the details of the proposed model, including mass balance, momentum balance and associated constitutive relations. In Section 6.4, the behavior and prediction of the model are investigated with the help of a finite element framework. Section 6.5 finally provides a discussion of the model and proceeds with recommendation for future improvements.

6.2 The physics of contractile fibroblasts and their interactions with an elastic substrate

Functions and health of biological tissues rely on the finely tuned architecture and shape of populations of cells and their surrounding extra-cellular matrix. This organization largely depends on many internal characteristics, including ECM and cell properties, deformation and orientations, as well as external factors such as the existence of external loads and their variation in time. Any change in cell behavior (due to disease, for instance), ECM properties (from aging or injuries) or external forces affects the balance of tissue. This may have large consequences on the tissue, which includes remodeling, reorganization, change in cell phenotype, angiogenesis or apoptosis. For this reason, a better understanding of cell-ECM interactions is the object of intense research activities in the bio-medical field. An increasing body of evidence now points out that besides bio-chemical interactions, cell-ECM communication is largely driven by mechanical processes. Cells are able to apply contractile forces to their surroundings, a behavior that is often associated with cell spreading and contractibility, two phenomena that may be studied by observing the behavior of fibroblasts on elastic substrates.

6.2.1 Cell spreading, contractility and substrate elasticity

The dynamical behavior of fibroblasts and their ability to attach and deform their surrounding matrix by applying mechanical tension has been recognized since the work of Bell^[26] and Harris et al.^[3,6,7] three decades ago. In fact, the role of mechanics in cell behavior was recognized as early as 1952 by Weiss and Garber^[27], where the nature and architecture of a filamentous substrate were shown to determine the morphological features of mesenchyme cells. More recent studies have further shown that cell contraction is dependent on the state of macroscopic stress and effective stiffness of the ECM. This can particularly be seen during the phenomenon of tissue compaction, which is described by very large contractile strains in collagen gels, few hours after encapsulating a population of fibroblasts in a collagen gel.^[28-30] A particularity of this process is that the level of tension developed by cells is dependent on gel's resistance to deformation. If the gel is free floating, small traction is applied, while if the gel is constrained and unable to deform, cells exert more significant traction. Local states of stress and strains are also known to have an influence on cell shape. For instance, Baxter et al.^[31] showed that the morphology of cardiac fibroblasts embedded in a collagen gels greatly differed according to their locations in the gel. In regions of oriented tensile stresses (near constrained boundaries), fibroblast displayed highly orientated and elongated shapes while they took stellate shapes in the center of the gels, where the state of stress was isotropic. These differences have been linked to

a cell's perception of apparent stiffness of the surrounding gel. It was also shown that as the effective stiffness of gels increases, fibroblast morphology evolves from dendritic to stellate and these changes are associated with the development of mature adhesion between matrix and cells^[3]. However, below a certain stiffness threshold, these phenomena are not observed and fibroblasts remain in their original round configuration.

While observations of cells in gels are very educational, they are often limited in terms of quantitative measurement due to the difficulties associated with three-dimensional complex geometries and access to individual embedded cell. Alternative techniques thus consist in studying the evolution of cells adhering to two-dimensional elastic substrates. Generally, those procedures have shown similar trends as those observed in three-dimensional gel, with the difference that there is no ECM remodeling. Recently, experimental procedures based on microcontact printing^[5] and beds of microneedles^[13] have shown promising ways to quantify shape and contractile forces in various conditions. Particularly, tractional forces, cell spreading area, stress-fiber and focal adhesion assembly, as well as substrate deformation can readily be measured. A brief summary of the observed behavior of fibroblasts on compliant substrate can be given as follows:

(1) Fibroblast spreading area, rigidity and traction forces increase with increasing substrate rigidity^[32]. They reach a maximal value after which they remain independent of substrate stiffness^[33].

(2) Fibroblast morphology depends on the elastic properties of their underlying substrate and tend to align in directions of maximal stiffness.

(3) Fibroblast react to the local deformation of the substrate^[34]. Since individual cells exert traction force and deform their surrounding substrate, deformation can be used as an effective means of communicating between cells^[35]. Observations show that cells attract one another on soft substrate while they tend to repulse one another on stiff substrates. The mechanical properties of the substrate therefore have a large effect on final tissue organization^[36].

(4) Fibroblasts migrate from low to high substrate stiffness and are not able to pass through an interface transitioning from high to low stiffness^[34]. Note that this observation is true for fibroblast but can greatly vary with different kinds of cells.

(5) Cell-substrate adhesion becomes more stable with rigidity, while soft substrates lead to unstable, highly dynamic adhesion. As a result, motility decreases with increasing substrate stiffness^[37].

(6) Cell growth and apoptosis are regulated by the rigidity of the underlying substrate. Healthy cells on soft substrates exhibit a higher rate of apoptosis, while this is not necessarily observed for transformed cells. This lack of regulation for transformed cells provides a clue on the uncontrolled growth rate of tumor cells, regardless of medium rigidity^[38].

These observations clearly emphasize the fact that fibroblast's behavior is highly dependent on mechano-sensitive processes originating from their inter-

actions with the substrate. Particularly, the above observations clearly show that mechanical properties and substrate structure are keys to cell spreading and contractility. In order to provide a more fundamental overview of the physics at play during cell contraction, we next provide a short discussion of the molecular mechanisms at the cellular level.

6.2.2 Molecular mechanisms of cell contractility

The cytoskeleton distinguishes itself by its highly dynamic behavior, allowing its architecture, contractile forces and shape to change rapidly in response to external stimuli. The communication of a cell with its surroundings is made possible through a population of free moving membrane proteins—the integrins—that provide a physical bridge between the extracellular matrix (or substrate) and the cytoskeleton. These complex integrins, also known as focal adhesion, revert two main functions: (a) they provide an anchor for the stiff mechanical environment in order to stabilize the cell and (b) they are the stage for cell-ECM crosstalks through their mechano-sensing capabilities. In other words, depending on the input they receive from the environment (ECM effective stiffness, force, etc), integrins may start a cascade of signaling activities that eventually result in cytoskeleton remodeling, cell contractility, spreading or migration. The fast response of cells is then possible thanks to its multi-phasic structure, in which mass transport, mass transfer and structural reorganization are facilitated. On the one hand, the solid skeleton (or cytoskeleton) is comprised of an intricate network of interconnected filaments (which include actin filaments, microtubules and intermediate filaments), that are able to assemble and dissociate during deformation. In addition, the capacity of certain filaments (such as stress-fibers) to undergo contraction also enable the cytoskeleton to quickly change its mechanical stiffness. The underlying mechanisms of these phenomena have been studied in the context of tensegrity structures^[15–18]. On the other hand, an interstitial fluid phase (or cytosol) contains a rich solution of ions and macromolecules that can readily diffuse and react with the solid cytoskeleton. Exchanges of mass between fluid and solid phases are keys to structural reorganization and adaptivity as described below.

In that context, cell contractility is associated with a significant evolution of cytoskeleton architecture. Particularly, contractility is associated with the appearance of a network of highly organized actin filaments that can span the entire length of the cell. This structure can be easily observed under a confocal microscope after using actin staining procedures on the cell as shown in Fig.6.1. These filaments, known as *stress-fibers*, are distinguished by two specific features: (a) they are usually aligned along specific directions and (b) they are capable of applying tensile force along the direction of their axes. Evidence of this can be observed during experiments of cell contractility on

a bed of microneedles that clearly show a correlation between the structure and orientation of stress-fibers and the force the cell applies on underlying needles. The origins of stress-fiber contraction can be explained in terms of interactions between two important cytoskeletal proteins: actin filaments and myosin II motor proteins.

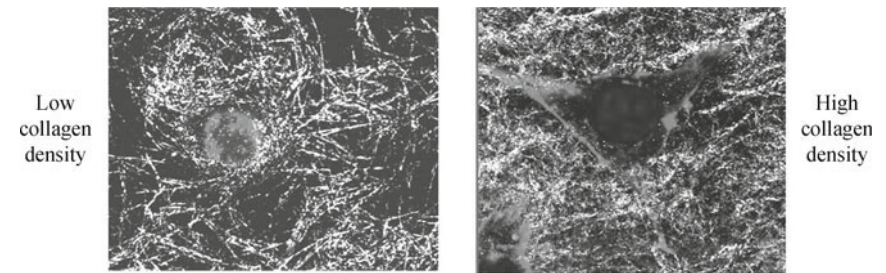


Fig. 6.1 NIH 3T3 fibroblast in a collagen network. For low density collagen, the network is too soft for the fibroblast to spread in exerting force. However, when the collagen concentration is increased, the rise in stiffness results in fibroblast spreading. Well-developed stress-fibers can be observed (red lines, color plot in the book end).

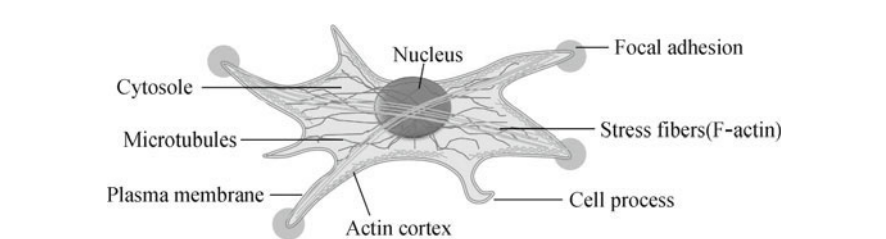


Fig. 6.2 Schematic of a contractile fibroblast.

6.2.2.1 Actin filaments structure and assembly

Actin filaments are comprised of two intertwining protofilaments and are characterized by a strong polarity as well as “barbed” and “pointed” ends. This feature plays an important role in contraction as explained in the next section. Stress-fibers are of very dynamic structures that can assemble and disassemble from a pool of globular actin proteins (known as G-actin) dissolved in the cytosol. It is very likely that the assembly of globular actin into stress-fibers heavily dependent on the presence of mechanical forces within the cytoskeleton. Indeed, experimental observations have shown the clear relationship between force generation and stress-fibers appearance on cells plated on nano-rods. However, upon force release, one could observe a clear dissociation of stress-fibers.

6.2.2.2 Myosin and cross-bridge dynamics

Similarly to actin proteins, myosin II can assemble into bipolar filaments and can bind to actin filaments at myosin binding sites. Upon binding, myosin proteins have the ability to travel toward the barbed end of actin filament, through a cycle of binding, ratcheting and release, powered by ATP. This mechanism, known as the acto-myosin cross-bridge, creates a relative motion between the two filaments and is at the origin of cell contraction. Indeed, the manifestation of cross-bridge dynamics into macroscopic cell contraction can be understood by looking at the architecture of stress-fibers, depicted in Fig. 6.3^[39]. At the molecular level, stress-fibers are comprised of five to twenty actin filaments bound together by cross-linking proteins (including α -actinin) and found in a sarcomeric-like structure. Each sarcomere is a symmetric structure that contains two oppositely oriented actin filaments whose barbed ends points away from the sarcomere's center. This structure enables myosin mini-filaments to bind to actin and generate sarcomere contraction by moving toward opposite ends through cross-bridge dynamics. The mechanism is then amplified by the combined action of the large number of sarcomeres in each stress-fiber (in series) in the cytoskeleton, in order to generate overall cell contraction.

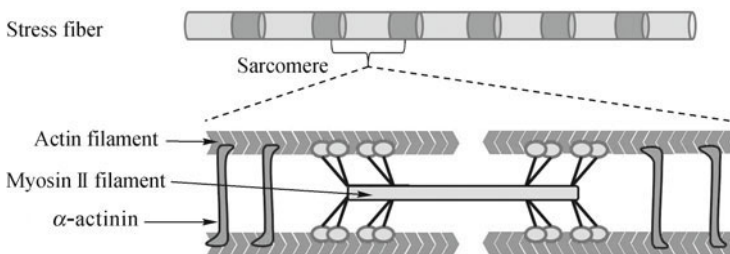


Fig. 6.3 Sarcomeric structure of a stress-fiber. A sarcomere is depicted as a symmetric structure composed of actin filaments, linked by α -actinin and put in tension by a central Myosin II filament.

6.2.2.3 Empirical model of stress-fiber contraction

Due to the strong analogy between stress-fibers and muscle contraction, it is very likely that stress-fibers follow a similar relationship between force and rate of deformation, as described by Hill^[40] in his seminal work on muscle dynamics. In a nutshell, the model describes the nonlinear increase of contractile forces generated by acto-myosin complex with the rate of axial lengthening of the fiber (Fig. 6.4). Particularly, the model predicts a total disappearance of contraction below a critical amount of fiber shortening, while the force converges to a maximum value for large rates of extension. In addition, when the rate of deformation vanishes, fiber contraction, a so-called state of *isometric tension*, is observed. The consequences of these phenomena on cell contractility are important; indeed considering a contractile cell adhering to

an elastic substrate, active contraction may lead to significant shortening (for soft substrate) or negligible deformation (for stiff substrate) of stress-fibers according to substrate stiffness. The Hill model therefore predicts that contractility decreases with substrate stiffness, which illustrates the dependency of cell contraction on its mechanical environment.

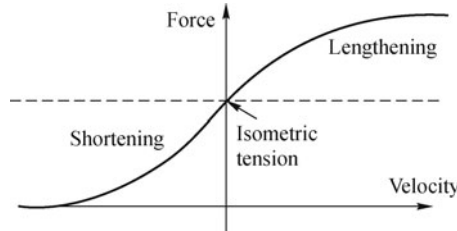


Fig. 6.4 Fiber shortening-fiber contraction relation according to the Hill model.

6.3 Multiphasic mixture theory and cell contractility

The evolution of contractility and associated morphological and structural changes are driven by the interactions between mechanical and chemical forces occurring in the cytoplasm. On the one hand, mechanical force must satisfy internal equilibrium (cytoplasm forces) and external equilibrium (balance of force between cell and substrate). On the other hand, chemical equilibrium (driving stress-fiber assembly and disassembly) must be verified in the cytoplasm. While the steady-state of a cell may be determined by considering these equilibrium considerations, the transient dynamics are dominated by rate dependent processes such as G-actin monomers diffusion, cytosol flow through the cytoskeleton and kinetic of stress-fibers formation. The objective of this chapter is to introduce the reader to a class of multiphysics continuum formulations that can be used to capture the above physics.

In this perspective, we take a view of the cell as a multiphasic structure, in which a multitude of individual constituents (e.g. actin filaments, microtubules, interstitial fluid, dissolved ions) constantly interact. Such media have traditionally been described by the theory of mixtures^[24,25] for which a material point (or particle) is representative of all constituents present in the mixture, weighted by their respective volume fractions. A number of formulations have been proposed to describe the behavior of a variety of materials including soils, charged hydrated tissues^[41], or hydrogels^[4]. Its application to the mechanics of cells has until now been limited to biphasic models (fluid-solid mixture) and triphasic (solid-fluid-ions) formulations^[20] in order to describe the flow dependent mechanical response and swelling-shrinking behavior of cells in response to their osmotic environment. The present section presents an alternative approach that has been developed to describe

the chemo-mechanical processes responsible for cell contraction.

6.3.1 The cytoplasm as a quadriphasic medium

In the light of the molecular mechanisms presented in Section 6.2, the cytoplasm of a contractile cell is assumed to be a mixture of four different constituents (Fig.6.5):

(1) A passive cytoskeleton, that consists of a filamentous network of microtubules, actin and intermediate filaments. This structure is highly porous and permeable, which facilitates the transport of interstitial fluid and macromolecules within the cell.

(2) The cytosol is the intracellular fluid that fills the porous space in the cytoplasm. This liquid is composed of a complex mixture of dissolved ions and macromolecules that play a role in maintaining the cell's metabolism. For the low pressure range a cell is subjected to, the cytosol may be considered as an incompressible fluid.

(3) Globular actin (G-actin) monomers are dissolved in the cytosol and may polymerize to form actin filaments. Particularly, the actin filament can combine with Myosin II motors to form stress-fibers.

(4) A network of stress-fibers, that are able to polymerize from G-actin under certain circumstances. As described in the previous paragraph, stress-fibers are able to produce a contractile stress along the filaments through cross-bridge dynamics.

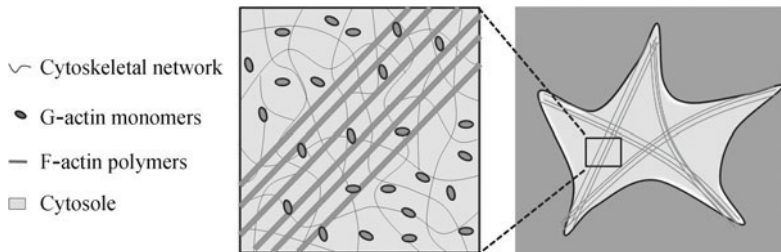


Fig. 6.5 In the cytoplasm, a material point is seen as a mixture of four phases, namely, the passive cytoskeletal network, the contractile stress-fibers, the cytosol and dissolved G-actin monomers.

While many more constituents exist in the cytoplasm, the above four phases are critical components of the physical mechanisms responsible for cell contractility. Indicating constituents by index α , we denote $\alpha = s$ for solid, $\alpha = f$ for fluid, $\alpha = p$ for filamentous actin polymers, and $\alpha = m$ for dissolved G-actin monomers. The respective volume fraction (in the current frame) of each constituent at a material point may be given in terms of a scalar quantity ϕ^α . Using the fact that the cytoplasm is saturated with the

four constituents, their volume fractions must satisfy:

$$\sum_{\alpha=1}^4 \phi^\alpha = \phi^s + \phi^f + \phi^m + \phi^p = 1 \quad (6.1)$$

where the volume fraction of each constituent may change during cell deformation as a result of volumetric deformation or mass exchange between constituents.

6.3.1.1 Continuum characterization of oriented stress-fibers

In general, the structure of stress-fibers is characterized by a strongly anisotropic distribution, in which fibers take root in regions of cell-substrate attachment and may span the entire length of the cell. In addition, the stress-fiber network is a highly dynamic structure, whose architecture can change in the lap of few seconds if mechanical forces act on the cell. The continuum characterization of such evolving anisotropic media has mainly been introduced in the context of biological tissues undergoing remodeling. In these approaches, the bridge between the structural scale (level of individual fibers) and the continuum scale (level of fiber assemblies) was established in terms of a statistical description of fiber orientations in small, elementary volume of materials. This leads to the introduction of the so-called *structure's tensor*, providing a homogenized representation of the orientation distribution of the fibrous network at continuum points. This representation could then be used to model the evolution of the fiber assemblies in terms of evolution laws relating the time derivative of the structure tensor to physical quantities such as states of stress and strain. The method has so far been successful to describe the evolution of anisotropy in soft tissue subjected to large deformation and remodeling^[42,43]. Its application to the continuum modeling of the formation and evolution of stress-fibers is given below.

Concentrating on the case of a two-dimensional planar fibrous assembly, the starting point of the method consists of isolating a representative volume element (RVE) located at point $\mathbf{X}$ and observing the fiber distribution within this domain. Assuming that the size of the RVE is small compared to the radii of curvature of fibers, each fiber can be considered as a straight line whose direction is given by a tangent unit direction vector $\mathbf{a}$ such as $\mathbf{a} = [\cos \theta \ \sin \theta]^T$ (where θ is the direction angle). A distribution ϕ_θ^p of fiber orientations is thus introduced as the volume fraction of fibers whose direction angle is comprised of between $\theta - \delta\theta$ and $\theta + \delta\theta$ where $\delta\theta$ is a small angular variation. This leads to the definition of the structure's tensor Φ^p ^[42] as an average measure of fiber distribution as follows:

$$\Phi^p = \langle\langle \phi_\theta^p \rangle\rangle \quad \text{where} \quad \langle\langle \bullet \rangle\rangle = \frac{1}{\pi} \int_{-\pi/2}^{\pi/2} \bullet \cdot \mathbf{a} \otimes \mathbf{a} d\theta \quad (6.2)$$

where $\langle\langle \bullet \rangle\rangle$ is defined as the directional average of the orientation density ϕ_θ^p and $\otimes$ denotes the vector multiplication defined as $(\mathbf{a} \otimes \mathbf{a})_{ij} = a_i a_j$. Note that

the above operation represents an average of fiber distribution, and as such, one should keep in mind that there are many different fiber distributions corresponding to the same tensor Φ^p . In addition, due to its very definition, the structure tensor can be shown to be symmetric and is related to the total volume fraction ϕ^p of stress-fibers at a continuum point by:

$$\phi^p = \frac{1}{\pi} \int_{-\pi/2}^{\pi/2} \phi^p d\theta = \text{trace}(\Phi^p). \quad (6.3)$$

Furthermore, in case of an isotropic fiber distribution (e.g. ϕ_θ^p is independent of θ), the structure tensor becomes a diagonal matrix of the form $\Phi^p = (\phi^p/2)\mathbf{I}$, where $\mathbf{I}$ is the identity tensor. It is particularly useful to realize that due to its symmetry, Φ^p may be represented in terms of three independent variables for a two-dimensional fiber distribution. A physically sound set of parameters is given by $\{\phi^p, \eta, \theta_0\}$, where η refers to the degree of anisotropy and θ_0 shows the principal direction of stress-fibers. The structure tensor may thus be reconstructed as follows:

$$\Phi^p = \phi^p[\eta\mathbf{I} + (1 - 2\eta)\mathbf{a}_0 \otimes \mathbf{a}_0] \quad (6.4)$$

where $\mathbf{a}_0 = [\cos \theta_0 \quad \sin \theta_0]^T$. It can be seen that if $\eta = 0$, all fibers are aligned in the same direction (defined by $\mathbf{a}_0$), whereas $\eta = 1/2$ for an isotropic distribution of stress-fibers (Fig.6.6).

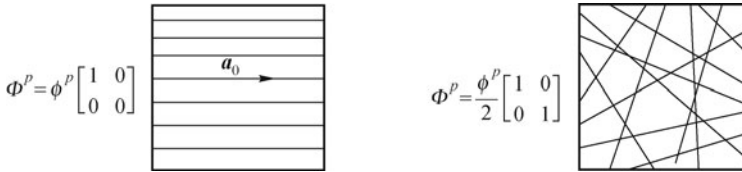


Fig. 6.6 Illustration of the distribution of stress-fiber orientations. The concentration ϕ_θ^p of stress-fibers in direction θ is interpreted by the ratio of the number of moles of F-actin in the shaded area (left figure) and the volume of the area. A simple anisotropic distribution is represented by the function depicted in the right figure.

6.3.1.2 Kinematics of cell deformation

To provide a continuum description of cellular deformation, it is necessary to introduce the various measures of motion, associated with each phase of the cytoplasm. For this, it is necessary to take a consistent measure of cell deformation. Referring to experimental methods, cell deformation is generally measured by the motion of its passive cytoskeleton (or non-evolving solid phase). A mathematical measure of cytoskeleton motion can then be introduced by considering a small volume element of the cell (comprised of four phases) in its reference and current configuration as depicted in Fig.6.7. Denoting $\mathbf{x}$ and $\mathbf{X}$ as the coordinates of the mass center of this volume (in

a Cartesian coordinate system) in the reference and current configuration, respectively, the displacement $\mathbf{u}$ and velocity $\mathbf{v}$ of this point are defined by the following vectors:

$$\mathbf{u} = \mathbf{x} - \mathbf{X} \quad \text{and} \quad \mathbf{v} = \frac{D\mathbf{u}}{Dt} \quad (6.5)$$

where the velocity is introduced in terms of the material time derivative with respect to the cytoskeleton motion. Following concepts of continuum mechanics, an appropriate measure of deformation is then given by the Green-Lagrange strain tensor:

$$\mathbf{E} = \frac{1}{2}(\mathbf{F}^T \cdot \mathbf{F} - \mathbf{I})$$

defined in terms of the deformation gradient $\mathbf{F} = \partial \mathbf{x} / \partial \mathbf{X}$ and the second order identity tensor $\mathbf{I}$. Let us now concentrate on the motion of other phases. Adopting an Eulerian point of view, the motion of stress-fibers, cytosol and G-actin monomers can be characterized by their respective velocities $\mathbf{v}^p$, $\mathbf{v}^f$ and $\mathbf{v}^m$ at material points located in $\mathbf{x}$ (Fig.6.7). Consistent with the theory of mixtures, it is convenient to describe the motion of fluid phases by their relative flux, with respect to cytoskeleton motion as:

$$\mathbf{J}^f = \phi^f (\mathbf{v}^f - \mathbf{v}), \quad (6.6)$$

$$\mathbf{J}^m = \phi^m (\mathbf{v}^m - \mathbf{v}). \quad (6.7)$$

In addition, assuming that stress-fibers are fixed to the passive cytoskeleton, we obtain $\mathbf{v}^p = \mathbf{v}$.

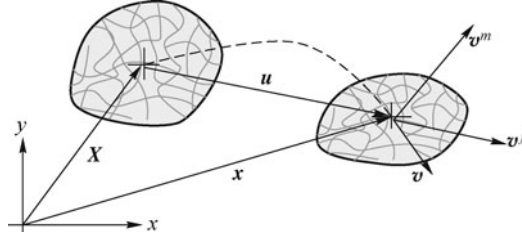


Fig. 6.7 Kinematics of cell motion.

6.3.2 Mass transport and mass exchange within the cell

As described previously, cell contractility relies on the mass exchange through the polymerization of G-actin monomers into actin filaments, that are a key structural component of stress-fibers. These reactions are possible through the transport of G-actin monomers to the site of reaction, a process that depends on the combination of cytosol flow and G-actin diffusion. This section discusses the mathematical description of these phenomena.

6.3.2.1 General mass conservation

To accurately describe mass transport and mass transfer, it is essential to ensure that mass is conserved throughout the process. For this, let us introduce two measures of mass densities for each phase: a real mass density ρ_R^α that measures the mass per unit volume of each constituent, taken independently from the mixture, and an effective density ρ^α that represents the mass of the α th constituent per unit mass of mixture: $\rho^\alpha = \phi^\alpha \rho_R^\alpha$. These definitions imply that the density ρ of the mixture is simply the sum of the effective densities of each constituent $\rho = \sum_{\alpha=1}^4 \rho^\alpha$. In the presence of mass exchange between constituents (due to the polymerization of G-actin monomer into stress-fibers), the local conservation of mass for each constituent is written as:

$$\frac{\partial \rho^\alpha}{\partial t} + \nabla \cdot (\rho^\alpha \mathbf{v}^\alpha) = \rho_R^\alpha \Gamma^\alpha \quad (6.8)$$

where $\mathbf{v}^\alpha$ is the velocity of the α th component and ∇ is the spatial differential operator in the current configuration. Furthermore, mass exchange between constituents is characterized by the quantity Γ^α , representing the rate of volume change of the α th component per current volume. When dealing with anisotropic stress-fiber formation, it is of particular interest to quantify this mass exchange along specific directions. This can be done by considering the orientation density distribution, represented by the tensor $\rho_r^p \Phi^p$, for which one can show that the balance of mass reads:

$$\frac{\partial \rho_r^p \Phi^p}{\partial t} + \nabla \cdot (\rho_r^p \Phi^p \mathbf{v}^\alpha) = \rho_R^\alpha \mathbf{\Gamma}^p \quad (6.9)$$

where the quantity $\mathbf{\Gamma}^p$ represents a source term for the anisotropic stress-fibers. The properties of the directional averaging operation introduced in Eq.(6.2) also imply that the total change in stress-fiber mass at a continuum point is given by the trace of $\mathbf{\Gamma}^p$. Further considering the mass conservation of the mixture,

$$\sum_{\alpha} \rho_R^\alpha \Gamma^\alpha = 0 \quad (6.10)$$

and using the fact that no mass exchange occurs for the passive cytoskeleton and cytosol ($\Gamma^s = \Gamma^f = 0$), the rate of G-actin production (or consumption) is related to $\mathbf{\Gamma}^p$

$$\Gamma^m = -\Gamma^p = -\text{tr}(\mathbf{\Gamma}^p) \quad (6.11)$$

where it is implicitly assumed that actin density is the same in its monomer or polymer form ($\rho_r^p = \rho_r^m = \rho^a$). In summary, for incompressible constituents, the mass balance for each phase is written as:

$$\text{Passive cytoskeleton} \quad \frac{D\phi^s}{Dt} + \phi^s \nabla \cdot \mathbf{v} = 0, \quad (6.12)$$

$$\text{Cytosol (fluid)} \quad \frac{D\phi^f}{Dt} + \nabla \cdot \mathbf{J}^f + \phi^f \nabla \cdot \mathbf{v} = 0, \quad (6.13)$$

$$\text{G-actin monomers} \quad \frac{D\phi^m}{Dt} + \nabla \cdot \mathbf{J}^m + \phi^m \nabla \cdot \mathbf{v} = -\text{tr}(\mathbf{\Gamma}^p), \quad (6.14)$$

$$\text{Stress-fibers} \quad \frac{D\Phi^p}{Dt} + \Phi^p \nabla \cdot \mathbf{v} = \mathbf{\Gamma}^p. \quad (6.15)$$

Note that the mass balance of stress-fibers is a tensor equation that describes the anisotropic evolution.

6.3.2.2 Stress-fiber formation

Let us now focus on the continuum modeling of stress-fibers formation, represented by the tensor $\mathbf{\Gamma}^p$. As stated in Section 6.2, the process of stress-fiber polymerization and dissociation is anisotropic and largely depends on the level of contractile stress in existing stress-fibers. We define G-actin/F-actin reaction by characterizing the rate of stress-fiber polymerization in the direction θ by a scalar quantity Γ_θ^p . To obtain Γ_θ^p , the following assumptions can then be made: (a) the chemical reaction between G-actin and F-actin (stress-fibers) is described by a first-order kinetic equation and (b) the rate of stress-fiber formation is affected by the magnitude of contractile stress T_θ^p in direction θ . This yields:

$$\Gamma_\theta^p = \frac{M^a}{\rho^a} (k^f c^m - k^d c_\theta^p) \quad \text{where} \quad k^f = k^f(T_\theta^p) \quad (6.16)$$

where M^a and ρ^a are the molar mass and density of actin, respectively, and k^f and k^d are the rates of formation and dissociation of F-actin, respectively. The concentration of actin filaments in the θ direction is c_θ^p and c^m is the concentration of G-actin monomers in the cytosol. Their relationship to volume fractions are

$$c^p = \frac{\rho^a}{M^a} \phi^p \quad \text{and} \quad c^m = \frac{\rho^a}{M^a} \frac{\phi^m}{\phi^f + \phi^m} \approx \frac{\rho^a}{M^a} \frac{\phi^m}{\phi^f} \quad (6.17)$$

where we assume that the volume fraction of G-actin is negligible compared to that of the cytosol, an assumption that is valid for dilute solutions. We note that the concentration of F-actin is taken as the number of moles per unit volume of mixture while the concentration of G-actin is the number of moles of G-actin per unit cytosol-G-actin mixture volume. To characterize the mechano-sensitivity of stress-fiber assembly, a rise in stress-fiber polymerization with contractile stress T_θ^{iso} , a linear approximation can be used for the function $k^f(T_\theta^p)$:

$$k^f(T_\theta^p) = k_0^f + k_1^f T_\theta^p. \quad (6.18)$$

Here, the constant $k_0^f > 0$ denotes the rate of F-actin formation in the absence of contractile stress and $k_1^f > 0$ is a measure of the acceleration of

polymerization rate in the presence of contraction T_θ^F . On the analogy of the structural tensor, the rate of stress-fiber formation in different directions can be expressed by a tensorial quantity $\mathbf{I} = \langle\langle I_\theta \rangle\rangle$ resulting from the directional average defined in Eq.(6.2). With the help of Eq.(6.16) and Eq.(6.18), it can be shown that the anisotropic stress-fiber formation is described by the following tensorial equation:

$$\mathbf{I} = \left(\frac{1}{2} k_0^f \mathbf{I} + k_1^f \mathbf{T}^p \right) \frac{\phi^m}{\phi^f} - k_0^d \Phi^p. \quad (6.19)$$

The above equation, together with Eq.(6.15), finally leads to the total knowledge of the evolution of the concentration and structure of stress-fibers represented by the tensor Φ^p . Indeed, the material time derivative of Φ^p is determined by:

$$\frac{D\Phi^p}{Dt} = \underbrace{\left(\frac{1}{2} k_0^f \mathbf{I} + k_1^f \mathbf{T}^p \right) \frac{\phi^m}{\phi^f}}_{\text{Force dependent formation}} - \underbrace{k_0^d \Phi^p}_{\text{Dissociation}} - \underbrace{\Phi^p \nabla \cdot \mathbf{v}}_{\text{Volume change}}. \quad (6.20)$$

This expression captures important processes of cell contractility. Particularly, the formation of stress-fibers in various directions depends on the availability of actin monomers (through ϕ^m), the concentration of existing stress-fibers (through Φ^p) and the level of contractile force (through $\mathbf{T}^p$). Finally, the last term in Eq.(6.20) characterizes the change in fiber concentration with volumetric deformation of the cell (through the term $\nabla \cdot \mathbf{v}$).

6.3.2.3 Mass transport through the cytoskeleton

Mass transport within the cytoplasm is an important player in both the passive mechanical response of cells and the dynamic of cell spreading and contraction. For instance, mechanical testing procedures, such as micropipette aspiration, clearly exhibit a time-dependency that is usually attributed to a combination of the intrinsic visco-elasticity of the cytoskeleton and the flow-driven deformation of the cytoplasm. In terms of cell morphology, it is likely that cytosol flow governs the number and size of cell protrusions. Indeed, Weiss^[27], in his analysis of the shape of mesenchymal cells, explained the development of cell extensions (or filopodia) in terms of competitive mechanisms based on the amount of cytosol each protrusion could take in.

To capture the behavior of flow and diffusion of species within the cytoskeleton, constitutive relations have to be introduced for cytosol and monomer transport. To keep the analysis simple, one makes a few realistic assumptions. The present model is based on the fact that the concentration of G-actin monomers in the cytosol is significantly small. In other words, it is acceptable to assume that G-actin monomer motion does not significantly affect that of the cytosol, and thus cytosol flux is independent of cytosol flow. Furthermore, considering that the volume fraction of cytoskeleton is high and can be considered as a porous and permeable medium in which fluid motion

is relatively small, cytosol flow can be expressed in terms of a differential of pressure through the well-known Darcy's law as follows:

$$\mathbf{J}^c = -\frac{\mathbf{K}}{\mu} \cdot \nabla p \quad (6.21)$$

where $\mathbf{K}$ is the permeability tensor that depends on the porosity and anisotropy of the cytoskeleton and μ is the viscosity of the cytosol. Globular actin transport then arises from two distinct sources: (a) convection with the cytosol and (b) diffusion through the cytosol. Assuming that the size of monomer is negligible compared to the characteristic mesh size of the cytoskeleton network, we may neglect the influence of cytoskeleton resistance on monomer flow and obtain the following relationship between the flux $\mathbf{J}^G$ and the spatial gradients of cytosol pressures and G-actin concentration:

$$\mathbf{J}^G = -c^G \frac{\mathbf{K}}{\mu} \cdot \nabla p - \phi^c D \nabla c^G \quad (6.22)$$

where D is the diffusion constant of G-actin monomers in the cytosol. Finally, considering an impermeable cell membrane, one must state that the flux of cytosol and G-actin across the membrane are null. This typically appears in terms of boundary conditions on the cell surface Γ as follows:

$$\mathbf{J}^c \cdot \mathbf{n} = 0 \quad \text{and} \quad \mathbf{J}^G \cdot \mathbf{n} = 0 \quad \text{on} \quad \Gamma \quad (6.23)$$

where $\mathbf{n}$ is the outward, unit normal vector to the surface Γ . The above equations show that cytosol viscosity and diffusivity of G-actin play a role in controlling the rate of cell contractility. Indeed, for high values of D (and small values of μ), G-actin monomers can rapidly diffuse to the site of stress-fiber polymerization and increase the speed of contractility. However, if D is small (or μ is high), G-actin diffusion slows down the process by limiting the amount of monomers on the site of polymerization.

6.3.3 Contractility and force balance

Let us now present a model for the second key component of cell contraction: force generation and cytoskeleton deformation. As mentioned earlier, active contraction originates from cross-bridge dynamics at the level of stress fibers. This creates a state of stress that results in cytoskeleton deformation and its resistance through the passive elasticity of the cytoskeleton and the pressure in the cytosol. These processes may readily be incorporated in the multiphasic continuum model presented in this chapter.

6.3.3.1 Balance of force in the cytoplasm

To characterize the material's deformation, we consider the equilibrium of forces (or balance of linear momentum) at any physical point $\mathbf{x}$ that belongs to the material domain. For a multiphasic medium such as the cell, let

us first consider the force equilibrium in each phase (cytoskeleton, cytosol, stress-fibers and G-actin monomers). To do this, it is important to note that due to the slow time scales associated with cell motion, the inertial force in each constituent may be neglected; this means that a quasi-static version of momentum balance can be developed for each phase. Introducing $\mathbf{T}^\alpha$ as the partial Cauchy stress associated with constituent α , the momentum balance can be written as:

$$\nabla \cdot \mathbf{T}^\alpha + \rho^\alpha \mathbf{b} + \mathbf{f}^\alpha = \mathbf{0} \quad (6.24)$$

where $\mathbf{b}$ is the body force per unit mass of constituent α and $\mathbf{f}^\alpha$ represents volumic forces acting on constituent α due to the interaction with other constituents. Noting that at the mixture level, the net force resulting from interactions between constituents must vanish ($\sum_\alpha \mathbf{f}^\alpha = \mathbf{0}$), the linear momentum balance for the entire mixture can be obtained by adding the equations to each constituent. This yields the conventional equation:

$$\nabla \cdot \mathbf{T} + \rho \mathbf{b} = \mathbf{0} \quad \text{where} \quad \mathbf{T} = \sum_{\alpha=1}^4 \mathbf{T}^\alpha. \quad (6.25)$$

Furthermore, using the fact that G-actin concentration is small, one may neglect the contribution of the monomer stress $\mathbf{T}^m$ compared to other stresses and obtain the following decomposition of the Cauchy stress $\mathbf{T}$:

$$\underbrace{\mathbf{T}}_{\text{Total stress}} = \underbrace{\mathbf{T}^p}_{\text{Contractile stress}} + \underbrace{\mathbf{T}^s}_{\text{Cytoskeleton resistance}} - \underbrace{p\mathbf{I}}_{\text{Cytosol pressure}} \quad (6.26)$$

where $\mathbf{T}^p$ is the contractile stress of stress-fibers, $\mathbf{T}^s$ is the passive stress in the cytoskeleton, p is the pressure in the cytosol and $\mathbf{I}$ is the second-order identity tensor (Fig.6.8). A set of constitutive relations for each stress is provided next.

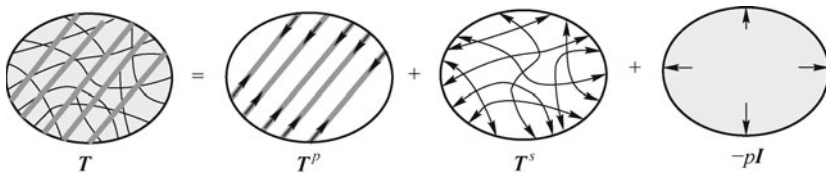


Fig. 6.8 Multiphasic stress decomposition.

6.3.3.2 Stress-fiber contraction and the Hill model

On the basis of our previous discussion on the origin and behavior of cell contractility in Section 6.2.2, it is natural to start the derivation of a constitutive relation for contractile stress $\mathbf{T}^p$ from the Hill model (Fig.6.4). For this, let us consider a bundle of stress-fibers, oriented in a specific direction, say θ , that is characterized by a volume fraction ϕ_θ^p . The Hill model then

allows us to establish a relationship between the axial contractile stress T_θ^p in the bundle and the axial strain $\dot{\epsilon}_\theta$ characterizing the elongation/shortening of this bundle. In other words, if $\dot{\epsilon}_\theta < 0$, fibers shorten, while if $\dot{\epsilon}_\theta > 0$, fibers lengthen. In general, it is possible to introduce a scalar function ψ , that we call contractile power function, so that T_θ^p is written as:

$$T_\theta^p = \phi_\theta^p \left(\bar{T} + \frac{\partial \psi}{\partial \dot{\epsilon}_\theta} \right). \quad (6.27)$$

This equation describes the linear increase of contraction T_θ^p with fiber volume fraction ϕ_θ^p . Furthermore, under the condition that $\psi(0) = 0$ and $\partial \psi / \partial \dot{\epsilon}_\theta(0) = 0$, Eq.(6.27) incorporates the existence of an *isometric stress* $\bar{T}$ when $\dot{\epsilon}_\theta$ vanishes. A variety of expressions can be introduced for the contractile power function in order to approximate the Hill model. For the sake of simplicity, we present a two-parameter function:

$$\psi(\dot{\epsilon}_\theta) = \bar{T} \left[-1 + \sqrt{\left(\frac{\dot{\epsilon}_\theta}{\dot{\epsilon}_0} \right)^2 + 1} \right] \quad (6.28)$$

that captures the trends of the Hill model relatively well as seen in Fig.6.9. In addition to the isometric stress $\bar{T}$, the function is written in terms of the parameter $\dot{\epsilon}_0$ representing a characteristic strain rate of fiber contraction. As illustrated in Fig.6.9, increasing $\dot{\epsilon}_0$ results in enlarging the range of strain rate sensitivity, while in the limit where $\dot{\epsilon}_0$ tends to zero, very small strain rate results in large variations of contraction stress. While the above model is valid for unidirectional fiber bundles, stress-fiber organization and contraction usually occur along various directions in the cell. To account for this, an generalization of the Hill model for multi-dimensional fiber assemblies is therefore presented. First of all, recognizing that the stress tensor $\mathbf{T}^p$ and the

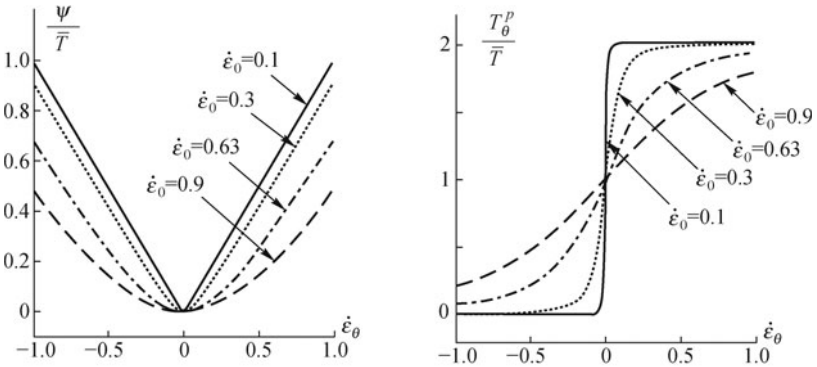


Fig. 6.9 Contractile power function ψ and associated contractile stress for different values of $\dot{\epsilon}_0$.

strain tensor $\dot{\mathbf{E}}$ are related to unidirectional measures T_θ^p and $\dot{\epsilon}_\theta$ respectively through the directional averaging operation

$$\mathbf{T}^p = \langle \langle T_\theta^p \rangle \rangle \quad \text{and} \quad \dot{\mathbf{E}} = \langle \langle \dot{\epsilon}_\theta \rangle \rangle, \quad (6.29)$$

one can extend Eq.(6.27) to the multidirectional case as follows^[44]:

$$\mathbf{T}^p(\dot{\mathbf{E}}) = \Phi^p : \left(\bar{\mathbf{T}}\mathbf{I} + \frac{\partial \Psi}{\partial \dot{\mathbf{E}}} \right) \quad \text{where} \quad \Psi = \langle \langle \psi \rangle \rangle \quad (6.30)$$

and the fourth-order identity tensor $\mathbf{I}$ is defined as $I_{ijkl} = \delta_{ik}\delta_{jl}$ where δ is the Kronecker delta. The attraction of the relation relies in that it keeps the same simple structure as in Eq.(6.27), and it is entirely defined by the knowledge of the tensor Ψ . Furthermore, the function Ψ can be simply obtained by performing the integration of the contractile power Eq.(6.28) as defined in Eq.(6.2). Several observations can be made with respect to Eq.(6.30). First of all, the constitutive relation relates the stress in stress-fibers to the strain rate in the cytoskeleton in a nonlinear fashion; such a behavior may be compared to a nonlinear Newtonian fluid. Second, the contraction response is anisotropic due to the presence of the structure tensor Φ^p in the equation. This captures the fact that oriented stress-fibers produce more contraction in the direction of their principal alignments. Finally, when the cell is in static equilibrium ($\dot{\mathbf{E}} = 0$), one observes that the contractile stress is non-zero and equal to $\mathbf{T}^p = \Phi^p \bar{\mathbf{T}}$. This clearly shows how contraction is related to stress-fiber distribution (through Φ^p) and isometric stress.

6.3.3.3 Passive elasticity of the cytoskeletal fibrous network

Let us now give a brief description of the passive cytoskeleton forces generated by cell deformation. Considering the passive cytoskeleton as an isotropic and incompressible fibrous network described by neo-Hookean elasticity, the large deformations occurring during contraction require that one write the Cauchy stress $\mathbf{T}^s$ in terms of the so-called second Piola-Kirshoff stress $\mathbf{S}^s$ by using a mapping operation between original and current configurations:

$$\mathbf{T}^s = \mathbf{F} \cdot \mathbf{S}^s \cdot \mathbf{F}^T. \quad (6.31)$$

In this equation, $\mathbf{F}$ is the deformation gradient and J is the Jacobian of the deformation, i.e. $J = \det(\mathbf{F})$. The stress $\mathbf{S}^s$ in the network may then be related to the deformation by the Green-Lagrange strain tensor $\mathbf{E}$ through a strain energy function that can capture the nonlinear behavior of the filamentous network. Particularly, it is now accepted that this network exhibits a strong stress-stiffening response but the underlying physics for these behavior are still not understood. Several explanations have been advanced, including the entropic elasticity and the transition from filament bending to filament stretching during deformation^[45,46]. While these aspects of cell deformation might play a role in the overall morphology and contractibility, the chapter only considers the case of linear, isotropic elasticity, for clarity. This means

that the relationship between $\mathbf{S}$ and $\mathbf{E}$ is written in terms of an elasticity matrix $\mathbf{C}$ in the form:

$$\mathbf{S}^N = \mathbf{C} : \mathbf{E} \quad (6.32)$$

where $\mathbf{C}$ is written in terms of Lamé constants λ and μ as $C_{ijkl} = \lambda\delta_{ij}\delta_{kl} + \mu(\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk})$. The lamé constants can be determined in terms of the Young's modulus E and Poisson's ratio ν given in Table 6.1 by $\lambda = E\nu/[(1 + \nu)(1 - 2\nu)]$ and $\mu = E/[2(1 + \nu)]$. Note that while the material response is linear, the relation between force and displacement is nonlinear due to the geometrical effects associated with finite deformation.

Table 6.1 Physical constants used in simulations

Constant	Value	Reference
E	77 Pa	[47]
ν	0.3	[47]
D	$10 \mu\text{m}^2/\text{s}$	[22]
κ	$10^{-15} \text{m}^4/\text{N} \cdot \text{s}$	[41]
k_0^f	10^{-5}s^{-1}	
k_1^f	10^{-1}s^{-1}	
k_0^d	10^{-1}s^{-1}	[22]
ϕ^s	0.7	
ϕ^f	0.25	
$\phi^m + \phi^p$	0.05	
$\dot{\epsilon}_0$	2.510^{-3}s^{-1}	[47]
$\bar{T}$	2000 Pa	[47]

6.3.4 Model's prediction for simple cases

At this point, it is useful to recap and illustrate how the above multiphase model is able to capture the essence of the active contractile response of cells. For clarity, we propose to investigate the qualitative behavior of the model for a simple one-dimensional case. Let us therefore consider an originally square shaped cell at initial time t_0 that does not contain any stress-fibers $c^p(t_0) = 0$ and whose properties are given in Table 6.1. The evolution and contraction of such a cell are then determined in two cases: (a) In the first case, the cell is not constrained in any direction and no forces are applied on its boundary. This situation corresponds to a free floating cell with no attachments to external matrices (or substrates). (b) In the second case, the cell is subjected to a prescribed (vanishing) vertical displacement on its top and bottom boundaries, while all other modes of deformation are permitted. This situation may be thought of as cell attached to a substrate on its top and bottom boundaries, for which the substrate has an infinite stiffness in the vertical direction and a vanishing stiffness in the horizontal direction (see subfigure of Fig.6.10). Let us now evaluate the behavior of a cell in each case.

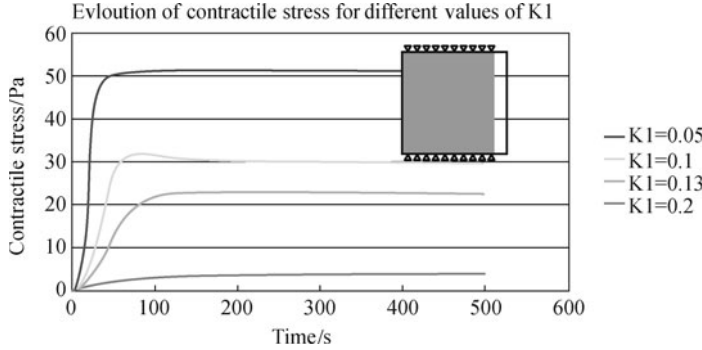


Fig. 6.10 Evolution of contractile stress force in the vertical direction for different values of k_1^f .

6.3.4.1 Free floating cell

Let us first provide an explanation of the series of events that characterize the evolution of a free floating cell.

(1) Due to the vanishing stress-fiber concentration at t_0 , the cell is not initially in a state of chemical equilibrium. In order to reestablish the equilibrium according to Eq.(6.19), the model predicts the nucleation of a small quantity of isotropic stress-fibers throughout the cell ($\Phi^p \neq 0$).

(2) According to Hill's model (6.30), the existence of a small isotropic concentration of stress-fibers engenders an isotropic contractile stress $\mathbf{T}^p$.

(3) This stress triggers an overall contraction $\epsilon < 0$ of the cell that is only resisted by the passive elasticity of the cytoskeleton (particularly, the presence of microtubules that can withstand compressive stresses).

(4) In agreement with the Hill model (Fig.6.9), the negative strain results in a decrease and eventually the disappearance of the contractile stress $\mathbf{T}^p$. As a consequence to these events, the equilibrium state of the free floating cell exhibits a very small quantity of isotropically distributed stress-fibers and negligible contractility. Particularly, the model shows that the contractility of a free floating cell depends on (a) the isotropic formation of stress-fibers (represented by the rate constant k_0^f in Eq.(6.19)) and (b) the resistance of the passive cytoskeleton to compression. In fact, in this case, cell contraction, supported by the compression of microtubules, may be necessary to maintain the structural integrity of the cytoskeleton as described by the tensegrity model^[16-18,48].

6.3.4.2 Unidirectionally constrained cell

In the case of a cell whose deformation is constrained in the vertical direction, the following processes will be observed, based on the model's assumptions:

(1) Similar to the case of a free floating cell, the first two stages involve the initial nucleation of stress-fibers and the appearance of contractile stress.

(2) The contractile stress will trigger a negative contraction $\epsilon < 0$ in

the horizontal direction, while no direction will be observed in the vertical direction due to the mechanical constraints applied to the cell.

(3) According to the Hill model (Fig.6.9), this deformation produces a constant isometric contraction in the vertical direction (since $\epsilon = 0$ in this direction) and a vanishing contraction in the horizontal direction (since $\epsilon < 0$ in this direction).

(4) Because stress-fiber formation in certain directions is promoted by the existence of a contractile stress (depending on the rate constant k_1^f in Eq.(6.19)), the state of contraction will result in: (a) no formation of stress-fibers in the horizontal direction and (b) generation of stress-fibers aligned in the vertical direction.

(5) The anisotropic stress-fiber formation will intensify contraction in the vertical direction, starting a self-feeding loop that will eventually come to an arrest when the quantity of G-actin monomers is too small to sustain F-actin (stress-fiber) formation.

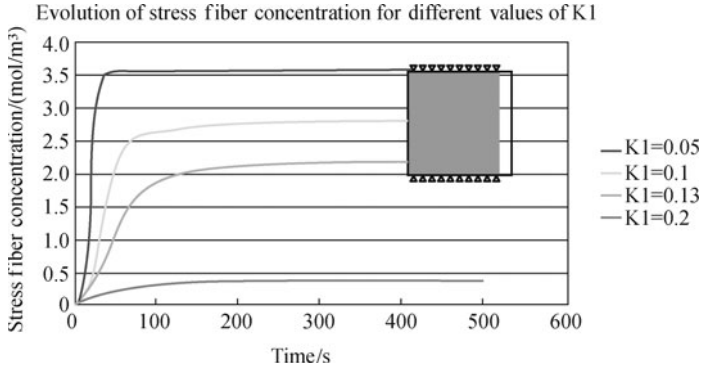


Fig. 6.11 Evolution of stress-fiber concentration in the vertical direction for different values of k_1^f .

To better illustrate the process, Figs.6.10 and 6.11 show the effect of the rate constant k_1^f (referring to Eq.(6.19)) on the evolution of stress-fibers and contractile stress in the vertical direction. The constant k_1^f governs the mechano-sensitivity of the cell response by increasing the rate of stress-fiber formation when contractile stress is present. The result clearly shows that increasing k_1^f results in (a) increasing cell contractility, (b) increasing the formation of stress-fibers and (c) increasing the rate of cell contractility. Furthermore the final cell configuration (shown in the subfigures of Figs.6.10 and 6.11) is characterized by a large amount of stress-fibers aligned in the vertical direction and a contraction in the horizontal direction due to the stress independent contraction (similar to a free floating cell).

6.4 Interaction between contractile cells and compliant substrates

While the first part of this chapter concerns the contractile behavior of cells, the second part describes how the above model can be used to characterize the mechano-sensitive behavior of contractile cells on elastic substrates of arbitrary stiffness. Modeling such problems usually involves solving the equations of cell contractility in the case of complex geometry, a task that is generally necessitated to resort to numerical methods. After formulating the problem of cell-substrate interaction, this section therefore describes a numerical strategy based on finite elements, that can be used to obtain the evolution of both cell morphology and substrate deformation for arbitrary stiffness. We finally present a few simulations illustrating the predictions of the model presented in this chapter.

6.4.1 Two-dimensional plane stress formulation

Let us consider a thin elastic substrate undergoing a deformation for which the displacement, deformation (Green-Lagrange strain) and Cauchy stress are denoted by $\mathbf{u}^s$, $\mathbf{E}^s$ and $\mathbf{T}^s$, respectively. Without going into details, the elasticity of the substrate is specified in terms of a Neo-Hookean model, parametrized in terms of the Young's modulus E^s and Poisson's ratio ν . Referring to Fig.6.12, it is now assumed that thin contractile cells are deposited on this substrate, so that two-dimensional plane-stress assumptions can be

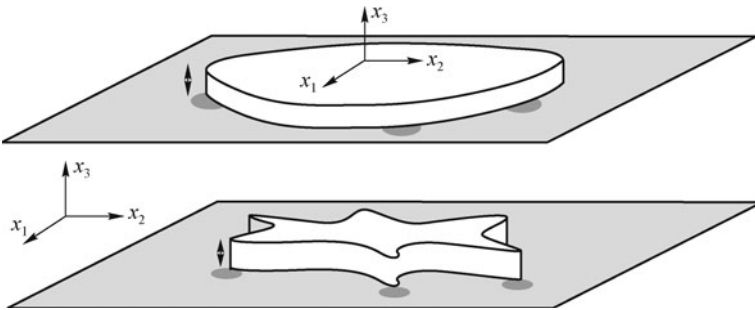


Fig. 6.12 Plane stress formulation of cell-substrate interactions.

used, allowing us to reduce the original three-dimensional problem to a two-dimensional equivalent formulation in the plane of the substrate. Plane-stress assumptions imply that (a) there are no out-of-plane shear stresses and (b) the normal stress T_{33} in the cell is equal to fluid pressure, while the normal stress T_{33}^s in the substrate vanishes.

$$T_{33} = p, \quad T_{33}^s = 0. \quad (6.33)$$

Note that due to the incompressibility assumption of the cell, an change in cell area will automatically result in a change of cell thickness. Particularly, the new cell thickness can be evaluated by computing the out-of-plane strain E_{33} .

Furthermore, the contractility and deformation of cells heavily depend on the location of cell-substrate adhesion complex, which, like the cytoskeleton, are of very dynamic structures. However, to simplify the problem, it is possible to assume that adhesion complexes do not change during cell evolution. This assumption is consistent with experiments of cell on adhesion islands as provided by arrays of micro-needles^[13] and micro-contact printing^[14]. In this context, cell-substrate adhesion can be considered as an elastic cohesive model. Thus, if $\mathbf{u}$ and $\mathbf{u}^s$ are the respective displacement of the cell and substrate at their initial contact points, the resulting in-plane cohesive force $\mathbf{f}^c$ is given by:

$$\mathbf{f}^c = \eta(\mathbf{X})\mathbf{K}^a(\mathbf{u} - \mathbf{u}^s). \quad (6.34)$$

In this expression, the quantity $\mathbf{K}^a$ is the stiffness of the adhesion and $\eta(\mathbf{X})$ is a binary function so that $\eta = 1$ on adhesion islands and $\eta = 0$ outside adhesion islands. Using the above assumptions, the state of the cell-substrate system is entirely determined through the knowledge of five continuum fields, the displacements $\mathbf{u}$ and $\mathbf{u}^s$ of cell and substrate, respectively, the cytosol pressure p , the volume fraction ϕ^m of G-actin monomers and finally, the stress-fiber structural tensor Φ^p . Accordingly, the determination of these variables is performed by solving a set of five coupled equations, that consist in this study, of mass balance Eqs.(6.13), (6.14) and (6.15), the momentum balance of the mixture Eq.(6.25) and the momentum balance of the substrate, that take a similar form to Eq.(6.25).

6.4.2 Numerical strategy: XFEM-level methods

A finite element method (FEM) is now presented to provide a numerical solution of the above equations. Challenges to modeling cell-substrate systems generally reside in (a) the derivation of an accurate and efficient formulation to solve nonlinear equations driving cell-substrate interactions and (b) handling the deformation of two interacting bodies and their interactions. These challenges may be met by combining two recent, yet powerful numerical methods: the extended finite element method (XFEM)^[49,50] that is capable of introducing boundary discontinuities within the traditional finite element method and the level-set method that naturally describes the evolution of interfaces with respect to time^[51-53]. Using these concepts, one may introduce a single finite element mesh used to describe both the cell and the substrate (Fig.6.13). The cell boundary may then be defined by an implicit *level-set* function $\phi(X_1, X_2)$ so that the sign of ϕ is opposite on two sides

of discontinuity. This function is then used to represent the cell contour, i.e. when $\phi(x, y) < 0$ (cell's interior), a node is associated with both cell and substrate degrees of freedom, while when $\phi(x, y) > 0$ (cell's exterior), nodes only contain degrees of freedom associated with substrate displacements.

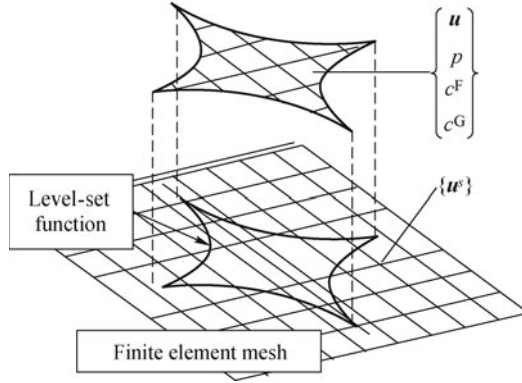


Fig. 6.13 Illustration of the XFEM-level-set formulation.

Because the same mesh is used for both substrate and cell, cell displacements and cell strain fields, calculated outside the cell domain must, vanish. This gives rise to discontinuities in strain and displacement between points, located inside and outside the cell. Many numerical techniques (including FEM) are developed for continuous fields and fail to describe such discontinuities. To address this issue, the XFEM was first originally introduced to incorporate a jump in displacement occurring as a result of a propagating crack in a continuous medium^[54,55]. A key feature of this method resides in that the description of the discontinuity is independent of spatial discretization. The method was further improved to model weak discontinuities, as described in^[56,57], making it a natural platform on which the equations of cell-substrate interaction can be solved with great flexibility and minimal computation cost. In a nutshell, the XFEM approximation of a continuum field $\tilde{f}(\mathbf{x})$ representing, for instance, cell displacement, cytosol pressure, F-actin or G-actin volume fraction is written in terms of three components, namely f , $\bar{f}$ and $\bar{\bar{f}}$, associated with continuous, strong, and weak discontinuous functions, respectively^[56]:

$$\tilde{f}(\mathbf{x}) = \sum_{I=1}^n N_I(\mathbf{x}) f_I + \sum_{J=1}^m N_J(\mathbf{x}) [H(\mathbf{x}) - H(\mathbf{x}_J)] \bar{f}_J + \sum_{J=1}^m N_J(\mathbf{x}) \chi_J(\mathbf{x}) \bar{\bar{f}}_J \quad (6.35)$$

where the functions $N_I(\mathbf{x})$ are finite element shape functions associated with node I , $N_J(\mathbf{x})$ are the shape functions associated with the nodes of an element that have been cut by the interface (Fig.6.14). Also, n is the total number of nodes per element while m is the number of enriched nodes ($m \leq n$) that belongs to elements cut by the interface (Fig.6.14). Finally, $H(\mathbf{x})$ and $\chi(\mathbf{x})$

are the Heaviside and Ridge enrichment functions with the required strong and weak discontinuities^[57,58], written in the form:

$$H(\phi) = \begin{cases} 1 & \phi > 0 \\ 0 & \phi < 0 \end{cases} \quad \text{and} \quad \chi_j(\mathbf{x}) = |\phi(\mathbf{x})| - |\phi(\mathbf{x}_j)|. \quad (6.36)$$

The above equation clearly shows that the Heaviside function describes a jump in displacement (strong discontinuity) while the ridge function causes a jump in strain field (weak discontinuity) across the interface.

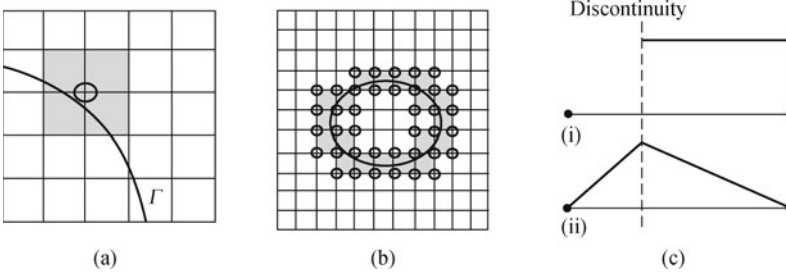


Fig. 6.14 Illustration of the enrichment technique with the extended finite element method. (a) Enriched nodes belong to elements cut by the interface(gray) and enriched elements (gray) possess at least one enriched node. (b) Example of enriched nodes (circles) in the case of an elliptic cell. (c) The two types of enrichment functions considered in this chapter: (i) the Heaviside function and (ii) the ridge function.

Upon substituting the XFEM approximation equation (6.35) into the governing equations (6.13), (6.14), (6.15) and (6.25), the final system is written in the form of a system of ordinary differential equations as (a detailed discussion of this procedure is given in [59]):

$$\mathbf{C} \cdot \dot{\mathbf{U}} + \mathbf{K} \cdot \delta \mathbf{U} = \mathbf{R} \quad (6.37)$$

where the solution vector $\mathbf{U}$ contains the degrees of freedom to be computed at each node:

$$\mathbf{U} = [\mathbf{u} \quad \mathbf{u}^s \quad p \quad \phi^m \quad \Phi^p]^T \quad (6.38)$$

where $\mathbf{u}$, p , ϕ^m and Φ^p implicitly contain their continuous, weakly discontinuous and strongly discontinuous contributions, respectively. Moreover, a superimposed dot denotes a material time derivative with respect to the cytoskeleton, $\mathbf{C}$ and $\mathbf{K}$ are the damping and stiffness matrices of the system, respectively, and $\mathbf{R}$ is the residual. This system of equations may be integrated in time using a backward-Euler scheme and the Newton-Raphson method to work out the nonlinear system at each time step.

6.4.3 Analysis of mechanical interactions between a contractile cell and an elastic substrate

In order to assess the capacity of the developed model in reproducing the main trends observed in experiments (sensitivity of contraction and stress-fiber formation on substrate stiffness, spatial stress-fiber distribution), this section presents key numerical results concerning the contraction of a simple-shaped cell (contractile fibroblast) adhering to an elastic substrate. Similar to the theoretical work of Deshpande et al.^[60] and the experimental work of Bischofs et al.^[5], we considered a square cell attached to an underlying substrate through adhesion islands located at the cell's corners and at the center of the cell (Fig.6.15). In this study, the effect of the cortical membrane was neglected and the cell properties given in Table 6.1 were considered. Initial conditions are taken as vanishing deformation and stress-fiber concentration $c^p = 0$ so that the cell is not in chemical equilibrium. In addition, the finite element discretization consists of an 11×11 nine-node square finite elements and the cell is roughly composed of 7×7 elements delimited by a square level-set function as depicted in Fig.6.15. Results are then shown after reaching a steady state solution upon solving the FEM equation.

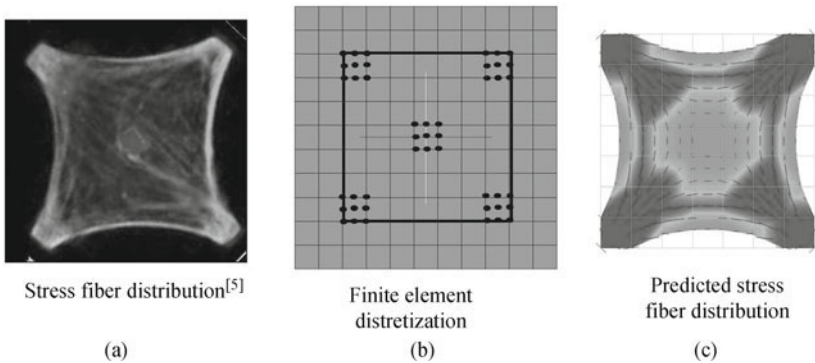


Fig. 6.15 Contraction of a fibroblast on a rigid substrate with periodically spaced adhesion islands. (a) Experimental observation from Bischofs et al.^[5] showing actin fiber distribution (green), (b) Finite element discretization, level-set definition (thick line) and cohesive region between cell and substrate (dots), and (c) Final cell configuration showing the principal direction of stress-fibers (vectors) and their concentration (high concentrations are depicted in red while low concentrations are shown in green, color plot in the book end).

Considering a rigid substrate, the figure on the right-hand side of Fig.6.15 shows the stress-fiber concentration, orientation, as well as cell deformation at steady state. One can make the following observations. First of all, cell deformation and stress-fiber distribution generally agree with experimental observation depicted on the left hand side of Fig.6.15. Furthermore, stress-fiber distribution is characterized by a strong alignment at 45 degrees with

respect to the horizontal direction. This can be explained by the fact that due to the constrained displacement at the cell's corners, it is impossible for the cell to contract along diagonal direction. This means that at all times, along these directions, $\epsilon = 0$ which implies large fiber formation consistent with our analysis of Section 6.3.4. Finally, it is important to note that high actin concentrations near the cell boundary observed in experiments are not predicted by the model since the actin cortex (made of a thin layer of dense actin meshwork) is not considered in the present model.

The next set of results concerns the contraction of a square cell upon modifying the stiffness (Young's modulus) of the substrate (Fig.6.16). In these simulations, the substrate is subjected to a “no-stress” boundary condition and is only constrained in terms of rigid body motion. The main trends are given as follows: When the substrate stiffness is small (1 Pa), the cell exhibits a very low concentration of stress-fibers and undergoes a general compaction due to the small amount of isotropic fibers. This is similar to the case of a free floating cell described in Section 6.3.4. However, as substrate stiffness is increased, the stress-fiber concentration increases dramatically until it reaches its final configuration obtained on a rigid substrate. This increase in cell concentration with substrate stiffness is also observed by measuring the adhesion force exerted by the cell on its substrate as shown in Fig.6.17 These results illustrate how well the multiphasic model, by coupling the chemistry of actin

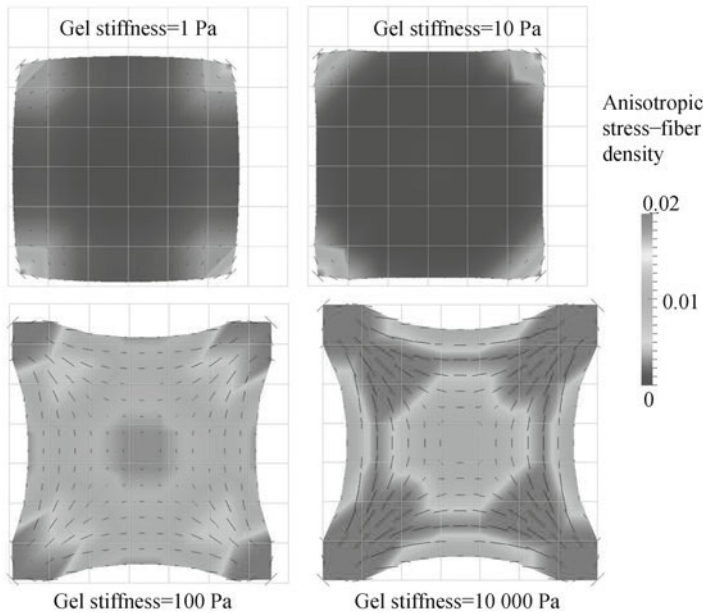


Fig. 6.16 Original (gray mesh) and final cell configuration and stress-fiber distribution for various values of substrate stiffness (varying from 1 Pa to 10 kPa) (color plot in the book end).

polymerization to the mechano-sensitive contraction of stress-fiber, captures the dependency of cell contraction and shape in its external mechanical environment (Fig.6.1).

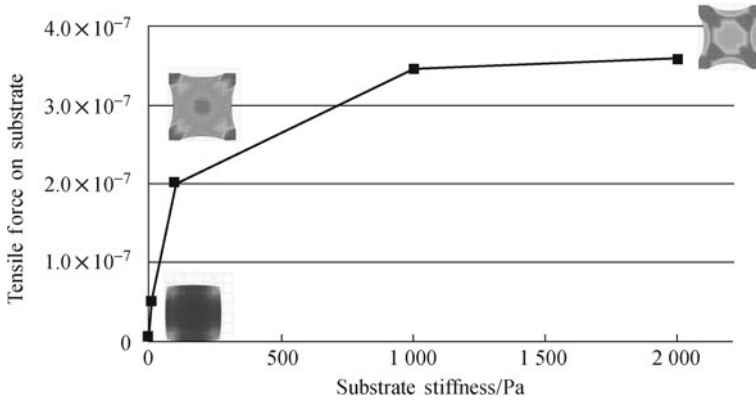


Fig. 6.17 Model prediction of the relationship between substrate stiffness of the tensile force exerted by the cell on substrate.

6.5 Summary and conclusion

To conclude this chapter, this section provides a brief summary of the development and findings, a discussion of the limitation of the multiphasic model and finally a concluding remark on the relevance of this work to medicine and biophysics.

6.5.1 Summary

Recent experimental investigations have shown that the adaptive behavior of soft biological tissues is determined by the mechanical interactions between contractile cells and their surrounding extracellular matrix. Because these interactions are dominated by the mechano-sensitive contractility of cells, research advances mainly rely on mathematical models that can accurately predict how cells react to their mechanical environment. Current knowledge in the process of active cell deformation indicates that such models must be capable of coupling multiple physics, including elastic deformation, fluid flow, chemical reaction and chemical transport.

Multiphasic approaches provide an ideal framework for such an endeavor as they naturally couple the behavior and interactions of several interpenetrating constituents in the continuum theory. Based on these considerations,

the present chapter introduces a quadriphasic continuum model, that aims at predicting the strong interactions between mechanical forces and actin cytoskeleton assembly during cell contraction. Decomposing the cytoplasm into individual constituents representing passive and active cytoskeleton, interstitial cytosol and free actin monomers, the model can capture the following key phenomena:

- (1) the force-dependent actin polymerization leading to stress-fiber formation;
- (2) the increase in cell contraction with support rigidity, through an extended version of the Hill model;
- (3) the transport and reactivity of cytosol and globular actin monomers through the cytoplasm.

The development of such a model, together with advances in computational approach, such as the extended finite element methods, has been shown to provide promising predictions concerning the relationship between cell contractility and substrate stiffness. Particularly, the model clearly predicts the increase in cell contractility and stress-fiber concentration with substrate stiffness and provides a fairly good description of the stress-fiber structure within the cell.

6.5.2 Limitations of the multiphasic approach

While the ultimate goal of this chapter is to demonstrate the applicability of multiphasic models to the description of the active response of cells, the application of these types of models to other situations is interesting. However, to do this, it is important to be aware of the model's limitations and possible ways to circumvent them. First of all, the multiphasic model involves a certain number of physical constants (rate constants, material constants, diffusion constants) that may be a challenge of measuring experimentally. This is currently hindering the validation of the model. To resolve this important issue, a full integration of mathematical modeling and experimental techniques (based on AFM, cytoindentation, micropipette aspiration or optical tweezers^[61]) is necessary. Another limitation of the multiphasic model arises from its continuum assumptions. Continuum theory is based on the idea that the characteristic length of the cell's structure (mesh size of the actin network, for instance) is small in comparison to the cell dimension and the typical variation of continuum fields. These assumptions are sometimes questionable^[15,16], especially when the local response of the cell is interesting. In such a case, structural models^[17] may be more adapted, despite the fact that they are less flexible in capturing multiphysics interactions and have a higher computational cost. Besides these two limitations, the described model is still limited in that it cannot describe the mechano-sensitive behavior of substrate/adhesion complex known to be key players in cell-matrix

interactions^[62-64]. However, this aspect can be incorporated into the continuum model in the spirit of the work performed by Deshpandes et al.^[60,65], who described the force-dependent dynamics and adhesion of cross-membrane integrins within the continuum theory. Finally, it is important to note that cell contraction is generally associated with cell spreading, a process that relies on the extension of surface protrusions that attach to the substrate, stretch the cell and result in an increase in cell area^[66-68]. While the model described in this chapter does not incorporate such physics, multiphasic models have been proposed for protrusion growth^[48,69,70]. An interesting future research direction therefore resides in coupling the above cell contractile model to protrusion growth and adhesion, in order to predict the combination of contraction and spreading that is essential to tissue remodeling.

6.5.3 Concluding remark

Contrary to traditional engineering disciplines such as materials science and structural engineering, medicine has traditionally been dominated by trial and error experimental approach. This trend can be explained by the fact that the high complexity and nonlinearity of biological systems have hindered the predictive power of mathematical models. However, with the increase in computer capacity and the development of increasingly powerful multiscale/multiphysics methods, it will soon become possible to use mathematical model to understand how phenomena at the molecular scale influence the active behavior of cells.

While the model described in this chapter does not explicitly establish the relation between molecular mechanisms (including myosin/actin interactions, cross-bridge dynamics for instance), it provides a critical step in this direction. Indeed, the multiphasic framework provides a flexible platform onto which accurate mechanistic models of the dynamics of actin and myosin association as well as cross-bridge dynamics of cell contraction can be used in place of the empirical Hill model. The model can further be improved by considering action potential and the effect of calcium ions on muscle contraction. By providing such a bridge between molecular and cellular scales, multiphasic formulation therefore has the potential to shine a light on complex interactions between molecular, diffusional and structural mechanisms that often lead to behavior that is difficult to apprehend through experimental procedures only. As such, one may hope that through a full integration with experiments, the use of multiphasic models and numerical analysis can help to provide the necessary piece to unravel fundamental mechanisms, and enable significant discoveries in modern medicine and biophysics.

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Chapter 7 Effect of Substrate Rigidity on the Growth of Nascent Adhesion Sites

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Abstract: Mechanical stiffness of bio-adhesive substrates is one of the major regulators of the cell adhesion and migration. In this contribution, we propose a theoretical model for the spontaneous growth of focal adhesion sites on compliant elastic substrates at the early stages of cellular adhesion. Using a purely thermodynamic approach, we demonstrate that the rate of membrane-substrate association decreases with increasing the compliance of the substrate. This can be considered as a reason for smaller spread area of the focal adhesion points after the stabilization of adhesion on compliant substrates, as reported by experiments. We also show that the extent to which the compliance of the substrate modulates the growth rate of adhesion site depends on the areal density of cell-adhesive ligands on the substrate.

Keywords: cell adhesion, membrane, kinetics, rigidity, diffusion

7.1 Introduction

Cell behavior is mediated by variety of physiochemical properties of extracellular matrix (ECM). Surface chemistry^[1,2], roughness^[3,4] and distribution pattern of cell adhesive proteins^[5-7] are among the ECM properties which are known to modulate various cellular physiological behavior of cells. Mechanical stiffness of ECM is also found to be a major regulator for multiple aspects of cellular function, ranging from cell motility to the lineage commitment and differentiation^[8]. Quantification of migration speed of motile cells cultured on substrate with variable stiffness reveals a biphasic dependence on substrate compliance, suggesting the existence of an optimal stiffness capable of supporting maximal speed of migration^[9]. Contractile cells, such as vascular smooth muscle cells (VSMC), become more proliferative and less

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apoptotic in response to the increasing substrate stiffness^[10]. Other studies have demonstrated the strong influence of substrate elasticity on the lineage commitment of naive stem cells and driving their differentiation to variety of mature cells^[11,12].

The acute molecular basis of the sensory mechanism through which the tissue cells sense the ECM elasticity and translate it into a downstream response is largely unknown. It is currently believed that the downstream signaling in response to the matrix stiffness is initiated at the molecular level and by transmembrane integrin *receptors* present on the surface of adherent cells. These mobile proteins can selectively associate with the complementary adhesive *ligand* molecules of ECM. Cell membrane conjugates to an adhesive substrate through islets of receptor-ligand assemblies, often referred to as focal adhesion (FA), which can grow to a lateral size of a few microns^[13]. Typically, the areal density of receptors distributed in membrane is insufficient for stable adhesion of these focal points with the substrate. Hence, for the nucleation and growth of stable adhesion, receptors must be recruited from non-adhered regions. The spontaneous segregation of mobile receptors results in the formation of tightly bound adhesion zones even at astonishingly low receptor densities^[14].

The rigidity of the substrate regulates the size and geometry of the FAs. Adhesion on stiff substrates generally gives rise to the formation of stable and large FA points whose size is on the order of several microns, whereas on very compliant substrates, FAs are mainly unstable with typical area $< 1 \mu\text{m}^2$ ^[15,16]. It has been found that the larger size of FA points on stiff substrates correlates with a larger traction force transmitted at the site of adhesion and consequently larger protein aggregation^[17,18].

In the following, we consider the effect of substrate rigidity on the dynamics of membrane spreading and growth of an FA zone. Particularly, we focus on the earliest events in FA formation, before the myriad of adaptor proteins are recruited and assembled on the cytoplasmic face to anchor to the cytoskeleton. From a thermodynamic perspective, spontaneous spreading at this stage is a kinetic process which is controlled by the diffusibility of receptor molecules^[19,20]. A few theoretical models are available to describe the kinetics of membrane spreading during the formation of FA sites on rigid substrates^[21,22]. The purpose of the present contribution is to extend the available models for adhesion on compliant substrates. Particularly, we aim to understand (a) how substrate compliance affects the membrane-substrate association and growth rate of the adhesion zone and (b) how substrate elasticity couples with its adhesivity to mediate the kinetics of spontaneous membrane spreading.

7.2 Model

Figure 7.1 shows a simplified 1D model for a growing FA on a planar soft elastic substrate, coated with surface conjugated ligands. The position along the membrane (width=unit of length) is described by an arc length coordinate, r . Receptors are assumed as ideal solute particles which can diffuse laterally within the plane of the membrane, until they are stabilized through association with immobilized ligands. Before adhesion, the distribution of receptors on the membrane is statistically uniform with an areal density of R_∞ . This density is assumed to be much lower than that of surface ligands and thus, spontaneous spreading of the membrane on the adhesive substrate is mediated by the diffusion of the receptors^[21,22]. The progressive adhesion zone is represented by a straight line, whose position is denoted by $a(t)$. At $t = 0$, the margin of the adhesion zone is assumed to be at $r = 0$.

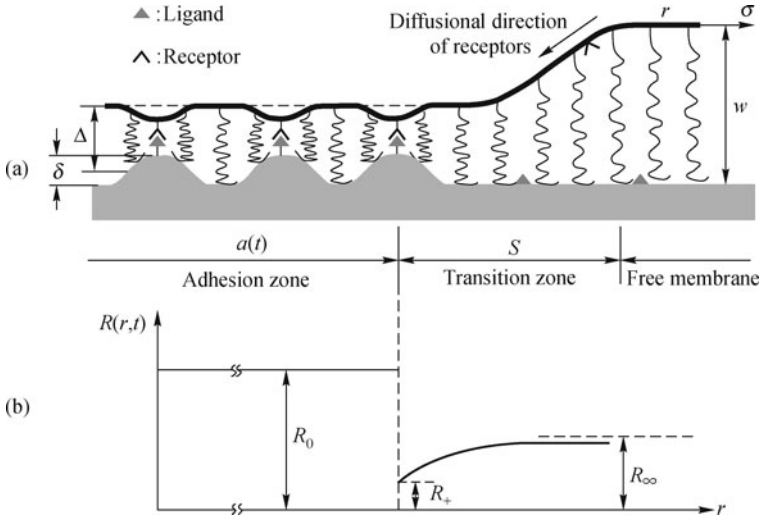


Fig. 7.1 (a) Schematic diagram of the 1D model of a focal adhesion of size $a(t)$, growing on a compliant bio-adhesive substrate. The adhesion between the membrane and substrate is formed by recruitment of the mobile receptors from the regions on the membrane far away from the adhesion zone. The specific adhesion between the receptors and ligands is resisted by a compressible layer of repeller molecules (with thickness w) and bending rigidity of the membrane. The adhesion zone and free membrane are separated by a transition zone where the membrane is deformed predominantly by bending. The ligand-receptor bonds are being stressed by the forces developed in compressed repelling layer. The stressed bonds can locally deform the membrane and the compliant substrate. (b) Schematic profile of the receptor molecules $R(r,t)$ at a given time along the membrane with an edge density of R_+ and an initial uniform density R_∞ . See the text for definition of other quantities.

The ligand-receptor specific interaction is resisted by nonspecific repulsions of repeller molecules (e.g., glycocalyx) on the ventral side of the membrane surface. When the membrane comes into contact with the surface, the repellers are compressed and lose conformational entropy (Fig.7.1). The resulting energy penalty for compressing a repeller to the height Δ is often represented by the following phenomenological function^[23]:

$$\Gamma(\Delta) = \frac{\gamma}{\Delta} \exp(-\Delta/w), \quad (7.1)$$

where γ and w characterize the stiffness and (un-deformed) thickness of the brush-like repelling layer, respectively. The ligand-receptor bonds are stressed by the non-specific forces tending to separate the membrane from the adhesive substrate. As a result, the forces exerted by bonds locally deform the membrane and compliant substrate. Due to these adhesion-induced deformations, the separating distance between the membrane and substrate changes along the adhesion zone. The detailed analysis of these local deformations although is possible, yet it leads to considerable complexity. Here, we adopt a simple analogue to account for these deformations. To this end, the mechanical properties of the membrane, ligand-receptor bonds, and substrate are represented by harmonic springs connected in series. The stiffness of the membrane, ligand-receptor bonds, and substrate is accounted for by linear springs with constant k_m , k_b , and k_s , respectively. Furthermore, let us assume that Δ is the average distance between the substrate and (undeformed) membrane. Assumption of mechanical equilibrium inside the adhesion zone implies

$$k_c(\Delta - L) + R_0^{-1} \partial \Gamma / \partial \Delta = 0, \quad (7.2)$$

where L shows the length of an unstressed ligand-receptor bond, R_0 is the areal density of bound receptors, and k_c is the combined stiffness of membrane and a ligand-receptor bond, i.e., $k_c^{-1} = k_m^{-1} + k_b^{-1}$. If δ represents the substrate deformation at each bonding point, then $k_c(\Delta - L) = k_s \delta$.

Cell-substrate adhesion changes the distribution of receptors inside and outside of the adhesion zone. The concentration profile of receptors along the membrane is shown schematically by Fig.7.1. For $r < a(t)$, the receptor density is constant and equal to R_0 . Consistent with the condition of maximum entropy, the distribution of receptors on non-adhered parts of the membrane, far from the adhesion zone, is taken to be statistically uniform with areal density of $R_\infty < R_0$. The local receptor density in immediate vicinity of the adhesion zone, $r = a_+$, is assumed to be R_+ which is less than R_∞ . The gradient of chemical potential induced by the difference in receptor density along non-adhered part of the membrane drives the diffusive motion of the receptors from more dense to less dense regions, thereby expanding the size

of the adhesion zone. For $r > a(t)$, the receptor density $R(r, t)$ is determined from Fick's second law, that is^[22],

$$\frac{\partial R}{\partial t} = D \frac{\partial^2 R}{\partial r^2}, \quad \text{for } r > a(t), \quad (7.3)$$

where D is the mobility constant. Equation (7.3) is subjected to boundary conditions $R(\infty, t) = R_\infty$, $R(a_+, t) = R_+$, and $R(r, 0) = R_\infty$ for $r > a(t)$. The solution can be expressed in terms of complementary error function as

$$R(r, t) = R_\infty + (R_+ - R_\infty) \text{Erfc} \left(\frac{r}{2\sqrt{Dt}} \right) / \text{Erfc}(\alpha), \quad (7.4)$$

where α is a constant to be determined. According to Eq.(7.4), the only plausible form for $a(t)$ is $a(t) = 2\alpha\sqrt{Dt}$. The receptor density can be related to the diffusive flux of receptors $j(r, t)$ using the Fick's first law of diffusion,

$$j(r, t) = -D \frac{\partial R}{\partial r} \quad \text{for } r > a(t), \quad (7.5)$$

where $j(r, t)$ is the flux of receptors along the membrane. The conservation of receptors requires

$$\frac{d}{dt} \left(\int_0^{a(t)} R_0(r) r dr + \int_{a(t)}^\infty R(r, t) dr \right) = 0, \quad (7.6)$$

from which one can deduce

$$(R_0 - R_+) \dot{a}(t) + j_+ = 0. \quad (7.7)$$

Substitution of Eq.(7.5) into Eq.(7.7) yields

$$\alpha \sqrt{\pi} \text{Erfc}(\alpha) \text{Exp}(\alpha^2) = \frac{R_\infty - R_+}{R_0 - R_+}. \quad (7.8)$$

The following components are assumed to contribute in the total free energy landscape of the system: the binding energy between ligands and receptors, the repulsive energy of repellers, change in entropy due to variation of free receptor density along the membrane, elastic deformation of unbound membrane, and elastic deformation of the membrane, ligand-receptor bonds, and compliant substrate within the adhesion zone. Assuming that membrane-substrate adhesive and repulsive interactions take place within the adhesion zone, the free energy (per unit width) of the system can be written as

$$\begin{aligned} F(t) = & \int_0^{a(t)} \left[-R_0 \gamma_b + \frac{\gamma}{\Delta} \exp\left(-\frac{\Delta}{\tau}\right) + \frac{1}{2} k_c R_0 (\Delta - L)^2 \right. \\ & \left. + \frac{1}{2} k_s R_0 \delta^2 + k_B T R_0 \ln \frac{R_0}{R_\infty} \right] dr + \int_{a(t)}^\infty \left[k_B T R(r, t) \ln \frac{R(r, t)}{R_\infty} \right. \\ & \left. + \frac{\gamma}{h(r)} \exp\left(-\frac{h(r)}{\tau}\right) + f_e(r) \right] dr, \end{aligned} \quad (7.9)$$

where γ_b is the binding energy of a receptor-ligand pair; f_e is the elastic energy density of deformed membrane; $k_B T$ is thermal energy (k_B is the Boltzmann constant and T is the absolute temperature). Under the assumption of small deformations, the elastic energy density f_e can be written as

$$f_e(r) = \frac{\kappa}{2} \left(\frac{\partial^2 h}{\partial r^2} \right)^2 + \frac{\sigma}{2} \left(\frac{\partial h}{\partial r} \right)^2, \quad (7.10)$$

where κ is the bending modulus of the membrane, σ is the surface tension, and $h(r)$ is the membrane profile. Within the transition zone, the membrane follows the Euler-Lagrange equation of deformation

$$\kappa \frac{\partial^4 h}{\partial r^4} - \sigma \frac{\partial^2 h}{\partial r^2} + \frac{\partial \Gamma}{\partial h} = 0. \quad (7.11)$$

Equation (7.11) is subjected to boundary conditions $h(a) = \Delta$, $h'(a) = 0$, $h(a+S) = w - \Delta$, and $h'(a+S) = 0$. This equation can be analytically solved after linearizing $\partial \Gamma / \partial h$ about $h = \Delta$ as $\Gamma'(h) \approx \Gamma'(\Delta) + \Gamma''(\Delta)(h - \Delta)$. The value of S , the length of the transition zone shown by Fig.7.1 can be obtained by minimizing $\int_a^{a+S} \left[\frac{\kappa}{2} \left(\frac{\partial^2 h}{\partial r^2} \right)^2 + \frac{\sigma}{2} \left(\frac{\partial h}{\partial r} \right)^2 \right] dr$ with respect to S . The results are not shown here for the sake of brevity.

The time derivative of Eq.(7.9) reads

$$\begin{aligned} \dot{F}(t) = \dot{a}(t) & \left[-R_0 \gamma_b + \frac{1}{2} k_c R_0 (\Delta - L)^2 + \frac{1}{2} k_s R_0 \delta^2 + k_B T R_0 \ln \frac{R_0}{R_\infty} \right] \\ & - \dot{a}(t) \left[k_B T R_+ \ln \frac{R_+}{R_\infty} + f_e(a) \right] + \int_{a(t)}^\infty k_B T \frac{\partial R(r, t)}{\partial t} \chi dr, \end{aligned} \quad (7.12)$$

where $\chi(r, t) = \ln(R(r, t)/R_\infty) + 1$ represents the local chemical potential. Applying the continuity condition $\dot{R} = -\partial j / \partial r$, it can be shown that

$$k_B T \int_{a(t)}^\infty \dot{R} \chi dr = k_B T j_+ \chi_+ - \dot{Q}(t), \quad (7.13)$$

where $\dot{Q}(t)$ is the rate of energy dissipation due to the diffusion of receptors, given by

$$\dot{Q}(t) = k_B T \int_{a(t)}^\infty D R(r, t) \left(\frac{\partial \chi}{\partial r} \right)^2 dr. \quad (7.14)$$

For a closed system, the rates of dissipation and change of free energy are equal, i.e., $\dot{F}(t) + \dot{Q}(t) = 0$. Hence,

$$\begin{aligned} R_0 \gamma_b - \frac{1}{2} k_c R_0 (\Delta - L)^2 - \frac{1}{2} k_s R_0 \delta^2 - f_e(a) - k_B T R_0 \ln \frac{R_0}{R_+} \\ + k_B T (R_0 - R_+) = 0 \end{aligned} \quad (7.15)$$

from which R_+ can be calculated. Having the value of R_+ , the constant α can be obtained by solving Eq.(7.8).

7.3 Results and Discussion

In this section, we use the proposed model to perform a numerical study and examine how the rigidity of the substrate affects the rate of membrane-substrate association. Table 7.1 represents the values of dimensional parameters used in the numerical solution. The following procedure is undertaken in order to predict the growth kinetics of the adhesion zone and the receptor density as a function of time. The value of the separation distance Δ , predicted by Eq.(7.2), is used to determine the membrane profile and the corresponding elastic energy density f_e , using Eqs.(7.10) and (7.11). This is implemented in Eq.(7.15) in order to evaluate the edge receptor density R_+ and consequently the constant α using Eq.(7.8). Then the time dependent density of receptors is calculated by Eq.(7.4) while the growing length of adhesion zone follows $a(t) = 2\alpha\sqrt{Dt}$.

Table 7.1 Numerical values used for dimensional parameters in the model

Parameter	Definition	Estimated value	Source
γ_b	Energy of single bond	$5k_BT$	[24]
R_∞	Initial receptor density	$10^3 \mu\text{m}^{-2}$	[25]
R_0	Density of bonds	$10^4 \mu\text{m}^{-2}$	–
κ	Bending modulus	$10^{-8} \text{ dyn}\cdot\mu\text{m}$	[26]
σ	Membrane tension	$10^{-5} \text{ dyn}/\mu\text{m}$	[27]
k_b	Rigidity of a single bond	$10^{-5} \text{ dyn}/\mu\text{m}$	[25]
k_m	Rigidity of membrane	$10^{-4} \text{ dyn}/\mu\text{m}$	[28]
L	Length of an unstressed ligand-receptor bond	$2 \times 10^{-2} \mu\text{m}$	[25]
γ	Compressibility coefficient of glycocalyx	10^{-6} dyn	[25]
w	Thickness coefficient of glycocalyx	$10^{-2} \mu\text{m}$	[25]

The profiles of receptor density on the free part of the membrane (normalized by the receptor density R_∞) are computed and shown by Fig.7.2a and 7.2b. Parameter $\tau = Dt$ represents the scaled time of spreading. The stiffness of the substrate and the distance are represented in dimensionless forms $\bar{k}_s = k_s/k_c$ and $\bar{r} = r\sqrt{R_\infty}$, respectively. Along the free part of a membrane adhered on a compliant substrate ($\bar{k}_s = 0.1$), receptors exhibit significantly difference density profiles compared with those adhered on a relatively stiff substrate ($\bar{k}_s = 10$). Comparison of receptor densities at the leading edges of the adhesion zones indicates a considerably larger R_+ value for the membrane adhered on the more compliant substrate. Since the diffusion of the receptors is mediated by the gradient in chemical potential ($\partial\chi(r, t)/\partial r$) between $r = a_+$ and $r \rightarrow \infty$, the adhesion zone is expected to grow slower on

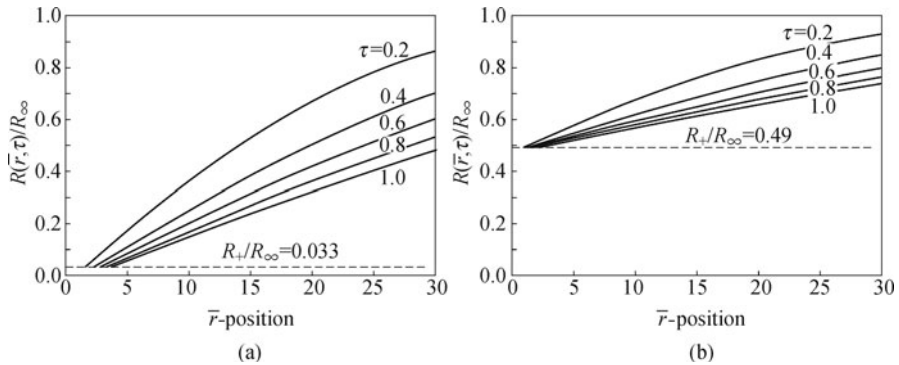


Fig. 7.2 Normalized density profile of the receptors, R/R_∞ , along the adhesive membrane interacting with (a) a stiff substrate with $\bar{k}_s = 10$ and (b) a soft substrate with $\bar{k}_s = 0.1$. The non-dimensional rigidity $\bar{k}_s$ is defined as the ratio of the substrate spring constant to that of the membrane. Profiles are shown at different snapshots after the initiation of adhesion. The distance along the membrane is represented in dimensionless form $\bar{r} = r\sqrt{R_\infty}$ and $\bar{\tau}$ is the dimensional elapsed time defined by $\tau = \sqrt{Dt}$. The dash lines show R_+/R_∞ , the (normalized) edge density of receptors. Comparison between the edge densities shows that the value of R_+ increases with decreasing substrate stiffness.

more compliant substrates. This is demonstrated by Fig.7.3a, representing the edge displacement of the adhesion zone as a function of time at different substrate rigidities. All graphs follow a square-root law dependence with time (i.e., $\bar{a} \sim \tau^{\frac{1}{2}}$); however, at any instant, the length of the adhesion zone gradually decreases on increasingly softer substrates. For $\bar{k}_s < 0.2$, the reduction of substrate rigidity significantly affects the edge displacement whereas for $\bar{k}_s > 1.0$ curves exhibit virtually identical values at each moment. This crossover in time-dependent length of adhesion zone is illustrated more clearly by Fig.7.3b, where the edge displacements at $\tau = 1.0$ are shown as a function of substrate rigidity. The sharp decrease of the edge displacement happens concurrently with an abrupt increase of the edge density of receptors for $\bar{k}_s < 0.2-0.3$, thereby reducing the gradient of chemical potential driving the membrane-substrate association. A threshold phenomenon of this kind has been reported in the recent experimental study on FA sites of myofibroblasts adhesion on silicone substrates^[29]. It was found that in general, the smaller size of myofibroblast FAs correlates with reduced stiffness of substrate. However, when the substrate stiffness is < 10 kPa, reduction of substrate stiffness leads to an abrupt decrease in the FA size. This crossover response is similar to the prediction of the presented model, shown by Fig.7.3b.

The areal density of ligand molecules is another controllable parameter in design of bio-adhesive substrates. It has been shown that the effect of stiffness can be coupled with adhesiveness of the substrate to upregulate the cellular

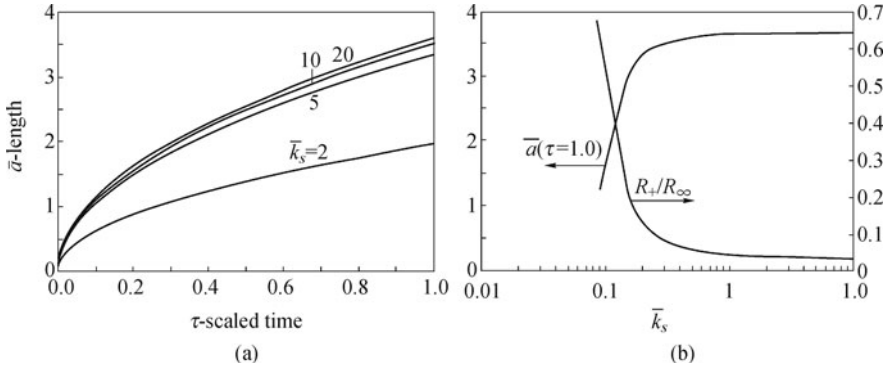


Fig. 7.3 (a) The effect of substrate rigidity on $\bar{a}(\tau)$, the (normalized) time-dependent length of the adhesion zone. (b) Variation of $\bar{a}(\tau = 10)$ and the edge density of receptors with the rigidity of the substrate.

adhesion. For example, measurement of the spreading area of smooth muscle cells on collagen coated poly(methyl methacrylate) gels shows that on relatively stiff substrates, the cell size exhibits much dependence on the changes in collagen density. On soft substrates, however, the variation of cell spread area is much less sensitive to bio-adhesivity of the substrate^[30]. To explore the possible concomitant effect of substrate stiffness and adhesivity on the growth of FA sites, we examine the model predictions for the length of an adhesion zone on a compliant substrate while the ligand density changes over a wide range. Figure 7.4 shows the variation of edge displacement with ligand density and substrate stiffness at $\tau = 1.0$. On relatively stiff substrates, increasing the ligand density is followed with the reduction of edge displacement at a given time. Such dependence between the edge displacement and ligand density is relatively insensitive to the stiffness of the substrate as long as $\bar{k}_s > 0.3$. For more compliant substrates, the edge displacement is smaller compared with that of a stiff substrate at similar ligand density and exhibits less sensitivity to the variation of ligand density.

The above results all indicate the significance of substrate elastic deformation in modulating the kinetics of membrane-substrate association, and suggest that the mechanism of rigidity sensing at bio-interfaces may be rooted, at least partly, in the nature of thermodynamic interactions between the substrates and changing the free energy landscape of the soft systems. As the compliance of the substrate increases, the elastic energy required to deform the substrate becomes increasingly important compared with (specific) adhesion energy. This leads to lower free energy for the closed system and consequently smaller gradient in chemical potential to drive the membrane spreading. This can be understood better by calculation of $j_+\chi_+$, the rate of energy transport by receptors across the advancing edge of the adhesion

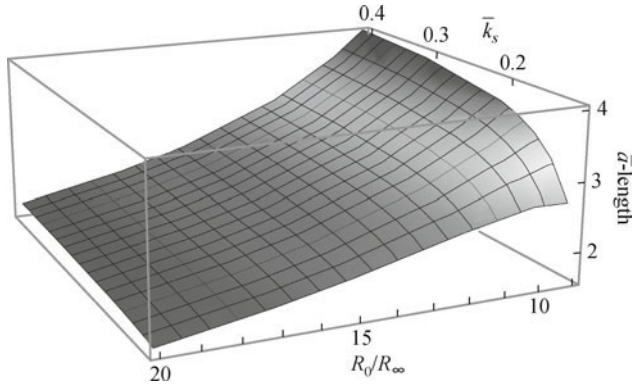


Fig. 7.4 Variation of the length of adhesion zone at a given time ($\tau = 1.0$) with the ligand density (R_0/R_∞) and rigidity of the substrate ($\bar{k}_s$). On relatively stiff substrates, increasing the ligand density is followed by the reduction of edge displacement, whereas on more compliant substrates the edge displacement is smaller and less sensitive to the variation of ligand density. The edge displacement of adhesion zone is independent of the rigidity of underlying substrate when the ligand density is high. The effect of substrate stiffness becomes more apparent as the surface adhesivity decreases.

zone, shown by Fig.7.5. The magnitude of energy transport per unit time decreases by increasing the compliance of the adhesive substrate, indicating the deceleration of growth process on softer substrates.

The proposed mechanistic scenario is supposed to regulate the early phase of FA growth on a compliant substrate; however, its effect can be envisioned to continue even after the adhesion sites mature into FA complexes by recruiting adaptor proteins from cytoplasm. Clustering of receptors in the FA site is accompanied by the assembly of the cytoplasmic plaque which leads to Rho-activation. Rho, a small GTPase protein, in turn activates the myosin II molecular motors, leading to increased tension in the actin cytoskeleton. This increased tension is accommodated by redistribution of receptors within the adhesion zone and increasing the size of the FA sites^[31]. The extracellular rigidity is expected to modulate this positive feedback loop. On very compliant substrates, the weaker thermodynamic driving force for receptor clustering (as described in this paper) may lead to weakening of the activation of Rho molecules and perhaps eventually cessation of the feedback loop.

There are several dynamic events which are not included in the proposed model which, in general, may affect the rate of membrane-substrate association. This includes the association and the dissociation rates of receptor-ligand bonds, the mobility of the repellers, and the Helfrich repulsion between the membrane and substrate. The kinetics of ligand-receptor associations is expected not to be a dominant process as long as the concentration of im-

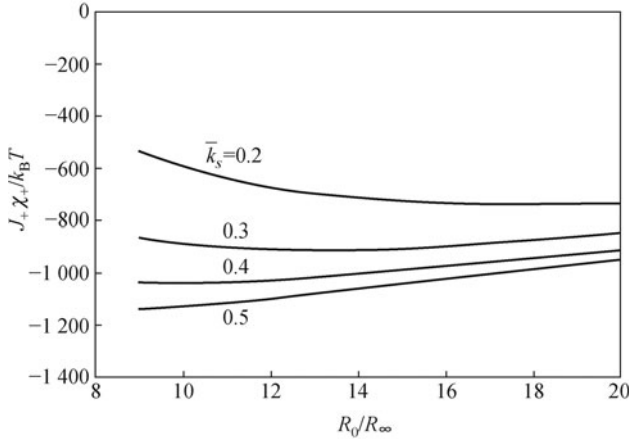


Fig. 7.5 The effect of ligand density (R_0/R_∞) and substrate rigidity ($\bar{k}_s$) on the rate of energy transport (per $k_B T$) by receptors across the advancing edge of the adhesion zone. The rate of energy transport is defined as $j_+ \chi_+$ where j_+ and χ_+ are the diffusive flux and the local chemical potential of receptors at the edge of adhesion zone, respectively. The magnitude of energy transport per unit time decreases by decreasing rigidity of the substrate. This implies that the growth rate of adhesion zone decreases on increasingly softer substrates.

mobilized ligands is much higher than that of mobile receptors. Under this condition, the diffusion time of receptors is much longer than the typical reaction time between a ligand and a receptor and hence, the growth of the adhesion zone is diffusion-dominated^[21]. The mobility of repellers can be accounted for simply by considering an extra entropic component in the free energy landscape, similar to the mobility of receptors. If the repulsion between the repeller molecules and substrate is large, the repellers are expected to substantially redistribute themselves within the adhesion zone, in order to facilitate the adhesion^[32]. This may significantly change their contribution in the free energy and their ability to deform the membrane and compliant substrate.

Finally, some comments are necessary with regard to the predictions of the presented model in comparison with that of Nicolas et al. ^[33]. Their mechanistic model is focused on the response of FA sites to cytoskeletal forces exerted by stress fibers (assuming that primary adhesion already exists). The major outcome of their model is analogous to the underlying mechanism for rigidity sensing of cells as described here. Their model demonstrates that the growth of FAs is a process that releases energy and is driven by the balance between the exothermic adsorption of new receptors and the mechanical cost of elastic deformations of the junction induced by cytoskeletal forces. Hence, reduction of substrate stiffness makes this thermodynamically driven process costly

and leads to reduction or even cessation of growth process. This conclusion is similar to what our kinetic model suggests. The main difference, however, is the origin of the mechanical force transmitted to the adhesion area. In their model that is the force actively exerted by actin filaments while in the model presented here, the substrate is deformed due to the non-specific forces separating the membrane from the adhesive substrate.

7.4 Conclusion

In this paper, we present a thermodynamic model which evaluates the effect of substrate rigidity on the dynamics of membrane spreading and growth of FA zone. We have shown that the growth rate of the adhesion zone is very sensitive to both rigidity and adhesivity of the substrate. In general, the growth rate decreases as the compliance of the substrate increases. The effect of substrate rigidity on the adhesion zone is coupled with adhesivity of the substrate and diminishes on more adhesive substrates. The model predictions suggest that the mechanistic pathways inherent to membrane-substrate thermodynamic interactions can be important to mediate the rigidity sensing by cells.

Acknowledgements

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Chapter 8 Opto-Hydrodynamic Trapping for Multiaxial Single-Cell Biomechanics

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Abstract: In this chapter, a unique experimental platform is described for singlecell biomechanics. An optical tweezer with minimal applied laser power has successfully suspended biologic cells at the geometric center of a microfluidic crossjunction. The resulting flow environment creates a unique multiaxial load application to isolated cells with site-specific normal and shear stresses resulting in a physically extended state. Computational fluid dynamics combined with multiphysics modeling has characterized the fluid environment and solid cellular strain response in three-dimensions to accompany experimental cell stimulation. These models will guide future microfluidic experiments as well as provide a framework for characterizing cytoskeletal structures influencing the stress on strain response.

Keywords: multiphysics, optical tweezer, microfluidics, cellular stress and strain, mechanotransduction

8.1 Introduction

In an approach to studying single-cell biomechanics, isolated cellular measurements require methods capable of suspending an individual cell for repeated dynamic manipulation of the entire membrane surface. Current available technologies include dielectrophoretic (DEP) traps creating a capturing force by acting on cell polarization induced in an oscillating electrical field^[1], acoustic tweezers (AT) applying an ultrasonic standing wave to create a pressure node attracting particles or cells^[2], and hydrodynamic tweezers (HT) suspending single cells with only secondary hydrodynamic forces^[3]. Advancements in laser technology have facilitated a popular means of manipulating isolated single cells, that of optical tweezers or traps (OT)^[4]. Distinct loads can be applied experimentally to single cells in culture to quantify cellular, membrane, and cytoskeletal biomechanics. This type of intracellular

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‘holding’ can apply forces in extracellular microenvironments on the order of 100 pN with resolution smaller than one picoNewton ($1 \text{ pN} = 10^{-12} \text{ N}$)^[5,6]. All of these currently available trapping methods apply conditions outside the physiologic range for cells, making their influence on cell behavior up for debate and a topic of continued investigation.

Direct or partial assessment of cellular stress and strain through non-contacting techniques have included the application of a rheoscope, which examined red blood cell properties by measuring blood viscosity as a function of cell deformation and cell aggregation^[7]. Applying the rheoscope and an ektacytometer, elliptocytic red blood cell deformation under varying shear stresses was also explored^[8]. However, these approaches examine cells as an aggregate slurry.

Through alternative optical tweezer design and associated technologies, novel testing of isolated cells with and without physical contact has become available. In an optical channel, hydrodynamic stresses caused elongation in red blood cells positioned within a focused beam^[9]. For optical stretching, a cell can be held directly^[5] or with attached beads^[10], and stretched through optically generated forces.

With innovations in microscale fabrication, microfluidic devices create opportunities to study dynamic mechanical behavior of individual cells under controlled conditions. These approaches foster experimental and mathematical platforms for relating mechanical perturbation to biological response (mechanotransduction) as well as defining biometrics for disease assay^[11,12]. Through fluid mechanics and optical physics, flow-based mechanical test sequences (applied shear and normal stresses) provide control of unique microenvironments when coupled with single-cell suspension techniques. This chapter describes the combined platform available through optical and hydrodynamic trapping as advancement in single-cell biomechanical testing.

8.2 Optical-hydrodynamic trapping

8.2.1 Optical physics and microfluidics

Single cell manipulation is now available by combining optical trap and microfluidics technologies. A novel instrument was recently co-developed by the present author and others which integrates two laser-based techniques for manipulating and characterizing the mechanical environment adjacent to cellular and biomolecular structures^[13,14].

Briefly, the optical tweezers or trap applies an infrared laser ($\lambda = 1\,064 \text{ nm}$) to suspending micron-sized objects with nanometer position control and constraining forces on the order of a pN. For translucent objects larger in dimension than the wavelength of the trapping laser (such as individual biolog-

ical cells), a ray optics approach indicates that the rays of light are refracted as they pass into and out of the trapped cell. This change in direction and the associated momentum changes impart an equal and opposite force on the “trapped” object^[15].

Micron-resolution particle image velocimetry is a high-resolution, two-dimensional full-field velocity measurement technique. Flow velocity measurements are obtained by seeding the flow domain with 275 nm diameter fluorescent nanoparticles, volume illuminating the region of interest with pulses from two frequency-doubled lasers ($\lambda = 532$ nm) near the nanoparticle excitation wavelength ($\lambda = 535$ nm), and imaging the emitted light ($\lambda = 575$ nm). The lasers are synchronized with a charge-coupled device camera so that the emitted particle light from each laser pulse is captured on consecutive image frames. The resulting image pairs are cross-correlated through ensemble averaging to obtain the resulting velocity field^[16].

As an enhancement to the optical trapping capabilities, a microfluidic chip configured with a silicone mold was prepared in the form of a cross-junction channel (Fig.8.1). This geometry created a hydrodynamic trapping environment with extensional flow and a stagnation point at the geometric center. A cell may be positioned at the channel centroid with optical trapping, thus cre-

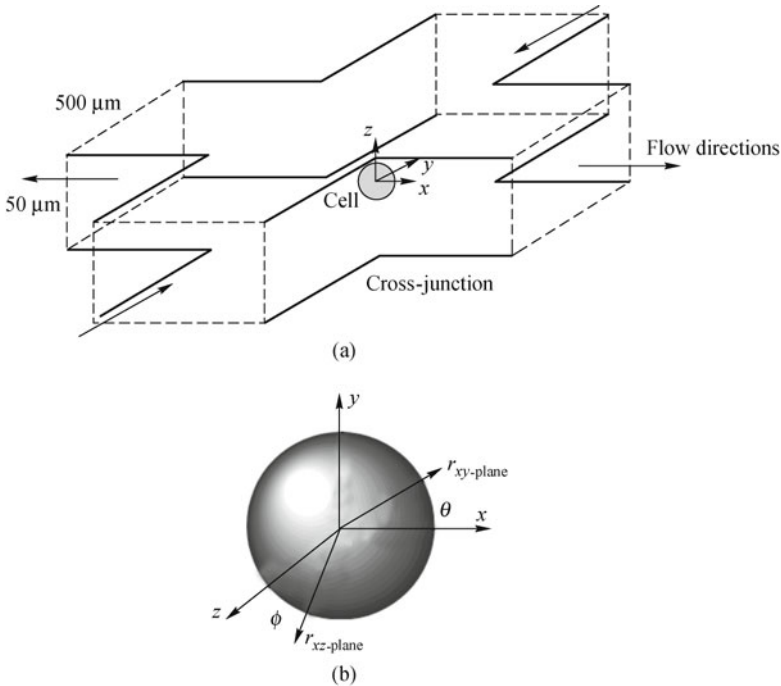


Fig. 8.1 Schematics of (a) microchannel configuration creating a hydrodynamic trap through multiaxial fluid loading and (b) relationship between Cartesian ($x - y - z$) and spherical polar ($r - \theta - \phi$) coordinate systems ^[14].

ating an opto-hydrodynamic trap. In this environment, the cell experiences a total drag force equal to zero, confirmed by integrating the stress tensor as defined by the normal (form drag) and shear (friction drag) stresses^[14]. This reflects the stability or saddle-point nature of the cross-junction's geometric center. Chips may be fabricated using a standard soft lithography approach using polydimethylsiloxane (PDMS) as the soft peeled material^[17]. PDMS is bonded to a glass cover slip, after having punched ~ 0.9 mm diameter holes for microport access. Here, input and output open reservoirs (syringes) were placed at different head-heights generating a gravity-driven flow. The output syringe height was held constant while the input syringe height was controlled in order to vary the flow rate.

8.2.2 Theoretical stress analysis

In the following mathematical development, bold upper-case letters indicate tensors while bold lower-case letters indicate vectors. Here, the three-dimensional stress state is characterized as applied to the surface of a non-rotating spheroid cell of radius a , optically suspended in the linear extensional flow field within the hydrodynamic trap as described in Section 8.2.1. For a non-rotating sphere suspended in a general linear flow with viscosity μ , the fluid velocity field vector $\mathbf{u}$ is^[18]:

$$\mathbf{u} = \mathbf{E} \cdot \mathbf{x} \left(1 - \frac{a^5}{r^5}\right) + \frac{1}{2}(\boldsymbol{\omega} \times \mathbf{x}) \left(1 - \frac{a^3}{r^3}\right) - \mathbf{x}(\mathbf{x} \cdot \mathbf{E} \cdot \mathbf{x}) \left(\frac{5}{2} \frac{a^3}{r^5} - \frac{5}{2} \frac{a^5}{r^7}\right)$$

including the corresponding pressure distribution:

$$p = p_\infty - 5\mu(\mathbf{x} \cdot \mathbf{E} \cdot \mathbf{x}) \left(\frac{a^3}{r^5}\right)$$

For the specific linear case of planar extensional flow (vorticity vector defined as $\boldsymbol{\omega} = \mathbf{0}$), the strain rate tensor $\mathbf{E}$ is reduced to that defined by the shear rate magnitude $\dot{\gamma}$ along the matrix diagonal:

$$\mathbf{E} = \frac{U}{a}(\mathbf{i}\mathbf{i} - \mathbf{j}\mathbf{j}) = \begin{bmatrix} \dot{\gamma} & 0 & 0 \\ 0 & -\dot{\gamma} & 0 \\ 0 & 0 & 0 \end{bmatrix}.$$

Using spherical coordinates for the general position vector $\mathbf{x}$ (Fig.8.1), the equational components of the fluid velocity vector are defined as:

$$\mathbf{E} \cdot \mathbf{x} = \frac{Ur}{a} \sin \phi (\sin \phi \cos 2\theta \mathbf{e}_r + \cos \phi \cos 2\theta \mathbf{e}_\phi - \sin 2\theta \mathbf{e}_\theta)$$

and

$$\mathbf{x} \cdot \mathbf{E} \cdot \mathbf{x} = \frac{Ur^2}{a} \sin^2 \phi \cos 2\theta.$$

Substituting these expressions into the generalized flow field gives the individual flow velocity components:

$$\begin{aligned}\mathbf{u} = & U \left[\frac{r}{a} - \frac{5}{2} \left(\frac{a}{r} \right)^2 + \frac{3}{2} \left(\frac{a}{r} \right)^4 \right] \sin^2 \phi \cos 2\theta \mathbf{e}_r \\ & + U \left[\frac{r}{a} - \left(\frac{a}{r} \right)^4 \right] \sin \phi \cos \phi \cos 2\theta \mathbf{e}_\phi \\ & + U \left[\frac{r}{a} - \left(\frac{a}{r} \right)^4 \right] \sin \phi \cos 2\theta \mathbf{e}_\theta\end{aligned}$$

as well as the pressure distribution not previously included in a two-dimensional stress analysis^[14]:

$$p = p_\infty - \frac{5\mu U}{a} \left(\frac{a}{r} \right)^3 \sin^2 \phi \cos 2\theta$$

These expressions now describe the fluidic environment surrounding the isolated and suspended cell. Translating the velocity gradients into applied mechanics, the constitutive equation for an incompressible, Newtonian fluid states that:

$$\mathbf{T} = -p\mathbf{I} + 2\mu\mathbf{E}$$

where $\mathbf{T}$ is the stress tensor and $\mathbf{I}$ is the identity matrix associated with the local isotropic (hydrostatic) pressure distribution p . $\mathbf{E}$ can be further represented by the flow velocity gradient tensor and its transpose, noted by the superscript T:

$$\mathbf{E} = \frac{1}{2}[\nabla\mathbf{u} + \nabla\mathbf{u}^T].$$

Again, separating the polar components of the volumetric stress state produces the following normal and shear stress definitions:

$$\begin{aligned}\mathbf{T}_{rr} = & -p_\infty + \frac{\mu U}{a} \left[2 + 15 \left(\frac{a}{r} \right)^3 - 12 \left(\frac{a}{r} \right)^5 \right] \sin^2 \phi \cos 2\theta \mathbf{e}_r, \\ \mathbf{T}_{r\phi} = & \frac{\mu U}{a} \left[2 - 5 \left(\frac{a}{r} \right)^3 + 8 \left(\frac{a}{r} \right)^5 \right] \sin \phi \cos \phi \cos 2\theta \mathbf{e}_\phi, \\ \mathbf{T}_{r\theta} = & -\frac{\mu U}{a} \left[2 - 5 \left(\frac{a}{r} \right)^3 + 8 \left(\frac{a}{r} \right)^5 \right] \sin \phi \sin 2\theta \mathbf{e}_\theta.\end{aligned}$$

Evaluating the stress tensor at the cellular surface, $r = a$, gives the following volumetric stress state on the cellular boundary:

$$\mathbf{t}_r = \mathbf{T}\mathbf{e}_r = \sigma_{rr}\mathbf{e}_r + \tau_{r\phi}\mathbf{e}_\phi + \tau_{r\theta}\mathbf{e}_\theta$$

where

$$\begin{aligned}\sigma_{rr} = & -p_\infty + \frac{5\mu U}{a} \sin^2 \phi \cos 2\theta, \\ \tau_{r\phi} = & \frac{5\mu U}{a} \sin \phi \cos \phi \cos 2\theta, \\ \tau_{r\theta} = & -\frac{5\mu U}{a} \sin \phi \sin 2\theta\end{aligned}$$

are the applied normal (σ_{rr}) and shear ($\tau_{r\phi}$, $\tau_{r\theta}$) stresses in three-dimensions. This resulting stress distribution is represented in both two- and three-dimensions where peak polar coordinate stresses are aligned with the Cartesian coordinates (Fig.8.2).

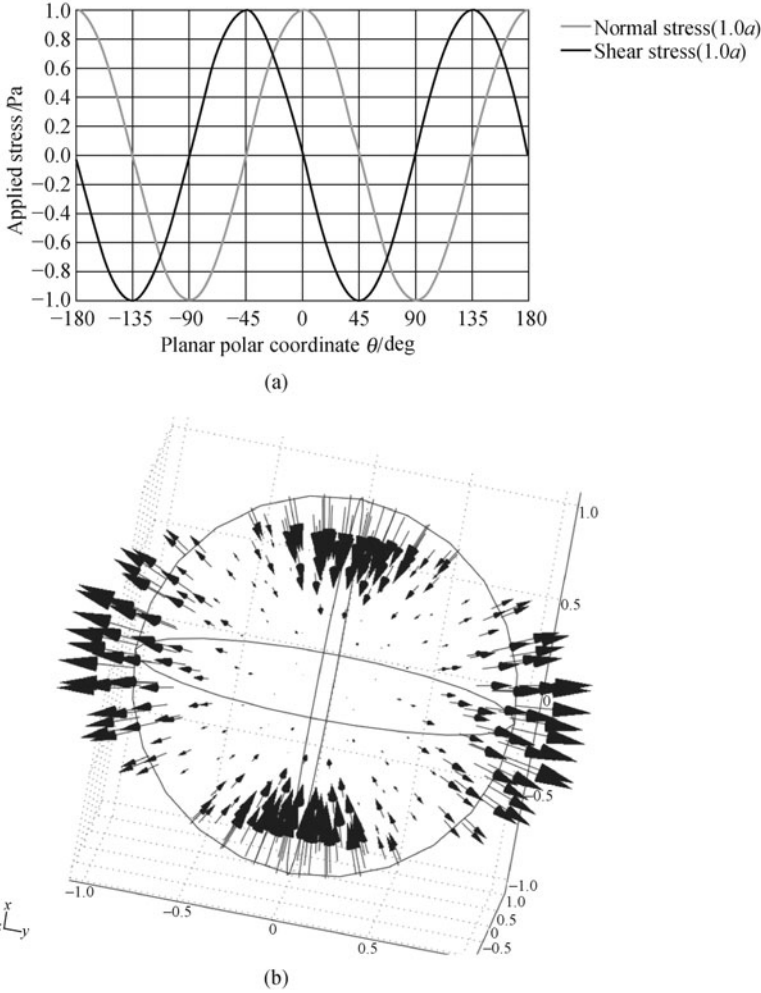


Fig. 8.2 Modeled (a) two-dimensional and (b) three-dimensional stress patterns applied to suspended cells optically and hydrodynamically trapped in an extensional flow with peak stresses similar to monolayer studies (~ 1 Pa)^[23,37]. The planar polar coordinates ($r = 1.0a$ and θ) are defined in the $x - y$ plane at $z = 0$. The three-dimensional normal stresses are isolated for clarity on the full cellular surface (shown as a sphere before deformation, color plot in the book end).

Principal stresses were then determined for the applied perimeter stress states as extended to intracellular elements representing specific cellular zones

(Fig.8.3). The principal stresses describe internal or whole-cell elements with a rotated perspective, which minimizes or eliminates local shear stresses. Maximum and minimum principal stresses act on mutually perpendicular planes, and are the algebraically largest and smallest normal stresses at a point in the described stress fields^[19]. Although the cellular biomechanical microenvironment is in a three-dimensional stress state, the two-dimensional maximum ($\sigma_1 = \sigma_{\max}$) and minimum ($\sigma_2 = \sigma_{\min}$) normal stresses are obtained from the following equation:

$$\sigma_{1,2} = \frac{\sigma_x + \sigma_y}{2} \pm \sqrt{\left(\frac{\sigma_x - \sigma_y}{2}\right)^2 + \tau_{xy}^2}$$

when expressed in Cartesian coordinates. Translating key stresses into polar

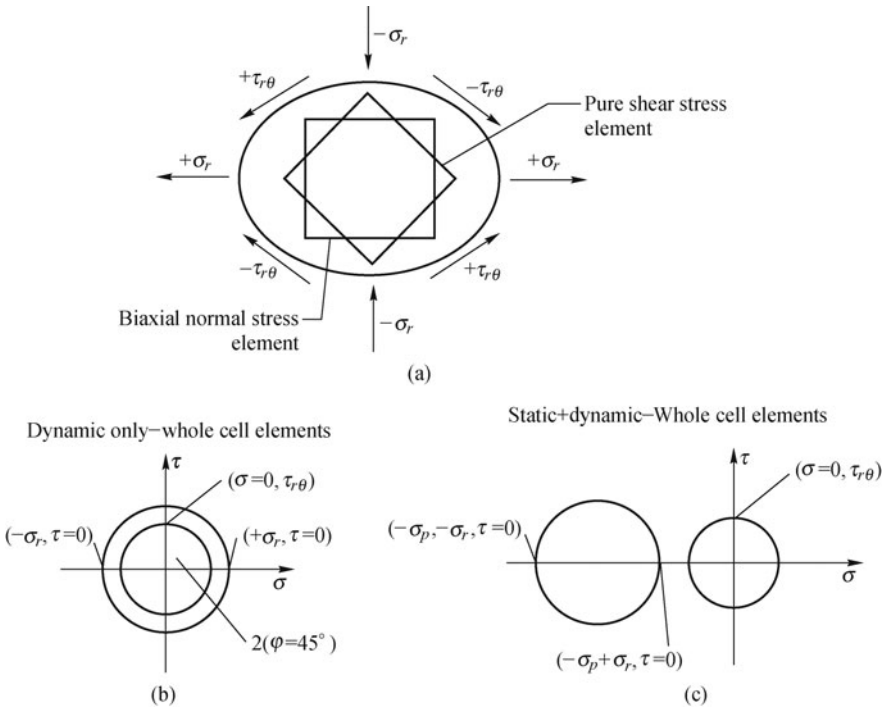


Fig. 8.3 (a) Localized theoretical elastic elements representing the applied stresses of the multiaxial flow field^[14]. Only the hydrodynamic state is shown with mild deformation into an ellipsoid shape: oblate, prolate or scalene spheroids. Mohr's circle characterizations of the two-dimensional normal (σ_r with or without σ_p) and shear ($\tau_{r\theta}$) stresses with conversion into planar principal stresses (σ_1 and σ_2) and maximum shear stress ($\tau_{\max}$) for (b) hydrodynamic only and (c) hydrostatic plus hydrodynamic cross-junctional channel flow conditions (axes not drawn to scale). The rotation of the element required to minimize or maximize shear stress is also shown (φ). Principal strains (ε_1 and ε_2) would be coincident with principal stresses and the $x - y$ coordinates.

coordinates with the cell positioned at $z = 0$ indicates:

$$\sigma_x = \sigma_r|_{\theta=0^\circ, \pm 180^\circ}, \quad \sigma_y = \sigma_r|_{\theta=\pm 90^\circ} \quad \text{and} \quad \tau_{xy} = \tau_{r\theta}.$$

The principal stress analysis was expressed graphically through a Mohr's circle construction (Fig.8.3) where the graphed circular components are identified as the center σ_c , projected segment s , and radius R , defined as:

$$\sigma_c = \frac{\sigma_x + \sigma_y}{2}, \quad s = \frac{\sigma_x - \sigma_y}{2} \quad \text{and} \quad R = \sqrt{s^2 + \tau_{xy}^2}.$$

The rotation of an internal or whole-cell principal element representing an orientation minimizing (φ) or maximizing ($\varphi + 45^\circ$) shear stress is then calculated as:

$$2\varphi = \arctan \frac{\tau_{xy}}{s}.$$

This overall analytical approach facilitates the prediction of magnitudes and orientations of the applied stresses and resulting cellular strains within the opto-hydrodynamic trap during single-cell biomechanics.

8.2.3 Experimental and computational flow validation

In experimental validation efforts, a 20.6 μm diameter microsphere was optically trapped in a 50 $\mu\text{m} \times 500 \mu\text{m}$ water-filled microchannel cross-junction fabricated in a similar manner just described^[14]. The radius of curvature at each of the channel wall intersections was maintained at 200 μm for laminar cornering flow. With a pressure head of 24.5 mm and $p_\infty = -239.5$ Pa, a gravity driven flow was generated with a far-field channel, centerline mid-plane velocity of 750 $\mu\text{m/s}$ and a local hydrostatic pressure of $p = \frac{1}{2}p_\infty = -119.75$ Pa.

Particle image velocimetry confirmed the extensional flow state predicted by the analytical solution (Fig.8.4). This experimental arrangement produced a mid-plane shear rate of $\dot{\gamma} = 12.4$ strain/s at the cross-junction (determined without microsphere perturbation) and thus a characteristic flow velocity ($U = \dot{\gamma}a$) of 128 $\mu\text{m/s}$ where “ a ” is the radius of the sphere. Experimental velocity gradients were assessed with linear regression and consistently indicated a strong linear fit ($|R| > 0.90$). Regression analysis compared experimental with theoretical stresses at coincident locations throughout the examined spherical perimeter region, indicating high correlations ($R^2 = 0.927$ to 0.974).

To assist in the validation of the defined stresses and extend the analysis beyond the capabilities of theoretical assumption, a two-dimensional computational model made a quantitative analysis of the cross-junction flow^[20]. With the measured local flow velocities as input, the flow scenario was modeled with computational fluid dynamics (CFD) software (mesh creation in

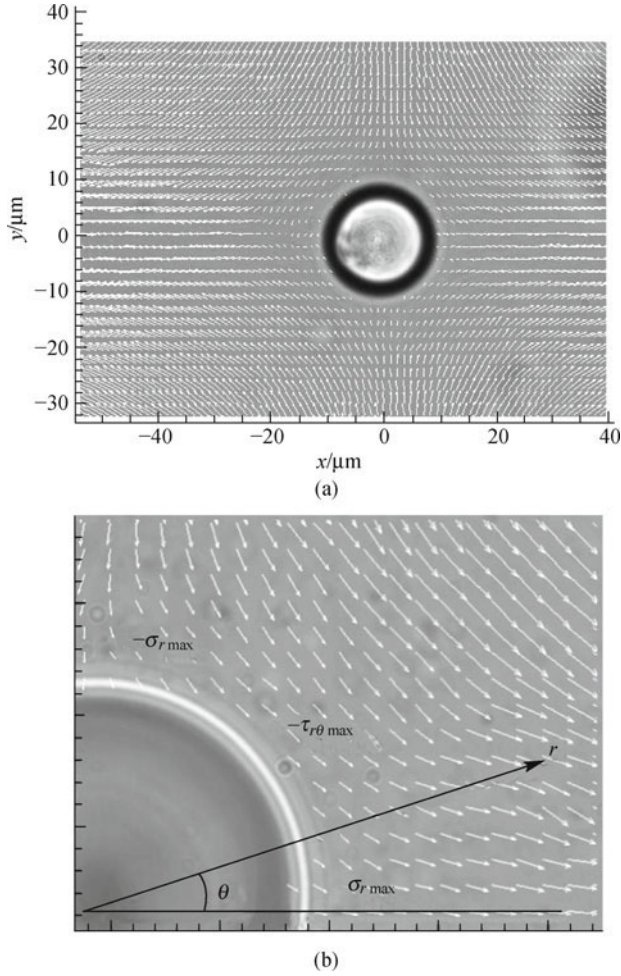


Fig. 8.4 Two-dimensional experimental flow velocities in (a) full-field and (b) isolated quadrant views surrounding an analogous cell (20.6 μm diameter polystyrene microsphere) as measured with micron-resolution particle image velocimetry^[14,26].

STAR-Design and solution computation in STAR-CCM+, CD-adapco, Seattle, WA). Symmetry of the cross-junction geometry reduced the computational domain to a quadrant section. Individual mesh properties were adjusted by setting a maximum triangle size and surface thickness while constructing a composite mesh. The mesh resolution on and around the sphere was constructed to exceed the resolution of the experimental velocimetry data, particularly on the $x - y$ symmetry plane at the channel center coincident with the flow stagnation point ($x, y, z = 0$). The resulting flow field velocities were generated by iteratively solving components within the Navier-Stokes and continuity equations, identifying the final solution based on a minimum

residual (Fig.8.5a). This computational modeling approach is now standard practice in CFD calculations^[21].

Initial three-dimensional CFD models were also constructed to character-

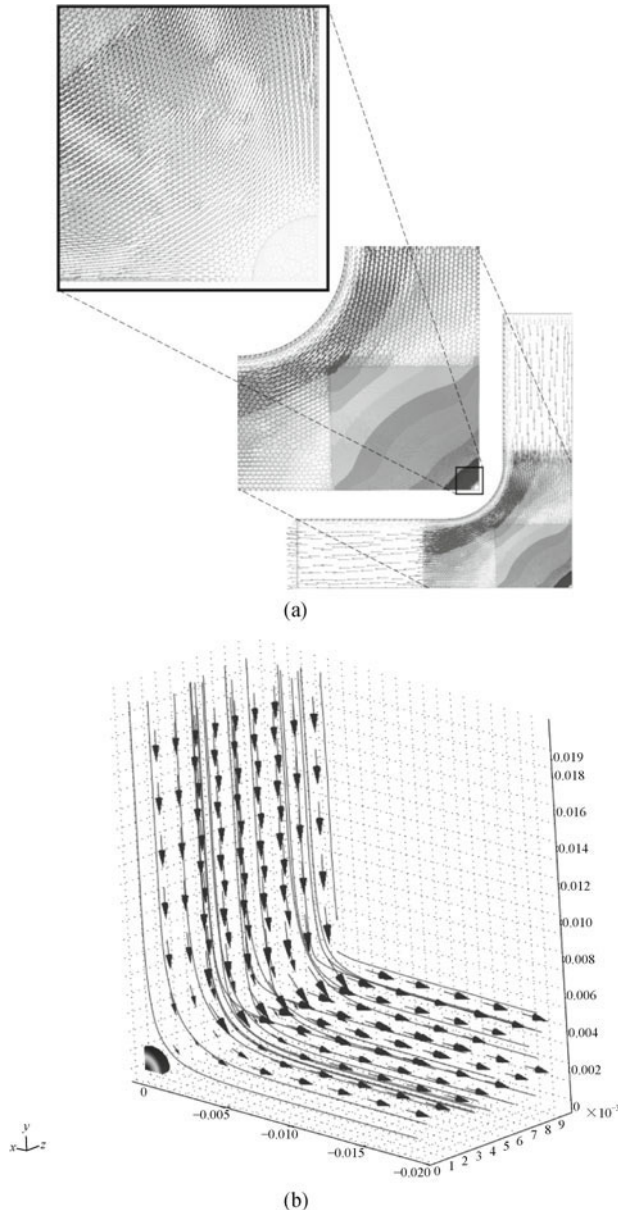


Fig. 8.5 Computationally derived (a) two-dimensional and (b) three-dimensional localized flow velocities in an opto-hydrodynamic trap^[20,22] (color plot in the book end).

ize and validate the influence of the z -direction flow, which would influence shear stresses in that orientation. This volumetric analysis was completed using multiphysics software (COMSOL v3.5a, COMSOL, Inc., Palo Alto, CA) and was applied to an octant section as a reduction due to symmetry (Fig.8.5b). This effort supports an ongoing effort in modeling the influence of microfluidics on cellular stress and strain^[22,23] in three-dimensions.

All of the computational models showed good qualitative and quantitative agreement with the experimental flow data and in turn the velocity gradients determined in the theoretical stress analysis. A representative quantitative comparison of predicted and measured velocities along the inflow axis for a uniform flow state yielded a correlation of $R^2 = 0.988$ and a root mean square error of $\text{RMS} = 13.04 \mu\text{m/s}$.

8.2.4 Applied stresses and strain response

In multiphysics stress-strain analyses, a solid mechanics modeling approach was applied in two- and three-dimensions (COMSOL v3.5a). A range in magnitude of locally applied stresses was included as boundary condition (100 mPa to 10.0 Pa) for a central cell section (872 nm thick slice at $z = 0$) as well as a whole cell modeled with a spectrum of mechanical properties representing healthy and diseased states (elastic moduli of $E = 0.1$ to 5.0 kPa and a Poisson ratio of $\nu = 0.4$). With this modeling approach, dominant hydrostatic stresses were separated from the more spatially sensitive hydrodynamic stresses. Separated strain responses to normal and shear stresses were also characterized regarding their individual contributions via superposition.

Multiaxial stress and strain analyses initially included the calculation of strain energy density (SED) by integrating the local multiaxial stress state over the resulting strains to achieve the relationship (shown here in Cartesian coordinates for two-dimensions)^[19]:

$$\text{SED} = \frac{1}{2E} (\sigma_x^2 + \sigma_y^2 - 2\nu\sigma_x\sigma_y) + \frac{\tau_{xy}^2}{2G}$$

incorporating linear elastic moduli, E and $G = E/2(1 + \nu)$, and the Poisson ratio ν . This relationship was further integrated over the volume of the cellular section (20 μm diameter $\times$ 872 nm thickness) as associated with twice the experimental planar (z -direction) resolution of the particle image velocimetry technique^[24].

Site-specific strain patterns due to a 1.0 Pa stress amplitude were characterized within the modeled single cells at a deformation resolution of ~ 100 nm (Fig.8.6). A characteristic flow velocity of $U = 2\,077 \mu\text{m/s}$ would be required within the hydrodynamic trap for this peak stress level producing a shear rate of $\dot{\gamma} = 202$ strain/s. Volumetric cellular strain energy density was calculated to characterize the influence of varying elastic modulus as a metric for disease assay (Fig.8.7) where moduli with lower orders of magnitude

have been indicated for diseased cells in comparison with healthy cells^[25]. This stress-strain modeling approach identified initial cellular biomechanical boundary conditions in terms of applied mechanics and material properties.

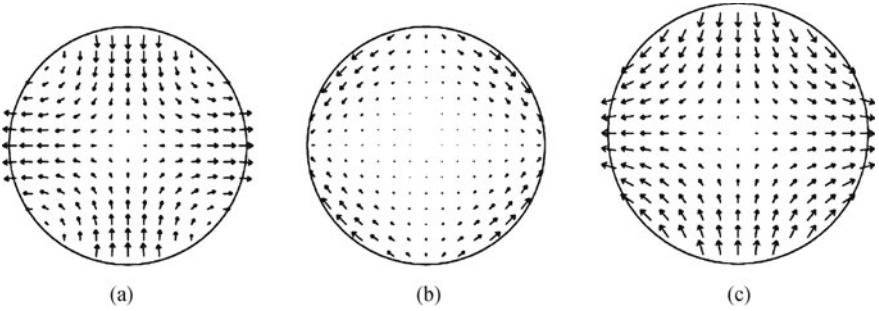


Fig. 8.6 Strain superposition within a modeled cellular section ($20\text{ }\mu\text{m}$ diameter $\times$ 872 nm thickness, with a 0.5 kPa elastic modulus) due to applied 1.0 Pa amplitude hydrodynamic trapping stresses^[37]. (a) Normal stress-induced strains in tension or compression (b) plus shear-induced strains equal (c) the total cellular strain state, where arrow-length represents magnitude.

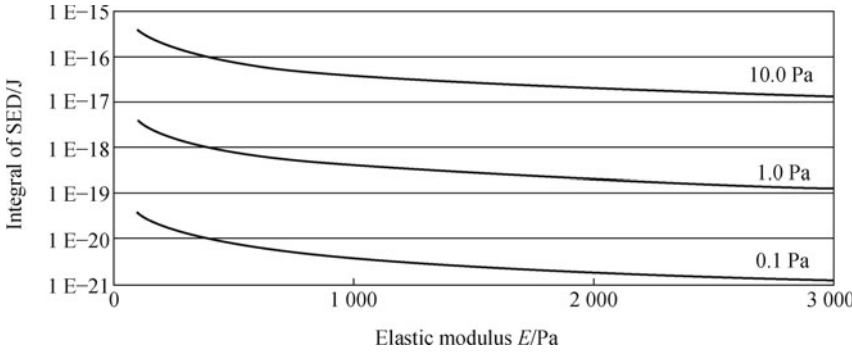


Fig. 8.7 The relationship between strain energy density (SED) integrated over a biological cell's sectioned volume due to a range of cellular elastic moduli. Applied hydrodynamic trapping stresses of 0.1 Pa , 1.0 Pa , and 10.0 Pa in amplitude are shown. For each order of magnitude increase in applied stress, the integrated SED increases by two orders of magnitude with similar curve shapes.

8.2.5 Multiaxial single-cell biomechanics

As a key component in the ongoing investigation of applied opto-hydrodynamic traps, living non-adhered, suspended osteoblasts, chondrocytes, fibroblasts, and myoblasts have been isolated and mechanically ma-

nipulated (Fig. 8.8)^[14,26,27]. Primary cultures of chondrogenic and osteogenic tissues were generated from rat long bones. The muscle cells were mouse-derived myoblasts (C2C12 cell line obtained from ATCC, CRL-1772; Manassas, VA). Cells were cultured in tissue culture flasks using α -MEM (Cellgro, Herndon, VA) supplemented with 10% fetal bovine serum (GIBCO, Gaithersburg, MD), 2% glutamine, 1%–2% penicillin-streptomycin (Cellgro), and 2% 1 M HEPES under standard culture conditions^[24] (37°C, 5% CO₂, pH = 7). Cells directly harvested from the cartilage tissue consisted mainly of chondrocytes (rounded cells) and chondroblasts (attached). Osteogenic cultures exhibited firmly attached osteoblasts with a typical epithelial morphology. Myoblasts were firmly attached elongated cells that retained the ability to form myotubes, if exposed to tissue culture medium with reduced serum content (2%). Harvested cells were diluted (10 000 cells/mL) in a solution containing 50 mL of artificial cerebrospinal fluid, 1 mL of HypoThermosol FRS (BioLife Solutions, Bothell, WA), and one micro-molar of EDTA to avoid clustering. Cells were tested at room temperature for all experiments ($\sim 20.5^\circ\text{C}$). The flow media consisted of a physiological buffer (127 mM NaCl, 5 mM KCl, 2 mM MgCl₂, 0.5 mM Na₂H PO₄, 2 mM CaCl₂, 5 mM NaHCO₃, 10 mM glucose, 10 mM HEPES, 0.1% BSA adjusted to pH 7.4).

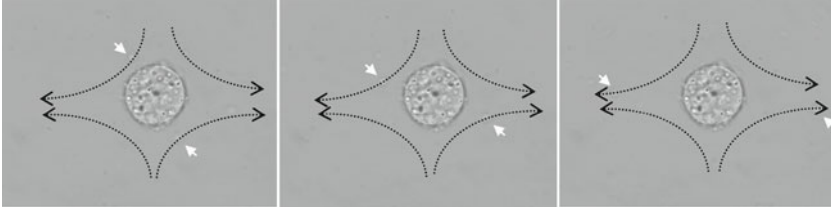


Fig. 8.8 Representative time-lapse image sequence of a living rat osteoblast suspended within the geometric center of the opto-hydrodynamic trap^[14,26]. The dash arrows follow the streamline path of a particle within the culture media as indicated by the white arrows.

Quantifying the mechanical state through three-dimensional stress versus strain relationships, the six deviatoric stresses were combined as an effective stress value, σ_{eff} ^[19]:

$$\sigma_{\text{eff}} = \frac{1}{\sqrt{2}} \left[(\sigma_x - \sigma_y)^2 + (\sigma_y - \sigma_z)^2 + (\sigma_z - \sigma_x)^2 + 6 (\tau_{xy}^2 + \tau_{yz}^2 + \tau_{zx}^2) \right]^{\frac{1}{2}}.$$

Volumetric strain e was defined to encompass the full-field deformation response of the cell to the multiaxial fluidic loads based on the strain invariants I_i , which coincide with the Cartesian axes as determined from the Mohr's circle analysis^[28]:

$$e = I_1 + I_2 + I_3 = \varepsilon_x + \varepsilon_y + \varepsilon_z + \varepsilon_x \varepsilon_y + \varepsilon_y \varepsilon_z + \varepsilon_z \varepsilon_x + \varepsilon_x \varepsilon_y \varepsilon_z.$$

Since the experimental approach is a planar measurement technique, the off-axis strain value ε_z can be determined through a transposition of the

planar loading scenario, again through a Mohr's circle analysis^[29]:

$$\varepsilon_z = -\frac{\nu}{1-\nu}(\varepsilon_x + \varepsilon_y).$$

The applied deviatoric stress state was related to volumetric strain and each of the individual principal strains (Fig.8.9). In addition, the droplet deformation strain $\varepsilon_{\text{drop}}$, a ratio incorporating the major ($A = 2a$) and minor ($B = 2b, C = 2c$) axes, was calculated:

$$\varepsilon_{\text{drop}} = \frac{A - B}{A + B}.$$

Under suspension and hydrostatic loading, the cell's volume can be defined as a sphere, $V = 3/4\pi abc$, where radii $a = b = c$. However, in the extensional loading state with applied hydrodynamics, the cell deforms into an oblate spheroid ($a = b < c$), a prolate spheroid ($a = b > c$), or a scalene ellipsoid ($a \neq b \neq c$). It makes intuitive sense that the resulting shape would replicate a scalene ellipsoid due to different maximum stresses applied in the three orthogonal axes. Ongoing experiments and models will attempt to clarify this multiaxial biomechanical response.

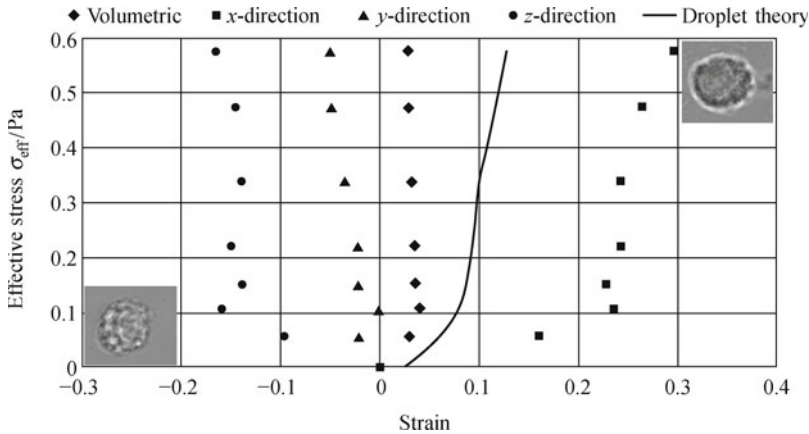


Fig. 8.9 Volumetric stress versus strain of a trapped myoblast. Volumetric strains are equivalent to that of the fractional change in volume of the spherical cell as well as those calculated with the individually plotted principal strains. Strain due to droplet deformation theory is shown for comparison^[27].

8.3 Discussion

Advancements in technologies for assessing biomechanics at the cellular level have led to discoveries in the relationship between mechanics and biology

(mechanotransduction). The aim of the described work is to directly measure cell biomechanical properties and provide a more complete knowledge of individual cellular biomechanics. Outcomes from this effort have included the continued validation of a novel instrument and the formation of a database archiving cellular biomechanical properties.

Although the opto-hydrodynamic trap provides a novel approach to cell biomechanics, significant limitations were identified in the presented instrument as currently designed^[13]. These limitations include:

Low applied stress levels: The described optical trap and microfluidic devices are currently only capable of applying mPa stresses ($\times 10^{-3}$ Pa) to cells with typical kPa stiffness ($\times 10^3$ Pa). Current cell biomechanics studies apply stresses on the order of a Pa^[30] and living joints experience healthy stresses of MPa ($\times 10^6$ Pa). As presented here in myoblasts (Fig.8.9), cells tested at low stress levels may demonstrate incompressible behavior. This phenomenon has also been demonstrated in osteoblast-like cells, suspended in media under hydrostatic pressures up to 7 MPa^[31].

Lack of cell incubation microenvironment: Environmentally sensitive cells need to be kept alive over lengthy time periods before, during, and after testing. A thermofluidic heat transfer analysis was conducted in the design of microscope stage incubator^[32]. Preliminary experiments identified a strategy for heating within the microfluidic device itself. Thin-film resistive heaters provided a suitable heating solution. Manufacture of prototype on-chip resistors is ongoing via Physical Vapor Deposition using an Electron-Beam Evaporator.

Non-adhered cell testing: Cells are typically adhered to surfaces in living physiologic environments. This interaction encourages normal cellular communication and biologic activity. While the suspended cell testing approach provides full-surface access to individual cells resulting in unique volumetric data, it is not physiologic. Ongoing efforts are exploring microfluidic configurations with biocompatible wall-surface characteristics (roughness, chemistry, etc.) as a means to enhance the biologic relevance of opto-hydrodynamic trapping.

Toxic nanoparticles: The blend of nanoparticles applied in the flow velocity measurements was not biocompatible and eventually contributed to cellular death. Unfortunately, current attempts to “clean” the anti-bacterial ingredients have failed. Cell-nanoparticle interactions have been successfully applied as a means to explore the mechanical deformability of living cytoplasm^[33] and will be adapted to this approach during future experiments.

Laser heating: Direct trapping of cells with the infrared laser leads to localized heating and cell death. Low laser power is viable for short periods and is a benefit when optically and hydrodynamically trapping living cells (< 30 mW required). As water is the main constituent of biological cells ($\sim 70\%$) and has minimal absorption properties, laser-induced heating may be relatively mild for laser wavelengths of 200–1100 nm. It has been observed that for a laser operating at 1064 nm, laser-induced heating of 1.15 °C was

produced for every 100 mW of applied laser power entering a trapped ovarian cell^[34]. A more automated approach to laser guidance may lessen the power required to center living cells within the equilibrium position created by the cross-junction channel design.

The volumetric strain analysis facilitated a comparison with the common assumption applied in cell biomechanics, basing the mechanical properties within the framework of liquid droplet models^[35]. This approach is typically applied to neutrophil biomechanics and has two main drawbacks. It treats the cytoplasm as a single phase rather than as multi-phase or composite material and it does not address the cell as a living entity having active cellular deformation and force generation. Of course the latter issue of passive versus active mechanics is a significant hurdle in any biomechanical analysis applied to a living system.

Despite the limitations in the technical components of this research endeavor, many opportunities are available through the described microscale bioengineering approach. Making cell-level knowledge relevant to tissue-level therapies will be a critical element for establishing future relevance to health-care^[36]. Thus an emphasis on multiscale experimentation and mathematical modeling amplifies the significance, extending the reach of the single-cell approach. In this philosophy, a strong link is made between cell engineering and tissue engineering. Ergo, cell population biomechanics and biomaterials science become important areas of applied research when harnessing the adaptability of the living system toward repair, regeneration, and healing. Ongoing efforts are addressing these multiple-scale links by exploring both spatial and temporal relationships.

Acknowledgements

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Chapter 9 Application of Nonlocal Shell Models to Microtubule Buckling in Living Cells

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Abstract: A version of nonlocal elasticity theory is employed to develop a nonlocal shear deformable shell model. The governing equations are based on higher order shear deformation shell theory with a von Kármán-type of kinematic nonlinearity and include small scale effects. These equations are then used to solve buckling problems of microtubules (MTs) embedded in an elastic matrix of cytoplasm subjected to bending. The surrounding elastic medium is modeled as a Pasternak foundation. The thermal effects are also included and the material properties are assumed to be temperature-dependent. The small scale parameter e_0a is estimated by matching the buckling curvature of MTs observed from measurements with the numerical results obtained from the nonlocal shear deformable shell model. The numerical results show that buckling loads are decreased with the increasing small scale parameter e_0a . The results reveal that the lateral constraint has a significant effect on the buckling moments of a microtubule when the foundation stiffness is sufficiently large.

Keywords: microtubules, nonlocal shell model, higher order shear deformable shell theory, buckling

9.1 Introduction

Microtubules (MTs) are important components of cytoskeletal structures, which, in conjunction with actin and intermediate filaments, provide both the static and dynamic framework that maintains cell structure. Determining their material properties, including physical, chemical, electrical and mechanical properties, is the topic of investigations^[1-6]. MTs are long, hollow cylinders made of $\alpha\beta$ -tubulin protein heterodimers and the length of which

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may vary from tens of nanometers to hundreds of micrometers. A microtubule buckles when subjected to a sufficiently large mechanical loading, such as axial compressive load, radial pressure, bending and torsion. The microtubule buckling has been observed in various types of living cells^[7-11]. It is reported that isolated microtubules may form an Euler buckling pattern with a long wavelength for very small compressive force *in vitro* experiments^[8]. In contrast, microtubules buckle with a short wavelength *in vivo* experiments^[9]. With the support of the cytoplasm, an individual microtubule can sustain a compressive force on the order of 100 pN. It is also reported that the critical pressure can be measured on the order of 600 Pa from a synchrotron small angle X-ray diffraction study of MTs subjected to osmotic stress^[10,12]. As experiments are expensive and difficult to conduct due to the very small size of MTs, the numerical simulation and the theoretical approaches are widely used to investigate buckling behavior of MTs. The buckling failure modes of a cylindrical tube can generally be categorized as global buckling (Euler pattern), and local buckling (shell pattern). In order to predict the short wavelengths buckling behavior of MTs, Li^[13] presented an Euler beam model for microtubule buckling in living cells. In his study, the surrounding cytoplasm was modeled as a viscoelastic medium. Das et al.^[14] presented a rod embedded in a nonlinear elastic medium of cytoplasm to determine both the buckling force and deformation of the MTs. Jiang and Zhang^[15] presented a cylindrical tube embedded in three types of viscous, elastic and viscoelastic medium for microtubule buckling. They also concluded that the final equilibrium configuration is completely determined by the elasticity of cytoplasm when the buckling occurs. In the aforementioned studies, researchers mainly focused on the column buckling of MTs based on 1D beam models. On the other hand, 1D ring models were adopted for the buckling analysis of MTs subjected to external radial pressure. However, 1D beam models as well as 1D ring models are too simple to describe 2D shell-like deformation of hollow MTs, such as localized indentation^[2] and shell-like buckling^[12]. In addition, local buckling of MTs of a fully constrained cytoskeleton can be the main source of cell nonlinearity^[16].

It has been reported in the literature that continuum shell model, which is familiar to engineers, may be applicable to the analysis of MTs. Wang et al.^[17] proposed an orthotropic thin shell model to study buckling behavior of MTs subjected to external radial pressure and axial compression. This work was then extended to study of MTs subjecting bending and torsion^[18]. In these studies, the classical shell theory, i.e. the theory based on the Kirchhoff-Love hypothesis, is used and therefore the transverse shear deformation is usually not accounted for. It is well known that the thin shell theory is adequate for cylindrical shells when the radius-to-thickness ratio is greater than 20. As shown by Wade et al.^[19], MTs have low values of radius-to-thickness ratio. Hereby, the continuum mechanics model for MTs requires the shear deformation shell theory.

The major difference between the macroscale structures and the nanoscale

structures, e.g. carbon nanotubes^[20–22], single layer graphene sheets^[23] and MTs^[24,25], lies in that the latter structure has a small scale effect. Although the classical continuum models are efficient in MTs buckling analysis through their relatively simple formulae, the small scale effects on the buckling and postbuckling behavior of MTs have been ignored. Most classical continuum theories are based on hyperelastic constitutive relations, which assume that the stresses at a point are functions of strains at that point. On the other hand, the nonlocal continuum mechanics assumes that the stress at a point is a function of strains at all points in the continuum. Such theories contain information about the forces between atoms, and the internal length scale is introduced into the constitutive equations as a material parameter. The nonlocal elasticity is initiated in the papers by Eringen^[26,27] and Eringen and Edelen^[28]. A nonlocal Timoshenko beam model was proposed^[29,30] to solve buckling problem of axially compressed MTs embedded in viscoelastic surrounding cytoplasm. In their studies, the surrounding medium is modeled as an elastic foundation of Winkler-type. Recently, Gao and An^[31] proposed a nonlocal thin shell model to solve buckling problem of MTs subjected to axial compression combined with radial pressure exerted by the cytosol motion.

Motivated by these ideas, a complete development of the classical and shear deformation shell theories using the nonlocal constitutive differential equations is presented. Material properties are assumed to be anisotropic and temperature dependent. The equations are then solved for bending buckling of MTs embedded in an elastic matrix of cytoplasm.

9.2 Nonlocal shell theories

Consider a microtubule modeled as a cylindrical shell which is subjected to a mechanical load in thermal environments. The length, mean radius and effective wall thickness of the shell are L, R and h , respectively, as shown in Fig.9.1. The shell is referred to a coordinate system (x, y, z) in which x and y are in the axial and circumferential directions of the shell and z is in the direction of the inward normal to the middle surface. The corresponding displacements are designated by $\bar{U}$, $\bar{V}$ and $\bar{W}$. $\bar{\Psi}_x$ and $\bar{\Psi}_y$ are the rotations of normals to the middle surface with respect to the y and x axes, respectively. The origin of the coordinate system is located at one end of the shell on the middle plane. The shell is assumed to be anisotropic and to be geometrically perfect. The outer surface of the shell is in contact with an elastic medium that acts as an elastic foundation represented by the Pasternak model, i.e. the reaction of the foundation is assumed to be $p_0 = K_1 \bar{W} - K_2 \nabla^2 \bar{W}$, where p_0 is the force per unit area, K_1 is the Winkler foundation stiffness and K_2 is the shearing layer stiffness of the foundation, and ∇^2 is the Laplace operator in x and y . Let $\bar{F}(x, y)$ be the stress function for the stress resultants defined by $\bar{N}_x = \bar{F}_{,yy}$, $\bar{N}_y = \bar{F}_{,xx}$ and $\bar{N}_{xy} = -\bar{F}_{,xy}$, where a comma denotes partial

differentiation with respect to the corresponding coordinates.

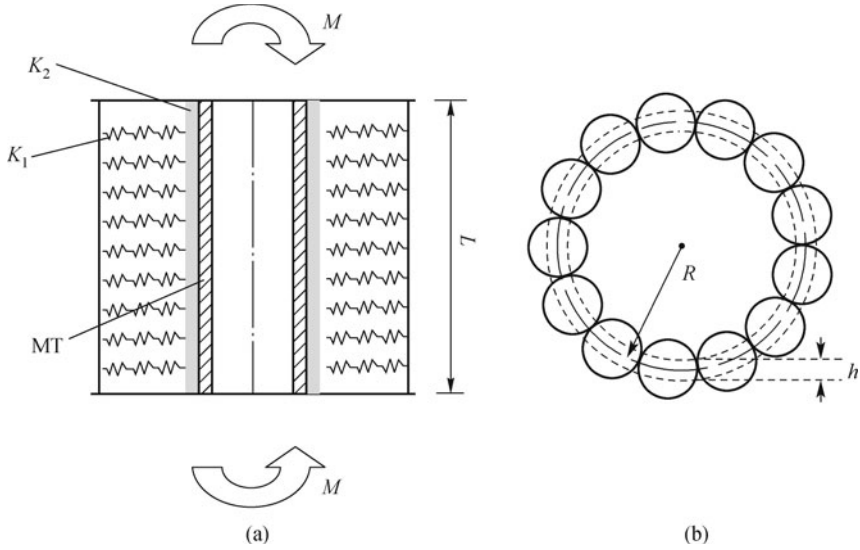


Fig. 9.1 Configurations of a microtubule surrounded by an elastic medium: (a) front view, (b) top view.

9.2.1 Constitutive relations

According to Eringen^[27], the stress field at a point $\mathbf{x}$ in an elastic continuum depends not only on the strain field at the point (hyperelastic case) but also on strains at all other points of the body. Eringen attributed this fact to the atomic theory of lattice dynamics and experimental observations on phonon dispersion. Thus, the nonlocal stress tensor σ at point $\mathbf{x}$ is expressed as

$$\sigma(\mathbf{x}) = \int_V K(|\mathbf{x}' - \mathbf{x}|, \tau) \mathbf{t}(\mathbf{x}') d\mathbf{x} \quad (9.1)$$

where $\mathbf{t}(\mathbf{x})$ is the classical, macroscopic stress tensor at point $\mathbf{x}$ and the kernel function $K(|\mathbf{x}' - \mathbf{x}|, \tau)$ represents the nonlocal modulus or attenuation function, in which $|\mathbf{x}' - \mathbf{x}|$ is the Euclidean distance and τ is a material constant and $\tau = e_0 a / L$, where L is the external characteristic length (such as the lattice spacing and wavelength, respectively), a is an internal characteristic length (such as the length of a C–C bond, lattice spacing, granular distance), and e_0 is a constant appropriate to each material. The macroscopic stress $\mathbf{t}$ at a point $\mathbf{x}$ in a Hookean solid is related to the strain ϵ at the point by the generalized Hooke's law

$$\mathbf{t}(\mathbf{x}) = \mathbf{C}(\mathbf{x}) : \epsilon(\mathbf{x}) \quad (9.2)$$

where $\mathbf{C}$ is the fourth-order elasticity tensor.

The constitutive Eqs.(9.1) and (9.2) together define the nonlocal constitutive behavior of a Hookean solid. Equation (9.1) represents the weighted average of the contributions of the strain field of all points in the body to the stress field at a point. The integral constitutive relation in Eq.(9.1) makes the elasticity problems difficult to solve. However, it is possible^[27] to represent the integral constitutive relations in an equivalent differential form as

$$(1 - \tau^2 L^2 \nabla^2) \sigma_{ij} = C_{ijkl} \varepsilon_{kl}. \quad (9.3)$$

The distinct difference between the classical and nonlocal elasticity theories lies in the presence of small scale parameter $e_0 a$ in the nonlocal theory. For carbon nanotubes the characteristic length a may be taken as the length of the C-C bond, i.e. $a=0.142$ nm. However, it is hard to find a physical relevance of the characteristic length a for MTs. It is a better way to use $e_0 a$ as a single scale coefficient that captures the small-scale effect on the response of structures in nanosize^[32,33].

9.2.2 Shear deformable shell model

Reddy and Liu^[34] developed a simple higher order shear deformation shell theory. This theory assumes that the transverse shear strains present a parabolical distribution across the shell thickness. The advantages of this theory over the first- order shear deformation theory are that the number of independent unknowns ($\overline{U}, \overline{V}, \overline{W}, \overline{\Psi}_x$ and $\overline{\Psi}_y$) is the same as in the first order shear deformation theory, and no shear correction factors are required. In the first order shear deformation theory, this shear correction factor was obtained by comparing the results with an exact elasticity solution and generally it heavily depends on various parameters such as material properties, geometric parameters and others^[35]. In the nanoscale structures the material properties are found to be size-dependent^[36]. It is questionable that the commonly used value $5/6$ or $\pi^2/12$ for shear correction factor^[37] is still correct in the nanoscale structures.

From Eq.(9.1), the nonlocal constitutive equations for shear deformation shells can be written as

$$\begin{bmatrix} \overline{N} \\ \overline{M} \\ \overline{P} \end{bmatrix} = \begin{bmatrix} \mathbf{A} & \mathbf{B} & \mathbf{E} \\ \mathbf{B} & \mathbf{D} & \mathbf{F} \\ \mathbf{E} & \mathbf{F} & \mathbf{H} \end{bmatrix} \begin{bmatrix} \epsilon^0 \\ \kappa^0 \\ \kappa^2 \end{bmatrix} + \begin{bmatrix} \overline{N}^t \\ \overline{M}^t \\ \overline{P}^t \end{bmatrix}, \quad (9.4a)$$

$$\begin{bmatrix} \overline{Q} \\ \overline{R} \end{bmatrix} = \begin{bmatrix} \mathbf{A} & \mathbf{D} \\ \mathbf{D} & \mathbf{F} \end{bmatrix} \begin{bmatrix} \epsilon^0 \\ \kappa^2 \end{bmatrix} \quad (9.4b)$$

where ϵ^0 , κ^0 , κ^2 are strains and higher order strains defined by (with $\chi = 1$)

$$\begin{aligned}
 \epsilon_1^0 &= \frac{\partial \bar{U}}{\partial X} + \frac{1}{2} \left(\frac{\partial \bar{W}}{\partial X} \right)^2, & \kappa_1^0 &= \frac{\partial \bar{\Psi}_x}{\partial X}, & \kappa_1^2 &= -\chi \frac{4}{3h^2} \left(\frac{\partial \bar{\Psi}_x}{\partial X} + \frac{\partial^2 \bar{W}}{\partial X^2} \right), \\
 \epsilon_2^0 &= \frac{\partial \bar{V}}{\partial Y} + \frac{1}{2} \left(\frac{\partial \bar{W}}{\partial Y} \right)^2, & \kappa_2^0 &= \frac{\partial \bar{\Psi}_y}{\partial Y}, & \kappa_2^2 &= -\chi \frac{4}{3h^2} \left(\frac{\partial \bar{\Psi}_y}{\partial Y} + \frac{\partial^2 \bar{W}}{\partial Y^2} \right), \\
 \epsilon_4^0 &= \bar{\Psi}_y + \frac{\partial \bar{W}}{\partial Y}, & \kappa_4^2 &= -\chi \frac{4}{h^2} \left(\bar{\Psi}_y + \frac{\partial \bar{W}}{\partial Y} \right), \\
 \epsilon_5^0 &= \bar{\Psi}_x + \frac{\partial \bar{W}}{\partial X}, & \kappa_5^2 &= -\chi \frac{4}{h^2} \left(\bar{\Psi}_x + \frac{\partial \bar{W}}{\partial X} \right), \\
 \epsilon_6^0 &= \frac{\partial \bar{U}}{\partial Y} + \frac{\partial \bar{V}}{\partial X} + \frac{\partial \bar{W}}{\partial X} \frac{\partial \bar{W}}{\partial Y}, & \kappa_6^0 &= \frac{\partial \bar{\Psi}_x}{\partial Y} + \frac{\partial \bar{\Psi}_y}{\partial X}, \\
 \kappa_6^2 &= -\chi \frac{4}{3h^2} \left(\frac{\partial \bar{\Psi}_x}{\partial Y} + \frac{\partial \bar{\Psi}_y}{\partial X} + 2 \frac{\partial^2 \bar{W}}{\partial X \partial Y} \right)
 \end{aligned} \tag{9.5}$$

In Eq.(9.4), $\bar{N}$ and $\bar{Q}$ are membrane and transverse shear forces, $\bar{M}$ is bending moment per unit length, and $\bar{P}$ and $\bar{R}$ are higher order bending moment and shear force, respectively, defined by

$$[\bar{N}, \bar{Q}, \bar{M}, \bar{P}, \bar{R}] = (1 - \tau^2 L^2 \nabla^2) \begin{bmatrix} \bar{N}_x & \bar{Q}_x & \bar{M}_x & \bar{P}_x & \bar{R}_x \\ \bar{N}_y & \bar{Q}_y & \bar{M}_y & \bar{P}_y & \bar{R}_y \\ \bar{N}_{xy} & 0 & \bar{M}_{xy} & \bar{P}_{xy} & 0 \end{bmatrix} \tag{9.6}$$

and $\bar{N}^t$, $\bar{M}^t$ and $\bar{P}^t$ are thermal forces, moments and higher order moments caused by elevated temperature, as defined in Appendix A.

Applying Eq.(9.4) to Reddy's higher order shear deformation shell theory, the equilibrium equations can be expressed as

$$(1 - \tau^2 L^2 \nabla^2) \left(\frac{\partial \bar{N}_x}{\partial X} + \frac{\partial \bar{N}_{xy}}{\partial Y} \right) = 0, \tag{9.7a}$$

$$(1 - \tau^2 L^2 \nabla^2) \left(\frac{\partial \bar{N}_{xy}}{\partial X} + \frac{\partial \bar{N}_y}{\partial Y} \right) = 0, \tag{9.7b}$$

$$\begin{aligned}
 (1 - \tau^2 L^2 \nabla^2) \left[\frac{\partial^2 \bar{M}_x}{\partial X^2} + 2 \frac{\partial^2 \bar{M}_{xy}}{\partial X \partial Y} + \frac{\partial^2 \bar{M}_y}{\partial Y^2} + \frac{\bar{N}_y}{R} \right. \\
 \left. + \left(\bar{N}_x \frac{\partial^2 \bar{W}}{\partial X^2} + 2 \bar{N}_{xy} \frac{\partial^2 \bar{W}}{\partial X \partial Y} + \bar{N}_y \frac{\partial^2 \bar{W}}{\partial Y^2} \right) + q \right] = 0,
 \end{aligned} \tag{9.7c}$$

$$\begin{aligned}
 (1 - \tau^2 L^2 \nabla^2) \left[\frac{\partial \bar{M}_x}{\partial X} + \frac{\partial \bar{M}_{xy}}{\partial Y} - \bar{Q}_x + \frac{4}{h^2} \bar{R}_x \right. \\
 \left. - \frac{4}{3h^2} \left(\frac{\partial \bar{P}_x}{\partial X} + \frac{\partial \bar{P}_{xy}}{\partial Y} \right) \right] = 0,
 \end{aligned} \tag{9.7d}$$

$$(1 - \tau^2 L^2 \nabla^2) \left[\frac{\partial \overline{M}_{xy}}{\partial X} + \frac{\partial \overline{M}_y}{\partial Y} - \overline{Q}_y + \frac{4}{h^2} \overline{R}_y - \frac{4}{3h^2} \left(\frac{\partial \overline{P}_{xy}}{\partial X} + \frac{\partial \overline{P}_y}{\partial Y} \right) \right] = 0. \quad (9.7e)$$

The partial inverse of Eq.(9.4) yields

$$\begin{bmatrix} \epsilon^0 \\ M^* \\ P^* \end{bmatrix} = \begin{bmatrix} \mathbf{A}^* & \mathbf{B}^* & \mathbf{E}^* \\ -(\mathbf{B}^*)^T & \mathbf{D}^* & (\mathbf{F}^*)^T \\ -(\mathbf{E}^*)^T & \mathbf{F}^* & \mathbf{H}^* \end{bmatrix} \begin{bmatrix} \mathbf{N}^* \\ \kappa^0 \\ \kappa^2 \end{bmatrix} \quad (9.8)$$

in which $\mathbf{N}^* = \overline{\mathbf{N}} - \overline{\mathbf{N}}^t$, $\mathbf{M}^* = \overline{\mathbf{M}} - \overline{\mathbf{M}}^t$ and $\mathbf{P}^* = \overline{\mathbf{P}} - \overline{\mathbf{P}}^t$.

It is assumed that the effective Young's moduli E_{11} and E_{22} , shear moduli G_{12} , G_{13} and G_{23} , and thermal expansion coefficients α_{11} and α_{22} of microtubule are temperature-dependent, whereas Poisson's ratio ν_{12} depends slightly on temperature change and is assumed to be a constant. Hence, in Eq.(9.8) the reduced stiffness matrices $[A_{ij}^*]$, $[B_{ij}^*]$, $[D_{ij}^*]$, $[E_{ij}^*]$, $[F_{ij}^*]$ and $[H_{ij}^*]$ are all functions of temperature and determined through relationships $\mathbf{A}^* = \mathbf{A}^{-1}$, $\mathbf{B}^* = -\mathbf{A}^{-1}\mathbf{B}$, $\mathbf{D}^* = \mathbf{D} - \mathbf{B}\mathbf{A}^{-1}\mathbf{B}$, $\mathbf{E}^* = -\mathbf{A}^{-1}\mathbf{E}$, $\mathbf{F}^* = \mathbf{F} - \mathbf{E}\mathbf{A}^{-1}\mathbf{B}$, $\mathbf{H}^* = \mathbf{H} - \mathbf{E}\mathbf{A}^{-1}\mathbf{E}$.

Substituting Eqs.(9.8) and (9.4b) into Eqs.(9.7a-e), and considering the condition of compatibility, the Kármán-Donnell-type nonlinear differential equations for the microtubule, including small scale effects and thermal effects, have readily been derived and can be expressed in terms of a stress function $\overline{F}$, two rotations $\overline{\Psi}_x$ and $\overline{\Psi}_y$, and a transverse displacement $\overline{W}$. They are

$$\begin{aligned} & \tilde{L}_{11}(\overline{W}) - \tilde{L}_{12}(\overline{\Psi}_x) - \tilde{L}_{13}(\overline{\Psi}_y) + \tilde{L}_{14}(\overline{F}) - \tilde{L}_{15}(\overline{N}^T) - \tilde{L}_{16}(\overline{M}^T) - \frac{1}{R}\overline{F}_{,xx} \\ & = (1 - \tau^2 L^2 \nabla^2)[\tilde{L}(\overline{W}, \overline{F}) + q], \end{aligned} \quad (9.9)$$

$$\begin{aligned} & \tilde{L}_{21}(\overline{F}) + \tilde{L}_{22}(\overline{\Psi}_x) + \tilde{L}_{23}(\overline{\Psi}_y) - \tilde{L}_{24}(\overline{W}) - \tilde{L}_{25}(\overline{N}^T) + \frac{1}{R}\overline{W}_{,xx} \\ & = -\frac{1}{2}\tilde{L}(\overline{W}, \overline{W}), \end{aligned} \quad (9.10)$$

$$\tilde{L}_{31}(\overline{W}) + \tilde{L}_{32}(\overline{\Psi}_x) - \tilde{L}_{33}(\overline{\Psi}_y) + \tilde{L}_{34}(\overline{F}) - \tilde{L}_{35}(\overline{N}^T) - \tilde{L}_{36}(\overline{S}^T) = 0, \quad (9.11)$$

$$\tilde{L}_{41}(\overline{W}) - \tilde{L}_{42}(\overline{\Psi}_x) + \tilde{L}_{43}(\overline{\Psi}_y) + \tilde{L}_{44}(\overline{F}) - \tilde{L}_{45}(\overline{N}^T) - \tilde{L}_{46}(\overline{S}^T) = 0 \quad (9.12)$$

where all linear operators $\tilde{L}_{ij}(\cdot)$ and the nonlinear operator $\tilde{L}(\cdot)$ are given in detail in Appendix B.

It is worth noting that Eqs.(9.9), (9.11) and (9.12) represent equilibrium conditions and Eq.(9.10) represents compatibility condition. The geometric

nonlinearity in the von Kármán sense is given in terms of $\tilde{L}(\cdot)$ in Eqs.(9.9) and (9.10). Small scale parameter e_0a is included in Eq.(9.9). The nonlocal shear deformable shell model described by Eqs.(9.9)–(9.12) reduces to the local shear deformable shell model when the small scale parameter e_0a vanishes.

Equations (9.9)–(9.12) are remarkable for the flexural-twist and extension-twist couplings indicated by D_{16}^* , D_{26}^* , A_{16}^* and A_{26}^* . Note that the microtubule is modeled as a single walled shell, no extension-flexural coupling exists, and B_{ij}^* and E_{ij}^* are all zero-valued, in such a case $\tilde{L}_{14}(\cdot) = \tilde{L}_{22}(\cdot) = \tilde{L}_{23}(\cdot) = \tilde{L}_{24}(\cdot) = \tilde{L}_{34}(\cdot) = \tilde{L}_{44}(\cdot) = 0$. If the thermal field is assumed to be uniform, from Eq.(A.1) we have $\overline{M}^T = \overline{S}^T = 0$ and $\overline{N}^T = \text{constant}$. In such a case, $\tilde{L}_{15}(\overline{N}^T) = \tilde{L}_{25}(\overline{N}^T) = \tilde{L}_{35}(\overline{N}^T) = \tilde{L}_{45}(\overline{N}^T) = \tilde{L}_{16}(\overline{M}^T) = \tilde{L}_{36}(\overline{S}^T) = \tilde{L}_{46}(\overline{S}^T) = 0$. Eqs.(9.9)–(9.12) are successfully used in solving postbuckling problems of MTs subjected to axial compression or external pressure^[24,25].

9.2.3 Thin shell model

For thin shell theory, we have $\chi = 0$, and $\overline{\Psi}_x = -\overline{W}_{,x}$, $\overline{\Psi}_y = -\overline{W}_{,y}$ in Eq.(9.5), then Eqs.(9.9)–(9.12) reduce to two equations and can be expressed by

$$\begin{aligned} \tilde{L}_{11}(\overline{W}) + \tilde{L}_{14}(\overline{F}) - \tilde{L}_{16}(\overline{M}^T) - \frac{1}{R}\overline{F}_{,xx} \\ = (1 - \tau^2 L^2 \nabla^2)[\tilde{L}(\overline{W}, \overline{F}) + q], \end{aligned} \quad (9.13)$$

$$\tilde{L}_{21}(\overline{F}) - \tilde{L}_{24}(\overline{W}) - \tilde{L}_{25}(\overline{N}^T) + \frac{1}{R}\overline{W}_{,xx} = -\frac{1}{2}\tilde{L}(\overline{W}, \overline{W}) \quad (9.14)$$

where

$$\begin{aligned} \tilde{L}_{11}(\cdot) &= D_{11}^* \frac{\partial^4}{\partial X^4} + 4D_{16}^* \frac{\partial^4}{\partial X^3 \partial Y} + 2(D_{12}^* + 2D_{66}^*) \frac{\partial^4}{\partial X^2 \partial Y^2} \\ &\quad + 4D_{26}^* \frac{\partial^4}{\partial X \partial Y^3} + D_{22}^* \frac{\partial^4}{\partial Y^4}, \\ \tilde{L}_{14}(\cdot) &= B_{21}^* \frac{\partial^4}{\partial X^4} + (2B_{26}^* - B_{61}^*) \frac{\partial^4}{\partial X^3 \partial Y} + (B_{11}^* + B_{22}^* - 2B_{66}^*) \frac{\partial^4}{\partial X^2 \partial Y^2} \\ &\quad + (2B_{16}^* - B_{62}^*) \frac{\partial^4}{\partial X \partial Y^3} + B_{12}^* \frac{\partial^4}{\partial Y^4}, \\ \tilde{L}_{21}(\cdot) &= A_{22}^* \frac{\partial^4}{\partial X^4} - 2A_{26}^* \frac{\partial^4}{\partial X^3 \partial Y} + (2A_{12}^* + A_{66}^*) \frac{\partial^4}{\partial X^2 \partial Y^2} \\ &\quad - 2A_{16}^* \frac{\partial^4}{\partial X \partial Y^3} + A_{11}^* \frac{\partial^4}{\partial Y^4}, \\ \tilde{L}_{24}(\cdot) &= \tilde{L}_{14}(\cdot). \end{aligned} \quad (9.15)$$

Most cellular microtubules have 13 protofilaments and 3 starts. They usually have an outer radius of 12.7 nm and an inner radius of 8.7 nm (this

means the mean radius $R=10.7$ nm). However, MTs with as few as 8 and as many as 19 protofilaments have been observed both *in vivo* and *in vitro*^[38]. The number of starts is also variable and can range from 2 to 4^[39]. MTs with N protofilaments and S start helix will be noted with N_S in the following. It is reported that^[40] MTs with different N_S configurations have different skew angles θ , except the 13.3 has $\theta=0^\circ$. As a result, only 13.3 MTs can be modelled as an orthotropic cylindrical shell for which all terms A_{16}^* , A_{26}^* , D_{16}^* and D_{26}^* are zero-valued. Otherwise, the anisotropy caused by skew angles affects the postbuckling response of MTs significantly, as reported in [25].

For linear buckling problem of orthotropic cylindrical shells (e.g. 13.3 MTs), Eqs.(9.13) and (9.14) reduce to equations

$$\begin{aligned} & \left[D_{11} \frac{\partial^4 \bar{W}}{\partial X^4} + 2(D_{12} + 2D_{66}) \frac{\partial^4 \bar{W}}{\partial X^2 \partial Y^2} + D_{22} \frac{\partial^4 \bar{W}}{\partial Y^4} \right] - \frac{1}{R} \bar{F}_{,xx} \\ & = (1 - \tau^2 L^2 \nabla^2) \left(\bar{N}_X^0 \frac{\partial^2 \bar{W}}{\partial X^2} + 2\bar{N}_{XY}^0 \frac{\partial^2 \bar{W}}{\partial X \partial Y} + \bar{N}_Y^0 \frac{\partial^2 \bar{W}}{\partial Y^2} + q \right), \end{aligned} \quad (9.16)$$

$$\left[A_{22} \frac{\partial^4 \bar{F}}{\partial X^4} + (2A_{12} + A_{66}) \frac{\partial^4 \bar{F}}{\partial X^2 \partial Y^2} + A_{11} \frac{\partial^4 \bar{F}}{\partial Y^4} \right] + \frac{1}{R} \bar{W}_{,xx} = 0. \quad (9.17)$$

If the material properties are assumed to be isotropic (i.e. $D_{11} = D_{22} = D$, $A_{11} = A_{22} = 1/Eh$), substituting Eq.(9.17) into Eq.(9.16) yields

$$\begin{aligned} & D \nabla^8 \bar{W} + \frac{Eh}{R^2} \frac{\partial^4 \bar{W}}{\partial X^4} = (1 - \tau^2 L^2 \nabla^2) \\ & \cdot \left\{ \nabla^4 \left(\bar{N}_X^0 \frac{\partial^2 \bar{W}}{\partial X^2} + 2\bar{N}_{XY}^0 \frac{\partial^2 \bar{W}}{\partial X \partial Y} + \bar{N}_Y^0 \frac{\partial^2 \bar{W}}{\partial Y^2} \right) + \nabla^4 [q] \right\}. \end{aligned} \quad (9.18)$$

Equation (9.18) is identical in form to that used in carbon nanotubes^[41,42].

9.3 Bending buckling analysis

For the application of nonlocal shear deformable shell model to microtubule buckling, we consider here 13.3 MTs embedded in an elastic matrix subjected to bending in thermal environments. The surrounding elastic medium is modeled as a Pasternak foundation, and in such a case $q = -p_0 = -(K_1 \bar{W} - K_2 \nabla^2 \bar{W})$. Now Eqs.(9.9)–(9.12) reduce to equations

$$\begin{aligned} & \tilde{L}_{11}(\bar{W}) - \tilde{L}_{12}(\bar{\Psi}_x) - \tilde{L}_{13}(\bar{\Psi}_y) - \frac{1}{R} \bar{F}_{,xx} = (1 - \tau^2 L^2 \nabla^2) \\ & \cdot \left[\bar{N}_X^0 \frac{\partial^2 \bar{W}}{\partial X^2} + 2\bar{N}_{XY}^0 \frac{\partial^2 \bar{W}}{\partial X \partial Y} + \bar{N}_Y^0 \frac{\partial^2 \bar{W}}{\partial Y^2} - (K_1 \bar{W} - K_2 \nabla^2 \bar{W}) \right], \end{aligned} \quad (9.19)$$

$$\tilde{L}_{21}(\bar{F}) + \frac{1}{R} \bar{W}_{,xx} = 0, \quad (9.20)$$

$$\tilde{L}_{31}(\bar{W}) + \tilde{L}_{32}(\bar{\Psi}_x) - \tilde{L}_{33}(\bar{\Psi}_y) = 0, \quad (9.21)$$

$$\tilde{L}_{41}(\overline{W}) - \tilde{L}_{42}(\overline{\Psi}_x) + \tilde{L}_{43}(\overline{\Psi}_y) = 0. \quad (9.22)$$

In Eq.(9.19)

$$\overline{N}_x^0 = -\frac{M}{\pi R^2} \cos \frac{Y}{R}, \quad \overline{N}_{xy}^0 = 0 \quad (9.23)$$

where M is the bending moment. Note that $N_y^0 \neq 0$, excepting temperature rise $\Delta T = 0$.

The two end edges of MTs are assumed to be simply supported or clamped, so that the boundary conditions are

$X=0, L$:

$$\overline{W} = \overline{V} = \overline{\Psi}_y = 0, \quad \overline{M}_x = \overline{P}_x = 0. \quad (\text{simply supported}) \quad (9.24a)$$

$$\overline{W} = \overline{V} = \overline{\Psi}_x = \overline{\Psi}_y = 0 \quad (\text{clamped}). \quad (9.24b)$$

Also, we have the closed (or periodicity) condition

$$\int_0^{2\pi R} \frac{\partial \overline{V}}{\partial Y} dY = 0 \quad (9.25a)$$

or

$$\int_0^{2\pi R} \left[\left(A_{22}^* \frac{\partial^2 \overline{F}}{\partial X^2} + A_{12}^* \frac{\partial^2 \overline{F}}{\partial Y^2} \right) + \frac{\overline{W}}{R} - (A_{12}^* \overline{N}_x^t + A_{22}^* \overline{N}_y^t) \right] dY = 0. \quad (9.25b)$$

Because of Eq.(9.25), the in-plane boundary condition $\overline{V} = 0$ (at $X = 0, L$) is not needed in Eq.(9.24). It is worthy to note that the thermal field is assumed to be uniform, and therefore, the thermal coupling in Eqs.(9.9)–(9.12) vanishes, i.e. $\tilde{L}_{16}(\overline{M}^T) = \tilde{L}_{25}(\overline{N}^T) = \tilde{L}_{36}(\overline{S}^T) = \tilde{L}_{46}(\overline{S}^T) = 0$, but the thermal forces $\overline{N}_x^t$ and $\overline{N}_y^t$ are still included in Eq.(9.25b).

Before proceeding, it is convenient first to define the following dimensionless quantities

$$\begin{aligned} x &= \pi \frac{X}{L}, \quad y = \frac{Y}{R}, \quad \beta = \frac{L}{\pi R}, \quad \varepsilon = \frac{\pi^2 R}{L^2} [D_{11}^* D_{22}^* A_{11}^* A_{22}^*]^{\frac{1}{4}}, \\ W &= \frac{\overline{W}}{[D_{11}^* D_{22}^* A_{11}^* A_{22}^*]^{\frac{1}{4}}}, \quad F = \frac{\overline{F}}{[D_{11}^* D_{22}^*]^{\frac{1}{2}}}, \\ (k_1, k_2) &= \left(\frac{L^4}{\pi^4 D_{11}^*} K_1, \frac{L^2}{\pi^2 D_{11}^*} K_2 \right), \\ (\Psi_x, \Psi_y) &= \frac{L}{\pi} \frac{(\overline{\Psi}_x, \overline{\Psi}_y)}{[D_{11}^* D_{22}^* A_{11}^* A_{22}^*]^{\frac{1}{4}}}, \quad (N_x^0, N_y^0) = \frac{R^2}{[D_{11}^* D_{22}^*]^{\frac{1}{2}}} (\overline{N}_x^0, \overline{N}_y^0), \\ \gamma_{14} &= \left[\frac{D_{22}^*}{D_{11}^*} \right]^{\frac{1}{2}}, \quad \gamma_{24} = \left[\frac{A_{11}^*}{A_{22}^*} \right]^{\frac{1}{2}}, \quad \gamma_5 = -\frac{A_{12}^*}{A_{22}^*}, \\ (\gamma_{T1}, \gamma_{T2}) &= (A_x^t, A_y^t) R \left[\frac{A_{11}^* A_{22}^*}{D_{11}^* D_{22}^*} \right]^{\frac{1}{4}} \end{aligned} \quad (9.26)$$

in which A_x^t and A_y^t are defined by

$$\begin{bmatrix} A_x^t \\ A_y^t \end{bmatrix} = - \int_{-\frac{h}{2}}^{\frac{h}{2}} \begin{bmatrix} A_x \\ A_y \end{bmatrix} dZ \quad (9.27)$$

where A_x and A_y are given in detail in Eq.(A.2).

Equations (9.20)–(9.23) may then be written in dimensionless form as

$$L_{11}(W) + (1 - \tau^2 \pi^2 \nabla^2)(k_1 W - k_2 \nabla^2 W) - L_{12}(\Psi_x) - L_{13}(\Psi_y) - \varepsilon^{-1} \gamma_{14} F_{,xx} = (1 - \tau^2 \pi^2 \nabla^2)[\gamma_{14} \beta^2 (N_x^0 W_{,xx} + N_y^0 W_{,yy})], \quad (9.28)$$

$$L_{21}(F) + \varepsilon^{-1} \gamma_{24} W_{,xx} = 0, \quad (9.29)$$

$$L_{31}(W) + L_{32}(\Psi_x) - L_{33}(\Psi_y) = 0, \quad (9.30)$$

$$L_{41}(W) - L_{42}(\Psi_x) + L_{43}(\Psi_y) = 0 \quad (9.31)$$

where the non-dimensional linear operators $L_{ij}(\cdot)$ are defined in Appendix C.

The boundary conditions of Eq.(9.24) become

$x = 0, \pi$:

$$W = \Psi_y = 0, M_x = P_x = 0, \quad (\text{simply supported}) \quad (9.32a)$$

$$W = \Psi_x = \Psi_y = 0, \quad (\text{clamped}) \quad (9.32b)$$

and the closed condition becomes

$$\int_0^{2\pi} \left[\left(\frac{\partial^2 F}{\partial x^2} - \gamma_5 \beta^2 \frac{\partial^2 F}{\partial y^2} \right) + \varepsilon^{-1} \gamma_{24} W + \varepsilon^{-1} (\gamma_{T2} - \gamma_5 \gamma_{T1}) \Delta T \right] dy = 0. \quad (9.33)$$

The solutions of Eqs.(9.28)–(9.31) satisfying the simply supported boundary conditions may be assumed to have the form

$$W = \sin mx \sum_{n=1} A_n \cos ny, \quad (9.34a)$$

$$F = \sin mx \sum_{n=1} B_n \cos ny, \quad (9.34b)$$

$$\Psi_x = \cos mx \sum_{n=1} C_n \cos ny, \quad (9.34c)$$

$$\Psi_y = \sin mx \sum_{n=1} D_n \sin ny \quad (9.34d)$$

and the initial in-plane loads are assumed to have the form

$$N_x^0 = -B_{00} \cos y, \quad N_y^0 = -b_{00}. \quad (9.35)$$

From Eq.(9.33) one has

$$b_{00} = \varepsilon^{-1} (\gamma_{T2} - \gamma_5 \gamma_{T1}) \Delta T. \quad (9.36)$$

Substituting Eqs.(9.34a) and (9.34b) into Eq.(9.29), yields

$$B_n = \varepsilon^{-1} \gamma_{24} \frac{m^2}{g_{210}^{(n)}}. \quad (9.37)$$

Substituting Eqs.(9.34a), (9.34c) and (9.34d) into Eqs.(9.30) and (9.31), yields

$$C_n = -m \frac{g_{04}^{(n)}}{g_{210}^{(n)}} A_n, \quad D_n = n \beta \frac{g_{03}^{(n)}}{g_{210}^{(n)}} A_n. \quad (9.38)$$

Substituting Eqs.(9.34)–(9.38) into Eq.(9.28), one has

$$\begin{aligned} & \sin mx \sum_{n=1} A_n \{ g_{08}^{(n)} + \varepsilon^{-2} \gamma_{14} \gamma_{24} \frac{m^4}{g_{210}^{(n)}} + [1 + \tau^2 \pi^2 (m^2 + n^2 \beta^2)] [k_1 + \\ & k_2 (m^2 + n^2 \beta^2) - \varepsilon^{-1} \gamma_{14} n^2 \beta^2 (\gamma_{T2} - \gamma_5 \gamma_{T1}) \Delta T] \} \cos ny \\ & - \frac{1}{2} \gamma_{14} \beta^2 B_{00} m^2 \sin mx \sum_{n=1} A_n \{ 1 + \tau^2 \pi^2 [m^2 + (n+1)^2 \beta^2] \} \cos(n+1)y \\ & - \frac{1}{2} \gamma_{14} \beta^2 B_{00} m^2 \sin mx \sum_{n=1} A_n \{ 1 + \tau^2 \pi^2 [m^2 + (n-1)^2 \beta^2] \} \cos(n-1)y \\ & = Q(B_{00}, A_n, x, y) = 0. \end{aligned} \quad (9.39)$$

Equation (9.39) may then be solved by Galerkin procedure. It has been reported that three terms of n are sufficient to accurately predict the critical bending moment [18]. Taking $n=1, 2$ and 3 , and let

$$\int_0^{2\pi} \int_0^\pi Q(B_{00}, A_1, A_1, A_3, x, y) (\sin mx \cos y) dx dy = 0, \quad (9.40a)$$

$$\int_0^{2\pi} \int_0^\pi Q(B_{00}, A_1, A_2, A_3, x, y) (\sin mx \cos 2y) dx dy = 0, \quad (9.40b)$$

$$\int_0^{2\pi} \int_0^\pi Q(B_{00}, A_1, A_2, A_3, x, y) (\sin mx \cos 3y) dx dy = 0. \quad (9.40c)$$

From Eq.(9.40), the existence of nontrivial solution for A_1, A_2 and A_3 leads to

$$\begin{vmatrix} a_{11} & a_{12} & 0 \\ a_{21} & a_{22} & a_{23} \\ 0 & a_{32} & a_{33} \end{vmatrix} = 0. \quad (9.41)$$

Solving Eq.(9.41), we obtain the buckling load in an explicit form

$$B_{00} = \frac{2}{\gamma_{14} \beta^2 m^2} \left[\frac{a_{11} a_{22} a_{33}}{R_{12} (R_{13} a_{11} + R_{11} a_{33})} \right]^{\frac{1}{2}} \quad (9.42)$$

Hence, the bending moment can be expressed by

$$M = \frac{2\pi[D_{11}^*D_{22}^*]^{\frac{1}{2}}}{\gamma_{14}\beta^2m^2} \left[\frac{a_{11}a_{22}a_{33}}{R_{12}(R_{13}a_{11} + R_{11}a_{33})} \right]^{\frac{1}{2}} \quad (9.43)$$

and the corresponding curvature can be written as

$$\frac{1}{\rho} = \frac{M}{\pi E_{11} R^3 h} \quad (9.44)$$

In the above equations all symbols are described in detail in Appendix D.

Equations (9.43) and (9.44) can be employed to obtain numerical results for buckling moments and corresponding curvatures of MTs subjected to bending in thermal environments. As is well known, the buckling of a perfect shell is a bifurcation phenomenon. In the present case, the minimum load (called buckling load) and corresponding buckling mode (m) can be determined by comparing bending moments [obtained from Eq.(9.43)] under various values of (m), which determines the number of half-waves in the axial direction.

9.4 Numerical results and discussion

Numerical results are presented in this section for MTs subjected to bending in thermal environments. The key issue is first to determine the material properties and effective wall thickness of a microtubule. However, a large variation of Young's modulus E was obtained and reported in the open literature. For example, Takasone et al.^[43] gave $E=0.1$ GPa by using a laser trapping technique and dark-field microscopy, while by using a nondestructive method Wagner et al.^[44] gave $E=2.55$ GPa which is found to be about 25 times as large as that given by Takasone et al.^[43]. It has been reported that the material properties of MTs are anisotropic and temperature-dependent^[1,4,45]. The longitudinal Young's modulus E_{11} varies from 0.12–0.46 GPa^[46], the circumferential modulus E_{22} varies from 1.0–4.0 MPa^[45] and the MTs have a very large ratio $E_{11}/G_{12} = 10^2\text{--}10^6$ ^[1,4].

MTs in living cells frequently bend and occasionally break. The experimental results of MTs with different values of length show that the mean curvature is about 0.4 rad/ μm in fibroblast cells before breaking and the broken curvature is about 1.5 rad/ μm ^[47]. This means the curvature of MTs should be greater than or equal to 0.4 rad/ μm , but must be less than 1.5 rad/ μm when buckling occurs.

We first examine the effect of material properties on the buckling loads of 13.3 MTs with $R=10.7$ nm, $h=1.6$ nm and $L/R=100$. Three cases, i.e. (a) isotropic material $E=0.8$ GPa, $\nu=0.3$ ^[2]; (b) $E_{11}=1.32$ GPa, $E_{22}=4.0$ MPa, $G_{12}=G_{12}=0.5$ and 0.01 MPa, $\nu_{12}=0.3$ ^[45]; (c) $E_{11}=1.0$ GPa, $E_{22}=1.0$ MPa, $G_{12}=1.0$ and 0.01 MPa, $\nu_{12}=0.3$ ^[48], are considered. In this example, the material properties are assumed to be independent of temperature. The buckling

moments and corresponding curvatures are calculated and are compared in Table 9.1. The results show that the buckling moments as well as curvatures are very sensitive to the shear modulus G_{12} . It can be seen that the buckling load is very high when the material properties are assumed to be isotropic, while two others predict too small curvatures. As a result, all these materials are unacceptable for the buckling analysis of MTs under bending. It is worthy to note that $h=1.6\text{ nm}^{[2]}$ is applicable, while the previously used value of 2.7 nm for wall thickness^[49,50] is thoroughly inappropriate to MTs.

Table 9.1 Buckling moments and curvatures for MTs subjected to bending ($R = 10.7\text{ nm}$ and $h = 1.6\text{ nm}$)

Source	$G_{12}\text{ /GPa}$	$L/R = 100$	
		$M_{cr}/(\text{nN}\cdot\text{nm})$	$1/\rho/(\text{rad}/\mu\text{m})$
de Pablo et al. (2003) ^a		57.446 7 (138) ^d	11.661 5
Tuszynski et al. (2005) ^b	0.5e-3	0.568 0 (52)	0.069 9
	1.0e-5	0.077 8 (33)	0.009 6
Shi et al. (2008) ^c	1.0e-3	0.688 7 (18)	0.111 8
	1.0e-5	0.046 3 (28)	0.007 5

^a $E=0.8\text{ GPa}$, $G = E/[2(1+\nu)]$, $\nu=0.3$.
^b $E_{11} = 1.32\text{ GPa}$, $E_{22} = 4.0\text{ MPa}$, $G_{12} = G_{13} = G_{23}$, $\nu_{12}=0.3$.
^c $E_{11} = 1.0\text{ GPa}$, $E_{22} = 1.0\text{ MPa}$, $G_{12} = G_{13} = G_{23}$, $\nu_{12}=0.3$.
^dThe numbers in brackets indicate the axial half wavenumber m .

In the following, the material properties are assumed to be anisotropic and temperature-dependent. Typical results are listed in Table 9.2 for 13.S MTs. Poisson’s ratio ν_{12} depends weakly on temperature change and is taken to be $\nu_{12} = 0.2^{[50]}$. The thermal expansion coefficient α_{11} varies from $(0.82\text{--}1.08)\times 10^{-4}/^\circ\text{C}$ (similar to nylon)^[51] under the thermal environmental conditions $T=5\text{--}37^\circ\text{C}$, and $\alpha_{22}/\alpha_{11}=10^{-2}$.

Table 9.2 Temperature-dependent material properties for MTs

Temperature	E_{11}	E_{22}	G_{12}	α_{11}	α_{22}
$/^\circ\text{C}$	$/\text{MPa}$	$/\text{MPa}$	$/\text{MPa}$	$/(\times 10^{-4}/^\circ\text{C})$	$/(\times 10^{-6}/^\circ\text{C})$
5	170	1.35	1.7	0.82	0.82
10	178	1.41	1.8	0.86	0.86
15	185	1.47	1.9	0.90	0.90
20	138	1.09	1.5	0.94	0.94
25	129	1.02	1.4	1.00	1.00
30	127	1.0	1.3	1.04	1.04
37	126	1.0	1.3	1.08	1.08

The key issue for successful application of the nonlocal continuum mechanics models to MTs is to determine the magnitude of the small-scale parameter e_0a . In most studies e_0 is usually taken to be 0.39 proposed by Eringen^[27]. It is suggested that e_0 (or e_0a) may be estimated by matching theoretical results obtained on the basis of nonlocal elasticity to those

from experiments, molecular dynamics (MD) or molecular mechanics (MM) simulations. For a single-walled carbon nanotube, the small-scale parameter e_0a is found to be less than 2.0 nm^[52]. However, there are no experiments conducted to determine the value of e_0a for MTs. Small scale effects on the mechanical behaviors of protein microtubules were produced analytically by assuming a range of e_0a values since its actual value is not known^[29-31]. It has been shown that the small scale parameter e_0a will have different values under different loading cases^[22,24,25]. In the present study, we give the estimation of parameter e_0a by matching the buckling curvature of MTs observed from measurements^[47] with the numerical results obtained from the nonlocal shear deformable shell model.

The buckling moments M_{cr} (in nN · nm) and corresponding curvatures $1/\rho$ (in rad/ μ m) for 13.3 MTs with $R=10.7$ nm, $h=1.6$ nm and $L/R=10, 50, 100, 200$ are calculated and compared in Table 9.3. The thermal environmental conditions are considered to be $T=5-37$ °C, and the reference temperature is assumed to be $T_0=27$ °C (~ 300 K) at which there are no thermal strains. In Table 9.3, $e_0a = 0$ means the local shear deformable shell model. It can be seen that the buckling moments are decreased with increasing the small-scale parameter e_0a . Through comparison, we find that the buckling curvature obtained from the nonlocal shear deformable shell model and existing results can match reasonably well if the small-scale parameters are properly chosen. It is found that the small-scale parameter e_0a varies within a small range of values. The reasonable range of e_0a is from 2.0–3.0 nm for 13.3 MTs under bending. It can be seen that the length of MTs has almost no effect on the buckling moments and curvatures when $L/R > 50$. Consequently, it is reasonable to state that the curvature of MTs should be greater than or equal to 0.4 rad/ μ m, but must be less than 1.5 rad/ μ m when buckling occurs. The wavelength is about 0.07 μ m which is smaller than that of 0.11 μ m in the case of axial compression^[24]. It is worthy to note that the wavelength in Euler buckling pattern^[9] is much greater than that in shell buckling pattern^[17] for compressive buckling of MTs.

Table 9.3 Comparisons of buckling moments and curvatures for 13.3 MTs with different values of L/R subjected to bending under thermal environmental conditions ($R=10.7$ nm and $h=1.6$ nm)

Temperature /°C	$L/R = 10$			$L/R = 50$		
	e_0a /nm	M_{cr} /nN·nm	$1/\rho$ /(rad/ μ m)	e_0a /nm	M_{cr} /nN·nm	$1/\rho$ /(rad/ μ m)
5	0.0	0.604 3 (3) ^a	0.577 3	0.0	0.604 3 (15)	0.577 3
	2.973	0.418 7 (3)	0.4	2.956	0.418 8 (17)	0.4
10	0.0	0.635 5 (3)	0.579 8	0.0	0.635 5 (15)	0.579 8
	2.994	0.438 4 (3)	0.4	2.976	0.438 5 (17)	0.4
15	0.0	0.665 7 (3)	0.584 3	0.0	0.665 7 (15)	0.584 3
	3.031	0.455 7 (3)	0.4	3.012	0.455 7 (17)	0.4

Continued

Temperature /°C	$L/R = 10$			$L/R = 50$		
	e_0a	M_{cr}	$1/\rho$	e_0a	M_{cr}	$1/\rho$
	/nm	/nN·nm	/(rad/μm)	/nm	/nN·nm	/(rad/μm)
20	0.0	0.508 6 (3)	0.598 5	0.0	0.508 6 (15)	0.598 5
	3.145	0.339 9 (3)	0.4	3.120	0.339 9 (17)	0.4
25	0.0	0.475 2 (3)	0.598 2	0.0	0.475 2 (15)	0.598 2
	3.143	0.317 8 (3)	0.4	3.118	0.317 7 (17)	0.4
30	0.0	0.455 1 (3)	0.581 9	0.0	0.455 1 (15)	0.581 9
	3.011	0.312 8 (3)	0.4	2.993	0.312 8 (17)	0.4
37	0.0	0.454 1 (3)	0.585 2	0.0	0.454 1 (15)	0.585 2
	3.038	0.310 3 (3)	0.4	3.018	0.310 3 (17)	0.4
$L/R = 100$			$L/R = 200$			
5	0.0	0.604 3 (30)	0.577 3	0.0	0.604 3 (60)	0.577 3
	2.955	0.418 7 (33)	0.4	2.955	0.418 8 (66)	0.4
10	0.0	0.635 5 (30)	0.579 8	0.0	0.635 5 (60)	0.579 8
	2.976	0.438 4 (33)	0.4	2.976	0.438 4 (66)	0.4
15	0.0	0.665 7 (30)	0.584 3	0.0	0.665 7 (60)	0.584 3
	3.012	0.455 6 (33)	0.4	3.011	0.455 7 (67)	0.4
20	0.0	0.508 6 (30)	0.598 5	0.0	0.508 6 (60)	0.598 5
	3.120	0.339 9 (33)	0.4	3.120	0.339 9 (68)	0.4
25	0.0	0.475 2 (30)	0.598 2	0.0	0.475 2 (60)	0.598 2
	3.118	0.317 7 (33)	0.4	3.118	0.317 7 (68)	0.4
30	0.0	0.455 1 (30)	0.581 9	0.0	0.455 1 (60)	0.581 9
	2.993	0.312 8 (33)	0.4	2.993	0.312 8 (66)	0.4
37	0.0	0.454 1 (30)	0.585 2	0.0	0.454 1 (60)	0.585 2
	3.018	0.310 3 (33)	0.4	3.018	0.310 3 (67)	0.4

^aThe numbers in brackets indicate the axial half wavenumber m .

Table 9.4 compares buckling moments of a 13.3 microtubule with $L/R = 150$ subjected to bending in thermal environments by nonlocal shear deformable shell model (NSDSM) and nonlocal thin shell model (NTSM). The results of nonlocal thin shell model are obtained analogously by solving Eqs.(9.16) and (9.17). It can be seen that the buckling moments from nonlocal thin shell model are about 8% higher than those of nonlocal shear deformable shell model. This is because in the present example the shell radius-to-thickness ratio ($R/h = 6.7$) is much smaller than 20, and in such a case the transverse shear deformation should be taken into account.

Table 9.5 presents the buckling moments and corresponding curvatures for 13.3 MTs surrounded by an elastic medium under bending in thermal environments. The small scale parameter e_0a is taken to be 2.30 nm and $L/R=100$ in this example. Three sets of foundation stiffness are considered. The stiffness is characterized by $(K_1, K_2) = (2.7, 0.27)$ (in kPa/nm, kPa·nm)

for the Pasternak foundation model, by $(K_1, K_2) = (2.7, 0)$ for the Winkler foundation model^[13], and by $(K_1, K_2) = (0, 0)$ for an unconstrained microtubule. The differences in brackets show the effect of lateral constraint on the buckling moments of MTs. Like in the case of axial compression^[20], the buckling moment of a microtubule increases when it is embedded in an elastic medium. The percentage of the increment is about 18.1%–22.7% for the Winkler foundation model, and 18.2%–22.8% for the Pasternak foundation model. Unlike in the compressive buckling case^[20], the effect of lateral constraint becomes less on the buckling loads of MTs under bending.

Table 9.4 Comparisons of buckling moments and curvatures for a 13.3 microtubule subjected to bending under thermal environmental conditions by two different nonlocal shell models ($L/R = 150$, $R = 10.7$ nm, $h = 1.6$ nm)

Temperature/°C	$e_0 a$ /nm	Theory	$M_{cr}/(\text{nN}\cdot\text{nm})$	$1/\rho/(\text{rad}/\mu\text{m})$
5	2.955	NSDSM ^a	0.418 8 (50) ^c	0.4
		NTSM ^b	0.451 7 (40) (+7.9%) ^d	0.431 5
10	2.976	NSDSM	0.438 4 (50)	0.4
		NTSM	0.472 8 (40) (+7.8%)	0.431 3
15	3.011	NSDSM	0.455 7 (50)	0.4
		NTSM	0.491 4 (40) (+7.8%)	0.431 3
20	3.120	NSDSM	0.339 9 (51)	0.4
		NTSM	0.366 1 (41) (+7.7%)	0.430 9
25	3.118	NSDSM	0.317 7 (51)	0.4
		NTSM	0.342 3 (41) (+7.7%)	0.430 9
30	2.993	NSDSM	0.312 8 (50)	0.4
		NTSM	0.337 1 (40) (+7.8%)	0.431 1
37	3.018	NSDSM	0.310 3 (50)	0.4
		NTSM	0.334 6 (40) (+7.8%)	0.431 2

^aNSDSM = nonlocal shear deformable shell model.

^bNTSM = nonlocal thin shell model.

^cThe numbers in brackets indicate the axial half wavenumber m .

^dDifference = $100\%[M_{cr}(\text{TSM}) - M_{cr}(\text{SDSM})]/M_{cr}(\text{SDSM})$.

Table 9.5 Comparisons of buckling moments and curvatures for a 13.3 microtubule embedded in an elastic medium subjected to bending under thermal environmental conditions ($L/R=100$, $R=10.7$ nm, $h=1.6$ nm, $e_0 a=2.30$ nm)

Temperature /°C	(K_1, K_2) /(kPa/nm, kPa·nm)	M_{cr} /(nN·nm)	$1/\rho$ /(rad/μm)
5	(0, 0)	0.476 8 (32) ^a	0.455 5
	(2.7, 0.0)	0.571 9 (53) (+19.9%) ^b	0.546 3
	(2.7, 0.27)	0.572 3 (53) (+20.0%)	0.546 7
10	(0, 0)	0.501 3 (32)	0.457 4
	(2.7, 0.0)	0.598 3 (51) (+19.3%)	0.545 8
	(2.7, 0.27)	0.598 7 (52) (+19.4%)	0.546 2

Continued			
Temperature	(K_1, K_2)	M_{cr}	$1/\rho$
/°C	/(kPa/nm, kPa·nm)	/(nN·nm)	/(rad/μm)
15	(0, 0)	0.525 1 (32)	0.460 9
	(2.7, 0.0)	0.623 3 (50) (+18.7%)	0.547 2
	(2.7, 0.27)	0.623 8 (51) (+18.8%)	0.547 5
20	(0, 0)	0.401 0 (32)	0.471 9
	(2.7, 0.0)	0.489 5 (55) (+22.1%)	0.576 0
	(2.7, 0.27)	0.489 9 (55) (+22.2%)	0.576 5
25	(0, 0)	0.374 7 (32)	0.471 7
	(2.7, 0.0)	0.460 7 (57) (+22.9%)	0.580 0
	(2.7, 0.27)	0.461 1 (57) (+23.0%)	0.580 5
30	(0, 0)	0.359 0 (32)	0.459 1
	(2.7, 0.0)	0.443 8 (60) (+23.6%)	0.567 4
	(2.7, 0.27)	0.444 1 (60) (+23.7%)	0.567 9
37	(0, 0)	0.358 1 (32)	0.461 6
	(2.7, 0.0)	0.442 5 (60) (+23.6%)	0.570 4
	(2.7, 0.27)	0.442 9 (60) (+23.7%)	0.570 8

^aThe numbers in brackets indicate the axial half wavenumber m .
^bDifference = $100\%[M_{cr}(k_1, k_2 \neq 0) - M_{cr}(k_1 = k_2 = 0)]/M_{cr}(k_1 = k_2 = 0)$.

It is worth noting that, from Tables 9.3–9.5, the variation of temperature reduces the elastic moduli and decreases the buckling moments of MTs unconstrained or surrounded by an elastic medium when $T > 15^\circ\text{C}$. It can also be seen that the effect of temperature change becomes less significant when $T > 30^\circ\text{C}$.

9.5 Conclusions

A version of nonlocal elasticity theory is employed to develop a nonlocal shear deformable shell model. The nonlocal stress incorporating the small-scale parameter e_0a is introduced into the governing equations. These equations are then analytically solved for the buckling problems of MTs embedded in an elastic matrix of cytoplasm subjected to bending in thermal environments. The surrounding elastic medium is modeled as a Pasternak foundation to account for not only the normal stress, but also the shear stress between the microtubule and the surrounding matrix. An approximate solution is obtained in an explicit form that can be easily developed in program code. The numerical results show that buckling moments are decreased with increasing the small-scale parameter e_0a . The results reveal that the lateral constraint has a significant effect on the buckling moments of a microtubule when the foundation stiffness is sufficiently large.

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Appendix A

In Eq.(9.4) the thermal forces $\overline{N}^t$, moments $\overline{M}^t$ and $\overline{S}^t$, and higher order moments $\overline{P}^t$ caused by elevated temperature are defined by

$$\begin{bmatrix} \overline{N}_x^t & \overline{M}_x^t & \overline{P}_x^t \\ \overline{N}_y^t & \overline{M}_y^t & \overline{P}_y^t \\ \overline{N}_{xy}^t & \overline{M}_{xy}^t & \overline{P}_{xy}^t \end{bmatrix} = \int_{-\frac{h}{2}}^{\frac{h}{2}} \begin{bmatrix} A_x(T) \\ A_y(T) \\ A_{xy}(T) \end{bmatrix} (1, Z, Z^3) \Delta T dZ \quad (\text{A.1a})$$

and

$$\begin{bmatrix} \overline{S}_x^t \\ \overline{S}_y^t \\ \overline{S}_{xy}^t \end{bmatrix} = \begin{bmatrix} \overline{M}_x^t \\ \overline{M}_y^t \\ \overline{M}_{xy}^t \end{bmatrix} - \frac{4}{3h^2} \begin{bmatrix} \overline{P}_x^t \\ \overline{P}_y^t \\ \overline{P}_{xy}^t \end{bmatrix} \quad (\text{A.1b})$$

where $\Delta T = T - T_0$ is the temperature rise from some reference temperature T_0 at which there are no thermal strains, and

$$\begin{bmatrix} A_x(T) \\ A_y(T) \\ A_{xy}(T) \end{bmatrix} = - \begin{bmatrix} \overline{Q}_{11} & \overline{Q}_{12} & \overline{Q}_{16} \\ \overline{Q}_{12} & \overline{Q}_{22} & \overline{Q}_{26} \\ \overline{Q}_{16} & \overline{Q}_{26} & \overline{Q}_{66} \end{bmatrix} \begin{bmatrix} c^2 & s^2 \\ s^2 & c^2 \\ 2cs & -2cs \end{bmatrix} \begin{bmatrix} \alpha_{11}(T) \\ \alpha_{22}(T) \end{bmatrix} \quad (\text{A.2})$$

in which α_{11} and α_{22} are the thermal expansion coefficients in the longitudinal and transverse directions, and $\overline{Q}_{ij}$ are the transformed elastic constants, defined by

$$\begin{bmatrix} \overline{Q}_{11} \\ \overline{Q}_{12} \\ \overline{Q}_{22} \\ \overline{Q}_{16} \\ \overline{Q}_{26} \\ \overline{Q}_{66} \end{bmatrix} = \begin{bmatrix} c^4 & 2c^2s^2 & s^4 & 4c^2s^2 \\ c^2s^2 & c^4 + s^4 & c^2s^2 & -4c^2s^2 \\ s^4 & 2c^2s^2 & c^4 & 4c^2s^2 \\ c^3s & cs^3 - c^3s & -cs^3 & -2cs(c^2 - s^2) \\ cs^3 & c^3s - cs^3 & -c^3s & 2cs(c^2 - s^2) \\ c^2s^2 & -2c^2s^2 & c^2s^2 & (c^2 - s^2)^2 \end{bmatrix} \begin{bmatrix} Q_{11} \\ Q_{12} \\ Q_{22} \\ Q_{66} \end{bmatrix}, \quad (\text{A.3a})$$

$$\begin{bmatrix} \overline{Q}_{44} \\ \overline{Q}_{45} \\ \overline{Q}_{55} \end{bmatrix} = \begin{bmatrix} c^2 & s^2 \\ -cs & cs \\ s^2 & c^2 \end{bmatrix} \begin{bmatrix} Q_{44} \\ Q_{55} \end{bmatrix} \quad (\text{A.3b})$$

where

$$Q_{11} = \frac{E_{11}(T)}{(1 - \nu_{12}\nu_{21})}, \quad Q_{22} = \frac{E_{22}(T)}{(1 - \nu_{12}\nu_{21})}, \quad Q_{12} = \frac{\nu_{21}E_{11}(T)}{(1 - \nu_{12}\nu_{21})},$$

$$Q_{44} = G_{23}(T), \quad Q_{55} = G_{13}(T), \quad Q_{66} = G_{12}(T) \quad (\text{A.4a})$$

and

$$c = \cos \theta, \quad s = \sin \theta \quad (\text{A.4b})$$

where θ is the skew angle with respect to the shell x axis.

Appendix B

In Eqs.(9.9)–(9.12)

$$\begin{aligned} \tilde{L}_{11}(\) &= \frac{4}{3h^2} \left[F_{11}^* \frac{\partial^4}{\partial X^4} + 2(F_{16}^* + F_{61}^*) \frac{\partial^4}{\partial X^3 \partial Y} \right. \\ &\quad \left. + (F_{12}^* + F_{21}^* + 4F_{66}^*) \frac{\partial^4}{\partial X^2 \partial Y^2} \right. \\ &\quad \left. + 2(F_{26}^* + F_{62}^*) \frac{\partial^4}{\partial X \partial Y^3} + F_{22}^* \frac{\partial^4}{\partial Y^4} \right], \\ \tilde{L}_{12}(\) &= \left(D_{11}^* - \frac{4}{3h^2} F_{11}^* \right) \frac{\partial^3}{\partial X^3} + \left[3D_{16}^* - \frac{4}{3h^2} (F_{61}^* + 2F_{16}^*) \right] \frac{\partial^3}{\partial X^2 \partial Y} \\ &\quad + \left[(D_{12}^* + 2D_{66}^*) - \frac{4}{3h^2} (F_{12}^* + 2F_{66}^*) \right] \frac{\partial^3}{\partial X \partial Y^2} \\ &\quad + \left(D_{26}^* - \frac{4}{3h^2} F_{26}^* \right) \frac{\partial^3}{\partial Y^3}, \\ \tilde{L}_{13}(\) &= \left(D_{16}^* - \frac{4}{3h^2} F_{16}^* \right) \frac{\partial^3}{\partial X^3} + \left[(D_{12}^* + 2D_{66}^*) - \frac{4}{3h^2} (F_{21}^* + 2F_{66}^*) \right] \\ &\quad \cdot \frac{\partial^3}{\partial X^2 \partial Y} + \left[3D_{26}^* - \frac{4}{3h^2} (F_{62}^* + 2F_{26}^*) \right] \frac{\partial^3}{\partial X \partial Y^2} \\ &\quad + \left(D_{22}^* - \frac{4}{3h^2} F_{22}^* \right) \frac{\partial^3}{\partial Y^3}, \\ \tilde{L}_{14}(\) &= B_{21}^* \frac{\partial^4}{\partial X^4} + (2B_{26}^* - B_{61}^*) \frac{\partial^4}{\partial X^3 \partial Y} + (B_{11}^* + B_{22}^* - 2B_{66}^*) \frac{\partial^4}{\partial X^2 \partial Y^2} \\ &\quad + (2B_{16}^* - B_{62}^*) \frac{\partial^4}{\partial X \partial Y^3} + B_{12}^* \frac{\partial^4}{\partial Y^4}, \\ \tilde{L}_{15}(\overline{N}^t) &= \frac{\partial^2}{\partial X^2} (B_{11}^* \overline{N}_x^t + B_{21}^* \overline{N}_y^t + B_{61}^* \overline{N}_{xy}^t) \\ &\quad + 2 \frac{\partial^2}{\partial X \partial Y} (B_{16}^* \overline{N}_x^t + B_{26}^* \overline{N}_y^t + B_{66}^* \overline{N}_{xy}^t) \end{aligned}$$

$$\begin{aligned}
& + \frac{\partial^2}{\partial Y^2} (B_{12}^* \bar{N}_x^t + B_{22}^* \bar{N}_y^t + B_{62}^* \bar{N}_{xy}^t), \\
\tilde{L}_{16}(\bar{M}^t) &= \frac{\partial^2}{\partial X^2} (\bar{M}_x^t) + 2 \frac{\partial^2}{\partial X \partial Y} (\bar{M}_{xy}^t) + \frac{\partial^2}{\partial Y^2} (\bar{M}_y^t), \\
\tilde{L}_{21}(\quad) &= A_{22}^* \frac{\partial^4}{\partial X^4} - 2A_{26}^* \frac{\partial^4}{\partial X^3 \partial Y} + (2A_{12}^* + A_{66}^*) \frac{\partial^4}{\partial X^2 \partial Y^2} \\
&\quad - 2A_{16}^* \frac{\partial^4}{\partial X \partial Y^3} + A_{11}^* \frac{\partial^4}{\partial Y^4}, \\
\tilde{L}_{22}(\quad) &= \left(B_{21}^* - \frac{4}{3h^2} E_{21}^* \right) \frac{\partial^3}{\partial X^3} + \left[(B_{26}^* - B_{61}^*) - \frac{4}{3h^2} (E_{26}^* - E_{61}^*) \right] \\
&\quad \cdot \frac{\partial^3}{\partial X^2 \partial Y} + \left[(B_{11}^* - B_{66}^*) - \frac{4}{3h^2} (E_{11}^* - E_{66}^*) \right] \frac{\partial^3}{\partial X \partial Y^2} \\
&\quad + \left(B_{16}^* - \frac{4}{3h^2} E_{16}^* \right) \frac{\partial^3}{\partial Y^3}, \\
\tilde{L}_{23}(\quad) &= \left(B_{26}^* - \frac{4}{3h^2} E_{26}^* \right) \frac{\partial^3}{\partial X^3} + \left[(B_{22}^* - B_{66}^*) - \frac{4}{3h^2} (E_{22}^* - E_{66}^*) \right] \\
&\quad \cdot \frac{\partial^3}{\partial X^2 \partial Y} + \left[(B_{16}^* - B_{62}^*) - \frac{4}{3h^2} (E_{16}^* - E_{62}^*) \right] \frac{\partial^3}{\partial X \partial Y^2} \\
&\quad + \left(B_{12}^* - \frac{4}{3h^2} E_{12}^* \right) \frac{\partial^3}{\partial Y^3}, \\
\tilde{L}_{24}(\quad) &= \frac{4}{3h^2} \left[E_{21}^* \frac{\partial^4}{\partial X^4} + (2E_{26}^* - E_{61}^*) \frac{\partial^4}{\partial X^3 \partial Y} \right. \\
&\quad + (E_{11}^* + E_{22}^* - 2E_{66}^*) \frac{\partial^4}{\partial X^2 \partial Y^2} \\
&\quad \left. + (2E_{16}^* - E_{62}^*) \frac{\partial^4}{\partial X \partial Y^3} + E_{12}^* \frac{\partial^4}{\partial Y^4} \right], \\
\tilde{L}_{25}(\bar{N}^t) &= \frac{\partial^2}{\partial X^2} (A_{12}^* \bar{N}_x^t + A_{22}^* \bar{N}_y^t + A_{26}^* \bar{N}_{xy}^t) \\
&\quad - \frac{\partial^2}{\partial X \partial Y} (A_{16}^* \bar{N}_x^t + A_{26}^* \bar{N}_y^t + A_{66}^* \bar{N}_{xy}^t) \\
&\quad + \frac{\partial^2}{\partial Y^2} (A_{11}^* \bar{N}_x^t + A_{12}^* \bar{N}_y^t + A_{16}^* \bar{N}_{xy}^t), \\
\tilde{L}_{31}(\quad) &= \left(A_{55} - \frac{8}{h^2} D_{55} + \frac{16}{h^4} F_{55} \right) \frac{\partial}{\partial X} + \left(A_{45} - \frac{8}{h^2} D_{45} + \frac{16}{h^4} F_{45} \right) \frac{\partial}{\partial Y} \\
&\quad + \frac{4}{3h^2} \left\{ \left(F_{11}^* - \frac{4}{3h^2} H_{11}^* \right) \frac{\partial^3}{\partial X^3} + \left[(F_{16}^* + 2F_{61}^*) - \frac{4}{h^2} H_{16}^* \right] \right. \\
&\quad \cdot \frac{\partial^3}{\partial X^2 \partial Y} + \left[(F_{21}^* + 2F_{66}^*) - \frac{4}{3h^2} (H_{12}^* + 2H_{66}^*) \right] \frac{\partial^3}{\partial X \partial Y^2} \\
&\quad \left. + \left(F_{26}^* - \frac{4}{3h^2} H_{26}^* \right) \frac{\partial^3}{\partial Y^3} \right\},
\end{aligned}$$

$$\begin{aligned}\tilde{L}_{32}(\) &= \left(A_{55} - \frac{8}{h^2} D_{55} + \frac{16}{h^4} F_{55} \right) - \left(D_{11}^* - \frac{8}{3h^2} F_{11}^* + \frac{16}{9h^4} H_{11}^* \right) \frac{\partial^2}{\partial X^2} \\ &\quad - 2 \left[D_{16}^* - \frac{4}{3h^2} (F_{16}^* + F_{61}^*) + \frac{16}{9h^4} H_{16}^* \right] \frac{\partial^2}{\partial X \partial Y} \\ &\quad - \left(D_{66}^* - \frac{8}{3h^2} F_{66}^* + \frac{16}{9h^4} H_{66}^* \right) \frac{\partial^2}{\partial Y^2},\end{aligned}$$

$$\begin{aligned}\tilde{L}_{33}(\) &= \left(A_{45} - \frac{8}{h^2} D_{45} + \frac{16}{h^4} F_{45} \right) - \left[D_{16}^* - \frac{4}{3h^2} (F_{16}^* + F_{61}^*) \right. \\ &\quad \left. + \frac{16}{9h^4} H_{16}^* \right] \frac{\partial^2}{\partial X^2} - \left[(D_{12}^* + D_{66}^*) - \frac{4}{3h^2} (F_{12}^* + F_{21}^* + 2F_{66}^*) \right. \\ &\quad \left. + \frac{16}{9h^4} (H_{12}^* + H_{66}^*) \right] \frac{\partial^2}{\partial X \partial Y} \\ &\quad - \left[D_{26}^* - \frac{4}{3h^2} (F_{26}^* + F_{62}^*) + \frac{16}{9h^4} H_{26}^* \right] \frac{\partial^2}{\partial Y^2},\end{aligned}$$

$$\tilde{L}_{34}(\) = \tilde{L}_{22}(\),$$

$$\begin{aligned}\tilde{L}_{35}(\overline{N}^t) &= \frac{\partial}{\partial X} \left[\left(B_{11}^* - \frac{4}{3h^2} E_{11}^* \right) \overline{N}_x^t + \left(B_{21}^* - \frac{4}{3h^2} E_{21}^* \right) \overline{N}_y^t \right. \\ &\quad \left. + \left(B_{61}^* - \frac{4}{3h^2} E_{61}^* \right) \overline{N}_{xy}^t \right] + \frac{\partial}{\partial Y} \left[\left(B_{16}^* - \frac{4}{3h^2} E_{16}^* \right) \overline{N}_x^t \right. \\ &\quad \left. + \left(B_{26}^* - \frac{4}{3h^2} E_{26}^* \right) \overline{N}_y^t + \left(B_{66}^* - \frac{4}{3h^2} E_{66}^* \right) \overline{N}_{xy}^t \right],\end{aligned}$$

$$\tilde{L}_{36}(\overline{S}^t) = \frac{\partial}{\partial X} (\overline{S}_x^t) + \frac{\partial}{\partial Y} (\overline{S}_{xy}^t),$$

$$\begin{aligned}\tilde{L}_{41}(\) &= \left(A_{45} - \frac{8}{h^2} D_{45} + \frac{16}{h^4} F_{45} \right) \frac{\partial}{\partial X} + \left(A_{44} - \frac{8}{h^2} D_{44} + \frac{16}{h^4} F_{44} \right) \frac{\partial}{\partial Y} \\ &\quad + \frac{4}{3t^2} \left\{ \left(F_{16}^* - \frac{4}{3h^2} H_{16}^* \right) \frac{\partial^3}{\partial X^3} + \left[(F_{12}^* + 2F_{66}^*) \right. \right. \\ &\quad \left. \left. - \frac{4}{3h^2} (H_{12}^* - 2H_{66}^*) \right] \frac{\partial^3}{\partial X \partial Y^2} + \left[(F_{26}^* + 2F_{62}^*) - \frac{4}{h^2} H_{26}^* \right] \frac{\partial^3}{\partial X \partial Y^2} \right. \\ &\quad \left. + \left(F_{22}^* - \frac{4}{3h^2} H_{22}^* \right) \frac{\partial^3}{\partial Y^3} \right\},\end{aligned}$$

$$\tilde{L}_{42}(\) = \tilde{L}_{33}(\),$$

$$\begin{aligned}\tilde{L}_{43}(\) &= \left(A_{44} - \frac{8}{h^2} D_{44} + \frac{16}{h^4} F_{44} \right) - \left(D_{66}^* - \frac{8}{3h^2} F_{66}^* + \frac{16}{9h^4} H_{66}^* \right) \frac{\partial^2}{\partial X^2} \\ &\quad - 2 \left[D_{26}^* - \frac{4}{3h^2} (F_{26}^* + F_{62}^*) + \frac{16}{9h^4} H_{26}^* \right] \frac{\partial^2}{\partial X \partial Y} \\ &\quad - \left(D_{22}^* - \frac{8}{3h^2} F_{22}^* + \frac{16}{9h^4} H_{22}^* \right) \frac{\partial^2}{\partial Y^2},\end{aligned}$$

$$\begin{aligned}
\tilde{L}_{44}(\) &= \tilde{L}_{23}(\), \\
\tilde{L}_{45}(\overline{N}^t) &= \frac{\partial}{\partial X} \left[\left(B_{16}^* - \frac{4}{3h^2} E_{16}^* \right) \overline{N}_x^t + \left(B_{26}^* - \frac{4}{3h^2} E_{26}^* \right) \overline{N}_y^t \right. \\
&\quad \left. + \left(B_{66}^* - \frac{4}{3h^2} E_{66}^* \right) \overline{N}_{xy}^t \right] + \frac{\partial}{\partial Y} \left[\left(B_{12}^* - \frac{4}{3h^2} E_{12}^* \right) \overline{N}_x^t \right. \\
&\quad \left. + \left(B_{22}^* - \frac{4}{3h^2} E_{22}^* \right) \overline{N}_y^t + \left(B_{62}^* - \frac{4}{3h^2} E_{62}^* \right) \overline{N}_{xy}^t \right], \\
\tilde{L}_{46}(\overline{S}^t) &= \frac{\partial}{\partial X} (\overline{S}_{xy}^t) + \frac{\partial}{\partial Y} (\overline{S}_y^t), \\
\tilde{L}(\) &= \frac{\partial^2}{\partial X^2} \frac{\partial^2}{\partial Y^2} - 2 \frac{\partial^2}{\partial X \partial Y} \frac{\partial^2}{\partial X \partial Y} + \frac{\partial^2}{\partial Y^2} \frac{\partial^2}{\partial X^2}.
\end{aligned} \tag{B.1}$$

In the above equations $[A_{ij}^*]$, $[B_{ij}^*]$, $[D_{ij}^*]$, $[E_{ij}^*]$, $[F_{ij}^*]$ and $[H_{ij}^*]$ are reduced stiffness matrices, defined as

$$\begin{aligned}
\mathbf{A}^* &= \mathbf{A}^{-1}, \quad \mathbf{B}^* = -\mathbf{A}^{-1} \mathbf{B}, \quad \mathbf{D}^* = \mathbf{D} - \mathbf{B} \mathbf{A}^{-1} \mathbf{B}, \quad \mathbf{E}^* = -\mathbf{A}^{-1} \mathbf{E}, \\
\mathbf{F}^* &= \mathbf{F} - \mathbf{E} \mathbf{A}^{-1} \mathbf{B}, \quad \mathbf{H}^* = \mathbf{H} - \mathbf{E} \mathbf{A}^{-1} \mathbf{E}
\end{aligned} \tag{B.2}$$

where A_{ij} , B_{ij} , etc., are the shell stiffnesses, defined by

$$\begin{aligned}
(A_{ij}, B_{ij}, D_{ij}, E_{ij}, F_{ij}, H_{ij}) &= \int_{-\frac{h}{2}}^{+\frac{h}{2}} \overline{Q}_{ij}(1, Z, Z^2, Z^3, Z^4, Z^6) dZ \\
&\quad (i, j = 1, 2, 6)
\end{aligned} \tag{B.3a}$$

$$(A_{ij}, D_{ij}, F_{ij}) = \int_{-\frac{h}{2}}^{+\frac{h}{2}} \overline{Q}_{ij}(1, Z^2, Z^4) dZ \quad (i, j = 4, 5) \tag{B.3b}$$

Appendix C

In Eqs.(9.28)–(9.31) the non-dimensional linear operators $L_{ij}(\)$ are defined by

$$\begin{aligned}
L_{11}(\) &= \gamma_{110} \frac{\partial^4}{\partial x^4} + 2\gamma_{112} \beta^2 \frac{\partial^4}{\partial x^2 \partial y^2} + \gamma_{114} \beta^4 \frac{\partial^4}{\partial y^4}, \\
L_{12}(\) &= \gamma_{120} \frac{\partial^3}{\partial x^3} + \gamma_{122} \beta^2 \frac{\partial^3}{\partial x \partial y^2}, \\
L_{13}(\) &= \gamma_{131} \beta \frac{\partial^3}{\partial x^2 \partial y} + \gamma_{133} \beta^3 \frac{\partial^3}{\partial y^3}, \\
L_{21}(\) &= \frac{\partial^4}{\partial x^4} + 2\gamma_{212} \beta^2 \frac{\partial^4}{\partial x^2 \partial y^2} + \gamma_{214} \beta^4 \frac{\partial^4}{\partial y^4},
\end{aligned}$$

$$\begin{aligned}
L_{31}(\quad) &= \gamma_{31} \frac{\partial}{\partial x} + \gamma_{310} \frac{\partial^3}{\partial x^3} + \gamma_{312} \beta^2 \frac{\partial^3}{\partial x \partial y^2}, \\
L_{32}(\quad) &= \gamma_{31} - \gamma_{320} \frac{\partial^2}{\partial x^2} - \gamma_{322} \beta^2 \frac{\partial^2}{\partial y^2}, \\
L_{33}(\quad) &= -\gamma_{331} \beta \frac{\partial^2}{\partial x \partial y}, \\
L_{41}(\quad) &= \gamma_{42} \beta \frac{\partial}{\partial y} + \gamma_{411} \beta \frac{\partial^3}{\partial x^2 \partial y} + \gamma_{413} \beta^3 \frac{\partial^3}{\partial y^3}, \\
L_{42}(\quad) &= L_{33}(\quad), \\
L_{43}(\quad) &= \gamma_{42} - \gamma_{430} \frac{\partial^2}{\partial x^2} - \gamma_{432} \beta^2 \frac{\partial^2}{\partial y^2}
\end{aligned} \tag{C.1}$$

and in the above equations

$$\begin{aligned}
(\gamma_{110}, \gamma_{112}, \gamma_{114}) &= \frac{\frac{4}{3h^2} \left(F_{11}^*, \frac{F_{12}^* + F_{21}^* + 4F_{66}^*}{2}, F_{22}^* \right)}{D_{11}^*}, \\
(\gamma_{120}, \gamma_{122}) &= \frac{D_{11}^* - \frac{4F_{11}^*}{3h^2}, (D_{12}^* + 2D_{66}^*) - \frac{4(F_{12}^* + 2F_{66}^*)}{3h^2}}{D_{11}^*}, \\
(\gamma_{131}, \gamma_{133}) &= \frac{(D_{12}^* + 2D_{66}^*) - \frac{4(F_{21}^* + 2F_{66}^*)}{3h^2}, D_{22}^* - \frac{4F_{22}^*}{3h^2}}{D_{11}^*}, \\
(\gamma_{212}, \gamma_{214}) &= \frac{A_{12}^* + \frac{A_{66}^*}{2}, A_{11}^*}{A_{22}^*}, \\
(\gamma_{310}, \gamma_{312}) &= \frac{\frac{4}{3h^2} \left[F_{11}^* - \frac{4H_{11}^*}{3h^2}, (F_{21}^* + 2F_{66}^*) - \frac{4(H_{12}^* + 2H_{66}^*)}{3h^2} \right]}{D_{11}^*}, \\
(\gamma_{320}, \gamma_{322}) &= \frac{D_{11}^* - \frac{8F_{11}^*}{3h^2} + \frac{16H_{11}^*}{9h^4}, D_{66}^* - \frac{8F_{66}^*}{3h^2} + \frac{16H_{66}^*}{9h^4}}{D_{11}^*}, \\
(\gamma_{31}, \gamma_{42}) &= \frac{\frac{L^2}{\pi^2} \left(A_{55} - \frac{8D_{55}}{h^2} + \frac{16F_{55}}{h^4}, A_{44} - \frac{8D_{44}}{h^2} + \frac{16F_{44}}{h^4} \right)}{D_{11}^*}, \\
\gamma_{331} &= \frac{(D_{12}^* + D_{66}^*) - \frac{4(F_{12}^* + F_{21}^* + 2F_{66}^*)}{3h^2} + \frac{16(H_{12}^* + H_{66}^*)}{9h^4}}{D_{11}^*}, \\
(\gamma_{411}, \gamma_{413}) &= \frac{\frac{4}{3h^2} \left[(F_{12}^* + 2F_{66}^*) - \frac{4(H_{12}^* + 2H_{66}^*)}{3h^2}, F_{22}^* - \frac{4H_{22}^*}{3h^2} \right]}{D_{11}^*},
\end{aligned}$$

$$(\gamma_{430}, \gamma_{432}) = \frac{D_{66}^* - \frac{8F_{66}^*}{3h^2} + \frac{16H_{66}^*}{9h^4}, D_{22}^* - \frac{8F_{22}^*}{3h^2} + \frac{16H_{22}^*}{9h^4}}{D_{11}^*}. \quad (C.2)$$

Appendix D

In Eq.(9.41)

$$\begin{aligned} a_{11} &= g_{08}^{(1)} + \varepsilon^{-2} \gamma_{14} \gamma_{24} \frac{m^4}{g_{210}^{(1)}} + R_{11} [F_{11} - \varepsilon^{-1} \gamma_{14} \beta^2 (\gamma_{T2} - \gamma_5 \gamma_{T1})] \Delta T, \\ a_{12} &= -\frac{1}{2} \gamma_{14} \beta^2 B_{00} m^2 R_{11}, \quad a_{21} = -\frac{1}{2} \gamma_{14} \beta^2 B_{00} m^2 R_{12}, \\ a_{22} &= g_{08}^{(2)} + \varepsilon^{-2} \gamma_{14} \gamma_{24} \frac{m^4}{g_{210}^{(2)}} + R_{11} [F_{12} - 4\varepsilon^{-1} \gamma_{14} \beta^2 (\gamma_{T2} - \gamma_5 \gamma_{T1})] \Delta T, \\ a_{13} &= -\frac{1}{2} \gamma_{14} \beta^2 B_{00} m^2 R_{12}, \quad a_{32} = -\frac{1}{2} \gamma_{14} \beta^2 B_{00} m^2 R_{13}, \\ a_{33} &= g_{08}^{(3)} + \varepsilon^{-2} \gamma_{14} \gamma_{24} \frac{m^4}{g_{210}^{(3)}} + R_{11} [F_{13} - 9\varepsilon^{-1} \gamma_{14} \beta^2 (\gamma_{T2} - \gamma_5 \gamma_{T1})] \Delta T \quad (D.1) \end{aligned}$$

and in the above equations

$$\begin{aligned} R_{11} &= 1 + \tau^2 \pi^2 (m^2 + \beta^2), \quad R_{12} = 1 + \tau^2 \pi^2 (m^2 + 4\beta^2), \\ R_{13} &= 1 + \tau^2 \pi^2 (m^2 + 9\beta^2), \\ F_{11} &= k_1 + k_2 (m^2 + \beta^2), \quad F_{12} = k_1 + k_2 (m^2 + 4\beta^2), \\ F_{13} &= k_1 + k_2 (m^2 + 9\beta^2), \\ g_{210}^{(n)} &= (m^4 + 2\gamma_{212} m^2 n^2 \beta^2 + \gamma_{214} n^4 \beta^4) \quad (n = 1, 2, 3, \dots), \\ g_{08}^{(n)} &= (\gamma_{110} m^4 + 2\gamma_{112} m^2 n^2 \beta^2 + \gamma_{114} n^4 \beta^4) \\ &\quad + \frac{m^2 (\gamma_{120} m^2 + \gamma_{122} n^2 \beta^2) g_{04}^{(n)} + n^2 \beta^2 (\gamma_{131} m^2 + \gamma_{133} n^2 \beta^2) g_{03}^{(n)}}{g_{00}^{(n)}} \\ &\quad (n = 1, 2, 3, \dots), \\ g_{00}^{(n)} &= (\gamma_{31} + \gamma_{320} m^2 + \gamma_{322} n^2 \beta^2) (\gamma_{42} + \gamma_{430} m^2 + \gamma_{432} n^2 \beta^2) - \gamma_{331}^2 m^2 n^2 \beta^2 \\ &\quad (n = 1, 2, 3, \dots), \\ g_{03}^{(n)} &= (\gamma_{31} + \gamma_{320} m^2 + \gamma_{322} n^2 \beta^2) (\gamma_{42} - \gamma_{411} m^2 - \gamma_{413} n^2 \beta^2) \\ &\quad - \gamma_{331} m^2 (\gamma_{31} - \gamma_{310} m^2 - \gamma_{312} n^2 \beta^2) \quad (n = 1, 2, 3, \dots), \\ g_{04}^{(n)} &= (\gamma_{42} + \gamma_{430} m^2 + \gamma_{432} n^2 \beta^2) (\gamma_{31} - \gamma_{310} m^2 - \gamma_{312} n^2 \beta^2) \\ &\quad - \gamma_{331} n^2 \beta^2 (\gamma_{42} - \gamma_{411} m^2 - \gamma_{413} n^2 \beta^2) \quad (n = 1, 2, 3, \dots). \quad (D.2) \end{aligned}$$

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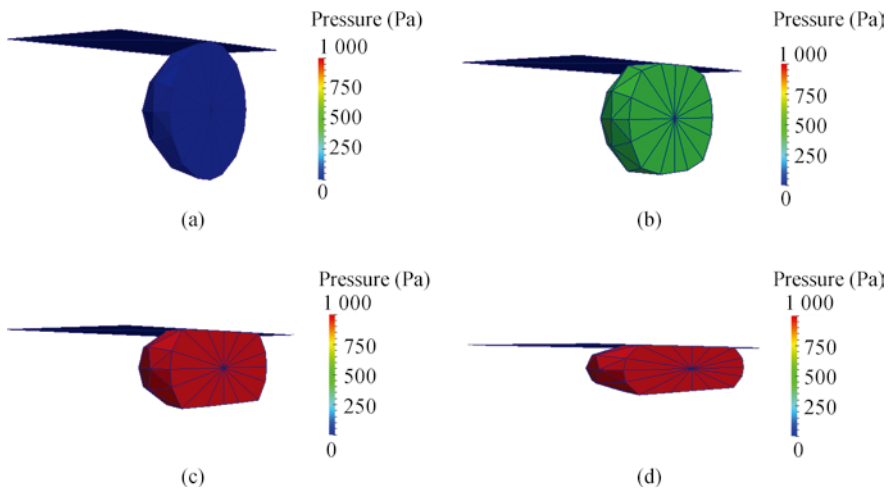


Fig. 1.3 Cell compression test. The cell is cut through a vertical plane to show the stress distribution in the inside.

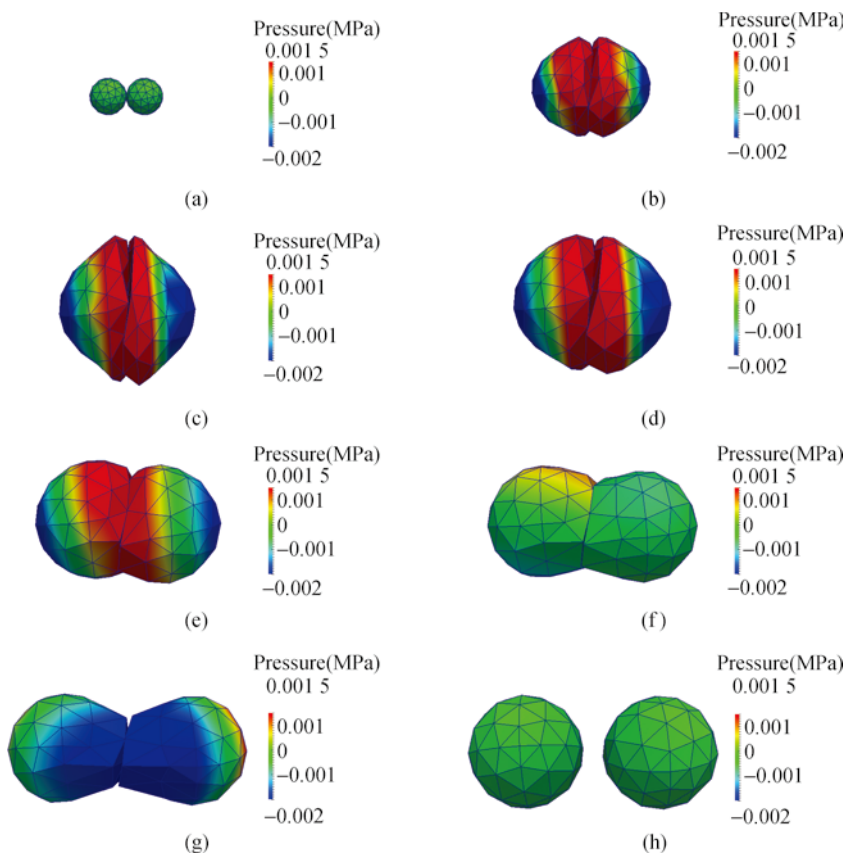


Fig. 1.5 Adhesion test. The centers of the two cells are initially fixed, and contact occurs due to cell growth. When the cell on the right is pulled apart from the one on the left the contact stresses are relaxed and adhesion helps to keep the interacting surfaces glued.

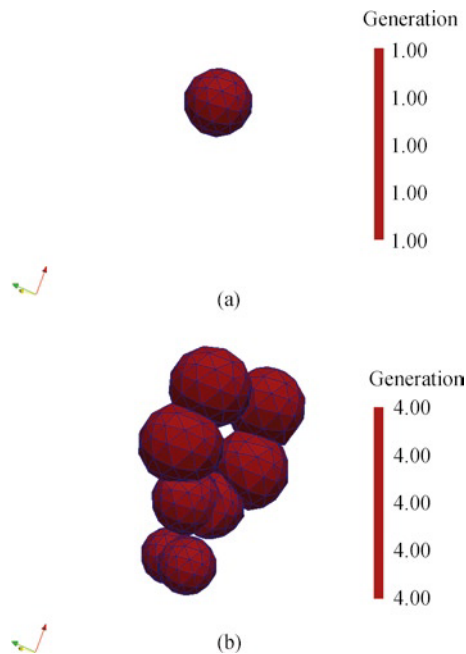


Fig. 1.9 Initial cell (a) and population on day 8 (b).

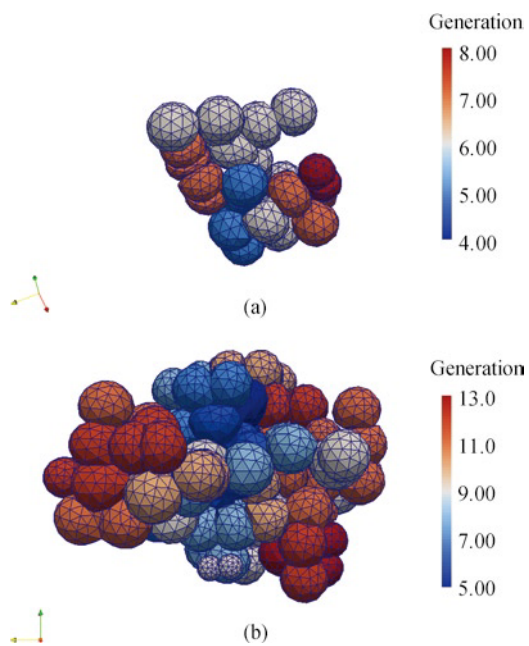
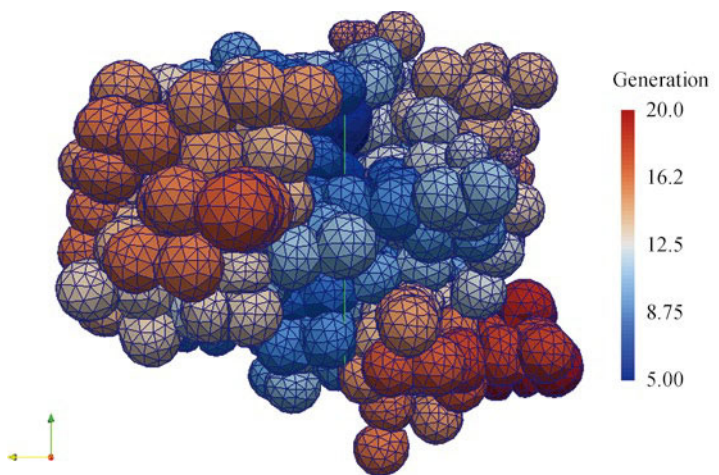
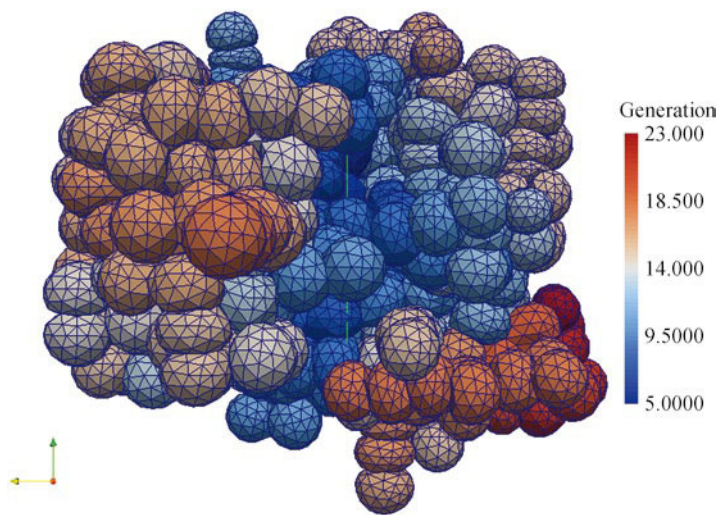


Fig. 1.10 Cell population on day 17 (a) and day 33 (b).



(a)



(b)

Fig. 1.11 Cell population on day 50 (a) and day 55 (b).

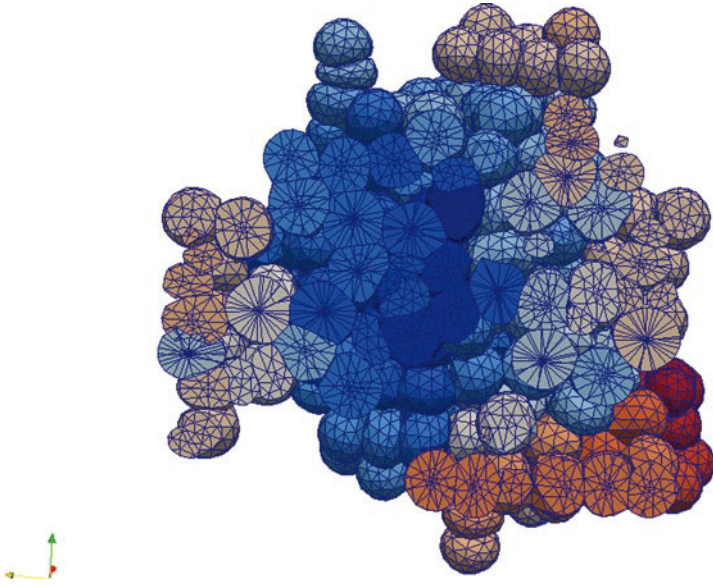


Fig. 1.12 Cluster section.

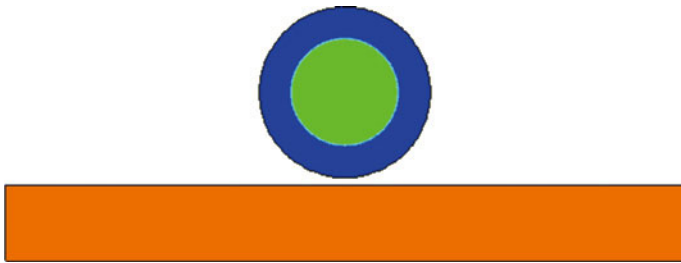


Fig. 2.7 Computational model of cellular spreading on different substrates (the colors represent different modeled materials).

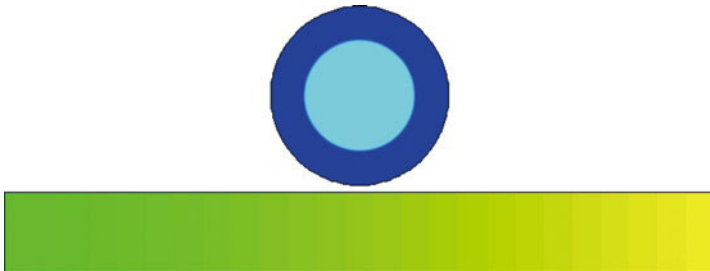


Fig. 2.11 Computational model of cell spreading on a stiffness-varying substrate, the color in the substrate stands for different stiffnesses, the yellow color at the right end indicates the highest stiffness.

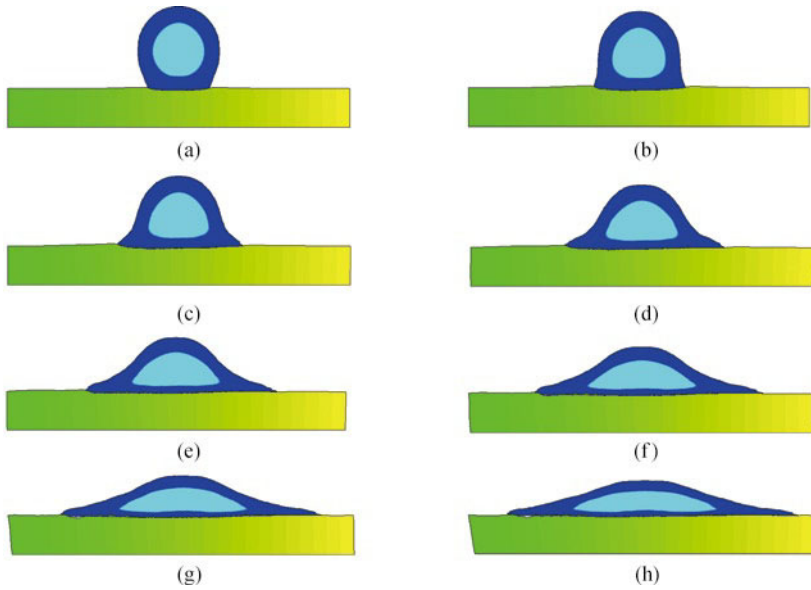


Fig. 2.12 Time sequence of cell contact with a stiffness-varying substrate (classical contact theory).

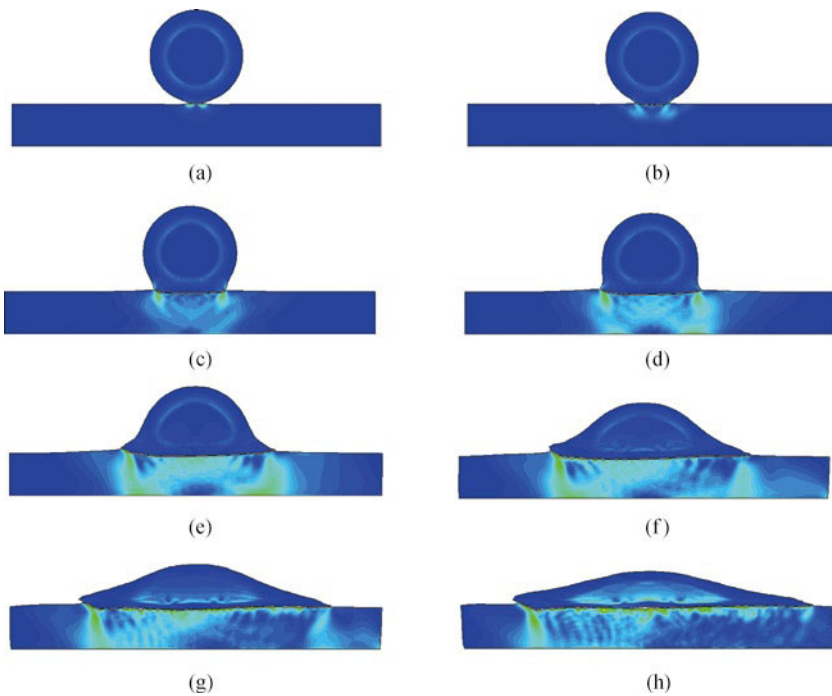


Fig. 2.13 Time sequence of cell contact with a stiffness-varying substrate (stress contour from the cohesive contact model).

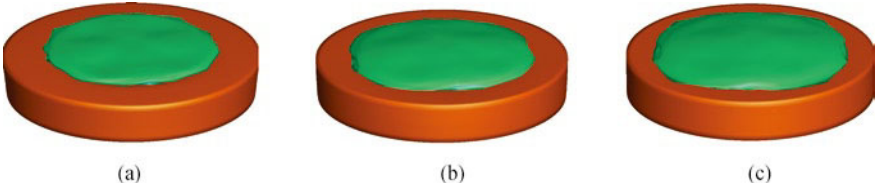


Fig. 2.15 Cell spreading over substrates with different stiffnesses: (a) Substrate-I, (b) Substrate-II, (c) Substrate-III.

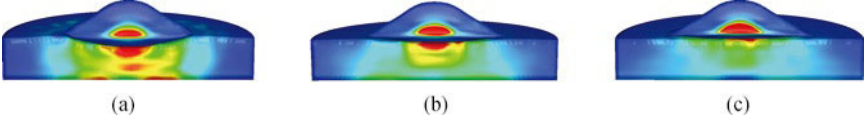


Fig. 2.16 Effective stress over substrates with different stiffnesses: (a) Substrate-I, (b) Substrate-II, (c) Substrate-III.

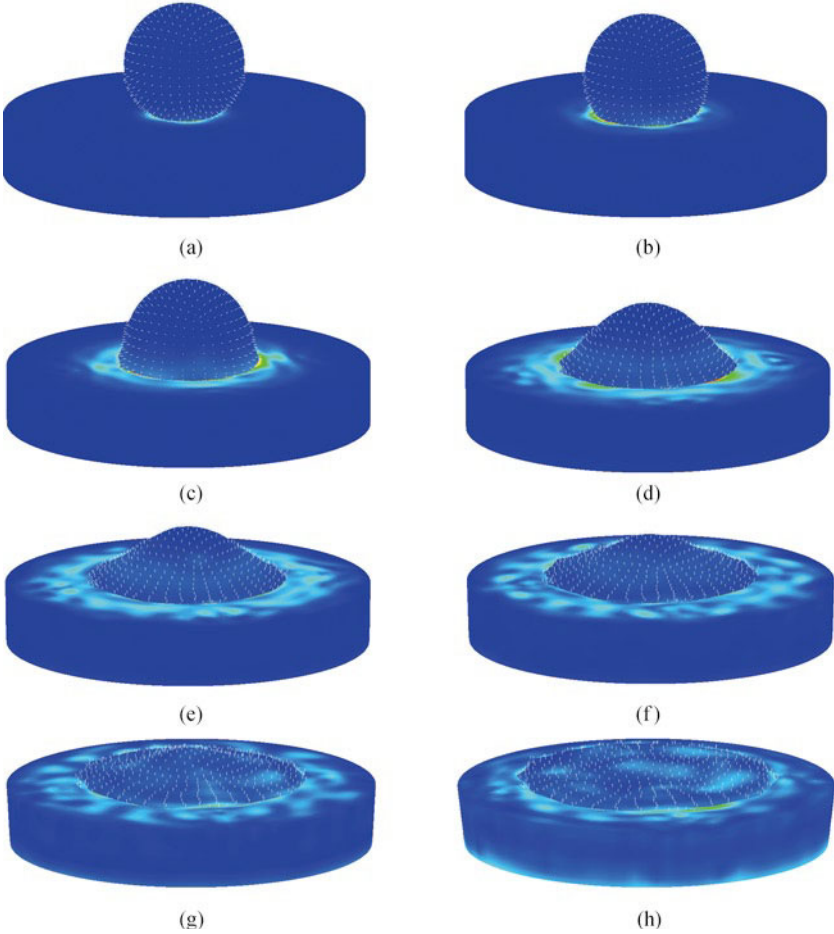


Fig. 2.18 Time sequence of cell contact with the soft Substrate-I.

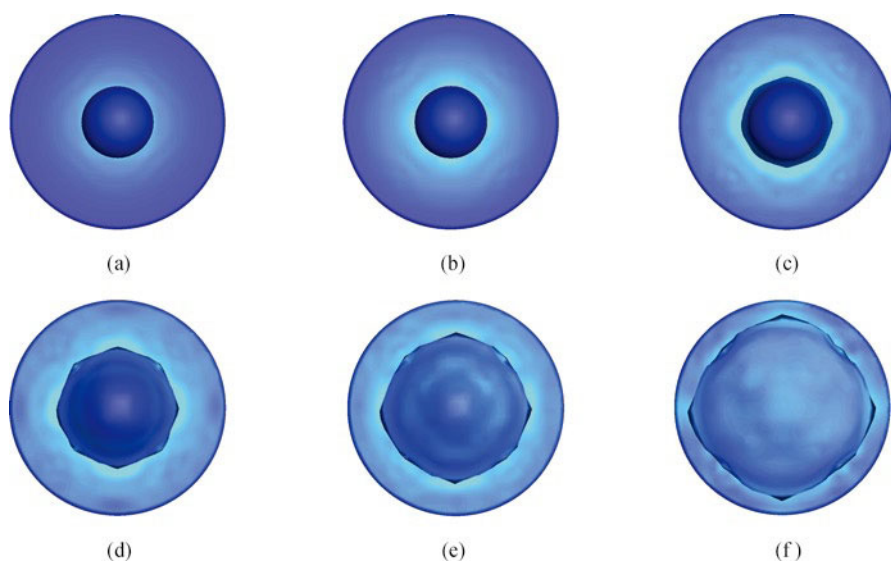


Fig. 2.19 Time sequence of cell contact with the soft Substrate-III (top view).

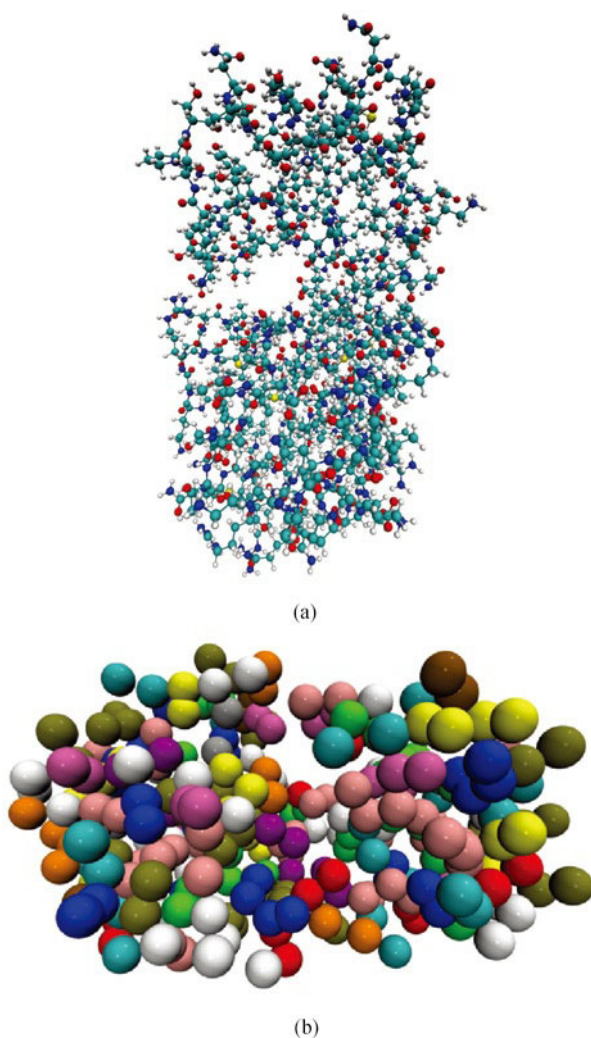


Fig. 3.1 Model T4 Lysozyme (PDB: 3lzm) with elastic network mode. Each bead stands for one residue and is connected with abstract springs within a cutoff distance. Colored by atom type: H white; O red; N blue; C cyan; S yellow; P tan: (a) molecular structure with CPK view, (b) elastic network model.

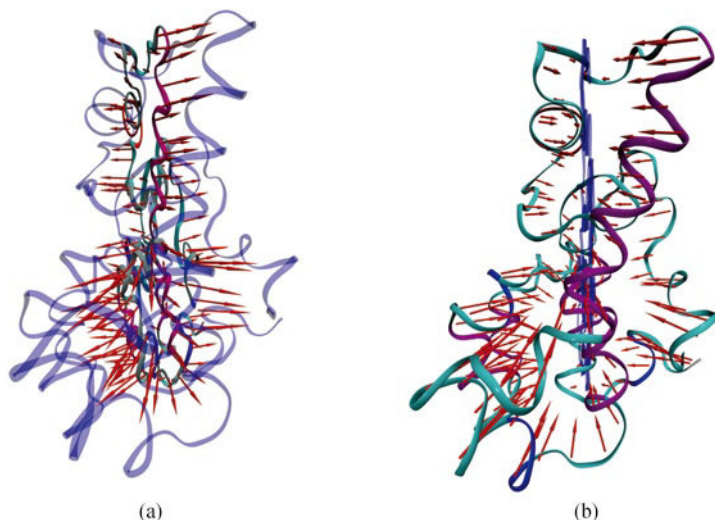


Fig. 3.12 Non-aligned PCA mode 1 displacement visualizations of MD simulation without solvent. The starting structure is shown in new-cartoon representation colored by structure. The destination structure is shown in blue ribbon. The red arrows indicate the direction of displacement based upon 1st PCA mode of MD simulation without solvent: (a) expansion, (b) contraction.

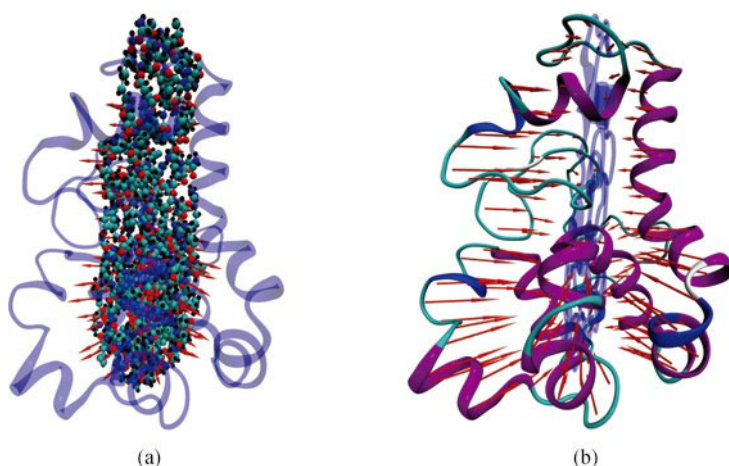


Fig. 3.13 Non-aligned PCA mode 2 displacement visualizations of MD simulation without solvent. In CPK representation colored by atom type: H black; O red; N blue; C cyan; S yellow; P tan. The starting structure is shown in new-cartoon representation colored by structure. The destination structure is shown in blue ribbon. The red arrows indicate the direction of displacement based upon 2nd PCA mode of MD simulation without solvent: (a) expansion, (b) contraction.

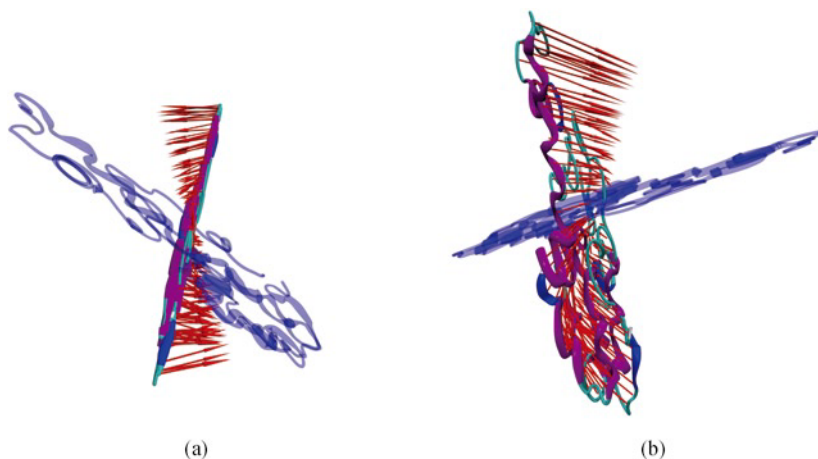


Fig. 3.14 PCA mode 3 displacement visualizations of MD simulation without solvent. The starting structure is shown in new-cartoon representation colored by structure. The destination structure is shown in blue ribbon. The red arrows indicate the direction of displacement based upon 3rd PCA mode of MD simulation without solvent: (a) expansion, (b) contraction.

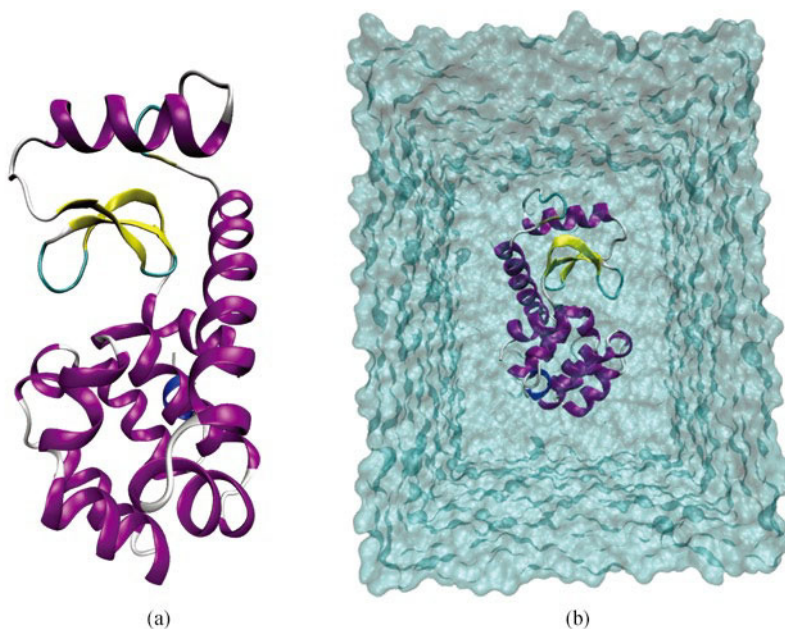


Fig. 3.16 Schematic representation of the energy-minimized molecular structure analyzed for T4 lysozyme with structure view, colored by structure type: Alpha-Helix purple, 3₁₀-Helix blue, Pi-Helix red, Extended-Beta yellow, Bridge-Beta tan, Turn cyan, Coil white: (a) molecular structure in vacuum, (b) molecular structure in water box.

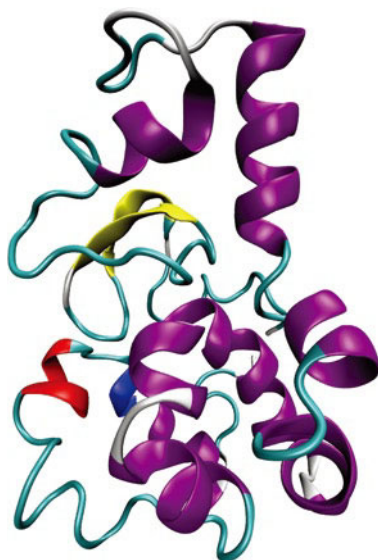


Fig. 3.17 Illustration of T4 lysozyme structure after 40 ns simulation in vacuum.

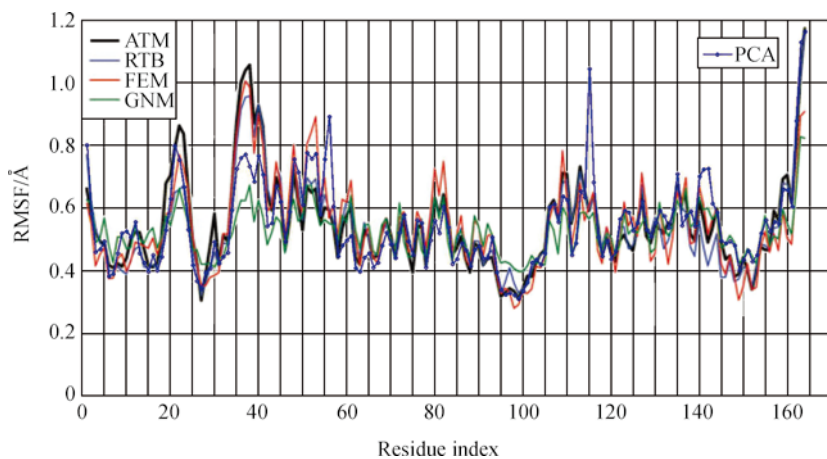


Fig. 3.19 RMSFs of the α -carbon atoms at 300 K for T4 lysozyme. Methods used are: All Atom Method (ATM), Rotational-Translational Block (RTB), Gaussian Network Model (GNM), Finite Element Method (FEM), Data generated from simulation (PCA).

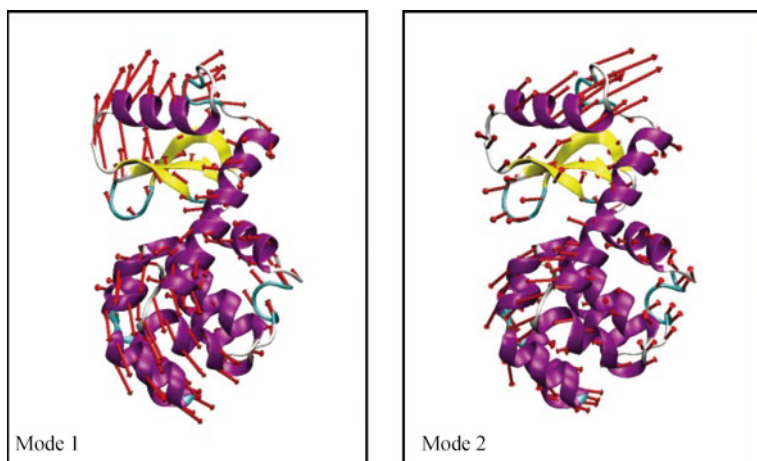


Fig. 3.27 Schematic representation of the two lowest eigenmodes of T4 lysozyme computed using the FEM.

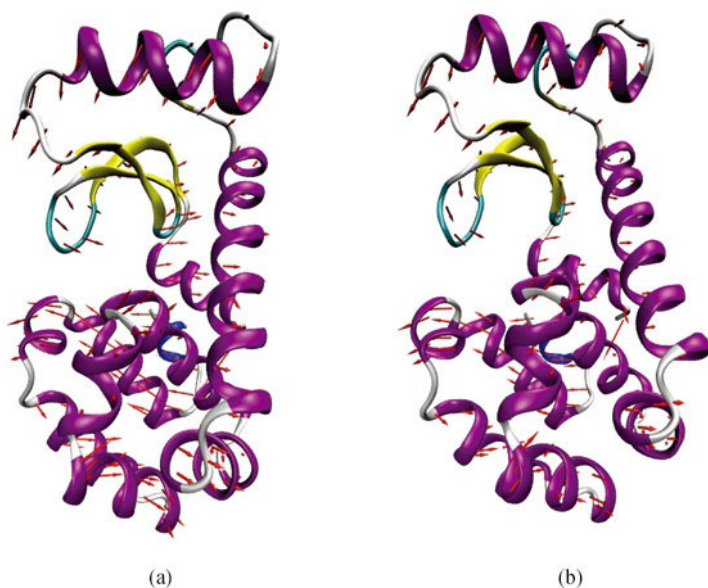


Fig. 3.28 The 1st eigenmode displacement visualization of T4 lysozyme MD simulation with solvent. The starting structure is shown in new-cartoon representation colored by structure. The red arrows indicate the direction of displacement based upon 1st eigenmode of MD simulation with solvent: (a) generated from PCA, (b) generated from QHA.

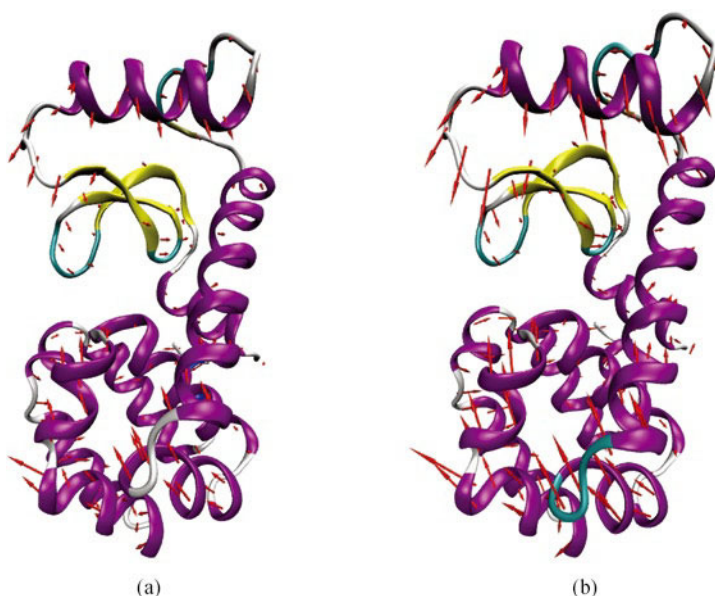


Fig. 3.29 The 2nd eigenmode displacement visualization of T4 lysozyme MD simulation with solvent. The starting structure is shown in new-cartoon representation colored by structure. The red arrows indicate the direction of displacement based upon 2nd eigenmode of MD simulation with solvent: (a) generated from PCA, (b) generated from QHA.

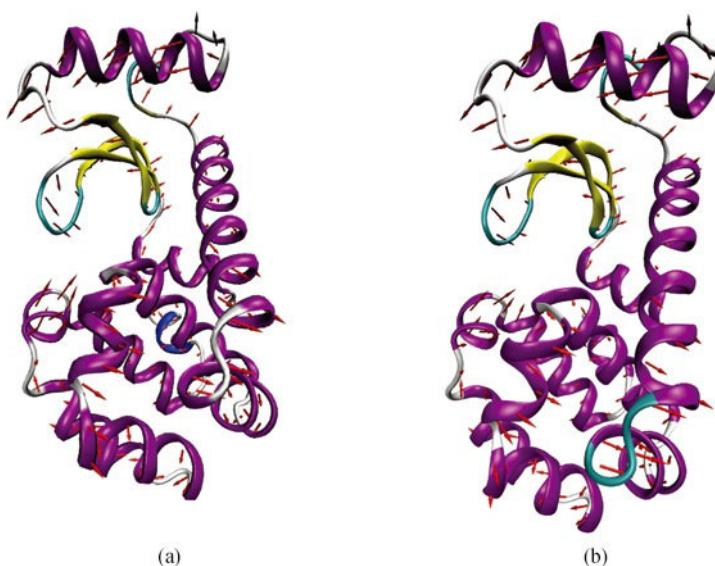


Fig. 3.30 The 3rd eigenmode displacement visualization of T4 lysozyme MD simulation with solvent. The starting structure is shown in new-cartoon representation colored by structure. The red arrows indicate the direction of displacement based upon 3rd eigenmode of MD simulation with solvent: (a) generated from PCA, (b) generated from QHA.

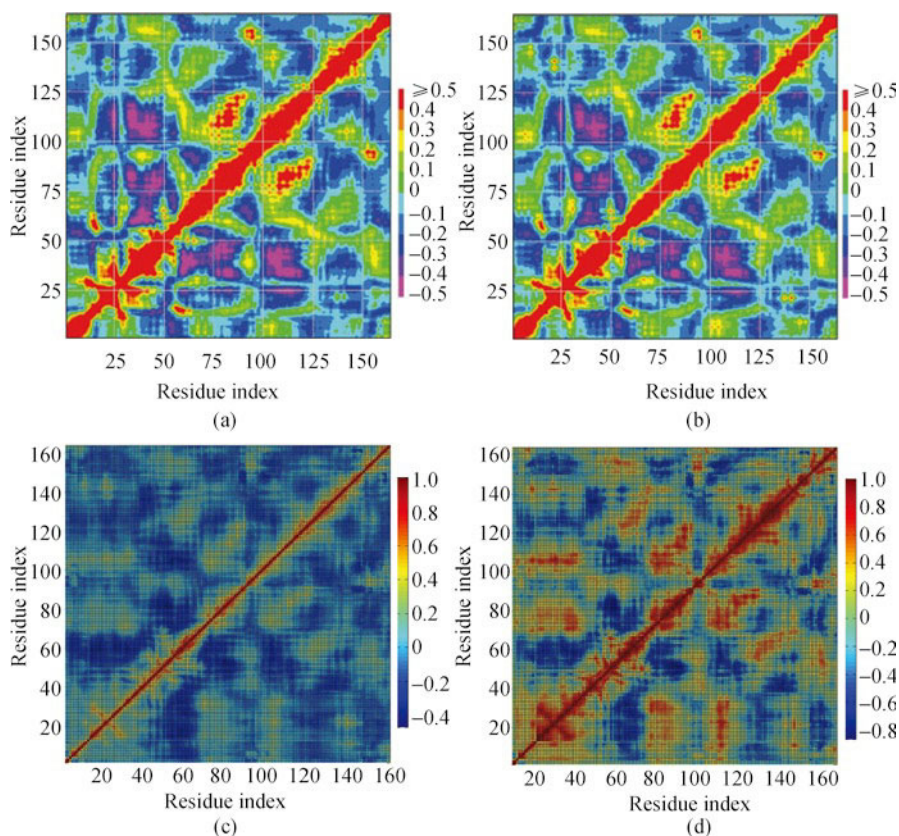


Fig. 3.31 Correlated fluctuation of α -carbon atoms computed for T4 lysozyme: (a) all Atom Method (ATM) vs. Normal Mode Analysis (NMA), (b) RTB procedure, (c) original simulation data, (d) PCA method.



Fig. 3.32 Residue clusters based on the correlation matrix for MD simulation without solvent. Clusters generated using the UPGMA algorithm were selected based on binding site residues, etc. These clusters are simply groups of residues with concerted conformational fluctuations that could be important for inter-domain communication. Different clusters are indicated by color.

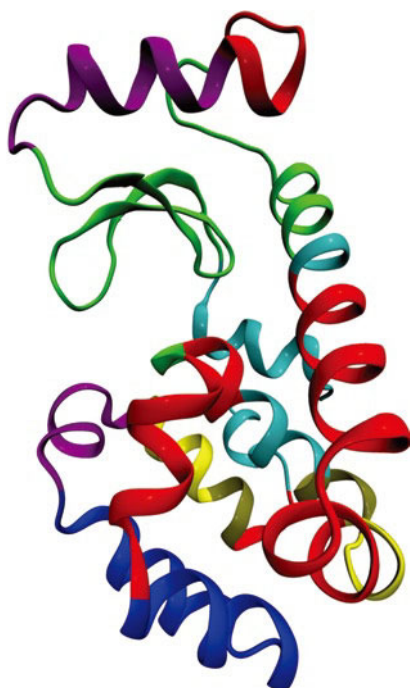


Fig. 3.33 Residue clusters based on the correlation matrix first 10 PCA modes. Clusters generated using the UPGMA algorithm were selected based on binding site residues, etc. These clusters imply groups of residues with concerted conformational fluctuations that could be important for inter-domain communication. Different clusters are indicated by color.

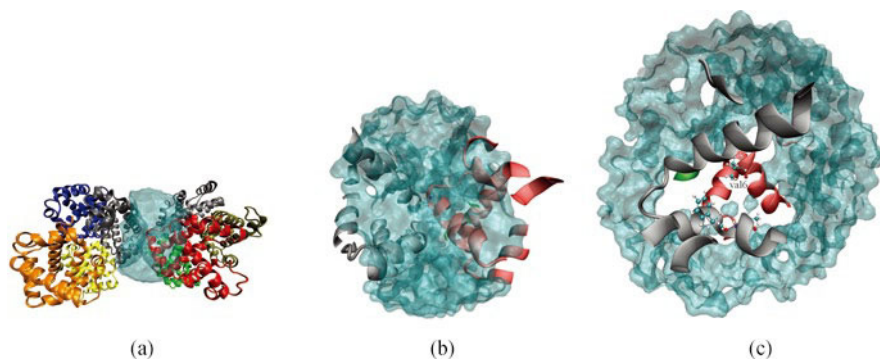


Fig. 3.39 Illustration for energy minimization position with HbS simulation: (a) global view, for illustration water was shown within 20 Å of residue $\beta 6$ only, (b) a closer look of the pocket, (c) rotation by 90°.

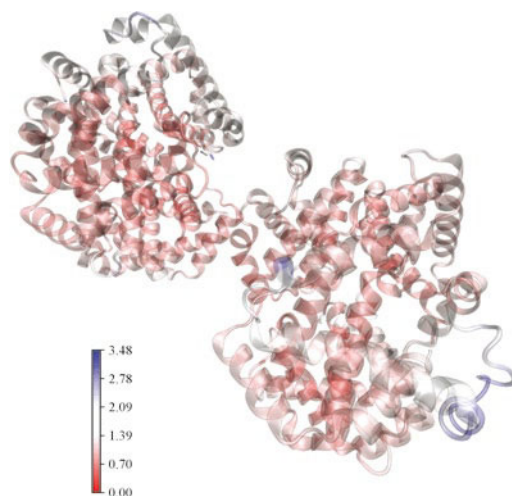


Fig. 3.42 Illustration of RMSFs for hemoglobin interaction simulation, colored by RMSF value for each residue.

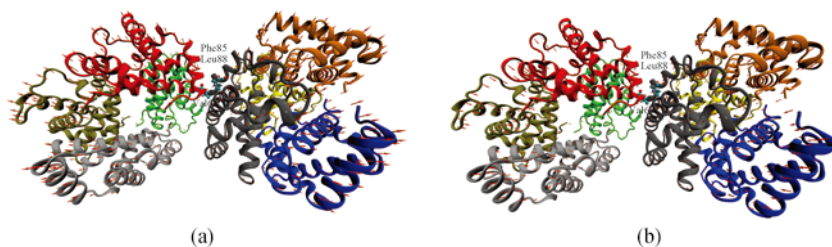


Fig. 3.45 Hydrophobic motion captured by PCA analysis, colored by chains: chain A blue, chain B gray, chain C orange, chain D yellow, chain E tan, chain F silver, chain G green, chain H red. The red arrows indicate the direction of displacement based on 1st eigenvector, the lengths of arrows indicate the amplitude: (a) eigenvalue, (b) cumulative energy content for each eigenvector.

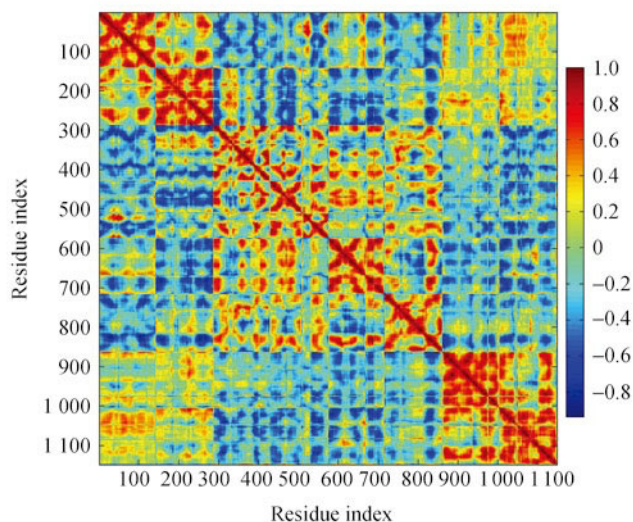


Fig. 3.46 Correlation of HbS result.

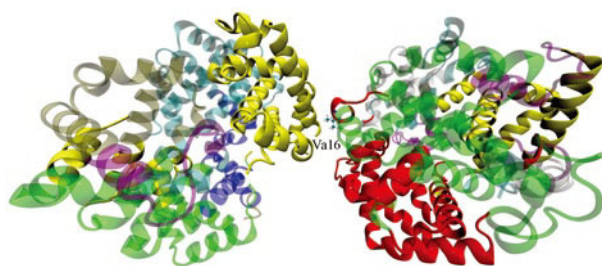


Fig. 3.47 Residue clusters based on the correlation matrix first 5 PCA modes. Clusters generated using the UPGMA algorithm were selected based on including binding site residues. These clusters imply groups of residues with concerted conformational fluctuation that could be important for inter-domain communication. Different clusters are indicated by color.

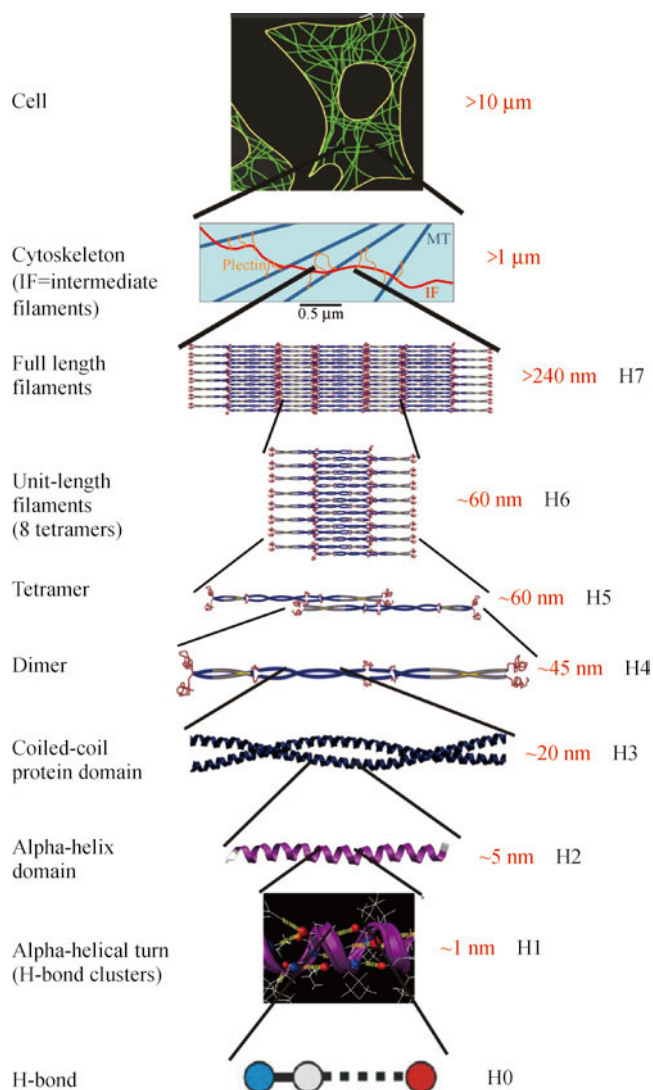


Fig. 4.1 The hierarchical structure of intermediate filaments, from atomic to cellular scales.

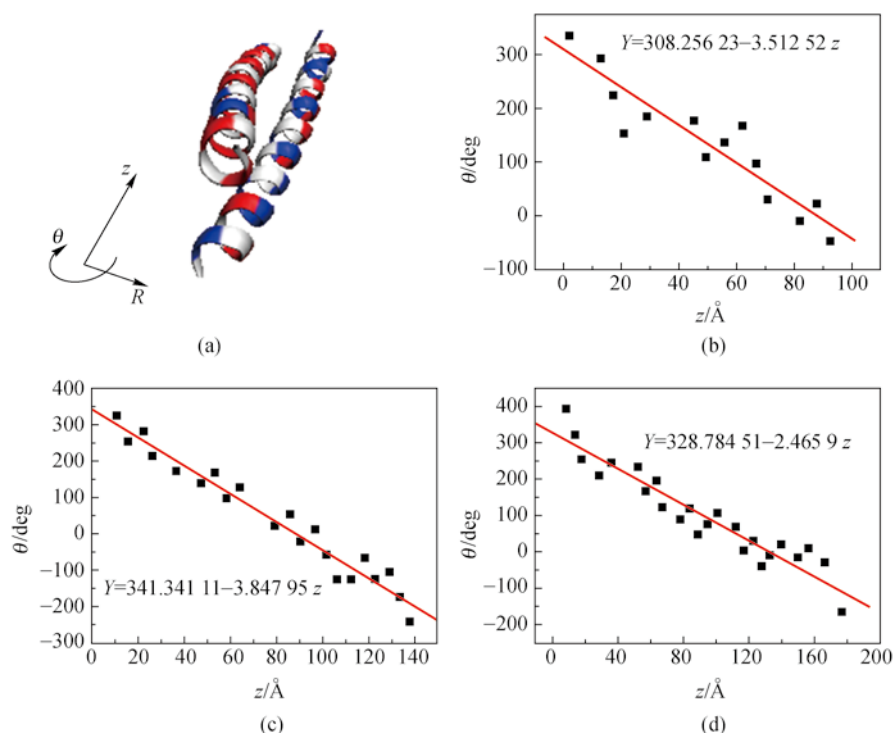


Fig. 4.3 The geometry character of each coiled-coil domain in a vimentin dimer. Panel A: the schematic representation of a coiled coil segment, and the relative coordinate used to form the model. The hydrophobic amino acids are white, while other amino acids are colored; Panel B: the coordinates of the hydrophobic stripe along a chain in 1A domain; Panel C: the coordinates of the hydrophobic stripe in 1B domain; Panel D: the coordinates of the hydrophobic stripe in 2B domain.

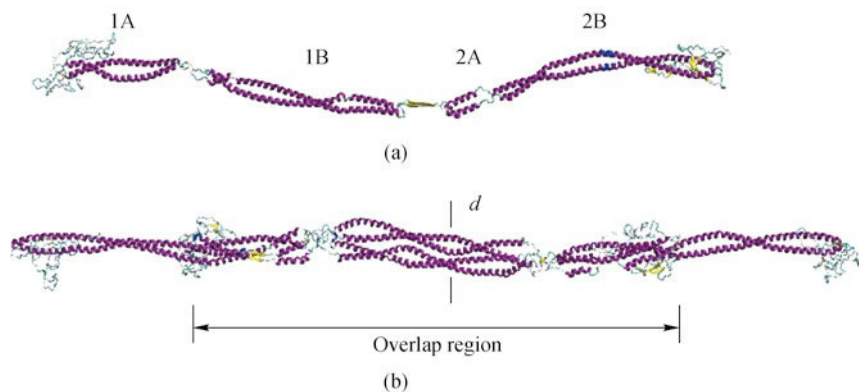


Fig. 4.4 Molecular structures of the vimentin dimer and tetramer. Panel A: snapshot of the atomistic model of the dimer. The blue part denotes the stutter part around the stutter (amino acids 345–351). Panel B: snapshot of the atomistic model of the tetramer.

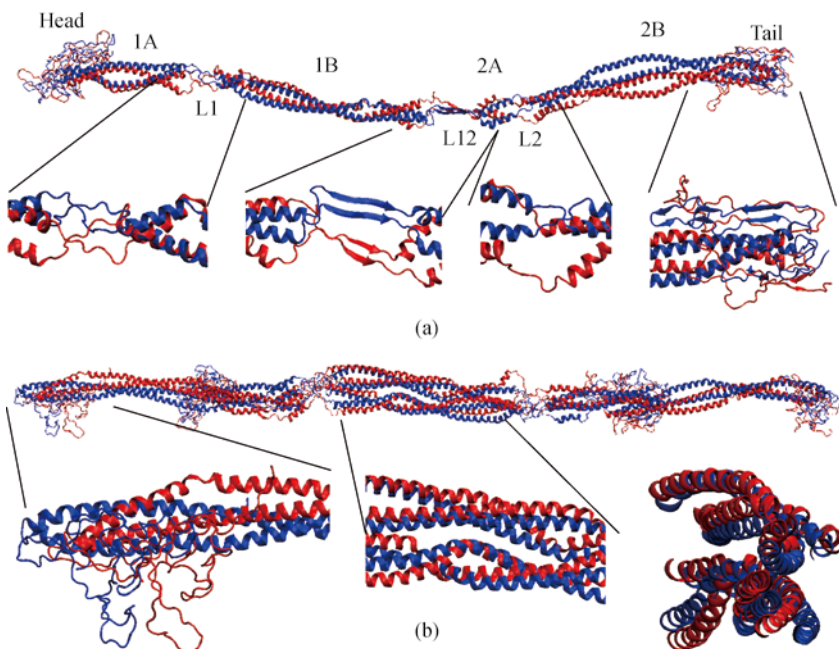


Fig. 4.7 Structure of the vimentin intermediate filament dimer (panel A) and tetramer (panel B), depicting a comparison between implicit and explicit solvent simulation results.

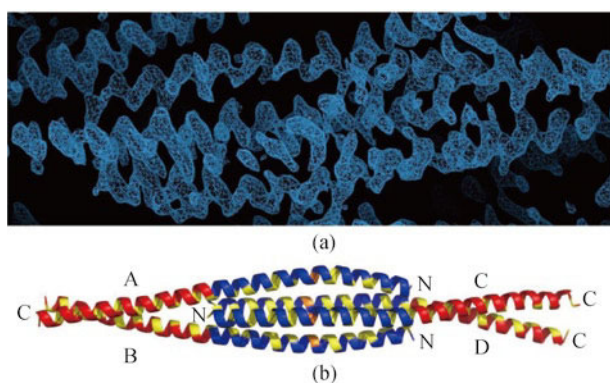


Fig. 4.9 The crystallized structure of the 2A, L2 and part of 2B domain close to the N terminal of vimentin dimer (AA263–AA334 with PDB ID 3klt).

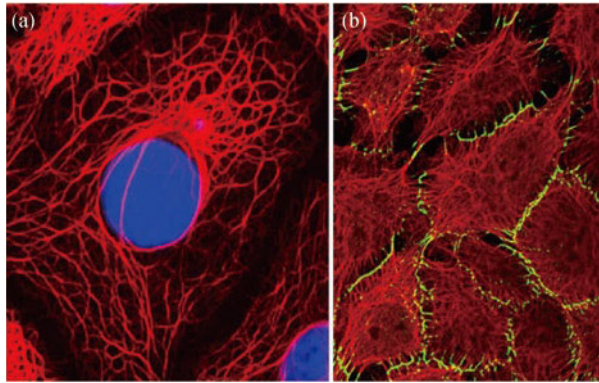


Fig. 4.11 Keratin intermediate filament in epithelial cells: (a) the keratin intermediate filament (K18) and the nucleus (liver carcinoma cell) are stained in red and blue respectively, (b) keratin filaments (in red) and the desmoplakin (in green), a component of desmosomes, are labeled in cultured keratinocytes of line HaCaT. The location of desmoplakin and desmosomes in a cell is shown in Fig.4.2. a and b are the immunofluorescence staining images.

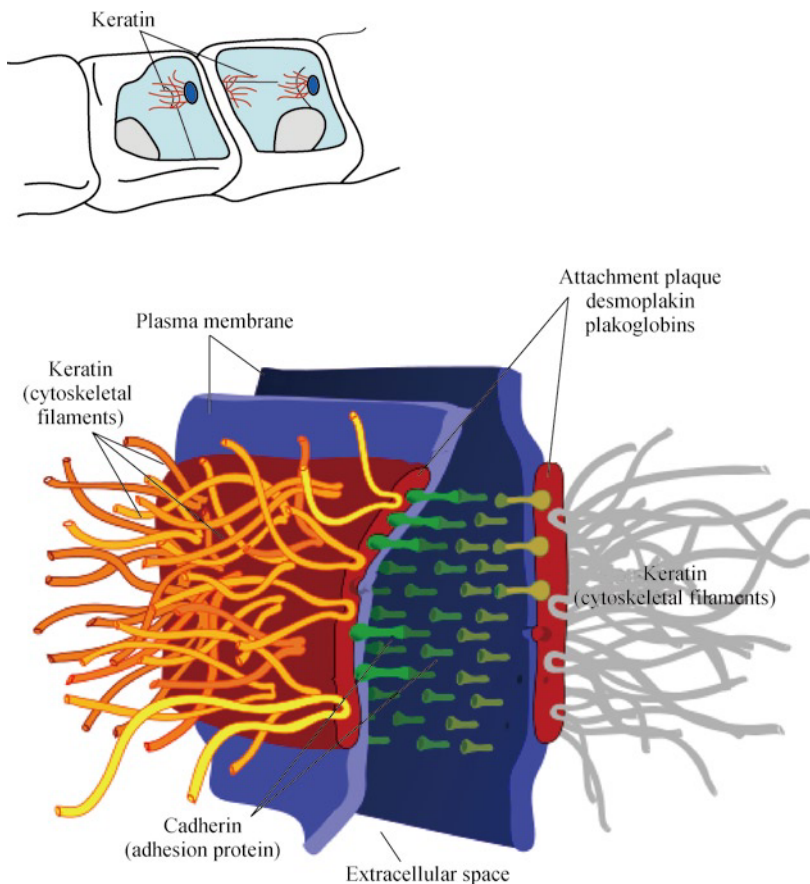


Fig. 4.12 Keratin intermediate filaments are attached to desmoplakin in the desmosome, which is known as the complex responsible for cell-to-cell adhesion in animal cells.

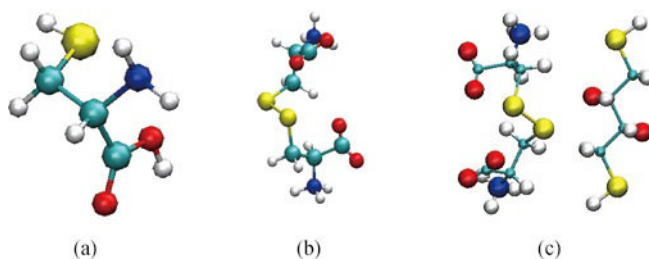


Fig. 4.13 Cysteine is an amino acid with the chemical formulation $C_3H_7NO_2S$. Panel A: image of one cysteine residue. Panel B: illustration of how a disulfide bond is formed between two cysteine residues. Panel C: model of a disulfide bond under a reducing agent, DTT, in explicit solvents water molecules are not shown in the figure. The carbon, hydrogen, nitride, oxygen and sulfur atoms are colored blue, white, dark blue, red and yellow respectively.

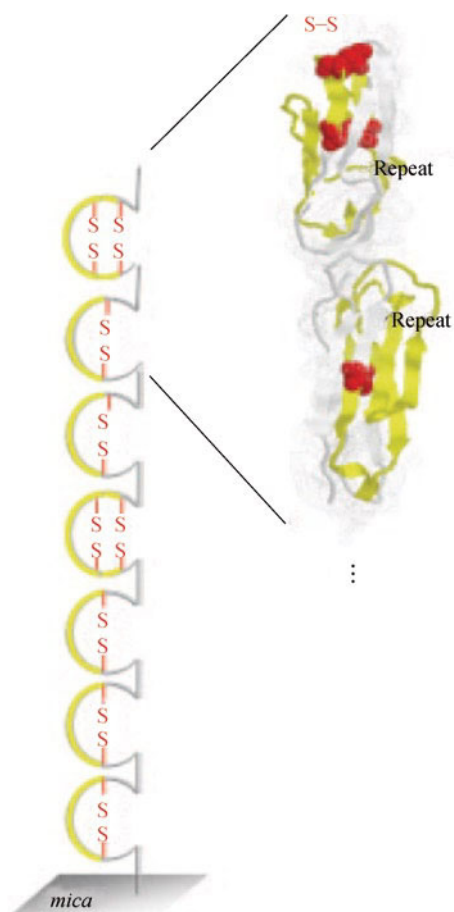


Fig. 4.14 The schematic diagram of force tension experiment by AFM. There are seven immunoglobulin domains of VCAM. The disulfide bonds, shown in red, stabilize the VCAM protein structure.

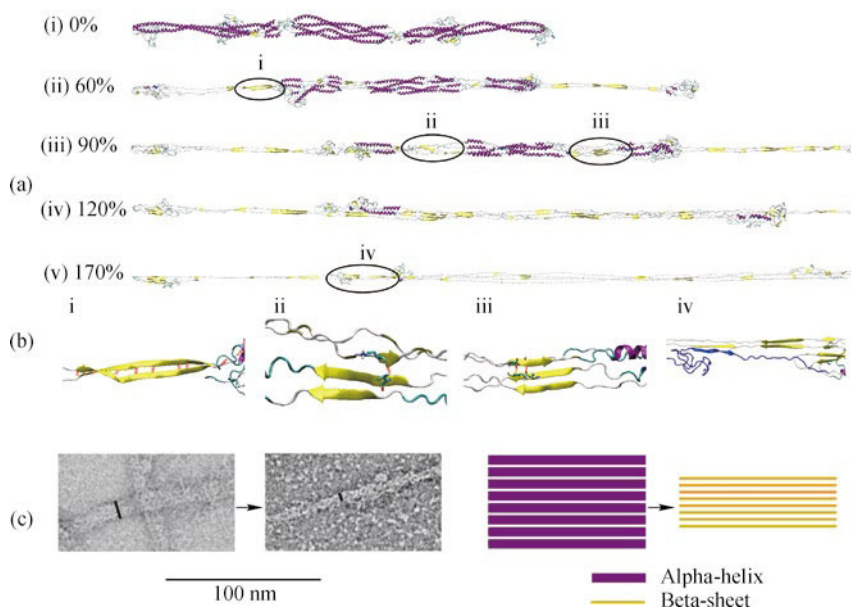


Fig. 4.22 Simulation snapshots during pulling of the dimer at different pull strain (a), (b) shows detailed views of the molecular structure at various levels of strain, (c) shows the experimental evidence that alpha-beta transition takes place during stretching.

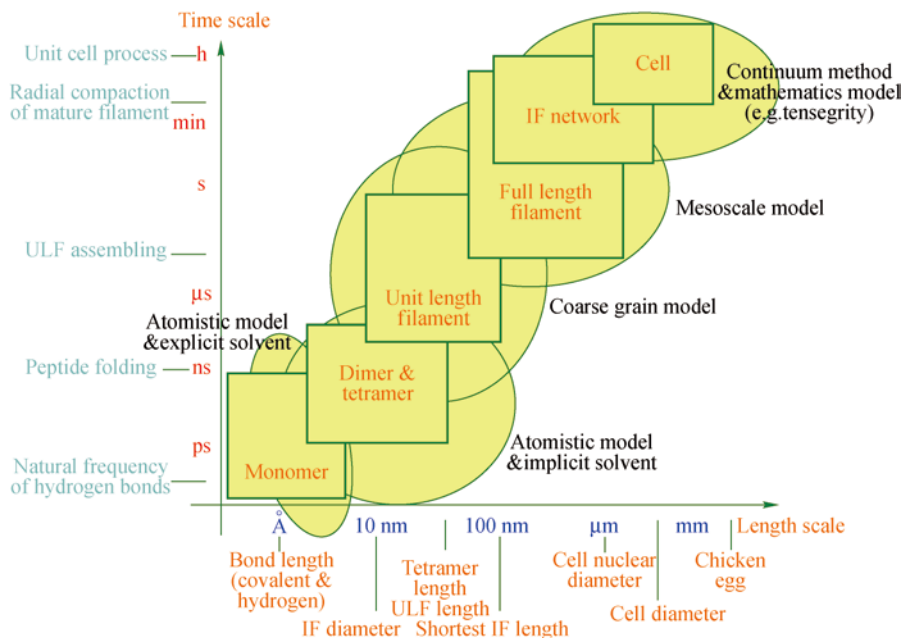
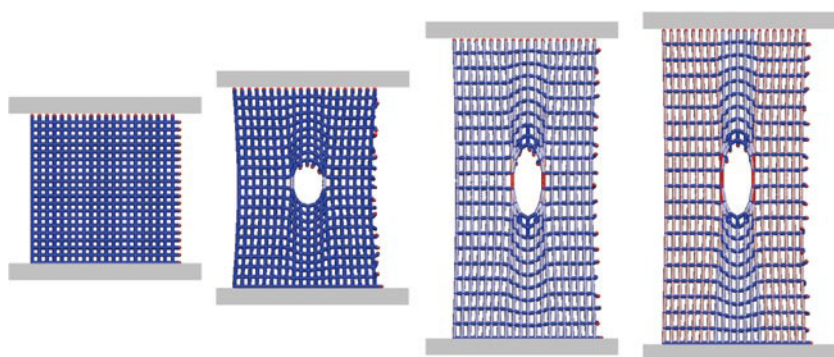
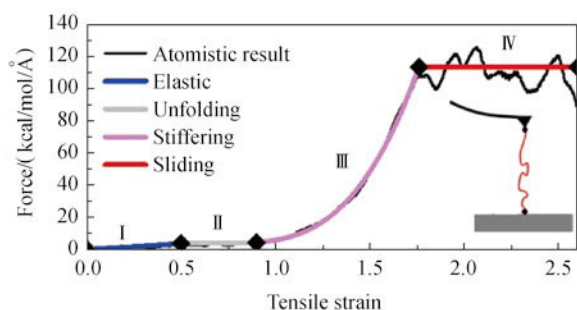


Fig. 4.28 The basic approach of the step-by-step procedure of studying the structural and mechanical properties of IF structures by using multi-scale experiment and simulation.



(a)



(b)

Fig. 4.29 Snapshots of a larger-scale intermediate filament network deformation (a). The mechanical response of each filament is fitted fully according to the result of full atomic modeling as given in Fig.4.23. Each filament under tension is colored according to the strain state, as shown in the force-strain curve (b).

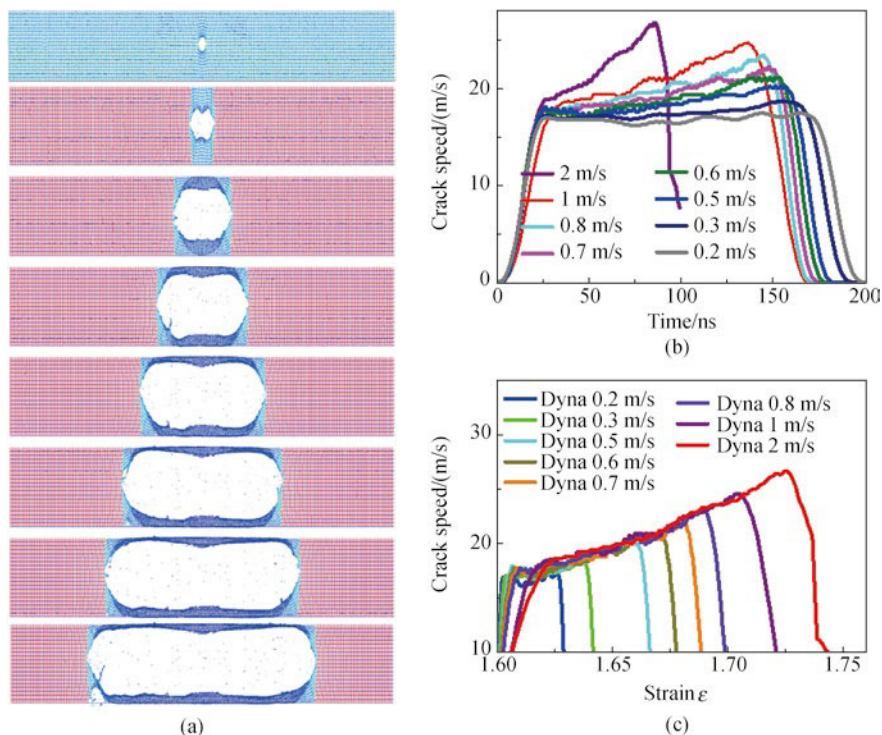


Fig. 4.30 Snapshots of the intermediate filament network under tension after the applied strain exceeds the strength limit as shown in Fig.4.30. We record the crack speed as a function of time with different loading rates and find that the crack speed is a function of applied strain rather than the loading rate.

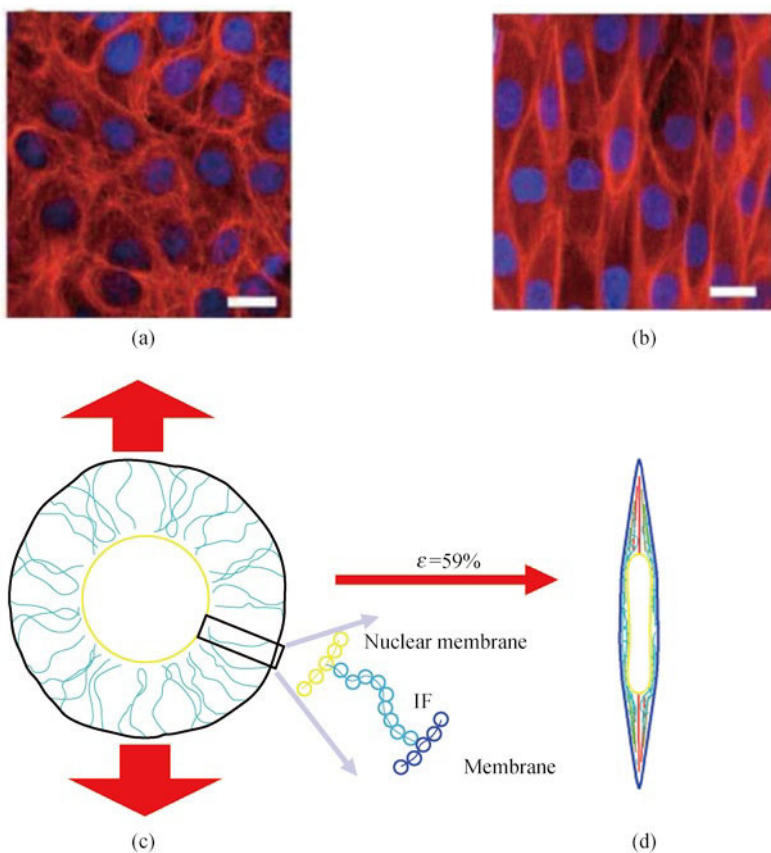


Fig. 4.31 Cell model geometry, boundary conditions and illustration of the role of intermediate filament network for maintaining cell integrity at large deformation. (a) and (b): intermediate filament network in MDCK cells (scale bar is approximately $25\ \mu\text{m}$) under static and strained conditions. (c) and (d): snapshots of the cell model deformation under mechanical load (at 0% and 59% strain, respectively).

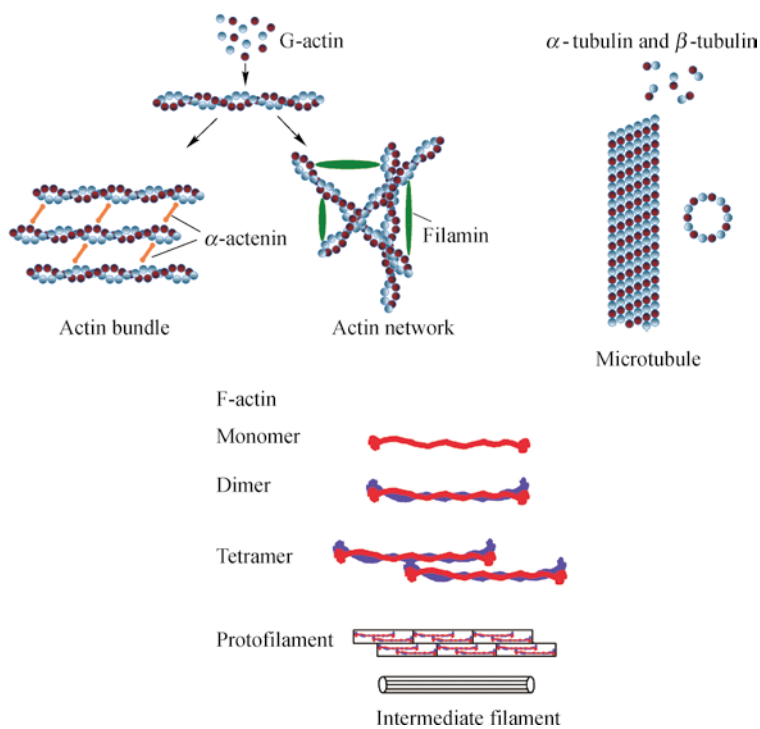


Fig. 5.1 Schematic plot for the structure of an actin filament, a microtubule and an intermediate filament.

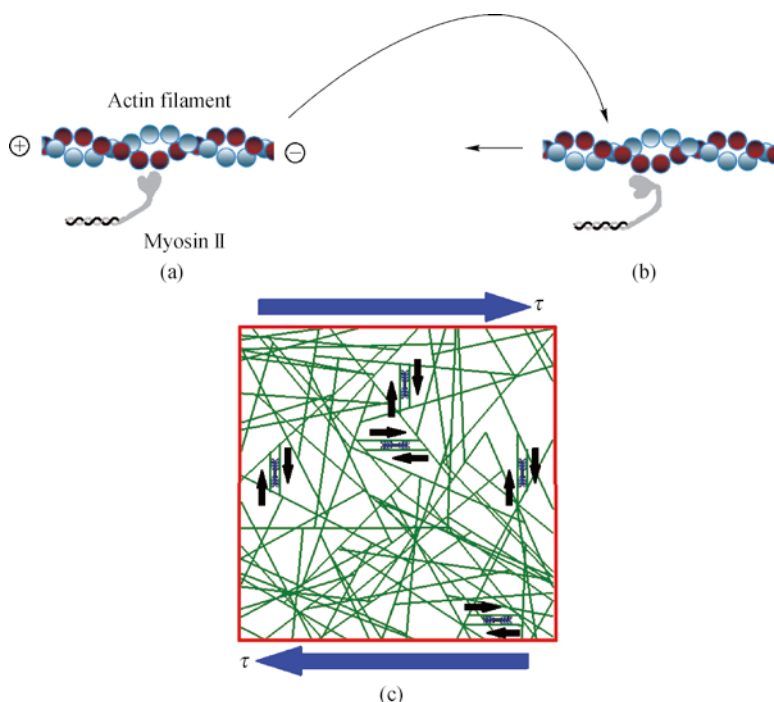


Fig. 5.2 Schematic of a bipolar myosin filament interacting with two actin filaments. The $\pm$ signs indicate the polarity of actin; myosin moves toward the plus end. (a) Myosin head attaches to the actin; high energy configuration. (b) The myosin head bends and pulls on the actin filament; power stroke. (c) Active networks subjected to external loadings. In active networks, myosin molecular motors control the network elasticity and induce a state of pre-stress inside the network.

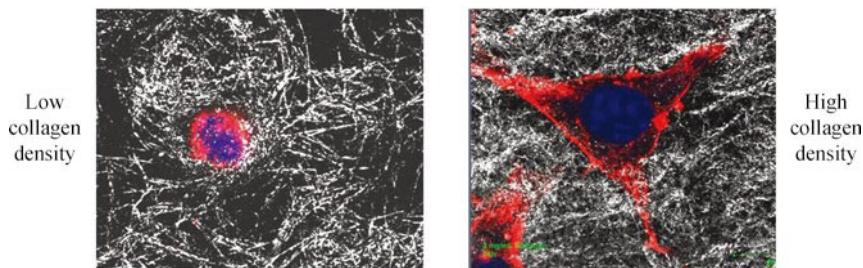


Fig. 6.1 NIH 3T3 fibroblast in a collagen network. For low density collagen, the network is too soft for the fibroblast to spread in exerting force. However, when the collagen concentration is increased, the rise in stiffness results in fibroblast spreading. Well-developed stress-fibers can be observed (red lines).

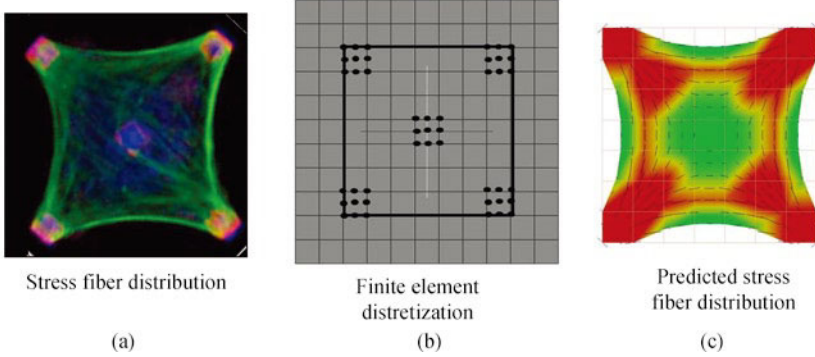


Fig. 6.15 Contraction of a fibroblast on a rigid substrate with periodically spaced adhesion islands. (a) Experimental observation from Bishofs et al. showing actin fiber distribution (green), (b) Finite element discretization, level-set definition (thick line) and cohesive region between cell and substrate (dots), and (c) Final cell configuration showing the principal direction of stress-fibers (vectors) and their concentration (high concentrations are depicted in red while low concentrations are shown in green).

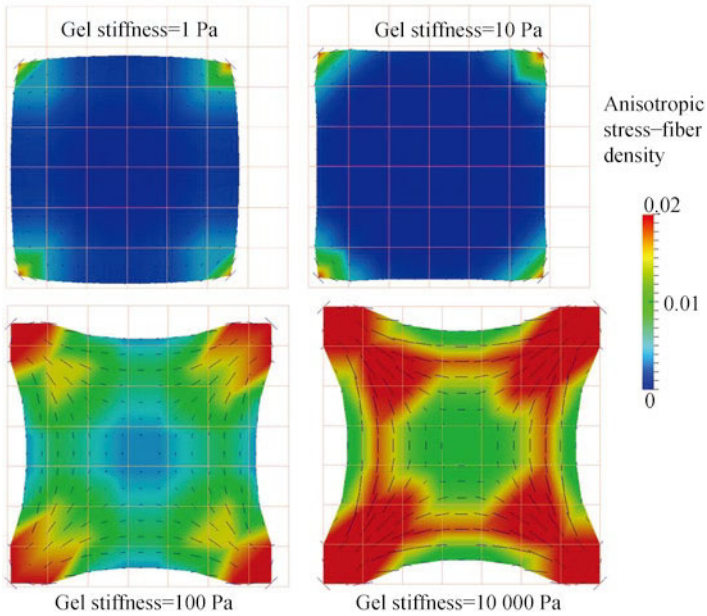
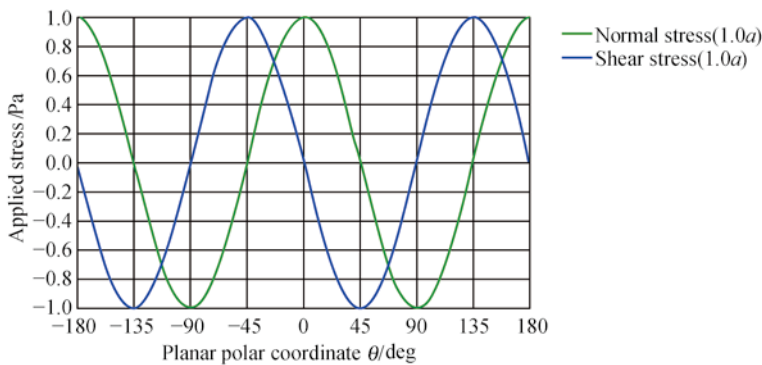
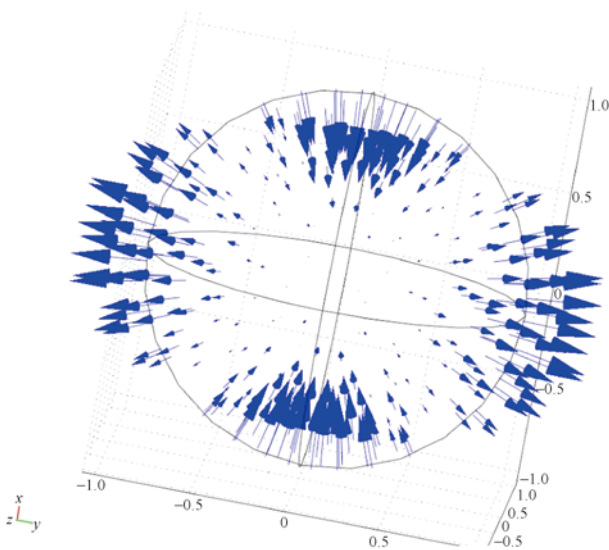


Fig. 6.16 Original (gray mesh) and final cell configuration and stress-fiber distribution for various values of substrate stiffness (varying from 1 Pa to 10 kPa).

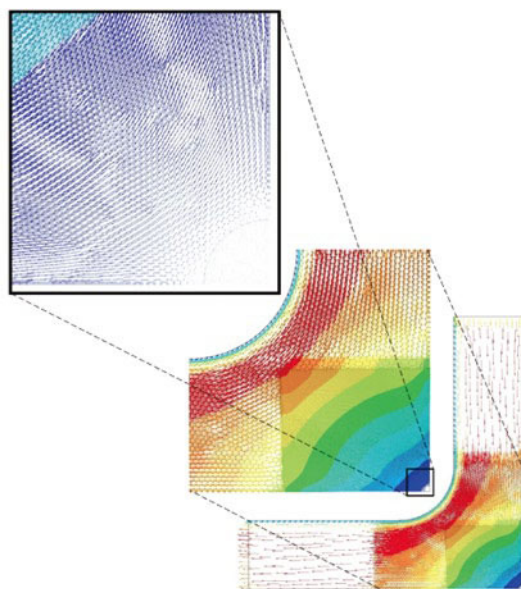


(a)

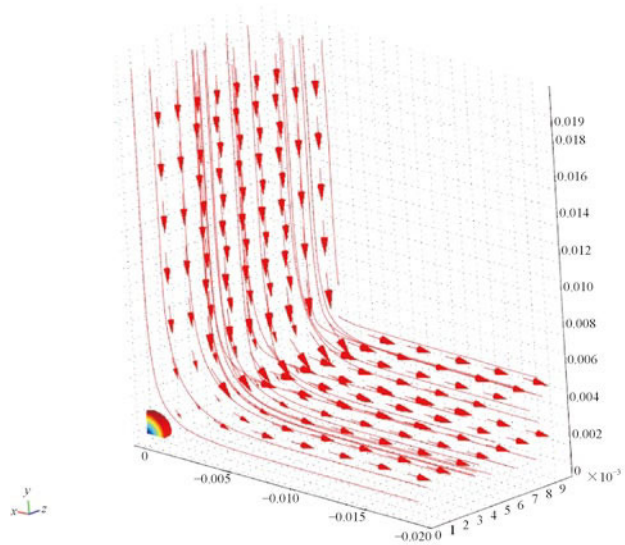


(b)

Fig. 8.2 Modeled (a) two-dimensional and (b) three-dimensional stress patterns applied to suspended cells optically and hydrodynamically trapped in an extensional flow with peak stresses similar to monolayer studies (~ 1 Pa). The planar polar coordinates ($r = 1.0a$ and θ) are defined in the $x - y$ plane at $z = 0$. The three-dimensional normal stresses are isolated for clarity on the full cellular surface (shown as a sphere before deformation).



(a)



(b)

Fig. 8.5 Computationally derived (a) two-dimensional and (b) three-dimensional localized flow velocities in an opto-hydrodynamic trap.