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Molecular Biomechanics: The Molecular Basis of How Forces Regulate Cellular Function

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Abstract

Recent advances have led to the emergence of molecular biomechanics as an essential element of modern biology. These efforts focus on theoretical and experimental studies of the mechanics of proteins and nucleic acids, and the understanding of the molecular mechanisms of stress transmission, mechanosensing and mechanotransduction in living cells. In particular, single-molecule biomechanics studies of proteins and DNA, and mechanochemical coupling in biomolecular motors have demonstrated the critical importance of molecular mechanics as a new frontier in bioengineering and life sciences. To stimulate a more systematic study of the basic issues in molecular biomechanics, and attract a broader range of researchers to enter this emerging field, here we discuss its significance and relevance, describe the important issues to be addressed and the most critical questions to be answered, summarize both experimental and theoretical/computational challenges, and identify some short-term and long-term goals for the field. The needs to train young researchers in molecular biomechanics with a broader knowledge base, and to bridge and integrate molecular, subcellular and cellular level studies of biomechanics are articulated.

Keywords

Mechanobiology; Force; Cytoskeleton; Mechanotransduction; Protein conformational change; Molecular motors

MOLECULAR BIOMECHANICS: AN EMERGENT FIELD

Living cells are dynamic systems that perform integrated functions including metabolism, control, sensing, communication, growth, remodeling, reproduction and apoptosis (programed cell death). During the past few decades, extensive studies have elucidated the structure, mechanical responses and biological functions of cells in different organs and tissues including, for example, lung, bone, cartilage, blood vessels, and skeletal and cardiac

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muscles. These studies have led to a better understanding of how the biological functions of a cell are regulated by mechanical forces or deformation. They have also demonstrated the central role that forces play in the initiation and progression of numerous diseases such as atherosclerosis, arthritis, asthma, to name a few. However, to decipher the fundamental mechanisms of force-induced control of cellular function, more systematic studies of deformation, structural dynamics and mechanochemical transduction in living cells and biomolecules are needed.

As the basic unit of life, living cells perform an enormous variety of functions through synthesis, sorting, storage and transport of biomolecules; expression of genetic information; recognition, transmission and transduction of signals; and conversion between different forms of energy. Many of these cellular processes involve mechanical force, or deformation, at the cellular, subcellular and molecular levels. For example, biomolecular motors and machines convert chemical energy into mechanical work in performing their diverse range of functions. During cell migration, contractile forces are generated within the cell in order for the cell body to move forward. These contractile forces, in combination with the adhesion of cells to extracellular matrix (ECM) through focal adhesion complexes enable cells to sense the stiffness of the surrounding substrate and respond to it. Many normal and pathological conditions are dependent upon or regulated by their mechanical environment. Some cells, such as osteoblasts and vascular cells, are subjected to specific forces as part of their `native' physiological environment. Others, such as muscle and cochlear outer hair cells,[61] perform their mechanical function either by converting an electrical or chemical stimulus into mechanical motion or vice versa.

Of particular importance is the ability of cells to sense mechanical force or deformation, and transduce these mechanical signals into a biological response.[96] For example, endothelial cells can recognize the magnitude, mode (steady or pulsatile), type (laminar or turbulent) and duration of applied shear flow, and respond accordingly, maintaining healthy endothelium or leading to vascular diseases including thrombosis and atherosclerosis.[17,54] Vascular smooth muscle cells in the arterial wall remodel when subjected to pressure-induced wall stress. Fibroblast cells `crawl' like an inchworm by pulling the cell body forward using contractile forces. Bone alters its structure to adapt to changes in mechanical environment as occurs, for example, in spaceflight. Stem cells sense the elasticity of the surrounding substrate and differentiate into different phenotypes accordingly.[22] These and other examples vividly demonstrate the ability of cells to sense and respond to their local mechanical environment. However, little is currently known about the fundamental molecular mechanisms by which cells sense mechanical force or deformation, and transduce the mechanical signal into a biological response. Ample evidence suggest that there are specialized `force sensors' on the cell membrane that detect the mechanical signal, however little is known about how these sensors work (see reviews in Kamm and Kaazempur-Mofrad[46] and Vogel and Sheetz[88]). As illustrated in Fig. 1 using numerical simulation of a mechanosensitive ion channel, one class of membrane-localized mechanosensors is stretch-activated ion channels on the cell membrane that change their conductance in response to forces.[29] These ion channels could sense increases in membrane tension as a result of applied mechanical load and open, thus converting mechanical force exerted on the cell membrane into electrical or biochemical signals.[39,78] However, it is not yet clear if the force-induced activation of ion channels is the dominant mechanism for mechanotransduction. Integrin molecules or other proteins such as vinculin and talin in the focal adhesion complex may serve as force sensors and transducers[41,53,60] as illustrated in Fig. 2 in which the molecular dynamics simulation of the force-induced activation of talin's vinculin binding site is shown. Talin, an essential structural protein in the focal adhesion complex, contains the N-terminal five-helix bundle in the rod domain with a known cryptic vinculin binding site 1 (VBS1). The perturbation of this stable structure through elevated

temperature, destabilizing mutation and mechanical force activates vinculin binding.[52] However the underlying molecular mechanism is only beginning to be explored. Yet another possibility is that forces are transmitted via the cytoskeleton to remote sites within the cell or nucleus where they elicit a response.[35,42] There is a critical need to identify definitively the molecular mechanisms of mechanotransduction in living cells that are common to most, if not all, cell types.

As an emerging field, molecular biomechanics integrates mechanics, molecular biology, biophysics, biochemistry and biomolecular engineering, and encompasses three broadly defined and inter-related areas: (1) the molecular mechanisms of mechanosensing and mechanotransduction; (2) the mechanics of biomolecules, either individually or as part of a larger complex; (3) molecular motors or machines and their use in bio/nano-devices, including mechanochemical coupling and interfacing issues. There is no doubt that progress in these areas will have a tremendous impact on the life sciences and our understanding of disease processes.

Studies of force sensing and mechanotransduction in cells are inevitably related to the mechanics of biomolecules (proteins and nucleic acids), and these include the molecular analysis and constitutive modeling of single biomolecules and multi-molecular complexes. Specifically, it is important to characterize how the three-dimensional structural rigidity of DNA, RNA and proteins control their deformation under various loading conditions—stretching, twisting, bending and shear conditions—and how such deformation alters DNA condensation, gene replication and transcription, DNA–protein/RNA–protein interactions, protein function, protein–protein interaction, and protein–ligand interaction.[5–7,14,48,51,53,71] These research topics encompass bond formation, reaction rates, and the thermodynamics and kinetics of biomolecular interactions. While there have been extensive single-molecule studies of the mechanics of DNA, to date only very limited theoretical and experimental studies of the mechanics of proteins have been conducted. A better understanding of the mechanical behavior of proteins, nucleic acids and other macromolecules will provide the opportunity to decipher the fundamental mechanisms of mechanotransduction, and to predict mechanical function of protein complexes (including filaments, focal adhesion complexes, and subcellular structures) in living cells.

With the recent advent of biotechnology and nanomedicine, there is an increasing need to understand mechanochemical coupling in biomolecular motors and enzymes, and to uncover the engineering design principles of proteins as nanomachines. Since DNA and proteins may be used as components of hybrid nanobiosystems, the optimal design of such systems inevitably requires an understanding of the mechanics of biomolecules, as well as how the individual components interact with each other. A specific example is the development of nanodevices utilizing, or powered by, biomolecular machines that can produce linear, rotary, or even reciprocating motions such as kinesin, dynein and F_1-ATPase[25](Fig. 3). The use of current technologies (e.g., batteries, electromagnetic motors or hydraulic energy sources) are often unable to meet the needs of functionalized nanoscale inorganic machines with moving parts, especially when the nanodevices are used *in vivo*. On the other hand, progress in molecular biology has revealed intriguing features of the structures, mechanisms and functions of many biomolecular motors, including kinesin, myosin, dynein, ATP synthase, DNA polymerase and RNA.[37] These biomotors directly convert chemical energy derived from ATPase activities into mechanical force or motion with high efficiency. With their nanometer size, they have the potential for use in multifunctional, self-powered nanosystems.[76] How to integrate biomolecular motors with other nanodevices to perform specific mechanical, biochemical or biological functions in a controlled fashion remains a significant challenge.

MAJOR ISSUES AND NEW OPPORTUNITIES

It is now widely accepted that mechanical and biochemical functions of living cells are highly integrated in controlling the phenotype of the cell in health and disease. How cells sense mechanical forces and deformation, and convert and combine such signals with biochemical responses are not well understood. Living cells are dynamic and their structures can change in response to mechanical load. This raises many fundamental questions essential to biomechanics: How do forces applied to a cell, either directly or through cell–cell or cell–matrix adhesion sites, induce reorganization of the cytoskeleton, thus changing its mechanical properties? How do the dynamics of cytoskeleton affect cell spreading, rounding, crawling, and adhesion? How does the interaction between ECM and focal adhesion complexes transduce a mechanical signal (force or deformation) into cells? Answering these and other questions will be crucial in understanding the structural basis of cellular function. To date, the molecular mechanisms of force sensing and mechanotransduction remain elusive. While numerous mechanisms have been proposed, a strong candidate for the molecular mechanism of mechanosensing and mechanotransduction is protein deformation, or protein conformational change under force.[41,51,53,87,95,96] The unique three-dimensional structure (i.e., conformation) of a protein largely determines its function. However, proteins in a cell are deformable and can assume different (altered) conformations under physical forces. Just as proteins can transform from a native or biologically active state to a denatured or inactive state in response to small changes in temperature or pH in its environment, the application of mechanical forces can lead to protein domain conformational change and even unfolding, thus affecting protein–protein and protein–DNA recognition, binding/unbinding, enzymatic activity, causing changes in downstream biochemical processes that trigger intracellular signaling pathways and ultimately control cellular behavior. The underlying reason is that the three-dimensional geometry and surface chemistry local to the binding pocket of a receptor–ligand pair or the protein–DNA binding site contributes significantly to the characteristics of their binding. Good conformational matches usually lead to strong and long-lasting bonds. However, the conformational match at the binding site may change when the protein domains are deformed or unfolded under mechanical forces. In certain cases, an applied force can alter the affinity and lifetime of a receptor–ligand pair. In some other cases, protein deformation can expose (or bury) the binding site, thus switching between the `on' and `off' states of protein, as illustrated by the extension and unfolding of fibronectin.[28] In vertebrate muscle, the giant elastic protein titin is involved in strain sensing via its C-terminal kinase domain (TK) at the sarcomeric M-band and contributes to the adaptation of muscle in response to changes in mechanical strain.[69]

To understand the essential roles of protein conformational change in mechanosensing and mechanotransduction, theoretical and experimental studies need to be carried out, first to understand how forces are distributed throughout the cell via its protein networks and molecular complexes. This information then needs to be coupled with studies to analyze the constitutive behavior of proteins, including how proteins deform under different mechanical loads, such as tension, compression, shear, torsion, and their combinations, and how such deformations are related to protein structural rigidities. It is necessary to study protein dynamics, including domain motion, the rate effect, and to quantify how the dynamics of proteins, i.e., the modes and time scales of protein motions and deformations, are determined by the structural features of proteins. It is necessary to demonstrate experimentally that protein deformation (i.e., conformational change under force) occurs *in live cells* under physiological conditions, and it is a key event of mechanosensing and mechanotransduction. It is also necessary to study how protein deformation affects protein–protein, protein-DNA, and receptor–ligand interactions, and how such deformation is directly related to human diseases.

In the past 10 years, enormous strides have been made in the field of molecular biomechanics, including most notably the development of methods to apply forces in the range of 0.001 pN–10 nN to single molecules or small molecular complexes (Table 1). The magnitude of the forces under consideration in molecular biomechanics studies is typically in the range of 0.1–1000 pN. During this time, a small number of model systems have been extensively studied, including elastic molecules such as titin[56] and fibronectin,[64] receptor ligand pairs such as streptavidin–biotin,[94] selectin-PSGL-1 and FimH-mannose and motor proteins such as kinesin and various myosins.[37] These studies have pushed the envelope for methods development and for understanding the principles underlying molecular biomechanics. This creates the opportunity for the community to apply these methods and approaches to current medical and technological problems.

Medical and Biological Applications

An opportunity exists to gain a better understanding of diseases in which single molecules or molecular complexes change function with the application of mechanical force. Many diseases in which molecular biomechanics plays a central role are diseases that involve pathologies in mechanotransduction. For example, it is well known that atherosclerotic plaques form in regions of low and oscillatory wall shear stress.[91] Even in patients who are biochemically susceptible to atherosclerosis (e.g. due to LDL levels), most of their arteries may be protected from the disease due to their shear stress environment. Understanding this mechanism may enable alternative treatments for the disease to be identified by providing targets for new drugs that would mimic the protective effects of high steady shear stress. Mechanotransduction may also play a role in the pathobiology of asthma. It has been shown that airway epithelial cells sense compressive forces and respond by activation of several intracellular signaling pathways including the epithelial growth factor (EGF) receptor pathway.[82] Airway smooth muscle cells respond to both external mechanical stress and their own contraction by remodeling and stiffening the cytoskeleton, which in turn increases stress. This suggests that a positive feedback loop might exist between stiffening and increased stress tending to exacerbate asthmatic conditions, and that bronchodilators function by decreasing contraction to break the cycle.[20] Mechanosensing by organelles such as cilia allow detection of fluid flow in the inner ear, kidneys and other locations and malfunctions in the cilia cause a variety of diseases.[11] For example, polycystic kidney diseases involve mutations in proteins that localize to the cilia,[32] suggesting that the detection of fluid flow by the cilia is essential to proper renal function. However, the molecular mechanism(s) of how the mutations affect cilia mechanobiological function is not known. Perhaps the most widely recognized example of mechanotransduction in disease is that bones or connective tissue require mechanical forces to heal. Loss of forces due to immobilization, bed rest or even low gravity can cause disease or limit healing.[73] Mechanosensing is also involved in cancer. First, matrix stiffness regulates the ability of cancer cells to invade.[2,66] Second, it has been hypothesized[21] that cancer cells may metastasize in part because they exhibit aberrant mechanosensing,[30] which allows them to become adhesion independent and able to travel around the body. A drug that interferes with the tension-independent signaling by this pathway may have fewer side effects than traditional chemotherapy drugs that kill all dividing cells.[8] In each disease mentioned here, the actual mechanotransducers—the proteins that sense mechanical force and transduce the mechanical signal to a biological response—are generally not known. What is known is that certain mechanical forces lead to the activation of many processes including signaling pathways, transcription factors and changes in gene expression. However, these same processes are also involved in a wide variety of other regulatory responses and thus would make risky drug targets due to numerous side effects. In contrast, identifying the actual mechanosensor and the mechanism of force sensing and mechanotransduction might provide for a drug target that is specific to the mechanosensing process.

Other diseases involve a wide range of proteins that are subjected to mechanical forces, often outside the cell, in various bodily compartments. In some cases, the proper functioning of these proteins is needed to maintain normal function in high-force situations. One example is the involvement of proteoglycans in disease. These molecules contain high molecular weight negatively charged polysaccharide chains called glycosaminoglycans (GAGs) attached to a core protein. Large proteoglycans containing 10–100 GAG chains are found in musculoskeletal and cardiovascular connective tissues where compressive or osmotic forces act.[43] Diseases involving these connective tissues often involve the proteolytic degradation of constituent proteoglycans, resulting in loss of tissue-level mechanical function. An example is the ~3 MDa proteoglycan, aggrecan, found in cartilaginous tissues and the intervertebral disc, shown in its monomeric form in Fig. 4.[63] The high negative charge density of the linear GAG chains results in molecular level electrostatic repulsion forces that significantly contribute to tissue compressive stiffness and osmotic fluid retention. Glycosaminoglycans such as hyaluronan are also found in synovial fluid and have been used for orthopedic therapy because of their role in joint lubrication.[89] It is currently unknown how the mechanical properties of these molecules and their molecular complexes are changed in disease states such as osteoarthritis. An important step in addressing these issues is to image the relevant molecules, using methods such as atomic force microscopy (AFM) (Fig. 4), and to directly measure molecular level nanomechanical function, using techniques such as optical tweezers or AFM.[18]

In other cases, proper mechanical functioning of extracellular proteins is necessary to respond to abnormal force conditions to initiate certain processes. For example, high shear stress induces platelets to bind to the blood serum protein von Willebrand factor (VWF) to mediate adhesion to the substratum and thrombosis.[15] This allows blood to clot upon injury to arteries to prevent bleeding but can also cause thrombotic occlusions leading to heart attack and stroke in patients with cardiovascular disease. It has been proposed that shear stress changes the 3D structure of the platelet glycoprotein Ib (GPIb), which is the receptor for VWF,[55,72] or that VWF may form catch bonds with GPIb.[50] The catch bonds between VWF and GPIb have been demonstrated by AFM experiments, their structural mechanism has been explained using SMD simulation results, and their relationship to von Willebrand diseases (a bleeding disorder) has been proposed.[92] Understanding the mechanism of shear-activation may allow novel therapies for both bleeding and thrombotic disorders. Another example is that passive forces in muscle are elevated in some disease states. This may reflect changes in the elasticity of titin, but while titin elasticity has been well-studied, changes due to disease have not been addressed.

Technological Applications

Another broad area of application is the incorporation of molecular biomechanics design principles into nano- and micro-scale devices. For example, there are many potential technologies based on the force-modulated adhesion of particles via biomolecules. These include adhesion of nanoparticles in the blood stream, intestines, or lungs for either drug delivery or molecular imaging.[80] Another example is the capture of viruses, bacteria, or cells in microfluidic devices, all of which must bind when biological bonds are subjected to tensile force due to drag on the particle. This tensile force may weaken adhesion for many bonds,[10,23] but strengthens adhesion for a variety of bonds termed catch bonds.[19,81,97] Another application of molecular mechanics in adhesion technology is the development of mechanically smart adhesives that respond to mechanical force. For example, there has been a great deal of interest in gecko adhesion[4] as a strong reversible adhesive that is mechanically regulated and thus appropriate for robotics. Can catch bonds be used to engineer smart nano-adhesives that respond to force rather than pH and other signals, for use in medical microrobotics? A final example of smart adhesives is the need to develop

imitation platelets and their cofactors that will enable patients with bleeding disorders to form thrombi in high shear conditions but will not clot and block arteries at physiological levels of shear stress.

Molecular biomechanics may also be used to design novel nanomechanical devices. For example, molecular machines can be used to move particles instead of fluid in nanofluidic devices.[33,79,86] Smart polymers may be designed to have actively regulated mechanical properties, or have specific functions activated by mechanical force.[59] For example, they might contract or expand with changes in pH, light, temperature, electrical current, or a chemical or biological compound. The biocatalytic activity of nano-assemblies consisting of polyelectrolyte multilayer stratum loaded with enzymes can be switched on/off reversibly by mechanical stretching.[59] One challenge over the next decade will be to design and incorporate force-sensing or force-generating components into larger devices in an oriented fashion. This will require many of the concepts of self-assembly that arise with the engineering and manufacture of all nanoscale devices. However, there will be the added challenge of engineering linkages that have the right mechanical properties to transfer and properly distribute force without breaking. It is even possible that the components can be made to reassemble into alternate complexes as well as change their individual structure and function in response to mechanical force.

Moving the field of molecular biomechanics toward solving biological, clinical and technological challenges will have many benefits. It will enhance the field of research by attracting more funding and interest from industry and by raising new challenges that will inspire novel research and methods. More importantly, the novel solutions provided by this field may benefit human health and the economy.

COMPUTATIONAL AND EXPERIMENTAL CHALLENGES

The study of single-cell and molecular biomechanics has expanded enormously with the advent and increasingly common usage of more precise experimental tools and techniques, including embedded particle tracking, micropipette aspiration, atomic force microscopy (AFM) and optical tweezers. Although these experimental techniques have led to significant progress in single-molecule biomechanics, they still lack atomic level resolution. Computational techniques, including Molecular dynamics (MD), Monte Carlo methods, and Brownian dynamics calculations, have the potential to complement the single-molecule experimental investigations, and provide insight into the underlying mechanisms with atomic resolution. For example, in the case of the motor protein kinesin, its force generation mechanism had been proposed by MD simulation[40] which agreed well with subsequent single-molecule experiments.[47] However, significant challenges exist in studying the conformational changes of biomolecules under force, including the nonequilibrium nature of conformational dynamics, and the emergence of collective behavior while individual proteins follow stochastic processes.

Computational techniques have been used to study a variety of issues in molecular biomechanics. For example, motivated by experimental studies using AFM and optical tweezers, MD and Monte Carlo calculations have been used to explore the dynamic properties of nucleic acids that determine their behavior during unfolding and refolding, and the structural basis underlying these properties, including the specific interaction among residues, and secondary structure rearrangements. Another example is the computational simulation of the mechanical properties of ATP synthase and other molecular motors. In the case of ATP synthase, MD simulations have revealed that the gamma subunit rotates as a consequence of torsional forces generated by electron transport across the membrane bound domain of the molecule.[1] One of the most important applications of computational

molecular biomechanics is the study of mechanotransduction, the process by which cells transduce mechanical signal into biological processes. Numerous experimental investigations have revealed that, when external forces are applied to the surface of a cell, cytoskeletal filaments and associated molecules rearrange to form focal adhesion complexes, which act to transmit mechanical signal into and out of cells through membrane-bound integrin molecules. MD simulations of locally applied forces to talin, one of the mechanically-sensitive molecules in a focal adhesion, have shown activation of its cryptic vinculin binding site, which is important to the linkage of actin filaments to integrin molecules[41,53] (Fig. 2). Other MD simulations have shown that the interaction between integrin and the arginine–glycine–aspartate (RGD) peptide sequence in ECM filaments is due to a multitude of stabilizing hydrogen bonds, which account for the strength of the interaction between the cell and the substrate. Still others have investigated the modulation of transport though ion channels.[31,93]

Several computational tools have been developed for molecular biomechanics studies. For example, steered molecular dynamics (SMD) has been used as a technique for simulating nonequilibrium conformational changes along a molecule's free energy landscape by overcoming energy barriers with externally applied forces. The umbrella sampling technique calculates the potential of mean force along the reaction coordinates of the system. Normal mode analysis has advanced as a technique for determining the natural vibrational states of molecules. Together these computational techniques are used to determine mechanical and physical properties of molecules that account for their functional roles. Although these computational techniques have demonstrated wide applicability to the study of molecular biomechanics, there are several limitations. The greatest limitation is the availability of protein structures solved by X-ray crystallography and other experimental techniques. Further, the force fields used in simulations are empirically based and the accuracy of the simulations is limited by the accuracy of these force fields. The treatment of solvent has also been a computational challenge: implicit solvent representations are more efficient but less accurate, whereas explicit solvent representations, although they better capture the solvent-induced conformational motion, are computationally very demanding.

A common question in MD simulations is how to mimic *in vivo* conditions. Specifically, how should the mechanical signal be applied to the cell, constant displacement (velocity) or constant force? What is the appropriate direction in which to apply the force? How do steric effects of protein complexes affect these studies? What boundary conditions are most appropriate in studying single protein molecules or multi-molecular complexes? How can one best bridge different length scales in molecular biomechanics simulations? Since biomechanical phenomena span a large range of time scales (from femtoseconds to days and even years) and length scales (from Angstroms to meters), one important challenge is to develop novel algorithms and modeling approaches that can bridge these disparate scales. Finally, while most of the biological processes in a living cell occur with a timescale longer than a few milliseconds, available computing power prevents simulations over times longer than microseconds. This severely limits the applicability of MD simulations.

In addition to computational challenges, many experimental challenges exist in molecular biomechanics studies of proteins and nucleic acids. Many of the experimental challenges in molecular biomechanics involve the need to study molecular complexes in conditions closer to their *in vivo* environment. Current methods allow researchers to apply forces to simple molecular complexes *in vitro* with an AFM,[26,70] optical traps,[12] biomembrane force probe (BFP),[24] or other single molecule force spectroscopy methods. Ideally, these methods require the complexes to be strongly bound to two surfaces via two distinct sites. While many cross-linkers can achieve covalent binding, they recognize functional groups such as cysteines that are increasingly common as the complexity of macromolecular structures

grow. Conversely, functional groups such as gold binding polypeptide,[67] His-tags, and streptavidin bind noncovalently and may detach under the applied force before the molecular event to be studied occurs. Therefore, a more specific method of linking proteins in an oriented fashion is needed depending on the types of macromolecular complexes that have a large number of components *in vitro*. It is also possible to apply forces to cells using beads that are tethered to cell receptors and manipulated with a magnet[36] or optical tweezers,[16] or by applying fluidic shear stress,[84] or stretching the substratum.[68] Finally, we need to improve and apply methods to measure or sense forces quantitatively on target molecules inside living cells, so that we can determine how forces are applied to molecules of interest *in vivo*.[58] It is possible to apply and sense forces using a single tool such as optical trapping of liquid droplets to characterize motor protein function *in vivo*.[74] Alternatively, forces that are applied by normal physiological processes may be sensed with a method such as a fluorescent force-sensitive probe[58,75] or using cysteine labeling to identify intracellular proteins that change their conformation under force.[45] Together, these methods will allow researchers to apply and measure forces on complicated molecular complexes to understand the force response of systems that bridge the length scale between single molecules and cells.

ROADMAP: SHORT-TERM AND LONG-TERM GOALS

Numerous open questions and opportunities exist on the roadmap of molecular biomechanics. One most relevant question is to specifically identify the mechanosensing proteins, i.e. what proteins are likely to be deformed? What is the extent and importance of mechanosensing characteristics of different proteins in the molecular machinery of the cell? How can we control molecular conformational changes and protein's modes of deformation to ascertain the biomechanical phenomena under study? To demonstrate experimentally that protein deformations actually occur *in vivo* and link them to certain biological functions, we need to distinguish with specificity the distinct molecular conformations. We also need to be able to alter the molecular conformation of mechanosensing proteins or nucleic acids with threshold specificity. Given that protein domain motions can often function as switches between distinct biological functions, experimental techniques are necessary to characterize and quantify how domain deformation and unfolding alter interactions. In order to ascertain relevance of these localized molecular events to overall biological phenomena, it is essential to identify how conformational changes at one location may potentially change binding affinity at another location. It is also important to characterize allosteric effects. Optical fluorescent techniques involving green fluorescent proteins (GFP), which can be genetically incorporated in a robust manner, have recently made major contributions to many biomechanical and biological discoveries, yet one must note that the GFP proteins themselves are large (approximately 5 nm in size), comparable in size to most proteins that are to be tagged. This caveat points out the need for less invasive approaches for marking proteins *in vivo*. Other new experimental techniques are required for mechanical regulation of proteins and other biomolecules. Similar to computational approaches, multiscale experimental settings are also essential to the study of molecular basis of macroscale mechanical behavior of the cells. Taking into account the spatial organization of proteins and cellular complexes, and how forces are distributed in molecular complexes proves critical here. Many of these phenomena require breakthrough thinking as in many cases one needs to identify, devise, and build the appropriate building blocks for such complexes and incorporate the suitable interactions and force fields, similar to what is needed for the computational approaches. It is vitally important to devise surrogate cell models *in vitro* where we can add one component at a time and study that by applying mechanical stimuli. Such cases rapidly become extremely complex, and will likely require a “systems biomechanics” approach.

New computational modeling techniques are needed to model protein clusters and apply forces that realistically mimic biologically meaningful settings. How do we discover alternative protein states and identify the effect of force on them? New computational methods are necessary to show (slow) allosteric changes and we need to address the critical role of allosteric regulation by force. Experimental methods are clearly essential. We need experimental methods to better elucidate how the structure is related to mechanical properties. In fact, it was revealed recently that integrin catch bonds provide a mechanism for mechanosensing and that force is required for outside-in signaling.[49] Mutation experiments represent a valuable tool to ascertain the effect of localized changes in the molecular constructs of the protein machinery on their overall mechanical behavior. Perhaps the most subtle step is to couple these computational/theoretical and experimental aspects. With the development of new techniques, theories and algorithms, it will become increasingly feasible to understand the biological phenomena over a range of time and length scales. This will also offer a unique platform for probing the underlying mechanisms involved in the initiation and development of many pathological conditions. Many disease examples remain to be explored, including atherosclerosis, cancer, infectious diseases, in which mechanics may play an important role. In atherosclerosis, for example, after more than 30 years of multifaceted investigations, we still do not know the molecular mechanisms involved in the initiation and development of this disease. In cancer, it remains unclear whether physical factors including force play a role in metastasis. Thus, there is a need to perform multiscale (tissue, cellular and molecular) studies of metastasis incorporating the key mechanical signaling pathways involved.

Enormous opportunities exist for making progress in molecular biomechanics and in its application more broadly to molecular and cell biology and bioengineering. Looking into the future, we can anticipate some of the advances that will be made, and the goals we might pose for future research. Many advances have occurred during the past decade in the visualization of molecular events within the cell (such as STED-4pi) or *in vitro* (see, Jares-Erijman and Jovin,[44] O'Hare *et al*.,[65] and Willig *et al*.[90] for recent advances), which is likely to continue. In connection with protein mechanics, the potential exists now to probe large-scale conformational changes using Fluorescence-Resonance Energy Transfer (FRET) or its close cousin, Fluorescence Lifetime Imaging Microscopy (FLIM) with the donor and acceptor fluorophores tethered to the same protein or to a pair of proteins. Using the theoretical dependence of FRET ratio on separation distance as a guide, it is possible to monitor changes in protein conformation. But many of the current fluorophores are quite large, and may influence protein conformation, and the number of probes is often limited to no more than one or two. New, small molecular weight fluorophores such as FLASH may prove more useful.[34,57] In any event, new and more precise methods for measuring protein conformational change are sorely needed. One especially promising new three-dimensional imaging technique, stochastic optical reconstruction microscopy, or STORM,[38] provides a means of obtaining resolution on the order of 20–30 nm of intracellular structures.

As a protein unfolds, the resisting force arises from a combination of entropic and enthalpic effects, where the latter involves rupture of internal non-covalent bonds. This can be seen in simulations and experiments on single proteins when rupture of an internal bond triggers complete unfolding, but more subtle transitions also occur each time an internal bond is broken. While thermal fluctuation is another essential element that complicates the picture, analysis of the force–extension curves can still provide insight into the internal structure of a protein or other macromolecule. These force–extension relations could thus be viewed as a “force signature” of a particular protein that arises from the internal structure and interactions. Taking this one step further, the force signature of a protein should change whenever the protein conformation changes, as due to normal conformational fluctuation, or, more importantly, by activation or binding to another protein. Thus, binding events

between a single pair of proteins might be investigated by observing changes in the force signature of a single protein. Even single molecule stretching can display diverse types of force–extension behavior (Fig. 5).

Several methods are currently used to obtain force–extension data on single proteins, and these generally employ either AFM or optical tweezers technologies (Table 1). Each has its advantages and disadvantages in terms of force and distance resolution and the complications of simultaneous force measurement and optical (e.g., single molecule fluorescence) measurements. Conventional AFM systems make it difficult to view the molecule under high magnification, but methods are being developed to circumvent that problem. Fluorescence measurements are compromised by the trapping laser, typically of high intensity and likely to induce photobleaching, but new methods that involve rapid switching between trapping and fluorescence measurement are being developed.[13] Force resolution tends to be better at the low end in optical traps. AFM is capable of applying higher forces, but the range of applicability of both methods is continually expanding. Still, room exists for new approaches that facilitate simultaneous manipulation and visualization of single molecules so that we can sense force and observe conformational changes in a single experiment.

Clearly, to make significant progress on the computational front, more atomic structures are needed, and these are becoming available at an accelerating rate. X-ray crystallography has been the “gold standard” for structures, but cryoelectron microscopy, that is capable of imaging proteins that are difficult to be crystallized, has made significant advances in resolution, and other methods, such as solution or solid-state nuclear magnetic resonance (NMR), and neutron or electron diffraction are also potentially useful tools.[83] Ultimately, we will need methods with Angstrom-level resolution that can determine the structure of single proteins, or even protein complexes.

As we probe conformational change in force transmitting proteins, it will be important to gain the ability to map the effective intramolecular stress distribution. This is somewhat analogous to the use of finite element programs to obtain stress distributions in macroscopic continua, but on an atomistic length scale. Current MD programs have the capability to calculate instantaneous or time-averaged force interactions, though, so taking the step of mapping these in 3D may be quite easily implemented. In a broader sense, however, new visualization schemes need to be developed that enhance the presentation of mechanical data. As with a macroscopic object with a complex geometry, regions of stress concentration in a protein may not be obvious, but knowledge of where these reside can help us to interpret force-induced changes in conformation.

One of the major limitations in using MD or SMD to investigate single molecule mechanics has been the enormous computational demands, so that most simulations are limited to no more than about 50–100 ns. Various improvements such as efficient implicit methods for incorporating solvation effects, and coarse-graining methods that reduce the number of degrees of freedom of the system have been developed, but there is no consensus as yet on the best approach to use for a given system, nor is there agreement in the research community regarding the accuracy of these methods. We will certainly see advances in computational methods during the next 5–10 years, and these should help to expand our capabilities to simulate the effects of force on protein conformation.

Coarse-graining or similar approaches might also make possible true multi-scale simulations. At present, time steps used in MD vs. Brownian Dynamics vs. finite element methods can span over 15 orders of magnitude, so simultaneous calculations tend to be unfeasible. Fortunately, they may be largely unnecessary, as well, except in a few

circumstances. At some point in the future, methods will be developed to simulate force transmission through the entire cell, incorporating events down to molecular scale. But detailed knowledge of protein conformation may only be necessary locally, at sites, for example, of mechanosensing. In such cases, network or even continuum structural models may suffice for the bulk of the computational domain, with MD being applied to a single protein or even a subdomain of a larger protein. Other situations, however, such as the simulation of multiple signaling molecules influenced by transmitted stresses, in which the response of one is fundamentally linked to conformational changes in another, may demand a true multi-scale, simultaneous simulation. Progress will certainly be made in this direction, but it will likely be slow given the enormous magnitude of the problem. One approach that shows promise is the use of coupled MD-FEM methods[9] (Fig. 6). In a sense, this is yet another example of coarse-graining, but given the maturity of finite element methods, effective and reliable coupling of these two approaches could prove highly beneficial.

Improvements are also inevitable in computational speed. But in addition to the anticipated progression along Moore's Law, new computers are being designed that specifically cater to the needs of MD simulation that could reap enormous benefits. These computers draw advantage from the use of a dedicated high-performance processor specifically for non-bonded, long-range interactions that can often consume over 99% of MD simulation times. Recent reports indicate that speed increases of several hundred fold can be realized. (Blue Gene: A vision for protein science using a petaflop supercomputer.[3])

So what does the future hold in molecular biomechanics? While we have no crystal ball, it can reasonably be predicted that we will develop new and greater capabilities to measure, monitor and model the response of single molecules to mechanical loads. At the same time, working with our colleagues in biology and chemistry, we will begin to gain a better understanding of the interactions between intracellular biochemical signaling pathways, and what might be termed, “mechanical signaling pathways”, the transmission of signals through the network of linked proteins by means of force transmission. Mechanical signaling has already been widely appreciated, but its full impact on biological function is just now being recognized. And once its role is better understood, one might envision “mechanical therapies” in which diseases may be treated by altering the mechanical properties of the relevant molecules, cells or tissues. This is already happening to a limited extent and having significant impact in cancer treatment in the context of molecular therapies that alter the ability of cells to generate new vascular networks to nourish a tumor, or the tendency of the tumor cells to migrate, extrasavate or intrasavate. Recognizing the pivotal role of mechanical signaling and mechanosensing opens new opportunities for therapeutic control in a wide range of pathologies.

Speculating further, one might envision “designer proteins” with desired mechanical properties, that can turn intracellular processes on or off through the application of external force (e.g., with the introduction of targeted magnetic particles). Since we know that the forces experienced by a cell can influence a multitude of cell functions, forces can conceivably be used to control cellular activity. Similarly, one might envision “mechanical beacons”, that produce a signal, optical, for example, when a certain mechanical stimulus is sensed. This, or a similar approach, might be used to detect arterial blood flow conditions that contribute to atherogenesis as a means of training individuals to avoid certain detrimental behaviors.

Finally, advances in these areas require individuals with a unique knowledge and skill set, bridging between chemistry, biology and bioengineering or biophysics. To prepare for these challenges, we need to ensure that a new cadre of researchers is trained in these fields. Cross-disciplinary teaching, which has been discussed extensively, needs to be

implemented. Students of biomechanics need to understand not only continuum, but also statistical mechanics, as well as having a solid grasp of physical chemistry. Our programs need to move to develop courses that span these disciplines and produce students who are truly multilingual, being comfortable working at this critical interface.

CONCLUDING REMARKS

In closure, we have presented a few of the challenges and milestones, both experimental and computational/theoretical, on the horizon for the advancement of molecular biomechanics. Experimental techniques are required to capture intracellular forces and molecular conformations. More sophisticated techniques are essential to dissect changes in molecular conformation. New computational modeling techniques are needed to be able to model protein clusters and apply forces on them to mimic biologically meaningful settings. These computational/theoretical and experimental aspects must be integrated to help understand the multiscale/multiphysics processes underlying biological phenomena over a range of time and length scales. This represents both a challenge and an opportunity to understand a disease or biological process in its entirety. This calls for a close coordination between efforts in molecular biomechanics and the investigations focused on cell, tissue and organ biomechanics, necessitating developments of theoretical and computational approaches to bridge the gap between these disparate scales.

Acknowledgments

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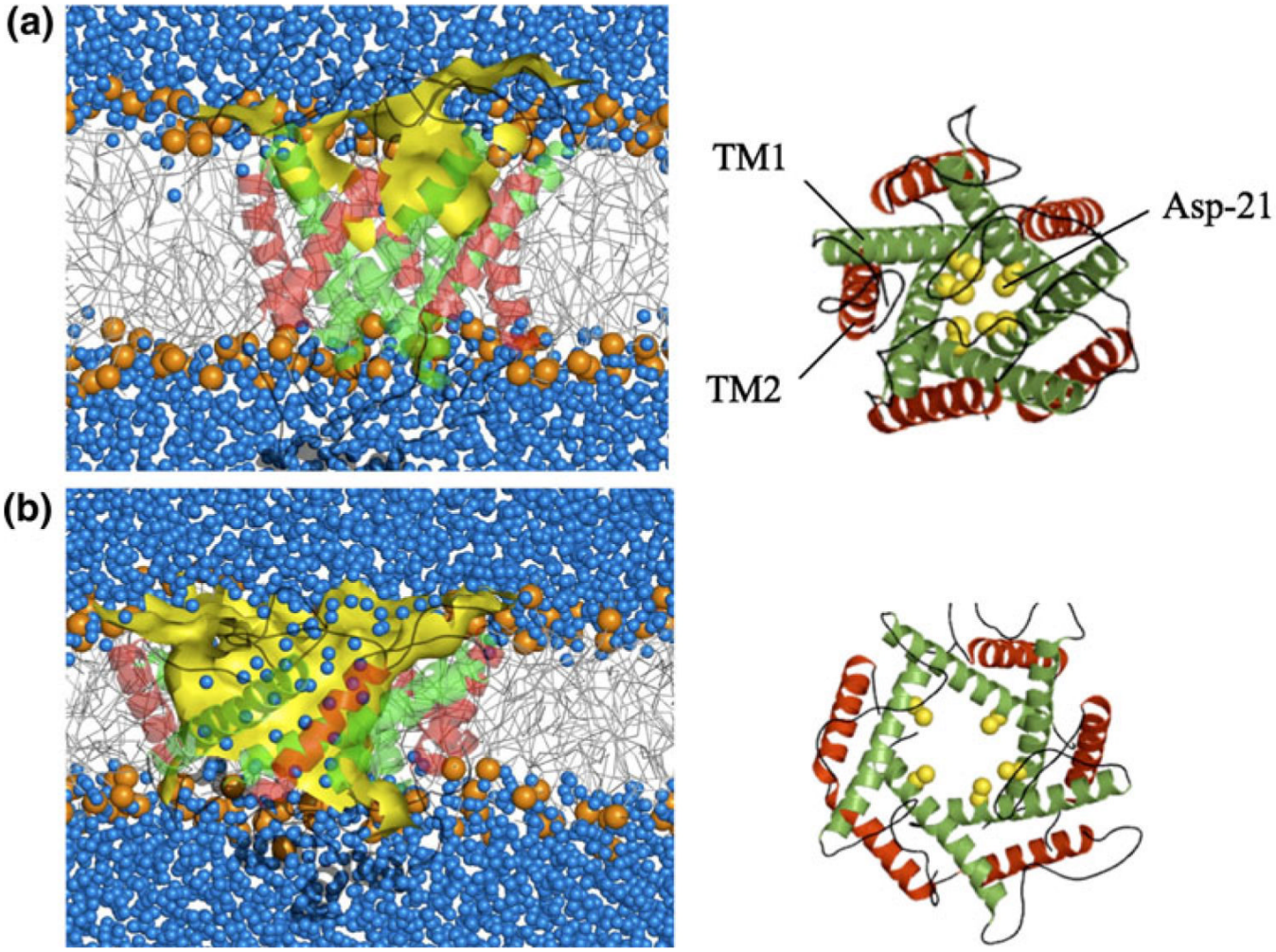

FIGURE 1.
Schematic of numerical simulation of a mechanosensitive ion channel with (a) small and (b) large opening under the action of membrane tension. Left: side view showing the lipid bilayer (white region with red phospholipid head groups) in an aqueous (blue) environment. Right: End view showing the membrane pore. Reproduced with permission from Yefimov *et al*.[93]

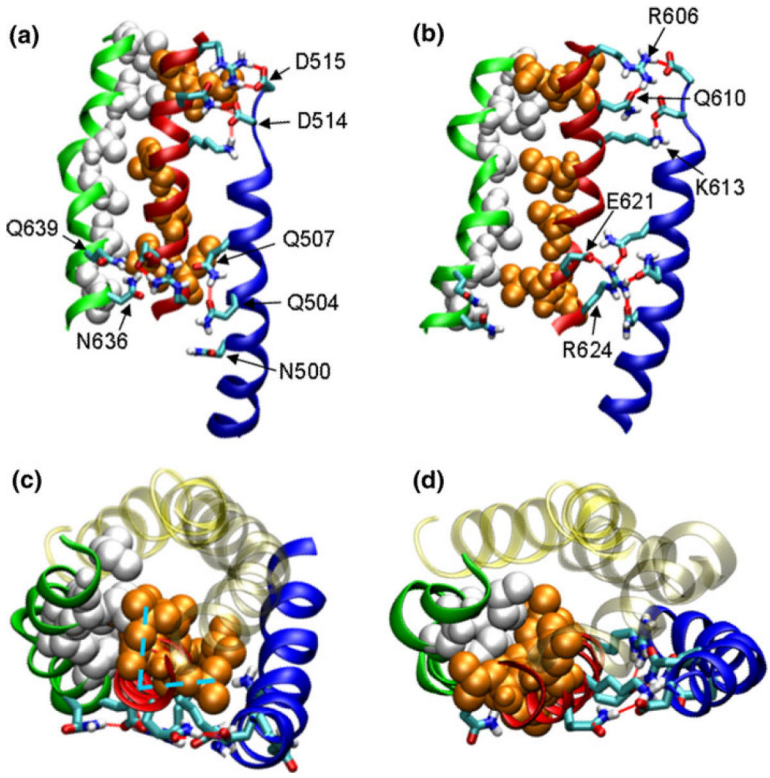

FIGURE 2.

Molecular dynamic simulation of the force-induced activation of talin's vinculin binding site (VBS1) (red ribbon with organ spheres). Helix H1 (blue ribbon), H2 (transparent yellow), H3 (transparent tan), VBS1 (red ribbon), H5 (green ribbon), hydrophobic residues of VBS1 (orange VDW; also the vinculin-binding residues), hydrophobic residues of H5 (white VDW), and some important polar residues (stick representation with color denoting the atom type). Polar residues are labeled on the figures. (a, b) side view; (b, d) top view. (a and c) Before the conformational transition, the hydrophobic residues of VBS1 are hidden in the hydrophobic core. (b and d) At increased force, the hydrophobic residues rotate and become exposed to solvent. Hydrogen bonds between H5 and VBS1 are broken. The hydrophobic residues, or the vinculin binding residues, point into the page in (a) and point to left in (b). (c) Conformation at t = 0.86 ns viewed from top. The V-shaped VBS1 hydrophobic residues are packed within the hydrophobic core of TAL5 (cyan dotted lines). (d) Conformation at t = 9.24 ns showing VBS1 rotation. The hydrophobic residues H5 (white VDW) fit into the `V' of the VBS1 hydrophobic residues (orange VDW). Reproduced with permission from Lee *et al.*[52]

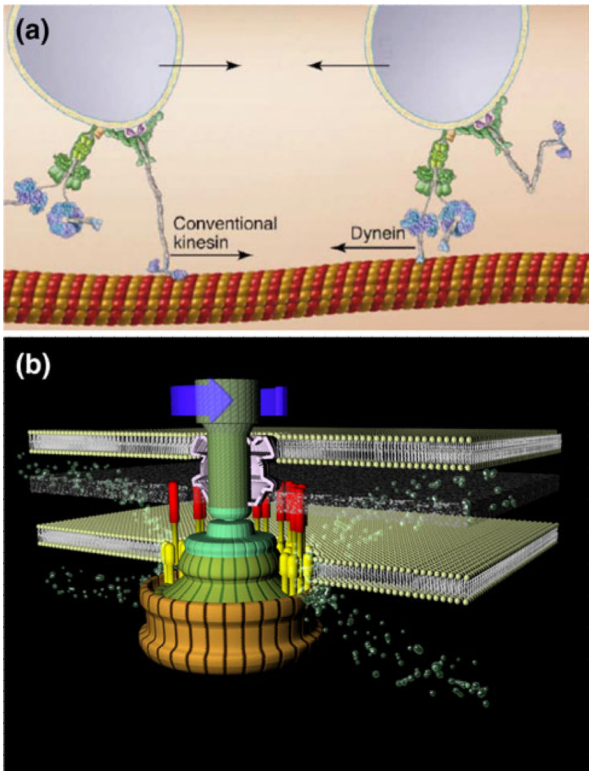

FIGURE 3.
Two types of molecular motors. (a) Linear motor: Two linear molecular motors, kinesin and dynein, are shown. Both walk along a microtubule, but in opposite directions. In this schematic the motors are shown attached to vesicles for intracellular transport along a neuronal process (reproduced with permission from Vale[85]). (b) Rotary motor: Schematic of a bacterial flagellar rotary motor. Rotational speed is over 100,000 rpm and it measures just 50 nm across (Image provided courtesy of A. Ishijima, Tohoku University, Japan).

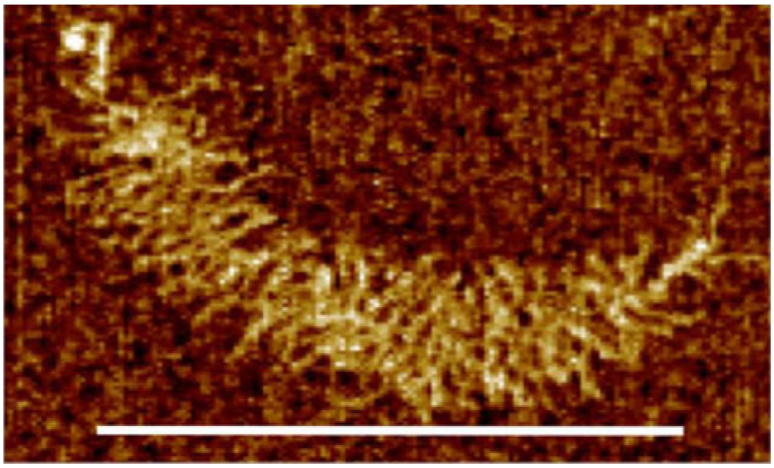

FIGURE 4.

AFM image of a single aggrecan monomer extracted from human articular cartilage, consisting of a core protein substituted with almost 100 chondroitin sulfate and 10–20 keratan sulfate glycosaminoglycan chains. Scale bar = 300 nm. The globular G1 domain at the left-most (N-terminal) end can bind to hyaluronic acid (HA), stabilized by co-binding of a 45 kDa link protein, thereby forming supramolecular aggregates containing as many as 100 aggrecan monomers. Enzymatic cleavage of aggrecan by aggrecanase enzymes (e.g., ADAMTS-4, -5) at 5 or more sites along the core protein causes degradation and loss of these monomers in diseases such as osteoarthritis (Image courtesy of H.-Y. Lee, A.J. Grodzinsky, and C. Ortiz).

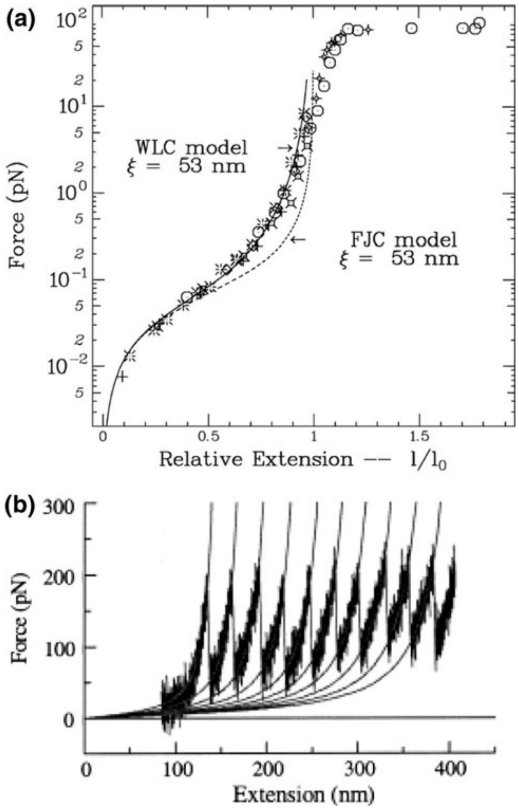

FIGURE 5.

The force–extension relationship for two biological molecules (DNA in (a) and protein in (b)) can be well described by a worm-like chain (WLC) model. (a) Force vs. relative extension curves of single DNA molecules. The dots correspond to several experimental measurements performed over a wide range of forces. The full line curve is a best fit to the WLC model for forces smaller than 5 pN. The dashed curve is the result of the freely jointed chain (FJC) model with the same persistence length. Above 70 pN, the length abruptly increases, corresponding to the appearance of S-DNA (reproduced with permission from Strick *et al*.[77]). (b) A force vs. extension curve of Filamin A protein in aqueous solution measured by AFM at room temperature. The WLC model fits the sawtooth pattern of the force vs. extension curve well where the force gradually increased after the abrupt decrease in force (reproduced with permission from Furuike *et al*.[27]).

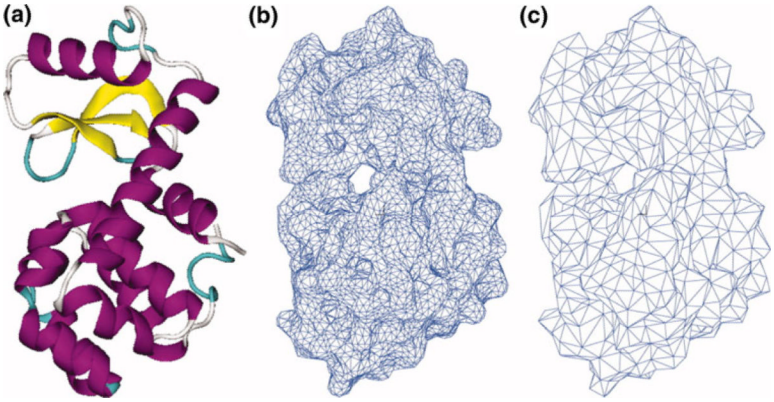

FIGURE 6.

Computational simulation of protein conformational changes using a coupled MD-FEM method. (a) Minimized energy molecular structure of the T4 lysozyme protein. (b) and (c) High and low resolution discretized meshes, respectively, based on the solvent-excluded surface of the structure in (a) and used for finite element simulations (reproduced with permission from Bathe[9]).

TABLE 1

Specifications for three methods commonly used to manipulate single molecules (adapted from Neuman *et al.* [62]).

Specification	AFM	Magnetic trap	Optical trap
Bandwidth (Hz)	1000	10–1000	50–5000
Stiffness (pN nm^{-1})	$1–10^5$	10^{-6}	0.005–1
Spatial resolution (nm)	0.1–1	2–10	0.1–5
Force range (pN)	$5–10^3$	$10^{-3}–10^4$	0.1–100