Multiscale Design and Modeling of Protein-based Nanomechanisms for Nanorobotics
Mustapha Hamdi and Antoine Ferreira
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What is This?
Abstract

In this paper, we present a novel approach that makes use of multiscale and multiphysics modeling coupled to virtual reality for nanorobotic prototyping systems. First, a CAD-assisted assembly system that integrates principles for a multiscale approach into a nanorobotics structure design is presented. Then, we focus on the different design levels, more specifically, the optimization of geometry structure carried out by quantum mechanics, molecular dynamics and continuum mechanics methodologies. As an illustration of the proposed multiscale modeling concepts, we test the dynamic characteristics of a molecular sarcomere mechanism through steered molecular dynamics (SMD) simulations. The nano-kinematic parallel platform is composed of protein-based passive kinematic chains and actuated by myosin actuators. Multiscale simulations and experiments prove the effectiveness and accuracy of the proposed design and modeling approaches.

KEY WORDS—bio-nanodevice, multiscale modeling, multiphysics, nanorobotics

1. Introduction

Bio-nanorobotics is a new and rapidly growing interdisciplinary field addressing the assembly, construction and utilization of biomolecular devices based on nanoscale principles and/or dimensions. A key application is for medical target identification in therapeutical diagnosis, medical therapies and minimally invasive surgery (MIS) (Hogg and Kuekes 2006; Requicha 2006; Calvacanti et al. 2008). Modern engineering actuation techniques inspired by nature have been implemented successfully in microrobots evolving in fluidic environments using external electromagnetic fields (Behkam and Sitti 2006; Mathieu et al. 2006; Yesin et al. 2006). In contrast, the biological approach shows that flagellated propulsion mechanisms of bacteria (Martel et al. 2006), DNA-based conformation devices (Hamdi et al. 2008) or magnetic stereotaxis systems (Steager et al. 2006) represent a fertile territory for untethered nanoscale machines without the need for external hardware for actuation. In this way proteins could act as motors, mechanical joints, transmission elements or sensors (Chirikjian et al. 2005). The successful implementation of bio-nanorobotics requires the merging of both research approaches in order to address nanorobot choices on sensing, hardware architecture design, manufacturing approaches and control methodology investigation (Mitsuya 2004). However, when size goes down to the nanoscale new physical properties and coupling effects between mechanical motion and physical, biochemical properties may become dominant, and multiphysics-scale problems arise. In addition, the experimental techniques usually employed for the characterization of motor proteins have one limitation consisting of the direct observation occurring during mechanical tests. Owing to the small scale structure of the proteins it is difficult to anchor them, assemble them and control their behavior during the pulling experiments.

As a consequence, realtime three-dimensional (3D) prototyping and simulation are important tools for biologically inspired nanorobot development. Simulation can anticipate performance and help in the development of new device design and manufacturing (Toth-Fegel 2000), nanomechatronics control design (Calvacanti and Freitas 2005) and hardware implementation. This includes techniques that mainly cover the range of multidisciplines from atomistic mechanics to continuum mechanics (CM): quantum mechanics (QM), multiparti-
multiscale models for non-organic materials do not integrate the hierarchical design and focus mainly on the multiscale modeling of single components, such as carbon nanotubes (CNTs) (Liu and Chen 2003; Maiti 2007) or electronic materials (Karakasidis and Charitidis 2007). Some multiphysics models have been proposed but they do not take into consideration all of the physical interactions, such as QM (Frenkel and Smit 2001), nanomechanics (Ghoniem et al. 2003) or multiphysics parametrization in order to ensure a connection between length scales (from atomistic to macroscopic) (Braatz et al. 2006). Recent approaches involve combining the multiscale modeling of macromolecular conformational changes to concepts from rigidity and elastic network theory (Feig et al. 2004; Ahmed and Gohlke 2006). Still, owing to their size, biomolecular nanorobotics characterized by computational methods such as molecular dynamics (MD) are often beyond the reach of realtime feedback for computer aided design (CAD) assisted assembly.

The objective of this work is to demonstrate a framework that couples multiscale, multiphysics and virtual reality advanced techniques for the design and modeling of bio-nanorobotic systems. A three-level modeling paradigm was developed for this purpose. QM for designed components parametrization, MD for mechanical properties characterization and CM efficiently integrated with MD. As a good illustration of the above multiscale and multiphysics models, we choose the biological actuation generated in a striated muscle sarcomere nanomechanism composed of serially linked tandem titin proteins. First, a linear nanorobotic parallel platform model composed of biological proteins and non-organic graphite material is adopted in order to mimic the elementary passive elasticity of tandem proteins. Its kinematic structure is designed, modeled and optimized. Simulation results clearly show the main advantages of the proposed method in terms of computational time, accuracy of simulation and energy optimization. Second, we apply the above techniques to the study of long serially linked kinematic chains composed of tens of elastic proteins. The multiphysics modeling results, carried out at the level of polypeptide chains, helped us to develop a better qualitative and quantitative understanding of actin-microtubule actuation forces generated by stretching/relaxation of protein-based nanomechanisms. Based on experiments in the literature, the proposed multiscale design and model is validated. This approach enables a “robot scale” to be reached making the design and modeling of conventional robot kinematics accessible to atomistic and molecular-scale modeling.

This paper is organized as follows. In Section 2 we present a CAD system for bio-nanorobotic multiscale prototyping. In Section 3 we describe a set of utilities and programming software for the implementation of computationally distributed and multiscale sampling methods based on existing simulation programs. In Section 4 we present the simulation results of a bio-nanorobotic platform. A comparison study between relevant experiments from the literature and steered molecular dynamics (SMD) simulations is then proposed in order to validate the modeling methodologies.

2. Design of Bio-nanomechanisms Mimicking Biological Structural Proteins

New nanostructures using synthetic strategies have emerged recently. Inspired by biological systems, concepts of self-organization and self-assembly of building blocks allow the self-association (in series or in parallel) of particular patterns to form higher-order organized biological nanomechanisms. Elementary biological structural components composed of protein-based nanosprings are of prime importance in the elasticity and stability of main biological functions at the macroscale, i.e. tendons, muscles, lungs and skin. Bioinspired nanomechanisms involved in nature are interesting for the design of protein-based nanorobotic structures. Figure 1 shows the giant multidomain fibrillar protein titin composing the sarcomeres of human striated muscles (Collinsworth et al. 2002) which creates the strong passive elasticity of muscle. Titin, a 1-μm-long protein found in striated muscle myofibrils, possesses unique elastic and extensibility properties owing to the serial assembly of coiled titin molecules. Titin is composed of around 300 repeats of two types of domains, fibronectin-type III-like (Fn-III) domains and immunoglobulin (Ig) domains. The Ig domain realizing the connection between the end of the thick filament and the Z-line constitutes the titin-based passive elastic nanomechanism. Figure 1(c) shows the molecular kinematic chain modeling the extensible part of the titin molecule used in this study: proximal tandem-Ig, PEVK unique sequence and distal tandem-Ig. The biologically inspired nanomechanism involves different phases of elasticity (Figure 1(b)). The I-band part of titin is coiled up when a sarcomere is at its back slack length due to the inherent flexibility of the molecule (i.e. the length to which relaxed muscle freely shortens). The first elasticity phase only involves straightening of the tandem-Ig segments. After straightening, further extension involves the unfolding of the titin polypeptide, giving rise to restoring force. Finally, extension of PEVK unique sequence dominates the stiffness of sarcomere. The magnitude of the force required to stretch titin must be put into the context of the forces operating in cells. The force generated by two to four motor molecules operating in parallel stretch titin completely. The stretching force is provided by elementary myosin motor molecules “walking” on actin filaments. It highlights that the sarcomere behaves as a versatile and adjustable molecular spring with a range of important functions in passive and restoring forces in kinematic nanostructures. Just as muscles magnify force and movements by using hierarchy mechanisms, bio-inspired nanomechanisms using similar principles.
Fig. 1. Molecular mechanism of sarcomere passive contraction/relaxation (adapted from Collinsworth et al. (2002)). Cardiac titin’s extensible I-band region acts as a biological inspired spring nanomechanism. (a) Regions of the titin molecule composed of an I-band part and an A-band part connected between the N- and C-termini. (b) Titin configuration at different sarcomere lengths. Upon sarcomere stretch, initially the extension of tandem Ig (immunoglobulin-like) segments dominates followed by a dominating PEVK unique sequence extension rich in proline (P), glutanate (E), voline (V) and adlysine (K). The stretching force is provided by elementary myosin motor molecules “walking” on actin filaments. (c) Molecular model of a titin–PEVK strand formed by a polypeptide chains.

could lead to the design of new class of bio-inspired nanorobotics. These nanoscale fibrillar nanostructures have equivalencies with macroscale robotic kinematic chains. The serial and/or parallel composition synthesis of protein-like springs allows the passive elasticity of biomechanisms found in nature to be mimicked for application in new designs of molecular robotic kinematic structures. The main challenges of the present work are the theoretical understanding, modeling and simula-
Fig. 2. Basic concept of virtual environment and haptics technology coupled to multiphysics computational methods for bio-nanorobot simulation.

3. CAD System for Bio-nanorobotic Multiscale Prototyping

3.1. Multiscale Platform

The developed simulation system presented in Figure 2 allows 3D manipulation and assembly of bio-nanorobotic components. Energy-based optimization of biological and non-biological interactions (equilibrium bond lengths, angles, energetic barriers) is performed by quantum chemistry. The system allows external constraints such as force, temperature and pH parameter levels to be controlled during MD simulations with realtime force feedback and 3D graphical display. It consists of three primary components: a haptic device controlled by a computer that generates the force environment, a MD simulation for determining the effects of force application, and a visualization program for the display of results. Communication is achieved through the IMD (Stone et al. 2001) protocol between the visualization program Visual Molecular Dynamics (VMD) (Humphrey et al. 1996) and the MD program NAMD (Nelson et al. 1996) running on multiple machines. A force-feedback PHANToM device measures a user’s hand position and exerts a precisely controlled force on the hand in order to apply different mechanical constraints, force and energy fields on the virtual model in order to prototype bio-nanorobotic components. The application of the mechanical constraints is applied by a virtual atomic force microscopy (AFM) microlever and can be manipulated with a mouse or a haptic interface with force-feedback. The interested reader is referred to Hamdi and Ferreira (2008) for further details.

3.2. Nanoassembly Methodology

Nanoassembly methodology requires the assembly process to be addressed systematically to design it optimally. Two major assembly processes are currently employed at the nanoscale. Robotic nanoassembly, much like its macroscopic counterpart, involves the positioning and joining of components. Nanocomponents are positioned by using AFM as a robot, essentially by pushing the components mechanically and joining them chemically, by using linkers such as DNA complementary strands, or by depositing additional material over the templates defined by the nanocomponents (Requicha 2003; Li et al. 2004). Mass production is best approached by programmed self-assembly,
Fig. 3. Theoretical and computational nanoassembly methodology.

using scaffolds designed by algorithmic methods. Several self-assembly approaches have been proposed based on various forces such as capillary force, hydrophobic force, electrostatic force and so on (Chi-Yuan et al. 2004; Yin 2005). The proposed multiscale design methodologies are well adapted to both robotic nanoassembly and self assembly processes. The controlled nanoassembly approach is based on hierarchical theoretical and computational assembly methods as depicted in the flowchart shown in Figure 3.

1. The designer can choose the desired organic and non-organic elements from a homemade component database (Hamdi et al. 2006). The database is composed of various nanorobotic components such as elastic proteins, carbon structures, protein-based actuators or molecular attachments. The different mechanical, dynamics and kinetics properties of the database structures have been characterized previously. The nanorobotic components are accessible via a 3D graphical user interface (GUI) developed through the VMD environment.

2. The attachment phase between the different links, structure or proteins can then be initiated through 3D virtual manipulation and stereo visualization. The user performs atomistic connections, i.e. carbon–carbon (C–C) or carbon–nitrogen (C–N) bonds, in order to create new residues.

3. Such residues are then parameterized through quantum dynamics (QD) simulation in terms of bond length, dihedral angles and Coulomb charges at around the equilibrium state. We use semi-empirical PM3 or Hartree–Fock molecular orbital models.

4. The parameterized attachment is then defined as new residue in a CHARMM27 force field (MacKerell et al. 2001). The assembled total nanorobot structure is energetically minimized through the steepest descendent algorithm (SDA) with the respect to two energetic barriers (E1 and E2) corresponding to the energies of acceptable robot geometry.

5. Thus, the program prevents the user from applying the tools in a way that is not chemically reasonable.

4. Multiscale Modeling and Design of Titin-based Nanomechanisms

The long kinematic polypeptide chain composing the I-band part (see Figure 1) possesses extensible and inextensible tandem-Ig repeats. In the simplest way, the sarcomere’s contraction/relaxation mechanism can be modeled by a guided hyper-redundant parallel mechanism actuated by multiple molecular myosin motors. As an illustration of the proposed multiscale nanorobotic design and modeling approach, we simplified the kinematic study to a one-degree-of-freedom (DOF) parallel protein-based robotic platform as shown in Figure 4. Its nanomechanical architecture is composed of three passive parallel links based on elastic and reversible proteins for the extensible tandem-Ig repeats. Each link is composed of two β-sheet titin proteins linked serially. In order to simulate the upward linear motion of the platform generated by the myosin motor, three controllable stretching forces are applied to the upper platform (Lu and Schulten 2000). The three β-sheet titin links acting as spring elements or as a restitution force will bring the platform back to its original position when the myosin motors are at rest. The attachment of the different components is constituted by three carbon atoms at the proteins/graphite interface. The dimensions for the triangular graphite sheet are 5.0 nm × 5.6 nm and the total length of the two proteins is 8.5 nm. In order to model and simulate the stretching/refolding serially linked titin Ig domain, we proposed a solution using several physics for particular length-scales and timescales.
4.1. Quantum Mechanics

A wide variety of different procedures or “models” have been developed to calculate molecular structure and energetics among other properties. Different QM models are currently developed in the literature, i.e. Hartree–Fock molecular orbital models (Cramer 2002), semi-empirical molecular orbital models (Stewart 2004) and molecular mechanics models (Kuhn and Kollman 2000). However, these models fail when identifying conformational minima and determining the geometries (bond lengths, angles and charges) of these minima. We developed a specific PM3 semi-empirical model when attaching leucine termini to a graphite layer as shown in Figure 5(a). The first task is to determine exactly which parameters are already known, and which will need to be developed from scratch. The unparameterized part of the new residue is the connection between the azote and the carbon. The connection between the azote of LEU and the carbonyl carbon of the ARM residue is called a thioester bond. Leucine-ARM linkage is defined as a new residue in the CHARMM27 topology file with the name LARM. In order to use this LARM residue in MD simulations, we need to make a topology file entry for it as well as develop a complete set of parameters describing it. The unparameterized part of the LARM residue is the connection between the leucine and the carbon. To actually determine the optimum parameter values for equilibrium bond lengths and angles, and to determine the energetic barriers associated with them, we need to use quantum chemistry. We calculate the values semi-empirically. With semi-empirical QM calculation, we optimize the geometry of the structure as shown in Figure 5(b), calculate electrostatic potential (ESP) charges and vibrational frequencies. The LARM linkage, which corresponds to a C–N bond, calculated by semi-empirical PM3 methods, is 1.449 Å and the C–N–C angle is 123.25°. The LARM linkage parameters (angles, bonds lengths and charges) will be added to CHARMM27 force field in order to be used at the MD level.

4.2. Molecular Dynamics Characterization

As shown previously, the structure of the elastic serial link consists of two domains of globular proteins. The titin protein (I27) is referenced as 1TIT in the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/) as entry code “1TIT”. The domains were solvated in a water box of 70 Å length. First, the system was minimized for 2,000 conjugate gradient steps. Following the minimization, the system was heated from 0 to 300 K in 10 ps and was coupled to a 300 K heat bath for an additional 10 ps. The temperature control was released, and the whole system was subsequently equilibrated for 1 ns. Finally, SMD simulations (see Figure 6) were carried out by fixing the C-atom of the N-terminus of I27 and applying external forces to the C-atom of the C-terminus. The forces were directed along the vector from the pulled atom to the fixed atom. A constant velocity protocol is used for the SMD simulations with a pulling speed of 0.5 Å ps⁻¹. In the latter case the pulling atom is harmonically constrained with a force $F = -k(x-\nu t)$, where $k$ is the spring constant, $x$ is the coordinate of the pulling atom, $\nu$ is the velocity of the atom, and $t$ is the time. The value of the spring constant $k$ was set to 500 pN nm⁻¹.

Figure 7 shows the typical force-extension curve by stretching two immunoglobulin-like proteins. In comparison to the previous SMD simulations of titin unfolding made by the authors in (Lu and Schulten 2000; Gao and Wilmanns 2002), our results differ from different viewpoints. First, we performed SMD simulation of titin repeats unfolding with optimized energy linkers through PM3 semi-empirical models.
Then, stretching forces applied to the parallel nanorobotic structure are in direct correspondence with those experienced in real biomolecular actin-kinesin actuation at the origin of sarcomere passive stretching/relaxation. Finally, we attempted to investigate whether pH changes and/or variable temperature might affect the region’s stiffness. Therefore, we determined titin-based stiffness from the force stretching under a variety of pH and temperature conditions.

4.3. Combined Molecular Dynamics and Continuum Approach

The main goal of MD studies is to understand the relation between molecular behavior and nanorobot response. MD computing time may take a very long time, thus evaluation of the nanorobotic platform design cannot be achieved through real-time simulation. A combination of MD and CM approaches is investigated. An analysis of Figure 7 shows that the initial part of the force-extension curve is fitted with the worm-like chain (WLC) model to obtain the entropic spring of “1TIT” serial module. After unfolding, the second module unfolds and can be also be fitted by a WLC model (Trombitas et al. 1998). The extension of “1TIT” serial domains can be represented by an elastic spring. Based on these comments, Figure 8 suggests a nanomechanical portrait model: its behavior during extension might be modeled as series of elastic springs with a viscous element corresponding to the unfolding of the individual titin domain. The number of force peaks arising in the profiles is equal to the number of 1TIT domains involved in the stretched protein. As shown in Figure 7, titin generates a restoring force based on the mechanism of entropic elasticity. The elasticity of entropic components allows titin to be extended fully reversibly at physiological forces, without the need to unfold the “1TIT” domains. The elastic component arises from the extension of the serially linked “1TIT” domain chain. The continuum nanomechanical model can be described as follows.

4.3.1. Force–Stretching Modeling

In CM, the stretching force with respect to the elongation $F(z)$ is given by the following relationship:

$$F(z) = \begin{cases} f_{\text{WLC}}(z) - f_1(z) & \text{if } z < L, \\ f_{\text{ENG}}(z) + f_2(z) & \text{if } z \geq L, \end{cases} \quad (1)$$

where $f_{\text{WLC}}(z)$ and $f_{\text{ENG}}(z)$ denote the entropic elasticity and tensile force. The boundary conditions are as follows:

$$f_1(z) = 0 \quad \text{for } z \to 0, \quad f_2(z) = 0 \quad \text{for } z \gg L, \quad (2)$$

$$f_1(z) \to \infty \quad \text{for } z \to 0, \quad (3)$$

$$f_2 \text{ reaches a finite value for } z \to L. \quad (4)$$
Table 1. Performance comparison.

<table>
<thead>
<tr>
<th>Simulation</th>
<th>Number of atoms</th>
<th>Simulation time (s)</th>
<th>Computational time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QM-MD</td>
<td>9,984</td>
<td>$9.984 \times 10^{-9}$</td>
<td>$52.56 \times 10^3$</td>
</tr>
<tr>
<td>QM-MD-CM</td>
<td>1,718</td>
<td>$1.718 \times 10^{-12}$</td>
<td>$58.052 \times 10^{-9}$</td>
</tr>
</tbody>
</table>

$$f(t) = f_0 + A_1 \times e^{-(t-t_0)/t_1} + A_2 \times e^{-(t-t_0)/t_2} + A_3 \times e^{-(t-t_0)/t_3},$$

where $f_0$ and $t_0$ are offset and center, respectively, $A_1$ through $A_3$ are decay amplitudes, and $t_1$ through $t_3$ are decay time constants. Curve fitting was done by using a non-linear least-squares method.

Based on these CM models, the tandem-Ig proteins are modeled by WLC models in MD without changing the graphite monolayer model (Figure 11). The WLC model is implemented as a TCL script, and communicates in realtime with NAMD program during simulations. In order to compare the performances of QM–MD and QM–MD–CM, we performed several calculations (see Table 1). It can be noticed that for acceptable results of simulation (Figure 10), the QM–MD–CM reduces the complexity of nanorobotic structures (decrease of number of atoms) to be simulated for smaller computational time (around few picoseconds) and smaller simulation times (of the order of a nanosecond).

4.3.3. Modulating the Spring Stiffness of Biological Nanomechanisms

We now consider the modulation of the strength of the molecular nanomechanism when variations of the medium environment are controlled through two parameters, i.e. (i) the temperature and (ii) the level of acidic pOH of medium environment. The strong sensitivity of the force–extension characteristics of the titin molecule to temperature plays an important role in the control of the protein stiffness. Indeed, the overstretching portions of the force–extension curves as a function of temperature decrease greatly as the temperature is increased in a narrow range of temperatures. As an example, Figure 12(a) shows the evolution of the Ig-tandem spring constant $K_{\text{stretch}}$ with respect to the temperature ranging from 273 to 550 K. In theory, the results show clearly a strong variation of spring constant with respect to the temperature. In practice, for such high temperatures the tandem-Ig becomes completely denatured and reversibility is altered by the strong hysteresis as shown by Collinsworth et al. (2002). In a realistic way, the adjustable stiffness can be adapted between 100 and 180 N m$^{-1}$.
Practically, the tandem-Ig repeat nanomechanism is able to perform repeatable motion controlled by variation of pH by adding protons (termed protonation). It is proposed to isolate this domain from the titin and trigger the stiffness change by varying the pH (lowering the melting point of denaturation). Figure 12(b) shows the change of the protein stiffness with respect to the pH 5.5 value. For a neutral acidic pH value, the titin protein is in a partial β-sheet configuration. An increase occurs at higher pH values, between 5.5 and 10, leading to a stiffness variation ranging from 182 to 175 N m\(^{-1}\). For this configuration, it is necessary to protonate the amino acid side chain of the protein by adding protons inside the native environment. These values are in good agreement with those that have been reported previously by (Linke et al. 1998). The authors showed that as pH is raised between 6.0 and 10, the shape of the force–extension curves changes very little but the value of the overstretching forces decreases greatly explaining the linear decrease of titin stiffness in Figure 12(b). At low pH levels ranging from 2 to 5.5, the simulations showed that the force–extension curves could be reproduced without any hysteresis indicating that the changes to titin molecule at low pH are fully reversible. These results are in good agreement with experiments carried out in (Linke et al. 2002).

4.4. Comparison with Experiments from the Literature

The experimental investigation of the molecular sarcomere elasticity mechanism driven by myosin–actin microfilaments is a challenging research issue. Most of the information about single-molecule mechanics of motor proteins arises from the \textit{in vitro} gliding and bead assays during which the proteins are in relation to the temperature control with a minimum at 300 K corresponding to nominal temperature conditions.
subjected to mechanical forces. These methods allow for the manipulation of single molecules, to observe single events in a chemical reaction such as ATP hydrolysis, to measure mechanic parameters during stretching and force generated by single molecules (Howard 2001). These techniques do not allow one to find out how different types of deformation are correlated with protein structure. Moreover the experimental techniques usually employed for motor protein characterization have one limitation consisting of the direct observation occurring during mechanical tests. Owing to the small-scale structure of the proteins it is difficult to anchor them, assemble them, and control their behavior during the pulling experiments (Bao 2002). A quantitative analysis of this is beyond the scope of the present work but the magnitude of the forces required to stretch titin repeats can be experimentally and theoretically estimated as a proof of concept. Micromechanical studies on isolated single titin molecules recently have demonstrated that the Ig domains and PEVK segment can unfold at high forces and thereby contribute to the extensibility of the sarcomere (Linke 2006). To understand how it is possible that titin’s distinct nanomechanism extends, we simulated the segmental extension with the multiscale QM–MD–CM methodology. Six double tandem Ig27 (see Figure 13) repeats composed of 154,476 atoms in a solute water medium (pH 5.5, \( T = 300 \) K) have been thermalized and solvated with the TIP3P model for water to apply stretching forces. During the first simulation steps, the poly-Ig chains lengthen mainly within a low sarcomere length (SL) range where passive force is very small. The first stretch would involve straightening out the tandem-Ig, disrupting the interfaces between adjacent domains, which are relatively small areas of contact, equivalent to weak protein–protein interaction. The second step of stretching simulation involves unravelling Ig27 Ig domains. The tensile force on titin operates on the N- and C-termini of all domains. A typical experimental force–extension curve of Ig27 is shown in Figure 14(a) through AFM force spectroscopy obtained from the data provided by Trombitas et al. (1998). A single molecule is identified by the correct spacing between force peaks (the contour-length increase, \( \Delta L \), measured by the WLC model, is 28.1 nm for one unfolding event) and the unfolding force magnitude of \( \sim 200 \) pN. The initial part of the force–extension curve is fitted with the WLC model to obtain the entropic spring parameters. The simulated results us-

![Fig. 12. Evolution of the spring stiffness of the Titin protein during stretching for changing medium properties: (a) temperature and (b) level of acidic pH.](image-url)

![Fig. 13. Molecular structure sarcomere elasticity nanomechanism: two extensible tandem-Ig repeats constituted by six Ig proteins in series with PEVK stiff protein form a closed-loop kinematic chain.](image-url)
Fig. 14. (a) AFM force spectroscopy of a heteropolyprotein containing six I27 Ig domains: representative force–extension curve obtained by stretching a single-molecule containing I27 Ig domains (reproduced with permission from Trombitas et al. (1998)). (b) Simulation results of a heteropolyprotein containing six I27 Ig domains.

The average contour length of a fully extended tandem-I27 Ig repeats fits the real values $\Delta L = 31.6$ nm. However, the mismatch between the real and simulated peak force values are due to the pulling speeds adopted in the simulations that were six to eight orders of magnitude higher than those in the experiments ($v = 0.01$ Å ps$^{-1}$).

Another typical feature of viscoelasticity is force hysteresis, usually observable as higher force during stretch than during release. We can compare this with the stretching–relaxation curve for both types of simulations, i.e. QM–MD simulation (Figure 15) and QM–MD–CM (Figure 16) for small extensions. The simulation results show a good reversibility of the extensible protein-based nanomechanism when relaxed completely. The error tends to be reduced (less than 6%) for small stretching deformations of the protein-based nanosprings. At high extensions, the QM–MD–CM modeling is revealed to be effective in predicting the force hysteresis of the sarcomere mechanism with an error of less than 12%. These simulation results are in good agreement with those experimented by the authors in Minajeva et al. (2001) for small deformations. We considered other complex systems with various kinds of forces to testify the performance and complement the approach. Similar results have been obtained on different reversible protein-based nanomechanisms such as four $\alpha$-helix ROP protein as a parallel spring nanomechanism and a tandem of four double-strand DNA proteins (Hamdi et al. 2006). Furthermore, superelastic series structures with high tensile strength have been experienced, i.e. a pair of double $\alpha$-helix tropomyosin filaments and sandwich-like $\beta$-sheet silk proteins (Ferreira and Mavroidis 2006). This allowed us to predict the type of force spectra, reversibility, hysteresis, viscoelasticity and irreversible work that may be expected from molecular nanomechanisms. However, the authors although validate the one-dimensional molecular link, did not validate the parallel platform for various directional SMD forces. It implies the introduction of additional SMD simulations, i.e. shearing, bending and compression tests, before to propose a complete molecular nanomechanical model.
5. Conclusion

Development of nanoscale components from biological systems is the first step towards the design and development of an advanced bio-nanorobot. A design based on a multiscale coupled with a virtual reality advanced technique approach is very promising in the domain of bio-nanorobotics. We have demonstrated that realistic design and simulations can be carried out by integrating the physics at several scales (atomic, nanometer, molecular and mesoscopic) for various timescales of simulation (from a few nanoseconds to several milliseconds). The results show that a reduction of $10^6$ seconds in computation time with the very short timescale length of simulation of $10^{-12}$ seconds can be obtained using multiscale modeling (MD and CM) approaches. While a more careful evaluation of the methodology is ongoing and will be continued in the future, the purpose of this paper has been to demonstrate the capabilities of the design and simulation tool set. The authors believe that this work is a huge proposition and that only an example among many potential possibilities is described to explain the concept. Further work with continuous upgrades will be required as the field of research progresses, notably to perform a faster development and control of nanodevices based on different nanomaterial structures: graphite monolayers or multilayers, electronic materials, proteins or lipids.

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