Prototyping Bio-Nanorobots using Molecular Dynamics Simulation and Virtual Reality

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This paper presents a molecular mechanics study using a molecular dynamics software (NAMD) coupled to virtual reality (VR) techniques for intuitive bio-nanorobotic prototyping. Using simulated bio-nano environments in VR, the operator can design and characterize through physical simulation and 3-D visualization the behavior of protein-based components and structures. The main novelty of the proposed simulations is based on the characterization of stiffness performances of passive joints-based proteins (\(\alpha\)-helix deca-alanine, repressor of primer protein and immunoglobulin protein) and active joints-based viral protein motor (VPL) in their native environment. Their use as elementary bio-nanorobotic components are also simulated and the results discussed.

1. INTRODUCTION

Recent advances in understanding how biomolecular motors work has raised the possibility that they might find applications as protein-based nanomachines. For example, they could be used as molecule-sized robots that are able to apply forces and manipulate objects in the nanoscale world. Thus biomolecular motors could form the basis of bottom-up approaches for constructing active structuring and maintenance at the nanometer scale. Protein is the most versatile of the natural bionanomachines. As example, the most familiar motor is the protein myosin \cite{1} or dynein \cite{2} which moves along filaments, formed through the protein actin, to drive the contraction of muscles. In addition, there are compliance devices such as spring-like proteins called fibronectin \cite{3} and vorticellids \cite{4}, as well as synthetic contractile plant polymers \cite{5} which can act as compliant joints in molecule size robotic systems. The idea is to use biomolecular motors as the actuators of such bio-nanorobots, where the structural elements are carbon nanotubes, while the joints are formed by appropriately designed biological spring elements \cite{6}.

To achieve these long-term goals, prototyping tools based on molecular dynamics (MD) simulators should be developed in order to understand the molecular mechanics of proteins and develop dynamic and kinematic models to study their performances and control aspects. The ability to visualize the atom-to-atom interaction in real-time and see the results in a fully immersive 3-D environment is an additional feature of such simulations \cite{7},\cite{8},\cite{9}. Virtual Reality (VR) technology is applied here, which not only provides immersive visualization but also gives an added functionality of CAD-based design, simulation, navigation and interactive manipulation of molecular graphical objects. Using virtual biological environments, the operator can design and characterize through physical simulation the behavior of bio-nanorobots. Adding haptic interaction, the operator can explore and prevent the problems of molecular robots in their native environment. Based on VR technology and MD simu-
lators, our long-term goal is to prototype virtually bio-nanorobotic systems and control their movements in their biological environment [10]. In this work, we consider real-time force-feedback technology for improving the methodology of studies of folding and unfolding proteins acting as passive or active joints in molecule-size robots. We therefore decided to begin our investigations by simulating the forces involved under various external mechanical stress (stretching, contraction, shearing, bending) to predict the type of force spectra and irreversible work that may be expected from single-molecule protein manipulation experiments.

These recent years, many mechanical proteins composed of multiple, individually folded protein domains have been studied using steered molecular dynamics (SMD)[11]. The available SMD tool has provided the basis for a cascade of studies on the unique role of proteins as passive springs providing restoring force either in shortened or stretched configurations: SMD simulates force-induced unfolding by applying force to the two termini of a protein module. Many SMD studies have been reported on the elasticity and structural properties of specific proteins, such as: giant muscle protein titin I1-I27 [12]-[15], extra cellular matrix protein fibronectin FN-III [16]-[18] or tropocollagen molecule [19]. Understanding the molecular mechanics behavior and control parameters of such proteins is an important challenge for interfacing protein nanostructures to structural elements [20] such as single walled carbon nanotubes (CNTs) [21] or highly conductive nanowires [22].

The paper is organized as follows: Section 2 presents the architecture of virtual molecular dynamics simulator using haptics and virtual reality. Section 3 presents the assembly of a protein-based robotic structure. Then, simulations of different active/passive proteins are analyzed in section 4.

2. MOLECULAR DYNAMICS SIMULATION USING HAPTICS AND VIRTUAL REALITY

2.1. CAD-design methodology using computational steering

Considering the above mentioned research issues, we decided to adopt VR technologies coupled to computational molecular dynamics and quantum molecular dynamics to simulate, analyze, model, and visualizes the complex architecture and interactions of bio-nanorobotic components. Real-time exploration is what attracts researchers to develop man-machine interfaces for nanoscale manipulation that use haptic display technologies. The developed simulation system presented in (Fig.1) permits manipulation, connection and assembly of bio-nanorobotic components in molecular dynamic simulations with real-time VR devices such as stereo glasses, 3D trackers, force-feedback devices and 3D graphical display.

The adopted CAD design methodology is based on computational steering techniques where the user is able to design or modify a simulation interactively in a virtual environment during runtime. It gives a tremendous advantage over post-simulation visualization and analysis of results. In run-time steering the user does not have to wait till the end of the simulation to see the results of his modifications, instead he can immediately see the result of the interactively changed parameters giving him an opportunity to detect and modify them to steer the simulation to a desired output. In computational steering the user can steer molecular dynamics simulation by applying external forces into the computations. These external forces can help a complex molecular nanorobotic system overcome a potential energy barrier and can even steer the system to a new geometric conformation for further analysis. The specific tasks are:

1. To connect and assemble interactively the molecular components of the bio-nanorobotic structure through a data glove (Ascension Technologies CyberGlove18 interface) which can be displayed in the virtual environment as a mapping of hand.
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It is composed of fully enclosed fiber optic bend sensors that provides up to 22 high-accuracy joint-angle measurements allowing to capture all assembly hand postures.

2. To virtually steer the protein molecules using a haptic device (SensAble Technologies PHANToM Desktop interface). The haptic device is virtually bound to an atom of a conformationally stable subset of the molecule. This system would allow applying stretching, shearing and bending mechanical constraints on the protein.

3. To distinguish possible conformational paths, feel the energy barriers associated with each path and choose the optimal path/strategy.

4. To control the interaction mechanism in a solvent. Protein folding and unfolding can be controlled by assessing the influence of pH value, temperature, electron density or salt concentration.

2.2. Software architecture

The software architecture is composed of three software modules: (i) a haptic device module controlled by a computer that generates the force environment, (ii) an interactive molecular environment (IME) module for manipulation, attachment and assembly of nanocomponents in 3-D virtual world and (iii) a molecular dynamics (MD) module for determining the effects of force application.

As shown in Figure 1, steering forces are issued from the the haptic device which 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 for prototype bio-nanorobotic prototyping. The haptics module sends command data (position, velocity, force) and receives force feedback signals from the IME module.

The main role of the interactive molecular environment module (IME) module is to gather the nanorobotic components (proteins, DNA, CNT, graphite) in a one 3-D representative space called visual molecular dynamics (VMD) [23]. VMD software is a molecular visualization program for displaying, animating and analyzing large biomolecular systems using 3-D graphics and built-in scripting. The VRPN software package [24] was used to connect VMD to the PHANToM Desktop device. The Data Glove input device is also supported by VRPN. The user manipulates in an intuitive way the attachment and assembly of the different molecular components within VMD environment. As the linkers and/or connectors between multi-domain proteins are not always clear during complex assemblies, Pdbswiss software allows the analysis of structural protein and/or nanostructure misalignments (rotation, translation, orientation). When the 3-D structures are aligned, the molecular attachment between the different components is achieved via VegaZZ software [25]. Finally, the psfgen tools generate a molecular structure file (.psf or .pdb format) associated to the CAD-designed bio-nanorobotic
structure: initial coordinates, force field parameters, structure solvation and ionization.

These data are sent to the molecular dynamics (MD) module through a communication protocol named interactive molecular dynamics (IMD) [26]. The IMD interface consists of a small set of C-callable functions which can be adapted to any molecular dynamics and/or visualization program. Periodically, VMD checks for a coordinate set from the MD program, updates the representation geometry if a new coordinate set was received, redraws the screen, and updates the restraint position of the haptic device. If the user has applied a force through the haptic device, VMD routes this force to NAMD2, which then integrates the force into the equations of motion for the molecule. Updates to the haptic restraint point are made when VMD receives a new coordinate set from NAMD2 software; while awaiting an update, the haptic server applies smooth feedback forces based on the most recent restraint point position.

The main program of the computational is NAMD2 [27], a fast, scalable, program which implements the popular CHARMM27 force field [28] for molecular dynamics. The absolute speed of NAMD2 is an essential ingredient of a responsive interactive system. It runs on a parallel cluster composed of 10 Pentium IV-4.1 GHz personal computers. NAMD's scalability enables us to use the computational module to study large bio-nanorobotic structures as well as small ones, given enough computational power. These computational resources allow the simulation of systems containing several thousand atoms at a speed sufficient for interaction (~400 Hz).

3. DESIGN OF A BIO-NANOROBOTIC PARALLEL PLATFORM

Computational steering of MD simulation with the aid of VR visualization can help exploration of new models and their structural behavior, and also to study by simulations the elastic/mechanical behavior of bionanotechnology-relevant molecular nanorobots. As example, we simulated a single degree-of-freedom parallel bionanorobotic platform composed of four passive parallel springs composed of proteins (Fig.2). Each spring link is composed of several α-helical proteins connected in series such as (a) α-helical deca-alanine protein, repressor of primer protein (ROP) or titin fibers. Three linear biomotors are inserted in the center of the platform (Viral Protein Linear actuator [29]) to join two graphite platforms. The four α-helix elastic protein links are acting as spring elements or as restitution forces that will bring the platform back to its original position (Fig.2)(b). The attachment of the different components are defined by 4 carbon atoms bounds (-COOH-) at the protein/graphite interface and 3 carbon atoms bounds(C-C) at the protein/protein interface. The overall dimensions of the rectangular graphite sheet are 14.8 nm × 8.5 nm and the total length of the three proteins is 13.1 nm.

4. ASSEMBLY OF PROTEIN SPRINGS AS PASSIVE JOINTS

In this section, the simulations of 3-D structures of biological proteins and nucleic acids have been found on the Protein Data Bank [30]. In order to study the mechanical characteristics of protein-based elastic springs, we applied the techniques described above to three exemplary elastic proteins: α-helix deca-alanine protein, repressor of primer protein and immunoglobulin-like proteins.

4.1. Methodology of simulation

All simulations were carried out at a temperature of 300 K (through Langevin damping with a coefficient of 5 ps⁻¹) with temperature rescaling performed every 10 timesteps and a pressure of 1 atm (using the Langevin piston method). In more detail, the protocol is as follows:

1. **Energy minimization:** The first step is the energy minimization of the biomolecular structure in order to remove any strong van der Waals interactions that may exist which might otherwise lead to unstable simulations. At this point, all proteins were solvated by a periodic box of dimensions covering the module by at least five layers of water molecules with the TIP3P model [31].
The water box dimensions were adjusted with respect to each protein structure. Non explicit medium representation, characterized by the dielectric constant $\varepsilon$ was considered. The aqueous medium was represented by $\varepsilon = 78$, as proposed by J. Israelachvili [11] for water at 27 degree Celsius (300 K). The entire box of water is overlayed onto the protein and those water molecules that overlap the protein are removed. Energy re-minimization is realized by means of the steepest-descent algorithm with a convergence of $10^{-5}$ kcal/mol.

2. Heating up process: Initial velocities at a low temperature are assigned to each atom of the system and Newton’s equations of motion are integrated to propagate the system in time. During the heating phase, initial velocities are assigned at a low temperature and the simulation is started periodically, new velocities are assigned at a slightly higher temperature and the simulation is allowed to continue. The entire system gradually was heated up to 300 K in increments of 30 K for time intervals of 1 ps, while leaving the box volume unchanged.

3. Thermalization: When the desired temperature is reached, the equilibration procedure consists to run the simulation until that the structure parameters, i.e., pressure, temperature and energy, become stable with respect to time. During equilibration at a temperature of $T=300$ K, the water molecules composing the box were harmonically restrained to their original positions to maintain the shape of the water bubble. An equilibrium simulation at least 1 ns in length was performed for each protein.

4. Steered Molecular Dynamics: The final simulation step was to run Steered Molecular Dynamics (SMD) simulations [11]. Various deformations properties of mechanical proteins can be simulated when subjected to external stretching. First, (i) in longitudinal stretching (Fig.3(b)), we fix one end of the molecule (the C-atom of the N-terminus) at the origin and constrain the other end (the capping nitrogen atom of the C-terminus) to move only along the longitudinal axis, thereby removing the irrelevant degrees of freedom, i.e., removing the overall translation and rotation. Then, (ii) in lateral bending (Fig.3(c)), the C- and
N-terminals are fixed and the α carbon at the center is harmonically pulled in lateral direction. Finally, (iii) in lateral shearing (Fig.3(d)), the C-terminal is held fixed and the N-terminal is harmonically pulled in lateral direction. The moving guiding potential:

\[ u(r, \lambda) = \frac{k}{2} [\xi(r) - \lambda]^2 \]  

(1)
is added to control the end-to-end distance \( x \) which is a function of the 3N-dimensional position \( r \) of the system. The moving guiding potential used in the pulling simulations is represented by a spring which is connected to the C-terminus and pulled with a constant velocity \( v \). For each protein, the values of the spring constant \( k \) and stretching velocity \( v \) were chosen to correspond pulling with a stiff spring in the drift regime.

4.2. α-helix deca-alanine protein

A canonical right-handed α-helix was built using oligopeptide composed of 10 alanine residues in order to study helix-to-coil transition of α-helix deca-alanine in vacuum and in solvent. In vacuum at room temperature (\( T=300 \) K), the stable configuration of deca-alanine is a α-helix. After energy minimization, deca-alanine protein was solvated in a water box of 50 Å length. The system was minimized for 1000 conjugate gradient steps, then the system was minimized and heated from 0 K to 300 K in 6 ps, the system was subsequently equilibrated for 1 ns. Stretching the molecule by an external force can induce its transition to an extended (coiled) form. This helix-to-coil transition represents a simple but basic folding system acting as a molecular spring. The parameter \( \lambda \) is varied from 15 to 35 Å with a constant pulling speed \( v \) of 0.1 Å/ps. A force constant of \( k=500 \) pN/Å is used in order to allow the end-to-end distance \( x \) closely follow the constraint center \( \lambda \). The external work curve is defined as [32]:

\[ \langle W \rangle = -k \nu \int_0^t dt' f(t') \]  

(2)

Fig.4 shows the maximal force (before breaking) as a function of stretching distance. Depending on the sign of \( v \), the external work can be defined for either stretching or contracting motion of the protein. Fig.4(a) shows two distinct conformational transitions that provoke the conversion of α-helix to an extended form approaching the coil conformation.

The force increases rather smoothly to almost 10 pN up to the length of the protein. Once this transition is completed, the hydrogen bonds start to break and the structure further extends toward the coil conformation (intrinsic elastic regime), as shown in Part II, of the force curve (\( \sim 700 \) pN). At the lowest forces, the molecule behaves as a Hookian spring and its extension is proportional to the force applied at the end with a reversible
motion. A useful approximation for spring constant $k_{\text{stretch}}$ is given by the inextensible worm-like chain (WLC) model [33]. The WLC model of entropic elasticity predicts the relationship between the relative extension of a polymer ($z/L$) and the entropic restoring force ($f$) through

$$f = \left( \frac{k_B T}{A} \right) \left[ \frac{z}{L} + \frac{1}{4(1 - \frac{z}{L})^2} - \frac{1}{4} \right] \quad (3)$$

where $k_B$ is the Boltzmann constant, $T$ is absolute temperature, $A$ is the persistence length, $z$ is the end-to-end length, and $L$ is the length. Then, if the stretching force increases gradually until it reaches its stretching limit ($\sim$3600 pN) with an irreversible motion in Part III. Fig.4(b) shows the nonlinear reversibility of the protein when relaxed. It shows clearly that the elastic behavior of the protein can be used as a passive control element. The previous results showed that the native structure is not destroyed under normal physiological conditions. In some unnatural conformations, lateral shearing and bending forces applied on a protein molecule has been simulated (see Fig.3). These tests simulate disturbances of the bio-nanorobotic component under various operating conditions. For these conformations, Fig.5 presents the lateral forces obtained. As shown in Fig.5(a), the lateral Hookian spring $k_{\text{shear}}$ has high stiff spring value that is able to counteract microenvironment variations and mechanical disturbances of a bio-nanorobotic platform. In this case, in contrast to stretching described above, the force variation is roughly monotonic with different plateaus leading to different Hookian spring values. The extension of the Hookian spring is proportional to the force applied at its end such as:

$$f = \left( \frac{k_B T}{A} \right) \left( \frac{x}{L} \right) \quad (4)$$

This is explained by a successive rather than a concurrent rupture of the hydrogen bonds joining the strands. Shearing is largely limited to breaking hydrogen bonds as there is little conformational change in the extended peptide backbones. Conversely, Fig.5(b) shows a linear variation of force behavior when considering pure bending deformation of the protein. It shows that for little length deformation, it requires low constant force. After a threshold value, lateral bending must break the van der Waals interactions of the entire surface, leading to high forces and strong length dependence. Finally, these results indicate that it may be possible to obtain uncoupled mechanical spring behavior of the protein: stiff in lateral and compliant in longitudinal direction.

We applied stretching forces at the upper graphite platform. Simulations showed that the

![Figure 4. α-helix to coil stretching. Force curve and reversibility done by forward pulling (stretching) and backward pulling (contracting) with $v=1 \text{Å/ps}$. For the forward pulling, the position of the constraint center $x$ is varied from 15 to 35 Å; for the backward pulling, from 35 to 15 Å.](image-url)
parallel connection of serially-linked deca-alanine proteins permits to augment considerably the resulting spring-force and to decrease the overall displacement. As example, Fig.6 shows the stretching force curve of two parallel links composed of four serial spring-like proteins (deca-alanine) for $v=1$ Å/ns, $k=500$ pN/Å and $T=300$ K in a water box. We connected two adjacent repeats by using rigid non-helical linkers composed of 3 carbon atom bounds (C-C).

The spring force is considerably increased symmetrically for both parallel links until to reach its point of rupture. As expected during stretching, the generated forces in parallel configuration is multiplied by 2 while in serial configuration the displacement is multiplied roughly by 4. The elongation of the serial configuration is mainly dominated by unfolding the α-helices of the different deca-alanine proteins. However, the rupture point is not due to the rupture of the protein but simply by the rupture of the linker between the proteins (1,1') and (2,2') as shown in Fig.6(b). It should be noted that the force behavior of each parallel link is quite similar.
Such serial multi-repeat deca-alanine proteins can be compared to spectrin, dystrophin, α-actinin, and related proteins sharing similar structures (called a spectrin repeat or domain). They share three antiparallel α-helices linked by long helices (∼8–9 nm) that extends from one domain to the next [34]. Forced unfolding in SMD has been reported by [35]. In comparison to Fig. 6, the force-extension data shows similar linear forces at the initial stage of end-to-end extension of a spectrin tandem repeat. At a more advanced stage, the extension of previously unfolded domains appears to fit a worm-like chain model with a sawtooth profile. Furthermore, the range of linear force-extension data is lower than 2–3 orders of magnitude. Unfortunately, the works reported in [34], [35] did not demonstrate reversible elasticity of spectrin multi-repeat. In [36], the authors used SMD to unfold stacks of 12-24 ankyrin repeats, each repeat has two short α-helices and a less order loop, with the helix pairs stacked in parallel in adjacent repeats. The constant-force SMD simulations demonstrate clearly that elongation under stretching require several hundred piconewtons to unfold. Refolding is partially reversible in a surprisingly short time (∼5 ns) but the end-to-end distance does not decrease to its initial value. As far we know, this is one of the first studies where a reversible α-helical protein over a long range end-to-end extension (∼355 Å) has been shown through molecular dynamics simulation. All the presented studies used SMD simulation to characterize forced stretching unfolding without consideration of other mechanical constraints. As a result of our SMD simulations (stretching, bending and shearing) the different stiffness differs strongly depending of the external disturbances applied to the nanorobotic platform: (i) stretching stiffness is strongly influenced by the native environment $k_{\text{stretch}} = 400$ pN/nm in air and $k_{\text{stretch}} = 70$ pN/nm in water; (ii) lateral shearing $k_{\text{shear}} = 700$ pN/nm is much more stiffer than lateral bending $k_{\text{bend}} = 30$ pN/nm which ensures a good mechanical stability of the platform against external disturbances.

4.3. Repressor of primer protein

In order to reduce the number of components, the serial configuration of multi-repeats deca-alanine proteins have been replaced by repressor of primer (ROP) proteins (PDB; entry code 1ROP). The repressor of primer protein structure is a small, dimeric molecule consisting of two identical chains of 63 amino acids. The two monomers pack together as a fully antiparallel four helix-bundle. The ROP protein was solvated in a water box of 50 Å length. The system was minimized for 1000 conjugate gradient steps, then the system was minimized and heated from 0 K to 300 K in 6 ps. Finally, the ROP protein was subsequently equilibrated for 1 ns. The bend region of ROP has attracted considerable interest as a parallel molecular spring due to its stability and elasticity properties [37]. The SMD simulations were carried out by fixing C atom of both N-terminus of molecule (short turn) and applying external forces to C atom of both C-terminus along the $z$ axis (see Fig. 7). Constant velocity protocol is used for the SMD simulations with pulling speed 1 Å/ps. The value of $k$ was settled to $k = 500$ pN/Å.

Fig. 7(a) shows two distinct conformational transitions that provoke the conversion of double α-helix to an extended form approaching the coil conformation. Both α-helix extensions are very similar and can not be distinguished in Fig. 7(a). Obviously, the single α-helix behaves as the α-helix deca-alanine structure of Fig. 4(a) with very high values of the restoring forces in the intrinsic linear regime (∼1000 pN). On contrary to deca-alanine structure, the double α-helix structure is naturally fully reversible in a wide domain of extension (see Fig. 7(b)) without the need of an external pulling force. While single α-helix deca-alanine respond with little change in end-to-end distance to moderate forces and requires several hundred piconewtons to unfold, the parallel elongation found in ROP protein seems to be more suitable for a mechanical function as a parallel biological spring. The elastic response of ROP protein in the first stage of elongation in well reproduced by simple mechanical model of two entropic parallel springs. The other lateral shearing and bending stiffness are $k_{\text{shear}} = 240$
4.4. Immunoglobulin-like domain

β-sandwich structures are found in a variety of mechanical proteins composed of multiple, individually folded protein domains. The forced folding and unfolding of proteins using SMD simulations have been mostly reported for two important examples: the immunoglobulin (Ig)-type fold [12],[15]-[16] and the fibronectin-type fold (the most common of which is fibronectin type 3 or FN-III) [17],[18]. Force-induced extension of the protein titin, for example, which is responsible for the passive elasticity of muscle, can cause its constituent Ig and FN-III domains to unravel. Currently, I27 is the only I-band Ig with an experimentally solved structure and hence has been selected for investigation (PDB; entry code 1TIT). The Titin I27 domain was placed in the center of the water box of 70 Å length and equilibrated with a thermal bath of $T=300$ K. First, the system was minimized for 2000 conjugate gradient steps. Following the minimization, the system was heated from 0 K to 300 K in 10 ps and was coupled to a 300 K heat bath for additional 10 ps. The temperature control was released, and the whole system was subsequently equilibrated for 1 ns. The SMD simulations 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 simulation began with an equi-
liberated folded structure and was stopped when a fully extended polypeptide was obtained. The extension of I27 was performed with a pulling speed $v=0.5$ Å/ns and was stopped when the extension reached 33 nm. The extension domain (Fig.8(a)) is divided into four sections: I. preburst at extension of 4 nm during which the protein maintains $\beta$-sheet structure and the external force remains smaller than 1500 pN; II. major burst immediately after the pre-burst at extension of 8 nm; III. post-burst at extension of 27 nm during which the protein unravels; IV. pulling of fully extended chain up-to an extension of 33 nm. The simulated force extension profile is in accordance with [12],[40].

Other simulations showed similar features of the unfolding process and force profiles with only small variations in force peak value and degree of extension at the force peak. Results of stretching-relaxation curve (Fig.8(b)) show a good reversibility of the protein motion when completely relaxed. Its behavior during extension might be modeled as series of elastic springs with a viscous element corresponding to the unfolding of the individual I27 domain. Stretch would result first in straightening of the Ig domain chain (corresponding to the pre-burst-part I) as an entropic spring. The tightly folded Ig domain might function as a "shock absorber" (parts II: major burst and III: post-burst) by reversible unfolding only in the case of extremely high stretching forces. This structure allows avoiding the complete rupture of the protein due to overstretching.

As example of serial Ig multi-repeat, Fig.9 shows the typical force-extension curve by stretching two immunoglobulin-like proteins (I1 and I27-Ig modules). The simulation parameters are $v=10$ Å/ns, $T=300$ K and a force constant $k=500$ pN/Å. The initial part of the force-extension curve is fitted with the WLC model to obtain the entropic spring of I27-Ig module. After the unfolding of the I27-Ig module, the second I1-Ig module unfolds and can be also fitted by a WLC model. The extension of (I27-I1) domains can be represented by an elastic spring. When considering several Ig modules connected in series, the force-distance curve behaves as a sequential unfolding of the Ig modules. The number of force peaks arising in the profiles is equal to the number of Ig domains involved in the stretched protein. Its behavior during extension might be modelled as series of elastic springs with different stiffness together with the viscous elements corresponding to the unfolding of individual domains.

These simulation results are in accordance with the mechanical properties of human myomesin domain experimented in [42]. Stretching of homomeric polyproteins, constructed of Ig and FN-III domains of human myomesin, produces a typical saw-tooth pattern in the force-extension curve which function as a passive reversible spring with adaptable elasticity.

As shown in (Fig.9), 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 Ig domains as shown in [41],[43]. The elastic component arises from the extension of the serially linked Ig domain chain. Subsequent re-extension of the protein repeat demonstrates a recovery of folded domains that is dependent on the time interval between consecutive extensions. In addition, lateral shearing and bending stiffness have been evaluated to $k_{\text{shear}} = 280 \text{ pN/nm}$ and $k_{\text{bend}} = 925 \text{ pN/nm}$, respectively.

![Figure 9. I27-I1 stretching simulations. Force-distance curve of molecular spring Ig modules connected in series.](image)

Figure 9. I27-I1 stretching simulations. Force-distance curve of molecular spring Ig modules connected in series.

5. PROTEINS AS BIOMOTORS

In this part, we study the molecular properties of viral proteins to change their 3D conformation depending on the pH level of environment. Thus, a new linear biomolecular actuator type called Viral Protein Linear motor (PDB; entry code 1HTM), developed originally in [29], is characterized as an active linear joint. The structure is like a hairpin composed of three coils, having one C terminal (carboxy-end) and undergoes a conformational change induced by mildly acidic conditions (i.e., pH around 5). With the change in pH, the N-terminals pop out of the inner side and the peptide acquires a straightened position. The VPL motor uses the Influenza virus protein Hemagglutinin (HA). Two known states are established, i.e., the native state and the fusogenic state of the 36-residue peptide of HA. The representative "open" structure can be generated by forcing the structure away from the native conformation (Fig.8a) to open state (conformation) (Fig.10) with constrained high-temperature molecular dynamics. Both the forward (closed to open) and the reverse (open to close) transformations are carried out for two end-point structures of 9 Å. This peptide is able to perform repeatable motion controlled by variation of pH. In order to study the mechanical limits of the reference end-point states with a reversible motion, simulations of the mechanical stretching through a guiding potential defined by Equ.(1) has been investigated.

![Figure 10. Loop 36 protein in the native state (left side). Open-state state during conformation of the VPL motor (right side) with van der Waals distribution force with constrained high-temperature molecular dynamics.](image)

The VPL motor was solvated in water box of 80 length. Simulations of VPL motor were carried out using the same protocol as Titin with
different conditions, e.g., with different value of constant force, $k$ was set to 694 pN/nm, and with 0.8 Å/ps pulling velocity. Here, we stretched the viral protein in an irreversible manner and examined the resulting distribution of force. The spring constant is settled at $k=500$ pN/nm and $T=300$ K. The molecule is stretched by changing the parameter $l$ from 15 to 40 Å with a constant speed. Figure 11(a) gives the results of displacement and force stretching ($k_{\text{stret}}=625$ pN/nm) of the VPL motor. (Fig.11(b-c)) shows the shearing ($k_{\text{shear}}=312$ pN/nm) and bending results ($k_{\text{bend}}=714$ pN/nm) when $v=1$ Å/ps, $k=500$ pN/nm and $T=300$ K. A more realistic environment of the VPL requires the inclusion of the effect of pH on the protein. In order to take into account the real-time effect of pH on the ionic stability of the protein, a current model will be considered in a next phase.

6. CONCLUSION

Our objective has been to interface molecular dynamics and kinematics computations with real-time truly VR simulations and measure the force, position and energy feedback for design evaluation of bio-nanorobots. The ability to interact with a computer-generated object in the same manner that a person would interact with a physical object - to investigate its structure by simply moving around it, to change its position by grabbing the object and moving the hand in space, without such artificial devices as computer mice. The preliminary mechanical force results given in this study corroborates the force results when stretching $\alpha$-helix, $\beta$-ribbon proteins. It will allow the roboticians to use mechanical force to control the dynamics, time evolution and fate of chemical and biochemical reactions when connecting in series or parallel different bionanorobotic components together. In the next step, we will integrate the experimental and computational process by making a peptide-AFM-Phantom-VMD-NAMD system.

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