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Computational Studies of a Protein-based Nanoactuator for Nanogripping Applications

Abstract

The design hypothesis, architectures, and computational modeling of a novel peptide-based nanoactuator are presented in this paper. We engineered the α-helical coiled-coil portion of the yeast transcriptional activator peptide called GCN4 to obtain an environmentally responsive nanoactuator. The dimeric coiled-coil peptide consists of two identical approximately 4.5 nm long and approximately 3 nm wide polypeptide chains. The actuation mechanism depends on the modification of electrostatic charges along the peptide by varying the pH of the solution resulting in the reversible movement of helices and, therefore, creating the motion of an actuator. Using molecular dynamics simulations we showed that pH changes led to a reversible opening of up to 1.5 nm which is approximately 150% of the initial separation of the nanoactuator. We also investigated the forces generated by the nanoactuator upon pH actuation, using a new method based on a modified steered molecular dynamics technique. Owing to its open and close motion resembling that of tweezers, the new nanoactuator can potentially be used as a nanogripper in various nanomanipulation tasks such as detection and removal of heavy metal ions during nanofabrication processes or as a molecular switch.

KEY WORDS—nanorobotics, bio-nanotechnology, protein engineering, molecular dynamics

1. Introduction

Nanotechnology can best be defined as a description of activities at the level of atoms and molecules that have applications in the real world. A nanometer is a billionth of a meter, that is, about 1/80,000 of the diameter of a human hair, or 10 times the diameter of a hydrogen atom. The size-related challenge is the ability to measure, manipulate, and assemble matter with features on the scale of 1–100 nm. In order to achieve cost-effectiveness in nanotechnology it will be necessary to automate molecular manufacturing. The engineering
of molecular products needs to be carried out by robotic devices, which have been termed as nanorobots. A nanorobot is essentially a controllable machine at the nanometer or molecular scale that is composed of nanoscale components (Freitas 1999). Nanorobots would constitute any passive or active structure capable of actuation, sensing, signaling, information processing, intelligence, or swarm behavior at the nanoscale. The field of nanorobotics studies the design, manufacturing, programming, and control of the nanoscale robots. Bionanorobots are nanorobots performing their tasks in a biological environment and some of their nanoelectromechanical components are made out of biological elements such as proteins and DNA (Ummat et al. 2005).

The concept of bionanorobots is thoroughly inspired by nature’s way of doing things at the nanoscale. Mother Nature has her own set of molecular machines that have been working for millennia, and have been optimized for performance and design over the ages. As our knowledge and understanding of these numerous machines continues to increase, we now see a possibility of using the natural machines, or creating synthetic machines from scratch, using nature’s components. Our approach is step-wise and initially envisions the use of various biomolecular elements (e.g. peptides, DNA strands, etc.) that are capable of generating motion, exerting force, or producing a signal as fundamental machine components. These components will be designed to perform different functions in response to specific physiochemical stimuli in a variety of native and non-native settings. In this manner, these individual biomolecular elements will serve as sensors, actuators, grippers, or modulating agents. By assembling different components together in the proper proportion and orientation, nanodevices with multiple degrees of freedom could be generated, able to apply forces and manipulate objects. Eventually, these devices will be evolved into robotic machines that are able to autonomously perform complex tasks. Nanorobotic devices will be a boon to the biomedical research, therapeutics, and diagnostics arenas, as these devices offer the promise to manipulate single cells (Li et al. 2005; Kometani et al. 2007), to deliver small amounts of material at precise locations (Mohanty and Gupta 2007), to detect target agents even at a low nanomolar concentrations level (Bontidean et al. 2003) and, in general, to perform useful physical work at the molecular level (Dubey et al. 2004; Grove et al. 2005). Such bionanorobotic devices will hopefully be the basis of an arsenal of future medical and nanotech devices that will automatically perform operations, inspections, and treatments while achieving ultra-high accuracy in time and space.

Given the vast body of knowledge in theoretical, applied, and analytical kinematics and dynamics, the robotics community can make a significant contribution to the understanding of biological systems and especially to protein engineering, which can lead to the design and engineering of novel bionanodevices. However, most of the work performed by the robotics community in this area has been in the field of motion prediction and path planning and solving protein folding problems using robot kinematics, which have implications in receptor–ligand docking and drug design. For a more detailed description the reader is referred to Kazerounian (2004), Zhang and Kavraki (2002), Chirikjian et al. (2005), Manocha et al. (1995) and Sharma et al. (2005).

In this study we present the design hypothesis, architectures, and computational results of a novel protein-based nanoactuator that could eventually serve as a component of a bionanorobotic system. Our goal was to engineer nanoactuators that are inspired by nature and could be used in various biological, chemical, and nanomanufacturing applications. We first characterized a natural peptide motif, the coiled-coil motif of the yeast transcriptional activator GCN4, that could act as a template for designing nanoscale actuators owing to its structure, stability, and specificity at various physiochemical conditions. We then altered and improved the functionality of these natural elements using protein engineering, which leads to protein-based nanoactuators with optimal performance characteristics. Owing to its open and close motion resembling that of tweezers, the new nanoactuator can potentially be used as a nanogripper in various nanomanipulation tasks.

2. Nanogrippers: State of the Art

The development of tools to precisely manipulate and sense objects at the nanoscale is vital to the advancement of nanoscale science and technology. For manipulation at the nanoscale, scanning tunneling microscopy (STM) and atomic force microscopy (AFM) are now widely used and have the capability to work at atomic length scales. The microscopes involved, however, are single probed which greatly restricts their ability to manipulate or grab nanoscale objects, measure their physical and electrical properties, or transfer them from one place to another. Also, the probes of these microscopes move in a lateral direction to push or pull nano-objects and hence cannot manipulate nano-objects three dimensionally. A two-probed device in the form of a gripper or tweezers can provide a second contact with the structure and thus might enable new manipulation tasks on the nanostructure. Molecular or nanoscale grippers/tweezers are a relatively new technology that offers an enormous potential for a variety of biological as well as nanomanipulation processes. The size of the molecular/nanoscale tweezers are of the order of nanometers and the basic structure consists of two “arms” joined at one end or separated by a “spacer”. Their working principle is based on controlled triggering of the molecule conformation by an external stimulus such as voltage, light, pH, ion gradient, or a specific molecule (ligand). This section outlines some of the existing molecular/nanoscale grippers and tweezers, their working principle, and their relevance to the scientific community.
A considerable amount of research has been done in the design and fabrication of microscale/nanoscale grippers or tweezers to form a basic nanoelectromechanical system (NEMS) (MacDonald et al. 1989; Yao and MacDonald 1992; Shi et al. 1995; Boggild et al. 2001; Carlson et al. 2007). These grippers work on the principle of electrostatic forces generated between the two “arms” through an externally applied voltage. The grippers were fabricated using conventional lithography techniques and the operating voltages for these grippers vary from 10 to approximately 150 V. Recently researchers have focused their attention on using carbon nanotubes (CNTs) to create robust nanogrippers that can be utilized for nanoscale manipulation and measurement (Kim and Lieber 1999; Nakayama and Akita 2002; Lee and Kim 2005). CNTs are hollow tubes of carbon with typical diameters of 1–100 nm and lengths from 100 nm up to several centimeters. The nanotubes have outstanding mechanical (Falvo et al. 1997; Wong et al. 1997) and electrical (Dai et al. 1996; Bockrath et al. 1997) properties which make them ideal materials for nanoelectromechanical devices.

In 1999 researchers at Harvard University proposed the first CNT-based nanotweezers (Kim and Lieber 1999). These tweezers were made by attaching nanotubes to glass electrodes under an optical microscope. The opening and closing of the tweezers arms was achieved by applying voltages to the electrodes. This electromechanical response of the tweezers was demonstrated by grabbing submicrometer clusters and nanowires and also by measuring the electrical properties of silicon carbide nanoclusters under the conducting arms of the nanotube tweezers. Another group of researchers in 2002 developed nanotweezers consisting of two arms of CNTs that operate electromechanically in AFM (Nakayama and Akita 2002). The two nanotubes were attached on the metal electrodes and fixed by carbon deposition. The principle of operation was similar to the previous nanotweezers, i.e., through the application of DC voltage to the two nanotube arms inducing their movement to approach each other. These nanotube tweezers installed on an atomic force microscope have potential applications in manipulating nanomaterials for constructing three-dimensional structures, for investigating the interaction between the nanomaterials, and for measuring the mass and electrical conduction of nanomaterials.

For both nanotweezers mentioned above the two CNTs are attached to the same substrate thereby limiting the tweezing range of the nanotweezers. Furthermore, a conducting material cannot be used as a substrate and it was necessary to deposit additional electrodes for the driving voltage. Lee and Kim (2005) proposed yet another type of nanotweezers using length controlled CNT arms. These nanotweezers have separated tungsten substrate for each CNT arm making it possible to close and open the nanotweezers repeatedly. Another advantage of these nanotweezers with separated substrate is that they can be used to grip large particles and a conducting material can be used as a substrate. The length of the CNT is controlled using electrochemical etching. These nanotweezers also work on the electrostatic forces generated between the two CNT arms due to the application of external voltages.

All three types of nanotweezers mentioned above are of relatively large size. The length of these nanotweezers is in the range of several micrometers whereas the separation between the nanotube tips varied from 250 to 780 nm. The need to shrink the size further lead scientists to build tweezers from DNA (Yurke et al. 2000). Each arm of this tiny tool is just 7 nm in length. These tweezers exploit the complementary nature of the two strands of a double helix DNA for their function. The tweezers comprise three single strands of synthetic DNA. Two strands act as the arms; one strand straddles the others and acts as a kind of backbone and hinge holding the whole V-shaped structure together. The arms extend far enough to leave a number of unpaired bases dangling free beyond the backbone. When a fourth DNA strand is added to the test tube, it grabs the unpaired bases and zips the tweezers shut. Again, just a few bases are allowed to hang unpaired, which permits a fifth strand to rip away the first fuel unit and open the tweezers. The two fuel units, now paired, then drift away as if they were “exhaust fumes” from this molecular motor. The advantage of using the DNA tweezers is their small size and ease of operation; no complex fabrication steps are required to build such a device. The drawback, however, lies with the need for the extra “fuel” strand for their actuation. This device may not be ideal for actual tweezing or “catch and release” kinds of mechanism since the potential grasping site of the tweezers is occupied by the “fuel” strand. This device has potential applications in molecular computing and molecular switching.

Many existing molecular tweezers are either supramolecular complexes (Nemoto et al. 2000; Petitjean et al. 2004; Schaller et al. 2007), mechanoenzymes (Danino and Hinshaw 2000; McNiven et al. 2000; Zhang and Hinshaw 2001) or photoresponsive molecular scissors (Muraoka et al. 2003, 2006) having a well preorganized yet flexible shape enabling variation in the size of the binding site. Their function can best be described as a receptor–ligand interaction where the tweezer molecule (receptor) binds a substrate molecule (ligand) via non-covalent interactions such as hydrogen bonding or ion pairing. The main drawback of the supramolecular tweezers is their specificity to bind only a few types of aromatic molecules. The supramolecular design also inhibits their ability to be conjugated to other substrates to create a more robust device.

The nanoactuator proposed in this study is designed to overcome the shortcomings of the above grippers. It is smaller in size and does not require any external voltage or “fuel strand” to function. It can be easily interfaced with other nanoscale components such as CNTs and quantum nanodots by simple chemical functionalization. Varying degrees of opening can be observed by varying the pH of the solution. It is smaller in size than the nanotube-based tweezers and more robust and versatile when compared with the DNA or supramolecular tweezers.
3. Protein-based Nanoactuator: Concept and Applications

Proteins are biopolymers that are made up from 20 different amino acids. Each of these amino acids is referred to as a residue. About 50 to 100 of these residues are connected together via peptide bonds to create a long chain. The chain is known as a polypeptide chain or simply a protein. We used a nanoscale length two-stranded parallel \( \alpha \)-helical coiled-coil protein/peptide to create a robust nanoactuator that can be used for nanoscale manipulation and sensing. The coiled-coil is a ubiquitous protein motif made up of \( \alpha \)-helices wrapped around each other forming a supercoil (Crick 1953). Coiled-coils are ideal candidates for protein design studies, as they represent probably the simplest secondary structure with physical properties that make them ideal for both nanoscale manipulation and measurement. They are found to be very stable in the native state largely due a repeat of hydrophobic core (hydrophobic residues that are spaced every four and then three residues apart) in their primary sequence (Cohen and Parry 1990; Krys-tek et al. 1991). The particular coiled-coil model studied here is the one corresponding to the leucine zipper (LZ) of the yeast transcriptional activator GCN4 (Landschultz et al. 1988).

From an engineering point of view, GCN4-LZ consists of two identical 33-residue polypeptide chains/helices and is approximately 4.5 nm long and about 3 nm wide. The helices wrap around each other to form approximately one-quarter turn of a left-handed supercoil. The pitch of the supercoil averages 181 Å, and the average distance between the helix axes is 9.3 Å (O’Shea et al. 1991). Figure 1 shows an enlarged view of the LZ with the corresponding dimensions.

Coiled-coil proteins have been extensively investigated using various experimental techniques such as nuclear magnetic resonance (NMR) spectroscopy (Junius et al., 1996), X-ray diffraction (XRD) (Rasmussen et al. 1991), circular dichroism (CD) and fluorescence spectroscopy (Suzuki et al. 1998), differential scanning calorimetry (DSC) (Yu et al., 1996) and electron spin resonance (ESR) spectroscopy (Columbus and Hubbell 2004) as well as theoretical and computational approaches using molecular dynamics (MD) simulation techniques (Mohanty et al. 1999; Missimer et al. 2005; Pineiro et al. 2005).

The GCN4-LZ peptide was engineered to obtain an environmentally responsive nanoactuator involving the reversible movement of helices towards and away from each other. The actuation mechanism depends on the creation of similar electrostatic charges along the peptide chain which forces the two coils to repel each other and move apart thus creating an opening tweezer-like motion of the nanoactuator. This motion can be reversed by neutralizing the charges. Creating of electrostatic charges depends on the differences in the ionization states of certain amino acids in the peptide chain, which in turn depends on the pH of the solvent. Of the 20 amino acid residues, histidine, glutamic acid, and asparatic acid ionize at pH range 4–7.4. Glutamic and asparatic acids are initially negatively charged at neutral pH (\( \sim 7.4 \)) and become neutral at lower pH due to the addition of a proton. On the other hand, histidine which is neutral at pH \( \sim 7.4 \) becomes positively charged at lower pH \( \sim 4 \). Thus, by the introduction of different ionizable residues along the peptide chain and varying the pH of the solvent different nanoactuator architectures with varying degrees of motion can be obtained. Figure 2 shows the schematic of one such nanoactuator.

The peptide nanoactuator can be employed in a wide variety of nanomanipulation and biomedical applications. For example, the nanoactuator can potentially be employed for the detection and removal of heavy metal ions during nanofabrication processes (see Figure 3) or as a molecular switch (see Figure 4). We would like to emphasize that the goal of this paper is not focused on a specific application, but rather prove \textit{in silico} the opening and closing of the nanoactuator and characterize some of its mechanical properties (displacement, force...
Fig. 2. Schematic of a nanoactuator showing the working principle: (a) nanoactuator in its initial closed state at neutral pH; (b) open configuration of the nanoactuator owing to the electrostatic repulsions of the positively charged histidine residues along the chain at low pH.

capability). The nanoactuator design for a specific application is beyond the scope of this paper.

4. Computational Design and Modeling

To predict the performance of the peptide nanoactuators, MD simulations were performed. The MD models used in this study are based on the calculation of the conformational energy that is released during the protein actuator conformational change. The calculation of the free energy facilitates the calculation of important parameters for the performance of the nanoactuator such as force, favorable conformations, and optimal environmental conditions. Using MD simulation techniques different operating conditions such as varying pH, temperature, and pressure variations can be readily applied on the peptide system. The response of the peptide system such as the conformational changes, free energy, force output, structural stability, and reversibility under the operating conditions are qualitatively and quantitatively studied. This gives a set of parameters on which to compare different peptide mutants and select that which gives the optimal performance result. The design of different peptide mutants is another area where MD is useful. Mutation is the process of replacing a residue in a protein chain by another residue resulting in the creation of a new character or trait not found in the parental protein. This change can alter the chemical and physical properties of proteins and can help design a new protein with desired properties. In the case of the proposed nanoactuator, the desired property was the creation of an electrostatic charge on the two helices and this was achieved by mutating several residues of the original GCN4 peptide with histidine residues.

The main challenge associated with computational modeling of bionanosystems is the size of the system that can be efficiently simulated. Even with the advancement in computer technology most MD approaches have been practically limited to simulation times and system sizes less than 100 ns and 10 nm, respectively. A coarse-grained (CG) molecular model (Arkhipov et al. 2006; Shih et al. 2006) can provide a possible way to extend molecular modeling technique to large systems and bridge the gap with experimental techniques. A CG model represents a system by a reduced number of degrees of freedom. Owing to this, a CG simulation requires fewer resources and goes faster when compared with a system with all-atom representation. As a result, larger systems can be simulated to extended time scales.

4.1. Sample Preparation

The X-ray crystallographic structure of the native GCN4 (PDB entry: 1YSA), complexed with AP-1 yeast DNA, was obtained from the Protein Data Bank (see Figure 1(a)). The DNA was removed by deleting the coordinates from the PDB structure and the two peptide chains (A and B) were truncated to contain 33 residues each numbered 249 to 281 corresponding to the coiled-coil portion of the peptide (henceforth referred to as GCN4-LZ). Two different nanoactuator mutants (M1 and M2) were designed using GCN4-LZ as a template. First, a pentaglycine tag was added at the N-terminus of GCN4-LZ, the glycine (Gly) residues were added in order to maintain the same number of residues as those in the nanoactuator mutants described below. The resulting structure is referred to as the wild-type (WT) peptide in subsequent discussions. Mutant M1 consists of a pentahistidine tag (His-tag) aligned with the \( \alpha \)-helix at the N-terminus of the GCN4-LZ. Mutant M2 consists of five mutations (L253H, K256H, E259H, L261H, and Y265H) in each of the helical chains in addition to the His-tag. At low pH upon histidine protonation the His-tags at the N-terminus of the mutants help in generating electrostatic repulsive forces thereby aiding the motion of the nanoactuator. Figure 5 shows the architecture of different nanoactuator mutants with the position of His-tags and histidine residues shown in dark color.

4.2. Simulation Parameters

The Nanoscale Molecular Dynamics (NAMD) (Phillips et al. 2005) program was used to perform MD simulations in this study. The protein was modeled with an all atom CHARMM22 force-field (MacKerell et al. 1998). The protein was solvated by placing it at the center of a box of water with approximate edge lengths of \( 50 \times 50 \times 70 \) Å and subtracting all water molecules within 2.4 Å of any protein atom. Water molecules were described by the TIP3P model (Jorgensen et al. 1983).
Fig. 3. One of the important challenges during a nanofabrication process is contamination due to the presence of heavy metal ions (such as Co or Ni) in the fabricated nanostructures. The nanoactuator is rich in histidine residues which have a special affinity towards bivalent metallic ions such as Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, etc., and can be used as a heavy metal ion capture mechanism. (a) Immobilization of the nanoactuator at low pH to the amide binding sites of the template nanotubes. (b) The Cu ion–nanoactuator peptide complexation on the nanotube surfaces. (c) The nanoactuator “closes” at neutral pH binding the Cu ions in the basic region formed by the histidine tags. Once the metal ions are captured the entire nanoactuator–nanotube assembly can be easily flushed out leaving the purified nanostructure.

Fig. 4. Schematic of a molecular switch or a pH sensor, based on the nanoactuator architecture, which relies on the change in conformation at low pH. (a) At pH 7 the peptide is in compact configuration and the ions have access to the gold substrate. (b) At low pH the peptide is in “open” configuration and the access of the ions is blocked by the passivating dodecanethiol layers resulting in reduced conductivity.
Salt (NaCl) was added to neutralize the excess charges generated due to the protonation of amino acids in the protein. Electrostatic interactions were computed using particle mesh Ewald (PME) method (Darden et al. 1993). Van der Waals interactions were truncated at a cutoff distance of 12 Å and a smooth switching function was used at a switching distance of 10 Å. The ShakeH (van Gunsteren and Berendsen 1977) algorithm was used to fix the bond between each hydrogen and its mother atom to the nominal bond length with a relative tolerance of $1.0 \times 10^{-8}$ Å and the timestep for integration was
2 fs. The temperature was regulated by coupling the system to an external bath with a damping coefficient of 5. An isobaric ($P = 1$ atm) and isothermal ($T = 298$ K) with constant number of atoms (the so-called NPT) ensemble was created using the approach developed by Nose and Hoover (Martyna et al. 1994). The Visual Molecular Dynamics (VMD) software package (Humphrey et al. 1996) was used as the visualization software for analyzing trajectories generated by NAMD. The steps involved in the preparation of the sample for MD simulations are shown graphically in Figure 6.

5. Results and Discussion

5.1. WT Structure and Mutant M1 are Stable

From previous experimental (Kohn et al. 1995; Yu et al. 1996; Hendsch and Tidor 1999) and computational studies (Rozzelle et al. 1994; Mohanty et al. 1999; Missimer et al. 2005; Pineiro et al. 2005), we expect that the WT GCN4-LZ will be stable at both neutral and low pH. To address this, simulations were performed starting with the GCN4-LZ crystal structure immersed in a box of water molecules. The backbone Ca root mean square deviation (RMSD) for residues 248–281 do indeed remain low (1.5 Å) over the course of simulation at neutral and low pH (result not shown). This value agrees well with the previously reported range of RMSD values from MD simulations (Nilges and Brunger 1993; Vieth et al. 1994; Mohanty et al. 1999) of GCN4-LZ. For the low-pH simulation we also plotted the opening of the WT peptide as measured by the distance between the His247 residues in the corresponding helices (Figure 7(a)). The objective of this plot is to provide a benchmark for comparing the opening from other nanoactuator mutants as described in the following sections. No net opening was observed between the two helices for the WT structure at low pH—the initial and final distance between the two helices was 11 and 12 Å, respectively. This behavior was expected since the WT GCN4 is in a very stable conformation owing to various hydrophobic and electrostatic interactions as explained earlier. Also under normal physiological conditions (pH~7) there was no protonation of the residues and hence no extra electrostatic charges strong enough to overcome the sta-
Fig. 8. Opening dynamics of mutant M1. The initial separation of 13 Å between the two helices increased to 16 Å over the simulation time thereby showing a total opening of only 3 Å which is insignificant and can be attributed to thermal fluctuations.

The pH-dependent actuation of mutant M1 which contains a 5-histidine tag at the N-terminus of each helical chain (see Figure 5) was next evaluated at low pH. It was hypothesized that the protonation of histidine residues in the N-terminal tags at pH 4 would result in significant electrostatic repulsive forces and “push” the two helices apart thus generating the closed-to-open mechanochemical actuation in the mutant peptide. Two atoms (Cα atoms of the His246 residues) were selected near the N-terminal of the individual chains in order to measure the opening between the helices and the distance between the two was plotted as a function of simulation time. Figure 8 shows the plot of opening dynamics of M1 while Figure 9 shows the snapshots from the simulation.

No significant opening was observed after a 4-ns simulation; the initial distance of 13 Å between the two atoms remained constant during the first nanosecond of simulation after which it increased to 16 Å and remained stable at this separation for the rest of the simulation. The increase of 3 Å is not significant and can be attributed to atomic fluctuations or the perturbation in the histidine residues due to repulsive forces rather than the overall displacement of the two chains. This implies that the electrostatic repulsive forces generated by the positively charged N-terminal histidines are not sufficient to overcome the strong hydrophobic interactions that stabilize the coiled-coil core of M1 mutant. We therefore designed mutant M2, which possesses histidine residues along the length of the coiled-coil in order to offset the attractive hydrophobic interactions in the core.

5.2. Mutant M2 Shows pH Dependent Conformational Change

Mutant M2 was designed next with the following point mutations in addition to the N-terminal histidine tag: L253H, K256H, E259H, L261H, and Y265H. M2 has a uniform distribution of His residues along the helical chain which results in a spatial distribution of electrostatic charges in addition to the concentrated charges from the distal His-tags. Further, the L253H, L261H, and Y265H mutations replace the hydrophobic leucine and tyrosine residues with polar His residues thereby significantly reducing the strength of the hydrophobic interactions towards the N-terminal and “middle” regions of the coiled-coil core while maintaining the strong hydrophobic core in the C-terminal region. This evolved design was therefore a balance between repulsive forces that can induce the actuation mechanism at acidic pH and strong hydrophobic interactions that can (i) maintain the coiled-coil structure and (ii) serve as the restituting force for the “hinge” action in order to restore the original conformation of the peptide at neutral pH.

Figure 10 shows the snapshots of a 4 ns simulation of M2. Large conformational changes were observed in the M2 system leading to a significant net opening between the two helices. The helices rapidly moved apart within the first nanosecond and continued to move apart steadily until 3 ns, before adopting a final stable conformation. The distance between the Cα atoms of the His246 residues in both chains was plotted as a function of the simulation time (Figure 11). The initial distance between the two atoms was 11 Å which gradually increased to 28 Å at the 2.5 ns stage. The distance then fluctuated due to the dynamic nature of the electrostatic forces but stayed near the 27 Å separation during the rest of the simulation (4 ns). Thus, a net opening of 16 Å, which is approximately 150% of the initial separation, was observed for M2 at low pH. This result verifies the hypothesis that selective mutations can be performed in the native GCN4-LZ that can induce large conformational changes without compromising its structural stability.

5.3. Conformational Change in Mutant M2 is Reversible upon pH Modulation

One of the key design goals of a nanoactuator is the reversibility of mechanochemical actuation. We therefore investigated whether the mutant M2 demonstrated a reversible open-to-closed transition when the pH was reverted back to neutral from acidic. The initial structure (the “open” state) for this simulation was taken from the final conformation generated from
Fig. 9. Snapshots from a 4-ns simulation of mutant M1. The N-terminal His-tags are shown in dark color. The opening was measured between the Cα atoms of His246 residues in chains (shown as spheres).

Fig. 10. Snapshots of mutant M2 at various time instances during a 4-ns simulation. The position of histidine residues is shown in dark color. Location of the His246 residue between which the opening is measured is shown as a sphere.

Fig. 11. Opening dynamics of M2 at low pH. The initial separation between the two chains was 11 Å which gradually increased to 27 Å at the 2.5 ns stage and then stabilized for the rest of the simulation giving a net opening of 16 Å.

the previous 4-ns closed-to-open simulation at low pH. Histidine, glutamic acid, and aspartic acid residues in M3 were unprotonated to simulate neutral pH. Figure 12 shows snapshots of a 5-ns long reversible motion simulation of M2. Increasing the pH back to neutral triggered the reversible transition of the mutant and the final conformation generated by this simulation resembles the initial starting structure from the closed-to-open simulation (Figure 10).

Figure 13 shows the dynamics of the reversible motion of M2 over the simulation time. The “open” state generated at the end of the close-to-open simulation of M2 at pH 4 was in a state of dynamic equilibrium. This means that the peptide was in a “tensed” state wherein the restituting forces due to helices elasticity and the hydrophobic attractions near the C-terminal of the peptide chains balanced the repulsive electrostatic forces of the ionized histidine residues. At pH 7, the force generating capability vanished due to histidine neutralization leading to the restitution of the “relaxed” state. The reversible transition
Fig. 12. Snapshots of a 5-ns simulation of open-to-close transition to show the reversible motion of mutant M2. Owing to the lack of ionic repulsions at neutral pH and also due to the attractive hydrophobic interactions the actuator chains rapidly closed back. The final state closely resembles the initial NMR structure.

Fig. 13. Reversible motion dynamics for mutant M2 at neutral pH. As with all elastic systems, mutant M2 shows motion hysteresis.

of the mutant at pH 7 was exactly as hypothesized and verifies the concept of designing a nanoactuator element whose actuation can be modulated by pH. Figure 13 also reveals that the new nanoactuator has some hysteretic behavior which is something very common in non-linear actuators such as that presented in this paper.

From the results it is obvious that mutant M2 is the best design for a nanoactuator element. It shows maximum pH-dependent opening while maintaining its structural rigidity. The motion of the M2 mutant was also shown to be reversible upon pH modulation. The next step is to calculate the mechanical force generated by the M2 nanoactuator upon pH actuation.

5.4. Mechanical Force Generated by the Nanoactuator

The GCN4-LZ peptide molecular actuator based on the M2 mutant design shows a spontaneous “opening” and “closing” mechanism upon pH modulation and can be considered a basic nanoactuator element capable of generating mechanical force during its closed-to-open actuation. In this section we show the results from computational studies employing statistical mechanics principle to investigate the nature and magnitude of the mechanical force generated by the M2 nanoactuator during its closed-to-open transformation at low pH. To do this we employed a modified steered molecular dynamics (SMD) technique within the conventional MD framework. This type of technique has also been previously employed to study the mechanical force generated in G proteins (Kosztin et al. 2002).

The closed-to-open transformation model of the nanoactuator is used as a computational platform to estimate the external force and hence the work done by the system. To measure this force, we repeated the low pH simulation of the M2 mutant but this time with an applied external constraint to its motion. This constraint was applied in the form of a harmonic spring of known stiffness $k$, attached to the center-of-mass (COM) of the His-246 backbone atoms in the corresponding chains (Figure 14).

The harmonic guiding potential and the corresponding exerted force for this system are of the form:

$$U = -k(x - x_0)^2/2, \quad F = k(x - x_0),$$

where $x$ is the distance between the COMs of the two His-246 residues at any given time instance $t$, and $x_0$ is the equilibrium value (at $t = 0$) of $x$. With a known value of the spring stiffness $k$, the time series of the reaction coordinate $x$ can be obtained from the MD simulations which can then be plugged in to the force equation above to obtain the force time series. A statistical analysis of the force time series can reveal the nature and
Fig. 14. Schematic representation of the structure of the (a) closed and (b) open states of the nanoactuator. To estimate the force generation capabilities of the nanoactuator a harmonic spring with a known spring constant was attached to the COM of His-246 residues (shown as spheres) near the N-termini of the two chains.

magnitude of the force exerted by the nanoactuator. Seven simulations were performed with the value of \( k \) varying between 0.2 and 3 kcal mol\(^{-1}\) Å\(^{-2}\). Figure 15 shows a representative result of a 4-ns SMD simulation of the nanoactuator peptide in the presence of an attached harmonic spring with \( k = 0.6 \) kcal mol\(^{-1}\) Å\(^{-2}\). The time series of force (top curve) exerted by the protein on the spring is calculated using the equation \( F(t) = k(x(t) - x_0) \) while the bottom curve shows the corresponding normalized force distribution histogram.

For the most part the nanoactuator exerts a stretching force on the spring the magnitude of which increases with the applied load (spring stiffness). For \( k = 0.2 \) kcal mol\(^{-1}\) Å\(^{-2}\) the mean force exerted is 20 pN (results not shown) which increases to 50 pN for \( k = 0.6 \) (Figure 15). The magnitude of mean force remained in the 40–50 pN range for larger force constants of \( k = \{0.8, 1.0, 2.0, 3.0\} \) (results not shown). This force output is comparable to that generated by other protein-based molecular motors such as flagella, ATPase, etc. (Yin et al. 1995) even though the nanoactuator is smaller in size and does not require any external fuel such as ATP to function.

6. Conclusions

In this paper we have presented the concept and computational evaluation of a pH-dependent coiled-coil molecular actuator using molecular dynamics. Two mutants were designed based on the parent coiled-coil GCN4-LZ protein containing histidine tags (five histidines) and up to five point histidine mutations along the helical chains. One of the mutants, M2, showed excellent performance characteristics (displacement, force) while maintaining its structural integrity during the entire operation. Upon pH actuation, M2 opened up to 27 Å which is a significant displacement considering that the initial separation between the two chains was only 11 Å. More importantly, this actuation behavior was shown to be reversible upon restoration of initial pH conditions. Furthermore, we analyzed the force capabilities of mutant M2 peptide due to pH actuation. For this we employed statistical mechanics principles. We devised a new method based on a modified SMD technique to estimate the biological force generated due to conformational changes in macromolecules. Using this method we were able to show that the nanoactuators based on M2 mutant can generate mechanical force upon pH modulation. The forces are generated due to the electrostatic repulsions at low pH between His-tag handles and other charged residues engineered into the peptide sequence. The biological force output of the nanoactuators is comparable to that generated by ATP-based molecular motors such as myosin and kinesin even though our molecular motor is smaller in size to these molecular motors.

Our computational results support the hypothesis of the peptide nanoactuator. The engineered nanoactuators are inspired by nature and were able to perform the specific function without the requirement for high-energy fuel molecules. Work is also in progress on the experimental front and we have expressed the peptide using recombinant means. We are now working to experimentally determine the opening of the nanoactuator using ESR spectroscopy (Hubbell et al. 1998). ESR spectroscopy is a powerful technique for probing structure and site-specific conformational dynamics in biopoly-
mers, primarily proteins. The standard approach is to attach spin-labels such as nitroxide reporter group to a specific amino acid such as cysteine in the protein chain. The unpaired electron on the spinlabels absorbs microwave radiation in the presence of a strong magnetic field, and the resulting change in electron spin state reveals information about the structure and dynamics of the molecule. Thus, it is possible to determine protein structures using a double labeling method in which distance between label sites is obtained by measuring the electron spin–spin dipolar interaction between them.

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