Design and Modeling of a Protein Based NanoGripper
Gaurav Sharma, Kaushal Rege, David Budil, Martin Yarmush, and Constantinos Mavroidis

Abstract— The design hypothesis, architectures, and computational modeling of a novel peptide based nanoGripper 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 nanoGripper. The dimeric coiled-coil peptide consists of two identical ~4.5nm long and ~3nm 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 a gripper. Using molecular dynamics simulations we showed that pH changes lead to a reversible opening of up-to 1.5nm which is approximately 150% of the initial separation of the nanoGripper. We also investigated the forces generated by the nanoGripper upon pH actuation. Using a new method based on a modified steered molecular dynamics technique we were able to show that the force output of the nanoGripper is comparable to that generated by ATP-based molecular motors such as myosin and kinesin even though our molecular tweezer is smaller in size to these molecular motors.

I. INTRODUCTION

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 nanoscale scanning tunneling microscope (STM) and atomic force microscope (AFM) are now widely used and have the capability to work at atomic length scales. These microscopes, however, are single probed which greatly restricts their ability to manipulate or grab nanoscale objects, measure their physical and electrical properties or transfer them one place to another. Also, the probes of these microscopes move in a lateral direction to push or pull nanobjects 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 nano-tweezers are a relatively new technology that offers an enormous potential for a variety of biological as well as nano-manipulation processes. The size of a molecular/nano tweezer is 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).

In this paper we present the design hypothesis, architectures and computational results of a novel protein based nanoGripper. Our goal was to engineer nanoGrippers that are inspired by nature and could be used in various biomedical and nano-engineering 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 grippers due to its structure, stability and specificity at various physico-chemical conditions. We then altered and improved the functionality of these natural elements using protein engineering, which leads to protein-based nanoGrippers with optimal performance characteristics.

The nanoGripper presented in this paper is designed to overcome the shortcomings of grippers currently found in literature. It is smaller in size and does not require any external voltage or ‘fuel strand’ for its functioning. It can be easily interfaced with other nanoscale components such as CNTs and quantum nanodots by simple chemical functionalization. Varying degree of opening can be observed by varying the pH of the solution. It is smaller in size than nanotube based tweezers and more robust and versatile when compared to the DNA or supramolecular tweezers.

II. PROTEIN BASED NANOGRIPPER: 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 fifty to hundreds 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 α-helical coiled-coil protein/peptide to create a robust nanoGripper that can be used for nanoscale manipulation and sensing. The coiled-coil is a ubiquitous protein motif made up of α-helices wrapping around each other forming a supercoil [1]. Coiled coils are ideal candidates for protein

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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 to a repeat of hydrophobic core (hydrophobic residues that are spaced every four and then three residues apart) in their primary sequence [2]. The particular coiled-coil model studied here is the one corresponding to the leucine zipper (LZ) of the yeast transcriptional activator GCN4 [3]. From an engineering point-of-view GCN4-LZ consists of two identical 33-residue polypeptide chains/helices and is ~4.5nm long and ~3nm wide. The helices wrap around each other to form approximately ¼ 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 [4]. Fig. 1 shows the enlarged view of the leucine zipper with the corresponding dimensions.

Fig. 1. (a) Coiled-coil GCN4 bound to DNA showing the basic and the leucine zipper regions; (b) Enlarged view of the leucine zipper region showing the side (Left) and top (Right) views and the corresponding dimensions.

The GCN4-LZ peptide was engineered to obtain an environmentally responsive nanoGripper involving the reversible movement of helices towards and away from each other. The actuation mechanism depends on the creation of like electrostatic charges along the peptide chain which forces the two coils to repel each other and move apart thus creating an opening motion of the tweezer. 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 aspartic acid ionize at pH range (4 - 7.4). Glutamic acid is initially negatively charged at neutral pH (~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 ~7.4 becomes positively charged at lower pH (~4). Thus by the introduction of different ionizable residues along the peptide chain and varying the pH of the solvent different nano-tweezer architectures with varying degree of motion can be obtained. Fig. 2 shows the schematic of one such nanoGripper.

III. COMPUTATIONAL DESIGN AND MODELING

To predict the performance of the peptide nanoGrippers, molecular dynamic simulations (MD) were performed. The MD models are based on the calculation of the conformational energy that is released during the protein tweezer conformational change. The calculation of the free energy facilitates the calculation of important parameters for the performance of the nanoGripper 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 is qualitatively and quantitatively studied. This gives a set of parameters on which to compare different peptide mutants and select the one 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 nanoGripper, 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.

Fig. 2. Schematic of a nanoGripper showing the working principle; (Left) nanoGripper in its initial closed state at neutral pH; (Right) open configuration of the tweezer due to the electrostatic repulsions of the positively charged histidines residues along the chain at low pH.

Fig. 3. NanoGripper mutants; wild-type (WT), mutants M1 and M2. The position of glycine tag in WT ‘bond’ representation. Position of His-tags and histidine mutations in other mutants is shown in dark color.

A. 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 Fig. 1a). 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.
Two different nanoGripper mutants (M1 and M2) were designed using GCN4-LZ as 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 molecular tweezer 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 α-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 nanoGripper. Fig. 3 shows the architecture of different nanoGripper mutants with the position of His-tags and histidine residues shown in dark color.

**Fig. 4. Steps involved in the sample preparation for MD simulation.**

**B. Simulation Parameters**

The Nanoscale Molecular Dynamics (NAMD) [5] program was used to perform molecular dynamics (MD) simulations in this study. The protein was modeled with an all atom CHARMM22 force-field [6]. The protein was solvated by placing it at the center of a box of water with approximate edge lengths of 50 x 50 x 70 Å and subtracting all water molecules within 2.4 Å of any protein atom. Water molecules were described by the TIP3P model [7]. 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 [8]. 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 [9] 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 x 10^-4 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 [10]. The Visual Molecular Dynamics (VMD) software package [11] 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 Fig. 4.

**IV. RESULTS AND DISCUSSION**

**A. Wild Type Structure and Mutant M1 Are Stable**

From previous experimental [12-14] and computational studies [15-17], we expect that the wild-type 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 [16,18,19] 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 (Fig. 5a). The objective of this plot is to provide a benchmark for comparing the opening from other molecular tweezer mutants as described in the following sections. No net opening was observed between the two helices for the WT structure; the initial and final distance between the two helices was 11 Å and 12 Å respectively. This behavior was expected since the wild-type GCN4 is in a very stable conformation due 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 stabilizing hydrophobic interactions were generated. Fig. 5b shows the snapshots of the WT peptide conformations from the simulation.

The pH-dependent actuation of mutant M1 which contains a 5-histidine tag at the N-terminus of each helical chain (see Fig. 3) 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 mechaenochemical 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. Fig. 6 shows the plot of opening dynamics of M1 while Fig. 7 shows the snapshots from the simulation.

![Fig. 6. 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.](image1)

![Fig. 7. Snapshots from a 4-ns simulation of mutant M1. The N-terminal His-tags are shown in red color. The opening was measured between the Cα atoms of His246 residues in chains (shown as sphere).](image2)

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 that possess histidine residues along the length of coiled-coil in order to offset the attractive hydrophobic interactions in the core.

**B. 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, 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.

Fig. 8 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 three nanoseconds, 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 (Fig. 9). The initial distance between the two atoms was 11 Å which gradually increased to 27 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 (4ns). Thus, a net average 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.

![Fig. 8. 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.](image3)
**C. Mechanical Force Generated by the NanoGripper**

The GCN4-LZ peptide molecular gripper based on the M2 mutant design shows a spontaneous ‘opening’ and ‘closing’ mechanism upon pH modulation and can be considered a basic nanoGripper 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 principles to investigate the nature and magnitude of the mechanical force generated by the M2 nanoGripper 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 [20]. The closed-to-open transformation model of the nanoGripper 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 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 (Fig. 10).

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 into the force equation above to obtain the force-time series. A statistical analysis of the force-time series can reveal the nature and magnitude of the force exerted by the molecular tweezer. Seven simulations were performed with the value of \( k \) varying between 0.2 – 3 kcal/mol/Å\(^2\). Fig. 11 shows a representative result of a 4-ns SMD simulation of the nanoGripper peptide in the presence of an attached harmonic spring with \( k = 0.6 \) kcal/mol/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.

**CONCLUSIONS**

This paper presented the concept and computational evaluation of a pH-dependent coiled-coil molecular gripper 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 molecular tweezers 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 molecular tweezers is comparable to that generated by ATP-based molecular motors such as myosin and kinesin even though our molecular tweezer is smaller in size to these molecular motors.

Our computational results support the hypothesis of the peptide nanoGripper. The engineered nanoGrippers 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 on to experimentally determine the opening of the nanoGripper using Electron Spin Resonance (ESR) spectroscopy. ESR spectroscopy is a powerful technique for probing structure and site-specific conformational dynamics in biopolymers 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.

REFERENCES