Abstract – A new nanoscale biomolecular motor is introduced: The Viral Protein Linear (VPL) Motor. The motor is based on a conformational change observed in a family of viral envelope proteins when a virus attempts to infect a cell by the process of membrane fusion. By developing similar proteins in vitro and subjecting them to appropriate conditions, a similar conformational change can be observed and controlled. The conformational change produces a motion of about 10 nm, making the VPL a basic linear actuator which can be further interfaced with other organic/inorganic nanoscale components such as DNA actuators and carbon nanotubes.

I. INTRODUCTION

Just as conventional macro-machines are used to develop forces and motions to accomplish specific tasks, bio-nano-machines can be used to manipulate nano-objects, to assemble and fabricate other machines or products and to perform maintenance, repair and inspection operations. The advantages in developing bio-nano-machines include: a) energy efficiency due to their intermolecular and interatomic interactions; b) low maintenance needs and high reliability due to the lack of wear and also due to nature’s homeostatic mechanisms (self-optimization and self-adaptation); c) low cost of production due their small size and natural existence. But to reach this state of technology, we must begin with the basics. We must understand the biological components that draw a parallel to current macro-scale designs. With this knowledge in hand we can continue forward and join these components into assemblies of bio-nano-robots. Some of these assemblies will execute specific tasks, while others perform a number of different operations. Eventually these bio-nano-robots will interact with one another, collaborating to build, repair, and manipulate other objects in the nano-world.

In this project, we are focusing on the mechanical properties of certain viral envelope proteins that change their conformation depending on the pH level of the environment. Based on this principle, a new linear biomolecular actuator type is obtained that we call: Viral Protein Linear (VPL) motor. So far, we know, to a great degree of accuracy, the role of envelope glycoproteins of various retroviruses for the process of membrane fusion, which is a process necessary for the virus to be able to infect a cell. During the process of membrane fusion, there is a distinct conformational change in the peptide on the viral surface as it ‘readies’ itself for infecting the cell. This change is due to a pH difference associated with the vicinity of the cell [1]. Given artificially created similar conditions, it is proposed to use this conformational change to produce VPL motors.

There is a group of retroviruses whose envelope glycoproteins undergo such a conformational change, and hence, each one, in principle, is a candidate for the creation of a different VPL motor. The most common of these are the Influenza Virus protein hemagglutinin (HA) and the Human Immunodeficiency Virus type 1 (HIV 1) protein gp41. We have selected the HA as the basis of our VPL motor. Our approach it two-pronged – experimental characterization, and computational modeling and simulation of VPL actuators. Once the individual motors are created and modeled, it will be possible to synthesize and control complex biomolecular systems consisting of these motors interfaced with other components such as DNA based actuators and carbon nanotubes.

II. BACKGROUND

There is an engineering interest in utilizing machines, which have always been an integral part of all life. These motors, which are called Biomolecular Motors have attracted a great deal of attention recently because they have high efficiency, they could be self-replicating, hence cheaper in mass usage, and they are readily available in nature. A number of enzymes such as kinesin [2,3], RNA polymerase [4], myosin [5], dynein [6] and adenosine triphosphate (ATP) synthase [7] function as nanoscale linear, oscillatory or rotary biological motors. Some other machines that have been extensively studied include the flagella motors [8] and the rotaxanes [9], which are an example of a purely chemical motor. In addition, there are compliance devices such as spring-like proteins called fibronectin [10] and vorticellids [11].

In addition to protein-based machines, several researchers are exploring the use of DNA in nanoscale mechanisms. Compared with protein structures, DNA is small, simple and homogeneous, and its structure and function is well understood. The generally predictable self-assembling nature of the double helix makes it an attractive candidate for engineered nanostructures. This property was exploited to build several complex geometric structures, including knots, cubes and various polyhedra [12]. Mathematical analyses of the elastic structure of DNA using energy minimization methods were performed to examine its molecular stability, wherein short DNA strands were treated as an elastic rod [13]. Initial physical experiments on DNA visualization and manipulation using mechanical, electrical, and chemical means have been underway for a decade [14, 15]. A dynamic device providing atomic displacements of 2-6 nm was proposed in [16], wherein the chemically induced transition between the B and Z DNA morphologies acts as a moving nanoscale device. A method for localized element-specific
motion control was seen in the reversible transition between four stranded topoisomeric DNA motifs (PX and JX) thereby producing rotary motion [17]. A very important, though simple DNA machine that resembles a pair of tweezers was successfully created, whose actuation, opening and closing, is also fueled by adding additional DNA fuel strands [18].

III. VPL MOTOR PRINCIPLE

The X-ray crystallographic structure of bromelain-released soluble ectodomain of Influenza envelope glycoprotein hemagglutinin (BHA) was solved in 1981 [19]. BHA and pure HA were shown to undergo similar pH-depandent conformational changes which lead to membrane fusion [20]. HA consists of two polypeptide chain subunits (HA1 and HA2) linked by a disulfide bond. HA1 contains sialic acid binding site which responds to the cell surface receptors of the target cells [21]. Out of the various theories proposed to explain the process of membrane fusion, the spring loaded conformational change theory [1] is the most widely accepted. According to this model, there is a specific region (sequence) in HA which tends to form a coiled coil. In the original X-ray structure of native HA, this region is simply a loop. It further states that a 36 amino-acid residue region, upon activation, makes a dramatic conformational change from a loop to a triple stranded extended coiled coil along with some residues of a short α-helix that precedes it. This process relocates the hydrophobic peptide (and the N-terminal of the peptide) by about 10 nm. Figure 1 shows the triple stranded coiled coil. VPL motor is based on the spring loaded conformational change model for the HA peptide as described above. In a sense of bio mimicking, we are engineering a peptide identical to the 36-residue long peptide mentioned above. Once characterized, the peptide will be subjected to conditions similar to what a virus experiences in the proximity of a cell, that is, a reduced pH. The resulting conformational change can be monitored by fluorescence tagging techniques and the forces can be measured using Atomic Force Microscope (AFM).

IV. COMPUTATIONAL STUDIES

In order to perform simulations of the VPL motor function, we used a Molecular Dynamics (MD) software called CHARMM (Chemistry at Harvard Molecular Mechanics) [23]. In MD, the feasibility of a particular conformation of the biomolecule in question is dictated by the energy constraints. Hence, a transition from one given state to another must be energetically favorable, unless there is an external impetus that helps the molecule overcome the energy barrier. When a macromolecule changes conformation, the interactions of its individual atoms with each other - as well as with the solvent - compose a very complex force system. The CHARMM energy function is given by:

\[ E = E_b + E_\theta + E_\phi + E_\omega + E_{vdW} + E_{el} + E_{hb} + E_{cr} + E_{cf} \]

The individual components of the total energy E are the Bond Potential \(E_b\), Bond Angle Potential \(E_\theta\), the Torsion Potential \(E_\phi\), Van der Waals \(E_{vdW}\), the electrostatic function \(E_{el}\), the Hydrogen Bonds energy \(E_{hb}\), the constraint term \(E_{cr}\), and the dihedral constraint potential \(E_{cf}\).

With CHARMM, we can model a protein based on its amino acid sequence and allow a transition between two known states of the protein using Targeted Molecular Dynamics (TMD) [24]. In our case, the two known states are the native and the fusogenic states of the 36-residue peptide of HA. The structural data on these two states was obtained from Protein Data Bank (PDB) [25]. These PDB files contain the precise molecular make up of the proteins, including the size, shape, angle between bonds, and a variety of other aspects. We used the PDB entries 1HGF and 1HTM respectively, as sources for initial and final states of the peptide. In order to study the energetic feasibility of the actuation process of VPL, we then allowed a targeted change of conformation for the protein from initial to the final state at constant temperature and guided by the root mean square difference (RMSD) in the atom positions between an instantaneous and the final required state. The RMSD was reduced step by step until the desired state or the peptide was attained. The energy plot using solvation function EEF1 [26] is shown in figure 2 and the simulation snapshots for the process are shown in figures 3 and 4.

**Figure 1.** Ribbon drawing of the trimer with the N and the C terminus of one molecule labeled. Also shown is the end view of the trimer, looking down the 3-fold axis. Image courtesy [22].

**Figure 2.** Energy variation for LOOP-36 peptide with salvation model EEF1.
where \(3N\) are the Cartesian coordinates of the position vectors \(\mathbf{x}_i\) at the end of the simulation, the final distance \(\Delta \rho\) is reached. In order to select the cells that were successfully transfected, we grew the cells in the presence of the antibiotic ampicillin. As the plasmids were ampicillin-resistant, only those cells containing the recombinant DNA survived.

Current work focuses on the purification and characterization of the single synthetic protein as an actuator. The bacterial cells will be harvested, lysed, and then clarified by centrifugation. Protein purification will be done by a novel system in which an internal protein segment, a self-cleavable protein-splicing element, is used to separate the target protein from an affinity tag. Protein splicing is a post-translational event in which the internal protein segment, or intein, catalyzes its own excision from the precursor protein and ligates the two flanking regions, exteins, together [27]. In our
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VII. REFERENCES


