Biomimetic Molecules as Building Blocks for Synthetic Muscles – A Proposal

A.H. Flood, J. Badjic, J.-Y. Han, C. Pentecost, B.H. Northrop, S.J. Cantrill, K.N. Houk and J.F. Stoddart

Department of Chemistry and Biochemistry, and the California NanoSystems Institute,
University of California, Los Angeles, 405 Hilgard Avenue, Los Angeles, CA 90095-1569,
stoddart@chem.ucla.edu

ABSTRACT

The goal of this proposal is to cast biomolecular motors as the model for designing wholly artificial motor-molecules to serve as the building blocks for synthetic muscles. At the level of the single molecule, the artificial machines will be constructed from chemically powered motor-molecules with mechanically interlocked components. On the molecular ensemble level, the motor-molecules will be coherently self-organized on to surfaces in order to harness the cooperative operation of each motor-molecule in unison, consuming chemical energy to perform mechanical work up to the micro scale. These structures could be stacked in series to form artificial muscle tissue displaying large distance contraction and extension and to serve as a standard module for analogous applications on the meso scale. In the first phase of this work, the synthesis of the motor-molecules for attachment on surfaces is proposed, together with demonstrable nanoscale actuation as the target deliverable.

Keywords: interlocked molecules, motor-molecules, NEMS, self-organization, switches

1 INTRODUCTION

1.1 Biomimcry – Concept Transfer

Biomolecular motors (BMMs), such as the myosin-actin complex,[1] are nature's machines that convert chemical energy into mechanical work. The performance and scale of these systems are thought[2] to derive from their precise positioning and alignment into organized hierarchical levels beginning at the nanoscale. Moreover, this complex organization is one of the reasons why biomolecular motors are believed to outperform human-made machines.

Mechanistically, the BMMs couple the binding, hydrolysis and release of ATP with concomitant stepwise mechanical movements within an aqueous in vivo environment. Generally, the BMM's cycle of force production occurs through a sequence of alternating power and diffusive strokes.[3] We propose (Figure 1) artificial motor-molecules that operate in non-aqueous solutions, over a range of temperatures, by converting chemical fuel in the form of a proton gradient, into linear mechanical nanometer-scaled movements by a power-diffusive two-stroke cycle. Furthermore, we propose amplifying these motions by self-organizing the motor-molecules on solid surfaces so that they can coherently push and pull much larger objects, like the muscles in our bodies.

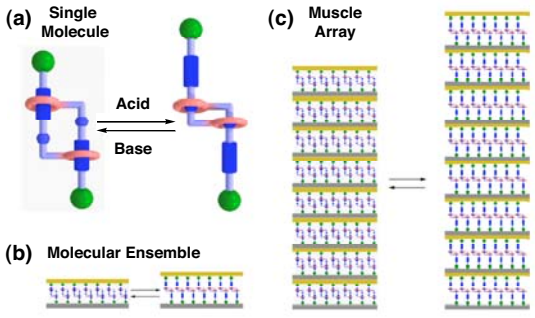


Figure 1. (a) The simple chemically powered contraction and extension of a linear molecular muscle could be (b) integrated into a monolayer assembly that will allow (c) higher order systems to amplify the nanoscale motion for macro scale applications.

1.2 Linear Motor-Molecules

The motor-molecules of interest rely on chemical energy provided by base and acid to switch in a linear manner.[4] This type of switching derives from turning off and on the recognition between a dialkylammonium center (dark blue) and an aromatic crown ether (red). The function of a muscle is to contract and elongate; this relative movement in a molecular context can only be defined when the molecule itself is dimeric.[5] It is known, that when the molecule has both electron rich and poor moieties, the self-assembly of the dimeric [c2]daisy chain[6] is preferred over the entropically unfavorable infinite supramolecular arrays. The initial design is based on a hermaphroditically-shaped muscle motif (Figure 2) with either end differently modified for attachment to gold (disulfide) and silicon (hydroxyl) surfaces accordingly.

2 INNOVATIVE CLAIMS

Wholly artificial molecular motors are a unique class of compounds – only recently developed[2] – that perform relative mechanical movements on the nanometer scale. Their potential has not yet been fully realized, and so, acknowledging that these are "new systems for new applications" it is important to assess all classes of motor-molecules available for the most viable candidates.

Utilization of linear motor-molecules will provide a range of properties and enable a variety of auxiliary functions that will ultimately facilitate their inclusion into an integrated system.

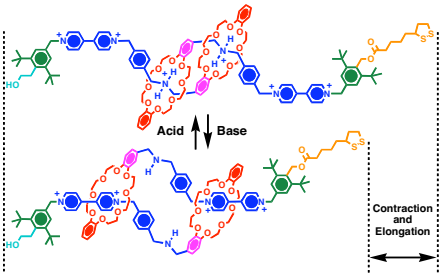


Figure 2. Chemical formulas of the hermaphroditic linear molecular muscle displaying the mechanical ring movement from one recognition station to another

(1) The integrated systems will be obtained by the organization at three hierarchical levels. *Single* linear motor-molecules are synthesized by employing self-organizing protocols. *Many* motor-molecules can be self-assembled into a molecular ensemble on a solid substrate for their coordinated movement and resulting cooperative force production. The force produced and strain observed can be *amplified* in the final level of organization by stacking the self-assembled monolayers on top of each other. The proposed hierarchical organization allows for optimization to be obtained at each level.

(2) The single motor-molecule – synthetically crafted – is tunable and customizable. Operationally, and as envisioned here, the molecules are predicted to produce (1) 10 pN of force, (2) 30% strain in the process of (3) repeatable (4) bistable mechanical switching, that is (5) chemically driven in a (6) "wet" non-aqueous environment, over (7) a range of temperatures (–38 to +62 C). Tunability is available for the strain, force, molecular rigidity and temperature ranges as directed from rational modifications to molecular structure. Customizability will allow (1) more than one length change, i.e., tristability, (2) for the system to be driven by light, such as a laser or sunlight, and (3) for attachment to a variety of substrates.

3 RELATED WORK

The proposed molecular design complements the work already undertaken[7] by Stoddart, Montemagno, Tai and Ho – however, the ammonium system presented here relies on a simple proton gradient or pH-switch, whereas the TTF systems are electrically actuated. Whereas the TTF system has a palindromic motif, the ammonium system is hermaphroditic, implying the optimal vectorial display of moving parts for linear movements. These molecular muscle designs are similar to those of Sauvage[8] but are more rigid, smaller, easier to make and rely on simpler chemical stimuli.

The nanoactuation of the proposed compounds relies on a uniquely different mechanism to other molecular systems. Liquid crystals operate[9] at ~micron scale by changes in the intermolecular alignment within molecular ensembles. Some electrically-activated polymers[10] and carbon nanotubes[11] rely on the diffusive migration of solvent (swelling) or ion migration (electrostatics) which are a phenomena associated with bulk materials rather than single molecular actuation. DNA hybridization[12] is neither reversible nor mechanistically well understood. An alternative contender to the molecular muscles is the photoactuation[13] of an azobenzene dye. The ammonium systems are ripe, however, for photoactivation utilizing excited-state acids and bases, such as $[Ru(bpy)_3]^{2+}$.[14]

4 TIMELINE AND BUDGET

4.1 Timeline

Phase I (18 Months)
(a) Synthesize prototype, characterize switching
(b) Synthesize tethered compounds and self-assemble
(c) Demonstrate surface switching
(d) Self-assemble 3D bulk network
(e) Demonstrate chemically-driven strain in 3D network
(f) Proof of principle light-driven motion
(g) Force spectroscopy and computational modeling
Phase II (18 Months)
(a) Interact with mechanical engineers
(b) Synthesize light-activated system and integrate like the chemically powered system (Phase I (a-d))
(c) Optimize strain
(d) Demonstrate force
(e) Demonstrate muscle module
(e) Demonstrate light-driven strain in 3D network
Phase III (12 Months)
Deliverable – Light-driven muscle module

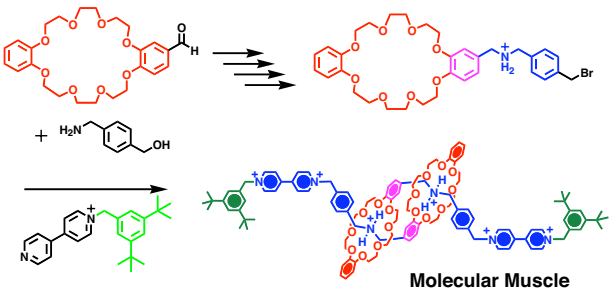
4.2 Budget

Phase I ($450K) – 2 Grad, 1 Postdoc, supplies
Phase II ($300K Chemistry) – 1 Grad student and 1 Postdoc plus supplies + $500K Engineering
Phase III ($300K Chemistry) – 1 Grad student and 1 Postdoc plus supplies + $500K Engineering
Total = $2.05M

5 TECHNICAL APPROACH

5.1 Synthesis of Linear Molecular Muscles

The molecular structure of the proposed molecular muscle consists of an electron rich ring in the shape of a dibenzo[24]crown-8 (DB24C8) macrocycle, and two electron poor sites – a secondary dialkylammonium (NH_2^+) center and a bipyridinium ($Bpym^{2+}$) unit – to which the electron rich ring can bind. A convergent synthetic route[4] (Scheme 1) was utilized to make the molecular muscle. Careful reaction conditions gave the DB24C8-based molecular muscle (DB-MM) in 14 % yield in five steps and



Scheme 1. Synthetic route used to obtain the molecular muscle in 14% yield.

following a chromatographic purification process. Compared to the molecular muscles made by Sauvage,[8] many fewer synthetic steps are required in order to assemble the final molecular muscle compound. Moreover, it still shows the same functions – contraction and elongation – upon the addition of stimulus, which, in this case, are acids and bases. Based on variable temperature ^{1}H-NMR spectroscopic studies, it has been concluded that the DB24C8 macroring is selectively bound to the NH_2^+ center from 235 to 335 K.

Fully reversible switching of the DB-MM dimer induces a length change, i.e., strain, of ~1 nm (29 % change).

The operation of this molecular muscle can be further extended into its self-assembly on solid surfaces by substituting the end groups, with disulfide or hydroxy substituted end groups.

5.2 Self-Assembly of Linear Molecular Muscles on Solid Surfaces

There is a growing range of molecular tethers for attachment[15] to solid substrates – disulfides on gold, polypodal carboxylates and phosphates on TiO_2 and SnO_2, and hydroxyl for silica (SiO_2) and chloro for silicon. Initially, disulfide-tethered molecular muscles will be utilized for self-assembly on gold using standard protocols.

5.3 Switching Analyses

Addition of a slight molar excess of a weak base, e.g., Et_3N or Bu_3N, which deprotonates the NH_2^+ center, causes the DB24C8 to move – i.e., the components slide with respect to each other – to the $Bpym^{2+}$ unit which can now provide stronger binding (Figure 2). This process can be observed either by UV-vis or ^{1}H-NMR spectroscopy. For example, the solution's appearance changed reversibly from clear to yellow when the base was added to the solution, indicating the creation of a charge-transfer interaction between the DB24C8 ring component and the $Bpym^{2+}$ unit.

Emission spectroscopy will used to probe the switching behavior of the system at the molecular level[16] on various surfaces. Fluorescein will be incorporated into the monomer unit to serve as both a stopper and fluorescent probe. A disulfide tether, allowing for the attachment to a gold surface, will be appended to this stopper at least six carbons long in order to prevent quenching of the fluorescent probe by the gold.[17] The propensity of the aryl groups on the crown ether to π-π stack with any neighboring aryl groups, such as fluorescein, will facilitate the distinction between the extended and contracted states, as such π-π stacking interactions give rise to wavelength shifts in the emission spectra. The displacement incurred upon switching will be measured quantitatively using florescence resonance energy transfer (FRET) on the molecular muscle system, assymetrically stoppered with fluoroscein/rhodamine as the donor/acceptor pair. To monitor switching on a topographical level, characterization of the functionalized surfaces will be obtained using X-ray reflectivity, AFM, and SPR.[18]

5.4 Force Spectroscopy and Simulation

The force exerted by molecular muscles will be measured by AFM under contracted (base) and extended (acid) conditions. Dynamic force spectroscopy[19] will be utilized to probe host-guest interactions under thermodynamic equilibrium. Computations using the AMBER force-field and dynamics will assess free energy changes upon switching under acid/base conditions, as well as the details of molecular motions occurring in these processes.

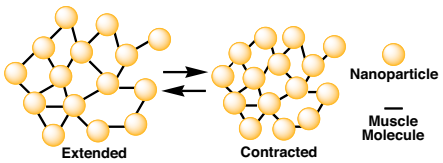


Figure 3. Extension and contraction of a bulk network of gold nanoparticles interconnected with muscle molecules.

5.5 Self-Assembly of Linear Molecular Muscles into a Random 3D Network

An interconnecting network (Figure 3) of muscle molecules and gold nanoparticles will be formed in order to provide a simple platform for the demonstration of strain in a bulk material. Two alternative protocols can be envisioned to self-assemble the network. The first relies on mixing bare gold nanoparticles with nanoparticles that are precoated with muscle molecules in which one of the two tethers is unbound. For example, a free disulfide could be displayed by utilizing either (1) two different tethers on the molecule, or (2) by optimizing the conditions (solvent, temperature, salt conc.) using a symmetrically tethered molecule so that one end is always free. Alternatively, stable solutions of nanoparticles coated with muscle molecules displaying hydroxyl groups may be esterified with disulfide tethers in situ and subsequently mixed with bare gold nanoparticles. Standard dynamic light scattering (DLS), TEM and UV-vis spectroscopic measurements will be employed to characterize the resulting assemblies.

5.6 Strain Demonstration in a Bulk Network

Changes in the size of the bulk network (Figure 3) will be determined by utilizing measurements during stimuli cycling that reflect (1) the overall size of the network, such as DLS or TEM samples, (2) the inter-nanoparticle distances, by UV-vis spectroscopy of the gold plasmon resonances, and (3) fluorescence to correlate the observed strain to molecular actuation.

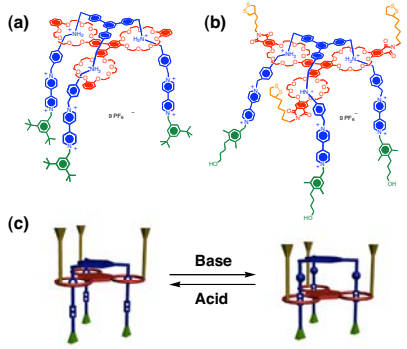


Figure 4. (a) Structural formulas of a molecular elevator,[20] (b) customized for (c) pulling two surfaces together.

5.7 Conceptual Extension

A second motif is conceptually formulated as a *molecular elevator* (**Figure 4a**).[20] The presence of three legs provides an internal amplification of the force produced from each extension and contraction cycle. The inclusion of the disulfide and hydroxyl units will again be utilized for surface attachment. In this way, not only is the force produced from a single motor enhanced.

Light-driven actuation is attractive for applications and so a series[14] of photogenerated acids and bases will be assessed for their facility to switch model systems, and then ultimately integrated into molecular muscles.

6 TECHNOLOGY TRANSFER

Success during Phase I anticipates the formation of a multidisciplinary team involving engineers and the emergence of commercial interests. These collaborations will rely upon constant feedback, allowing for an integrated systems-oriented approach to evolve an actuating muscle module for delivery into a niche product in the marketplace.

7 RELEVANCE TO DOD

In the first generation, pH-driven, single use modules with tunable force may deliver fast (ms) unparalleled, ultra-high density, light weight actuating material for deployment in the field. Recycling of the system will be possible upon addition of base. In the second generation, utilizing a photo-activated pH gradient, the contraction and extension could be cycled many times. These modules will serve niche or bulk deployment across a range of length scales.

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