SELF-ASSEMBLED NANOMACHINES FOR PROGRAMMABLE SENSING AND ACTUATION

Gabriel Lavella and Michel M. Maharbiz
University of California, Berkeley, California, USA

ABSTRACT

We demonstrate a Brownian ratchet architecture that can be used to create molecular machines capable of sensing and actuation. The machines are programmable and autonomous in the sense that the chemomechanical functionality and energy source can be readily tailored. Machines were self-assembled in a one pot reaction from a set of oligonucleotides and hapten molecules using honeycomb array DNA origami.[1], [2] This technique, allowed us to obtain a sophisticated level of control over the structural topology and consequently the energy landscapes that give rise to Brownian ratchet transduction phenomena. The self-assembled devices measure ~ 100 nm in length and we set to transduce the detection of a streptavidin ligand into mechanical motion.

INTRODUCTION

Brownian ratcheting is a mechanism that can be used to create mechanically functional and autonomous molecular machines. Evidence for this exists in the multitudes of chemomechanical ratchets in nature and the wide range of tasks they perform[3], [4]. Well known examples include the actin ratchets used in filopodia protrusion[5], [6] and *Lysteria monocytogenes* locomotion[7], [8]. They distinguish themselves from molecular motors, such as myosin and kinesin, which operate on mechanochemical cycles, and acquire energy for work from the hydrolysis of ATP[9]. Instead, ratchets are non-cyclic, obtain energy from polymerization reactions, and achieve directed motion by rectifying random substrate movements with asymmetries in the energy landscape[10], [11]. While many natural ratchets have been studied in the context of their biological function, the technological pursuit of synthetic chemomechanical ratchets has garnered little attention. By comparison, molecular motors are being heavily pursued with applications in nanoscale transport in mind[10], [12]. Several synthetic motors have been produced; many of these are walking devices that move along artificial tracks and are fueled in artificial environments with DNA strands through hydrolysis[13], [14] or hybridization[15], [16]. The most ambitious efforts toward synthetic chemomechanical machine development focus on understanding how to design machines from scratch through direct engineering of macromolecules; ideally designing for function, operating environment and energy source. Nevertheless, before this vision is realized a multitude of challenges in molecular structure[17], [18] and function prediction[19] must be overcome.

Here we demonstrate a molecular machine based on a synthetic Brownian ratchet architecture that senses, mechanically reacts and extracts energy from a streptavidin ligand. Our architecture avoids the complexities of macromolecule design by using molecular assemblies. In addition it can allow user selection of the chemomechanical functionality and the energy source. These two characteristics result in a machine that can be adapted for the desired task and the application environment, respectively. We achieve this through topology, tailoring of energy landscapes, and a cascade of ligand-receptor binding events that serve as the Brownian ratchet polymerization reaction. This distinguishes our architecture from natural systems, where the polymerization species is often structural and enables the chemomechanical functionality to be chosen during design by selecting a ligand-receptor pair.

Figure 1: Device topology and operational mechanism. The backbone is shown in grey, receptors molecules are shown in orange, and the sensed ligand is shown in blue. (State X_1) device and notional energy landscape prior to encountering a ligand; (State X_2) after ligand introduction and sandwich bond formation; (State X_n) device in a completely ratcheted state. (bottom) Transition between local equilibriums at the level of a single sandwich bond complex.

THEORY

Our architecture consists of a clip shaped backbone (shown in grey, Fig. 1) conjugated with receptor molecules (orange, Fig. 1). Before the introduction of ligand molecules, this structure thermally vibrates in accord with the flexure characteristics of the backbone and the temperature of the bath (Fig. 1, State X_1). The

thermal vibrations, introduce potential energy shifts, ΔU , originating from the strain energy in the backbone and stretched molecular complexes. These changes cause the ratchet to move along the Brownian coordinate, χ , which we have defined as the symmetric displacement of the tips; χ increases as the device moves toward a closed state.

An alternative path in the energy landscape is created though the bonding of a ligand to one of the receptors from the first pair. This event enables a transition between states, X_{1-2} by presenting the possibility of the formation of a sandwich bond (such as in Fig. 1, III). For this transition to occur the random thermal input must surmount the forward energy barrier, $U_{1,for}$. In addition a bound/unbound receptor configuration exists between opposing receptors. These configurations arise when the reaction rate for sandwich bond formation is sufficiently higher than the reaction rate for ligand-receptor binding.

Upon the a new potential well, State X_2 is created, with a new equilibrium position, $\chi_{2,eq}$, advanced in the closing direction and bound by the reverse, $U_{2,rev}$, and forward, $U_{2,for}$, energy barriers. Energy is extracted for each of these transitions from the ligand-receptor binding free energy. This extraction can be accounted for with an additional term in the Gibbs free energy equation [20–22] and occurs during the simultaneous relaxation of the backbone and tensioning of the sandwich bond (Fig 1, bottom). As a result of the distribution of random thermal vibrations, not all receptor pairs need to form bonds, ratcheting can proceed by leapfrogging. Sequential transitions proceed until the ratchet is closed (Fig. 1, State X_n). In essence the ratchet moves through a progression towards a lower global equilibrium with the aid of thermal vibrations.

SELF-ASSEMBLY AND DEVICE TOPOLOGY

We created devices with similar topologies to those presented in Fig. 1. Device were made using the honeycomb array DNA origami[1], [2] wherein a backbone was formed from 10 B-form DNA helices stressed by base pair insertions and deletions (Fig. 2, top). Each helices was joined to its three neighbors through Holiday junction crossovers every 21 bp. The backbone design was modified by eliminating sequences along the center helix and inserting toehold strands (44 bp long) 5' modified with biotin (receptors).

Self-assembly reactions were performed in a 100 μ l reaction composed of 5 mM Tris, 1 mM EDTA and 18 mM of $MgCl_2$ and contained 200 nM of all staple and toehold strands and 20 nM of the scaffold strand[23]. The reaction was thermally annealed (Applied Biosystems 2720, Thermal Cycler) over a linear temperature gradient from 80°C to 60°C for two hours and over linear gradient from 60°C to 24°C for 156 hours.

Negative stain TEM images of self-assembled device ensembles (Fig 2, middle) show correctly formed devices. These devices were extracted from the gel band highlighted in (Fig 2, middle). Extraction was conducted by cutting the band from the gel, pesteling, and conducting freeze and squeeze purification. The purified devices could then be stored for several months and later used in the experiments.

Using this approach, four device variants with different receptor/toehold configurations were assembled; two active device sets, and two controls. The active device, with receptor spacings of 7nm and 14nm (Fig. 2, bottom) were expected to ratchet. While the two control sets (Fig. 2, bottom), possessed a high forward energy barrier such that closing could not occur in the absence of ratcheting phenomena. In control A, the middle 6 receptors were eliminated, while in control B we removed the first nine receptor pairs.

Figure 2: Molecular topology and receptor configurations for device variants. (top) Backbone topology schematic showing B-form DNA helix configuration and placement of toeholds/receptors; (middle) TEM of fabricated device ensemble, excised from gel and negatively stained, scale bar = 100 nm and agarose gel characterization of all device variants before ligand introduction; (bottom) device variant receptor/toehold configurations

RESULTS

In order to investigate the response we introduced the tetravalent streptavidin ligand into a solution containing devices in a ratio of $1:10^3$ (device:streptavidin). The reaction was carried out in a solution containing 0.25x TBE and 0.5x PBS pH 7.4; the temperature was controlled at 25°C and was allowed to proceed for twenty minutes before aliquots were removed placed on a TEM grid for inspection. Negative stain TEM images show three primarily reaction products; ratcheted devices, devices which did not ratchet and devices which formed dimers (Fig. 3, bottom). In all devices the tetravalent streptavidin is visibly bound to the

Figure 3: Analysis of device response to streptavidin ligand. Concentration ratios (device:ligand) were $1:10^3$ (top) agarose gel image of reaction products for all device variants, the ratcheted band is highlighted (bottom) Negative stain TEM images of reaction products: ratcheted devices (~ 475 bp), devices that failed to ratchet (~ 2.2 kbp), and device dimers (~ 4.4 kbp) all scale bars are 50 nm. In all images the ligand can be seen as a white dot.

toehold linked receptors. On the ratcheted device, streptavidin can be seen down the centerline.

Following identification of the products, gel analysis was performed on the reaction solution. Gels contained 1.75% Agarose and 11 mM MgCl_2 . We used a 0.5x Tris/Borate/EDTA (45mM Tris-borate, 1 mM Na_2EDTA) running buffer containing 11 mM MgCl_2 . Electrophoresis was run for 2.5 hours at 4 V/cm (Thermo Scientific Owl B2 EasyCast Mini Gel System). After electrophoresis, the gel was removed and then stained using SYBR GREEN II (Invitrogen | Molecular probes) and imaged.

Inspection of the gel shows the primary product bands for the device variants with 7 nm and 14 nm receptor spacings. The dimer band (~ 4.4 kbp) can be seen at approximately twice the position of the unreacted band (~ 2.2 kbp); a smearing of this band occurs as the presence of streptavidin slows the device migration in the gel. The ratcheted device band (~ 475 bp) migrates at a significantly faster speed in the gel. This occurs as a result of the streamlined configuration of the ratchet device. We excised and imaged this

Figure 4: Agarose gel analysis of reacted devices with 7nm receptor spacing over a range of concentration ratios (ligands:devices) from $1:10$ to $1:10^5$ (top) gel image; (middle) intensity analysis for trimer band, b1, and dimer band, b2; (bottom) intensity analysis for ratcheted bands b3 and b4.

band and confirmed the presence of ratchet devices. Control devices, which presented high forward energy barriers, did not ratchet as indicated by the lack of a forward band. The absence of closed devices for the controls strongly indicates that ratcheting must present for device actuation.

We subsequently inspected the 7 nm device response over a logarithmic concentration ratio range from $1:10^1$ (Fig 4, lane c1) to $1:10^5$ (Fig 4, lane c5). The device response is compared to the reference lane, c0, for which the reaction contained no streptavidin. The aggregate of the ratcheted bands, b4 + b3 (more heavily bound with streptavidin), reaches a maximum at $1:10^3$ and then remains relatively constant at higher concentrations. This result demonstrates a robustness of the device operation even in the presence of a high ligand concentration. The expectation that large quantities of ligand might cause receptor saturation may be offset by faster reaction kinetics from sandwich bond formation than the kinetics of ligand binding from the bulk solution. Interestingly, the contrary condition would conceivably result in a device that operated over a concentration range. Dimers, band b2, and trimers, band b3, peak and gradually from concentrations of $1:10^2$.

DISCUSSION

Ratchet designs, such as these, can become more sophisticated with improvements in our ability to model the energy landscapes and control molecular assemblies. There applications are apparent when considering the sensing and actuation can be coupled to technologically useful outputs. For instance, the coupling of a secondary molecule release can yield new technologies such as programmed artificial chemical to chemical transduction; a space which holds the promise of chemical logic, artificial signaling between cells, signal amplification, and new forms of diagnostics.

REFERENCES

- [1] H. Dietz, S. M. Douglas, and W. M. Shih, "Folding DNA into twisted and curved nanoscale shapes.," *Science (New York, N.Y.)*, vol. 325, no. 5941, pp. 725-30, Aug. 2009.
- [2] S. M. Douglas, H. Dietz, T. Liedl, B. Högberg, F. Graf, and W. M. Shih, "Self-assembly of DNA into nanoscale three-dimensional shapes.," *Nature*, vol. 459, no. 7245, pp. 414-8, May 2009.
- [3] S. Cruz and C. Biology, "Ratchets , power strokes , and molecular motors," vol. 323, pp. 315-323, 2002.
- [4] L. Mahadevan, "Motility Powered by Supramolecular Springs and Ratchets," *Science*, vol. 95, no. 2000, 2008.
- [5] C. S. Peskin, G. M. Odell, and G. F. Oster, "Cellular motions and thermal fluctuations: the Brownian ratchet.," *Biophysical journal*, vol. 65, no. 1, pp. 316-24, Jul. 1993.
- [6] a. Fiasconaro, W. Ebeling, and E. Gudowska-Nowak, "Active Brownian motion models and applications to ratchets," *The European Physical Journal B*, vol. 65, no. 3, pp. 403-414, Jul. 2008.
- [7] J. a Theriot, T. J. Mitchison, L. G. Tilney, and D. a Portnoy, "The rate of actin-based motility of intracellular *Listeria monocytogenes* equals the rate of actin polymerization.," *Nature*, vol. 357, no. 6375, pp. 257-60, 21-May-1992.
- [8] L. G. Tilney and D. a Portnoy, "Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*," *The Journal of cell biology*, vol. 109, no. 4 Pt 1, pp. 1597-608, Oct. 1989.
- [9] E. R. Kay, D. A. Leigh, and F. Zerbetto, *Molecular Devices Synthetic Molecular Motors and Mechanical Machines Angewandte*. 2007, pp. 72 - 191.
- [10] P. Hänggi, "Artificial Brownian motors: Controlling transport on the nanoscale," *Reviews of Modern Physics*, vol. 81, no. 1, pp. 387-442, Mar. 2009.
- [11] L. Mahadevan, "Motility Powered by Supramolecular Springs and Ratchets," *Science*, vol. 288, no. 5463, pp. 95-99, Apr. 2000.
- [12] H. Hess, H. Hess, G. D. Bachand, and V. Vogel, "Powering Nanodevices with Biomolecular Motors," 2004.
- [13] Y. Tian, Y. He, Y. Chen, P. Yin, and C. Mao, "A DNAzyme that walks processively and autonomously along a one-dimensional track.," *Angewandte Chemie (International ed. in English)*, vol. 44, no. 28, pp. 4355-8, Jul. 2005.
- [14] J. Bath, S. J. Green, and A. J. Turberfield, "A free-running DNA motor powered by a nicking enzyme.," *Angewandte Chemie (International ed. in English)*, vol. 44, no. 28, pp. 4358-61, Jul. 2005.
- [15] W. B. Sherman and N. C. Seeman, "A Precisely Controlled DNA Biped Walking Device," *Nano Letters*, vol. 4, no. 7, pp. 1203-1207, Jul. 2004.
- [16] T. Omabegho, R. Sha, and N. C. Seeman, "A bipedal DNA Brownian motor with coordinated legs.," *Science (New York, N.Y.)*, vol. 324, no. 5923, pp. 67-71, Apr. 2009.
- [17] C. a. Floudas, H. K. Fung, S. R. McAllister, M. Mönnigmann, and R. Rajgaria, "Advances in protein structure prediction and de novo protein design: A review," *Chemical Engineering Science*, vol. 61, no. 3, pp. 966-988, Feb. 2006.
- [18] a K. Dunker et al., "The unfoldomics decade: an update on intrinsically disordered proteins.," *BMC genomics*, vol. 9 Suppl 2, p. S1, Jan. 2008.
- [19] S. M. Lippow and B. Tidor, "Progress in computational protein design.," *Current opinion in biotechnology*, vol. 18, no. 4, pp. 305-11, Aug. 2007.
- [20] C. Bustamante, Y. R. Chemla, N. R. Forde, and D. Izhaky, "Mechanical processes in biochemistry.," *Annual review of biochemistry*, vol. 73, pp. 705-48, Jan. 2004.
- [21] I. Tinoco and C. Bustamante, "The effect of force on thermodynamics and kinetics of single molecule reactions.," *Biophysical chemistry*, vol. 101-102, pp. 513-33, Dec. 2002.
- [22] D. Keller and C. Bustamante, "The Mechanochemistry of Molecular Motors STOCHASTIC MACHINES," *Biophysical Journal*, vol. 78, no. February, pp. 541-556, 2000.
- [23] C. E. Castro et al., "a primer to scaffolded DNA origami," *Nature Methods*, vol. 8, no. 3, pp. 221-229, 2011.

CONTACT

*Gabriel J. Lavella, tel: 510.664.4144; glavella@eecs.berkeley.edu