The Tumbleweed: towards a synthetic protein motor

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Biomolecular motors have inspired the design and construction of artificial nanoscale motors and machines based on nucleic acids, small molecules, and inorganic nanostructures. However, the high degree of sophistication and efficiency of biomolecular motors, as well as their specific biological function, derives from the complexity afforded by protein building blocks. Here, we discuss a novel bottom-up approach to understanding biological motors by considering the construction of synthetic protein motors. Specifically, we present a design for a synthetic protein motor that moves along a linear track, dubbed the “Tumbleweed.” This concept uses three discrete ligand-dependent DNA-binding domains to perform cyclically ligand-gated, rectified diffusion along a synthesized DNA molecule. Here we describe how *de novo* peptide design and molecular biology could be used to produce the Tumbleweed, and we explore the fundamental motor operation of such a design using numerical simulations. The construction of this and more sophisticated protein motors is an exciting challenge that is likely to enhance our understanding of the structure-function relationship in biological motors. [DOI: 10.2976/1.3111282]

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Biomolecular motors are predominantly multi-subunit proteins, which transduce chemical energy into mechanical work. They are central to many cellular processes and functions, including DNA replication and repair, protein synthesis, cell division, intracellular transport, cell motility, and ion pumping. It is therefore a major goal in structural molecular biology to develop a clear biochemical and biophysical understanding of the general operational principles of such motors.

Experimental studies of biological motors using techniques such as protein-structure analysis, kinetic studies, genetic modification, and single-molecule experiments (Svoboda *et al.*, 1993; Finer *et al.*, 1994; Noji *et al.*,

1997; Kitamura *et al.*, 1999; Greenleaf *et al.*, 2007) have provided the basis for several models of biological motors and machines (Howard, 1997; Jülicher *et al.*, 1997; Vale and Milligan, 2000; Bustamante *et al.*, 2001; Kolomeisky and Fisher, 2007). These include models of several types of myosin (Finer *et al.*, 1994; Kitamura *et al.*, 1999; Rief *et al.*, 2000; Sellers and Veigel, 2006; Craig and Linke, 2009), ATP synthase (Noji *et al.*, 1997; Wang and Oster, 1998), kinesin (Rice *et al.*, 1999; Schnitzer *et al.*, 2000; Block, 2007), and polymerases (Guajardo and Sousa, 1997; Jülicher *et al.*, 1997; Wang *et al.*, 1998; Bar-Nahum *et al.*, 2005; Kafri *et al.*, 2005).

In a more general approach, the physics community has established the conditions under which a molecular-scale object exposed to substantial random (thermal) motion can perform directed motion in the absence of a macroscopic chemical or force gradient (Astumian and Hänggi, 2002). In this context, molecular motors are classified as ratchets (Vale and Oosawa, 1990; Astumian, 1997; Jülicher *et al.*, 1997; Nishiyama *et al.*, 2003) or Brownian motors (Astumian, 1997; Reimann and Hänggi, 2002), that is, systems that combine local asymmetry, thermal noise, and a deviation from thermal equilibrium to induce directed motion in the absence of macroscopic forces or gradients. The operational principles of Brownian motors, including the constructive role of thermal noise, as well as their relationship to biological motors are now well understood (Jülicher *et al.*, 1997; Astumian, 2007).

Inspired by biological motors and by the physics of Brownian motors, several approaches to synthetic molecular motors are being developed, either via the synthesis of small molecules (Kottas *et al.*, 2005; Balzani *et al.*, 2006; Feringa, 2007; Kay *et al.*, 2007) or by using oligonucleotides as building blocks (Bath and Turberfield, 2007; Yurke, 2007). The latter approach has already led to “spider” designs, consisting of a hub with DNA legs capable of moving along a DNA-functionalized surface (Pei *et al.*, 2006), as well as DNA “walkers” capable of performing steps along a DNA track in either a non-autonomous (Shin and Pierce, 2004) or free-running fashion, fueled by DNA or RNA strands (Yin *et al.*, 2004; Bath *et al.*, 2005; Bath and Turberfield, 2007). These nucleic-acid-based approaches to artificial molecular motors are very promising because relatively straightforward design rules can be used to construct nanoscale devices by deterministic self-organization. At the same time, however, the properties and design rules of RNA and DNA motors are likely to be different from those of protein motors, limiting their usefulness as model systems for biological motors.

Here we introduce and develop a pathway to synthetic protein-based motors, with the goal of establishing a system that will allow us to perform controlled experiments on the relationship between structure and function in protein motors. This approach is challenging, but lays a foundation for taking advantage of the great mechanical, kinetic, and structural versatility and specificity afforded by Nature’s material of choice for nanoscale machines. Our strategy uses rational peptide design and molecular biology to combine nonmotor protein domains in such a way that motor function results. Since the design details are known precisely, the resulting motor designs are amenable to detailed numerical and analytical modeling, even though their complexity may ultimately approach that of a biological system. Models of these motors can then be compared to results from single-molecule experiments. This novel bottom-up route tests our understanding of structure-function relationships in natural

motors, in the spirit of the Richard Feynman quote “*What I cannot create, I do not understand*” (Feynman, 1989).

We begin by outlining general requirements for the construction of a linear protein motor. We then present an example of a synthetic protein motor design that could be constructed using currently accessible methods in peptide chemistry and molecular biology. Specifically, we introduce the “Tumbleweed,” a three-legged self-assembled protein complex designed to move by cyclically ligand-gated, rectified diffusion along a DNA track, using three discrete ligand-dependent DNA-binding domains to control binding and unbinding events. Next, we explore a specific approach for the synthesis and self-assembly of what may become the first synthetic protein motor. Finally, we present numerical simulations of this concept that demonstrate processivity, we estimate the expected motor speed, and we establish design criteria.

DESIGNING A MOTOR

We begin by considering what it would take to construct a synthetic, linear motor akin to kinesins, dyneins, or myosin V. The following are the hallmarks and general properties of this broad class of natural motors: (i) they perform linear motion along polymeric tracks with built-in polarity, in the absence of macroscopic forces or gradients; (ii) their processivity is typically enabled by coordinated binding and unbinding of multiple track-binding sites; (iii) they tolerate, or even exploit, thermal motion within their degrees of freedom; (iv) driven by an enzymatic cycle, they move autonomously through ligand-binding states coupled to mechanical states; and (v) some of these motors use long-range interactions and/or mechanical levers to create a working stroke that increases motor performance (Howard, 2006).

Natural motors achieve the above properties necessary for processive motion via a series of conformational rearrangements that correspond to different ligand-binding states. For example, the motor domain of kinesin has subtly different conformations in up to five different ligand-dependent states (involving three different possible nucleotide bound states and two track binding states) (Schief and Howard, 2001). It is these conformational changes that are thought to provide both the symmetry-breaking information and the mechanical stress required to move the motor forward efficiently (Asbury, 2005). To design a synthetic protein-based motor with all or most of the above hallmarks, entirely from the bottom up, challenges the current capabilities of protein engineering and design.

The protein-design field has recently matured to a point where novel amino-acid sequences that fold to a single conformation can be designed reliably (Bromley *et al.*, 2008). Globular protein folds have been made using both sequence-based (Poole and Ranganathan, 2006) and structure-based algorithms such as Rosetta Design (Liu and Kuhlman, 2006). The latter of these has also been used to design novel globu-

lar protein structures (Kuhlman *et al.*, 2003) as well as enzymes for non-natural substrates (Jiang *et al.*, 2008). There are, however, relatively few examples of sequences designed to fold to, or switch between, more than one conformation (Lippow and Tidor, 2007). Of these, examples include switches from alpha-helix to beta-sheet; from ordered to disordered; and conformational rearrangement on metal binding (Ciani *et al.*, 2002; Pandya *et al.*, 2004; Cerasoli *et al.*, 2005; Ambroggio and Kuhlman, 2006). However, in each of these cases the switch is between only two states and is triggered by significant changes in solution conditions.

As it is not currently possible to design an amino-acid sequence that encodes the full complexity of conformations and activity necessary for motor function, we focus on an approach that combines *de novo* protein design and traditional protein engineering. We propose the use of natural protein domains that possess the ability to bind the track selectively, and combine them with novel domains that specify self-organization in such a way that the resulting assembly realizes the motor hallmarks (i)–(iii) defined above. Figure 1 shows the basic conceptual idea that we will develop herein: the Tumbleweed (TW) will be a self-assembled protein complex designed to move by cyclically ligand-gated, rectified diffusion along a DNA track.

The key design features of the TW are three discrete ligand-dependent DNA-binding domains (R_A , R_B , R_C), which are to be mechanically coupled via a hub to define a step size. Specific target sites (A, B, C) are spaced and ordered appropriately along a synthesized DNA track. Directed, processive tumbleweedlike motion is expected to be induced by externally supplying ligands (a, b, c) in the temporal order [a, b], [b, c], [c, a], such that at all times at least one domain is bound to the track. For instance, if the motor is initially bound to the DNA track in the presence of ligand pulse [a, b], domains R_A and R_B will be bound to sites A and B, respectively. As domain R_A unbinds from site A during the subsequent pulse [b, c], R_B stays bound to site B while the motor executes diffusional motion such that it will eventually bind to the downstream site C as dictated by the track polarity. The resulting motor uses no working stroke, and motion along the track is by stepwise rectified diffusion, powered by the externally controlled changes in chemical potential.

SYNTHESIS ROAD MAP

To illustrate that the synthesis of an artificial molecular motor is feasible with the current state of the art, we develop a road map for the experimental realization of a TW motor. First, we identify “feet”—ligand-gated, DNA-binding protein domains—that interact specifically and controllably with a DNA track. Next, we discuss the design of a “hub” and an approach to linking the feet to the hub in the correct configuration and with appropriate flexibility. This is followed by remarks on design criteria for the DNA track.

The TW feet must comprise three different DNA-binding

Figure 1. Illustration of the basic concept of the Tumbleweed. Three different DNA-binding protein domains R_A , R_B , R_C bind only in the presence of their respective ligand. By externally cycling the concentration of ligands in appropriate time sequence, the TW can be made to perform directed diffusion along a DNA track with the specific recognition sequences.

protein domains whose binding to distinct sequences is orthogonally gated by three different ligands. In addition, their native oligomeric state should be no larger than a dimer to facilitate coupling to a hub using standard molecular-biology techniques. Based on a comprehensive search of the Protein Data Bank (Berman *et al.*, 2000), the following three

Figure 2. The proposed TW feet and hub. (a) The crystal structures of repressor proteins bound to DNA: (i) the MetJ repressor taken from PDB (Berman *et al.*, 2000) accession code 1MJM; (ii) the TrpR repressor taken from 1TRO; and (iii) the PurR repressor taken from 1QPZ. (b) A Y-shaped hub design and a triangular hub design, each comprising three sequences designed to self-assemble into three coiled-coils.

repressors from *Escherichia coli* form a suitable set: a mutant form of the methionine repressor MetJ (Q44K) (Garvie and Phillips, 2000); the tryptophan repressor TrpR; and the purine repressor PurR [Fig. 2(a)]. These fulfill our orthogonality criterion: first, by binding to different recognition sequences on DNA, and, second, by the dependence of binding on three different ligands, S-adenosyl methionine, L-tryptophan, and hypoxanthine or guanine, respectively. Furthermore, each of these three repressors adopts a different mode of binding to operator DNA [TrpR: helix-turn-helix major groove binding (Otwinowski *et al.*, 1988); PurR: major and minor groove binding (Glasfeld *et al.*, 1999); MetJ: beta ribbon binding major groove (Garvie and Phillips, 2000)], which is useful in optimizing cognate DNA sequences to minimize nonspecific binding.

Ligand-repressor binding is thought to modulate the affinity of these repressors for specific binding to DNA either allosterically by conformational change in the repressor [TrpR (Schevitz *et al.*, 1985; Zhang *et al.*, 1987) and PurR (Nagadoi *et al.*, 1995; Schumacher *et al.*, 1995)], or by altering the electrostatic potential between the repressor and the DNA backbone and “freezing in” a given conformation [MetJ (Cooper *et al.*, 1994; Parsons *et al.*, 1995)].

Thermodynamic analyses have shown that $K_d \sim \text{nM}$ for specific binding of these repressors to DNA in the presence of excess ligand (Carey, 1988; Rolfes and Zalkin, 1990; Borden *et al.*, 1991; Phillips and Phillips, 1994). In the absence of available ligand, the binding affinity of the repressor for DNA decreases 100–1000-fold (Carey, 1988; Borden *et al.*, 1991; Phillips and Phillips, 1994). This nonspecific

binding in the absence of ligand is weak and can likely be controlled by ionic strength (Reedstrom *et al.*, 1997). This modulation of binding affinities by ligand concentration suggests that we can both bind our chosen repressor to the track during a ligand pulse and unbind it rapidly by removing the bulk ligand supply.

To specify the self-assembly of the motor and create the mechanical coupling between the feet we consider a hub constructed of linked dimeric coiled-coil segments. Coiled coils are naturally occurring oligomerization domains in which two or more amphipathic α -helices wrap around each other in a super coil (Lupas, 2005). The individual α -helices can be considered as building blocks (or *tectons*) that contain the information necessary for self-assembly such as oligomer state and partner specificity, for example (Bromley *et al.*, 2008). They lend themselves well to this application as sequence-to-structure rules have been extensively explored, and coiled coils with a range of oligomerization states (dimer, trimer, tetramer), orientations (parallel or antiparallel), and partner specificities (homo or hetero) can be designed with considerable success (Woolfson, 2005). Additionally, coiled-coil structures are well suited for the hub because of their structural rigidity.

Figure 2(b) shows two possible hub configurations: the first Y-shaped hub comprises three polypeptide sequences designed to fold such that two parallel and one antiparallel dimeric coiled coils are made; the second triangular hub consists of three sequences all designed to form parallel dimeric coiled coils. Although both of these hubs would assemble the repressors in the required configuration, they have different geometrical constraints. Flexibility in the linking regions between the coiled-coil units will allow much greater movement of the tethered repressors in the Y-shaped hub than the triangular hub. This increase in flexibility may facilitate the binding of the repressors to the track, but could also be expected to reduce the overall motor speed.

The TW motor can be synthesized using existing molecular-biology methods. The DNA repressors are readily over expressed in *Escherichia coli* to produce milligram quantities of protein, and standard genetic manipulation techniques can be used to link synthetic oligonucleotides encoding for linkers and coiled-coil sequences to repressor gene sequences. This will result in the creation of single constructs to encode for modules consisting of a coiled coil and a repressor unit joined by a linker. The linkers must be sufficiently long to minimize disruption to the function of the domains and may either be rigid or flexible in nature. Again, some flexibility may be required to allow binding of the feet to the track, but will also influence diffusion times as will be discussed in the modeling section below. Constructs where each monomer in the repressor dimer requires a different coiled-coil extension can be made using the pET-DUET system (Novagen).

The DNA track can be synthesized by cloning a repeating

Figure 3. Motor models used in simulations. (a) The Y-motor model consists of three rigid bonds joined at a node of controllable angular flexibility. Repressor proteins are treated as spherical monomers at the extremities of the bonds and simulations are performed in two dimensions. (b) To explore the role of additional degrees of freedom, repressor proteins are modeled as dimeric feet with steric regions (equilateral triangles plus rigid leg) and are linked to an equilateral triangular hub with flexible joints. Simulations are performed in three dimensions. In both models, full circles represent inflexible joints (large K_{bend}), and open circles represent joints of tunable flexibility (variable K_{bend}) (see [Supplementary Materials](#)).

sequence of the recognition sites A, B, C (MetJ, TrpR, PurR) separated by short spacer sequences ([Kemp et al., 1985](#)). The footprints of the repressors are between 16 and 19 base pairs ([Otwinowski et al., 1988](#); [Lawson, 1993](#); [Glasfeld et al., 1999](#); [Garvie and Phillips, 2000](#)) [see Fig. 2(a)]. These should be spaced to enable binding to the same side of the DNA helix. As an initial input to our numerical model below, we use a spacing of three helical turns (31 bp; 11 nm). This is the minimum separation likely to avoid disrupting the binding site of the neighboring repressor; a small separation is preferred for fast diffusion-driven motion of the TW.

MODEL

To test the fundamental behavior of the TW concept we have developed coarse-grained simulation models that explore geometric requirements and allow estimates of important time scales. Two- and three-dimensional, numerical simulations were performed of motor models with the basic symmetry, geometry, steric size, and mechanical degrees of freedom of the TW motor concept (Fig. 3).

For details of modeling techniques please refer to the [Supplementary Materials \(S1.A\)](#). In brief, rigid components of the motor were represented by spheres, each with an associated drag coefficient corresponding to its size. To define motor geometry (see Fig. 3), the distances between spheres were maintained by very stiff axial spring potentials. Motion of angular internal degrees of freedom is possible through a number of semi-flexible joints (indicated by open circles in Fig. 3) represented by a moderate bending potential. The dynamics of motor parts in their fluid environment were modeled using the overdamped Langevin equation, which includes a stochastic, thermal force term, and neglects inertial motion. The track was modeled as a periodic line of bind-

Figure 4. Two-dimensional simulation results for the center-of-mass motion of a Y-motor as shown in Fig. 3(a), for $d=11$ nm. A completely rigid Y-motor with a fixed $\theta=120^\circ$ and a size exactly commensurate with the binding site separation [$l=d/(2 \sin(60^\circ))$] takes one step for each cyclical change in ligand composition (Trace I). The diffusional search for the next binding site is visible as positional noise immediately after the ligand exchange. When a 25% size mismatch is introduced ($l=d/(2.5 \sin(60^\circ))$) the motor is barely large enough to reach into two 2-nm binding potentials simultaneously and can detach from the track; the first such event is highlighted by an arrow in Trace III. However, if, with the same geometric mismatch, free thermal fluctuations of θ around 120° are permitted, processivity is reestablished (Trace II).

ing potentials, where each potential had a radial extent comparable to typical electrostatic interaction distances in solution (2 nm). To increase computational efficiency, we limited the volume in which a completely detached motor could diffuse by confining the motor within hard walls that did not change the dynamics of a motor bound to the track.

First we considered the most basic representation of the TW concept [Fig. 3(a)], referred to as the Y-motor, which uses a single, central node to which three feet are attached with tunable bending flexibility. The simulation was carried out in two dimensions, and ligands were supplied in periodic pulses of duration T_{lig} in the temporal order [a, b], [b, c], [c, a]. Binding of a foot to the track was allowed only in the presence of its specific ligand, and unbinding occurred immediately upon pulse change (see [Supplementary Materials](#) for details). Trace I in Fig. 4 shows calculated stepping data for the motor center of mass. These illustrate that the Y-motor model can easily be tuned to be perfectly processive provided T_{lig} is long enough for the motor to diffuse rotationally to the next binding site. However, model details are important. A mismatch between motor size and binding site separation d caused the motor to miss binding events, resulting in detach-

ment from the track (Fig. 4, Trace III). However, processivity could be reestablished by increasing the motor's internal flexibility and thereby its diffusional reach, highlighting the diffusive nature of the motor (Trace II).

The Y-motor trajectories shown in Fig. 4 confirm the validity of the basic concept of a TW motor and highlight the importance of details such as internal molecular degrees of freedom. This model, however, does not account for the steric hindrances of all motor components and was only simulated in two dimensions. To examine how the performance of the motor changes when steric hindrance and internal flexibility are included, we incorporated a central triangular hub with legs attached at each corner by a fully flexible “hip” joint, [Fig. 3(b)]. We also represented the general shape of the dimeric repressor proteins (feet) by symmetric, steric areas that were attached to the legs by rigid “ankle” joints. The finite size of each component was modeled by a screened, repulsive Lennard-Jones potential whose screening length was comparable to the steric size of the physical components. Numerical simulations of this model were performed in three dimensions.

The steric regions immediately introduce basic size requirements for the motor components. For two feet to be able to bind next to each other, the hub side length h must be longer than the steric extent of the foot, f . By setting $d=h=2f$ we observed processivity (Fig. 5, inset). Figure 5 shows the distribution of first passage times, defined as the time required for the motor to perform a diffusional search for the next binding site. The statistical distribution of first passage times indicates a characteristic diffusion time to the next binding site of $\tau_{\text{diff}} \approx 150 \mu\text{s}$.

We now discuss the expected performance of the motor and consider first the general requirements for processivity, that is, the motor's ability to take many steps without detaching from its track. For a successful step, the trailing foot of the TW needs to release its ligand and detach from its recognition sequence (we define a combined rate k_{off} for the process of dissociation in the absence of bulk ligand), while the free foot needs to acquire its specific ligand and diffusively search and attach to its recognition sequence (this time is at least τ_{diff}). Both these operations have to occur on a timescale faster than the average dissociation time $(k_{\text{off}}^*)^{-1}$ of the bound third foot in the presence of its excess ligand. Together, these timescales set a lower and upper limit for the rate at which the ligand concentration in the buffer needs to be changed for each foot:

$$\frac{1}{k_{\text{off}}^*} \gg T_{\text{lig}} > \frac{1}{k_{\text{off}}} + \tau_{\text{diff}}. \quad (1)$$

While there is little detailed information available on k_{off}^* for our chosen repressors, it is generally found to be around 0.005 s^{-1} (Parsons *et al.*, 1995; Stockley *et al.*, 1998; Finucane and Jardetzky, 2003; Wang *et al.*, 2009). We can therefore expect a single foot in the presence of ligand to

Figure 5. Three-dimensional simulation results for the model in Fig. 3(b). Main figure: histogram of the first passage times for the diffusional search, taken as the time from when the trailing foot releases until the leading foot reaches the next binding site ($d=h=2f=11 \text{ nm}$), which yields a characteristic diffusion time of $\tau_{\text{diff}} \approx 150 \mu\text{s}$. Inset: stepping traces of the center of mass. When ligands are supplied for sufficiently long pulses ($T_{\text{lig}}=525 \mu\text{s}$ for Trace I), the motor is processive. For shorter pulses ($T_{\text{lig}}=440 \mu\text{s}$ in Trace II), the motor can dissociate and may then need multiple attempts to rebind in synchrony with ligand supply. Note that the simulation used a finite sample volume with hard walls, limiting diffusion and facilitating rebinding.

be attached on the order of 100 s. In general, the off rate in the absence of ligand is on the order of 100 times faster than that in the presence of ligand, suggesting $(k_{\text{off}})^{-1} \approx 1 \text{ s}$. Thus, both $(k_{\text{off}}^*)^{-1}$ and $(k_{\text{off}})^{-1}$ far exceed the expected τ_{diff} , and to ensure processivity one should choose a T_{lig} comparable to a few $(k_{\text{off}})^{-1}$.

The expected speed of the motor in an experiment will thus be limited either by $(k_{\text{off}})^{-1}$ or the technically achievable T_{lig} , whichever is slower. In a microfluidic system, T_{lig} as short as 0.1–1 s can be achieved. Assuming a step size of $d=11 \text{ nm}$, consistent with a repressor dimension of $f \approx 6 \text{ nm}$ (Otwinowski *et al.*, 1988; Schumacher *et al.*, 1994; He *et al.*, 2002), and T_{lig} of order $(k_{\text{off}})^{-1} \approx 1 \text{ s}$, one can expect an observable speed of order 10 nm/s. This value, while significantly slower than some biological motors [kinesin and myosin V both walk at around 600 nm/s (Wolenski *et al.*, 1995; Kojima *et al.*, 1997)] will still produce processive motion on an experimentally observable scale. It is interesting to note that the diffusion-limited speed d/τ_{diff} of the TW motor (that is, ignoring the limiting time scales k_{off}^{-1} and T_{lig}) is much faster, on the order of 70 $\mu\text{m/s}$.

For the models in Fig. 3, our results highlight that the dimensions of a rigid motor must be well matched to the track geometry, in order to ensure that binding can take

place. It is likely that experimental variations in binding site separation will occur, due to flexibility of the DNA and modifications of the DNA track induced by the binding process. For example, certain repressor proteins can cause a local kink in DNA while bound [as much as 45° in the case of PurR (Schumacher *et al.*, 1994)], which could change the relative position/orientation of the neighboring binding sites on the track. Thus, it may be necessary to build in additional degrees of freedom into the TW design, such as flexible linkers to the hub and/or feet. Even though such additional molecular degrees of freedom can be expected to slow diffusion, the above discussion suggests that diffusion is not likely to limit the motor speed in an experiment.

CONCLUSION

The design and eventual construction of synthetic protein motors at a level of sophistication comparable to that of biology is a significant and exciting challenge to the molecular biology, peptide design, synthetic biology, and biophysics communities. Here we have illustrated how the first steps toward this goal might be taken with state-of-the-art capabilities, forming the foundation for an approach to the understanding of the structure-function relationship in biological motors from the bottom up.

Although not discussed in detail here, the observation of the motion of a single TW motor should be feasible using existing methods for the observation of single-molecule diffusion along synthesized DNA tracks, either stretched in nanochannels (Wang *et al.*, 2005) or elongated by flow after anchoring to a surface (Wang *et al.*, 2007). Techniques such as TIRF (Total Internal Reflection Fluorescence) microscopy and FIONA (Fluorescence Imaging with One-Nanometer Accuracy) localization analysis, used to detect the hand-over-hand motion of myosin V with better than 2 nm precision (Yildiz *et al.*, 2003), could be expanded to account for three different fluorophores, one for each TW foot. Attachment of a bead to the TW would enable the use of optical or magnetic tweezers to probe additional motor characteristics, such as its force-velocity relationship and efficiency (Bustamante *et al.*, 2004). As the motor and its components are designed *de novo*, attachment points for the covalent or non-covalent binding of fluorophores or beads can be incorporated into the motor design.

In summary, we have outlined how the construction of a linear protein motor and the observation of its motion along a track are within reach of current capabilities. Note, however, that the design of TW is still rudimentary compared to highly evolved and well-studied biological examples such as myosin V and kinesin. The TW would move by rectified diffusion, coordinated by externally controlled, cyclical changes in chemical potential. In contrast to myosin and kinesin it does not run autonomously in a given chemical environment, it does not employ conformational changes that could create a working stroke, and it lacks an internal gating mechanism

for the control of binding and unbinding. Following a path that will eventually lead to the realization of synthetic protein motors with some or all of the advanced features of a biological motor will enable us to understand in even greater detail the fundamental design rules of biological motors, such as the nature and the biophysical role of conformational changes and flexibility (Mickler *et al.*, 2008). Furthermore, it will push forward the boundaries of protein/peptide-based assembly and nanostructure design.

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