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A bioinspired design principle for DNA nanomotors: Mechanics-mediated symmetry breaking and experimental demonstration

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ABSTRACT

DNA nanotechnology is a powerful tool to fabricate nanoscale motors, but the DNA nanomotors to date are largely limited to the simplistic burn-the-bridge design principle that prevents re-use of a fabricated motor-track system and is unseen in biological nanomotors. Here we propose and experimentally demonstrate a scheme to implement a conceptually new design principle by which a symmetric bipedal nanomotor autonomously gains a direction not by damaging the traversed track but by fine-tuning the motor's size.

1. Introduction

A direction of DNA nanotechnology is to develop designed DNA structures into functional devices. A target is nanoscale motors [1] capable of continual directional motion beyond switch-like devices. A central problem to solve in designing and fabricating a nanomotor is how to rectify a net directional motion for a motor as a whole given the fact that its molecular components are each alone incapable of any directional drift. Artificial nanomotors [2–18] to date mostly use burn-the-bridge methods [3,7,8,10,13–16] to gain motor-level directionality. Namely, the traversed track (or an equivalent structural component) is chemically damaged in the wake of the moving motor to prevent its backward motion. The burn-the-bridge methods render the track not re-usable and are not seen in biological nanomotors [19,20] that inspired the artificial ones. Other methods to render a motor a net direction are also reported. An ordered administration of multiple strands is shown [4,5] to drive a motor with chemically different pedal components. A physically more sophisticated method [9,12] is also reported that enables a symmetric bipedal nanomotor to autonomously consume a fuel preferentially at the rear leg and thereby to gain a direction. Interestingly, a latest theory [21] suggests the possibility of using mechanical effects to improve this method, e.g., to add a forward bias for leg placement.

A conceptually new design principle was proposed [22] and experimentally developed [17,18] recently for motor-level direction rectification in a non-destructive way [17] and with an integration [18] of preferential rear leg dissociation and biased forward leg placement. This design principle, termed mechanics-mediated symmetry breaking [22], was extracted from biomotos [19,20,23,24] and generalized for implementation in artificial motors. By this design principle, the motors may be symmetric bipeds that can be fabricated by dimerizing identical synthetic pedal components, and the tracks may be a periodic array of merely two species of footholds (i.e., the minimum number of different foothold species required for an asymmetric track). But such a symmetric bipedal motor may gain a direction on such a minimally heterogeneous track by simply fine-tuning the size of the motor, under the condition that a track-bound pedal component induces a local alignment along the track. Then the motor may be driven into continual directional run under random execution of a single operation designed to break the foot-track binding associated with the alignment.

In this paper, we outline and experimentally demonstrate a general scheme to implement the design principle based on local migration, which is applicable not only to DNA motors as shown in this study, but potentially also to nanomotors constructed from peptides and synthetic polymers.

2. Materials and methods

2.1. Strands and sequences for the DNA motor-track system

The nucleotide sequences for the DNA strands were first selected using the NuPack server (www.nupack.org). Secondary structures

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were manually checked and eliminated by sequence adjustment. The azo-carrying strands (MS1, MS2) were purchased from Nihon Techno Service Co. Ltd (Japan) (presence of azo-moieties confirmed by photoabsorption) and the non-azo strands from 1st Base Pte Ltd (Singapore). Below are the sequences used to fabricate the motor-track system.

(1) Motor strands (X indicates azo-moiety covalently inserted into the backbone of the oligonucleotides)

MS1 = D3 – S1 – D2 – D1 (20 + 4 + 20 + 5 = 49mer) with dye:

5′-GAGTTACCATCTAGGTA-GAG+AGTC+CTXTTXAAXGAXAGXGAXGAXTAXTAXCC+ATTCC-3′-FAM

MS2 = D3* – S1 – D2 – D1 (20 + 4 + 20 + 5 = 49mer) with dye:

5′-CTCTACCTAGATGGTA-ACTC+AGTC+CTXTTXAAXGAXAGXGAXGAXTAXTAXCC+ATTCC-3′-FAM

(2) Track strands

TS1 = D1* – D4* – D5* (6 + 75 + 15 = 96mer):

5′-GGAATG+AATTTCTGCGAGAGGCTCCGAGCTAGTCCAAGGGGATCGTAGTATTTTGCATGACAAAGCCCCAGCCATTATAGC+AGCGAT-TACTTGTGC-3′

TS2 = D4 – D5 – S2 – D2* (75 + 15 + 4 + 20 = 114mer):

5′-GCTATAATGGCTGGGGCTTTGTCATGCAAAATACTAC-GATCCCCTTGGACTAGCTCGGAGCCTCTCGCAGAAATT+GCACAAG-TAATCGCT+TAAA+GGTATATCTCCTTCTTAAAG-3′

(3) End strands

ES1 = D4 – D6 – S2 – D2* (75 + 15 + 4 + 20 = 114mer):

5′-GCTATAATGGCTGGGGCTTTGTCATGCAAAATACTACGATCCCCTT-GGACTAGCTCGGAGCCTCTCGCAGAAATT+TAG-CCGTGGGTCGGT+TAAA+GGTATATCTCCTTCTTAAAG-3′

ES2 = D6* (15mer) with Quencher: IBRQ-5′+ACCGACCCACGGC-TA-3′

ES3 = D5 (15mer): 5′-GCACAAGTAATCGCT-3′

2.2. Motor-track assembly

To assemble the motor, the azo-DNA stock solutions (40–60 μM) were prepared in TAE/Mg^{2+} buffer (40 mM Tris (pH 8.0), 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate) and the concentrations were determined by measuring the absorbance at 260 nm with a UV–visible spectrophotometer. An equimolar mix of motor monomers (MS1, MS2) was pre-annealed in a heating block from 90 °C to 25 °C over a period of 3 h, and analyzed for the duplex formation using native polyacrylamide gel electrophoresis (PAGE) and purified using Qiagen gel extraction kit. In the PAGE, the gels (4–15%, 19:1, acrylamide: bisacrylamide) were run in TBE buffer (Tris–borate–EDTA) at 4 °C. The gels were stained with Sybr–gold stain (from Invitrogen Technologies), and scanned using a Gel-documentation system.

The tracks shown in Fig. 2B is fabricated by a three-step assembly procedure. First, the two track strands (TS1, TS2) were mixed stoichiometrically at 0.6 μM in TAE/Mg^{2+} buffer, and then annealed in a heating block from 90 °C to 25 °C over a period of 3 h to form tracks of different lengths without the protection of ending strands. Second, end strands (ES1, ES2 and ES3) were added to terminate growth of the tracks. Third, the two-site or three-site tracks protected by end strands were then purified from one of multiple bands of the unpurified samples by the right molecular weight. The final two-site and three-site tracks appear each as a single band in Fig. 2 (lane 2 and lane 3) because they had been purified to remove the free end strands and other unwanted components. To form the motor-track binding complex, an equimolor ratio of motor sample and gel-purified track sample were mixed, incubated (3 h) and then analyzed by PAGE.

2.3. Fluorescence detection of motor motility

The motor shown in Fig. 2A was labeled with two light-emitting dye molecules (FAM, excitation/emission wavelength as 495/520 nm) at 5′ ends of the legs. The track's plus end was labeled with a quencher (Iowa Black RQ) at the 5′ end of strand ES2 so as to reduce the fluorescence emission of a motor upon its arrival at the plus end. The fluorescence measurements were conducted using a Cary eclipse spectrophotometer (Varian, Inc.) equipped with a 100 W xenon flash lamp (Agilent Technologies, 0.1 s/flash). For each round of irradiation operation, a motor-track sample was first irradiated by UV light through a filter of narrow wavelength window (350–380 nm) for a period of time (30 min), followed by visible irradiation ($\lambda > 400$ nm, ~20 s) and fluorescence measurement. The filtered UV flash has a low power (~10 μW as measured by a power meter), effectively suppressing photobleaching of the dye-labeled sample. The fluorescence experiments were all done at 25 °C for submicromolar concentrations in a working volume of ~600 μl. The motility experiments used the same TAE/Mg^{2+} buffer as for the motor-track assembly (i.e., 40 mM Tris (pH 8.0), 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate).

2.4. Motor-track variations

The two elongated motors shown in Fig. 3A contain extra nucleotide sequences in the D3–D3* duplex, which are 5′-GGGGATTTCG and 5′-ATGTCGGGGATTTCGTCACA from the 3′ end of the normal motor's D3. The motors now carry two quenchers (BHQ-2) at its legs, and the track is re-designed to carry two different dyes (FAM, and TAMRA with excitation/emission wavelength as 559 nm/583 nm) at the plus and minus ends. For the site-specific labelling, the D5 segment at the minus-end site is mutated into a new sequence D5′ (5′-CGAACATTGCTGTTG-3′). The new motors and a two-site track with the two-dye labelling are fabricated using the self-assembly method. Since the track contains only four DNA strands (a template strand hybridized with three short strands, see Fig. 3A), it is formed by a one-step assembly without need of purification; annealing a stoichiometric mix of the strands produces a single band of expected molecular weight from PAGE.

2.5. A simplistic mechanical model

To illustrate general features of the design principle, we constructed a mechanistic model for bipedal nanomotors made of linear polymer molecules, which may be DNA, peptide or synthetic polymers. Following the basic design of the motor-track system illustrated in Fig. 1, the total free energy for a two-leg motor-track binding state shown is a sum of foot-anchorage binding free energy and the free energy associated with the motor's inter-pedal polymer chain, i.e.,

$$E_i = U_i + F_i \tag{1}$$

The binding free energy (U_i) and the chain free energy (F_i) are

$$U_i = 2U_0 + n_{M,i}\Delta U \tag{2}$$

$$F_i = (k_B T)\left(\frac{L_i}{l_p}\right)\left[\frac{(d_i/L_i)^2(3-2d_i/L_i)}{4(1-d_i/L_i)}\right] \tag{3}$$

Here i marks one of two-leg binding states I–IV; U_0 is the free energy associated with the weak binding, $U_0 + \Delta U$ is the free energy for the strong binding. Then ΔU is the extra free energy driving the leg migration from the weak to the strong binding. $n_{M,i}$ is the number of the strong binding (e.g., $n_{M,i} = 1, 0, 2, 1$ for states I–IV, respectively).