Coordinated chemomechanical cycles: a mechanism for autonomous molecular motion

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Supplementary Material

0. General Information.

DNA strands were supplied by Integrated DNA Technologies (IDT). T4 DNA ligase, Exo I and Exo III, were supplied by New England Biolabs (NEB) and used in the ligase buffer provided. A hybridization buffer containing 25 mM NaCl, 5 mM MgCl$_2$, 10 mM Tris•HCl (pH 8.0) was used for all experiments. PAGE gels (29:1 acrylamide:bisacrylamide) were run in TAE buffer (40 mM Tris•acetate, 1 mM EDTA, pH 8.3) at 4 °C. All gels were 15% polyacrylamide except the gel presented in Figure 3(c) and Figure S4 which was 9% polyacrylamide.
1. Interaction between H1 and H2 in the absence of motor

Figure S1. Investigation of kissing interactions between fuel loops H1 and H2. Samples were incubated at 23°C; the concentration of each component was 100 nM. After 2, 4 and 16 hours samples were analyzed by PAGE. The H1•H2 duplex accumulates over the course of 16 hours. A faint band that migrates more slowly than the H1•H2 duplex is visible after 4 hours and has increased in intensity after 16 hours. We attribute this faint band to a kissing interaction between H1 and H2.

In the description of the motor presented in the accompanying manuscript we assume that loops H1 and H2 arrive sequentially at the motor. Some loops, especially those with longer loop domains, form metastable ‘kissed’ complexes held together by base pairing between complementary loop domains1,2,3. Figure S1 shows that under our experimental conditions H1 and H2 can form a kissing complex, but that it is slow to form and only accounts for a small fraction of the total DNA (<10% after 16 hours: lane 11). The observation that the timescale for accumulation of the kissed complex is slow compared to that of the formation of the H1•H2 duplex demonstrates that it is not a necessary intermediate in the hybridization of the complementary hairpins. We conclude that, under the conditions that we have used in our experiments, H1 and H2 arrive sequentially at the motor.

Note that the motor mechanism does not depend upon sequential arrival of the fuel loops and that design of a stronger interaction between H1 and H2 might allow a mechanism where both fuel components are delivered to the motor simultaneously3.

2. Control Experiments (Figure 3a)

Figure S2. PAGE analysis of the motor $f^2/f^2$ on two-site track $t$. Lane 1: Gel-purified motor bound to the track (40 nM). Lane 2: After incubation with fuel $H1$ (200 nM) for 2 mins at 23°C. Lane 3: After annealing with fuel by heating to 96°C and cooling to 23°C over 20 mins. Lane 4: control with fixed right foot and wild-type left foot displaced by $H1$. Lane 5: control with fixed left foot and wild-type right foot displaced by $H1$. Samples in lanes 4 and 5 were assembled by mixing annealed $f^2/H1$ with annealed $f^5/t15$ (lane 4) or $f^5/t51$ (lane 5) in a stoichiometric ratio at room temperature. Lane 6: $f^1/f^2/t$ after incubation with fuel $H1$ (200 nM) for 30 mins at 23°C. Minor bands that have not been annotated are attributed to imperfect stoichiometry: the electrophoretic mobilities are consistent with those of individual component strands.

The experiment presented in Figure 3(a) demonstrates that the left foot can be lifted from the track in preference to the right foot. The entire gel, complete with controls, is reproduced in Figure S2.

The track-bound motor migrates as a single band (lane 1). Upon incubation with an excess of $H1$, two higher-mobility bands appear that correspond to the motor with its left or right foot lifted by interaction with $H1$ (lane 2). Controls that position a wild-type foot to the left or right of a fixed foot (lanes 4, 5) confirm that the faster of the new bands corresponds to lifting the left foot from the track and the slower corresponds to lifting the right foot. Analysis after incubation with $H1$ for 2 minutes shows that the left foot is lifted in preference to the right (lane 2), as designed. After incubation for 30 minutes, approximately equal populations of the two configurations are observed (lane 6) indicating that the feet can rearrange on the track. We attribute slow rearrangement of the feet to the flexibility of the test track which allows the empty binding site to compete directly for hybridization to a bound foot in a strand displacement reaction initiated by hybridization to a competition domain $C$. The motor remains bound to the track even after long incubation times: free motor and track do not appear until the sample is annealed (lane 3).

The difference in mobility between complexes in which the either the left or right foot is raised is increased by the addition of tails of 10 T’s to the 5’ and 3’ ends of the track.

Additional nucleotide sequences required for the controls are given below.

$f5$ \[5′-\text{GTATTGAGGGAATCGCACTTTTTTTCAGTGACATCTTGATGCGTGCTAGATCAGT-3′}\]

$t15$ \[5′-\text{TTTTTTTTACCTGAACGAGACCGTCAAATCTAGCACGCTCAACTGAAATTTTTTTTTTTTT-3′}\]

$t51$ \[5′-\text{TTTTTTTTACCTGAATCTAGCACGCTCAACTGAAACGAGACCGTCAAATT TT TT TT TT TT TT TT TT TT TT TT TT TT TT-3′}\]
3. Fluorophores used to monitor foot-fuel interactions (Figure 3b)

**Figure S3.** Fluorophores used to monitor foot-fuel interactions for the experiment shown in Figure 3b. Heterodimeric motor \((f_1\cdot f_4\cdot t_{R/L})\) with \(f_4\) labeled at the 5’ end with tetrachlorofluorescein (TET; filled circle) was used together with \(H1\) labeled at the 3’ end with carboxytetramethylrhodamine (TAMRA; filled square).
4. Driving the motor out of equilibrium (Figure 3c)

![Figure S4](image)

**Figure S4.** PAGE analysis of a heterodimeric motor f1•f3 bound to a gel-purified circular track tC, demonstrating that fuel can drive the motor out of equilibrium. Lane 1: Motor + track ([f1•f3•tC] = 100 nM). Schematics indicate bands in which the wild-type foot is raised or bound to the left or right of the fixed foot (colored black). Lane 2: After incubation with fuel H1 (100 nM; 90 mins at 23°C). Lane 3: After annealing with fuel H1 by heating to 96°C and cooling to 23°C over 20 mins. Lane 4: Annealed sample after incubation with complementary fuel H2 (100 nM; 90 mins at 23°C). Lane 5: Motor + track incubated with excess of both fuel components H1 (200 nM) and H2 (250 nM) (90 mins at 23°C).

The ability of the hybridization motor to use fuel to move away from equilibrium, and thus its potential to do work, is demonstrated in Figure 3(c). The entire gel is reproduced in Figure S4.

The covalently-closed circular track was made by ligation of 11 µM tC using 11 µM f3 as a splint. The oligonucleotides were incubated with T4 DNA ligase (NEB) in the supplied buffer for 90 minutes at room temperature. The splint was then digested by incubation with exonucleases Exo I and Exo III (NEB) for 30 minutes at 37°C. Circular track bound to a heterodimeric motor (f1•f3•tC) was purified by PAGE.

Use of a circular track inhibits rearrangement of the feet on the track because the track has no unhybridized domain that could bind to the exposed competition domain on a foot to initiate a strand displacement reaction. The heterodimeric motor f1•f3 can bind to the track in one of two configurations, with its wild-type foot to the left or to the right (lane 1). The two configurations have different electrophoretic mobilities because (unlike f1) the fixed foot f3 has no loop. We make the assumption, based on the results of the experiments presented in Figure 3(a) and 3(b), that we can discriminate between the two possible configurations on the basis of their reactivities with H1. Lane 2 contains the track-bound motor after incubation with a stoichiometric amount of fuel H1. The slower of the two bands present in lane 1 is absent in lane 2: we conclude that this corresponds to the reactive configuration with a wild-type left foot. The faster of the two bands in lane 2 reacts slowly, if at all, with H1: we conclude that it corresponds to the unreactive configuration with a wild-type right foot.

Track-bound motor was annealed with fuel H1 (lane 3) then incubated with fuel H2 (lane 4). Finally, track-bound motor was incubated with both H1 and H2 (2 fold and 2.5 fold excess respectively) (lane 5). The equilibrium bias toward the slower of the two track-bound motor configurations (observed in lanes 1 and 4) was reversed upon addition of H1 and H2 (lane 5), demonstrating that fuel can drive the motor away from equilibrium and thus that the motor is capable of doing work. Note that this conclusion does not depend on the correct indentification of the two motor configurations, but follows from the observation that the relative intensities of the two bands is switched when both fuel components are added.
4. Supplementary Experiment on Processivity

Figure S5. Investigation of motor processivity. *H*1 was purchased with fluorescent labels 5’ carboxyfluorescein (FAM) and 3’ carboxytetramethylrhodamine (TAMRA). *H*1 was added to gel-purified, track-bound motor (*f1*•*f2*•*t’) at 23°C to give final concentrations [H1] = 50 nM, [f1•f2•t’] = 12.5 nM (fuel to feet ratio of 2:1). After 5.6×10^4 sec. the sample was annealed *in situ* by heating to 85°C then cooling to 23°C. Note that the track *t’* is identical to *t* except that it lacks the *T*10 tails at the 5’ and 3’ ends.

The motor is designed such that once one foot is lifted from the track the remaining foot is lifted much more slowly because there is no longer any competition between feet to expose a toehold for interaction with fuel. Figure S5 shows the results of an experiment in which wild-type motor bound to a two-site track was incubated with an excess of *H*1. The fuel is fluorescently labelled such that foot-fuel hybridization increases the separation between fluorophores and thus the intensity of donor (FAM) fluorescence. The FAM signal rises relatively quickly to one half of the equilibrium value as the first foot is lifted from the track by reaction with *H*1 with a rate constant of approximately 1.5×10^4 M⁻¹ s⁻¹, similar to that measured for lifting the left foot of a heterodimeric motor (k₀₁L = 5.8×10^4 M⁻¹ s⁻¹). The remaining foot is lifted at a much slower rate: no measurable increase in fluorescence was observed until the sample was annealed (indicated by the break in the x-axis). We estimate an upper limit of 10 M⁻¹ s⁻¹ to the rate constant for lifting the second foot.

The measured rate constant for replacing the lifted foot (Fig. 3b) is k₂₀L ≈ k₂₀R = 9.8×10^3 M⁻¹ s⁻¹. Once the first foot has been lifted from the track, the ratio of rates for replacement on the track and for dissociation is therefore 10^7:1. This experiment confirms that the mechanism has the characteristics required for processivity: it is unlikely that interaction with fuel will cause the motor to dissociate from the track.