Molecular Motors

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Mechanism for a Directional, Processive and Reversible DNA Motor**

*Jonathan Bath, Simon J. Green, Katherine E. Allen and Andrew J. Turberfield**

We present a mechanism for a directional and processive synthetic molecular motor whose energy is provided by hydrolysis of a single-stranded DNA fuel. The motor mechanism relies on coordination between two identical feet[1]: its directional bias can be switched from forward to reverse by changing the nucleotide sequence of the fuel.

Conformational change in mechanical devices built from DNA[2] can be triggered by addition of DNA control strands[3]: sequential addition of control strands can be used to direct movement along a track[4]. The requirement for external intervention is overcome by motors that couple motion to catalysis of a non-equilibrium reaction. Some free-running motors, driven by DNA hydrolysis[5] or hybridization[6], have obtained energy by assembling[6a] or damaging[5][6b] their tracks. Motors that operate continuously on a reusable track require a separate fuel: linear motion has been successfully coupled to ATP hydrolysis[7] and, using a motor architecture similar to that presented here, to hybridization of a metastable DNA fuel[1,8].

Our motor has two identical single-stranded feet attached via 4-nucleotide (nt) linkers to the 3′ ends of a 16 base-pair (bp) double-stranded spacer (Fig. 1 and Table 1). The track is a single-stranded DNA that consists of alternating binding and competition domains *B* and *C* that are 10 and 6 nt respectively. Each foot has the domain sequence *cbc* (domain sequences are written 5′ to 3′ and upper and lower case are used to indicate complementarity such that *cb* is the complement of *BC*). The feet are constrained by the length of the spacer to bind to overlapping sites on the track where they compete for binding to the overlap domain *C*. This competition is the basis for communication between feet[1]. When the 5′ domain *c* of the left foot (the foot nearer the 5′ end of the track) is transiently displaced from the track by competition it can act as a toehold[9] to initiate hybridization with the forward fuel (*BC*) which displaces 5′ domains *cb* from the track. Dissociation of the remaining 3′ domain *c* can occur spontaneously: the loss of 6 bp is offset by the increase in entropy resulting from the opening of the loop formed when both feet are bound to the track[10] and by the repair of two G•T mismatches in the foot-track duplex (which become G•C base pairs in the foot-fuel duplex - see below). Forward fuel can also bind the competing domain *c* at the 3′ end of the right foot but this transient complex cannot readily react further. The left foot is therefore lifted by hybridization to the fuel in preference to the right foot.

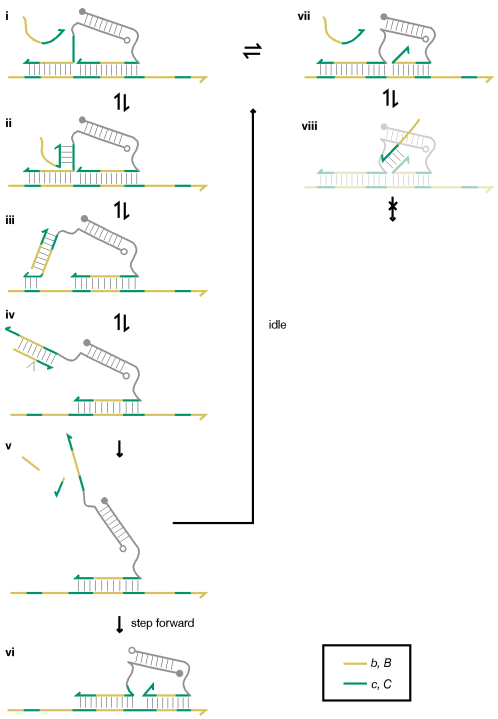

Figure 1. Mechanism. Identical single-stranded feet, linked by a double-stranded spacer (grey), bind a single-stranded track. The track is a repeating sequence of binding and competition domains (*B* and *C*, yellow and green respectively). A barb indicates the 3′ end of each strand. Adjacent binding sites overlap, causing the feet to compete for hybridization to a competition domain (green) (**i, vii**). When the 5′ end of the left foot is lifted from the track by this competition, it can serve as a toehold to initiate binding of the 'forward' fuel (**ii**) which partially displaces the foot from the track (**iii**); the remaining 6 nt dissociates spontaneously (**iv**). Fuel bound to the foot can be cut by the nicking enzyme N.BbvC IB (**v**): dissociation of the fragments allows the foot to rebind the track (**vi** or **i**/**vii**). The transient complex (**viii**) formed between the fuel and the 3′ end of the right foot cannot readily react further. The motor is designed to idle and step to the right with equal probability but to step to the left only rarely.

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

Table 1. DNA sequences[a].

Name	Sequence, 5′ to 3′
Motor 1	GTATTATCGTTAGTCTtttttGATGCTGAGGCTGAGGGATGCT
Motor 2	AGACTAACGATAATACttttGATGCTGAGGCTGAGGGATGCT
Motor 3[b]	AGACTAACGATAATACttttGATGCTGGCGGTATGCGATGCT
Track 1	AGCATCCTTCAGCTTCAGCATCCTTCAGCTTCAGCATC
Track 2	AGCATCCTTCAGCTTCAGCATCGCATACCGCCAGCATC
Track 3	AGCATCGCATACCGCCAGCATCCTTCAGCTTCAGCATC
Forward fuel [c]	CCTCAGCCTCAGCATC
Reverse fuel	AGCATCCCTCAGCCTC

[a] Complementary sequences are indicated by light and bold typeface. Colours correspond to those used in Figure 1. Spacer regions that remain single-stranded throughout the operating cycle are shown in lower case. Mismatches between track and motor are underlined. [b] Labelled with 5′ tetrachlorofluorescein (TET) for Fig 4. [c] Labelled with 3′ carboxytetramethylrhodamine (TAMRA) for Fig. 4 and with 5′ carboxyfluorescein (FAM) and 3′ TAMRA for Figs. 3 and 5.

The nicking enzyme N.BbvC IB recognises a 7 bp sequence in the foot:fuel duplex and catalyzes hydrolysis of the fuel[11] ($\Delta G°_{hydrolysis}$ = -5.3 kcal.mol^{-1})[12]. Dissociation of the spent fuel allows the foot to rebind the track. The corresponding sequence in the foot-track duplex is protected from the enzyme by the inclusion of mismatched nucleotides in the track[5a]: the presence of these mismatches also helps to tip the thermodynamic balance away from foot-track binding towards foot-fuel binding. By binding the fuel to form the duplex substrate recognised by the enzyme, the motor also plays a catalytic role in the hydrolysis of the fuel. The motor is not designed to influence whether the lifted foot rebinds to the left or to the right of the stationary foot. However, the bias to picking up the left foot allows directional motion with a ratchet mechanism, stepping to the right and idling with approximately equal probability but rarely stepping to the left.

The directional bias of the motor results from the preferential interaction of the forward fuel with the left foot. The bias can be reversed by changing the domain sequence of the fuel from *BC* (forward fuel) to *CB* (reverse fuel). The nucleotide sequence of the foot contains overlapping recognition sequences for the nicking enzyme so that both the forward and reverse fuel are cut into equal 8-nt fragments when bound to the foot.

Wild-type motor bound to a two-site test track runs as a single band on a polyacrylamide gel (Fig. 2). Addition of fuel generates two new bands corresponding to motor with either left or right foot lifted from the track: the forward and reverse fuels produce a bias towards lifting the left or right foot, respectively. The ability to discriminate between two identical feet based upon their relative positions on the track gives the motor mechanism intrinsic directionality.

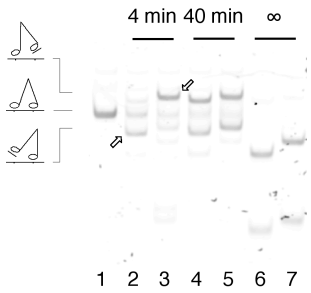

Figure 2. Directional bias. Motor, bound to a two-site track, runs as a single band on a polyacrylamide gel (lane 1). Addition of fuel generates two new bands. After a 4 minute incubation with forward fuel a bias toward the faster of the two bands, corresponding to a raised left foot, is observed (lane 2). Incubation with reverse fuel produces the opposite bias (lane 3). (Arrows indicate the dominant products.) After a 40 minute incubation, both fast and slow bands are present in equal quantities (forward fuel in lane 3, reverse fuel in lane 4), demonstrating that the initial asymmetries indicate non-equilibrium discrimination between feet, as designed. Both feet are lifted from the track upon annealing (forward fuel in lane 6, reverse fuel in lane 7). Controls that confirm the identity of the fast and slow bands are shown in Supporting Information.

Interaction between fuel and motor can be followed by labelling the fuel at the 5′ end with FAM and at the 3′ end with TAMRA. When the fuel is bound to the motor the separation between the fluorophores increases, decreasing fluorescence resonance energy transfer (FRET) between them and thus increasing FAM fluorescence. Measurement of FRET between fluorophores attached to the forward fuel demonstrates that the first foot is lifted from the track quickly compared to the remaining foot (Fig. 3): we estimate that the rates differ by ~100-fold. A similar result was obtained using the reverse fuel (Supporting Information). Once one foot is lifted there is no longer any competition between feet for track binding: the remaining foot is lifted from the track slowly because it offers no single-stranded toehold to initiate interaction with the fuel. Such control of the reactivity of each foot as the motor moves through its operating cycle is necessary for processive motion: a motor that takes many steps during each diffusional encounter with its track must remain bound by at least one foot with high probability.

The directional bias of the motor depends on the bias toward lifting the back foot and on the bias toward replacing it ahead of the stationary foot[1]. To quantify the bias, reaction kinetics for the two feet were measured separately. Modified motors with one wild-type foot and one fixed foot were used, together with tracks that position the wild-type foot to the left or to the right of the fixed foot. Fuel binding was observed by measuring FRET between TET attached to the 5′ end of the fixed foot and TAMRA attached to the 3′ end of the fuel. The TET signal decreases upon fuel binding. As anticipated[9], the interaction between forward fuel and the left foot fits well to second-order kinetics (Fig. 4, solid line) giving a rate constant of 4.5×10^5 M^{-1}s^{-1}. Interaction with the right foot is 30-fold slower as judged by time to one-quarter completion. Interaction with the right foot does not fit well to second-order kinetics, consistent with the absence of a toehold to initiate strand exchange: picking up the ′wrong′ foot probably requires spontaneous dissociation of at least part of the foot from the track. Similar results for the reverse fuel demonstrate the opposite bias (Supporting Information).

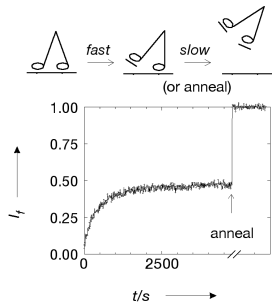

Figure 3. Coordination to control the reaction rates of identical feet makes motor detachment unlikely. Motor bound to a two-site track was incubated with a four-fold excess of forward fuel labelled with FAM and TAMRA. Fuel binding is accompanied by an increase in donor fluorescence. The signal rises relatively quickly to approximately half of the equilibrium value as the first foot is lifted from the track. The remaining foot is lifted from the track slowly. It is not until the sample is annealed that the remaining foot is lifted from the track.

The same assay shows that there is no measurable bias when the left or right foot is replaced on the track as the fuel is hydrolyzed by the enzyme. Replacement of the foot is rate-limiting (the slow step is likely to be release of the cut fuel fragments[11b]). The strong bias toward picking up the left foot, together with the lack of bias in putting the foot down ensures directionality[1].

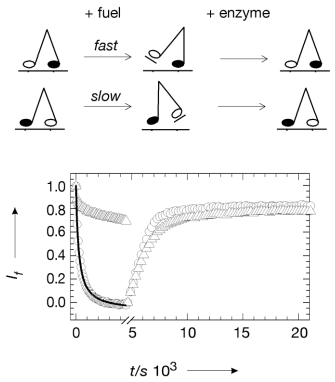

Figure 4. Kinetics of lifting and replacing left and right feet. The rate at which left (circles) and right feet (triangles) are lifted from the track by forward fuel was measured using a heterodimeric motor with one wild-type foot and one fixed foot (carrying wild-type competition domains) that cannot be lifted from the track by fuel. The 5′ end of the fixed foot was labelled with TET and the 3′ end of the fuel was labelled with TAMRA. The solid line shows a fit to second order kinetics. After 5000 s samples were annealed to ensure that all wild-type feet were lifted, then enzyme was added.

The ability of the motor to catalyse the hydrolysis of ≥64 fuel molecules was demonstrated by incubating heterodimeric motor with an excess of fuel labelled at 5′ and 3′ end with FAM and TAMRA (Fig. 5).

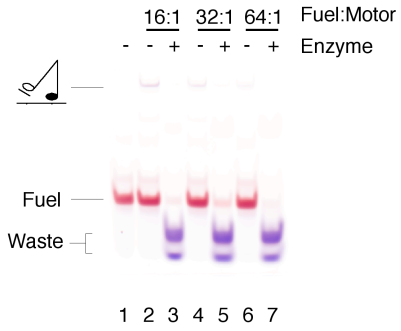

Figure 5. Polyacrylamide gel electrophoresis (PAGE) analysis of turnover of fuel by the motor. Forward fuel labelled with FAM (donor) and TAMRA (acceptor) was incubated in excess over track-bound heterodimeric motor for 16 hours. Two channels are overlaid: red is the FRET signal (donor excitation at 488 nm / acceptor detection at 590 nm), blue is the donor signal (488 nm / 530 nm). The random-coil configuration of the fuel produces a high FRET and low donor fluorescence. Motor binding to fuel gives a slowly migrating low-FRET band (faint because fuel is in excess over motor). Enzyme cleaves the fuel to produce low-FRET, high-mobility waste products. The motor can turn over ≥64 fuel molecules in 16 hours.

The motor mechanism is not limited by the sequence constraint imposed by this particular nicking enzyme. We have tested motors designed to work with two other enzymes that allow a fuel, but not the track, to be cut when hybridized to a lifted foot. RNAse H hydrolyses the RNA component of a DNA:RNA duplex[13]. When the DNA fuel is replaced with an RNA fuel, the bias towards lifting the selected foot is mantained; hydrolysis of the fuel by RNAse H allows the lifted foot to be replaced on the track (Supporting Information). The repair enzyme APE I, which cleaves to the 5′ side of an abasic site in double-stranded DNA[14], can also be used in combination with a DNA fuel containing an abasic (1′,2′ dideoxyribose) site (Supporting Information). Auxiliary enzymes could be dispensed with altogether by incorporating a deoxyribozyme that cuts an RNA fuel into each foot of the motor[5b]. These systems could be used to allow several motors to operate independently, each using a different fuel.

We have demonstrated that a bipedal motor mechanism that coordinates the chemical and mechanical cycles of two identical feet[1] can be generalized to obtain energy from hydrolysis of a DNA or RNA fuel. This mechanism has the properties required to create a chemically fuelled molecular motor that is both directional and processive. The directional bias of these motors can be reversed by changing the fuel. Future work will be directed towards single-molecule measurements of the motor as it steps along an extended track.

Experimental Section

PAGE purified DNA strands were purchased from Integrated DNA Technologies. Enzymes were purchased from New England Biolabs. (Note that the enzyme N.BbvC IB and Nt.BbvC I are alternative names for the same enzyme.) A buffer containing 10 mM $MgCl_2$, 50

mM NaCl, 10 mM Tris-HCl and 1 mM EDTA (pH 8.0) was used throughout. The temperature for all experiments was 37 °C except for Fig. 2 which was at 21 °C. PAGE gels were 12% (Fig. 1) or 15% (Fig. 5) 29:1 acrylamide in TAE run at 4 °C. In Fig. 2, fuel and motor were at 100 nM. In Fig. 3, fuel was at 32 nM and motor was at 8 nM. In Fig. 4, fuel and motor were at 8 nM. In Fig. 5, fuel was at 1 μM and motor was at 1/16, 1/32 or 1/64 μM. Wild-type motor ≡ [*Motor 1*•*Motor 2*]; heterodimeric motor ≡ [*Motor 1*•*Motor 3*].

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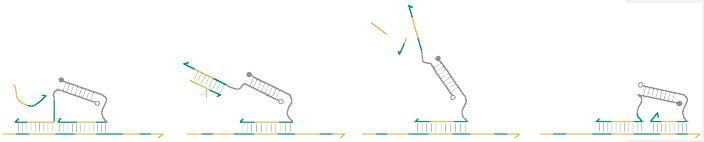
Mechanism for a Processive, Directional and Reversible DNA Motor.



Directional motion of a molecular motor requires both asymmetry in the component parts and the dissipation of energy. We present a mechanism for a directional and processive DNA motor in which directionality is established by coordinating the reactivities of two identical feet and energy is provided by hydrolysis of a single-stranded DNA fuel. The directional bias of the motor can be controlled through the choice of fuel.

Mechanism for a Directional, Processive and Reversible DNA Motor.

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Supporting Information contains the following data:

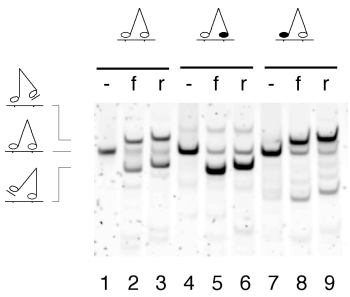

Fig. S1. Controls to confirm identity of bands in Fig. 1. Samples were run on a 12% polyacrylamide gel. Lane 1 contains motor bound to a two-site track. Lanes 2 and 3 show the slow and fast bands produced after a 40 minute incubation with fuel (f = forward, r = reverse). Lanes 2 and 3 are equivalent to lanes 4 and 5 of Fig. 2 and have been annotated the same way. Heterodimeric motor with a wild-type left foot [Motor 1 • Motor 3 • Track 2] produces faster migrating bands when the left foot is lifted from the track by incubation with forward or reverse fuel (lanes 5 and 6). Heterodimeric motor with a wild-type right foot [Motor 1 • Motor 3 • Track 3] produces slower migrating bands when the right foot is lifted from the track by incubation with forward or reverse fuel (lanes 5 and 6). The difference in mobility between complexes with forward and reverse fuel can be attributed to the 6 nt difference in position in which forward and reverse fuels bind to the feet (forward fuel leaves 6 nt at the 3´ end of the foot unhybridized; reverse fuel leaves 6 nt at the 5´ end of the foot unhybridized). Note that the relative mobilities of motor bound to track and motor with left or right foot lifted from the track depend on the composition of the polyacrylamide gel: on a 9% gel complexes with fuel run slower than the motor (see Figs. S4 and S5) whereas on a 15% gel they run faster (data not shown).

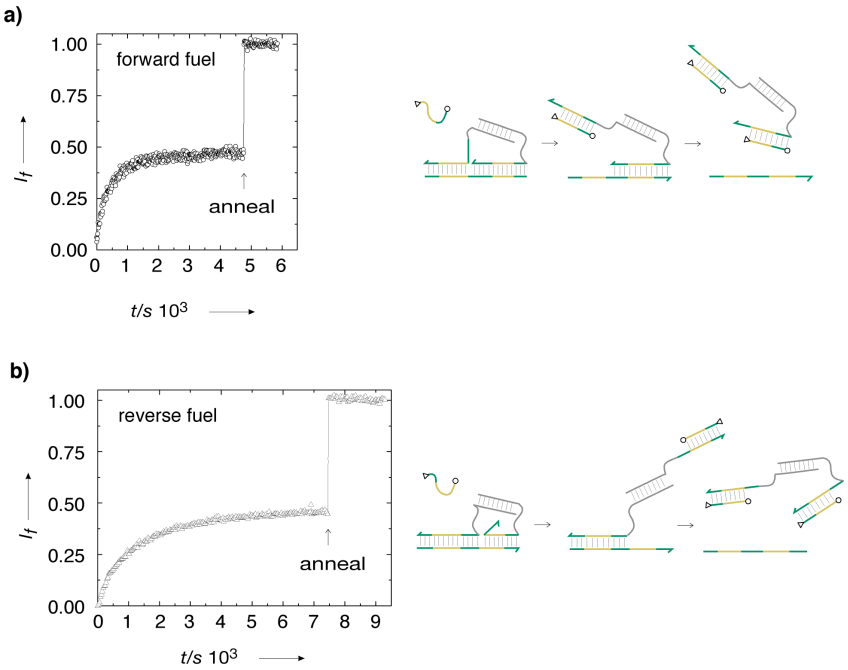

Fig. S2. Hydrolysis motor processivity.

Gel-purified wild-type motor bound to a two-site track was incubated with an excess of (**a)** forward or **(b)** reverse fuel. The first foot was lifted relatively quickly from the track whereas the second foot remained bound for the duration of the experiment (it was only lifted from the track when the sample was annealed).

Motor ([Motor 1 • Motor 2]) bound to a track with two sites [Track 1] at 8 nM was incubated with a four-fold excess of dual-labelled forward fuel or reverse fuel in a buffer containing 10 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris•HCl and 0.5 mM EDTA at pH 8.0 at a temperature of 37 °C. The fuel was labelled at the 5´ end with FAM (triangle) and at the 3´ end with TAMRA (circle). The rate of fuel binding was measured by measuring FAM fluorescence: fluorophore separation increases when the fuel binds to the foot, reducing FRET and increasing the FAM fluorescence intensity. Samples were annealed by heating to 85 °C for 10 minutes then cooling to 37 °C over 45 minutes. Part **(a)** (forward fuel) contains the same data as Fig. 3.

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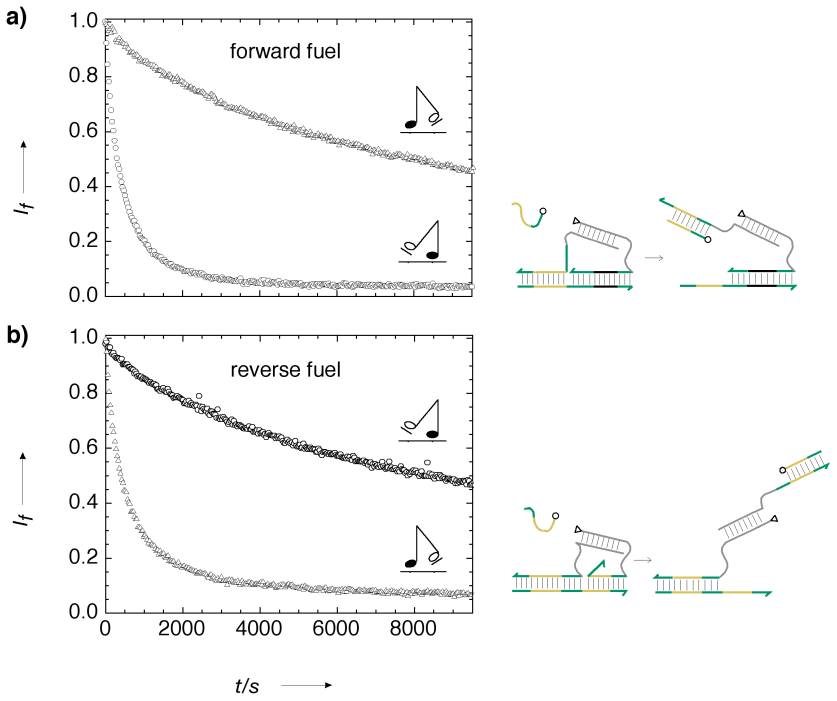

Fig. S3. Kinetic measurement of bias in lifting the left and right feet of the hydrolysis motor.

The rates at which the left and right foot were lifted from the track were measured using a heterodimeric motor with one wild-type foot and one fixed foot that offered competition for track binding but could not be lifted from the track.

Motor ([Motor 1 • Motor 3]) was bound to a track that positions the wild-type foot to the left of the fixed foot (Track 2: circles) or to the right (Track 3: triangles). The fixed foot was labelled at the 5´ end with TET (triangle). The TET fluorescence intensity was measured as a funtion of time after addition of forward fuel (**a**) or reverse fuel (**b**). In each case the fuel was labelled at the 3´ end with TAMRA (circle). There is a decrease in TET (donor) fluorescence as fuel binds to the foot because donor and acceptor fluorophores are brought into close proximity. Data from **(a)** (forward fuel) are also shown in the first part of Fig. 4.

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The forward fuel shows a bias toward lifting the left foot (**a,** Fig. 4). The fitted second-order rate constant for interaction of the forward fuel with the left foot is 4.5×10^5 M^{-1} s^{-1}. An assumption of second-order kinetics for the slower interaction of the forward fuel with the right foot does not give a good fit, but we estimate a corresponding rate constant of 1.4×10^4 M^{-1} s^{-1}. The forward fuel can therefore lift up the left foot approximately 30 times faster than the right foot.

The reverse fuel shows a bias toward lifting the right foot (**b**). The fitted second-order rate constant for interaction of the reverse fuel with the right foot is 3.7×10^5 M^{-1} s^{-1}. By assuming second order kinetics, we estimate a rate constant of 1.3×10^4 M^{-1} s^{-1} for interaction of the reverse fuel with the left foot. The reverse fuel can therefore lift up the right foot approximately 30 times faster than the left foot.

Motor and fuel (8 nM) were incubated at 37 °C in 10 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris•HCl and 0.5 mM EDTA at pH 8.0.

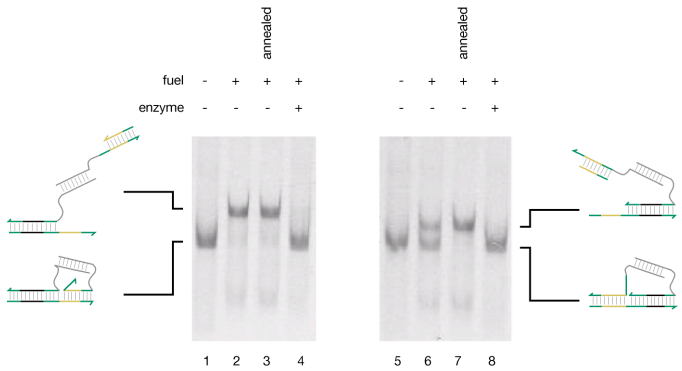

Fig. S4. Motor driven by RNAse H hydrolysis of an RNA fuel.

Heterodimeric motor ([Motor 1•Motor 3]) bound to Track 3 (left-hand gel: lanes 1 to 4) or Track 2 (right-hand gel: lanes 5 to 8) was incubated with an RNA version of the reverse fuel (5´ AGCAUCCCUCAGCCUC 3´). Track 2 positions the wild-type foot to the left of the fixed foot, Track 3 positions the wild-type foot to the right of the fixed foot. After 1 hour at 37 °C, all of the wild-type right foot was lifted from the track (lane 2) whereas less than half of the wild-type left foot was lifted from the track (lane 6) demonstrating that the RNA fuel discriminates between right and left feet. When annealed, all of the wild-type feet were lifted from the track (lanes 3 and 7). Upon addition of RNAse H and subsequent incubation for 1 hour at 37 °C, the lifted feet are returned to the track (lanes 4 and 8).

The buffer used for RNAse H contains 75 mM KCl, 3 mM $MgCl_2$, 50 mM Tris•HCl (pH 8.3) and 10 mM dithiothreitol. The gels are 9% polyacrylamide.

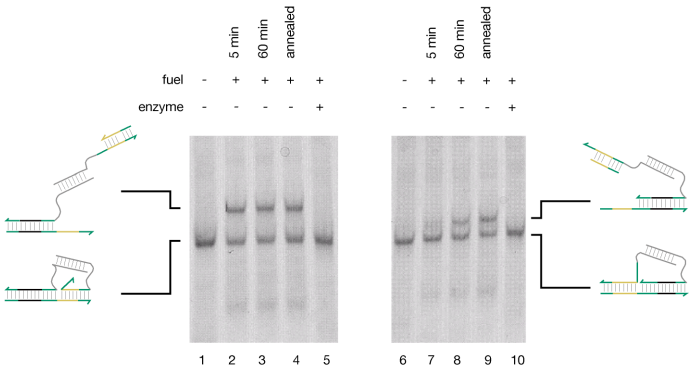

Fig. S5. Motor driven by APE I hydrolysis of an abasic fuel.

Heterodimeric motor ([Motor 1•Motor 3]) bound to Track 3 (left-hand gel: lanes 1 to 5) or Track 2 (right-hand gel: lanes 6 to 10) was incubated with an abasic version of the reverse fuel (AGCATCCC/*/CAGCCTC, where * is 1′,2′ dideoxyribose). Track 2 positions the wild-type foot to the left of the fixed foot, Track 3 positions the wild-type foot to the right of of the fixed foot. After 5 minutes at 37 °C, approximately half of the wild-type right foot was lifted from the track (lane 2) and no further increase was seen after 1 hour (lane 3). In contrast, relatively little of the wild-type left foot was lifted from the track after 5 minutes (lane 7) and the reaction was still not complete after 1 hour (lane 8), demonstrating that right and left feet can be distinguished by abasic fuel. When annealed, approximately half of the wild-type feet were lifted from the track (lanes 4 and 9) (the abasic fuel binds to the foot more weakly than the RNA or DNA fuels). Upon addition of APE I and subsequent incubation for 1 hour at 37 °C, the lifted feet are returned to the track (lanes 5 and 10).

The buffer used for APE I contains 50 mM potassium acetate, 10 mM magnesium acetate 20 mM Tris-acetate (pH 7.9) and 1 mM dithiothreitol. The gels are 9% polyacrylamide.