

## Molecular Devices

**A Free-Running DNA Motor Powered by a Nicking Enzyme\*\***

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Herein, we present a simple linear motor, built from DNA and a restriction enzyme, which moves a DNA cargo in discrete steps along a DNA track. Movement is powered by a nicking enzyme that cuts the track. Damage to the track in the wake of the cargo imposes directionality.

The specificity of base-pairing, the rigidity of short segments of the double helix, and the flexibility of single-stranded segments make DNA an ideal material for construction of nanometer-sized mechanical devices.<sup>[1–7]</sup> Such devices can generate forces of the order of picoNewtons,<sup>[2]</sup> in the same range as the forces developed by single-molecule

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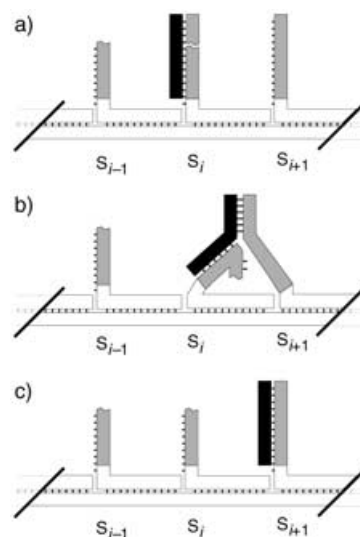


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protein motors.<sup>[8,9]</sup> A typical device relies on the binding of a fuel strand of DNA to trigger a conformational change. The device can be restored to its initial conformation by subsequent addition of a reset strand that strips the fuel strand from the device. The operation of these devices can be coupled to stepwise movement along a track.<sup>[10,11]</sup> There is interest in designing free-running devices that operate continuously while the supply of fuel lasts. One approach is to design a secondary structure to slow the conversion of DNA fuel into waste unless catalyzed, and to couple the catalysis of fuel to the operation of a device.<sup>[12]</sup> Another approach is to use the hydrolysis of the phosphodiester backbone of DNA<sup>[6]</sup> or a combination of DNA and ATP hydrolysis.<sup>[7]</sup> We have used the hydrolysis of DNA, catalyzed by a restriction enzyme, as the energy source for our motor. Autonomous, directional motion is achieved by making DNA cleavage conditional on the state of our device. The device moves a cargo stepwise along a one-dimensional track; damage caused to the track by the restriction enzyme prevents the cargo from stepping backwards and therefore imposes directionality—a “burnt bridges” mechanism.<sup>[13]</sup>

A suitable track is a rigid self-assembled structure with many identical single-stranded stators attached periodically along its length. Motion is driven by cleavage of the stators by the restriction endonuclease N.BbvC IB (New England Biolabs). N.BbvC IB binds to double-stranded DNA at a specific 7-bp (bp = base pair) recognition sequence and catalyzes cleavage of the DNA backbone of one strand only to introduce a nick at a precisely determined position. This nicking property has been specifically engineered by modification of the naturally occurring heterodimeric BbvC I.<sup>[14]</sup> The cargo is an oligonucleotide that can hybridize to any one of the stators. It is only when the cargo is hybridized to a stator that N.BbvC IB can cut the stator to release a short fragment of the stator and promote movement of the cargo onto the next stator along the track.

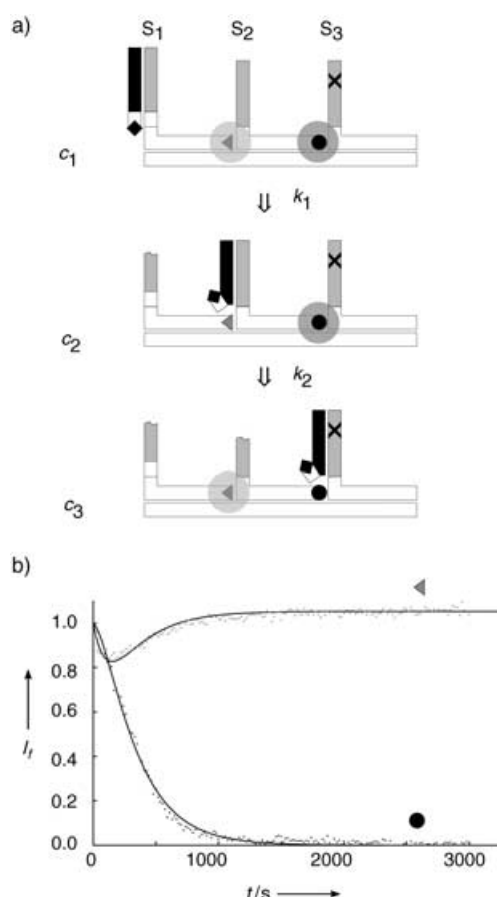
Figure 1 shows one step of the motor. In our experiments, the stators are 24-nt (nt = nucleotide) sequences attached to a double-stranded track every 21 bp (7 nm) by a 3-nt flexible linker. Stators are attached at the 3' end and, when hybridized with the cargo, can be cut by N.BbvC IB at 8 nt from the 5' end. The melting temperature of the 8-nt stator fragment generated by cutting is calculated to be between 13°C and 20°C, well below the operating temperature of the motor (37°C). Figure 1a shows the cargo bound to stator  $S_i$ . Cutting by N.BbvC IB releases an 8-nt fragment of the stator and promotes movement of the cargo onto an adjacent stator. Release of the short stator fragment leaves the cargo with an 8-nt single-stranded overhang that can bind to the intact stator  $S_{i+1}$ , initiating an energetically favorable step to  $S_{i+1}$  (Figure 1b). The backwards step to  $S_{i-1}$  is strongly inhibited because  $S_{i-1}$ , which was cut in the previous step, lacks the 8-nt section that is complementary to the exposed overhang of the cargo. The step from  $S_{i+1}$  to  $S_i$  is a simple branch-migration reaction. The initial part of this reaction involves a random walk of the branch point that marks the junction between sections of the cargo bound to the competing stators  $S_i$  and  $S_{i+1}$ . For each base pair broken between cargo and  $S_i$ , a base pair can be formed between cargo and  $S_{i+1}$ . However, the



**Figure 1.** Design of a linear motor powered by a nicking enzyme. DNA strands are represented as black and gray ribbons, with black lines indicating base pairs. The track is an array of periodically spaced single-stranded stators. The cargo (black ribbon) is an oligonucleotide that can bind to any one of the stators. a) The cargo is bound to stator  $S_i$  which enables enzyme N.BbvC IB to cut the stator and release a short stator fragment and to leave the cargo with a single-stranded overhang free to bind to the adjacent stator  $S_{i+1}$ . The cargo cannot bind to  $S_{i-1}$  which was cut in the previous step. b) The cargo then steps onto  $S_{i+1}$  by a simple branch-migration reaction. For each base pair broken between cargo and  $S_i$ , a base pair can be formed between cargo and  $S_{i+1}$ . The tethers that hold the stators to the rigid track limit this process, and stepping to  $S_{i+1}$  requires spontaneous dissociation of the last few nucleotides of the cargo from  $S_i$ . c) Upon dissociation, the cargo is bound to  $S_{i+1}$  and the operational cycle is complete.

lengths of the tethers that hold the stators to the rigid track limit this process, and stepping to  $S_{i+1}$  requires thermally activated, spontaneous dissociation of the last 3–6 nucleotides of the cargo from  $S_i$ . After spontaneous dissociation of the last few nucleotides, the cargo arrives at  $S_{i+1}$  (Figure 1c) and the operational cycle is complete.

Our test track is composed of three stators,  $S_{1-3}$  (Figure 2a, Table 1). The cargo is labeled at the 5' end with the nonfluorescent quencher, Iowa Black. The track is labeled with Cy3 and Cy5 cyanine dyes, which are arranged such that when the cargo is bound to  $S_2$  the signal from Cy3 is quenched and when the cargo is bound to  $S_3$  the signal from Cy5 is quenched. The test track incorporates modifications to stators  $S_1$  and  $S_3$ . The interaction between the cargo and  $S_1$  spans 2 nt more than the interaction between the cargo and either  $S_2$  or  $S_3$  which ensures that the cargo binds more stably to  $S_1$  than to either of the other stators:  $\Delta G_{37}^\circ$  is calculated to be  $-30.4$ ,  $-27.1$ , and  $-23.6$  kcal mol $^{-1}$  for binding to  $S_1$ ,  $S_2$ , and  $S_3$  respectively.<sup>[15]</sup> At equilibrium, under our experimental conditions, we calculate that the cargo is distributed between the stators  $S_1$ ,  $S_2$ , and  $S_3$  in a ratio of 94:6:≪1. This distribution allows us to prepare the track with the cargo bound predominantly to  $S_1$ . The complex that forms between  $S_3$  and the cargo contains a single mismatch within the N.BbvC IB recognition site that renders  $S_3$  resistant to cutting.



**Figure 2.** Operation of a linear motor powered by a nicking enzyme. a) The five strands of DNA used to assemble the test track are represented as ribbons. The black square represents the quencher Iowa Black carried by the cargo; as the cargo steps along the track, its position can be determined from the fluorescence signal of the two reporter fluorophores, Cy3 (gray triangle) and Cy5 (black circle). b) Normalized fluorescence intensities ( $I_i$ ) from Cy3 (gray triangle) and Cy5 (black circle) showing the movement of the cargo when the motor was incubated with N.BbvC IB at 37°C. The solid lines show the fit to a simple model described in the text. Fluorescence from Cy3 is transiently quenched when the cargo resides on  $S_2$ ; fluorescence from Cy5 is permanently quenched when the cargo arrives at  $S_3$ .

This ensures that when the cargo reaches the “mismatch” stator  $S_3$  it is trapped and the motor stops.

The test track was prepared by self-assembly followed by purification by polyacrylamide gel electrophoresis (PAGE) as described in the Supporting Information. To test the operation of the motor, a purified track with cargo on  $S_1$  at a

concentration of approximately 8 nM was incubated at 37°C. Movement of the cargo from  $S_1$  was triggered by the addition of N.BbvC IB in approximately eightfold excess relative to the concentration of the track. The position of the cargo on the track was measured by recording the fluorescence signals from Cy3 and Cy5 (Figure 2b). Quenching of the signal from Cy3 indicates that the cargo is bound to  $S_2$ ; quenching of the signal from Cy5 indicates that the cargo is bound to  $S_3$ . As the cargo moves from  $S_1$  to  $S_2$  and then to  $S_3$  there is a transient reduction in the signal from Cy3 corresponding to the state in which the cargo is bound to  $S_2$ . The signal from Cy5 is permanently quenched when the cargo arrives at  $S_3$ .

The movement of cargo along the test track can be modeled with two first-order rate constants— $k_1$ , for stepping between  $S_1$  and  $S_2$ ; and  $k_2$ , for stepping between  $S_2$  and  $S_3$ —according to Equation (1), where  $c_i(t)$  is the fraction of stator  $i$  occupied by the cargo at time  $t$ .

$$\begin{aligned} \frac{dc_1(t)}{dt} &= -k_1 c_1(t) \\ \frac{dc_2(t)}{dt} &= k_1 c_1(t) - k_2 c_2(t) \\ \frac{dc_3(t)}{dt} &= k_2 c_2(t) \end{aligned} \quad (1)$$

We have assumed that the cargo moves along a track (rather than between tracks), that it cannot hop directly from  $S_1$  to  $S_3$ , and that the probability of a backward step is negligible. The relationships between the fluorescence intensities of Cy3 and Cy5 and the fractions  $c_2$  and  $c_3$  are described in the Supporting Information. The data can be fitted very well by this simple model (solid lines in Figure 2b) by using values of  $0.003 \text{ s}^{-1}$  and  $0.009 \text{ s}^{-1}$  for  $k_1$  and  $k_2$ , respectively, to give a cargo speed of the order of  $0.1 \text{ nm s}^{-1}$ .

We performed a control experiment in which an excess of free stator is able to compete with track-bound stators for hybridization to the cargo. At a 100-fold excess of competitor, we could still see changes in the fluorescence of Cy3 and Cy5 that are characteristic of stepping of the cargo along the track. We conclude that movement of the cargo between tracks is not significant at the concentration of track used during normal operation of the motor (see Supporting Information).

Direct stepping from  $S_1$  to  $S_3$  is unlikely. For  $S_1$  to approach closer to  $S_3$  than to  $S_2$  would require a bend of the track at  $S_2$  of more than  $120^\circ$ . The fit to the data is not improved by the addition of a third rate constant corresponding to stepping of the cargo directly from  $S_1$  to  $S_3$  which suggests that the cargo does step sequentially along the track.

**Table 1:** DNA strands.

Strand	Modification	Sequence
cargo	5'-(Iowa Black)	CGATGTTAGTTGGCTGAGGTTTCGAT
$S_1$	3'-Cy3	ATCGAACC/ <b>TCAGCCCA</b> CTAACATCGTTTCCGTCTTTAATGCCTTTCTGT
$S_2$	3'-Cy5	ATCGAACC/ <b>TCAGCCCA</b> CTAACATTTTCCGGTAGTCGCTATATCCGT
$S_3$	none	ATCGAAGT* <b>TCAGCCCA</b> CTAACATTTTCCGTATTGTGCGTCAAGAGT
track	none	ACTCTTGACGCACAAATACGGACGGATATAGCGCACTACCGGACAGAAAGGCATTAAAGACGG

The N.BbvC IB recognition site is shown in bold; / indicates the position at which N.BbvC IB cuts; \* indicates the single nucleotide change that prevents N.BbvC IB from cutting.

The probability of a backwards step from an intact stator to a cut stator is negligible as the free energy change is extremely large:  $\Delta G_{37}^{\circ} = 10.3 \text{ kcal mol}^{-1} \approx 17 RT$ . We expect the probability of a backwards step between cut stators to be similar to that of a forwards step from an uncut stator: both processes involve blunt-strand exchange—that is, exchange without a toehold<sup>[16]</sup>—whose rates depend weakly on the length of the strand.<sup>[17]</sup> The system has been designed to ensure that both of these processes are much less probable than a forwards step from a cut to an intact stator: the cargo has an 8-nt single-stranded toehold that can bind only to an intact stator to initiate strand displacement. Such toeholds greatly increase the rate of strand displacement.<sup>[16]</sup>

Stepping requires spontaneous dissociation of the last 3–6 nucleotides of the cargo (Figure 1b).  $S_1$  has two extra nucleotides that hybridize to the proximal end of the cargo, so stepping from  $S_1$  to  $S_2$  requires the spontaneous dissociation of approximately one nucleotide more than stepping from  $S_2$  to  $S_3$ . The difference in activation energy between the two steps may account for the threefold difference in the rate constants,  $k_1$  and  $k_2$ .

The rate at which N.BbvC IB cuts its substrate has been measured as  $0.17 \text{ s}^{-1}$ ,<sup>[18]</sup> which is much faster than the rate at which the cargo moves along the track: motion of the cargo along the track without first cutting it can therefore be neglected. Dissociation of the 8-nt stator fragment once this has been released by the enzyme is also fast: the melting temperature of this fragment is well below the operating temperature of the device, and the dissociation rate, once the fragment has been released by the enzyme, is estimated to be  $> 0.01 \text{ s}^{-1}$ .<sup>[19]</sup> The rate-limiting step is either the release of product by N.BbvC IB or stepping from the cut stator to the intact stator. In a control experiment in which there was competition between free (not track-bound) stators for binding to the cargo, we have shown that in the presence of an excess of N.BbvC IB the stepping rate between stators is proportional to the concentration of competing stators (see Supporting Information). Extrapolation to the effective stator concentration on the track (see Supporting Information) gives an estimate of the order of magnitude of the stepping rate of  $0.01 \text{ s}^{-1}$ , which is of the same order as that measured. In contrast, the rate constant for stepping from a truncated (precut) stator is four orders of magnitude higher (see Supporting Information). We tentatively conclude that the release of product by N.BbvC IB is the rate-limiting step,<sup>[18]</sup> although the fact that  $k_1$  and  $k_2$  are different suggests that the spontaneous dissociation of the last few nucleotides of the cargo may also play a role.

The results presented here demonstrate the design, construction, and operation of a directional linear DNA motor driven by an enzyme that nicks DNA. The motor can be halted at a desired location by a simple modification to the track. This nanometer-scale motor moves at a speed of order  $0.1 \text{ nm s}^{-1}$  along a self-assembled track.

**Keywords:** DNA · enzymes · molecular devices · nanostructures · self-assembly

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