Communication

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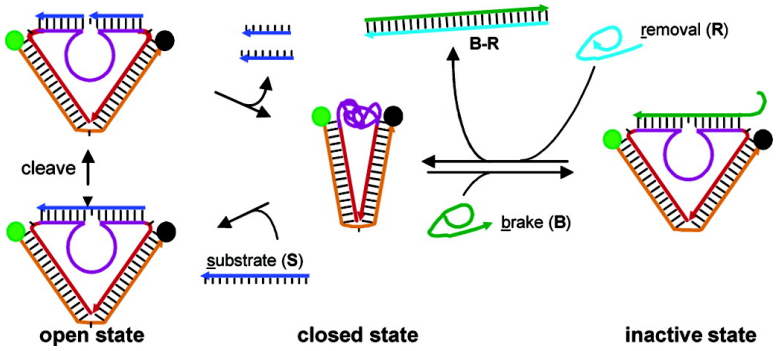
J. Am. Chem. Soc., **2004**, 126 (28), 8626-8627• DOI: 10.1021/ja047991r • Publication Date (Web): 25 June 2004



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Published on Web 06/25/2004

Putting a Brake on an Autonomous DNA Nanomotor

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It is important to control nanoscale motion and construct nanomachines for many applications, such as nanorobotics, molecular computation, dynamic nanomaterials, biosensors, and smart materials.[1−3] Ideally, a nanomotor should operate autonomously and under tight control. For example, human-made macroscale mobile devices such as cars have brakes. Cellular protein motors are controlled by regulatory signals. Motors can function properly only if they follow instructions. However, engineering of such nanomotors remains elusive. Herein, we report an autonomous nanomotor that is reversibly controlled by a brake. The motor, fuel, and brake are all nucleic acid molecules.

DNA has been well recognized for its capability in nanoconstructions.[4] Besides static nanostructures, a number of DNA nanomotors have been reported.[5] Very recently, we have constructed an autonomous DNA nanomotor based on a DNAzyme.[5j] Herein, we incorporate a reversible brake into the DNA motor. When the brake is absent, the DNA motor can constantly perform open/close motion. Addition of the brake locks the motor and stops the motion. The brake can be removed, and the motor will resume its motion. Thus, the motor not only moves autonomously but is also controlled by a brake.

Most cellular protein motors are powered by enzymatic activities and perform mechanical motion by extracting chemical energy from fuel molecules,[7] as does the reported DNA motor (Figure 1). The DNA motor (M) contains a 10−23 DNA enzyme, which can cleave RNA substrate (S).[6] The binding, cleavage, and dissociation of the DNA enzyme with strand S produce the power to open and close the DNA motor. Multiple turnovers of the enzyme contribute autonomy to the DNA motor. To stop the DNA motor, a brake molecule (B) is used. The brake (B) is a DNA analogue of strand S, which cannot be cleaved by the enzyme. Like strand S, strand B can bind to the recognition arms of the enzyme. However, strand B slightly extends complementary segments into the catalytic core by two bases. Strand B can form a longer bulged duplex with the enzyme than strand S does. Thus, the enzyme will bind to strand B instead of strand S because of differing affinity. After binding to strand B, the enzyme becomes inactivated, and the motor stops moving. To reactivate the motor, strand B has to be removed, which can be accomplished by a strand-displacement mechanism.[5b−h] For this purpose, a 10-base-long tail is added to strand B. The tail is not involved in the brake function but does in its removal. A brake removal (R) strand is fully complementary to strand B, and they form a long duplex (R−B). Strand R first base-pairs with strand B at the tail region and then pulls strand B out of the motor through branch migration. When strand B is removed from a motor, the enzyme becomes activen and the motor resumes motion. The brake can be reversibly added to and removed from the motor.

The DNA motor was formed by cooling an equimolar mixture of its component strands (E and F) from 95 °C to room temperature over 2 h. We initially used gel electrophoresis to confirm the addition/removal of the brake and the brake effect on the DNA enzyme (Figure 2). The result showed that strand B could associate

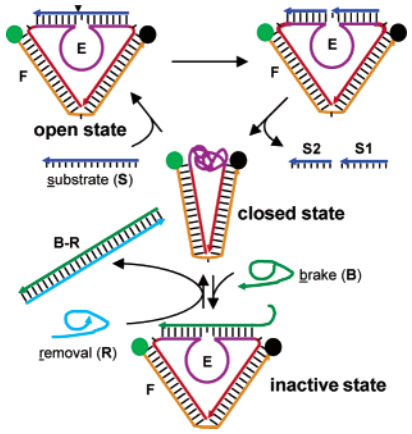

Figure 1. Scheme of a DNA nanomotor. The DNA motor consists of two single strands: E and F. The E strand contains a 10−23 DNA enzyme domain, which is colored purple. The F strand has a rhodamine green fluorophore at the 5′-end (labeled as a solid green circle) and a black hole quencher-1 (BHQ-1) at the 3′-end (labeled as a solid black circle).

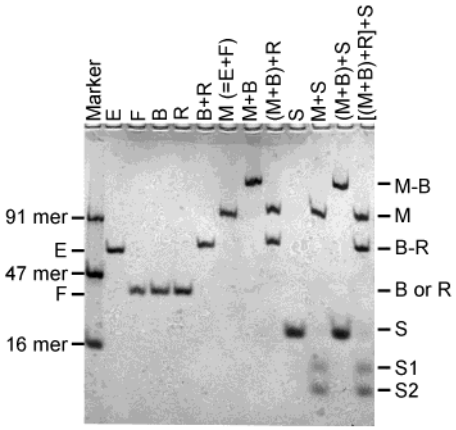

Figure 2. Gel demonstration of the brake effect. The left eight lanes show the formation of the DNA motor, and the right four lanes show that the brake inhibits the enzyme. DNA strands in any bracket were incubated at 22 °C for 30 min before adding the next strand.

with a DNA motor to form a stable M−B complex (lane M + B) and effectively stop the DNA enzyme activity (lane M + B + S). Strand B could also be effectively removed from an M−B complex by addition of strand R at an equimolar ratio. Strands R and B formed a long B−R duplex. Consequently, a motor was freed from the control of the brake (lane M + B + R) and resumed its motion (lane M + B + R + S). As a result of the enzyme activity, the RNA substrate (S) became cleaved into short fragments (S1 and S2).

We further confirmed the brake effect with a fluorescence spectroscopy study (Figure 3). Fluorescence resonance energy transfer (FRET) is an ideal tool to study nanoscale motion.[5] The energy transfer efficiency is very sensitive to the distance between

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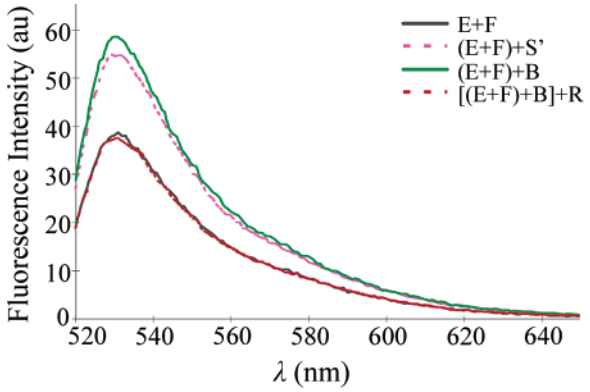

Figure 3. Fluorescence spectra demonstration of the brake effect. Strand S′ is a DNA analogue of strand S. Strands S and S′ have exactly the same sequence and length, but the DNA enzyme cannot cleave S′. Strand S′ brings the motor to the open state.

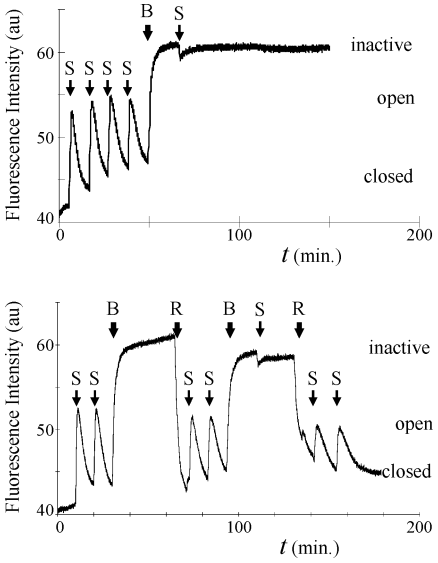

Figure 4. The brake effect on motor cycling. Addition of the brake locks the DNA motor in the inactive state; when the brake is removed, the DNA motor resumes motion. Arrows indicate the times when strands S, B, or R were added. Fluorescence signal was monitored at 531 nm, the maximium emission wavelength of rhodamine green.

a pair of fluorophores as $[1 + (R/R_0)^6]^{-1}$ (R is the distance between the two fluorophores, and R_0 is a fluorophore-dependent constant).[8] In the closed state, energy was efficiently transferred from rhodamine green to BHQ-1, and the fluorescence signal of rhodamine green (the peak at 531 nm) was of low intensity. While in the open state, the energy transfer efficiency was low, and the fluorescence was strong. After addition of strand B, the motor was locked in the inactive state (M−B) whose two fluorophores were separated from each other. Thus, the fluorescence was strong. The brake effect could be reversed by addition of strand R. Strand R removed strand B from the motor and led the motor to return to an active form (closed state) whose fluorescence was quenched as for the original motor.

Finally, we demonstrated the brake effect with a motor cycling experiment (Figure 4).[5b−j] We manually added an equal amount of strand S to the motor solution. Immediately after addition of the substrate, the DNA motor switched to the open state and gave a strong fluorescence signal. As time lapsed, strand S was cleaved into small fragments and dissociated from the DNA motor. The motor consequently returned to the closed state, and the fluorescence was effectively quenched. The open−closed cycle repeated with repeated addition of strand S. However, addition of strand B locked the DNA motor in the inactive state whose fluorescence would not change even if substrate was added. Upon removal of strand B from the locked motor by adding strand R, the motor resumed the motion. The fluorescence signal decreased over the course of the experiment, which was presumably caused by photobleaching of fluorescence dyes.

In summary, we have developed a strategy to reversibly switch an autonomous DNA nanomotor on/off. Such a motor also can be regarded as a motor that can move with two different fuels by two different mechanisms. One is an autonomous mode mediated by a DNA enzyme activity; the other is manually controlled by hybridization through alternative addition of fuel strands (the brake strand [B] here) and removal strands (R). The two modes interplay and result in better control over a nanomotor. It is worth pointing out that the motion of the reported motor involves formation of a bulged DNA duplex, whose length could vary from 14 bp (with a bulge of 15 bases, as in this study) to at least 29 bp (with no bulge). Thus, at least 16 distinct open states could be achieved, which would be far beyond the motion complexities previously reported.[2,5]

Acknowledgment. This work was supported by NSF (EIA-0323452), DARPA/DSO (MDA 972-03-1-0020), and Purdue University. We thank Prof. D. R. McMillin and M. H. Wilson for help with fluorescence measurements, and Prof. J. J. Wilker and Ms. E. E. Hewitt for help with manuscript preparation.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA047991R