DNA Fuel for Free-Running Nanomachines

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We describe kinetic control of DNA hybridization: loop complexes are used to inhibit the hybridization of complementary oligonucleotides; rationally designed DNA catalysts are shown to be effective in promoting their hybridization. This is the basis of a strategy for using DNA as a fuel to drive free-running artificial molecular machines.

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Molecular motors require an energy source. Biological motors typically extract energy from the hydrolysis of nucleoside triphosphates, usually adenosine triphosphate (ATP) (e.g., myosin [1], kinesin [2], dynein [3], DNA processing enzymes [4], ion pumps [5]) or by transporting ions down an electrochemical potential gradient across a membrane (e.g., F_O ATP synthase [6], the bacterial flagellar motor [7]). If their environment is *constant* (and both energy sources are actively maintained in biological systems), then these motors can run indefinitely. In contrast, most synthetic molecular machines change state in response to *changes* in external conditions—they act as switches and are closer to regulatory proteins that undergo conformation changes driven by guanosine triphosphate hydrolysis [8] than to ATP-driven motors. Such synthetic machines must be clocked around an operating cycle—e.g., by repeated photoisomerization [9] or by cyclic changes of the chemical or electrochemical environment [10]. Some progress towards a unidirectional, chemically driven rotor has been reported [11], though the molecule described completes only one third of a revolution. The first molecular device capable of cycling freely under continuous stimulation is light driven [12]: its operating cycle is driven by reversible photoisomerizations and made irreversible by dissipative thermal isomerization. We introduced the use of DNA as a chemical fuel [13] and demonstrated that the free energy of hybridization of complementary oligonucleotides (short strands of DNA) may be used to produce repetitive motion of a molecular machine. The first DNA-fuelled devices [13,14] are clocked around their operating cycle: the machine's conformation is changed by interaction with a fuel strand, and the change is reversed when the first fuel strand is displaced by a complementary strand (if both components of the DNA fuel are added simultaneously, they will react directly without affecting the machine). In this Letter, we demonstrate essential elements of kinetic control of hybridization [15] that permit operation of a *free-running* DNA-fuelled machine. (i) Direct hybridization of the DNA fuel, without involvement of the machine, is inhibited by using a protective strand to tie the ends of one or both fuel strands together to form a loop complex. (ii) A rationally designed DNA catalyst (a new class of deoxyribozyme [16]) promotes hybridization of the modified fuel by partially displacing the protective strand and opening the loop. A machine that catalyzes the reaction of DNA fuel in this way will be able to run continuously until the fuel is exhausted.

We introduce our discussion of control of DNA hybridization by considering the simple strand-displacement reaction shown in Fig. 1(a). Hybridization between complementary strands S and $\overline{S}$ is inhibited by a protective strand P which has already hybridized to $\overline{S}$ [17]. Because P is a truncated copy of S, strand displacement can be

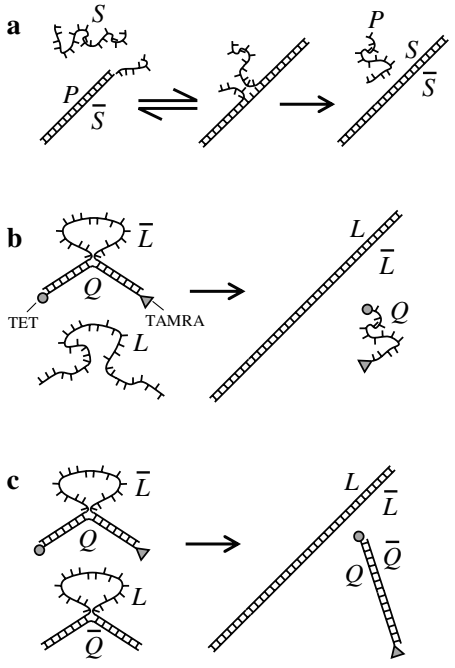

FIG. 1. Reactions in which the hybridization of two complementary strands is inhibited by a protective strand, already hybridized to one of them.

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initiated by hybridization at the overhang of unpaired bases and proceed by branch migration, a random walk of the junction between competing strands S and P, until P is completely displaced [18]. The nucleation of a hybridized region joining S and $\overline{S}$ is an activated process [19] and for oligonucleotide lengths considered here (<100 bases) this, not the displacement of P, is the rate-limiting step [19,20]. For overhangs longer than eight bases the presence of P has little effect on the reaction rate; the rate constant for the $S - \overline{S}$ reaction is 10^5–10^6 $M^{-1}\,s^{-1}$, giving a time to half-completion of $\sim$1 s for 1 μM reactant concentrations [21]. Protective strand P is ineffective at inhibiting hybridization between S and $\overline{S}$.

Our strategy for inhibiting hybridization is shown in Fig. 1(b). Protective strand Q (30 bases) is designed to hybridize with 15-base sections at either end of 70-base strand $\overline{L}$ to form a loop. Figure 2 gives nucleic acid sequences for the system studied. The reaction of the $Q\overline{L}$ complex with the complementary strand L, shown in Fig. 1(b), is driven by the free energy released by hybridization of the 40 unpaired bases in the belly of the loop. Although we expect that hybridization between L and $\overline{L}$ will be readily initiated at unpaired bases near the center of $\overline{L}$, its progression is inhibited in two ways. (i) To form a double helix one strand of DNA must twist around the other: for the reaction to proceed an end of L must be threaded through the loop. Although single-stranded DNA is relatively flexible (persistence length $\sim$3 bases [22]), the loop opening is small (< 4 nm) and this process is hindered. (ii) The perimeter of the loop is less than one-third of the persistence length of double-stranded DNA [23], so hybridization creates strain that can be relieved only by opening the loop. Protective strand Q is effective in inhibiting the reaction between L and $\overline{L}$ even though the initial and final states differ by 40 base pairs or $\sim$70 kcal mol^{-1} [24].

We use dye labels TET (5′ tetrachloro fluorescein phosphoramidite) and TAMRA (carboxy tetramethyl rhodamine) on the ends of Q to follow the reactions shown in Fig. 1(b). TAMRA's absorption spectrum overlaps the emission of TET: resonant Förster energy transfer from TET to TAMRA quenches TET fluorescence with an efficiency that depends on the inverse sixth power of their separation [25]. The ends of Q are further apart in the $Q\overline{L}$ complex than in the random coil configuration of displaced Q, so the TET fluorescence is quenched as the reaction proceeds. TET fluorescence was excited with the 514.5 nm line of an argon ion laser and selected by an interference filter with bandpass 10 nm centered at 540 nm. Reactants (L and the $Q\overline{L}$ complex) were mixed to give equal initial concentrations $c = 0.5$ μM [26]. The reaction was typically followed for 10^5 s, after which the reactants were annealed for 4 min at 80 °C then cooled to 20 °C to check the fluorescence intensity at the end point I_∞. Second-order rate constants k_2 were determined by fitting the fluorescence intensity decay curve to the function $I(t) = \{I(0) - I(\infty)\}/\{1 + k_2 ct\} + I(\infty)$. Figure 3(a) shows the fluorescence decay as L reacts with the $Q\overline{L}$ complex with 0.5 μM reactant concentrations. The second-order rate constant for this reaction is $k_2 = 420(\pm 60)$ $M^{-1}\,s^{-1}$ corresponding to a time to half-completion of 4.8×10^3 s. The rate constant is not significantly decreased if Q, L, and $\overline{L}$ are extended by ten bases such that the $Q\overline{L}$ complex is held together by two 20-base sections; this suggests that the reaction rate is determined by competition between L and Q for binding to $\overline{L}$, not by spontaneous loop opening. The reaction of L with unprotected $\overline{L}$ is 100× faster ($k_2 = 4.4 \times 10^4$ $M^{-1}\,s^{-1}$).

Figure 1(c) shows an even more effective protection strategy; both L and $\overline{L}$ are protected (complex $\overline{Q}L$ is

Q	5′ AA1GAGGG1GAC1T1CGACCG1AAACGACGA 3′
$\overline{Q}$	5′ TCGTCGTTTACGGTCGAAGTCACCCTCATT 3′
L	5′ AA1GAGGG1GAC1T1CAACC1AGCGAGCGAACG1GCCA ATTCTGATCTACTGTGTGGACCGTAAACGACGA 3′
$\overline{L}$	5′ TCGTCGTTTACGGTCCACACAGTAGATCAGAATTGGC ACG1T1CGC1CGC1AGG1T1GAAG1CACCC1CA1T1 3′
$\overline{L}'$	5′ GCATCTCTGGAATCGTCGTTTACGGTCCACACAGTAG ATCAGAATTGGCACGTTCGCTCGCTAGGTTGAAGTCA CCCTCATT 3′
$M_{10,6}$	5′ CTACTGTGTGGACCGTAAACGACGATTCCAG 3′
$M_{10,0}$	5′ CTACTGTGTGGACCGTAAACGACGA 3′
$M_{6,0}$	5′ TGTGTGGACCGTAAACGACGA 3′
$M_{0,10}$	5′ GACCGTAAACGACGATTCCAGAGAT 3′
$M_{0,6}$	5′ GACCGTAAACGACGATTCCAG 3′
$M_{0,0}$	5′ GACCGTAAACGACGA 3′

FIG. 2 (color). Oligonucleotide sequences.

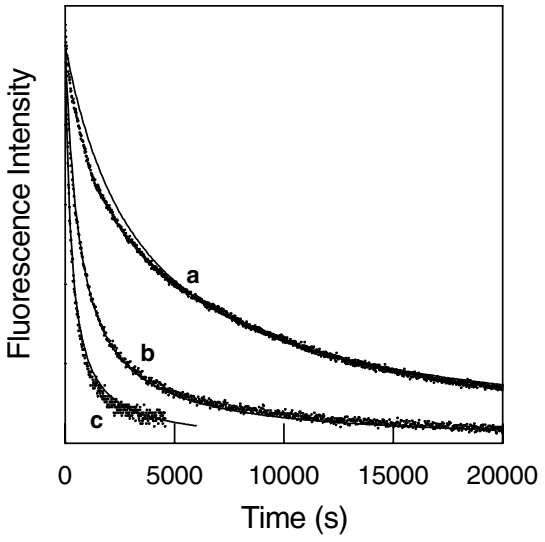

FIG. 3. Time-dependence of TET fluorescence during reactions of the $Q\overline{L}$ loop complex. The residual quenched intensity from free Q has been subtracted, so the signal is proportional to the concentration of unreacted loops. Solid lines are fits assuming second-order kinetics. (a) $Q\overline{L} + L \rightarrow Q + L\overline{L}$; (b) $Q\overline{L}' + L \rightarrow Q + L\overline{L}'$ catalyzed by 0.125 μM $M_{0,6}$; (c) $Q\overline{L}' + L \rightarrow Q + L\overline{L}'$ catalyzed by 0.5 μM $M_{0,6}$.

complementary to $Q\overline{L}$) and cannot twist around each other without opening at least one loop. We have established a lower limit to the time to half-completion for reaction between the complexes at 5 μM concentration of 6×10^4 s: we deduce that $k_2 < 3\ \mathrm{M}^{-1}\,\mathrm{s}^{-1}$, more than $10^4\times$ slower than for the unprotected reaction.

We have established above that it is possible to increase the reaction time for hybridization of complementary strands of DNA by 2 and 4 orders of magnitude by use of geometrical and topological constraints induced by loop structures. This creates the opportunity to design free-running machines that catalyze the reaction of a long-lived two-component fuel. Figure 4 shows how we have implemented catalytic control of reaction 1(b). The loop complex has been modified by adding a 12-base overhang to $\overline{L}'$. $M_{x,y}$ is designed to catalyze the reaction by displacing part of protective strand Q and thus opening the loop. $M_{x,y}$ is complementary to a section of $\overline{L}'$ starting with x bases in the belly of the loop (the "internal toehold"), continuing with a 15-base section which competes with Q for binding to $\overline{L}'$, and ending with y bases that hybridize to the overhang section of $\overline{L}'$ (the "external toehold"). Hybridization of $M_{x,y}$ to the $Q\overline{L}'$ complex may be initiated at either toehold: hybridization at the internal toehold is only weakly hindered because it is designed to be short compared to the loop perimeter; interaction at the external toehold is not hindered by the loop. $M_{x,y}$ then displaces half of Q from $\overline{L}'$, opening the loop. Once the loop is open, hybridization of L to $\overline{L}'$ is no longer impeded: L displaces $M_{x,y}$ (regenerating the catalyst) and completes the displacement of Q. Figures 3(b) and 3(c) are fluorescence decay curves for reactions catalyzed by two different concentrations of the catalyst $M_{0,6}$.

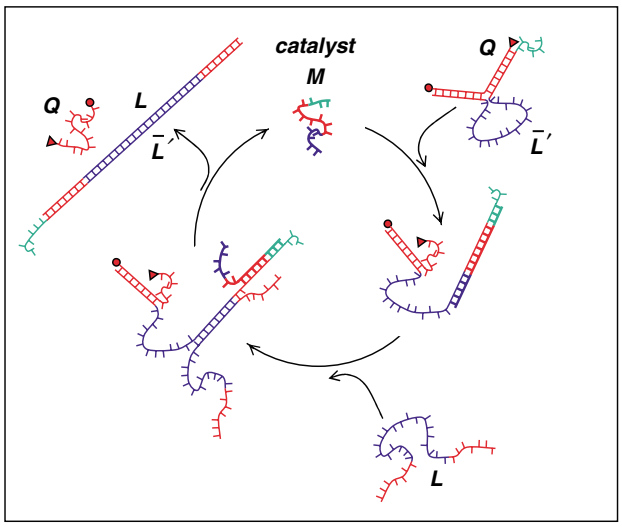

FIG. 4 (color). Catalysis of the reaction of a DNA loop with a complementary strand of DNA. The catalyst hybridizes with the $Q\overline{L}'$ loop complex, opening the loop and lowering the free energy barrier to reaction with complementary strand L.

Figure 5 shows the dependence of the reaction rate on the concentration of catalysts $M_{x,y}$. All catalysts with 6- and 10-base internal and external toeholds (and both) perform similarly. The initial linear increase in rate constant with catalyst concentration $[M_{x,y}]$, followed by saturation at approximately stoichiometric $[M_{x,y}]$, is consistent with the calculated stability of catalyst/loop complexes: for substoichiometric $[M_{x,y}]$ the reaction rate is approximately proportional to the concentration of loop complexes that have bound a catalyst strand [27]; for above-stoichiometric $[M_{x,y}]$ most loops have a bound catalyst, so the reaction rate is approximately independent of catalyst concentration. Small differences in performance may be partly due to unintended intramolecular interactions: self-hybridization of single-stranded sections impedes the designed interactions and reduces the reaction energy. Such secondary structure is sequence specific; our sequences are designed to ensure that it is weaker than the weakest designed interaction [24] (a 6-base overlap at the external toehold). The maximum reaction rate is increased by $\sim 30\times$, corresponding to a reaction time to half-completion of 170 s. A control "catalyst" $M_{0,0}$ with no toehold had no significant effect on the reaction rate.

We have demonstrated kinetic control of hybridization between DNA oligomers: hybridization may be inhibited by the deliberate formation of loop complexes, and triggered by a DNA catalyst that opens the loop by a strand-displacement reaction. Kinetic control is a new

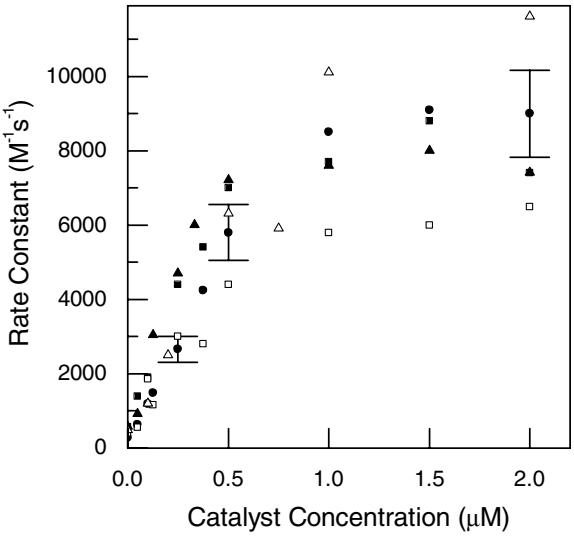

FIG. 5. Reaction rate constants as a function of the concentration of DNA catalysts $M_{x,y}$ ($[Q] = [L] = [\overline{L}'] = 0.5\ \mu$M). Catalysts have the same 15-base section that competes with Q to open the $Q\overline{L}'$ loop but different toeholds for initial interaction with the loop complex: $M_{x,y}$ has an internal toehold complementary to x bases in the belly of the loop and an external toehold complementary to y bases in the overhang section of $\overline{L}'$. ●, $M_{10,6}$; ▲, $M_{0,6}$; △, $M_{0,10}$; ■, $M_{6,0}$; □, $M_{10,0}$.

degree of freedom in the design of DNA nanostructures. It opens the way to the production of DNA machines that obtain energy by catalyzing the reaction of a fuel consisting of metastable loop complexes; such machines can run freely while the supply of unreacted fuel lasts. We may regard our catalyst strand as a primitive example of such a machine: in the presence of fuel it undergoes repeated transitions between ordered (hybridized) and random coil configurations in a tightly coupled chemomechanical cycle [28] (Fig. 4) in which each step is to a state of lower free energy. The transition from random coil to ordered occurs when the catalyst binds its substrate (the $Q\overline{L}'$ complex). The resulting intermediate complex has a lower free energy barrier for hybridization of L with $\overline{L}'$. The reaction releases waste products ($L\overline{L}'$ and Q) and is coordinated with the catalyst's return to a random coil. The stability of the duplex waste product means that there is no significant back action of accumulated waste (waste could be removed by flowing reactants past a tethered molecular motor). We note that the conformation changes of a similar device could be harnessed to create a time-dependent potential driving a isothermal ratchet [29]. We also note that catalytic control of the kinetics of DNA hybridization has the potential to increase the flexibility and reliability of DNA self-assembly by making the rapid formation of hybridization bonds conditional on the presence of catalytic oligonucleotides anchored to one or more correctly positioned adjacent elements of the structure [15].

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[27] For substoichiometric concentrations of catalysts $M_{x,0}$ which have only internal toeholds, there are deviations from second-order reaction kinetics: these catalysts can bind only to unreacted $Q\overline{L}'$ complexes (not to the $L\overline{L}'$ product), so the catalyst concentration effectively increases as the reaction proceeds.
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