# A molecular machine made of and powered by DNA

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The predictable and specific hybridization of complementary strands of DNA allows construction on a nanometre length scale: (i) DNA tags may be used to organize the assembly of colloidal particles[1,2]; (ii) DNA may be used as a structural material in its own right to make complicated objects, e.g. ordered arrays of tiles[3], linked rings[4] and polyhedra[5]; (iii) DNA may act as a template to direct the growth of semiconductor nanocrystals[6] and metal wires[7]. These are static structures: Seeman and co-workers have produced an active device, a nanomechanical switch[8] whose conformation is changed by inducing a B-Z transition (from a right- to a left-handed double helix) by changing the composition of the buffer solution. Conformational changes caused by the binding of oligonucleotides or other small molecules have been shown to change the enzymatic activity of ribozymes[9,10,11,12] We report the construction of a new class of active nanostructure: a machine in which DNA is used not only as a structural material but also as a fuel.

Single strands of DNA composed of complementary sequences of the bases adenine, cytosine, guanine and thymine (A,C,G,T) hybridize to form a stable duplex (double helix) bound together by hydrogen bonds between complementary base pairs (A-T and C-G). Our machine is prepared by mixing stoichiometric quantities of three strands, A, B and C, in SPSC buffer at 20 °C to give a final

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concentration of 1 µM; the base sequences chosen for the three strands are given in Table 1. The machine has the form of a pair of tweezers; its structure and operation are shown in Figure 1. Strand A consists of two 18-base sequences which hybridize with complementary sequences at ends of strands B and C to form two stiff[13] arms; the hinge is formed from a four-base single-stranded region of A between the regions hybridized to strands B and C. In the machine's rest state the remaining unhybridized 24-base portions of the 44-base strands B and C dangle floppily from the ends of the tweezers: double-stranded DNA has a persistence length of order 100 base pairs[13,14] whereas at 1 M salt concentration single-stranded DNA has a persistence length ~1 nm[15] or approximately three bases.

Strand A is labelled at the 5' and 3' ends with dyes TET (5' tetrachloro fluorescein phosphoramidite) and TAMRA (carboxy tetramethyl rhodamine) respectively. When TET is excited by the 514.5 nm emission of an Argon ion laser it fluoresces with a peak emission wavelength of 536 nm; this emission is quenched by resonant intramolecular energy transfer from TET to TAMRA (a longer-wavelength dye whose absorption band overlaps the emission band of TET) with an efficiency that decreases rapidly as the distance between the dyes increases[16]. Fluorescence quenching is used as an indicator[17] to titrate strands B and C against A; as half of A is straightened from a random coil by hybridization with B (or C) the mean separation between the dye molecules on A increases leading to a five-fold increase in the fluorescence intensity. The cumulative effect of hybridization with both B and C is a seven-fold increase in fluorescence intensity in the rest state.

The assembled tweezers are opened and closed with fuel and removal strands F and $\overline{\overline{F}}$. The 56-base fuel strand F consists of two consecutive 24-base sections, which are complementary to the dangling ends of B and C, with an additional 8-base overhang section. Figure 1b shows how fuel strand F hybridizes with the free ends of strands B and C, pulling the ends of the tweezers together. The average free energy change associated with the hybridization of a complementary base pair is -78 meV (-1.8 kcal mol$^{-1}$) at 20 °C [18] and the separation between base pairs in single-stranded DNA is

0.43 nm[19] giving an average closing force ~15 pN which is consistent with that required to pull apart double-stranded DNA[20]. This is at the upper end of the range of measured forces exerted by single-molecule kinesin[21,22] and myosin[23,24] motors. Removal strand $\overline{F}$ is the complement of F: the additional free energy gained when the overhang (single-stranded in the initial state) hybridizes with $\overline{F}$ ensures that when a stoichiometric quantity of $\overline{F}$ is added it removes F from the machine to form a double-stranded waste product $F\overline{F}$ and returns the tweezers to the open state. Hybridization between the fuel and removal strands is expected to occur first at the exposed overhang[25] and to proceed by branch migration[26], a random walk of the junction between the region of F newly hybridized to the removal strand $\overline{F}$ and the region still hybridized to the tweezer components B and C, that continues until both B and C have been completely displaced and the $F\overline{F}$ duplex diffuses away. The random walk occurs sufficiently quickly that the rate-limiting step in such strand-displacement reactions is the endothermic nucleation of a region in which complementary bases are joined[25,27,28] (in this case, the nucleation of a hybridization between the F and $\overline{F}$ strands): this is consistent with our observation that the measured second-order rate constants for opening and closing the tweezers are approximately equal.

Dye quenching is used to titrate the fuel and removal strands and to determine the state of the machine. The fluorescence intensity drops by a factor of six when the tweezers are closed, back to approximately the same level as from unhybridized, randomly-coiled A strands. Figure 2 shows the fluorescence intensity variation as the tweezers are cycled seven times between the open and closed states by successive additions of strands F and $\overline{F}$. The switching time for the machine is approximately 13 s.

To calibrate the motion of the device we measure the fluorescence intensity as a function of the separation between TET and TAMRA dye labels. A 24-base oligonucleotide labelled at the 5' end with TET and a separate 24-mer labelled at the 3' end with TAMRA are hybridised to complementary sequences at either end of a (48+$n$)-mer ($n$ = 0, 10, 15, 20, 40) whose central $n$-base spacer section is

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straightened by hybridisation to a complementary *n*-mer. The dye-labelled ends of the two 24-mers are separated only by the spacer section; its length is much less than the persistence length of double-stranded DNA so it acts as a rigid rod holding the TET and TAMRA end groups at a fixed separation. The fluorescence intensity from open tweezers (with free ends) is equal to the interpolated value for a 17-base spacer: we deduce an approximate average value for the difference in separation between the ends of the tweezers in the open and closed states of 5.8 nm, corresponding to an average angle between the open tweezer arms of 50º.

The low fluorescence intensity in the closed state tells us only that TET and TAMRA dye molecules are relatively close together. To confirm our model of closed tweezers, in which the stiff arms of the tweezers are held together by the fuel strand (the 'properly closed' configuration), it is necessary to demonstrate that quenching is due to the proximity of the two dye molecules attached to a single strand of DNA, and not to the formation of dimers or larger complexes (referred to collectively as dimers below) in which fuel strands tie the TET molecule on one strand close to the TAMRA on another. To do this we have made use of a modified tweezer structure made from strands A, ß, ? (Table 1). Strands ß, ? have the same 18-base sequences complementary to A as strands B, C but have different 24-base free ends. Strands $F_{B?}$, $F_{Cß}$ cannot close either tweezer structure, but can hybridize to link free ends of strands B to those of strand ?, and free ends of strands C to those of strands ß, joining one of the original tweezers to one of the modified tweezers to form a dimer. We have used polyacrylamide gel electrophoresis to compare 'closed tweezers' (A,B,C) + F with 'dimers' (A,B,C) + (A,ß,?) + $F_{B?}$ + $F_{Cß}$. Figure 3 shows an image of the gel recorded using laser-excited dye fluorescence. Lanes a-e are controls containing incomplete structures; lane l contains dimers. Lanes f-k contain open (f,h,j) and closed (g,i,k) tweezers corresponding to successive additions of fuel and removal strands F and $\overline{F}$. Bright bands in lanes b-f,h,j correspond to structures in which quenching of TET fluorescence is minimum; their intensities in this image have saturated. The dominant band in the

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'closed tweezer' lanes is absent from the 'dimer' lane, consistent with our interpretation that the dominant component of 'closed tweezers' does indeed correspond to single, 'properly closed' tweezers as designed. Low-intensity bands in the 'closed tweezer' lanes match bands in the 'dimer' lane l, confirming that some dimer formation does occur when F is added to open tweezers; their integrated intensity is ~30% of the total from closed tweezers. Quantitative analysis of this image is unreliable as both excitation intensity and the degree of fluorescence quenching vary from band to band.

To estimate the yield of 'properly closed' tweezers we have compared the quenching of the fluorescence intensity, $Q = \Delta I / I$ in three cases: *a*) $Q_a = 0.83$ when a stoichiometric quantity of F is added to a sample of open tweezers (control); *b*) $Q_b = 0.77$ when a stoichiometric quantity of F is added to a sample of open tweezers in which 50% of strands A are not dye-labelled; *c*) $Q_c = 0.23$ when a stoichiometric quantities of $F_{B?}$ and $F_{C\beta}$ are added to a mixture of equal quantities of original tweezers (with dye labels) and modified tweezers (without dye labels). In case *c*) strands $F_{B?}$ and $F_{C\beta}$ can only link tweezers to form dimers in which dye-labelled strands A are joined to unlabelled strands; we interpret the slight quenching in this case as evidence that the TET and TAMRA molecules on the same strand A move closer together when it is incorporated in a dimer. We expect ~50% of dimers formed in case *b*) to link a dye-labelled strand to an unlabelled strand reducing fluorescence quenching relative to the control: if only dimers were formed we would expect $Q_b = (Q_a + Q_c)/2 = 0.53$. From our measured value $Q_b = 0.77$ we infer that ~80% of tweezers are 'properly closed' by the fuel strand F.

To test whether the duplex structures formed by hydrogen bonding between A, B and A, C survive the forces exerted by hybridization with the fuel strand we have used an additional strand $\overline{A}$, the complement of A, to test the integrity of the machine by displacing B and C from A. In the absence of other strands $\overline{A}$ hybridizes with A (producing a eight-fold increase in fluorescence intensity) with a time to half-completion of 17 s for $[A] = [\overline{A}] = 1\,\mu M$. When a stoichiometric quantity of $\overline{A}$ is introduced to the same concentration of ready-formed tweezers in the open state the time to half-

completion for interaction is increased to $4.8 \times 10^3$ s by the need for thermally-activated displacement of parts of B and C from A before hybridization with $\overline{A}$ can begin. The interaction of $\overline{A}$ with tweezers held in the closed state is an order of magnitude slower than in the open state (time to half-completion $5 \times 10^4$ s); the closed structure inhibits the winding and unwinding of strands that accompanies strand exchange. The high stability of the tweezers, even in the closed state where the hinge region is likely to be strained, indicates that its structural integrity is maintained. The recovery of a high fluorescence intensity after each cycle of closing and opening indicates that the dye-labelled ends of A are relatively far apart. We have used polyacrylamide gel electrophoresis to check that strand A is still intact after three such cycles (Figure 3 lanes 6-11): we deduce that the initial configuration, in which the two halves of A are straightened by hybridization with B and C, is recovered after each cycle. We have also checked that when one of the tweezer components B, C is left out then changes in fluorescence intensity due to successive additions of F and $\overline{F}$ are less than 8% of the change achieved with the complete structure, and that there is no observable interaction between unhybridized strand A and either F or $\overline{F}$. We conclude that unanticipated interactions between strands are weak and that the machine operates as designed.

We have demonstrated a simple nanomachine constructed of DNA that uses DNA as a fuel. Our tweezer structure allows the separation between two dye molecules to be changed repeatably on a nanometre length scale; a similar structure could be used to investigate the interaction between chemically active components attached to the ends of the tweezers or, if combined with a DNA cage[5], to alternately hide and reveal a target molecule. More sophisticated devices based on this principle may include, for example, levers made of the rigid double-crossover structures demonstrated by Seeman and co-workers[29] to increase the force exerted, rotary motors in which the rotor is pulled sequentially to points spaced around a stator, and free-running machines using metastable DNA loops as fuel[30].

Because the binding between fuel and machine is sequence-specific the DNA strands that act as fuel may also serve as information carriers to coordinate components of a complex machine or to carry signals between machines. The use of DNA fuel allows precise control of movements on a nanometre length scale without prodding by a scanning microscope tip; it makes possible coordinated motion of between 2 and $10^{18}$ (or more) devices which may incorporate elements chosen for their biochemical, optical or electrical properties. Controlled motion provides exciting new degrees of freedom in the design of nanostructure devices.

Methods

Oligonucleotides were supplied by Integrated DNA Technolgies, Inc; strand A was HPLC purified, other strands were PAGE purified. Stock solutions were prepared by resuspending the lyophilized oligonucleotides in TE buffer (10 mM Tris pH 8.0, 1 mM EDTA) at a nominal 25 µM concentration. Fluorescence quenching was used as an indicator to titrate strands B, C against A and strands F, $\overline{\text{F}}$ against open tweezers (formed of stoichiometric quantities of A,B and C) in SPSC buffer (50mM $Na_2HPO_4$ pH 6.5, 1M NaCl). TET fluorescence was excited with the 514.5nm line of an argon ion laser (mechanically chopped at 130 Hz), selected by an interference filter with bandpass 10nm centred at 540nm and detected by a Si photodiode and phase-sensitive detector. We estimate an error of 10% in determining relative concentrations. Absolute concentrations were obtained from uv absorption measurements by the commercial supplier of the oligonucleotides; from our measurements of relative concentrations we estimate that the error in absolute concentrations is of order 30%. To form open tweezers 2 µl of stock solution of strand A was diluted to 1 µM using 48 µl SPSC buffer, then stoichiometric quantities of the stock solutions of strands B and C were added; the mixture was left for >20 mins. to allow the reaction to reach near-completion. Each addition of a stoichiometric quantity of a stock solution of strand F or $\overline{\text{F}}$ resulted in ~3% further dilution of the reactants; after each addition (Figure 2) reactants were mixed by rapid pipetting up and down lasting <8 s. All measurements were performed at 20°C.

Polyacrylamide gel electrophoresis (Figure 3) was carried out using a Multiphor II flatbed electrophoresis system with an ExcelGel 48S (T=12.5%, C=2%) precast gel (Amersham Pharmacia Biotech). Reactions were carried out and 5 µl samples loaded in SPSC buffer with an approximate DNA concentration of 5 µM for each species except $F_{B?}$, $F_{Cß}$ (2.5 µM). Each reaction was allowed a minimum of 20 mins to proceed to near-completion. Fluorescence was excited by 514.5nm line of an argon ion laser, selected by an interference filter with bandpass 10nm centred at 540nm and recorded by a CCD camera: the figure is a composite of four images.

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**Table 1  Oligonucleotide Sequences**

| Name | Sequence |
|------|----------|
| A | 5' TGCCTTGTAAGAGCGACCAT CAACCTGGAATGCTTCGGAT 3' |
| B | 5' GGTCGCTCTTACAAGGCA <span style="color:blue">CTGGTAACAATCACGGTCTATGCG</span> 3' |
| C | 5' <span style="color:green">GGAGTCCTACTGTCTGAACTAACG</span> ATCCGAAGCATTCCAGGT 3' |
| F | 5' <span style="color:blue">CGCATAGACCGTGATTGTTACCAG</span> <span style="color:green">CGTTAGTTCAGACAGTAGGACTCC</span> <span style="color:red">TGCTACGA</span> 3' |
| ß | 5' GGTCGCTCTTACAAGGCA CAGCTAGTTTCACAGTGGCAAGTC 3' |
| ? | 5' GCAGGCTTCTACATATCTGACGAG ATCCGAAGCATTCCAGGT 3' |
| $F_{B?}$ | 5' CGCATAGACCGTGATTGTTACCAG CTCGTCAGATATGTAGAAGCCTGC ACGTCGAT 3' |
| $F_{Cß}$ | 5' GACTTGCCACTGTGAAACTAGCTG CGTTAGTTCAGACAGTAGGACTCC TGTCCAGA 3' |

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**Figure Captions**
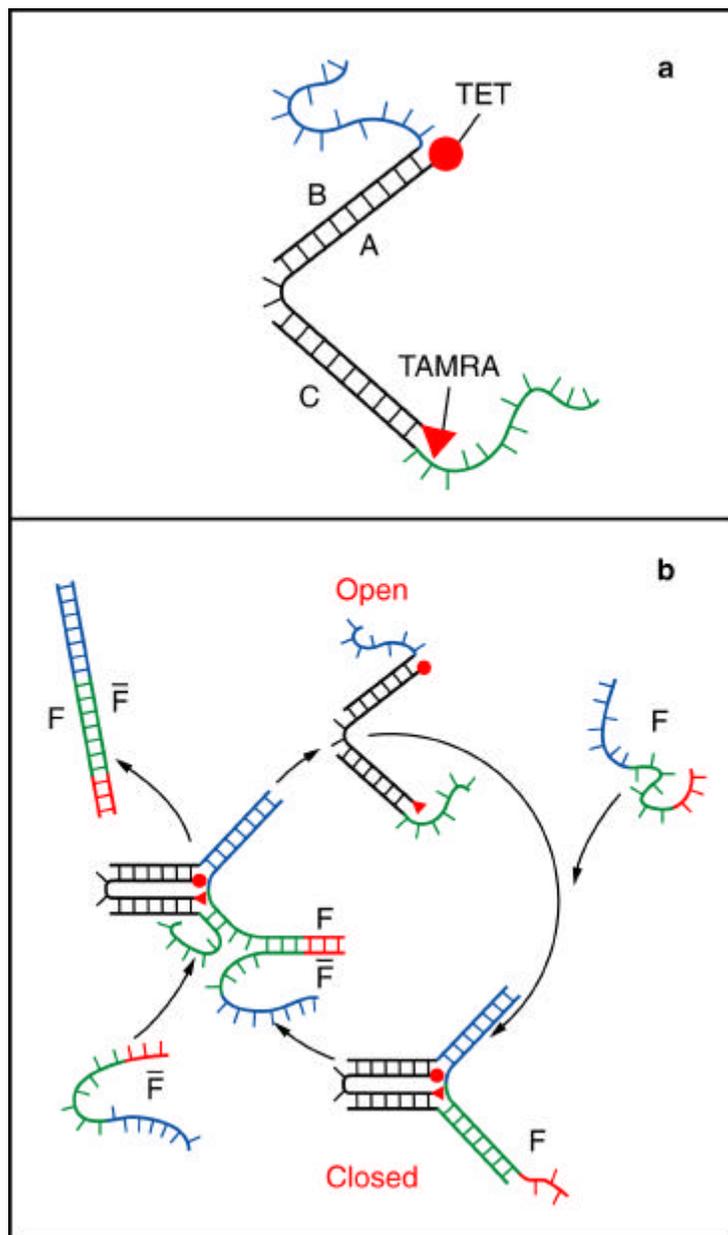


**Figure 1**

**a** Molecular tweezer structure formed by hybridization of oligonucleotide strands A, B and C

**b** Closing and opening the molecular tweezers. Fuel strand F hybridizes with the dangling ends of strands B and C (shown in blue and green) to pull the tweezers closed. Hybridization with the overhang section of F (red) allows strand $\overline{F}$ to remove F from the tweezers forming a double-stranded waste product F$\overline{F}$ and allowing the tweezers to open.
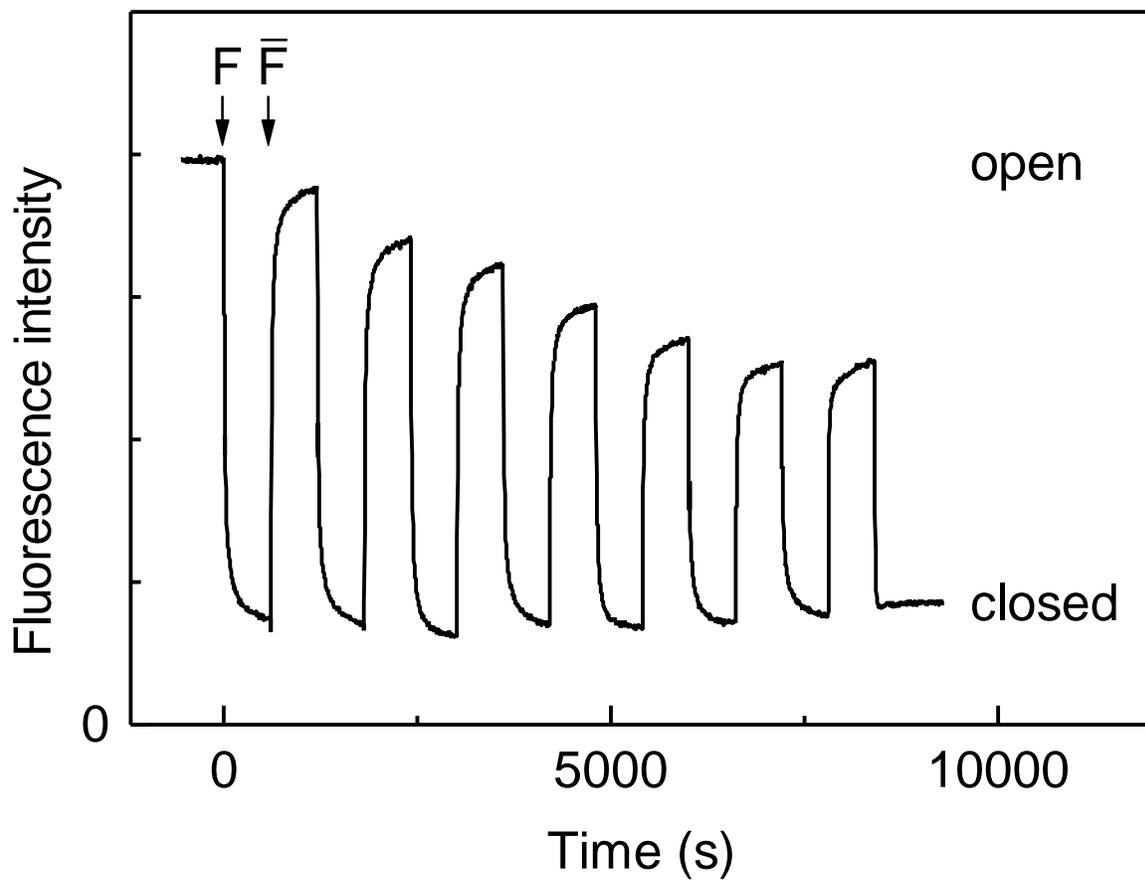
**Figure 2**

Cycling the molecular tweezers. By adding stoichiometric quantities of fuel and removal strands F and $\bar{\bar{F}}$ in sequence the tweezers may be closed and opened repeatedly. When the tweezers are closed, resonant energy transfer from the TET dye to the TAMRA quencher molecule reduces the fluorescence intensity.
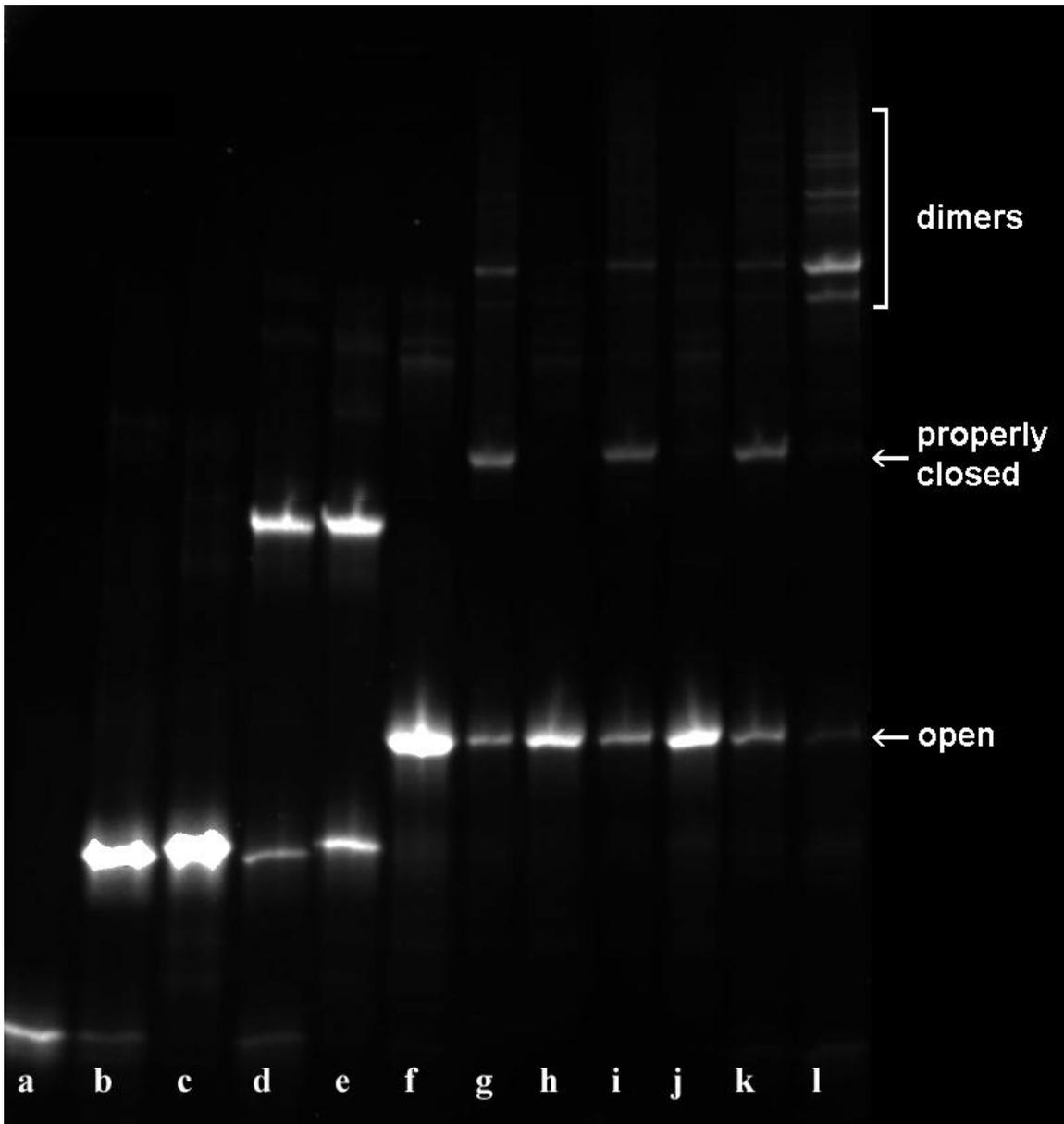
**Figure 3**

Analysis of tweezer formation by polyacrylamide gel electrophoresis. Lanes:a) strand A; b) A+C; c) A+B; d) A+C+F; e) A+B+F; f) A+B+C (open tweezers); g) (A+B+C)+F (closed tweezers); h)-k) two further cycles of opening and closing produced by successive additions of $\overline{F}$ and F to closed tweezers; i) (A,B,C)+(A,ß,?)+$F_{B?}$+$F_{Cß}$ (only dimers can form).