

DNA Nanomachines

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We are learning to build synthetic molecular machinery from DNA. This research is inspired by biological systems in which individual molecules act, singly and in concert, as specialized machines: our ambition is create new technologies to perform tasks that are currently beyond our reach. DNA nanomachines are made by self-assembly, using techniques that rely on the sequence-specific interactions that bind complementary oligonucleotides together in a double helix. They can be activated by interactions with specific signalling molecules or by changes in their environment. Devices that change state in response to an external trigger might be used for molecular sensing, intelligent drug delivery or programmable chemical synthesis. Biological molecular motors that carry cargoes within cells have inspired the construction of rudimentary DNA walkers that run along self-assembled tracks. It has even proved possible to create DNA motors that move autonomously, obtaining energy by catalyzing the reaction of DNA or RNA fuels.

The remarkable specificity of the interactions between complementary nucleotides makes DNA a useful construction material: interactions between short strands of DNA can be controlled with confidence through design of their base sequences (Box 1). The construction of branched junctions between double helices¹ makes it possible to create complex three-dimensional objects^{2,3,4,5}, such as the tetrahedron⁵ shown in Figure 1, by self-assembly. One way to exploit this extraordinarily precise architectural control is to use self-assembled DNA templates to position functional molecules: examples include molecular electronic circuits^{6,7}, near-field optical devices⁸ and enzyme networks⁹. It is an obvious extension of this research to convert static DNA structures into machines. DNA is not the natural choice of material with which to build active structures because it lacks the structural and catalytic versatility of proteins and RNA*. If we could cope with the complex repertoire of interactions required for a three-dimensional fold we would design more competent machines made, as in nature, from RNA and proteins^{11,12}. We make nanomachines from DNA because the simplicity of its structure and interactions allows us to control its assembly.

In this review we concentrate on research that is leading towards the development of synthetic molecular motors. We start by showing how DNA nanostructures can be made to switch between two states in response to molecular or environmental signals; we describe how a device can be moved along a track by operating molecular switches in the correct sequence; we finish with an account of the current state of development of autonomous molecular motors that are inspired by the natural motors myosin and kinesin. Closely-related work on DNA sensors and DNA-templated chemistry is described briefly in Boxes 2 and 3.

* For both DNA and RNA, Watson-Crick base pairing is the strongest interaction determining inter- and intra-molecular interactions, but RNA has a much richer repertoire of weaker non-covalent interactions that can stabilize complex three-dimensional structures¹⁰.

Molecular Switches

The simplest active DNA nanostructures are switches or actuators that can be driven between two conformations. Motion is induced by changes in temperature or ionic conditions or by the binding of a signalling molecule, often a DNA strand.

Conformation changes induced by changes in environment

Rotary motion can be produced by changing the twist of DNA. Double-stranded DNA with the sequence $(CG)_n$ can be flipped from the usual right-handed helix (B-DNA) to a left-handed conformation (Z-DNA); this transition is favoured by high salt concentrations and low temperatures¹³. One of the earliest nanomechanical DNA devices¹⁴ used this transition to change the angle between two rigid DNA tiles connected by a $(CG)_{10}$ stem. Each tile carried a reporter fluorophore. Förster resonant energy transfer (FRET) between fluorophores allows sensitive measurement of their separation on a nanometre length scale: the efficiency of energy transfer, mediated by a dipole-dipole interaction, scales as the inverse sixth power of their separation¹⁵. When the B-Z transition was induced by an increase in ionic strength, FRET measurements showed an increase in the separation between the fluorophores which was consistent with the expected relative rotation of the tiles by $\sim 3\frac{1}{2}$ turns.

Yang and co-workers converted changes in the twist of DNA into linear motion¹⁶. Their device consisted of a closed loop of double-stranded DNA attached to opposite arms of a four-arm Holliday junction (Box 1)¹⁷. A Holliday junction can migrate (isomerize) by breaking identical base pairs in one pair of opposite arms and remaking them in the other pair. A change in the conformation of the DNA within the loop was initiated by adding ethidium bromide: this intercalating dye binds between adjacent base pairs, lengthening and partially unwinding the double helix. The resulting stress was relieved by junction migration: by shortening the protruding arms of the junction the loop was allowed to lengthen without changing the total number of twists within it.

Environmentally-driven changes in the conformation of single-stranded DNA can induce linear motion. Under slightly acidic conditions a single strand with appropriately spaced cytosine bases folds into an i-motif, a compact three-dimensional structure that is held together by cytosine – protonated cytosine base pairs¹⁸. In the presence of a near-complementary strand of DNA there is competition between the i-motif and an extended double helix formed by hybridization of the two strands (note that a perfectly complementary strand would itself fold into a stable structure called the G-quadruplex¹⁹). The i-motif device can be switched between compact and extended states by changing the pH^{20,21}. Cyclic switching can be driven by an oscillating chemical reaction^{22,23}.

The i-motif to duplex transition has been made to do mechanical work. One surface of a silicon cantilever was coated with tethered cytosine-containing strands; formation of the i-motif induced a compressive surface stress that bent the cantilever²⁴. The origin of the surface stress, which is also observed when complementary strands hybridize to tethered probes²⁵, is not understood: electrostatic repulsion between the compact i-motifs plays a part, but the effect persists at high salt concentrations at which interactions over a distance comparable to the separation between strands are effectively screened.

It has also been suggested that the conformational change resulting from pH -dependent binding of a single strand of DNA to a duplex to form a triple-helical structure could be used as the basis of a nanomechanical actuator^{26,27}.

Devices such as those described above can be used to monitor and report on their environment. Box 2 describes how active DNA nanostructures are being developed as sensors, how elementary logical operations can be performed on their outputs, and how the combination of sensor and computation might be used to create smart drug delivery systems.

Conformation changes induced by signalling

Yurke and co-workers²⁸ constructed a pair of DNA tweezers with two rigid double-stranded arms connected at one end by a flexible single-stranded hinge (Figure 2). In the open configuration, the arms rotate freely about the hinge. Single-stranded tails extend from the free end of both arms and serve as attachment points for a control strand, the 'set' or 'fuel' strand, that can pull the arms together by hybridizing to both of them. A short region of the set strand remains single-stranded even when hybridized to the device: it serves as a toehold for hybridization of a complementary 'unset' or 'antifuel' strand that strips the set strand from the device by branch migration (Box 1). Displacement of the set strand generates a double-stranded waste product and resets the device to its initial open configuration. The device can be driven through many cycles of operation simply by repeated sequential addition of set and unset strands. The time to half completion of a single switching operation is ~10 s at typical (micromolar) control strand concentrations: the rate constant for toehold-mediated strand exchange is ~10⁵ M⁻¹s⁻¹ (ref 29). Operation of the DNA tweezers has recently been characterised using single molecule FRET measurements³⁰.

Strand displacement has been used to effect conformation changes in a wide variety of systems. A number of variations on the tweezers have been published including a device where the arms are pushed apart instead of being pulled together³¹ and a three-state device³². Yan and co-workers³³ constructed a device consisting of a linear array of rigid DNA tiles; by stripping away and replacing control strands an extra 180° twist could be added to the connecting region, flipping adjacent tiles between *cis* and *trans* conformations which were easily distinguished by atomic force microscopy. The same device has recently been incorporated into a two-dimensional DNA array³⁴. Feng *et al.*³⁵ created a two-dimensional array which could be switched between states with different lattice spacings. Hazarika and co-workers³⁶ used strand displacement to reverse the aggregation of gold nanoparticles held together by DNA bridges³⁷. A number of groups have used DNA control strands to switch a section of DNA between a single-stranded state, designed to fold into a compact G-quadruplex stabilized by hydrogen-bonded tetrads of guanine¹⁹, and an extended duplex^{38,39,40}.

Motion of DNA devices can be triggered using RNA control strands⁴¹. By using a specific mRNA as a control strand, a tweezers device has been used to sense *in vitro* transcription^{42,43}. These experiments show how DNA devices could be controlled by transcriptional circuits⁴⁴⁻⁴⁷, and point to future applications in which the presence of a specific mRNA within a cell might trigger an event such as the release of a caged drug⁴⁸.

The maximum force exerted by hybridization of a signalling strand of DNA can be estimated as the free energy change on hybridization divided by the distance through which the hybridizing strands must move together and is of the order of 10 pN (ref. 28). The fact that protein motors generate similar forces⁴⁹⁻⁵² encourages speculation that DNA hybridization could be used to drive DNA devices that mimic natural molecular motors.

Clocked walkers

Machines that can be driven through more than one conformational transition are capable of relatively sophisticated tasks, like programmable synthesis of oligomers (Box 3) or directed motion along a track. They can be controlled by means of a set of DNA instructions that set the state of the machine by addressing each of the component devices independently. The simplest example is a 3-bit rewritable memory consisting of three single-stranded anchorages attached to a rigid double-stranded scaffold⁵³. The anchorages have unique base sequences so that they can be individually addressed. Each control strand delivers an instruction to the device to change its state: set strands hybridize to straighten the anchorages and are removed by unset strands.

Shin and Pierce⁵⁴ used a similar strategy to build a device with two distinguishable feet that walks on a track with four different anchorages. Each step requires the sequential addition of two instruction strands: the first lifts the back foot from the track and the second replaces it ahead of the stationary foot (Figure 3A). Directional movement, which is possible because the sequence of anchorages has no inversion symmetry, requires a set of eight DNA instructions. A similar scheme was used to roll two DNA 'gears' against each other⁵⁵. The Sherman and Seeman walker⁵⁶ uses a different stepping mechanism: The front foot steps forward then the back foot catches up (the front foot always remains ahead of the back foot). Both walkers could step indefinitely along a track composed of a repeating sequence of anchorages provided that the free foot is never offered a choice of anchorages. For the Shin and Pierce device, walking along a repeating sequence of four anchorages, this could be achieved by ensuring that the free foot can only bind to an anchorage adjacent to the bound foot (the sequence of anchorages could be reduced to three if the number of control strands were increased to twelve). For the inchworm mechanism of the Sherman and Seeman walker the feet are sometimes bound to non-adjacent sites: continuous operation requires either a sequence of more than four anchorages or the constraint that the feet cannot swing past each other on the track. If the latter condition were met an inchworm device with distinguishable feet could walk on a repeating sequence of only two anchorages, although if it did manage to reverse it would not correct itself. Extended tracks for DNA walkers might be made from DNA nanotubes, which are straight structures with persistence lengths of many micrometres⁵⁷⁻⁶⁰, or from arrays built on periodic⁶¹⁻⁶³ or non-periodic⁶⁴ DNA templates.

The walking devices described in this section are not true molecular motors because they cannot complete a cycle of motion without external intervention. Lack of autonomy brings the advantage of increased controllability: the device can be stopped at a desired location or reversed simply by reversing the order that instruction strands are added. However, the construction of a free-running DNA motor that does not require external intervention would be a significant accomplishment.

Molecular Motors

The ambition to use DNA to construct autonomous motors that step along linear tracks is inspired by biological motors like myosin, kinesin and dynein that use free energy from hydrolysis of ATP to drive directional movement. Biological motors are astonishingly competent: they can be very fast, moving loads at speeds of up to $60 \mu\text{m s}^{-1}$ (ref 65), and processive, travelling distances of up to $1 \mu\text{m}$ before dissociating from their tracks^{66,49}.

A chemically driven molecular motor is necessarily a catalyst for the reaction of the fuel from which it obtains energy. Biological motors couple conformational changes, including track binding and unbinding and rotation amplified by lever arms, to the binding and hydrolysis of ATP and release of ADP⁶⁷. Three different sources of energy for synthetic DNA motors have been explored: hydrolysis of the DNA backbone and ATP hydrolysis (both of which involve making and breaking covalent bonds), and DNA hybridization.

Motors powered by DNA and RNA hydrolysis

The free energy released on hydrolysis of the phosphodiester backbone of a DNA or RNA fuel can drive an autonomous device that is capable of catalyzing this reaction. RNA hydrolysis can be catalyzed by a limited set of DNA sequences including the '10-23' DNA enzyme⁶⁸, a short DNA strand consisting of a catalytic loop that cuts a substrate that is held in place by hybridization to two arms that flank the loop. This reaction was used to drive autonomous conformational changes of a tweezer-like device⁶⁹. The arms of the tweezers are tethered together by a single strand of DNA that incorporates the catalytic sequence. Hybridization of a single-stranded fuel to the tether pushes the arms apart and positions two RNA bases in the fuel close to the catalytic motif of the DNA enzyme. The DNA enzyme cuts the fuel between the RNA bases to create two short fragments that dissociate spontaneously, restoring the random coil configuration.

Two groups have used DNA hydrolysis to power a motor that moves along a track (Figure 3B). In both cases the track consists of repeated identical single-stranded anchorages attached to a double-stranded backbone. The cargo is a strand which can hybridize to any anchorage and, in conjunction with a catalyst, enables its cleavage. In the motor constructed by Tian and co-workers⁷⁰ each anchorage contains two RNA bases and can be cleaved when hybridized to the cargo which contains the '10-23' catalytic domain⁶⁸. In the motor created by Bath and co-workers⁷¹ the cargo – anchorage duplex contains a recognition site for a genetically modified nicking restriction enzyme (Box 1), N.BbvC IB⁷², that cuts only the anchorage strand. The outer segment that is cleaved from the anchorage is short enough to dissociate spontaneously from the cargo, leaving the complementary section of the cargo as a single-stranded overhang that can hybridize with the next anchorage. The cargo is transferred completely to the new anchorage by a branch migration reaction and the operating cycle can be repeated. (It is important that the rate of stepping *without* cleavage be small, as such a step creates the possibility that after the next cleavage the cargo will step backwards and be marooned.) The free energy input is provided by the hydrolysis of the DNA backbone and includes the configurational entropy of the liberated fragment. Autonomous, directional motion is achieved by making cleavage of the anchorage conditional on the presence of the cargo, and rapid motion of the cargo along the track conditional on cleavage. Unidirectionality is achieved by the simple expedient of destroying the track that the cargo has passed over, although the

track does not impose an initial direction on the motion unless it is prepared with the cargo at one end. At all stages in the cycle the cargo is hybridized to one or two anchorages: the stronger the interaction the lower the probability of dissociation of the cargo from the track. Unfortunately, strong binding of the cargo also has the effect of slowing down transfer of the cargo between anchorages: the strength of the cargo – track interaction must be tuned to achieve an appropriate balance between speed of operation and processivity. Ultimately the rate is limited by the turnover of the catalyst (0.003 s^{-1} for N.BbvC IB⁷³ and 0.06 s^{-1} for the 10-23 ribozyme⁶⁸). The destruction of the track after a single passage limits the potential applications of these devices.

Motors powered by ATP hydrolysis

Several designs for motors⁷⁴⁻⁷⁶ and computing devices^{77,78} involve cycles of restriction (backbone cleavage) and ligation (backbone joining). Bonds broken by a restriction enzymes are repaired by DNA ligase (Box 1) which converts one molecule of ATP to AMP for every phosphodiester bond made. The free energy input to a cycle of operation of one these devices is thus provided by ATP hydrolysis. Yin and co-workers⁷⁶ constructed an autonomous DNA motor that passes its cargo down a track from one anchorage to the next (Figure 3C). Its operating cycle is designed to prevent dissociation of the cargo from the track: the cargo is attached covalently, rather than by hybridization, and release from one anchorage is made conditional on attachment to the next. The track consists of a double-stranded DNA backbone to which double-stranded anchorages are attached by short single-stranded tethers. Each anchorage has a 3-base 3' overhang. The cargo consists of two 3-base DNA fragments, one of which is ligated to each strand of one double-stranded anchorage. The track, which is intrinsically directional, consists of an infinitely repeated sequence of four anchorages A-D (only a three-anchorage track has been tested⁷⁶). The operating cycle of the motor is as follows (an anchorage to which the cargo has been ligated is indicated by an asterisk - A* etc.). The sticky end (overhang) of A* is complementary to that of B; the two sticky ends hybridize and are ligated by T4 DNA ligase. The A*B complex contains a sequence of base pairs that is recognized by a restriction enzyme which cuts it to create A and B*, transferring the cargo to anchorage B. Similarly, the cargo can be transferred from B* to C, C* to D and D* to A etc. Two restriction enzymes (PflM I and BstAP I), which have different recognition sites, are used, one for the first and last steps in the sequence and one for the middle two steps[†]. The motor is unidirectional: if (say) B* is religated to A in an idling step then the B*A complex can be cut to recreate B* and A (repeating the previous forwards step) but not B and A* (a backwards step). Energy is input at each ligation step by ATP hydrolysis, and dissipated at each enzymatic cleavage (hydrolysis of the DNA backbone releases free energy). Passage of the cargo does not permanently change the track. The motor is processive: the cargo is always covalently attached to at least one if not two anchorages. In principle the motor is capable of indefinite unidirectional, processive, autonomous motion. In practice, this scheme is complicated by the need to use three enzymes simultaneously.

[†] The four-anchorage sequence, that allows the track for this motor to be extended indefinitely, can be obtained by adding a fourth anchorage D 5'-CTGG-3' / 5'-CCAGCAG-3' to the three-anchorage track published by Yin and co-workers⁷⁶.

Catalysis of DNA hybridization as an energy source

The energy that drives most of the two-state devices and clocked walkers described in the first part of this review is provided by DNA hybridization. In general their operating cycles involve a conformation change driven by interaction with at least one fuel strand which is later removed by hybridization with the corresponding antifuel strand. The fuel-antifuel duplex is a waste product. Both processes are driven by the decrease in free energy on forming additional base pairs, which can be substantial: the free energy change on creation of ten basepairs is comparable to that for ATP hydrolysis ($\Delta G^\circ_{ATP} = -7.7 \text{ kcal mol}^{-1}$ (ref 79) and under cellular conditions $\Delta G_{ATP} \approx -14 \text{ kcal mol}^{-1}$; $\Delta G^\circ_{hybridization} \approx -1.4 \text{ kcal M}^{-1}$ per basepair⁸⁰).

In order to use DNA hybridization as an energy source for a free-running molecular motor it is necessary to create both a metastable DNA fuel, whose spontaneous hybridization rate is slow compared to the cycle time of the motor, and a means of catalyzing its hybridization. Turberfield and co-workers introduced the idea that DNA loops could be used as a fuel⁸¹. In order to simplify FRET measurement of reaction rates they used two-strand loop complexes; hairpin loops, formed by hybridization of complementary domains at either end of a single strand, are also suitable⁸²⁻⁸⁴. Figure 4 uses hairpin loops as an example to introduce the ideas of a metastable DNA fuel and of a hybridization catalyst. Two hairpins with identical necks and complementary loop domains can hybridize to produce a continuous duplex (Figure 4). This reaction is driven by the free energy of hybridization of the loop bases (bases in the necks are hybridized both before and after the reaction, although to different partners) which more than compensates for the loss in configurational entropy attendant on dimer formation. Although hybridization can be initiated by interaction of unpaired bases in the loop domains, it is strongly hindered by closure of the necks: hybridization of the loop bases is limited by the topological impossibility of winding one loop around another to form a double helix. The reaction can be speeded up by competing open at least one neck by means of an ‘opening strand’ that incorporates a neck sequence. A useful opening strand also incorporates a toehold domain⁸¹ that can initiate the strand-displacement reaction that opens the neck by hybridizing either to the first few bases in the loop domain or to an unprotected single-stranded extension at the free end of the neck (Box 1). The opened loop can react more rapidly with its complement: it can, for example, thread through the loop of an unopened hairpin to facilitate hybridization in the loop domain. Complete hybridization of the hairpin with its complement displaces the opening strand (but not from an external toehold, which must be short enough to allow spontaneous dissociation). The freed opening strand can then hybridize to another loop and accelerate its reaction with its complement: it is a catalyst for loop – loop hybridization. If a single strand of DNA that undergoes a transition from random coil to duplex can be described as a machine then a hybridization catalyst was the first autonomous DNA machine^{81, ‡}.

Interactions between complementary pairs of hairpins^{82,84} and of two-strand loops^{81,85,86} have been studied with reference to their potential use as fuel. Dirks and Pierce have demonstrated a triggered amplification reaction⁸³ in which a loop-opening initiator

[‡] It might be helpful to restrict the use of ‘machine’ to mean ‘an apparatus constructed to perform a task’ (OED).

triggers a cascade of hairpin-hairpin reactions. Seelig and co-workers⁸⁶ pointed out that the loops studied in reference 81 can form a stable ‘kissed’ complex held together by partial base-pairing between loop domains without disruption of the necks. The kissed complex was purified and shown to be particularly long-lived ($t_{1/2} \sim 10^6$ s); catalysis of the hybridization of this complex by a neck-opening strand can speed up their reaction to form a duplex by a factor of 5000 (ref 86). The authors point out that the kissed complex may be suitable for use as a one-component fuel.

We show in Figure 5 an early, unpublished design (AJT) which demonstrated that it is possible to create a motor from a hybridization catalyst. A single step of a cargo along a track is driven by hybridization of one pair of complementary hairpins. Successful operation of such a free-running, hybridization-powered motor has not yet been reported.

The use of DNA hybridization as an energy source is attractive because the rates of different reactions can be tuned independently by adjusting the base sequences and concentrations of the corresponding fuel strands. The demonstration of an autonomous molecular motor made from DNA that is capable of extracting energy from a metastable DNA fuel remains a significant challenge.

Outlook

Research objectives are suggested by the example of natural molecular machines, the drive to miniaturize existing devices and the possibility of implementing models of computation in molecular systems. Biological systems set challenging – perhaps impossibly high – benchmarks.

- The use of static DNA templates (Box 3) is only the first stage in the development of DNA-directed synthesis. The example of the ribosome suggests that the track down which a synthetic molecular motor moves could be used as an instruction tape to control the stepwise addition of different monomers to create an oligomer of arbitrary length and sequence.
- The direct experimental realization of a Turing machine as a molecular machine, moving on and altering a DNA instruction tape, according to its internal state and transition rules, could be used as a challenge to drive the development of active DNA devices.
- DNA machines are built by rational design whereas biological machines evolve. We should explore the combination of rational design with *in vitro* selection or evolution as a way to build more competent machines.
- The simplicity of DNA is both an advantage and a drawback when making molecular machines. Where chemical versatility is required synthetic DNA bases⁸⁷ and backbone linkages^{88,89} and RNA and peptide components should be considered.
- Many DNA sensors and other active devices are inspired by potential medical applications. We should investigate the behaviour of DNA devices *in vivo* and explore techniques for introducing them into cells.

As our knowledge of how to engineer self-assembling systems improves so the fields of DNA, RNA and peptide design will converge; as the resolution of semiconductor

lithography improves so self-assembly and top-down nanofabrication will be integrated. In constructing functional molecular devices we have the opportunity to learn about biology while imitating its processes. We have hardly begun to explore the potential of synthetic molecular machinery.

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Competing financial interests

The authors declare that they have no competing financial interests.

References

1. Seeman, N.C. Nucleic Acid Junctions and Lattices. *J. Theor. Biol.* **99**, 237-247 (1982).
2. Chen, J.H. and Seeman, N.C. Synthesis from DNA of a molecule with the connectivity of a cube. *Nature* **350**, 631-633 (1991).
3. Zhang, Y. W. and Seeman, N. C. Construction of a DNA-Truncated Octahedron. *J. Am. Chem. Soc.* **116**, 1661-1669 (1994).
4. Shih, W. M., Quispe, J. D. and Joyce, G. F. A 1.7-kilobase single-stranded DNA that folds into a nanoscale octahedron. *Nature* **427**, 618-621 (2004).
5. Goodman, R.P., Schaap, I.A.T., Tardin, C.F., Erben, C.M., Berry, R.M., Schmidt, C.F. and Turberfield, A.J. Rapid chiral assembly of rigid DNA building blocks for molecular nanofabrication. *Science* **310**, 1661-1665 (2005).
6. Robinson, B. H. and Seeman, N. C. The design of a biochip: a self-assembling molecular-scale memorydevice. *Protein Eng.* **1**, 295–300 (1987).
7. Keren, K., Krueger, M., Gilad, R., Ben-Yoseph, G., Sivan, U. and Braun, E. Sequence-specific molecular lithography on single DNA molecules. *Science* **297** 72-75 (2002).
8. Heilemann, M., Tinnefeld, P., Mosteiro, G.S., Parajo, M.G., Van Hulst, N.F. and Sauer, M. Multistep energy transfer in single molecular photonic wires. *J. Am. Chem. Soc.* **126**, 6514-6515 (2004).
9. Niemeyer, C.M., Koehler, J. and Wuerdermann, C. DNA-directed assembly of bienzymic complexes from in vivo biotinylated NAD(P)H:FMN oxidoreductase and luciferase. *Chembiochem* **3**, 242-245 (2002).
10. Cate, J.H., Gooding, A.R., Podell, E., Zhou, K.H., Golden, B.L., Kundrot, C.E., Cech, T.R. and Doudna, J.A. Crystal structure of a group I ribozyme domain: Principles of RNA packing. *Science* **273**, 1678-1685 (1996).
11. DeGrado, W.F., Summa, C.M., Pavone, V., Nastri, F., Lombardi, A. De novo design and structural characterization of proteins and metalloproteins. *Annu. Rev. Biochem.* **68**, 779-819 (1999).
12. Chworos, A., Severcan, I., Koyfman, A.Y., Weinkam, P., Oroudjev, E., Hansma, H.G., Jaeger, L. Building programmable jigsaw puzzles with RNA. *Science* **306**, 2068-2072 (2004).
13. Pohl, F.M. and Joyin, T.M. Salt-induced co-operative conformational change of a synthetic DNA: equilibrium and kinetic studies with poly (dG-dC). *J. Mol. Biol.* **67**, 375-396 (1972).
14. Mao, C., Sun, W., Shen, Z. and Seeman, N.C. A nanomechanical device based on the B-Z transition of DNA. *Nature* **397**, 144-146 (1999).
15. Stryer, L. and Haugland, R.P. Energy transfer: a spectroscopic ruler. *Proc. Natl. Acad. Sci. USA* **58**, 719-726 (1967).

16. Yang, X., Vologodskii, A.V., Liu, B., Kemper, B and Seeman, N.C. Torsional control of double-stranded DNA branch migration. *Biopolymers* **45**, 69-83 (1998).
17. Holliday, R. A mechanism for gene conversion in fungi. *Genet. Res.* **5**, 282-304 (1964).
18. Gehring, K., Leroy, J.L. and Gueron, M. A tetrameric DNA structure with protonated cytosine-cytosine base-pairs. *Nature* **363** 561-565 (1993).
19. Aboul-ela, F., Murchie, A.I.H., Lilley, D.M.J. NMR study of parallel-stranded tetraplex formation by the hexadeoxynucleotide d(TG₄T). *Nature* **360**, 280-282 (1992).
20. Liu, D., and Balasubramanian, S. A proton-fuelled DNA nanomachine. *Angew. Chem. Int. Ed.* **42**, 5734-5736 (2003).
21. Liu, D., Bruckbauer, A., Abell, C, Balasubramanian, S., Kang, D-J., Klenerman, D. and Zhou, D. A reversible pH-driven DNA nanoswitch array. *J. Am. Chem. Soc.* **128**, 2067-2071 (2006).
22. Liedl, T. and Simmel, F.C. Switching the conformation of a DNA molecule with a chemical oscillator. *Nano Lett.* **5**, 1894-1898 (2005).
23. Liedl, T., Olapinski, M. and Simmel, F.C. A surface-bound DNA switch Driven by a Chemical Oscillator. *Angew. Chem. Int. Ed.* **45**, 5007-5010 (2006).
24. Shu, W., Liu, D., Watari, M., Reiner, C.K., Strunz, T., Welland, M.E., Balasubramanian, S. and McKendry, R.A. DNA molecular motor driven micromechanical cantilever arrays. *J. Am. Chem. Soc.* **127**, 17054-17060 (2005).
25. Baller, M.K., Lang, H.P., Fritz, J., Gerber, C., Gimzewsk, J.K., Drechsler, U., Rothuizen, H., Despont, M., Vettiger, P., Battiston, F.M., Ramseyer, J.P., Fornaro, P., Meyer, E. and Guntherodt, H.J. A cantilever array-based artificial nose. *Ultramicroscopy* **82** 1-9 (2001).
26. Chen, Y., Lee, S-H. and Mao, C. A DNA nanomachine based on a duplex-triplex transition. *Angew. Chem. Int. Ed.* **43**, 5335-5338 (2004).
27. Brucale, M., Zuccheri, G. and Samori, B. The dynamic properties of an intramolecular transition from DNA duplex to cytosine-thymine motif triplex. *Org. Biomol. Chem.* **3**, 575-577 (2005).
28. Yurke, B., Turberfield, A.J., Mills Jr, A.P., Simmel, F.C. and Neumann, J.L. A DNA-fuelled molecular machine made of DNA. *Nature*, **406**, 605-608 (2000).
29. Yurke, B. and Mills, A.P., Jr. Using DNA to Power Nanostructures. *Genetic Programming and Evolvable Machines* **4**, 111-122 (2003).
30. Muller, B.K., Reuter, A., Simmel, F.C. and Lamb, D.C. Single-Pair FRET characterization of DNA tweezers. *Nano Lett.* **6** 2814-2820 (2006).
31. Simmel, F.C. and Yurke, B. Using DNA to construct and power a nanoactuator. *Phys. Rev. E* **63**, 041913 (2001).

32. Simmel, F.C. and Yurke, B. A DNA-based molecular device switchable between three distinct mechanical states. *Appl. Phys. Lett.* **80**, 883-885 (2002).
33. Yan, H., Zhang, X., Shen, Z. and Seeman, N.C. A robust DNA mechanical device controlled by hybridization topology. *Nature* **415**, 62-65 (2002).
34. Ding, B. and Seeman, N.C. Operation of a DNA Robot Arm Inserted into a 2D DNA Crystalline Substrate. *Science* **314**, 1583-1585.
35. Feng, L., Park, H., Reif, J.H. and Yan, H. A two-state DNA lattice switched by DNA nanoactuator. *Angew. Chem. Int. Ed.* **42**, 4342, 4346 (2003).
36. Hazarika, P., Ceyhan, B. and Niemeyer, C.M. Reversible switching of DNA-gold nanoparticle aggregation. *Angew. Chem. Int. Ed.* **43**, 6469-6471 (2004).
37. Mirkin, C.A., Letsinger, R.L., Mucic, R.C., Storhoff, J.J. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature* **382** 607-609 (1996).
38. Li, J.J. and Tan, W. A single DNA molecule nanomotor. *Nano Lett.* **2**, 315-318 (2002).
39. Alberti, P. and Mergny, J-L. DNA duplex-quadruplex exchange as the basis for a nanomolecular machine. *Proc. Natl. Acad. Sci. USA* **100**, 1569-1573 (2003).
40. Wang, Y. Zhang, Y, and Ong, N.P. Speeding up a single-molecule DNA device with a simple catalyst. *Phys Rev. E* **72**, 051918 (2005).
41. Zhong, H. and Seeman, N.C. RNA used to control a rotary device. *Nano Lett.* **6**, 2899-2903 (2006).
42. Dittmer, W.U. and Simmel, F.C. Transcriptional control of DNA-based nanomachines. *Nano Lett.* **4**, 689-691 (2004).
43. Dittmer, W.U., Kempter, S., Radler, J.O. and Simmel, F.C. Using gene regulation to program DNA-based molecular devices. *Small* **7**, 709-712 (2005).
44. Gardner, T.S., Cantor, C.R. and Collins, J.J. Construction of a genetic toggle switch in *Escherichia coli*. *Nature* **403**, 339-342 (2000).
45. Elowitz, M.B. and Leibler, S. A synthetic oscillatory network of transcriptional regulators. *Nature* **403**, 335-338 (2000).
46. Becskei, A. and Serrano, L. Engineering stability in gene networks by autoregulation. *Nature* **405**, 590-593 (2000).
47. Kim, J., White, K.S. and Winfree, E. Construction of an in vitro bistable circuit from synthetic transcriptional switches. *Molecular Systems Biology* **2**:68 (2006).
48. Erben, C.M., Goodman, R.P. and Turberfield, A.J. Single-molecule protein encapsulation in a rigid DNA cage. *Angew. Chem. Int. Ed.* **45**, 7414-7417 (2006).
49. Svoboda, K., Schmidt, C.F., Schnapp, B.J. and Block, S.M. Direct observation of kinesin stepping by optical trapping interferometry. *Nature* **365**, 721-727 (1993).
50. Kuo, S.C. and Sheetz, M.P. Force of single kinesin groups measured with optical tweezers. *Science* **260**, 232-234 (1993).

51. Finer, J.T., Simmons, R.M. and Spudich, J.A. Single myosin group mechanics: piconewton forces and nanometre steps. *Nature* **368**, 113-119 (1994).
52. Ishijima, A., Harada, Y., Kojima, H., Funatsu, T., Higuchi, H. and Yanagida, T. Single-molecule analysis of the actomyosin motor using nano-manipulation. *Biochem. Biophys. Res. Commun.* **199**, 1057-1063 (1994).
53. Shin, J-S. and Pierce, N.A. Rewritable memory by controllable nanopatterning of DNA. *Nano Lett.* **4** 905-909 (2004).
54. Shin, J-S. and Pierce, N.A. A synthetic DNA walker for molecular transport. *J. Am. Chem. Soc.* **126**, 10834-10835 (2004).
55. Tian, Y. and Mao, C. A pair of DNA circles continuously rolls against each other. *J. Am. Chem. Soc.* **126**, 11410-11411 (2004).
56. Sherman, W.B. and Seeman, N.C. A precisely controlled DNA biped walking device. *Nano Lett.* **4**, 1203-1207 (2004).
57. Mitchell, J.C., Harris, J.R., Malo, J., Bath, J. and Turberfield, A.J. Self-assembly of chiral DNA nanotubes. *J. Am. Chem. Soc.* **126**, 16342-16343 (2004).
58. Rothermund, P.W.K., Ekani-Nkodo, A., Papadakis, N., Kumar, A., Fygenson, D.K. and Winfree, E. Design and characterization of programmable DNA nanotubes. *J. Am. Chem. Soc.* **126**, 16344-16352 (2004).
59. Liu, D., Park, S.H., Reif, J.H. and LaBean, T.H. DNA nanotubes self-assembled from triple-crossover tiles as templates for conductive nanowires. *Proc. Natl. Acad. Sci. USA* **101**, 717-722 (2004).
60. Mathieu, F. Liao, S., Kopatscht, J., Wang, T., Mao, C. and Seeman, N.C. Six-helix bundles designed from DNA. *Nano Lett.* **5**, 661-665 (2005).
61. Lubrich, D., Bath, J. and Turberfield, A.J. Design and assembly of double-crossover linear arrays of micrometre length using rolling circle replication. *Nanotechnology* **16**, 1574-1577 (2005).
62. Beyer, S., Nickels, P. and Simmel, F.C. Periodic DNA nanotemplates synthesized by rolling circle amplification. *Nano Lett.* **5**, 719-722 (2005).
63. Deng, Z., Tian, Y., Lee, S.H., Ribbe, A.E. and Mao, C. DNA-encoded self-assembly of gold nanoparticles into one-dimensional arrays. *Angew. Chem. Int. Ed.* **44**, 3582-3585 (2005).
64. Rothermund, P.W.K. Folding DNA to create nanoscale shapes and patterns. *Nature* **440**, 298-302 (2006).
65. Higashi-Fujime, S., Ishikawa, R., Iwasawa, H., Kagami, O., Kurimoto, E., Kohama, K. and Hozumi, T. The fastest actin-based motor protein from the green algae, *Chara*, and its distinct mode of interaction with actin. *FEBS Lett.* **375**, 151-154 (1995).
66. Howard, J., Hudspeth, A.J., Vale, R.D. Movement of microtubules by single kinesin molecules. *Nature* **342**, 154-158 (1989).

67. Vale, R.D., Milligan, R.A. The way things move: Looking under the hood of molecular motor proteins. *Science* **288**, 88-95 (2000).
68. Santoro, S.W. and Joyce, G.F. A general purpose RNA-cleaving DNA enzyme. *Proc. Natl. Acad. Sci. USA.* **94**, 4262-4266 (1997).
69. Chen, Y., Wang, M. and Mao, C. An autonomous DNA nanomotor powered by a DNA enzyme. *Angew. Chem. Int. Ed.* **43**, 3554-3557 (2004).
70. Tian, Y., He, Y., Peng, Y. and Mao, C. A DNA enzyme that walks processively and autonomously along a one-dimensional track. *Angew. Chem. Int. Ed.* **44**, 4355,4358 (2005).
71. Bath, J., Green, S.J. and Turberfield, A.J. A free-running DNA motor powered by a nicking enzyme. *Angew. Chem. Int. Ed.* **44**, 4358-4361 (2005).
72. Heiter, D.F., Lunnen, K.D. and Wilson, G.G. Site-specific DNA-nicking mutants of the heterodimeric restriction endonuclease R.BbvCI. *J. Mol. Biol.* **348**, 631-640 (2005).
73. Bellamy, S.R.W., Milsom, S.E., Scott, D.J., Daniels, L.E., Wilson, G.G. and Halford, S.E. Cleavage of Individual DNA Strands by the Different Subunits of the Heterodimeric Restriction Endonuclease BbvCI. *J. Mol. Biol.* **348**, 641-653 (2005).
74. Reif, J.H. The design of autonomous DNA nanomechanical devices: Walking and rolling DNA. *Lecture Notes in Computer Science* **2568**, 22-37 (2003).
75. Yin, P., Turberfield, A.J., Sahu, S. and Reif, J.H. Designs for autonomous unidirectional walking DNA devices. *Lecture Notes in Computer Science* **3384**, 410-425 (2005).
76. Yin, P., Yan, H., Daniell, X.G., Turberfield, A.J. and Reif, J.H. A unidirectional DNA walker that moves autonomously along a DNA track. *Angew. Chem. Int. Ed.* **43**, 4906-4911 (2004).
77. Benenson Y., Paz-Elizur, T., Adar, R. Keinan, E., Livneh, Z. Shapiro, E. Programmable and autonomous computing machine made of biomolecules. *Nature* **414** 430-434 (2001).
78. Yin, P., Sahu, S., Turberfield, A.J. and Reif, J.H. Design of autonomous DNA cellular automata. *Lecture Notes in Computer Science* **3892**, 399-416 (2006).
79. Alberty, R.A. and Goldbert, R.N. Standard Thermodynamic Formation Properties for the Adenosine 5'-Triphosphate Series. *Biochem.* **31**, 10610-10615 (1992).
80. SantaLucia, J. A unified view of polymer, dumbbell, and oligonucleotide nearest neighbour thermodynamics. *Proc. Natl. Acad. Sci. USA.* **95**,1460-1465 (1998).
81. Turberfield, A.J., Mitchell, J.C., Yurke, B., Mills Jr., A.P., Blakey, M.I. and Simmel, F.C. DNA fuel for free-running nanomachines. *Phys. Rev. Lett.* **90**, 118102 (2003).

82. Bois, J.S., Venkataraman, S., Choi, H.M.T., Spakowitz, A.J., Wang, Z-G. and Pierce, N.A. Topological constraints in nucleic acid hybridization kinetics. *Nucleic Acids Res.* **33**, 4090-4095 (2005).
83. Dirks, R.M. and Pierce, N.A. (2004). Triggered amplification by hybridization chain reaction. *Proc. Natl. Acad. Sci. USA* **101**, 15275-15278.
84. Green, S.J., Lubrich, L. and Turberfield, A.J. DNA hairpins: fuel for autonomous DNA devices. *Biophys. J.* **91**, 2966-2975 (2006).
85. Seelig, G., Yurke, B. and Winfree, E. DNA Hybridization Catalysts and Catalyst Circuits. *Lecture Notes in Computer Science* **3384**, 329-343 (2005).
86. Seelig, G., Yurke, B. and Winfree, E. Catalysed relaxation of a metastable fuel. *J. Am. Chem. Soc.* **128**, 12211-12220 (2006).
87. Kool, E.T. Replacing the nucleobases of DNA with designer molecules. *Acc. Chem. Res.* **35**, 936-943 (2002).
88. Nielsen, P.E., Egholm M., Berg R.H. and Buchardt, O. Sequence-selective recognition of a DNA by strand displacement with a thymine-substituted polyamide. *Science* **254**, 1497-1500 (1991).
89. Koshkin, A.A. Singh, S.K. Neilson, P., Rajwanshi, V.K., Kumar, R., Meldgaard, M., Olsen, C.E. and Wengel, J. Synthesis of the adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil bicyclonucleotide monomers, oligomerisation, and unprecedented nucleic acid recognition. *Tetrahedron* **54**, 3607-3630 (1998).
90. Smith, S.B., Finzi, L. and Bustamante, C. Direct Mechanical Measurements of the Elasticity of Single DNA Molecules by Using Magnetic Beads. *Science* **258**, 1122-1126 (1992).
91. Smith, S.B., Cui, Y. and Bustamante, C. Overstretching B-DNA: The Elastic Response of Individual Double-Stranded and Single-Stranded DNA Molecules. *Science* **271**, 795-799 (1996). Seeman, N.C. De novo design of sequences for nucleic-acid structural engineering. *J. Biomol. Struct. Dyn.* **8** 573-581 (1990).
92. Seeman, N.C. De novo design of sequences for nucleic-acid structural engineering. *J. Biomol. Struct. Dyn.* **8**, 573-581 (1990).
93. Dirks, R.M., Lin, M., Winfree, E. and Pierce, N.A. Paradigms for computational nucleic acid design. *Nucleic Acids Res.* **32**, 1392-1403 (2004).
94. Goodman, R.P. NANEV: a program employing evolutionary methods for the design of nucleic acid nanostructures. *Biotechniques* **38**, 548-550 (2005).
95. Tashiro, R. and Sugiyama, H. A nanothermometer based on the different stackings of B- and Z-DNA. *Angew. Chem. Int. Ed.* **42**, 6018-6020 (2003).
96. Shen, W., Bruist, M.F., Goodman, S.D. and Seeman, N.C. A protein-driven DNA device that measures the excess binding energy of proteins that distort DNA. *Angew. Chem. Int. Ed.* **43**, 4750-4752 (2004).
97. Tyagi, S and Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* **14**, 303-308 (1996).

98. Wilson, D.W, and Szostak, J,W. In vitro selection of functional nucleic acids. *Ann. Rev. Biochem.***68**, 611-648 (1999).
99. Dittmer, W.U., Reuter, A. and Simmel, F.C. A DNA-based machine that can cyclically bind and release thrombin. *Angew. Chem. Int. Ed.* **43** 3550-3553 (2004).
100. Rusconi, C.P., Scardino, E., Layzer, J., Pitoc, G.A., Ortel, T.L., Monroe, D. and Sullenger, B.A. RNA aptamers as reversible antagonists of coagulation factor IXa. *Nature* **419**, 90-94 (2002).
101. Beyer, S. and Simmel, F.C. A modular DNA signal translator for the controlled release of a protein by an aptamer. *Nucleic Acids Res.* **34**, 1581-1587 (2006).
102. Chelyapov, N. Allosteric Aptamers Controlling a Signal Amplification Cascade Allow Visual Detection of Molecules at Picomolar Concentration. *Biochem.* **45**, 2461-2466 (2006).
103. Liu, J and Lu, Y. A colorimetric lead biosensor using DNA enzyme-directed assembly of gold nanoparticles. *J. Am. Chem. Soc.* **125**, 6642-6643 (2003).
104. Porta H, and Lizardi, P.M. An allosteric hammerhead ribozyme. *Biotechnology* **13**, 161-164 (1995).
105. Stojanovic, M.N., de Prada, P., Landry, D.W. Catalytic molecular beacons. *Chembiochem* **2**, 411-415 (2001).
106. Robertson, M.P. and Ellington, A.D. In vitro selection of an allosteric ribozyme that transduces analytes to amplicons. *Nat. Biotechnol.* **17**, 62-66 (1999).
107. Weizmann, Y., Beissenhirtz, M.K., Chaglakov, Z., Nowaski, R., Kotler, M. and Willner, I. A virus spotlighted by an autonomous DNA machine. *Angew. Chem. Int. Ed.* **45**, 7384-7388 (2006).
108. Van Ness, J., Van Ness, L.K. and Galas, D.J. Isothermal reactions for the amplification of oligonucleotides. *Proc. Natl. Acad. Sci USA* **100**, 4504-4509 (2003).
109. Stojanovic, M.N., Mitchell, T.E. and Stefanovic, D. Deoxyribozyme-based logic gates. *J. Am. Chem. Soc.* **124**, 3555-3561 (2002).
110. Penchovsky, R. and Breaker, R.R. Computational design and experimental testing of oligonucleotide-sensing allosteric ribozyme. *Nat. Biotechnol.* **23**, 1424-1433 (2005).
111. Stojanovic, M.N. and Stefanovic, D. Deoxyribosome-based half adder. *J. Am. Chem. Soc.* **125** 6673-6676 (2002).
112. Stojanovic, M.N. and Stefanovic, D. A deoxyribozyme-based molecular automaton. *Nat. Biotechnology* **21**, 1069-1074 (2003).
113. Seelig, G., Soloveichik, D., Zhang, D.Y. and Winfree, E. Enzyme-Free Nucleic Acid Logic Circuits. *Science* **314**, 1585-1588 (2006).

114. Benenson, Y., Gil, B., Ben-Dor, U., Adar, R. and Shapiro, E. (2004). An autonomous molecular computer for logical control of gene expression. *Nature* **429**, 423-428.
115. Isaacs, F.J., Dwyer, D.J., Ding, C., Pervouchine, D.D., Cantor, C.R. and Collins, J.J. Engineered riboregulators enable post-transcriptional control of gene expression. *Nat. Biotechnol.* **22**, 841-847 (2005).
116. Bayer, T.S. and Smolke, C.D. Programmable ligand-controlled riboregulators of eukaryotic gene expression. *Nat. Biotechnol.* **23**, 337-343 (2005).
117. Halpin, D.R. and Harbury, P.R. DNA Display I: Sequence-Encoded Routing of DNA Populations. *PloS Biology* **2**, 1015-1021 (2004).
118. Halpin, D.R. and Harbury, P.R. DNA Display II: Genetic Manipulation of Combinatorial Chemistry Libraries for Small-Molecule Evolution. *PloS Biology* **2**, 1022-1030 (2004).
119. Liao, S. and Seeman, N.C. Translation of DNA signals into polymer assembly instructions. *Science* **306**, 2072-2074 (2004).
120. Gartner, Z.J. and Liu, D.R. The generality of DNA-templated synthesis as a basis for evolving non-natural small molecules. *J. Am. Chem. Soc.* **123**, 6961-6963 (2001).
121. Snyder, T.M. and Liu, D.R. Ordered multistep synthesis in a single solution directed by DNA templates. *Angew. Chem. Int. Ed.* **44**, 7379-7382 (2005).
122. Gothelf, K.V., Thomsen, A., Nielsen, M., Clo, E. and Raymond S. Brown, R.S. Modular DNA-programmed assembly of linear and branched conjugated nanostructures. *J. Am. Chem. Soc.* **126**, 1044-1046 (2004).
123. Eckardt, L.H., Naumann, K., Pankau, W.M., Rein, M., Schweitzer, M., Windhab, N., von Kiedrowski, G.. DNA nanotechnology: chemical copying of connectivity. *Nature* **420**, 286 (2002).
124. Chen, Y. and Mao, C. Reprogramming DNA-directed reactions on the basis of a DNA conformational change. *J. Am. Chem. Soc.* **126**, 13240-13241 (2004).
125. Chhabra, R., Sharma, J., Liu, Y. and Yan, H. Addressable molecular tweezers for DNA-templated coupling reactions. *Nano Lett.* **6** 978-983 (2006).
126. Goodman, R.P., Heilemann, M., Kapenidis, A.N. and Turberfield, A.J. *manuscript in preparation.*

Box 1 The Building Material

a, The DNA backbone, shown in green, is an alternating string of deoxyribose sugars and phosphate groups whose polarity is indicated by the end-labels 5' and 3'. One of the four bases G, A, T, C (guanine, adenine, thymine, cytosine) is attached to each sugar (bases are shown in grey). Hydrogen bonding between complementary CG and AT base pairs holds two strands of DNA together to form a right-handed double helix. (Formation of

the double helix by base-pairing between complementary strands is described as hybridization.) For clarity, the helical character of DNA is omitted from the figures that follow: the backbone is represented as a coloured line and the bases (paired or unpaired) as short grey lines. Complementary sequences are indicated by light and dark shades of the same colour.

Double-stranded DNA has a persistence length of 50nm (ref 90): on the length scale of most devices described here (a few tens of basepairs, or ~10nm) it can be considered to be stiff and straight. Single-stranded DNA is very flexible: its persistence length is of order three backbone units⁹¹.

Enzymes can be used to break the phosphodiester backbone of DNA: a typical restriction enzyme recognizes a specific sequence of bases within double-stranded DNA and cuts both strands within the recognition sequence (black triangles indicate cuts made by the enzyme EcoRI).

b, Short single-stranded ‘sticky ends’, shown in red, can be used to join two segments of double-stranded DNA. Hybridization between complementary sticky ends leaves nicks in the backbone where the 3’ end of one strand meets the 5’ end of another. Nicks can be repaired by DNA ligase, an enzyme that catalyses formation of a phosphodiester bond between adjacent 3’ hydroxyl and 5’ phosphate groups.

c, A four-arm junction made from four strands of DNA illustrates the principle that self-assembly can be controlled through design of the component strands. The interactions that hold together the four double-helical arms are encoded in the base sequences of the strands by ensuring that sections of DNA that should hybridize to each other have complementary sequences. Undesired interactions are ‘designed out’ by adjusting the sequences to reduce the stability of competing structures⁹²⁻⁹⁴. For a simple structure, this design process will ensure that when the component strands are annealed the desired structure will be formed in high yield. An analogous procedure was used to create the tetrahedron shown in Figure 1 in a single assembly step⁵.

A Holliday junction is a four-arm structure in which opposite arms have the same nucleotide sequences¹⁷. The position of the junction can migrate in a process in which base pairs in one opposite pair of arms break and are reformed in the other pair.

d A short single-stranded sticky end can also serve as a toehold for strand exchange²⁹. A complementary strand which binds first to the toehold can displace the shorter strand of the original duplex: strand exchange proceeds by means of a random walk of the branch point separating regions hybridized to the competing strands. Strand exchange is driven by the greater stability of the duplex formed with the invading strand, which results from the additional base pairs in the toehold region. Several devices that use strand exchange drive conformation changes are described in this review.

Box 2 Sensors that can process information

A DNA device that changes configuration in response to a chemical or physical input, and couples that change to a useful output is a sensor. Temperature⁹⁵ - and pH²⁰ -sensors have been described. FRET changes have been used to detect the deformation of a double helix when bound by a protein and to estimate the DNA-protein interaction energy⁹⁶.

Molecular beacons⁹⁷ are routinely used to detect specific DNA sequences: hybridization to the target opens a hairpin loop and separates fluorophores attached to the neck domains. Aptamers are oligonucleotides that are capable of binding specific targets; they are generated from random sequence libraries by repeated *in vitro* selection and amplification⁹⁸. DNA sensors that incorporate aptamers can be designed to detect small molecules or proteins^{83,99}. Binding and release of the blood clotting protein thrombin from a DNA aptamer can be triggered using a DNA control strand⁹⁹⁻¹⁰¹.

DNA-based sensors can incorporate gain. An ATP-binding aptamer has been used to trigger the polymerization of DNA hairpin loops⁸³. An RNA aptamer has been used to couple detection of a protein to release and activation of viper venom enzyme that triggers a blood-clotting cascade, generating a visible signal by precipitating polystyrene microspheres¹⁰². A colour-based lead sensor has been constructed from a lead-dependent DNA enzyme that cleaves the DNA linkages holding together a network of gold nanoparticles¹⁰³. Various schemes for detecting target DNA sequences make use of DNA enzymes to amplify the input signal: the target sequence can switch on a DNA enzyme^{104,105} or a ribozyme¹⁰⁶, or act as a primer to trigger the production of a DNA enzyme¹⁰⁷ by isothermal amplification¹⁰⁸.

Ribozymes whose activity is regulated by control strands can be used to perform logical operations^{109,110}. The DNA enzyme shown in **a** binds to a DNA substrate (dark green) incorporating a ribobase (white) and cuts it. A simple modification to the DNAzyme produces a NOT gate (shown in **b**): the DNA enzyme will not cut in the presence of strand *x* (blue) because its binding disrupts the active site. Further modification produces a DNA enzyme (shown in **c**) that will not cut in the absence of strand *y*: the substrate will only bind in the presence of strand *y*. Simple programs can be executed by combinations of gates^{111,112}.

Seelig *et al* have built logic gates that are based on DNA hybridization rather than DNA hydrolysis¹¹³. For example, their AND gate uses hybridization of the first input strand to the gate complex to uncover a toehold that allows binding of a second input strand, releasing the output strand. Outputs from one operation can be used as inputs to another, opening the way to the creation of multilayer circuits.

In principle, molecular computation could be used to control a therapeutic device: for example, a device operating within cells could trigger programmed cell death in response to a specific set of mRNAs. Working towards this goal, Benenson *et al.* constructed system of enzymes and DNA fragments that can release a short strand of DNA (which could have a therapeutic effect) after successful completion of a series of tests for the presence or absence of DNA signals¹¹⁴. Control of gene expression has been demonstrated using engineered riboregulators, noncoding RNA fragments that can interfere with transcription and whose interaction with the target gene can be modulated by binding of a signal RNA or small molecule^{115,116}.

Box 3 Controlling chemical Synthesis

The ribosome creates proteins by linking amino acids according to instructions encoded in mRNA. Could a DNA nanomachine be used to build an oligomer specified by instructions encoded in DNA? The challenge is to read and process a sequence of

instructions in the correct order and to respond by promoting the appropriate chemical reactions.

Halpin and Harbury used DNA-encoded instructions to synthesize short polypeptides. DNA instruction strands consisted of a string of unique sequence tags (codons) that specified both the identity of an amino acid and its position in the polypeptide product^{117,118}. This strand remained covalently attached to the growing chain and was used to direct its path between reaction vessels, in each of which one amino acid was added. At each step, molecules with the appropriate tag for a given reaction were selected by hybridization to complementary sequence tags (anticodons) immobilized on a solid support.

Liao and Seeman constructed a reconfigurable device that can template assembly of four different DNA products¹¹⁹. At the heart of the device are two conformational switches that can be rotated by 180° by interaction with DNA instruction strands. The device promotes the ligation of double-crossover tiles. The four states of the device offer up four different combinations of splints that bind and hold together single-stranded sticky ends protruding from different tiles, and thus catalyze ligation of four different tile sequences. This device does not read instructions in sequence, instead its switches are initialized independently before synthesis begins. The length of the product is limited by the length of the device.

It is possible to promote other chemical reactions simply by attaching reactants to complementary DNA strands and using hybridization to hold them together and thus to increase the reaction probability¹²⁰. Multistep synthesis can be carried out by controlling the secondary structure of the DNA template: masks that prevent reagents from coming into contact can be removed by increasing the temperature¹²¹. DNA-templated chemistry can also be used to assemble linear and branched organic precursors into complex structures whose connectivity is programmed by the template^{122,123}. DNA devices can be used to trigger chemical reactions by changing the effective concentration of reagents in response to a change in pH¹²⁴ or to the addition of a control strand¹²⁵.

Progress in the construction of linear motors built from DNA, together with the demonstration of DNA-templated chemistry, sets the following challenge: to construct a DNA device that acts, like the ribosome, as a chemical assembler that can process an instruction tape of arbitrary length.

Figure 1. Self-assembly of a nanometre-scale object. The DNA tetrahedron⁵ has relatively stiff double-stranded edges linked by flexible single-stranded hinges. A cargo, for example a protein⁴⁸, can be trapped in the central cavity of the tetrahedron. Mechanical devices built from DNA could be used to open the tetrahedron¹²⁶ to control access to the cargo.

Figure 2. A DNA nanomachine driven by repeated sequential addition of DNA control strands. DNA tweezers²⁸ have two double-stranded arms connected by a flexible single-stranded hinge. The ‘set’ strand pulls the arms into a closed conformation by hybridizing to single-stranded tails at the ends of the arms. A short region of the set strand remains single-stranded even when it is hybridized to the tweezers: this region

serves as a toehold that allows the ‘unset’ strand to hybridize to the set strand and strip it from the device, returning the tweezers to the open configuration and generating a double-stranded waste product. The state of the device can be determined by measuring the separation between donor and acceptor fluorophores (represented by the green triangle and red circle) using FRET.

Figure 3. DNA Nanomachines that execute directional stepwise movement along linear tracks.

a, The Shin and Pierce walker⁵⁴ has two distinct feet and steps along a track that displays a sequence of four distinct single-stranded anchorages (the first three are shown). Each step is driven by sequential addition two control strands, one that lifts the back foot from the track and one that binds it to a new anchorage ahead of the stationary foot. The single step shown here requires two instruction strands. A total of eight instruction strands are required for extended operation of the motor.

b, Autonomous movement can be driven by enzymatic hydrolysis of the DNA⁷¹ or RNA⁷⁰ backbone. Binding of the cargo to an anchorage enables an enzyme to cleave the anchorage (the cleavage site is indicated by a black triangle). A short fragment of the anchorage is released, leaving the cargo with a single-stranded toehold that can bind to the intact anchorage ahead of it; the cargo can then step forward by a branch migration reaction. Destruction of the track in the wake of the cargo imposes directionality.

c, A cargo can be passed autonomously from one anchorage to the next by a repeated cycle of enzymatic ligation and hydrolysis⁷⁶. The cargo consists of two short fragments of DNA. It is passed along a track with four distinct double-stranded anchorages. In the top panel the cargo is covalently attached to anchorage *A*. Anchorage *B* ahead of it has a sticky end that is complementary to the free end of the cargo. DNA ligase joins the two anchorages covalently with the cargo bridging the gap between them (middle panel). This creates a sequence of bases that is recognized by a restriction enzyme which cleaves the cargo from *A*, leaving it covalently attached to *B* (bottom panel), and so on. Cleavage sites are represented by black triangles.

Figure 4. Hairpin loops to fuel DNA motors⁸¹. DNA loop complexes can be used as an energy source. The DNA hairpin has a single-stranded loop trapped by a double-stranded neck. Hybridization of complementary hairpins releases free energy of $\sim 1.4 \text{ kcal mol}^{-1}$ per additional base pair formed⁸⁰, but is hindered because the loop domains cannot wind round one another without opening at least one neck. The reaction can be catalyzed by a short strand that binds to a single-stranded toehold, either in the loop or at the far end of the neck, and opens the hairpin by hybridizing to the neck along its entire length⁸¹⁻⁸⁶. Once opened a loop interacts relatively quickly with its complement and releases the catalyst strand.

Figure 5. Scheme for a hybridization-powered molecular motor.

This scheme shows how stepwise movement of a single-stranded DNA cargo along a rigid track might be coupled to hybridization of complementary hairpin loops. The cargo

moves between single-stranded anchorages. An empty anchorage is designed to bind to the first few bases of the loop domain of the \bar{l} hairpin (red) and to compete open the hairpin's neck (blue) by a strand-displacement reaction, in the same way as the hybridization catalyst shown in Figure 4. The cargo is complementary to the anchorage sequence and has an extended toehold region which is designed to interact with the loop domain of the complementary hairpin l . The track-bound cargo can open an l hairpin in a strand-exchange reaction involving nucleation and migration of a four-arm Holliday junction (Box 1). In this reaction the blue domains in the hairpin neck, the cargo and the anchorage exchange partners, displacing the cargo from its initial anchorage but keeping it securely attached to the track. By forcing open the necks of complementary hairpins l and \bar{l} the track-bound cargo and adjacent empty anchorage catalyze their hybridization. When the open loops hybridize to produce an: l - \bar{l} duplex (a waste product) the cargo is deposited on the next anchorage by strand exchange. One loop-loop reaction is thus coupled to one step down the track. This motor could be made directional by preparing all anchorages with bound (open) \bar{l} hairpins then removing excess \bar{l} . Motion of the cargo would leave empty anchorages behind, preventing backwards steps.

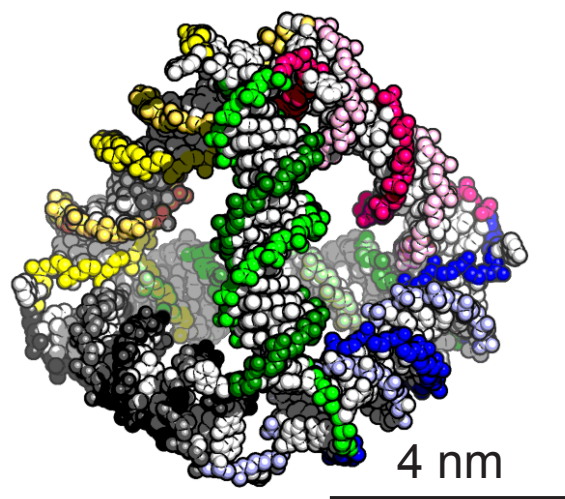


Figure 1

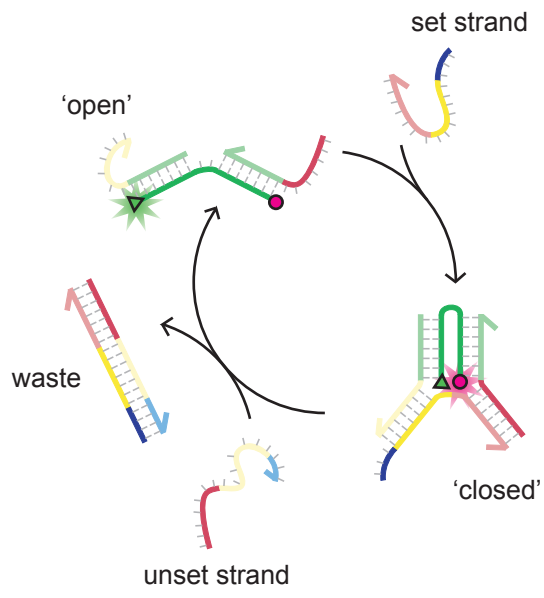


Figure 2

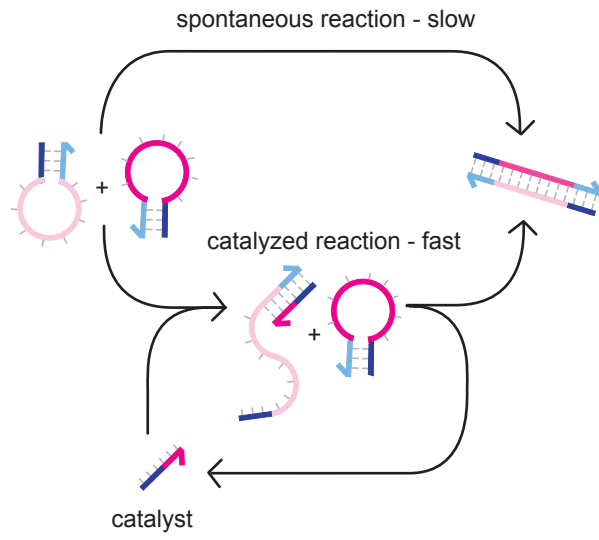


Figure 4

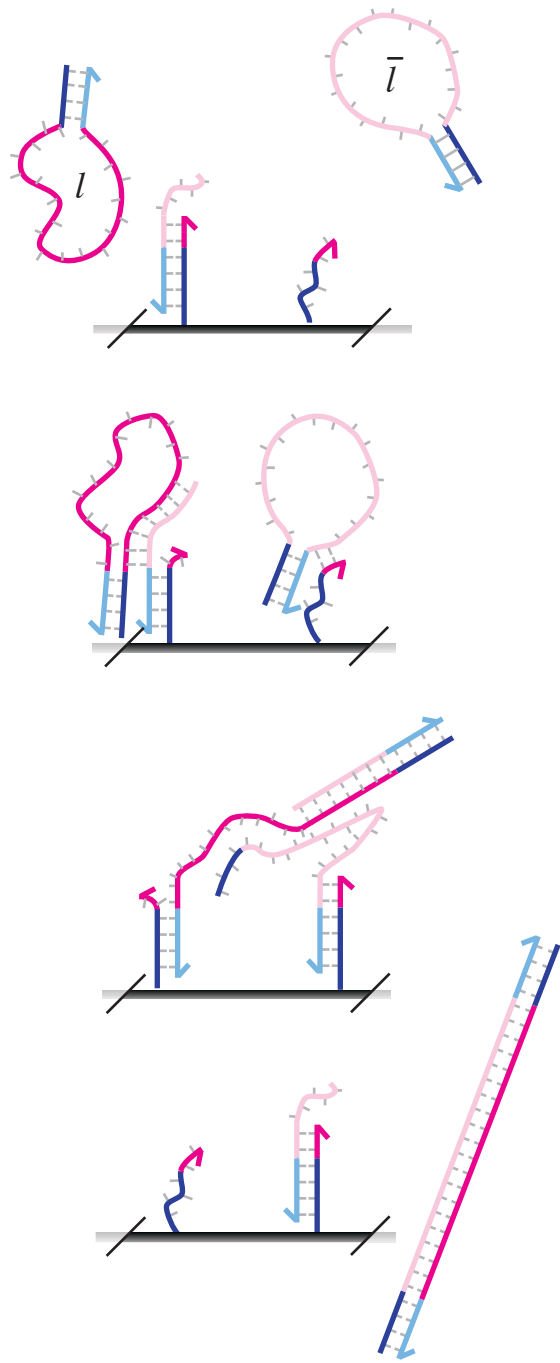
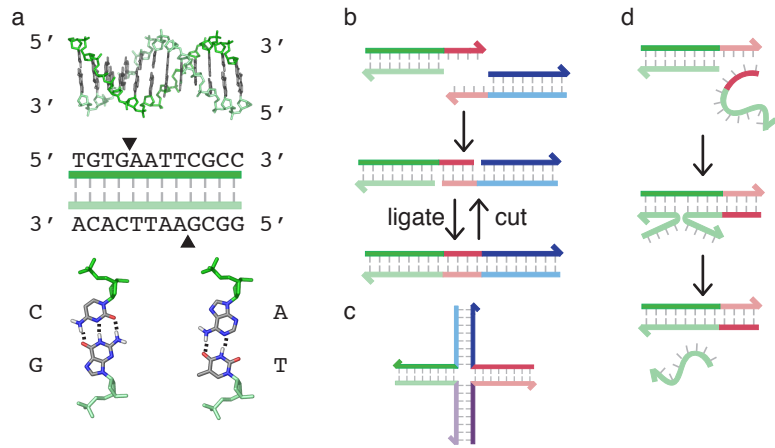
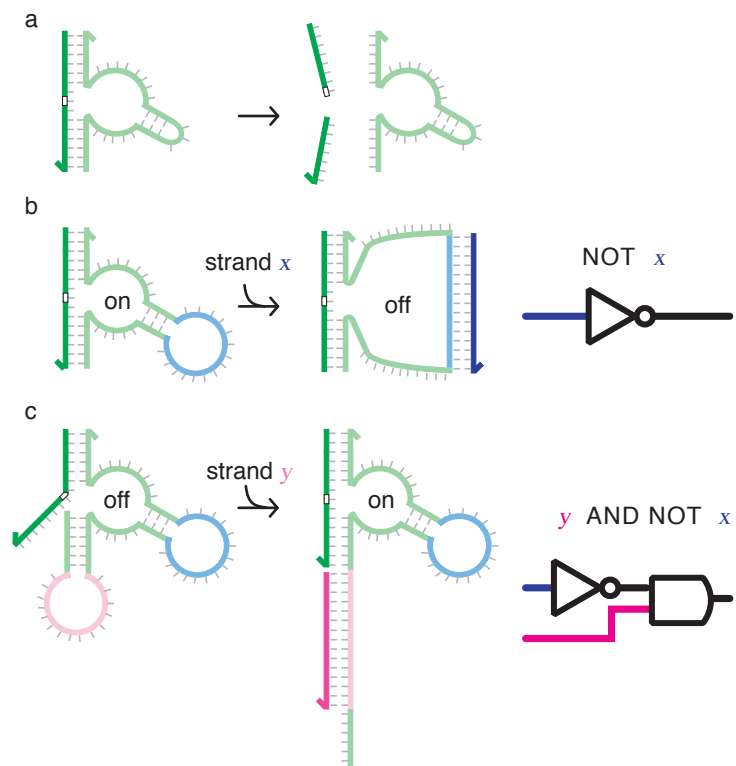


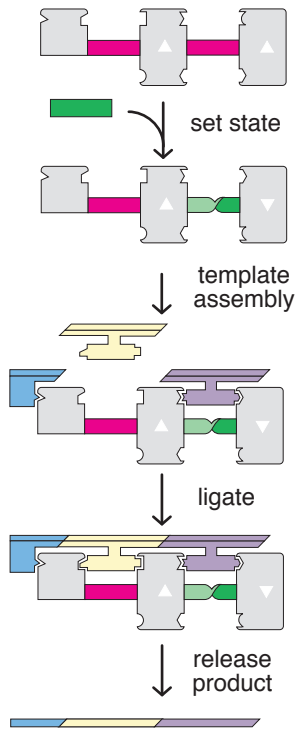
Figure 5



Box 1



Box 2



Box 3