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Dynamic DNA nanotechnology using strand-displacement reactions

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The specificity and predictability of Watson–Crick base pairing make DNA a powerful and versatile material for engineering at the nanoscale. This has enabled the construction of a diverse and rapidly growing set of DNA nanostructures and nanodevices through the programmed hybridization of complementary strands. Although it had initially focused on the self-assembly of static structures, DNA nanotechnology is now also becoming increasingly attractive for engineering systems with interesting dynamic properties. Various devices, including circuits, catalytic amplifiers, autonomous molecular motors and reconfigurable nanostructures, have recently been rationally designed to use DNA strand-displacement reactions, in which two strands with partial or full complementarity hybridize, displacing in the process one or more pre-hybridized strands. This mechanism allows for the kinetic control of reaction pathways. Here, we review DNA strand-displacement-based devices, and look at how this relatively simple mechanism can lead to a surprising diversity of dynamic behaviour.

The thermodynamics of DNA hybridization have been carefully characterized within the past few decades[1,2], allowing the quantitative prediction and design of structures and interactions. The use of DNA as an engineering material has also been aided by the exponentially decreasing cost of oligonucleotide preparation and purification[3]. These developments have led to new non-biological uses of DNA as a material for self-assembly[4,5] and molecular computation[6], and provided the foundation for the field of DNA nanotechnology.

DNA nanotechnology uses DNA strands to manipulate the spatial and temporal distribution of matter, and can be broadly divided into structural and dynamic DNA nanotechnology. Structural DNA nanotechnology has achieved the construction of two- and three-dimensional objects of varying sizes and complexity using 'bottom-up' DNA self-assembly, and has culminated in the development of macroscopic materials with nanometre-scale addressability[7–9]. In contrast, dynamic DNA nanotechnology is exemplified by reconfigurable and autonomous devices in which the 'interesting part' is the non-equilibrium dynamics rather than the equilibrium end-states.

Here, we review dynamic DNA devices whose operation is based on DNA strand displacement. We show how the systematic use of this simple and robust mechanism makes it possible to produce molecular logic circuits, catalytic amplifiers, autonomous molecular walkers and reprogrammable DNA nanostructures. Although we focus on work using strand displacement in which no covalent bonds are modified, dynamic DNA devices have also been engineered using ribozymes and deoxyribozymes[10,11].

The specifics of the work described here are unique to DNA nanotechnology, but many goals and ideas are shared with other fields: both synthetic biology[12–14] and DNA nanotechnology aim to engineer molecular devices and circuits that can perform specific tasks as efficiently and reliably as their counterparts in living systems. DNA nanotechnology, like supramolecular chemistry[15], uses non-covalent interactions to design higher-order assemblies with new functions. Both dynamic DNA nanotechnology and nonlinear chemical dynamics[16] are concerned with potentially large numbers of molecular species, and with the complex spatial and temporal dynamics that can arise from interactions among them.

DNA strand displacement

Strand displacement is the process through which two strands with partial or full complementarity hybridize to each other, displacing one or more pre-hybridized strands in the process. Strand displacement can be initiated at complementary single-stranded domains (referred to as toeholds) and progresses through a branch migration process that resembles a random walk (Box 1). By varying the strength (length and sequence composition) of toeholds, the rate of strand-displacement reactions can be quantitatively controlled over a factor of 10^6 (refs 17–19). Importantly, this feature allows engineering control over the kinetics of synthetic DNA devices.

In molecular biology, strand displacement frequently denotes a process mediated by enzymes such as polymerases[20], but the reaction as defined above is guided by the biophysics of DNA and occurs independently of enzymes. Enzyme-free strand displacement and branch migration have been studied since the 1970s[21–28], but have only been applied to DNA nanotechnology within the past decade.

Switchable devices and structures

The systematic use of toehold-mediated strand displacement in DNA nanotechnology was pioneered by Yurke *et al.*[29], who observed that the same strand of DNA can undergo multiple hybridization and strand-displacement cycles through the use of toeholds. Using this crucial idea, Yurke demonstrated a set of DNA tweezers—two double-helical arms connected by a single-stranded flexible hinge—that could be repeatedly cycled between an open and a closed state through successive additions of two specific single-stranded DNA 'fuel' molecules (inputs A and B in Fig. 1a).

Yurke's tweezers showed that DNA hybridization and strand displacement can be used to engineer molecular-scale changes in structure. In contrast to previous demonstrations of molecular devices that were switched by changes in environmental conditions (salt, pH, temperature)[30–33], this mechanism makes it possible to address individual devices in a sequence-specific manner.

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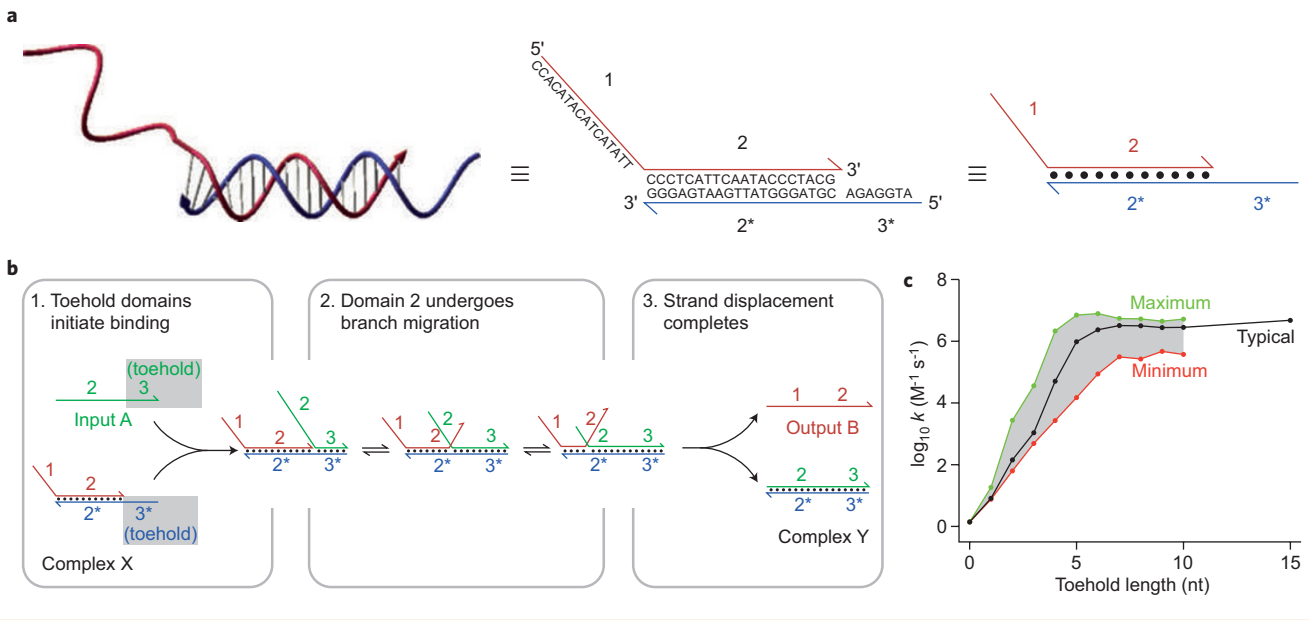
Box 1 | Overview of DNA strand displacement

DNA is represented as directional lines, with the hook denoting the 3′ end (panel **a**). For many strand-displacement-based designs, it is convenient to abstract contiguous DNA bases into functional DNA domains that act as a unit in hybridization, branch migration or dissociation. Domains are represented here by numbers; a starred domain denotes a domain complementary in sequence to the domain without a star (for example, domain 2* is complementary to domain 2). The sequences of the nucleotide bases are not typically shown because it is expected that DNA devices based on strand displacement will work for many if not most choices of domain sequences.

The key reaction that has allowed the construction of the dynamic assemblies shown in this review is DNA strand displacement. Panel **b** shows one example of this reaction. Single-stranded DNA molecule A reacts with multi-stranded DNA complex X to release strand B and complex Y. Throughout the text we will refer to single-stranded reactants (such as A) that initiate a reaction as 'inputs' and to single-stranded reactants that are released from a complex (such as B) as 'outputs'. The strand-displacement reaction is facilitated by the 'toehold' domains 3 and 3*: the hybridization of these single-stranded toeholds co-localizes A and X, and allows the 2 domain to 'branch migrate'. Branch migration is the random walk process in which one domain displaces another of identical sequence through a series of reversible single nucleotide dissociation and hybridization steps[24]. At the completion of branch migration, complex Y is formed and strand B is released. The concept of toeholds was introduced to DNA nanotechnology by Yurke *et al.*[29], and studied in detail by Yurke and Mills[17], Li *et al.*[18] and Zhang and Winfree[19].

Panel **c** shows that the kinetics of strand displacement can be accurately modelled and predicted from the length and sequence of the toehold domain[19] (nt = nucleotide). The rate constant of the strand-displacement reaction varies over a factor of 10^6, from 1 M^{-1} s^{-1} to 6×10^6 M^{-1} s^{-1}. The green trace shows the kinetics of using a strong toehold composed of only G/C nucleotides, the red trace shows the kinetics of using a toehold composed only of A/T nucleotides, and the black trace shows the kinetics of a toehold composed of roughly equal numbers of all four nucleotides. The grey region spanned by the green and red traces roughly shows the range of potential kinetics based on toehold length. The progress of strand-displacement reactions is typically assayed using fluorescence, either by means of reporter complexes that stoichiometrically react with the output, or by using dual-labelled probes as output strands. Part **c** reproduced with permission from ref. 19, © 2009 ACS.



Several subsequent works used Yurke's basic reaction sequence (a hybridization step followed by strand displacement to reverse the effect of the initial hybridization) for controlling complex nanoscale structures. Simmel and Yurke[34] demonstrated a nanoactuator related to Yurke's original tweezer design. Addition of a first input strand pushed the two arms of the nanoactuator apart; addition of a second input strand set them free. In further work they built a device that could be switched between three distinct states using two pairs of fuel strands[35]. Tian and Mao[36] built a device consisting of two DNA complexes reminiscent of interlocking gears that could be repeatedly cycled through three different states.

Reconfiguring self-assembled structures. Strand displacement can be combined with structural self-assembly to enable dynamic reconfiguration of larger DNA nanostructures post-assembly, and can be used to induce changes at macroscopic scales. A first example of this was described by Yan and co-workers[37] who used the toehold-mediated cycling technique of Yurke *et al.* to construct a rotary DNA device. Their device could be switched between two states corresponding to different DNA tile motifs, called PX and JX_2 (Fig. 1b). They also assembled multiple devices into a linear structure large enough to be visualized with an atomic force microscope and demonstrated switching of a DNA multi-stranded structural motif relative to the main axis of a larger structure (Fig. 1c). Their device was based on an earlier example of a switchable DNA nanomachine[30] that responded to ambient salt concentration rather than DNA inputs.

Chakraborty *et al.*[38] later extended this basic design to a system that could be switched between three different states, and Zhong and Seeman[39] demonstrated that switching could be indirectly controlled

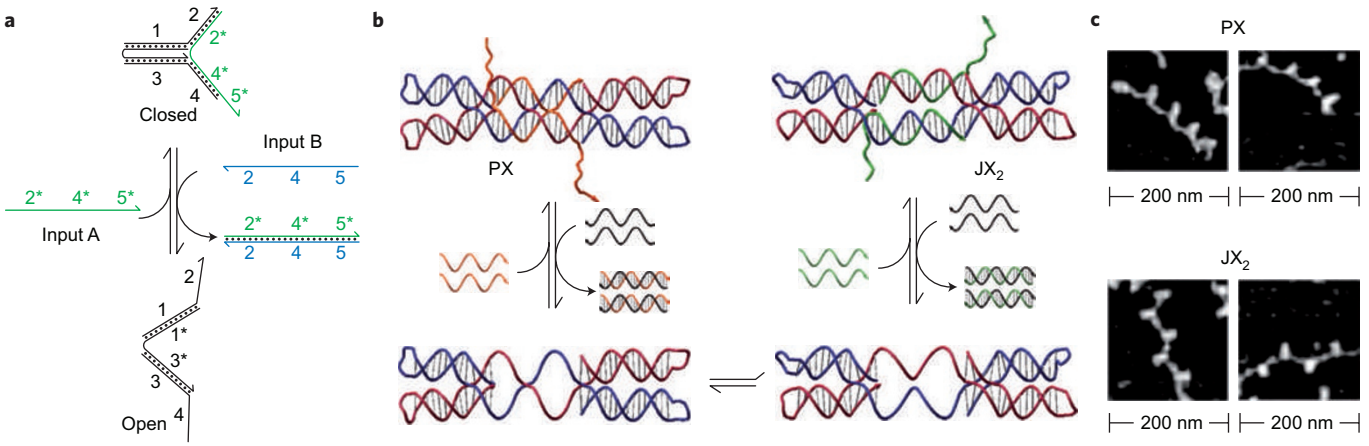


Figure 1 | DNA switches. a, DNA tweezers[29]. Input A binds to distal domains 2 and 4 of the tweezers, causing the tweezers to adopt a closed configuration. Input B displaces the tweezers in binding to input A, releasing the DNA tweezers and allowing them to relax into an open configuration. Each reaction step is driven by the formation of additional base pairs and each full cycle leads to the production of an inert double-stranded by-product. **b**, A rotational DNA switch[37]. Different DNA strands are indicated in colour. PX and JX_2 are DNA tile motifs based on the reciprocal exchange of strands between two adjacent helices. In the PX molecule, strands of identical polarity cross over at all possible positions, whereas two exchanges are missing in the JX_2 molecule. This causes the two ends of the red strand to be on the opposite sides of the PX configuration while being on the same side in the JX_2 configuration. Hybridization and strand displacement by introduced DNA inputs can be used to interconvert the two motifs. **c**, Atomic force microscope images of a rotary device using PX–JX_2 junctions. In this system, there is a PX–JX_2 junction between each DNA triangle; switching the state of the junction causes alternative DNA triangles to flip with respect to the centre axis. Part **c** reproduced with permission from ref. 37, © 2002 NPG.

with RNA rather than DNA. Ding and Seeman[40] integrated PX–JX_2 switches into a two-dimensional crystal and demonstrated controllable motion of DNA 'arms' relative to the stable lattice.

Feng *et al.*[41] reported the self-assembly of a two-dimensional DNA lattice that dynamically changed aspect ratio through the addition of single-stranded DNA inputs that effect expansion and contraction of the lattice's monomer units. Lubrich *et al.*[42] used strand displacement to controllably change the length of a one-dimensional DNA polymer. Goodman *et al.*[43] reported the self-assembly of DNA tetrahedra, in which one edge adopted one of two different lengths depending on the presence of an effector strand.

Several recent advances in structural DNA self-assembly have been based on the DNA origami technology[44], which uses short oligonucleotide 'staple' strands to fold a long single-stranded 'scaffold' (typically the m13 viral genome) into a two- or three-dimensional shape of interest[9]. Andersen *et al.*[45] used DNA origami to construct a DNA box with a 'lid' that could be opened and closed by strand displacement. Constructions like those by Goodman and Andersen could potentially be used as vehicles for the sequestration and conditional release of molecular cargo.

Strand displacement can also be used to control the reconfiguration of macroscopic assemblies of other materials, for instance the reversible aggregation of gold nanoparticles[46], or dynamical modulation of the stiffness of a DNA-functionalized polyacrylamide gel[47].

Stepped DNA walkers. The controlled nanomechanical actuation provided by strand displacement was also used to construct molecular devices that could continuously move along a predefined trajectory rather than switching between a limited number of fixed configurations. Sherman and Seeman[48] as well as Shin and Pierce[49] used strand displacement to implement DNA 'walkers' that could be induced through the external addition of reagents to move directionally along a one-dimensional DNA track, the walker taking one step with every input added. Their designs were inspired by motor proteins such as kinesin that similarly move in a step-by-step fashion. DNA walkers could potentially act as a method for active molecular transport: in fact, Gu *et al.*[50] demonstrated a DNA walker moving along a specific path that could pick up, transport and drop off gold nanoparticle 'cargo'. This multi-component DNA device integrated structural elements with stepped walkers and rotary DNA switches.

Strand-displacement cascades

In the previous section, we saw how toehold-mediated strand displacement can give rise to mechanical devices controlled by DNA. However, these devices require the external addition of single-stranded reagents for continued operation. Strand-displacement reactions can be cascaded to eliminate this need for external triggers at every step; this allows the engineering of complex autonomous systems.

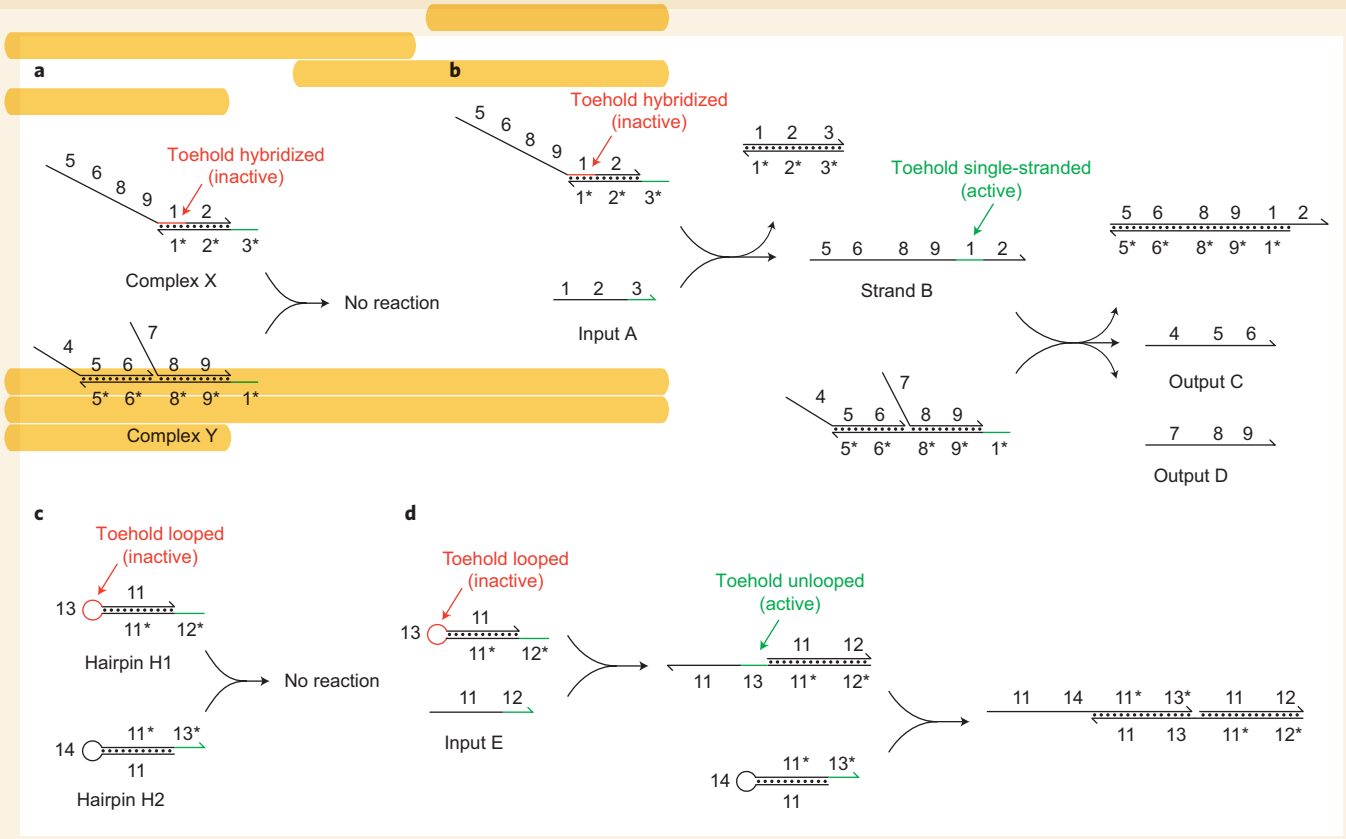
One key feature of strand-displacement reactions that was not fully used in the works previously described is that strand displacement releases at least one single-stranded nucleic acid product, the output. In a DNA strand-displacement cascade, this output serves as the input to a downstream reaction. In Box 2 panel a, complexes X and Y do not directly react with each other, owing to toehold 1 in complex X being inactive. Reaction between the input A and the complex X releases strand B with active toehold 1, which serves as input to the second reaction with complex Y to produce outputs C and D (Box 2 panel b). Compared with the direct reaction of complexes X and Y, the cascaded reaction in the presence of the input strand A is 10^6 times faster.

Shown in Box 2 panels c and d is an example of a strand-displacement cascade using hairpins (H1 and H2) rather than multi-stranded complexes. Toeholds are initially inactive because they are constrained in a short hairpin loop. Again, addition of an input strand (strand E in Box 2 panel d) greatly accelerates the reaction.

The free energy that drives strand-displacement cascades is derived from the potential of forming base pairs (enthalpy gain) or releasing strands (entropy gain), and is provided by the reactants. The reaction is therefore limited by the amount of reactants that are supplied initially, and once the system reaches equilibrium, no more information processing or physical work can be done. This is similar to other closed reaction systems such as the polymerase chain reaction, which depends on DNA primers that deplete over the course of a reaction.

Unlike electrical or biological circuits that are powered by a standardized energy source (electrical voltages or ATP concentrations),

Box 2 | Programming strand-displacement cascades.



The toehold is instrumental in controlling the kinetics of strand-displacement reactions (Box 1). One powerful concept that makes use of this observation is toehold inactivation — that is, preventing strand-displacement reactions by rendering toeholds inactive. Toeholds must hybridize to each other to serve their purpose of co-localizing DNA for branch migration; thus any mechanism that inhibits the hybridization of complementary domains will serve to inactivate toeholds. The most common method of inactivating toeholds is to make them double-stranded[64], as shown in panel **a**. Another method of inactivating toeholds is to constrain them geometrically in a hairpin loop[74], shown in panel **c**.

In panel **a**, the toehold domain 1 is inactive, and no reaction occurs between complexes X and Y. In panel **b**, input strand A reacts by strand displacement with complex X to release strand B. The toehold domain 1 is now activated, and B reacts with complex Y to release the two output strands, C and D. This cascade of reactions serves as a stoichiometric translator of the nucleic acid sequence: the input DNA strand is completely independent in sequence of the output strands. This translator design was introduced by Seelig *et al.*[64] and studied in detail by Picuri *et al.*[66]. The second reaction (that between B and Y) also demonstrates that one input strand can cause the release of multiple output strands. In panels **c** and **d**, input strand E reacts by strand displacement to open hairpin H1, exposing toehold domain 13. Subsequently, the intermediate reacts with — and opens — hairpin H2. This method of toehold inactivation and activation was introduced by Dirks and Pierce[74].

circuits based on strand displacement cannot be easily recharged because the reactant species for each strand-displacement reaction is different. If necessary, a chemostat or mechanisms such as transcription can be used to continuously replenish reactants. However, as we will argue below, DNA strand-displacement cascades can realize a variety of useful functions despite their intrinsically limited lifetime.

DNA circuits and reaction networks. Synthetic molecular circuits that are capable of complex information processing and computation have been built using a range of approaches; examples include synthetic gene regulatory and signalling networks[12,14,51], computational networks using *in vitro* transcription[52,53], digital logic circuits based on small molecules[54] or peptides[55], and the nonlinear chemical reaction networks underlying the Belousov–Zhabotinskii reaction and related phenomena[16]. In these circuits, information is stored in the concentrations, spatial localizations and/or chemical properties of molecules; chemical reactions between molecules implement molecular information processing.

For many of these technologies it is inherently difficult to increase the number of components in a circuit beyond a very small number or to control and modify the kinetics of the reactions involved. In this section we review how cascades of strand-displacement reactions enable the design of potentially large circuits capable of complex behaviours. The biological importance of nucleic acids means that synthetic circuits that sense and conditionally modulate nucleic acids could be used as a powerful tool for programming biology.

Initial demonstrations of nucleic acid logic circuits did not rely exclusively on strand displacement but took advantage of enzyme or deoxyribozyme catalysis[10,11]. For example, Benenson *et al.* proposed and developed a DNA and enzyme-based molecular automaton that could perform a computation[56] where the outcome (the release of an antisense drug mimic) was dependent on the absence or presence of specific inputs (ssDNA with sequence analogous to diagnostically relevant mRNA)[57]. Stojanovic and collaborators developed deoxyribozyme-based logic gates[58] and used these gates in combination to form a variety of logic circuits[59–61]. Penchovsky and Breaker[62]

developed allosteric ribozymes that could implement cascaded logic using DNA inputs and RNA outputs.

Enzyme or deoxyribozyme catalysis is not necessary for complex information processing. Using only strand displacement, Takahashi *et al.*[63] and Seelig *et al.*[64] proposed and tested designs for Boolean logic gates that used short DNA oligonucleotides as input and output signals. Seelig *et al.* further demonstrated cascaded logic circuits that exhibited a complete set of logic functions (AND, OR and NOT). Thresholds are used to suppress small leaky signals to be 'off' and amplification is used to restore attenuated signals to the correct digital 'on' state. Together, these components thus implemented signal restoration and enable digital abstraction (Fig. 2). With signal restoration, a multi-component circuit was able to produce the correct digital output even when the concentrations of the inputs deviated from their ideal values (Fig. 2c).

Frezza *et al.*[65] developed surface-bound logic gates that release diffusible single-stranded signals. Notably, they used spatial separation rather than toehold inactivation to control interactions between gates. Picuri *et al.*[66] extended this approach to include toehold inactivation, and constructed a two-layer translator system for diagnostic applications. Qian and Winfree[67] proposed a standardized method of constructing logical AND and OR gates from a basic gate motif with amplification and thresholding (see 'Non-covalent DNA catalysis' for amplification mechanism). They further showed how arbitrary feed-forward digital logic circuits, relay contact circuits, and various analog circuits could be systematically built.

The digital abstraction is useful for constructing reliable circuitry, but chemical reactions are intrinsically analog and their kinetics enable a much broader class of behaviour including but not limited to oscillations, chaos and pattern formation. Soloveichik *et al.*[68] suggested a systematic approach for approximating arbitrary mass action kinetics through DNA strand-displacement cascades (Fig. 3). They treated chemical reaction kinetics as a prescriptive 'programming language' and suggested an automated process for implementing a system of coupled chemical reactions with strand-displacement cascades. Furthermore, Oishi and Klavins[69] showed how arbitrary linear input/output systems can be implemented with DNA.

Cardelli and collaborators[70] developed a stochastic model of molecular computation that is similar to the computer science concept of process algebras, which is used to coordinate actions of multiple independent agents. Their formalism allows hierarchical abstraction of strand-displacement reactions into functional modules, which can be used to program reaction networks to yield complex behaviours[71]. They also proposed several new reaction designs based on strand displacement, using nicked double-stranded DNA[72].

Although it often takes advantage of ideas from engineering, the goal of the work reviewed here is not to compete with electronics but to implement molecular information processing similar to that which occurs in cells. This work is thus notably different in outlook and motivation from earlier demonstrations of DNA computation based on Adleman's work[6]. For deeper discussion of this point we refer the reader to the review by Chen and Ellington[73].

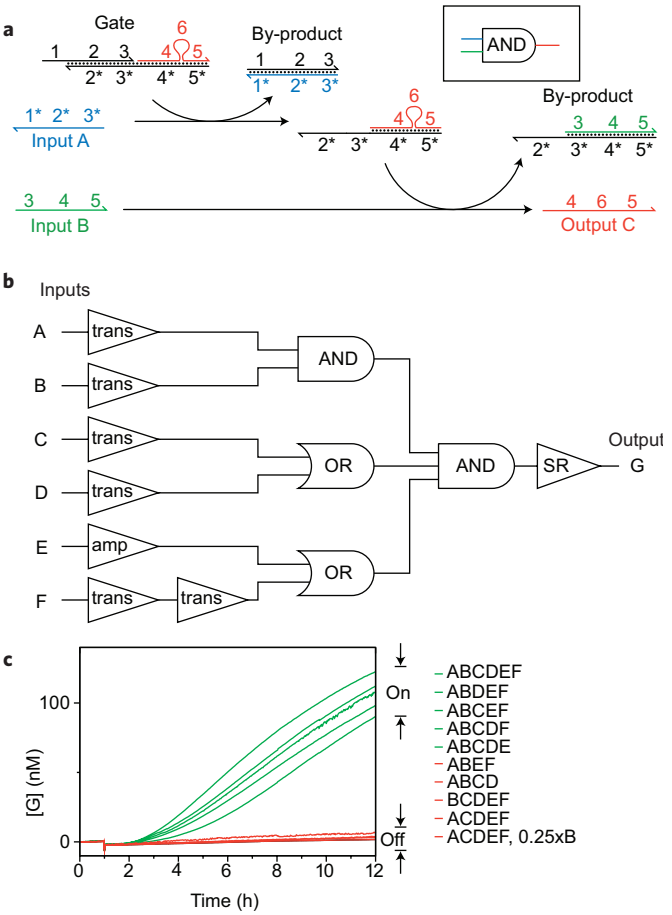


Figure 2 | Logic gates and cascaded circuits[64]. a, AND logic gate. In AND logic, an output C is produced only if the two inputs A and B are present. No output is produced if only one input, or none, is available. Input A binds to the three-stranded complex (gate) at the top left to release a by-product and activate the 3* toehold on the remaining two-stranded complex. Input B then binds to the two-stranded complex to release output C and another by-product. **b**, A 12-component logic circuit. Inputs A to F (DNA analogues of the let-7c, mir-124a, mir-15a, mir-10b, mir-143 and mir-122a microRNAs, respectively) are present at 200 nM or 0 nM concentration, denoting Boolean true (on) and false (off), respectively. Translation (trans) is realized with a method similar to that shown in Box 2. OR logic uses translators with different inputs but the same output. In an OR logic gate, an output strand is released as long as either one of two inputs is present. Amplification (amp) is implemented using strand-displacement-based catalysis[86]. Signal restoration (SR) uses amplification and thresholding. **c**, Experimental results of the 12-component circuit. The output is measured by fluorescence, and increases with time when the input concentrations are such that the logical circuit evaluates to 'on'. The data also demonstrate signal restoration: an intermediate value of input (0.25 × mir-124) is correctly recognized as a 'leak' and evaluated as 'off'. Part **c** reproduced with permission from ref. 64, © 2006 AAAS.

Controlling DNA self-assembly. Strand-displacement cascades can be used to kinetically control DNA self-assembly pathways, and may offer practical advantages in assembly fidelity, speed and complexity over traditional self-assembly that uses thermal annealing.

Kinetically controlled self-assembly has been initially demonstrated with strand-displacement cascades that use hairpins rather than multi-stranded complexes[74–76]. In these systems, strand displacement leads to hairpin opening and the resulting output strands remain covalently attached. This co-localization of products and reactants enables the hierarchical assembly of larger nanostructures. Kinetically controlled self-assembly is reminiscent of algorithmic tile-based self-assembly[77–79] and even of the biological development process. In all of these cases, the information encoded in an emerging structure guides subsequent growth steps.

Initial work by Dirks and Pierce[74] demonstrated a hybridization chain reaction. They used hairpins with overlapping partial complementarities to construct a reaction cascade that resulted in the formation of double-stranded DNA polymers up to thousands of base pairs long (Fig. 4a). They also coupled the initiation of the chain reaction to an ATP aptamer[74], and were able to trigger DNA polymer formation specifically and conditionally in the presence

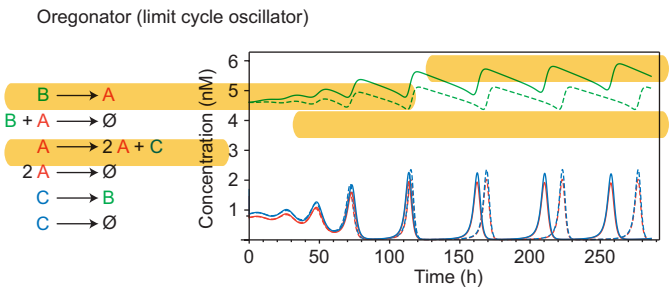


Figure 3 | Complex dynamics (in this case, a limit cycle oscillator) with DNA strand-displacement reaction networks. The reactions on the left are implemented using DNA strand-displacement reactions[68] similar to those shown in Box 2 panel a and in Fig. 2a. The notation B + A → Ø means that B stoichiometrically reacts with A to form an inactive by-product not relevant to this system. The plot shows simulations of the ideal chemical reaction network (dashed lines) and of the corresponding DNA reaction network (solid lines). The colours of the traces correspond to the colours of the species (that is, the red trace denotes the concentration of A, green denotes B and blue denotes C). Figure reproduced with permission from ref. 68, © 2010 NAS, USA.

of ATP. A related kinetically controlled linear polymerization process used multi-stranded monomers rather than hairpins as building blocks[80].

Yin *et al.*[76] expanded the hybridization chain reaction to demonstrate self-assembly of branched DNA dendrimers (Fig. 4b). Inactive toeholds were sequestered in the double-stranded stem of the hairpin, and each hairpin opening could trigger two downstream reactions. Combined with demonstrations of catalytic formation of multi-arm DNA structures, autonomous walkers and exponential growth circuits, this work demonstrated the versatility of a simple hairpin motif in constructing many different types of behaviour and devices.

Non-covalent DNA catalysis

In typical strand-displacement reactions, the single-stranded input is consumed in the course of the reaction, and ends up in an inert double-stranded by-product. Here, we will describe mechanisms through which the same input molecule can participate in multiple strand-displacement reaction cycles, thereby leading to the release of many outputs. The input can then be thought of as acting catalytically, even if no covalent bonds are made or broken. Motivation for this work came from the goals of developing molecular devices for isothermal detection and engineering stable molecular fuels for autonomous molecular walkers.

The reactants (other than the catalyst) of these non-covalent DNA catalysis systems generally to consist of DNA strands or complexes that are kinetically trapped in metastable configurations. They conceptually act as 'fuels' because they collectively store the energy that thermodynamically drives the catalysed reaction forward.

Interaction between the catalyst and these fuels (by strand displacement) opens a fast pathway for the rearrangement of the fuels into products. The products can yield a fluorescence signal for detection, can lead to DNA nanostructure formation, or can be inputs for downstream strand-displacement reactions.

Turberfield *et al.*[81] first explored this approach by demonstrating a system in which the hybridization of two complementary strands was slowed by constraining one or both of the strands via hybridization to shorter auxiliary strands. A specific input strand could controllably reverse this constraint, and catalytically accelerate the formation of the double-stranded product. Turberfield and co-workers later expanded this approach to demonstrate mechanisms for fully autonomous molecular walkers where the walker acts as a catalyst[82,83] (see 'Autonomous DNA nanomachines').

Bois *et al.*[84], Green *et al.*[85] and Seelig *et al.*[86] reported formation of double-stranded products catalysed by a single-stranded DNA, using reactant complexes with complementary hairpin or bulge structures. These systems showed significantly lower uncatalysed (leak) reaction rates than the initial design by Turberfield *et al.*, and thus were more suitable for applications as stable fuels for autonomous motors. Furthermore, the amplification afforded by these catalytic systems enabled enzyme-free nucleic acid detection mechanisms with improved detection sensitivity over hybridization-based methods (such as molecular beacons[87]). Finally, these catalyst systems could be used for achieving signal gain and restoration in the context of cascaded DNA circuits that perform information processing[64].

The catalysis system by Seelig *et al.*[86] showed the catalytic release of a single-stranded output with sequence unrelated to the input. Because of the standardized nature of the inputs and outputs in this design, this motif could be modularly incorporated into the construction of multilayer reaction cascades[64].

Zhang *et al.*[88] rationally designed a catalytic system that is driven by the entropy gain of additional released molecules, rather than enthalpy gain of base-pair formation (Fig. 5). The sequence of the output for this system can be completely independent of that of the catalytic input. For example, in the system shown in Fig. 5, the output was designed to be identical to that of the input so that the kinetics of the reaction show exponential growth. The authors also demonstrated experimentally cascades of two catalytic reactions, and this system featured significantly higher gain and kinetic speed-up than previous designs. Zhang and Winfree[89] further improved this entropy-driven catalyst system to allow dynamic allosteric modulation. The logic circuits of Qian and Winfree[67] make extensive use of a related catalytic motif[19].

Yin *et al.*[76] demonstrated a reaction in which single-stranded DNA catalysed the formation of multi-arm branched structures from hairpin monomers, in addition to seeding branched dendrimers as described previously. Each arm of the product structures contained single-stranded regions, and could act in downstream reactions. Using this technique, Yin *et al.* also built a two-component system in which two partially duplexed structures catalysed each other's formation and showed exponential growth kinetics.

Compared with commonly used deoxyribozymes[90], the strand-displacement-based catalysis reactions described in this section show lower increases in reaction rate, being of the order of 10^4 rather than 10^6. The lower catalytic speed-up is probably due to a higher uncatalysed (leak) reaction rate, caused by blunt end strand exchange[28]. The advantage of using strand-displacement-based catalysts over deoxyribozymes is that the former generally have far fewer sequence constraints, and are robust across a greater range of environmental conditions, such as pH, temperature and salt concentrations.

For purposes of amplification and signal gain, catalysis-like kinetic behaviour can be achieved with strand-displacement cascades[68,71,74,80]. For example, a strand-displacement reaction in which a product is identical to the initial input closely approximates the kinetics of a catalytic system in which the same input is released and reacts multiple times. The previously introduced polymerization reactions[74,80] could consequently also be considered catalysis-like in kinetics.

Autonomous DNA nanomachines

Protein-based molecular motors couple the release of chemical energy (for example hydrolysis of ATP) to mechanical work, and are essential to many cellular functions. Synthetic molecular motors[31,32] that can operate autonomously by coupling the free energy of hybridization to mechanical work could play similarly important roles in choreographing molecular processes in nanotechnology applications.

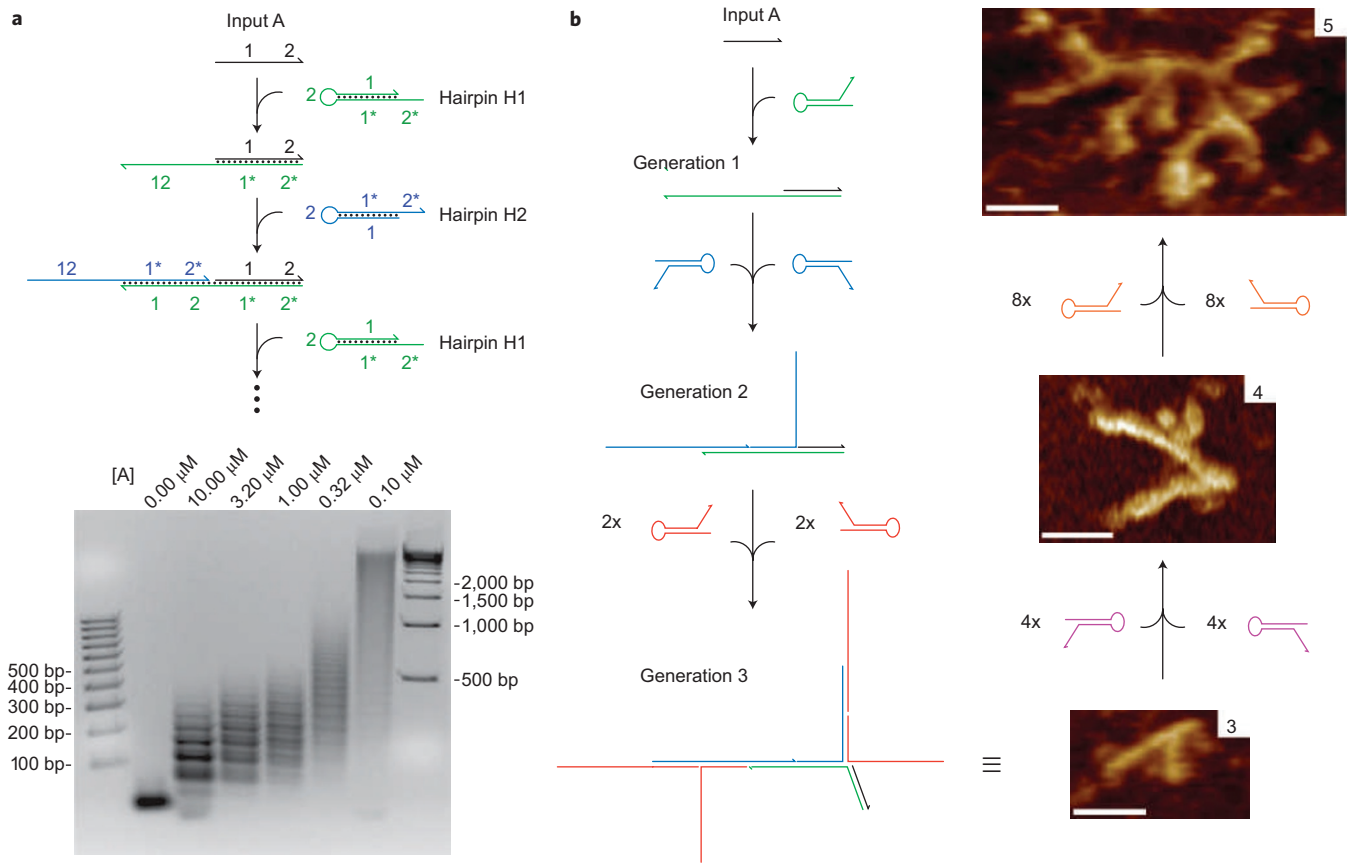


Figure 4 | Controlling the self-assembly of DNA nanostructures with strand displacement[74]. a, Nucleated growth of linear DNA polymers, using the hybridization chain reaction. Input A reacts with hairpin H1 to activate domain 2, which then allows the polymer to react with H2, and so on. As shown in Box 2 panel b, the two hairpins are metastable and do not react in the absence of input. Polyacrylamide gel electrophoresis analysis verifies that polymerization requires input. The size of the polymers varies inversely with the concentration of the input, because higher input concentration implies a lower hairpin to input ratio. **b**, Nucleated growth of branched DNA dendrimers[76]. The extra domains on the hairpin allow the resulting linear polymer to bind to two additional hairpins. Each open hairpin in turn provides two hairpin binding sites. Thus, the size of the polymer roughly doubles with every additional generation. The right side shows atomic force microscope images of the third-, fourth- and fifth-generation dendrimers. Scale bars represent 30 nm. Figures reproduced with permission from: **a**, ref. 74, © 2004 NAS, USA; **b**, ref. 76 © 2008 NPG.

The catalytic systems introduced in the previous section could be considered first examples of hybridization-driven motors. In each reaction cycle a catalyst strand undergoes a transition from a random coil configuration into a stretched double-stranded state and back. However, it is difficult to couple the state changes of the catalyst molecules to useful nanomechanical work. Several groups have tackled the challenge of designing motors with more desirable properties, and in particular have constructed autonomous walking motors that can directionally move along a track and transport molecular cargo.

Initial constructions of autonomous DNA walkers and other motors used enzymes or ribozymes for their operation and were driven by the formation or cleavage of covalent bonds rather than by hybridization[91–94]. Only recently have researchers developed mechanisms for strand-displacement-based DNA walkers with autonomous and directed movement[76,82,83,95].

Yin *et al.*[76] developed a walker that moves autonomously and directionally, but has intrinsically limited processivity (there is a roughly 50% chance that motion is terminated at every step). Green *et al.*[82] proposed and tested a mechanism for a walker that is autonomous, processive and directional. Omabegho *et al.*[95] demonstrated a walker (see Fig. 6) that autonomously and processively moved three steps; there does not seem to be an intrinsic limitation on the number of steps this walker can take.

There are three essential components for any DNA walker design: the motor that physically moves, the fuel that provides the chemical energy, and the track that prescribes the direction of motion. In many of the above designs, the track also serves as the fuel, so tracks cannot be used by more than one walker without further processing. In contrast, walkers such as kinesin use a diffusible fuel (ATP), and multiple different kinesin molecules can walk simultaneously along the same microtubule.

Of the synthetic DNA walkers, the enzyme-based design by Yin *et al.*[92] and the strand-displacement-based mechanism by Green *et al.*[82] achieve clear separation of motor, fuel and track, so that the track can be reused. These walkers typically demonstrated two to three steps of autonomous movement along a track; at the time, part of the difficulty of demonstrating extended autonomous movement was the difficulty of preparing long rigid DNA tracks. Since then, DNA origami technology[44] has allowed the construction of considerably longer tracks with more complex geometry[50,96] leading to correspondingly longer processive walks and integration of many different kinds of DNA nanomotors.

Although we have focused on DNA walkers, there are other ways in which strand displacement can be used to engineer devices that perform mechanical work. For instance, Venkataraman *et al.*[75] implemented a synthetic DNA-based version of a polymerization motor that is both autonomous and processive. In a reaction

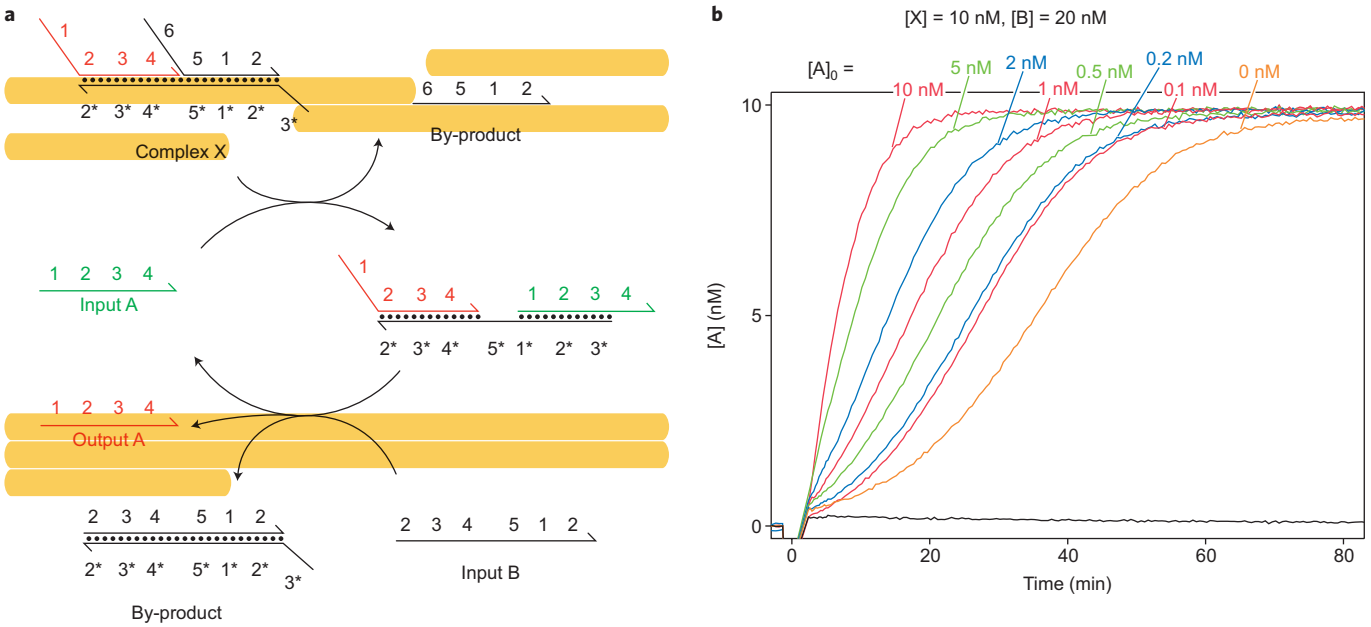


Figure 5 | DNA amplification by non-covalent catalysis[88]. a, Input A catalytically expedites the release of an output A (identical to the input) from complex X through the pathway shown. Strand B and complex X serve as the fuels for this reaction. Output A is released at the end of the cycle and can subsequently participate in further reaction cycles as input. The concentration of A grows exponentially in time until X and/or B is exhausted. **b**, Experimental demonstration of catalysis. The concentration of A is plotted against time, with different colour traces corresponding to different initial concentrations of input A. Figures reproduced with permission from ref. 88, © 2007 AAAS.

pathway reminiscent of the hybridization chain reaction but using four-way branch migration, up to 20 monomers were sequentially inserted into a growing polymer exerting force against a cargo.

Design tools and experimental considerations

Up to this point, we have considered the idealized behaviour of DNA strand-displacement devices with the assumption that hybridization is perfectly specific and that no unintended hybridization occurs. Unfortunately, sequence crosstalk is common in actual DNA systems and hinders the kinetics of hybridization and strand displacement[26,97,98]. Consequently, careful sequence design is necessary to suppress crosstalk[99–101].

Sequence design becomes increasingly important but also more difficult as the complexity of DNA systems increases; this has motivated the development of automated sequence design software[99–102]. These methods generally use the thermodynamic parameters of DNA[2,103] to design sequences with maximal probability of forming the desired structures and complexes at equilibrium. Several web-based programs are available that can evaluate the thermodynamics of DNA oligonucleotides[103,104]. Although not always applicable, the use of a three-letter alphabet has proved to be a useful heuristic that balances maximizing sequence space with minimizing crosstalk[67,88,105]. In this approach all input and output strands are designed using only the bases A, C and T, because G is known to be the most promiscuous nucleotide in terms of non-Watson–Crick hybridization[2].

Another reality that DNA nanotechnologists face is the imperfection of oligonucleotide synthesis[106]. The DNA that we work with contains synthesis errors such as single-base deletions and chemical damage (for example, deamination or depurination). Such impurities are not always completely removed even by post-synthesis purification techniques such as HPLC or PAGE and can negatively affect system performance. For example, in Box 2 panel a, if the bottom strand of complex X suffered deletions in the 1* domain or if the top right strand of complex Y suffered deletions in the 9 domain, then complex X could spontaneously interact with complex Y, even in the absence of input A.

The design of several systems based on strand displacement[64,67,76,86,88] accounted for the imperfection of oligonucleotide synthesis and used 'clamp' domains to combat unintended strand-displacement reactions. For example, in Box 2 panel a, insertion of an extra double-stranded domain 10:10* between domains 9 and 1 in both complexes X and Y would serve as a clamp. With this clamp, it is less likely that the X and Y complex can react with each other even if there is a deletion at the 3′ end of the 1* domain. Similarly, in Fig. 5, the domains labelled 2 are clamps that prevent multiple molecules of X spuriously undergoing strand displacement.

The predictability of nucleic acid hybridization and strand-displacement kinetics gives rise to the hope that *in silico* experiments could partially replace *in vitro* experiments in the near future. For this, kinetic simulations of DNA reaction networks must reliably capture the behaviour of DNA strands and complexes, including all potential reactions. Kinetic simulations of DNA interactions at the individual base-pairing level are one promising approach[107]. Simulations of this type should capture the dynamics of not only all designed reaction pathways, but also unintended reactions, such as from sequence crosstalk. However, such simulations are computationally expensive and may not be currently practical for large reaction networks with tens or hundreds of components.

On the other hand, even large systems can still potentially be modelled and simulated at the higher domain level. The DNA Strand Displacement (DSD) simulator by Phillips and Cardelli[71] is a design and simulation toolbox that automatically generates and simulates all possible strand-displacement reactions given a set of strands and complexes that exist in solution. This simulator, based on embedded stochastic and differential equations, plots the concentration trajectories of all initial and generated species, using experimentally measured rate constants[19].

Outlook and applications

The strand-displacement devices reviewed here are proof-of-concept systems that demonstrate a rational design approach to programming complex dynamical behaviours using only nucleic acids.

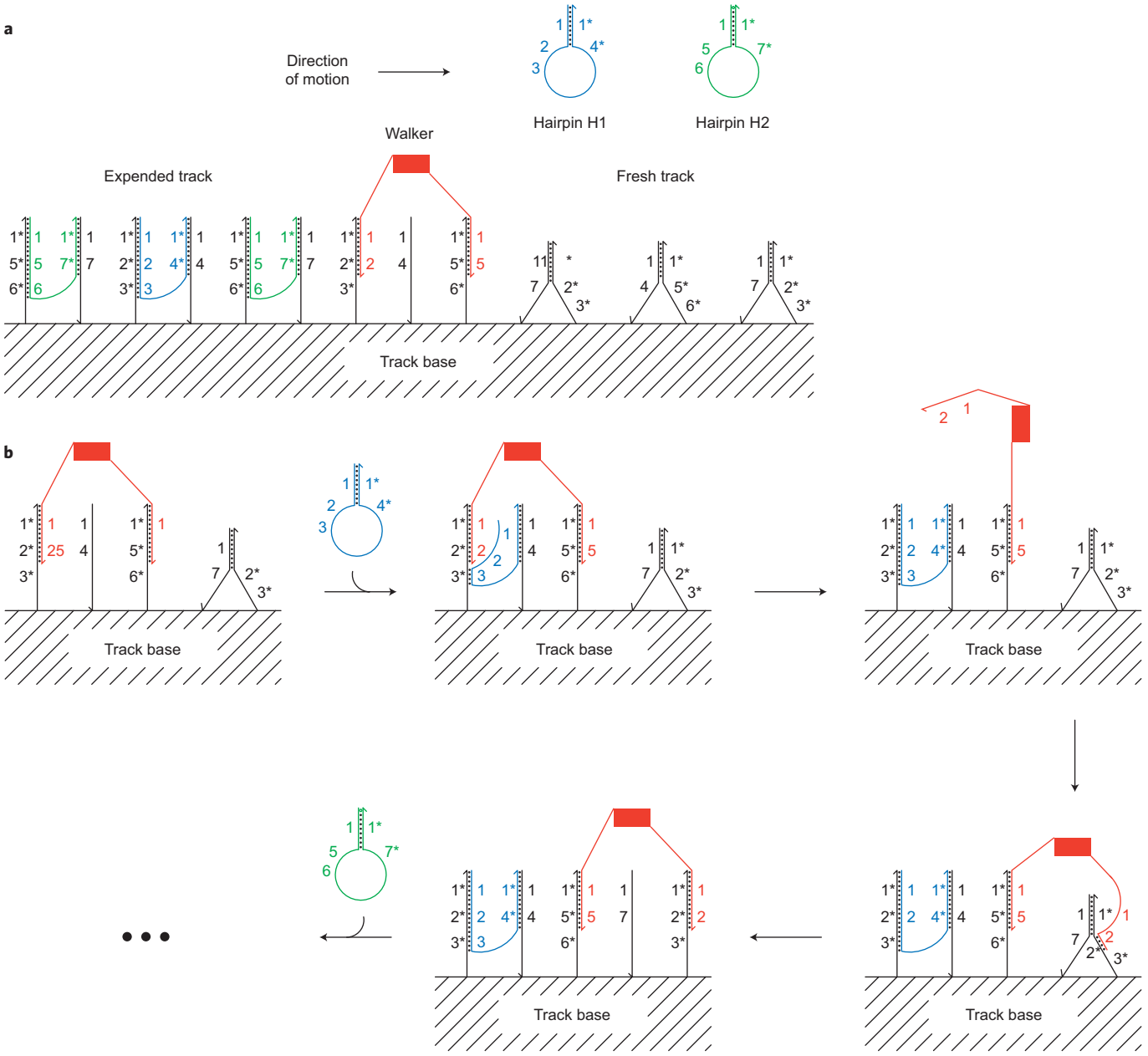


Figure 6 | An autonomous, processive and directional DNA walker based on strand displacement[95]. a, Fuel hairpins H1 and H2 are present in solution, and react with the track to push the walker forward. In the absence of the walker, however, the fuels do not react with the track. The track behind the walker is different from that ahead of the walker. **b**, Schematic of the walker taking one step. Hairpin H1 displaces the hind leg of the walker through a series of strand-displacement reactions. The lengths of the walker legs constrain the walker such that the freed hind leg cannot bind any track molecule other than that directly in front of it. The hind leg is now the leading leg, and H2 can initiate a similar reaction to drive the new hind leg forward.

The focus on a single material and the systematic application of a few basic design principles means that different devices are compatible with each other and can be modularly integrated into increasingly complex assemblies.

Functional nucleic acids, such as ribozymes and aptamers, can be used to broaden the set of chemistries that can be controlled by strand-displacement circuits[62,66,74]. Systems combining protein function with strand displacement further increase the variety of behaviours that can be programmed[52,57].

Devices with practical applications may benefit from integrating the programmability of nucleic acids with physical and/or chemical properties of other materials[8]. Liu and co-workers, for example, used the specific hybridization of nucleic acids to speed up the reactions of organic molecules functionalized to the complementary strands, in a process known as DNA-templated synthesis[108]. Mirkin and co-workers directed the aggregation of gold nanoparticles using DNA hybridization[109]. Le *et al.* showed improved control over gold nanoparticle positioning by using DNA self assembly[110]. Maune *et al.* assembled crossbar connections between carbon nanotubes by using DNA origami as a template, and demonstrated an ensemble with field-effect transistor properties[111]. Inclusion of strand displacement in the design of these DNA scaffolds could potentially allow precise modulation of metamaterial behaviour[46].

Control of gene expression is a primary goal of synthetic biology; dynamic DNA nanotechnology provides a practical approach

for achieving this through the programmable binding and release of biologically relevant nucleic acids. Smart therapeutics applications[57,112] are a particularly promising area. For example, the hybridization chain reaction using RNA hairpins can function inside living cells and can even be used to selectively kill cancer cells via the protein kinase R pathway[113]. It is likely that strand-displacement-based sensors and logic circuits similarly can be integrated with molecular actuators based on antisense oligonucleotides, siRNA or ribozymes. First steps in this direction have been taken[114].

RNA synthetic biology methods and tools[14,115] could potentially be integrated with strand-displacement-based systems to construct devices with improved performance. *In vivo* operation could further benefit from the use of chemically modified nucleic acids such LNA[116], PNA[117] or even expanded nucleic acid alphabets[118].

DNA is a powerful nanoscale engineering material, and recent research has shown that it can be used to build not only complex static nanostructures but also dynamic nanodevices capable of autonomous actuation. Applications in materials and biotechnology will continue to drive improvements in DNA strand-displacement-based devices.

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Additional information

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