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Dissipative DNA nanotechnology

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DNA nanotechnology has emerged as a powerful tool to precisely design and control molecular circuits, machines and nanostructures. A major goal in this field is to build devices with life-like properties, such as directional motion, transport, communication and adaptation. Here we provide an overview of the nascent field of dissipative DNA nanotechnology, which aims at developing life-like systems by combining programmable nucleic-acid reactions with energy-dissipating processes. We first delineate the notions, terminology and characteristic features of dissipative DNA-based systems and then we survey DNA-based circuits, devices and materials whose functions are controlled by chemical fuels. We emphasize how energy consumption enables these systems to perform work and cyclical tasks, in contrast with DNA devices that operate without dissipative processes. The ability to take advantage of chemical fuel molecules brings dissipative DNA systems closer to the active molecular devices that exist in nature.

Nucleic acid nanotechnology has produced a rapidly expanding toolkit of engineered DNA and RNA systems[1]. The high predictability of non-covalent interactions, primarily Watson–Crick–Franklin base-pairing, makes it possible to program how individual and multiple nucleic-acid strands fold and self-assemble, determining their equilibrium and transient configuration[1]. A growing platform of customizable nucleic-acid scaffolds includes assemblies with sizes ranging from a few nanometres (kilodaltons) to hundreds of micrometres (megadaltons), and a complexity that often exceeds that of naturally evolved scaffolds in cells[1]. Less complex yet easier to control nucleic-acid devices have been developed to sense, or respond to, stimuli such as other nucleic acids, small molecules or proteins[2]. Finally, by taking advantage of conformational changes in multi-stranded nucleic-acid duplexes (gates), systematic and scalable frameworks have been developed to build logic circuits, dynamic systems, strand-displacement cascades and neural networks that often include hundreds of components[3]. These technical achievements, combined with the low cost of synthesis, biocompatibility and ease of conjugation with other compounds, make nucleic-acid systems particularly attractive as designable materials and machines for applications that include sensing[4], computing[5], molecular transport[6] and drug delivery[7,8].

An emerging frontier in nucleic acids nanotechnology is the synthesis of devices and materials with the capacity to exhibit life-like behaviour, such as directional motion, transport, communication and adaptation[9,10]. Yet, in the majority of cases the system's response is determined by a change in the free energy landscape induced by the input, which causes the system to progress towards a new stable thermodynamic equilibrium[4,11]. Once that equilibrium state is reached, these systems become permanently non-responsive to the original stimulus. This is in strong contrast with the non-equilibrium nature of living systems, which maintain their responsiveness thanks to chemically fuelled processes. Energy is stored in thermodynamically activated but kinetically stable molecules[12], such as adenosine triphosphate (ATP), and is used to repeatedly power biomolecular machines such as pumps and motors, and to drive the dynamic formation of self-assembled structures such as actin and microtubules[13]. Energy dissipation provides a way to regulate chemical processes with exquisite spatiotemporal control, and is at the basis of the phenomena associated with life[14,15].

In this Review we describe functional DNA systems that exploit chemical energy for their operation, and we propose to use the term 'dissipative DNA nanotechnology' to describe this emerging field. We first clarify the concept of energy dissipation, introducing relevant definitions and terminology, and we put a particular emphasis on the conceptual differences between dissipative DNA nanotechnology and the consolidated field of dynamic DNA nanotechnology. A substantial part of the Review is dedicated to an illustration of applications ranging from out-of-equilibrium reactions to nanodevices and responsive polymer systems. We conclude by discussing open challenges and promising research directions that will enable taking full advantage of dissipative DNA nanotechnology for the design of artificial systems with life-like properties.

Dynamic versus dissipative DNA systems

In DNA nanotechnology the majority of responsive DNA systems rely on the activation of a series of DNA-hybridization and DNA-exchange reactions upon the addition of an 'input' DNA strand. With this concept, a variety of nanoscale devices made of synthetic DNA sequences have been rationally designed to undergo controlled motion or reconfiguration and exert a specific function. These devices and systems respond to the addition of input single-stranded DNA or RNA (ssDNA/ssRNA) sequences or other molecular cues and they are conventionally classified under the term 'dynamic DNA nanotechnology'[11]. Considering the existence of dynamic DNA nanotechnology as a well-established field, we start by discussing the conceptual differences between dynamic and dissipative DNA nanotechnology (Box 1).

A key strategy that has enabled the development of dynamic DNA systems is the toehold-mediated (or toehold-exchange) strand-displacement reaction, a process through which an 'input' DNA strand is able to react with a preformed duplex, leading to the displacement of another ssDNA sequence in a highly controlled manner[4,11,16]. Strand-displacement reactions are highly programmable and sequence-specific and can be used to activate DNA-based devices[17] and also to trigger the assembly and disassembly of DNA nanostructures[18–20] with high accuracy and precision. From a

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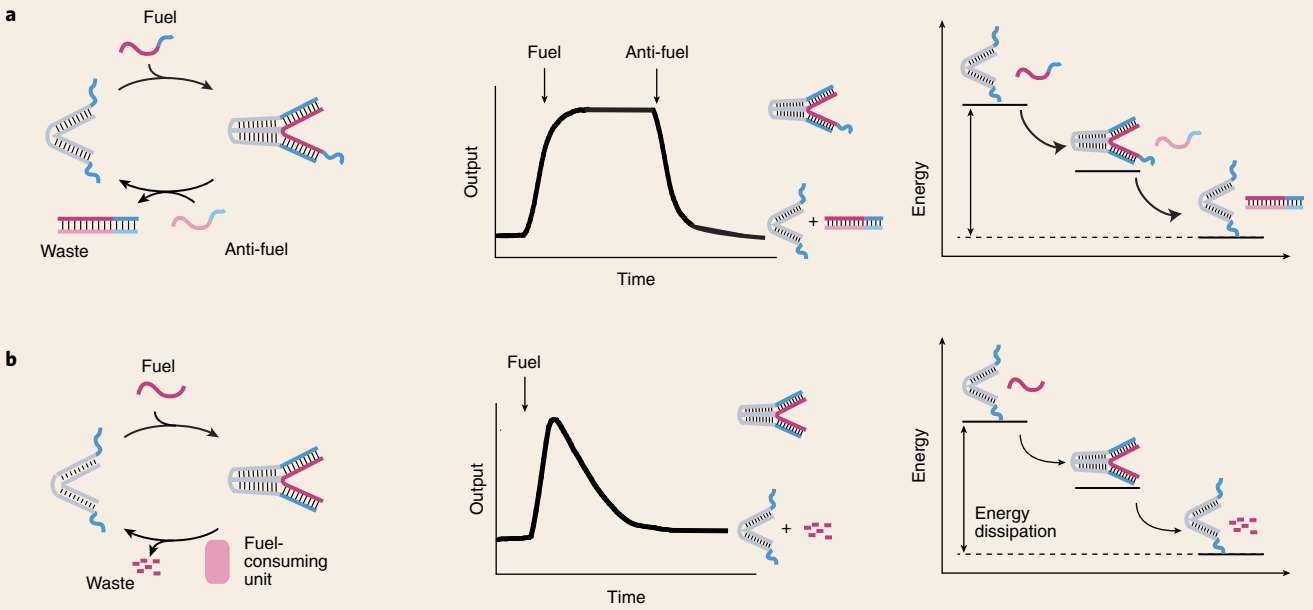
Box 1 | Dissipative versus dynamic DNA systems

We illustrate the conceptual difference between dynamic and dissipative DNA systems using the DNA-based molecular tweezer developed by Yurke, Turberfield and colleagues[23] as an example. The tweezer can be operated either as a dynamic device or as a dissipative device depending on the reactions introduced to control its open/closed state. To realize a dynamic DNA device, the tweezer is cycled between open and closed conformations by alternating additions of so-called DNA 'fuel' and 'anti-fuel' (panel **a** in the figure). Both the opening and closing of the DNA tweezer are thermodynamically controlled processes: the tweezer adapts to the change in the free energy landscape induced when the fuel and anti-fuel strands are added. The change in free energy between start and end of one open–close–open cycle corresponds to the hybridization energy of the fuel/anti-fuel duplex. Because the operation of the tweezer is controlled by thermodynamics, a full cycle does not take place spontaneously and the system resides indefinitely in either the open or the closed state until a new stimulus is provided.

A dissipative version of the same device can be achieved by creating conditions in which a fuel molecule is chemically degraded over time and converted to waste. This can be done, for example, by introducing a nuclease that degrades the nucleic-acid fuel closing the tweezers (panel **b**). As a result of energy dissipation, the energy landscape gradually changes over time: the tweezer adapts to that change and yields a spontaneous open–closed–open cycle triggered by the addition of fuel. The energy that is consumed in one cycle corresponds to the difference in chemical energy potential between fuel and waste. The tweezer resides in the closed state for an interval of time that is determined by the rate at which fuel is consumed, allowing control of the kinetic operation of the tweezers.



conceptual point of view, the addition of the 'input' DNA strand (in the literature generally referred to as 'fuel') alters the free energy landscape and the system adapts spontaneously by shifting its composition towards a set of complexes that reside at a new global minimum. Activation of the 'input' DNA strand via a molecular input (such as other DNA strands or biomolecules) or environmental stimuli (pH or light) provides a means to indirectly control the exchange reaction and, consequently, the function exerted by the DNA device[21,22]. Alternatively, the subsequent addition of a DNA strand that is able to sequester the input DNA strand (a so-called 'anti-fuel') switches the system back to the original state, but always controlled by a thermodynamically driven process[23]. In dynamic DNA systems of higher complexity, input 'fuel' molecules activate key DNA components for the release of oligonucleotides that then participate further in downstream steps. These components are DNA fuel gates that power many strand-displacement cascades by the triggered release of output for downstream processing[11]. Such fuel gates therefore play a dual role as information carrier and as activator for additional processes by releasing secondary DNA fuel strands. After the cascade has been triggered by the initial addition of the DNA fuel, these fuel gates are gradually consumed as the system progresses towards the final thermodynamic state. Consequently, on arrival at the final state, not only has the initial trigger become thermodynamically deactivated, but so also have these fuel gates. This implies that in many complex dynamic DNA systems, a new cycle of operation cannot be reinitiated just by adding a new batch of the initial trigger; it also requires a replenishment of functional system components (that is, the fuel gates). To summarize these observations, we can say that dynamic DNA nanotechnology operates strictly under thermodynamic control, and the observed kinetic behaviour, which may be very sophisticated, is defined by the mechanistic pathway that brings the system to the thermodynamically stable final state.

In contrast to the thermodynamic control in dynamic DNA nanotechnology, the approach of dissipative DNA nanotechnology requires the presence of an energy-dissipating chemical reaction that converts molecules with high chemical potential (fuel) into molecules with lower chemical potential (waste)[12]. In these systems, the addition of fuel alters the free energy landscape; however, this continues to change in time as a result of fuel-to-waste conversion. The system's components respond to the addition of fuel, but this response is only transient, and the system returns to the initial state after the fuel-to-waste conversion is complete. This behaviour is autonomous and, unlike most dynamic DNA systems, is not

Box 2 | Glossary

Dynamic DNA nanotechnology
DNA systems that dynamically respond to the addition of fuel strands by adapting in a thermodynamically controlled fashion to the new energy landscape.

Fuel (in this context): a molecular input (such as nucleic-acid strand, pH or small molecule) that serves as 'input' for activation of the system by initiating a strand-displacement reaction or other fuel-induced downstream processes.

Anti-fuel: a molecular input able to displace the fuel (such as a DNA strand complementary to the fuel strand).

Waste (in this context): a thermodynamically stable anti-fuel/fuel complex (for example, a fuel/anti-fuel duplex).

Fuel gate: a DNA system able to process input molecules to release secondary fuels.

Dissipative DNA nanotechnology
DNA systems that incorporate a chemical fuel-to-waste conversion. Chemical energy stored in the fuel is used to drive the system out of equilibrium.

Fuel (in this context): a molecular input (such as nucleic-acid strand, ATP) with high chemical potential that activates a DNA-based system.

Waste (in this context): product of lower chemical potential obtained after chemical degradation of the fuel molecule.

Energy consumption: exploitation of the free energy released from the conversion of fuel into waste.

directed stepwise by the sequential addition of multiple inputs. In addition, once the fuel is consumed and the system's components have returned to their original state, it is possible to restart a cycle of operation by simply adding a new batch of fuel, without having to replenish the pool of functional components (Box 1 figure, panel **b**). A characteristic feature of dissipative DNA nanotechnology is that kinetic, rather than thermodynamic, processes control the active state of the system. A fundamental implication is that the presence of an energy-dissipating process enables operation under non-equilibrium conditions, which is an essential feature of living systems.

In light of these observations, it is important to clearly disambiguate the concepts of fuel and waste in dynamic and dissipative DNA nanotechnology (Box 2). Fuel in dynamic DNA nanotechnology is used to describe not only the initial nucleic-acid input that activates the fuel gates described above, but also the strands that are released from the fuel gates and trigger downstream processes. At the end of the process, the fuel ends up in a thermodynamically stable inert nucleic-acid complex (waste) that often also includes (part of) the fuel gates. In dissipative DNA nanotechnology, the term 'fuel' describes an input of the system that is chemically or enzymatically converted to waste. Examples of fuels described in the later sections are nucleoside triphosphates, which serve either for the polymerization or for the ligation of nucleic-acid strands, which subsequently activate the system through hybridization with systemic nucleic-acid strands. On occasion, pre-synthesized nucleic-acid strands themselves are also directly used as chemical fuels.

It is also important to clarify the concept of 'non-equilibrium', because the term is used with some ambiguity in the literature related to dissipative self-assembly[24–26]. A non-equilibrium state of a chemical process occurs when the rate of the forward reaction is different from that of the backward reaction. Transient non-equilibrium conditions can be installed in a rather trivial way simply by adding a chemical fuel to the system. An increase in fuel concentration will enhance the forward reaction rate and create a transient non-equilibrium state of the process. The vast majority of systems that will be presented in this Review rely on the installation of such a transient non-equilibrium state, which can be regenerated by adding a new batch of fuel.

A non-equilibrium steady state can only occur in open systems in which fuel is continuously replenished and waste is eliminated. However, this in itself is not sufficient to ensure that the composition of the system is not dictated solely by the relative thermodynamic stabilities of its components. A non-equilibrium steady state implies that at constant fuel (and waste) concentrations, the rate of the forward reaction that describes the equilibrium process is also different from the backward reaction rate. In the general context of chemically fuelled self-assembly, we and others have shown that kinetic asymmetry in the energy-dissipation process is an essential prerequisite[24,27–29]. This aspect has so far not been considered in the context of dissipative DNA nanotechnology, which implies that examples are not yet available in the literature. In the Perspective section we return to this discussion and illustrate the importance of designing DNA-based systems that can operate under non-equilibrium steady-state conditions.

Dissipative nucleic-acid-based circuits

Life is sustained by out-of-equilibrium processes that are often directed by the outputs of dynamic reaction networks. A canonical example is given by oscillators: in biology, periodic behaviours, such as circadian rhythms, usually occur in the presence of a negative feedback loop that is destabilized by delays or ancillary positive feedback[30]. Another canonical example of dynamic networks is given by bistable systems, which require the presence of positive feedback. In these systems, dissipation must be present for the existence of non-equilibrium states. Building on demonstrations of dynamic circuits in synthetic biology[31,32], DNA nanotechnology provides many strategies to build dissipative dynamic networks.

Taking inspiration from the architecture of gene networks, Winfree and Kim[33,34] devised in vitro 'genelets' that are regulated only via RNA transcripts and strand displacement. A genelet is a short linear DNA template that includes a nicked bacteriophage T7 promoter (Fig. 1a). If an activator DNA strand is bound to the promoter, transcription by T7 RNA polymerase (RNAP) proceeds. If the activator is displaced by an inhibitor strand, T7 RNAP binding is unsuccessful, and transcription is turned off. RNA transcripts produced by genelets can be designed to serve as inhibitors or activators to other genelets (Fig. 1a,b). When bound to a DNA molecule, RNA is degraded by the enzyme RNase H. Genelet circuits are fuelled by nucleoside triphosphates, which permit transcription to give high-energy RNA. As RNA bound to DNA is degraded, the chemical energy is dissipated, pushing a spontaneous return to the original state. With this strategy, genelets have been used to build a repertoire of feedback networks with complex temporal dynamics such as self-activation[35], bistability[33,36] and oscillations[34]. The simplest transcriptional oscillator (Fig. 1a,b) includes two genelets connected in a negative feedback loop. The amplitude and frequency of this circuit typically fall in the range of 50–100 nM and 1–3 h, respectively (Fig. 1c), and can be tuned by changing the concentrations of DNA and enzymes. The progressive loss of enzyme activity, as well as the accumulation of abortive transcripts and partially degraded RNA, cause oscillations to have fluctuating amplitude and frequency that cannot be sustained for more than 16–20 h, as shown in Fig. 1c[34,37,38]. Recent work by the Schulman group has demonstrated modified synthetic genelets with improved modularity that can be used to program multi-state systems[36]. Genelet networks

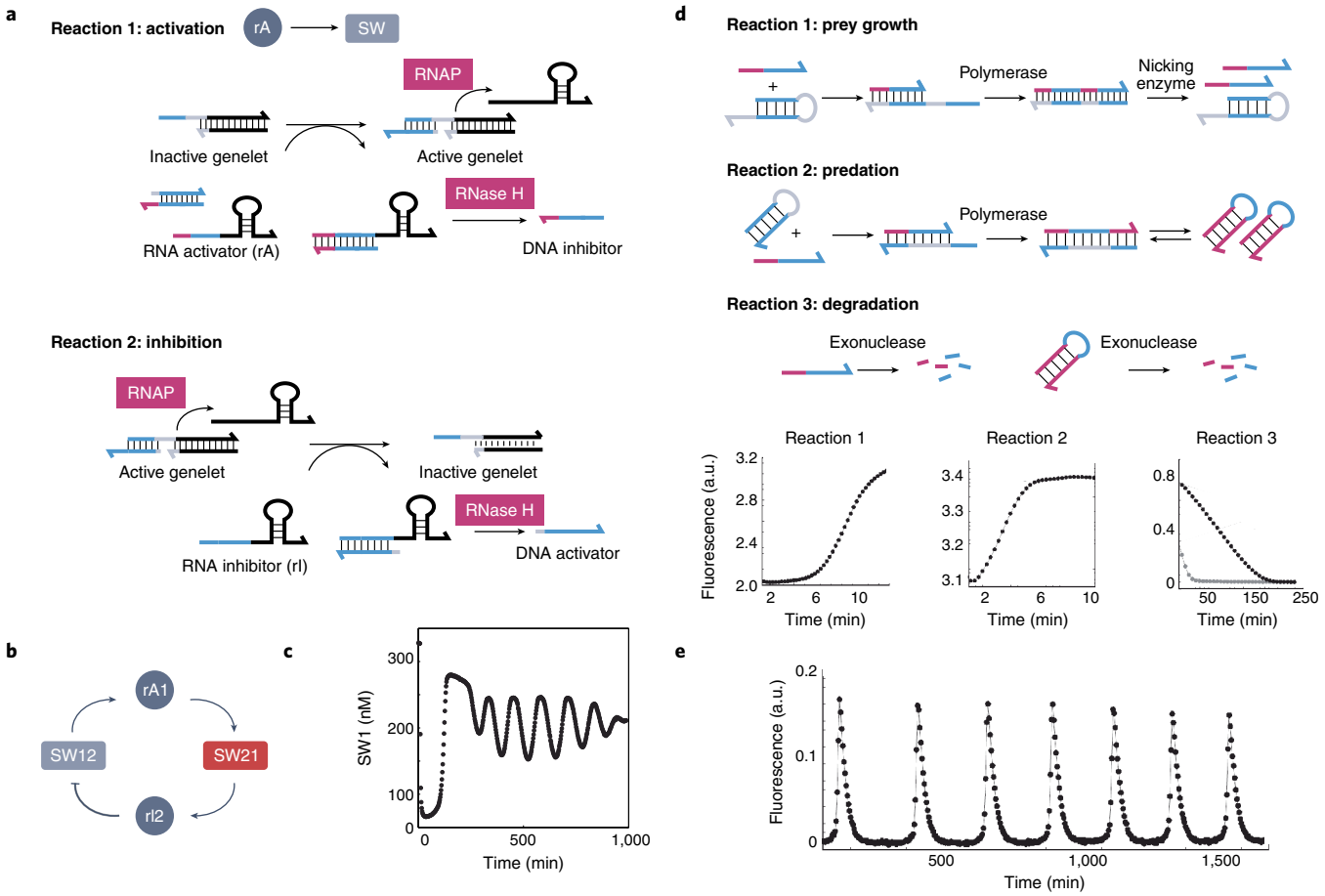


Fig. 1 | Nucleic-acid circuits operating via dissipative reactions. a, Genelets are short linear genes transcribing RNA using T7 RNA polymerase (RNAP). Genelets can be activated (reaction 1) or repressed (reaction 2); they include a nicked promoter (grey domain), so that a domain of the template strand (light blue) can be displaced or released through toehold-mediated strand displacement (light blue and red domains). Dissipation in the system is introduced by the enzyme RNase H, which degrades RNA regulators bound to DNA inhibitors or activators. **b**, RNA transcripts produced by a genelet (SW12, dark grey molecules) can be designed to operate as an activator (rA1) for a second genelet (SW21). This last genelet can then transcribe for another RNA strand that acts as an inhibitor (rI2) for the first genelet (SW12), generating networks such as in the oscillator[34]. **c**, Example data showing the temporal evolution of the active state of the genelet SW21 in **b**. **d**, The PEN toolkit is based on three different processes. In reaction 1 (prey growth), the incomplete DNA templates are activated by inputs (blue–red strand) and extended by DNA polymerase; the templates produce outputs upon nicking by a nickase. The fluorescence trace (bottom left) shows the exponential increase of the prey. In reaction 2 (predation), the palindromic predator is duplicated, using and consuming prey. The fluorescence trace (bottom middle) shows the exponential growth of the palindromic predator until all prey is converted into predator. In reaction 3 (degradation), the excess inputs and outputs are degraded by exonucleases, as shown by the fluorescence trace (bottom right). **e**, The particular PEN reactions illustrated here, which represent the data in ref. [42], generate sustained predator–prey oscillations that are seen as repeated on/off fluorescence. Credit: adapted with permission from ref. [37], PNAS (**a**–**c**); ref. [42], ACS (**d**,**e**).

have the potential to serve as a minimal transcription-based regulatory system in synthetic cells, as shown in studies conducted within droplet systems[38,39].

An alternative strategy to build dissipative kinetic circuits was developed by Rondelez, Padirac and Fujii and takes advantage of polymerase–exonuclease–nickase (PEN) reactions[40]. The PEN reactions are used to dynamically connect templates that can be activated or repressed by hybridization (Fig. 1d). DNA polymerase extends single-stranded domains of active templates; the extended domain is recognized and cleaved by nickases, causing the 'replicated' short strand to be released. These short replicated strands can be used to activate or inhibit other templates, thereby building circuits. Dissipation is introduced by the nickase and by the exonuclease, which respectively release and degrade ssDNA fuelling the dynamics; in the absence of these enzymes, the system would reach thermodynamic equilibrium. The PEN toolbox is flexible and has been used to build amplifiers, toggle switches and negative feedback oscillators. The molecular implementation of a Lotka–Volterra predator/prey system, as described by Montagne, Fujii and Rondelez[41–44], sustains remarkably robust oscillations for more than 24 h (Fig. 1e). The PEN system has been used in the context of pattern formation, multi-agent systems, and can be tuned to operate in cell media[45–47].

Recent work by Deng and Walther[48] introduced dissipative reactions in dynamic strand displacement (DSD) systems through DNA restriction enzymes (BsaI) and ligases (T4). Here, a transient DSD was obtained by calibrating a DNA gate composed of two components (the complex and reporter) to release an output strand upon ligation of the components; after the parts are ligated, excision of the gate by a restriction enzyme introduces a conformational change in the reporter that recaptures the output, generating a feedback loop. Because the ligase operation is fuelled by ATP, the system produces

a transient pulse of output, reverting to a stable state (output not released) when ATP is depleted.

In two recent examples, Willner et al. demonstrated nucleic-acid-based dissipative networks using a nickase enzyme (Nt.BbvCI) as fuel-consuming unit. In an initial study, they introduced the concept of nucleic-acid-based dissipative constitutional dynamic networks, demonstrating the possibility to transiently control the catalytic property of a Mg^{2+}-ion-dependent DNAzyme and a G-quadruplex-based DNAzyme[49]. More specifically, the authors designed two independent constitutional dynamic networks that were each composed of four duplex DNA structures. The addition of a fuel strand could introduce the time-dependent reconfiguration of the network and up- or down-regulate the different constituents that include two DNAzyme reporter systems. This is thus a demonstration of a constitutional dynamic network coupled to a dissipative behaviour in the field of nucleic-acid nanotechnology and, as such, could open the field to systems of enhanced complexity such as cascaded dissipative networks and dynamic combinatorial library reactions. An attempt in this direction was recently proposed by Willner and colleagues that demonstrated dissipative cascaded DNA networks[50].

The classes of circuit we have just described are dissipative systems due to the presence of (enzymatic) reactions that convert fuel into waste molecules of lower chemical potential that do not—or to a lower extent—interact with the system's components. The conceptual difference with dynamic DNA nanotechnology emerges from a comparison with a circuit that has been described in that context. A highly intricate DNA circuit was devised that is able to develop damped oscillations (rock-paper-scissors oscillator) triggered by the simultaneous addition of three inputs[51]. However, contrary to the dissipative systems described above, this system relies entirely on thermodynamically controlled toehold-displacement reactions. The oscillations are a result of a highly sophisticated kinetic pathway that brings the system to the thermodynamic end state. The new thermodynamic equilibrium is reached when all fuel species are converted into waste (that is, hybridized fuel) and, at that stage, cannot be reactivated.

Chemically fuelled DNA walkers

DNA walkers take inspiration from motor proteins such as kinesin and myosin that move along one-dimensional cytoskeletal filaments[52,53]. A DNA walker is a specific complex or molecule (usually a DNA strand) that achieves processive motion by binding to multiple DNA-binding sites placed along a molecular track, also made of DNA[4,11]. Several variants of DNA walkers achieve motion exclusively through toehold-mediated strand displacement and are not dissipative systems[54]. These walkers operate through thermodynamically controlled processes that are designed so as to permit sorting of cargo, processive movement along a programmed path[7,55–58] or walks up to 300 steps long[59].

Dissipative reactions make it possible to build simple DNA walkers that achieve autonomous processive motion by exploiting chemical fuels. To obtain motor-protein-like processive motion exclusively via strand displacement it was necessary to introduce bipedal walkers[55–57] or multiple distinct fuel strands that each activate a particular displacement event in a sequence[7,58,60]. The need for multicomponent walkers was bypassed by introducing catalytic reactions to control the walker operation[61–63]. The energy-dissipating reactions occur at the interface between the walker and its track: the track bound to a foot of the walker is degraded either by an enzyme or by catalytic nucleic-acid sequences. Degradation of the path already covered funnels autonomously future steps of the walker in a specific forward direction. The first example of an autonomous walker by Yin et al.[61] employs T4 DNA ligase and two restriction endonucleases (that is, BstAP I and PflM) to enable the movement of a 6-nt DNA strand along a DNA track. The DNA track contains three binding sites for the walker, labelled A, B and C (Fig. 2a). In the absence of ATP, the walker is inactive and binds at site A. In the presence of the walker, A and B have complementary sticky ends that can hybridize. Addition of ATP, which acts as the fuel, makes it possible for T4 ligase to catalyse the ligation of the sticky ends (process I) and create the recognition site for the endonuclease PflM I. At the next step (process II) the PflM I cleaves the duplex formed between anchorages A and B, resulting in movement of the walker at anchorage B. After that, the sticky ends of anchorages B and C can hybridize and can also be ligated by T4 ligase (process III). Also in this case, the ligation results in the formation of a recognition site for another endonuclease (BstAP I) that can cut this new duplex, leading to the movement of the walker at anchorage C (process IV).

After this first breakthrough work, several other examples of autonomous walkers were reported based on nicking enzymes (N.BbvC IB), making it possible to processively navigate networks of tracks[62,64,65]. An alternative approach was followed by Tian et al.[66], who developed a very simple DNA walker to move along an RNA track by using an RNA-cleaving deoxyribozyme (Fig. 2b); the DNA walker could repeat a number of steps limited exclusively by the number of anchoring sites available. This work was later extended to show processive motion along a DNA origami track with a multi-legged DNA spider[63]. An important observation is that, even in the presence of an energy-dissipation process, the walker cannot proceed indefinitely and its motion is constrained by the length of the track. Additionally, a limitation of these approaches is that the track becomes inert and cannot be used again once the walker has reached the endpoint.

Dissipative nucleic-acid-based devices

Chemically fuelled dissipative DNA switches. Thanks to the high programmability of DNA–DNA interactions, it is fairly straightforward to rationally design synthetic DNA-based sequences (here denoted as switches) that are able to undergo a conformational change in response to a specific input (a DNA sequence, an environmental cue, a small molecule or a protein)[67]. Switches usually operate under strict thermodynamic control, but several examples have recently appeared in the literature where their operation can be triggered in a dissipative way. One of the first examples of dissipative DNA nanoswitch[68] employs a short nucleic-acid strand as a fuel and takes advantage of specific DNA sequences that have enzyme-like catalytic activity[69]. More specifically, the authors employed a well-known DNAzyme to control the opening and closing of a DNA tweezer in an autonomous way. A key characteristic of the DNAzyme is the ability to spontaneously cleave the complementary nucleic-acid strand upon hybridization. The tweezer is composed of two different synthetic DNA strands (Fig. 3a): one that contains the sequence encoding for the DNAzyme (purple, Fig. 3a) and two 15-nt tails at the two ends of this sequence, and a second DNA strand fully complementary to the above-described tails. The hybridization of the two strands forms a tweezer-like structure that brings together these two portions in a random-coil conformation, so that the structure is in a 'closed state'.

The external addition of a specific DNA–RNA chimera fuel strand complementary to the DNAzyme portion can open the tweezer. Upon binding of such fuel, the catalytic cleavage activity of the DNAzyme is activated and the fuel is cleaved into two short fragments. Because such fragments have a lower affinity for the tweezer strand, they dehybridize from it, resulting in the time-dependent return of the system to the original random-coil conformation and the system becomes available for a new cycle. The system shows reversible behaviour upon successive additions of the RNA substrate for up to eight cycles, even if a fluorescence intensity drift is observed over time. The elegance of this system is that energy dissipation is an intrinsic property of the DNA-based system and does not require any kind of enzyme. Using a very similar conformational-change

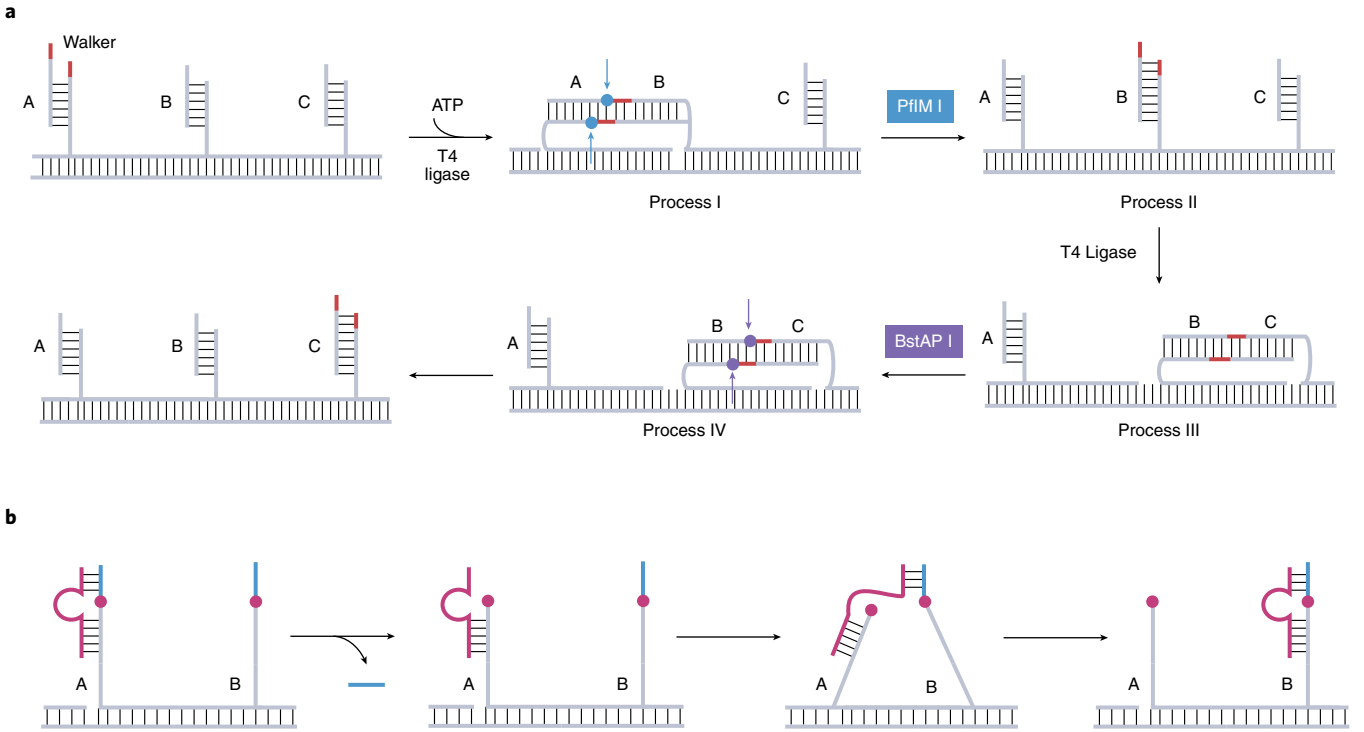


Fig. 2 | DNA walkers. DNA molecules can be designed to move along a predefined path via dissipative reactions. **a**, A DNA walker strand (red) can processively change location on a track thanks to the concerted action of the T4 ligase (able to ligate consecutive strands) and two restriction nucleases[61] (PflM I and BstAP I) that are able to cut their specific recognition sites (light blue and purple dots, respectively) so that, after each ligation–cutting process, the walker strand (red) finds itself located to a strand that is adjacent to its original one. **b**, A DNA walker strand (pink) can proceed across multiple locations on an RNA track. The walker strand is designed to have an RNA-cleaving ribozyme sequence that can cut the walker binding sites[66] (red dots), inducing the spontaneous dehybridization of the cleaved portion. The free single-stranded domain of the walker strand can thus move towards the next location (B) through a strand-displacement reaction. In both examples, motion is autonomous yet limited by the number of binding sites, and a particular track can only be used once as it is degraded during the walker operation. Credit: adapted with permission from ref. [61], Wiley (**a**); ref. [66], Wiley (**b**).

tweezer-like DNA switch, Bishop and Klavins reported the opening of a tweezer fuelled by an RNA molecule[70], where dissipative conditions were installed by the presence of a nuclease enzyme (RNase H). A DNA-based oscillating circuit from the genelet toolbox was also demonstrated by Simmel, Winfree and Franco as a molecular clock to direct motion of the tweezer[37] (Fig. 3c).

Cargo loading and release via chemically fuelled dissipative DNA devices. Conformational-change mechanisms similar to those described above can also be employed to induce the capture or release of a molecular cargo from a DNA-based system. Many responsive DNA ligand-binding devices have been engineered by designing DNA sequences that, in the presence of an effector, undergo a conformational change that affects their affinity for the ligand[2]. As an example, a ligand-binding device can load a specific molecular cargo (a DNA sequence, a small molecule and so on) and release it in the presence of a specific allosteric effector (a different DNA sequence or an environmental cue such as pH)[71]. This mechanism resembles what happens in nature where biological receptors (that is, enzymes and proteins) are allosterically controlled by different effectors that change their affinity towards a specific substrate.

Recently, a versatile strategy to design a synthetic ligand-binding DNA device that can transiently load and release a molecular cargo was demonstrated[72]. The device is a clamp-like DNA-based receptor that is able to recognize and bind a specific DNA sequence cargo through the formation of a triplex structure (a secondary DNA motif that involves both Watson–Crick–Franklin and Hoogsteen interactions; Fig. 4a)[73]. The device is rationally designed so that the addition of a specific fuel RNA strand (purple strand, Fig. 4a), which is able to bind to the loop portion of the device, induces a conformational change, resulting in the unfolding of the triplex structure and the consequent release of the DNA cargo. A nuclease enzyme (that is, RNase H) can specifically recognize the RNA/DNA heteroduplex formed and hydrolyse the RNA fuel strand. This will allow the DNA device to rebind the DNA cargo with a kinetic that depends on the enzyme (Fig. 4b) and fuel concentration. Multiple load–release cycles can be achieved through repetitive fuel additions, demonstrating the reversibility of the system (Fig. 4c).

To further demonstrate the versatility of this strategy, a similar approach was also applied to aptamers[74], in vitro selected DNA and RNA sequences able to bind a variety of targets with high affinity and specificity. A dissipative aptamer-based system for the transient release of small molecules (that is, ATP and cocaine)[75] was obtained by rationally designing an allosterically regulated aptamer that, in the presence of a specific DNA strand (fuel), releases its molecular ligand. The fuel, once bound to the aptamer, is specifically degraded through the action of an enzyme (Nt-BsmAI), and this restores the original binding ability of the aptamer (Fig. 4d,e). These examples point to the fact that the scope of dissipative DNA nanotechnology goes beyond the demonstration of autonomous DNA systems, as it provides adaptable devices with the capacity to control a multitude of biological and non-biological components.

Most systems in dissipative DNA nanotechnology rely on enzymes for the conversion and dissipation of chemical energy. However, dissipative DNA devices that do not rely on enzymatic

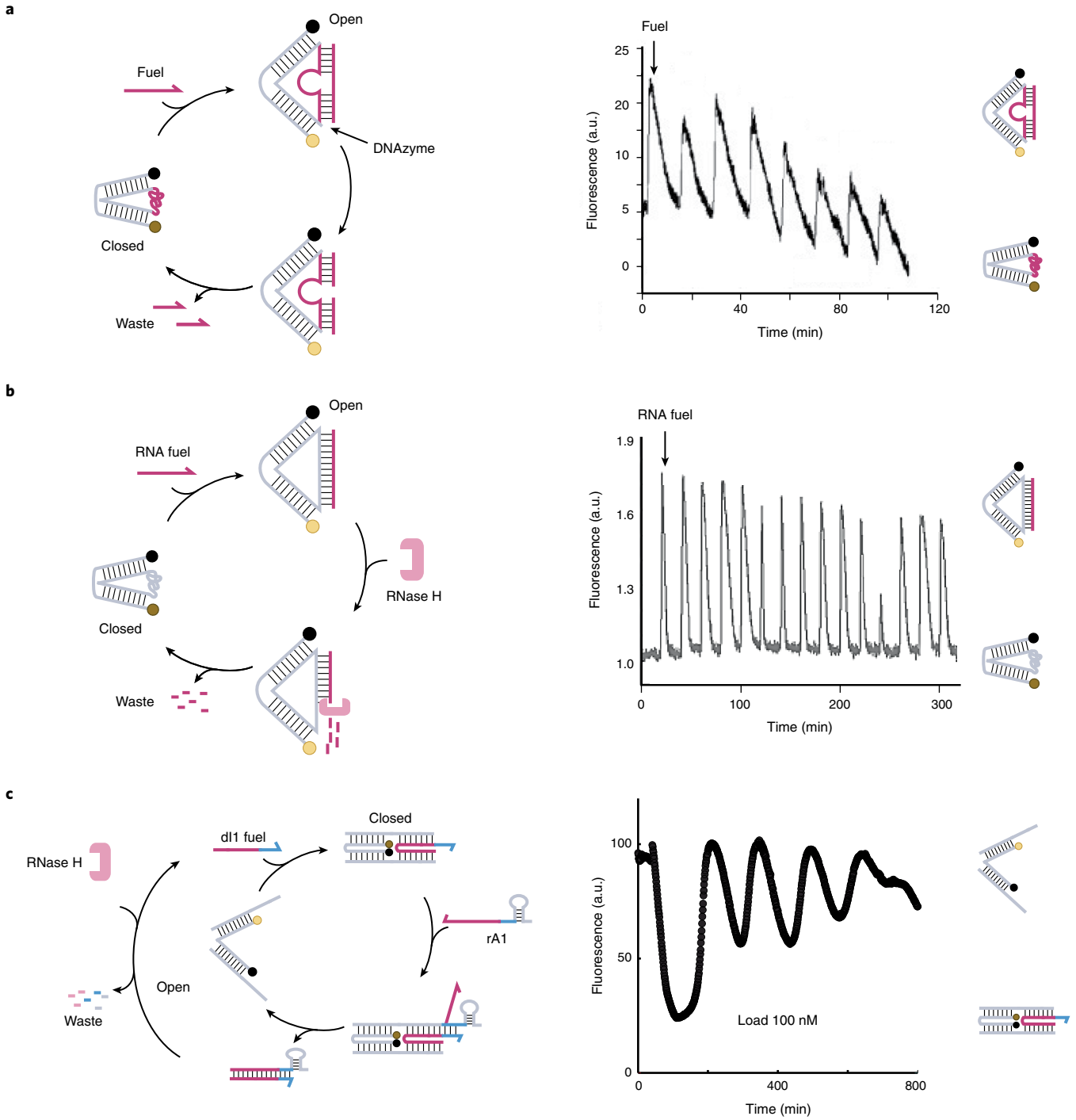


Fig. 3 | Dissipative tweezer-like DNA-based switches. a, In a first example, a molecular tweezer made of synthetic DNA strands is designed to contain a self-cleaving DNAzyme domain[68]. Left: schematic representation of the tweezer. Right: its fluorescence trace. In the absence of fuel the tweezer is in a closed state (low fluorescence signal, brown dot). Addition of the specific DNA–RNA chimera fuel can open the tweezer structure (leading to a high fluorescence signal, yellow dot) and trigger the DNAzyme self-cleavage activity. This results in catalytic cleavage of the fuel and return of the system to the initial condition. Reversibility of the tweezer for consecutive cycles is measured via the fluorescence change associated with the opening and closing of the structure. **b**, A similar dissipative behaviour in controlling the opening and closing of a tweezer can be achieved by using an RNA strand as fuel and the enzyme RNase H as a fuel-consuming unit[70]. In this case, the tweezer also shows reversibility for several consecutive cycles. **c**, Alternatively, dissipation can be installed in the tweezer dynamics through coupling with a genelet-based oscillator (Fig. 1b,c)[37]. Credit: adapted with permission from ref. [68], Wiley (**a**); ref. [70], ACS (**b**); ref. [37], PNAS (**c**).

processes were also described using pH-dependent motifs coupled with chemical pH oscillating networks[76–80] or enzymatic catalysis[81] and disulfide/thiol reactions using disulfide-linked dimer DNA strands as fuels[82].

Dissipative DNA-based materials

Thanks to the predictability of base-pairing interactions and the ease of synthesis, synthetic nucleic acids can be conveniently used to build nanoscale structures with quasi-ångstrom precision[1]. Initially,

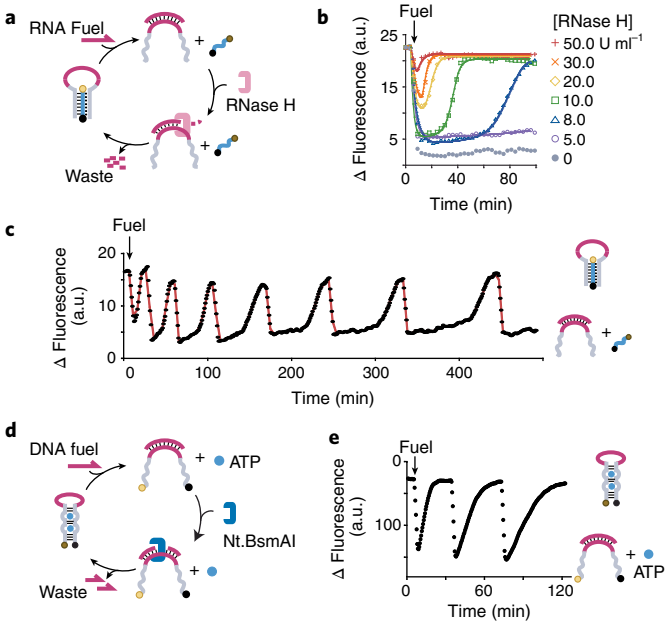


Fig. 4 | Ligand-binding DNA-based devices for the transient load and release of a molecular cargo. a, A clamp-like DNA-based device able to recognize a specific DNA sequence (ligand) through the formation of a triplex structure[72]. A RNA fuel strand binds the loop portion of the device, causing its opening and the consequent release of the DNA cargo. An endoribonuclease enzyme (RNase H) is used as the fuel-consuming unit. The enzyme binds the RNA/DNA heteroduplex and selectively hydrolyses the RNA portion of the duplex, restoring the initial loading condition. **b**, The corresponding fluorescence traces show modulation of the transient cargo release by varying the concentration of the RNase H (with decreasing concentration of enzyme, a slower reloading of the cargo is observed). **c**, The device in **a** shows a highly reversible transient behaviour. **d**, Synthetic DNA-based receptor for the transient release of small molecules[75]: re-engineered ATP-binding aptamer for the reversible transient release of ATP. The aptamer is split into two functional units joined together with a linker domain. The DNA fuel strand is fully complementary to this portion, and its binding to it can cause the opening of the aptamer and release of the ATP (resulting in a high fluorescence signal). Nt.BsmAI is able to recognize the fuel/linker domain duplex and selectively cleave the DNA fuel, restoring the original affinity of the aptamer for the ATP (leading to a low fluorescence signal). **e**, Kinetic fluorescence traces showing the reversibility of the transient release of ATP following consecutive addition of the fuel strand. Credit: adapted with permission from ref. [72], Wiley (**a**–**c**); ref. [75], Wiley (**d**,**e**).

DNA-based nanostructures (DNA origami) were essentially 'static' assemblies built by folding long DNA strand backbones through short DNA staples[83,84]. More recently, these structures have been re-engineered to undergo structural reconfigurations in response to specific chemical inputs[22,85] or environmental triggers such as temperature[86,87] and pH[19,21]. These 'dynamic' DNA structures, however, often operate at thermodynamic equilibrium (that is, the external trigger causes a change in the free energy landscape), a feature that lacks the adaptability and transientness of naturally occurring materials. In the next paragraphs we will thus describe chemically fuelled dissipative DNA-based materials in which the assembly/disassembly process can be controlled by the concurrent presence of an anabolic and catabolic reaction, the former leading to the self-assembled material, and the latter destroying it.

Dissipative DNA-based nanostructures. The first examples of DNA structures controlled through dissipative reactions were proposed by Franco and her group, who demonstrated the possibility of using synthetic genelets to enzymatically produce nucleic-acid fuels controlling the assembly and disassembly of DNA structures (Fig. 5)[88]. RNA produced by genelets was used to activate or deactivate double-crossover DNA tiles, forming nanotubes. The DNA tiles were activated by RNA strands designed to complete the tile connector domains or sticky ends (Fig. 5a)[89]. Degradation of the connectors by RNase H causes the nanotubes to disassemble, and by varying the concentration of RNase H, at a fixed concentration of polymerase, it was possible to control the kinetics of the transient assembly (Fig. 5b). A similar strategy makes it possible to control disassembly of DNA tiles using RNA 'invaders' that block their sticky ends[90] (Fig. 5c). A synthetic genelet was designed to transcribe the RNA invader, causing the disassembly of tubular nanostructures[90]. RNase H in the system hydrolyses RNA, thereby reactivating the tile and promoting the reassembly of the DNA structures. Yet, the RNA invader may be produced with any circuit, so the process of assembly and disassembly was made completely autonomous by adopting a synthetic genelet oscillator (section 3). The oscillator was accompanied by an insulator circuit to modularly couple the circuit and assembling tiles. This complex system produced two cycles of nanotube assembly and disassembly, but loss of enzyme activity and side reactions pose major challenges in achieving more sustained operation (Fig. 5d). Recently, a similar strategy based on the use of RNase H was also employed to achieve spontaneous reconfiguration of DNA-based polymers[91]. These examples highlight how the transient assembly of DNA nanostructures can be achieved by using enzymes and external circuits that may impart a variety of dynamic behaviours to the downstream assemblies.

Dissipative control of the assembly/disassembly of DNA nanostructures can also be achieved through non-enzymatic chemical reactions. To do this, Ricci, Prins and co-workers[92] employed the same DNA tiles self-assembling into tubular structures described in the example above[90] and designed DNA activators or inhibitors split into two halves and joined together by a disulfide bond. Such disulfide controllers used in a reducing environment can lead to a transient self-assembly (activator) or disassembly (inhibitor) controlled by the reduction of the disulfide bond that inactivates the controller's function (Fig. 5e,f). Moreover, by varying the concentration of the reducing agent (that is, tris (2-carboxyethyl) phosphine) it is possible to control the associated lifetime of the transient behaviour.

Dissipative DNA polymer systems fuelled by ATP. Many DNA-recognizing enzymes employ ATP as their cofactors. This feature has been used by Walther et al. to achieve dissipative behaviour in different DNA-based systems using ATP as the fuel[93,94]. In one example, short double-stranded (ds) segments bearing single-stranded sticky ends on each side can be ligated by ATP-dependent T4 DNA ligase and make long DNA polymers[95] (Fig. 6a). The ligated segments form a domain recognized by the restriction enzyme BamHI, which induces fragmentation of the polymer. Immediately after the addition of ATP, the DNA polymer grows, because ligation is the predominant reaction. Once the ATP level becomes limiting and the ligase activity declines, the restriction enzyme activity becomes prevalent and the DNA polymers become shorter. As a result, the concurrent action of the two enzymes makes it possible to obtain transient formation of dsDNA polymers. The transient lifetime of the DNA polymer can be controlled by modulating the concentration of ATP. For example, by varying the concentration of ATP from 0.1 to 1.0 mM, the polymer lifetime can be extended from a few minutes to ten days (Fig. 6b). It was also possible to control the speed of the transient formation of polymers by changing the enzyme concentration: by increasing the T4 DNA ligase/BamHI ratio, for example, faster kinetics for the dynamic elongation during the growth phase could be achieved.

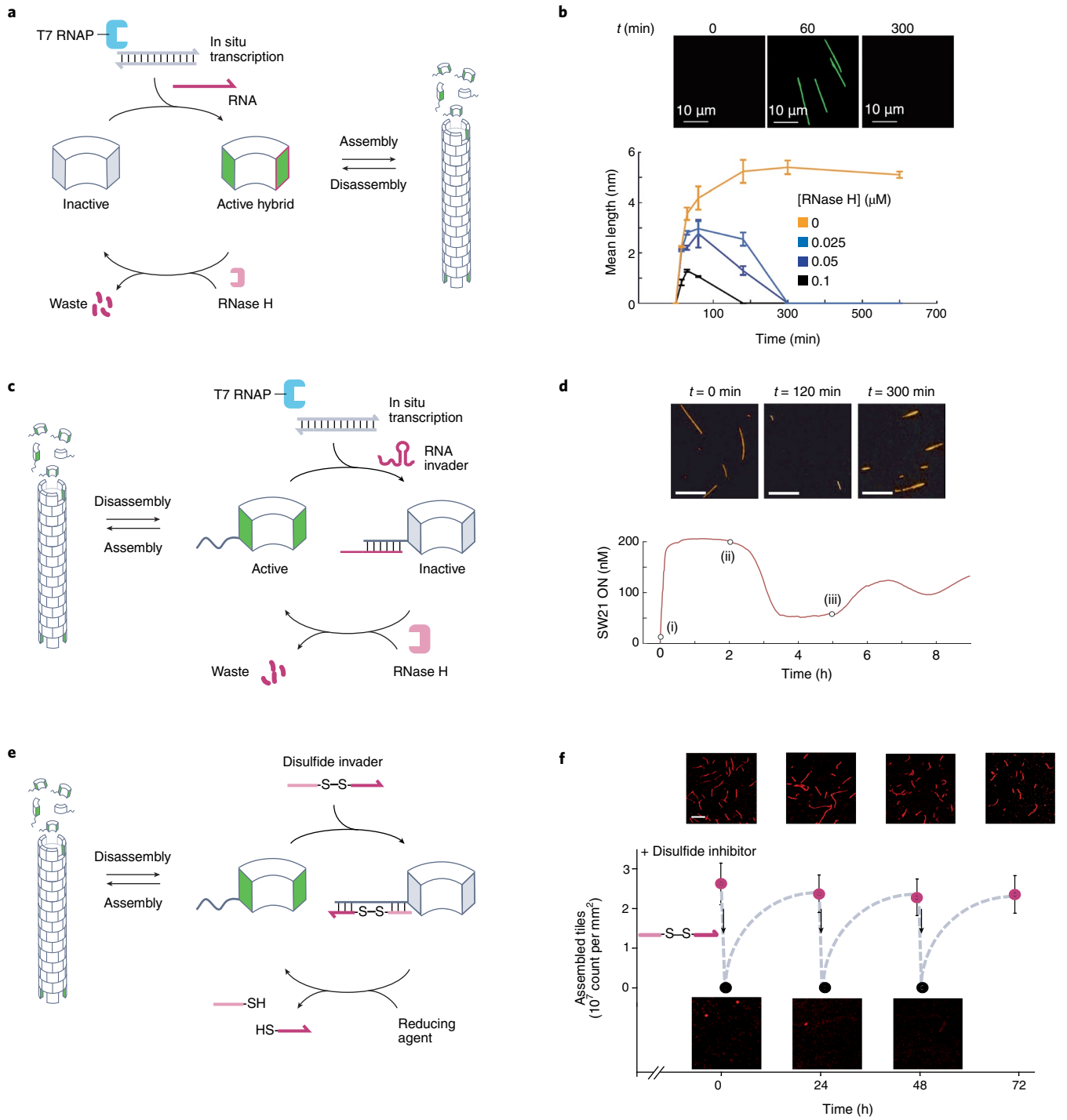


Fig. 5 | Dissipative DNA-based structures. a,**b**, Transcriptional control of the dynamic assembly and disassembly of DNA micrometre-scale structures. **a**, DNA nanotubes assemble from tiles made of short DNA strands[88]. In ref. [89], a hybrid DNA–RNA tile was designed for activation via RNA transcribed by an external genetic circuit, inducing the self-assembly of nanotubes. The RNA strand bound to DNA is hydrolysed by RNase H, causing the nanotubes to disassemble. **b**, Top: microscope images at 0, 60 and 300 min show the transient nanotube growth, which can also be controlled by varying the concentration of RNase H in the presence of a fixed concentration of RNA polymerase. Bottom: kinetic traces showing the transient increase in nanotube length. Different concentrations of RNase H induce different kinetics of the disassembly process. **c**, DNA tiles were engineered by Green et al. to include a binding site (black toehold) allowing for invasion of inter-tile bonds by an RNA or DNA molecule[90]. In the absence of invader, the tiles self-assemble and form nanotubes. In the presence of invader, the tiles dissociate and the tubes disassemble. If the invader is an RNA strand, it is hydrolysed by RNase H, enabling reassembly of the tubes. With this idea, RNA outputs of transcriptional synthetic circuits such as the oscillator described earlier (Fig. 3c) can be used to direct the assembly and disassembly of nanotubes. **d**, Top: microscope images show the transient disassembly of DNA nanotubes. Scale bars, 10 μm. Bottom: fluorescence trace reporting the fraction of active genelet SW21 (see also Fig. 1b) that is coupled to the nanotube assembly. **e**, Dissipative redox control over the disassembly of DNA-based nanostructures achieved by designing a disulfide invader strand (pink) that induces disassembly in the oxidized form but spontaneously dehybridizes from the tiles, allowing their consequent reassembly, upon reduction. **f**, Microscope images and number of assembled tiles (count per mm^2) measured immediately after the addition of the disulfide invader (black dots) and after 24 h (pink dots). Scale bars, 5 μm. The reversibility is demonstrated by cyclic additions of the disulfide invader[92]. Credit: adapted with permission from ref. [89], ACS (**b**); ref. [90], Springer Nature Ltd (**d**); ref. [92], Wiley (**e**,**f**).

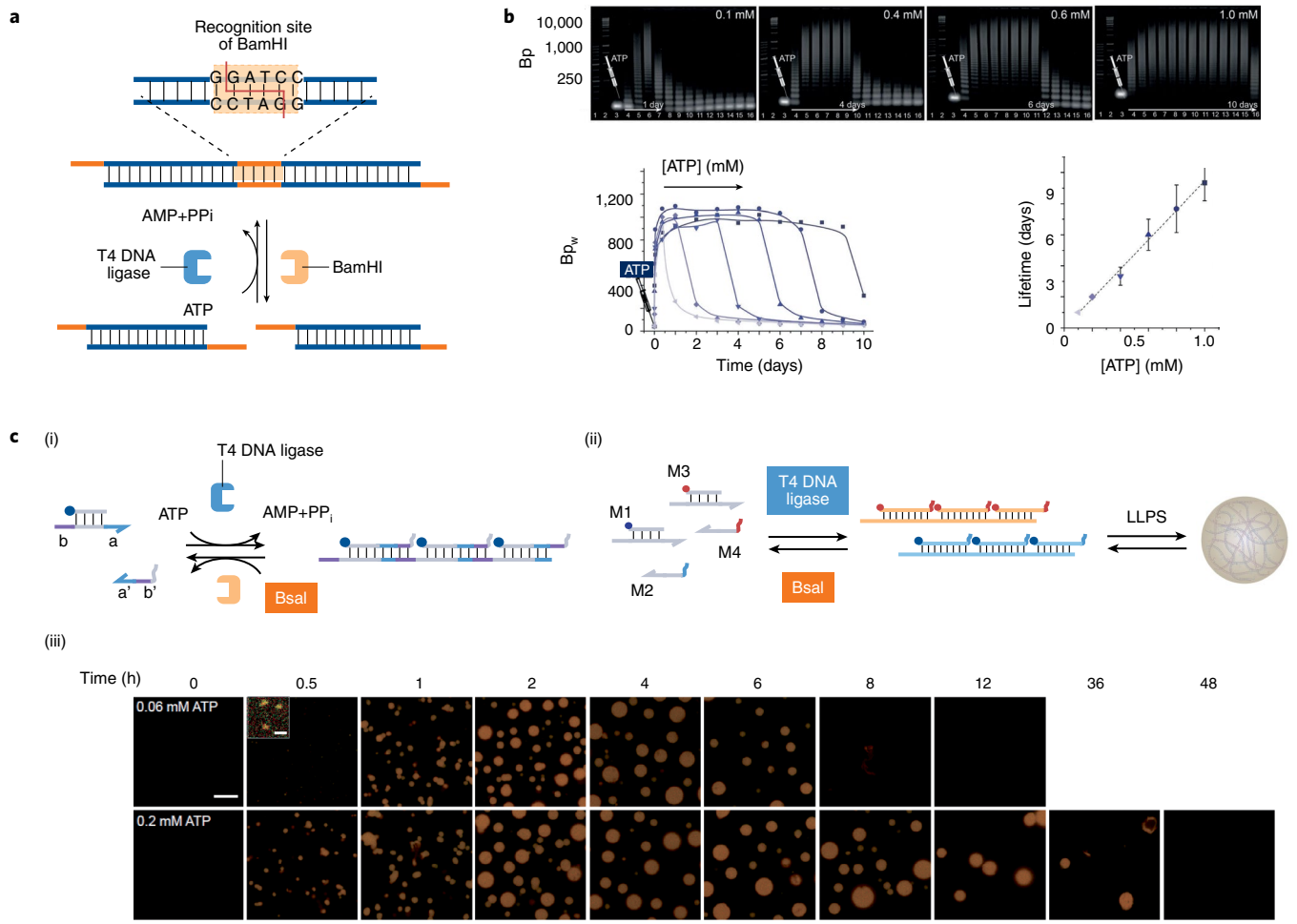


Fig. 6 | ATP-fuelled activation and dynamization of a covalent and transient dsDNA-based chain growth. a, The system is based on a rigid duplex with ssDNA portions at both sides allowing hybridization and dsDNA chain growth. The transient polymerization and the consequent DNA-based chain growth is triggered with an enzymatic reaction network composed of two enzymes (T4 DNA ligase and BamHI) able to work simultaneously. **b**, Gel electrophoresis shows modulation of the transient lifetime of the chain growth obtained by varying the concentration of ATP (0.1, 0.4, 0.6 and 1 mM), which controls the activity of T4 DNA ligase. Kinetic traces obtained by gel electrophoresis experiments show how the transient lifetime of chain length (bp_w) changes with different concentrations of ATP. The lifetime of the dsDNA chain shows a linear correlation with ATP concentration. **c**, (i) Schematic representation of ATP-driven non-equilibrium reaction networks of concurrent ligase action (using T4 DNA ligase) and endonuclease-restriction action (using BsaI) employed to build sequence-defined functionalized DNA polymers. (ii) Illustration of ATP-driven generation of DNA coacervates with programmable lifetime, which form through liquid–liquid phase separation (LLPS) of transient orthogonal sequence-defined functionalized DNA polymers. (iii) Confocal laser scanning microscopy images at two different concentrations of ATP (0.06 and 0.2 mM). The DNA coacervates show a longer transient lifetime at higher concentration of ATP (0.2 mM). Scale bar, 20 μm; scale bar for inset, 2 μm. Credit: adapted with permission from ref. [95], AAAS (**a**,**b**); ref. [97], Elsevier (**c**).

By using a photolysable caged ATP derivative, Walther et al. demonstrated that fuel availability can be regulated through light, making it possible to control transient polymerization by changing the light intensity and illumination time[96]. Similar ATP-driven non-equilibrium reaction networks were also employed by Walther et al. to build self-resetting strand-displacement systems[48], DNA coacervates with programmable lifetime[97] (Fig. 6c), DNA oligomers with transient catalytic functions[98] and autonomous DNA-based nanostructures[99]. A higher level of complexity was also recently demonstrated by Walther's research group when designing dissipative hierarchical multicomponent systems[100].

Perspective

The implementation of dissipative processes in DNA-based systems is rapidly gaining traction[101–103,116] and the examples highlighted in this Review illustrate the potential for introducing new modes of operation that are a step closer to those exploited by nature. When compared to other biomaterials and abiotic materials[104–106], nucleic acids present unique advantages for designing dissipative systems. First, the high programmability and predictability of DNA/RNA interactions provides a very precise control over the ground states in the free energy landscape as well as control over reaction kinetics (for example, the toehold-exchange reaction). Second, the availability of a wide family of enzymes that can cleave DNA or RNA sequences with very high selectivity provides exquisite control over the energy-dissipating process, which is hard to achieve in abiotic systems. Third, DNA-based dissipative systems are generally more tolerant to waste products when compared to most synthetic dissipative systems, because of the multivalent nature of DNA hybridization. Fourth, polymerization and transcription are well-understood reactions that can be easily harnessed in vitro to control the production of RNA or DNA strands serving as fuel molecules. Fifth, all the components of these systems are biocompatible, making them relevant to in vivo applications. These advantages indicate that

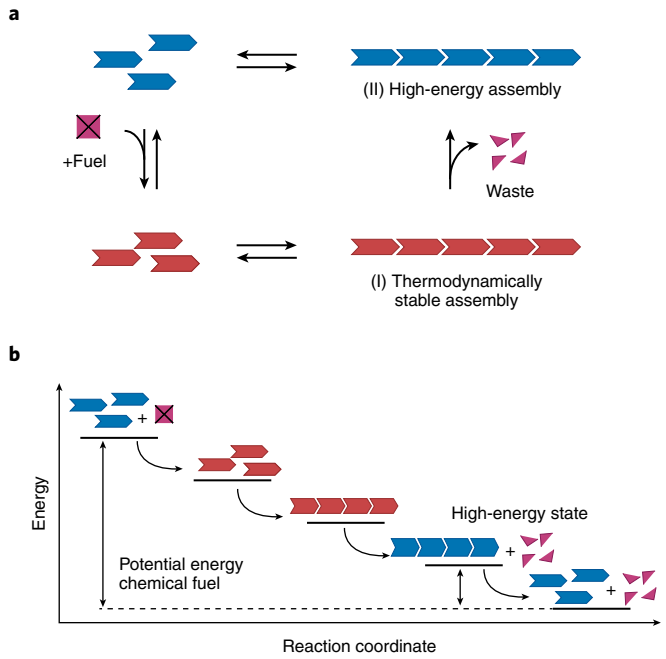


Fig. 7 | A route toward the design of active DNA materials based on the operation of microtubules. a, System components (blue tiles) need interaction with a fuel molecule to start the assembly of a thermodynamically stable structure (I). Fuel-to-waste conversion of the fuel thus turns the stable assembly into a high-energy material (II). **b**, Energy transfer from fuel to material, illustrated by the energy-consumption pathway.

DNA-based systems are poised to play a dominant role in the field of non-equilibrium chemistry. In this section we outline additional practical and conceptual steps that are required to make progress towards building synthetic life-like systems.

Open systems. The vast majority of examples of dissipative DNA nanotechnology rely on the batch-wise addition of fuel to trigger activation in a closed system. To match nature's capacity to sustain processes for long periods of time, synthetic DNA dissipative systems need to be engineered to manage fuel intake and waste elimination. This could be addressed, for example, through microfluidic methods generating artificial vasculature[39,107] or via compartmentalization in vesicles or droplets with selective nanopores, or in semi-permeable hydrogels[108,109]. Alternatively, new ways to control non-equilibrium DNA systems with abiotic fuels (similar to the disulfide/thiol reactions or pH oscillators described in this Review) could further improve the tolerance against waste products and avoid loss of activity as a result of enzyme degradation. These abiotic reactions may also provide better control over the kinetics and increase the stability of the system under non-physiological conditions.

Non-equilibrium operation of molecular devices. The most intriguing feature of life is its non-equilibrium nature. An entropically disfavoured high level of organization is maintained at the expense of energy. Examples of non-equilibrium operations are the directional motion of motor proteins and the uphill transport of species against a concentration gradient. A fundamental step towards life-like DNA systems will rely on the ability to install non-equilibrium conditions. In the majority of examples discussed in this Review, non-equilibrium conditions are installed in a trivial way: the addition of a batch of fuel transiently alters the energy landscape of the system's components, which is maintained until all fuel is converted to waste. Although the future development of open systems will allow the fuel-induced energy landscape to be maintained indefinitely, it is unlikely that this will bring us conceptually closer to the desired 'life-like' properties. Most reported dissipative DNA systems rely on transient shuttling between two states (open–closed; unassembled–assembled), which by themselves are thermodynamically stable states that can also exist in the absence of energy dissipation. This implies that, at constant fuel concentration, such a system will reside in a thermodynamically stable state; fuel consumption brings no additional benefit apart from the possibility to turn off the system—and its functional properties—by stopping the injection of fuel.

Recent analyses of the physical–chemical origins of the functioning of molecular motors and pumps have revealed that the persistence of a non-equilibrium steady state at constant fuel concentrations requires the presence of kinetic asymmetry in the energy consumption pathways[25,110–112]. This implies the presence in the system of a kinetically preferred pathway along which the chemical energy of the fuel is consumed. For example, kinesin moves preferentially towards the (+)-end of microtubules because fuel conversion (ATP into adenosine diphosphate) is more efficient—that is, it occurs at a higher rate—when the motor protein moves in that direction.

Development of non-equilibrium DNA-based devices will require a higher level of design. It is not sufficient to just implement an energy-dissipating step in the system; attention should rather be focused on designing the kinetic pathway along which energy is consumed. A successful implementation will give access to molecular DNA-based devices, such as walkers and motors, able to perform their non-equilibrium operation autonomously under stationary conditions.

Active materials. The transition towards 'active' matter, defined as material that can perform work, requires the design of systems that consume energy to create a spatial displacement of target components. This means that the material should store at least part of the energy being consumed to reach an energetically activated state. Microtubules are the archetypal biological example of a chemically fuelled active material[113]. Microtubules are composed of tubulin dimers, which require complexation of guanosine-5'-triphosphate (GTP) to be activated for self-assembly. In the assembled state, tubulin·GTP becomes catalytically active and converts GTP to guanosine-5'-diphosphate (GDP). This conversion is crucial, because the presence of tubulin·GDP dimers in the assembled state is energetically disfavoured and causes a build-up of strain in the polymer lattice. Structural integrity is maintained if a GTP cap is present, and the microtubules rapidly collapse if the GTP cap is lost. This sequence of events illustrates that the fuel-to-waste conversion is directly coupled to a self-assembly and disassembly process, and part of the chemical energy is stored in the polymer. Storage of energy in the structure enables active cycles of growth and shrinkage, which allow microtubules to carry out mechanical work by generating pushing and pulling forces and the fast search and capture of kinetochores during cell division[24].

The lesson that we can take from the microtubule example is that the design of active DNA-based materials that can perform work should include an energy-consumption pathway associated with the production of matter residing at a high-energy state (Fig. 7a)[27,28,112]. Essential in such a design is the use of system components that are not prone to structure formation and need interaction with a fuel, for example RNA, to be activated for (thermodynamically controlled) self-assembly. Fuel-to-waste conversion by the thermodynamically stable assembly (Fig. 7a, I) then leads to the population of a high-energy material (II), which is composed of native building blocks trapped in an energetically disfavoured conformation until a stimulus is received that will allow the material to relax back to

the stable state (I). The energy transfer from fuel to material can be illustrated by following the overall energy consumption pathway (Fig. 7b). Starting from the high energy level of building blocks and fuel, a cascade of energetically downhill steps leads to the formation of a high-energy material. The material finally releases the stored energy by relaxing to the unassembled state. If its release is regulated by chemical or physical triggers (GTP concentration in the case of microtubules), the energy may be exploited for a functional purpose such as generating rapid push or pull forces.

Complexity. In dynamic DNA nanotechnology, the use of fuel gates (DNA constructs that process fuel strands to release secondary fuel strands as output for further downstream processing) has permitted the design of DNA circuitry including hundreds of strands to perform sophisticated operations. In comparison, the level of complexity in dissipative DNA nanotechnology is far lower. The vast majority of systems exploit chemical fuels to transiently shift the system between two thermodynamically stable states. Small circuits are now emerging in which the addition of fuel leads to the release of DNA strands from fuel gates for subsequent activation of secondary events[114,115]. Yet, these circuits are operated through a single dissipative process and the cascades rely on the release of stable secondary DNA strands for downstream processing, which are not consumed by a dissipative process. An essential step forward towards systems that can rival the complexity of thermodynamically controlled DNA circuits, while maintaining the advances of system reactivation by refurnishing fuel, requires the exploitation of fuel gates that contain dormant fuels, which, upon arrival of a trigger, are released for downstream processing and, at the same time, activated for conversion into waste.

Conclusions

DNA nanotechnology offers a repertoire of tools to create synthetic devices and materials with the complexity and sophistication of living matter. Research in this direction is rapidly expanding, by taking advantage of the programmability of nucleic-acid interactions, of the ease of conjugation of different responsive molecules on nucleic-acid scaffolds, and of the wide choice of sequence-specific DNA-binding enzymes that can operate in vitro. Within the framework of dynamic DNA nanotechnology, many systems take advantage of synthetic DNA to build large reaction networks relying on the design of thermodynamic states and of the kinetic pathways leading to them. Although these systems may display a very complex dynamic behaviour, the overall temporal evolution of the system is determined by an irreversible progress towards a final thermodynamically stable state. This marks a stark contrast with nature, in which complex machines, structures and reaction networks are maintained in a non-equilibrium state at the expense of the consumption of chemical energy. In this Review we have discussed dissipative DNA nanotechnology as an approach to develop artificial nucleic-acid-based systems that require chemical fuel for activation.

Dissipative DNA nanotechnology marks a transition from functional systems governed by thermodynamics towards systems that rely on the availability of energy for functional activation. This allows the development of life-like matter that is activated only when energy is spatiotemporally provided. On the horizon, we envision the development of 'intelligent' matter that can autonomously move, respond and act in response to stimuli provided by the environment. Systems will emerge for which the functional efficiency is no longer determined by thermodynamic or kinetic stability, but by the efficacy with which such systems are able to exploit available energy. We can also see the emergence of complex DNA-based circuitry with the potential to compute a large variety of complex operations, the activation of which depends on the types of fuel that have been added.

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Competing interests

The authors declare no competing interests.

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