Chem Soc Rev

Cite this: *Chem. Soc. Rev.*, 2011, **40**, 3656–3676

www.rsc.org/csr

CRITICAL REVIEW

Walking molecules

Max von Delius and David A. Leigh*

Received 7th January 2011, Accepted 17th February 2011
DOI: 10.1039/c1cs15005g

Movement is intrinsic to life. Biologists have established that most forms of directed nanoscopic, microscopic and, ultimately, macroscopic movements are powered by molecular motors from the dynein, myosin and kinesin superfamilies. These motor proteins literally walk, step by step, along polymeric filaments, carrying out essential tasks such as organelle transport. In the last few years biological molecular walkers have inspired the development of artificial systems that mimic aspects of their dynamics. Several DNA-based molecular walkers have been synthesised and shown to walk directionally along a track upon sequential addition of appropriate chemical fuels. In other studies, autonomous operation—*i.e.* DNA-walker migration that continues as long as a complex DNA fuel is present—has been demonstrated and sophisticated tasks performed, such as moving gold nanoparticles from place-to-place and assistance in sequential chemical synthesis. Small-molecule systems, an order of magnitude smaller in each dimension and 1000× smaller in molecular weight than biological motor proteins or the walker systems constructed from DNA, have also been designed and operated such that molecular fragments can be progressively transported directionally along short molecular tracks. The small-molecule systems can be powered by light or chemical fuels. In this *critical review* the biological motor proteins from the kinesin, myosin and dynein families are analysed as systems from which the designers of synthetic systems can learn, ratchet concepts for transporting Brownian substrates are discussed as the mechanisms by which molecular motors need to operate, and the progress made with synthetic DNA and small-molecule walker systems reviewed (142 references).

School of Chemistry, University of Edinburgh, The King's Buildings, West Mains Road, Edinburgh EH9 3JJ, UK.
E-mail: David.Leigh@ed.ac.uk; Fax: +44 131 650 6453;
Tel: +44 131 650 4721

Max von Delius

Max von Delius studied chemistry at Friedrich-Alexander Universität Erlangen-Nuremberg and at the Université Louis Pasteur Strasbourg, where he spent a research semester in the lab of Prof. J.-M. Lehn. He was awarded the Helmut-Zerweck Prize for his Master thesis (with Prof. A. Hirsch). From 2007–2010 he carried out his PhD at the University of Edinburgh under the super vision of Prof. D. A. Leigh. His PhD Thesis focussed on the design, synthesis and operation of the first bipedal small-molecule motors.

David A. Leigh

David Leigh is the Forbes Professor of Organic Chemistry at the University of Edinburgh. His research interests include the development of new methods for interlocked molecule synthesis and new paradigms and mechanisms for synthetic molecular-level motors and machines. He was elected to the Fellowship of the Royal Society in 2009.

1. Introduction

Nature has evolved a vast range of protein-based molecular motors,†[1] including ion pumps,[2] translocation pores,[3] DNA helicases,[4] DNA and RNA polymerases,[5,6] the rotating motor F_1 ATPase[7] and the flagellar motor.[8] A plethora of molecular walkers from the dynein, myosin and kinesin families travel either short or long distances along the cell's 'motorway network', performing tasks such as organelle transport, muscle contraction, mitosis and sensory transduction.[9–12]

Although their molecular architectures and cellular roles can differ significantly, all naturally occurring motor proteins share several important characteristics. First, they convert an energy input (supplied by either nucleotide hydrolysis or ionic gradients) into mechanical and/or chemical work. Second, their molecular design restricts the degrees of freedom of the motor and/or the substrate. For example, DNA and RNA polymerases 'pull' the blueprint nucleic acid strand in only one direction through a narrow clamp and molecular walkers migrate along rigid, essentially one-dimensional, tracks. Third, all molecular motor proteins operate under conditions of low Reynold's number where inertia and momentum are irrelevant and viscous forces and random thermal motion dominate dynamics.[13–15] In other words they must "*swim in molasses and walk in a hurricane*".[13] Brownian motion is not necessarily a problem, however, but a randomizing element that is utilized in the ratchet mechanisms (see Section 3.2) that allow the motors to move directionally or to drive chemical systems away from equilibrium.[14,15]

This critical review aims to provide an overview of one particular class of molecular motors: molecules that can walk along tracks. The naturally occurring molecular walkers from the dynein, myosin and kinesin families are discussed from the perspective of what can be achieved with molecular systems. General principles for the design of synthetic molecular walking systems are outlined and the progress made to date with synthetic molecular walkers based on DNA or small-molecule building blocks reviewed.

What defines a 'molecular walker'? In addition to the obvious structural characteristic of multiple contact points ('feet') that can associate with distinct binding sites on a track, there are fundamental characteristics of dynamic behaviour with respect to its interaction with the substrate:

(i) *Processivity*: the ability of a molecular walker to remain attached to its track during its operation, *i.e.* to migrate along a molecular scaffold for more than a single motor cycle. Mechanistically this is not trivial to achieve since molecular walkers, unlike their macroscopic counterparts, cannot use gravity to remain attached to their track. Not all of the naturally occurring molecular motors operate in a processive manner, but non-processive motors can only perform work in large ensembles (so that multiple motors are attached to the track at any one time).

† In the scientific literature the terms 'molecular motor' and 'molecular machine' are not used in a consistent manner. Here, the term molecular motor is used for any system that is able to progressively (*i.e.* without undoing that work upon resetting) convert energy derived from a chemical fuel or proton gradient into mechanical work (see ref. 15).

(ii) *Directionality*: migration of the molecular walker preferentially or exclusively towards one end of a molecular track.

(iii) *Repetitive operation*: the motor's ability to repeatedly perform similar mechanical cycles.

(iv) *Progressive operation*: the capability of the molecular motor to be reset at the end of each mechanical cycle without undoing the physical task that was originally performed.

(v) *Autonomous operation*: the ability of the molecular motor to continually function as long as an energy input is present, *i.e.* no external intervention such as the application of a sequence of stimuli is required. All biological walkers operate autonomously, using ATP as their fuel.

In this review article, any molecular moiety that is associated to a track through one or more distinct points of contact and exhibits characteristics i–iv is considered a molecular walker. Autonomous operation (v) can be a desirable additional characteristic (although it can come at the cost of reduced operational control) but is not essential for a functional molecular walker system.

1.1. Molecular shuttles: useful properties and limitations

Mechanically interlocked molecules, such as rotaxanes and catenanes (Fig. 1a), offer some useful structural features that

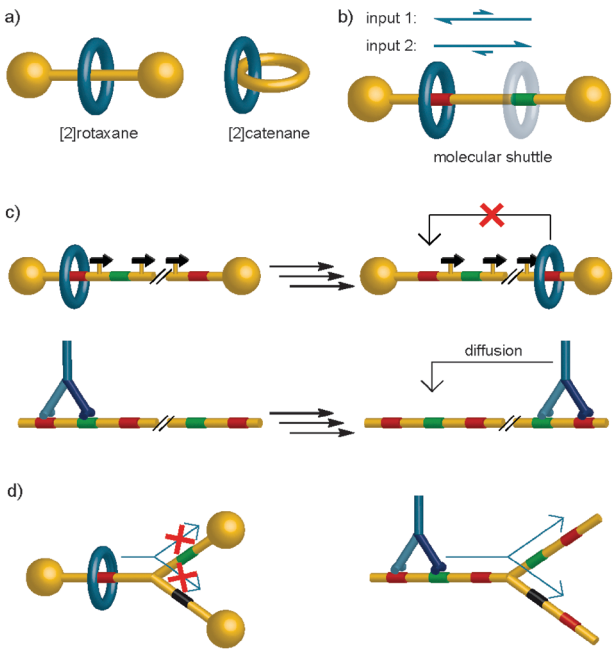


Fig. 1 (a) Schematic illustration of two types of mechanically interlocked molecules: a [2]rotaxane and a [2]catenane. (b) A molecular shuttle: a [2]rotaxane featuring two or more binding sites for the ring on the thread. In some systems the equilibrium distribution can be switched through application of appropriate chemical or physical stimuli which alter the chemical structure of the ring or thread. (c) Directional ring movement in a compartmentalised molecular shuttle (top) and the problem to 'reset' the location of the macrocycle without it following a reciprocal path, whereas a molecular walker (bottom) can be reset by detachment and subsequent reattachment at the original starting position. (d) A molecular walker (right hand side) can, in principle, choose between alternative pathways, perhaps under the influence of a Boolean logic gate.[15] The ring in a molecular shuttle (left hand side) cannot pass a junction.

can be exploited in the design of synthetic molecular motors.[15] The interlocked nature of the components provides inherent processivity (the individual components cannot exchange with the bulk) and at the same time restricts the degrees of freedom of their relative motion. Catenanes have been successfully used to create synthetic rotary motors in which small rings can be induced to rotate directionally[16] (and reversibly)[17] around a larger ring.

The macrocyclic components of molecular shuttles (Fig. 1b) can be considered as molecular units that move processively along tracks. Rotaxane switches—systems that change between different positional states of the rotaxane—are often described as 'motor-molecules'. However, unlike a motor, each time the position of a switch is changed it undoes any work done during the previous change of position of the switch.[15] Unfortunately, calling simple switches 'motors' has probably held up the development of genuine molecular motor systems, at least in the rotaxane field. Few rotaxanes have been reported[18–20] in which the location of the macrocycle can be manipulated away from its equilibrium distribution. Such dynamics requires compartmentalisation of the system and a Brownian ratchet mechanism[15–20] which can be achieved by introducing at least one addressable blocking group on the thread (Fig. 1c). In a molecular walker such a manipulation of the track is unnecessary, since a walker possesses more than one point of contact with the substrate, a feature that intrinsically compartmentalises the system[15,18] (see Section 3.2 for a discussion of the Brownian ratchet behaviour of molecular bipeds).

The processivity inherent to the threaded architecture of molecular shuttles (a covalent bond has to be broken for the ring to dissociate from the track) makes it difficult for such systems to operate progressively. Even if a macrocycle can be transported directionally (*i.e.* away from equilibrium) along a thread featuring several potential binding sites, the shuttle cannot be reset to its original location without undoing the original task the device had performed (*e.g.* cargo transport) (Fig. 1c). The finite processivity of a molecular walker is of advantage here since it can return to its original position by the nonreciprocal pathway of detachment followed by reattachment. A further limitation of an interlocked architecture is that a threaded ring cannot select between two or more alternative pathways, a limitation that does not apply to walker–track systems (Fig. 1d).

Overall, although catenanes and rotaxanes have been shown to have utility as switches and as rotary motors,[21] for linear molecular motors there can be advantages in employing architectures and mechanisms more similar to those exploited in biology.

2. Biological protein walkers

2.1 Nature's pack horses: myosins, dyneins and kinesins

Motor proteins from the myosin,[22,23] dynein[24,25] and kinesin[26–28] superfamilies move along cytoskeletal polymers to transport various cellular cargos, including membranous organelles, protein complexes and mRNAs. The cytoskeleton is formed from three types of polymers: intermediate filaments, microtubules and actin microfilaments. The last two, amongst other functions, act as tracks for various types of motor proteins. Microtubules[29,30] are linear rigid structures, consisting of 13 linear polymeric protofilaments, which when wrapped together create a cylindrical tube. Each protofilament is formed by polymerisation of α and β tubulin units, a process that is reversible and highly dynamic. Actin filaments are formed by dynamic polymerisation of actin units, which leads to a rigid helical scaffold. Transport along microtubule filaments is mediated by kinesins (mostly moving towards the 'plus' ends, *i.e.* the cell periphery) and dyneins (which move towards the 'minus' ends, *i.e.* the cell nucleus), whereas myosins transport cargo along actin filaments.[31]

Myosin, dynein and kinesin motors are ATPases, *i.e.* they convert chemical energy derived from ATP hydrolysis into mechanical work. In light of their diverse cellular roles, it is not surprising that defects in motor-dependent transport are associated with a large range of diseases, including neurodegeneration, tumorigenesis and developmental defects.[11] In mammals, 40 distinguishable kinesins, 40 myosins and more than 12 dyneins can be found.[12]

To date the investigation of the molecular mechanisms behind the complex operation of cytoskeletal protein motors has sought to answer two fundamental questions:

(i) How do (almost all) cytoskeletal protein motors achieve near-perfect directionality?

(ii) How do (most) cytoskeletal protein motors achieve high processivity?

Not all members of the myosin, kinesin or dynein families exhibit the same motor characteristics. Conventional myosin (myosin-II), for example, is a directional but non-processive motor. The motor mechanism features attachment to the actin track, exertion of a force of several pN on the filament,[11] and rapid detachment from the track after only one cycle of ATP hydrolysis. Thus, to perform a useful task (in this case: force generation for muscle contraction), it must work in large groups, constituting the so-called 'sarcomeric ensemble'.[11] If myosin remained attached to its track for more than one cycle the whole process would be slowed. Thus it is the *lack* of processivity in this case that makes the motor protein effective at performing its task.

Myosin-V (Fig. 2a), on the other hand, is a processive member of the myosin family which remains attached to its actin track over a large number of consecutive steps.[32] This behaviour reflects the cellular function of the motor, as myosin-V is responsible for long-range organelle transport towards the minus-end of the actin filaments. The main mechanistic difference between these two myosins is that in myosin-V both motor heads (or 'feet') are involved in track binding, while in myosin-II only one head binds to the track.

The presence of two active feet is, however, neither a necessary nor a sufficient requirement for processivity. KIF1A[33] is a monomeric (single-footed) kinesin motor protein that walks processively along microtubules. KIF1A maintains processivity through secondary interactions between its only foot and the track and its translocation resembles a sliding movement rather than walking. Myosin-II, despite having two feet, operates in a non-processive manner. Many other dimeric motor proteins, such as kinesin-I (Fig. 2c) and myosin-V (Fig. 2a), achieve high processivity by coordinating the actions

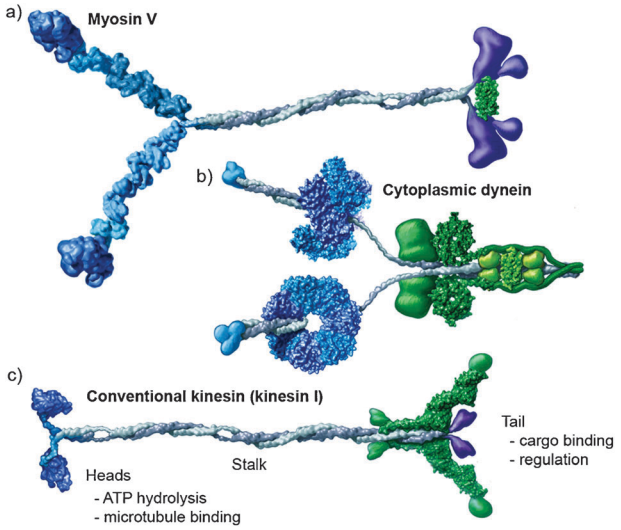


Fig. 2 Examples of processive motor proteins from the three major superfamilies: (a) Myosin-V. (b) Cytoplasmic dynein. (c) Kinesin-1. Representations based on atomic resolution structures.[10] Blue: motor domains; light blue: mechanical amplifiers; purple: cargo attachment site; green: associated motor units. Adapted by permission from Elsevier: *Cell* (ref. 10), copyright (2003).

of their two feet, so that at any time during the catalytic cycle at least one of the two feet is bound strongly to the track. This is particularly remarkable in the case of kinesin-I, which achieves high processivity despite having two chemically identical feet (see Section 2.2).

The mechanism that confers directional bias to the walker locomotion is similar in all myosins (Fig. 2a), dyneins (Fig. 2b) and kinesins (Fig. 2c). In each case, a small conformational change that occurs at the catalytic motor domain during a specific stage of the ATP hydrolysis cycle is transduced into a large-amplitude motion in the forward direction. In myosins (Fig. 2a) the release of inorganic phosphate (P_i) triggers a conformational change in the motor domain that is conveyed to a long and rigid lever arm (light blue region in Fig. 2a). As a result, this lever arm is immediately propelled 25 nm towards the next forward binding site. Thermal diffusion of the detached foot is believed to play only a minor role in this 'power-stroke' mechanism.[12] The mechanism found in kinesins is very similar and the motor domains in kinesins and myosins have been shown to be virtually identical,[34] with only two notable differences. (i) For kinesins the crucial conformational change occurs during ATP binding, not phosphate release; and (ii) in kinesin the element that acts as a mechanical amplifier is flexible (light blue region in Fig. 2a), which indicates that a power-stroke mechanism can only operate to a limited degree.

Kinesin-I is perhaps the prototypical processive motor protein, and the basic characteristics of its dynamics is the model for most artificial molecular walkers described to date. Accordingly, we shall discuss the molecular basis for its walking mechanism in more detail.

2.2 Kinesin-I

Kinesin-I (also referred to as 'conventional kinesin' or just 'kinesin') was first isolated in 1985 by Vale and co-workers[35] and can be extracted in relatively large quantities from the brain.[26] For this practical reason kinesin-I is by far the most studied member of the kinesin superfamily.[26–28,36–56] Kinesin-I is a homodimeric protein consisting of two chains of 120–130 kDa each. The dimer comprises three distinct regions (see Fig. 2c): (i) a tail that binds cargo and most likely plays a part in the regulation of the motor activity;[57,58] (ii) an intertwined stalk, responsible for the dimeric nature of kinesin-I but which may also play a subtle role in the motor mechanism; and (iii) two identical heads (350 amino acids each) which are responsible for ATP hydrolysis and binding to the microtubular track. From a mechanistic point of view, the ATP hydrolysis domain and the neck and hinge region (light blue domain in Fig. 2c) are particularly important.

Soon after its discovery it was established that kinesin-I migrates along microtubular tracks with high processivity. An average run length of $\sim$1 mm, which corresponds to $\sim$100 steps, has been determined using optical tweezers.[36,37,59–64] Similar experiments have shown that the length of individual steps is 8 nm,[43] which corresponds exactly to the smallest distance between two binding sites (α/β-tubuline dimers) on the polymeric track. The mechanism of kinesin-I's gait has, however, remained controversial until recently, due to initially conflicting experimental results.

Theoretically, a molecular biped with two identical feet can walk along a track in three different ways: (i) symmetric hand-over-hand (where the two feet exchange leading and trailing positions, but alternate steps are identical); (ii) asymmetric hand-over-hand (where the two feet exchange leading and trailing positions, but alternate steps differ mechanistically); and (iii) inchworm (where one head is always leading).

Kinesin-I does not rotate its stalk while walking along the microtubule, a discovery that was interpreted as possibly indicative of an inchworm mechanism.[43] Fluorophore foot-labelling experiments, however, demonstrated that each motor domain moves in steps of 16 nm,[51] an observation that strongly points towards a hand-over-hand mechanism (kinesin-I's centroid would still move 8 nm per step, as earlier experiments had shown). The asymmetric hand-over-hand mechanism supports both observations and, indeed, evidence has been found for a 'limping' that occurs during every second step.[50]

Two key questions remain, however, that biophysicists are currently trying to answer: What is the molecular basis for kinesin-I's behaviour and in particular, how does kinesin-I, with two identical feet, achieve processivity and directionality?

While many details are still under debate, Block has recently proposed a "consensus model"[27] for the kinesin-I walking mechanism, illustrated in Fig. 3:

Resting state (Fig. 3, I and II). For the situation where one motor domain is bound to ADP and the other motor domain is unbound, an equilibrium between two conformers has been found (I and II in Fig. 3). In both conformers, the foot including the vacant motor domain (yellow in Fig. 3, left hand side) binds strongly with the track. In conformer I, the ADP bound foot interacts with the track, which results in a certain amount of ring strain in the neck-linker region. In conformer II, which represents the only unstrained intermediate in the catalytic cycle, the ADP bound foot is detached from the

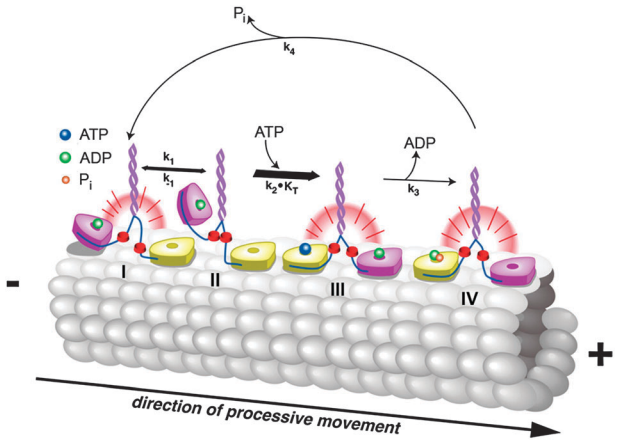


Fig. 3 The mechanochemical cycle of kinesin-I. Adapted by permission from the National Academy of Sciences of the United States of America: *Proc. Natl. Acad. USA* (ref. 56), copyright (2010).

microtubule. Toprak *et al.*[56] have determined an equilibrium constant between I and II of $\sim$1.4 (k_1/k_{-1}).

ATP binding (Fig. 3, II $\rightarrow$ III). There is evidence to suggest that ATP binding in the leading foot is only possible in the absence of strain (only in conformer II in Fig. 3). This strain gate coordinates the actions of the two feet, which is key to kinesin-I's high processivity and directionality.

Neck-linker docking (Fig. 3, II $\rightarrow$ III). The binding of ATP releases significant energy, which drives a conformational change that results in docking of the neck linker to the motor domain. This leads to a small ($\sim$1–2 nm) movement of the rear foot towards the plus-end of the microtubule (power-stroke).

Diffusional search (Fig. 3, II $\rightarrow$ III). The unbound foot can now undertake a diffusional search for the next forward binding site, in order to complete the $\sim$16 nm step. At this stage, there is a finite probability of the foot attaching to the rear binding site again, but the neck-linker docking[45] renders the forward stepping energetically more favourable. Such a mechanism, which makes use of a combination of an asymmetric energy potential (here: through neck-linker docking) and random thermal motion (here: foot diffusion) is a Brownian ratchet mechanism.[15] The feet have now swapped the relative position and the centroid of kinesin-I has moved by 8.3 nm. The transition from state II to state III (Fig. 3) proceeds much faster than all the other steps, which is an important factor for the high processivity of kinesin-I.

ADP release (Fig. 3, III $\rightarrow$ IV). After the purple foot has reached the forward binding site (state III in Fig. 3), ADP is released, which leads to tight binding to the microtubule and the generation of ring strain (communicated through the neck linker or the microtubule). The strain suppresses premature binding of ATP to the leading foot before the rear foot can hydrolyse its bound ATP.

P_i release (Fig. 3, IV $\rightarrow$ I). The release of inorganic phosphate completes the catalytic cycle.

Kinesin-I's high processivity is a result of the strain-related gating mechanism that keeps the biochemical cycles of its two feet strictly out of phase. The directional bias stems mainly from a Brownian ratchet mechanism that is powered by the ATP-induced docking of the neck-linker to the leading head. Since both feet are identical, it is the interaction between the feet and the track that is crucial for both the achievement of processivity (the important strain-gate is only possible since both feet do not fit perfectly on two neighbouring α/β-tubulin dimers) and directionality (neck-linker docking creates a bias for a step towards the plus end of the polymer).

The complex motor proteins presented above have emerged over the course of hundreds of millions of years through a process of random molecular mutations and natural selection. This continuous optimisation process has enabled motors such as kinesin and myosin to operate with efficiencies beyond 50%.[65,66] In the last decade, a large number of hybrid biological-artificial devices have been created with the aim of exploiting biological walkers in a technological context (*e.g.* for the transport and release of artificial cargo). The progress made with such hybrid systems was recently reviewed elsewhere.[67,68]

3. Design principles for synthetic molecular walkers

Experimental scientists intending to create entirely synthetic molecular walkers must design their systems with consideration to the motor characteristics of processivity and directionality (see Introduction for definitions).

3.1. Processivity—interaction between feet, fuel and track

Nature has evolved both processive and non-processive translational motor proteins. The latter can, however, only perform useful tasks when operating in large ensembles.

For a molecular walker to be processive, complete detachment of the walker moiety is prevented by having at least one foot connected to the molecular track at all times. This can be realised by having two chemically different feet and two mutually-exclusive conditions, or fuels, for detachment of each foot (Fig. 4a). Alternatively, the two feet could be chemically equivalent (as in kinesin-I), provided the interaction of the feet with an unsymmetric track renders the two feet chemically inequivalent (*e.g.* if the back foot reacts with a fuel at a different rate to the front foot for steric reasons, Fig. 4b). Another requirement for processivity is that a walker should not form a 'bridge' between two tracks (and then eventually swap tracks). In biological systems this is guaranteed by the polymeric nature of microtubule or actin filaments. In artificial non-polymeric systems this can be realised by operating under very dilute conditions or perhaps by tethering the track or the walker to a surface.

For molecular walkers with only one foot (*e.g.* KIF1A,[33] see Section 2.1), secondary interactions between the foot and the track can confer processivity (Fig. 4c). Finally, although biology only employs molecular walkers with two or one legs, processivity could also be realised in molecules with more than two points of contact with the track. A 'spider-walker' with several simultaneously dynamic feet could migrate with relatively high processivity along a track with the probability for complete detachment dependent on the number of legs and the kinetics of the individual binding events.

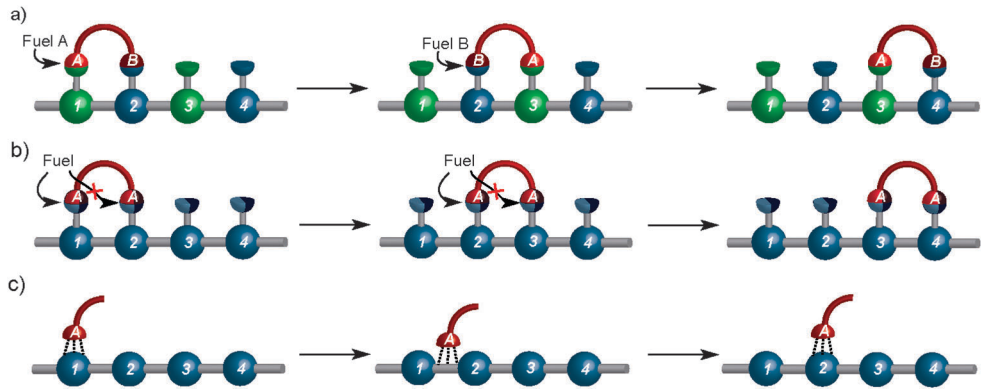


Fig. 4 Three possible strategies to confer processivity on the migration of a molecular walker: (a) two different feet and two different reaction conditions/fuels. (b) Two identical feet, but an interaction with the track that breaks their symmetry and makes one foot significantly more susceptible to the fuel. (c) One-legged walker that relies on secondary (tether) interactions (indicated by dashed lines) with the track.

3.2 Directionality—Brownian ratchet mechanisms

All biological molecular walkers operate with directional bias towards one end of their respective tracks, an indication of the importance that directionality plays in the function of translational molecular motors. In fact, a molecule that migrates in both directions of a track with equal probability cannot be considered a motor (it does not perform net-mechanical work) and could not carry out any useful task (such as transporting cargo to the periphery of the cell).

Although biological molecular walkers, such as kinesin-I, myosin-V and KIF1A, can provide valuable ideas for how to realise processivity in artificial analogues (see Section 3.1), it is doubtful that at the present time they can offer much insight into how to achieve significant directional bias in purely synthetic systems.[66] This is because the molecular structure and operating mechanisms in nature's protein motors are so complex that, at present, they are not understood in sufficient detail to be useful design models. In our view, the most valuable information for devising synthetic molecular motors comes from theoretical physics and in particular from the field of non-equilibrium statistical mechanics.[15]

The Principle of Detailed Balance[69] states that, at equilibrium, transitions between any two states take place in either direction with no net flux. For molecular walkers, this implies that at equilibrium no net-directional walker migration can occur and that, for the emergence of directionality, detailed balance must be broken for which an energy input is necessary. At first sight, one might think that this energy input could be provided by Brownian motion. This would, however, correspond to a molecular version of a perfect heat engine, which cannot be realised according to the second law of thermodynamics[70] that precludes the spontaneous emergence of heat gradients.

During the past decade, theoretical physicists have developed theorems (usually referred to as Brownian ratchet theory),[71–80] that successfully explain the underlying behaviour of both biological and synthetic molecular motors.[13,14,80–84] There are three fundamental requirements for the emergence of directed transport of a Brownian particle.[15]

A randomizing element: this is provided by random thermal motion in all molecular-scale motors (biological or synthetic).

An energy input: in biological systems this is commonly supplied by ATP hydrolysis; however in synthetic systems many other forms of energy input are conceivable.

An asymmetric potential of energy or information in the direction in which the motion occurs.

Many different theoretical variations based on these three requirements have been discussed in the physics literature, with a particularly extensive account provided by Reimann in 2002.[77] For the design of synthetic molecular motors, it is beneficial to distinguish between two general classes of Brownian ratchets: energy ratchets and information ratchets.[15]

In an energy ratchet mechanism the potential energy surface is periodically or stochastically varied irrespective of the position of the particle in order to cause directional transport (for example, the relative depths of two pairs of minima and the relative heights of the maxima that connect them could be repeatedly switched, as shown in Fig. 5). By simultaneously (or sequentially) reversing the relative position of the two pairs of thermodynamic minima and maxima, the particle is transported predominantly from left to right (occasional transport over a barrier in the wrong direction can occur). The direction of particle migration depends on the order of the changing thermodynamic minima and maxima. In Fig. 5 the purple and blue relative minima always have the higher kinetic barrier to their left. Changing the position of the kinetic barriers so that the higher barrier was always on the right would result in particle transport in the opposite direction.

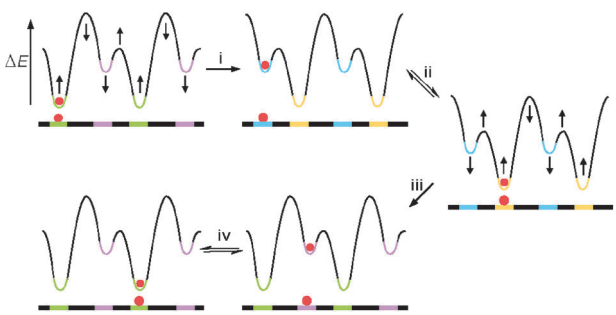


Fig. 5 A flashing type of Brownian energy ratchet mechanism. Sequential manipulation of the thermodynamic minima and kinetic barriers experienced by a Brownian particle (indicated by red dot).

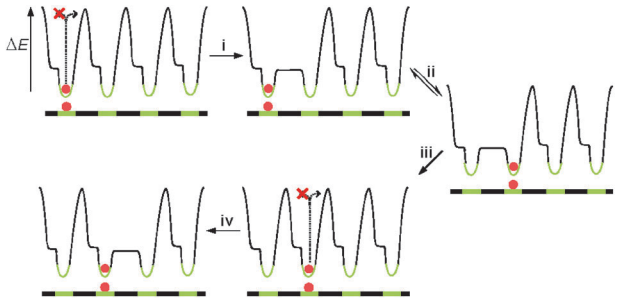


Fig. 6 An information ratchet mechanism leading to directed transport of a Brownian particle (in bright red) along an asymmetric potential energy surface. Asymmetry in the relative height of the kinetic barriers results from information transfer between the particle and the barrier.

In an information ratchet mechanism the position of the particle on the potential energy surface causes the potential energy surface to change (at an energetic cost) leading to directional transport of the particle. In Fig. 6 information regarding the position of the particle is communicated to the potential energy surface. The particle starts in one of the energy wells and communicates its presence only to the kinetic barrier to its right (the selectivity perhaps occurring by the particle being closer to the barrier to its right than the one to its left). This communication results (by some mechanism) in that barrier being lowered and Brownian motion allows the particle to sample both wells. Eventually the barrier is raised again, trapping the particle either in the original well or in the one to the right and the process can be repeated leading inexorably to transport to the right. Although one thermodynamically unbiased reversible step is involved (ii), this mechanism leads to directed movement, because a backward step is impossible (probability for step forward = $\frac{1}{2}$; probability for step backward = 0).

When describing the physical aspects of motor proteins, cell biologists tend to distinguish between 'Brownian ratchet' and 'power-stroke' mechanisms. The main criterion leading to this distinction is whether the track-binding domain ('foot') of a protein motor arrives at the forward binding site on the track through biased diffusion (Brownian ratchet) or through a mechanism, in which the foot, mediated by a rigid lever arm, is directly propelled forward (power-stroke). In many cases it is, however, difficult to draw a distinction between the two mechanisms,[85] which merely represent two mechanistic extreme cases. Crucially from the point-of-view of a synthetic molecular machine design, the underlying types of potential energy surface manipulations are almost certainly the same in both cases. Directional behaviour emerges as a result of the same type of asymmetric potential energy landscapes (see Fig. 5 and 6), with the only difference that in a Brownian energy ratchet mechanism the local free energy minima correspond to the track-binding domains of the motor ('feet'), whereas in a power-stroke mechanism they correspond to elements in the catalytic (ATP-binding) domain. These types of Brownian ratchet mechanisms were introduced into small-molecule systems in 2003 to make catenane-based rotary molecular motors[16,17] and later in linear energy ratchet[18] and light-[19] and chemically-[20]driven rotaxane-based information ratchets.

Having established the theoretical aspect of the mechanisms that can lead to unidirectional walker migration, the question arises as to how to realise either of the two different types of ratchet mechanisms in practice. Four different mechanistic possibilities for achieving directional bias in bipedal walker–track systems are illustrated in Fig. 7.

The first possibility (Fig. 7a) features fully reversible foot detachment and rebinding processes and corresponds to the mechanism adopted by biological walkers such as kinesin. Key for achieving directional bias is the conversion of a high-energy fuel X to low-energy fuel waste product Y, which must be coupled to a stride of the walker moiety in the forward direction. The driving force behind directional bias is the corresponding gain in free energy from the formation of Y. This mode of action, where the walker unit acts as a catalyst for the reaction of X to Y is viable for bipeds with two different (Fig. 7a; A $\neq$ B; X $\neq$ X′; Y $\neq$ Y′; autonomous or sequential operation) or with two identical feet (Fig. 7a; A = B; X = X′; Y = Y′; autonomous operation).

Directional bias can also be realised in a so called 'burnt-bridges' walker (Fig. 7b). Starting from one end of the track, the walker unit upon forward movement consumes the footholds it was attached to. This effectively prevents strides in the backward direction, which will eventually result in the walker unit being transported to the opposite end of the track. However, a walker–track system whose directionality is entirely based on a burnt-bridges mode of action (formally an information ratchet mechanism) has two important disadvantages. Firstly, the track cannot be reused (or at least it needs a reset operation) and, secondly, if the walker unit starts from a position somewhere in the middle of the track rather than at one end, it will migrate in one direction but that direction will depend on which step, forward or backward (destroying the original foothold), the walker initially takes at random.

In a walker with two different feet, directional bias is also viable when each foot stepping process is fully reversible (Fig. 7c) if there is a switchable element in either the track or the walker unit. The switch could be used to raise the energy of states where one particular foot is leading (*e.g.* the state where foot A is leading and the walker resides on the purple track linkage; middle of Fig. 7c), which, together with control of the kinetic barriers, leads to movement with net directionality. This corresponds to a classic energy ratchet mechanism (see Fig. 5).

Finally, directionality can also be achieved if one or more of the feet migration processes are irreversible (Fig. 7d). The outcome of a stride would then be kinetically controlled and not a function of the ground state energies of the two walking states but instead a function of the different activation energy barriers. For example, if during the irreversible step (Fig. 7d, I) a high-energy macrocycle could be formed, which in the subsequent reversible step (Fig. 7d, II) is mostly converted to a low-energy macrocycle, a net-directional bias towards the right end of the track would result. Such a behaviour, which corresponds to an information ratchet mechanism (see Fig. 6),

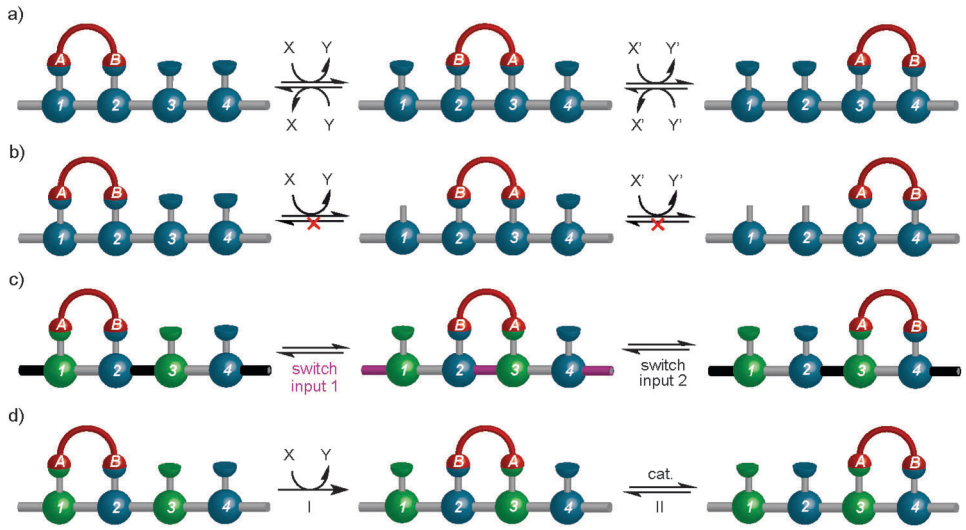


Fig. 7 Four possible strategies to confer directionality on the migration of a molecular walker: (a) reversible foot exchange coupled to consumption of stoichiometric complex fuel X (an information ratchet mechanism). The driving force for directional transport is provided by the reaction X → Y. The feet can be identical (coordination required) or different (in which case a second fuel, X′, may be required). (b) A 'burnt-bridges' walker catalyses the decomposition of the footholds, thus rendering any step in the forward direction essentially irreversible (an information ratchet mechanism). Feet can be identical (coordination required) or different. (c) Reversible migration processes that can be induced by catalytic reagents, but require energy input through switching stimulus (an energy ratchet mechanism). Feet cannot be identical. (d) One (or more) irreversible, kinetically controlled, migration process (I; an information ratchet mechanism). Feet cannot be identical.

could only be realised in a walker system where the two feet can be addressed separately.

Many rotary motors[21,86] achieve directional dynamic behaviour due to the helical chirality inherent to their molecular design.[87] Similarly, introducing chirality into the walker moiety and track (or fuel) of a molecular walker system could, when coupled to fuel consumption, potentially lead to directional walker locomotion (not shown in Fig. 7).

3.3 Autonomous operation

The fuel for biological motors (ATP) is present in the cell at all times, enabling them to operate in an autonomous manner. If autonomous operation *and* processivity are to be achieved in a synthetic molecular walker, the action of the two feet must be coordinated (otherwise both feet could interact with the excess fuel and detach). This means that a structural gate (for example in the track) must guarantee that, when one foot is detached, the other cannot dissociate from the track at the same time. This has been cleverly achieved for several DNA-based synthetic walker systems.

However, sequential operation can also offer useful benefits: (i) walker migration can proceed in a controlled manner in *either* direction of the molecular track (no natural or artificial autonomous walking devices achieve this); (ii) the rate of oscillation of the reaction conditions, and thus the speed of walker migration, can be controlled; and (iii) walker migration can be stopped at any time which allows the distance travelled by the walker to be governed precisely.

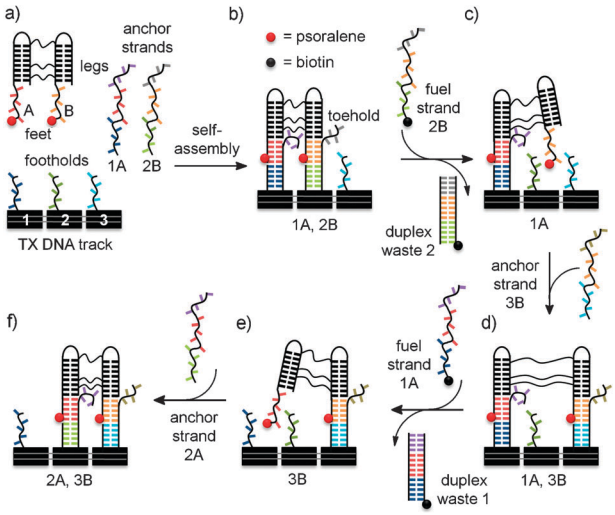
4. DNA-based molecular walkers

Since 2004, a number of molecular walker–track systems which are either largely or entirely assembled from DNA building blocks have been reported.[88] Many of these DNA-walkers are genuine molecular motors since they exhibit all four of the fundamental motor characteristics: progressive, repetitive, processive and directionally biased transport of a molecular fragment (walker unit) along a track. Synthetic DNA walkers are generally of a similar size, or even larger than, biological motor proteins such as kinesin-I.

4.1 Non-autonomous DNA walkers

The first example of a non-autonomous DNA walker, *i.e.* one that relies on the sequential addition of suitable chemical fuels, was described in 2004 by Sherman and Seeman.[89] With the exception of psoralene and biotin tags, which both serve particular functional roles, the entire walker–track system is made of DNA oligonucleotides. A double-stranded triple crossover (TX)[90] DNA structure (in solid black; Scheme 1a) serves as the rigid backbone of the track from which three single-stranded footholds of differing nucleotide sequence protrude (dark blue, green and light blue; Scheme 1a). The biped is constructed of two double-stranded 'legs' (black; Scheme 1a), two different single-stranded 'feet' (red and orange; psoralene tags in bright red; Scheme 1a) and three flexible linker strands (black curved lines) that connect the two legs and remain single-stranded throughout the experiments.

The starting position of the walker on the track was established in buffered aqueous solution at 16 °C by the addition of anchor strands that were partially complementary to the two strands in one foot/foothold pair: anchor strand 1A (Scheme 1a) is complementary to the single-stranded regions of foothold 1 and foot A, whereas anchor strand 2B matches foothold 2 and foot B. In contrast to biological walkers, such as kinesin-I, this DNA device does not rely on direct interactions between the walker and the track. Instead, the anchor



Scheme 1 Non-autonomous inchworm walker described by Sherman and Seeman.[89] (a) Self-assembly of the system from three components: rigid triple-crossover track featuring three protruding single-stranded footholds; walker including two single-stranded feet (featuring psoralene tags), separated by three single-stranded spacers; two anchor strands which act as 'molecular Velcro' to attach the feet to the footholds. (b) Initial position of the walker on the track established by self-assembly. (c) Foot B released from track after detachment procedure (competitive hybridisation). (d) Foot B attached to foothold 3 after addition of anchor strand 3B. (e) Foot A released from foothold 1. (f) Foot A anchored to foothold 2. Matching colours indicate complementary sequences between strands; the lines indicating base pairing do not represent a particular number of bases.

strands act as 'molecular Velcro', holding the walker and the track together through the effect of 20 cooperative base pair interactions.

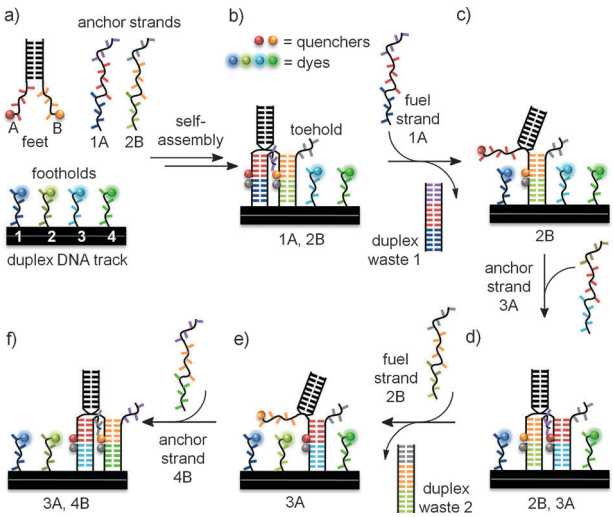
After the self-assembly step, the biped, anchor strands 1A and 2B and the track form a metastable aggregate. The instability is caused by the anchor strands having an overhang ('toehold' in Scheme 1b) of eight bases that remain unpaired. The overlapping toehold regions allow the removal of an anchor strand through competitive hybridisation (branch migration), which is rather like the closing of one zipper at the cost of opening another. Fuel strand 2B, for example, can bind to the toehold region of the anchor strand and completely displace it from the foot, leaving a free foot behind. This process is powered by the free energy gained through the formation of eight new base pairs in the stable duplex waste (the average free energy gain per base pair is $\sim$1 kcal mol^{-1})[91] that can be removed from the mixture through the interaction of the biotin-tag with magnetic streptavidin-coated beads.

Once foot B is detached from the track (Scheme 1c) it is free to diffuse within the restrictions imposed by the three single-stranded spacer strands (length *ca.* 2 nm). Addition of anchor strand 3B leads to reattachment of the leading foot to foothold 3 (Scheme 1d). Repetition of similar detachment and anchoring procedures leads to a walker–track conjugate in which both feet have moved one foothold in the forward direction (Scheme 1f). Seeman and coworkers also demonstrated that their device can be induced to walk backwards.

The composition of the mixtures was analysed by means of polyacrylamide gel electrophoresis (PAGE). Non-denaturing gels confirmed that the monomeric walker–track conjugates were stable and represented the major component of the mixture throughout the experiments. Psoralene cross-linking led to the formation of characteristic fragments at each stage of the experiment, as observed by denaturing PAGE.

From a mechanistic perspective the walker migration depicted in Scheme 1 is different from kinesin-I or myosin-V since it corresponds to an inchworm gait (foot A is always leading), not a hand-over-hand (or 'passing-leg') gait.[15] The walker is, however, processive because complete walker detachment does not occur to any significant extent at 16 °C and scrambling of the walker moiety between different tracks is unlikely at the low concentrations employed (0.26 to 0.5 μM). The driving force for directional migration is supplied by the free energy gain resulting from the pairing of eight bases in the toehold region of an anchor strand with the complementary bases in a fuel strand.

Shin and Pierce reported on a conceptually very similar DNA-walker that, like kinesin-I, walks in a hand-over-hand gait.[92] The design of the walker–track system is minimalistic, with the backbone of the track consisting of only one linear double helix and the walker comprising two partially complementary oligonucleotides (see Scheme 2a). The walker–track conjugate was assembled by the sequential addition of anchor strand 1A and anchor strand 2B to the walker and the track (Scheme 2b). Foot A could be detached from the track



Scheme 2 Hand-over-hand DNA-walker described by Shin and Pierce.[92] (a) Self-assembly of the system from three components: rigid, double-stranded track featuring four protruding single-stranded footholds functionalised with fluorescence dyes; walker including two single-stranded feet (functionalised with quenchers), held together by duplex domain; two anchor strands which act as 'molecular Velcro' to attach the feet to the footholds. (b) Initial position of the walker on the track established by stepwise self-assembly. (c) Foot A released from track after detachment procedure (competitive hybridisation). (d) Foot A attached to foothold 3 after addition of anchor strand 3A. (e) Foot B released from foothold 2. (f) Foot B anchored to foothold 4. Matching colours indicate complementary sequences between strands; lines indicating base pairing do not represent a particular number of bases.

through competitive hybridisation upon addition of a fuel strand (Scheme 2c). Using four different anchor and fuel strands, Shin and Pierce were able to control their device and induce it to walk in either direction down the track (Scheme 2f).

The progress of the walking experiments was analysed by non-denaturing PAGE and fluorescence spectroscopy. The terminus of each foothold was functionalised with a fluorescent dye of a characteristic emission wavelength while each foot of the walker unit was functionalised with an appropriate quencher (Scheme 2b). Visualizing the gels with different fluorescent scans allowed the position of the walker on the track to be deduced. For example, when foot A is attached to foothold 1 the fluorescence of the dye on foothold 1 is almost entirely quenched while the fluorescence of the dye on foothold 2 remains unaltered. Using multiplexed real-time fluorescent monitoring the walking process could be followed *in situ*.

More recently, Seeman and coworkers[93,94] demonstrated that sequentially fuelled DNA-based molecular walkers can perform sophisticated tasks at the nanometre scale. For a track they employed a large (*ca.* 300 nm wide) two-dimensional DNA origami[95] tile assembled from a total of 202 DNA oligonucleotides. The origami tile featured 18 protruding single-stranded footholds and three large slots (Fig. 8a). The footholds were positioned in a precise pattern on the DNA origami tile that allowed a four-legged triangular DNA walker to perform an essentially one-dimensional migration of its centroid, while the walker's extremities rotate by 120° during each step. The structure of the walker moiety, a tensegrity triangle organisation constructed from seven oligonucleotides, is shown in Fig. 8b. The walker has four 'feet' and three 'hands', all consisting of single-stranded DNA segments. As in the previously discussed systems, the walker is attached to the track through complementary anchor strands featuring toehold regions. Fig. 8c illustrates the transition of the walker from a three-foot-bound state to a two-foot-bound state, while the whole triangular shape rotates by an angle of 120°.

At three positions on its linear trajectory, the walker comes into proximity of one of three cassettes which carry different DNA-bound gold nanoparticles. The DNA machines on these cassettes are positional ON–OFF switches which can be induced through the addition of fuel strands to either offer, or not offer, the gold cargo to a bypassing walker. The mechanism of the cargo handover, a competitive hybridisation event, is illustrated in Fig. 8d. The role of the fourth foot (F4) is to keep the walker in a conformation where one hand of the walker is in proximity to the cargo (F4 is not needed for translation/rotation of the walker). Depending on the state of the DNA cargo delivery machines (ON or OFF), the walker can assemble eight (2^3) different cargo combinations during the course of its journey.

Seeman and coworkers studied the complex behaviour of their system by PAGE, atomic force microscopy (AFM) and scanning electron microscopy (SEM). AFM could not resolve several gold nanoparticles attached to one walker but could be performed during the course of the experiments, whereas SEM delivered higher resolution but required isolation of the cargo-bound walkers after the experiments were completed.

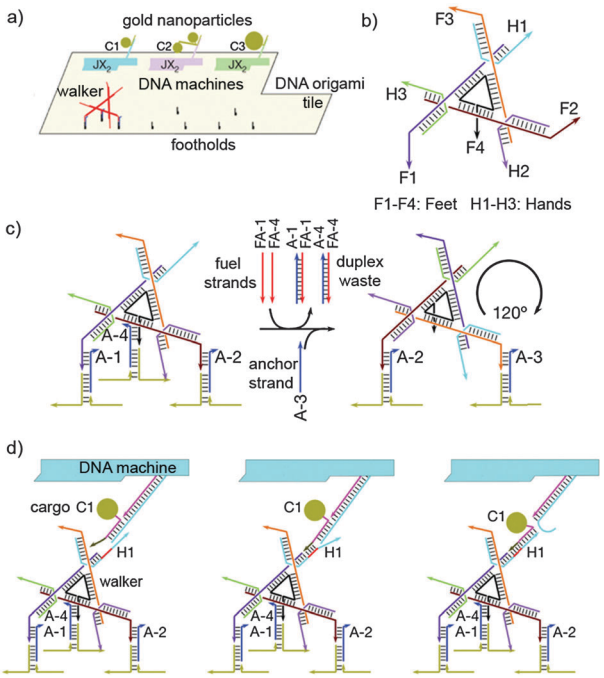
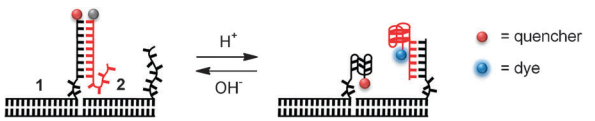


Fig. 8 Nanoscale assembly line published by Seeman and coworkers.[93] (a) Schematic illustration of the entire assembly, consisting of a large DNA origami tile (track), three cassettes each including a DNA ON–OFF switch which carry three different DNA-bound gold nanoparticles; triangular walker moiety. (b) Structure of the walker moiety featuring seven single-stranded domains: four feet (F1 to F4) and three hands (H1 to H3). (c) One 'stride' of the walker moiety, requiring the sequential addition of two fuel strands and one anchor strand (walker rotated by 120°). (d) Handover of DNA-bound cargo (C1) from the DNA machine to the walker. Adapted by permission from Macmillan Publishers Ltd: *Nature* (ref. 93), copyright (2010).

Through a statistical analysis of the SEM data, the researchers were able to show that in each of the eight experiments the expected walker–cargo products were formed in yields of 75% to >90%.

Two other non-autonomous systems based solely or partially on DNA are worthy of note. Bromley *et al.* have described the concept of a peptide-based three-legged "tumbleweed" walker that, upon sequential addition of three different chemical fuels, could progress directionally along a synthetic DNA track.[96] Although the operation of the system was successfully simulated *in silico*,[97] no corresponding experimental study has been published to date. Ren and coworkers have described a single-stranded DNA device that responds to changes in pH by migrating processively between two different single-stranded footholds on a rigid DNA track (Scheme 3).[98]



Scheme 3 Positional DNA switch reported by Ren and coworkers.[98] Reversible, pH-induced translocation of an oligonucloetide strand along a track constructed from DNA. The system is not a motor, since it is not operating progressively or directionally (on a longer track, no net transport of the DNA device would occur).

Although the authors call their device a DNA walker, the system is more accurately classed as a molecular switch since it lacks a mechanism (and a fuel input) which would allow it to operate directionally and progressively.

4.2 Autonomous DNA walkers

In 2004 and 2005 three reports of autonomous DNA walkers appeared in the literature (Schemes 4 and 5).[99–101] In all three cases the directional bias relied on the enzymatic cleavage of DNA or RNA strands and the walker locomotion resembles more "*a bucket being passed along a fire brigade*"[102] rather than a hand-over-hand or inchworm gait.

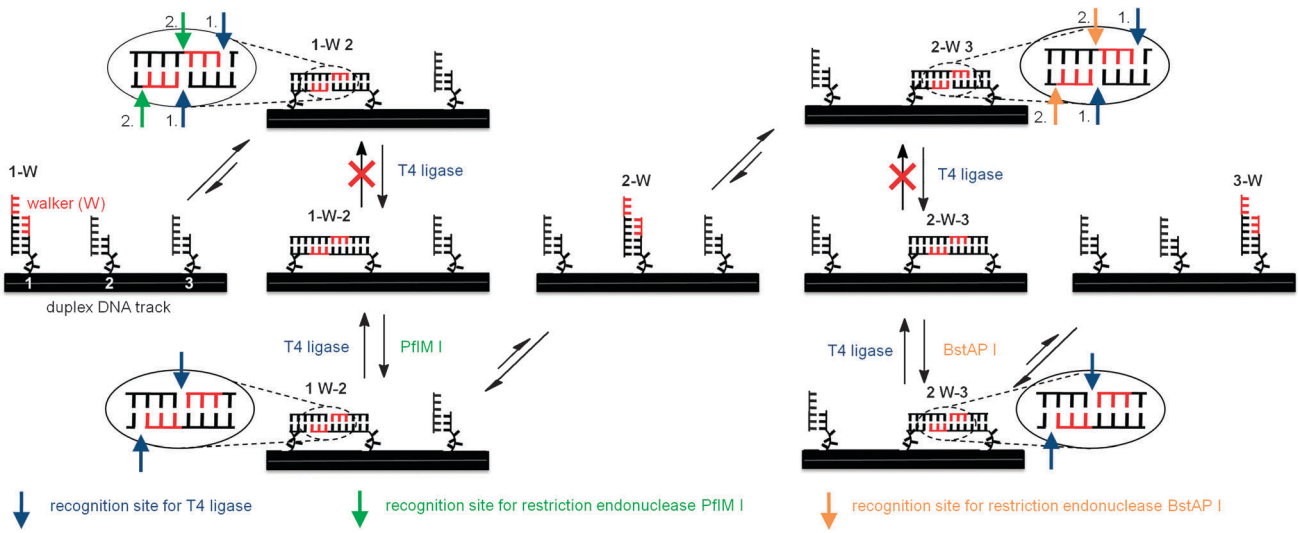
The operation of the first autonomous DNA walker, reported by the groups of Turberfield, Reif and Yan,[99] is illustrated in Scheme 4. The track consists of a DNA duplex to which three mainly double-stranded footholds are connected through a short single-stranded hinge (see Scheme 4). The walker (shown in red) consists of only six DNA nucleotides and is initially ligated to foothold 1 (Scheme 4, state 1-W). In addition to the walker–track adduct, three enzymes were added to the buffer solution: a ligase (T4 ligase) and two restriction enzymes (PfIM I and BstAP I). Owing to the flexibility of the hinges, footholds 1 and 2 can hybridise their toehold regions (Scheme 4, 1-W 2) which allows T4 ligase to form a covalent bond between the walker and foothold 2 (Scheme 4, 1-W-2). The ligation step creates a recognition site for one of the restriction enzymes (PfIM I), which selectively cuts the walker from foothold 1 (Scheme 4, 1 W-2). The energy required for this step, which represents the crucial process for the emergence of net-directionality, is supplied by ATP hydrolysis of the restriction enzyme. Repetition of the ligation and cleavage processes (with only restriction enzyme BstAP I recognising a cleavage site) leads to the partial formation of walker state W-3 (Scheme 4). The walker migration occurs with overall net-directionality as there is no mechanism for the walker to step backwards.

Turberfield and colleagues provided evidence for the processive and directional behaviour of their system using denaturing PAGE and a radioactively (γ-P^{32}) labelled walker unit.

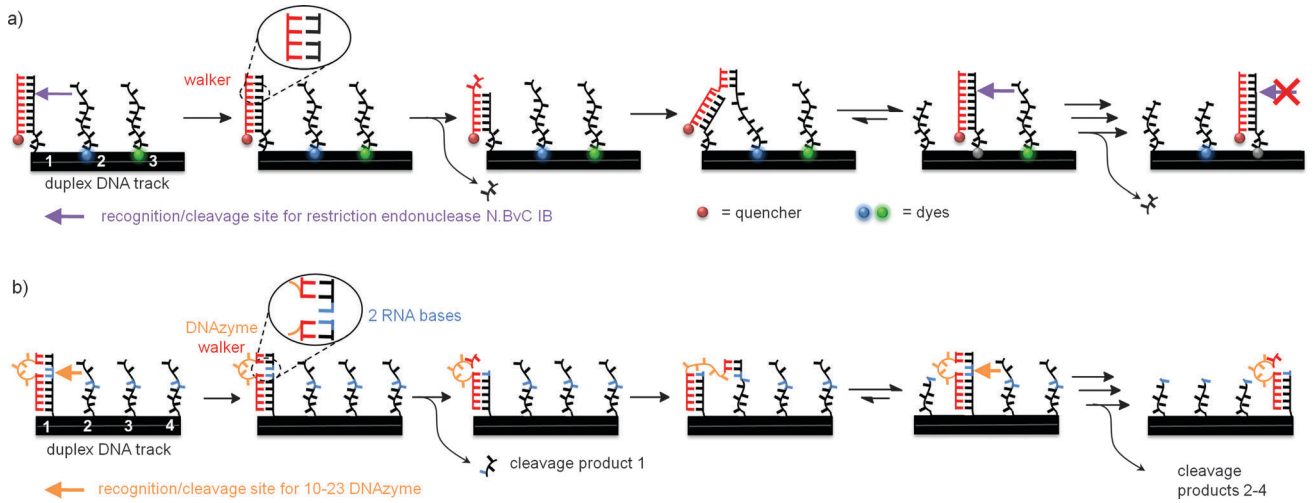
In 2005, Turberfield and coworkers reported the design and operation of an autonomous 'burnt-bridges' walker that achieves directionality by consuming the track as it moves forward.[100] The system consists of a track with three almost identical footholds, a single-stranded DNA walker (shown in red in Scheme 5a) and a single enzyme that cuts off the terminal part of a foothold only when the walker is attached to it. The employed enzyme, restriction nuclease N.BbvC IB, accomplishes this task by first recognising a particular sequence in a walker–foothold duplex and then catalysing the hydrolysis of the foothold strand.

When the walker is located on the left hand side of the track (Scheme 5a), the action of the restriction enzyme leads to the cleavage of a short (eight base) duplex at the terminus of the foothold which is released into the solution due to its melting temperature being considerably lower than the operating temperature of the motor. As the walker unit now possesses a toehold region that can reach the second foothold, competitive hybridisation results in the attachment of the walker to foothold 2. A backward step is extremely unlikely because attachment to the longer footholds is associated with a $\sim$10 kcal mol^{-1} gain in free energy. The process is then repeated so that the walker is eventually located on foothold 3 which, due to a sequence mismatch, is not cleaved by the enzyme (Scheme 5a, right hand side). The partial hydrolysis of the first two footholds of the track provides the energy source for directional walker transport. Turberfield and coworkers functionalised footholds 2 and 3 with different fluorescence dyes and the walker with a quencher, which allowed the verification of the behaviour of their system as well as the extraction of kinetic data (stepping rate $\sim$0.01 s^{-1}).

Mao and coworkers published a conceptually very similar burnt-bridges walker (Scheme 5b),[101] however in their case no



Scheme 4 Autonomous walker transport mediated by three enzymes reported by the groups of Turberfield, Reif and Yan.[99] The walker consists of six DNA bases (displayed in red). In the descriptions of the states, covalent attachment of the walker (W) to a foothold is indicated by a hyphen; lines indicating base pairing do not represent a particular number of bases.



Scheme 5 Two other enzyme-dependent autonomous DNA walkers. (a) Turberfield's autonomous 'burnt-bridges' walker, mediated by restriction endonuclease N.BvC IB.[100] A walker unit consisting of 26 DNA bases (in red) indirectly consumes the track (provides recognition site for enzyme) while it is moving forward; lines indicating base paring do not represent a particular number of bases. (b) Mao's autonomous 'burnt-bridges' walker, mediated by a DNAzyme in the walker unit.[101] The DNAzyme walker directly consumes the track while it is moving forward; lines indicating base pairing do not represent a particular number of bases.

external enzyme needs to be added as the walker itself acts as the enzyme. The track is constructed of duplex DNA (shown in blue in Scheme 5b) with four evenly spaced slightly different footholds consisting of 21 DNA (shown in black) and 2 RNA (shown in blue) nucleotides. The walker is a DNAzyme[103] capable of cleaving RNA strands with sequence specificity (catalytic core shown in orange in Scheme 5b). Once the system is assembled, a reaction cascade occurs that is essentially the same as the one described for Turberfield's system (Scheme 5a). One notable difference is that in Mao's system all footholds are susceptible to recognition by the DNAzyme, so that the final foothold is also hydrolysed. Mao and coworkers verified the directional behaviour of their system by monitoring the order in which the short single-stranded cleavage products appear over time (using denaturing PAGE).

In 2007, Winfree, Rothemund and Pierce reported an autonomous polymerisation motor[104] reminiscent of the Rickettsia bacteria;[105] an intracellular pathogen which achieves locomotion by polymerising actin moieties at their surface. The active motor component is a single-stranded DNA oligonucleotide that continuously catalyses the polymerisation of two hairpin fuels.[106] Although the movement of the motor domain is processive, the direction in which the DNA catalyst is propelled forward is random.

An enzyme-free, autonomous DNA biped was reported by Pierce and coworkers in 2008.[107] A burnt-bridges type of walker locomotion was achieved through the use of DNA hairpin[106] strands but, due to the lack of coordination between the two feet, the process occurred without processivity.

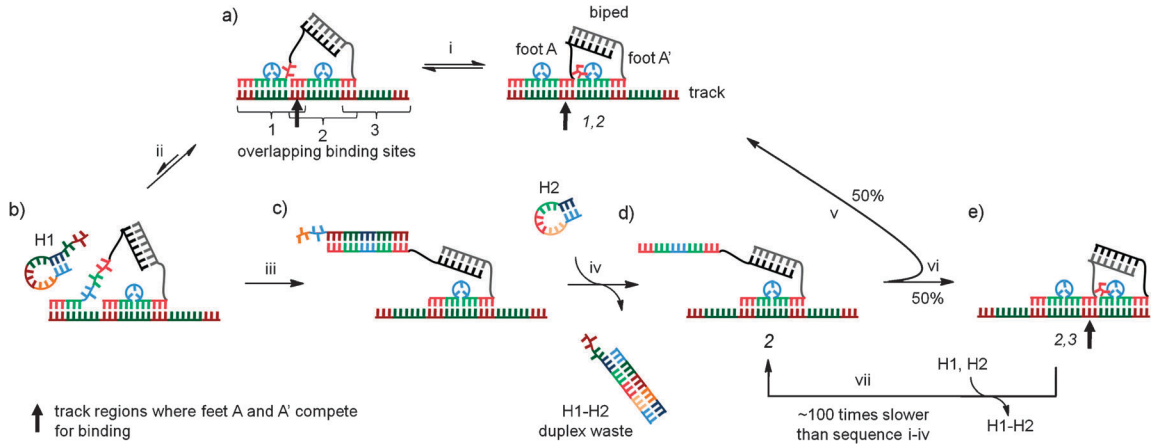
The first enzyme-free, autonomous, processive and directional DNA walker was reported by the group of Turberfield in 2008 (Scheme 6).[108] Their system is reminiscent of kinesin-I in that it uses a structural gate in order to discriminate between two identical feet. Directionality is achieved (see Scheme 6) due to the impossibility for both feet to hybridise completely to the track, which results in the two feet consuming the supplied chemical fuel at different rates.[109]

The track consists of one single DNA oligonucleotide that features three partial binding sites for the walker feet (dark colour tones in Scheme 6a). The walker consists of two identical oligonucleotides that assemble to a joint double- (black and grey) and two single-stranded regions (feet A and A′; light colour tones).

When the two single-stranded feet of the walker are hybridised to the left hand side of the track (Scheme 6a) the two feet compete for a small region of the track (indicated by the black arrow in Scheme 6a). Starting from the equilibrium that results from this competition, part of the left foot is lifted from the track (Scheme 6b) which reveals a toehold region that is complementary to the toehold region of one of the hairpin fuels (H1, Scheme 6b). In a branch migration process, hairpin H1 displaces the left foot from the track which in turn reveals a binding site for hairpin fuel H2 (Scheme 6c). In the next step, hairpin H2 displaces hairpin H1 from the left foot (Scheme 6d).

During this reaction sequence (Scheme 6, i–iv) the left foot of the walker has catalysed the formation of duplex waste H1–H2 (in the absence of the walker–track-conjugate, H1–H2 is formed only very slowly). The energy released by this process has enabled the left foot to detach from the track‡ (Scheme 6d) so that it can either bind on the first or the third site of the track. Although this reattachment occurs without bias (50% attachment on either side) the overall process is directional because the stepping mechanism (steps i–iv in Scheme 6) is not possible when starting from state 2,3 (where the feet are attached to the right hand side of the track;

‡ Note that the left foot does not necessarily need to be fully detached from fuel strand H1 (as shown for clarity in Scheme 6d) in order to be able to reattach to track binding sites 1 or 3.



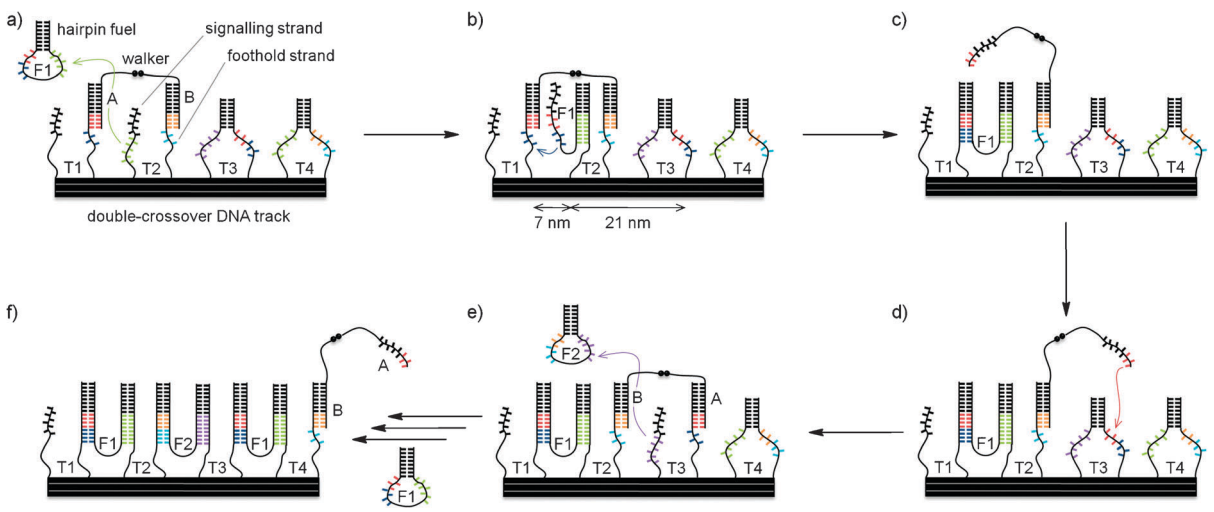
Scheme 6 Turberfield's autonomous bipedal DNA walker, powered by two DNA hairpin fuels (H1 and H2).[108] (a) Equilibrium between two starting states results from competition of the two walker feet for the binding site indicated with an arrow. (b) The left foot can partially lift from the track and expose a toehold, which is complementary to the toehold in fuel H1. (c) Branch migration leads to detachment of the left foot. (d) Fuel H2 can displace H1 from the left foot; intermediate 2 (shown for reasons of clarity) or an intermediate of the previous branch migration process can either rebind at site 1 or site 3, which occurs with statistical product distribution (50 : 50). (e) Starting from the 2,3 state, steric reasons preclude the mechanism shown in steps i–iv; process 2,3 → 2 is therefore 100 times slower than 1,2 → 2; complementary nucleotide sequences are indicated by the use of light and dark colour tones (*e.g.* a dark red region is complementary to a light red region); lines indicating base pairing do not represent a particular number of bases.

Scheme 6e). This is due to steric reasons (the toehold region that was exposed in the left foot in step 'ii' is sterically shielded in the case of the right foot) and results in the process 2,3 → 2 being 100 times slower than the process 1,2 → 2. Turberfield and coworkers studied the dynamic behaviour of the full three-site system with PAGE and the kinetic behaviour of a smaller two-site model system with fluorescence spectroscopy.

A particularly impressive, albeit less minimalistic, example of an autonomous DNA biped with coordinated feet was published by the Seeman group in 2009.[110,111] Their system (Scheme 7a) consists of a rigid track and a biped with two different single-stranded feet (A and B). The track is a ~49 nm double-crossover (DX) DNA structure decorated in a directionally polar manner with different metastable DNA stem–loop motifs (T1–T4; each consisting of one 'signalling' and one 'foothold' strand). The walker comprises two different single-stranded feet that, unlike the previously discussed bipeds, are not joined by a duplex region but by a covalent 5′,5′ linkage.

Overall, processivity is guaranteed by signalling strands that mediate the interaction between feet and fuel strands in a way that only one foot can detach from the track. Directionality is achieved through the hybridisation of metastable hair pin fuel strands to the track (a 'burnt-bridges' mechanism).

In the starting position (Scheme 7a), where the walker is located on the left end of the track, foot B is hybridised to the



Scheme 7 An autonomous DNA biped in which coordination between the feet is achieved through signal strands on the track.[110] (a) Resting state 1; walker in starting position (foot B leading); signal strand T2 hybridises with hairpin fuel F1. (b) Activated fuel strand F1 hybridises with toehold on T1 foothold strand and displaces foot A from foothold T1. (c) Foot A is free. (d) Foot A diffuses to stem–loop T3, hybridises with its toehold and frees signal strand T3. (e) Resting state 2 (foot A leading); signal strand T3 hybridises with hairpin fuel F2. (f) Final resting state where foot B is bound to foothold T4 and foot A is free. Matching colours indicate complementary sequences between strands; lines indicating base pairing do not represent a particular number of bases.

foothold strand of stem–loop T2. The free signalling strand T2 hybridises with one half of hairpin fuel F1 (Scheme 7b) whereupon the other half of the fuel displaces foot A from foothold strand T1. Fuel strand F1 then forms a stable aggregate with the track (Scheme 7c) and foot A is free to diffuse to a forward binding site (Scheme 7d). After foot A has hybridised with foothold strand T3, a second resting state is formed (Scheme 7e) in which the order of the feet has reversed (hand-over-hand gait). The interaction of signalling strand T3 with fuel strand F2 initiates a second motor cycle that leads to a third resting state where the walker is attached to T3 and T4 (not shown in Scheme 7). In the third motor cycle, signalling strand T4 activates fuel F1, leading to displacement of the trailing foot from foothold strand T3. The walker thus takes two and a half steps and in the final resting state only foot A is attached to foothold strand T4 (Scheme 7f).

Seeman and coworkers demonstrated a full walking cycle of their device by covalently cross-linking a radioactively labelled walker (^{32}P) to its track in successive walking states and observing fragments with characteristic mobilities during autoradiogram analysis of denaturing PAGE.

DNA-based walkers with more than two feet have also been reported. A study published in 2006 by Stojanovic described autonomous processive multipedal walkers with 2–6 DNAzyme feet.[112] The movement of some of these 'spider' walkers occurred with reasonable processivity but the direction of the movement occurred in random directions on the nucleotide matrix that served as the track (the authors later termed these systems 'random walkers').[113]

In 2010 a more sophisticated use of molecular 'spiders' was reported by the groups of Stojanovic, Winfree, Walter and Yan.[113] Through their interaction with appropriately designed two-dimensional DNA origami[95] tiles the multipedal walkers could carry out robotic actions such as 'start', 'follow', 'turn' or 'stop'. The molecular spider used in the study is illustrated in Fig. 9a. Four single-stranded DNA oligonucleotides are attached to an inert streptavidin core. Three of the single strands (F1 to F3)—the spider's legs—are DNAzymes that can recognise and cleave oligonucleotide substrates containing one RNA base. The fourth unpaired strand (F4) acts as a 'capture leg' that is used to position the molecular spider with precise control on the 'START' position of the track (Fig. 9d).

The interaction between the DNAzyme legs and the DNA/RNA-chimeric footholds that decorate the track is shown in Fig. 9b. Similar to Mao's autonomous walker, the employed 8–17 DNAzyme first hybridises with the foothold (substrate) strand and then cuts off an eight base section that is released into solution. The DNAzyme binds significantly weaker to the resulting shorter product strand which results in its dissociation and quick reattachment to a nearby product or substrate strand. The device operates processively (average step number ~200), because the DNAzyme legs spend a relatively long time on substrate strands but only a short time on product strands and even less time in an unbound state. Consequently, at any time the probability is very high that at least one of the legs is attached to the track.

Three pathways for the directionally-biased migration of a molecular spider are illustrated in Fig. 9c. If placed on a pathway decorated with substrate strands the spider will move

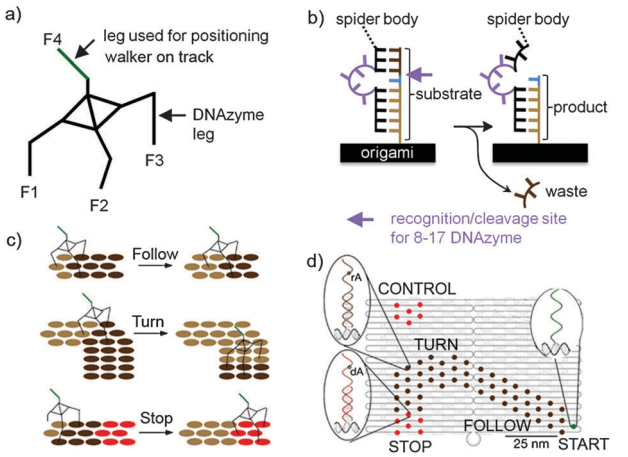
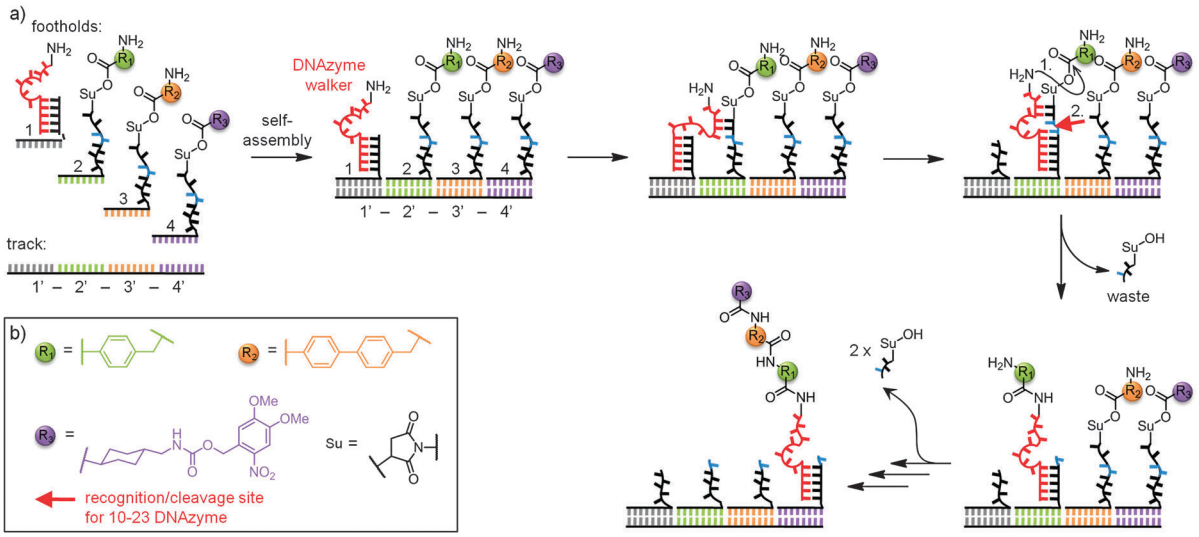


Fig. 9 A molecular spider with three DNAzyme legs that displays autonomous robotic behaviour on a DNA origami landscape.[113] (a) Components of the employed molecular spider: inert streptavidin body; three 8–17 DNAzyme feet that can cleave RNA substrates; one capture leg which is used to position the spider on the origami tile; (b) illustration of the interaction between the spider legs and the footholds on the origami tile (DNAzyme leg shown in black, foothold shown in brown, RNA base at which cleavage by the DNAzyme occurs highlighted in blue; lines indicating base pairing do not represent a particular number of bases); (c) robotic behaviour of the spider as determined by the foothold sequence (substrate footholds shown in brown; hydrolysed product footholds shown in beige; 'STOP'-footholds lacking the RNA base are shown in red); (d) representative origami landscape including 'START', 'FOLLOW', 'TURN', 'STOP' and 'CONTROL' sites. Adapted by permission from Macmillan Publishers Ltd: *Nature* (ref. 113), copyright (2010).

directionally towards the substrate region and will also follow turns. Foothold strands that lack the RNA base (shown in red in Fig. 9c) act as a thermodynamic trap for the spider because the DNAzyme leg is not able to hydrolyse these footholds. The effect of these thermodynamic traps is so powerful that they can even impose net-directionality on a spider that performs a fully random walk along a pathway constructed exclusively from product strands. The behaviour of the spiders on 48 and 90 nm pathways on the origami landscape (Fig. 9d) was analysed by atomic force microscopy (AFM) and real-time total internal reflection fluorescence microscopy. Statistical analysis of the AFM data showed that on the 90 nm track, 70% of the spiders reached the 'STOP' site within 60 minutes. Very few spiders were found on a control site (red, top of Fig. 9d) on the origami tile, illustrating the processivity of the walker locomotion.

An autonomous DNA walker that progressively performs three consecutive amine acylation reactions while moving forward on a DNA track has been created by Liu and He.[114] The system is similar to the autonomous DNA walker described by Mao (Scheme 5b), with the crucial difference that footholds 2, 3 and 4 are functionalised with activated esters of three different non-natural amino acids (Scheme 8). The use of non-natural amino acids, in which the acyl and amino functionalities are separated by a rigid spacer moiety (Scheme 8b), was necessary to prevent spontaneous intramolecular cyclisation.



Scheme 8 An autonomous molecular walker capable of performing consecutive intramolecular acylation reactions.[114] (a) Schematic illustration of the operation sequence of the device. Matching colours indicate complementary sequences between strands; lines indicating base pairing do not represent a particular number of bases. (b) Chemical structures of spacer groups R_1, R_2, R_3 and *N*-succinimide active ester Su.

Once the starting position of the device is established *via* self-assembly (Scheme 8a), the single-stranded part of the walker unit can hybridise with a complementary toehold region on foothold 2. This is followed by a competitive hybridisation process that results in the translocation of the entire walker moiety onto foothold 2. The migration of the walker unit from foothold 1 to foothold 2 triggers two processes. First, intramolecular nucleophilic attack of the walker-bound amino functionality on the activated acyl group on foothold 2 occurs. This reaction, whose rate is significantly enhanced by the high effective molarity of the two reagents, results in the transfer of the first amino acid building block (shown in green in Scheme 8) from foothold 1 onto the walker moiety. The second process is the recognition and subsequent cleavage of the ribonucleotide linkage (in blue in Scheme 8a) in foothold 2 by the DNAzyme motif present in the walker moiety. After the dissociation of a short single-stranded fragment of foothold 2, the system is in a state which is essentially identical to its starting state. After two further cycles of walker translocation, intramolecular acylation and DNAzyme foothold cleavage, the walker is located on foothold 4 and has assembled a triamide on its *N*-terminus.

High-resolution mass spectroscopy of the crude reaction mixtures and comparison of the resulting spectra with mass spectra of authentic product mixtures of known composition suggested that walker–triamide conjugate(s) (Scheme 8a) constituted the major reaction product (estimated yield: 45%). It is crucial that the DNA foothold cleavage reactions are chosen to be significantly slower than the transacylation reactions or errors will be introduced into the sequence of the amide bonds constructed. Indeed, walker–monoamide and walker–diamide conjugates were found as predominant side products that result when the walker moiety moves forward to the next foothold before the walker-bound-amine has picked up the amino acid from the previous foothold.

Using mass spectrometry, Liu and He could only indirectly assess the extent to which the predominant triamide product was indeed formed with sequence-specificity (*i.e.* in the order R_1–R_2–R_3 as shown in Scheme 8a). In a control experiment, a walker–track conjugate was prepared in which the relative positions of footholds 2 and 4 were swapped. Foothold 4 lacks a terminal amine group and thus serves as a synthesis terminator. As a result only a monoamide product (walker-R_3) was detected by mass spectrometry, which helped to confirm the processivity of the device as well as the lack of scrambling between reactive groups on separate footholds.

5. Small-molecule walkers

As impressive as some of the DNA systems are, the ultimate in miniaturisation of translational motors is to make walker systems with small molecules. Just as synthetic catalysts can be made that are far smaller than enzymes, it should not be necessary to construct molecules the size of kinesin-I (as most DNA walkers are) in order to achieve functional walker systems. However to do so requires the development of suitable chemical systems and mechanisms from first principles. Unfortunately, unlike DNA—for which even the most complex structures can be ordered from commercial suppliers or made with automated synthesizers—small-molecule systems must be constructed from scratch, often using complex chemistry and bespoke synthetic routes.

5.1 Processive migration of small molecular fragments along tracks

There are many chemical reactions, including a multitude of sigmatropic rearrangement reactions,[115] that feature the migration of molecular fragments. However, relatively few of these appear intrinsically well-suited for developing into a processive, directional and repetitive motor mechanism.

In many sigmatropic rearrangement reactions, such as the Payne rearrangement[116] (Fig. 10a), only bonds—rather than molecular fragments—actually migrate. In others, such as the Cope or Claisen rearrangements, a processive (*i.e.* intramolecular)

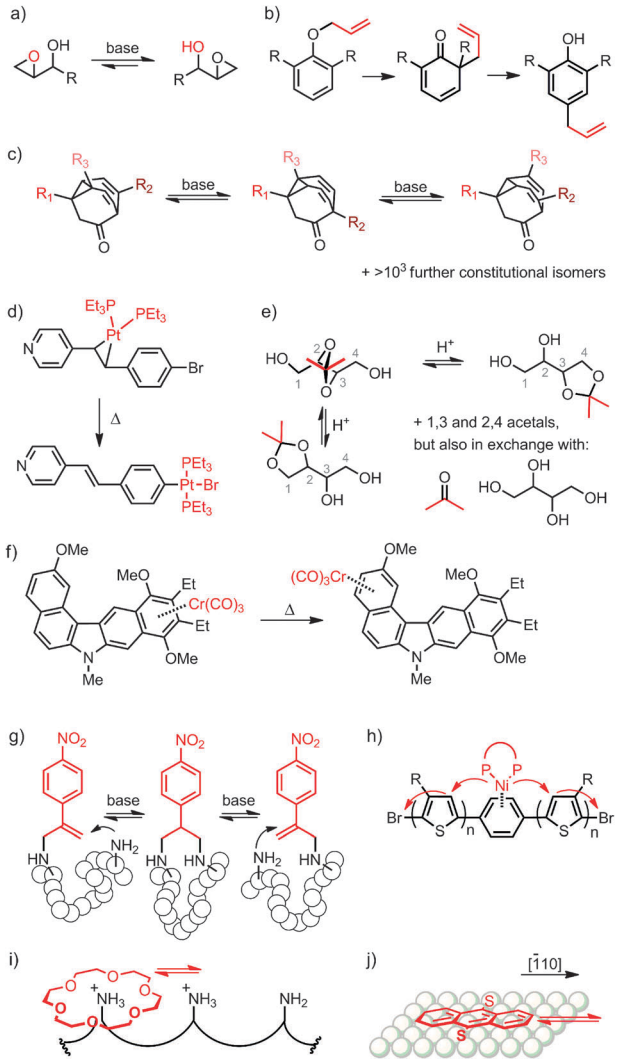


Fig. 10 Selected examples of the migration of small molecular fragments. (a) In the Payne rearrangement no atoms, only bonds migrate.[116] (b) The Claisen rearrangement occurs processively and in some instances repetitively.[117] (c) Base-induced interconversion of bullvalone isomers *via* successive Cope rearrangements.[118] Shown are only three of $>10^3$ bullvalone constitutional isomers. (d) Metal atoms can migrate processively along some molecular scaffolds.[121] (e) Reversible, non-processive migration of acetone along a threitol substrate (acetal exchange with the bulk competes).[123] (f) Haptotropic migration of a chromium complex.[122] (g) Reversible processive migration of a three-carbon fragment along nucleophilic sites of a protein *via* Michael/retro-Michael reactions.[124] (h) A Kumada catalyst that randomly migrates along a π-system while the oligothiophene chain is extended in both directions.[128] (i) Non-directional but (under high vacuum conditions) processive migration of a crown ether along an oligolysine chain.[131] (j) One-dimensional diffusion of 9,10-dithioanthracene on a high-symmetry metal surface [Cu(111)].[133]

migration of atoms does occur. However, such migrations are generally neither repetitive (the 1,4-Cope rearrangement of an allyl ether can occur twice in a row if rearomatisation on the 2-position is impossible; Fig. 10b)[117] nor progressive. An interesting example of multiple repetitive group migrations, recently studied by the Bode group,[118] is the interconversion of a large number of constitutional isomers of derivatised bullvalones[119,120] with base (Fig. 10c).

In haptotropic rearrangements, metal complexes migrate along extended π-systems (see Fig. 10d and f for two examples),[121,122] but these are not generally directional in a sense that can be repetitively propagated.

In other reactions, for example the exchange of acetals between the hydroxyl groups of carbohydrates[123] (Fig. 10e), the migration of the carbonyl fragments is intrinsically non-directional and non-processive (intramolecular exchange competes with exchange with the bulk).

There are, however, some reports of small molecular units that move processively (*i.e.* without detaching) along molecular frameworks. Lawton and coworkers have described a series of reagents for the cross-linking of biomolecules under thermodynamic control.[124–127] These molecules are transferred between accessible nucleophilic (amine and thiol) sites on proteins such as ribonuclease.[124] The transport proceeds intramolecularly (*i.e.* processively) due to the clever construction of the cross-linking moiety: only one or two—never zero—of the nucleophilic footholds are attached to the cross-linking molecule at any one time as it is passed from one nucleophilic site to another intramolecularly towards the thermodynamic minimum (Fig. 10g). Loss of processivity in these systems may still occur, however, through intermolecular bridges, the presence of which has been reported.[124]

During polymer synthesis, catalytic metal species have been shown to migrate intramolecularly for considerable distances along polymer chains growing by Kumada catalyst-transfer polycondensation[128] (Fig. 10h) and during ethylene polymerization.[129,130]

Schalley and coworkers have described systems in which crown ethers apparently migrate processively along linear[131] or dendritic[132] oligoamine scaffolds under the extreme dilution present in a FTICR mass spectrometer (Fig. 10i).

The one-dimensional diffusion of 9,10-dithioanthracene (DTA) molecules (Fig. 10j) on a copper surface has been reported by Bartels and coworkers.[133] Although the restriction of the movement to only one dimension (exclusively in the [1̄10] direction) is a remarkable feature, and the mechanism through which this is achieved resembles the gait of a biped, the system lacks directionality. For the DTA motion to be directional, coupling of the forward motion to the consumption of an energy input would be necessary.[134,135]

5.2 Synthetic small-molecule walkers

The first small-molecule walkers able to operate in a processive, directional, repetitive and progressive manner (see Introduction for definitions) were recently described.[136–139]

The synthesis and operation of a 21-atom two-legged molecular unit (shown in red in Fig. 11) that is able to walk along a four-foothold molecular track was reported in early 2010.[136,137] A walker unit with two chemically different feet was connected to a track in such a way that each foot could act as a temporarily fixed pivot while the other engaged in a dynamic covalent[140] exchange reaction. Under acidic conditions the disulfide bond between one foot of the walker and the track remains kinetically locked while the hydrazone unit that

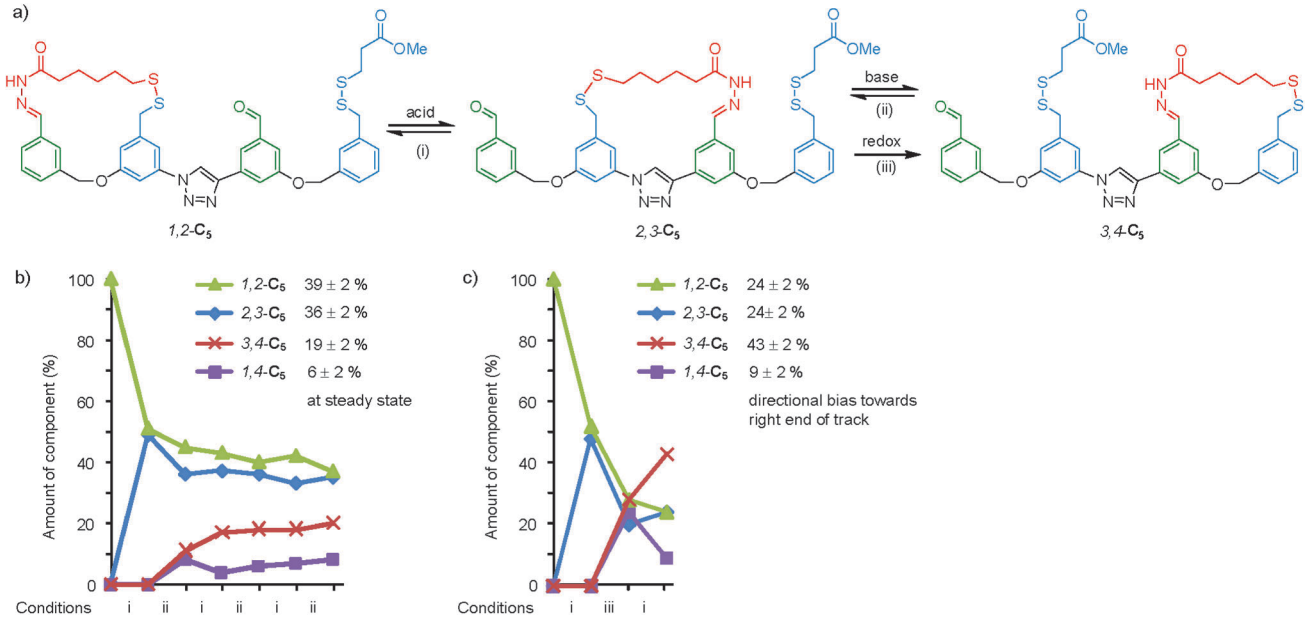


Fig. 11 (a) Oscillation of pH induces the migration of a synthetic small-molecule walker (shown in red) along a four-foothold molecular track (footholds shown in green and blue; linker groups shown in black).[136,137] Replacing the reversible base-induced disulfide exchange reaction with an irreversible two-step redox process transports the walker predominantly to the right hand side of the track (away from the minimum energy distribution). (b) Product distribution during three cycles of directionally-non-biased acid–base operation starting from pristine *1,2*-$\mathbf{C_5}$. Note: the minor isomer *1,4*-$\mathbf{C_5}$ results from folding of the track. (c) Product distribution during 1.5 cycles of directionally-biased acid-redox operation starting from pristine *1,2*-$\mathbf{C_5}$.

joins the other foot to the track is labile allowing that foot to sample two different ('forward' and 'backward') footholds through hydrazone exchange (left hand side in Fig. 11). Under basic conditions the relative kinetic stabilities of the foot–track interactions are reversed and the disulfide foot samples forward and backward binding sites on the track while the hydrazone foot is locked in place (right hand side in Fig. 11).[141] The walker molecule thus randomly and processively takes zero or one steps along the track using primarily a 'hand-over-hand' gait each time the environment is switched between acid and base.

After several such acid–base oscillations, for an ensemble of walker–track conjugates a steady-state distribution of walkers on the four-foothold tracks is reached (Fig. 11b). The same steady-state, minimum energy, distribution is reached irrespective of which end of the track the walker starts from. This mode of operation lacks an energy source and is not directional (at the steady state, the probability for a forward step is equal to that for a backward step).

Replacing the basic step with a redox-mediated disulfide exchange reaction carried out under kinetic control, however, leads to a different population distribution of the sulfur foot between the footholds (Fig. 11c). Alternating between acidic conditions and the redox sequence thus causes the two-legged molecule to walk directionally down the track, away from the minimum energy distribution, by an information ratchet type of Brownian ratchet mechanism (see Section 3.2). A moderate directional bias (a forward step is roughly 1.5 times more likely than a backward step) was achieved in this way.

An investigation of a series of walker–track conjugates,[138] differing in the length of the spacer that separates the feet in

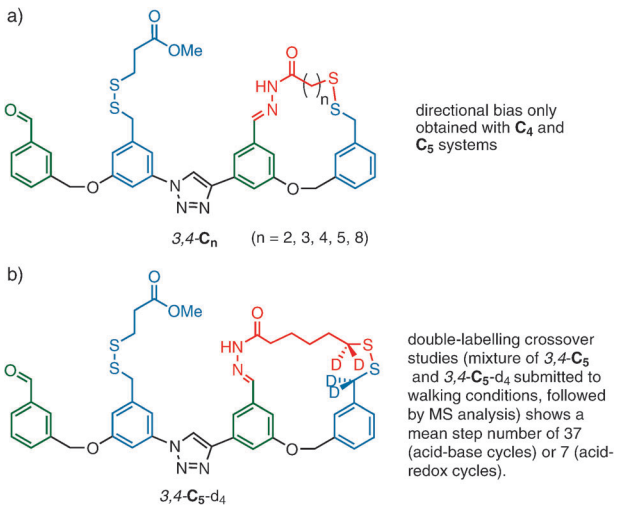


Fig. 12 (a) Chemical structure of a series of small-molecule walker–track conjugates.[138] (b) Chemical structure of fourfold-deuterated walker–track conjugate *3,4*-$\mathbf{C_5}$-d_4, which was used in a double-labelling crossover study to determine the average processivity of walker migration.

the walker (Fig. 12a), revealed that the stride lengths of the two shortest studied walker units (n = 2 and 3) were too short to allow them to bridge the internal footholds. Only for the two systems of intermediate walker length (n = 4 and 5) was significant directional bias observed under acid-redox conditions, interestingly in opposite directions. These results indicate that a certain amount of ring strain is crucial for the emergence of directional dynamics in these systems, a requirement that was

best met by the $\mathbf{C_4}$ system in which a step forward was roughly two times more likely than a step backward.

In a double-labelling crossover study,[136,138] the processivity of the walking process in the $\mathbf{C_5}$ system was determined quantitatively. The double-labelled walker–track conjugate *3,4*-$\mathbf{C_5}$-d_4 (Fig. 12b) was mixed with unlabelled *3,4*-$\mathbf{C_5}$ (Fig. 11) and the mixture subjected to conditions for both directional and non-directional walker migration. Mass spectrometric analysis of the product mixtures revealed an average step number (the number of steps after which 50% of the walkers are no longer attached to their original track) of 37 under acid–base (non-directional) conditions and an average step number of seven under acid-redox (directional) conditions. Loss of processivity does not occur *via* complete detachment of the walker moiety,[141] but through oligomers in which a walker unit bridges two tracks (these are formed in larger quantities during the kinetically-controlled redox step).

A light-driven small-molecule walker, *E*/*Z*-**1**, in which the walker unit can be transported in either direction along a four-foothold track was recently described (Fig. 13).[139] The design is closely related to the original small-molecule walker–track systems, with the crucial addition of a stilbene unit between the internal aldehyde and disulfide footholds of the track.

The key to achieving directionality lies in the isomerisation of the stilbene moiety, through which significant ring strain can be induced in the positional (constitutional) isomer in which the walker unit bridges the stilbene linkage (*E*-*2,3*-**1** in Fig. 13a). $E \rightarrow Z$ isomerisation provides a driving force for the walker to step onto the central stilbene unit, while subsequent $Z \rightarrow E$ isomerisation results in a majority of the walkers being transported away from the stilbene group in a direction determined by which foot–track interaction is labilised next. The overall direction in which walker transport occurs is thus dependant on the sequence of the four applied stimuli: acid or base for mutually-exclusive 'foot' dissociation; UV light or visible light (plus iodine) to induce or release ring strain between the walker and the track.

Such a manipulation of the thermodynamic minima (here by strain induction through stilbene isomerization) and kinetic barriers (here by addition of either base or acid) experienced by a substrate corresponds to an energy ratchet type of Brownian ratchet mechanism (see Section 3.2). The energy required to fuel directional transport in this system (a forward step occurs roughly 1.5 times more likely than a backward step) is all supplied through the $E \rightarrow Z$ photoisomerisation reaction (i; Fig. 13a), that creates configurational strain in the track. The other three reactions (ii–iv; Fig. 13a)—all under thermodynamic control—each dissipate some of that energy in a way designed to achieve the desired directional migration of the walker.

6. Conclusions and outlook

Nature employs linear motor proteins to extraordinary effect, performing numerous complex tasks in the cell and driving chemical systems away from equilibrium. The diversity in the structures and dynamic properties of members of the kinesin, myosin and dynein families illustrate the many different forms that molecular motors can take. In going from simple switches that toggle between two or more states to motors that change the position of their components (or substrates) progressively and repetitively, the incorporation of ratchet mechanisms into chemical structures is required.[15] Molecular bipeds, with their two distinct sites of interaction with a track, offer a logical and effective means of achieving this.

Remarkable progress has been made in a relatively short period in the development of synthetic molecules that can walk along tracks.[142] Some of the recently developed DNA systems, particularly those that move autonomously or can carry out sophisticated tasks (Fig. 8 and Schemes 4–8), are

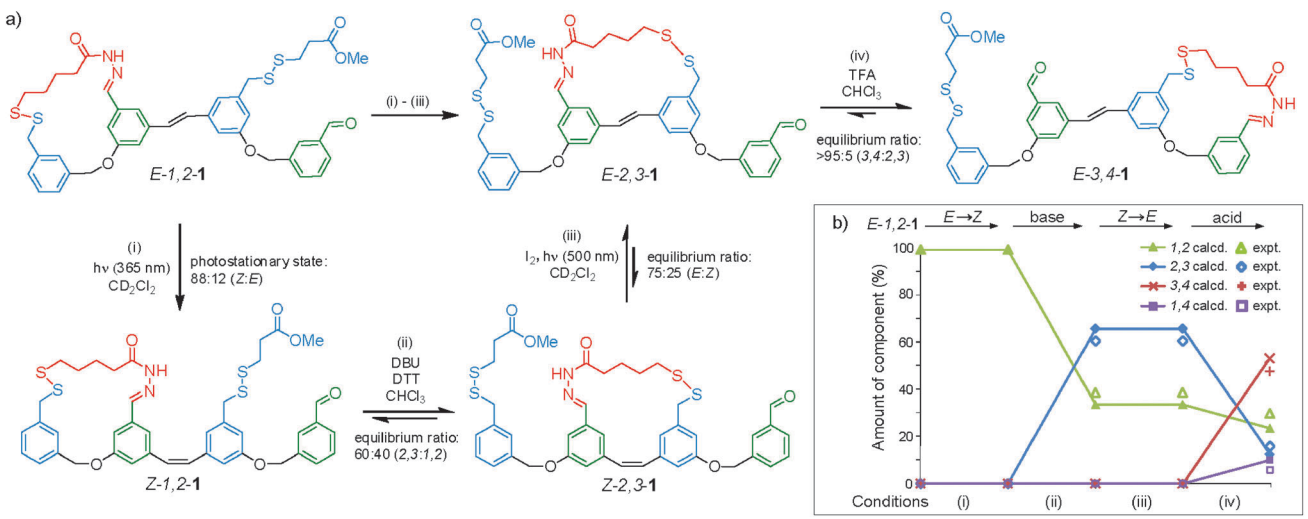


Fig. 13 A light-driven small-molecule walker.[139] (a) Operating mechanism of light-driven walker–track system based on selectively labile feet and adjustable ring strain between the walker (red) and track in one positional isomer. The reaction sequence shown transports the walker from left-to-right; switching steps (ii) and (iv) would cause the walker to be transported preferentially from right-to-left. Stimuli: (i) UV light; (ii) base; (iii) visible light, iodine; (iv) acid. (b) Composition of the positional isomer mixture during one cycle of operation applying stimuli sequence: i–ii–iii–iv (leads to bias towards the right end of the track).

extraordinary feats of molecular-level design. They employ ratchet mechanisms that enable them to exhibit dynamic behaviour that no other synthetic molecular systems have yet achieved. When compared to biological motor proteins a crucial weakness of all the artificial molecular walking motors developed to date is their relatively low efficiency and poor performance characteristics. However, until faster synthetic walkers are developed, hybrid (biological/artificial) devices can still offer a useful compromise. Possible disadvantages in using biological or hybrid walkers for technological applications could be their constraints with respect to fuel (ATP), operating environment (aqueous), modest stability (at least compared to small-molecule systems) and operating characteristics (walking speed or size).

Artificial DNA walkers offer the great practical advantage over small-molecule systems that their synthesis can be achieved by automated synthesizers (often carried out to order by commercial suppliers) and their design, although far from trivial, can be aided by computer programs. Their size is, however, generally similar to that of kinesin-I and, in comparison to small-molecule walkers, chemical stability might be a limitation. Future advances in DNA walkers are likely to feature ever-more extended two- and three-dimensional DNA origami tracks, more sophisticated task performance and attempts to measure physical parameters such as the velocity or the stalling force of artificial motors at the single-molecule level.

Only a few authentic small-molecule bipedal motors have been described to date and they are still slow, inefficient and exhibit modest directional bias. Furthermore, the tracks employed are not particularly rigid, leading to the formation of isomers in which the walker units bridge non-adjacent footholds. However, small-molecule walkers are more fundamental systems than DNA walkers and may ultimately offer some distinct advantages. Control over the sense of the walking direction, already demonstrated in the most recent small-molecule walker study (Fig. 13), is a feature not found even in biological motor proteins. The use of light (or other non-ATP fuels) as an energy source, their small size (typically 10× smaller in each dimension—1000× smaller in terms of molecular weight—than biological or DNA walkers), chemical stability, the ability to operate in different environments and with different chemistries, and the possibility of investigating the detailed nature of their dynamics represent significant potential advantages of small-molecule systems. In the near future studies on small-molecule walkers are likely to focus on new mechanisms for improved directional bias, different walker–track binding chemistries, walker units that can migrate along tracks autonomously, and on the design of new tracks that are rigid, polymeric, have junctions or can be tethered to surfaces.

Artificial molecular walkers have some way to go before their unique dynamic properties can be exploited in practical technological applications, but they have taken their first tentative steps along that path. Perhaps their greatest significance, however, is that for the first time chemists have learnt how to go beyond switches and the properties of molecules based simply on functions of state, and can now start to design molecules that can progressively drive chemical systems away from equilibrium.[15]

Acknowledgements

D.A.L. thanks the European Research Council (ERC) and the Engineering and Physical Sciences Research Council (EPSRC) for their funding of his group's research on synthetic molecular motors and machines.

Notes and references

1 *Molecular Motors*, ed. M. Schliwa, Wiley-VCH, Weinheim, 2003.
2 For a recent review article on the Na/K ion pump see: D. C. Gadsby, *Nat. Rev. Mol. Cell Biol.*, 2009, **10**, 344.
3 For a review article on translocation pores see: Y. Yoneda, *J. Biochem. (Tokyo)*, 1997, **121**, 811.
4 For a recent review article on DNA and RNA helicases see: M. Pyle, *Annu. Rev. Biophys.*, 2008, **37**, 317.
5 For a recent review article on DNA polymerases see: A. J. Berdis, *Chem. Rev.*, 2009, **109**, 2862.
6 For a recent review article on RNA polymerases see: K. S. Murakami and S. A. Darst, *Curr. Opin. Struct. Biol.*, 2003, **13**, 31.
7 For a recent review article on the F_0F_1 ATP synthase motor see: C. von Ballmoos, G. M. Cook and P. Dimroth, *Annu. Rev. Biophys.*, 2008, **37**, 43.
8 For a recent review article on the flagellar motor see: Y. Sowa and R. M. Berry, *Q. Rev. Biophys.*, 2008, **41**, 103.
9 R. D. Vale and R. A. Milligan, *Science*, 2000, **288**, 89.
10 R. D. Vale, *Cell*, 2003, **112**, 467.
11 M. Schliwa and G. Woehlke, *Nature*, 2003, **422**, 759.
12 L. A. Amos, *Cell. Mol. Life Sci.*, 2008, **65**, 509.
13 R. D. Astumian, *Phys. Chem. Chem. Phys.*, 2007, **9**, 5067.
14 R. D. Astumian, *Biophys. J.*, 2010, **98**, 2401.
15 E. R. Kay, D. A. Leigh and F. Zerbetto, *Angew. Chem., Int. Ed.*, 2007, **46**, 72.
16 D. A. Leigh, J. K. Y. Wong, F. Dehez and F. Zerbetto, *Nature*, 2003, **424**, 174.
17 J. V. Hernandez, E. R. Kay and D. A. Leigh, *Science*, 2004, **306**, 1532.
18 M. N. Chatterjee, E. R. Kay and D. A. Leigh, *J. Am. Chem. Soc.*, 2006, **128**, 4058.
19 V. Serreli, C.-F. Lee, E. R. Kay and D. A. Leigh, *Nature*, 2007, **445**, 523.
20 M. Alvarez-Pérez, S. M. Goldup, D. A. Leigh and A. M. Z. Slawin, *J. Am. Chem. Soc.*, 2008, **130**, 1836.
21 For other examples of rotary molecular motors, see: E. M Geertsema, S. J. van der Molen, M. Martens and B. L. Feringa, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 16919 and references therein.
22 J. R. Sellers and C. Veigel, *Curr. Opin. Cell Biol.*, 2006, **18**, 68.
23 H. L. Sweeney and A. Houdusse, *Annu. Rev. Biophys.*, 2010, **39**, 539.
24 M. P. Koonce and M. Samsó, *Trends Cell Biol.*, 2004, **14**, 612.
25 K. Oiwa and H. Sakakibara, *Curr. Opin. Cell Biol.*, 2005, **17**, 98.
26 N. J. Carter and R. A. Cross, *Curr. Opin. Cell Biol.*, 2006, **18**, 61.
27 S. M. Block, *Biophys. J.*, 2007, **92**, 2986.
28 N. Hirokawa, Y. Noda, Y. Tanaka and S. Niwa, *Nat. Rev. Mol. Cell Biol.*, 2009, **10**, 682.
29 T. Mitchison and M. Kirschner, *Nature*, 1984, **312**, 237.
30 F. Kozielski, I. Arnal and R. H. Wade, *Curr. Biol.*, 1998, **8**, 191.
31 K. C. Holmes, D. Popp, W. Gebhard and W. Kabsch, *Nature*, 1990, **347**, 21.
32 K. Shiroguchi and K. Kinosita, *Science*, 2007, **316**, 1208.
33 N. Hirokawa, R. Nitta and Y. Okada, *Nat. Rev. Mol. Cell Biol.*, 2009, **10**, 877.
34 F. J. Kull, E. P. Sablin, R. Lau, R. J. Fletterick and R. D. Vale, *Nature*, 1996, **380**, 550.
35 R. D. Vale, T. S. Reese and M. P. Sheetz, *Cell*, 1985, **42**, 39.
36 J. Howard, A. J. Hudspeth and R. D. Vale, *Nature*, 1989, **342**, 154.
37 S. M. Block, L. S. Goldstein and B. J. Schnapp, *Nature*, 1990, **348**, 348.
38 K. Svoboda, C. F. Schmidt, B. J. Schnapp and S. M. Block, *Nature*, 1993, **365**, 721.

39 S. M. Block, *Trends Cell Biol.*, 1995, **5**, 169.
40 E. Berliner, E. C. Young, K. Anderson, H. K. Mahtani and J. Gelles, *Nature*, 1995, **373**, 718.
41 R. D. Vale, T. Funatsu, D. W. Pierce, L. Romberg, Y. Harada and T. Yanagida, *Nature*, 1996, **380**, 451.
42 W. Hua, E. C. Young, M. L. Fleming and J. Gelles, *Nature*, 1997, **388**, 390.
43 M. J. Schnitzer and S. M. Block, *Nature*, 1997, **388**, 386.
44 S. M. Block, *Cell*, 1998, **93**, 5.
45 S. Rice, A. W. Lin, D. Safer, C. L. Hart, N. Naber, B. O. Carragher, S. M. Cain, E. Pechatnikova, E. M. Wilson-Kubalek, M. Whittaker, E. Pate, R. Cooke, E. W. Taylor, R. A. Milligan and R. D. Vale, *Nature*, 1999, **402**, 778.
46 K. Kawaguchi and S. Ishiwata, *Science*, 2001, **291**, 667.
47 W. R. Schief and J. Howard, *Curr. Opin. Cell Biol.*, 2001, **13**, 19.
48 W. Hua, J. Chung and J. Gelles, *Science*, 2002, **295**, 844.
49 S. Rice, Y. Cui, C. Sindelar, N. Naber, M. Matuska, R. Vale and R. Cooke, *Biophys. J.*, 2003, **84**, 1844.
50 C. L. Asbury, A. N. Fehr and S. M. Block, *Science*, 2003, **302**, 2130.
51 A. Yildiz, M. Tomishige, R. D. Vale and P. R. Selvin, *Science*, 2004, **303**, 676.
52 T. Mori, R. D. Vale and M. Tomishige, *Nature*, 2007, **450**, 750.
53 R. Dixit, J. L. Ross, Y. E. Goldman and E. L. F. Holzbaur, *Science*, 2008, **319**, 1086.
54 V. Bormuth, V. Varga, J. Howard and E. Schaffer, *Science*, 2009, **325**, 870.
55 N. R. Guydosh and S. M. Block, *Nature*, 2009, **461**, 125.
56 E. Toprak, A. Yildiz, M. T. Hoffman, S. S. Rosenfeld and P. R. Selvin, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 12717.
57 G. Woehlke and M. Schliwa, *Nat. Rev. Mol. Cell Biol.*, 2000, **1**, 50.
58 K. J. Verhey and J. W. Hammond, *Nat. Rev. Mol. Cell Biol.*, 2009, **10**, 765.
59 D. D. Hackney, *Nature*, 1995, **377**, 448.
60 R. D. Vale, T. Funatsu, D. W. Pierce, L. Romberg, Y. Harada and T. Yanagida, *Nature*, 1996, **380**, 451.
61 R. B. Case, D. W. Pierce, N. Hom-Booher, C. L. Hart and R. D. Vale, *Cell*, 1997, **90**, 959.
62 L. Romberg, D. W. Pierce and R. D. Vale, *J. Cell Biol.*, 1998, **140**, 1407.
63 K. S. Thorn, J. A. Ubersax and R. D. Vale, *J. Cell Biol.*, 2000, **151**, 1093.
64 J. Yajima, M. C. Alonso, R. A. Cross and Y. Y. Toyoshima, *Curr. Biol.*, 2002, **12**, 301.
65 S. M. Block, *Trends Cell Biol.*, 1995, **5**, 169.
66 For a recent article suggesting a more bio-inspired approach for the design of synthetic molecular motors see: T. Hugel and C. Lumme, *Curr. Opin. Biotechnol.*, 2010, **21**, 683.
67 D. J. G. Bakewell and D. V. Nicolau, *Aust. J. Chem.*, 2007, **60**, 314.
68 A. Agarwal and H. Hess, *Prog. Polym. Sci.*, 2010, **35**, 252.
69 L. Onsager, *Phys. Rev.*, 1931, **37**, 405.
70 *The Second Law of Thermodynamics.* Memoirs by Carnot, Clausius and Thomson: Harper's Scientific Memoirs, ed. W. F. Magie, Harper & Brothers, New York, 1899.
71 R. D. Astumian and M. Bier, *Biophys. J.*, 1996, **70**, 637.
72 M. Bier, *Contemp. Phys.*, 1997, **38**, 371.
73 R. D. Astumian and I. Derényi, *Eur. Biophys. J.*, 1998, **27**, 474.
74 L. Mahadevan and P. Matsudaira, *Science*, 2000, **288**, 95.
75 C. Bustamante, D. Keller and G. Oster, *Acc. Chem. Res.*, 2001, **34**, 412.
76 R. D. Astumian, *Appl. Phys. A: Mater. Sci. Process.*, 2002, **75**, 193.
77 P. Reimann, *Phys. Rep.*, 2002, **361**, 57.
78 B. J. Gabryś, K. Pesz and S. J. Bartkiewicz, *Physica A (Amsterdam)*, 2004, **336**, 112.
79 M. Kurzynski and P. Chełminiak, *Physica A (Amsterdam)*, 2004, **336**, 123.
80 P. Hänggi and F. Marchesoni, *Rev. Mod. Phys.*, 2009, **81**, 387.
81 F. Jülicher, A. Ajdari and J. Prost, *Rev. Mod. Phys.*, 1997, **69**, 1269.
82 R. D. Astumian, *Science*, 1997, **276**, 917.
83 A. Mogilner and G. Oster, *Curr. Biol.*, 2003, **13**, R721.
84 G. Oster and H. Y. Wang, *Trends Cell Biol.*, 2003, **13**, 114.
85 K. Shiroguchi and K. Kinosita, *Science*, 2007, **316**, 1208.
86 T. R. Kelly, H. De Silva and R. A. Silva, *Nature*, 1999, **401**, 150.
87 D. A. Leigh and E. M. Pérez, *Top. Curr. Chem.*, 2006, **265**, 185.
88 For a recent highlight article on selected DNA-based molecular walkers that includes an insightful discussion of their strengths and limitations see: F. C. Simmel, *ChemPhysChem*, 2009, **10**, 2593.
89 W. B. Sherman and N. C. Seeman, *Nano Lett.*, 2004, **4**, 1203.
90 T. LaBean, H. Yan, J. Kopatsch, F. Liu, E. Winfree, J. H. Reif and N. C. Seeman, *J. Am. Chem. Soc.*, 2000, **122**, 1848.
91 J. SantaLucia, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 1460.
92 J.-S. Shin and N. A. Pierce, *J. Am. Chem. Soc.*, 2004, **126**, 10834.
93 H. Gu, J. Chao, S.-J. Xiao and N. C. Seeman, *Nature*, 2010, **465**, 202.
94 L. M. Smith, *Nature*, 2010, **465**, 167.
95 P. W. K. Rothemund, *Nature*, 2006, **440**, 297.
96 E. H. C. Bromley, N. J. Kuwada, M. J. Zuckermann, R. Donadini, L. Samii, G. A. Blab, G. J. Gemmen, B. J. Lopez, P. M. G. Curmi, N. R. Forde, D. N. Woolfson and H. Linke, *HFSP J.*, 2009, **3**, 204.
97 N. J. Kuwada, G. A. Blab and H. Linke, *Chem. Phys.*, 2010, **375**, 479.
98 C. Wang, J. Ren and X. Qu, *Chem. Commun.*, 2011, **47**, 1428.
99 P. Yin, H. Yan, X. G. Daniell, A. J. Turberfield and J. H. Reif, *Angew. Chem., Int. Ed.*, 2004, **43**, 4906.
100 J. Bath, S. J. Green and A. J. Turberfield, *Angew. Chem., Int. Ed.*, 2005, **44**, 4358.
101 Y. Tian, Y. He, Y. Chen, P. Yin and C. Mao, *Angew. Chem., Int. Ed.*, 2005, **44**, 4355.
102 T. R. Kelly, *Angew. Chem., Int. Ed.*, 2005, **44**, 4124.
103 R. R. Breaker and G. F. Joyce, *Chem. Biol.*, 1994, **1**, 223.
104 S. Venkataraman, R. M. Dirks, P. W. K. Rothemund, E. Winfree and N. A. Pierce, *Nat. Nanotechnol.*, 2007, **2**, 490.
105 E. Gouin, M. D. Welch and P. Cossart, *Curr. Opin. Microbiol.*, 1999, **8**, 35.
106 A. J. Turberfield, J. C. Mitchell, B. Yurke, A. P. Mills, M. I. Blakey and F. C. Simmel, *Phys. Rev. Lett.*, 2003, **90**, 118102.
107 P. Yin, H. M. T. Choi, C. R. Calvert and N. A. Pierce, *Nature*, 2008, **451**, 318.
108 S. J. Green, J. Bath and A. J. Turberfield, *Phys. Rev. Lett.*, 2008, **101**, 238101.
109 J. Bath, S. J. Green, K. E. Allen and A. J. Turberfield, *Small*, 2009, **5**, 1513.
110 T. Omabegho, R. Sha and N. C. Seeman, *Science*, 2009, **324**, 67.
111 W. Sherman, *Science*, 2009, **324**, 46.
112 R. Pei, S. K. Taylor, D. Stefanovic, S. Rudchenko, T. E. Mitchell and M. N. Stojanovic, *J. Am. Chem. Soc.*, 2006, **128**, 12693.
113 K. Lund, A. J. Manzo, N. Dabby, N. Michelotti, A. Johnson-Buck, J. Nangreave, S. Taylor, R. Pei, M. N. Stojanovic, N. G. Walter, E. Winfree and H. Yan, *Nature*, 2010, **465**, 206.
114 Y. He and D. R. Liu, *Nat. Nanotechnol.*, 2010, **5**, 778.
115 For details on rearrangement reactions, see: strategic applications of named reactions in organic synthesis, L. Kürti and B. Czakó, Elsevier, Oxford, 2005 and references therein.
116 G. B. Payne, *J. Org. Chem.*, 1962, **27**, 3819.
117 See: J. P. Ryan and P. R. O'Connor, *J. Am. Chem. Soc.*, 1952, **74**, 5866 and references therein.
118 A. R. Lippert, J. Kaeobamrung and J. W. Bode, *J. Am. Chem. Soc.*, 2006, **128**, 14738.
119 A. R. Lippert, V. L. Keleshian and J. W. Bode, *Org. Biomol. Chem.*, 2009, **7**, 1529.
120 A. R. Lippert, A. Nagawana, V. L. Keleshian and J. W. Bode, *J. Am. Chem. Soc.*, 2010, **132**, 15790.
121 D. Strawser, A. Karton, O. V. Zenkina, M. A. Iron, L. J. W. Shimon, J. M. L. Martin and M. E. van der Boom, *J. Am. Chem. Soc.*, 2005, **127**, 9322.
122 K. H. Dötz and H. C. Jahr, *Chem. Rec.*, 2004, **4**, 61.
123 For an example of a competition between acetal hydrolysis and acetal migration on a pyranose scaffold see: N. A. Hughes, *Carbohydr. Res.*, 1968, **7**, 474.
124 S. Mitra and R. G. Lawton, *J. Am. Chem. Soc.*, 1979, **101**, 3097.
125 F. A. Liberatore, R. D. Comeau, J. M. McKearin, D. A. Pearson, B. Q. Belonga, S. J. Brocchini, J. Kath, T. Phillips, K. Oswell and R. G. Lawton, *Bioconjugate Chem.*, 1990, **1**, 36.

126 R. B. del Rosario, S. J. Brocchini, R. G. Lawton, R. L. Wahl and R. Smith, *Bioconjugate Chem.*, 1990, **1**, 50.
127 R. B. del Rosario, L. A. Baron, R. G. Lawton and R. L. Wahl, *Nucl. Med. Biol.*, 1992, **19**, 417.
128 R. Tkachov, V. Senkovskyy, H. Komber, J.-U. Sommer and A. Kiriy, *J. Am. Chem. Soc.*, 2010, **132**, 7803.
129 M. Möhring and G. Fink, *Angew. Chem., Int. Ed. Engl.*, 1985, **24**, 1001.
130 L. K. Johnson, C. M. Killian and M. Brookhart, *J. Am. Chem. Soc.*, 1995, **117**, 6414.
131 D. P. Weimann, H. D. F. Winkler, J. A. Falenski, B. Koksch and C. A. Schalley, *Nat. Chem.*, 2009, **1**, 573.
132 H. D. F. Winkler, D. P. Weimann, A. Springer and C. A. Schalley, *Angew. Chem., Int. Ed.*, 2009, **48**, 7246.
133 K.-Y. Kwon, K. L. Wong, G. Pawin, L. Bartels, S. Stolbov and T. S. Rahman, *Phys. Rev. Lett.*, 2005, **95**, 166101.
134 For a similar study demonstrating one-dimensional surface diffusion of an anthraquinone molecule and its ability to reversibly bind CO_2 molecules, see: K. L. Wong, G. Pawin, K.-Y. Wong, X. Lin, T. Jiao, U. Solanki, R. H. J. Fawcett, L. Bartels, S. Stolbov and T. S. Rahman, *Science*, 2007, **315**, 1391.
135 For a related quadrupedal surface-walker, see: Z. Cheng, E. S. Chu, D. Sun, D. Kim, M. Luo, G. Pawin, K. L. Wong, R. Carp, M. Marsella and L. Bartels, *J. Am. Chem. Soc.*, 2010, **132**, 13578.
136 M. von Delius, E. M. Geertsema and D. A. Leigh, *Nat. Chem.*, 2010, **2**, 96.
137 S. Otto, *Nat. Chem.*, 2010, **2**, 75.
138 M. von Delius, E. M. Geertsema, D. A. Leigh and D.-T. D. Tang, *J. Am. Chem. Soc.*, 2010, **132**, 16134.
139 M. J. Barrell, A. G. Campaña, M. von Delius, E. M. Geertsema and D. A. Leigh, *Angew. Chem., Int. Ed.*, 2011, **50**, 285.
140 P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders and S. Otto, *Chem. Rev.*, 2006, **106**, 3652.
141 M. von Delius, E. M. Geertsema and D. A. Leigh, *Org. Biomol. Chem.*, 2010, **8**, 4617.
142 For an account of recent developments in synthetic small molecule walkers, see:E. M. Pérez, *Angew. Chem., Int. Ed.*, 2011, DOI: 10.1002/anie.201006946.

