FEBRUARY 2010 VOL 2 NO 2
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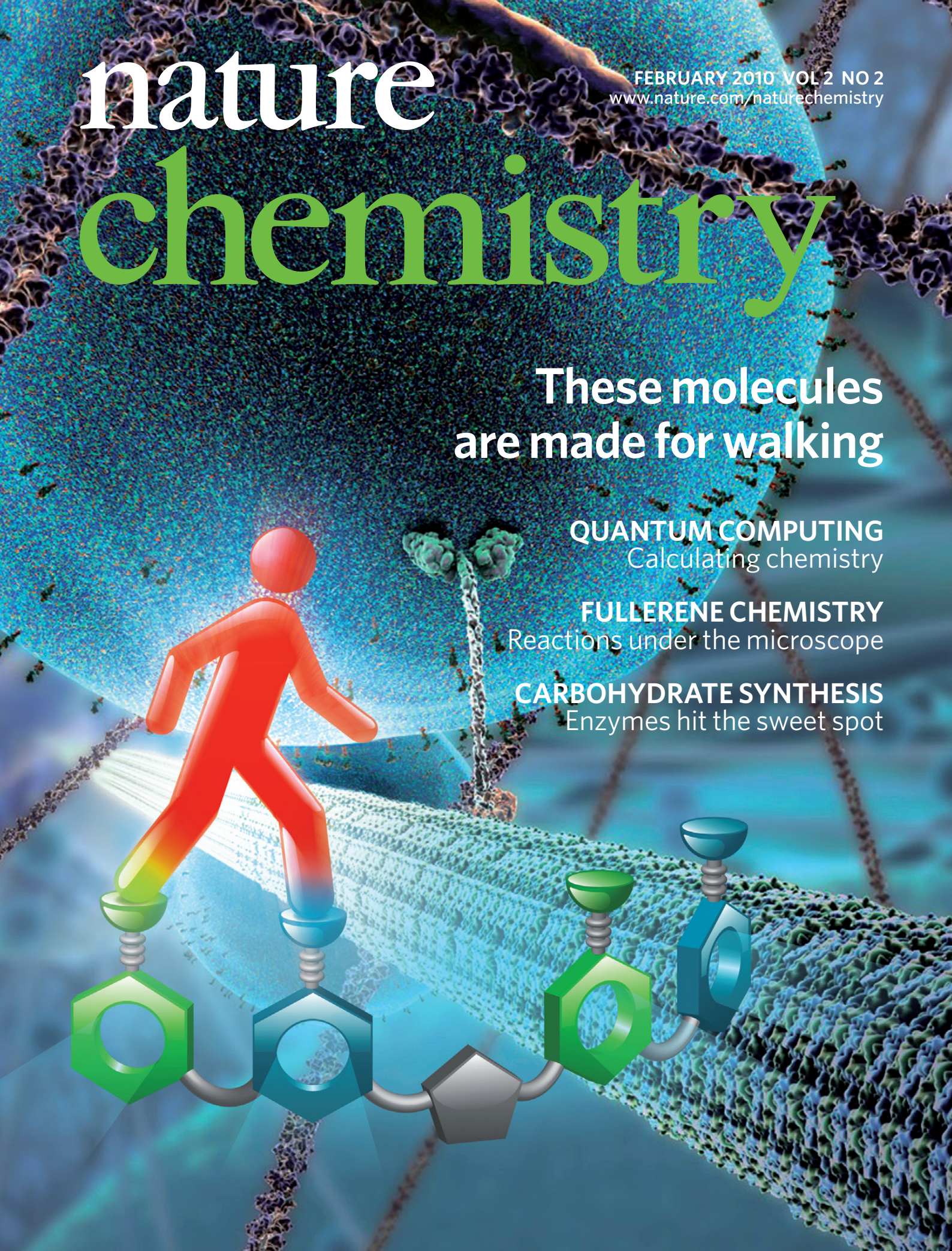

These molecules are made for walking

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PUBLISHED ONLINE: 20 DECEMBER 2009 | DOI: 10.1038/NCHEM.481
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A synthetic small molecule that can walk down a track

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Although chemists have made small-molecule rotary motors, to date there have been no reports of small-molecule linear motors. Here we describe the synthesis and operation of a 21-atom two-legged molecular unit that is able to walk up and down a four-foothold molecular track. High processivity is conferred by designing the track-binding interactions of the two feet to be labile under different sets of conditions such that each foot can act as a temporarily fixed pivot for the other. The walker randomly and processively takes zero or one step along the track using a 'passing-leg' gait each time the environment is switched between acid and base. Replacing the basic step with a redox-mediated, disulfide-exchange reaction directionally transports the bipedal molecules away from the minimum-energy distribution by a Brownian ratchet mechanism. The ultimate goal of such studies is to produce artificial, linear molecular motors that move directionally along polymeric tracks to transport cargoes and perform tasks in a manner reminiscent of biological motor proteins.

Molecular motors are used throughout biology to drive chemical systems away from equilibrium, and thereby enable tasks to be performed, cargoes to be transported directionally and work to be done[1]. Spectacular examples include the kinesin, myosin and dynein bipedal motor proteins, which are directionally driven along microtubule or actin filament tracks by adenosine triphosphate hydrolysis[1]. Despite fundamental advances in recent years[2–10], most of the artificial molecular machines prepared so far fall well short of the degree of control over motion exhibited by such biomolecules[11]. To date the only synthetic structures that can, even in principle, take repetitive, processive (that is, without detaching or exchanging with other molecules in the bulk) steps along a molecular track are systems constructed from DNA[12–19].

Here we report the synthesis and operation of a synthetic small molecule that is able to walk repetitively up and down a four-foothold molecular track in response to a changing chemical environment. High processivity is conferred by designing the track-binding interactions of the two feet to be labile under different sets of conditions (acidic and basic). Under acidic conditions the disulfide bond between one foot of the walker and the track is locked kinetically, and the hydrazone unit that joins the other foot to the track is labile, which allows that foot to sample two different (forward and backward) footholds through hydrazone exchange. Under basic conditions the relative kinetic stabilities of the foot–track interactions are reversed, so the disulfide foot samples the forward and backward binding sites on the track, and the hydrazone foot is locked in place. The walker molecule thus randomly and processively takes zero or one step along the track using a passing-leg gait each time the environment is switched between acidic and basic. For an ensemble of walker–track conjugates a steady-state, minimum-energy distribution of walkers on the four-foothold tracks is reached after several acid–base oscillations, irrespective of which end of the track the walkers start from. Alternating between acidic conditions and a kinetically controlled redox reaction causes the two-legged molecule to walk down the track with inherent directionality through an information-ratchet[10,11,20,21] type of Brownian ratchet mechanism.

Results

Design of a dynamically processive, small-molecule walker–track system. Motors and machines at the molecular level operate by (and must be designed according to) chemical laws and statistical mechanisms, not the Newtonian laws for momentum and inertia that dictate the mechanisms of mechanical machines in the macroscopic world[11,20]. The design outline for the small-molecule walker–track system intended to mimic some of the basic characteristics of linear motor protein dynamics, namely progressive, processive and repetitive motion of a walker unit up and down a molecular track without fully detaching or exchanging with others in the bulk, is shown in Fig. 1. The walker unit (shown in red) traverses the track by a passing-leg gait that involves two chemically different 'feet' ('A' and 'B'), which reversibly bind to different regions of the track. Linear motor proteins and the synthetic DNA walkers use non-covalent interactions for track binding, but our understanding of how to lock and release sequentially and kinetically different artificial hydrogen-bond recognition sites (for example) in the desired manner is beyond the capabilities of present-day synthetic supramolecular chemistry.

We instead chose to use dynamic covalent chemistry[22–24] for this purpose, which combines some of the features of supramolecular chemistry (reversibility, dynamics) with those of covalent bond chemistry (bond strength, robustness). To prevent the walker from completely detaching from the track during the walking process, the different feet form covalent bonds that are labile or kinetically locked under different sets of conditions[25–27]. Under condition I (Fig. 1a), foot A can dissociate from the track and then rebind, either to its original foothold or to another one within reach, and foot B remains in position on the track. Under condition II (Fig. 1b) the situation is reversed and foot B can detach from the track and rebind, with the bond between foot A and the track locked kinetically. Switching repeatedly between conditions I and II should cause the walker to move randomly up and down the track, taking zero or one step each time the conditions change. If the reactions employed are fully reversible then, without the consumption of any additional fuel, the oscillation in conditions eventually leads to a steady-state, energy-minimum distribution of the walker on the track. Interestingly, although this distribution is arrived at through a process analogous to that of attaining a thermodynamic equilibrium (it is the average of a statistical sampling of exchanging chemical entities), each walker–track positional (constitutional) isomer is actually only ever in chemical equilibrium with a limited number of the other isomers. For

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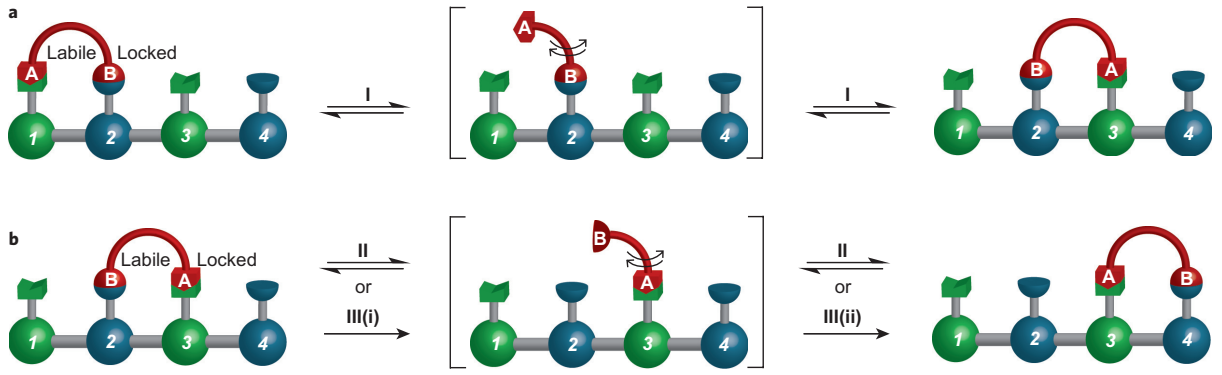


Figure 1 | Binding requirements for the processive migration of a two-legged walker molecule (red) along a track that features two possible binding sites (green and blue) for each foot. For the walking action to be processive, the two feet of the walker (A and B) must not be disconnected from the track at the same time (in the two key intermediate states, shown in square brackets, one or other foot is disconnected from the track). A way to achieve this is to design the foot–track bond-forming and -breaking events to occur under different sets of conditions (I and either II or III) for each foot. If conditions are used in which the chemical reaction that generates one of the two steps proceeds with a different forward–backward ratio to the other step (pathway I + III), directional processive transport of the walker along the track occurs.

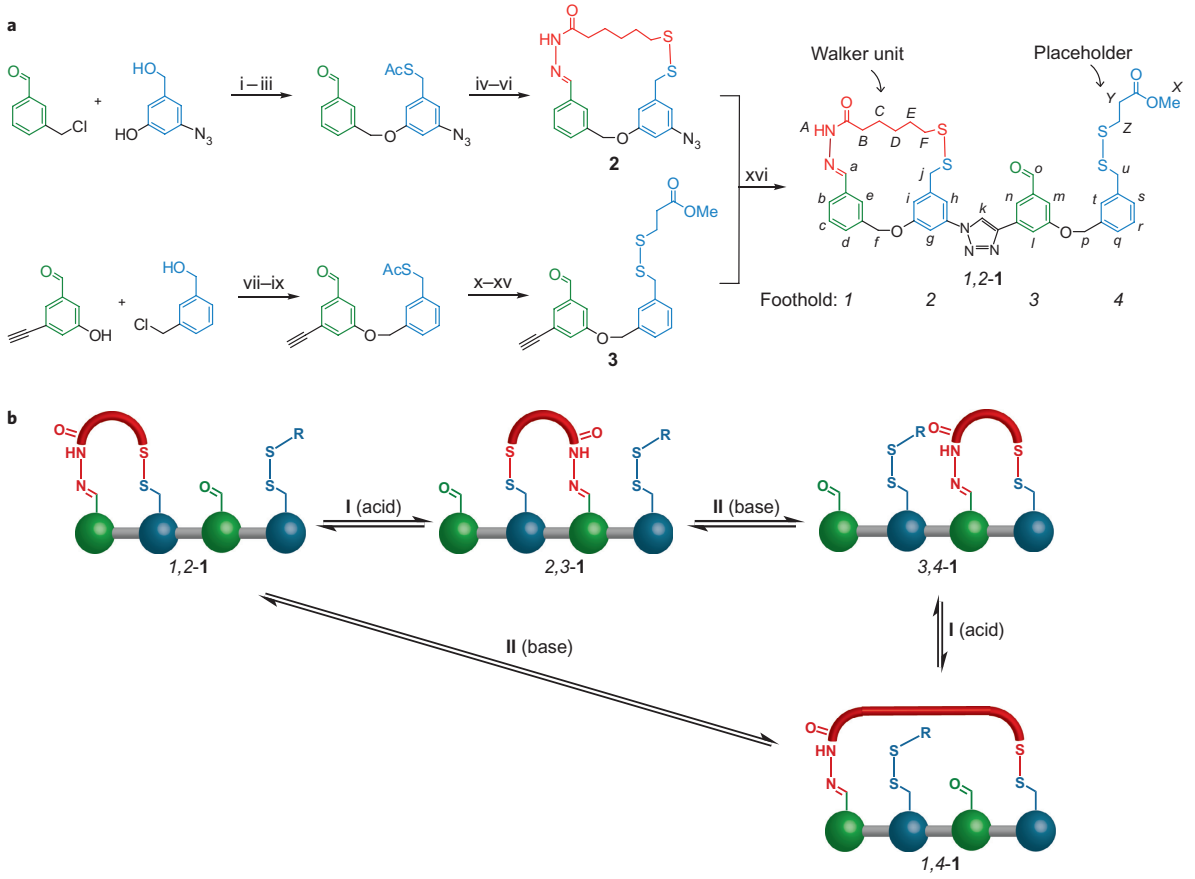


Figure 2 | Synthesis and operation of molecular walker–track conjugate *1,2*-1 under different sets of conditions (acid–base) for reversible covalent bonding of each foot with footholds on the track. a, Synthesis of *1,2*-**1**: i, NaH, dimethylformamide (DMF), room temperature (RT), 16 h, 88%; ii, methanesulfonyl chloride (MsCl), Et_3N, CH_2Cl_2, 0 °C, 30 min; iii, potassium thioacetate (KSAc), DMF, RT, 3 h, 77% (over two steps); iv, 6-mercaptohexanoic acid hydrazide, AcOH, MeOH, RT, 2 h, 78%; v, NaOMe, MeOH, RT, 2 h; vi, I_2, KI, CH_2Cl_2, RT, 5 min, 32% (over two steps); vii, NaH, DMF, 0 °C to RT, 16 h, 65%; viii, MsCl, Et_3N, CH_2Cl_2, RT, 16 h; ix, KSAc, DMF, RT, 3 h, 66% (over two steps); x, $HC(OMe)_3$, *p*-toluenesulfonic acid, MeOH, RT, 30 min; xi, NaOMe, MeOH, RT, 30 min; xii, 3-mercaptopropionic acid, I_2, KI, CH_2Cl_2, RT, 5 min; xiii, TFA, CH_2Cl_2, RT, 30 min, 58% (over four steps); xiv, H_2SO_4, MeOH, RT, 16 h; xv, TFA, CH_2Cl_2, RT, 30 min, 40% (over two steps); xvi, $Cu(MeCN)_4PF_6$, tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine, CH_2Cl_2–tetrahydrofuran–MeOH, RT, 16 h, 79%. **b**, Local equilibria that connect the four positional isomers *1,2*-**1**, *2,3*-**1**, *3,4*-**1** and *1,4*-**1** under various conditions. The upper pathway represents the major passing-leg mechanism from *1,2*-**1** to *3,4*-**1** (through *2,3*-**1**), and the lower pathway (through *1,4*-**1**) is a minor double-step mechanism. Oscillation between acid (condition I) and base (condition II) leads to a steady-state distribution of 39:36:19:6 (±2) for *1,2*-**1**:*2,3*-**1**:*3,4*-**1**:*1,4*-**1** (see Fig. 4).

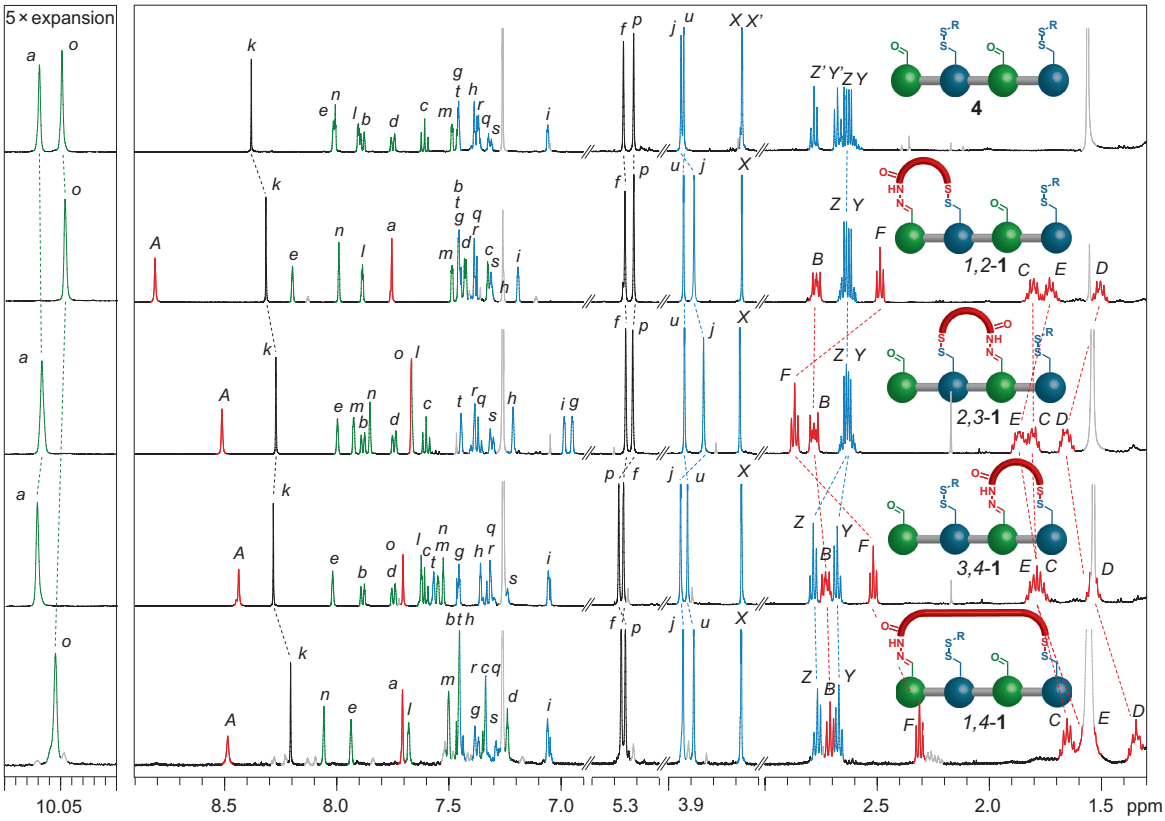


Figure 3 | Partial ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) spectra of molecular track 4 and the four walker–track positional isomers *1,2*-1, *2,3*-1, *3,4*-1 and *1,4*-1. Dotted lines connect signals that differ significantly in chemical shift as a result of the position of the red walker unit. For example, the expanded aldehyde region (framed) shows distinctive shifts for the two chemically different protons labelled *o* and *a* (see chemical structures in Fig. 2 for the labelling system).

example, there is no single set of conditions whereby the *1,2*-isomer (Fig. 1a) is able to exchange with the *3,4*-isomer (Fig. 1b). Their interconversion only occurs through the *2,3*-isomer, which under condition I is in equilibrium with the *1,2*-isomer and under condition II is in equilibrium with the *3,4*-isomer. This unusual property of the system confers processivity on the walking process.

Synthesis, characterization and directionally unbiased walking. A walker–track conjugate, *1,2*-**1**, in which the molecular walker is attached to a four-foothold track by hydrazone (labile in acid, locked in base) and disulfide (labile in base, locked in acid) linkages[26,27], was constructed as shown in Fig. 2 (see Supplementary Information for the full experimental procedures and compound characterization). The four-foothold track contains two potential sites of attachment for the hydrazide foot (position *1* and the aldehyde at position *3*, shown in green) and two potential sites of attachment for the sulfur foot (position *2* and the benzylic mercaptan at position *4* (shown in blue), masked with a 'placeholder' aliphatic unit as a disulfide in *1,2*-**1**).

The starting position of the walker at footholds *1* and *2* in the walker–track conjugate was established by synthesizing macrocycle **2** and coupling this to the building block **3** that contained footholds *3* and *4* (Fig. 2a, step xvi). We also prepared isomer *3,4*-**1** unambiguously through synthesis (see Supplementary Information) and isolated and characterized isomers *2,3*-**1** and *1,4*-**1** from reactions that could not produce unknown positional isomers. The partial ^{1}H NMR spectra of the four walker–track positional isomers *1,2*-**1**, *2,3*-**1**, *3,4*-**1** and *1,4*-**1** confirmed their structures and, together with that of the parent track **4**, are shown in Fig. 3. Although the structural similarity between the compounds is immediately evident from the spectra, differences in chemical shift occur for the protons of the methylene groups in the five-carbon spacer unit (shown in red, H_B and H_F), which reflect the difference in the shape and environment of the macrocycle formed between the walker and track in each isomer. A useful probe for the position of the walker is shown in the expanded aldehyde region at a chemical shift around 10.05 parts per million (ppm) (Fig. 3). In the four walker–track positional isomers the aldehyde proton (H_o in *1,2*-**1** and *1,4*-**1**; H_a in *2,3*-**1** and *3,4*-**1**) is diagnostic of the aldehyde foothold that is free. Similarly, the methylene protons (H_Z and H_Y) serve as distinctive markers for the position of the placeholder disulfide.

The dynamic behaviour of the walker–track conjugate system on cycling between acid and base is shown in Fig. 4. When a dilute solution of *1,2*-**1** in chloroform ($CHCl_3$) was treated with a catalytic amount of trifluoroacetic acid (TFA), intramolecular hydrazone exchange gave rise to a mixture of *1,2*-**1** and its positional isomer *2,3*-**1** in a 51:49 ratio (Fig. 4b, cycle 1, condition I). Treatment of this mixture with a strong base, *1,8*-diazabicyclo[5.4.0]undec-7-ene (DBU), D,L-dithiothreitol (DTT) (which promotes disulfide exchange by acting as a source of a nucleophilic thiolate anion[28]) and dimethyl 3,3′-disulfanediyldipropanoate ($(MeO_2CCH_2CH_2S)_2$), the placeholder disulfide, generated a mixture of all four positional isomers, *1,2*-**1**, *2,3*-**1**, *3,4*-**1** and *1,4*-**1**, in a 45:36:11:8 ratio, as determined by high-performance liquid chromatography (HPLC) (Fig. 4b, cycle 1, condition II; see Supplementary Information for details). Switching between conditions I and II over several cycles (Fig. 4a,i) led to convergence of the distribution of the positional isomers to a ratio for *1,2*-**1**:*2,3*-**1**:*3,4*-**1**:*1,4*-**1** of 39:36:19:6 (±2) (Fig. 4b). A very similar distribution of isomers was obtained by starting from pristine *3,4*-**1** and carrying out the operation sequence over two or three cycles (Figs 4a,ii and 4b). Thus, irrespective of which end of the track the walker starts

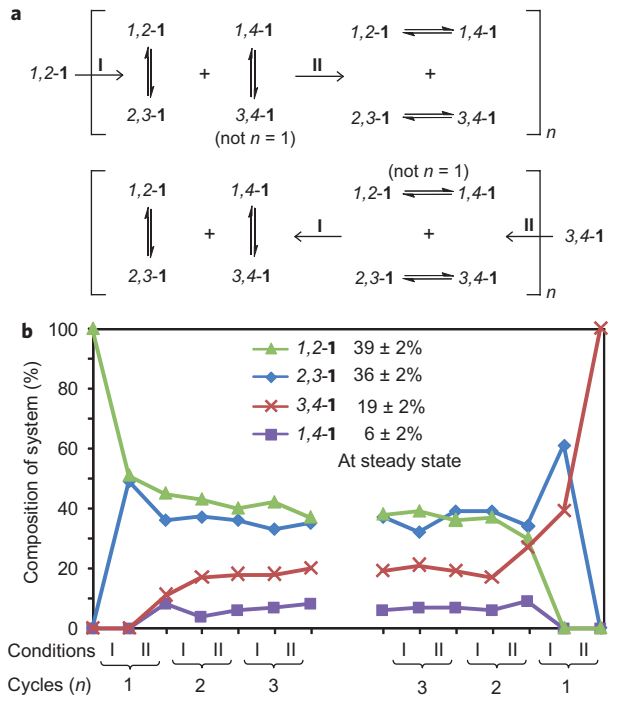


Figure 4 | Dynamic behaviour of molecular walker–track conjugates *1,2*-1 and *3,4*-1, each under cycling of the conditions (acid–base) for reversible covalent bonding of each foot with pairs of footholds on the track. a, Experimental sequence that, over a number of operational cycles (*n*), leads to a steady-state, minimum-energy distribution of the walker on the track. Condition I (reversible hydrazone exchange): 0.1 mM, TFA, $CHCl_3$, RT, 6–96 h (allowed to continue until the distribution no longer changed, as monitored by HPLC). Condition II (reversible disulfide exchange): 0.1 mM, DTT (10 equiv.), DBU (40 equiv.), $(MeO_2CCH_2CH_2S)_2$ (20 equiv.), $CHCl_3$, RT, 12–48 h (allowed to continue until the distribution no longer changed, as monitored by HPLC). **b**, Product distribution. Under each set of conditions (I and II) two different pairs of positional isomers are in equilibrium. Values are based on HPLC integration and are corrected for the absorbance coefficients at 290 nm (see Supplementary Information).

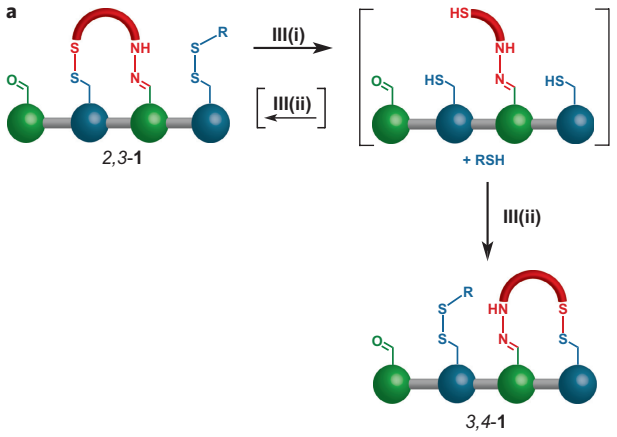
from, the effect of the passing-leg gait operations is to reach the same (global minimum energy) distribution of positional isomers of the walker on the track.

In addition to the major passing-leg gait mechanism for the route from *1,2*-**1** to *3,4*-**1**, the somewhat unexpected presence of *1,4*-**1** in the product distributions (albeit in small amounts, 4–10%) (we originally suspected it might be too strained to be present in observable quantities using reversible chemical reactions) provides a minor 'double-step' route from *1,2*-**1** to *3,4*-**1** (Fig. 2b). It is interesting that occasional statistical 'errors' to the major pathway mechanisms also occur with some biological motor proteins[1,29].

Processivity studies of directionally unbiased walking. To determine the processivity of the walking experiments shown in Fig. 4, we carried out double-labelling cross-over experiments (see Supplementary Information for details). Deuterium labels were introduced into both the walker moiety (d_2 unit at position *F*, Fig. 2) and the track part (d_2 unit at position *u*, Fig. 2) of the molecule in *3,4*-**1** (the labelling experiments were performed on *3,4*-**1** rather than *1,2*-**1** solely for synthetic convenience). The doubly labelled walker–track conjugate was mixed with an unlabelled sample of *3,4*-**1** in a 1:1 ratio. This mixture was subjected to the walking-cycle conditions shown in Fig. 4 and the product distribution analysed by HPLC-MS. The small amount (typically less than 1%) of singly labelled products obtained (a labelled walker on an unlabelled track or an unlabelled walker on a labelled track) can occur only by the swapping of a walker between tracks, and so by comparing the amount of doubly labelled and unlabelled products with that of the singly labelled products the processivity of the walking process could be determined quantitatively. The results (see Supplementary Information) show that the small-molecule walker is highly processive under the operating conditions, with a mean step number of 37 before losing its processivity, which corresponds to an average run length of ~26 nm on a hypothetical infinite track. In comparison, wild-type kinesins typically exhibit a mean step number in the range 75–175 (refs 30,31) and an average run length of ~1 μm (refs 30,31) (the stride lengths of wild-type kinesins are ~10 times longer than that of the 21-atom walker reported here).

Directionally biased walking. Starting with the walker at one end of the track (that is, pristine *1,2*-**1** or *3,4*-**1**) and cycling between conditions I and II results in some walkers moving from one end of the track to the other (Fig. 4). However, this is a consequence of the initial distribution of walker–track conjugates not being at the minimum-energy distribution and the system relaxing towards it, so the walking sequence outlined in Fig. 4a is not intrinsically directional. (By this we mean that in the internal region of a polymeric track made of alternating benzaldehyde and benzylic disulfide footholds, the walker would move, over several acid–base oscillations, in each direction with equal probability.) The lack of intrinsic directionality in the movement of the walker may appear counterintuitive given the stimuli-induced changes in its position reported in Fig. 4b, but (ignoring substituents and the minor *1,4*-isomer) only two fundamentally different types of macrocycles are formed between the walker and the track—one in which the track ether linkage is internal to the macrocycle (present in *1,2*-**1** and *3,4*-**1**) and one in which the track triazole unit is internal to the macrocycle (*2,3*-**1**). Each operation I or II thermodynamically equilibrates this pair of macrocycles about the pivot (kinetically locked) foot and, unless the macrocycles happen to have different relative thermodynamic stabilities in acid and base, the ratio produced should not depend on which foot, disulfide or hydrazone, is fixed to the track. The difference in the amount of *1,2*-**1** and *3,4*-**1** present in the minimum-energy distribution is, therefore, largely a consequence of the different substitution patterns on the macrocycles in these compounds. In the internal region of a polymeric track made of alternating benzaldehyde and benzylic disulfide footholds no net directionality of walking would occur using a pair of operations that give the same ratio (for example, both give the thermodynamic ratio) of the two different macrocycles.

To create a system in which the walker can be transported with a directional bias, even in the middle of a polymeric track, and therefore be able to transport cargo or generate a progressively increasing force, it is necessary to replace one of the walking operations with a step that proceeds with a different forward–backward ratio to the other step. This was achieved for the four-foothold track by replacing the base-promoted disulfide exchange reaction with a two-stage redox process (Fig. 5a,b) in which the ring-opened intermediate (shown in square brackets) is generated in the absence of the placeholder disulfide (1 mM walker–track conjugate, DBU (3 equiv.), DTT (6 equiv.), $CHCl_3$, reflux), and then reoxidized rapidly with iodine (0.1 mM walker–track conjugate, I_2 (12 equiv.), $MeO_2CCH_2CH_2SH$ (8 equiv.), Et_3N, $CHCl_3$/MeOH 1:1) to regenerate the walker–track disulfide linkages. As the reoxidation occurs virtually instantaneously and is irreversible under the reaction conditions, reattachment of the sulfur foot to the track occurs under kinetic control and gives a product ratio different to that achieved by the reversible disulfide-exchange reaction. The effect of introducing this operation into the walking sequence is shown in Fig. 5c. In just 1.5 cycles starting from 100%, *1,2*-**1** (the walker) moves down the track to give 43% *3,4*-**1**, compared to the 19% of



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Cycles	0	0.5	1	1.5	Steady state from Fig. 4b, *n* = 3
Conditions		I	III	I	
1,2-**1**	100	52	28	24	39
2,3-**1**	0	48	20	24	36
3,4-**1**	0	0	28	**43**	**19**
1,4-**1**	0	0	24	9	6

Figure 5 | Processive directionally biased walk from *1,2*-1 under cycling of conditions (acid–redox) for covalent bonding of each foot with pairs of footholds on the track. a, Redox-mediated reaction sequence illustrated for *2,3*-**1**. III(i): reductive ring-opening quantitatively generates the trithiol. III(ii): rapid oxidation recyclizes to give the disulfides. The redox sequence is carried out under kinetic control and gives a different product ratio to the base-promoted, reversible disulfide exchange shown in Figs 2 and 4. This bias in the product distribution leads to an inherently directional transport of the walker away from the minimum-energy distribution. **b**, Reaction sequence. Condition I (reversible hydrazone exchange): 0.1 mM, TFA, $CHCl_3$. Condition III (kinetically controlled disulfide exchange): III(i) 1.0 mM, DTT (6 equiv.), DBU (3 equiv.), $CHCl_3$, reflux, 2–12 h (allowed to continue until the reduction of all disulfide bonds was complete, as monitored by ^{1}H NMR spectroscopy); III(ii) $MeO_2CCH_2CH_2SH$ (8 equiv.), I_2, Et_3N, $CHCl_3$/MeOH 1:1, RT, 5 min. **c**, Evolution of the mixture of positional isomers over three acid–redox operations compared to the acid–base sequence. The right-hand column shows the composition of the mixture at its steady state using the acid–base conditions (see Fig. 4b). The remarkable difference in the amount of *3,4*-**1** after only one biased step is highlighted in bold. Percentages are based on HPLC integration and are corrected for the absorbance coefficients at 290 nm (see Supplementary Information). RSH = $MeO_2CCH_2CH_2SH$.

3,4-**1** present at the steady state using the two sets of reversible conditions. As long as the product ratio obtained from condition III is different to that obtained from condition II on a polymeric (or cyclic) track, then directionally biased walking occurs at any point (although the bias on a polymer may not be as great as that for the four-foothold track, the disulfide foothold of which is unsubstituted at the end of the track). The biased walking mechanism reported in Fig. 5 relies on the increased rate of accessibility of a foothold given the particular position of the walker on the track (that is, which is the pivot foot). As such it corresponds to an information ratchet[10,11,20,21] type of Brownian ratchet mechanism.

Conclusions

We describe here a system in which a 21-atom molecular walker moves up and down a four-foothold track primarily through a passing-leg gait mechanism, each step induced by an acid–base oscillation. The feet–track interactions feature covalent bonds that are dynamic under mutually exclusive sets of conditions and ensure a level of processivity (mean step number 37) that is 20–50% that of wild-type kinesins. Replacing one of the reactions with a kinetically controlled redox operation biases the directionality of one of the steps. This is sufficient to transport the walker directionally away from its minimum-energy distribution on the four-foothold track. The two passing-leg gait steps taken by the walker to go from one end of the four-foothold track to the other are the full repeat cycle necessary for the molecule to walk down a hypothetical polymeric track made of alternating benzaldehyde and benzylic disulfide footholds. Such extended tracks, and walkers that can carry cargoes along them, are currently under construction in our laboratory.

Methods

General procedure for acid-catalysed hydrazone exchange (condition I). To a 0.1 mM solution of *1,2*-**1** (typically 1 mg in ca. 10 ml; 1.0 equiv.) in $CHCl_3$ (HPLC grade) were added five drops of a solution that contained 20% vol/vol TFA and 1% vol/vol H_2O in $CHCl_3$ (HPLC grade). The mixture was stirred at room temperature and the progress of equilibration followed by analytical HPLC (see Supplementary Information). When the relative ratios of the isomers were stable (6–96 hours), the mixture was washed with an aqueous solution of $NaHCO_3$. The layers were partitioned and the aqueous layer extracted with $CHCl_3$. The combined organic layers were dried over $MgSO_4$. The solvents were removed under reduced pressure and the amount and constitution of the mixture determined by weight and, after dissolving in a defined amount of $CHCl_3$, analytical HPLC.

General procedure for base-catalysed disulfide exchange (condition II). To a 0.1 mM solution of *3,4*-**1** (typically 1 mg in 10 ml; 1.0 equiv.) in $CHCl_3$ (HPLC grade) was added DBU (40 equiv.), DTT (10 equiv.) and $(MeO_2CCH_2CH_2S)_2$ (20 equiv.) from stock solutions. The mixture was stirred at room temperature and the progress of the equilibration followed by analytical HPLC. When the relative ratios of the isomers were stable (12–48 hours), the excess of DTT was oxidized by dropwise addition of a solution of iodine in $CHCl_3$ until a slightly brown colour persisted. An aqueous solution of NH_4Cl and Na_2SO_3 was added and the mixture stirred vigorously until decolourization was complete. The layers were partitioned and the aqueous layer extracted with $CHCl_3$. The combined organic layers were dried over $MgSO_4$. After work up the product distribution was no longer dynamic (free of DTT and DBU) and the solvent could be removed to allow analysis by weight and analytical HPLC (see Supplementary Information).

General procedure for redox-mediated disulfide exchange (condition III). (i) Reduction step. DTT (6 equiv.) and DBU (3 equiv.) were added from stock solutions to a 1 mM solution of *2,3*-**1** (1 equiv.) in $CDCl_3$ or $CHCl_3$. The mixture was heated under reflux until ^{1}H NMR spectroscopy showed that all disulfide bonds had been reduced (2–12 hours). (ii) Oxidation step. The solution from step (i) was diluted to 0.1 mM with a 1:1 mixture of $CHCl_3$ and MeOH (both HPLC grade). Et_3N (five drops) and methyl 3-mercaptopropionate (8 equiv.) were added. At room temperature, a solution of iodine in $CHCl_3$ was added to the mixture until the brown colour persisted. An aqueous solution of NH_4Cl and Na_2SO_3 was added and the solution stirred vigorously until decolourization was complete. The layers were partitioned and the aqueous layer extracted with $CHCl_3$. The combined organic layers were dried over $MgSO_4$. At this stage a broad-window preparative HPLC was carried out (retaining the products in a window of 5–12 minutes retention time, see Supplementary Information) to remove excess reagents, waste products and other impurities (the two-step redox sequence produces more by-products than the acid- or base-catalysed procedures). The samples were analysed by HPLC before and after to confirm that the walker–track isomer ratios remained unchanged by this procedure.

Note on molar ratios. The molar ratios of DBU and DTT used during the base-catalysed disulfide exchange experiments (40 and 10 equiv., respectively) are higher than those used during the reduction step of the redox disulfide-exchange experiments (3 and 6 equiv., respectively) because there is a tenfold difference in concentration at which the experiments were carried out (0.1 mM and 1.0 mM, respectively). The DBU:DTT ratio is also reversed (from 4:1 to 1:2) in the two experiments. The rationale behind these changes reflects the different objectives of the two types of exchange experiments: During the base-catalysed exchange experiment, DTT is added to increase the rate of disulfide exchange for which thiolates are a necessary intermediate; during the redox operation the excess DTT results in quantitative reduction of the disulfide bonds. The resulting thiols were

subsequently oxidized rapidly by iodine to effect a kinetically controlled reaction outcome.

Received 9 October 2009; accepted 11 November 2009; published online 20 December 2009

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Acknowledgements

We thank the Engineering and Physical Sciences Research Council (EPSRC) National Mass Spectrometry Service Centre (Swansea, UK) for high-resolution mass spectrometry and Juraj Bella for assistance with high-field NMR spectroscopy. This research was funded through the European Research Council Advanced Grant *WalkingMols*. D.A.L. is an EPSRC Senior Research Fellow and holds a Royal Society–Wolfson Research Merit Award.

Author contributions

M.v.D. and E.M.G. carried out the experimental work. All the authors contributed to the design of the experiments, the analysis of the data and the writing of the paper.

Additional information

The authors declare no competing financial interests. Supplementary information and chemical compound information accompany this paper at www.nature.com/naturechemistry. Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/. Correspondence and requests for materials should be addressed to D.A.L.

MOLECULAR MACHINES

Tiny steps

A molecular 'walker' can be made to move up and down a molecular 'track' by alternately locking and unlocking the two different types of covalent bonds that join the two components together. By changing the conditions under which one of the bond-forming/bond-breaking processes occurs, a directional bias for walking can be achieved.

Sijbren Otto

The molecules that nature has evolved to perform crucial biological tasks continue to inspire awe and admiration — especially in the synthetic chemists who strive to make molecules capable of mimicking these functions. Take, for example, the kinesin motor-protein that can be thought of as a railway engine inside the cell — it is able to transport cargo along extended tracks made from another protein, tubulin. Unlike a railway engine, however, kinesin does not have wheels, but two little 'feet' with which it 'walks' on the tubulin track. The 'engine' is fuelled[1] by the hydrolysis of adenosine triphosphate (ATP) and it travels in just one direction because of the unsymmetric architecture of the track. Moreover, the presence of either ATP or adenosine diphosphate (ADP) in the binding pocket of each of the kinesin molecule's feet determines which foot is released from the track during the walking process. A beautiful animation of a kinesin molecule walking down a tubulin track can be viewed online[2].

As reported in *Nature Chemistry*, a team of researchers led by David Leigh at the University of Edinburgh have now developed a small synthetic molecule–track system that mimics the action of kinesin[3]. In their design, Leigh and co-workers had to take a number of factors that affect movement at the molecular level into consideration. For example, whereas gravity will ensure that the feet of a walker remain firmly on the ground in the macroscopic world, in the molecular world gravity is of little importance and an alternative force has to be introduced to keep the walker on its track. In nature, non-covalent interactions are used to serve this purpose, but in a synthetic system it is difficult to achieve the necessary tight binding, while at the same time maintaining the ability to unbind the feet before taking another step.

Leigh's team has solved this problem by making clever use of reversible covalent bonds to achieve controlled binding and unbinding of the feet to the track (Fig. 1). The walker has one hydrazide foot and one thiol foot. The hydrazide can react with an aldehyde at either position 1 or 3 to form a hydrazone, and the thiol foot can form a disulfide linkage at either position 2 or 4. Depending on the acidity of the medium, either one or the other bond type becomes labile, but not both at the same time[4,5]. This orthogonality ensures that one foot is always bonded to the track and that the walker does not fall off. Under acidic conditions the hydrazone bonds readily fragment and re-form, allowing one foot to move, whereas under basic conditions the disulfide bonds break and form, allowing the other foot to move.

Starting from the compound in which the walker is bonded to positions 1 and 2 on the track (the 1,2-isomer), adding acid causes

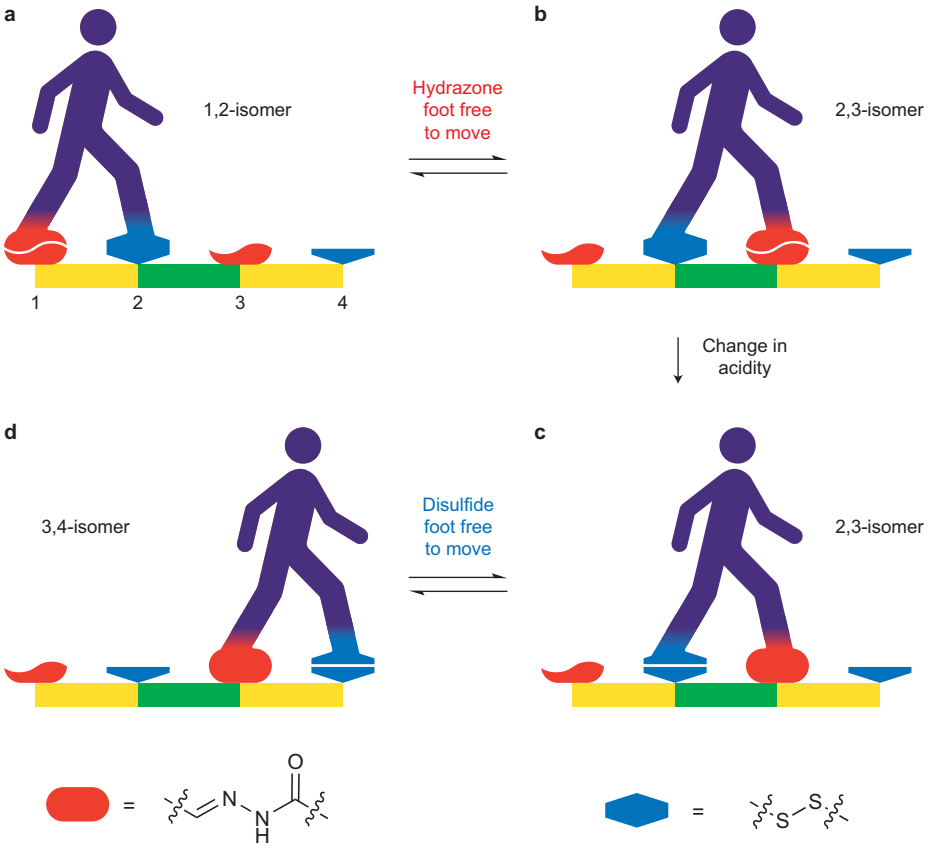

Figure 1 | Schematic representation of how a molecule featuring a thiol foot (blue) and a hydrazide foot (red) can walk along a short linear track with four stepping stones (numbered 1–4). **a**,**b**, Starting on the left of the track with the 1,2-isomer (**a**) switching on the reversible hydrazone chemistry under acidic conditions gives the walker the choice between stepping to position 3 (**b**), or remaining at position 1. **c**,**d**, Switching off the hydrazone chemistry and switching on disulfide exchange fixes the hydrazone foot in place (**c**), while allowing the disulfide foot to choose between two blue stepping stones, position 2 and position 4 (**d**). The unsymmetrical nature of the track is denoted by the coloured sections. For example, when the disulfide bond is cleaved in the 2,3-isomer, the thiol foot must either step across the yellow section to form the 3,4-isomer or back across the green section to reform the 2,3-isomer.

the hydrazone bond at position 1 to break. The newly freed hydrazide foot of the walker can then react at either position 1 to reform the 1,2-isomer, or at position 3 to form the 2,3-isomer, during which the walker has taken one step along the track. The process of hydrazone cleavage and formation occurs under thermodynamic control and the relative amounts of the 1,2- and 2,3-isomers (51:49) reflects the stability of these two different macrocyclic compounds. This mixture is then treated with base, which locks the hydrazone bonds in place, but enables the disulfide bond at position 2 to break and exchange with the disulfide at position 4 to form the 3,4-isomer (from the 2,3-isomer) — the walker has now taken two steps along the track from its original position. Cycling between acidic and basic conditions gives a mixture of the 1,2-, 2,3- and 3,4-isomers (and also a small amount of the 1,4-isomer) in a ratio of 39:36:19:6.

The bias for moving from left to right can be increased by replacing one of the reversible bond-breaking/bond-forming steps with a kinetically controlled sequence of reactions. Instead of performing the disulfide exchange reactions under reversible conditions, the disulfides are controllably cleaved to give free thiols, and then subsequently re-oxidized. This process gives a different product distribution of walker–track isomers than the reversible reaction, and further biases the direction of the walker. For example, after cycling the 1,2-isomer under the biasing conditions, 43% of the 3,4-isomer is formed, in contrast with only 19% being produced under the conditions where all of the bond-forming/bond-breaking steps are reversible.

In the present study the track is relatively short, allowing for just two steps. In principle, however, it should be possible to extend the approach to longer tracks and even cyclic tracks, although such systems may become increasingly difficult to analyse in as much detail as the present construct. Much of the beauty of the present system is in its (relative) simplicity. Unlike previously reported DNA-based walkers[6] it does not feature any components borrowed from nature. It also operates with a simple energy source — a change in acidity of the medium. One exciting possibility that is now almost within reach is to couple this type of walker to an oscillating reaction that rhythmically switches between two different pHs. Once such a system is set going, the walker should continue its journey without any human intervention.

On a more general note, the system is a clever new entry in the as yet limited number of man-made systems that operate out-of-equilibrium. All life forms as we know them are far from equilibrium and can only exist by coupling processes that are thermodynamically uphill to ones that go downhill. The design of new chemical systems that feature such coupling is a challenging endeavour. It pushes the boundaries of supramolecular chemistry and will be at the heart of the emerging fields of systems chemistry and synthetic biology. ❐

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QUANTUM COMPUTING

Chemistry from photons

The use of conventional computers to calculate molecular properties is hindered by the exponential increase in computational cost on increasing the size of the molecules studied. Using quantum computers could be the solution and the initial steps are now being taken.

Kenneth R. Brown

Calculating ground- and excited-state energies of molecules with high accuracy and precision remains a daunting task. Exact solutions for molecules consisting of more than about ten atoms remain beyond the scope of computational chemists even with the latest multicore-processing technology. The essence of the problem is the disparity between our classical description of molecules and the underlying quantum mechanics. A quantum computer — a quantum simulator that actually uses a quantum system to process data — offers the possibility of overcoming this mismatch[1] and nowhere is its potential application greater than for the calculation of molecular properties[2]. Writing in *Nature Chemistry*, Aspuru-Guzik, Lanyon and co-workers describe an initial experimental step down this path[3].

The challenge of quantum chemistry — applying quantum theory to the calculation of molecular properties — comes from the interactions and correlations of electrons. Simply placing electrons into the lowest available molecular orbitals and then calculating the energy can give a reasonable approximation, but because of the interactions between electrons, calculating exact energies requires including configurations where the electrons are in higher-energy orbitals. The number of possible electron configurations grows exponentially with the size of the molecule and so, therefore, do the resources required for an exact calculation. Despite the progress made in quantum chemistry — through the development of approximation methods that look at only a few-electron configurations[4] — an exponential increase in classical computational power leads to only a linear increase in the size of molecules considered. Conversely, an exponential improvement in quantum computer hardware would transform quantum chemistry.

A quantum computer is composed of two-level quantum systems, qubits, with variable interactions. Their theoretical power means that a quantum computer with 500 reliable qubits could perform an exact calculation on large and important molecules, such as caffeine[2], for example. Based on an extrapolation of Moore's law, this calculation would become possible on a classical computer in the next millennia, long after Moore's law is actually expected to end. Unfortunately, present quantum computers are composed of only a few qubits, but a linear improvement in their hardware would have the same effect on quantum chemistry as an exponential improvement in conventional computers.