NANO LETTERS
LETTER
pubs.acs.org/NanoLett

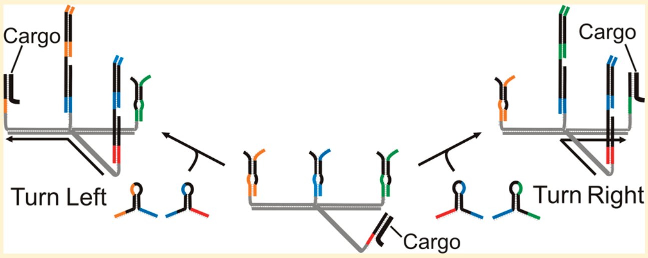
A Programmable Molecular Robot

Richard A. Muscat, Jonathan Bath, and Andrew J. Turberfield*

Clarendon Laboratory, Department of Physics, University of Oxford, Parks Road, Oxford OX13PU, U.K.

S Supporting Information

ABSTRACT: We have developed a programmable and autonomous molecular robot whose motion is fueled by DNA hybridization. Instructions determining the path to be followed are programmed into the fuel molecules, allowing precise control of cargo motion on a branched track.



KEYWORDS: DNA, molecular motors, programmable machines, self-assembly

Molecular machines built from DNA[1] can be actuated by DNA control strands (oligonucleotides) that trigger a hybridization reaction, switching the device between two states.[2,3] In simple, nonautonomous, DNA walking devices,[4,5] feet are anchored by adding "binding" control strands that hybridize to single-stranded domains on both foot and track, and freed by adding complementary "lifting" strands that remove binding strands to form double-stranded waste products. Sequential addition of instructions by the operator drives the cycle of foot release and reattachment at a forward site that causes the device to walk along its track. A similar mechanism has been used to control a nanoscale assembly line.[6]

The strand-exchange reactions responsible for these transitions are typically mediated by short, single-stranded "toeholds" to which the invading strand can hybridize to initiate strand displacement.[7,8] Toeholds give the invading strand a thermodynamic advantage and can increase the rate of strand exchange by several orders of magnitude.[8−10] Toeholds can be made unreactive by sequestering them within a loop domain, for example, of a DNA hairpin[11−13] or two-strand complex,[7,9] or in a duplex.[10,14−17] Unreactive toeholds can be activated by a strand displacement reaction that opens the loop[7,9,11−13] or displaces the blocking strand.[10,14−17] In both cases, the toehold-revealing reaction can be initiated by hybridization of the invading strand to a different, exposed toehold. The requirement for an external operator to control the order in which control strands are added can therefore be overcome by designing an autonomous reaction cycle in which the toeholds required to initiate a hybridization reaction are progressively revealed by the preceding reactions in the cycle. Control of reaction kinetics through toehold availability makes it possible for a hybridization-fueled motor or reaction network to operate in the presence of a nonequilibrium mixture of control strands while maintaining control of the sequence of reactions taking place. For example, in hybridization chain reactions[11,18,19] the incorporation of each strand at the end of a growing polymer reveals the next toehold required to extend the chain. More elaborate assembly sequences and reaction networks can be programmed using the same principle.[16,17]

Sequential release of toeholds is the design principle behind recent autonomous two-footed walking devices.[16,20−22] Autonomy can be dangerous if insufficiently regulated: if the reactions that bind and release the feet occur independently, the biped rapidly dissociates from the track or becomes stranded.[16] Some biped motors[20−22] have achieved the coordination between feet that is essential to ensure directional and processive motion. In one system, binding of the lead foot triggers a sequence of two hybridization reactions that lead to displacement of the back foot and blockage of the anchorage from which it is lifted.[22] This device is one of a class[23,24] referred to as "burnt bridges" devices[25] because directional movement is imposed by inactivation of the track behind the device. Other bipeds can move on a reusable track: competition between their feet is used to bias their reactivities toward the DNA fuel that lifts them from the track, ensuring that the foot in the back position is lifted preferentially.[20,21] Coordination can be regarded as transfer of information—the information that the front foot of a processive biped is bound to the track must be transferred backward to trigger release of the back foot.

Here, we demonstrate a molecular motor that transfers *forward* the information that it is bound to a specific anchorage in order to trigger binding to the next anchorage in a programmed sequence. In contrast to bipedal walkers, this motor is normally bound to the track by a single anchorage, although when stepping it does not dissociate from one anchorage until it is stably attached to the next. This motor can be programmed to choose between branches of a track junction while operating autonomously. The "fuel" hairpins whose hybridization powers the motor also encode the motor's instructions.

The track consists of anchorages tethered to a double-stranded DNA backbone (Figure 1). Each anchorage (e.g., X, Y) contains a common binding domain ($\bar{c}\bar{b}$, 12 nt) and an

Received: October 22, 2010
Revised: January 15, 2011
Published: January 28, 2011

dx.doi.org/10.1021/nl1037165 | *Nano Lett.* 2011, 11, 982–987

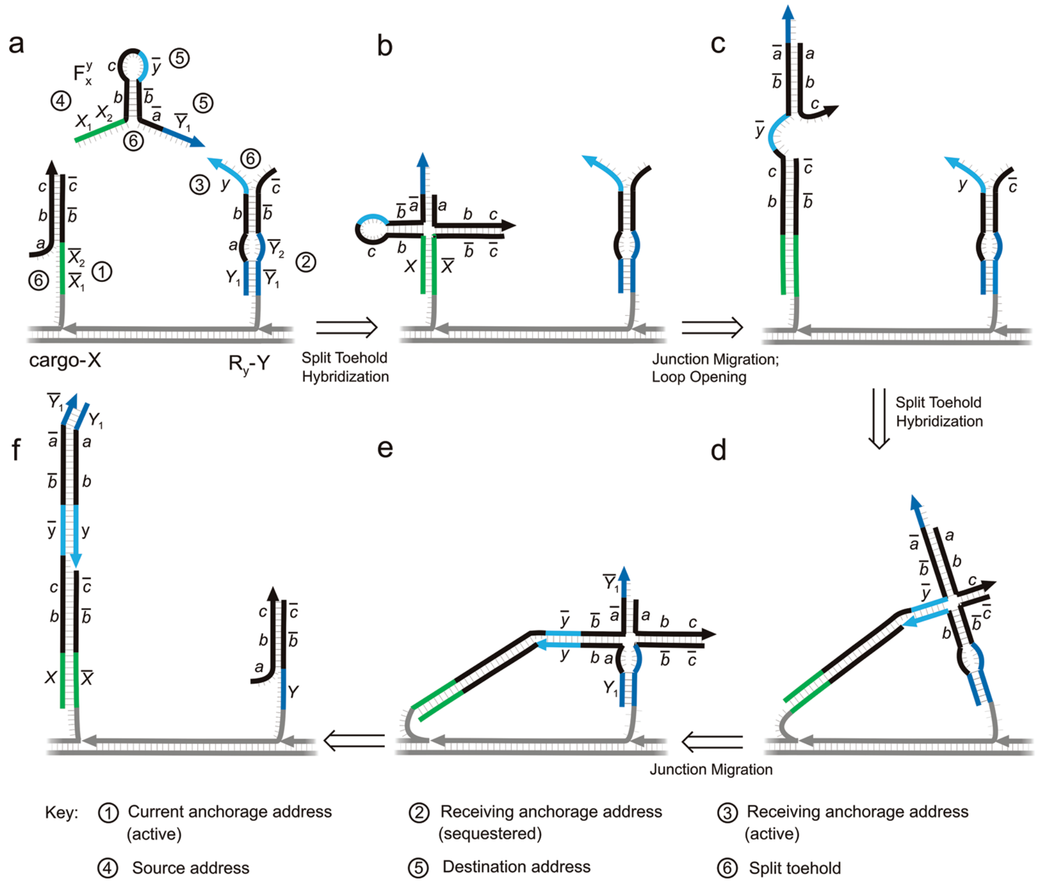


Figure 1. Motor scheme with detail of cargo displacement from anchorage X to Y. (a) A track consists of addressable anchorages attached to a dsDNA backbone. The cargo is shown bound to anchorage X in a complex that displays the source address domain $\overline{X}$; anchorage Y is blocked by removal strand R_y. (b) Fuel F_x^y binds to the split toehold created by the cargo-anchorage duplex, forming a Holliday junction. (c) Junction migration leads to the opening of the fuel hairpin loop and displacement of the cargo from source anchorage X, leaving it attached to the track through the fuel strand. The destination address domain $\overline{y}$ is activated by opening the fuel loop. (d) Interaction between the fuel—cargo duplex and the split toehold on the adjacent destination anchorage forms a new Holliday junction. (e) Junction migration effects transfer of the cargo to anchorage Y. (f) A waste product, consisting of the fuel strand F_x^y and removal strand R_y, is left on anchorage X blocking backward motion. The new complex between cargo and anchorage Y displays the new source address $\overline{Y}$, capable of initiating the next step.

identifying address domain ($\overline{X}$,$\overline{Y}$ etc., 10 or 11 nt). (In all figures common domains are shown in black and domains specific to a single anchorage are colored. Roman letters identify anchorage locations and strands and italic letters identify their component domains; domain x is complementary to $\overline{x}$, etc.) The cargo is a single strand that can hybridize to any anchorage (Figure 1a, left). In this complex the address domain of the current anchorage ($\overline{X}$) and part of the cargo (domain a, 5 nt) remain single-stranded: they are held together by the cargo/anchorage duplex, forming a structure that we refer to as a "split toehold" that signals the presence of the cargo and identifies the current anchorage by displaying its address. Split toeholds have been used previously to control a hybridization chain reaction and move a catalytic cargo.[18]

An anchorage in its active, receiving, state, before passage of the cargo, is hybridized to a "removal strand" (e.g., anchorage Y, Figure 1a, right). The removal strand (R_y in the case shown) contains a domain (b, 7 nt) that hybridizes to part of the common binding domain and another (Y_1, 7 nt) that hybridizes to part of the address domain of the anchorage ($\overline{Y} \equiv \overline{Y}_2\overline{Y}_1$) and is therefore specific to the anchorage. Some bases within the address domain are left unpaired but are held in an internal loop. The removal strand bears a second address domain (y, 6 nt) which has a different sequence to $\overline{Y}$ but is also uniquely associated with anchorage Y; this forms a split toehold with unpaired bases of the binding domain of the anchorage ($\overline{c}$, 5 nt). The receiving anchorage Y thus carries two unique identifying addresses: domain $\overline{Y}$ is effectively sequestered within the anchorage/removal strand duplex and is therefore inactive; domain y of the associated removal strand, R_y, forms part of an active split toehold, signaling that this anchorage is ready to receive the cargo.

Transfer of the cargo onto an adjacent active anchorage is mediated by a fuel hairpin. A fuel hairpin is both an energy source and a routing instruction; it contains address domains identifying the anchorage from which the cargo should be displaced and the active receiving anchorage to which it should be transferred. F_x^y denotes the fuel hairpin that transfers the cargo from anchorage X to anchorage Y (Figure 1a). The source address X, which is complementary to the address that is incorporated in the source anchorage ($\overline{X}$), is exposed and reactive. The hairpin incorporates domains complementary to both address domains carried by the removal strand on the destination anchorage: domain $\overline{Y}_1$, complementary to the address sequestered in the anchorage/removal strand duplex, is exposed and reactive; domain $\overline{y}$, complementary to the exposed address displayed by the removal

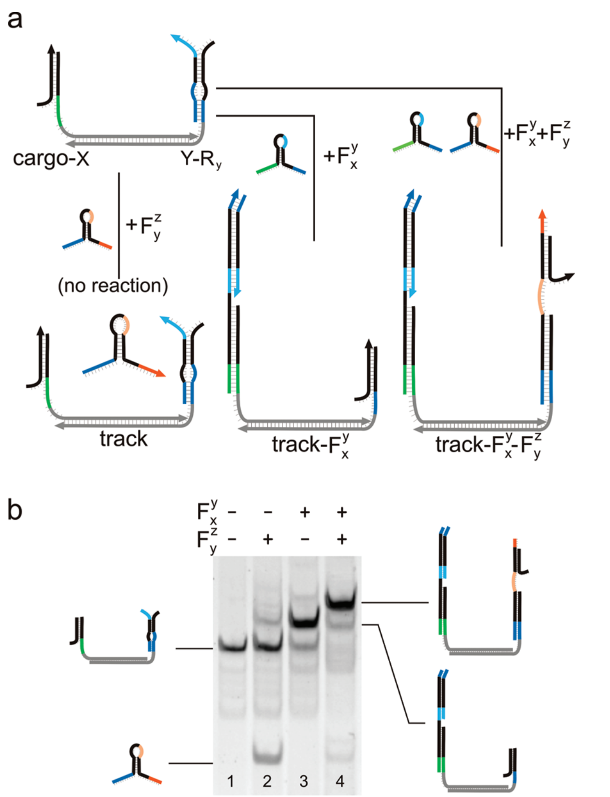


Figure 2. Control of the reactions of fuel hairpins. (a) Summary of designed interactions with fuels F^y_x and F^z_y. When the cargo is bound to anchorage X, no toehold is available to initiate interaction with F^z_y. F^y_x interacts with the cargo−anchorage X duplex through an active split toehold incorporating the source address domain $\overline{X}$, transferring the cargo to anchorage Y (see Figure 1). The new cargo−anchorage Y duplex displays a new source toehold that can interact with F^z_y, so if both fuel strands are added simultaneously they will react sequentially. (b) Polyacrylamide gel electrophoresis (PAGE) analysis of reactions with fuel. Fuel and cargo-laden track (100 nM) were incubated for 30 min (see Figures S3 and S4 in the Supporting Information for controls): lane 1, initial configuration with cargo bound to anchorage X; lane 2, addition of F^z_y results in little interaction with track; lane 3, F^y_x reacts readily, transferring cargo to anchorage Y; lane 4, simultaneous addition of F^y_x and F^z_y leads to a complex containing both fuel strands, completion of the first step from X to Y, mediated by F^y_x, enables reaction with F^z_y to initiate a second step, as designed.

strand, is sequestered in the loop of the hairpin and is therefore inactive. Address domains act as toeholds to initiate the reactions that move the cargo along the track. In the absence of cargo, no toehold-mediated interactions are possible because one of each pair of complementary address domains is sequestered, either in the loop of a fuel strand, by hybridization to a removal strand, or (after passage of the cargo) by hybridization to a spent fuel hairpin. The cargo−anchorage duplex is an exception: its active address toehold can initiate interaction with an appropriate fuel.

The movement of the cargo between two anchorages occurs in two stages (Figure 1). Each involves the reassortment of a pair of largely homologous duplexes that proceeds via the formation and migration of a four-arm Holliday junction (HJ).[26] HJs are nucleated by hybridization of complementary split toeholds (Figure 1b): the two pairs of single-stranded domains hybridize to form two of the arms of the junction, and the duplexes at the center of the split toeholds (which are, by design, homologous) become the other two arms. The reaction proceeds by reciprocal strand exchange between the homologous arms, which is equivalent to migration of the HJ. The reaction sequence is controlled through the progressive release of toehold sequences from complexes in which they are sequestered. The motor mechanism relies on the fact that HJ-mediated strand exchange occurs much more quickly if initiated by hybridization of both arms of a split toehold to form a complete HJ. Hybridization of one arm only, to form a three-arm junction, can also initiate HJ formation, but if the toehold sequences are short enough, the complex is more likely to dissociate (Figure S2, Supporting Information).

The split toehold formed by hybridization of the cargo to anchorage X can bind to the split toehold of fuel hairpin F^y_x (Figure 1b). Migration of the junction leads to the opening of the fuel hairpin and the displacement of the cargo from the anchorage, but the cargo remains connected to the anchorage through the fuel strand (Figure 1c). This reaction activates the destination address domain $\bar{y}$, that had been sequestered in the loop, which now forms part of a new split toehold with cargo domain *c*. If there is an adjacent anchorage displaying matching address *y* (and, by design, there will be), then a second HJ can be formed by association of split toeholds (Figure 1d), leading to transfer of the cargo onto anchorage Y (Figure 1e,f). In exchange, the corresponding removal strand is transferred backward to form a complex with spent fuel F^y_x and the previous anchorage X. The complex on anchorage X prevents it from taking part in any further hybridization reaction (Figure 1f), ensuring that the cargo can only be transferred forward (a burnt bridges mechanism). In the new complex between cargo and anchorage Y the new source address ($\bar{y}$) is exposed and ready to react with a fuel containing source address *Y* to initiate the next step (see Supporting Information for further details of strand sequences and motor design).

The autonomous operation of the motor is controlled by the release of previously sequestered toeholds to initiate subsequent reactions. Hybridization of toeholds also provides the energy required to drive directional motion: binding of a fuel hairpin to an anchorage (Figure 1b,c) creates 16 new base pairs (bp), and the subsequent interaction with the adjacent removal strand creates a further 11 bp (Figure 1d−f). No base pairs are gained or lost in the junction migration responsible for the intermediate strand-exchange reactions. The total energy released per step is approximately 37 kcal mol^{-1} (60 k_BT).[27]

This system is capable of autonomous motion, programmed by the layout of addresses along the track and the mixture of fuel hairpins present. All fuel hairpins (the complete program of motion) can be added simultaneously—there is no need for additional external control of the reaction sequence. Motion down a linear track with alternating anchorages X, Y can be programmed by adding fuel hairpins F^y_x and F^x_y. This system is directional after an initial symmetry-breaking step because backward motion is blocked by the products of the previous reaction. The symmetry between initial directions can be removed by using three or more addresses (X → Y → Z → X ...), in which case directionality is programmed by the choice of fuel hairpins and the burnt bridges mechanism is redundant. The direction chosen by the cargo at a branch point X → (P,Q) can be programmed by adding either F^p_x or F^q_x.

A two-anchorage track (X,Y) was used to demonstrate that the reaction of fuel hairpins can be coupled to the controlled movement of cargo (X → Y) by sequential activation of toeholds (Figure 2). In the initial configuration with cargo on anchorage X (Figure 2b, lane 1) F^z_y reacts poorly because anchorage address *Y* is inactive (Figure 2b, lane 2). However, fuel F^y_x reacts readily

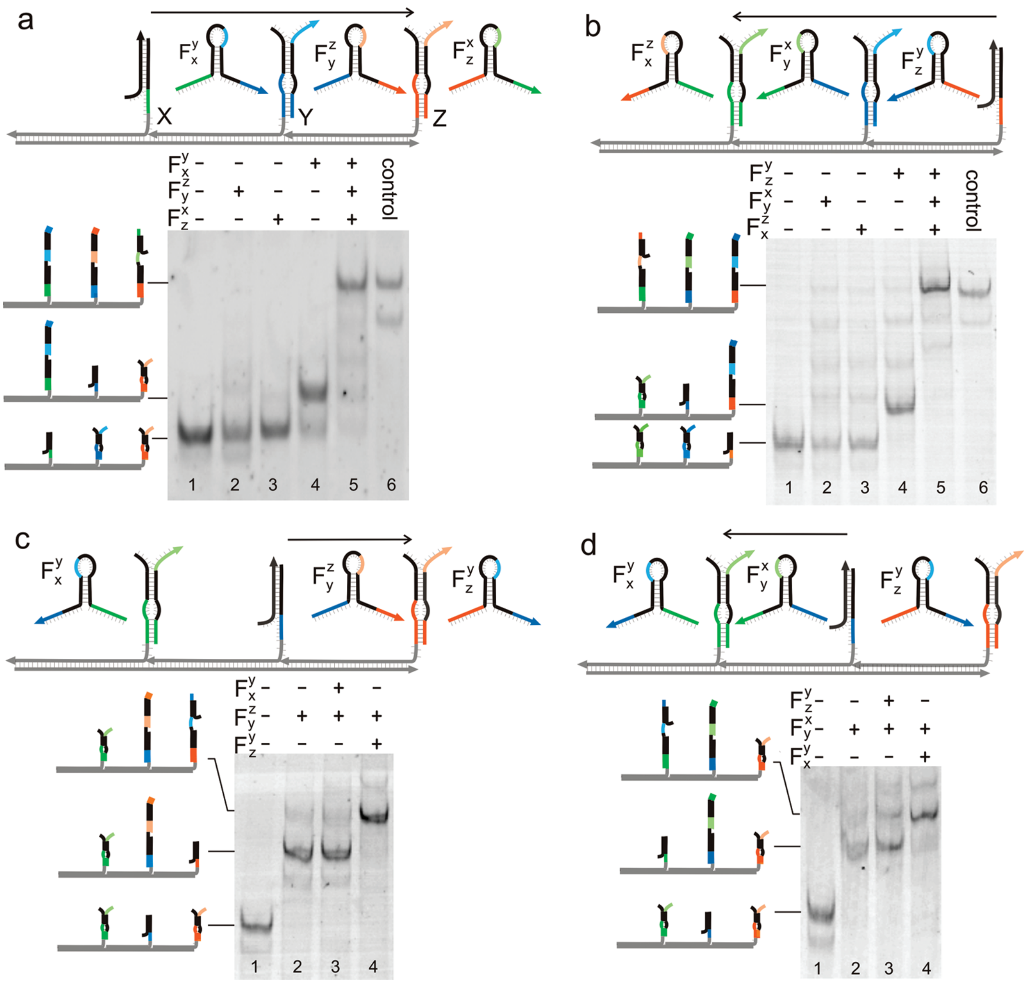


Figure 3. Operation of motor on a linear track consisting of anchorages X, Y, and Z (green, blue, and orange). Fuel and cargo-laden track (50 nM) were incubated for 90 min (parts a, b) or 30 min (parts c, d). (a) Movement left to right: lane 1, the cargo is initially bound to anchorage X; lanes 2−4, if fuels are added individually, the cargo only interacts significantly with fuel F_x^y only; lane 5, if all fuels are added simultaneously, then all can react in the designed sequence, transferring the cargo to anchorage Z; lane 6, the control is produced by annealing track, fuels, and removal strands such that each anchorage is blocked by waste products: this is a close mimic of the designed final state (the cargo is replaced by a removal strand). (b) Movement right to left. As (a) but with the cargo initially bound to anchorage Z. (c) Movement from the middle to the right: lane 1, the cargo is initially bound to the middle anchorage Y; lane 2, F_y^z moves the cargo to anchorage Z; lanes 3 and 4, controls showing that F_y^z moves the cargo from Y to Z, not X; lane 3, no additional interaction is observed when F_x^y is added with F_y^z, demonstrating that there is no cargo on anchorage X (which would enable F_x^y to bind to the track); lane 4, Addition of F_z^y with F_y^z causes an additional band shift, demonstrating that F_y^z has moved all cargo to anchorage Z (where it can interact with F_z^y). (d) Movement from the middle to the left. As (c) but demonstrating movement from middle anchorage Y to X.

with the cargo bound at X (Figure 2b, lane 3) resulting in transfer of the cargo from X to Y and activation of anchorage address *Y* (see Figures S5 and S6 in the Supporting Information for measurements of reaction rates). Simultaneous addition of the fuels F_x^y and F_y^z results in their sequential reaction, with an overall reaction yield of approximately 70% (Figure 2b, lane 4): first F_x^y transfers the cargo to Y, then F_y^z lifts the cargo from Y to form the complex that, on a longer track, would initiate the next step.

A three-anchorage track (X,Y,Z) was used to demonstrate that the direction of transport can be controlled by information encoded in the fuel hairpins. Addition of F_x^y, F_y^z, F_z^x results in the movement of the cargo from X → Y → Z (Figure 3a, lane 5). The reaction of F_z^x leaves the cargo ready to step to an anchorage of type X such that the cargo could move continuously on repeating track $(X,Y,Z)_n$ if it were sufficiently rigid to prevent stepping between nonadjacent anchorages (see Figure S7, Supporting Information). The direction of movement can be reversed by encoding movement with fuels F_z^y, F_y^x, F_x^z that specify movement of the cargo from Z → Y → X (Figure 3b, lane 5). We attribute weak bands in Figure 3b, that correspond to out-of-sequence interactions with fuel strands, to incorrectly assembled tracks with the cargo initially bound to the wrong anchorage (see Figure S8, Supporting Information).

When the cargo is loaded in the middle of the track (at Y) the decision to move to right or left is made by adding F_y^z (move right; Figure 3c, lane 2) or F_y^x (move left; Figure 3d, lane 2). Cargo that has moved right (Y → Z) can subsequently react with F_z^y (Figure 3c, lane 4) whereas cargo that has moved left cannot (Figure 3d, lane 3). These experiments demonstrate that the information encoded in the fuel hairpins is sufficient to control the direction taken by the cargo.

The ability of the motor to navigate more complex track configurations that include branch points was demonstrated using a 'T' junction track (W, X, Y, Z) where the cargo was

dx.doi.org/10.1021/nl1037165 | *Nano Lett.* 2011, 11, 982–987

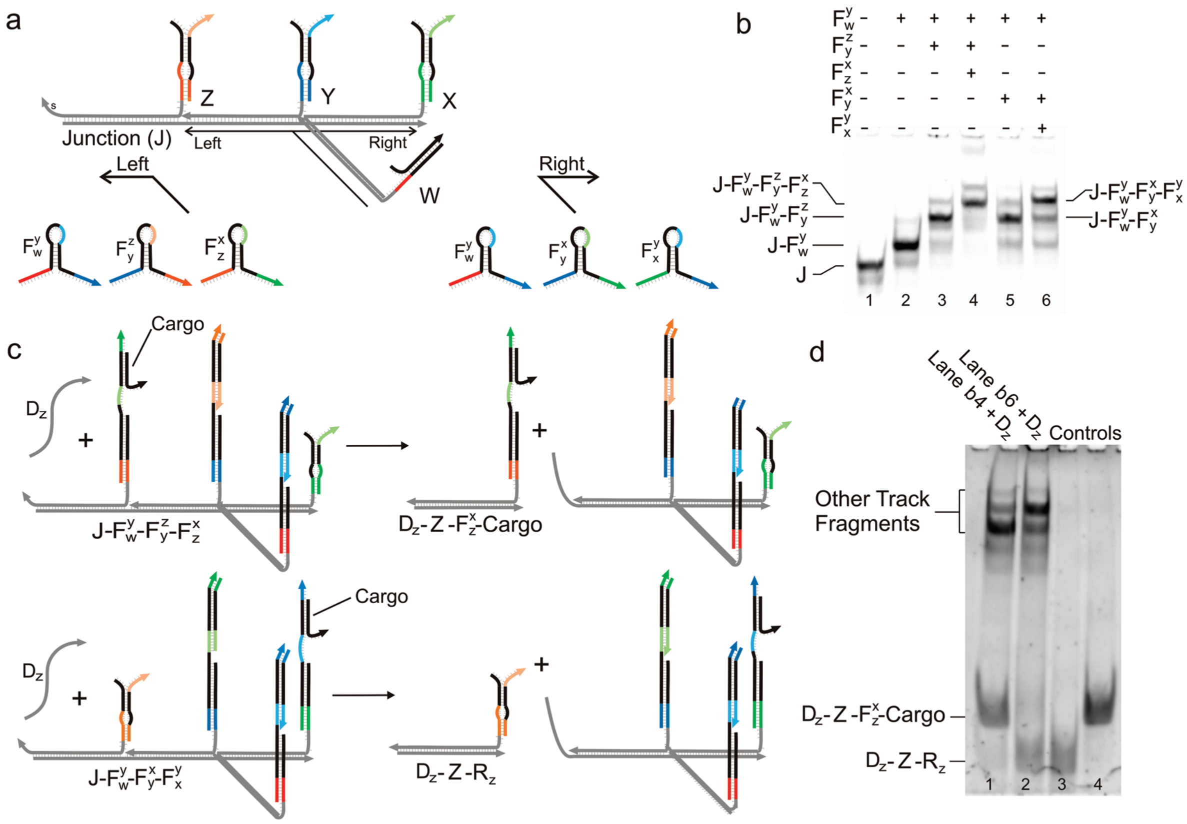


Figure 4. Programmed motion at a junction. (a) The cargo is initially on anchorage W (red). Depending on the program embodied in the set of fuel strands, the cargo can be sent to either exit. (b) PAGE analysis of programmed motion on the branched track (J). All fuels required to program the desired pattern of movement across the junction are added simultaneously to cargo-laden track (18 nM) and incubated for 90 min. The cargo is moved to the junction point by F_w^y (lane 2). It can be moved beyond the junction, in either direction, by adding F_w^y and F_y^z or F_y^x (lanes 3, 5). The presence of the cargo at the correct end of the track is demonstrated by adding either F_z^x or F_x^y to lift it from the final anchorage (lanes 4, 6). (c) The final location of the cargo can be confirmed by splitting the track in two using displacing strand D_z. (d) PAGE analysis of the fragments formed by addition of D_z. Comparison of the mobile track fragment incorporating anchorage Z with controls confirms that the cargo is present on Z only when programmed to move to this anchorage (lane 1).

loaded at W, transferred to Y by F_w^y, then sent left or right by F_y^x or F_y^z (Figure 4a). Figure 4b shows PAGE analysis of the motion of the cargo. Addition of the "left" fuel set F_w^y, F_y^z sends the cargo to the left, finishing on Z (lanes 2, 3); its presence on Z is demonstrated by adding F_z^x (lane 4) which binds to the cargo-activated anchorage (the flexibility of the track may allow further transfer of the cargo to anchorage X at the other end of the track —see Figure S8, Supporting Information). Addition of the "right" fuel set F_w^y, F_y^x sends the cargo to the right (lanes 2, 5) where its presence on X is demonstrated by adding F_x^y (lane 6), leaving the cargo bound to anchorage Y via the fuel strand. The route taken by the cargo is confirmed by adding displacement strand D_z which is designed to separate anchorage Z from the rest of the track (Figure 4c). PAGE analysis (Figure 4d) shows that anchorage Z reacts only when the left fuel set is added.

A recent study demonstrated a motor whose direction of motion is controlled by the stratagem of altering the path of its linear track.[28] We have made a further step in the development of molecular robotics by showing that the behavior of an autonomous motor on a branched track can be programmed by a rewritable external program encoded in DNA.

ASSOCIATED CONTENT

S Supporting Information. Strand sequences and motor design, methods, split toehold characterization, stepping on a two-anchorage track observed using fluorescent labels, two-anchorage tracks, kinetics of stepping mechanism, comparison between designed and "leakage" fuel reactions, stepping of cargo between nonadjacent anchorages, and PAGE purification of track components. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: a.turberfield@physics.ox.ac.uk.

ACKNOWLEDGMENT

This work was supported by EPSRC Grants EP/F056605/1 and EP/G037930/1.

REFERENCES

(1) Bath, J.; Turberfield, A. J. *Nat. Nanotechnol.* **2007**, *2*, 275–284.
(2) Yurke, B.; Turberfield, A. J.; Mills, A. P., Jr; Simmel, F. C.; Neumann, J. L. *Nature* **2000**, *406*, 605–608.
(3) Yan, H.; Zhang, X.; Shen, Z.; Seeman, N. C. *Nature* **2002**, *415*, 62–65.
(4) Sherman, W. B.; Seeman, N. C. *Nano Lett.* **2004**, *4*, 1203–1207.
(5) Shin, J. S.; Pierce, N. A. *J. Am. Chem. Soc.* **2004**, *126*, 10834–10835.

(6) Gu, H.; Chao, J.; Xiao, S.-J.; Seeman, N. C. *Nature* **2010**, *465*, 202–205.
(7) Turberfield, A. J.; Mitchell, J. C.; Yurke, B.; Mills, A. P.; Blakey, M. I.; Simmel, F. C. *Phys. Rev. Lett.* **2003**, *90*, No. 118102.
(8) Yurke, B.; Mills, A. P. *Genet. Program. Evolvable Mach.* **2003**, *4*, 111–122.
(9) Seelig, G; Yurke, B.; Winfree, E. *J. Am. Chem. Soc.* **2006**, *128*, 12211–12220.
(10) Zhang, D. Y.; Winfree, E. *J. Am. Chem. Soc.* **2009**, *131*, 17303–17314.
(11) Dirks, R. M.; Pierce, N. A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15275–15278.
(12) Green, S. J.; Lubrich, D.; Turberfield, A. J. *Biophys. J.* **2006**, *91*, 2966–2975.
(13) Bois, J. S.; Venkataraman, S.; Choi, H. M. T.; Spakowitz, A. J.; Wang, Z-G; Pierce, N. A. *Nucleic Acids Res.* **2005**, *33*, 4090–4095.
(14) Seelig, G.; Soloveichik, D.; Zhang, D. Y.; Winfree, E. *Science* **2006**, *314*, 1585–1588.
(15) Zhang, D. Y.; Turberfield, A. J.; Yurke, B.; Winfree, E. *Science* **2007**, *318*, 1121–1125.
(16) Yin, P; Choi, H. M. T.; Calvert, C. R.; Pierce, N. A. *Nature* **2008**, *451*, 318–322.
(17) Soloveichik, D.; Seelig, G.; Winfree, E. *Proc. Natl. Acad. Sci. U.S. A.* **2010**, *107*, 5393–5398.
(18) Venkataraman, S.; Dirks, R. M.; Rothemund, P. W. K.; Winfree, E.; Pierce, N. A. *Nat. Nanotechnol.* **2007**, *2*, 490–494.
(19) Lubrich, D.; Green, S. J.; Turberfield, A. J. *J. Am. Chem. Soc.* **2009**, *131*, 2422–2423.
(20) Green, S. J.; Bath, J.; Turberfield, A. J. *J. Phys. Rev. Lett.* **2008**, *101*, No. 238101.
(21) Bath, J.; Green, S. J.; Allen, K. E.; Turberfield, A. J. *Small* **2009**, *5*, 1513–1516.
(22) Omabegho, T.; Sha, R.; Seeman, N. C. *Science* **2009**, *324*, 67–71.
(23) Bath, J.; Green, S. J.; Turberfield, A. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 4358–4361.
(24) Tian, Y.; He, Y.; Peng, Y.; Mao, C. *Angew. Chem., Int. Ed.* **2005**, *44*, 4355–4358.
(25) Mai, J.; Sokolov, I. M.; Blumen, A. *Phys. Rev. E* **2001**, *64*, No. 011102.
(26) Thompson, B. J.; Camien, M. N.; Warner, R. C. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2299–2303.
(27) SantaLucia, J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1460–1465.
(28) Lund, K.; Manzo, A. J.; Dabby, N.; Michelotti, N.; Johnson-Buck, A.; Nangreave, J.; Taylor, S.; Pei, R.; Stojanovic, M. N.; Walter, N. G.; Winfree, E.; Yan, H. *Nature* **2010**, *465*, 206–210.