Published in final edited form as:

Nano Lett. 2009 July ; 9(7): 2641–2647. doi:10.1021/nl901008k.

Reciprocal DNA Nanomechanical Devices Controlled by the Same Set Strands

Chunhua Liu¹, Natasha Jonoska², and Nadrian C. Seeman^{1,*}

¹Department of Chemistry, New York University, New York, NY 10003, USA

²Department of Mathematics, University of South Florida, Tampa, FL 33620, USA

Abstract

Reciprocating devices are key features in macroscopic machines. We have adapted the DNA PX-JX₂ device to a reciprocal format. The PX-JX₂ device is a robust sequence-dependent nanomachine, whose state is established by a pair of control strands that set it to be either in the PX state or in the JX₂ state. The two states differ by a half-turn rotation between their ends. Here we report the construction of a pair of reciprocal PX-JX₂ devices, wherein the control strands leading to the PX state in one device lead to the JX₂ state in the other device, and *vice versa*. The formation, transformation and reciprocal motions of these two device systems are confirmed using gel electrophoresis, and atomic force microscopy. This system is likely to be of use for molecular robotic applications where reciprocal motions are of value, in addition its inherent contribution to molecular choreography and molecular aesthetics.

Keywords

Robust DNA Nanomechanical Devices; Reciprocal Motion; Gel Electrophoresis; Atomic Force Microscopy; Molecular Choreography

Reciprocating motion is central to many macroscopic machines. For example, pistons are often powered by forces that push first in one direction and then in the other. The nanoscale analog of a piston is not yet available, but the type of reciprocal motion that characterizes such a device certainly can be designed. Here, we demonstrate reciprocal motion using two DNA devices, one that moves reporter structures in one direction, while *the same signal* moves reporter structures in the opposite direction.

DNA has been shown to be a convenient basis for the construction of nanomechanical devices.¹ Its strengths in this regard include its structural robustness² and, in particular, its programmability. The addition of individual strands to DNA devices can lead to their assuming specific structural states, as a consequence of the addressability inherent in DNA sequences. The PX-JX₂ device is a robust sequence-dependent nanomechanical machine.³ Its mechanism of action depends on the addition of one or another pair of set strands that put it into two different structural states, termed either PX or JX₂; these two states differ by having one end of the device rotated relative to the other by a half turn. Recently, we have reported the development of a cassette whereby it is possible to insert a PX-JX₂ device into a 2D DNA construct, either a lattice built from small tiles,⁴ or a DNA origami tile.⁵ We have used the PX-JX₂ device as the basis for constructing a pair of reciprocal devices, wherein

*Address correspondence to this author at ned.seeman@nyu.edu. cl660@nyu.edu; jonoska@math.usf.edu.

Supporting Information Available. The experimental methods and the sequences of all the molecules used in this letter. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

one device assumes one state, while the other device assumes the opposite state. This capability has not been demonstrated yet on the nano-molecular scale. The key point of these two PX-JX₂ devices is that they share the same set strands, so that they make reciprocal motions synchronously: With the same control strands, one device is set to the PX state, and the other is set to the JX₂ state; the opposite set strands switch the two states. It is clear that synchronicity refers to operation during the same cycle of operation: The length of a cycle to reach equilibrium was overnight.

The two PX-JX₂ devices are illustrated in Figure 1a. In system I (left part of Figure 1a), each PX or JX₂ molecule contains two outer frame sections on the ends that flank a set strand section in the middle, similar to a previous design.⁶ The hybridization topology of the set strand section determines the state (PX or JX₂) of the device. In the molecule on the far left, the dark blue set strands interrupt the paths of the dark green and purple frame strands in the middle region; they cross over between helical domains, thereby setting the device to the PX state. By contrast, when the dark blue set strands are replaced by the light blue set strands, two juxtaposed duplex molecules without crossovers result, yielding the JX₂ structure. This change results in a half-turn rotation of the ends of the molecule relative to the PX structure; this change can be followed by examining the letters that label the ends of the helices, A, B, C and D. Each PX or JX₂ molecule contains 7.5 turns of DNA. The molecules labeled PX' and JX₂' in system II have exactly the same structural designs as those in system I, although they contain slightly different outer frame sequences near the set strands. These molecules also contain four DNA strands: The long outer frame strands are drawn in black and red; two short light blue set strands produce the PX' state (our notation for the PX state of System II), or two short dark blue set strands produce the JX₂' state (our notation for the JX₂ state of System II). Thus, the same dark blue set strands produce the PX state in system I, and the JX₂ state in system II. Likewise, the same light blue set strands produce the JX₂ state in system I, and the PX state in system II.

The machine cycles of these two devices are illustrated in Figure 1b. In process I, biotinylated dark blue unset strands are added to the molecules in the PX and JX₂' states to remove the dark blue set strands, leaving the amorphous frames. In process II, the light blue set strands are applied, and the frames are transformed into the JX₂ and PX' states. In process III, light blue unset strands are added, again leaving the amorphous frame as the product. Process IV shows that the addition of the dark blue set strands results in the restoration of the PX and JX₂' states. Thus, the opposite states are produced in the two different systems by the addition of exactly the same set strands.

The formation, transformation and reciprocal motion of the devices have been demonstrated by non-denaturing gel electrophoresis, as shown in Figure 2a. Lane M contains a 10 bp DNA ladder marker. Lanes 1, 4, 7 and 10 contain the annealed initial states JX₂, PX, PX' and JX₂' of the devices, respectively. Lane 2 contains the product obtained by converting the PX state of the device in Lane 4 to the JX₂ state, and lane 8 contains the product obtained by converting the JX₂' state of the device in Lane 10 to the PX' state, using the same control strands as those used to produce the material in Lane 2. Likewise, Lane 3 contains the product obtained by converting the JX₂ state of the device in Lane 1 to the PX state, and Lane 9 contains the product obtained by converting the PX' state of the device in Lane 7 to the JX₂' state by using the same control strands as those used to produce the material in Lane 3. Lanes 5 and 6 are the unstructured intermediates Frame and Frame', respectively. The single sharp target bands in Lanes 2, 3, 8 and 9 imply that the device states are well-formed and robust. The similar positions (~180-bp) of the PX and PX' devices suggest that they adopt similar conformations. So do the JX₂ and JX₂' devices (~190-bp). The retarded mobilities of the JX₂ and JX₂' devices are likely due to a less compact time-average structure, compared with that of the PX and PX' devices, as seen previously.^{3,6}

Cycling the operation of the devices is shown in Figures 2b and 2c. In panel (b), beginning with the annealed initial JX_2 state of system I (Lane 4) or the PX' state of system II (Lane 8), followed by three steps of operation, the same set strands convert the devices to the PX state (Lane 3) or the JX_2' state (Lane 7), then the opposite set strands further convert these devices to the JX_2 state (Lane 2) or PX' state (Lane 6), and then the original set strands yet further convert these devices to the PX state (Lane 1) or the JX_2' state (Lane 5). Figure 2c shows that the initial point of operation can be changed by starting from the annealed PX state of system I (Lane 4) or the JX_2' state of system II (Lane 8), followed by three steps of operation, to the JX_2 state of system I (Lane 1) or the PX' state of system II (Lane 5). In each step of the operation, the biotinylated unset strands for the PX or JX_2 states of the devices were added to the initial state of the complex. The solutions were then treated with magnetic streptavidin beads to remove the unset-strand/set-strand hybrids, followed by the addition of the new set strands to obtain the next state of the device complexes. Figures 2d and 2e show that the two devices can be operated together in the same solution. System II is present in twice the concentration of System I, so that transitions can be distinguished: Lane 1 of panel (d) contains the JX_2 state of System I and the PX' state of System II, which appears darker, because of its higher concentration. Following transitions of both species, the darker species in lane 2 is the JX_2 band. This alternation is visible as well in lanes 3 and 4. Panel (e) shows the same alternation beginning from the opposite starting point.

The absence of multimer bands or breakdown products indicates the absence of multimerization or dissociation during the transition. In contrast to other studies,⁷⁻⁹ we found it unnecessary to take special precautions to prevent these eventualities, arguably because of the robust nature of the PX - JX_2 device under ambient conditions.³ Following separate annealing of the system I and system II devices, it was possible to mix them and cause them to undergo transitions through isothermal strand replacement without multimerization. At the relatively low device concentrations used here (1.0 μ M or less), there seems to have been no cross-talk between or among the devices. Of course, as in all previous studies involving the PX - JX_2 device,³ we removed set strand-unset strand complexes using magnetic streptavidin beads (see Methods in Supplementary Information).

The Ferguson plot^{10,11} is a plot of the electrophoretic mobility as a function of gel concentration. We have used this method to compare the friction constants of the devices, because the friction constant is proportional to the slope of the plot. Figure 3a shows the Ferguson plots for all the molecules in system I, including the Frame, PX and JX_2 , as well as an additional 180 nt single duplex marker. The 180 nt duplex control has the smallest friction constant with a slope of -0.086, and an intercept of 1.707. The (slopes, intercepts) of the Frame, JX_2 and PX molecules are (-0.133, 2.172), (-0.133, 2.127) and (-0.126, 2.086), respectively. The Ferguson plots for device system II and the same 180 nt duplex marker are shown in Figure 3b. The slopes and intercepts for the molecules are the Frame' (-0.136, 2.157), JX_2' (-0.132, 2.111), PX' (-0.129, 2.118) and the 180 nt duplex (-0.086, 1.707), respectively. The values and trends of the slopes are all comparable to those in device system I, suggesting similar structural features.

To obtain direct visualization of the transformation, and of the reciprocal motion of the constructed devices, we have designed a one-dimensional system visible by atomic force microscopy. We connect a series of DNA PX triangles¹² with the system I and system II devices in the PX or JX_2 states using a pair of doubly cohesive sticky-ends.^{13,14} The PX triangle, containing 76 nucleotide pairs per edge, is large enough to be observed by atomic force microscopy (AFM), and the strand phasing in it matches that in the system I and system II devices in all their states. Schematic drawings of the two systems and the PX triangles are shown in Figure 4a. System I devices in the PX and JX_2 states contain two different 12 nt sticky ends A (orange), B (magenta) on one side, and two other 13 nt sticky

ends C (dark green), D (dark red) on the other. Likewise, sticky ends E (brown), F (dark purple) of 12 nt are on one side of the System II devices in their PX' and JX₂' states, and sticky ends G (light green) and H (bright red) of 13 nt are on the other side. The bottom edge of the PX triangle T1 contains two pairs of the sticky ends A' (orange), B' (magenta) of 12 nt and G' (light green), H' (bright red) of 13 nt, which complement the sticky ends of A, B, G, H of the devices. Similarly, the sticky ends of PX triangle T2, C' (dark green, 13 nt), D' (dark red, 13 nt) on one side, and E' (brown, 12 nt), F' (dark purple, 12 nt) on the other, are complementary to C, D, E and F in the devices. Thus, by designing different sticky ends in these two device systems, and the corresponding different PX triangle markers T1, T2, we are able to control the alternate connections of these two device systems in a one-dimensional array. This enables us to use AFM to observe reciprocal motion through visualizing the differences of the two pairs of states before and after the transformation.

Schematics showing the design of the one-dimensional array are illustrated in Figure 4b. The upper panel of Figure 4b shows the 1D array in the states formed by the dark blue strands (see Figure 1). At one end of the PX state in system I, sticky ends A and B, connect with one end of triangle T1, at sticky ends A' and B'. At the other end of the PX state in system I, sticky ends C and D, connect with one end of triangle T2, at sticky ends C' and D'. The other end of triangle T2 is connected to a system II device, in the JX₂' state; this is done through sticky ends E and F of the device, which are connected to sticky ends E' and F' of Triangle T2. The other end of the system II device in the JX₂' state contains sticky ends G and H, which connect with the other T1 triangle, through sticky ends G' and H'. The 1D array repeats, forming a periodic arrangement. The connections of the system I and system II devices are alternated by being interspersed between two distinct triangles. In this panel, we have a repetitive pattern of -T1-PX-T2-JX₂'-T1-PX-T2-JX₂'-T1-, because the system I device is in the PX state and the system II device is in the JX₂' state. This arrangement leads to both markers T1 and T2 pointing in the same direction. The transformation to the light-blue-strand states is shown in the middle of Figure 4b, and the post-transformation state is shown in the lower panel. It is evident from panel 4a that the light-blue-strand states flip the ends of the devices by a half turn, relative to the dark-blue-strand states. The consequence of this is that the PX state is converted to the JX₂ state and the JX₂' state is converted to the PX' state. The upshot of these transformations is that the disposition of the triangles switches from parallel orientations in the top panel of Figure 4b to alternating orientations in the bottom panel. If either of the devices, system I or system II does not switch, then the patterns depicted in Figure 4 will not be seen: Both devices flanking a triangle must switch to get the designed patterns. Although this design aims to produce parallel and alternating patterns that are similar to a previous result,³ the reasons for the triangle arrangements are different, because two devices are used here, and only one device was used previously. The period of the arrays containing two PX triangles and two devices (Figure 4b) is expected to be about 125 nm. Double cohesion^{13,14} is used here to connect the markers and the devices, owing to the structure of the PX triangle.

The AFM images shown in Figure 5 confirm the operation of the reciprocal PX-JX₂ devices. These are images are representative of those obtained from all experiments. The upper panel of Figure 5a displays a 1D assembly containing the PX, JX₂' arrangement of devices shown schematically in the upper panel of Figure 4b; this arrangement leads to parallel PX triangles, as can be seen readily. The lower panel of Figure 5a shows the JX₂, PX' arrangement of devices schematized in the lower panel of Figure 4b, leading to an alternating arrangement of PX triangles. The operation of the devices with a distinct starting point, is shown in Figure 5b. The upper panel shows the cycling of the system from an initial PX-JX₂' (dark blue strands) state with parallel triangles, moving to the PX'-JX₂ (light blue strands) state with oppositely oriented triangles (Step I), and then back to the PX-JX₂' state (Step II). The lower panel shows motion from the opposite starting point, starting from an

initial JX₂-PX' (light blue strands) state with oppositely oriented triangles, and moving to the PX-JX₂' (dark blue strands) state (Step I), and then back to the JX₂-PX' state (Step II). The distance of one period of the array, -T1-PX-T2-JX₂'-T1- or -T1-JX₂-T2-PX'-T1 (middle of first T1 to middle of second T1), has been measured as 124 (±12) nm, based on the average of thirty periodicities, in agreement with the estimated value of 125 nm mentioned above.

We have successfully constructed a pair of the programmable PX-JX₂ device systems that have a reciprocal relationship with each other, so that they can operate oppositely and synchronously with the same control strands in the same environment. Both gel electrophoresis and AFM images provide evidence supporting the notion that the devices form and are able to function as designed. The strategy of using the same control strands to set distinct states of two devices lends a new element to the toolbox for nanorobotics and for machines whose states are set by computational processes. It enables many nanoscale capabilities, from reciprocating machines to molecular choreography. The ability to correlate the motions of molecular devices can lead to complex behavior on the nanoscale. For example, in a recent device,¹⁵ the legs of a bipedal walker communicate with each other, producing an autonomous walker.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research has been supported by grants GM-29544 from the National Institute of General Medical Sciences, CTS-0608889 and CCF-0726378 from the National Science Foundation, 48681-EL and W911NF-07-1-0439 from the Army Research Office, N000140910181 from the Office of Naval Research and a grant from the W.M. Keck Foundation.

References

1. Seeman NC. Trends Biochem. Sci. 2005; 30:119–125. [PubMed: 15752983]
2. Hagerman PJ. Biochemistry. 1985; 24:7033–7037. [PubMed: 4084556]
3. Yan H, Zhang X, Shen Z, Seeman NC. Nature. 2002; 415:62–65. [PubMed: 11780115]
4. Ding B, Seeman NC. Science. 2006; 314:1583–1585. [PubMed: 17158323]
5. Gu H, Chao J, Xiao SJ, Seeman NC. Nature Nanotech. 2009; 4:245–249.
6. Chakraborty B, Sha R, Seeman NC. Proc. Nat. Acad. Sci. (USA). 2008; 105:17245–17249. [PubMed: 18474862]
7. Seelig G, Yurke B, Winfree E. J. Am. Chem. Soc. 2006; 128:12211–12220. [PubMed: 16967972]
8. Liu H, Chen Y, Yu H, Ribbe AE, Mao C. Angew. Chemie Int. Ed. 2006; 45:1942–1945.
9. Venkataraman S, Dirks RM, Rothmund PWK, Winfree E, Pierce NA. Nature Nanotech. 2007; 2:490–494.
10. Ferguson KA. Metabolism. 1964; 13:985–1002. [PubMed: 14228777]
11. Rodbard D, Chrambach A. Anal. Biochem. 1971; 40:95–134. [PubMed: 5550151]
12. Liu WY, Wang X, Wang T, Sha R, Seeman NC. Nano Lett. 2008; 8:317–322. [PubMed: 18069876]
13. Ding B, Sha R, Seeman NC. J. Am. Chem. Soc. 2004; 126:10230–10231. [PubMed: 15315420]
14. Constantinou PE, Wang T, Kopatsch J, Israel LB, Zhang XP, Ding B, Sherman WB, Wang X, Zheng J, Sha R, Seeman NC. Org. Biomol. Chem. 2006; 4:3414–3419. [PubMed: 17036134]
15. Omabegho T, Sha R, Seeman NC. Science. 2009; 324:67–71. [PubMed: 19342582]
16. Yurke B, Turberfield AJ, Mills AP Jr, Simmel FC, Newmann JL. Nature. 2000; 406:605–608. [PubMed: 10949296]

Table of Contents Graphic

Figure 1. Schematics of the Reciprocal PX-JX₂ devices

(a). *Two PX-JX₂ Device Systems.* Each motif of the device systems involves a 1.5-turn set strand section in the middle, and two 3-turn outer frame sections at the ends. Arrowheads indicate the 3' ends of strands. The strands of System I contain magenta and green frame strands, and the strands of System II contain red and black frame strands. Both are controlled by dark blue and light blue set strands: the dark blue strands put System I in the PX state and System II in the JX₂ state, termed JX₂', because it is in System II. Similarly, the light blue set strands put System I in the JX₂ state and System II in the PX' state. The set strands contain 8-nucleotide toeholds¹⁶ (drawn as horizontal lines) that are used for their removal. (b). *The Machine Cycles of the Two Systems Directed by the Same Set Strands.* The PX state of System I and JX₂' state of System II are shown at the left. In process I, the

dark blue unset strands with biotin (indicated by black circles) are added, and the unset strand/set strand complexes are removed by magnetic streptavidin beads, leaving naked frames (top). In process II, the light blue set strands are added, pairing with the exposed parts of the frames, and transforming them into the JX_2 and PX' states. In process III the unset strands for the light blue set strands are added to leave the naked frame again. In process IV, the dark blue set strands are added, so the system contains System I in the PX state and System II in the JX_2' state.

Figure 2. Non-denaturing gel electrophoresis showing the transformations of the components Lane M in all panels contains a 10 bp DNA ladder marker. All gels contain 10% polyacrylamide. (a) The Formation and Transformation of the PX, JX₂ and PX', JX₂' Device States. Lanes 1, 4, 7 and 10 contain the annealed initial JX₂ state of System I, the initial PX state of System I, the initial PX' state of System II and the initial JX₂' state of System II, respectively. Lane 2 contains the conversion of the PX material in Lane 4 to the JX₂ state, and lane 8 contains conversion of the JX₂' material in Lane 10 to the PX' state. Likewise, lane 3 contains the conversion of the JX₂ material in Lane 1 to the PX state, and Lane 9 contains the conversion of the PX' material in Lane 7 to the JX₂' state. Lanes 5 and 6 are the unstructured system I intermediate Frame and the unstructured system II

intermediate Frame', respectively; we do not understand why the mobilities of the two frame molecules are different. (b) *Cycling the States of the System*. The system begins with the annealed initial JX₂ state of System I (Lane 4) and the PX' state of System II (Lane 8). Proceeding leftwards for System I, the material in lane 3 contains the result of converting the material in lane 4 to the PX state, and the material in lane 2 contains that material further converted to the JX₂' state, and lane 1 contains the material in lane 2 yet further converted to the PX state. Similarly, for System II, the material in lane 7 contains the material in lane 8 converted to the JX₂' state, the material in lane 6 contains the material in lane 7 converted back to the PX' state, and the material in lane 5 contains the material in lane 6 reconverted to the JX₂' state. (c) *Cycling the System by Starting at a Different Point*. The same conversions take place as in (b), but lane 8 begins in the JX₂' state of System II and lane 4 begins in the PX state of System I. Following three steps of conversion, leftward on the gel, the material in lanes 5 and 1 is in the opposite state. (d) *Cycling Both Systems in the Same Solution*. This panel shows the cycling of the systems simultaneously through 3 steps. The concentration of the complexes in System II (1.0 μM) is twice as that of the complexes in System I (0.5 μM), so that the two cycle states can be distinguished. Lane 1 contains the annealed initial JX₂ state of System I and the PX' state of System II. Proceeding rightwards, lanes 2 to 4, respectively, show alternating transformations to the other states for Systems I and II. (e) *Cycling the Systems in the Same Solution by Starting at a Different Point*. The same conversions take place as in (d), except that the operation starts from the annealed initial PX state of System I and the JX₂' state of System II (Lane 1). Following by three steps of conversion, lanes 2 to 4 contain the alternating transformation products.

Figure 3. Ferguson Analysis of all the Complexes in System I and II

(a) Ferguson plots of the Frame (indicated as F), PX and JX₂ molecules in system I, compared with that of an 180 nt duplex. (b) Ferguson plots of the Frame' (indicated as F'), PX' and JX₂' molecules in system II, with the same 180 nt duplex control as (a). The values and trends of the slopes are all comparable to those in device system I.

Figure 4. Schematic Drawings of the One-Dimensional Self-Assembly System

(a). *The Devices and PX Triangles used in the 1D Array.* The devices used here are the same as those in Figure 1a, except that each loop of Figure 1a is substituted by a pair of sticky ends: A (orange, 12 nt) and B (magenta, 12 nt) on one side of the PX and JX₂ device, C (dark green, 13 nt) and D (dark red, 13 nt) on the other; E (brown, 12 nt) and F (dark purple, 12 nt) on one side of devices PX' and JX₂', G (light green, 13 nt) and H (bright red, 13 nt) on the other. The sticky ends A' (orange, 12 nt), B' (magenta, 12 nt) and G' (light green, 13 nt), H' (bright red, 13 nt) in the bottom edge of the PX triangle T1, complements those in the PX/JX₂ and PX'/JX₂' devices, A, B, G, H. Similarly, the sticky ends of PX triangle T2, C' (dark green, 13 nt), D' (dark red, 13 nt) and E' (brown, 12 nt), F' (dark purple, 12 nt) are

complementary to those in the devices, C, D, E, F. (b). Schematics of the Self-Assembly of the 1D Array. In the upper panel, the devices in the PX and JX_2' states successively connect the PX triangles T1 and T2, which point in the same direction. In the lower panel, the PX and JX_2' states switch to JX_2 and PX' states simultaneously, resulting T1 and T2 pointing in opposite directions. The period of the arrays involving two PX triangles and two devices, as shown in Figure 4b, is expected to be about 125 nm.

Figure 5. AFM Evidence for the Operation of the Reciprocal PX-JX₂ Devices

(a). 1D tiles containing PX and JX₂' molecules with a parallel arrangement of PX triangles (upper panel), and JX₂ and PX' molecules with an alternating arrangement of PX triangles (lower panel). The number of triangles seen is a function of the number of molecules that associated to form the 1D array. (b). The two-step transformations showing reciprocal motions of the PX-JX₂ devices. The arrays of the upper section illustrate the cycling operation, from the initial PX-JX₂' state to the JX₂-PX' state (Step I), and then back to the PX-JX₂' state (Step II). In the lower panel, the cycling operation starts at a different point, from an initial JX₂-PX' state to the PX-JX₂' state (Step I), and then back to the JX₂-PX' state (Step II). The distance of one period of the array, two triangles with two devices, is measured as 124 nm, with a standard deviation of ± 12 nm, based on the average of thirty periods, in agreement with the calculated value of 125 nm. These images depict successive aliquots taken from solutions containing the various states of the device array.