A Precisely Controlled DNA Biped Walking Device

William B. Sherman and Nadrian C. Seeman*

Department of Chemistry, New York University, New York, New York 10003

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ABSTRACT

We present an experimental realization of a molecular walking motor built from DNA. The device consists of a biped and a well-defined track where it walks. Input DNA strands act as a convenient interface to allow precise control of the biped walking forward or backward. Psoralen molecules are attached to the ends of the feet in synthesis. When irradiated, the psoralen molecules cross-link strands covalently, allowing unambiguous determination of the state of the walker.

The recent centennial of the Wright brothers' invention of the airplane reminded the world not only of the fundamental importance of traveling machines but also that the great achievement of the Wright brothers was not so much getting off the ground, but controlling their biplane so it could fly steadily. One hundred years later, we find ourselves facing many of the same challenges on the nanometer scale. Numerous techniques have been devised for building or isolating molecular machines and motors, but the powerful combination of a wide range of motion with precise control has proved elusive.

Several synthetic DNA devices have been reported recently.[1−7] These devices are characterized by having fixed links between all their primary elements, so that the motion is fundamentally intramolecular. Motion relative to an external substrate has not been demonstrated, although nothing precludes adapting some of the previously developed systems for that purpose. Many of the DNA devices described to date depend on the addition of fuel strands to power them.[3−7] Two useful classes of fuel strands are "set" and "unset" strands. Set strands join single stranded segments by hybridizing with them but can be removed by the addition of unset strands for which they have greater affinity.

We present here a DNA-based nanodevice where the two parts of the system are connected only via labile hydrogen bonds. The system, illustrated in Figure 1, has been proto-typed with two components: a triple crossover (TX) molecule,[8] shaded blue, which is referred to as the footpath, and a biped region, shaded tan, which consists of two double-helical domains connected by three flexible, 9-nucleotide linker strands of DNA. The three tan linker strands are flexible because they are not complementary to any of the other strands in the system, and thus they remain single stranded throughout the operation of the device. Each helical

domain in the biped has a single stranded portion, called a "foot", on its end; the foot is available to pair with complementary strands of DNA. Similarly, each domain in the footpath has a single stranded region, called a "foothold", which is also available for hybridization with complementary strands. The sequences of the feet and the footholds are all carefully selected to minimize complementarity between them.[9] A foot attaches to a foothold when a set strand complementary to both is added to the solution. Thus, when the black and white strand labeled "SS2B" is present, it connects the biped Foot 2 (labeled 'F2,') to the Foothold B (labeled 'H B') as shown.

Each set strand has an 8-base overhang, or "toehold," which is not complementary to any of the feet or footholds. The toehold allows the set strand to be removed by the unset strand by the method of Yurke et al.[3] Each unset strand is synthesized with a biotin on the 5′ end so that after an unset procedure, all the unset strands and the set strands with which they are paired, can be removed from the solution by the use of streptavidin coated magnetic beads.[4]

Figure 2 cartoons the operation of the biped. The sequences of each foot, foothold, and toehold are represented in the cartoons by unique colors. Complementary strand sections are shown with the same color. Thus, the magenta and aqua sections of Set Strand 1A complement Foot 1 and Foothold A, respectively. Panel a shows state 1A,2B of the system. In this state, Foot 1 is set to Foothold A, and Foot 2 is set to Foothold B. The biped is initially prepared in this state.

For the biped to take a step, the first thing that needs to happen is for Foot 2 to be released from Foothold B. Figures 2b and c show this event happening. First, Unset Strand 2B is introduced into the solution. It binds to the toehold of Set Strand 2B (Figure 2b), followed by branch migration that results in the complete hybridization of Set Strand 2B and Unset Strand 2B. This state, shown in Figure 2c, leaves Foot
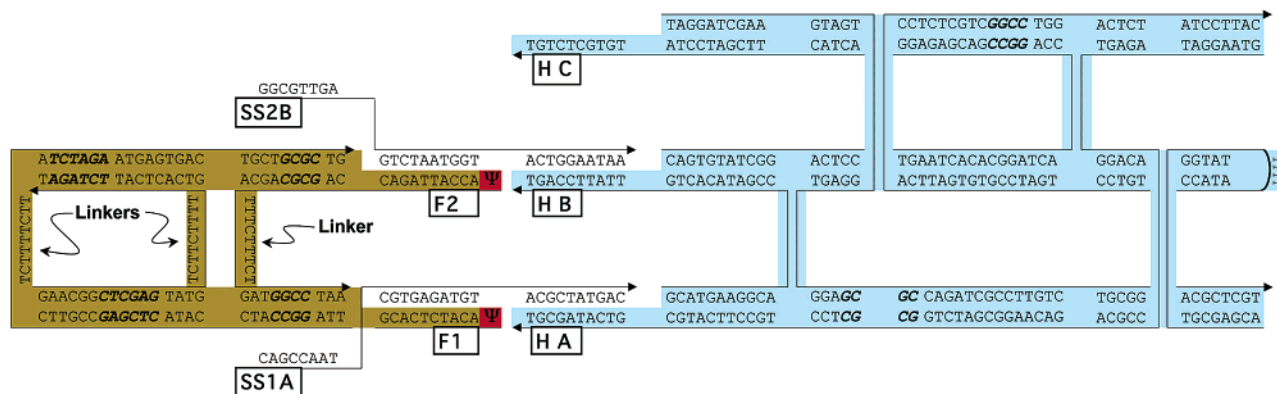
Figure 1. Cartoon of the biped system with DNA base sequence included. The entire system is constructed from conventional DNA nucleotides, except for the two psoralen molecules attached to the 5′ ends of strands F1 and F2. The blue section is the TX footpath, which is essentially a rigid structure with three single stranded "Footholds" on its left side, labeled HA, HB, and HC. The tan section is the biped, with the two double helical domains connected via three single stranded flexible linkers. Each domain of the biped has a "Foot" on the right side that is a single stranded segment; these are labeled F1 and F2. The set strands that attach the foot to the TX footpath are labeled SS1A and SS2B. This state of the device is therefore called 1A,2B. Psoralen groups for cross-linking are indicated by "Ψ" in a red box. Sites available for restriction analysis are shown italicized and bold. The 3′ end of each strand is indicated by a solid arrowhead.

2 connected to the footpath only through the flexible linkers to Foot 1 and Set Strand 1A. The Unset complex can be removed with streptavidin beads as mentioned above.

Once Foot 2 has been freed from Foothold B, it is free to be set back down either on Foothold B, which would return the system to State 1A,2B, or on Foothold C, which would move the system to State 1A,2C as shown in Figure 2d. Note that the flexible linkers need to extend across Foothold B to reach from Foothold A to Foothold C. Thus the shortest the linkers can be is about 2 nm. Similar unset and set operations can be used to move Foot 1 from Foothold A to Foothold B, bringing the system to state 1B,2C as shown in Figure 2f. The total distance traveled by the biped relative to the footpath during this step is approximately the 2 nm width of one DNA helical domain.

Minimizing sequence symmetry is a standard approach to designing systems in structural DNA nanotechnology.[9] All the strands used here were designed using the program *SEQUIN*[10] to achieve this result. The particular difficulty of designing multistate systems, such as this one, comes from the fact that the various set strands share some sequences. Set Strands 1A and 1B both need to be constructed so that they do not fold up on themselves. A change in the sequence of Foot 1, which might improve Set Strand 1A, may, unfortunately, introduce problems in Set Strand 1B, so numerous rounds of sequence checking were necessary to find a satisfactory solution for all the single stranded regions. All of the strands in the system were purified via poly-(acrylamide) gel electrophoresis (PAGE), stained with ethidium bromide, and briefly exposed to UV light to cut from the gel. This brief exposure leaves the bulk of the psoralen available for further UV activation. All the psoralenated strands run as single bands on a denaturing gel after purification. At all other times prior to UV treatment, light exposure to the psoralenated strands was minimized to prevent any additional disturbance of the psoralen.

The movement of the biped is observed using psoralen to link the foot strands covalently to their respective set and
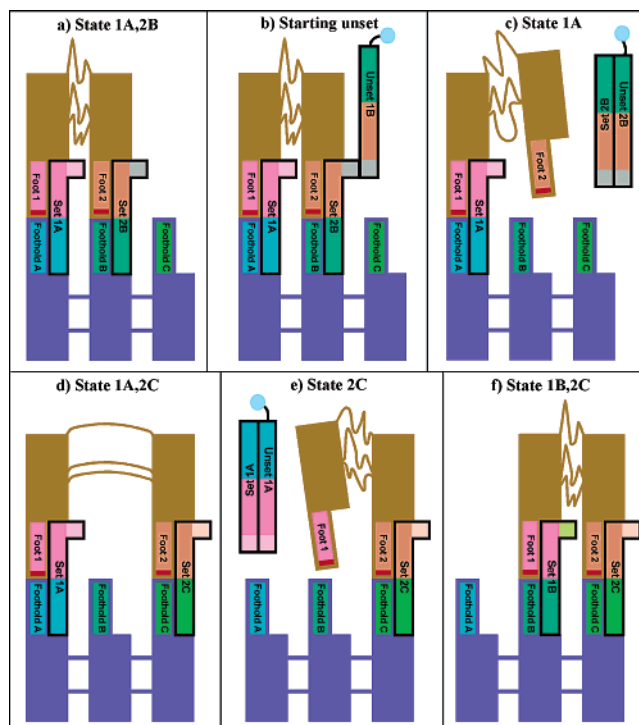

Figure 2. Cartoon depiction of biped system taking a full step. The states through which the system passes sequentially are shown in the panels of the drawing. The system is rotated 90° clockwise relative to Figure 1. Matching colors indicate complementary sequences between strands. The red section at the end of each foot indicates psoralen. Panels b and c depict Unset Strand 2B first attaching to the toehold section of Set Strand 2B (drawn in gray), and then removing it, setting Foot 2 free. The Unset complex is then removed via the biotin group (depicted as a blue circle) attached to the unset strand.[4] The initial phase of this detachment is not shown for the Unset 1A. Note that the connections between the domains of the footpath have been enlarged for simplicity; they are only about 1 nt long.

foothold strands (see Figure 3). To optimize the psoralen cross-linking efficiency, the 5′ two bases of each foot need to be 5′-AC-3′, and the 3′ two bases of each foothold need

Figure 4. 6.4% nondenaturing PAGE of the biped walk. The gel was run at 16 °C and stained with Stains-All dye. The left lane contains a 10-base marker ladder with dark bands at 10, 100, and 330 bases. Each state of the biped is shown to be stable at 16 °C.
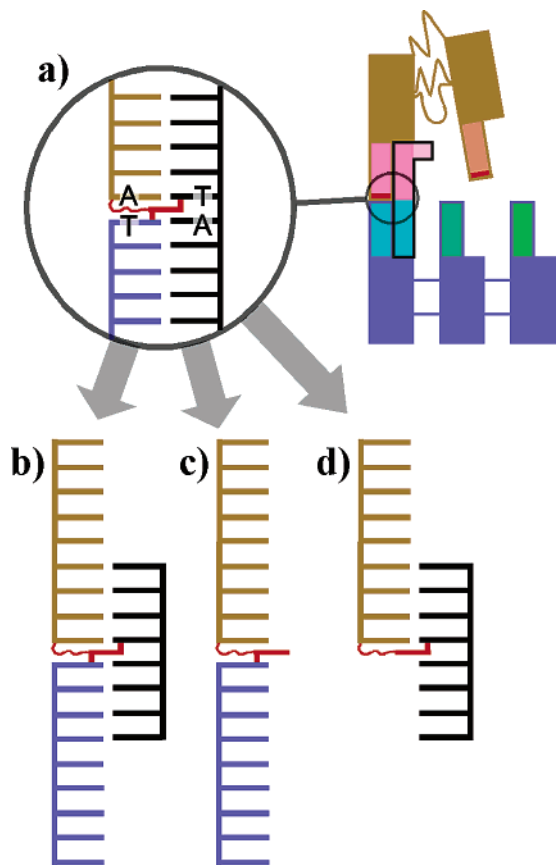
Figure 3. (a) The inset circle shows a detailed picture of a foot strand (tan), a set strand (black), and a foothold strand (blue). The psoralen (red) is covalently attached to the foot, but upon UV irradiation, it can form covalent links to either the set strand, the foothold, or both. This results in three possible structures in a denaturing environment. The target complex (b) is the largest, with all three strands covalently linked. The two failure complexes (c and d) have only two of the three strands linked.

to be 5′-GT-3′.[11] Restriction sites were incorporated into the design for trouble-shooting purposes (Figure 1).

The primary steric consideration of the system is that the flexible linkers need to be long enough to reach across the largest stride the biped will take. One factor that influences this calculation is the rotation of the feet as they move from one foothold to the next. This system was designed to minimize rotation of the feet; they only translate as they move from one foothold to the next, and the flexible linkers always connect the closest portions of each foot. Minimizing the lengths of the flexible linkers lowers the entropic barrier to setting a foot and it helps to minimize the chance that one foot will set on a different sidewalk from the other foot. We chose 9-nucleotide flexible linkers to be certain they were long enough. A flexible linker separated from the other two by 16 nucleotide pairs was used to help maintain the alignment of a detached foot facing toward the same footpath as the attached foot.

The state of the system is observed by taking an aliquot and exposing it to UV light. As shown in Figure 3, if a foot is attached to a foothold, then the psoralen on the end of the foot can link to the T on the end of the foothold and/or to the T in the set strand. The target product, shown in Figure 3b, is a three-strand complex which is formed with nearly
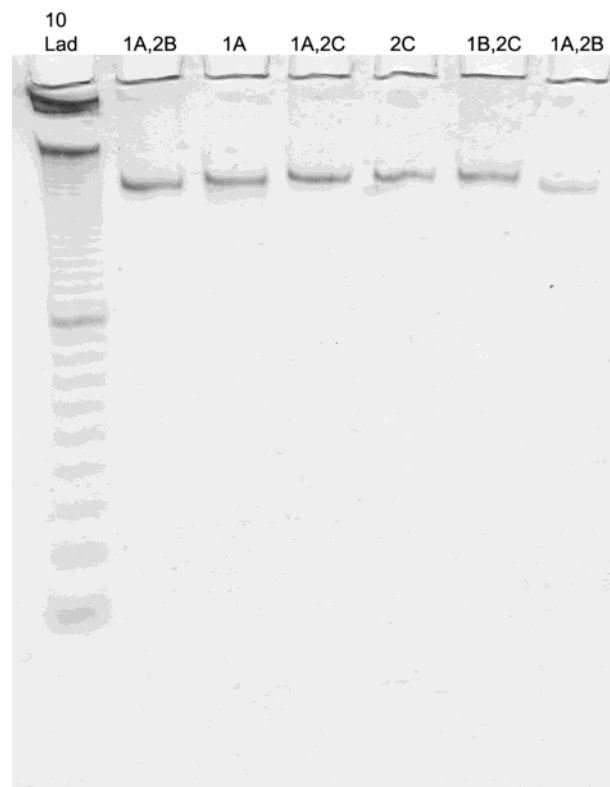
50% yield for an attached foot, but which cannot form for an unattached foot. Since each foot and foothold has a unique length, denaturing PAGE of the UV treated aliquot reveals which feet were detached and which feet were attached to which footholds.

A walk of the biped starts by annealing the biped and the footpath separately in a nondenaturing buffer, consisting of 40 mM Tris-HCl (pH 8.0), 2 mM EDTA, 12.5 mM magnesium acetate. The complexes are cooled from 95 °C to room temperature over 48 h in a 2 L water bath in a styrofoam box. They are then combined, and Set Strand 1A is added to the solution. After 20 min, Set Strand 2B is added. All subsequent operations are carried out at 16 °C unless otherwise noted. The biped starts in state 1A,2B, as shown in Figure 2a. Two aliquots were taken, one for nondenaturing gel analysis, the other for UV exposure and then denaturing gel analysis. The biped was then walked through states 1A; 1A,2C; 2C; and 1B,2C, (Figure 2c–2f) with two aliquots taken at each of those steps. After that, the reverse operations were carried out, and the biped walked back to the initial state 1A,2B, where the remaining solution was divided up for nondenaturing and denaturing analysis. The resulting gels are shown in Figures 4 and 5.

The nondenaturing gel shown in Figure 4 shows that the entire biped-footpath complex is stable at 16 °C. A single clear band is visible in each lane in the expected 235−255 base pair range. The concentration decreased from 0.5 μM to 0.26 μM through the walk as each fuel strand addition increased the solution volume.
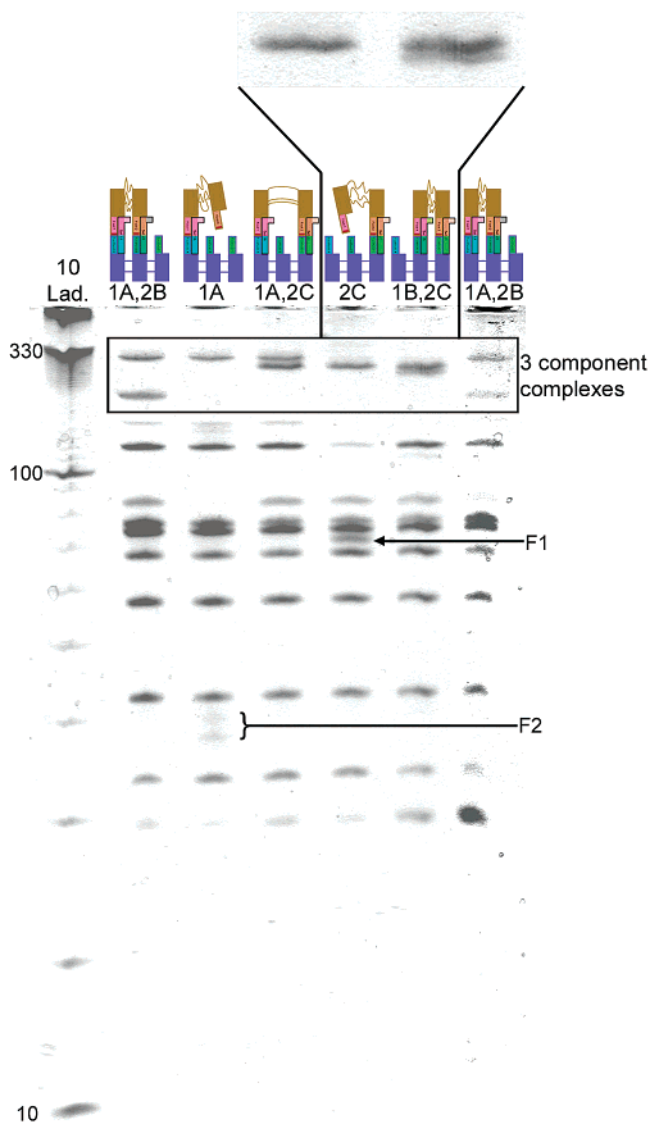
Figure 5. Record of a biped walk. This is a denaturing 13% polyacrylamide gel stained with Stains-All dye. The left lane is a 10-base ladder. The right lane has been contrast-enhanced to show fainter bands. The states progress in chronological order from left to right across the gel proceeding through the states indicated (see Figure 2, or the thumbnail images above each lane). The target three-strand complexes appear in the boxed area, showing where the feet have been cross-linked to the footpath. In the left-most experimental lane, the two Feet (1 and 2) of state 1A,2B are visible, indicating that they are attached to Footholds A and B. Next, in state 1A, Foot 2 has been detached, leaving only the complex corresponding to Foot 1 attached to Foothold A. In the next lane, Foot 2 has been attached to Foothold C, while Foot 1 remains attached to Foothold A. In the next lane over, Foot 1 has been released from Foothold A, so the only band corresponds to Foot 2 attached to Foothold C. In the following lane, Foot 1 has been attached to Foothold B. The bands run closely together, so the band has been magnified in the inset for comparison with the band in the 2C lane. In the final lane, the biped has been walked all the way back to the beginning, showing the initial arrangement of bands. Detached feet run largely as single strands, marked F2 and F1. See the Supporting Information for a complete analysis of the partially cross-linked products below the box.

Figure 5 shows a walk of the biped via denaturing PAGE analysis of the psoralen-linked complexes. The bands in the boxed region are all three-strand complexes such as the one illustrated in Figure 3b. The gel clearly shows one and only one three-strand complex for each foot attached to a foothold. The largest complex, which comes from Foot 1 on Foothold A (164 nucleotides), gives the highest band, and appears in states 1A,2B, 1A, and 1A,2C but not in any of the other states. Similarly, the second largest complex, Foot 2 on Foothold C (141 nucleotides), gives the second highest bands, and appears in states 1A,2C; 2C; and 1B,2C but none of the other states. Proceeding in chronological order from left to right, starting with the lane labeled 1A,2B: the two feet are shown to be on the correct footholds as indicated by the band corresponding to the cross-linking of Foot 1 and Foothold A, which contains 164 nt, and the other band, Foot 2 cross-linked to Foothold B, containing 99 nt. This latter band disappears in state 1A (lane 1A), where Unset Strand 2B has removed Set Strand 2B; this is expected, because Set Strand 2B is no longer properly positioned for cross-linking. The next state is state 1A,2C. The uppermost band is retained, but a new one is now seen, corresponding to Foot 2 cross-linked to Foothold C. This band contains 141 nt, and is intermediate in mobility between the other two bands in the lanes to its left. Lane 2C contains the cross-linking results after the Foot 1 has been detached from Foothold A: The upper band disappears, and only the lower band is retained. The final state is 1B,2C, in which the 141 nt band is retained, and a band containing 131 nt appears. This band corresponds to the cross-linking of Foot 1 to Foothold B. At the far right of Figure 5, the device has been walked all the way back to its starting state, 1A,2B, and the appropriate bands show up again. Thus, as each foot is attached and detached from each foothold, a band with the expected relative mobility appears or disappears. Ferguson analysis of these three-strand cross-linked molecules indicates that their friction constants on denaturing PAGE are three to four times as high as they are for a linear single strand of the corresponding length; this difference explains why the three-strand complexes migrate anomalously on the gel relative to linear markers. Unattached foot strands run primarily as single strands, marked F1 and F2 lower in the gel. Nonspecific binding leads to some intra- and interstrand psoralen cross-links, which explains the splitting of band F2, for example, which also appears when the Foot 2 strand is irradiated in isolation (data not shown). An analysis of all of the bands on this gel is available in the Supporting Information.

We have produced a device similar to the walking proteins kinesin and myosin V, albeit with precise bidirectional control and an inchworm-type gait instead of a foot-passing gait.[12,13] Longer footpaths would presumably accommodate a foot-passing gait, as well as multiple, independently address-able bipeds and multipeds. Further experimentation will be needed to establish whether a small set of footholds in a repeating pattern can provide the same precise control as the TX footpath used here. Such a track would be reminiscent of the walker proposed by Reif,[14] except that in our system, movement would be nonrandom and matter, not only infor-mation, would travel across multiple steps. The flexible na-ture of the biped mechanism should enable it to walk on

multidimensional footpaths. A small, circular footpath could be used to generate rotational as well as linear motion. Potential applications include transporting loads possibly connected to the biped and removed from it via fuel strands, as well as winding or threading polymers with one end attached to the biped and the other end affixed to the footpath (or another biped). Algorithmically generated set strands could drive a device on a 2D footpath so that the positions of the feet could represent the state of a DNA-computational machine.

Supporting Information Available: Strand sequences. Detailed gel analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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