Electronic Supplementary Information

A DNA bipedal nanowalker with a piston-like expulsion stroke
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S1. DNA Strands and sequences (Figure S1)
S2. Extra data (Figures S2, S3, S4, S5)
S3. Results of simulation study (Figures S6, S7, S8)

S1. DNA strands and sequences
The constituent strands of the walkers and tracks are marked in Fig. S1. The nucleotide sequences of the strands are given below following the sequence segments marked in Fig. S1 (from 5’ to 3’ end; with asterisk marking a complementary sequence).

**A. Walker**

![Diagram of Walker]

**B. 4-site track**

![Diagram of 4-site Track]

**C. 3-site track**

![Diagram of 3-site Track]

**D. 2-site track**

![Diagram of 2-site Track]

**Figure S1.** The DNA strands for the walkers and tracks with the segments with different sequences marked.
(1) Walkers
Walker with 35bp bridge: strand 1 = BHQ-1-L-LK-B35-C; strand 2 = BHQ1-L-LK-G4-B35*
Walker with 30bp bridge: strand 1 = BHQ-1-L-LK-B30-C; strand 2 = BHQ1-L-LK-G4-B30*
Walker with 25bp bridge: strand 1 = BHQ-1-L-LK-B25-C; strand 2 = BHQ1-L-LK-G4-B25*
Leg segment: L = TGGAATGACT
Linker segment: LK = TTTT
Cytosine-rich overhang (27nt): C = CCCTAACGCTAACGCTAACCCTAAGC
G-quadruplex segment (21nt): G4 = GGGTTAGGGTTAGGGTTAGGG
Bridge segments
B35 = CGGAATGCATCGCTCAGTGTTGATCTGATTAACTT
B30 = CGGAATGCATCGCTCAGTGTTGATCTGATT
B25 = CGGAATGCATCGCTCAGTGTTGATC

(2) Light-responsive fuel (with azobenzene indicated by X)
Fuel = ACXGTXTAXCGXGTXTAXGCGXGTXTAXGCGXGTXTA

(3) Tracks
Template strand for 3-site track: T3 = S4*-L*-S3*-L*-S2*-L*-S1*
Template strand for 4-site track: T4 = L*-S3*-L*-S2*-L*-S1*-L*
Template strand for 2-site track: T2 = S4*-L*-S3*-L*-S1*
Spacer strands (each 20nt long)
S1 = GAAACGCTTCATGGTGAGCG-TYE
S2 = TGTTATCCCGGTGCTTTTTG-CY5
S3 = CCATACCACGGGGCCAGCGC-FAM
S4 = GTATCATCGCCAGTCCATCA
S2. Extra data

Figure S2. Reversible formation of G-quadruplex. The data are from a fluorescence resonance energy transfer (FRET) experiment carried out using the same spectrophotometer as for the fluorescence motility experiments. The FRET experiment is done using a modified version for the walker’s strand containing the G4 sequence: A donor dye (FAM, with excitation/emission at 495nm/520nm) and an acceptor dyes (TAMRA, with excitation/emission at 559nm/583nm) are chemically tethered at two ends of the G4 sequence (i.e., intra-strand labelling for both dyes, with the quencher removed from the strand’s 5’ end). The excitation is done only for the donor (at 495nm, excitation slit width 5nm), and the emission is collected from both donor and acceptor (at 520nm and 583nm respectively, emission slit width 5nm). Since the excitation is selectively for the donor, the acceptor emission occurs only via energy transfer from the donor when it is near. Hence the G-quadruplex formation or opening is indicated by a high or low FRET efficiency, which is defined as the acceptor emission divided by the combined emission from the acceptor and donor. Panel A. Change of FRET efficiency after the strand with donor/acceptor is mixed with an ‘opener’ strand at a ratio of two openers per donor/acceptor strand. The opener strand is a 12nt-long sequence (CCCTAACCTCAA) that is part of the cytosine-rich overhang from the walker’s strand that doesn’t contain the G4 sequence. The efficiency drop indicates that the quadruplex structure can be opened by two openers, which are equivalent of the main body of the walker’s cytosine-rich overhang designed to open the quadruplex. Panel B. Change of FRET efficiency when the buffer containing the donor/acceptor strand (no opener strand) is heated from 25 °C to 75 °C (red dots) and when it is cooled reversely back to 25 °C (black dots). The efficiency decrease upon heating indicates quadruplex opening. The complete recovery of efficiency upon cooling indicates the reversible formation of the G-quadruplex when it is not interfered by the opener or the cytosine-rich overhang.
Figure S3. Change of control-calibrated fluorescence for the walkers with 35bp and 25bp bridge operated on a three-site track. The experimental procedure is same as for Fig. 2.
**Figure S4.** The fluorescence from the track-only control experiment accompanying the operation experiments on the two-site track, which are shown in Fig. 4A, B.
Figure S5. Control-calibrated fluorescence for the walker of 25 bridge operated on the two-site track. The experimental procedure is same as for Fig. 4A, B.
S3. Results of Simulation study

Figure S6. Free-energy profile for the walker of 35bp bridge on a two-site track. From the right to left, the reaction coordinate indicates the path way for dissociation of the front or rear leg when the walker adopts an elongated state (i.e., state ii in Fig. 1D, which is formed under UV when the G-quadruplex sequence hybridizes with the complementary overhang into a duplex). For a common reference, the zero free energy is chosen as the fully bound state (i.e., 10bp) for the walker’s either leg. A leg dissociation occurs when the number of leg-site base pairs drops to zero, which corresponds to a free-energy peak. This free-energy peak is the barrier of dissociation for the front or rear leg. The barrier is higher for the front leg than the rear leg, suggesting a bias for preferential rear leg dissociation for the walker of 35bp bridge.
Figure S7. The same as for Fig. S6 but for the walker of 30bp bridge. For this walker, the barrier is higher for the front leg than the rear leg, suggesting a bias for preferential rear leg dissociation.
Figure S8. The same as Fig. S6 for the walker of 25bp bridge. For this walker, the dissociation barrier is virtually the same for the front leg and rear leg, suggesting no bias for either leg’s dissociation.