Supporting Information

A Well-Directional Three-Dimensional DNA Walking Nanomachine That Runs in an Orderly Manner

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Supporting Figures

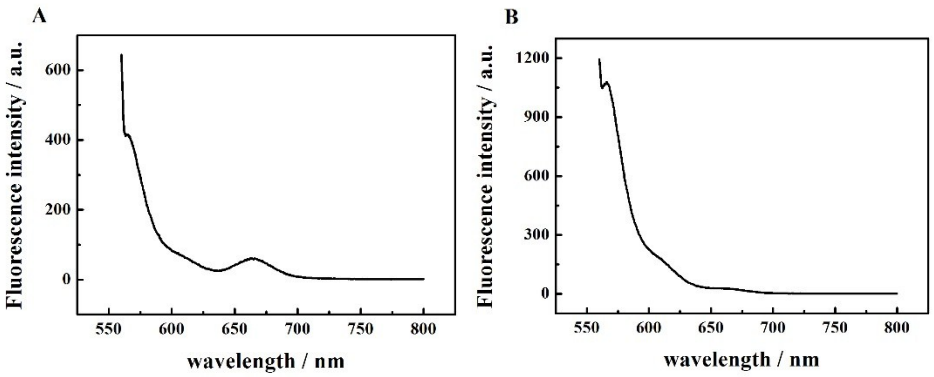

Figure S1. (A, B) The fluorescent spectra from the correct fluorometric 3D DNA walking nanomachine before and after walking.

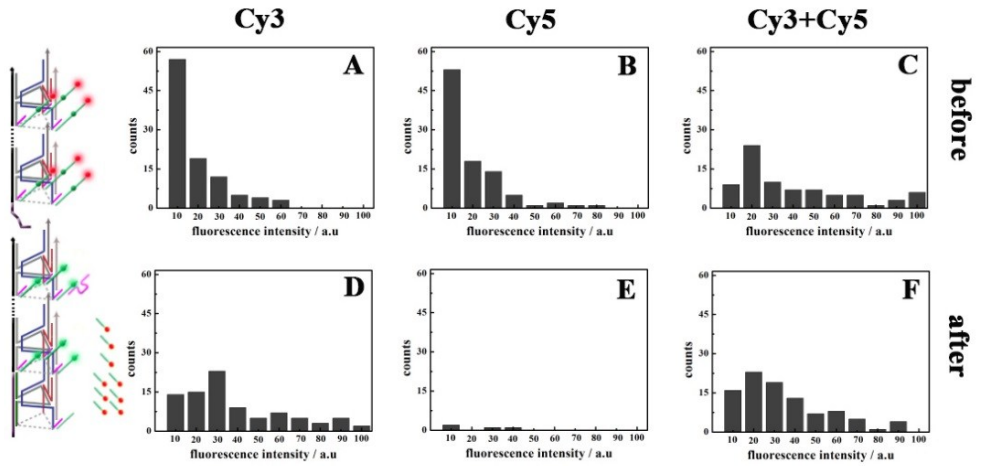

Figure S2. (A, D) Fluorescence intensity histograms of Cy3 spots from images A and D of Figure 4, respectively. (B, E) Fluorescence intensity histograms of Cy5 spots from images A and D of Figure 4, respectively. (A, D) Fluorescence intensity histograms of colocalized spots from images A and D of Figure 4, respectively.

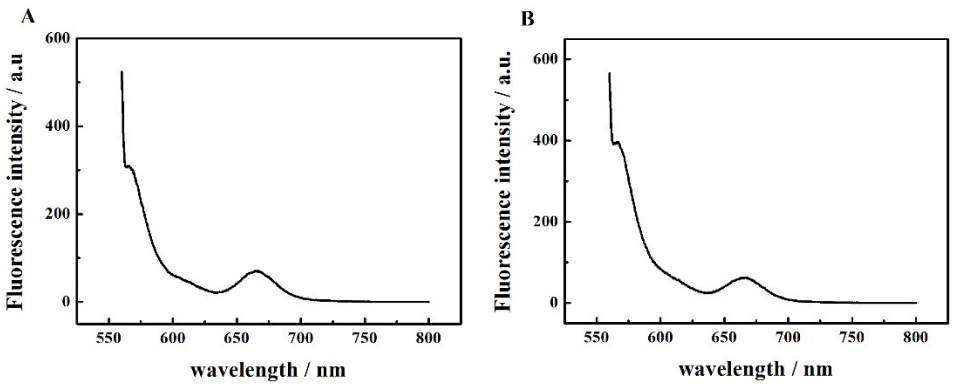

Figure S3. (A, B) The fluorescent spectra from the unassembled 3D DNA walking nanomachine before and after walking.

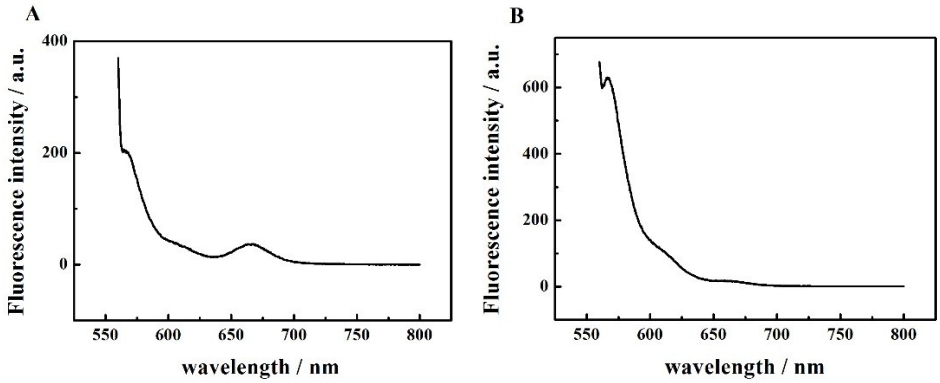

Figure S4. (A, B) The fluorescent spectra from the walker-unset 3D DNA walking nanomachine before and after walking.

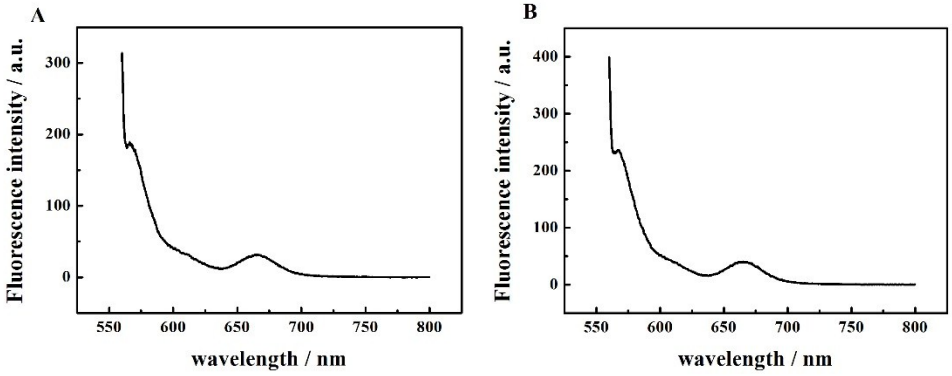

Figure S5. (A, B) The fluorescent spectra from the no-walker 3D DNA walking nanomachine before and after walking.

The digestion mechanism of 10-23 DNAzyme

The 10-23 DNAzyme, activited by the presence of Mg^{2+} ion, is comprised of a catalytic domain of 15 deoxynucleotides (GTCCGAATCAGCACT), flanked by two substrate-recognition domains of seven (GTCACTC) to eight deoxynucleotides each. The RNA substrate is bound through Watson–Crick base pairing and is cleaved at a particular phosphodiester located between an unpaired purine (A) and a paired pyrimidine residue (U).[1, 2]

Atomic force microscopy (AFM) measurement and analysis

Typically 3.0 μL of the assembled nanomachine solution (with 12.5 mM Mg^{2+}) was deposited on freshly cleaved mica sheet and allowed to incubate under clean condition (10 - 20 min). Then the sample was wash by 18-MΩ water and allowed to dry under clean condition (10 - 20 min). The scanasyst mode was used to perform the AFM image of the single DNA nanomachine with a AFM probe whose model is SCANASYST – AIR.

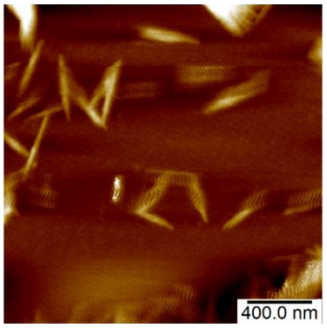

Figure S6. The AFM image of the single DNA nanomachine. Phase image: scale bar, 400 nm.

Confocal measurement and analysis

The glass slide was cleaned with NaOH (2 M) and ethanol (99%), respectively. The cleaned slide then was incubated in a solution of 12.5 mM Mg^{2+}, followed by washing with 18-MΩ water and allowed to dry under clean condition (10 - 20 min). Then 10.0 μL of the assembled nanomachine solution was deposited on the modified slide. Pulsed Lasers (561 nm, 514 nm) are used to excite the generation of fluorescence resonance

energy transfer (FRET) between the Cy3 (donor) and Cy5 (acceptor). The corresponding channels of filters are 557 nm - 578 nm and 654 nm - 676 nm.

Table S1. Sequences Information of All DNA Oligonucleotides Used in Our Work

Name	Oligonucleotides Sequences (5' to 3')
V	TCCTAAAGCATGACCTTCCGAACATTCGAGGCACGTTGTACGTCCACACTTGGAAC CTCATCGCACATCCGCCTGCCACGCTCTTGTTTCAAGCGCAGCCAGATTGGGCCCA ACCCGGG
C1	TGTTATCTCCGACGGTACTTCGTACAACGTGCCTCGAATGTAGAGGCAGGCGGATG TGAAGCAGTTGCACCGGCATTGTCGGGCCCAACCCGGG
C2	GTCACTACTAATACACCTGTCGATGAGGTTCCAAGTGTGGATAGCTAGGTAAGACC GCATCTCGGGCCCAACCCGGG
R1	AATGCCGGTGCAACTGCTACCAGGTGTATTAGTAGTGACGAC
R2	ATGCGGTCTTACCTAGCTCCAGTACCGTCGGAGATAACAGAG
V'	TCCTAAAGCAGCGAACTTTGTTCTCGCACCGTCCGCCTACACGCTACTCCAAGGTTC ACACCTGCATGTTGCACGGAGCTTACAAGCCTTCCAGTACGAAATCCT
V''	TCCTAAAGCAGCGAACTTTGTTCTCGCACCGTCCGCCTACACGCTACTCCAAGGTTC ACACCTGCATGTTGCACGGAGCTTACAAGCCTTCCAGTACGAAATCCTGGGGCCCA ACCCGGG
C1'	CTGTTACGGCCACGTTGACGAAGTGTAGGCGGACGGTGCGAGATGTAAGCTCCGTG CAACATGCTTCATGGCAGCCTCTATTGTGGGGCCCAACCCGGG
C2'	CTCTACGCCAGAATGGATCGATAGGTGTGAACCTTGGAGTAGCTGTCCACA TAATCATCACTG
R1'	CAGCAGTGATGATTATGTGGACCATCGTCAACGTGGCCGTAA
R2'	GAGACAATAGAGGCTGCCATGACCTCGATCCATTCTGGCGTA
Padlock probe	P-TCCTAAAGCATGACCTTCCGGTATTTCAAGCGCAGCCAGATT
linker	GGATTTCGTACTGGAAGGCAAAGTTCGCTGCTTTAGGAAATCTGGCTG
Short-backbone	GGATTTCGTACTGGAAGGCAAAGTTCGCTGCTTTAGGAAATCTGGCTGCGCTTGAA ACTACGGAAGGTCATGCTTTAGGA
h-S	GTCCGAATCAGCACTCCCGGGTTGGGCCC
Cy3/5-S	/iCy5/GTCACTCrArUGTCCGAAT/iCy3/CAGCACTCCCGGGTTGGGCCC
walker	AGTGCTGATTCGGACAGGCTAGCTACA ACGAGAGTGAC

REFERENCE

1. SANTORO, S. W.; JOYCE, G. F. *Proc. Natl. Acad. Sci. USA* 1997, 94, 4262–426.
2. He, Y.; Liu, David, R. *Nat. Nanotechnol*. 2010, 5, 778-782.