Supporting Information:

Building a Nanostructure with Reversible Motions Using Photonic Energy

*Mingxu You,[†] Fujian Huang, [†] Zhuo Chen,[‡] Ruowen Wang, [†] and Weihong Tan[†,‡,]**

† Department of Chemistry, Department of Physiology and Functional Genomics, Center for Research at the Bio/Nano Interface, Shands Cancer Center, University of Florida, Gainesville, FL 32611-7200, USA Fax: (+1)352-846-241; ‡ Molecular Science and Biomedicine Laboratory, State Key Laboratory of Chemo/Bio-Sensing and Chemometrics, College of Biology and College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082

E-mail: tan@chem.ufl.edu

Within this supporting information are found:

Experimental Section:

Chemicals and reagents employed, the synthesis of azobenzene phosphoramidite and DNA oligonucleotides, and the gel electrophoresis method.

Scheme S1: Sequences of DNA for constructing walking system.

Figure S1: Native gel electrophoresis to demonstrate walking system construction.

Figure S2: FRET between Dabcyl/TAMRA and between Dabcyl/FAM.

Figure S3: Processive motion of the walker along the track.

Figure S4: Fluorescence measurement and calibration curve for right/left turning choice.

Experimental Section:

Chemicals and reagents: The materials for DNA synthesis were purchased from Glen Research (Sterling, VA), including 6-(3', 6'-dipivaloylfluoresceinyl-6-carboxamido)-hexyl-phosphoramidite (6-FAM), 5'-Dimethoxytrityloxy-5-[N-((tetramethylrhodaminyl)-aminohexyl)-3-acrylimido]-2'-deoxyuridine phosphoramidite (TAMRA-dT), and 5'-dabcyl phosphoramidite. Other chemicals were purchased from Sigma-Aldrich. All reagents for buffer preparation and HPLC purification came from Fisher Scientific. Unless otherwise stated, all chemicals were used without further purification.

Azobenzene phosphoramidite: The synthesis of azobenzene phosphoramidite has been reported before[1,2]. The purity of the product has been proven by ^{1}H NMR ($CDCl_3$): δ 8.00-6.79 (m, 22H), δ 6.62 (d, 1H), δ 4.48 (m, 1H), δ 4.39 (m, 1H), δ 4.21-4.10 (m, 2H), δ 3.77 (s, 6H), δ 3.57-3.34 (m, 4H), δ 2.76-2.72 (m, 2H), δ 1.30-1.25 (m, 15H). ^{31}P NMR ($CDCl_3$): δ 149.

DNA synthesis: All oligonucleotides were synthesized using an ABI 3400 DNA synthesizer (Applied Biosystems, Inc., Foster City, CA) at the 1.0 micromolar scale. After complete cleavage and deprotection, the DNA sequences were purified on a ProStar HPLC system (Varian, Palo Alto, CA) with a C-18 reversed-phase column (Alltech, 5μm, 250mm × 4.6 mm). The eluent was 100mM triethylamine-acetic acid buffer (TEAA, pH 7.5) and acetonitrile (0-30min, 10-100%). All DNA concentrations were characterized with a Cary Bio-300UV spectrometer (Varian) using the absorbance of DNA at 260nm.

Native PAGE analysis: The binding and three-dimensional structures of the DNA walking system were observed using native PAGE gel. The gel was run in 8-10% acrylamide (containing 19/1 acrylamide/bisacrylamide) mixture with 1×TBE/15mM Mg^{2+} buffer, at 100V constant

voltage for 1 hour (4°C). After that, the gel was stained 30 min using Stains-All (Sigma-Aldrich) to image the position of DNA. Photographic images were obtained under visible light with a digital camera.

Scheme S1: Sequences of DNA for constructing walking system.

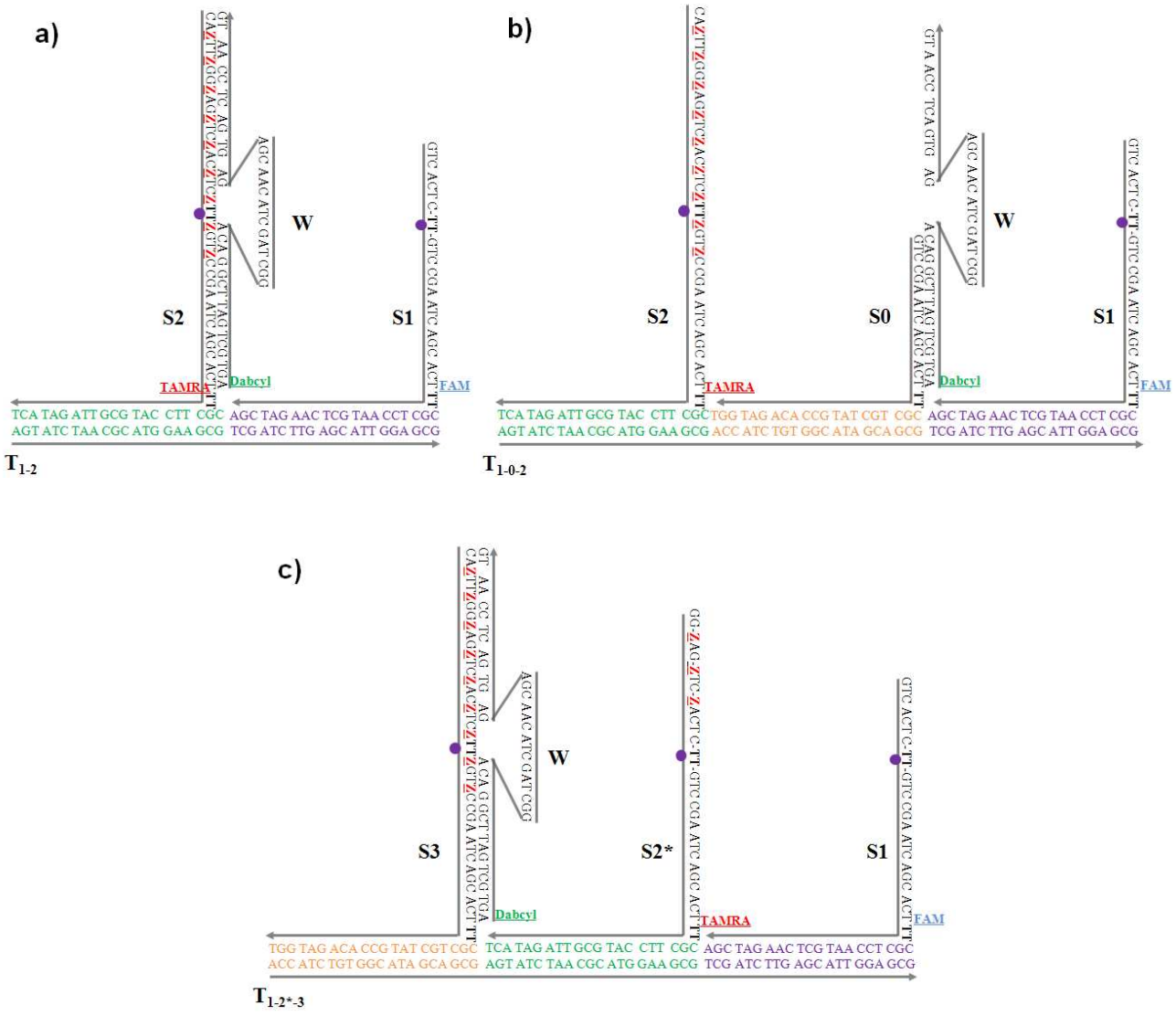


Figure S1: Native gel electrophoresis to demonstrate the construction of walking system. The higher molecular weight structure results in a slower movement in gel electrophoresis and an upper band. The construction of both **S1-S2-W-T_{1-2}** conjugate (right) and **S0-S1-S2-W-T_{1-0-2}** conjugate (left) are proven.

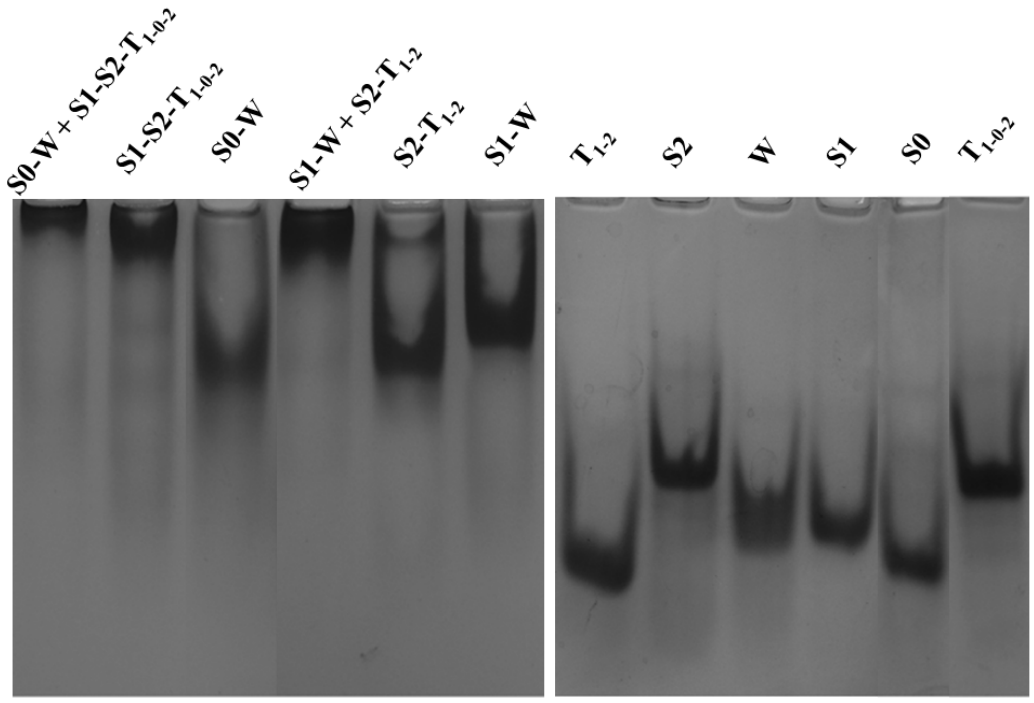


Figure S2: (a) Fluorescence resonance energy transfer (FRET) between the Dabcyl-labeled Walker (**W**) and FAM-labeled **S1**; λ_{ex} = 488 nm; (b) FRET between Dabcyl-labeled Walker (**W**) and TAMRA-dT-labeled **S2**; λ_{ex} = 550 nm.

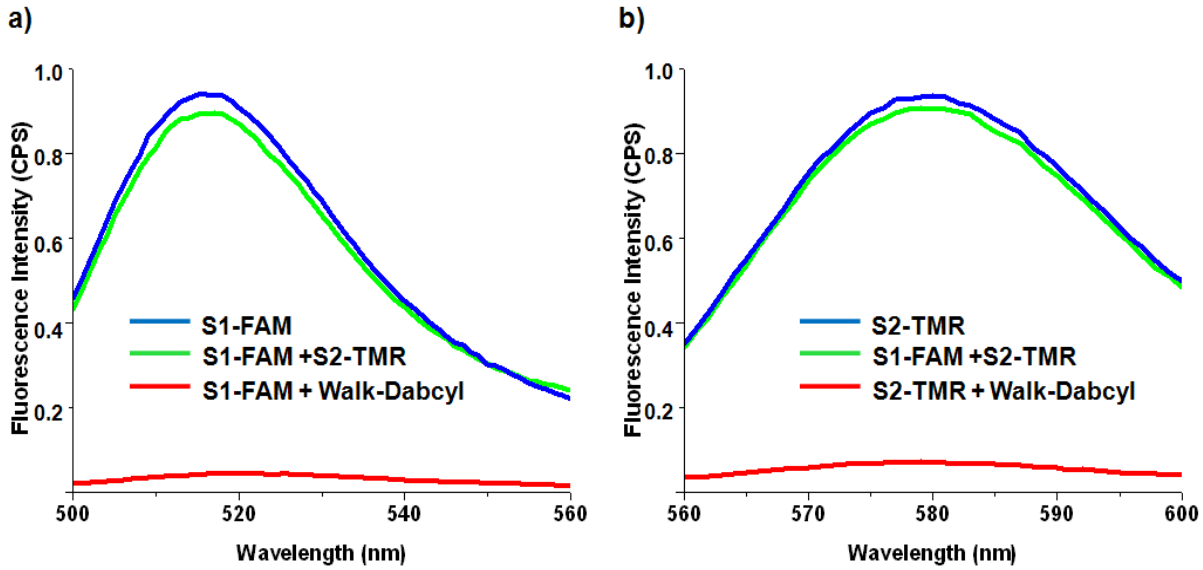


Figure S3: Processive motion of DNA walker. If free **S3** could displace the walker strand from the track to which it is bound, the TAMRA fluorescence would be recovered, but only a negligible effect was observed in the experiment. Preannealed **S1**-FAM/ **W**-Dabcyl sample was mixed separately with **S2**-TAMRA/$\mathbf{T_{1\text{-}2}}$ (red line) and **S3**/**S2**-TAMRA/$\mathbf{T_{1\text{-}2}}$ (blue line) sample. The TAMRA fluorescence was monitored post-mixing (λ_{em} = 580 nm, with λ_{ex} = 550 nm). The decrease of the TAMRA fluorescence signal resulted from the motion of Dabcyl-labeled walker (**W**) strand to the **S2** site (FRET between TAMRA and Dabcyl). If residual walker binds with the non-labeled free **S3** strand (instead of the labeled **S2** site), the TAMRA fluorescence would not be quenched. As shown in the figure, only slight fluorescence enhancement occurred after adding the **S3** strand, indicating that the free **S3** did not displace the walker.

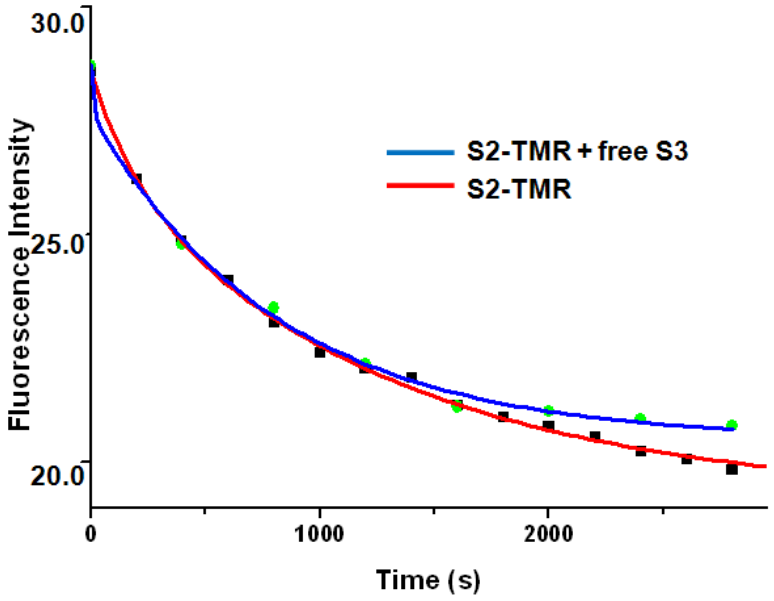
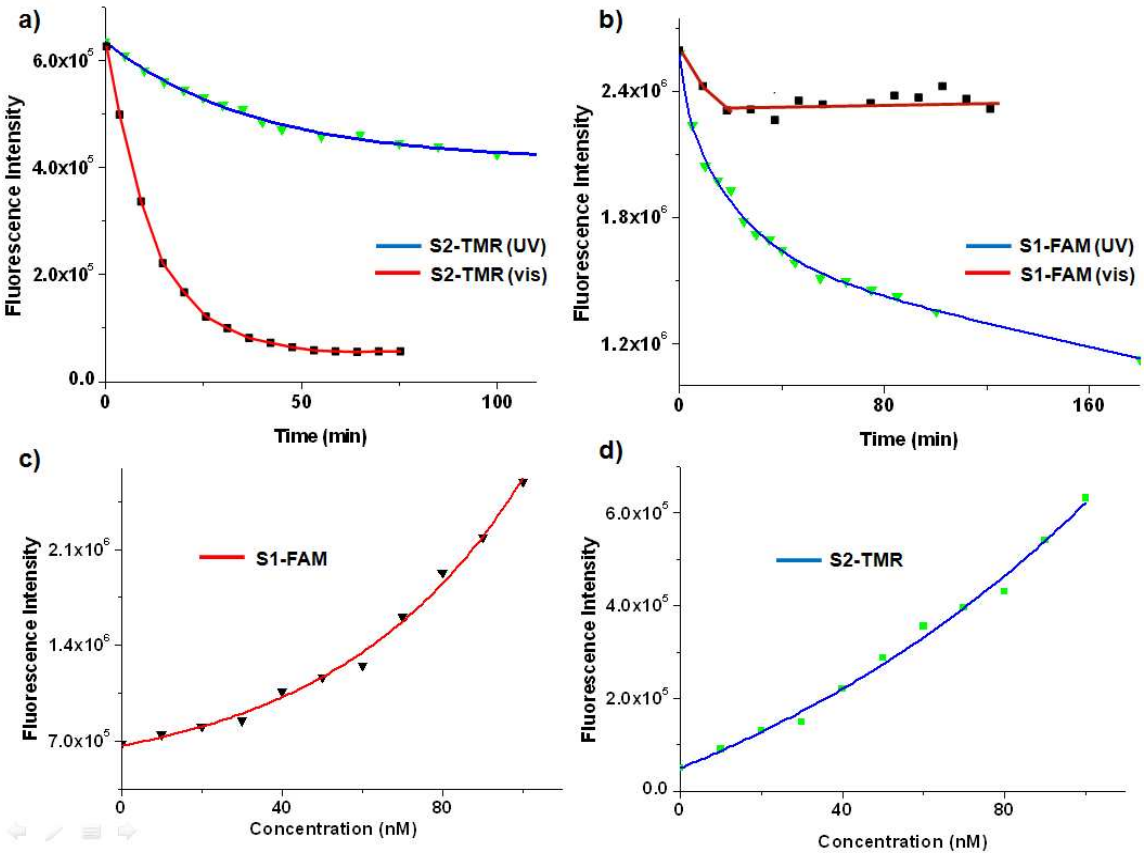


Figure S4: Fluorescence study confirming the photocontrollable direction selectivity. Both (a) TAMRA and (b) FAM fluorescence were recorded after periods of UV or visible light irradiation; at time=0 min, preannealed **S0-W** and **S1-S2-T** sample were mixed. Based on the standard calibration curve of either (c) FAM or (d) TAMRA, the percentage of walker steps towards each direction was calculated. The calibration curve was prepared by monitoring the fluorescence intensities of **S1**-FAM (or **S2**-TAMRA) during the titration by **W**-Dabcyl. The concentration of **S1**-FAM (or **S2**-TAMRA) was fixed as 100nM, while the concentration of **W**-Dabcyl was varied among samples in the range of 0-100nM [the X-axis of figure (c) and (d) is the concentration of the left-over free **S1** or **S2** strand after **W** binding].



References:

1. You, M.; Wang, R.; Zhang, X.; Chen, Y.; Wang, K.; Peng, L.; Tan, W. Photo-Regulated DNA-Enzymatic Nanostructures by Molecular Assembly. *ACS Nano* **2011**, *5*, 10090-10095.
2. Kang, H.; Liu, H.; Phillips, J.-A.; Cao, Z.; Kim, Y.; Chen, Y.; Yang, Z.; Li, J.; Tan, W. A Single-DNA Molecule Nanomotor Regulated by Photons. *Nano Lett.* **2009**, *9*, 2690-2696.