Supplementary Material (ESI) for Chemical Communications

Supplementary information:

A stimuli responsive DNA walking device

Chunyan Wang, Jingsong Ren* and Xiaogang Qu

Laboratory of Chemical Biology and State Key laboratory of Rare Earth Resources Utilization, Changchun Institute of Applied Chemistry,
Chinese Academy of Sciences, Changchun, 130022, China.

Experimental section:

Materials. Tris was purchased from USB, $MgCl_2$, NaOH, NaCl and Stains all were purchased from Sigma. All the DNA oligonucleotides were purchased from Sangon (ShangHai, China) without further purification. Concentration of each strand was estimated by absorption at 260 nm. Molar extinction coefficients were estimated by the nearest neighbor method. Sequences used in the experiments are listed below.

T1: 5'-ACTCTTGACGCACTAGTACGGGATCGTATTCATTGAGTTACA-3'

S1:

5'-CCGTACTAGTGCGTCAAGAGTTTAGTAGCCTACCACACCAACCATCGTC-3'

5'-CCGTACTAGTGCGTCAAGAGTTTAGTAGCCAACCACACCAACCATCGTC-3'-BHQ-2

S2: 5'-TGTAACTCAATGAATACGATCTTTATAGCGAGATTGACAGCCTA-3'

W1:

5'-GACGATGGTTGGTGTGGTTGGTAGGCTACTGTCAATCTCGCTAAT-3'

ROX-5'-GACGATGGTTGGTGTGGTTGGTAGGCTACTGTCAATCTCGCTAAT-3'

Electrophoresis. The native PAGE experiments were carried out on a 7.5 cm×7.5cm plate. 14% polyacrylamide (19:1 acrylamide: bisacrylamide) on non denaturing gel at 10 V/㎝ for 3.0 hour at 4 ℃. The running buffer consisted of 50 mM Tris HCl, pH

8.0, 20 mM acetic acid, and 2 mM EDTA (TAE). 1 μl of tracking dye solution, which contained 50% glycerol, and 0.2% each of Bromophenol Blue and Xylene Cyanol FF, was added to each sample. Gels were stained with stains-all.

Fluorescence spectra. Fluorescence spectra measurements were carried out on Jasco-FP-6500 spectrofluorometer (Jasco International Co. LTD. Tokyo, Japan) using a quartz cell of 1 cm path length. With an excitation wavelength of 580 nm, and fluorescence emission spectra was monitored from 590 nm to 650 nm at room temperature. The slits for the excitation and emission monochromators were both set to 5 nm.

Fluorescence time course measurements. The kinetics of fluorescence intensity changing with time was measured at excitation wavelength of 580 nm, monitored at wavelength of 603 nm. The slits widths were both set to 5 nm. After the addition of diluted HCl or NaOH, solution was mixed within 4s by rapidly drawing up with a pipette and releasing it. All measurements were performed at room temperature.

Formation of the track. The track was formed by mixing strand T and strand S1, S2 at equal molar ratio. Stoichiometric quantity of each strand designed in the complex was fixed at a concentration of 5 μM in TAE/Mg buffer (40 mM Tris·HCl, pH 8.0, 20 mM acetic acid, 2 mM EDTA, and 10 mM magnesium acetate). The solution was cooled slowly from 95 ℃ to 20 ℃ over 48 hours.

Formation of the walking system. Track solution was incubated at 4 □ for hours, after the hybridization reached a plateau, walker strand (W) was added in. An equal molar ratio of strand W will hybrid with strand S1 via 24nt complementary bases at its 5' end which incorporate aptamer sequence. Then solution pH was adjusted to facilitate the switch between two addresses. With the solution pH switching between 8.0 and 5.5, walker strand moved back and forth between S1 and S2. 5 μl aliquot was taken out for analysis after each switch.

Transportation of thrombin in the walking system. The walker and track mixture was incubated with thrombin in an aptamer binding buffer solution consisting of 20 mM Tris-HCl (pH 7.4), 140 mM NaCl, 5 mM KCl, 5 mM $CaCl_2$ and 1 mM $MgCl_2$ to make sure thrombin binding. The reaction mixture was carried out at room

temperature for 3 hrs.

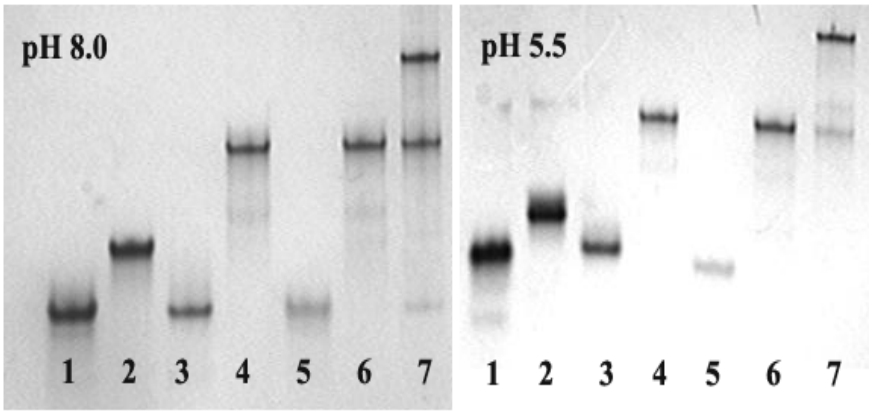

Figure S1. Electrophoresis performed at pH 8.0 and 5.5. Lane 1: strand T; lane 2: strand S1; lane 3: strand S2; lane 4: strand (T+S1); lane 5: strand W; lane 6: strand (T+S2); lane 7: strand (T+S1+S2).

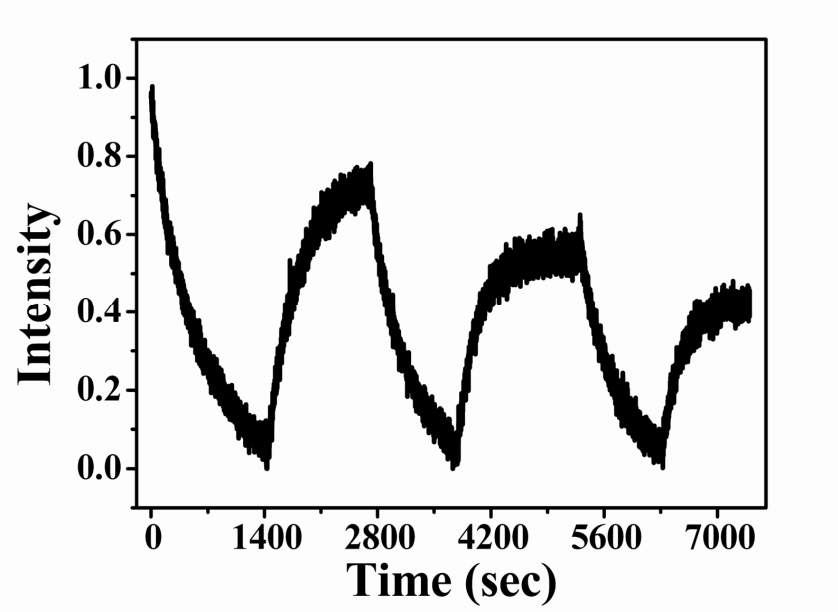

Figure S2. Cycling the walking device in the presence of thrombin. The intensity change at 603 nm was recorded.