

Supporting Information

Sensitive detection of protein by aptamer-based label-free fluorescing molecular switch

Bingling Li,^{a,b} Hui Wei^{a,b} and Shaojun Dong^{a,*}

^aState Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin, 130022, P. R. China.

^bGraduate School of the Chinese Academy of Sciences, Beijing, 100039, P. R. China.

Experimental Section

Materials. Oligonucleotides containing specific sequences (A1, 5' GGT TGG TGT GGT TGG 3' and C2, 5' CCA ACC ACA CCA ACC 3') were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The concentrations of oligonucleotides were determined using the 260 nm UV absorbance and the corresponding extinction coefficient. All the fluorescence experiments were operated at a temperature of 27°C.

Instrumentation. Fluorescent emission spectra were recorded on a Perkin Elmer LS55 Luminescence Spectrometer (Perkin Elmer Instruments U.K.). Thermal denaturation profiles were recorded on a Cary 500 Scan UV-Vis-NIR Spectrophotometer.

Thermal Denaturation Profiles

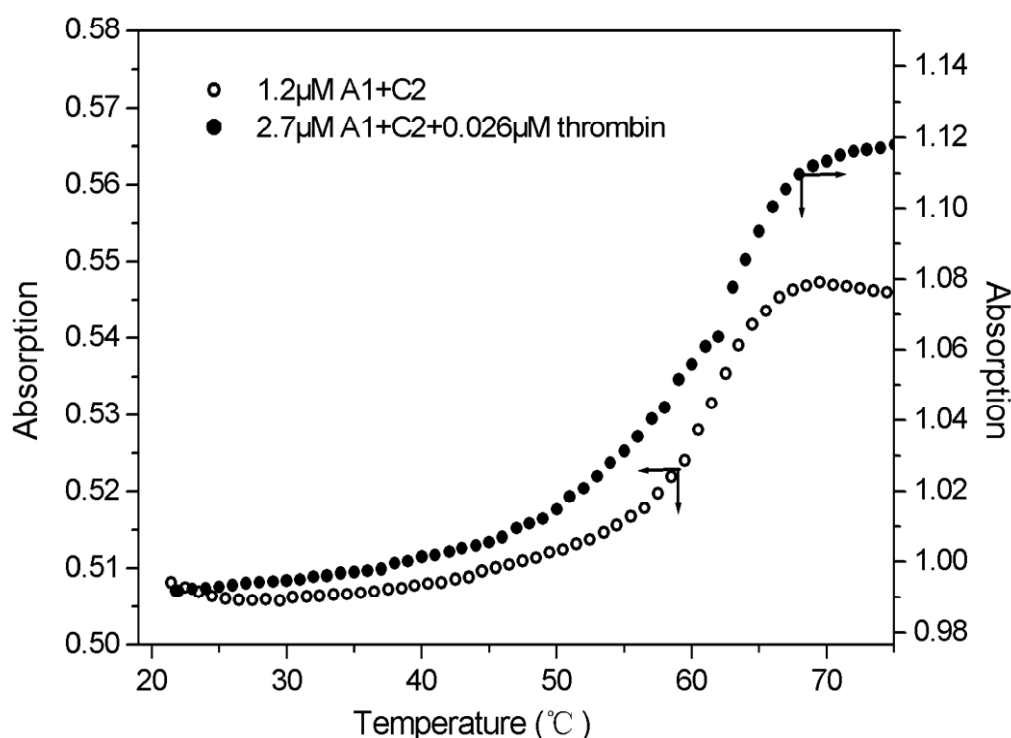


Figure S1 Thermal denaturation profiles before and after the thrombin was added to the dsDNA system. Circle: 1.2 μM hybridisation solution of the anti-thrombin aptamer and its complementary strand (20 mM Tris-HCl, 140 mM NaCl, 5 mM KCl, 5 mM MgCl_2 , pH 7.4). Dot: 2.7 μM hybridisation solution of the anti-thrombin aptamer and its complementary strand containing 0.026 μM thrombin (20 mM Tris-HCl, 140 mM NaCl, 5 mM KCl, 5 mM MgCl_2 , pH 7.4).

Explanation of the Reason for Relatively Long Equilibrium Time

In fact, the fluorescence signal did not change to a constant value as soon as adding the protein, but presented a gradual decrease in a period of time before it reached a stable state. This phenomenon may be assigned to the following two factors. Firstly, the interaction of thrombin with its aptamer was not a process finished instantaneously. Secondly, there was a competition between thrombin/A1 and A1/EB/C2, which was a

stable structure relatively hard to damage.

Actually, it had already been proven that sometimes over 10-mer duplex could also be damaged by the interaction between the target and its aptamer.^[1] We have compared the UV thermal denaturation profiles (Figure S1) after and before adding thrombin to the dsDNA solution, in the absence of EB. When thrombin was added, a trend in which increase of profile slope from 20°C to 30°C was observed, which implied that the addition of thrombin could indeed affected the dsDNA system at the temperature we did the experiment. Furthermore, EB would decrease the stabilization of the dsDNA system as well.^[2] So, it was reasonable that the interaction of thrombin and its aptamer was strong enough to compete with the EB/dsDNA system at some extent (even at the temperature we did the experiments), but the interacting time would be prolonged accordingly.

1. J. W. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2006, **45**, 90.
2. A. E. Wood, G. R. Bishop, *Molecules*, 2004, **9**, 67.