# Electronic Supplementary Material

## The aptasensor for label-free detection of thrombin based on turn-on fluorescent DNA-templated Cu/Ag nanoclusters

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**Table S1** Names and sequences of the oligonucleotides.

| Oligonucleotids | Sequences (5′- 3′) |
|---|---|
| TBA1 | **CCCTTAATCCCC**TTTTT*GGTTGGTGTGGTTGG*TTTTT**CCCTAACTCCC C** |
| TBA2 | *GGTTGGTGTGGTTGG*TTTTT**CCCTAACTCCCC** |

**Table S2** Comparison of different strategies for the detection of thrombin.

| Detection methods | LOD | Linear range | References |
|---|---|---|---|
| Surface plasmon resonance | 0.10 nM | 0.10-75 nM | 34 |
| Fluorescence | 0.18 nM | 0.50-20 nM | 35 |
| Fluorescence DNA-Ag NCs | 1.0 nM | 0.0-50 nM | 36 |
| UV-vis absorbance | 3.0 nM | 5.0-30.4 nM | 37 |
| Fluorescence | 30 pM | 0.28-86 nM | 9 |
| Fluorescence | 31.3 pM | 62.5-187.5 PM | 10 |
| Fluorescence DNA-Cu/Ag NCs | 1.6 nM | 1.6-8.0 nM | this work |

**Table S3** The lifetimes of TBA1-Cu/Ag NCs in the absence and presence of different concentration of thrombin.

| Samples | [TB] (nM) | $\tau_1$ (ns) | $\tau_2$ (ns) | $\tau_3$ (ns) | $\tau_{avg}$ (ns) | $\chi^2$ |
|---|---|---|---|---|---|---|
| TBA1-Cu/Ag NCs | 0 | 0.43 (50%) | 2.8(27%) | 13 (23%) | 3.9 | 1.090 |
| TBA1-Cu/Ag NCs + TB | 3.2 | 0.39 (46%) | 2.6 (26%) | 12 (28%) | 4.1 | 1.093 |
| | 6.4 | 0.37 (44%) | 2.5 (27%) | 10 (29%) | 3.8 | 1.100 |
| | 8.0 | 0.36 (53%) | 2.5 (25%) | 11 (22%) | 3.3 | 1.096 |

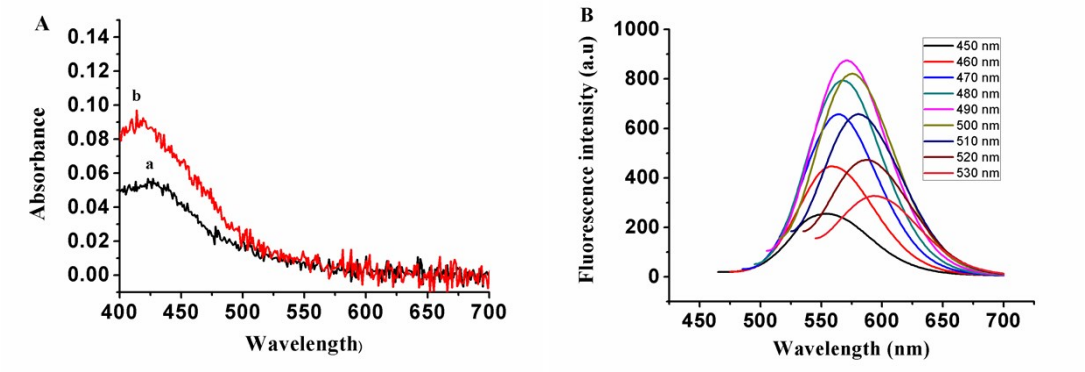


**Fig. S1** (A) UV-vis spectra of TBA2-Ag NCs without (a) and with 10 U/L thrombin (b). (B) Fluorescence emission spectra of TBA2-Cu/Ag NCs under different excitation wavelength. *c*(DNA) = 3 μM, Tris-HAc (10 mM, pH 7.0)

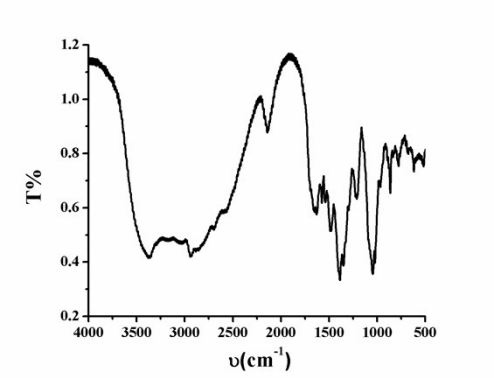


**Fig. S2** The IR spectrum of TBA1-Cu/Ag NCs.

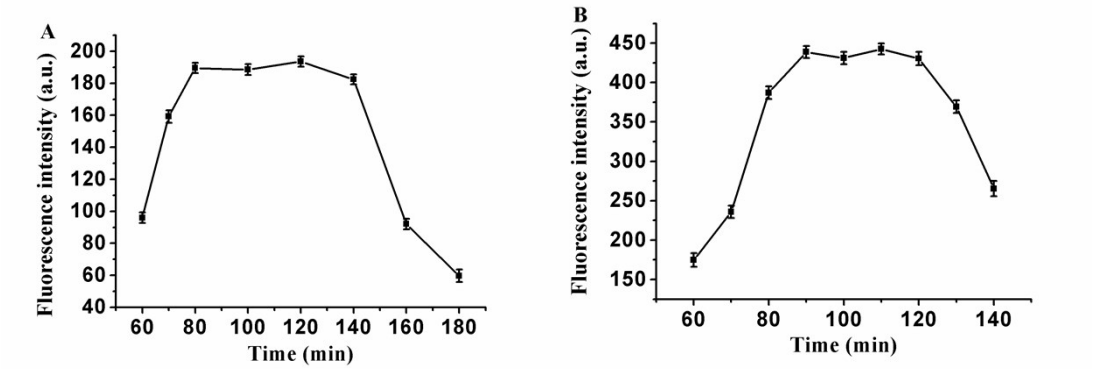


**Fig. S3** Stability of Cu/Ag NCs. The changes of fluorescence intensities of TBA1-Cu/Ag NCs at 560 nm (A) and TBA2-Cu/Ag NCs at 575 nm (B) against the increasing time. The error bars represent the standard deviation of three independent measurements. *c* (DNA) = 1.5 μM.

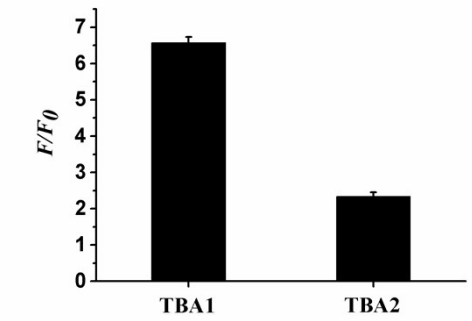


**Fig. S4** Relative fluorescence intensity ($F/F_0$) of different DNA-Cu/Ag NCs. $F_0$ and $F$ are the maximum emission intensity of the DNA-Cu/Ag NCs before and after the addition of 8.0 nM thrombin, respectively. The error bars

represent the standard deviation of three independent measurements.

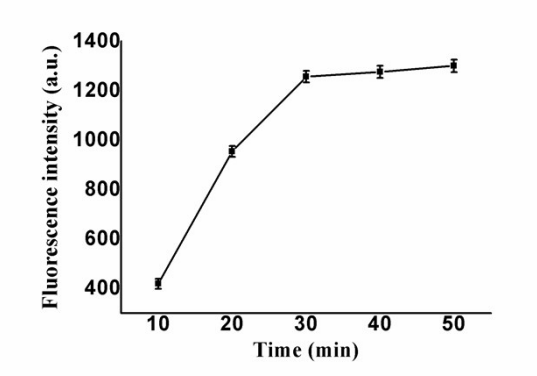


**Fig. S5** Fluorescence intensity of TBA1-Cu/Ag NCs in the presence of 8.0 nM thrombin against the increasing reaction time. The error bars represent the standard deviation of three independent measurements. *c*(DNA) = 1.5 μM.

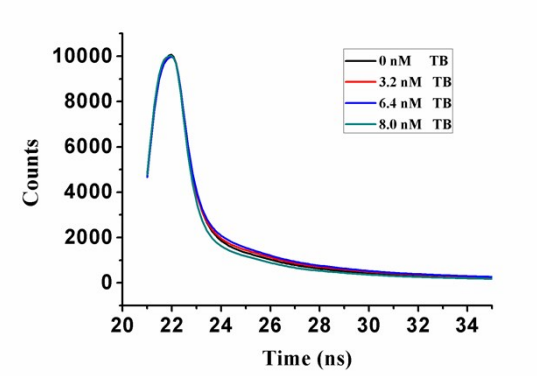


**Fig. S6** The fluorescence lifetime curves of TBA1-Cu/Ag NCs (excitation at 405 nm and emission at 560 nm) incubating with the different concentration of Thrombin.