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

Baozhu Zhang a and Chunying Wei b

a College of Chemistry and Chemical Engineering, Jinzhong University, Yuci 030619, Shanxi, China

b Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education, Institute of Molecular Science, Shanxi University, Taiyuan 030006, Shanxi, China

*Corresponding author.
E-mail address: weichunyi@sxu.edu.cn (C. Wei)
Table S1 Names and sequences of the oligonucleotides.

| Oligonucleotids | Sequences (5´-3´) |
|----------------|-------------------|
| TBA1           | CCCTTAATCCCCTTTTTTGTTGGTGGTGGTTTTTCCCTAACTCCC |
| TBA2           | GGTGGTGGTGGTGGTTTTTCCCTAACTCCC |

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 DNA-Cu/Ag NCs   | 31.3 pM   | 62.5-187.5 PM   | 10         |

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      |
|                   | 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 \(\mu\)M, 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. \( c \, (\text{DNA}) = 1.5 \, \mu\text{M} \).

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.