Electronic Supplementary Information

A peptide with a cysteine terminus: Probe for label-free fluorescent detection of thrombin activity

Jingjing Feng,†Caixia Zhuo,†Xuejuan Ma,†Shuangqin Li,†Yaodong Zhang*,†

†Key Laboratory of Applied Surface and Colloid Chemistry, Ministry of Education, Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi’an 710062, PR China

*Corresponding author: E-mail: ydzhang@snnu.edu.cn Phone: +86-29-81530726; Fax: +86-29-81530727

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S1. Synthesis of dC12-AgNCs

The synthesis of DNA-AgNCs was based on a previously reported method. AgNCs were synthesized by mixing AgNO₃ with a DNA (dC₁₂) solution and by adding NaBH₄ with continuous shaking. In brief, 150 µl of the DNA (dC₁₂) template (200 µM) was mixed with 2724 µl of phosphate buffer (2.0 mM, pH 7.4). Freshly prepared AgNO₃ aqueous solution (90 µl; 2.0 mM) was added to this solution. The resulting solution was vigorously shaken for 30 s. After 15 min in an ice bath, 36 µl of freshly prepared NaBH₄ aqueous solution (5.0 mM) was added to the solution. The mixture was vigorously shaken for 1 min. The solutions were kept in the dark at room temperature for 2 h and then incubated overnight at 4°C.

S2. Sequence-dependent responses of dC₁₂-AgNCs to peptides

Fig. S1. Fluorescence responses of dC₁₂-AgNCs on different peptides (0.2 µM). (a) dC₁₂-AgNCs. (b) dC₁₂-AgNCs + peptide 1. (c) dC₁₂-AgNCs + peptide 2. (d) dC₁₂-AgNCs + peptide 3.

S3. Optimizing the amount of GO.

Fig. S2. Fluorescence responses of the dC₁₂-AgNCs-peptide 1 conjugate to different GO concentrations. 0.0, 10.0, 20.0, 30.0, and 40.0 µg/mL. Inset: Changes in
fluorescence intensity at 635 nm as a function of GO concentration.

**S4. Zeta potentials of GO**

![Zeta potentials of GO](image)

**Fig. S3.** Zeta potentials of 0.25 mg/mL GO in PBS (2.0 mM, pH = 7.4).
Fluorescence responses of dC_{12}-AgNCs to peptides

Fig. S4. Fluorescence responses of the dC_{12}-AgNCs to peptides. Peptide 1 (A), peptide 2 (C) and peptide 3 (E). Changes in fluorescence intensity at 635 nm versus the concentrations of peptides. Peptide 1 (B), peptide 2 (D) and peptide 3 (F).
S6. Fluorescence spectra of the ensemble of dC₁₂-AgNCs-peptide 2/GO incubated with thrombin

![Fig. S5.](image)

**Fig. S5.** Fluorescence spectra of the ensemble of dC₁₂-AgNCs-peptide 2/GO incubated with thrombin (0.1 μM) at 37°C in different periods. Inset: Changes in fluorescence intensity at 635 nm as a function of incubation time.

S7. Fluorescence response of dC₁₂-AgNCs to 0.2 μM peptides 1.

![Fig. S6.](image)

**Fig. S6.** (A) Fluorescence spectra of the dC₁₂-AgNCs reacted with peptide1 (0.2 μM) at room temperature in different periods. (B) Changes in fluorescence intensity as a function of reacted time.
S8. Analytical performance of different platforms for the detection of thrombin.

**Table S1 Analytical performance of different platforms for thrombin detection**

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S9. Reference SI