Supplementary Information

A new FRET nanoprobe for trypsin using bridged β-cyclodextrin dimer-dye complex and its biological imaging applications

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Preparation and Characterization of GNPs, MGNPs, MGNPs-Lys-bis(β-CDs) and MGNPs-Lys-bis(β-CDs)-FL

Formation of Lys-bis(β-CDs)-FL complex and Determination of the apparent association constant

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Preparation and Characterization of GNPs, MGNPs, MGNPs-Lys-bis(β-CDs) and MGNPs-Lys-bis(β-CDs)-FL

Preparation Near-monodisperse GNPs of 10 ± 2.0 nm diameter were prepared using the classical citrate reduction route pioneered by Frens.[1] The concentration of the as-prepared GNPs was calculated to be approximately 5.4×10^{15}particles/L, ~9.0 nM.

MEA-modified gold nanoparticles[2] (MGNPs) were prepared by stirring an aqueous mixture of MEA (1.0 mL, 2.0×10^{-6} M) and citrate-stabilized 10-nm gold nanoparticles (1.0 mL, 9.0 nM) for 24 h at room temperature. Excess MEA was removed by repeated centrifugation at 11000 rpm using a Sigma 1-14 centrifuge (Sigma), and resuspension in 1.0mL ultra-pure water.

Then Lys-bis(β-CDs) (1.0×10^{-5} M) was added to the MGNPs solution, the conjugation proceeds by NHS and EDC forming active esters to conjugate the carboxyl groups of Lys-bis(β-CDs) to the primary amine groups of MGNPs. Briefly, 0.20 mg EDC and 0.10 mg NHS were added to 1.0 mL of the stock Lys-bis(β-CDs) solution to activate Lys-bis(β-CDs) in PBS buffer solutions (pH 7.4) with the final concentration of 5.0 μM and incubated for 30 min in the dark at room temperature with continuous gentle stirring. After that, the activated Lys-bis(β-CDs) and 1.0 mL of MGNPs solution were incubated in the dark at room temperature for another 2 h with continuous gentle stirring, and then stored at 4 ºC overnight. Excess reagents were then removed via centrifugation at 11000 rpm for 10 min at 4 ºC. The precipitate was washed two times with 10 mM PBS solution (pH 7.4), with repetitive centrifugation and dispersion. The final concentrated MGNPs-Lys-bis(β-CDs) was diluted to 1.0 mL with ultrapure water to give the fixed concentration (1.0×) ready to use in the following fluorescence assays. The product was then stored at 4 °C. For simplicity, we denote the concentration of MGNPs-Lys-bis(β-CDs) to be 1.0×. [3]

Characterization The modified GNPs can be characterized by IR spectrum, transmission electron microscopy (TEM) as well as UV-vis spectroscopy. From the IR spectra in Fig. S1, for lys-bis(β-CDs)-modified MGNPs, it displays the characteristic peak of amide, which suggests that lys-bis(β-CDs) has been modified to MGNPs through the intense condensation reaction between carboxyl and amino group. The average size of the GNPs increases from 9.0 ± 2.0 nm to 16 ± 2.0 nm after modified by MEA, Lys-bis(β-CDs) and Lys-bis(β-CDs)-FL step by step as measured by TEM (Fig. S2). UV–vis spectrum (Fig. S3a) of GNPs shows a characteristic absorption of the

surface plasmon resonance absorption at 520 nm in aqueous solution, and this maximum obviously shifts to 528 nm(Fig. S3b) and 536 nm(Fig. S3c), corresponding to modification by MEA and lys-bis(β-CDs), respectively. These results jointly provide the evidences for the attachment of MEA and lys-bis(β-CDs) to the gold nanoparticles.

Trypsin is specific for the hydrolysis of amide bond of lysine. Therefore, the addition of trypsin to the MGNPs-Lys-bis(β-CDs) solutions induces rupture of the amide bond. Furthermore, a concomitant narrowing of the optical absorption spectrum is associated with the blue-shift, which is a strong indication of dissociation of lys-bis(β-CDs) from MGNPs(Fig. S3d). Moreover, the changes in absorbance intensity are also observed. Formation of MGNPs-Lys-bis(β-CDs) induces aggregation of MGNPs and increased absorbance. After dissociation by the addition of trypsin solution, the absorbance intensity decreases, but is still slightly higher than the initial intensity before binding of Lys-bis(β-CDs). This indicates that the trypsin does not dissociate the MGNPs and Lys-bis(β-CDs) complexes completely, which is also confirmed by the slightly more broadened absorbance spectrum of MGNPs.

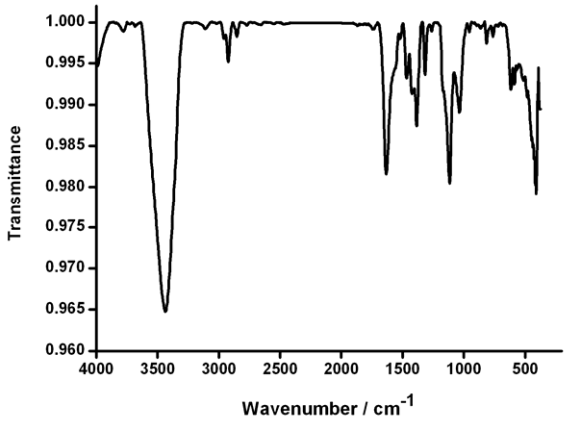

Fig. S1. IR spectra of MGNPs-Lys-bis (β-CDs) in a KBr pellet. The modified colloidal GNPs were mixed with KBr and dried under vacuum at 60 ℃.

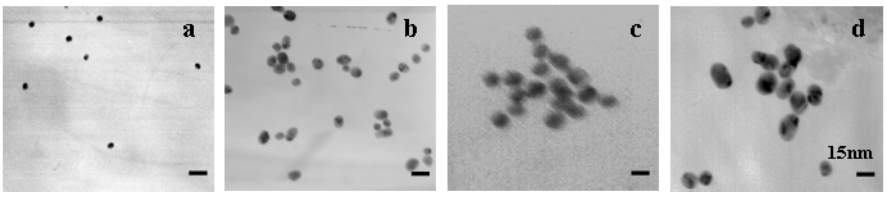

Fig. S2. TEM micrograph of GNPs (a), and MGNPs (b), MGNPs-Lys-bis(β-CDs) (c), MGNPs-Lys-bis(β-CDs)-FL (d).

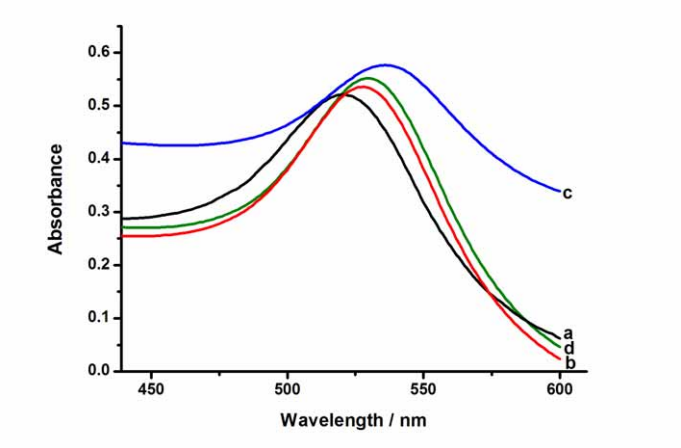

Fig. S3 Optical absorption spectra of aqueous solutions of (a)GNPs, (b)MGNPs, (c)MGNPs-Lys-bis(β-CDs) and d) solution in (c)following addition of trypsin.

Formation of Lys-bis(β-CDs)-FL complex and Determination of the apparent association constant

1.0 mL of 0.10 μM fluorescein (FL) were added into a series of 10 mL colorimetric tube, varied amounts of 1.0×10^{-3} M Lys-bis(β-CDs) and 2.0 mL Tris-HCl buffer solution (pH 7.4) were added sequentially. The mixed solution was diluted to 10 mL with doubly distilled water, shaken thoroughly and equilibrated at room temperature for 10 min. Then the fluorescence intensity of the solution was measured at 490/515 nm against a reagent blank. The relative fluorescence intensity of FL is increased proportionally with the concentration of Lys-bis(β-CDs) increased (Fig. S4a).

The apparent association constant of the inclusion complex can be determined by the following method: assuming that the composition of the complex is 1:1, it can be written as:

$$\text{FL} + \text{Lys-bis(β-CDs)} \longleftrightarrow \text{Lys-bis(β-CDs)-FL}$$

The formation constant of the complex (K) is given by:

$$K = [\text{Lys-bis(β-CDs)-FL}] / [\text{FL}][\text{ Lys-bis(β-CDs)}]$$

Herein, [FL], [Lys-bis(β-CDs)] and [Lys-bis(β-CDs)-FL] are equilibrium concentrations. An apparent association constant value for the inclusion complex can be determined through the typical double reciprocal plots: [4]

$$1 / (F - F_0) = 1 / [(F_\infty - F_0)\, K\, C_{\text{Lys-bis(β-CDs)}}] + 1 / (F_\infty - F_0) \qquad (1)$$

Herein, F is the observed fluorescence intensity of the FL solution at each Lys-bis(β-CDs) concentration tested, F_0 and F_∞ are the fluorescence intensity when Lys-bis(β-CDs) are absent and all the FL molecules are complex, respectively. It is taken into

account that (1) Lys-bis(β-CDs) is in a large excess with respect to FL and therefore its free and analytical concentration are same; (2) the variations in the fluorescence intensity are proportional to the complex concentrations and (3) at high Lys-bis(β-CDs) concentration essentially all of the FL molecules are complex.

The good linear relationship obtained when 1/ (F-F_0) is plotted against 1/ $C_{\text{Lys-bis(β-CDs)}}$ indicates the existence is a 1:1 complex (R=0.9974, Fig. S4b). Its apparent association constant was determined to be 1.31×10^5 M^{-1}. While the apparent association constant of β-CDs-FL complex was 3.6×10^2 M^{-1}.[5] All these indicated that a sandwich complex with FL was obtained.

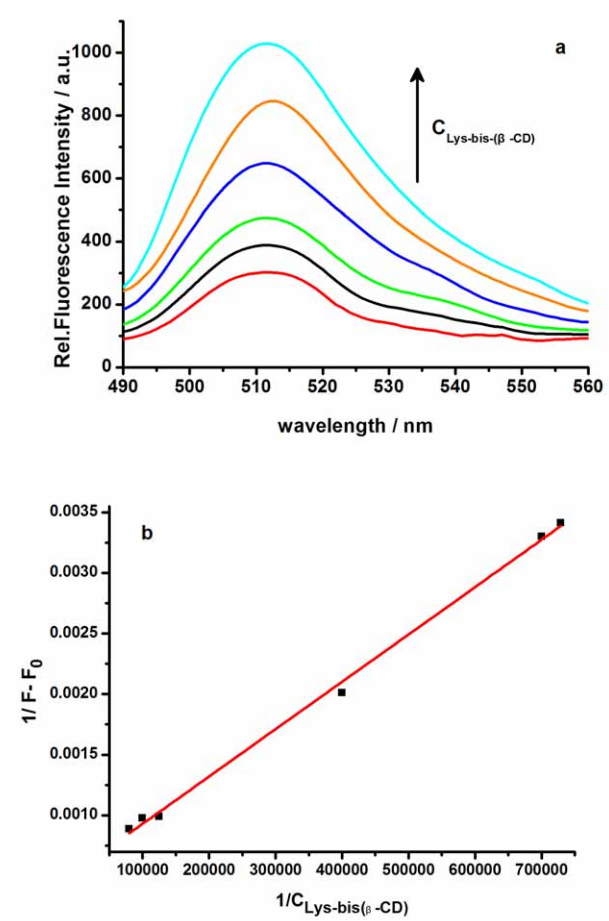

Fig. S4. a: Changes on the relative fluorescence intensity of 10 nM FL in the presence of different concentrations of Lys-bis(β-CDs) (final concentration: 1.0, 1.4, 2.5, 8.0, 10, 12.5 μM).The reaction solution was kept at 37 °C. b: Plot of 1/ (F-F_0) versus $1/C_{\text{Lys-bis(β-CDs)}}$.

Supplementary spectral figure of optimal experimental conditions

In order to improve the sensitivity of the determination, concentrations of Tris-HCl buffer solution, the effect of pH and concentrations of the MGNPs-Lys-bis(β-CDs) were optimized respectively, which were shown in following figures. And the final optimal conditions were 20mM Tris-HCl at pH 7.4, MGNPs-Lys-bis(β-CDs) 0.2 ×.

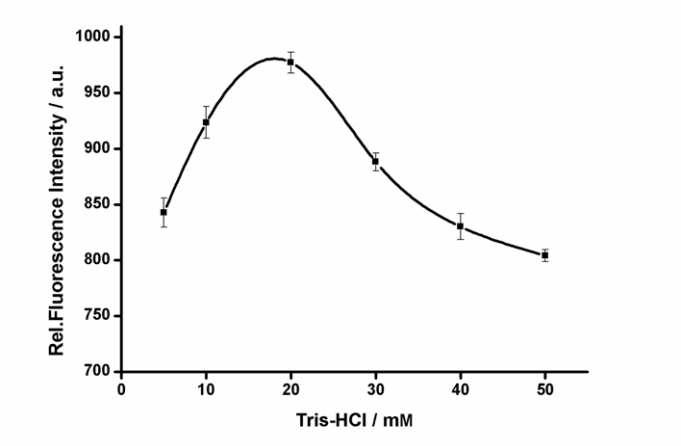

Fig. S5. The effect of different concentrations of Tris-HCl buffer solution on the relative fluorescence intensity changes in the presence of 25 μg·mL^{-1} trypsin, 0.2× MGNPs-Lys-bis(β-CDs), FL, 10 nM , the pH of reaction mixture was 7.4. The reaction solution was kept at 37 °C.

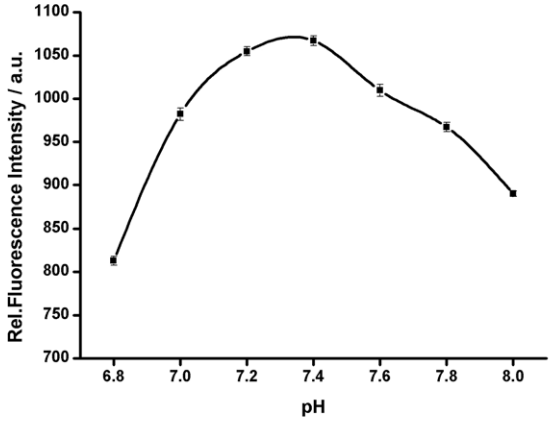

Fig. S6. Effect of pH on the relative fluorescence intensity. Reaction mixture contained: FL, 10 nM; Trypsin, 25 μg·mL^{-1}, MGNPs-Lys-bis(β-CDs), 0.2×; and different pH values were adjusted with Tris-HCl buffer, 20 mM. The reaction solution was kept at 37 °C.

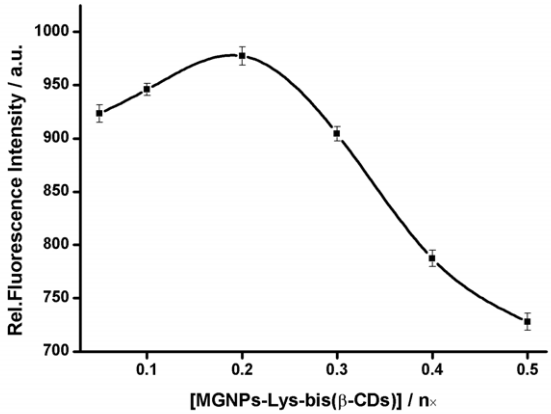

Fig. S7. Effect of MGNPs-Lys-bis(β-CDs) concentration on the relative fluorescence intensity. Reaction mixture contained: FL, 10 nM; Trypsin, 25 μg·mL^{-1}; MGNPs-Lys-bis(β-CDs), at different concentrations; and pH 7.4 Tris-HCl buffer, 20 mM. The reaction solution was kept at 37 °C.

Kinetic assays To test the stability of the probe, the kinetic behavior of the reaction was investigated. The fluorescence signal was recorded as a function of reaction time for 30 min. Fig. S8 showed the time courses of the fluorescence intensity (λ_{em}=515 nm) of the

probe at 37 °C with or without trypsin. The fluorescence intensity increased strongly after trypsin addition and remained almost constant with passage of time, which indicated that MGNPs-Lys-bis(β-CDs)-FL could instantly respond to the trypsin, had good photostability and it was not susceptible to photobleaching.

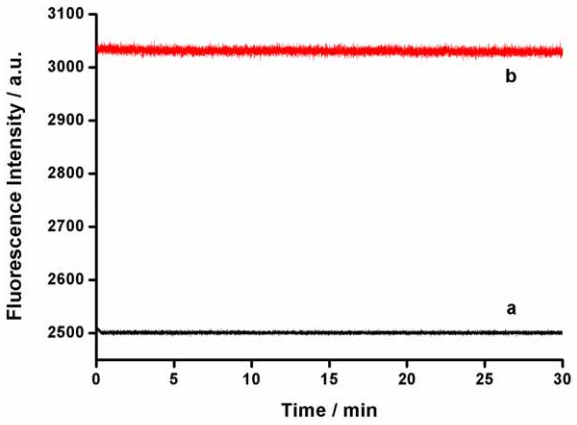

Fig. S8. Time course for the fluorescence intensity of probe when trypsin (18 μg·mL^{-1}) was absent(a) and present(b) with MGNPs-Lys-bis (β-CDs) 0.2 × and FL 10 nM in Tris-HCl buffer solutions (pH 7.4). The reaction solution was kept at 37 °C.

The cellular uptake of the probe

To observe the uptake of the AuNP-complex FRET probe by cells, we detected the Au concentration in living cells by the technique of ICP-AES. In the experiments, we incubated PANC-1 cells (1× 10^5/mL) with the nanoprobe in Dulbecco Minimum Essential Media (DMEM) supplied with 10 % fetal bovine serum. After 50 min, the cells were detached using the enzyme trypsin, and then homogenized. Next, we measured the amount of Au using the technique of ICP-AES (Thermo, IRIS Advantage, 242.795 nm), which was found to be 460 ng·mL^{-1} in the experimental PANC-1 cells.

References

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