SUPPORTING INFORMATION

Green synthesis of peptide-templated gold nanoclusters as novel fluorescence probes for detecting protein kinase activity

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Experimental Section

Reagents and chemicals

cAMP-dependent protein kinase (PKA) was obtained from New England Biolabs (UK). The arginine-terminated peptide Cys-Cys-Tyr-Leu-Arg-Arg-Ala-Ser-Leu-Gly (CCYLRRASLG) was purchased from GL Biochem (Shanghai, China). Adenosine 5′-triphosphate (ATP) disodium salt, 4,4′,5,5′,6,6′-hexahydroxydiphenic acid 2,6,2′,6′-dilactone (ellagic acid), NaOH, carboxypeptidase Y (CPY) were purchased from Sigma-Aldrich (USA). $HAuCl_4 \cdot 4H_2O$ was purchased from Sinopharm Chemical Rengent Co., Ltd. Other regents of analytical grade were obtained from Beijing Chemical Company (China) and were used as received without further purification. All solutions were prepared and diluted using ultrapure water (18.2 MΩ·cm) from the Millipore Milli-Q system. Human serum samples were provided by Jiangxi provincial people's hospital.

Preparation of peptide-Au nanoclusters

In a typical test, a 16 μL $HAuCl_4$ aqueous solution (25 mM) was slowly added to a 376 μL aqueous solution of peptide (CCYLRRASLG, 1.06 mM) in a glass vial with vigorous stirring. Then 8 μL 0.5 M NaOH was added within 30 seconds to adjust the solution pH approaching about 9. The sample was stored and sealed for 13 h in the dark with vigorous stirring to produce the peptide-AuNCs. The as-synthesized peptide-AuNCs were concentrated by a 3 kDa ultrafiltration device to remove free peptides.

Detection of protein kinase activity and its inhibition

A 120 μL solution of peptide-AuNCs was mixed with the 80 μL of 20 mM Tris buffer (containing 50 mM KCl and 10 mM $MgCl_2$, pH 7.5). Then, 40 μL PKA solution with the specified concentration was added into 20 μL of 1.0 mM ATP into the solution of peptide-AuNCs-Tris buffer. Ultrapure water was added into the mixture solution reaching 360 μL. The mixture was incubated at 37 ^oC for phosphorylation reaction by PKA. Finally, 40 μL of 100 U mL^{-1} CPY was added into the above mixture solution to hydrolyze the unphosphorylated peptide-AuNCs by incubating the mixture at 25 ^oC. Subsequently, the fluorescence spectra of the consequence solutions were recorded on an F-7000 spectrophotometer.

For detecting the IC_{50} value of ellagic acid, which is a potent inhibitor for PKA, some texts were carried out. A 80 μL of 20 mM Tris buffer (containing 50 mM KCl and 10 mM $MgCl_2$, pH 7.5) was added into 80 μL of 1% human serum sample, then 20 μL of different concentrations of ellagic acid and 40 μL of 400 U mL^{-1} PKA were

mixed. Then, 120 μL of peptide-AuNCs and 20 μL of 1.0 mM ATP were added to the ellagic acid-pretreated PKA solution and the mixture was incubated at 37 °C. Finally, 40 μL of 100 U mL^{-1} CPY was added into the mixture solution to hydrolyze the unphosphorylated peptide of peptide-AuNCs by incubating the mixture at 25 °C and the fluorescence spectra were measured.

Characterization

The UV-vis absorption spectra were collected on a Shimadzu UV-2450 spectrophotometer. The fluorescence spectra of the resulting solutions were recorded on a Hitachi F-7000 spectrophotometer. Transmission electron microscopy (TEM) images were obtained using a JEOL Ltd JEM-2010 transmission electron microscopy. Atomic force microscopy (AFM) images were recorded in ScanAsyst mode using a Bruker MultiMode-8 atomic force microscopy. X-ray photoelectron spectroscopy (XPS) studies were conducted by using a VG Multilab 2000X instrument.

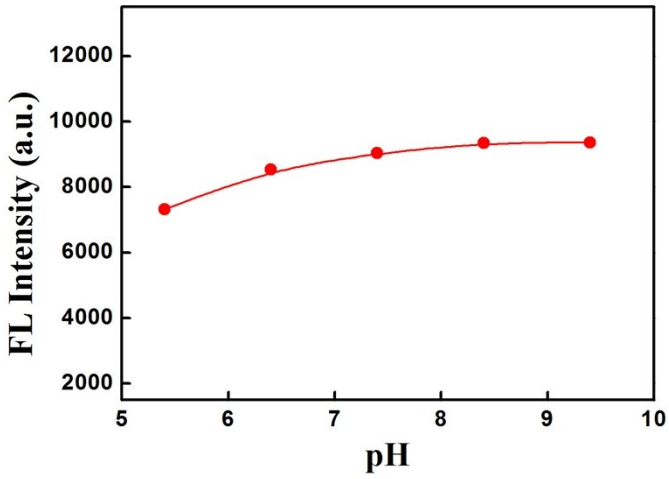

Fig. S1. The fluorescence intensity of peptide-AuNCs which were stored in the different pH.

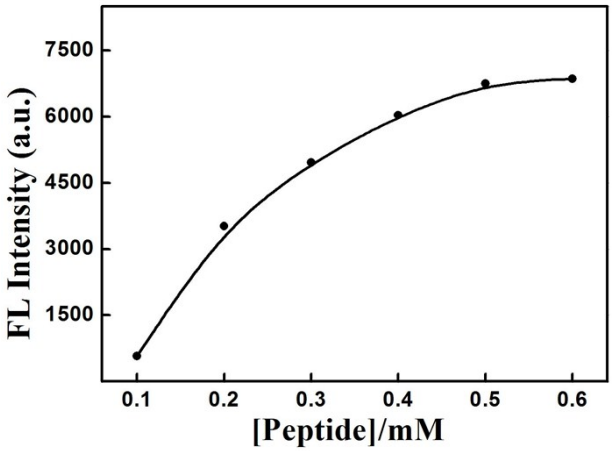

Fig. S2. Effect of the concentration of peptide on the fluorescence intensity of the fluorescent peptide-AuNCs. The concentration of $HAuCl_4$ was fixed to 1mM.

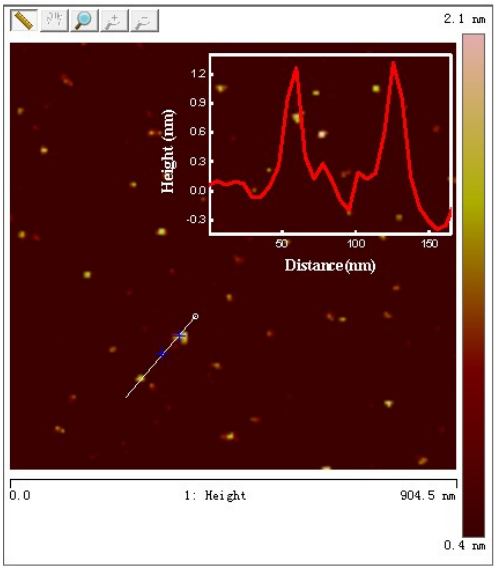

Fig. S3. The AFM images of the peptide-AuNCs. The inset shows the height distribution of the peptide-AuNCs.

Measurement of the quantum yield for peptide-AuNCs. Quinine sulphate was used as a reference.[1,2] The absorbance for the standard and peptide-AuNCs samples at the excitation wavelengths and the fluorescence spectra were measured, respectively. The areas of the integrated fluorescence vs. absorbance were plotted. The gradient of the line is M, which was used to calculate the QY of peptide-AuNCs from the

following eqation:

$$\varphi_X = \varphi_{ST}\left(\frac{M_X}{M_{ST}}\right)\left(\frac{\eta_X}{\eta_{ST}}\right)^2$$

Where the subscripts ST and X denote standard and peptide-AuNCs sample respectively, φ is the quantum yield, η is the refractive indices of the solvents. According to this equation, the quantum yield of peptide-AuNCs was 14.6%.

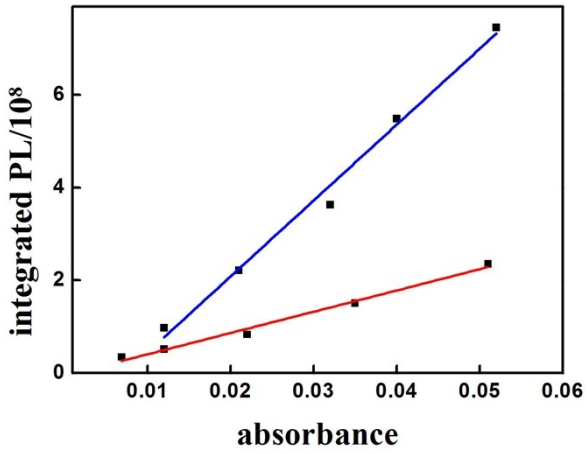

Fig. S4. Plot of the integrated fluorescent intensity versus the absorption for the standard (blue) and peptide-AuNCs (red). The gradient for each sample is proportional to that sample's fluorescence quantum yield.

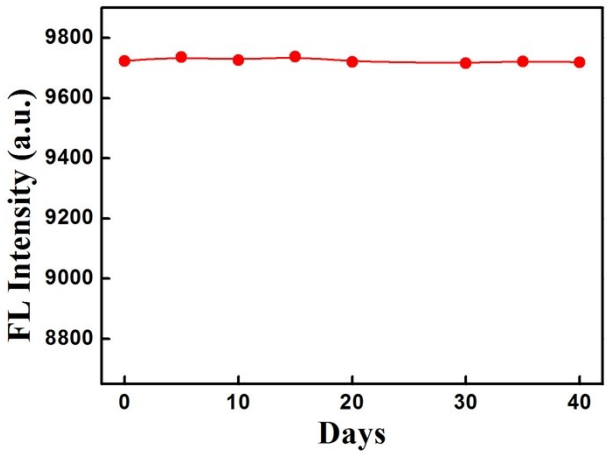

Fig. S5. Effect of the stability of peptide-AuNCs on the fluorescence intensity of the fluorescent peptide-AuNCs.

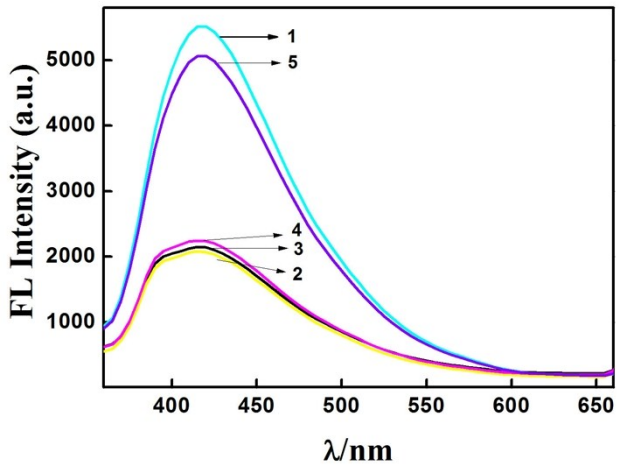

Fig. S6. The fluorescence spectra of peptide-AuNCs solution before (curve 1) and after (curve 2) incubated with CPY, respectively. The fluorescence spectra of peptide-AuNCs incubated with PKA alone (curve 3), ATP alone (curve 4), ATP and PKA (curve 5) followed by the incubation of CPY, respectively.

Optimization of Conditions. The time for CPY-conducted peptide hydrolysis and for phosphorylation reaction by PKA were shown in Fig. S7. As shown in Fig. S7a, with increasing the reaction time of CPY, the fluorescence intensity of AuNCs shows a decrease tendency until it reaches a plateau approximately at 30 min. Therefore, 30 min was chosen for CPY-conducted peptide hydrolysis. As shown in Fig. S7b, the fluorescence intensity of the AuNCs drastically recovers with increasing the incubation time of PKA. When the time of phosphorylation incubation is higher than 45 min, the fluorescence intensity is almost maintained at constant value. Therefore, 45 min was chosen for phosphorylation reaction by PKA.

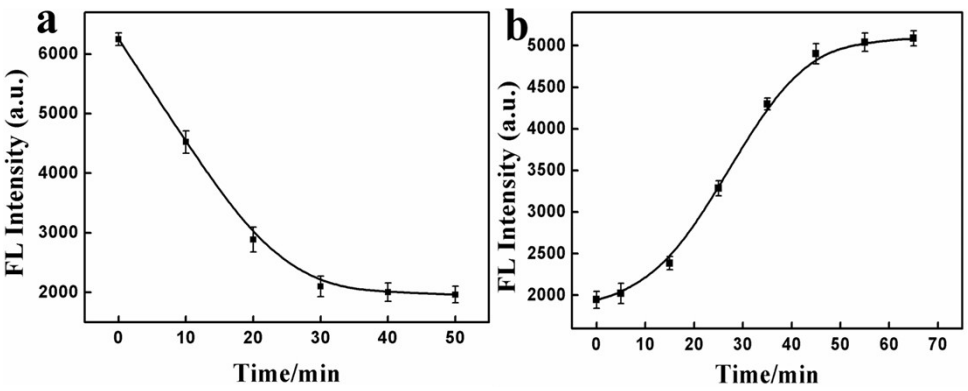

Fig. S7. (a) The fluorescence intensity of the AuNCs incubated with CPY for different time. (b) The fluorescence intensity of AuNCs incubated with PKA and ATP for different time, followed by the addition of CPY.

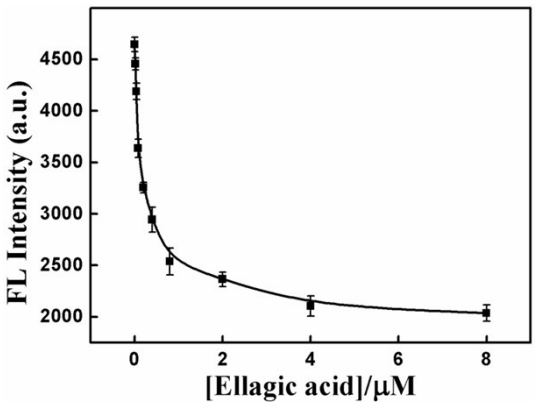

Fig. S8. Plot of the fluorescence intensity vs ellagic acid concentration.

References

1. Q. Wen, Y. Gu, L. J. Tang, R. Q. Yu and J. H. Jiang, *Anal. Chem.*, 2013, **85**, 11681-11685.

2. Y. Gu, Q. Wen, Y. Kuang, L. Tang and J. Jiang, *RSC Adv.*, 2014, **4**, 13753-13756.