Supporting Information

Peptide-Templated Gold Nanocluster Beacon as a Sensitive, Label-Free Sensor for Protein Post-translational Modification Enzymes

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EXPERIMENTAL DETAILS

Reagents and Materials. Histone deacetylase 1 (HDAC 1) and its inhibitor trichostatin A (TSA) were obtained from Cayman Chemical Company (Michigan, USA). The catalytic subunit of cAMP-dependent protein kinase (PKA) that is active in the absence of cAMP, PKA holoenzyme that is inactive in the absence of cAMP, and N-[2-(p-Bromocinnamylamino)ethyl]-5-isoquinoline-sulfonamide·2HCl hydrate (H-89) were purchased from Promega Corporation (Madison, USA). Sodium borohydride, cAMP, α-cyano-4-hydroxycinnamic acid (CHCA) and $HAuCl_4 \cdot 3H_2O$ were purchased from Sigma-Aldrich (Missouri, USA) and 3-mercaptopropionic acid (MPA) was from Thermo Fisher Scientific (New Jersey, USA). All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). The peptides used in the experiments were chemically synthesized by a solid phase method from A-Peptides Co. Ltd. (Shanghai, China). The sequences of the synthesized peptides are given in Table S1. All solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) and had an electric resistance >18.3 MΩ. The glassware were washed with aqua regia ($HCl:HNO_3$ in a volume ratio of 3:1), and then rinsed with ultrapure water for three times.

Synthesis and Characterization of Peptide-Templated AuNCs. In a mixture of 200 μL of 62 μM peptide aqueous solution, 1.5 mL of 50 mM $HAuCl_4$ aqueous solution and 1.5 mL methanol, 300 μL of 30 μM MPA aqueous solution was added under stirring on a magnetic stirrers. The pH value of the mixture was adjusted with 150 M NaOH (~60 μL) to pH 8. Then, the mixture was stirred for 10 min followed by the addition of 300 μL of 1 M $NaBH_4$ that was freshly prepared in ice-cooled water. The reaction mixture was stirred for another 10 min to allow the formation of the peptide-templated AuNCs. The resulting AuNCs (~3.6 mL) were concentrated and washed with ultrapure water for three times by ultrafiltration using a centrifugal filter unit (Amicon Ultra-0.5 mL with MWCO of 3000, Millipore) so as to remove excessive peptide and MPA. The concentrated AuNCs were diluted with ultrapure water to 1.8 mL.

For MALDI-TOF MS analysis, the AuNCs solution was firstly concentrated by 10 fold by ultrafiltration using a centrifugal filter unit (Amicon Ultra-0.5 mL with MWCO of 3000, Millipore). Then, a 10 μL aliquot of the AuNCs solution was mixed with an equal volume of methanolic solution of CHCA (0.1 mg/10 μL). The mixture was cast on a stainless steel plate and dried in air for 1 h. MALDI mass spectra were recorded with a TOF mass spectrometer (Applied Biosystems Voyager-DE Pro) operated with a N2 laser (337 nm, 3 Hz, <100 μJ) in the positive ion mode with delayed extraction. According to the mass of the parent ion peak, the peptide-templated AuNCs were determined as Au_8 clusters with surface ligands consisting of two peptides and two MPA molecules.

To determine the peptide concentration in the as-prepared AuNCs solution, a mixture of 0.2 M KCN and 2 mM $K_3Fe(CN)_6$ was added in the AuNCs solution to final concentrations of 0.08 M KCN and 0.8 mM $K_3Fe(CN)_6$. After the AuNC cores were etched, a 10 μL aliquot the released peptide solution was directly subjected to electrospray ionization (ESI) mass spectrometric analysis on a linear trap quadropole (LTQ) orbitrap Velos mass spectrometry equipped with a nanoelectrospray source (Thermo Fisher Scientific, Bremen, Germany). The concentration of the released peptide was determined to be ~100 nM according to its intensity of the parent ion peak with reference to three standard solutions of the peptide. According to the above determined ligand number, the concentration of the peptide-templated AuNCs was estimated to be ~50 nM.

The fluorescence spectra were measured at room temperature in a 100 μL quartz cuvette on a Fluorolog-Tau-3 spectrofluorometer (Jobin Yvon Inc., NJ) with the slit set to be 5 nm for both the excitation and the emission. The ultraviolet-visible (UV-vis) absorption spectra were measured using a UV-2550 spectrometer (Shimadzu, Japan) with a wavelength interval of 2 nm. Zeta potentials of the AuNCs were determined using a Malvern Zetasizer (Nano-ZS, USA). The morphologies of the AuNCs were obtained using a JEOL JEM-2100 transmission electron microscope (TEM). The X-ray photoelectron spectroscopy (XPS) analysis was performed using an X-ray photoelectron spectroscope (Thermo Fisher Scientific, USA).

Peptide-Templated AuNCs Beacon for Label-Free Detection of HDAC 1. In a typical assay, in a 100 μL reaction buffer containing 50 nM peptide **1**-templated AuNCs, 25 mM Tris-HCl (pH 8.0), 137 mM sodium chloride, 2.7 mM potassium chloride and 1 mM magnesium chloride, 2 μL of HDAC 1 of a given concentration was added. The mixture was incubated at 37 ºC for 45 min to allow a complete deacetylation reaction. After the reaction, the resulting solution was immediately subjected to fluorescence spectral measurements. Time-dependent fluorescence responses of the reaction were taken at a time interval of 30 s. The fluorescence spectra were recorded in the emission wavelength range from 440 to 560 nm with an excitation wavelength at 360 nm.

The control experiments were performed under the same conditions except the replacement of a given reagent by a control reagent, such as replacing peptide **1**-templated AuNCs by an equal volume of AuNCs templated by another peptide, or replacing HDAC 1 by an equal volume of another protein or mixture of HDAC 1 and TSA.

Peptide-Templated AuNCs Beacon for Label-Free Detection of PKA. In a typical assay, in a 100 μL reaction buffer containing 50 nM peptide **3**-templated AuNCs, 40 mM Tris-HCl (pH 7.4), 20 mM magnesium acetate and 0.2 mM ATP, 2 μL PKA of a given concentration was added. The mixture was incubated at 30 ºC for 45 min to allow a complete deacetylation reaction. After the reaction, the

resulting solution was immediately subjected to fluorescence spectral measurements. Time-dependent fluorescence responses of the reaction were taken at a time interval of 30 s. The fluorescence spectra were recorded in the emission wavelength range from 440 to 560 nm with an excitation wavelength at 350 nm.

The control experiments were performed under the same conditions except the replacement of a given reagent by a control reagent, such as replacing peptide **3**-templated AuNCs by an equal volume of peptide **2**-templated AuNCs, replacing PKA by an equal volume of cAMP-dependent PKA holoenzyme or mixture of PKA holoenzyme and cAMP, replacing PKA by an equal volume of mixture of PKA and H-89, or replacing ATP by an equal volume of ultrapure water.

Table S1. Synthesized peptide sequences used in the experiments [a].

Sequence **1**	CCIH*K*(Ac)
Sequence **2**	CCGKE
Sequence **3**	CCLRRA*S*LG
Sequence **4**	CCGG*K*(Ac)
Sequence **5**	CCLI*K*(Ac)

[a] Sequence **1**、**4**、**5** were substrate peptides of HDAC 1; Sequence **3** was substrate peptide of PKA; Sequence **2** was control peptide that was not the substrate of both HDAC 1 and PKA. The italic typed letter in each peptide sequence is the reaction site of corresponding enzyme.

Figure S1. Determination of quantum yield (QY) of the peptide-templated AuNCs. a) Absorption spectra of the AuNCs of different concentrations. b) Fluorescence spectra of the AuNCs of corresponding concentrations. c) Plot of the peak absorbances versus the integrated fluorescence intensities. Quinine sulphate (QY = 55%) was used as the reference (blue line). The QY of the AuNCs was calculated to be 21.7 %.

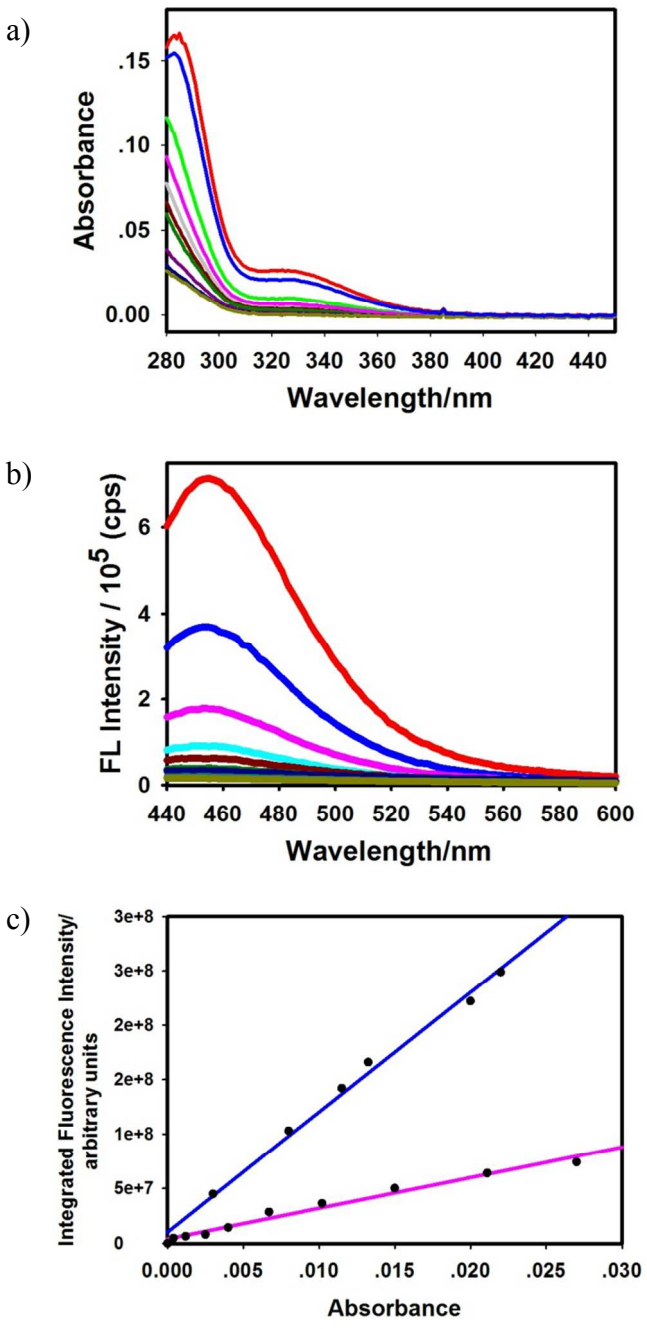
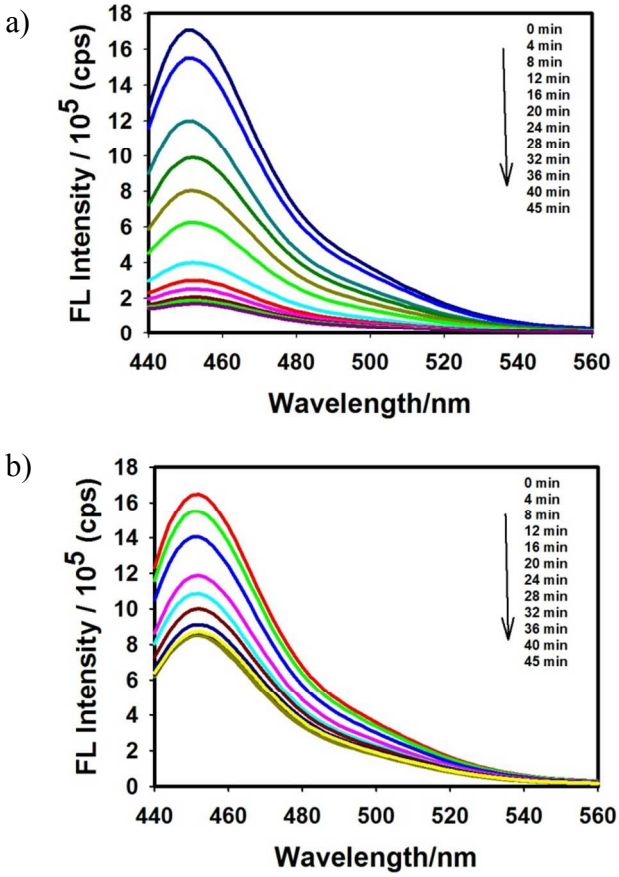


Figure S2. a) Time-dependent fluorescence spectral responses during the reaction between the peptide **1**-AuNCs (50 nM) and 30 nM HDAC 1. b) Time-dependent fluorescence spectral responses during the reaction between the peptide **1**-AuNCs (50 nM) and 750 pM HDAC 1.



Nonlinear fitting of the time-dependent fluorescence responses to 30 nM and 750 pM HDAC 1 gave the estimates of the Michaelis-Menten constant K_m and the catalytic constant k_{cat} as 0.17 μM and 21.6 × 10^9 min^{-1}, and 0.34 μM and 9.8 × 10^9 min^{-1}, respectively. The average Michaelis-Menten constant K_m and the average catalytic constant k_{cat} were calculated as 0.25 μM and 15.7 × 10^9 min^{-1}, respectively, which were approximate to the corresponding values reported for K_m (0.69 μM) and k_{cat} (8.2 × 10^9 min^{-1}).

Figure S3. MALDI-TOF MS spectra in the low-molecular weight range for peptide **1** templated AuNCs (500 nM) with CHCA matrix. a) before HDAC 1 reaction, b) after HDAC 1 reaction. HDAC 1 concentration was 30 nM.

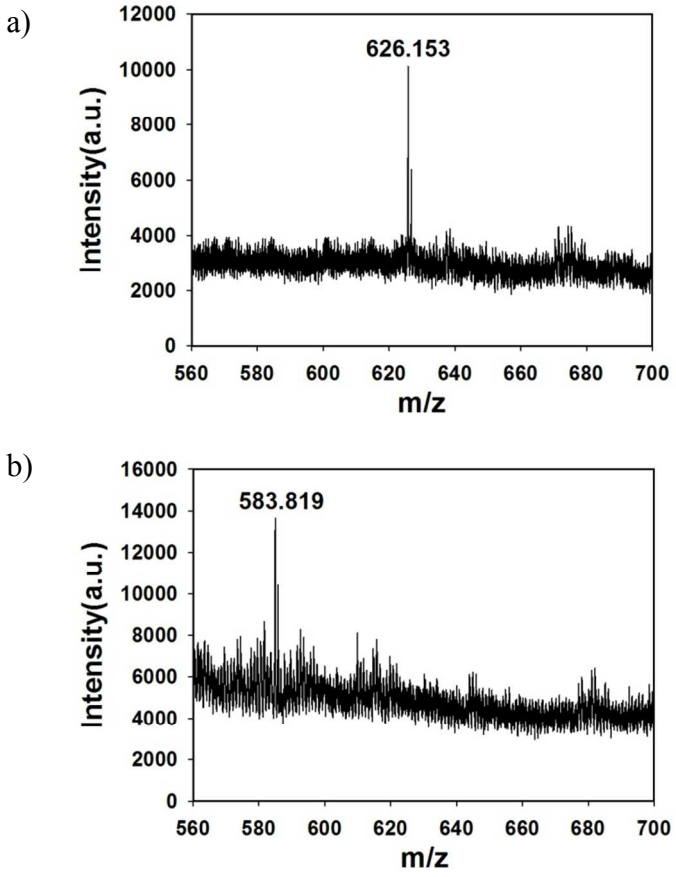


Figure S4. ESI MS spectra for peptide **1** templated AuNCs (50 nM) obtained after decomposing the AuNC cores. a) before HDAC 1 reaction, b) after HDAC 1 reaction. HDAC 1 concentration was 30 nM.

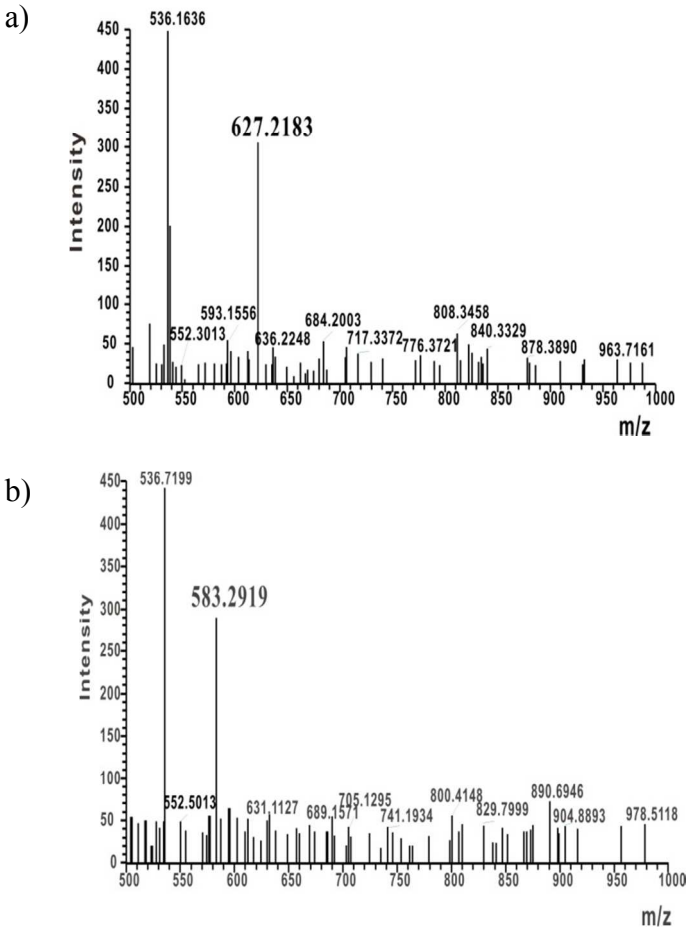
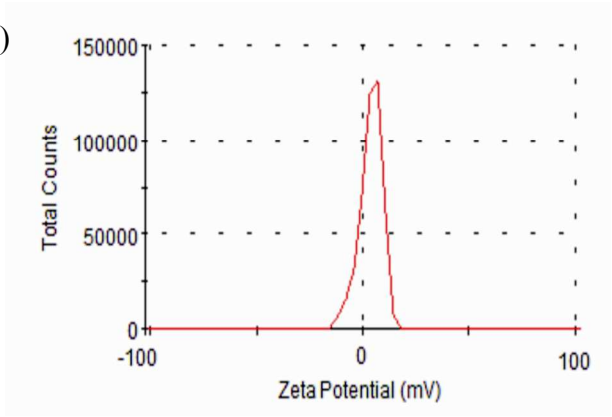


Figure S5. Zeta potential analysis of peptide **1** templated AuNCs (50 nM). a) before HDAC 1 reaction, b) after HDAC 1 reaction. HDAC 1 concentration was 30 nM.

a)



b)

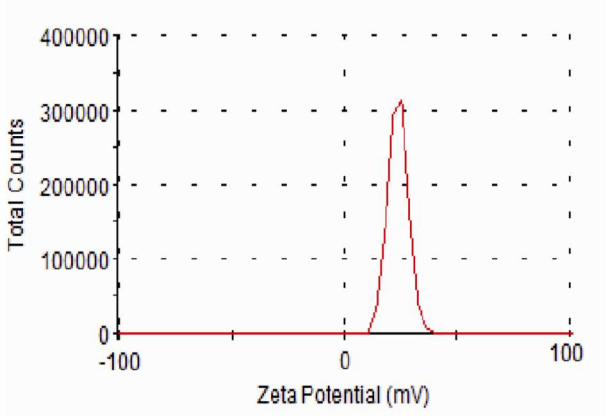
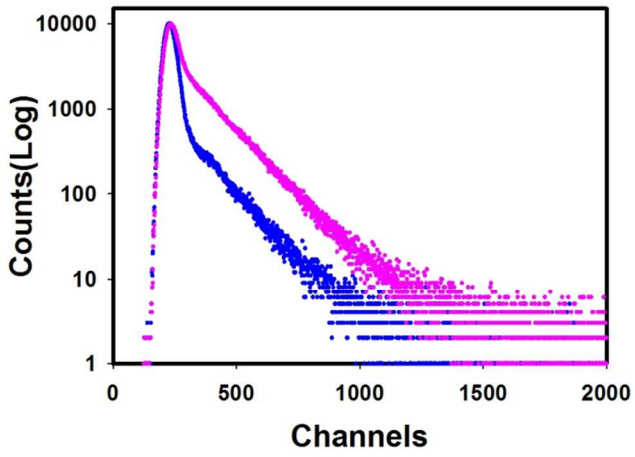


Figure S6. Fluorescence lifetime measurements of peptide **1** templated AuNCs (50 nM) before HDAC 1 reaction (pink) and after HDAC 1 reaction (blue). HDAC 1 concentration was 30 nM.



By fitting to a biexponential fluorescence decay, the lifetime for the AuNCs before HDAC 1 reaction was determined as 24.5 ns (80.1%) and 3.9 ns (19.9%), and after the reaction the lifetime was determined as 1.0 ns (48.6%) and 3.8 ns (51.4%).

Figure S7. Fluorescence anisotropy profiles of peptide **1** templated AuNCs (50 nM) during the reaction with 30 nM HDAC 1.

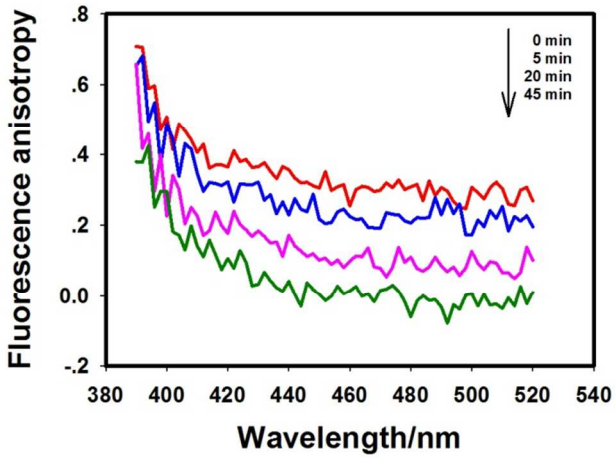


Figure S8. X-ray photoemission spectroscopy (XPS) analysis of peptide **1**-templated AuNCs before the reaction (top) and after the reaction (bottom) with 30 nM HDAC 1. The raw XPS spectra, $4f_{7/2}$ (right) and $4f_{5/2}$ (left), could be deconvolved into two distinct components corresponding to the binding energies for Au (0) (blue) and Au(I) (green). The deconvolved Au $4f_{7/2}$ spectra gave two binding energies of 83.75 and 85.47 eV, which corresponded to Au(0) and Au(I), respectively. The intensities of these two peaks indicated that the AuNCs contained ~95% Au(0) and ~5% Au(I) before the reaction, while the percentage of Au(I) increased to ~93% after the reaction with HDAC 1.

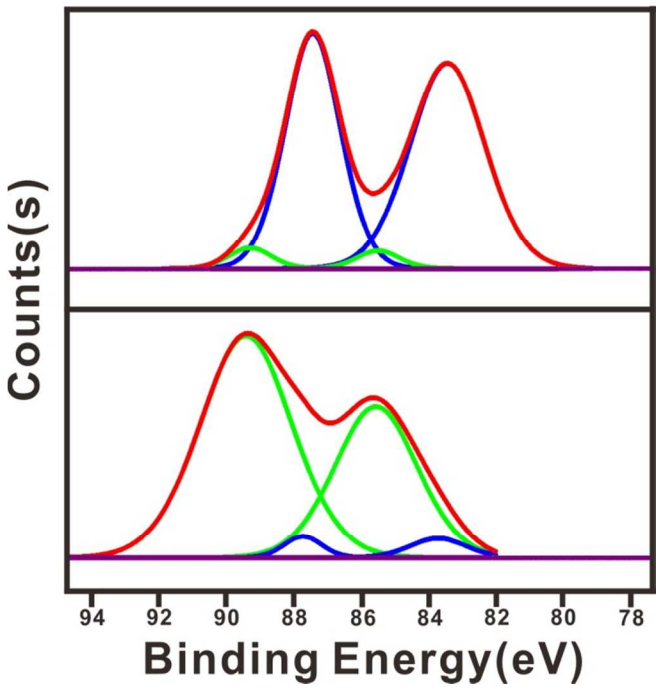


Figure S9. Fluorescence spectral responses of peptide **1** templated AuNCs to 30 nM HDAC 1: blank (red), under atmospheric condition (violet), under deoxygenation condition (green).

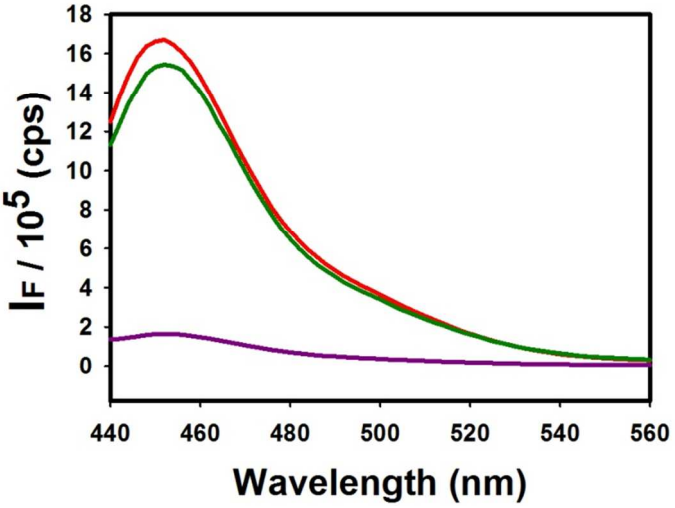
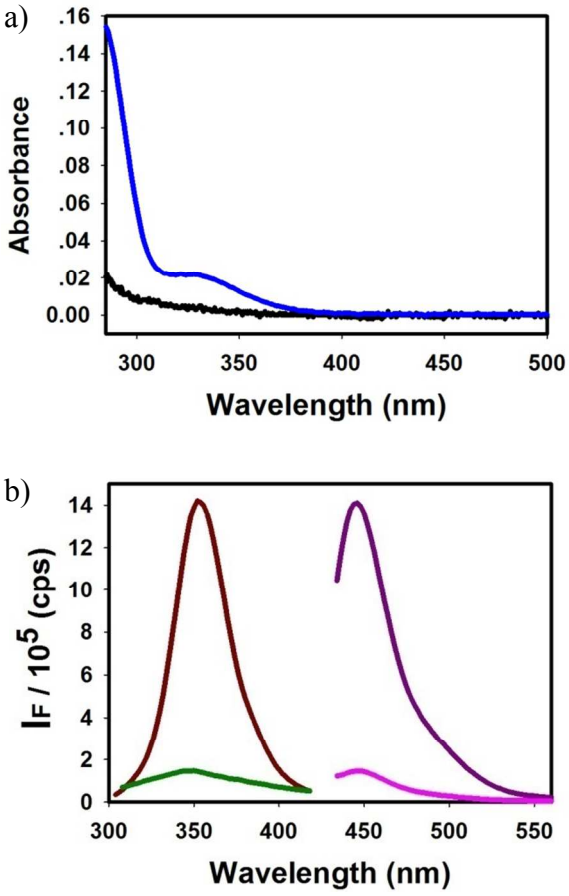
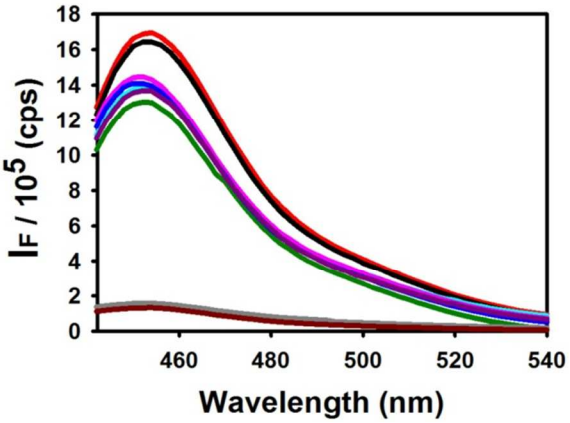


Figure S10. a) UV-Visible absorption spectra of AuNCs templated by peptide **2** (blue) and without peptide **2** (black). b) Fluorescence excitation and emission spectra of AuNCs templated with peptide **2** (brown and violet) and without peptide **2** (green and pink).



To demonstrate the generality of the peptide-templated synthesis method for fluorescent AuNCs and the label-free biosensor platform for PTM enzymes, we designed substrate peptide **3**, CCLRRASLG, for PKA. This peptide was used for the templated synthesis of AuNCs according to the developed procedure, and the resulting AuNCs were observed to give a strong fluorescence peak at 450 nm with an excitation maximum at 350 nm. The fluorescence maxima displayed slight shifts as compared with those from the peptide **1** templated AuNCs, indicating possible charge transfer interactions between AuNC cores and the surface peptide sequences that altered the HOMO−LUMO gaps of AuNCs.

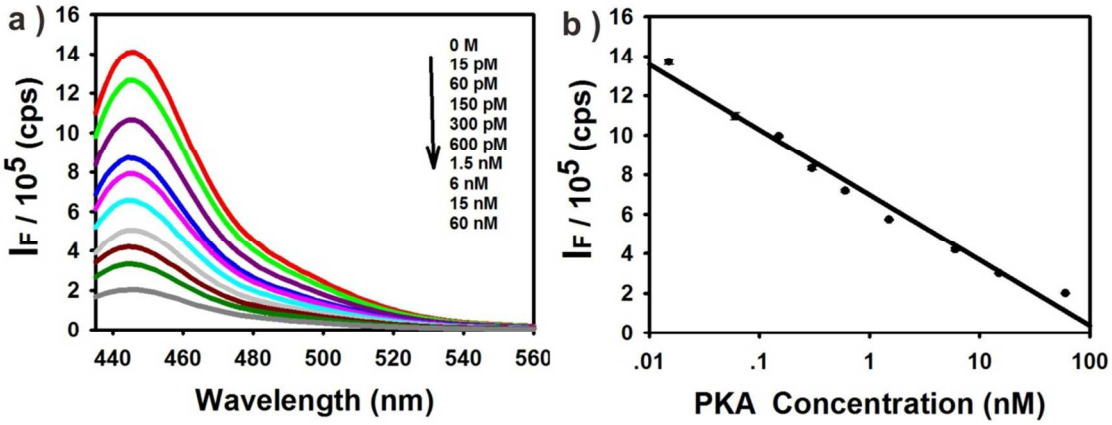
Figure S11. Fluorescence spectral responses of peptide **2** templated AuNCs: blank (red), PKA plus ATP (black), and responses of peptide **3** templated AuNCs: blank (blue), ATP (pink), PKA without ATP (cyan), c-AMP dependent PKA holoenzyme plus ATP (violet), PKA plus ATP and H-89 (green), c-AMP dependent PKA holoenzyme plus ATP and cAMP (gray), and PKA plus ATP (brown).



It was found that the peptide **3** templated AuNCs also showed fluorescence quenching response to active PKA. After incubated with 60 nM PKA in the presence of the co-substrate (200 μM ATP) for 45 min, the AuNCs exhibited a largely (~92%) quenched fluorescence. In control experiments in which the AuNCs were incubated with 60 nM PKA in the absence of ATP or with 200 μM ATP in the absence of PKA, there was no appreciable quenching of the fluorescence signals from the AuNCs. These observations implied that the fluorescence quenching of the peptide **3** templated AuNCs was selective to the active PKA reaction rather than the interactions of the AuNCs with PKA or ATP. In another control experiment using AuNCs synthesized with peptide **2** that was not the PKA substrate, no remarkable fluorescence quenching appeared after treated with 60 nM PKA in the presence of ATP for 45 min. This result confirmed the selectivity of the fluorescence quenching in response to PKA mediated reactions.

In addition, activation of a cAMP-dependent PKA holoenzyme by cAMP and inhibition of PKA by its inhibitor N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89) were examined.[S1] After the peptide **3** templated AuNCs incubated with 60 nM PKA in the presence of 200 μM ATP and 10 μM H-89, we obtained a slightly decreased fluorescence signal, This finding validated the selectivity of the fluorescence quenching response to active PKA reactions, and suggested the possibility of using the AuNCs-based label-free sensor for inhibitor screening for PKA. On the other hand, when the AuNCs incubated with the cAMP-dependent PKA holoenzyme, which was inactive in the absence of cAMP, even in the presence of ATP, no remarkable change was obtained in the fluorescence signal. In contrast, a substantially (~92%) quenched fluorescence peak was obtained for the reaction mixture containing the AuNCs with the PKA holoenzyme, 200 μM ATP and 1 mM cAMP. These data implied the ability of the label-free sensor for the assays of PKA activation and cAMP signaling.

Figure S12. a) Typical fluorescence spectral responses of peptide **3** templated AuNCs to PKA of varying concentrations. b) Fluorescence peak intensities versus PKA concentrations in logarithmic scale. Error bars indicated SDs across four repetitive assays.



For the fluorescence responses of the peptide **3** templated AuNCs to PKA of varying concentrations, the fluorescent peaks were found to be dynamically decreased with increasing PKA concentration ranging from 15 pM to 60 nM (0.001 to 4 U/mL). The peak responses also exhibited a linear correlation to the logarithmic PKA concentrations in this range and a detection limit of 6 pM was achieved.

REFERENCES

(1) Nagai, Y.; Miyazaki, M.; Aoki, R.; Zama, T.; Inouye, S.; Hirose, K.; Iino, M.; Hagiwara, M. *Nat. Biotechnol.* **2000**, *18*, 313-316.