Label-Free Fluorescent Detection of Protein Kinase Activity Based on the Aggregation Behavior of Unmodified Quantum Dots

Xiahong Xu, Xin Liu, Zhou Nie,* Yuliang Pan, Manli Guo, and Shouzhuo Yao

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P. R. China

Herein, we present a novel label-free fluorescent assay for monitoring the activity and inhibition of protein kinases based on the aggregation behavior of unmodified CdTe quantum dots (QDs). In this assay, cationic substrate peptides induce the selective aggregation of unmodified QDs with anionic surface charge, whereas phosphorylated peptides do not. Phosphorylation by kinase alters the net charge of peptides and subsequently inhibits the aggregation of unmodified QDs, causing an enhanced fluorescence with a 45 nm blue-shift in emission and a yellow-to-green emission color change. Hence the fluorescence response allows this QD-based method to easily probe kinase activity by a spectrometer or even by the naked eye. The feasibility of the method has been demonstrated by sensitive measurement of the activity of cAMP-dependent protein kinase (PKA) with a low detection limit (0.47 mU μL⁻¹). On the basis of the fluorescence response of QDs on the concentration of PKA inhibitor H-89, the IC₅₀ value, the half maximal inhibitory concentration, was estimated, which was in agreement with the literature value. Moreover, the system can be applicable to detect the Forskolin/3-isobutyl-1-methylxanthine (IBMX)-stimulated activation of PKA in cell lysate. Unlike the existing QD-based enzyme activity assays in which the modification process of QDs is essential, this method relies on unmodified QDs without the requirement of peptide labeling and QDs’ modification, presenting a promising candidate for cost-effective kinase activity and inhibitor screening assays.

Protein phosphorylation by kinases is an important post-translational modification mechanism, which plays vital regulatory roles in most metabolic pathways and in cell communication. The overexpression of protein kinases is responsible for various diseases such as cancer, diabetes, or Alzheimer’s disease.¹,² As a result, the identification of kinase activities and their potential inhibitors is not only necessary for basic biology to clarify molecular mechanisms of signal transduction but also valuable for clinical pharmacology and drug discovery.³,⁴ Compared with several kinase assays based on radioactive,⁵–⁷ electrochemical,⁸–¹³ and surface-plasmon resonance techniques,¹⁴,¹⁵ which require a surface-confined process and multistep washing, homogeneous fluorescence-based assays are very attractive due to their high sensitivity, short detection time, and readiness for high-throughput screening (HTS). In order to achieve the phosphorylation-dependent modulation of the fluorescence, most of existing fluorescence-based kinase assays rely on the use of fluorescent labeling techniques and phosphorylation-specific recognition proteins (e.g., antibodies or phosphate binding proteins).¹⁶–²⁰ Such assays are effective but require labor-intensive labeling procedures or expensive recognition proteins. Therefore, it is still a challenge to develop convenient, cost-effective, and label-free protein kinase assays.

Quantum dots are nanometer-size luminescent semiconductor crystals that exhibit unique optical and electronic properties.²¹,²² Because QDs’ emission wavelength can be tuned simply by modification, presenting a promising candidate for fluorescence-based assays.

[Note]: *To whom correspondence should be addressed. Phone: +86-731-88821626. Fax: +86-731-88821848. E-mail: niezhou.hnu@gmail.com.

varying their size and morphology, QDs as fluorescent probes attract substantial research interest in bioanalysis and have been widely used for sensing various biorecognition events mediated by DNA, aptamers, antibodies, and specific binding proteins. In recent years, biocatalytic processes sensing has become an emerging field in QD-based bioanalysis. Medintz et al. and Rosenzweig et al. designed a series of QD-peptide bioconjugates to detect proteases activity by fluorescence resonance energy transfer (FRET). Recently, they expanded their peptide-QD conjugates system to monitor kinase activity in combination with phosphorylation-specific antibodies labeled by fluorophores. These QD-based methods achieved the sensitive detection of enzyme-catalyzed events by FRET or ET quenching processes, which utilized quantum dots as donors of energy or electrons. Since the fabrication of peptide-QD bioconjugates to keep the proximity of QDs donors to the acceptors in the peptides is essential for the high efficiency of FRET or ET, all the above-mentioned QD-based methods involved a modification process for tethering peptides to QDs. Moreover, most of these methods, especially the methods based on FRET, require labeled peptide or antibody. Hence, in order to simplify the detection process of biocatalytic events, we expect to develop a QD fluorescent sensor without the modification process of QDs and peptide labeling.

Herein, we present a simple and novel method for profiling protein kinase activity using unmodified CdTe QDs as fluorescent probes. Unlike the existing bioanalysis platforms of QDs based on FRET or ET quenching mechanism, the present work relies on the stability of unmodified QDs which is susceptible to the surface charge change. The unmodified QDs, like other colloidal particles, are very sensitive to the presence of additional charges either on their surface or in the surrounding environment. The surrounding charge change of QD can easily affect the stability of QDs suspension, resulting in the aggregation of QDs. The aggregation of quantum dots can cause the significant change of nanocrystal fluorescence of QDs because of the mutual interparticle interactions, which has been demonstrated by various QD assembly systems. This is a potential mechanism for sensing but largely neglected. Taking advantage of these characteristics, we use the charge switch of peptide substrates, which is induced by kinase-catalyzed phosphorylation, to mediate the aggregation of unmodified QDs, eventually resulting in an enhanced fluorescence and an emission color change of QDs as a read-out. Compared with the existing kinase assays, the present assay can detect protein kinase activity without a multistep washing treatment, peptide labeling, and the use of expensive antibodies or binding proteins. Additionally, the common steps used in QD bioanalysis systems such as modification and purification of QDs composites probe are avoided in this method. It is worth noting that although there are many examples of QDs-based detection for biocatalytic process, the application of QD in kinase sensing is scarce. Thus this method will potentially broaden the applicability of QD in bioanalysis.

**EXPERIMENTAL SECTION**

**Materials and Measurements.** Cyclic adenosine 3',5'-monophosphate-dependent protein kinase (PKA, catalytic subunit) was obtained from New England Biolabs Inc. (Beverly, MA). Substrate peptide (S-peptide, LRRASLGGGGG) and phosphorylated peptide (P-peptide, LRRAPSLGGGGG) for PKA were purchased from GL Biochem Ltd. (Shanghai, China). ATP was purchased from General Biotech Co., Ltd. (Shanghai, China). Water-soluble (carboxyl coated) CdTe quantum dots were purchased from ZhongDS (Shenzhen, China). H-89 was obtained from EMD Biosciences (Calbiochem-Novabiochem, La Jolla, CA). Forskolin and 3-isobutyl-1-methylxantine (IBMX) were purchased from Sigma-Aldrich (St. Louis, MO). Protease inhibitor and improved Bradford protein assay dye reagent kit were obtained from Sangon (Shanghai, China). Other reagents including bovine serum albumin (BSA), Tris, Glycerol, DTT, and EDTA were purchased from Bio Basic Inc. (Ontario, Canada). Human breast cancer cells (MCF-7) were obtained from the Cell Bank of Xiangya Central Experiment Laboratory of Central South University (Changsha, China). All solutions were prepared using ultrapure water (18.3 MΩ cm) from the Millipore Milli-Q system.

Fluorescence measurements were performed on Synergy Mx multimode microplate reader (BioTek Instruments, Inc.). All samples were illuminated at an excitation wavelength of 365 nm, and the fluorescence emission was scanned from 400 to 700 nm at 25 °C. Except for the specific cases mentioned in the text, the fluorescence intensity of all the spectra was measured as the emission intensity at the maximum emission peak. The fluorescence measurements were performed three times for each sample (n = 3). The results show the average of measurements with the error bars indicating the relative standard deviation. Transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) measurements were conducted on a JEOL JEC-3010 electron microscope. Potential measurement was used to monitor the surface potential of the QDs with a
Peptide-Induced QDs Aggregation. In each well of 96-well plates, the sample consisted of 10 \( \mu \text{L} \) of peptide solution (4 \( \mu \text{M} \) final concentration) and 40 \( \mu \text{L} \) of QDs dispersion (5 \( \mu \text{g} \text{ mL}^{-1} \) final concentration) in 50 mM Tris-HCl buffer, pH 7.5, 25 °C. Tris-HCl buffer (10 \( \mu \text{L} \)) was used instead of 10 \( \mu \text{L} \) of peptide solution for the control. The microwell plate loaded with the sample was placed into a Synergy Mx multimode microplate reader, and the protocol was set by the procedure of shaking for 1 min with medium intensity and then measuring the fluorescence emission spectra from 400 to 700 nm at 25 °C (excitation at 365 nm).

For investigation of the effect of QDs concentration on the fluorescence response of QDs in the presence of S-peptide or P-peptide, a series of solutions containing 10 \( \mu \text{L} \) of S-peptide or P-peptide solutions (4 \( \mu \text{M} \) final concentration) and 40 \( \mu \text{L} \) of QDs dispersions (1, 2, 5, 8, and 10 \( \mu \text{g} \text{ mL}^{-1} \) final concentration, respectively) in 50 mM Tris-HCl buffer (pH 7.5) were prepared. To study the influence of the concentration of S-peptide or P-peptide on fluorescence response of QDs, a series of samples consisting of 40 \( \mu \text{L} \) of QDs dispersions (5 \( \mu \text{g} \text{ mL}^{-1} \) final concentration) and 10 \( \mu \text{L} \) of S-peptide or P-peptide solutions (final concentrations, 0, 1, 2, 4, 6, 8, and 10 \( \mu \text{M} \), respectively) in 50 mM Tris-HCl buffer (pH 7.5) were prepared. For investigating the fluorescence response of QD with different S-peptide/P-peptide concentration ratios, the procedure of this experiment was as follows: (1) preparing a series of peptide mixed solutions with various S-peptide/P-peptide concentration ratios \( (C_{\text{S-peptide}}/C_{\text{P-peptide}} = 5:0, 4:1, 3:2, 2:3, 1:4, \text{and } 0:5) \), total peptide concentration was 20 \( \mu \text{M} \) in 50 mM Tris-HCl buffer (pH 7.5); (2) mixing 10 \( \mu \text{L} \) of each peptide mixed solution with 40 \( \mu \text{L} \) of QDs solution (5 \( \mu \text{g} \text{ mL}^{-1} \)) in each microwell. The fluorescence measurement protocol was set as the above-mentioned procedure.

Detection of Activity and Inhibition of PKA. The PKA storing solutions were composed of 50 mM NaCl, 20 mM Tris-HCl buffer (pH 7.5, 25 °C), 1 mM EDTA, 2 mM DTT, and 50% glycerol. The PKA reaction solutions were composed of PKA (0–4 U \( \mu \text{L}^{-1} \)), S-peptide (20 \( \mu \text{M} \)), MgCl\(_2\) (10 mM), ATP (40 \( \mu \text{M} \)), and 50 mM Tris-HCl buffer (pH 7.5, 25 °C). After 60 min of incubation at 30 °C for PKA-catalyzed reaction, the resulting solution of kinase-phosphorylated peptide (10 \( \mu \text{L} \), 4 \( \mu \text{M} \) final peptide concentration) was added to the 40 \( \mu \text{L} \) of QDs dispersion (5 \( \mu \text{g} \text{ mL}^{-1} \) final concentration). For PKA inhibition assays, the experiments were conducted under the above-mentioned conditions, except for the involvement of 4 U \( \mu \text{L}^{-1} \) PKA and different concentrations of H-89 (0–8 \( \mu \text{M} \)) in the reaction solutions. The S-peptide was then added to the H-89-pretreated PKA solution and incubated for 60 min. After incubation, the reaction solution (10 \( \mu \text{L} \)) was mixed with 40 \( \mu \text{L} \) of QDs (5 \( \mu \text{g} \text{ mL}^{-1} \) final concentration) in each well. The fluorescence intensity was measured by a Synergy Mx multimode microplate reader, and the protocol was set by the procedure of shaking for 1 min with medium intensity and then measuring the fluorescence emission spectra.

MCF-7 Cell Culture and Lysate Preparation. MCF-7 cells (1 \( \times 10^5 \) cells) were supplemented with 10% fetal bovine serum, MEM nonessential amino acid solution (0.1 mM), 1% insulin-transferrin-selenium-A supplement, penicillin (100 U mL\(^{-1}\)), streptomycin (100 mg mL\(^{-1}\)), and amphotericin B (0.25 mg mL\(^{-1}\)). The cells were incubated under a humidified atmosphere containing 5% CO\(_2\) at 37 °C. The culture medium was replaced by serum-free medium (1 mL) for 4 h before stimulation. The mixtures with various concentrations of Forskolin and IBMX in DMSO (10 \( \mu \text{L} \)) were added to the medium to activate intracellular PKA. DMSO (10 \( \mu \text{L} \)) instead of Forskolin/IBMX solution was added to the medium for unstimulated samples. After 30 min of stimulation, the cultured cells were removed by scraping and lysed in Dulbecco’s phosphate-buffered saline (D-PBS) including protease inhibitor by sonication (200 W) for 2 s × 60 times at a interval of 3 s for each time. The cell lysates were clarified by centrifugation at 22 000 rpm for 60 min at 4 °C, and the clarified lysates were ready for phosphorylation reactions.

The total protein concentration of cell lysate was assessed by using the improved Bradford protein assay dye reagent kit with BSA as the standard. Briefly, standard solutions with different concentrations of BSA (5–30 \( \mu \text{g} \text{ mL}^{-1}\)) were incubated with the Bradford reagent for 10 min. The absorbance of resulting solutions was recorded at 595 nm using a UV–vis spectrophotometer, and the calibration curve of the standard concentration versus the absorbance was obtained by using a linear-regression program. The correlation coefficient of the absorbance with respect to the concentration was 0.998. Finally, an aliquot of the cell extract was mixed with Bradford reagent and detected as described above. Its total protein concentration was then calculated by reference to the calibration curve. All cell lysates were diluted to 8 \( \mu \text{g} \text{ mL}^{-1} \) total protein concentration for kinase activity assays.

Kinase Activity Assay with Cell Lysate. Phosphorylation reactions with cell lysates were achieved by incubation of the S-peptide with cell lysates (MCF-7 breast cancer cell) for 60 min at 30 °C. The phosphorylation reaction solutions were composed of MCF-7 cell lysates (8 \( \mu \text{g} \text{ mL}^{-1} \), with different degrees of stimulation), S-peptide (20 \( \mu \text{M} \)), MgCl\(_2\) (10 mM), ATP (40 \( \mu \text{M} \)), 50 mM Tris-HCl buffer (pH 7.5 at 25 °C). The control samples were prepared with unstimulated cell lysate or stimulated cell lysate (treated by 50 \( \mu \text{M} \) Forskolin and 100 \( \mu \text{M} \) IBMX) in the absence of ATP or in the presence of kinase inhibitor H-89 (10 \( \mu \text{M} \)). After the reaction, the resulting solutions (10 \( \mu \text{L} \)) were added to the QDs dispersions (40 \( \mu \text{L} \), 5 \( \mu \text{g} \text{ mL}^{-1} \) final concentration) for fluorescence detection using a Synergy Mx multimode microplate reader, and the measurement protocol was set by the same procedure.

RESULTS AND DISCUSSION

Discrimination of S-Peptide and P-Peptide Based on the Fluorescent Response of QDs. Since substrate peptide (S-peptide) and phosphorylated peptide (P-peptide) are the reactant and product of kinase-catalyzed phosphorylation, we investigate the effect of S-peptide or P-peptide on the fluorescence of
unmodified CdTe QDs at first. AMP-dependent protein kinase (PKA) was used as a model in this study. The PKA-specific S-peptide (LRRASLGGGGC) has a positive charge (2+), and its phosphorylated counterpart P-peptide (LRRApSLGGGGC) has a net charge of zero because of the introduction of a phosphate group with two negative charges. In order to reinforce the adsorption of peptide to the surface of the QDs, a cysteine residue was attached to the carboxy-terminal end of the peptide. Thus, when the peptides absorb on the surface of the QDs, the peptides would be anchored on the surface of QDs by thiol group-mediated chemisorptions.

Figure 1 shows fluorescence emission spectra of the QDs dispersions (5 µg mL⁻¹) in the presence of 4 µM S-peptide (1) and 4 µM P-peptide (2). The addition of the S-peptide induced a significantly decreased fluorescence of QDs as well as a red-shift in emission from 550 to 575 nm. Correspondingly, as shown in the inset of Figure 1, the green-to-yellow emission color change of QDs suspensions with S-peptide was observed under the ultraviolet irradiation (at the excitation wavelength of 365 nm), and this color change took place very rapidly (within 10 s). However, in the case of P-peptide, a blue-shifted emission band of QDs centered at 530 nm was observed and the emission color of QDs remains green. Figure 2 displays the TEM and HRTEM images of resulting QD/S-peptide sample (A), QD/P-peptide sample (B) and CdTe QD (C). In CdTe QDs sample, the QDs are dispersed with average diameter of 3.8 ± 0.6 nm (Figure 2C). The aggregation of CdTe QDs was observed upon addition of S-peptide (Figure 2A) and the average diameter of the aggregate grew up to a size on the order of 13.8 ± 3.0 nm. Other than the aggregation, we also observed that the core size of individual QDs in the QDs aggregates (2.5 ± 0.3 nm) decreased. In the presence of P-peptide (Figure 2B), the QD/P-peptide complex was well dispersed with a relative small average diameter of 2.1 ± 0.2 nm compared with that of CdTe QDs sample. The ζ potential measurement was used to monitor the surface charge of the QDs. As illustrated in Figure S1 in the Supporting Information, the initial ζ potential for a solution of negatively charged CdTe QDs was about −25 mV. The addition of S-peptide with positive charge (2+) causes a significant change of ζ potential from −25 to 0 mV. Nevertheless, a negligible change in ζ potential was found in the case of mixing QDs with the P-peptide. The results of TEM and ζ potential measurement can help us clarify the significantly different fluorescence response of CdTe QDs to S-peptide and P-peptide. The S-peptide induces the formation of QDs aggregates because the binding of cationic S-peptide on QDs surface neutralizes the negative surface charge of QDs to zero (ζ potential approaches zero) and diminishes the colloidal stability of QDs because of the reduced interparticle electrostatic repulsion. The red-shift and drastic fluorescent quenching of the CdTe emission by S-peptide are probably caused by electronic energy transfer between interdot in the QDs aggregate, which have been proven in various QDs aggregate systems where assembly in solution or formation of a solid QDs film from solution takes place.35–37 The blue shift of the CdTe emission by P-peptide corresponds to mean QDs size reduction. The reduced QDs particle size, which is observed in both P-peptide and S-peptide cases, may be due to the chemical interactions of cysteine residue with heavy metal
on the surface of QDs, leading to etch the core of the QDs.  
Therefore, on the basis of all these facts, we suggest that the  
S-peptide induced QDs aggregation is the main reason for the  
discrepancy in QD fluorescent response between the S- and  
P-peptide. The S-peptides and the P-peptides could be clearly  
differentiated not only by the enhancement of fluorescence  
intensity of peptide/QDs mixtures but also by the obvious color  
change from yellow to green with an obvious blue-shift in emission  
under the ultraviolet irradiation.

To obtain the best sensing performance of CdTe QDs, the  
concentrations of QDs and peptides were optimized. The effect  
of QDs concentration (1–10 µg mL⁻¹) on the fluorescence signal  
of CdTe QDs was tested with a fixed concentration of S-  
peptide or P-peptide (4 µM), and the results are shown in  
Figure S2 in the Supporting Information. The best concentration  
of QDs for distinguishing the S-peptide and P-peptide was found  
to be 5 µg mL⁻¹. Figure S3 in the Supporting Information shows  
the peptide-concentration-dependent fluorescence response of  
QDs. The fluorescence intensity of QDs drastically decreases to  
about 13% with increasing S-peptide concentration from 0 to 4 µM  
and approaches a minimum at S-peptide concentrations higher  
than 4 µM. However, P-peptide retains a relatively high fluores-  
cence intensity of QDs (above 80%) unless the concentration is  
higher than 4 µM. The results indicate that 4 µM is the optimal  
peptide concentration to differentiate between S-peptide and  
P-peptide.

Furthermore, a series of peptide mixtures with various  
concentration ratios of S-peptide to P-peptide (20 µM total peptide  
concentration), which mimics the peptide mixtures treated by  
kinase, were mixed with QDs dispersions. As shown in Figure  
3A, the fluorescence intensity at 530 nm gradually enhances and  
the emission peak shifts gradually from 575 to 530 nm when the  
P-peptide proportion in the P-peptide/S-peptide mixture increases.  
Figure 3A inset exhibits the dependence of the intensity ratios of  
emission at 530 nm to that at 575 nm (I₅₃₀/I₅₇₅) on the P-peptide  
proportion in the mixture. The color of these solutions under  
the ultraviolet irradiation changes progressively from yellow  
to green along with the increase of the P-peptide proportion in  
the mixture (as shown in the Figure 3B), which is in accordance  
with the blue-shift in emission from 575 to 530 nm.

**Detection of the Activity and Inhibition of Protein Kinase.**  
Scheme 1 shows the detection mechanism of the kinase activity  
assay based on unmodified CdTe QDs. In this detection system,  
the substrate peptides (S-peptides) possessing two positive  
charges function as “coagulants” of QDs. When carboxyl-coated  
green CdTe QDs (emission peak at 550 nm) are mixed with  
cationic S-peptides, the anchoring of peptides significantly  
decreases the negative charge density on each QD surface and  
correspondingly reduces the colloidal stability of QDs, inducing  
the aggregation of QDs. The interparticle interactions between  
CdTe QDs in the aggregate greatly quench the fluorescence of  
QDs and change the emissive wavelength of QDs to 575 nm,  
causing an observable green-to-yellow color change. After the  
treatment of PKA, the phosphate groups are transferred from ATP  
to S-peptide, generating phosphorylated peptide (P-peptide). Under  
the optimized experimental conditions, the electrically neutral  
P-peptides, which play a role as “stabilizers” of QDs, have a slight  
influence on surface charge of QDs and maintain the well  
dispersion of QDs, hence the CdTe QDs exhibit a strong  
fluorescence emission at 530 nm and the color of QDs remains  
green. Therefore, the phosphorylation process catalyzed by  
kinases can be monitored by a fluorescence spectrometer or  
directly visualized by the naked eye.

Detection of kinase activity was further studied under the  
preoptimized conditions (5 µg mL⁻¹ CdTe QDs, 4 µM S-  
peptide) with different amounts of PKA (0–4 U µL⁻¹). As  
an indicator of QD aggregation, fluorescence intensity was
measured after mixing the enzyme-treated solution with CdTe QDs suspension (Figure 4). The fluorescence intensity increased with increasing concentration of the PKA, indicating that a higher degree of phosphorylation induced a smaller quantity of aggregation. Meanwhile, control experiments were also carried out with increasing PKA concentrations in the absence of substrate peptide, indicating that the PKA and its storage buffer had no effect on the fluorescence of QDs. Phosphorylation time was optimized, and the result is shown in Figure S4 in the Supporting Information; the fluorescence intensity reached a plateau when the enzyme reaction time was longer than 60 min (PKA, 4 U µL⁻¹), so 60 min was employed in the subsequent assay of enzyme activity. On the basis of Figure 4, the dependence of the ratiometric fluorescence change \(I_{530}/I_{575}\) on the PKA concentration can be obtained and is plotted in the inset of Figure 4. The EC₅₀ value (enzyme concentration at which 50% substrate is converted) for PKA was determined to be 0.108 U µL⁻¹. The detection limit of PKA was 0.47 mU µL⁻¹ estimated from 3 (Sᵇ/m), where Sᵇ is the standard deviation of the fluorescent signal in the absence of PKA (n = 3) and m is the slope of the analytical curve in the linear region. The detection limit of this assay was not only close to that of our previous electrochemical PKA assay (0.15 mU µL⁻¹)¹² but also highly comparable with the recently reported PKA assays.¹¹⁻¹³,¹⁰

To further demonstrate the potential application of this method in the inhibition assay, the experiments were performed in the presence of the kinase inhibitor H-89, a potent and cell-permeable inhibitor of PKA, with different concentrations. As shown in Figure 5A, with increasing the concentration of H-89, the fluorescence intensity of peptide/QDs mixtures decreased, which revealed the inhibition of PKA and low levels of peptide phosphorylation. When the fluorescence intensity versus the H-89 concentration was plotted, a sigmoidal profile was obtained (Figure 5B). The IC₅₀ value, the half maximal inhibitory concentration, was determined to be 138 nM, which is comparable with that reported in the literature.³⁹ Additionally, it is interesting to note that, other than fluorescence quenching, no obvious red-shift in H-89 PKA inhibition system was observed. The TEM result of H-89 inhibition sample confirmed the generation of QDs aggregates because of inhibition of kinase (Figure S5 in the Supporting Information), demonstrating that the fluorescence quenching was related to the aggregation-induced interparticle interactions. The absence of red-shift is probably due to H-89, but the detailed mechanism required further study to clarify.

Since protein kinase requires divalent cation Mg²⁺ and ATP as cofactors, which may induce undesirable influence on the fluorescence of QDs, their effects on QDs were investigated carefully in our experiments. As shown in Figure 6A, the fluorescence of QDs suspension remains unaffected in the presence of divalent cation Mg²⁺ unless the Mg²⁺ concentration is higher than 2.5 mM. Figure 6B shows the QDs fluorescence intensity as a function of ATP concentration.

---

When the concentration of ATP is higher than 10 µM, a significant decrease of fluorescence is observed. Since we employed 2.5 mM Mg²⁺ and 10 µM ATP for each enzyme reaction, the effect of divalent cations and ATP on the QDs aggregation is negligible.

Detection of the Drug-Induced Activation of PKA in Cell Lysates. Because protein kinases have profound effects on the signal transduction and cell signaling, their activity is highly regulated in a cell. The turn-on or off of kinases caused by extracellular stimulation can trigger a series of important cellular processes including transcription, apoptosis, and differentiation. Hence, kinase assays available for activity measurement in cell lysate are important for the study of the regulation of kinases in the cell system. We examined whether our method can be applied to detect kinase activity in cell lysates. It is well-known that Forskolin, an activator of adenyl cyclase, combined with IBMX, a phosphodiesterase inhibitor, can efficiently increase the intracellular levels of cAMP, causing the activation of PKA. Thus, in this work, MCF-7 breast carcinoma cells were treated with various concentrations of Forskolin and IBMX for stimulation (the concentration of Forskolin and IBMX are shown in the inset table in Figure 6A), and the activity of PKA in cell lysate was monitored by the fluorescence of QDs. The stimulated cell lysates (the total protein concentration was measured to 8 µg mL⁻¹) were applied to S-peptide in the presence of substrate ATP, and the resulting mixtures were added to the QDs dispersions. As shown in Figure 6A, in the samples treated by stimulated MCF-7 cell lysate, the fluorescence intensity of QDs increased with increasing concentrations of stimulants (Forskolin and IBMX). However, the fluorescence signal of QDs remained unaffected by the cell lysate without stimulation. The control samples without ATP or with H-89 inhibitor (10 µM) also present a weak fluorescence emission, indicating that various charged molecules in the cell lysate solution have no significant influence on the performance of the QDs sensor. The inset of Figure 6B shows the corresponding emission color of QDs mixed with the peptides phosphorylated by stimulated lysate (1–6) and untreated lysate (0). The stimulant-concentration-dependent fluorescence intensity change (see Figure 6B) indicates that, with the increasing concentration of stimulant, the kinase activity in cell lysate shows an initial quick increase \( C_{\text{Forskolin}} = 0–2.5 \mu M, C_{\text{IBMX}} = 0–5 \mu M \) followed by a slow increase \( C_{\text{Forskolin}} = 2.5–25 \mu M, C_{\text{IBMX}} = 5–50 \mu M \), and then turning to decrease after reaching the maximum \( C_{\text{Forskolin}} = 25–50 \mu M, C_{\text{IBMX}} = 50–100 \mu M \). Therefore, the activation of PKA in MCF-7 cells by the drugs is clearly detected through the fluorescence and color change of the QDs dispersions, and the specificity of this sensing platform is feasible for in vitro cell kinase assay.

CONCLUSIONS

We have developed a simple, sensitive, and label-free fluorescence assay using unmodified CdTe QDs to probe the activity of protein kinase. In this method, kinase-catalyzed peptide phosphorylation was monitored via a novel mechanism based on the selective aggregation of unmodified QDs by changing the surface charge of QDs, which provides a new and attractive alternative to the commonly used mechanisms such as FRET and ET quenching in the design of QDs-based enzyme assays. Our method using unmodified QDs possesses several notable advantages such as quick detection (less than 3 min), no need for labor-intensive QDs modification, enhanced fluorescence, and visible detection by the naked eye. Moreover, in comparison with existing fluorescence kinase assays, our method can significantly simplify the experimental process because tedious labeling procedures and complicated protein recognition treatment are avoided. Since the measurement of protein kinase activity by our method is conducted in a multwell plate system with purified kinase and stimulated MCF-7 cell lysate, this method presents a promising technique for high-throughput screening in kinase-related drug discovery and the direct kinase analysis in signal-transduction pathways.
ACKNOWLEDGMENT

This work was financially supported by the National Natural Science Foundation of China (Grant Numbers 20975032, 20805013, 20905024, and J0830415), the National Basic Research Program of China (973 Program, Grant No. 2009CB421601), the Hunan Provincial Natural Science Foundation of China (Grant Numbers 09JJ4006, 09JJ4007), and the open fund of State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (Grant No. KP2007-03). The authors thank Prof. Mingqiang Zhu and Mr. Jinhua Liu of the Biomedical Engineering Center, Hunan University for helpful discussions. We also thank the reviewers for their constructive and valuable suggestions to improve the quality of the manuscript.

SUPPORTING INFORMATION AVAILABLE

Additional information including extensive figures as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review June 18, 2010. Accepted November 21, 2010.

AC102786C