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Gold nanoclusters-based dual-emission ratiometric fluorescence probe for monitoring protein kinase

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ABSTRACT

A novel and sensitive ratiometric fluorescent assay for monitoring kinase activity based on carboxypeptidase Y (CPY) digestion by using Au nanoclusters (AuNCs) and CdSe/ZnS QDs@SiO_2 as the dual-emission nanoparticles is developed. This approach can not only sensitively monitor the kinase activity with affording built-in correction which avoids environmental interferences, but also realize visual detection. Under optimal conditions, a linear relationship between the fluorescence intensity ratio (I_{415}/I_{630}) of CdSe/ZnS QDs@SiO_2@peptide-AuNCs and the protein kinase A (PKA) concentration in a range of 0.01 to 40 U mL^{-1} with a detection limit of 0.004 U mL^{-1} (3σ) is obtained. The feasibility of this CdSe/ZnS QDs@SiO_2@peptide-AuNCs-based ratiometric sensor has been further demonstrated by assessment of kinase inhibition by ellagic acid in cell lysates. The IC_{50} value (inhibitor concentration producing 50% inhibition) for ellagic acid is estimated to be 0.08 μM. The detection of other enzymes can also be realized with precise design of the peptide substrate sequences. The present assay is universal and visual for kinase assay and promises potential application in kinase-related biochemical fundamental research and inhibitor screening.

1. Introduction

PKA (protein kinase A) is a kind of protein kinase, which can catalyze the phosphorylation of proteins. Phosphorylation is a process that plays an especially important role in metabolic pathways that regulate many cellular biological processes, including signal cell life termination, gene expression, and transduction [1,2]. Aberrations in the expression of PKA activity is closely related to many diseases including HIV [3], cardiac diseases, cancer [4] and diabetes. As a result, a sensitive, simple operation detection method of PKA plays an important role in discovering cancer-related phosphorylation, screening of new drugs and providing the information for the inchoate diagnosis of cancers. Up to now, there have been reported several methods for the detection of activity of PKA, such as colorimetric [5], fluorescent [6,7], electrochemical [8], radioactive and magnetic resonance imaging (MRI). With the advantages of diversified design, convenient operation, low sample volume and high-throughput capability, fluorescence technique has attracted extensive attentions in comparison with other assays. In our recent research, peptide-AuNCs were used as efficient optical probes in a label-free and antibody-free fluorescence assay, which were available for kinase activity detection by means of signal transition of Zr^{4+} [9]. The sensing approach was sensitive and simple but environmental conditions might influence the result. However, the background noise of the sample media could adversely affect the expected signal in these methods. Therefore, it still remains a great challenge to develop stable, accurate, simple and sensitive methods to detect protein kinase activity.

Ratiometric fluorescent sensors have attracted much attention recently due to their excellent advantages. Most traditional chemosensors respond to signal through the change of fluorescence intensities in fluorescence methods, which are easily influenced by other factors, such as the drift of light source or detector and environmental conditions in complex samples. Using the ratiometric fluorescent sensors, these problems can be solved well [10]. Gold nanoclusters have drawn great interest recently in the fields of biomedical imaging, sensors and catalysis due to the outstanding properties, such as well-defined molecular structure [11], strong photoluminescence [12,13], good biocompatibility [14] and optical chirality. Lu's group developed a ratiometric fluorescent method for the detection of hROS which used gold nanoclusters and organic dye@SiNP as the dual-emission fluorescent nanoparticles [15]. However, organic dyes suffer from a lot of known drawbacks,

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such as broad emission bands and weak photostability [16]. Hence, to establish high performance ratiometric fluorescent assays, one of the key issues is to design and prepare the stable, accurate and sensitive dual-emission fluorescent nanoparticles.

Herein, we developed a novel and versatile ratiometric fluorescent assay for monitoring the kinase activity based on carboxypeptidase Y (CPY) digestion by using Au nanoclusters and CdSe/ZnS QDs@SiO_2 as the dual-emission nanoparticles. This assay can eliminate the environmental interference by built-in correction. As shown in Scheme 1, a dual-emission fluorophore (QDs@SiO_2@peptide-AuNCs nanohybrids) is syntheized through a combination of the peptide-AuNCs (emitting blue fluorescence) and CdSe/ZnS QDs (emitting red fluorescence). In order to eliminate the environmental interference, the CdSe/ZnS QDs embedded in silica shells (QDs@SiO_2) are served as reference signals for built-in correction. A one-step peptide biomineralization process is applied to synthesize the peptide-AuNCs under mild conditions without any strong reducing agents. The fluorescence quenching of peptide-AuNCs of QDs@SiO_2@peptide-AuNCs nanohybrids (λ_{em} = 415 nm) occurs due to the consecutive exocleavage of peptide by CPY without peptide phosphorylation. And the fluorescence of CdSe/ZnS QDs@SiO_2 of QDs@SiO_2@peptide-AuNCs nanohybrids (λ_{em} = 630 nm) remains stable. On the contrary, when the protein kinase catalyzed the phosphorylation of the peptide at the amino residue, the phosphate group prevents the fluorescence quenching of the peptide-AuNCs and the fluorescence of QDs@SiO_2 is remained. Based on the change of the two fluorescence intensities ratio (I_{415}/I_{630}), the continuous color changes can be easily monitored using the UV lamp by the naked eyes. The activity of protein kinase can be facilely monitored with high sensitivity by ratiometric fluorescent method.

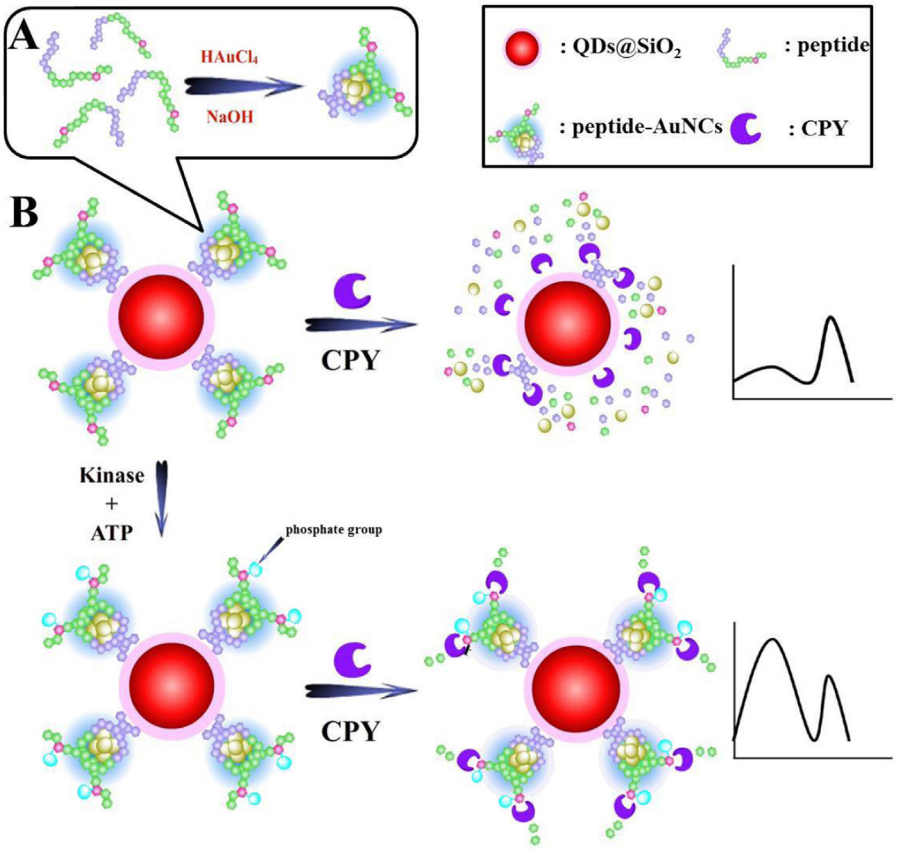
2. Experimental

2.1. Reagents and chemicals

PKA was obtained from New England Biolabs (UK). The carboxyl-functionalized CdSe/ZnS QDs@SiO_2 nanoparticles were purchased from Xintong Bio Technology Co., Ltd (Shanghai, China). The arginine-capped peptide Arg-Arg-Gly-Gly-Cys-Cys-Tyr-Gly-Gly-Ala-Leu-Arg-Arg-Ala-Ser-Leu-Gly (RRGGCCYGGALRRASLG) 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), 5,6-Dichloroben-zimidazole-l-β-D-ribofuranoside (DRB), NaOH, carboxypeptidase Y (CPY) were purchased from Sigma-Aldrich (USA). $HAuCl_4 \cdot 4H_2O$, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-flavone (quercetin), were 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.

2.2. 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 (RRGGCCYGGALRRASLG, 1.06 mM) in a glass vial with vigorous stirring. Then 8 μL 0.5 M NaOH was added within 30 s 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 [9].



Scheme 1. (A) Schematic illustration of the preparation of peptide-Au nanoclusters. (B) Schematic representation of ratiometric fluorescent assay for monitoring kinase activity based on carboxypeptidase Y (CPY) digestion by using Au nanoclusters and QDs@SiO_2 as the dual-emission nanoparticles.