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Time-resolved phosphorescent sensor array based on quantum dots for recognition of proteins

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ABSTRACT

The photoluminescence sensor array has attracted many researchers' attention because of highly sensitive response to protein analytes. Nevertheless, applying this technology to proteins detection suffers interferences from high short-lived autofluorescence emission and scattering light in real biological sample matrixes. To address this issue, a time-resolved phosphorescent (TRP) sensor array based on Mn-doped ZnS quantum dots (QDs) capped by different ligands was designed. The long lifetime of phosphorescence generated from QDs allows an appropriate time delay to effectively eliminate short-lived fluorescent and scattering light background. Thus, the signal-to-noise ratio of the detection and discrimination capability of the sensing system was improved distinctly for the analysis of proteins. Ten proteins at 500 nM in the buffer containing Rhodamine B (Rh B) were completely distinguished by using steady-state phosphorescent (SSP) measure, which were easily identified at the concentration as low as 10 nM by using TRP mode. The detection limit was 50-fold improved over that by using SSP assay. Most importantly, the identification ability of the same protein at the various concentrations was not sacrificed in the high fluorescent background using this sensing platform. Therefore, proteins spiked in human urine were successfully identified at 500 nM.

1. Introduction

The sensor array strategy often termed 'chemical nose/tongue' has received significant attention in recent years due to implications in important areas such as medical diagnostics, industrial product, and food security [1–4]. In this approach, an array of differential receptors generate the distinct response pattern that can be statistically analyzed and used for the discrimination of many target analytes. Generally, the sensing element consists of two components including the binding unit and the report transducer. The transducer translates the binding event into a readable signal (e.g. optical, electrochemical, and mass-sensitive) [5,6]. Particularly, the sensor array based on fluorescent transducer signal has been widely focused because of highly sensitive response to analytes. Recently, the various scaffolds based on the fluorescent array have been employed for discriminating many target analytes (protein, cell, tissue, bacteria, and so on) [7–11], including functionalized resins [12], graphene conjugated with fluorescent reporter molecules [13,14], porphyrin derivatives [15], polymers [16], complexes of gold nanoparticles with polymers/fluorescent proteins [17,18], quantum dots [19–24], and ionic liquid-quantum dots conjugates [25]. Unfortunately, for these fluorescent senor arrays, the sensitivity and discrimination ability of proteins is severely compromised by autofluorescence emission and scattering light, which limits practical application of these techniques for the real biological samples matrix. To avoid the autofluorescence and scattering light, some novel phosphorescence assays techniques have emerged. Among various luminescence materials, Mn-doped ZnS QDs have been regarded as a new promising class of nanophosphor and frequently used for biosensors and bioimaging applications [26–28]. Energy transfer from the band gap of ZnS to Mn^{2+} dopant and subsequent transition from the triplet state ($^4T_1$) to the ground state ($^6A_1$) of the Mn^{2+} incorporated into the ZnS host lattice, resulting in an orange phosphorescence emission (about 590 nm) [29]. It is worth noting that the longer wavelength belongs to room temperature phosphorescent emission. The phosphorescent signal transduction is considered to be excellent property for sensor

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due to larger Stokes shifts, longer excited-state lifetimes, and suitable excitation in the visible region in comparison to most purely organic fluorophores and quantum dots [30]. By using TRP technique, these phosphorescent features of QDs for optical sensing can effectively eliminate the short-lived background fluorescence and scattering light in biological samples by appropriate time delay, because the long-lifetime of phosphorescence allows an appropriate delay time to avoid the interferences from autofluorescence and scattering light [31–33]. TRP feature of Mn-doped ZnS QDs has been used for sensing many analytes [27,34–38], which concentrate on specific 'lock-and-key' recognition. Although these phosphorescent functional materials have shown potential and unique superiority, the sensor arrays based on QDs for analytes detection have been seldom explored using TRP technique to improve discrimination ability. In addition, the surface of QDs can be easily modified by many ligands with different groups [26,39–43]. Subtle changes of the surface properties of QDs can result in dramatic changes in their optical properties [44,45]. Therefore, these features are beneficial for the development of desired cross-receptors [25,46]. It is worthwhile to develop the time-resolved phosphorescent sensor array based on Mn-doped ZnS QDs for the detection of proteins and to further effectively utilize TRP measure for the lowest interference of the short-lived autofluorescence and scattering light in the complex biological matrix.

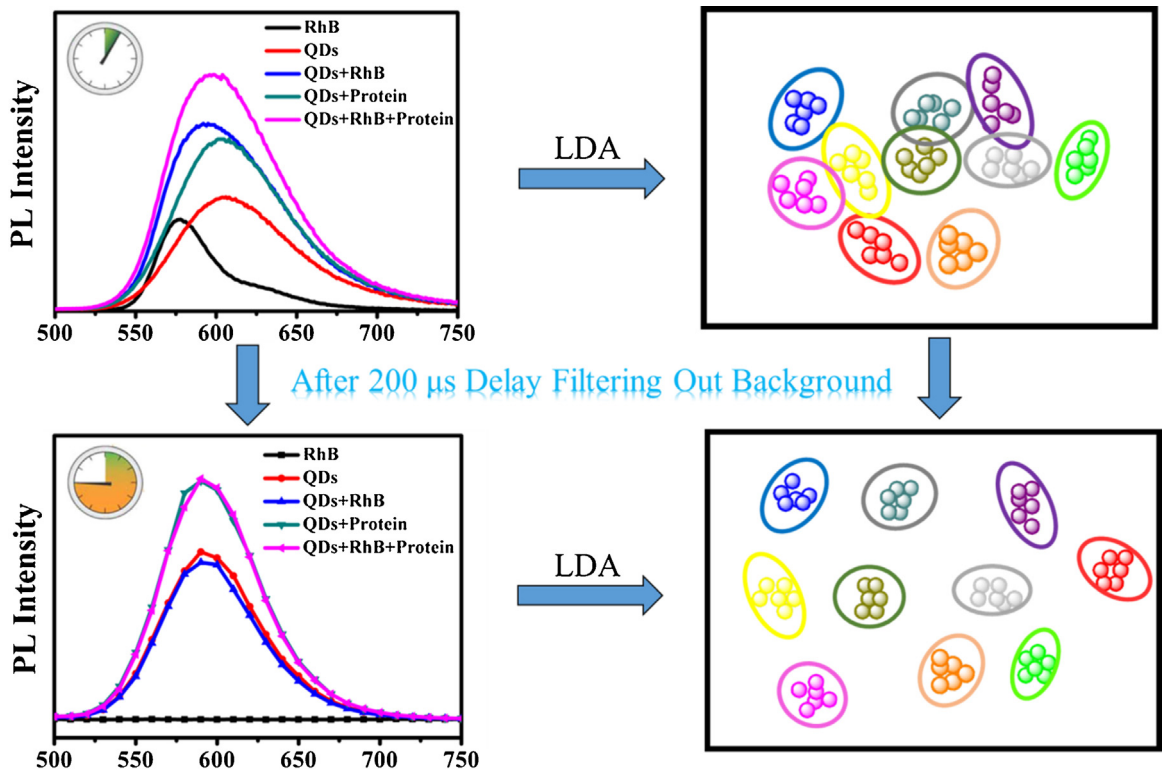
In the present work, we proposed TRP patter-sensing strategy for analysis of proteins in real samples. We designed and synthesized four kinds of Mn-doped ZnS QDs capped by different ligands as receptors for phosphorescent sensing of proteins (Scheme 1). The variation in the phosphorescent change could be employed as a fingerprint to accurately distinguish ten proteins. The sensitivity and discrimination ability for proteins was greatly improved in buffer containing Rhodamine B using TRP technique. Furthermore, this sensing platform facilitated simple and convenient approach to detect various proteins in complex biological and environmental samples since tedious sample pretreatment procedures can be avoided. Our work may also offer a new direction to enhance the discriminating efficiency of the sensor arrays.

2. Experimental

2.1. Materials and instrument

$ZnSO_4{\cdot}7H_2O$ and $Na_2S{\cdot}9H_2O$ were purchased from Tianjin Fuchen Chemical Reagent Factory. Mn $(CH_3COO)_2{\cdot}4H_2O$, 3-mercaptopropionic acid (MPA), *N*-acetyl-L-cysteine (NAC), L-glutathione (GSH), and alpha-thioglycerol (TG) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All reagents and chemicals were at least analytical reagent grade.

The steady-state phosphorescent (SSP) measure was carried out on a FLS-920 fluorescence spectrophotometer (Edinburgh, U.K.). Time-resolved phosphorescent (TRP) spectra were also performed through a time-correlated single photon counting (TCSPC) technique by using a FLS-920 spectrophotometer. Photoluminescent spectra at a delay time of 200 μs were chosen. The luminescence signal from 500 to 750 nm was collected at a step size of 10 nm. The slit widths of excitation and emission were 5 and 5 nm, respectively. The photoluminescence experiments were recorded while a 395 nm filter being inserted in front of the sample cell to avoid the interference of double frequency. The phosphorescence lifetime of as-prepared QDs was tested on FLS-920 spectrophotometer equipped with millisecond lamp. The luminescent quantum efficiency of QDs was determined using an integrating sphere (150 mm diameter) from FLS-920 spectrophotometer. The quantum yield can be defined as the integrated intensity of the luminescence signal divided by the integrated intensity of the absorption signal. The absorption intensity was calculated by subtracting the integrated intensity of the light source with the sample in the integrating sphere. The hydrodynamic sizes and zeta potentials were measured in a Malvern Zetasizer Nano-ZS90 analyzer (Malvern). The water



Scheme 1. Schematic illustration of the time-resolved phosphorescent sensor array based on QDs for the discrimination of protein.