Protein Sensors

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A Multidimensional Sensing Device for the Discrimination of Proteins Based on Manganese-Doped ZnS Quantum Dots**

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Multidimensional sensing devices have received increasing attention because they are able to read out the target analytes with more than one transduction channel and thus offer increased accuracy and/or diversity.[1] Generally, multidimensional sensing devices are constructed either by mechanically incorporating several different transducers (e.g. colorimetric, mass-sensitive, and capacitive) on a single-chip microsensor system (smart chip),[2] or by chemically integrating multiple reporter units (e.g. fluorescent, absorbing, and electrochemiluminescent) in a molecule (lab-on-a-molecule).[3–5] In the past few years, compounds enabling dual-,[3] triple-,[4] and quadruple-channel[5] sensing have been elegantly designed in a lab-on-a-molecule approach. Nanoparticles typically used in sensing applications also have the potential to give multidimensional sensory information, such as the dual-channel properties (conductivity and chemiluminescence) of SnO_2 nanoparticles that enable the discrimination of volatile organic compounds.[6] It is still a challenge to extract multidimensional information from a single material by utilizing different transduction principles to achieve high-order sensing.

Quantum dots (QDs) are robust inorganic chromophores that combine an efficient broadband absorption with a narrowband fluorescence spectrum. Particularly, doping the QDs with trace impurities enables an alteration or an increase of the number of emission centers.[7] Mn-doped ZnS (Mn–ZnS) QDs are the most studied doped QDs that exhibit both defect- and dopant-related emissions (Figure 1).[8] The defect-related emission leads to fluorescence (FL) in a wavelength range of 360–520 nm (Figure 1c) that originates from the band-gap transition of ZnS (Figure 1b) with a ns-scale lifetime (Figure S1 in the Supporting Information), whilst the dopant-related emission shows phosphorescence (Ph) in a wavelength range of 500–700 nm (Figure 1c) , which results from the triplet transition of Mn^{2+} ($^4T_1$-$^6A_1$) that is incorporated into the ZnS host lattice (Figure 1b) and has much longer decay times (ms-scale) than the FL (Figure S1). In addition, Mn–ZnS QDs are capable of scattering the incident light and the light scattering (LS) intensity can be greatly increased upon particle aggregation.[9a–c] The LS based on target-induced aggregation of nanoparticles is attractive for sensing.[9d–f]

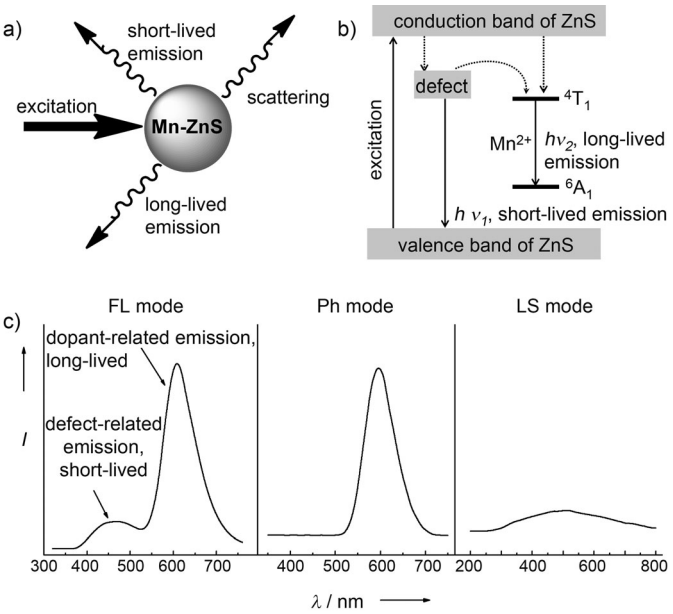

Figure 1. a) Schematic illustration of triple-channel optical properties of Mn–ZnS QDs; b) electronic transitions involved in the short- and long-lived emission of Mn–ZnS QDs; c) the fluorescence (λ_{ex} = 300 nm), phosphorescence (λ_{ex} = 300 nm), and light-scattering spectra of Mn–ZnS QDs.

Herein we report a multidimensional sensing device based on simultaneous utilization of the triple-channel optical properties (FL, Ph, LS) of Mn–ZnS QDs for the discrimination of proteins in a "lab-on-a-nanoparticle" approach. Large-scale proteomics projects require facile and robust detection of proteins in response to cellular stimuli.[10] Cross-reactive array-based sensing of proteins helps a great deal in such projects.[11] Recently, an enzyme-amplified array was developed for the sensing of proteins in urine, showing the potential of the method for diagnostic applications.[12] QDs provide versatile and sensitive scaffolds for the targeting of biomacromolecules that have sizes commensurate with proteins.[13] The complex non-specific interactions of proteins with QDs have been widely studied previously, but mostly with single signal transduction mode.[14] However, such a single signal transduction mode makes sensing/differentiation of proteins without specific functionalization of the QDs

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difficult. Here we explored the nonspecific interactions of Mn–ZnS QDs with proteins by reading out the triple channel optical properties of Mn–ZnS QDs to develop a sensor array for the discrimination of proteins. In a "lab-on-a-nanoparticle" manner, such a triple-channel sensing system can be considered as a virtual sensor array based on only one sensor unit but on multidimensional information from the sensor unit. Besides, monitoring the triple-channel optical properties can be easily achieved on a spectrofluorometer in different detection modes (Figure 1; Table S1), thereby greatly simplifying experimental procedures.

As a proof-of-concept system, we chose eight proteins that have diverse structural characteristics (i.e. metal/nonmetal-containing, molecular weight (MW), and isoelectric point (p*I*)) as the sensing targets (Table 1). For the sensing

Table 1: Basic properties of the target proteins.

Protein	Metal	pI	MW (kDa)
Cytochrome *c* (Cyt c)	Yes	10.7	12.3
Hemoglobin (Hb)	Yes	6.8	64.5
Human serum albumin (HSA)	No	5.2	69.4
Lysozyme (Lys)	No	11.0	14.4
Myoglobin (Mb)	Yes	7.2	17.0
Papain (Pap)	No	9.6	23.0
Transferrin (Tf)	Yes	9.6	80.0
Ovalbumin (Ob)	No	4.7	45.0

investigation, the intensities of the FL (440 nm), Ph (595 nm), and LS (500 nm) of mercaptopropionic acid-(MPA)-capped Mn–ZnS QDs (Figure S2) in PBS buffer (10 mM, pH 7.4) were recorded before and after addition of various concentrations of proteins (Figure S3-S10). The signal change constant (k) was defined as:

$$(I-I_0)/I_0 = k\,c \qquad (1)$$

where I and I_0 are the intensity of FL, Ph, or LS of Mn–ZnS QDs in the presence and absence of the target proteins, respectively, and c is the concentration of the target proteins (μM). Based on the calculated values of k, fingerprints for the proteins were generated (Figure 2).

All of the proteins studied enhanced the LS signal of Mn–ZnS QDs to various extents (Figure 2, S3–S10). Dynamic light scattering measurements demonstrated that the proteins induced aggregation of the Mn–ZnS QDs (Figure S3–S10) and the LS signals were well correlated to the hydrodynamic diameter of Mn–ZnS QDs in the presence of various proteins (Figure S11).

The spectroscopic response patterns obtained from the FL and Ph of Mn–ZnS QDs could categorize the eight target proteins into three groups (Figure 2). The first group contains cytochrome c (Cyt c), myoglobin (Mb), and hemoglobin (Hb), all of which quenched both the FL and the Ph of Mn–ZnS QDs. All three metalloproteins contain the Fe heme, which is an effective electron-transfer quencher for CdSe@ZnS QDs.[15] The FL and Ph lifetimes of Mn–ZnS QDs were greatly shortened in the presence of these proteins (Figure S5–S7), thus the quenching mechanism became

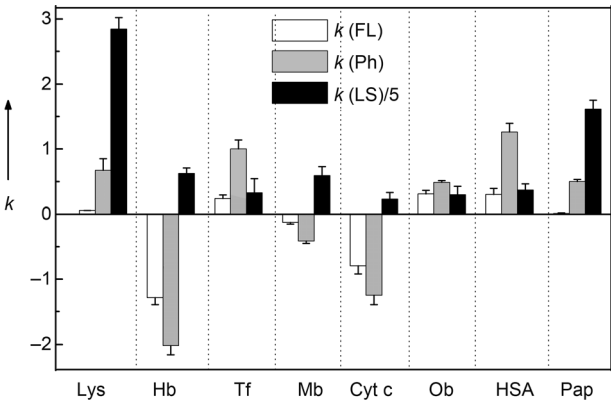

Figure 2. Fingerprints of eight selected proteins based on the patterns of the corresponding values of k obtained from the FL, Ph, and LS of Mn–ZnS QDs.

possible through electron transfer and the quenching differences depended on the structure diversity of these proteins.[16] Human serum albumin (HSA), transferrin (Tf), and ovalbumin (Ob) enhanced both the FL and the Ph of Mn–ZnS QDs, and were categorized as the second group. Previous studies revealed that HSA or Ob could enhance the fluorescence of CdTe QDs because of the diminishing of surface defects.[17] The FL and Ph lifetime of Mn–ZnS QDs increased somewhat in the presence of HSA, Tf, and Ob (Figure S8–S10). We thus speculated that the interactions of HSA, Tf, and Ob with Mn–ZnS QDs also resulted in a decrease of surface defects, thereby enhancing the FL and Ph intensity. Lysozyme (Lys) and papain (Pap) belonged to the third group, which effectively increased the Ph of Mn–ZnS QDs, but led to minimal variations of the FL. Particularly, the LS signal of Mn–ZnS QDs in the presence of Lys and Pap was obviously larger than that in the presence of other proteins (Figure 2). Highly aggregated Mn–ZnS QDs were reported to give an improved Ph signal.[18] Thus, the Ph increment of Mn–ZnS QDs in the presence of Lys and Pap exceeded the FL change.

To expose the fingerprints more clearly, the triple-channel response patterns were subjected to principal component analysis (PCA; 3 channels × 8 proteins × 6 replicates; Figure 3).[19] At a protein concentration of 0.5 μM, PCA demon-

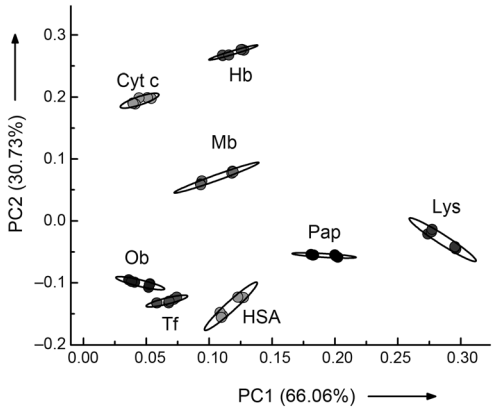

Figure 3. PCA plot for the discrimination of eight proteins (0.5 μM) based on the triple-channel optical properties of Mn–ZnS QDs.

strates that the canonical response patterns of the proteins to the triple-channel properties of Mn–ZnS QDs were clearly clustered into eight groups that correspond to each specific protein (95% confidence ellipses), thus showing the capability of our multidimensional sensing device based on the triple-channel optical properties of Mn–ZnS QDs to discriminate proteins. Further PCA examination of the triple-channel response data at various protein concentrations (i.e. 0.25, 0.5, 0.75, 1.0, and 1.25 μM) reveals that all eight proteins were discriminated at concentrations no less than 0.5 μM (Figure 3 and S12). The robustness of the developed triple-channel sensing device was tested using unknown samples randomly taken from the training set. The identification accuracy of the unknown samples was found to be 93.8% (75 out of 80) at the 0.5 μM level, and 96.3% (77 out of 80) at the 0.75 μM level (Tables S2–S4).

The performance of our triple-channel sensing device was further demonstrated for the discrimination of proteins in the presence of human urine. For this purpose, the triple-channel sensing device was applied to PBS buffer (control), urine, and urine samples with added physiologically relevant proteins (0.5 μM each). As shown in Figure 4, the urine behaved

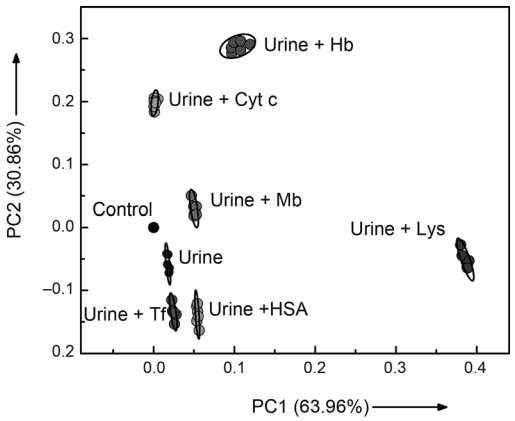

Figure 4. PCA plot for the discrimination of physiologically relevant proteins (0.5 μM) in the presence of human urine based on the triple-channel optical properties of Mn–ZnS QDs.

differently with the control sample, possibly because of the proteins present in urine. Although human urine with its protein and electrolyte content and multianalyte nature generates a complex matrix that is challenging for the identification of proteins, each of the physiologically relevant proteins present in the urine sample still generated distinct responses, thus demonstrating that the developed triple-channel sensing device also offers great potential for the discrimination of proteins in a biological matrix.

In summary, we have reported the simultaneous exploration of the FL, Ph, and LS properties of Mn–ZnS QDs as a multidimensional sensing device for the discrimination of proteins. The changes in such triple-channel optical properties of Mn–ZnS QDs after interaction with proteins lead to distinct patterns related to each specific protein, which can be incorporated into a new protein sensor array with a single sensor unit in a "lab-on-a-nanoparticle" manner. Application of PCA to the triple-channel optical properties of Mn–ZnS QDs allows the identification of proteins even in the presence of a biological matrix in a rapid and efficient fashion. We regard this QD-based triple-channel sensing system as a preliminary step for further exploration and application of the lab-on-a-nanoparticle concept.

Experimental Section

All reagents used were of analytical grade. Mercaptopropionic acid (MPA) (Aladin, Shanghai, China), $ZnSO_4 \cdot 7H_2O$, $Mn(Ac)_2 \cdot 4H_2O$ and $Na_2S \cdot 9H_2O$ (Tianjing Guangfu Chemical Co., Tianjing, China) were used for the preparation of Mn-doped ZnS QDs. All proteins used in this work were purchased from Newprobe Biotechnology Co. (Beijing, China). Ultrapure water (18.2 MΩ cm) was obtained from a WaterPro water purification system (Labconco Corporation, Kansas City, U.S.).

MPA-capped Mn–ZnS QDs were prepared based on a previous publication with minor modifications.[20] In brief, an aqueous solution of $ZnSO_4$ (7.5 mL, 0.1M), $Mn(Ac)_2$ (0.1 mL, 0.1M), and MPA (0.26 mL) was added to a three-necked flask. The mixed solution was filled up to 50 mL with ultrapure water, and was adjusted to pH 10 with 1M NaOH. After the removal of air by argon bubbling for 30 min at room temperature, Na_2S (7.5 mL, 0.1M) was quickly injected into the solution. The mixture was stirred for 20 min, and then stirred at 50°C for 2 h to form MPA-capped Mn–ZnS QDs. For purification, the obtained QDs were precipitated with ethanol, separated by centrifuging, washed with ethanol, and dried in vacuum. The quantum yield of as-prepared Mn–ZnS QDs was 8.2%, as determined with a PTI QM/TM/NIR spectrometer (Birmingham, U.S.). The weight contents of Zn and Mn in the as-prepared Mn–ZnS QDs were determined by flame atomic absorption spectrometry to be 47.4% and 0.42%, respectively.

FL, Ph, and SL measurements were performed on an F-4500 spectrofluorometer (Hitachi, Japan) equipped with a plotter unit and a quartz cell (1 cm × 1 cm) under the conditions listed in Table S1. In order to eliminate the potential interference of the SL on the FL intensity, a long-pass filter (> 380 nm) was positioned in front of the emission window in the FL mode. PBS buffer (pH 7.4, 1 mL, 0.1M), Mn–ZnS QDs (45 μL, 10 mg mL^{-1}) and various amounts of proteins (250 μM in 0.01M PBS buffer) were mixed, and diluted to a total volume of 10 mL with ultrapure water. The solution was mixed thoroughly, and was left to stand for 10 min to measure the FL, Ph, and LS signal intensity. All measurements were repeated to generate six replicates of each protein, so that for a given concentration, a 3 channels × 8 proteins × 6 replicates data matrix could be generated. The raw data were first normalized $[(I-I_0)/I_0]$ to eliminate the potential bias caused by the difference in the original signal intensity of Mn–ZnS QDs. Then, the data was processed using PCA in Matlab (The MathWorks Inc., U.S.).

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