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Quantum Dots for Monitoring Choline Consumption Process of Living Cells via an Electrostatic Force-Mediated Energy Transfer

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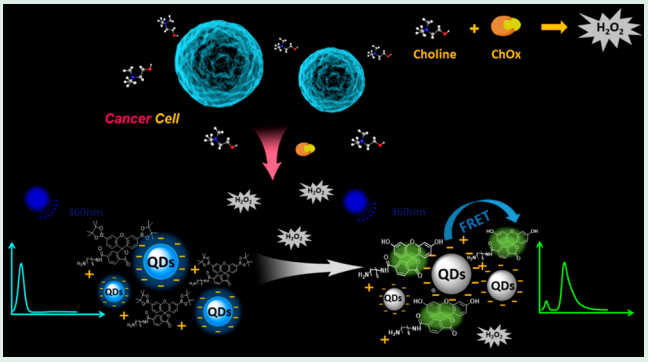
Supporting Information

ABSTRACT: In this work, a ratiometric nanoprobe CdS/ZnS-FB was designed for H_2O_2 detection based on FRET assay. Furthermore, CdS/ZnS-FB could work for detecting choline (Ch) and acetylcholine (ACh) since H_2O_2 is the enzyme cascade reaction product. Significantly, the Jurkat T's choline consumption could also be quantitatively measured by monitoring FRET ratio (I_{522}/I_{426}). Thus, the biosensor could be applied as a universal tool for the detection of choline consumption of living cells, which provides a good potential for the applications in detecting chemical transmitter and cancer diagnosis.



KEYWORDS: *quantum dots, FRET, H_2O_2, enzyme cascade reaction, living cells*

Semiconductor quantum dots (QDs) with excellent photophysical properties have emerged as a novel class of fluorophore within recent decades because of their broad absorbance range, large molar absorption coeffcient, narrow size-tunable emission, and unique photochemical stability.[1−3] With the aid of these advantages, QDs as donors or acceptors have received more and more research interest in Förster resonance energy transfer (FRET) processes.[4,5] Recently, QDs have been widely applied in the analysis of biological components including delivery agents,[6,7] antibodies,[8] cellular proteins,[9−11] or DNA,[12] and enzyme sensing[13,14] or cell imaging.[15−18] The superior performances of QDs in the related sensing process via FRET have motivated scientists to greatly improve and expand application in biosensor field.[19]

Choline, as an indispensable intermediate in the various activities of life, is involved in constructing cell membranes and adjusting insulin level.[20] In addition, the abnormal choline level may cause a series of diseases, such as Parkinson's, Alzheimer's disease, and other neurological related diseases.[21] Hence, developing a sensitive sensing strategy is urgently wanted to detect the choline in serving disease diagnosis and therapy or regulations of life process. Up to now, there are some methods such as the chemiluminescence, coupled-enzymatic spectrophotometric method,[22] colorimetric assays,[23] liquid chromatography,[24,25] and electrochemistry[26,27] that have been employed to detect choline. Among these methods, the couple-enzymatic reaction displayed better sensitivity due to the enzymatic cascade amplification. Inspired by this, our group previously reported a conjugated polymer material to detect the choline and acetylcholine by detecting H_2O_2,[28] a final product of an enzymatic cascade reaction where choline and acetylcholine are the start materials. However, the synthetic route of the conjugated polymer was complicated. Herein, we presented a novel electrostatic complex composed of anionic QDs (CdS/ZnS) and cationic boronate-protected fluorescein (FB), which could be easily acquired by simply mixed the two oppositely charged components and avoided the sophisticated synthesis.

In this work, a sensitive, rapid and selective approach was designed to detect choline by QDs-based FRET system. Scheme 1a depicted the principle of the electrostatic force mediated ratiometric fluorescent assay for H_2O_2. We presented a new electrostatic complex composed of anionic CdS/ZnS and cationic FB. Through electrostatic interaction, CdS/ZnS and FB were close to each other, which favored the process of energy transfer. Without H_2O_2, FB is stable, the emission of fluorescein is blocked due to the boronate protecting groups, and CdS/ZnS-FB shows blue fluorescence only. In the presence of H_2O_2, FB could be transformed into green fluorescent fluorescein due to the hydrolysis of boronate ester group caused by H_2O_2, and the corresponding fluorescence changes from blue to green. By this mechanism, not only the

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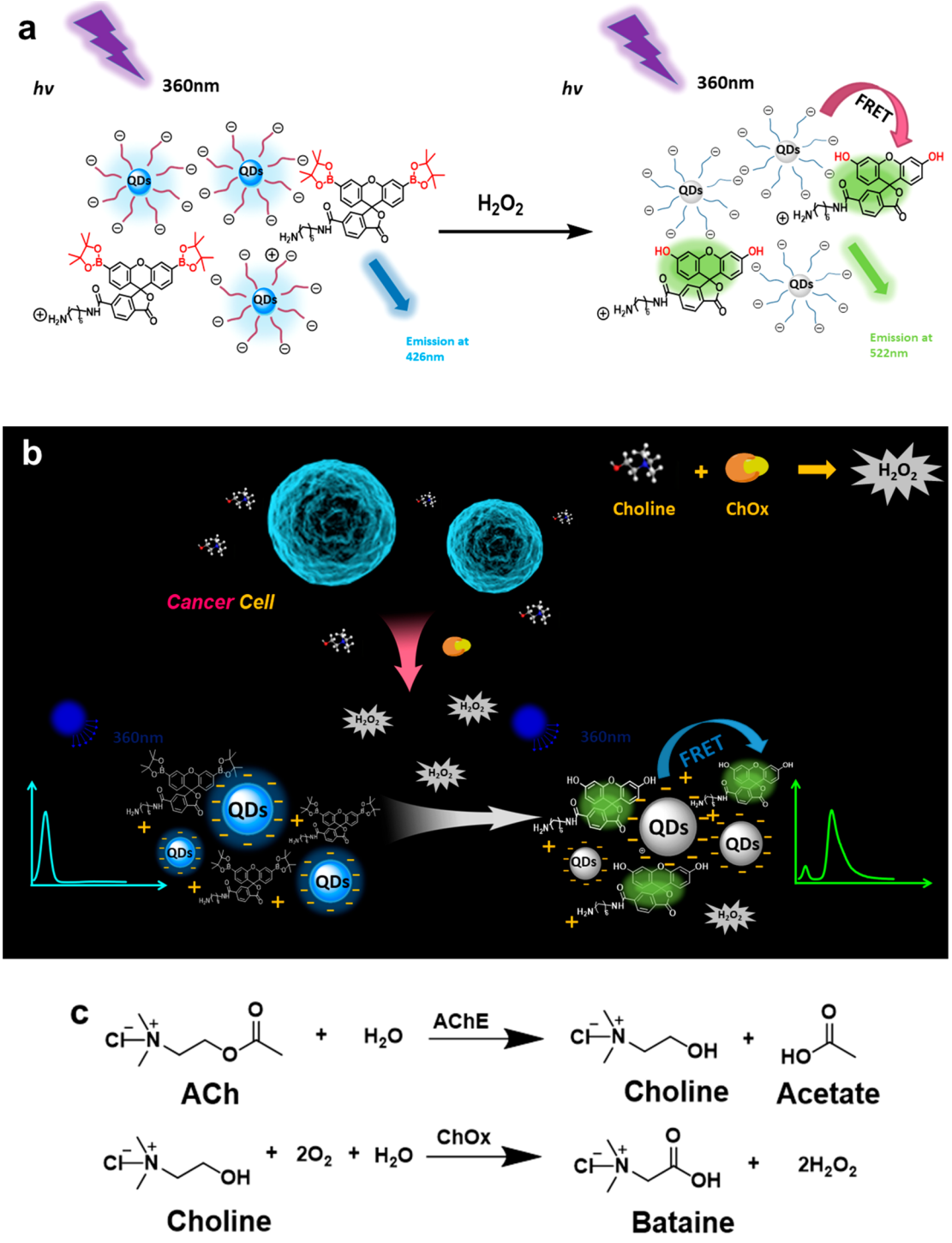
Scheme 1. (a) Reaction of CdS/ZnS-FB with H_2O_2; (b) Illustration for Ratiometric Fluorescence Assay Using CdS/ZnS-FB To Detect Living Cells' Choline Consumption Process; (c) AChE/ChOx Enzyme Cascade Reaction



H_2O_2 itself but also the enzymatic and chemical reactions where H_2O_2 was the final product could be monitored. As shown in Scheme 1b, to meet the needs of cell growth, the cell culture medium is usually equipped with a certain amount of choline. As choline oxidase (ChOx) could catalyze choline to generate betaine aldehyde and H_2O_2,[29,30] a novel biosensing platform was developed to detect choline, which successfully realized the dynamic monitoring of cancer cells' choline consumption process.

The corresponding spectral properties of CdS/ZnS-FB were tested in aqueous solution. As exhibited in Figure 1a, an efficient spectral overlap between the emission spectra of CdS/ZnS (from 400 to 450 nm) and the absorption spectra of fluorescein (from 400 to 550 nm) was observed, which was prerequisite for FRET phenomenon. Furthermore, the strong electrostatic interaction significantly shorted the distance between anionic CdS/ZnS and cationic FB, which favored the FRET process (Figure S3). To demonstrate the coupling of the FB with CdS/ZnS via electrostatic interaction, the zeta

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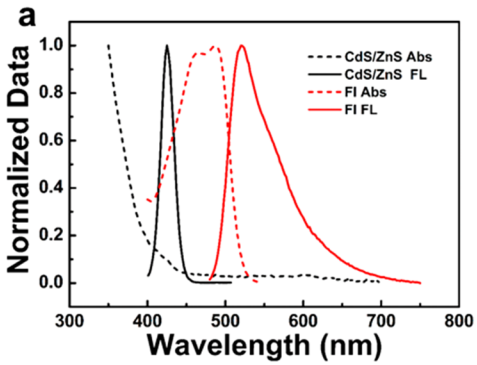

b

Samples	Size (nm)	Zeta potentials (ζ mv)
FB	44.0±2.3	14.2±4.3
CdS/ZnS	14.4±2.0	-18.8±5.0
CdS/ZnS-FB	72.0±1.3	-7.7±0.1

Figure 1. (a) Normalized absorption as well as fluorescence spectra of CdS/ZnS and FI in aqueous solution. (λ_{ex} = 360 nm and λ_{ex} = 480 nm, respectively). (b) Zeta potentials and size for FB, CdS/ZnS, and CdS/ZnS-FB.

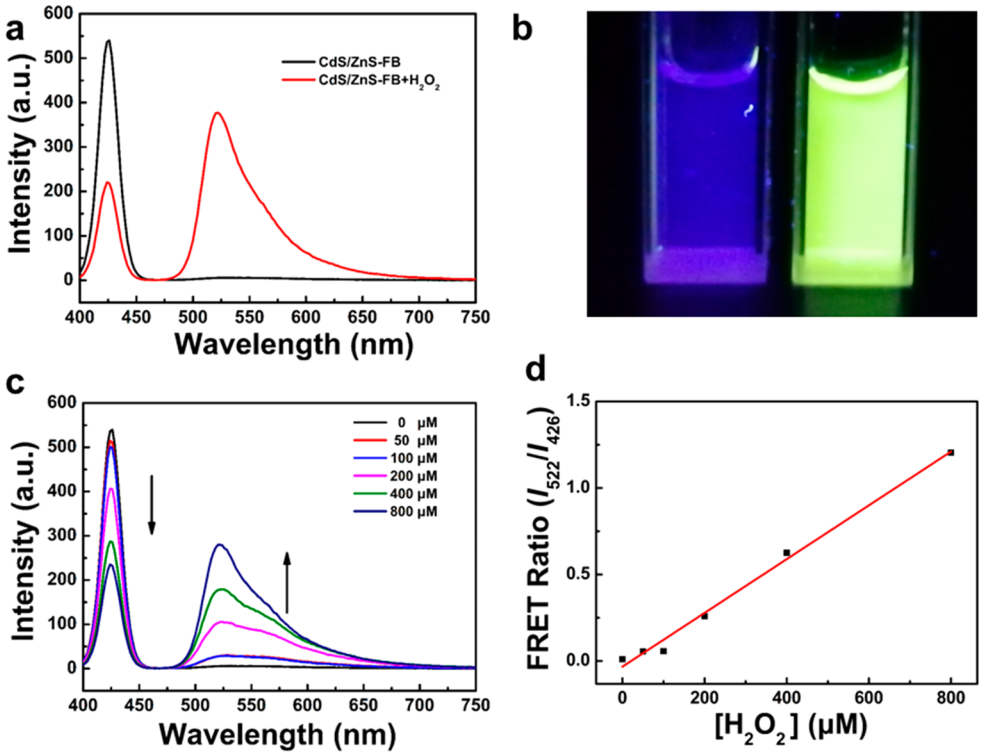

Figure 2. (a) Fluorescence spectra change of CdS/ZnS-FB with and without treatment of H_2O_2. (b) Color change of CdS/ZnS-FB solution when treated with H_2O_2 under UV light (λ_{ex} = 365 nm). (c) CdS/ZnS-FB treated with various concentrations of H_2O_2. (d) Curve of relationship between the value of I_{522}/I_{426} and concentration of H_2O_2.

potential of CdS/ZnS-FB was measured in PBS. Figure 1b and Figure S2 exhibited that the FB modified with PEG-NH_2 was positively charged (14.2 ± 4.3 mV); negatively charged CdS/ZnS (−18.8 ± 5 mV) was designed to improve water solubility. Meanwhile, CdS/ZnS and FB exhibited sizes of 14.4 and 44.0 nm, respectively. The CdS/ZnS-FB complex displayed a size of 72.0 nm and zeta potential of −7.73 ± 0.1 mV.

The average diameter increased and zeta potential of CdS/ZnS-FB sharply deceased owing to the neutralization interaction of opposite charges, and transmission electron microscopy (TEM) (Figure S1) also demonstrated that formation of the CdS/ZnS-FB complex.

To prove the feasibility of the sensing strategy, we studied the optical response of CdS/ZnS-FB to H_2O_2. The spectra of CdS/ZnS-FB only showed the emission peak at 426 nm by 360 nm excitation (Figure 2a). However, after treated with H_2O_2, the emission peak at 426 nm gradually reduced at the same time the emission peak at 522 nm arose, indicating H_2O_2 significantly changed the FB transform into FI. Meanwhile, the fluorescence color of the sensing system correspondingly changed from blue to green when treated with H_2O_2 under a UV lamp, realizing a visual detection (Figure 2b). As shown in Figure 2c, the spectra of CdS/ZnS-FB change under various concentrations of H_2O_2. Accompanied by increasing the H_2O_2 concentration, the emission peak at 426 nm reduced, while that emission peak at 522 nm significantly improved. As shown in Figure 2d, the linear change (R^2 = 0.991) between the FRET ratio (I_{522}/I_{426}) of CdS/ZnS-FB and H_2O_2 concentration indicated that CdS/ZnS-FB could be employed as a H_2O_2-sensitive probe. Moreover, the change of the FRET ratio (I_{522}/I_{426}) with the incubation time of H_2O_2 from 0 to 6 h is shown in Figure S4.

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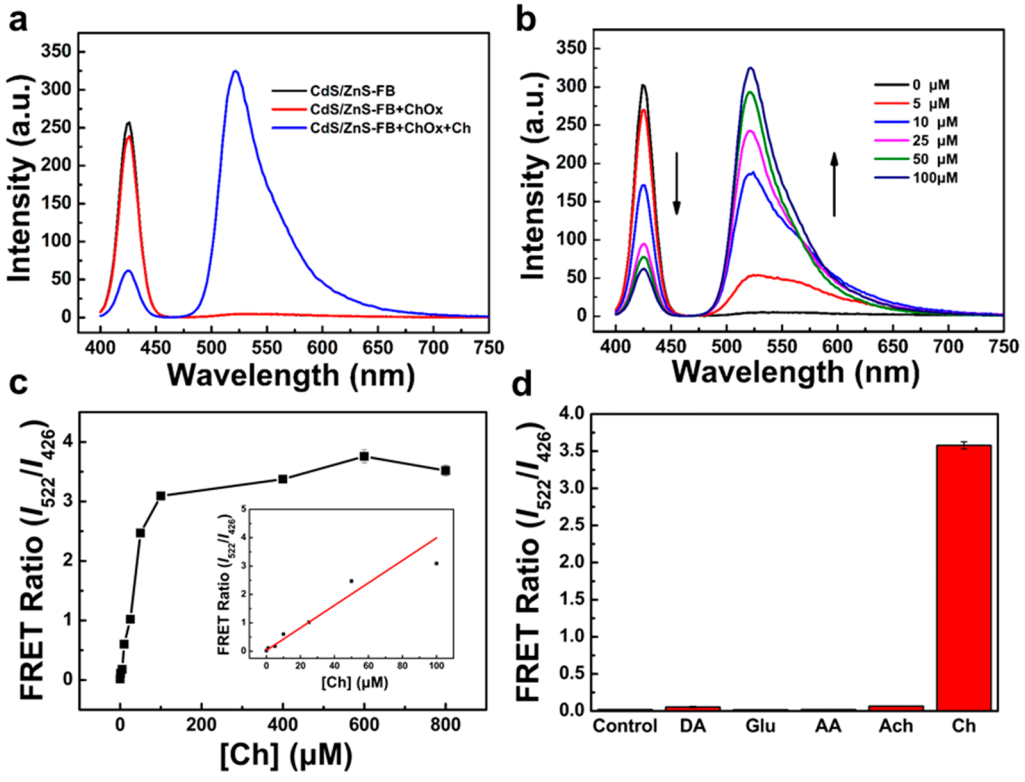

Figure 3. (a) Fluorescence spectra change of CdS/ZnS-FB/ChOx after treated with choline. (b) Fluorescence spectra change CdS/ZnS-FB/ChOx treated with various choline concentrations. (c) Value of FRET ratio (I_{522}/I_{426}) increased with the increase of the choline concentration. The inset demonstrates the corresponding linear relationship between the value of I_{522}/I_{426} and concentration of choline. (d) Selectivity of CdS/ZnS-FB probe against various analytes.

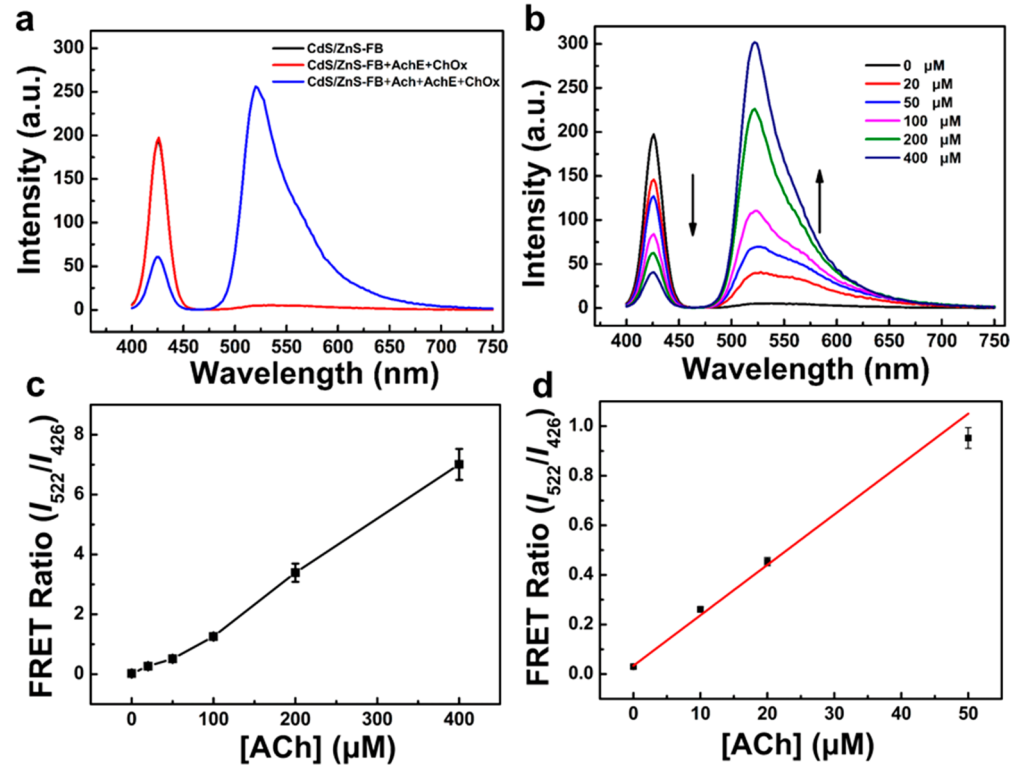

Figure 4. (a) Fluorescence spectra change of CdS/ZnS-FB/AChE/ChOx after treated with ACh. (b) Fluorescence spectra obtained from various concentrations of ACh. (c) Value of FRET ratio (I_{522}/I_{426}) as a function of the acetylcholine concentration. (d) Relationship between the value of I_{522}/I_{426} and acetylcholine concentration.

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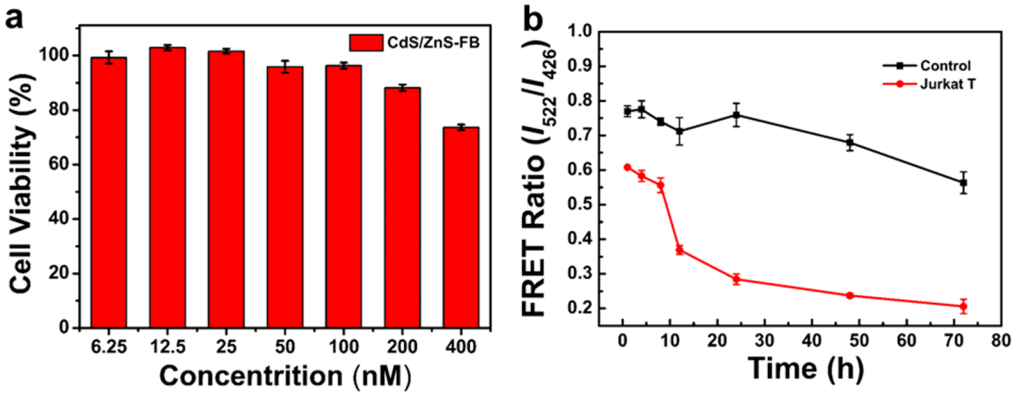

Figure 5. (a) Jurkat T cells viability against various CdS/ZnS-FB concentrations. CdS/ZnS-FB was made by the ratio of 1:33.6 (CdS/ZnSFB). (b) Change of FRET ratio (I_{522} /I_{426}) of CdS/ZnS-FB probe to detect the Jurkat T's choline consumption.

To verify the application of CdS/ZnS-FB in biosensors, an enzyme reaction model that produced H_2O_2 was constructed, and corresponding enzymes could catalyze many substrates. Because ChOx could catalyze choline to produce H_2O_2 and betaine aldehyde, H_2O_2-sensitive CdS/ZnS-FB probe combined with ChOx was further studied based on this strategy to detect choline. Physiological condition (pH 7.4, 37 °C) was chosen as the optimal reaction condition to favor the enzyme reaction. As shown in Figure 3a, with the catalysis of ChOx, adding the choline and ChOx to the CdS/ZnS-FB solution could lead to efficient FRET from CdS/ZnS to FI while ChOx itself could not. These observations demonstrated ChOx could result in a desired FRET phenomenon. Moreover, we studied the relationship between the spectral change of CdS/ZnS-FB and the amount of choline. Figure 3b showed the change of emission spectra of CdS/ZnS-FB when treated with different concentrations of choline. With the increasing concentrations of choline, the fluorescence emission intensity of FI at 522 nm was increased, while that of CdS/ZnS at 426 nm decreased. As shown in Figure 3c, the value of FRET ratio (I_{522}/I_{426}) gradually increased with the increase of choline concentration. Accompanied by increasing the choline concentration, the FRET ratio gradually increased and then achieved a platform at 100 μM. The inset of Figure 3c showed that there was a good linearity relationship between FRET ratio (I_{522}/I_{426}) and the choline concentration (from 0 to 100 μM) with a correlation coefficient of 0.979. Because the choline concentration in blood was usually found as 10.8 μM, CdS/ZnS-FB could be used for the detection of choline in biological samples. To demonstrate the specificity of the proposed method, we chose four possible interfering agents (dopamine, glucose, acetylcholine, and aspartic acid) that are usually found in cell culture environment for the selectivity studies (Figure 3d). Because of the specificity of the enzyme, CdS/ZnS-FB showed slight FRET response to these four substrates, suggesting that the CdS/ZnS-FB detection system had a good specificity to choline.

To further explore the enzymatic reaction of CdS/ZnS-FB with coupled multiple enzymes in bioassays, AChE and ChOx were selected as the conceptual models to detect ACh. Figure 4a shows the fluorescence spectra change of CdS/ZnS-FB with AChE/ChOx and Ach/AChE/ChOx, respectively. AChE catalyzed hydrolysis of ACh to generate choline, and then ChOx furtheroxidized choline to produce H_2O_2, resulting in efficient FRET response in CdS/ZnS-FB (Scheme 1c). Noted that in the absence of ACh, AChE/ChOx alone could not facilitate the FRET process between CdS/ZnS and FB. Figure 4b shows the fluorescence spectra change of CdS/ZnS-FB with the increasing concentration of Ach. Upon addition of Ach, the emission peak at 426 nm gradually decreased, while the peak at 522 nm dramatically increased. As shown in Figure 4c, the FRET ratio (I_{522} /I_{426}) gradually increased with the increase of the acetylcholine concentration, and the curve shows a good linear relationship (R^2 = 0.991) (Figure 4d). Therefore, with the aid of AChE/ChOx, CdS/ZnS-FB showed a good ability to detect ACh. Thus, we successfully realized the detection of raw material based on coupled multiple enzymes.

As an important molecule, choline is indispensable to the important physiological processes of living cells. On the basis of the method established above, this hybrid material is expected to be applied to detect the choline consumption of cells. To demonstrate this strategy, we investigated the biocompatibility of CdS/ZnS-FB, and the cell viability test was conducted by a traditional CCK8 assay. The cell viability by incubating Jurkat T cells with CdS/ZnS-FB for 24 h was examined. As shown in Figure 5a, the cell viability slightly decreased as the CdS/ZnS-FB concentration increased. The cell viability was still at about 80% when the concentration of CdS/ZnS-FB probe reached to 200 nM. Hence, CdS/ZnS-FB basically showed low cytotoxicity and served as a cell-friendly nanoparticle. The change of the FRET ratio (I_{522}/I_{426}) as a function of incubation time up to 72 h for the Jurkat T cells is shown in Figure 5b. The FRET ratio gradually decreased in a culture medium with cells, while the FRET ratio in the medium without cells exhibited a slight variation. These results confirmed that the proposed CdS/ZnS-FB probe could detect the choline consumption process of cells dynamically.

In summary, we developed a highly efficient FRET system based on ratiometric probe to detect H_2O_2. Combined with enzyme reaction, the CdS/ZnS-FB probe could realize the dynamic monitoring of the living cells' choline consumption process. This work brings forth three original ideas as follows: (i) the original electrostatic complex of quantum dots and FB was used in H_2O_2 detection; (ii) this complex also has the advantage of facile preparation process over other methods; (iii) this complex was further used in a coupled enzymatic reaction enriching the application of enzymatic biosensors. This work not only provides a simple and effective way to detect H_2O_2-produced by enzyme cascade reaction but also has ability to detect choline consumption of cells, which exhibits a promising potential in biomedical research and early clinical analysis or diagnoses of cancer.

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■ ASSOCIATED CONTENT

Supporting Information

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Materials and instruments; detailed experimental procedures (PDF)

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Author Contributions

H.Y., J.B., L.L., and S.W. designed experiments, analyzed data, and organized the manuscript. H.Z., K.P., and F.L. analyzed data.

Notes

The authors declare no competing financial interest.

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