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Fabrication of a Tyrosine-Responsive Liquid Quantum Dots Based Biosensor through Host−Guest Chemistry

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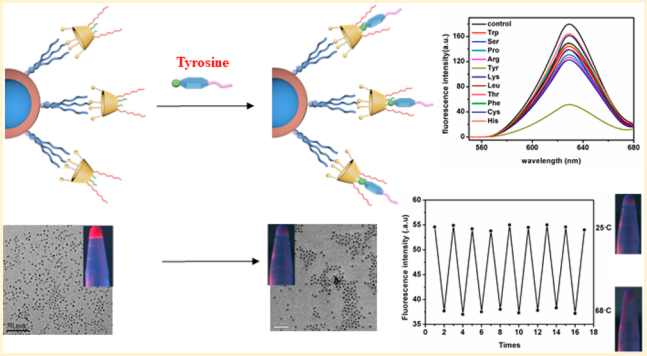
Supporting Information

ABSTRACT: Design and fabrication of smart liquid quantum dots (LQDs) with high biomolecule selectivity and specificity remains a challenge. Herein, a multifunctional calix[4]arene derivative (PCAD) was rationally designed and applied to fabricate a Tyr-responsive CdSe-LQD system through host−guest chemistry. Such a biosensor displays an outstanding fluorescence/macroscopic response for Tyr and reversible fluidic features due to the hydrogen interaction between the PCAD of CdSe-LQDs and Tyr. These excellent results highlighted CdSe-LQDs as a promising platform for biological molecule recognition and separation in the future.



Tyrosine (Tyr) is a well-known aromatic building block in proteins for the regulation of signal transportation.[1,2] As a precursor of dopamine and melanin, Tyr is widely distributed in many living organisms and always applied to keep the balance of nutrition for living subject.[3−5] Numerous studies indicated that several diseases such as Parkinson's has close relations with the deficiency of Tyr.[6,7] In contrast, a high level of Tyr in vivo often leads to diabetes mellitus and several kinds of cancers.[8,9] Therefore, the detection of Tyr levels is critical for early detection and treatment of diseases. Up to now, the traditional techniques for the determination of Tyr such as chromatography and electrophoresis methods suffer from either expensive equipment/reagent or time-consuming preparation.[10−13] Thus, developing a more facile and reliable strategy for the detection of Tyr levels with high sensitivity and reduced cost is crucial and highly demanded.

In the past decades, liquid type quantum dots (LQDs) with unique chemical/physical and optical features have attracted increasing interest from broad scientific fields.[14−20] Beyond the traditional QDs, LQDs demonstrated unique liquid-like behavior and fluidic properties, which could greatly expand the applications of LQDs in heat-transfer fluids, fluorescent sensors, and microfluidic sensors.[21−28] Despite these pioneering results, the research on the design of smart LQDs in response to the external stimuli for biosensor applications is still in its infancy.[29,30] Calixarenes, well-known cavity-adjustable, and rim-modified host scaffolds, have been widely incorporated into the surfaces of various materials to endow them a specific response to external stimuli including pH, molecules, and ions.[31−37] For example, Pochini et al. fabricated a pyridinium-responsive device based on a dihydroxycalix[4]-arene-modified Au surface, demonstrating remarkable specific and ultrasensitive recognition of pyridinium.[38] More recently, our group constructed a photoresponsive biosensor through chiral azo-calix[4]arene-modified silicon materials.[39] From both the results of fluorescence and wettability, such a biosensor showed high sensitivity/selectivity and excellent reversibility toward (1*R*,2*S*)-1-amino-2-indanol. However, the utilization of calixarene to regulate the fluorescence and fluidic properties of LCQs in the response to a specific biological molecule such as an amino acid is still rarely reported, so this work will greatly expand the applications of LCQs in biomolecule sensing and separation.

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Herein, we developed a highly fluorescent and selectively Tyr-responsive LCQ system based on PEGylated calix[4]arene derivatives (PCAD) incorporated onto the surfaces of CdSe QDs, which could selectively respond to Tyr through host–guest chemistry (Figure 1). In this Tyr-responsive system,

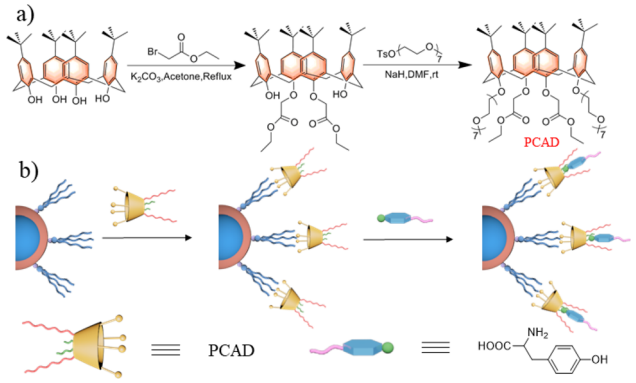

Figure 1. (a) The synthetic scheme of PCAD. (b) A schematic illustration to show the PCAD coated on the CdSe-QDs to produce CdSe-LQDs and its responses to tyrosine.

calix[4]arene derivatives were designed in terms of the following factors. First, calix[4]arene could be facilely assembled onto the surfaces of CdSe QDs via the hydrophobic interaction between their lower rim of *tert*-butyl groups and the aliphatic chains of QDs. Furthermore, the PEG chain modified on the upper rims of calix[4]arene could endow CdSe QDs with soft organic shells and a further tunable fluidic feature. Finally, the ester group and PEG chain of the upper rims of calix[4]arene provide the potential recognition sites for Tyr. This Tyr-responsive QDs nanofluid opens a way to design smart fluidic materials for biological molecule determination and separation.

EXPERIMENTAL SECTION

Synthesis and Characterization of PEGylated Calix[4]arene Derivatives (PCAD). PEGylated calix[4]arene derivatives (PCADs) were synthesized from *tert*-butylcalix[4]-arene starting materials by several steps (Figure 1a, 22% overall yield, see Supporting Information). The desired product (PCAD) was achieved after silica gel purification as a yellow oil and characterized by NMR (Figure S1). In order to endow PCAD with multiple functions, the upper rims of PCAD were asymmetrically modified with both ester and PEG molecules.

Synthesis and Characterization of CdSe-QDs. CdSe-QDs were synthesized according to the previously reported procedures with little modification.[40] The obtained OA-stabilized CdSe-QDs were dispersed in chloroform and stored at 4 °C for further use. The as-synthesized fluorescence emission spectra of CdSe-QDs were recorded on an Applied NanoFluorescence Spectrometer.

Preparation and Characterization of the PCAD-Modified CdSe-QD Nanofluids. The as-synthesized calix[4]arene derivative PCAD was coated onto the surfaces of OA-stabilized CdSe-QDs to obtain CdSe-LQDs through the hydrophobic interaction between the OA chains and *tert*-butyl groups of PCAD (Figure 1b).[41,42] The as-synthesized CdSe-LQDs were characterized by transmission electron microscope (TEM) and infrared spectra (IR).

Responsiveness and Selectivity of CdSe-LQDs to Amino Acids. 0.5 mL aliquots of 11 amino acid solutions (Trp, Ser, Tyr, Pro, Arg, Lys, Leu, Thr, Phe, Cys, and His, 10^{-4} M) were added into the PCAD-modified CdSe-LQDs solution (H_2O:CH_3CN = 3:1, 5 × 10^{-4} M), and the fluorescence spectrum was recorded after incubation for 10 min.

Preparation of Solid State of CdSe-LQDs. CdSe-LQDs were obtained as a waxy solid through evaporation of an aqueous solution under reduced pressure.[29]

Contact Angle (CA) Measurement. The CA was determined on an OCA 20 contact angle system at room temperature. The PCAD-modified LQDs were coated on the glass, and different amino acids were added. Then, the sample was dried over N_2. The measurement of CA was repeated three times

Differential Scanning Calorimetry (DSC) and Heat–Cool Cycle Experiments. The DSC of PCAD-modified LQDs was carried out by the following protocol: The cycle of LQDs in the presence of each amino acid (His, Phe, Arg, Trp and Tyr) was heated from 25 to 70 °C and then cooled to 25 °C (heating rate of 10 K/min) under N_2.

RESULTS AND DISCUSSION

OA-stabilized CdSe-QDs were prepared following the previous literature[41] and then modified with PEGylated calix[4]arene derivatives (PCAD) to obtain the desired CdSe-LQDs via hydrophobic interactions (Figure 2a). In order to keep the

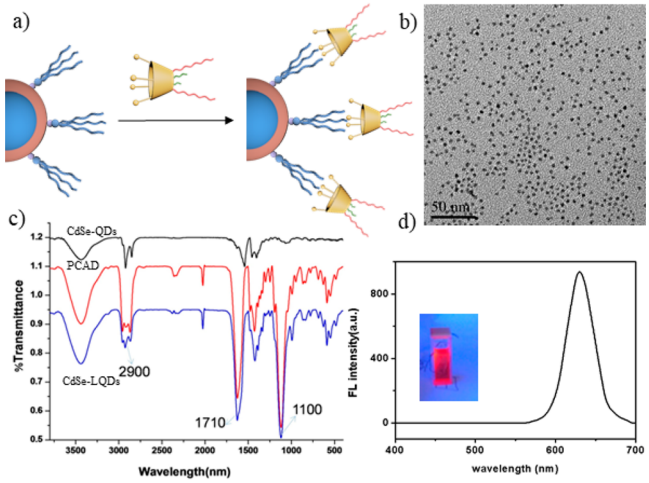

Figure 2. (a) A schematic illustration of preparing CdSe-LQDs via hydrophobic interaction. (b) TEM image of CdSe-LQDs. (c) IR spectrum of CdSe-QDs, PCAD, and CdSe-LQDs. (d) Fluorescence emission of CdSe-LQDs.

optimal fluorescence, the coating ratio of PCAD and CdSe-QDs was also investigated. As shown in Figure S2, the fluorescent intensity of CdSe-QDs is strongest when the ratio is 2. The soft PEG linkers from the PCAD endue the desired CdSe-LQDs with excellent mobility. The microstructure/size of the CdSe-LQDs was characterized by TEM, which indicated that the as-synthesized CdSe-LQDs were monodispersed and uniform with an average size of ∼7 nm (Figure 2b). Moreover, FT-IR also verified the PCAD had been successfully coated on the surfaces of the CdSe-QDs (Figure 2c). The characteristic bands at 1100 and 1710 cm^{-1} belonged to the stretching vibrations of C–O–C and C═O of the ester group from the PCAD. Finally, the fluorescence emission spectra of the CdSe-

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LQDs was measured, and they demonstrated a maximum peak emission wavelength at 625 nm (Figure 2d).

To evaluate the responsive ability of CdSe-LQDs toward nature amino acids, 11 different types of amino acids including Trp, Ser, Tyr, Pro, Arg, Lys, Leu, Thr, Phe, Cys, and His (10^{-4} M) were chosen. The fluorescence spectrum indicated that the addition of different amino acids could all lead to different degrees of fluorescence quenching for CdSe-LQDs (Figure 3a). It was worth noting that the CdSe-LQDs displayed

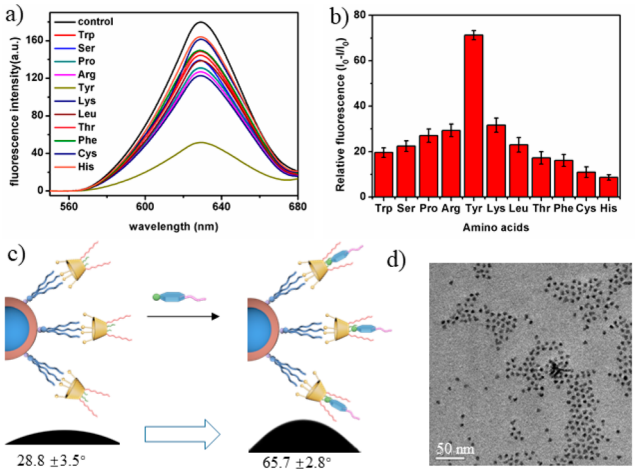

Figure 3. (a) Fluorescence spectra showing the response of CdSe-LQDs to 11 amino acids in CH_3CN/H_2O. (b) Relative fluorescence intensity $((I_0 - I)/I_0)$. I_0 and I are the fluorescence intensities without and with amino acids. (c) Profiles of water droplets on the CdSe-LQDs on quartz substrates and treated with Tyr, and the Tyr induced CA of CdSe-LQDs changes from 28.8 to 65.7°. (d) TEM image of CdSe-LQDs in the presence of Tyr.

relatively strong fluorescence quenching after incubation with Tyr, which reveals the high selectivity of CdSe-LQDs for Lys in the aqueous solution. The fluorescence change ratios, $(I_0 - I)/I_0$, of the CdSe-LQDs also confirmed the high Tyr selectivity of the CdSe-LQDs (Figure 3b). Moreover, the detection limit of the LQDs for Tyr is 2.0 μM (Figure S3). The mechanism of fluorescence quenching in the presence of Tyr may be attributed to the interaction between the Tyr and PCAD of CdSe-LQDs. The high selectivity for Tyr was also supported by CA measurement. The CA of the CdSe-LQDs loaded on a glass slide was 28.8°, indicating the hydrophilic feature of the CdSe-LQDs (Figure 3c). As seen in Figure S4, the slight increase on CA was observed after adding other amino acids. In contrast, the significant enhancement of the CA was observed after adding Tyr to the CdSe-LQDs, indicating the excellent selectivity for Tyr (Figure 3c). The state of CdSe-LQDs doped with Tyr tuning from hydrophilic to hydrophobic could be explained by the hydrogen interaction between the phenolic hydroxyl group of Tyr and the ester and PEG chain from PCAD, which hindered PEG stretching and resulted in the hydrophobic surface. From the TEM image results, the aggregated phenomenon was also clearly observed for CdSe-LQDs in the presence of Tyr (Figure 3d), which could be attributed to the interaction between Tyr and PEG chains inducing CdSe-LQDs to entangle with each other and self-assemble.

Furthermore, the fluidic behavior and fluorescent response of amino acid-doped CdSe-LQDs in the solvent-free state was also observed (Figure 4A). As seen in the digital photographs

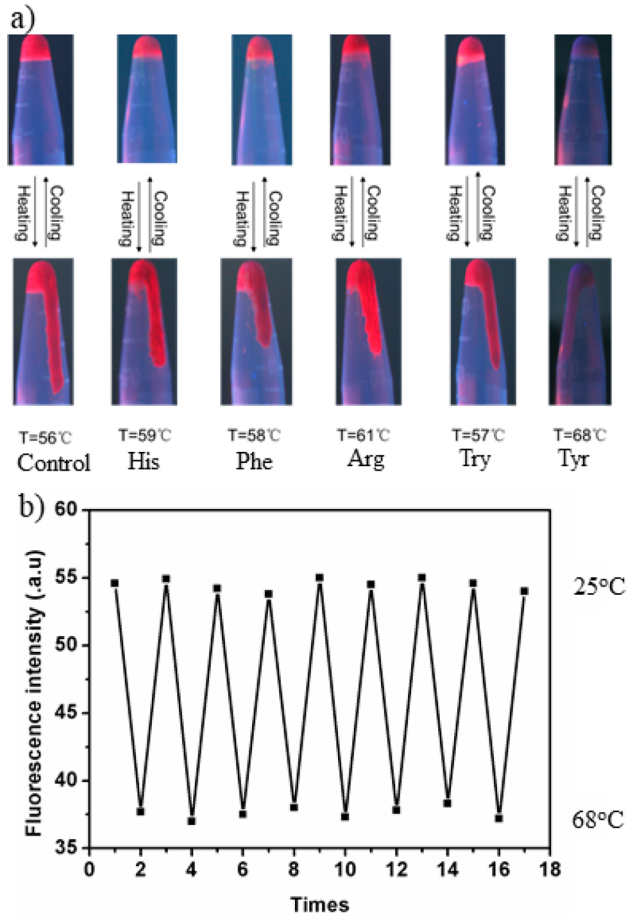

Figure 4. (a) The amino acid-doped CdSe-LQDs exhibited excellent fluidity after heating, and only the fluorescence intensity of Tyr-doped nanofluids was almost completely quenched. (b) The cycling experiment of Tyr-doped CdSe-LQDs from heating and cooling.

of Figure 4A, it seems that CdSe-LQDs in the presence or absence of amino acids remained in the solid state at room temperature, and only the Tyr induced remarkable fluorescent quenching (Figure 4A). Meanwhile, the nondoped CdSe-LQDs and amino acid-doped CdSe-LQDs both exhibited excellent flowability when heating. Among them, the Tyr-doped CdSe-LQDs demonstrated a higher rheological temperature, which was 12 °C higher than that of the nondoped CdSe-LQDs (Figures 4A and S5). More importantly, the liquid-like CdSe-LQDs could be easily recovered to the solid state with the decrease of temperature. Hence, we also investigated the flow reversibility of the Tyr-responsive CdSe-LQDs. The cycling performance of this system was investigated by recording the fluorescence intensity change between the solid states and liquid states of the CdSe-LQDs. As shown in Figure 4B, Tyr-doped CdSe-LQDs exhibited excellent reversibility even after 10 cycles. Therefore, liquid−solid transition Tyr-responsive CdSe-LQDs have been acquired with excellent reversibility.

To further prove the responsive mechanism based on the host−guest chemistry between the Tyr and PCAD of CdSe-LQDs, the interaction of Tyr and PCAD was then investigated by ^{1}H NMR spectroscopy. As seen in Figure 5A, after the equivalent ratio of Tyr was added into the PCAD solution, the H_a and H_b aromatic protons from Tyr shifted downfield by 0.02 and 0.12 ppm due to the hydrogen interaction between

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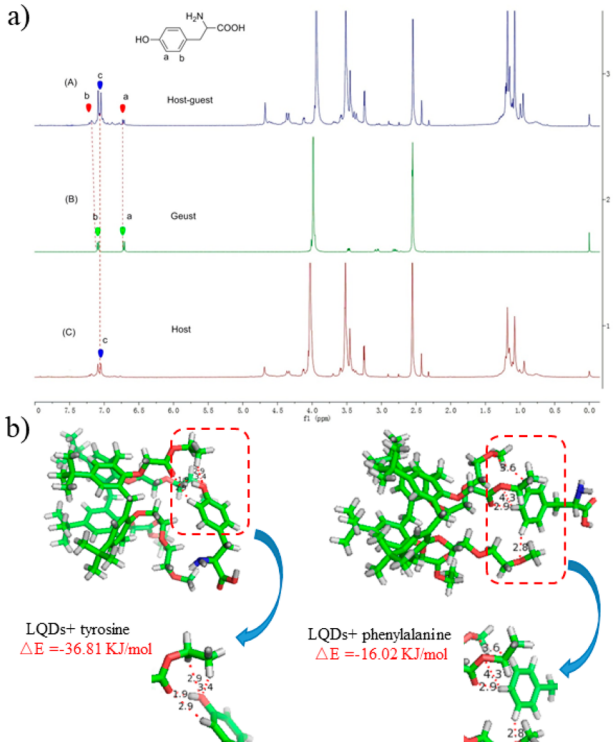

Figure 5. (a) ^{1}H NMR spectra of (a) PCAD (5 mM) + Tyr (5 mM), (b) free Tyr (5 mM), and (c) PCAD (5 mM). (b) The Gaussian calculation of the binding energy of PCAD + Tyr (PCAD + Phe as a control).

the phenolic OH and PEG or ester group. Meanwhile, the H_c aromatic protons from PCAD also showed a downfield shift of 0.10 ppm. All these data confirmed the coordination of hydrogen bonding between the host and guest molecules. Moreover, the possible interaction modes were verified by Gaussian simulations (Figures 5B and Figure S6). These model analyses revealed the critical role of hydrogen boding between Tyr and PCAD. The binding energy is −36.81 kJ/mol for Tyr. In contrast, it is only −16.02 kJ/mol for Phe (as a control), which indicated that the PCAD prefers binding with Tyr. Both the results of NMR and Gaussian simulations demonstrated that such CdSe-LQDs displayed high selectivity for Tyr. Therefore, the results of TEM, CA, NMR, and Gaussian simulations could both confirm that the possible mechanism is the hydrogen interaction between the phenolic hhdroxyl group of Tyr and the ester group/PEG chain of PCAD of the LQDs.

CONCLUSIONS

In short, we have demonstrated a Tyr-responsive CdSe QD biosensor by virtue of the hydrogen binding between PCAD of QDs and Tyr. This biosensor system exhibited a excellent selective fluorescence/macroscopic response for Tyr and reversible fluidic features. These excellent results highlighted CdSe-LQDs as a platform for biological molecule recognition and separation in the future.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.9b04034.

NMR, fluorescence spectrum, contact angle, Gaussian calculation, and chemical synthesis (PDF)

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Notes

The authors declare no competing financial interest.

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