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Fabrication of a Tyrosine Responsive Liquid Quantum dots based Biosensor Through Host-Guest Chemistry

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KEYWORDS: Biosensor, Liquid Quantum dots, Tyrosine detection, Host-Guest Chemistry, Calix[4]arene,

ABSTRACT: Design and fabrication of a smart liquid quantum dots (LQDs) with high biomolecules selectivity and specificity remains a challenge. Herein, a multifunctional calix[4]arene derivatives (PCAD) was rationally designed and applied to fabricate a Tyr-responsive CdSe-LQDs system through host-guest chemistry. Such this 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 molecules recognition and separation in the future.

Tyrosine (Tyr) is a well known aromatic building blocks 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, an 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 determination of Tyr such as chromatography and electrophoresis methods suffer from either an expensive equipment/reagent or time-consuming preparation.[10-13] Thus, developing more facile and reliable strategy for detection of Tyr levels with high sensitivity and reduced cost is crucial and highly demanded.

In the past decades, a 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, that could greatly expand the applications of LQDs in heat-transfer fluids, fluorescent sensors and microfluidic sensors.[21-28] Despite these pioneer results, the research on the design of smart LQDs in response to the external stimuli for biosensor applications are still on its infancy.[29-30] Calixarenes, a well known cavity-adjustable and rim-modified host scaffolds has been widely incorporated into the surface of various materials to endow them specific responding to external stimuli including pH, molecules and ions.[31-37] For example, Pochini et al. fabricated a pyridinium-responsive device based on dihydroxy-calix[4]arene modified Au surface, demonstrating remarkable specific and ultra-sensitive recognition of pyridinium.[38] More recently, our group constructed a photo-responsive biosensor through chiral azo-calix[4]arene modified silicon materials.[39] From the both results of fluorescence/wettability, such biosensor showed high sensitivity/selectivity and excellent reversibility toward (1R,2S)-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 amino acid is still rarely reported, which will greatly expand the applications of LCQs in biomolecules sensing and separation.

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

responsive system, calix[4]arene derivatives were designed in terms of the following factors. Firstly, calix[4]arene could be facilely assembled onto the surface 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 further tunable fluidic feature of QDs. Finally, the ester group and PEG chain of upper rims of calix[4]arene provide the potential recognition sites for Tyr. This Tyr-responsive QDs nanofluids opens a way to design smart fluidic materials for biological molecules determination and separation.

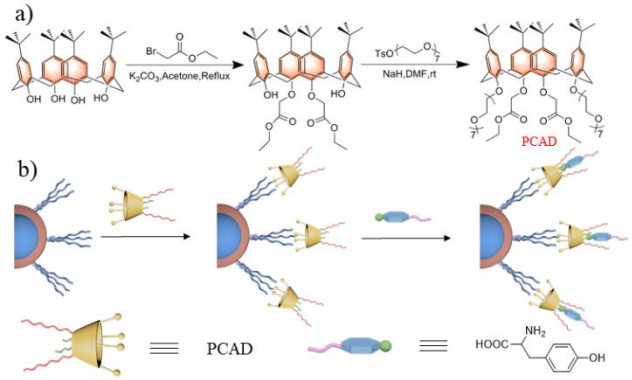

Figure 1. a) The synthetic scheme of PCAD. b) A schematic illustration to show that the PCAD coated on the CdSe-QDs to produce CdSe-LQDs and it responses to Tyrosine.

EXPERIMENTAL SECTION

The synthesis and characterization of PEGylated calix[4]arene derivatives (PCAD). PEGylated calix[4]arene derivatives (PCAD) was synthesized from Tert-butylcalix[4]arene starting materials by several steps (Figure 1a, 22% overall yield, see supported information). The desired product (PCAD) was achieved after silica gel purification as yellow oil and characterized by NMR (Figure S1, See supported information). In order to endow PCAD with multifunction, the upper rims of PCAD was asymmetry modified with both ester molecules and PEG molecules.

The synthesis and characterization of CdSe-QDs. CdSe-QDs was synthesized according to the previous reported procedures with little modification.[40] The obtained OA-stabilized CdSe-QDs was dispersed in chloroform and stored in the 4 ℃ for further use. The as-synthetic fluorescence emission spectra of CdSe-QDs was recorded on an Applied NanoFluorescence Spectrometer.

The preparation and Characterization of the PCAD modified CdSe-QDs Nanofluids. The as-synthesized calix[4]arene derivatives PCAD was coated onto the surface of OA-stabilized CdSe-QDs to obtain the CdSe-LQDs through the hydrophobic interaction between the OA chains and *tert*-butyl groups of PCAD (Figure 1b)[41]. The as-synthesized CdSe-LQDs were characterized by Transmission electron microscope (TEM) and Infrared spectra (IR).

The responsive and selectivity of CdSe-LQDs to amino acids. 0.5 mL of 11 amino acid solutions (Trp, Ser, Tyr, Pro, Arg, Lys, Leu, Thr, Phe, Cys and His, 10^{-4} M) added into the PCAD modified CdSe-LQDs solution (H_2O:CH_3CN=3:1, $5*10^{-4}$ M) and the fluorescence spectrum were 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. CA were recorded on an OCA 20 contact angle system at room temperature. The PCAD modified LQDs was coated on the glass, then different amino acids were added. Then the sample was dried over N_2. The measurement of CA was repeated for 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) heated from 25 ^{0}C to 70 ^{o}C and then cooling to 25 ^{0}C (heating rate of 10 K/min) under N_2 condition.

RESULTS AND DISCUSSION

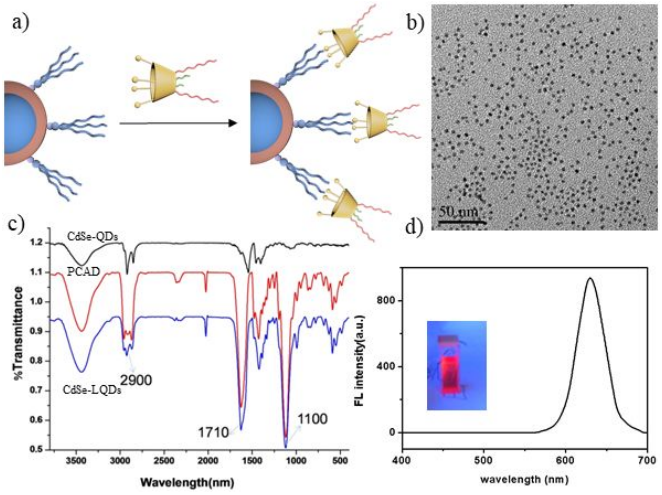

Figure 2. a) A schematic illustration of preparing CdSe-LQDs via hydrophobic interaction. b) TEM image of CdSe-LQDs. c) IR spectrum of CdSe-QDs, PCAD and CdSe-LQDs. d) Fluorescence emission of CdSe-LQDs.

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 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 excellent mobility. The microstructure/size of CdSe-LQDs was characterized by TEM. TEM image indicated that the as-synthesized CdSe-LQDs were monodispersed and uniform with an average size of ~7 nm (Figure 2b). Moreover, FT-IR spectrum also verified the PCAD has been successfully coating on the surface of CdSe-QDs (Figure 2c). The characteristic bands at 1100 cm^{-1} and 1710 cm^{-1} belonged to the stretching vibrations of C-O-C and C=O of ester group from the PCAD. Finally, the fluorescence emission spectra of CdSe-LQDs was measured and they demonstrated a maximum peak emission wavelength at 625 nm (Figure 2d).

To evaluate the responsive ability of CdSe-LQDs towards 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 phenomenon for CdSe-LQDs (Figure 3a). It was worth noting that the CdSe-LQDs displayed 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 $(I_0\text{-}I)/I_0$ of CdSe-LQDs also confirmed the high Tyr selectivity of CdSe-LQDs (Figure 3b). Moreover, the detection limit of 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 Tyr and PCAD of CdSe-LQDs. The high selectivity for Tyr was also supported by contact angel (CA) record. The CA of CdSe-LQDs loaded on a glass slide was 28.8^0, indicating the hydrophilic feature of 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 CA was observed after adding Tyr to CdSe-LQDs, indicating that 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 surface hydrophobic. 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 entangle with each other and self-assembled.

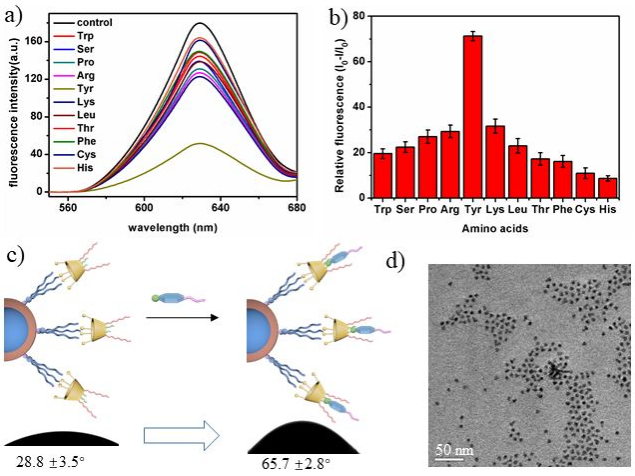

Figure 3. a) Fluorescence spectra showing the response of CdSe-LQDs to 11 amino acids in CH_3CN/H_2O. b) Relative fluorescence intensity ($(I_0\text{-}I)/I_0$), I_0 and I are the fluorescence intensities without and with amino acids. c) Profiles of water droplets on the CdSe-LQDs on quartz substrates and treated with Tyr and Tyr induced CA of CdSe-LQDs change from 28.8^0 to 65.7^0. d) TEM image of CdSe-LQDs in the presence of Tyr.

Furthermore, the fluidic behavior and fluorescent response of amino acid-doped CdSe-LQDs in solvent-free state was also observed (Figure 4A). As seen in digital photographs of Figure 4A, it seems that CdSe-LQDs in the presence or absence of amino acids kept solid-state at room temperature and only the Tyr induced the remarkable fluorescent quenching (Figure 4A). Meanwhile, the non-doped CdSe-LQDs and amino acid-doped CdSe-LQDs both exhibited excellent flowability when heating. Among them, the Tyr-doped CdSe-LQDs demonstrated higher rheological temperature, which was 12 ^{0}C higher than that of non-doped CdSe-LQDs (Figure 4A and Figure 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 show in Figure 4B, Tyr-doped CdSe-LQDs exhibited excellent reversibility even after 10 cycles. Therefore, a liquid-solid transition Tyr-responsive CdSe-LQDs has been acquired with a excellent reversibility.

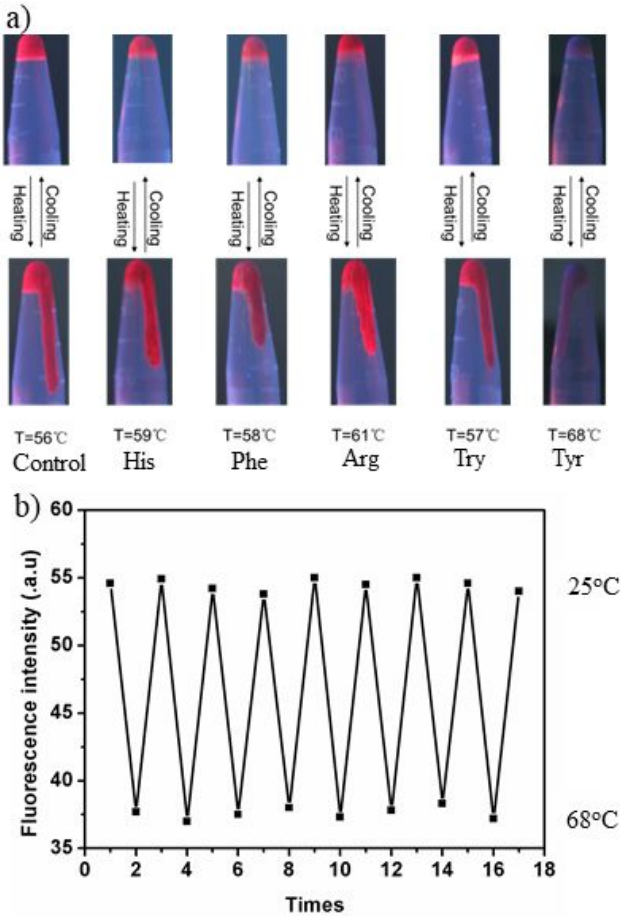

Figure 4. a) The amino acid-doped CdSe-LQDs exhibited excellent fluidity after heating and only the fluorescence intensity of Tyr-doped nanofluids was almost completely quenched. b) The cycling experiment of Tyr-doped CdSe-LQDs from heating and cooling.

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 the phenolic OH and PEG or ester group. Meanwhile, the Hc

aromatic protons from PCAD also showed a downfield shift with 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 (Figure 5B, Figure S6 and supported information). These model analyses revealed the critical role of hydrogen boding between Tyr and PCAD. The binding energies 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, these results of TEM, contact angle, 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 from PCAD of LQDs.

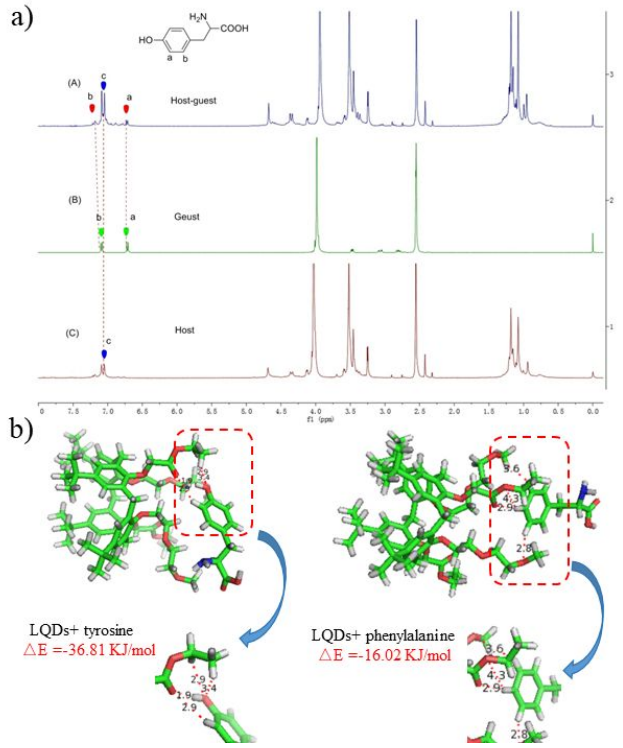

Figure 5. a) 1HNMR spectra of (a) PCAD (5 mM) + Tyr (5 mM), (b) free Tyr (5 mM), (c) PCAD (5 mM). b) The Gaussian calculation of binding energy of PCAD + Tyr (PCAD + Phe as a control).

CONCLUSIONS

In short, we have demonstrated a Tyr-responsive CdSe QDs 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 molecules recognition and separation in the future.

ASSOCIATED CONTENT

Supporting Information

NMR, fluorescent spectrum, Contact angle, Gaussian calculation and chemical synthesis.

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Notes

The authors declare no competing financial interest.

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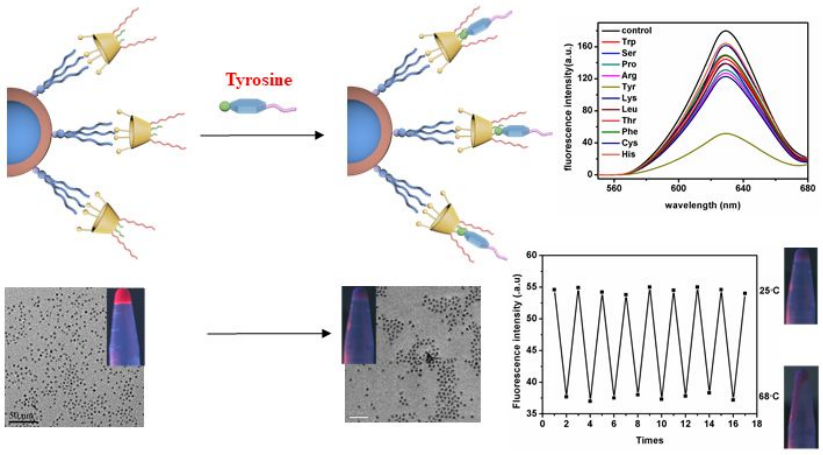

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