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Herein we developed a new approach for CdSe quantum dots (QDs) synthesis. These bovine serum albumin (BSA) and Glutathione (GSH) conjugated CdSe QDs (BSA-GSH-CdSe QDs) are low cytotoxic and NADPH responsive. The whole synthetic process can be completed within 8 minutes at room temperature.

Quantum dots (QDs) play an important role in various fields such as sensing, catalysis, solar cell, and bioimaging studies due to their unique electrooptical properties. However, several factors greatly limited application of QDs in broader areas. First of all, QD synthesis normally carried out at oil phase by heating under nitrogen protection and longtime incubation. On the other hand, it remains challenge to design and synthesize QDs that can response to various cellular metabolites with high selectivity and low cytotoxicity. To overcome these limitations, it is critical to find a new synthetic method for QD production. Indeed, during the last few years several methods were developed concerning the synthesis of QDs at relatively milder conditions in contrast to classical approach. Nevertheless, most of these approaches require multiple times of pH adjustment, long time incubation, high temperature, and are frequently only suitable for small volume of QD synthesis. For comparison, methods of synthesizing QDs at mild conditions were summarized (Table S1).

CdSe crystalline is among the most known QDs. Therefore, CdSe QD was chosen as a model system to explore the possibility of modification and improvement of QD synthetic protocol. It is known that a stable supply of active selenium group (Se\(^\text{2-}\)) is a critical and time-consuming step in classical CdSe QD synthetic approach. Se\(^\text{2-}\) ions can be easily oxidized by the trace amount of oxygen in the aqueous solution. Thus, finding of alternative ways to produce and stabilize Se\(^\text{2-}\) might be the key to green synthesis of QDs. To this end, we reasoned that a powerful ROS scavenging system may be introduced into the reaction solution of QD synthetic system to stabilize Se\(^\text{2-}\). It was found that selenium nanoparticles (SeNPs) contain ROS removal function which is partially attributed to their large surface area and small size. Very recently, a green synthetic route for SeNP synthesis was established by using Glutathione (GSH) as a reducing agent and bovine serum albumin (BSA) as a stabilizer. Notably, GSH is a free radical eliminating compound, widely distributed in almost all the organisms. This sulphur group containing molecule can interact with various metal ions and thus was frequently used for QD synthesis. Similarly, BSA was also sometimes used for HgS, ZnS and CdSe QD synthesis, which is likely due to its capability of interaction with variety of inorganic molecules and stabilize them.}

![Fig. 1 Synthetic route for BSA-GSH-CdSe QDs](image)

Herein this study, we developed an alternative route by using SeNPs as selenium source to initiate CdSe QD synthesis. This new system was exempted from N\(_2\) protection, low temperature and longtime incubation at oil phase, as compared to classical QD synthetic approach. The process can be divided into two phases: SeNP and CdSe QD synthesis (Fig. 1).

The first part of this synthetic process mainly concerns the production of SeNPs and this step was completed in 5 minutes. When selenite (Fig. 1-A) and BSA was mixed, there is no colour change observed, suggesting no SeNP was formed (Fig. 1-B\(_1\)). While GSH was added into selenite solution, the mixture was changed from clear colourless to turbid red solution, indicating the formation of selenium aggregates (Fig. 1-B\(_2\)). SeNP synthesis was initiated by mixing of selenite, GSH and BSA. The colour of this mixture was rapidly switched from colourless to red, indicating the formation of SeNPs (Fig. 1-B\(_3\)). Sodium borohydride was then introduced into the SeNP solution followed by 3-minute-incubation. The solution was switched back to colourless again within 3 minutes, hinting the Se\(^\text{2-}\) formation (Fig. 1-C\(_1\)). Cd\(^\text{2+}\) was then added into this Se\(^\text{2-}\) containing solution. The colour of the solution was shifted to light yellow-green within one second, suggesting the formation of CdSe QDs, as also...
shown in the images taken under UV irradiation (360 nm) (Fig. 1-D). This rapid synthetic process can be completed within approximately 8 minutes. In the absence of BSA, this process somehow can also produce relatively unstable CdSe QDs (Fig. 1-D). Synthesis of CdSe QDs at RT using SeNPs showed advantages over other selenium sources tested so far (Fig. S1).

Fig. 2 Schematic synthetic route and spectroscopic characterization of SeNPs and BSA-GSH-CdSe QDs. a. schematic presentation of BSA-GSH-CdSe QD synthesis. b. representative SEM image and DLS analysis of SeNPs. c. representative TEM image and a size histogram of BSA-GSH-CdSe QDs. Inset: enlarged images with visible lattice fringes. d. UV-visible spectra of GSH (black), BSA (red), BSA-GSH-CdSe QDs (blue), and SeNPs (green). e. fluorescent excitation (red) and emission (black) curves of BSA-GSH-CdSe QDs. f. substrates, intermediate products and synthesized BSA-GSH-CdSe QDs under visible (upper) and UV (lower) lights.

This SeNPs based approach for QD synthesis was summarized in Fig. 2a. SeNPs via this BSA-GSH assisted route was approximately 70 nm in size, as shown in scanning electron microscopy (SEM) images and dynamic light scattering (DLS) measurements (Fig. 2b). The core size of the BSA-GSH-CdSe QDs was approximately 4.5 nm with clear visible lattice fringes, as shown in transmission electron microscope (TEM) images (Fig. 2c). UV-vis spectra showed a strong shoulder peak centered at 425 nm (Fig. 1d). Fluorescence spectra showed that the main excitation and emission peaks of BSA-GSH-CdSe QDs were localized at 398 and 535 nm, respectively (Fig. 2e). As expected, BSA-GSH-CdSe QDs emitted yellow-green fluorescence under UV irradiation (Fig. 1f).

These CdSe QDs were conjugated with BSA and GSH, as confirmed by FT-IR spectra (Fig. S2). Based on literatures, the size of SeNPs was partially dependent on the ratio of BSA/GSH/SeO2. As a consequence, a mild blue shift of BSA-GSH-CdSe QDs was observed along with the increase of BSA concentration in the reaction mixture (Fig. S3), suggesting that BSA might act as a “size regulator” of CdSe QDs. Compared to other proteins such as mucin and collagen, BSA appeared to be an excellent candidate for synthesizing CdSe QDs in that BSA conjugated QDs show much higher fluorescence under the same synthetic condition (Fig. S4). BSA-GSH-CdSe QDs are fairly stable in different buffers (Fig. S5a), pH (Fig. S5b) and resistant to high salt condition (Fig. S5c) and can tolerate long time incubation at RT (Fig. S5d).

Recent years the design of biosensors for the recognition of biological active small molecules has received considerable attention, especially for nucleotides and their derivatives such as adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP(H)). NADP(H) are structurally similar to ATP and are cofactors for hundreds of cellular enzymes for nearly all the living organisms, where NADPH is the reduced form and NADP+ is the oxidized form. Cellular redox condition is largely associated with the status of these cofactors, for instance, the ratio of [NADP+] / [NADPH]. Therefore, it is highly important to generate sensors that can detect the content of these cofactors in aqueous solution. Several excellent methods have been developed for ATP recognition, whereas the detection method for NADP(H) are relatively less and suffer from laborious and low selectivity, such as electroanalysis and spectrum detection. It is highly valuable to find new approaches that can improve the simplicity, sensitivity and selectivity of the NADP(H) probing system.

Interestingly, BSA-GSH-CdSe QDs can respond to NADPH in aqueous solutions likely through forming a supramolecular complex (Fig. 3a). In contrast to most of currently available NADPH nano-sensors that detection mechanism lies in fluorescent quenching, a clear change of fluorescence emission wavelength was observed when BSA-GSH-CdSe QDs were mixed with NADPH (Fig. 3b). The blue shift of emission of BSA-GSH-CdSe QDs was accompanied by an increase of fluorescence intensity along with the raise of NADPH concentration (Fig. 3b, Fig. S6). There is a linear relationship between the fluorescence intensity and NADPH concentration at the range of 0 - 13 mM with a detection limit of 250 µM (Fig. 3c). In contrast, when MPA-CdSe QDs synthesized using classical method was incubated with NADPH, there is no blue shift event observed, instead the emission peaks of MPA-CdSe QDs and NADPH were separated, indicating the absence of physical interaction between MPA-CdSe QDs and NADPH (Fig. S7).
fluorescent intensity at 482 and 535 nm, respectively). d. the selectivity of BSA-GSH-CdSe QDs toward NADPH and other related compounds and biomolecules (5 mM). e. there was no clear cytotoxicity of BSA-GSH-CdSe QDs observed toward E. coli cells. OD600 stands for the optical density (OD) at 600 nm of the bacterial cells.

To investigate the selectivity of BSA-GSH-CdSe QDs based nano-sensor, the fluorescence changes of this nanomaterial were carefully analysed upon adding of NADPH and its derivatives and several other biomolecules or compounds as follows: NADP, ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP), 2'-deoxyadenosine-5'-triphosphate (d-GTP), 2'-deoxyadenosine-5'-triphosphate (d-ATP), 2'-deoxyguanosine-5'-triphosphate (d-CTP), 2'-deoxyadenosine-5'-triphosphate (d-ATP), 2'-deoxycytidine-5'-triphosphate (d-TTP), 2'-deoxycytidine-5'-triphosphate (d-CTP), and D-galactose, glycine, GSH, mucin, Na+, and H2O (Fig. 3d and Fig. S8). Except for NADPH, all the other reagents tested so far did not show obvious blue shift and fluorescence enhancement at 482 nm, suggesting a high selectivity of BSA-GSH-CdSe QDs toward NADPH. The zeta potential of CdSe QDs and NADPH was +14 and +12.3 mV, respectively, which was shifted to -18.7 mV when they were mixed, hinting a stable interaction between them. The cytotoxicity of this material was evaluated by using bacteria Escherichia coli cells as a model system. The growth of E. coli cells was not affected by adding of 2 µM of BSA-GSH-CdSe QDs, as measured at OD600 (Fig. 3e).

Human serum is a complex mixture containing large amount of ions and biological molecules. Therefore, it provides a good platform to evaluate sensor function of BSAOGSHOCdS e QDs and biological molecules. Therefore, it provides a good platform to evaluate sensor function of BSAOGSHOCdSe QDs and other related compounds and biomolecules (5 mM). e. there was no clear cytotoxicity of BSA-GSH-CdSe QDs observed toward E. coli cells. OD600 stands for the optical density (OD) at 600 nm of the bacterial cells.

In summary, we have developed a new approach for facile and rapid synthesis of CdSe QD at RT using eco-friendly molecule and biological molecules. Therefore, it provides a good platform to evaluate sensor function of BSAOGSHOCdSe QDs and other related compounds and biomolecules (5 mM). e. there was no clear cytotoxicity of BSA-GSH-CdSe QDs observed toward E. coli cells. OD600 stands for the optical density (OD) at 600 nm of the bacterial cells.

† Electronic Supplementary Information (ESI) available: Materials and methods, FT-IR spectra, fluorescence spectra, stability measurements, and several other experiments. See DOI: 10.1039/c000000x/
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