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PAPER

Synthesis of water-soluble Ag_2Se QDs as a novel resonance Rayleigh scattering sensor for highly sensitive and selective conA detection

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Ag_2Se quantum dots (QDs) have attracted a lot of interests due to their potential applications in biosensing and bioimaging. A strategy is presented that involves coupling selenium powder reduction with the binding of silver ions, and thioglycollic acid (TGA) and glycine as stabilizer to obtain ultrasmall Ag_2Se QDs at 85 ^oC in aqueous solution. This strategy avoids high temperatures, high pressures and organic solvents so that water-soluble 3 nm Ag_2Se QDs can be directly obtained. The conjugation of conA to TGA stabilized Ag_2Se QDs by hydrogen bonds leads to the adsorption of conA to Ag_2Se QDs and forms the aggregation and leads to the generation of resonance Rayleigh scattering (RRS) as a readout signal for the sensing events. The reaction mechanism of Ag_2Se QDs RRS enhancement is studied in this work. The resulting RRS sensor enables the detection of conA with limit of detection reaching 0.08 μg/mL concentration in a wide linear range from 0.27 μg/mL to 35 μg/mL. The recovery of spiked conA in human serum samples ranges from 94% to 106%. The relative standard deviation (RSD) for eleven replicate detections is 3.6%. Our results correlate many important experimental observations and will fuel the further growth of this field.

Introduction

Semiconductor nanocrystals (quantum dots, QDs) with fantastic optical properties, such as broad excitation, size-dependent photoluminescence, unusual photochemical stability, and single excitation/multiple emission, have attracted much attention in biosensing[1]. Besides biocompatibility and nontoxicity, small size is also important for labeling nanomaterials. In general, existing QDs are close to or larger than most biological macromolecules in size[2]. Thus, use of QDs in biological labeling may be limited due to their large size, which would interfere with both the recognition between QD-labeled bioprobes and target molecules because of steric hindrance and the movement of the bioprobes[3]. Therefore, it is still a great challenge to construct new QDs with less toxicity and small size for bioimaging and biodetection[4].

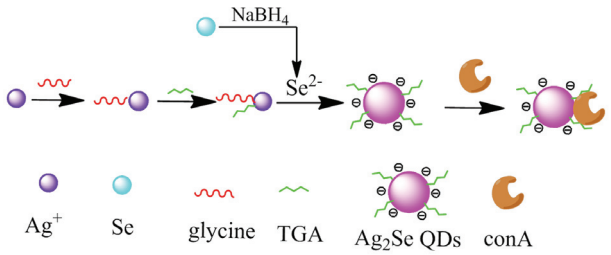
Ag_2Se QDs, without toxic heavy metal and usually smaller than 3 nm, are one kind of the most important NIR QDs[5,6]. The ultrasmall size makes Ag_2Se QDs have enormous surface area-to-volume ratios, which may be highly susceptible to heterogeneous redox chemistry with the surrounding environment. Such properties make Ag_2Se QDs suitable for surface chemistry research, biosensing and bioanalysis. It has been reported that ultrasmall and low cytotoxic of Ag_2Se QDs has been synthesized. For instance, Pang groups[7,8] reported the organic reagents synthetic route with TOPO (trioctylphosphine oxide) and TOP (trioctylphosphine) as stabilizing reagents. Generally, with water soluble thiols as transferring reagents, Ag_2Se QDs are transferred to water soluble semiconductor nanoparticles. It is difficult to efficiently maintain the Ag_2Se QDs fluorescence quantum yields on the transfer from the organic soluble Ag_2Se QDs to water soluble ones[9]. Besides, they are relatively complicated and require additional high cost instruments. Therefore, it is great significant to develop a fast and simple method for synthesis water soluble Ag_2Se QDs.

Concanavalin A (ConA), a legume lectin from Jack beans, can specifically bind to the glucose moiety of cell membrane glycoprotein[10], thereby initiating T-cell activation[11], cell mitogenesis[12], agglutination and apoptosis[13]. Many approaches have been developed to detect ConA because it is an important target for studying the carbohydrate-protein interactions[14,15]. Recent efforts have led to the development of carbohydrate chips by either covalent or noncovalent immobilization strategies have successfully fabricated a glycan chip by immobilizing various carbohydrates on nitrocellulose or nitrocellulose-coated glass slides, which may be used for high-throughput analysis of carbohydrate-protein interactions[16]. However, the process is complex and time-wasting. Therefore, developing a fast and simple method for homogeneous detection of ConA is of great significance.

Resonance Rayleigh scattering (RRS) has been known for its sensitivity and simplicity as an analytical technique developed in recent years[17]. This technique has been applied successfully to study macromolecules[18,19] and the determination of some metal ions[20], nonmetals[21], physicochemical constants[22]. RRS is very sensitive to the interaction caused by weak binding forces such as intermolecular electrostatic attraction, hydrogen bonding, hydrophobic interaction, and aggregation interaction of biological macromolecules[23]. The spectral characteristics and scattering

intensity are strongly influenced by the molecular size, shape, conformation, and interfacial properties, which further provide favorable new information for the study of the interaction of biological macromolecules and the molecular recognition[24].

Herein we demonstrate a new method that synthesis Ag_2Se QD and a new concept that RRS between Ag_2Se QD and conA. Accompanied by an increase in the RRS of Ag_2Se QDs can be used to develop a biosensor for the detection of target biomolecules with high selectivity and sensitivity. In this RRS process, Ag_2Se QD sand conA display weak RRS. The complex is formed between Ag_2Se QDs and conA by intermolecular hydrogen bonding, and then leads to change of the structure of conA and enhance of the RRS of the Ag_2Se QDs and conA (scheme 1). It is the first time that ConA is detected by means of RRS sensor based on Ag_2Se QDs. This scheme would open a new opportunity for design of more novel RRS-based sensing strategies for other biomolecules.



Scheme 1 Schematic illustration for fabricating TGA and glycine modified Ag2Se QDs for RRS detection of conA

Experimental Section

Reagents

$AgNO_3$ and TGA were purchased from Shanghai Chemicals Reagent Co., Shanghai. Se powder, $NaBH_4$ and glycine were purchased from Tianjin kemiou Fine Chemical Co., Tianjin. ConA was purchased from sigma-Aldrich (shanghai) trading Co. Led. Sodium citrate buffer solution (SCBS) was used to control the acidity of the aqueous medium. All the reagents were used of analytical regent grade without further purification and deionized water with conductivity of 18.2 $M\Omega cm^{-1}$ was used in this experiment from a water purification system (ULUPURE, Chengdu, China).

Instrumentations

Transmission electron microscopy (TEM) of TGA-stabilized Ag_2Se QDs were carried out on a Tecnai G2 F20 S-TWIN transmission electron microscope at an accelerating voltage of 200 kV (FEI Co., America). X-ray diffractometer (XRD) patterns of the samples were recorded using X' Pert Pro XRD (Philips) with Co Kα radiation (λ 1.79 Å). X-ray photoelectron spectroscopy (XPS) was performed with a XSAM 800 electron spectrometer (Kratos) using monochromatic Al Ka radiation for the analysis of the surface composition and chemical states of the product. The UV-vis spectra and RRS spectra were obtained with a U-2910 UV-vis spectrophotometer and an F-7000 fluorescence spectrophotometer (Hitachi Co., Tokyo, Japan).Fourier Transform Infrared spectra (FTIR) from 4000 to 400 cm^{-1} was recorded in KBr discs on a Nicolet IS10 FTIR spectrometer (Thermo Inc., America) for evaluating the encapsulation of Ag_2Se QDs.

Preparation of TGA-stabilizedAg_2Se QDs

Aqueous colloids TGA-Ag_2Se QDs solution was prepared at low temperature according to the reference[25,26]. It was described in detail as follows: Under N_2 atmosphere, deionized water (10 mL) was added to Se powder (0.0078 g) and excessive $NaBH_4$ under magnetic stirring at room temperature. After about 0.5 h, the colorless solution of NaHSe was prepared.

0.0680 g $AgNO_3$ was dissolved in 150 mL of deionized water and 110 μL of TGA and 0.0375 g glycine as stabilizer was added under stirring, followed by adjusting to pH = 11 by dropwising addition of 1.0 mol/L NaOH solution. The solution was placed in a tree-necked flask and deaerated by N_2 bubbling for about 30 min. Under magnetic stirring, H_2Se gas generated by the reaction of the solution of NaHSe with diluted HCl (1mol/L) was passed through the oxygen-free original solution together with a slow nitrogen flow for 30 min. Ag_2Se QDs precursors were formed at this stage. The molar ratio of Se/Ag+/TGA/glycine was fixed at 1:4:16:5. Then the resulting mixture was subjected to reflux at 85°C for 1 h under oxygen-free condition with condenser. TGA-Ag_2Se QDs were obtained. The concentration of TGA-Ag_2Se QDs was 6.67×10^{-3} mol/L (determined by the HSe^- concentration)[27].

Experimental procedure

150 μL above prepared TGA-Ag_2Se QDs, 300 μL SCBS and appropriate amounts of conA were added into a 2 mL colorimetric tube, then diluted with deionized water to the mark and mixed thoroughly with gentle shake. After incubated for 10 min, the RRS and UV-vis spectra of solution were examined.

Results and Discussion

The growth process of the Ag_2Se QDs

The strategic point in preparing the Ag_2Se QDs was to obtain Ag and Se precursor in the appropriate valence states. In previous reports[28], Se powder was reduced to low-valence selenium by $NaBH_4$, which can react with Cd^{2+} to form fluorescent CdSe QDs. This process of Se powder reduction from Se to HSe^- was using $NaBH_4$, as described in scheme 1. Besides the Se precursor, preparing an appropriate form of Ag precursor was of importance for preparing the monodispersed Ag_2Se QDs. Glycine and TGA, which can form an Ag^+-glycine-TGA complex[29], were chosen as the stabilizers for Ag_2Se QDs. The freshly prepared Se precursors were injected into the solution of the fresh Ag^+-glycine-TGA complex precursors, and the mixture was stirred for 1h at 85°C, obtaining Ag_2Se QDs.

Characterization of TGA-stabilized Ag_2Se QDs

TEM images displayed that the products obtained were spherical particles with good size distributions (Fig. 1A). The nanocrystals with sizes of 3 nm (100 particles measured in each image) were prepared at the reaction time of 1 h. XRD pattern (Fig.2) indicated that the diffraction peaks of the products matched with orthorhombic Ag_2Se (JCPDS Card No. 24-1041). The broadened peaks may be attributed to the low crystallinity of small-sized particles. XPS was used to analyze the surface composition of the

as-prepared products. The survey-scan spectra of the Ag 3d and Se 3d are shown in Fig. 3. The high-resolution XPS spectra of Ag 3d and Se 3d were referenced to the C 1s of aliphatic carbon at 284.9 eV. The peaks at 367.8 and 373.8 eV correspond to Ag $3d_{5/2}$ and $3d_{3/2}$, the peak at 53.8 eV corresponds to Se 3d of Ag_2Se QDs and the peak at 161.4 eV corresponds to S 2p of Ag_2S. The binding energy obtained from XPS analysis is consistent with a previous report[30]. The above results indicated that the products prepared were indeed TGA stabilized Ag_2Se QDs.

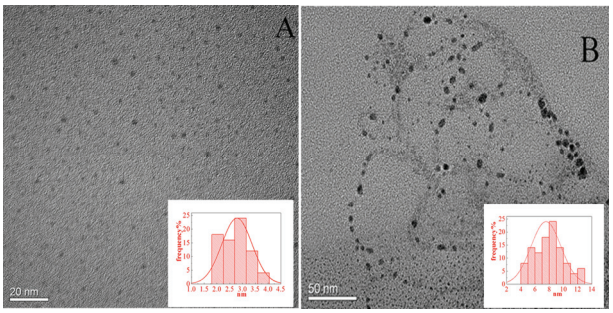


Fig.1 (A) TEM image of Ag_2Se QDs. (B) TEM image of Ag_2Se QDs-conA complex.

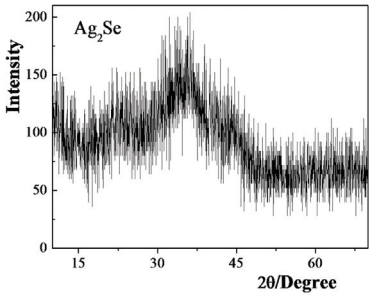


Fig.2 XRD pattern of Ag_2Se QDs.

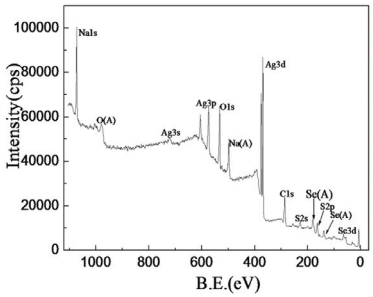


Fig. 3 XPS pattern of Ag_2Se QDs

The interaction between Ag_2Se QDs and conA and reasons for the enhancement of RRS

The RRS spectra of Ag_2Se QDs, conA and the Ag_2Se QDs-conA complex were shown in Fig.4. The experimental results showed that the RRS intensities of TGA-Ag_2Se QDs and conA were very weak. However, when TGA-Ag_2Se QDs was mixed with trace amounts of conA, the RRS intensity was enhanced greatly and a new RRS spectrum appeared. The maximum RRS peak was observed at 380 nm. RRS increased with an increase in concentration of conA in a certain range.

As for the enhancement mechanism, in theory, RRS enhancing in this system could occur by resonance enhanced Rayleigh scattering effect, increase of the molecular volume and change of

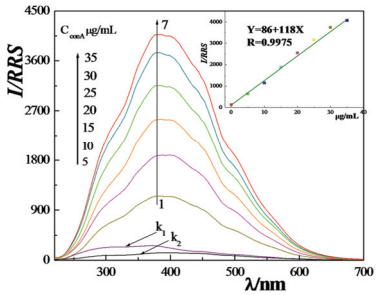


Fig. 4 Validation of the use of Ag_2Se QDs as probes for the detection of Con A. Ag_2Se QDs 5×10^{-4}mol/L; pH=6.0; SCBS buffer 300μL.

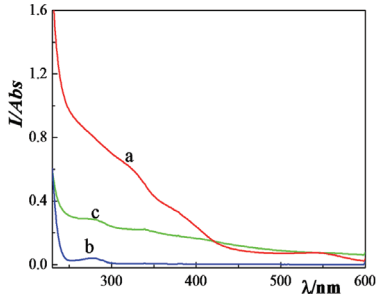


Fig. 5 UV-vis spectra of (a) Ag_2Se QDs, (b) conA and (c) Ag_2Se QDs in the presence of conA (Ag_2Se QDs as reference). k_1: Ag_2Se QDs (5×10^{-4} mol/L), k_2: conA (conA, 35 μg/mL), Ag_2Se QDs, 5×10^{-4} mol/L; SCBS buffer 300μL, pH=6.0, conA, 35 μg/mL

the conformation of the proteins. Here, several observations proved that RRS occurs in this enhancing process: (1) RRS was an absorption re-scattering process produced by the resonance between the Rayleigh scattering and the light absorption with the same frequency when the wavelength of Rayleigh scattering was located at its absorption band. Therefore, RRS spectrum was closely related to the absorption spectrum. From the comparison of RRS spectrum of the Ag_2Se QDs-conA complex with its absorption spectrum, from Fig. 5 it can be seen that the RRS peaks at 380 nm were close to the absorption bound, which would result in the resonance enhanced scattering[31]. (2) It is well known that the increase of the volume of the scattering molecule was advantageous to the enhancement of scattering intensity. It was clear that conA was negatively charged owing to the amino acid residue skeleton of conA (pI_{conA}=5.7) while the surface of TGA-Ag_2Se QDs charged negatively in the weak acidic buffer solution (pH=6). Besides, added electrolytes into the Ag_2Se QDs-conA solution, the intensity of RRS did not change obviously, which further suggested that hydrogen bond is very important in the interaction between Ag_2Se QDs and conA. The most important point, though the Fig.6 it was found that The -NH_2 (3388 cm^{-1}) of conA changed obviously. So it was speculated that conA molecules bind onto the surface of TGA-Ag_2Se QDs via the intermolecular hydrogen bonds[32], which resulted in the increase of the diameter up from about 3 nm to about 8 nm. Thus, the increase of the molecular volume was one of the reasons for the RRS enhancements. It was proved by the TEM image of Fig. 1B. The way how the conA attached to Ag_2Se QDs was shown in scheme 2. (3) Proteins are stable spherical and small in the aqueous, so the scattering of the proteins are weak, however, when the carbonyl (C=O) of the peptide chain of proteins bind with $-COO^-$ on the surface of a Ag_2Se QDs by hydrogen bonds, the original regular and repeating secondary structure of the

protein held together by a peptide chain and a hydrogen bond was destroyed and the structure became extended and loose, which was similar to the denaturing of the protein[33]. This can enhance the scattering.

Optimization of general procedure

The effect of Ag_2Se QDs concentration on the RRS intensity of the system was investigated. The system had the highest sensitivity and the system was stable when the concentration of Ag_2Se QDs was 4-7×10^{-4} mol/L for the system (Fig.7a). Therefore, the concentration of Ag_2Se QDs 5×10^{-4} mol/L was suitable. The influence of buffer solution on the RRS intensity of the system was investigated. Such as SCBS, Tris-HCl, sodium acetate, and PBS on the reaction system was studied. The results indicated that SCBS was the best buffer solution of the reaction system. Therefore, in this case, we used the SCBS of pH 5.6 and 6.6 to investigate the effect of pH on the RRS intensity (Fig.7b). The SCBS (0.1 M) buffer solution 300 μL (Fig.7c) of pH 6.0 was then chosen as reaction acidity for the system. We investigated the factors of reaction time influencing the RRS of the system. The RRS of Ag_2Se QDs increased quickly in the presence of conA, and reached stability in 10 min. Therefore, 10 min was chosen for further experiments (Fig.7d). The salt effect on the system was also investigated. The intensity of RRS did not change obviously when some NaCl was added (the figure was not showed), which suggested that electrostatic attraction is not very important in the interaction between Ag_2Se QDs and conA.

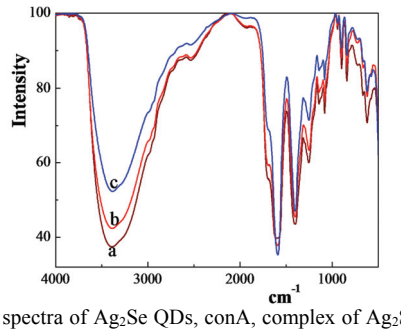
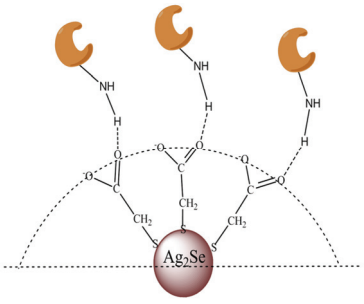


Fig. 6 FTIR spectra of Ag_2Se QDs, conA, complex of Ag_2Se QDs and conA. a: Ag_2Se QDs; b: conA; c: complex of Ag_2Se QDs and conA.



Scheme 2 The structure of Ag_2Se QDs-conA and the model of the hydrogen bonds between CdSe QDs-conA.

The sensitivity and selectivity of Ag_2Se QDs to conA

Under the optimum conditions, the enhanced RRS intensities of the system are determined at the maximum scattering wavelength. As indicated in Fig. 4, the RRS of the Ag_2Se QDs is sensitive to conA and linearly increase with the concentration of conA from

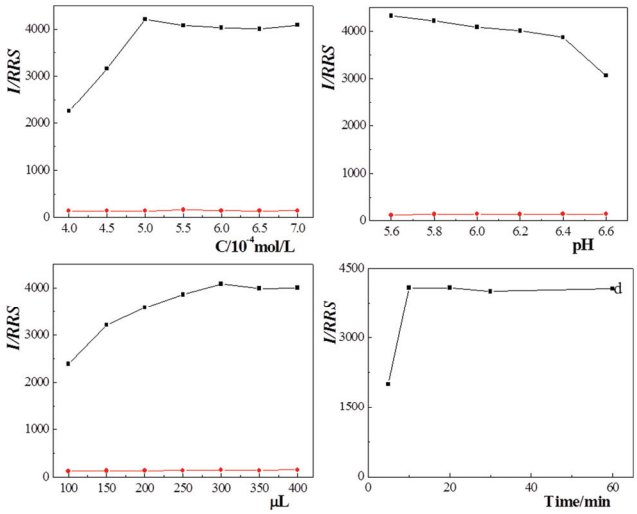


Fig.7 a: The effect of Ag_2Se QDs concentration on RRS intensities. SCBS buffer 300μL; pH=6.0; conA 35 μg/mL. b: The effect of pH on RRS intensities. Ag_2Se QDs 5×10^{-4} mol/L; SCBS buffer 300 μL; conA, 35 μg/mL. c: The effect of amount of SCBS on RRS intensities. Ag_2Se QDs, 5×10^{-4} mol/L; pH=6.0, conA, 35 μg/mL. d: The time-dependent RRS of the system after addition of conA.Ag_2Se QDs, 5×10^{-4} mol/L; pH=6.0; SCBS buffer 300 μL; conA35 μg/mL.

0.27 μg/mL to 35 μg/mL and the limit of detection is 0.08 μg/mL. The linear regression equation is $I = 86 + 118C$ (where C is the concentration of conA, μg/mL).The relative standard deviation (RSD) for eleven replicate detections is 3.6%. It can be seen that this method has a low detection limit and will be a valuable tool for the determination of conA.

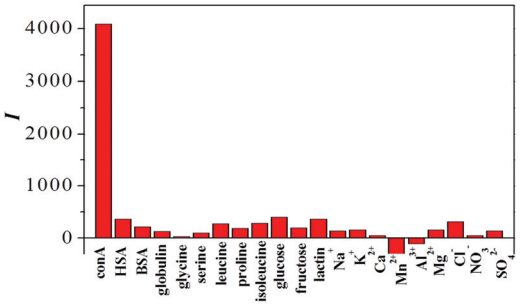


Fig. 8 Selectivity of Ag_2Se QDs probe towards conA. Ag_2Se QDs, 5×10^{-4}mol/L; pH=6.0; SCBS buffer 300μL; conA35 μg/mL.The concentrations of all samples were 5mmol/L.

Further study on the RRS response of the Ag_2Se QDs to various analytes shows good selectivity of the present assay for conA. As shown in Fig.8, only conA cause a significant increase in relative RRS intensity of Ag_2Se QDs, while other species have no evident effect on the RRS intensities. The results demonstrate that physiological levels of cation, anion, amino acid and small biomolecules do not interfere with the detection. Specially, large molecules such as BSA and HSA level of 5 mmol/L do not interfere with the detection, under the optimum condition, conA, BSA and HSA have negative charges, besides, isoelectric point of BSA and HSA is lower than conA. Therefore, there is a strong electrostatic repulsion between Ag_2Se QDs and BSA/HSA and the electrostatic repulsion may be stronger than other weak binding forces such as hydrogen bonding, hydrophobic interaction, and

aggregation interaction of biological macromolecules.

Application of the Ag_2Se QDs to the detection of conA in human serum samples

The potential application of the proposed bioassay is further demonstrated by detecting conA in human serum samples. The serum samples are ultrafiltrated to eliminate the RRS background of serum. The quantitative recoveries (94-106%) of spiked conA also indicate no interference from such ultrafiltrated serum samples (Table 1). The above results demonstrate that the developed biosensor offers great potential for specific detection of conA in biological fluids.

Table 1 Results of recoveries of heparin in serum samples

sample	Found (μg/mL)	Added (μg/mL, n =5)	Total found (μg/mL, n =5)	Recovery %
1	0	15	14.06	94
2	0	25	25.06	100
3	0	35	37.28	106

Conclusions

In summary, we have coupled Se powder reduction with the binding of silver ions and glycine to successfully realize the synthesis of small size (3 nm), less cytotoxic, and water-dispersible Ag_2Se QDs at 85 °C. A RRS bioassay for conA determination is established via a strategy of the target involved assembly of the prepared Ag_2Se QDs. This method exhibits favorable in a range and satisfactory selectivity and successfully applied and conA determination in human serum samples. The Ag_2Se QDs will be used as excellent scaffolds for the foundation of RRS-based protocols to directly detect target molecules.

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Notes and references

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The authors declare no competing financial interest.

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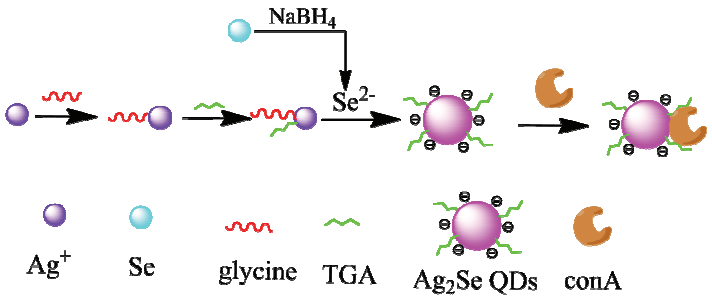
Graphical Abstract



Schematic illustration for fabricating TGA and glycine modified Ag_2Se QDs for RRS detection of conA