Functionalized ZnSe Quantum Dots as Luminescent Tags in High-Throughput Biological Assays

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Introduction

Nanocrystals (quantum dots) of II-VI semiconductors, such as CdSe and ZnSe, are exotic materials exhibiting size-dependent properties [1-5]. Of great interest is their size-dependent luminescence which arises from the confinement of optically-excited electron-hole pairs (excitons) by the boundary of nanocrystals whose size is smaller than the wavelength of an exciton. As the particle size decreases below a certain threshold (e.g. ~9nm for ZnSe) the luminescence peak shifts towards lower wavelengths. This property has sparked significant research activity on the use of such materials as luminescent tags for biomolecules [6-10]. Luminescence from quantum dots can be excited by all wavelengths lower than the emission wavelength. These materials also exhibit high brightness, excellent photo stability and narrow emission, making them superior to common fluorophores used for biological tagging. Furthermore, the tunability of their luminescence has sparked interest for developing multiplexed biosensors that can simultaneously track several biomolecular interactions [11-14].

One of the essential requirements for bioconjugation of nanocrystals and biomolecules is the aqueous solubility. Several methods are discussed in the literature for solubilizing the high-quality colloidal semiconductor quantum dots that are traditionally synthesized in hydrophobic solutions into an aqueous environment [6, 11, 14-23]. The most common method is exchanging the nanocrystal hydrophobic surface with a thiolated molecule having a free carboxyl group facing the solution that warrants water solubility [11, 13-19]. Quantum dots encapsulated by surface-capping molecules can selectively attach to a substrate via covalent interactions, i.e., chemical bonding, and noncovalent interactions, such as hydrophobic, ionic, van der Waal forces, and specific hydrogen bonding. Biological molecules such as biotin or oligonucleotides can be coupled to the surface of water-soluble semiconductor quantum dots using mono- or bifunctional cross-linker molecules, or they can be adsorbed by electrostatic and hydrophobic attraction. There are many strategies that have been developed for bioconjugation of quantum dots [6, 13-15, 23-26].

In this work we describe several techniques to make ZnSe quantum dots water-soluble. We are also developing techniques for coupling water-soluble ZnSe quantum dots with various oligonucleotide sequences.

Experimental Section

Water-soluble ZnSe nanocrystals synthesis

Highly luminescent ZnSe nanocrystals with hydrophobic surface capping were synthesized in a well-mixed batch reactor which contained hexadecylamine (HDA) as the coordinating solvent. The precursors were diethylzinc diluted in heptane and selenium mixed with trioctylphosphine (TOP). The mixture of precursors was injected into the reactor at 310ºC and the temperature was subsequently dropped to 280ºC and kept constant during nanocrystal growth. In this process, the processing time determines the average size and luminescence wavelength of the nanocrystals, while reaction temperature and mixing efficiency control the growth rate of the nanocrystals. Samples of the reaction solution (0.5ml) were removed at regular intervals (3-5mins) and injected into butanol (BuOH, 4ml) to form crude nanocrystals-

BuOH solution; the growth of nanocrystals was monitored by taking the emission spectra of these sample solutions. The fluorescence emission of the ZnSe nanocrystals capped by HDA/TOP was measured by using the SPECTRAmax GEMINI Dual-Scanning Microplate spectrophotometer, while the excitation wavelength was 280nm and the measuring temperature is 27~29ºC. To improve the photoluminescence quantum yields of ZnSe QDs, ZnSe/ZnS core-shell structure nanocrystals were synthesized which contained HAD, tri-n-octylophosphine oxide (TOPO) as coordinating solvent. The synthesis was performed by the following method: the mixture of HAD/TOPO (3:1) was heated to 190°C under vacuum for 1hr and then cooled to 60°C after which 0.5ml of TOP was added. 4ml ZnSeQDs dispersed in hexane was transferred into the reaction vessel and pumped off the solvent. Equimolar amounts of the precursors were dissolved in 2ml of TOP, when the desired temperature of coordinating solvents was reached; the Zn and S precursors were added dropwise to the vigorously stirring reaction mixture over a period of 5-10min. After the addition was complete, the mixture was cooled to 90°C and left stirring for several hours.

To prepare water-soluble ZnSe quantum dots with a positively charged hydroxyl-capping, the original solution of ZnSe quantum dots in the HDA/TOP coordinating mixture was first dissolved in chloroform. The chloroform-ZnSe quantum dots- HDA/TOP solution was subsequently transferred into a methanol-mercaptoethanol-HCl solution until the particles flocculated. Immediately after the flocculation, deionized water was added to the suspension, resulting in a two-phase system (with water forming the upper phase and chloroform the lower). To transfer the nanocrystals into the water phase, the solution mixture was stirred 10mins using magnetic stirrer with stirring speed 600rpm and subsequently allowed to phase separate. The synthesis of ZnSe quantum dots capped with HDA/TOP and their subsequent transfer into water were performed under nitrogen atmosphere. No additional steps were required for purification of the aqueous solution of ZnSe quantum dots, since both TOP and HDA remain in the chloroform rather than in the water phase. Ligands exchange with mercaptocarboxylic acids were performed by mixing ZnSe QDs with mercaptocarboxylic acids, then heating and stirring the mixture with appropriate temperature and time. After the surface exchange was complete, suitable solvent and deprotonation reagent were added to the mixture, then taking centrifugation to separate the water-soluble ZnSe QDs from the solvents. Dissolving the ZnSe QDs in hexane then adding sufficient surfactants such as sodium lauryl sulfate or Span 60 can also make the QDs water-soluble. After evaporate hexane, the resulting solid residue can be dissolved in water to give a clear solution whose quantum yield was approximately the same as the initial sample.

Bioconjugation

Several strategies will be tried to combine biomolecules with the water-soluble ZnSe quantum dots. One method is activating the hydroxyl-capped quantum dots with carbonyl diimidazole (CDI) to form imidazole-carbamate groups at the quantum dots surface, then these activated quantum dots can be coupled with aminated oligonucleotieds directly by covalent bonding. Another ways are using different bifunctional cross-linkers. These cross-linkers normally have one functional end group which can reacts with a primary amine to form an amide bond (connecting with DNA) and the other end group which can reacts with the thiol group on the nanocrystal surface while covalently linking the oligonucleotide to the quantum dots surface.

Results and Discussion

Synthesis of Quantum Dots

The production of monodisperse colloids requires uniform mixing and a temporally discrete nucleation event followed by slower controlled growth of the existing nuclei. Rapid injection of precursors raises the precursor concentration in the reactor leading to super-saturation. As a result, the nucleation process occurs very rapidly. As long as the consumption of precursors by the growing colloidal nanocrystals is not exceeded by the rate of precursor addition to solution, no new nuclei form and the growth of small crystals is controlled by diffusion. The production of monodisperse nanocrystal populations under such conditions requires instantaneous nucleation and perfect mixing. During the ZnSe QD synthesis process, samples were collected at specific time intervals and were transferred into vials containing butanol. The optical fluorescence emission of the butanol solutions was subsequently measured. Fig.1 shows the evolution of the fluorescence emission peak as function of reaction time. The wavelength of each peak increases with reaction time which means that the size of the nanocrystals increases with the reaction time. And the emission yields also become stronger, that is because with the reaction time increases, more small clusters coalesce into nanocrystals that increase the concentration of nanocrystals in the reaction medium. Growing ZnS shell on ZnSe QDs has improved the strength of luminescence obviously, as showing in Fig.2. The critical factors are the precursor's amount and injection temperature which were determined on the size of ZnSe QDs. Otherwise; it is difficult to obtain crystalline structure.

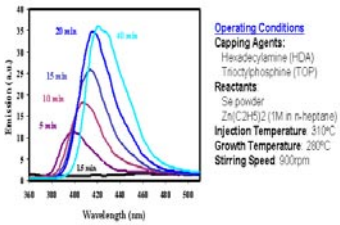

Fig.1 Evolution of ZnSe Nanocrystals UV-Violet-Blue Fluorescence Shifts with Reaction Time

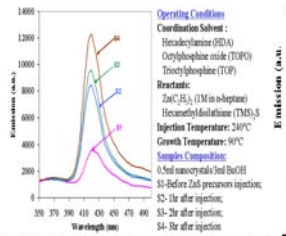

Fig.2 Evolution of ZnSe/ZnS Core-Shell Nanocrystal Fluorescence with Reaction Time

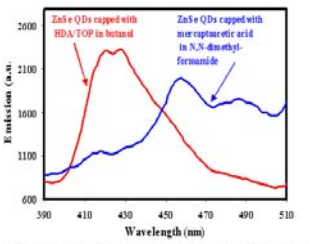

Fig. 3 ZnSe QDs Surface Modification with Mercaptoacetic Acid

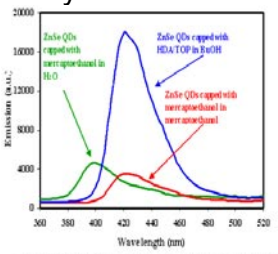

Fig.4 ZnSe QDs Surface Modification with Mercaptoethanol

Preparation of water-soluble ZnSe nanocrystals

Water-soluble ZnSe nanocrystals were prepared using both a reported technique involving mercaptoacetic acid as capping agent [18] and a new technique involving mercaptoethanol. A ligand exchange method was used to functionalize the ZnSe quantum dots with macaptoacetic acid. The acid reacted immediately with a suspension of HDA/TOP-stabilized ZnSe quantum dots in N, N,-dimethylformamide to form carboxylic-acid-functionalized nanocrystals. Unfortunately, the resulting nanocrystals were not stable in water and coalesced to form larger particles as demonstrated by the observed red shift in the fluorescence peak shown in Fig.3. The structural quality of the particles also deteriorated resulting in a broad fluorescence peak.

To prepare stable and luminescent water-soluble ZnSe quantum dots, a procedure using mercaptoethanol as the capping agent was developed. Since the Zn-thiol bond is much stronger than the Zn-amine bond, mercaptoethanol will replace the HDA/TOP capping layer of the ZnSe nanocrystals as soon as it is added to the original sample. Fig.5 shows the surface functionalization result with mercaptoethanol. We can see that the surface exchange resulted in nanocrystal coalescence and recombination, but the majority of nanocrystals keep their properties. The mercaptoethanol was mixed with same molar amount HCl before it was used as capping agent, then the mercaptoethanol-capped nanocrystals are surface charged and therefore stabilized in polar medium. When trying to transfer the nanocrystals capped with

mercaptoethanol into water, the small size particles tend to go to the water phase. The difference in emission strength is because of the difference in the nanocrystal concentration. The concentration of nanocrystals in water and in the mercaptoethanol-chloroform mixture are much lower than the initial concentration of HDA/TOP-capped nanocrystals in butanol.

Conclusions

In this study, a flexible synthesis of water-soluble ZnSe quantum dots capped with mercaptoethanol has been described. A new procedure that uses mercaptoethanol as surface stabilizer was developed. The resulting nanocrystals were arrested by precipitation in water and no post-preparative steps were required to clean the crude nanocrystals solution. This method is faster and simpler than reported techniques for synthesis of water-soluble quantum dots. Experiments are in progress for functionalizing the ZnSe quantum dots with short oligonucleotides. To monitor DNA hybridization by measurements of fluorescence intensity, several bioconjugation strategies will be investigated. Ongoing experiments in our laboratory aim to investigate the kinetics of DNA hybridization and extend our findings to simultaneous detection of multiple DNA probes functionalized with nanocrystal of different sizes. Successful attainment of these goals will have important implications for the use of nanocrystals in high-throughput biological assays such as real time PCR and DNA microaarys.

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