127e Synthesis, Functionalization and Clinical Diagnostic Applications of Znse Quantum Dots

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Fluorescent labeling of biological molecules is a technique that is used widely for analytical purposes in biotechnology and bioengineering. It typically involves the use of an organic dye molecule linked to a moiety that selectively bonds a particular biological molecule, allowing the detection of the latter by the fluorescence of the dye molecule. Semiconductor nanocrystals or quantum dots have emerged as a new class of fluorescent markers with distinct advantages over the traditional organic dyes [1-3]. Their attractive properties include narrow, symmetric, and very bright emission, continuous excitation by any wavelength smaller than the emission wavelength, resistance to photobleaching, as well as excellent optical and chemical stability that allows their use in lengthy experiments. The ability to synthesize different populations of quantum dots with narrow emission spectra permits multiplexing, a property that is very important for simultaneous detection of several analytes, that would be very tedious and expensive if done sequentially.

The focus of this work is the development of new strategies for functionalizing the surface of II-VI nanocrystals (e.g., ZnSe, CdSe, etc.) and their use in new applications in biological sensing and DNA analysis. Highly luminescent ZnSe quantum dots have been synthesized using a liquid-phase technique utilizing a hot coordinating solvent in which the nanocrystals are grown by injection of suitable precursors [4]. The synthesis of ZnSe quantum dots is carried out in a stirred batch reactor containing liquid hexadecylamine at 260 degrees C. The precursors are diethylzinc diluted in heptane and selenium powder dispersed in trioctylphosphine. The mixture of reactants is injected into the batch reactor and the time of reaction is used to determine the size and luminescence emission wavelength of the quantum dots. Capping of the ZnSe quantum dots with a ZnS layer to obtain a core-shell structure was found to increase their quantum yield and, as a result, the luminescence intensity, without significantly affecting the emission wavelength.

Surface ligand exchange reactions with mercapto-alcohols and mercapto-acids, as well as surface modification with surfactants were investigated to identify the best approach for obtaining water-soluble ZnSe quantum dots that are stable in an aqueous solution, highly luminescent and suitable for biological applications. The effects of the operating conditions during post-processing, such as temperature, sequence of chemical treatments, relative amount of reactants used, etc., were studied to develop optimal processing techniques yielding functionalized quantum dots that retain their attractive luminescence characteristics over several days.

Conjugating water-soluble ZnSe and (ZnSe)ZnS core-shell quantum dots with oligonucleotides was found to increase their fluorescence emission intensity 5 to 8 times. Quantum dots conjugated with longer DNA strands exhibited stronger fluorescence emission intensity than the ones conjugated with shorter strands. At the same time, the stability of quantum dots in water was improved. Hybridization kinetics for water-soluble quantum dots functionalized with short oligonucleotides were investigated in order to identify the relationship between DNA structures and fluorescence emission intensity. The results can be very useful for developing new DNA detection strategies.

Ongoing experiments in our laboratory aim to investigate and develop multiplexed assays for the simultaneous detection of multiple DNA probes functionalized with quantum dots of different sizes and emitting at different wavelengths. Substitution of fluorescent proteins by quantum dot labels in ELISA-type immunoassays is also under investigation aiming to improve the sensitivity and range of such assays. Successful attainment of these goals will have important implications for the use of quantum dots in high-throughput clinical diagnostic applications, such as real time PCR, DNA microarrays, and immunodiagnostics.

References

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