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QD barcodes for biosensing and detection

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Abstract— **Multiplexed nanobarcodes have been prepared with quantum dots (QDs) and amphiphilic copolymers consisted of hydrocarbons and maleic anhydride groups. In homogenous solution, the QD-polymer complexes self-assemble into nanobeads with narrow size dispersity, which has been previously achieved only for micrometer-sized beads in the presence of solid supports. More than 250 QDs can be loaded into a nanobead of 100 nm in diameter. Using this new generation of nanoprobe, sensitive detection of human prostate specific antigen (PSA) has also been demonstrated. This new technology is expected to open new opportunities in nanoparticle-based ultrasensitive and multiplexed detection and sensing.**

Keywords—**nanoparticles, quantum dots, barcodes, molecular, imaging, sensing, fluorescence, self assembly**

I. Introduction

The development of fluorescent probes that are stable, compact, and significantly brighter than traditional fluorophores (*e.g.* organic dyes and fluorescent proteins) is of considerable interests to many research areas including DNA sequencing, gene expression profiling, molecular imaging, fundamental biophysics, as well as biosensing. Despite recent success with semiconductor QDs, which are 20-50 times brighter than single dye molecules, fluorescent probes with improved brightness and multiplexing capability are highly desirable for analysis of low-abundance targets in bioassays including single molecule detection, immunoassays, and fluorescence in situ hybridization, and for understanding of complex human diseases involving a large number of genes and proteins (*e.g.* cancer and atherosclerosis).

In this context, optical encoding technologies by multiplexing colors (wavelengths) and fluorescence intensities of fluorophores have become an attractive strategy because a large number of high-brightness probes can be readily produced. Compared with organic fluorophores, semiconductor QDs are of particular importance to this application because of their favorable optical properties such as simultaneous excitation of multiple colors with a single light source, minimal spectral overlap between adjacent colors, and remarkable photostability. For example, we have previously reported the preparation of QD-tagged optical barcodes by incorporating multicolor QDs into mesoporous microspheres at predefined intensity ratios. The use of 10 intensity levels of 6 colors has a theoretical coding capacity of one million. The encoded microspheres are highly fluorescent and uniform, because they typically contains 5,000 – 10 million QDs per bead depending on the microbead size and doping level. Unfortunately, because of the large size (typically 1-15 μm), these QD-doped microspheres are not suitable for applications such as gene, protein and cell labeling. Toward the development of uniform and bright QD-encoded beads in the nanometer regime, multicolor doping with tunable fluorescence intensity ratios, low level of fluorophore self-quenching, precise control of the location and spacing of embedded nanoparticles has not been achieved.

II. Methodology

We have developed a new approach for preparation of QD-tagged nanobeads based on self-assembly of nanoparticle-amphiphilic polymer complexes in homogeneous solution. This new generation of fluorescent probe is uniform in size, thousands of times brighter than single organic dyes, stable against photobleaching, and free of 'blinking' effect. Furthermore, this self-assembly procedure is versatile in preparation of a variety of nanostructures in that the distribution and location of the embedded QDs can be precisely controlled.

An important finding was an amphiphilic alternating copolymer that is not only capable of encapsulating multicolor QDs but also capable of preventing them from forming irregular aggregates. In contrast to nanoparticles clustered inside block copolymer micelles, the QDs are pre-protected by the amphiphilic alternating polymers and thus preventing them from touching to each other. The encapsulated QD-polymer complexes do not phase separate into hydrophobic core and hydrophilic shells. Instead, they self-assemble into nanobeads with QDs distributed inside homogeneously. In this process, QDs and poly(maleic anhydride-octadecene) (PMAO) bond to each other via multivalent hydrophobic interactions. The QD-PMAO conjugates are highly soluble in tetrahydrofuran (THF) but form aggregates in polar solvents such as dimethylformamide (DMF). A solvent gradient created by slow addition of DMF leads to self-assemblly of highly fluorescent nanobeads with narrow size dispersity. The PMAO polymer plays a critical role in controlling the nanobead size and size distribution.

III. Results

Using single color QDs, we systematically investigated the conditions for nanobead formation. Dynamic light scattering measurements indicate that QDs remain single in

THF/DMF solvent mixture when DMF concentration is under 20% in volume. Increasing DMF concentration from 20% to 30% leads to spontaneous formation of QD-nanobeads as indicated by the size shift from approximately 10 nm to 100 nm. Higher concentration the nanoparticles can result in formation of nanobeads up to 500 nm in size. This self-assembly process is highly efficient at encapsulating QDs. Fluorescence measurement of single QDs left in the supernatant after isolation of nanobeads by centrifugation indicates that more than 95% of QDs are incorporated into the nanobeads. To further enhance the nanobead stability, such as preventing potential QD leaching or release in bioassays, the polymer chains are crosslinked with small-molecule diamines. Due to the rich anhydride contents in PMAO polymer and the high reactivity between anhydrides and primary amines, no catalytic reagents are needed to crosslink the polymers into a stable network. Similar reaction of crosslinking of neighboring polymer chains has been previously demonstrated with block copolymers for enhanced micelle stability. Following the nanobead formation, the resulting fluorescent nanobeads must be made water-soluble for biological applications. We found that the nanobeads isolated by centrifugation cannot be directly suspended in aqueous buffers. This is understandable because majority of the anhydride groups are not hydrolyzed into carboxylic acids, and thus the nanobeads are not sufficiently hydrophilic. We solved this problem by using a slow dialysis procedure against Tris buffer, for efficient hydrolysis of the anhydride groups as the solvents gradually change from THF/DMF mixture to aqueous solution. The resulting nanobeads are stable in aqueous buffers for at least several months.

We have further probed the possibility of making QD-nanobeads of different internal structures. Because block-copolymer based micelle formation is an efficient method for making core/shell structures with nanoparticles clustered inside the hydrophobic core and hydrophilic polymer segments forming a corona layer, we focused our effort on the homogeneous structure and core/shell structure with QDs in the shell layer. When amphiphilic polymers are first used to quickly nucleate into plain nanobeads (without QDs), subsequent deposition of QD-polymer complex will render the QDs to localize in the shell layer. By using different concentrations of polymer and polymer-QD building blocks, more complicated 3-D structures, such as a gradient distribution of QDs, could be fabricated. This kinetics-based manipulation of particle internal structure without changing the chemistry of the building blocks is highly desired in nanomaterial self-assembly.

IV. Discussion

We have developed a new generation of encoded nanobeads using QD-alternating copolymer complexes. As a result of the new bead formation mechanism, a large number of QDs can be loaded into a nanobead and the spatial distribution of the embedded nanoparticles can be manipulated. We envision that if additional types of nanoparticles are used (*e.g.* polymer coated metallic nanoparticles and magnetic nanoparticles), multi-functional nanobeads with spatially separated functionalities could be prepared. For example, QDs can be embedded in the core, and gold nanoparticles or iron oxide nanoparticles can be put in the shell layer. This spatial separation might offer advantages over the homogenous distribution because it's known that gold nanoparticles and iron oxide nanoparticles are efficient quenchers of fluorescent QDs.

Reference

Yang J., Dave S.R., and Gao X.H., Quantum dot nanobarcodes: epitaxial assembly of nanoparticle-polymer complexes in homogeneous solution, *J. Am. Chem. Soc.* **130**, 5286-5292, 2008.

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