J|A|C|S COMMUNICATIONS

Published on Web 03/17/2010

Small and Stable Sulfobetaine Zwitterionic Quantum Dots for Functional Live-Cell Imaging

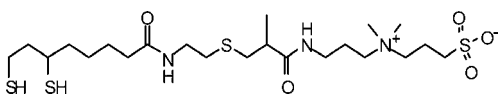
Eleonora Muro,[†] Thomas Pons,[†] Nicolas Lequeux,*[,†] Alexandra Fragola,[†] Nicolas Sanson,[‡] Zsolt Lenkei,[§] and Benoit Dubertret[†]

Laboratoire Physique et Etude des Matériaux, CNRS UPR5, Laboratoire de Physico-chimie des Polymères et Milieux Dispersés, UMR 7615, and Laboratoire de Neurobiologie, CNRS UMR7637, Ecole Supérieure de Physique et Chimie Industrielle, Université Pierre et Marie Curie, 10 rue Vauquelin, 75005 Paris, France

Received February 1, 2010; E-mail: nicolas.lequeux@espci.fr

Strategies to make quantum dots (QDs) that are water-soluble and biocompatible are guided by several criteria: (*i*) small size, enabling access to confined biological compartments; (*ii*) stability over a large pH range and at elevated salt concentrations; (*iii*) low nonspecific adsorption; and (*iv*) easy functionalization. Here we describe a novel QD surface chemistry based on bidentate sulfobetaine zwitterionic ligands that meets all of these criteria. Indeed, cap exchange with dithiol ligands such as dihydrolipoic acid (DHLA) provides a stable and compact QD surface,[1,2] but the current DHLA-based ligands suffer from several disadvantages. DHLA QDs are negatively charged and exhibit strong nonspecific electrostatic interactions with their biological environment. These interactions can be limited by coupling polyethylene glycol (PEG) strands to DHLA.[3] However, PEG QDs tend to aggregate in high-salinity buffers, and the PEG polymer substantially increases the size of the probe, which may restrict access to confined spaces and prevent renal elimination in *in vivo* applications.[4] In addition, the effect of PEG density and conformation at the nanoparticle surface on its antibiofouling capacity is still not well understood.[5] Zwitterionic groups present an alternative solution to PEG as an antibiofouling coating.[6−9] Liu et al.[9] showed that cysteine-coated quantum dots (Cys-QDs) exhibit much smaller diameters, weak nonspecific interactions with proteins, and good resistance to pH variations and salinity. However Cys-QDs quickly aggregate after a few days, even in the presence of a large ligand excess, because of the low stability of monothiol anchor groups. Here we present the synthesis and properties of QDs capped with a novel DHLA−sulfobetaine (DHLA−SB) ligand (Scheme 1). These DHLA−SB QDs are small and exhibit excellent stability with time, pH, and salinity. These zwitterionic QDs also can easily be functionalized with biotin or streptavidin by mixing DHLA−SB with other functional ligands in controlled ratios, allowing specific staining of membrane receptors and their tracking during recycling into living cells.

Scheme 1. Chemical Structure of the DHLA−Sulfobetaine Ligand



DHLA−SB ligands were synthesized in three reaction steps: First, *N*,*N*′-dimethyl(acrylamidopropyl)ammonium propanesulfonate (Ralu@Mer SPP, RASCHIG GmbH, Germany) was functionalized with a terminal amine by addition of 2-aminoethanethiol to the allyl group. This was then coupled to thioctic acid via an amide bond. Finally, DHLA−SB was obtained by ring opening of the 1,2-dithiolane end group using sodium tetrahydridoborate. Cap exchange of CdSe/ZnS and CdSe/CdS/ZnS QDs (emitting at ∼600 nm) with DHLA−SB was performed using a biphasic exchange method: QDs were dispersed in chloroform and stirred at room temperature with a 20 mM aqueous NaCl solution of DHLA−SB in large excess. Phase transfer of QDs from the organic phase to the aqueous phase occurred in less than 30 min, and the solution was incubated at 60 °C overnight to complete the cap exchange. The QDs were purified by ultrafiltration and ultracentrifugation according to previously described protocols [see the Supporting Information (SI)].

DHLA−SB QDs retained high fluorescence quantum yields in water and pH 8 borate buffer, with typical values of 20−30% for CdSe/ZnS and 50−60% for CdSe/CdS/ZnS multishell QDs (vs ∼70% in hexane). In contrast to Cys-QDs, DHLA−SB QDs were stable for at least several months when stored at 4 °C thanks to the more stable dithiol anchor group. Alternatively, these QDs could be dried, stored, and resuspended in water before use. DHLA−SB QDs showed excellent stability even in saturated NaCl solutions, while PEG-capped or charge-stabilized nanoparticles aggregate (Figure 1 and Table S1 in the SI). Another interesting feature of the DHLA−SB ligand is that the charges of the zwitterionic components are insensitive to pH, providing colloidal stability over the pH range 4−13. The hydrodynamic diameters of DHLA−SB QDs were typically 10−10.5 nm for ∼4.5 nm CdSe/ZnS QDs, as measured by dynamic light scattering (DLS); these are comparable to the values for DHLA-capped QDs.[10] The contrast with the much smaller hydrodynamic sizes reported for Cys-QDs[9] is probably due in part to methodological differences (see the discussion in the SI).

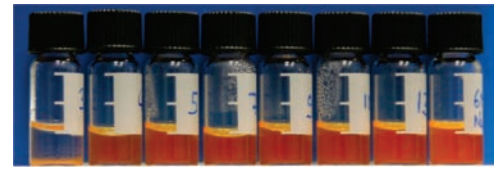


Figure 1. DHLA−SB QDs in pH 3, 4, 5, 7, 9, 11, 13, and saturated NaCl solutions (left to right).

Application of QDs to biological imaging requires the capacity to stably and specifically conjugate a wide variety of biomolecules. Sulfobetaine presents a crucial advantage in that, in contrast to cysteine, it does not contain any primary amines or carboxylic acids. This greatly simplifies subsequent conjugation to biomolecules, as sulfobetaine is inert with respect to most bioconjugation reaction schemes. Control over the number and nature of reactive functional

[†] Laboratoire Physique et Etude des Matériaux.
[‡] Laboratoire de Physico-chimie des Polymères et Milieux Dispersés.
[§] Laboratoire de Neurobiologie.

10.1021/ja1005493

groups can be achieved by introducing specific proportions of functional ligands during cap exchange. For example, the QDs could be exchanged with different mixtures of DHLA−SB and DHLA−PEG−COOH. The resulting QDs showed different mobilities under gel electrophoresis, consistent with a progressive incorporation of negatively charged carboxylic acids on their surfaces (Figure S3).

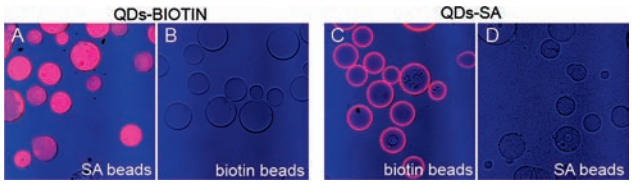


Figure 2. Specificity of functionalized QDs. QDs−biotin specifically bound streptavidin agarose beads (A) but did not bind biotin agarose beads (B). Similarly, conjugated QDs−SA specifically bound biotin agarose beads (C) but did not bind streptavidin agarose beads (D). The presented images are merged fluorescence (red) and transmission (blue) images.

We next demonstrated the functionalization of DHLA−SB QDs with biomolecules such as biotin and streptavidin (SA) and the absence of nonspecific adhesion *in vitro*. We coated the QDs with mixtures of DHLA−SB ligands and either DHLA−PEG−biotin or DHLA−PEG−COOH for subsequent coupling to SA using the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide (NHS) standard bioconjugation chemistry. This commonly used coupling technique allowed us to obtain stable bioconjugates that retained the high quantum yield and stability of the nanoparticles as well as the functionality of the conjugated biotin molecule and SA protein. Figure 2 shows the high specificity of (A, B) QDs−biotin and (C, D) QDs−SA tested on (B, C) biotinylated and (A, D) streptavidin agarose beads. Specific-to-nonspecific fluorescence ratios were on the order of 3000.

We finally demonstrated the application of zwitterionic QDs−SA as probes in a biological living system by specifically labeling the cannabinoid receptor 1 (CB1R) and tracking its intracellular traffic. CB1R is one of the most abundant G-protein-coupled receptors (GPCRs) in the central nervous system and is a good model system since it produces constitutive internalization in many cellular types. Here we showed the tracking of CB1R recycling with zwitterionic QDs−SA in living HEK cells that permanently express FLAG−CB1R−GFP.[11] One of the main challenges in nanoparticle cell staining is the mitigation of nonspecific interactions. These zwitterionic QDs−SA showed remarkably minimal nonspecific binding to cell membranes, as demonstrated by control cells exposed to QDs−SA (Figure S5B), which exhibited a fluorescence signal indistinguishable from the autofluorescence (Figure S5A). We attribute this "furtivity" to the high water solubility and global neutral charge of the DHLA−SB surface coating. In the presence of the biotinylated antibody, QDs−SA specifically recognized the pool of receptors (FLAG−CB1R−GFP) blocked at the cellular membrane at 4 °C (Figure 3A,B squares and merge). Furthermore, they retained the high brightness property and the functionality of SA after incubation at 37 °C, as they were still colocalized with the internalized pool of CB1R−GFP (Figure 3C,D squares and merge). The cellular activity was apparently unaffected by the probe, since the CB1R recycling process proceeded normally.

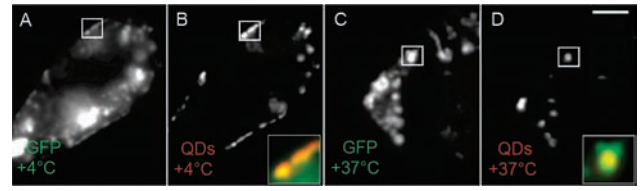


Figure 3. Specificity of QDs−SA in living cells. QDs−SA specifically bound the biotinylated CB1R−GFP receptor. Just after staining at 4 °C, the membranal pool of the receptor was recognized by QDs−SA: (A) CB1R−GFP; (B) CB1R·QDs−SA. At 37 °C, these QD-stained receptors were internalized, and they were still colocalized with the same pool of CB1R−GFP: (C) CB1R-GFP; (D) CB1R·QDs−SA. Scale bar 2 μm.

In conclusion, DHLA−SB represents a novel class of QD surface chemistry that combines preserved QD optical properties, excellent stability, and small size. It can easily be mixed with controlled ratios of functional ligands for subsequent efficient bioconjugation. In addition, the zwitterionic coating provides excellent antibiofouling properties, as demonstrated by the absence of nonspecific adhesion in live-cell imaging (Figure S6). We expect that these promising features will enhance the performance of QDs as probes in many biological imaging applications, such as long-term single-molecule tracking or simultaneous multicolor imaging. It may also be used in other types of semiconducting or metal nanoparticles and may benefit *in vivo* applications, where small size and stability are crucial parameters for targeting and renal elimination.

Acknowledgment. This work was funded by ANR.

Supporting Information Available: Experimental procedures for ligand and QD synthesis, cap exchange, purification, DLS, gel electrophoresis, bioconjugation, and cell imaging. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA1005493