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# Hydrophilic colloidal quantum dots with long peptide chain coats

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ABSTRACT

Here, the transition of colloidal CdSe quantum dots (QDs) from hydrophobic to hydrophilic environments after coating the surface with long peptide chains of membrane scaffold proteins (MSP) is reported. The intermediate step included the solubilization of QDs with detergents, where *n*-octyl glucoside was the most promising ligand. Furthermore, size analysis by fluorescence correlation spectroscopy, gel filtration and atomic force microscopy suggested that the obtained QD-MSP conjugates were primarily discoidal and were likely formed from single QDs tightly encircled by helix belts. In addition, Fourier-transformed infrared spectroscopy analysis confirmed the preservation of the secondary structure of most proteins during conjugate formation, with no signs of denaturation. The obtained QD-MSP conjugates were optimal in terms of stability in water environments, suggesting that it is possible to obtain QDs with single peptide coats and providing the first guidelines for future research in this direction.

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## 1. Introduction

There is significant pressure on nanotechnology to provide quantum dot-based optical probes for biological studies [1]. The primary reason for using fluorescent quantum dots (QDs) as active probes is the unique luminescent properties of these molecules. The emission signal is narrow and directly depends on the QD size, but the extinction spectrum is broad, suggesting that different probes, based on QDs of various sizes, are simultaneously present in a sample and can be excited using a single laser lane at 405 or 480 nm. The selective excitation of QD populations, using longer wavelengths or two-photon excitation, can still be performed when necessary. QDs can also be decorated with biological molecules, facilitating specific interactions with targets by antibodies [2,3], forming a sensor based on fluorescence energy transfer between the QD and the protein-bound fluorophore [4], or even powering the biological reaction by illumination [5]. QD might also be involved in the light-dependent generation of radicals, particularly oxygen derivatives, for phototherapy; however, these molecules might negatively influence biological systems. Moreover, controlling the surface properties of quantum dots is possible.

One of the major problems in using nanocrystals in biological research is their lack of dispersity in water or instability in water solution. Typical "water soluble" quantum dots (QD), for example, CdTe nanocrystals, comprise a metal core coated with a layer of organic molecules, with a hydrophilic region exposed to water. This coat is typically effective for a short time but, as observed in several studies, does not actually confer long-term protection to nanocrystals [6–8]. The short-term protection of this process might reflect the composition of the QD coat, comprising several molecules. This surface readily interacts with most biomaterials, but not always in a defined manner [9,10]. Therefore, a better coat might be obtained by substituting several small molecules with one or only a few longer molecules enclosing the nanocrystal. This approach would provide a clear advantage, thermodynamically influencing the cover stability. The best candidates for use in cover molecules are long peptide chains. The amino acid composition of polypeptide chains defines the further 3D organization and properties of proteins, that is, hydrophobic and hydrophilic and charged regions. Indeed, amphipathic proteins ensure coverage of the QD surface by the hydrophobic regions, while simultaneously exposing the hydrophilic regions to the environment. Moreover, proteins contain both carboxyl and amino groups and other potential reactive moieties, such as thiols, compared with typical organic QD covers, which contain only one type of group. Currently, most proteins are obtained by expression of heterologous systems, facilitating the control and influence of the properties of the desired protein. Furthermore, it is possible to obtain chimeric proteins with domains that are intended to cover QD and a second domain bearing specific activity (e.g., enzymatic). This strategy might be useful

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in the application of QD both as a fluorescent label and an electron donor.

Proteins have been previously used to functionalize QDs, as mentioned above. However, in such complexes, the protein molecules are attached (e.g., by electrostatic interactions) to QD surfaces that have been previously coated with an organic layer (e.g., mercapropionic acid). To our knowledge, the present study is the first to report the use of peptide chains as direct covers for QDs.

In designing an advantageous QD cover, we were inspired by biology. Nature has evolved a mechanism to stabilize nanometre structures of mixed hydrophobicity in human blood. These structures include lipoproteins that contain various lipids, particularly triglycerides and cholesterol [11]. Apolipoproteins, the protein scaffold of these structures, comprises amphiphilic helices, facing the hydrophobic regions towards lipids and the hydrophilic regions towards the water environment. Original human apolipoprotein (AP-1) has a length of 4 nm and appears as a bended circle. Expressed and purified in slightly modified version, after mixing with lipids, AP-1 forms nanodiscs [12]. These nanodiscs are flat lipid membranes encircled by a protein belt. Derivatives of AP-1, called membrane scaffold protein (MSP), are present in different lengths, enabling the formation of nanodiscs of various sizes, from ~10 to 17 nm in outer diameter [13,14]. Such variation ideally fits the size of QDs, for example, CdSe/ZnS quantum dots with emissions ranging from 500 to 700 nm [15]. Because the morphology of QDs is approximately a sphere, we expect that the organization of MSP on the surface of these molecules might be different than during formation of flat cylindrical nanodiscs. However, we might still take advantage of the wide belting structure with hydrophobic and hydrophilic surfaces to obtain a spherical structure, as occurs in nature for high-density lipoproteins [16].

Covering hydrophobic QDs using water-soluble MSP proteins required the identification of an environment in which both partners might coexist, at least for a time, enabling complex formation. Proteins are denatured and strongly aggregated in typical hydrophobic QDs solvents (e.g., chloroform, toluene, and hexane), but we observed that QDs might be mixed with detergents and suspended in water. Detergents are amphipathic molecules that are used for the isolation and stabilization of membrane protein and protein complexes in water solutions [17], such as photosystem II [18], respiratory mitochondrial super complexes [19] or ion pumps [20]. In water solution, detergents might form micelles. In general, detergent molecules comprise hydrophobic and hydrophilic regions, which might differ between different detergents. The hydrophobic region might be a hydrocarbon unit with several carbon atoms, for example, 8 hydrocarbons in *n*-octyl glucoside (nOG) or 10 hydrocarbons in β-dodecyl maltoside (bDM) and sodium dodecyl sulphate (SDS). There are also detergents that are derivatives of cholane (sodium cholate) or polyethylene chains (e.g., Triton X-100). The hydrophilic region might be charged (e.g., SDS) or neutral (e.g., nOG, bDM). There are also zwitterionic detergents, such as 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate hydrate (CHAPS). The chemical composition influences properties, such as the critical micellization concentration (CMC) and micelle size. Both properties define the feasibility of further detergent removal when necessary. The application of detergents also depends on the preservation of the native structure of protein for activity studies (so-called mild detergents) or complete solubilization for composition analyses. In some applications, as in the present study, the possibility of detergent removal is also important. The best detergent for a given application is typically experimentally selected [17].

Here, we showed a new approach to obtain MSP-QD complexes, enabling the transfer of QDs with hydrophobic ligand coats into aqueous environments. We determined whether the length of MSP influenced the formation of the hybrid complex and provided information on the size and fluorescence properties of the obtained products.

## 2. Experimental methods

### 2.1. Quantum dots

CdSe QDs were synthesized using a protocol similar to that of Nyk et al. [21]. In a typical synthesis, the selenium precursor was produced after mixing 0.4 g (~5 mmol) of selenium powder (95%, Sigma Aldrich) and 10 g of TOP (trioctylphosphine, Sigma Aldrich) in a dry box under an inert atmosphere. Separately, 20 g of TOPO (90% trioctylphosphine oxide, Sigma Aldrich) and 0.25 g of cadmium acetate (Sigma Aldrich) were added to a three-necked flask. The cadmium salt was heated to 100 °C under vacuum with stirring for ~30 min. To remove water and oxygen, the flask was purged with dry nitrogen at 10-min intervals. The transparent solution was subsequently heated to 320 °C under nitrogen and vigorous stirring. At this temperature, the selenium stock solution was swiftly injected into the reaction vessel in a single step. The reaction was stopped at 30 s after the injection by removing the heating mantle and cooling to room temperature. The mixture was precipitated using methanol in an ultrasonic bath and collected by centrifugation at 12,000 rpm for 10 min. The precipitate was dried and dispersed in anhydrous chloroform. The above-mentioned procedure was applied to obtain hydrophobic CdSe QDs using the TOPO ligand on the surface. The concentration of QD the chlorophormic solution was spectrophotometrically determined based on experimental extinction coefficients [15]. The morphology of the CdSe QDs was examined using a FEI Tecnai $G^2$ 20 X-TWIN transmission electron microscope (TEM). For the TEM study, a droplet of the as prepared CdSe QDs dispersed in chloroform was placed onto a TEM copper grid covered with ultrathin carbon (Ted Pella, Inc.) and subsequently dried.

### 2.2. Proteins

Plasmids containing the MSP2N2 (35 kDa) and MSP1D1 (24.7 kDa) genes were obtained from the plasmid repository Addgene (USA). The proteins were expressed in *E. coli* strain BL21. Induction was initiated after the addition of 1 mM IPTG, followed by incubation for 3 h at 37 °C. The proteins were purified according to a previously published method [12] using nickel bead-loaded HisTrap columns (GE Healthcare). The protein purity was electrophoretically determined by SDS-PAGE [22]. The MSP concentration was calculated based on the theoretical extinction coefficient, estimated by using PROTPARAM [23]: 21000 $M^{-1}$ $cm^{-1}$ for MSP1D1 and 37400 $M^{-1}$ $cm^{-1}$ for MSP2N2.

### 2.3. Other chemicals

The detergents used in the present study were derived from Carl Roth GmBh (noctyl glucoside – nOG, sodium cholate – NaCh, sodium dodecyl sulphate – SDS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate hydrate – CHAPS) and Glycon Gmbh (β-*n*-dodecyl maltoside – βDM). Polystyrene beads for detergent removal were obtained from BioRad Int. Other chemicals were obtained from Carl Roth Gmbh and Sigma-Aldrich Int. and were of analytical grade.

Solubilization of QDs and preparation of conjugates using MSP proteins.

Typically, 200 μl of a chlorophormic solution containing 50 μM QDs with a TOPO surface was placed in an agate mortar and dried under a gentle nitrogen stream to obtain a powder. Detergent was added as a powder (~9 mg SDS/~20 mg NaCh/~0.23 mg βDM/~11 mg CHAPS/~22 mg nOG) and mixed with QD powder

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