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Bioconjugation of quantum dots: Review & impact on future application

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ABSTRACT

Nowadays luminescent semiconductor quantum dots (QDs) are widely applied in different areas due to their unique optical properties. QDs can be used as photoluminescent labels with excellent possibilities for high-throughput detection and diagnostics. For most of such applications QDs must be coupled to biomolecules, which often represents a fundamental challenge. Although QDs have a lot of advantages over organic dyes, most of the techniques that have been developed for QD functionalization and bioconjugation, are more complicated than the corresponding techniques for organic fluorescent dyes. Here, the importance of choosing a suitable bioconjugation strategy in different applications, such as imaging and assays is described. The main goal of this review is to give a structured and detailed overview and comparison of the most widely used conjugation strategies in function of the active groups (carboxyl, amine, thiol, epoxy, hydroxyl and aldehyde groups) present on QD surface.

Contents

Abbreviations: ADH, adipic acid dihydrazide; bp, base pair; BSA, bovine serum albumin; CDI, N, N′-carbonyldiimidazole; CNBr, cyanogen bromide; dBSA, denatured BSA; DCC, N,N′-dicyclohexyl carbodiimide; DSP, dithiobis(succinimidylpropionate); DTT, dithiothreitol; DHLA, dihydrolipoic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; EG7, hepta(ethyleneglycol) succinimidylpropionate disulfide; ELISA, enzyme linked-immunosorbent assays; FRET, fluorescence resonance energy transfer; GSH, glutathione; His, histidine; Ig, immunoglobulin; K_D, dissociation constant; LFIA, lateral flow immunoassays; LC-SPDP, long chain SPDP; MAA, mercaptoacetic acid; 2-MAE, 2-mercaptoethylamine; MBP, maltose-binding protein; MPA, with 3-mercaptopropionic acid; MPA-NHS, 3-maleimidopropionic acid NHS; MPS, mercaptopropyltris(methoxy)silane; Ni-NTA, nickelnitrilotriacetic acid; PEG, polyethylene glycol; pI, isoelectric point; PL, photoluminescence; PMAO, poly(maleic anhydride 1-octadecene); PMPI, p-maleimidophenyl isocyanate; QD, quantum dot; QD-COOH, carboxyl functionalized QDs; QD-NH_2, amine functionalized QD; QD-OH, hydroxyl functionalized QD; QD-SH, Thiol functionalized QD; QY, quantum yield; SA, streptavidin; scFv, single chain Fv fragment; SMCC, succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate; SPDP, N-succinimidyl-3-(2-pyridyldithio)propionate; sulfo-SMCC, sulfosuccinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate; sulfo-NHS, N-hydroxysulfosuccinimide; TCEP, Tris(2-carboxyethyl)phosphine hydrochloride; TGA, thioglycolic acid; TOPO, trioctylphosphine oxide.

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1. Introduction

Colloidal quantum dots (QDs) are nanocrystals of a semiconducting material with diameters in the range of 1–20 nanometers [1,2], constructed from elements of Group II (Zn, Cd, Hg)-VI (Se, S and Te), III-V and IV-VI of the periodic table. Until the last decade, most studies focused on II-VI QDs (CdSe or CdTe) but toxicity of Cd and the regulation [3] on its use restricts their implementation. Nowadays I-III-VI_2 semiconductor QDs (e.g. $CuInS_2$) are considered as one of the main alternatives of Cd-based QDs [4]. Due to QDs small size, the electrons are confined in a limited space, and when the radii of the semiconductor nanocrystal is smaller or equal than the excitation Bohr radius, there is quantization of the energy levels. This is responsible for the unique spectral characteristics and positions the QDs properties between the properties of atoms and bulk materials.

QDs represent a special class of inorganic luminophores and have certain advantages over conventional fluorescent dyes (e.g. rhodamine 6G) [5] (Fig. 1). First, they have a high photostability due to a better resistance to chemical degradation and so they are less susceptible for photobleaching. Second, their broad absorption spectra make them ideal to excite photoluminescence (PL) with different colors of multiple QDs with a single excitation source. In addition, their narrow sharply-defined symmetrical emission spectra make it possible to combine different colored QDs without PL spectral overlap to perform multiplexing experiments [1,2,5–7]. Fourth, QDs have a much larger biochemically-accessible surface area compared to commonly used organic dyes, and subsequently facilitating the incorporation of multiple biomolecules [8].

The first colloidal QDs were synthesized in 1993 as 'core-only' CdSe QDs. It is well known that 'core-only' QDs are characterized by a low PL quantum yield (QY) (less than 10% [5]), a limited resistance to photobleaching and their PL intensity is easily affected by charges and free radicals present in their environment. Later, in 1996, core/shell CdSe/ZnS nanoparticles were synthesized. The shell is responsible for an enhancement of the QY up to 18% [1,7] and photostability [6]. In addition, multishelling gives a better control on the shell quality and therefore the overall optical properties and stability of the QDs. Nowadays, QDs consist of three parts i.e. a core, shell and hydrophobic capping layer. To obtain a high-ordered structure, which is important to obtain a high PL QY (gradient alloyed QDs: 27–61% and homogenous alloyed QDs: 72–93% [10]), QDs are usually synthesized in high boiling organic solvents. Next, the QDs need to be made water-soluble (hydrophilization) for bio- and analytical applications.

2. Hydrophilization of QDs

PL QDs are one of the most promising nanoprobes for any kind of bio-application, such as chemical, biomedical and therapeutic labeling and imaging, cell targeting etc. But for these kinds of applications the particles should meet some requirements, e.g. QDs should be (i) stable in aqueous solutions over a wide pH and ionic strength range while (ii) maintaining their optical properties. Furthermore, QDs should (iii) have functional groups available for conjugation on their surface. The synthesis of QDs results in very hydrophobic nanoparticles that are only soluble in non-polar solvents. The highest PL QYs are usually observed in organic solvents, and introduction of QD into aqueous media is usually accompanied with a decrease of their luminescence QY [11]. Solubilization of QDs also makes a future conjugation to biomolecules, such as proteins, immunoglobulins (Igs), aptamers, oligonucleotides etc. possible. Over the years surface chemistry underwent a refinement which led to a decrease of non-specific binding and subsequently to great improvement of the QD specificity [5]. Eventually bioconjugation results in a multifunctional nanoparticle that combines the optical/electrochemical properties of QDs with the biological function of the biomolecule [1]. For example, in bio-imaging applications the QDs serve as imaging tag while the attached Igs may serve as the unique targeting agent through the specific antigen binding action [12].

There are three main strategies employed for hydrophilization of QDs (Fig. 2). The first method involves ligand exchange, where the original hydrophobic coating (e.g. trioctylphosphine oxide (TOPO)) is removed and replaced with water-soluble bifunctional

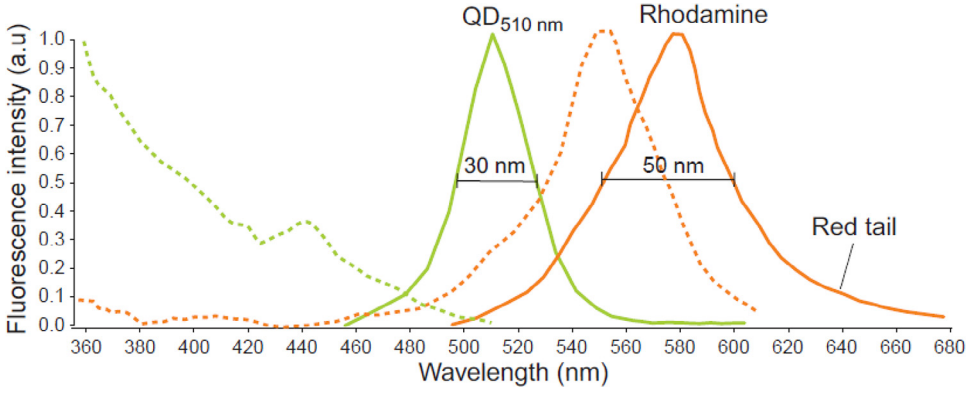

Fig. 1. Comparison of the excitation and emission behavior of green CdSe QDs with Rhodamine 6G dye. The excitation spectrum (green dashed line) of a QD is very broad, whereas the spectrum of Rhodamine 6G (orange dashed line), is narrow. The QD emission spectrum (green line) is almost symmetric and much narrower in comparison with the Rhodamine 6G dye (orange line) [9].