

Preparation of DNA-functionalised CdSe/ZnS Quantum Dots

Boon-Kin PONG¹, Bernhardt L. TROUT,^{1,3} and Jim-Yang LEE^{1,2}

¹ Singapore-MIT Alliance, National University of Singapore, 4 Engineering Drive 3, Singapore 117583, Singapore

² Department of Chemical and Biomolecular Engineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Singapore

³ Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, USA

Abstract : We functionalised core-shell CdSe/ZnS quantum dots (QDots) with short-chain 3-mercaptopropionic acid (3MPA) to render these nanocrystalline semiconductor water-soluble. The ligand-exchange reaction was significantly improved with the use of an organic base to first remove the thiolic hydrogen. Non-bound 3MPA could be removed from the colloid by dialysis, but it was found that the choice of membrane is important. Cellulose membrane obliterated the photoluminescence of the QDots, while cellulose-acetate membrane worked well. Amine-modified DNA was then attached to the QDots through amide bond linkage, using EDC and NHS as reaction promoters. The pH of the reaction medium has an important impact on the successful attachment of functional DNA on the QDots.

Index Terms — CdSe/ZnS, DNA, quantum dots.

I. INTRODUCTION

Quantum dots (QDots) offer a number of advantages over conventional dyes for applications in sensing / detection. They are brighter, photo-bleach resistant and highly suited for multiplex platform because of their narrow emission bandwidths. In recent years, there has been growing interests in the use of DNA-functionalised QDots for biosensing [1-3]. As fluorophores, QDots may be used as a passive tag/label. On the other hand, the ability of QDots to participate in resonance energy transfer (RET) [4] makes them potential candidates in other sensing formats e.g. molecular beacons.

High quality QDots are synthesized in organic media and have no intrinsic aqueous solubility. They are usually protected with hydrophobic capping agents such as tri-octyl phosphine oxide (TOPO). To solubilise

QDots in water, they must be coated with a solubilising shell such as phospholipids, polymers, proteins and thiols. However, these solubilising shells lead to substantial increase of particle diameters. Since RET processes are highly sensitive to the distance between the energy emitter and acceptor, these solubilising shells place a limit to the efficiency of energy transfer.

Another commonly adopted method to solubilise QDots in water is the use of ligand-exchange reaction with short-chain mercapto carbonic acids [2, 5-7]. This provides a thin solubilising shell on the nanoparticles. Unfortunately, current ligand exchange methods only managed to produce mercapto-acid functionalised QDots with limited colloidal stability and diminished quantum yields [8-10]. Nonetheless, functionalising CdSe/ZnS QDots with short-chain mercapto carbonic acid remains a promising strategy to adapt these QDots for RET applications in water if the associated loss of colloidal stability and quantum yield can be overcome. We have developed a synthetic strategy to efficiently functionalise CdSe/ZnS QDots with 3-mercaptopropionic acid (3MPA). The QDots exhibit good colloidal stability (>24 weeks) in water and remained highly dispersed as individual particles without any signs of cluster formation. Furthermore, the optical properties and the quantum yield remained unchanged.

Functionalised with carboxylic groups on the surface, the QDots may be attached to other amine-containing molecules via the formation of amide linkages. We attached amine-modified DNA onto the QDots surface and determine the hybridization efficiency of the immobilized DNA. It was found that the control of pH in the coupling reaction is important in ensuring the hybridisability of the immobilised DNA.

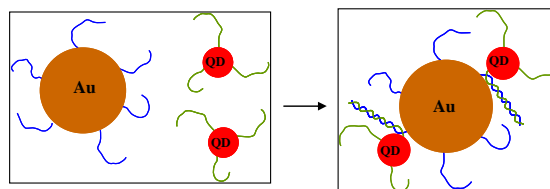
II. EXPERIMENTAL PROCEDURES

(a) **Ligand-exchange reaction to solubilise QDots in water.** The TOPO-capped QDots (in toluene) were first precipitated with methanol and re-dispersed in CHCl_3 . In a separate preparation, 500 μL of 3-mercaptopropionic acid (**3MPA**) was dissolved in 10 mL of CHCl_3 and added to 1g of the organic base tetramethylammonium hydroxide pentahydrate, (**TMAH**, $(\text{CH}_3)_4\text{N}\cdot\text{OH}\cdot 5\text{H}_2\text{O}$). A two-phase mixture was formed. The bottom organic phase, containing deprotonated **3MPA**, was collected. 1 mL of TOPO-capped QDots (1 μM) was added to this solution, and allowed to stand at room temperature for 48 hours.

(b) **Removal of unbound 3MPA from colloid.** Alkyl thiols are deactivators of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (**EDC**), an important promoter used to couple amine-modified DNA onto the QDots (see next section). It is therefore important that excess **3MPA** is removed from the QDots solution. These dissolved solutes were removed from the colloid by dialysis against a PBS buffer (10mM, pH 7).

(c) **Attachment of DNA onto QDots.** With the surface functionalized with carboxylic groups, amine-modified DNA can be coupled to the QDots surface via the formation of an amide linkage. The coupling is promoted by **EDC** and *N*-hydroxysuccinimide (**NHS**). Briefly, 100 μL of a solution of the QDots (200nM) was activated with 320 μL of EDC (0.1mM) and 160 μL of NHS (0.1mM). After incubating for 15 minutes at room temperature, 10 μL (100 μM) of amine-modified ssDNA ($5'\text{-TTG ACC TTG AGA AGC AAA AAA-C}_6\text{H}_{12}\text{-NH}_2\text{-3}'$) was added to the QDots. The solution was incubated at 30°C for at least 30 minutes. Dissolved solutes were removed by dialysis against a PBS buffer (10mM, pH 7.0) using cellulose-acetate membrane.

(d) **Hybridization efficiency of the immobilized DNA.** XPS (nitrogen) analysis was used to ascertain the successful attachment of DNA on the QDots. To evaluate the ability of these immobilized DNA to hybridize, we prepared gold nanospheres (**AuNS**, 13 nm diameter) functionalized with the complementary DNA sequence [11]. Successful hybridization will bring about the assembly of the particles to close proximity. This will be accompanied by a reduction in the QDots' fluorescence intensity. **Scheme 1** below illustrates how the approach used to determine the hybridizability of the DNA immobilized on QDots.



Scheme 1. Hybridization of the complementary DNA leads to an assembly of QDots and AuNS, causing a reduction of the QDots' fluorescence intensity due to energy transfer. This is used to determine the hybridizability of the DNA immobilized on the QDots.

III. RESULTS & DISCUSSIONS

(a) **Ligand-exchange reaction to solubilise QDots in water.** After reacting with deprotonated **3MPA** in chloroform for 48 hours at room temperature, the QDots separated out from the CHCl_3 solution and adhered to the walls of the glass container. We found it convenient to carry out the reaction in a vessel made of hydrophobic material (e.g. polypropylene) such that the hydrophilic QDots formed an oily droplet that floats above the chloroform solution. This fluorescent liquid droplet could then be easily transferred and washed 3 times with fresh chloroform before being re-dispersed in 1 mL of distilled water. **Fig.1** depicts the separation of the QDots from the CHCl_3 and dispersal into the water.

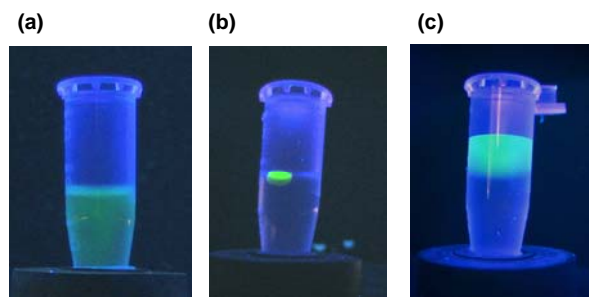


Fig. 1. Ligand-exchange reaction carried out at room temperature. (a) The TOPO-capped QDots were allowed to react with deprotonated 3MPA in chloroform. (b) After 48 hrs, the QDots separated out from the chloroform solution and gathered as an oily droplet, suspended above the heavy chloroform solvent. (c) Distilled water was added, into which the QDots dispersed spontaneously.

The size of the nanoparticles in water is 5.8 ± 2.8 nm in diameter (dynamic light scattering). In contrast, our attempts to solubilise QDots *without* the use of the organic base [12] produced QDots that formed large clustered particles 20-50 nm in diameter. The absorption and fluorescence properties of the QDots modified with our method also remained unchanged in the aqueous media. In contrast, other methods using thiol-acids for

the phase transfer produced only QDots with significantly reduced photoluminescence performances.[8-10]

(b) Removal of unbound 3MPA from colloid. The as-prepared QDots in water contains excess **3MPA**, which must be removed because it inhibits the coupling reaction for DNA attachment. These ions can be removed by dialysis of the colloid against a PBS buffer. However, our dialysis attempts using *cellulose* membrane always led to a severe loss of quantum yield. This has been reported by others, who attributed this to the desorption of **3MPA** from the QDots during dialysis [13]. We have, however, identified the problem to material incompatibility: *cut pieces* of cellulose membrane were found to exhibit the same detrimental effects when they were placed in a solution of the QDots. Clearly, the loss of quantum yield arises not from the dialysis process, but from the membrane material. On the other hand, we found that *cellulose-acetate* membrane was able to dialyse the QDots without any effects on the quantum yield. All dialyses were subsequently carried out with cellulose-acetate membrane.

A key difference between cellulose and cellulose-acetate membrane is that the former contains free hydroxyl groups, which has been esterified in the latter. However, it is unlikely that these hydroxyl groups are responsible for the loss of quantum yield because methanol and ethanol had no effect on the QDots' fluorescence. It is possible that heavy metals in the cellulose membrane, which were introduced during manufacture, were responsible for the loss of fluorescent activity.

(c) Hybridizability of QDots-immobilised DNA. The EDC-NHS linking chemistry is typically carried out under buffered acidic (or near neutral) conditions. However, initial attempts to attach amine-modified DNA onto the QDots in acidic and neutral buffers (pH 5.5 to 7.0) resulted in very poor hybridization activity. Since DNA contains amines in its bases (dA, dC & dG in **Fig. 2**), it is possible that competitive attachment via these internal amines resulted in the poor hybridization efficiency of the immobilized DNA.

The EDC-NHS coupling reaction occurs via the free amines, and not the protonated ones. The basicity of the DNA base amine ($pK_b \sim 9.5$) is significantly weaker than that of the terminal alkyl amine ($pK_b \sim 4$). In acidic/neutral pH, unprotonated amines are largely those located on the DNA bases, while the terminal alkyl amines are mostly protonated. Hence, acidic/neutral pH favours coupling at the DNA base amines. Alkaline pH should increase coupling at the terminal alkyl amine.

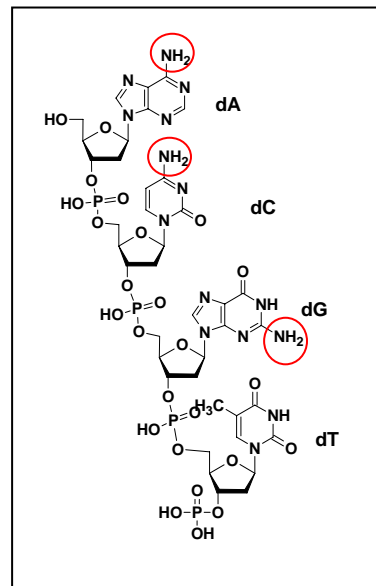


Fig. 2. Three of the four DNA bases (dA, dC & dG) contains amines that can compete with the terminal amine (not shown here) to bind onto the QDot surface.

We examined the effect of media pH on the attachment of DNA. The efficiency of the attachment is determined by the ability of the DNA-modified QDots to assemble with AuNS that has been modified with complementary DNA. The formation of the assembly, achieved through the hybridization of the complementary DNAs on the nanoparticles, is indicated by a reduction of fluorescence intensity. This arises from the quenching of QDots by AuNS when they are at close proximity.

The success of attaching DNA in its hybridizable form on QDots is strongly dependent on the pH used in the coupling step. **Table 1** summarizes the effect of pH on the hybridizability of the attached DNA.

Table 1. Effect of coupling pH (10mM HEPES buffer) on the ability of the QDots-immobilised DNA to hybridise with its complementary sequence on AuNS.

Coupling pH	5.5	7.0	8.0	9.5
Hybridization efficiency of attached DNA on QDots	Poor	Poor	Moderate	Good

Fig 3 shows the TEM micrograph of a successful assembly of QDots and with AuNS. In this case, the DNA was coupled to the QDots at optimal pH of 9.5 (10mM HEPES buffer). On the other hand, when the coupling reaction was performed at pHs 5.5 and 7.0, no

assembly was observed.

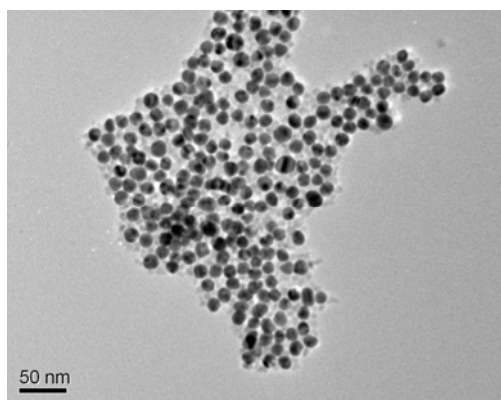


Fig. 3. TEM micrograph of AuNS-QDots assembly. Darker spheres are AuNS, while the light ones are QDots. The two types of nanoparticles are functionalized with complementary ssDNA.

IV. CONCLUSIONS

The use of a chloroform-soluble base, tetramethylammonium hydroxide, has greatly improved the ligand-exchange reaction to prepare QDots capped with 3-mercaptopropionic acid. The 3MPA-capped QDots retained its high quantum yield after being transferred to water, and has high colloidal stability. Cellulose membrane has been found to be incompatible with the 3MPA-capped QDots, causing a severe reduction in the quantum yield. However, *cellulose acetate* membrane can be used to purify the QDots without affecting any of the undesirable effects.

Amine-modified DNA can be coupled to the carboxylic surface of the QDots by using the EDC-NHS amide chemistry. However, to ensure the hybridizability of the attached DNA, an alkaline medium (pH 9.5) has been determined to be optimal. Apparently, this optimal pH ensures coupling take place preferentially with the terminal alkyl amine over the amino bases in DNA.

REFERENCES

- [1] D. J. Zhou, J. D. Piper, C. Abell, D. Klenerman, D. J. Kang, and L. M. Ying, "Fluorescence resonance energy transfer between a quantum dot donor and a dye acceptor attached to DNA," *Chem. Commun.*, pp. 4807-4809, 2005.
- [2] J. H. Kim, D. Morikis, and M. Ozkan, "Adaptation of inorganic quantum dots for stable molecular beacons," *Sens. Actuators, B*, vol. 102, pp. 315-319, 2004.
- [3] S. Hohng and T. Ha, "Single-molecule quantum-dot fluorescence resonance energy transfer," *Chemphyschem*, vol. 6, pp. 956-960, 2005.
- [4] D. M. Willard, T. Mutschler, M. Yu, J. Jung, and A. Van Orden, "Directing energy flow through quantum dots: towards nanoscale sensing," *Anal. Bioanal. Chem.*, vol. 384, pp. 564-571, 2006.
- [5] I. L. Medintz, H. T. Uyeda, E. R. Goldman, and H. Mattoussi, "Quantum dot bioconjugates for imaging, labelling and sensing," *Nat. Mat.*, vol. 4, pp. 435-446, 2005.
- [6] W. C. W. Chan, D. J. Maxwell, X. H. Gao, R. E. Bailey, M. Y. Han, and S. M. Nie, "Luminescent quantum dots for multiplexed biological detection and imaging," *Curr. Opin. Biotechnol.*, vol. 13, pp. 40-46, 2002.
- [7] G. P. Mitchell, C. A. Mirkin, and R. L. Letsinger, "Programmed assembly of DNA functionalized quantum dots," *J. Am. Chem. Soc.*, vol. 121, pp. 8122-8123, 1999.
- [8] T. Jin, F. Fujii, H. Sakata, M. Tamura, and M. Kinjo, "Calixarene-coated water-soluble CdSe-ZnS semiconductor quantum dots that are highly fluorescent and stable in aqueous solution," *Chem. Commun.*, pp. 2829-2831, 2005.
- [9] R. Gill, I. Willner, I. Shweky, and U. Banin, "Fluorescence resonance energy transfer in CdSe/ZnS-DNA conjugates: Probing hybridization and DNA cleavage," *J. Phys. Chem. B*, vol. 109, pp. 23715-23719, 2005.
- [10] V. Stsiapura, A. Sukhanova, A. Baranov, M. Artemyev, O. Kulakovich, V. Oleinikov, M. Pluot, J. H. M. Cohen, and I. Nabiev, "DNA-assisted formation of quasi-nanowires from fluorescent CdSe/ZnS nanocrystals," *Nanotechnology*, vol. 17, pp. 581, 2006.
- [11] J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin, and R. L. Letsinger, "One-Pot Colorimetric Differentiation of Polynucleotides with Single Base Imperfections Using Gold Nanoparticle Probes," *J. Am. Chem. Soc.*, vol. 120, pp. 1959-1964, 1998.
- [12] W. C. W. Chan and S. M. Nie, "Quantum dot bioconjugates for ultrasensitive nonisotopic detection," *Science*, vol. 281, pp. 2016-2018, 1998.
- [13] W. J. Parak, D. Gerion, T. Pellegrino, D. Zanchet, C. Micheel, S. C. Williams, R. Boudreau, M. A. Le Gros, C. A. Larabell, and A. P. Alivisatos, "Biological applications of colloidal nanocrystals," *Nanotechnology*, vol. 14, pp. R15-R27, 2003.