Chapter 7

Synthesis of Amino-Acid Modified Fluorescent Quantum Dots in Aqueous Solution and their Interaction with Biological Macromolecules

7.1. Introduction

Semiconductor quantum dots (QDs) have attracted great interest over the past decade due to their unique optical properties, such as a bright, narrow and tunable fluorescence signatures, broad excitation but specific emission spectra and good photochemical stability [1-3]. Because of these distinct optical properties, QDs are being extensively explored with respect to biomedical use as imaging contrast agents, traceable therapeutic vectors and for energy applications including photovoltaic solar cells [4-7]. However, their advantageous properties are undermined by the inherent insolubility of QDs in aqueous solution. While, water solubilization of QDs is essential for many biological applications, it presents a significant challenge. Use of mercaptoacetic acid ligands was one of the first strategies applied to produce water soluble QDs [8]. Since then, a number of other thioalkyl acid ligands have been used, including 3-mercapto-propionic acid [9, 10] and dihydrolipoic acid [11]. These ligands form a self-assembly on the surface of the QDs that proceeds via a metal-thiol affinity interaction and other polar groups of the ligands are exposed to the surrounding aqueous solution [12]. Other approaches using non-thiol based organic ligands have also been employed including 4-substituted pyridine, oligomeric phosphine, poly (dimethylaminoethyl) methacrylate [13], polymers [14], amphiphilic polymers [15, 16] and phospholipids [17]. There has been a growing emphasis on assembling biological molecules to the water-soluble QDs through different types of interactions. In most cases these interactions involve covalent conjugation or simple adsorption of the biological molecules to the solubilizing layer around the QDs [8, 10, 14, 18]. In addition to these, the formations of nanobioconjugates through various nonspecific interactions (electrostatic, hydrogen-bonding interactions etc) between biological molecules and nanoparticles have also been explored [1, 19-21].
To date, CdSe/ZnS core/shell QDs remain among the best available for many biological applications [6, 22, 23]. However, QDs synthesized in organic solvents contain hydrophobic surface ligands such as trioctylphosphine oxide (TOPO), trioctylphosphine (TOP) [24], tetradecylphosphonic acid (TDPA) or oleic acid [25]. As a result they are insoluble in water and in other protic solvents namely methanol or ethanol [26]. So, their biological applications are restricted, where water solubility is highly desirable. Hence, the main challenge, to make quantum dots soluble in water for their further prospective bioconjugate reactions, remains.

Amino acids are inherently biocompatible and among common amino acids L-arginine along with lysine are positively charged. Upon functionalization of nanoparticle with these amino acids, the nanoparticles become positively charged and their interaction with the negatively charged biomolecules is much more efficient. However, in comparison with lysine, due to the presence of a guanidyl group, arginine molecules can highly facilitate the interaction of nanoparticle with biological macromolecules [27].

In the present chapter, we have exploited the toluene/water interface to replace the original TOPO capping of CdSe/ZnS core/shell QDs dispersed in toluene, with a natural amino acid L-arginine (Arg) using the reactivity of the amine groups. This allows a dispersal of the QDs in aqueous solutions with a quantum yield of 14%. We have confirmed the conjugation of arginine molecules with the QDs by using FTIR spectroscopy. The structural integrity of the QDs upon water solubilization has been confirmed with HRTEM. Using picosecond-resolved photoluminescence measurements, we have explored an efficient ultrafast energy transfer from arg-capped CdSe/ZnS QDs (donor) to ethidium bromide-labeled DNA (EB-DNA, acceptor) applying the sensitivity of FRET. Employing the kinetic model developed by Tachiya (for the quenching of luminescent probes), we have also analyzed the picosecond-resolved photoluminescence measurement results to understand the kinetics of energy transfer with the dye labeled DNA and the distribution of acceptor molecules around the donor QDs, as it is a driving factor for efficient energy transfer and for the accurate donor-acceptor measurements. In order to confirm any structural perturbation of dodecamer DNA in the nanobioconjugate, circular dichroism (CD) studies have also been performed on both the DNA and DNA-QD conjugate. To investigate in more details the type of interaction taking place between the
QDs and DNA, using CD we have monitored the melting and rehybridization pathways of the dodecamer DNA conjugated to the QDs. This reveals that hydrogen bonding is the accompanied mechanism involved during the formation of this QD-DNA nanobioconjugate.

Scheme 7.1. Trioctylphosphine oxide (TOPO) stabilized CdSe/ZnS quantum dots (QD) were modified with L-arginine via ligand exchange. Phase transfer of arginine-modified QDs from toluene phase into water was achieved by using the reactivity of amine group of arginine. Upon replacement of the initial TOPO ligand with arginine the emission of the QDs is decreased.

7.2. Result and Discussion

7.2.1. Preparation of Water Soluble L-Arginine Capped CdSe/Zns QDs and their Interaction with Synthetic DNA: Picosecond-Resolved FRET Study [28]:

As Scheme 7.1 illustrates, the addition of QDs toluene suspension into the aqueous solution of L-arginine (pH~9) under vigorous stirring condition results in the formation of toluene microdroplets, and the QDs in toluene get the chance to interact strongly with the arginine molecules through the liquid-liquid interface [29, 30]. Photo images of the QDs
under UV excitation before and after ligand exchange clearly indicate the successful phase transfer of the QDs from toluene into the aqueous medium. Efficient ligand exchange through this process is driven by an interaction between the amine group of the stabilizing amino acid in the aqueous phase and the ZnS shell of the QDs in the toluene phase at the interface [31, 32]. Computational studies have shown that primary amines have greater surface binding energy than carboxylic acids, though lower binding energy compared to TOPO and phosphonic acids [33-35]. However, primary amines have the advantage of more complete surface coverage which can theoretically reach 100% - over TOPO (30% coverage) due to reduced steric effects [36].

To obtain direct evidence for the arginine functionalization of QDs, FTIR measurements were performed on both the free arginine molecules and arginine molecules attached to the QDs. The FTIR spectra of arginine capped QDs and free arginine molecules are shown in Figure 7.1. For arginine, the characteristic band at 3161 cm$^{-1}$ (Figure 7.1b) corresponding to the N-H stretching mode [37], is broadened and red-shifted to 3152 cm$^{-1}$, suggesting its interaction with the QD surface. Moreover, as shown in Figure 7.1a, the significant perturbation of other characteristic bands of arginine at 790 cm$^{-1}$ (C-N-H stretching), 847 cm$^{-1}$ (C-C stretching), 1095 cm$^{-1}$ (C-N stretching), 1174 cm$^{-1}$ (C-C-C symmetric stretching) and 1406 cm$^{-1}$ (COO$^-$ symmetric stretching) [37, 38] also confirm the binding of arginine molecules to the QD surface. The direct interaction of arginine molecules with the QDs surfaces ensured that the overall size of the QDs remains unchanged, with a thin solubilizing shell. Inset of Figure 7.2a shows the HRTEM images of arg-capped QDs in water, which reveal the diameters of the QDs to be 3.2 nm. The existence of lattice fringes illustrates the highly crystalline nature of the QDs. We have employed FRET to study the interaction of the synthetic dodecamer DNA (EB labelled) with the water soluble QDs (pH~7). Figure 7.2a shows the spectral overlap between the emission spectrum of arginine capped QDs (donor) and the absorption spectrum of EB-labeled DNA (acceptor) suggesting the possibility of efficient Förster resonance energy transfer (FRET) between the donor and the acceptor, when EB-labeled DNA becomes adsorbed at the surface of the arginine capped QDs. Figure 7.2b represents the steady state photoluminescence (PL) quenching of the donor (arginine capped QDs) in presence of EB-
Figure 7.1. FTIR spectra of free arginine molecules and arginine molecules attached to the QDs. (a) Spectral broadening of C-N-H, C-C and C-C-C stretching frequencies of arginine upon interaction with the QDs. Perturbation of C-N and COO\(^{-}\) stretching frequencies of arginine is also observed after interaction with the QDs. (b) Spectral broadening and red shift of N-H stretching frequency of arginine upon interaction with the QDs.
Figure 7.2. (a) Spectral overlap between emission spectrum of arginine-capped CdSe/ZnS core/shell QDs and the absorption spectrum of EB-labeled DNA (the extinction coefficient value is for the acceptor, EB-labelled DNA). Inset shows the HRTEM image of QD in toluene. (b) Steady-state fluorescence quenching of arginine-capped QDs in presence of the acceptor EB-DNA (c) Picosecond-resolved PL transients of arginine-capped CdSe/ZnS QDs and (EB-DNA)-QD complex monitored at $\lambda_{em} = 485$ nm. (d) Picosecond-resolved PL transients of arginine-capped CdSe/ZnS QDs and (EB-DNA)-QD complex, fitted with Tachiya kinetic model. The fitted curves are shown in black.

labeled DNA. Picosecond resolved PL transients (Figure 7.2c) of both donor and donor–acceptor systems monitored at 485 nm, shows significant shortening in the QDs fluorescence lifetime upon adsorption of EB-labeled DNA at the QDs surface. The picosecond resolved fluorescence decay of arginine capped QDs (donor) in buffer revealed multiexponential time constants of 0.08 ns (45%), 1.15 ns (25%) and 8.50 ns (29%) giving an average time constant ($<\tau>$) of 2.80 ns. For the donor–acceptor system (arginine capped QDs- EB labeled DNA) time constants are obtained as 0.09 ns (72%), 1.23 ns (18%) and 5.40 ns (8%) giving an average time constant ($<\tau>$) of 0.72 ns (Table 7.1). The substantial
shortening in the QDs excited state lifetime upon conjugate formation indicates conclusively that efficient FRET occurs from the QD donor to the EB-DNA acceptor. Taking the calculated quantum yield of Arg-capped QDs in absence of acceptor as 0.14 and based on the spectral overlap, we have estimated a FRET efficiency of 74% using Eq. 2.7b. The measured Förster distance, $R_0$, for the QD-DNA nanobioconjugate is 2.88 nm. The donor–acceptor distance (R) calculated using Eq. 2.6 is 2.42 nm (Table 7.1).

<table>
<thead>
<tr>
<th>System</th>
<th>$\tau_1$[ps]</th>
<th>$\tau_2$[ps]</th>
<th>$\tau_3$[ps]</th>
<th>$&lt;\tau&gt;$[ps]</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD</td>
<td>85 (45)</td>
<td>1153 (26)</td>
<td>8527 (29)</td>
<td>2799</td>
</tr>
<tr>
<td>QD-EB-DNA</td>
<td>98 (73)</td>
<td>1239 (19)</td>
<td>5403 (8)</td>
<td>726</td>
</tr>
</tbody>
</table>

For better understanding of the energy transfer between the excited state of QDs with EB-DNA, it is essential to know the distribution of acceptor molecules around the QDs because this is a governing factor that can influence the efficient energy transfer as observed from the time resolved fluorescence studies. In this regard, we have applied a kinetic model developed by Tachiya for the quenching of luminescent probes [39, 40]. We have determined the values of the parameters $m$, $k_q$, $k_0$, $m$, and $k_q$ by fitting Eq. 2.14 and 2.15 to the decay curves in the absence and presence of acceptor EB-DNA molecules.

Figure 7.2d shows the time resolved fluorescence transients of CdSe/ZnS QDs in absence and presence of EB-DNA molecules and black curves represents the result of fitting the curves with Eq. 7.7 and 7.8. The observed fluorescence transients were fitted using a nonlinear least squares fitting procedure (software SCIENTIST™) to a function

$$X(t) = \int_0^\infty E(t') P(t-t')dt'$$

comprising of the convolution of the instrument response function (IRF) $E(t)$ with exponential $P(t,m) = P(0)\exp\{-k_q t - m[1-\exp(-k_q t)\] - m[1-\exp(-k_q t)]\}$. The purpose of this fitting is to obtain the decays in an analytic form suitable for further data analysis. As evident from the Figure 7.2d, the fitting of the decay curves according to the model is
reasonably well. The quenching parameters are summarized in Table 7.2. The quenching rate constant \( k_{eq} \) due to unidentified traps on the surface of the nanocrystals are the same even after addition of acceptor (EB-DNA) molecules, and this indicates the average number of unidentified trap states to be the same. However, it is observed from Table 7.2 that the average number of unidentified traps state increases with addition of acceptor molecules. Since, there are still many unknown parameters in the QDs excitation dynamics, for an accurate interpretation of this observation a more complex model and a larger data set is required. As summarized in Table 7.2, the mean number of acceptor (EB-DNA) molecules associated with the QDs is 1.15 and the estimated rate constant for energy transfer \( k_q \) per acceptor molecules is 0.20 ns\(^{-1}\). The energy transfer rate calculated from conventional FRET model is found to be somewhat different (1.01 ns\(^{-1}\)) from the value obtained using Tachiya’s model (0.20 ns\(^{-1}\)). However, as shown in Table 7.1, the contribution of the longer lifetime (8.52 ns) in the overall average lifetime of the donor is significant. Other lifetime values of 1.15 ns and 0.08 ns could be associated with the unidentified trap states on the QDs surface [41]. Thus, considering 8.52 ns to be excited state lifetime of the donor QDs, the estimated energy transfer rate is found to be 0.33 ns\(^{-1}\), which is consistent with that from Tachiya model.

Table 7.2. Overview of the value of quenching parameters using a kinetic model.

<table>
<thead>
<tr>
<th>System</th>
<th>( k_o [\text{ns}^{-1}] )</th>
<th>( m_t )</th>
<th>( k_{eq} [\text{ns}^{-1}] )</th>
<th>( m )</th>
<th>( k_q [\text{ns}^{-1}] )</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD</td>
<td>0.28</td>
<td>0.40</td>
<td>4.60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>QD-EB-DNA</td>
<td>0.28</td>
<td>1.06</td>
<td>4.67</td>
<td>1.15</td>
<td>0.20</td>
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</table>

In order to confirm any structural perturbation in the native structure of the dodecamer DNA adsorbed onto the QDs surface, we have performed circular dichroism (CD) studies. As revealed from Figure 7.3a CD spectrum, the hybridized DNA used in our studies were in a B-form, evidenced by a negative band at 248 nm and a positive band at 280 nm [19, 42], also the structural integrity of DNA B-form is almost retained in the QD-DNA nanobioconjugates. Figure 7.3b shows the overall secondary structure of the QD conjugated dodecamer DNA at 20°C and 70°C temperatures. It is clear that both the peaks
at 252 nm and 280 nm are affected by the temperature-induced melting of the QD conjugated dodecamer DNA. The change in the molar ellipticity associated with the 252 nm peak has been monitored to construct the temperature-induced melting and rehybridization profiles of the QD conjugated dodecamer DNA, as shown in Figure 7.3c (melting) and 7.3d (rehybridization). The melting of DNA is accompanied by structural changes involving unwinding of the helix, destruction of major and minor grooves, and finally the separation of the two strands resulting in the formation of two single strands of complementary sequence. The melting and rehybridization temperatures have been estimated to be 42.6°C and 41.0°C, respectively, for the QD conjugated dodecamer DNA and this is in good agreement with the dodecamer DNA alone reported previously [43].

**Figure 7.3.** (a) Circular dichroism (CD) spectra of dodecamer DNA and dodecamer DNA conjugated to QDs. Structural integrity of the DNA in the QD-DNA conjugate is clearly evident. (b) CD spectra of dodecamer DNA-QD conjugates at two different temperatures. (c) and (d) the melting and rehybridization of dodecamer DNA conjugated to QDs. Solid lines are the fitted sigmoidal curve.
Figure (7.3c and 7.3d) show that the dodecamer is rehybridized into its original form maintaining the same hysteresis as it follows during its melting, which indicates that the π stacking interaction between the complementary base pair of the two strands is greater than the electrostatic interaction of each strand with the QDs. Moreover, an electrostatic interaction between the positively charged groups of the arg-capped QDs and the negatively charged DNA dodecamer, could have changed its conformation as well as its melting temperature and rehybridization pathway [44]. However, as revealed from the CD study, all of its characteristic conformational features remain the same, before and after conjugation with the QDs. So, it appears that hydrogen-bonding interactions (instead of electrostatic interactions) are playing the dominant role in the adsorption of DNA onto the surface of arg-capped QDs involving the protonated carboxyl surface groups of the thin solubilizing layer of amino acids around the QDs [45].

7.3. Conclusion

In conclusion, we report on a convenient approach for preparing water-soluble, biocompatible QDs following a liquid-liquid interfacial ligand exchange method, where L-arginine acts as a capping ligand. The successful conjugation of arginine with the QDs has been confirmed by FTIR spectroscopy. We have employed picosecond-resolved spectroscopic measurements, to demonstrate a highly efficient FRET from arginine-capped CdSe/ZnS QDs (donor) to EB-DNA (acceptor). The corresponding donor-acceptor distance has been calculated to be 2.42 nm, which suggest an adsorptive interaction between the dodecamer DNA molecules and arginine-capped QDs. From CD spectroscopic studies it is found that the dodecamer DNA retained their structural integrity upon conjugation with the QDs. Moreover, temperature induced melting and rehybridization of the QD conjugated dodecamer DNA suggest that hydrogen-bonding interaction could be the associated mechanism operating during the formation of QD-DNA nanobioconjugates. Considering the spread in use of QDs and the number of applications employing QD bioconjugates, understanding the interactions between QDs and biomolecules is of considerable importance and multidisciplinary interest. So, it is expected that this study may prove to be useful in making sensitive FRET-based sensors.
References


