SUPPORTING INFORMATION

Quantum Dot and Gold Nanoparticle Immobilization for Biosensing Applications using Multidentate Imidazole Surface Ligands

Eleonora Petryayeva and Ulrich J. Krull*

Chemical Sensors Group, Department of Chemical and Physical Sciences, University of Toronto Mississauga, 3359 Mississauga Rd. North, Mississauga ON, Canada L5L 1C6

*Author to whom correspondence should be addressed: ulrich.krull@utoronto.ca

Detailed Experimental Methods

Materials and Reagents

3-Aminopropyltrimethoxysilane (APTMS, 97%), polyacrylic acid (MW 2000), 1-(3-aminopropyl)imidazole (98%), N,N’-diisopropylcarbodiimide (DIC, >98%), N-hydroxysuccinimide (NHS, 98%), N-(3-dimethylaminopropyl)-N’-ethyldiethylcarbodiimide (EDC, ≥97%), 3-mercaptopropionic acid (MPA, 99%), lipoic acid (99%), sodium borohydride (≥98%), reduced glutathione (GSH, >98%), anhydrous dimethylformamide (DMF, 99.8%), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), tetramethylammonium hydroxide pentahydrate (TMAH, ≥97%), tetramethylammonium hydroxide solution in methanol (25% w/v), bovine serum albumin (fraction V, 96%), sodium dodecyl sulfate (SDS, 99%), sodium citrate and hydrogen tetrachloroaurate were from Sigma-Aldrich (Oakville, ON, Canada). Chloroform, dichloromethane, diethyl ether, methanol, 2-propanol and hydrochloric acid (38%) were from
Caledon (Georgetown, ON, Canada). Ammonium hydroxide (30%), hydrogen peroxide (30%) and glacial acetic acid were from EMD Chemicals (San Diego, CA, USA). Fused silica fibers (400 mm diameter, F-100 Suprasil high OH synthetic silica) were from Innova Quartz (Phoenix, AZ, USA) and were pre-cut to 48 mm length and polished by manufacturer. The fibers were free of coatings and cladding. Silicon wafers (<100>, single side polishing) were from International Wafer Service (Colfax, CA, USA). Borosilicate glass beads (2 mm) were from Sigma Aldrich.

Semiconductor nanocrystals with emission maxima at 525 nm and 624 nm dispersed in toluene were from Cytodiagnostics (Burlington, ON, Canada) and were of a CdS/Se/ZnS core/shell structure. Organic CdSe/ZnS core/shell QDs dispersed in toluene with emission maxima at 518 and 605 nm were from eBioScience Inc. (San Diego, CA, USA). QDs were dispersed in organic solvents and rendered water soluble by ligand exchange with MPA, GSH or DHLA. CdSe/ZnS Qdot® 525 ITK™ Streptavidin Conjugates were from Invitrogen by Life Technologies (Oakville, ON, Canada). Modified oligonucleotides were from Integrated DNA Technologies (Coralville, IA, USA) and were HPLC purified by manufacturer. His-tag appended peptide was obtained from Canpeptide (Montreal, QC, Canada) and purified by the manufacturer (purity >99%). Alexa Fluor 647 maleimide functionalized dye was from Invitrogen (Oakville, ON, Canada). Buffers were prepared with water purified by a Milli-Q water purification system (Millipore Corp., Mississauga, ON, Canada) and sterilized by autoclaving prior to use. Buffers included borate buffer (50 mM, pH 9.25), borate buffer (100 mM, pH 8.3), Tris-Borate (TB) buffer (50 mM, pH 7.44, 50 mM NaCl) and Gly-HCl buffer (100 mM, pH 3.0).
Instruments and Characterization Methods

Ultraviolet-visible absorption spectra were collected using a HP8452A Diode-Array Spectrometer (Hewlett Packard Corp., Palo Alto, CA, USA). PL spectra from fused silica fibers were obtained using a QuantaMaster PTI Spectro fluorometer and Felix Software (Photon Technology International, Lawrenceville, NJ, USA). The fibers were illuminated by total internal reflection using 402 nm diode laser (Radius 405, 25 mW, Coherent, Santa Clara, CA, USA). PL lifetime was measured with a dye laser pumped by a pulsed nitrogen laser (GL-3300, Photon Technology International) and analyzed using TimeMaster software. The laser dye used was 4,4’-(1,2-ethyenediyl)bis-1,1’biphenyl (DPS, Exciton, Dayton, OH, USA) in dioxane, with a laser output tuned to 402 nm. Brightfield and epifluorescence microscope images of glass beads were obtained using a custom-built microscope based on Nikon Eclipse L150 (Nikon Instruments Canada, Mississauga, ON, Canada). The microscope was equipped with a 407 nm diode laser (Radius 405, 25 mW, Coherent), 4x objective lens and monochrome CCD camera (Retiga 200R, Q-imaging, Surrey, BC, Canada) accompanied with QCapture Pro software (ver. 6.0, QImaging). The filter cube was equipped with 395-415 band-pass excitation filter (EXC, z405/20x, Chroma, Rockingham, VT, USA), dichroic mirror (DM, z405rdc, Chroma) and interchangeable emission filters. The gQD PL emission was collected using a 515-535 nm band-pass filter (D525/20, Chroma), and rQD emission was collected with a 600-660 nm band-pass filter (BA 600-660, Nikon). A schematic of the instrument can be found elsewhere.\(^1\) The images were processed using ImageJ software. PL spectra from glass beads were collected by replacing the CCD camera with a fiber-coupled diode array spectrometer (QE65000, Ocean Optics, Dunedin, FL, USA), with spectral processing using Spectra Suite software (Ocean Optics). Monochromatic ellipsometry measurements were made using an AutoEL III ellipsometer.
(Rudolf Research, Flanders, NJ, USA) equipped with a HeNe laser source (632.8 nm emission), and operated at an angle of incidence of 70 degrees. The refractive index of a silicon wafer standard that was used to estimate film thickness was 1.463. The X-ray photoelectron spectroscopy (XPS) analysis was done using a Thermo Scientific K-Alpha XPS spectrometer (Thermo Fisher, E. Grinstead, UK) at Surface Interface Ontario, University of Toronto. The X-ray source was monochromatic Al Ka. The survey spectra were collected at a 60 degree angle. Charge compensation was provided using an e−/Ar+ flood gun. The software used to process the XPS data was Avantage, as provided with the instrument. Scanning electron microscopy images were obtained using a Hitachi S-5200 SEM (Hitachi High Technologies America, Pleasanton, CA, USA). Nuclear magnetic resonance (NMR) spectra were collected using a Bruker Avance 400 MHz spectrometer (Bruker Biospin, Milton, ON, Canada). FTIR spectra were acquired using a Nicolet 6700 and an Avatar 360 FT-IR (Thermo Electron Corp., Thermo Scientific, Mississauga, ON, Canada).

**Preparation of water soluble QDs**

The MPA ligand (300 µL) in 3 mL of chloroform was mixed vigorously with 300 mg of TMAH and allowed to stand to achieve layer separation. Organic QDs (25 µL, 1 – 5 µM) were mixed with 800 µL of deprotonated MPA ligand in chloroform. The mixture was allowed to stand for 12-24 hours to obtain MPA coated QDs at the top. The organic layer was decanted and 100 mL of borate buffer (100 mM, 20 mM NaCl) was added to re-dissolve QDs. The QDs were washed by precipitation with ethanol three times, re-dissolved in borate buffer and stored at 4°C. The concentration of QDs was determined using UV-visible spectroscopy, using the first exciton
absorption peak (518 QDs $\varepsilon_{502} = 54700\ \text{M}^{-1}\text{cm}^{-1}$, 525 QDs $\varepsilon_{503} = 350000\ \text{M}^{-1}\text{cm}^{-1}$ 605 QDs $\varepsilon_{590} = 251000\ \text{M}^{-1}\text{cm}^{-1}$ and 624 QDs $\varepsilon_{611} = 350000\ \text{M}^{-1}\text{cm}^{-1}$).

DHLA-coated QDs were prepared according to previously published protocols with some modifications.\(^2\) The mixture (100 $\mu$L, 5 $\mu$M QD in toluene + 100 $\mu$L neat DHLA) was incubated in a glass culture tube for 2 hours at 70°C under Ar. Subsequently, the mixture was cooled on ice and 1 mL of cold borate buffer was added. To facilitate transfer into the aqueous phase, 200 $\mu$L of TMAH in methanol (25%) was added. The aqueous layer was transferred into an Eppendorf tube and excess ligand was removed by precipitation with ethanol (three times) in the presence of 250 mM NaCl. Finally, QDs were dispersed in borate buffer and stored at 4 °C.

GSH coated QDs were prepared by biphasic ligand exchange. Typically, 100 $\mu$L (5 $\mu$M) of QDs in toluene were mixed with 500 $\mu$L of CHCl\textsubscript{3} in a vial equipped with stir bar. A 1 mL aliquot of borate buffer containing 150 mg of GSH was added to the vial, which was then purged with argon and sealed. Subsequently, 100 $\mu$L of TMAH in methanol (25%) was injected with rapid stirring. Within 5-10 minutes QDs were transferred into aqueous layer. The mixture was allowed to stir for 2-3 hours and QDs were purified from excess ligand by centrifugation using a Amicon 50 kDa membrane filter (Millipore Corporation, Billerica, MA) at 7400 rpm (3 x 4 min) with borate buffer.

**Synthesis of citrate capped Au NPs**

Au NPs were prepared according to Turkevich method.\(^3\) Briefly, gold chloride was added to an Erlenmeyer flask for a final concentration of 1 mM in 50 mL of water from the Milli-Q system. The solution was brought to a boil with stirring and 5 mL of 1% (w/v) of sodium citrate tribasic
dihydrate in water was injected. After 10 minutes of stirring the solution was allowed to cool to room temperature.

**Characterization of Poly-Imidazole Polymer (PAAI)**

For characterization 5-10 mL of the reaction mixture in DMF was dried under high vacuum. PAA-NHS was precipitated with cold isopropanol, filtered, washed with cold methanol (3 x 10 mL) and dried under vacuum to obtain white powder. FT-IR (KBr, cm⁻¹): 2949 (aliphatic C-H), 1812, 1782, 1736 (NHS, C=O), 1430, 1384 (aliphatic C-H), 1207 (C-O). ¹H NMR (CDCl₃, ppm): 1.2-2.17 (PAA, −CH₂−), 2.82 (NHS, −CH₂−), 2.87-3.2 (PAA, −CH−).

PAAI in DMF was triturated with ether and collected precipitate was re-dissolved in a minimal amount of methanol (ca. 5-10 mL), diluted with an equal volume of DCM, and then cold ether was added until precipitation was observed. PAAI was collected as white solid by filtration. FT-IR (KBr, cm⁻¹): 3115 (aromatic C-H), 2941 (aliphatic C-H), 1655 (amide), 1447 (CH2), 1384 (aliphatic C-H), 1231 (C-N) and other imidazole relate peaks 3297, 1554, 1515, 1086, 664. ¹H NMR (DMSO-d₆, ppm): 1.1-1.98 (PAA, −CH₂−), 2.97-3.4 (PAA, −CH−), 3.54-3.93 (−CH₂−), 6.88, 7.15, 7.64.
Figure S1. Schematic illustration of solution synthesis of PAAI (a) and corresponding FT-IR spectra of (i) starting material (PAA), (ii) intermediate NHS activated (PAA-NHS) and (iii) PAAI (b).
Figure S2. $^1$H NMR spectra of PAAI in DMSO-d$_6$. Inset shows spectra of starting material, PAA.

XPS results for stepwise surface modifications

<table>
<thead>
<tr>
<th></th>
<th>Relative atomic percent, %</th>
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<tr>
<td></td>
<td>Si (2p)</td>
</tr>
<tr>
<td>RCA</td>
<td>53.5</td>
</tr>
<tr>
<td>APTMS</td>
<td>42.7</td>
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<tr>
<td>PAAI</td>
<td>37.4</td>
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Characterization of Photoluminescent Properties of QDs Immobilized using Multidentate Imidazole surface Ligands.

Table 2S. Photoluminescence properties of immobilized QDs.

<table>
<thead>
<tr>
<th>QDs</th>
<th>QY (soln)</th>
<th>$\lambda_{\text{max}}$ (soln), nm</th>
<th>$\Delta$PL, nm</th>
<th>FWHM (soln)</th>
<th>FWHM (immob)</th>
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<tbody>
<tr>
<td>518 MPA</td>
<td>46%</td>
<td>522.1 ± 0.4</td>
<td>3.5 ± 0.5</td>
<td>29.5 ± 0.5</td>
<td>31.8 ± 0.6</td>
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<td>525 MPA</td>
<td>18%</td>
<td>525.3 ± 0.3</td>
<td>2.4 ± 0.5</td>
<td>29.5 ± 0.6</td>
<td>30.9 ± 0.6</td>
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<tr>
<td>525 DHLA</td>
<td>18%</td>
<td>527.6 ± 0.2</td>
<td>3.1 ± 0.5</td>
<td>29.3 ± 0.4</td>
<td>29.8 ± 0.5</td>
</tr>
<tr>
<td>525 GSH</td>
<td>49%</td>
<td>524.3 ± 0.2</td>
<td>1.5 ± 0.5</td>
<td>29.1 ± 0.2</td>
<td>30.3 ± 0.3</td>
</tr>
<tr>
<td>605 MPA</td>
<td>56%</td>
<td>607.8 ± 0.5</td>
<td>3.3 ± 0.6</td>
<td>26.8 ± 0.5</td>
<td>28.0 ± 0.6</td>
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<tr>
<td>623 MPA</td>
<td>34%</td>
<td>622.1 ± 0.3</td>
<td>2.3 ± 0.5</td>
<td>29.3 ± 0.5</td>
<td>29.9 ± 0.1</td>
</tr>
<tr>
<td>623 DHLA</td>
<td>16%</td>
<td>620.2 ± 0.1</td>
<td>2.8 ± 0.6</td>
<td>29.6 ± 0.1</td>
<td>30.2 ± 0.8</td>
</tr>
<tr>
<td>623 GSH</td>
<td>30%</td>
<td>623.1 ± 0.3</td>
<td>0.8 ± 0.4</td>
<td>29.4 ± 0.2</td>
<td>31.4 ± 0.2</td>
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$^a$ peak emission wavelength in toluene and corresponding ligand to obtain water-soluble QDs. The 518 and 605 QDs were comprised of a CdSe/ZnS core/shell structure, and were estimated to have diameters of 2 nm and 6 nm, respectively. The 525 and 623 QDs were comprised of a CdSe$_x$S$_{1-x}$/ZnS core/shell structure, and were estimated to have a relatively constant diameter of 5 - 6 nm. $^b$ $\lambda_{\text{max}}$ = peak photoluminescence wavelength. $^c$ Spectral shift upon immobilization. FWHM = full-width-at-half-maxima.
Data Analysis

Förster Formalism and FRET Pairs

The 525 MPA QD - Cy3 and 624 DHLA QD – Alexa 647 FRET pairs were characterized using the Förster formalism. The Förster distance ($R_0$, in units of cm) was calculated from solution based measurements using Eq. 1:

$$R_0^6 = 8.79 \times 10^{-28} \text{ mol} \times (n^{-4} \kappa^2 \Phi_D J)$$  \[1\]

where $n$ is the refractive index of the surrounding medium and was approximated as 1.335, $\kappa^2$ is the orientation factor, assumed as 2/3, $\Phi_D$ is the quantum yield of the donor and $J$ is the spectral overlap which was calculated according to Eq. 2:

$$J = \frac{\int F_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda}{\int F_D(\lambda) d\lambda}$$  \[2\]

where $F_D$ is the fluorescence intensity of the donor and $\epsilon_A$ is the molar absorptivity coefficient of the acceptor as a function of wavelength $\lambda$. The spectral overlap was determined to be $5.66 \times 10^{-10}$ M$^{-1}$cm$^6$ and the Förster distance was calculated to be 4.82 nm for green QD - Cy3, while spectral overlap $1.94 \times 10^{-9}$ M$^{-1}$cm$^6$ and the Förster distance 5.80 nm for red QD - Alexa 647.

PL decay curves of immobilized QDs, QD-probe conjugates and QD – dsDNA conjugates were measured and the data was fit against the instrument response function while minimizing the $\chi^2$. PL decay curves were fit using a two component lifetime and average lifetime calculated using Eq. 3:
\[ \tau_{av} = \frac{A_s^2 \tau_s^2 + A_l^2 \tau_l^2}{A_s \tau_s + A_l \tau_l} \]  

where subscripts \( s \) and \( l \) denote short and long components respectively.

The FRET efficiency was calculated from the time-resolved PL measurements using Eq. 4:

\[ E = 1 - \frac{\tau_{DA}}{\tau_A} \]  

where \( \tau_D \) and \( \tau_{DA} \) correspond to the lifetime of the QD probe conjugates (D – Donor) before and after hybridization (DA – Donor in the presence of Acceptor) with labeled target, respectively.

The FRET ratio was used for ratiometric data analysis. All spectra were normalized to 450 nm, background corrected and then normalized to QD PL maxima. The FRET ratio was calculated using Eq. 5.

\[ FRET\ Ratio = \frac{\left( \sum_{\lambda=a}^{\lambda=b} PL(\lambda) \right)_{DA}}{\left( \sum_{\lambda=a}^{\lambda=b} PL(\lambda) \right)_{D}} - \frac{\left( \sum_{\lambda=c}^{\lambda=d} PL(\lambda) \right)_{DA}}{\left( \sum_{\lambda=c}^{\lambda=d} PL(\lambda) \right)_{D}} \]  

where a wavelength range in the numerator corresponded to acceptor PL, while a wavelength range in the denominator of each term was associated with donor PL.

For green QD – Cy3 FRET pair: \( a = 510 \) nm and \( b = 534 \) nm, \( c = 560 \) nm and \( d = 590 \) nm

For red QD – Alexa 647 FRET pair: \( a = 615 \) nm and \( b = 645 \) nm, \( c = 655 \) nm and \( d = 695 \) nm
The subscript $DA$ in Eq. 4 corresponds to the measurement made in the presence of the donor (QD) and the acceptor (Cy3/Alexa 647), while the subscript $D$ corresponds to the measurements made in the absence of acceptor.

References