Chapter 7

Phase Transfer and DNA Functionalization of Quantum Dots Using an Easy-to-Prepare, Low-Cost Zwitterionic Polymer

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Abstract

Small, stable, and bright quantum dots (QDs) are of interest in many biosensing and biomedical imaging applications, but current methodologies for obtaining these characteristics can be highly specialized or expensive. We describe a straightforward, low-cost protocol for functionalizing poly(isobutylene-*alt*-maleic anhydride) (PIMA) with moieties that anchor to the QD surface (histamine), impart hydrophilicity [(2-aminoethyl)trimethylammonium chloride (Me_3N^+-NH_2)], and provide a platform for biofunctionalization via click chemistry (dibenzocyclooctyne (DBCO)). Guidelines to successfully use this polymer for QD ligand exchange are presented, and an example of biofunctionalization with DNA is shown. Stable QD–DNA conjugates are obtained with high yield and without requiring additional purification steps.

Key words DNA labeling, Copper-free click chemistry, Biofunctionalization, Nanoparticle, Carboxybetaine, Poly(isobutylene-*alt*-maleic anhydride)—PIMA

1 Introduction

Quantum dots (QDs) are semiconductor nanocrystals that exhibit size-tunable fluorescence, which is bright, narrow, and photostable. This can be advantageous in biological applications that require a high signal-to-noise ratio or long-term exposure to light. However, the majority of quantum dots are synthesized in organic solvents and must be postprocessed to impart water solubility. The methods for achieving this are vast and varied [1–3]. Commercially available, hydrophilic QDs often employ an amphiphilic polymer that encapsulates the QD with its original organic ligands. While quite stable, their large hydrodynamic size can negatively affect energy transfer [4, 5], and the robustness of the steric coating precludes the use of histidine-based self-assembly of peptides and proteins to the QD, one of the simplest ways to prepare QD bioconjugates [5, 6]. Ligand exchange strategies that displace the native organic ligands create smaller particles. The first iterations of this method used small molecule ligands, but reports have shown

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that increasing the number of chemical handles bound to the QD surface improves stability [7, 8]. Building on that knowledge, several groups have reported the synthesis of multidentate polymers [9–11] for QD water dispersion that provide excellent colloidal stability. The Mattoussi group has developed a particularly compelling strategy using a poly(anhydride maleic-*alt*-isobutylene) (PIMA) as a polymer backbone for facile functionalization with primary amines [12]. They have shown effective grafting of amine-bearing imidazole, lipoic acid, and phosphonates for anchoring the polymer to the QD surface, as well as sulfobetaine and polyethylene glycol (PEG) derivatives for hydrophilicity [13–15]. Interest in zwitterionic or charge-based surface coatings, like the PIMA variant displaying sulfobetaine, is high as the surface charges are reported to confer excellent colloidal stability and reduce protein adsorption or opsonization [16]. Unfortunately, synthesizing and handling sulfobetaine or the similar zwitterionic molecule carboxybetaine with a primary amine is nontrivial, as is the synthesis of zwitterionic polymers from zwitterionic monomers. To expand access to those who are not organic chemists, we outline a straightforward protocol for preparing a low-cost, charged polymer (Subheading 3.1) and using it in a straightforward ligand exchange (Subheading 3.2) to obtain an aqueous colloidal solution of bright, small, and stable QDs [17].

Rather than using polyethylene glycol (PEG; expensive) or sulfobetaine (difficult to synthesize) derivatives to functionalize the PIMA, we use a commercially available quaternary amine (2-aminoethyl) trimethylammonium chloride (Me_3N^+-NH_2) to impart a persistent positive charge [18]. Combined with the carboxylic acid produced on each PIMA moiety formed during the amide coupling between the maleic anhydride and the primary amine-containing molecules, the Me_3N^+-NH_2 produces a carboxybetaine-like zwitterionic coating without the need to synthesize a zwitterionic agent. Histamine, used as the anchoring group mediating the interaction between the QD surface and the polymer, is also readily available. To make the polymer relevant to a variety of biological applications, we include dibenzocyclooctyne-amine (DBCO-amine), which can react with any azido biomolecule of interest (e.g., derivatives of ligands like folic acid or biotin as well as larger peptides or proteins). Specifically, we detail our purification-free protocol for grafting DNA to the QD surface (Subheading 3.3). Research in creating QD–DNA conjugates is ongoing, and historically, methods have been low-yield with the highest grafting efficiencies reaching only ~65% [9, 18], necessitating downstream purification steps [19]. The protocol we provide is simple and highly efficient (>95%), precluding the need for purification and increasing the overall yield of the QD–DNA conjugates obtained.

2 Materials

2.1 Polymer Synthesis, Purification, and Characterization

1. Polymer backbone: Poly(isobutylene-*alt*-maleic anhydride) (PIMA; molecular weight (MW) ~6000 g/mol). PIMA has been largely shelf-stable in our experience, but since water can hydrolyze the rings needed for functionalization, long-term storage in a desiccator or in a glove box at room temperature is preferred.
2. Primary amine-containing molecules to functionalize polymer: (2-aminoethyl) trimethylammonium chloride hydrochloride (Me_3N^+-NH_2) with triethylamine (Et_3N) for solubility; histamine; dibenzocyclooctyne-amine (DBCO-NH_2) (*see* **Note 1**).
3. Anhydrous dimethylsulfoxide (DMSO) (*see* **Note 2**).
4. Polymer precipitation solution: preweigh two 50 mL conical tubes, taking care to mark which lid belongs to which tube. Prepare 50 mL of ethyl acetate/diethyl ether (1:1) by mixing 25 mL of ethyl acetate and 25 mL diethyl ether and divide between the two tubes. Mixing the ethyl acetate and diethyl ether in one of the tubes and pouring half into the other is precise enough for this purpose and ensures that the solvent ratio is the same in both tubes.

2.2 Quantum Dot Preparation and Ligand Exchange

1. Quantum dots (*see* **Note 3**).
2. 0.1 M sodium bicarbonate ($NaHCO_3$): mix 8.401 g of $NaHCO_3$ with 1 L diH_2O.

2.3 DNA Labeling and Characterization

1. Azide-modified oligonucleotides (ssDNA-N_3) and their complement strands, plain and biotinylated (csDNA & csDNA-bt), can be custom ordered, for example from IDT Technologies).
2. 1× Duplex Buffer: 100 mM potassium acetate, 30 mM HEPES, pH 7.5.
3. Streptavidin-coated agarose beads.
4. 2 M sodium chloride (2 M NaCl): Add 1.17 g of NaCl to a graduated cylinder and increase the volume to 10 mL with diH_2O. Add stir bar and leave on a stir plate until completely dissolved (~1 min).

3 Methods

3.1 Polymer Preparation

All synthesis steps should be carried out under air-free conditions (https://www.chemistryviews.org/details/education/4360441/Tips_and_Tricks_for_the_Lab_Air-Sensitive_Techniques_3.html). Either nitrogen or argon can be used as the inert gas in this protocol.

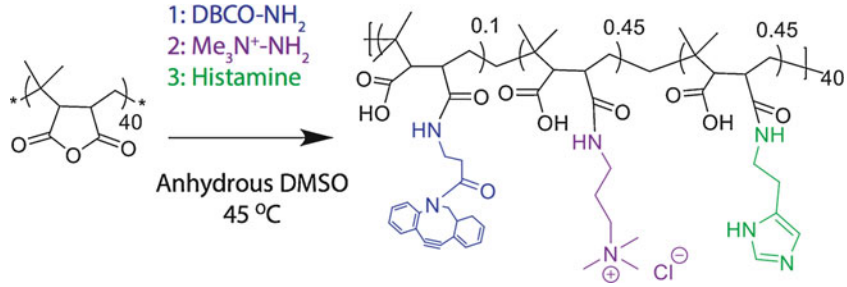


Fig. 1 Polymer functionalization scheme. Maleic anhydrides react with primary amines to functionalize the PIMA backbone. Hydrophilic or charged moieties are added in combination with anchoring groups for use as a coating to impart water solubility to QDs. Specifically, histamine is used to self-assemble the polymer to the QD surface, Me_3N^+ provides hydrophilicity and counterbalances the negative charges of carboxylic acids on the PIMA backbone, and DBCO enables further bioconjugation via copper-free click chemistry

Table 1
Calculation of reagent amounts needed for a reaction consisting of 45% histamine, 45% Me_3N^+, and 10% DBCO-NH_2

Reagent	Reagent Fraction	Molar equiv.[a]	Moles[b] (mmol)	MW (g/mol)	Mass (mg)	Density (g/mL)	Volume (μL)	[Final] (mM)	DMSO (mL)
PIMA		1	0.04	6000	240			6.7	6
histamine	0.45	18	0.72	111.15	80			268	11.5
Me_3N^+-NH_2	0.45	18	0.72	175.10	126				
DBCO-NH_2	0.1	4	0.16	276.33	44				
Et_3N		36	1.44	101.49	146	0.73	200		

[a]Molar equivalents obtained by multiplying desired grafting fraction by the number of monomer/PIMA polymer (~40)
[b]Millimoles (mmol) of each reagent needed, calculated by multiplying N by the number of mmol of PIMA. PIMA is first weighed and the number of mmol calculated by dividing mass (m) in mg by MW (molecular weight)

This procedure is specific to preparing a polymer for ligand exchange that is functionalized with 45% histamine, 45% Me_3N^+-NH_2, and 10% DBCO-NH_2 (Fig. 1). Different grafting percentages can be obtained by adjusting the molar ratios of each reagent. Additional or different functional groups can be included as long as they contain a primary amine (R-NH_2). It can be convenient to prepare a table (Table 1) for calculating the amount of each reagent needed. For PIMA with MW ~6000 g/mol there are on average ~39 monomers (MW = 154 g/mol) per polymer. We calculate our molar equivalents by assuming 40 monomers/polymer in order to provide slight excess of amine bearing reagents.

3.1.1 Solution 1

1. Weigh 0.240 g of PIMA into a 25 mL round bottom flask equipped with a magnetic stir bar and sealed with a septum.
2. Insert a needle (*see* **Note 4**) in the septum and degas either by (a) actively flowing nitrogen through the flask (using a second needle to allow nitrogen to escape, creating flow) while gently stirring the powder for 20 min or (b) applying three cycles of vacuum/nitrogen for 5 s each (*see* **Note 5**).
3. Add 6 mL of anhydrous DMSO to the dry powder with a syringe. The PIMA should dissolve easily in DMSO.
4. Heat Solution 1 to 45 °C while stirring.

3.1.2 Solution 2

1. In a separate septum-sealed container (glass vial or round bottom flask), combine histamine (80 mg), Me_3N^+-NH_2 (126 mg), and DBCO-NH_2 (44 mg) at 18, 18, and 4 molar equivalents to PIMA, respectively. Include a magnetic stir bar.
2. Add Et_3N at a 2:1 molar ratio to Me_3N-NH_2 (200 μL) to help solubilize Me_3N^+ in DMSO.
3. Insert a needle in the septum and degas by either (a) actively flowing nitrogen through the flask while gently stirring the mixture for 5 min or (b) applying three cycles of vacuum/nitrogen for 5 s each. Be careful not to evaporate the triethylamine.
4. Add anhydrous DMSO with a syringe such that the final concentration of all reagents in solution is 268 mM in 11.5 mL.
5. Heat Solution 2 to 50 °C while stirring to fully dissolve reagents.

3.1.3 Mixing Solutions

1. Add Solution 2 to Solution 1 (stirring at 45 °C) dropwise with a syringe.
2. Allow the mixture to stir overnight at 45 °C.

3.1.4 Polymer Purification and Characterization

1. Add the polymer–DMSO solution (18 mL total) dropwise to the two preweighed 50 mL conical tubes each containing ~25 mL of precipitating solvent (~9 mL polymer solution in each tube). The solution will become turbid (cloudy), indicating that the polymer is precipitating.
2. Centrifuge the 50 mL conical tube (~6000 × *g*, 5–15 min) to obtain a pellet of the PIMA polymer (*see* **Note 6**). Discard the supernatant containing unreacted amines and triethylamine.
3. Set the conical tubes upside down on a laboratory wipe and let sit for ~5 min to dry the pellet.
4. Add methanol (MeOH—~1–5 mL) directly to each tube to redisperse the polymer pellet (*see* **Note 7**).
5. Precipitate the polymer from MeOH by adding ethyl acetate until the solution becomes turbid (~10–20 mL).

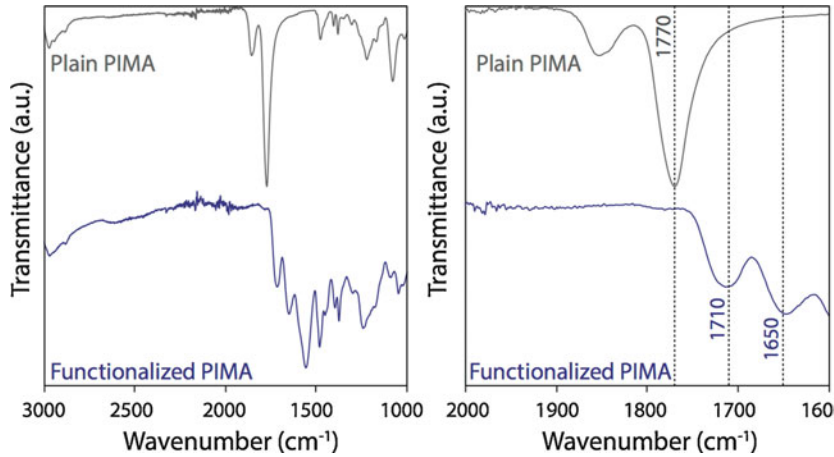
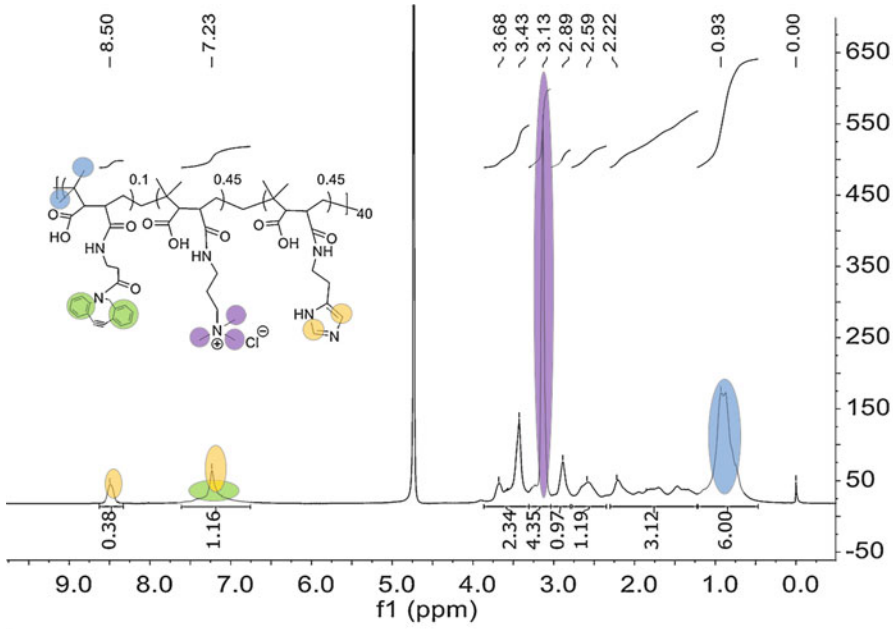


Fig. 2 FTIR spectra of the as-shipped (grey) and functionalized PIMA polymer (blue). The full spectrum is plotted on the left, while a narrower range is plotted on the right to highlight the region of interest

6. Centrifuge to obtain a pellet of the purified polymer and discard the supernatant.
7. Dry the purified polymer under vacuum. Septa (24/40 or 29/32) can be used to seal the 50 mL conical vials and attached to the vacuum/gas manifold with a syringe and needle. Alternatively, a vacuum desiccator can be used, if available.
8. The mass of the polymer is obtained by weighing the tube with polymer and subtracting the original weight of the tube. The chemical yield is estimated by dividing the end weight by the original total mass of the reaction. The yield should be higher than 70%.
9. After purification and drying, a white powder is obtained and should be stored at 4 °C in the dark for further use.
10. Acquire an infrared spectrum of the powder using an attenuated total reflection (ATR) probe on a Fourier-transform infrared (FTIR) spectrophotometer to confirm the disappearance of the C=O stretch of the anhydride at 1770 cm^{-1} and appearance of the C=O stretch of carboxylic acid and amide at 1710 cm^{-1} and 1650 cm^{-1}, respectively (Fig. 2).
11. Run ^{1}H NMR in D_2O to confirm the purity of the polymer and its molar composition (example calculations provided in Fig. 3; *see* **Note 8**).

3.2 Quantum Dot Preparation and Phase Transfer

1. Aliquot ~150 μL of QDs in organic solution into a 2 mL centrifuge tube and add ~150 μL of hexanes. Commercially sourced QDs ship at ~1 μM. The QDs used in this procedure started at 4.8 μM.



Proton	f1 (ppm)	Integral (H)	Proton Count	% R-NH_2 grafted
isobutyl	0.93	6	6	reference
3 x methyl	3.13	4.35	9	48%
imidazole	8.50 & 7.23	0.38 x 2 = 0.76	2	38%
aromatics	6.8 - 7.8	1.16 - 0.38 = 0.78	8	10%

Fig. 3 Analysis of ^{1}H NMR of the polymer in D_2O. The isobutyl group is used as reference (six protons). Percentage of R-NH_2 grafted is calculated by dividing the measured integral by each molecule's proton count. The 1H signal of the imidazole at 7.23 overlaps the 8H signal from the aromatics on the DBCO. For the imidazole, the 1H signal at 7.23 is estimated to be the same as that at 8.5, so the DBCO integral is estimated by subtracting the integral from 6.8 to 7.8 by that of the 1H from imidazole at 8.5

2. Add ethanol (EtOH) until the solution becomes cloudy (*see* **Note 9**).
3. Centrifuge to obtain a pellet of the "cleaned" QDs (*see* **Notes 10** and **11**).
4. Redisperse the QDs in chloroform ($CHCl_3$) to a final concentration of ~100 nM (7 mL).
5. Syringe filter (0.1 μm, polyvinylidene fluoride—PVDF) and transfer the 1 mL of the QDs in $CHCl_3$ to a glass vial with a stir bar (*see* **Notes 12** and **13**).
6. Prepare ligand solution: weigh polymer in a glass vial and add anhydrous DMSO to a final polymer concentration of 10 mg/mL. Use ~10 mg of polymer per nmol of QD (~800:1 molar

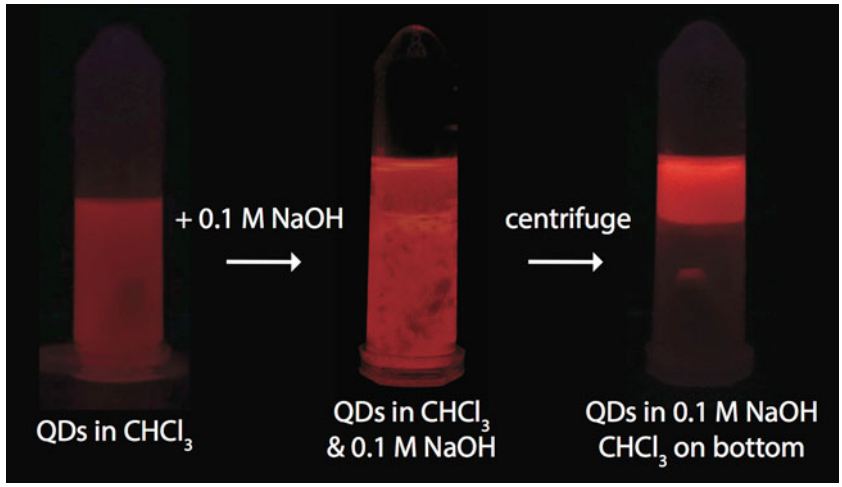


Fig. 4 Quantum dots ligand exchanged with the functionalized PIMA. Successful ligand exchange and phase transfer is indicated by the QDs moving from the bottom (organic) phase to the upper (water) phase

excess of polymer) (*see* **Note 14**). Heating with a heat gun or at 50 °C in an oil or water bath is usually required to fully dissolve the polymer.

7. Add the ligand solution dropwise to the QD solution while stirring at room temperature (ca. 22 °C). For 1 mL of QDs at 100 nM, 100 μL of polymer at 10 mg/mL should be added. The final solution should consist of 10:1 $CHCl_3$:DMSO.
8. Purge the vial with nitrogen and allow the solution to stir for at least 1 h at room temperature (ca. 22 °C) and protected from light (foil wrapped) (*see* **Note 15**).
9. For transfer to water, add 0.5 mL of 0.1 M NaOH to the QD–PIMA–DMSO–$CHCl_3$ mixture while stirring (*see* **Note 16**).
10. Turn off stir plate and allow the aqueous phase and organic phases to separate. In a successful ligand exchange, the QDs appear in the top (aqueous) phase (Fig. 4) (*see* **Notes 17–19**).
11. Transfer to a centrifuge tube and centrifuge at ~2000 × *g* for 1 min to obtain two nicely separated phases (Fig. 4) and precipitate any aggregates. Separate the supernatant and collect the top, aqueous layer containing QDs in 0.1 M NaOH.
12. Filter the QDs in 0.1 M NaOH on a 0.1 μm (or 0.25 μm) PVDF syringe filter into a 100 kDa centrifugal filter (0.5 mL capacity) (*see* **Note 20**).
13. Wash the QDs with 0.5 mL of 0.1 M $NaHCO_3$ and centrifuge at ~9000 × *g* for 2 min.
14. Discard the flow-through and refill the centrifugal filter with 0.1 M $NaHCO_3$.
15. Centrifuge at ~9000 × *g* for 2 min.

16. Repeat **steps 14** and **15** at least two more times.
17. Recover the QDs in a minimal amount of buffer (0.1 M $NaHCO_3$) and store concentrated at 4 °C in the dark.

3.3 DNA Functionalization

3.3.1 DBCO Stoichiometry Determination

1. The number of DBCO moieties per QD can be estimated with UV-Vis absorbance. DBCO absorbs strongly in the UV and has a molar extinction coefficient (ε) of 12,000 M^{-1} cm^{-1} at 309 nm. Scale QD absorbance before ligand exchange to the absorbance spectra of the QD after ligand exchange at 400 nm. Calculate QD concentration using the Beer–Lambert Law and the QD molar extinction coefficient given by the vendor or using published size-dependent molar extinction coefficient formulas [20].

   Beer–Lambert Law:

$$A_\lambda = \varepsilon_\lambda \tilde{n} c \tilde{n} l$$

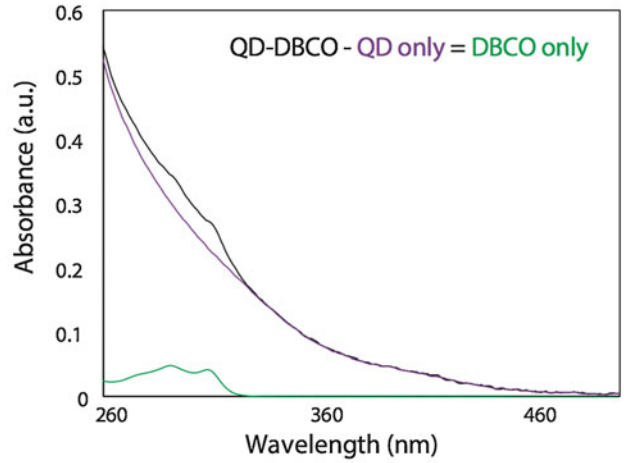
   where A_λ is absorbance at wavelength λ, ε_λ is molar extinction coefficient (M^{-1} cm^{-1}) at wavelength λ, c is concentration (M), and l is pathlength (cm).
2. Subtract the absorbance before ligand exchange from the absorbance after ligand exchange to obtain the DBCO absorbance. Use the value of the absorbance at 309 nm to calculate the DBCO concentration using the Beer–Lambert Law.
3. Divide the DBCO concentration by the QD concentration to obtain the DBCO:QD ratio (Fig. 5). The DNA grafting reaction relies on DBCO–azide coupling, so the DBCO:QD ratio can be used to ensure that there are a sufficient number of biofunctional handles on the QD.

3.3.2 DNA Conjugation and Hybridization

1. Add PIMA-coated QDs (0.2 nmol; 70 μL at ~3 μM) and DNA-N_3 (4 nmol; 20 μL at ~200 μM) to a 500 μL PCR tube at a DNA:QD ratio of 20:1 (*see* **Note 21**).
2. Add NaCl to the QD–DNA mixture to obtain a final NaCl concentration of 1 M (90 μL of 2 M NaCl).
3. Wrap the tube in aluminum foil and shake on an agitation plate for 4 days at room temperature.
4. Centrifuge with a tabletop centrifuge for ~ 1 min to pellet any aggregates (*see* **Note 22**). Collect the supernatant.

3.3.3 DNA Hybridization

1. Add 4 nmol (i.e., an equimolar amount) of the DNA complement to the QD/ssDNA conjugates (*see* **Note 23**).
2. Heat to 95 °C for 2 min and gradually cool. A cooling ramp can be used, but simply transferring samples from a heat block/water bath to the benchtop at room temperature is usually sufficient (*see* **Note 24**).
3. The QD-dsDNA can now be stored at 4 °C in the dark for further use. Do not freeze.



	QD @ 400 nm	DBCO only @ 309 nm
Absorbance	0.04	0.04
ε_λ (M^{-1} cm^{-1})	1.5×10^6	1.2×10^4
pathlength (cm)	1	1
Concentration (M^{-1})	2.7×10^{-8}	3.3×10^{-6}
DBCO/QD = $3.3 \times 10^{-6} / 2.7 \times 10^{-8} = 125$		

Fig. 5 DBCO/QD Analysis. Absorbance spectra of the QDs in $CHCl_3$ is normalized at 400 nm to the absorbance of the QDs coated with the functionalized PIMA ligand to obtain the contribution to absorbance from DBCO. The concentration of the QDs and DBCO can then be calculated separately and used to calculate DBCO:QD ratio

3.3.4 Verification of Hybridization

1. For simple verification of hybridization, a small aliquot of the QD-DNA can be hybridized with a biotinylated complement (QD-dsDNA-bt) so hybridization can be verified in a bead pull-down assay.
2. Aliquot ~200 μL of streptavidin-agarose bead slurry into a 500 μL PCR tube.
3. Centrifuge for 30 s using a tabletop centrifuge (~2000 × *g*) to pellet beads.
4. Pull off the supernatant (~100 μL) with a pipette and add 100 μL of 0.1 M $NaHCO_3$. Pipet up and down 5–10 times to mix.
5. Repeat **steps 3** and **4** two more times to fully wash the streptavidin beads.
6. Split bead slurry into two 500 μL PCR tubes (100 μL each).
7. To one tube (Tube 1) add 2–10 μL of the QD-dsDNA. Pipet up and down 5–10 times to mix.
8. To the other tube (Tube 2) add 2–10 μL of the QD-dsDNA-bt. Pipet up and down 5–10 times to mix.

9. Centrifuge for 30 s using a tabletop centrifuge (2000 × *g*) to pellet beads.
10. Repeat **steps 3** and **4** two times to wash unbound QDs from the slurry.
11. Check for fluorescence under UV illumination (lamp, flashlight, etc.) The QD-dsDNA should not bind to the beads, so Tube 1 should be nonfluorescent, while the opposite is true of QD-dsDNA-bt.

4 Notes

1. Histamine and DBCO-NH_2 should be stored at −20 °C. When using reagents from the freezer, they should be taken out prior to the reaction and allowed to warm to room temperature (ca. 22 °C) before opening to avoid water condensation.
2. When taking anhydrous DMSO from a commercial septum-sealed bottle, use an argon or nitrogen balloon to compensate for the volume of solvent taken with a syringe and needle. Do not use needles larger than 16-gauge to puncture the septum.
3. This protocol can be used with QDs from a wide variety of sources, including in-house lab-made QDs of any number of compositions (CdSe, InP, etc.) capped with a ZnS shell or commercially available QDs sold in organic solvents. The key QD characteristic critical to successful encapsulation is a ZnS capping shell, as this passivation layer ensures photoluminescence after water transfer. If using QDs synthesized in-house, it is helpful to terminate the QD surface with cation (zinc) because the histamine (imidazole) has affinity for divalent cations.
4. Consider the inner diameter of the tubing on the manifold and roughly match to the outer diameter of a plastic syringe, likely either the 1 or 3 mL size. Remove and discard the syringe plunger. Cut the syringe barrel just below the flanges on the back end to produce a plastic tube with a needle connection. Insert the straight barrel of the cut syringe into the manifold tubing; attach a needle and insert needle into septum.
5. If vacuum/inert gas cycling is used to degas the flasks relatively quickly, be sure to gradually switch from nitrogen to vacuum, as sudden introduction can cause the PIMA powder to fly about and potentially be drawn up the vacuum line.
6. If the polymer does not precipitate easily, place the conical tubes in the freezer (−20 °C) for 1 h and recentrifuge. If a refrigerated centrifuge is available, centrifuge at −20 °C.

7. If the polymer does not dissolve readily, gentle sonication, gentle heating (warning: boiling point of MeOH is 65 °C), or adding acetonitrile can help.
8. If purification by precipitation does not yield high purity, the polymer can be further purified by dialysis (<10 kDa membrane). This should be done *after* FTIR as any un reacted maleic anhydride rings will open in this process and confound FTIR results. Resuspend the product in a minimal amount of water and load into dialysis tubing or cassette. Place in a beaker filled with ultrapure water (100–1000 mL); add a couple drops of 1 M NaOH to the dialysis bath to help swell the polymer and remove impurities. Let dialysis proceed for at least 1 h (longer is not harmful to the product, but will extend the time of the procedure). Replace the dialysis bath with ultrapure water and again allow the dialysis to proceed for at least one hour. The dialysis bath should be replaced at least five times with ultrapure water to remove all impurities.
9. Although many labs, including ours, have wash bottles of commonly used solvents available for convenience, we find that using ethanol freshly aliquoted from the reagent bottle has a significant effect on maintaining the brightness of the final QD product. Ethanol is hygroscopic and will draw water out of the air, gradually contaminating the solvent.
10. Sometimes extra washing steps (redispersing in decane or hexanes and repeating **step 2** in Subheading 3.2) can help if the ligand transfer does not work for a specific QD sample. It is very important to remember to perform **step 5** (Subheading 3.2) if several QD precipitations are done because QDs will start to aggregate as more organic ligands are cleaned from their surface.
11. QDs that have not been successful in ligand transfer may benefit from a post-synthesis treatment with zinc stearate. After **step 3** (Subheading 3.2), the QD pellet can be redispersed directly in a small amount (~1 mL) of 0.2 M zinc stearate in octadecene (ODE). Transfer the QD–Zn solution to a septum-fitted glass vial with a stir bar. Degas the solution by bubbling nitrogen through the solution. Heat while stirring at 240 °C for 2–3 h. The QDs should then be precipitated from solution by addition of hexane and ethanol and centrifuging. Extra washing steps are necessary following the zinc treatment. Alternatively, the Smith group outlines several additional surface treatment approaches available to those who are more comfortable with chemistry [9]. To prepare 0.2 M zinc stearate in ODE, weigh 6.3233 g of zinc stearate into a 100 mL round bottom flask. Add a stir bar. Heat while stirring under vacuum to 80 °C until fully dissolved. Once dissolved, fill the flask with

nitrogen and stir for 15 min before switching back to vacuum and degassing for 15 min. Perform at least three vacuum/nitrogen cycling steps to ensure that the solution is air-free.

12. The rest of the QDs can be stored in a tightly sealed vial at 4 °C in the dark for up to a week or long term (~2 months) at −20 °C. Be sure to syringe filter the QD sample and check the concentration by UV-Vis absorption before ligand transfer.
13. For samples that are sensitive to oxidation, the vial can be septum capped and nitrogen gently bubbled through the solution (~5 min) while stirring. Dip the vial in an ice bath to avoid evaporation of the $CHCl_3$.
14. If nanoparticle size is known, scaling to nanoparticle surface area can help to reduce waste of polymer. We find that ~300 anchoring groups (imidazoles) per nm^2 QD surface (QD concentration multiplied by the surface area of 1 QD) works consistently.
15. One hour is normally sufficient for ligand exchange. Overnight ligand exchange has been qualitatively observed to impart better stability but at the cost of decreased quantum yield.
16. Alternatively, the QDs can first be precipitated from the DMSO–$CHCl_3$ solution by addition of ethyl acetate and centrifugation (~9000 × *g*, ~5 min). The supernatant should be discarded and the pellet recovered directly into 0.1 M NaOH.
17. Sometimes a white precipitate is seen at the solvent interface. This can be pelleted through centrifugation and discarded.
18. If the phase transfer failed and QDs are in the bottom organic phase, they can be recollected and the ligand exchange reattempted. The QDs should be first filtered through a 0.1 μm PVFD syringe filter to remove any aggregates that may have formed, and a higher polymer:QD ratio should be used.
19. If the QDs are stuck between the water and organic phases, sometimes they can be recovered. Remove the $CHCl_3$–DMSO layer with a syringe and pour the aqueous layer + QDs into a 2 mL centrifuge tube. This can be gently sonicated to disperse the QDs in the water phase. Once relatively dispersed, hexanes can be added to the tube to wash off any excess organic ligands that may have gotten "stuck" between the polymer and QD during ligand exchange. The tube should then be sonicated (~5 min) and centrifuged (~2000 × *g* for 1 min). QDs in the water phase (bottom) can be collected and used. If this does not work, the ligand transfer should be repeated from the beginning using freshly prepared QDs in $CHCl_3$ and a higher polymer ratio.
20. Sometimes at **step 12** (Subheading 3.2), the QDs are trapped on the PVDF filter, indicating that large aggregates were

solubilized rather than single QDs. Double check that the QD concentration in $CHCl_3$ is ~100 nM; if the solution is too concentrated, multiple QDs can be wrapped together during ligand exchange. Alternatively, increasing the $CHCl_3$:DMSO ratio by using a ligand solution at 20 mg/mL rather than 10 mg/mL may avoid QD aggregation during DMSO addition.

21. Higher or lower DNA:QD ratios can be used, but the DBCO:DNA ratio should be higher than 2:1.
22. We have consistently observed nearly quantitative (>95%) reaction efficiency in the DNA to QD conjugation reaction at the conditions described [17]. Most downstream applications do not need further purification. For applications extremely sensitive to free DNA, dialysis or centrifugal filters can be used to separate QD–DNA conjugates from free DNA.
23. High salt concentration can affect DNA hybridization. Double check your oligo melting temperature at 1 M NaCl before proceeding with hybridization (https://www.idtdna.com/calc/analyzer/). If the salt concentration needs to be lowered, the solution can be diluted with 1× duplex buffer (100 mM potassium acetate; 30 mM HEPES, pH 7.5).
24. https://www.idtdna.com/pages/education/decoded/article/annealing-oligonucleotides can be referred to for more detailed instructions.

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