Supporting Information

Photoligation of an Amphiphilic Polymer with Mixed Coordination Provides Compact and Reactive Quantum Dots

Wentao Wang,[1] Anshika Kapur,[1] Xin Ji,[1] Malak Safi,[1] Goutam Palui,[1] Valle Palomo,[2] Philip E. Dawson,[2] and Hedi Mattoussi,[1]

[1]Florida State University, Department of Chemistry and Biochemistry, Tallahassee, FL 32306, United States

[2] Department of Chemistry and Department of Cell Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037, United States

Materials

Poly(isobutylene-*atl*-maleic anhydride) (PIMA) (average MW: ~6000 Da), poly(ethylene glycol) (PEG) (average MW: ~600 Da), poly(ethylene glycol) methyl ether (average MW: ~750 Da), lipoic acid, histamine, dopamine hydrochloride, ethylenediamine, tetramethylammonium hydroxide (TMAH), dimethylformamide (DMF), tetrahydrofuran (THF), RPMI-1640 Medium, along with most of the chemicals used were purchased from Sigma Aldrich (St Louis, MO). Sulfo-Cy3 NHS ester and PD10 columns were purchased from Lumiprobe (Hallandale Beach, FL). Solvents were purchased from Sigma Aldrich (St Louis, MO). Column purification chromatography performed using silica gel (60 Å, 230-400 mesh) was acquired from Bodman Industries (Aston, PA). Deuterated solvents used for NMR experiments were purchased from Cambridge Isotope Laboratories (Andover, MA). The chemicals and solvents were used as received unless otherwise specified.

The syntheses were carried out under N_2 passed through an O_2 scrubbing tower unless otherwise stated. Air sensitive materials were handled in an MBraun Labmaster glovebox, and standard Schlenk techniques were used in manipulation of air-sensitive solutions.

Instrumentation

^{1}H NMR spectra of the ligands and hydrophilic QDs were recorded using a 600 MHz spectrometer (Bruker SpectroSpin, Billerica, MA). The photoligation experiments were carried out using a UV photoreactor Model LZC-4V (Luzchem Research Inc., Ottawa, Canada) containing fourteen lamps, 6 of them loaded on top and four loaded on the two sides (4 each). The reactor provides a UV signal with maximum peak at 350 nm and an intensity of 4.5 mW/cm^2. The optical absorption spectra for various QD dispersions were collected using a UV-Vis absorption spectrophotometer (UV 2450 model, Shimadzu, Columbia, MD). The fluorescence emission spectra were collected using a Fluorolog-3 spectrofluorometer (HORIBA Jobin Yvon, Edison, NJ) equipped with PMT and CCD detectors. The dynamic light scattering measurements were carried out using ALV/CGS-3 Compact Goniometer System (ALV-GmbH, Langen, Germany). This system is equipped with a HeNe laser (illumination at 632.8 nm), ALV photon correlator and an avalanche photodiode for signal detection. Solvent evaporation was carried using a rotary evaporator R-215 (Buchi, New Castle, DE). The absorption data for the MTT assay were collected using Infinite M1000 microplate reader (from Tecan, Durham, NC).

Synthesis of poly(ethylene glycol) precursors, amine-terminated lipoic acid and dopamine-isothiocyanate (dopamine-ITC)

The precursors H_2N-PEG_{750}-OCH_3 and H_2N-PEG_{600}-NH_2, were respectively synthesized in our laboratory starting from poly(ethylene glycol) methyl ether (average MW ~750 Da), and poly(ethylene glycol) (average MW ~600 Da), following the procedures detailed in previous publications.[1-4] The amine-terminated lipoic acid (LA-NH_2) was synthesized following a

previously reported protocol.[5] The synthesis of dopamine isothiocyanate was carried out following the schemes described in reference.[6]

Synthesis of LA-PIMA-PEG (30% LA and 70% PEG-OMe)

0.385 g of Poly(isobutylene-*alt*-maleic anhydride) (PIMA, MW ~6000 g/mol, 2.5 mmol monomer units) was dissolved in 10 mL of DMF using a 50 mL three-neck round-bottom flask equipped with an addition funnel and a magnetic stirring bar. The solution was purged with nitrogen and then heated to 70 ˚C. In a separate vial, a mixture of LA-NH_2 (0.187 g, 0.75 mmol) and H_2N-PEG-OMe (1.31 g, 1.75 mmol) were dissolved in 4 mL of DMF. This content was loaded into the addition funnel and added dropwise to the flask containing the PIMA solution. When the addition was complete, the reaction mixture was further stirred at 70 ˚C overnight. After removing the DMF under vacuum, 3 mL of chloroform was added to disperse the residue, which was further purified using silica column and chloroform as the eluent. The final product was gel-like yellow compound, with yield ~83%.

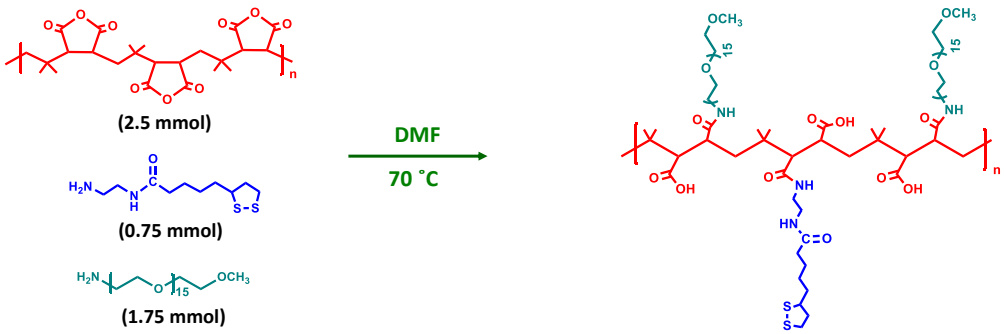
Synthesis of His-PIMA-PEG (50% His and 50% PEG)

Here we follow the same synthetic scheme used for preparing LA-PIMA-PEG above, but substitute LA-NH_2 with histamine. Briefly, in a 50 mL three-neck round-bottom flask equipped with an addition funnel and a magnetic stirring bar, we dissolved 0.385 g of PIMA (2.5 mmol monomer units) in 10 mL of DMF. The flask was purged with nitrogen for 10-15 minutes and the solution was heated to 40 ˚C. In a separate vial, 0.139 g of histamine (1.25 mmol) and 0.938 g of H_2N-PEG-OMe (1.25 mmol) were mixed in 4 mL of DMF and then slowly added to the PIMA solution through the addition funnel. When the addition was complete, the reaction mixture was further stirred overnight at 40 ˚C. The solvent was removed under vacuum, and the product was dissolved in 3 mL of chloroform. The product was purified using a silica column with chloroform as the eluent, yielding gel-like compound with slightly yellow color. To synthesize His-PIMA-PEG with of 30% His and 70% PEG insertions, a mixture of 0.75 mmol histamine and 1.75 mmol H_2N-PEG-OMe was used. The overall reaction yield for these His-PIMA-PEG ligands was ~84%.

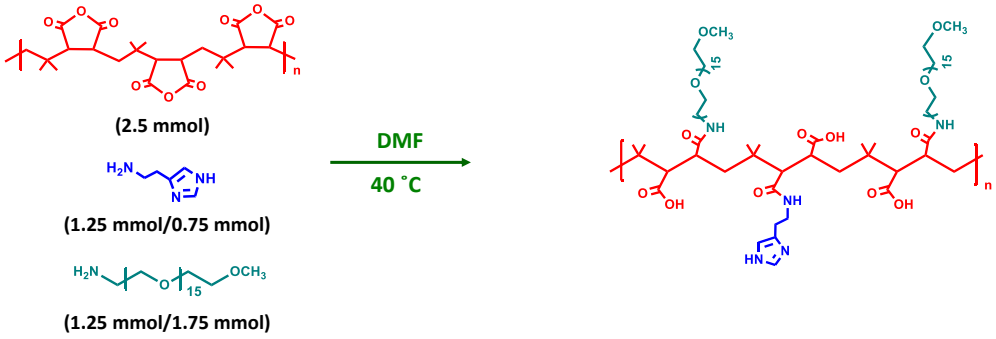
Gel electrophoresis

Gel electrophoresis experiments were conducted using QDs ligated with LA/His-PIMA-PEG. The nanocrystal dispersions were run on 0.6% agarose gel using tris borate EDTA buffer (TBE, 89 mM tris, 89 mM boric acid, 1 mM EDTA). QD samples were diluted to 0.5 μM using phosphate buffer with different pH (3-13). The solutions were incubated for 1 hour at room temperature and then mixed with 2 μL of loading buffer made of Ficoll 400 immediately prior to use. The total loading volume was 20 μL. The experiments were conducted for 30 min using a voltage of 5.5 V/cm and fluorescence images were captured using a Gel Doc XR+ System.

Scheme 1: Synthesis of LA-PIMA-PEG



Scheme 2: Synthesis of His-PIMA-PEG



Scheme 3: Synthesis of LA/His-PIMA-PEG and LA/His-PIMA-PEG-R

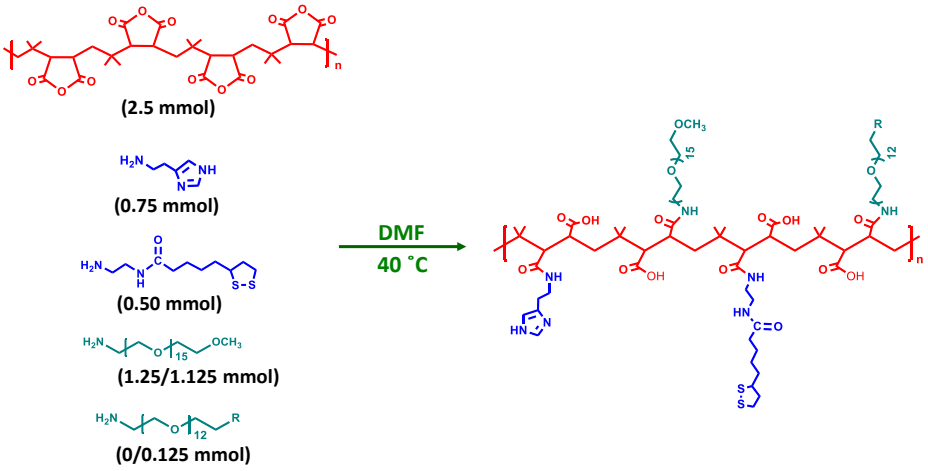


Figure S1. Synthetic schemes used to prepare a few additional LA-PIMA-PEG, His-PIMA-PEG, LA/His-PIMA-PEG and LA/His-PIMA-PEG-R ligands.

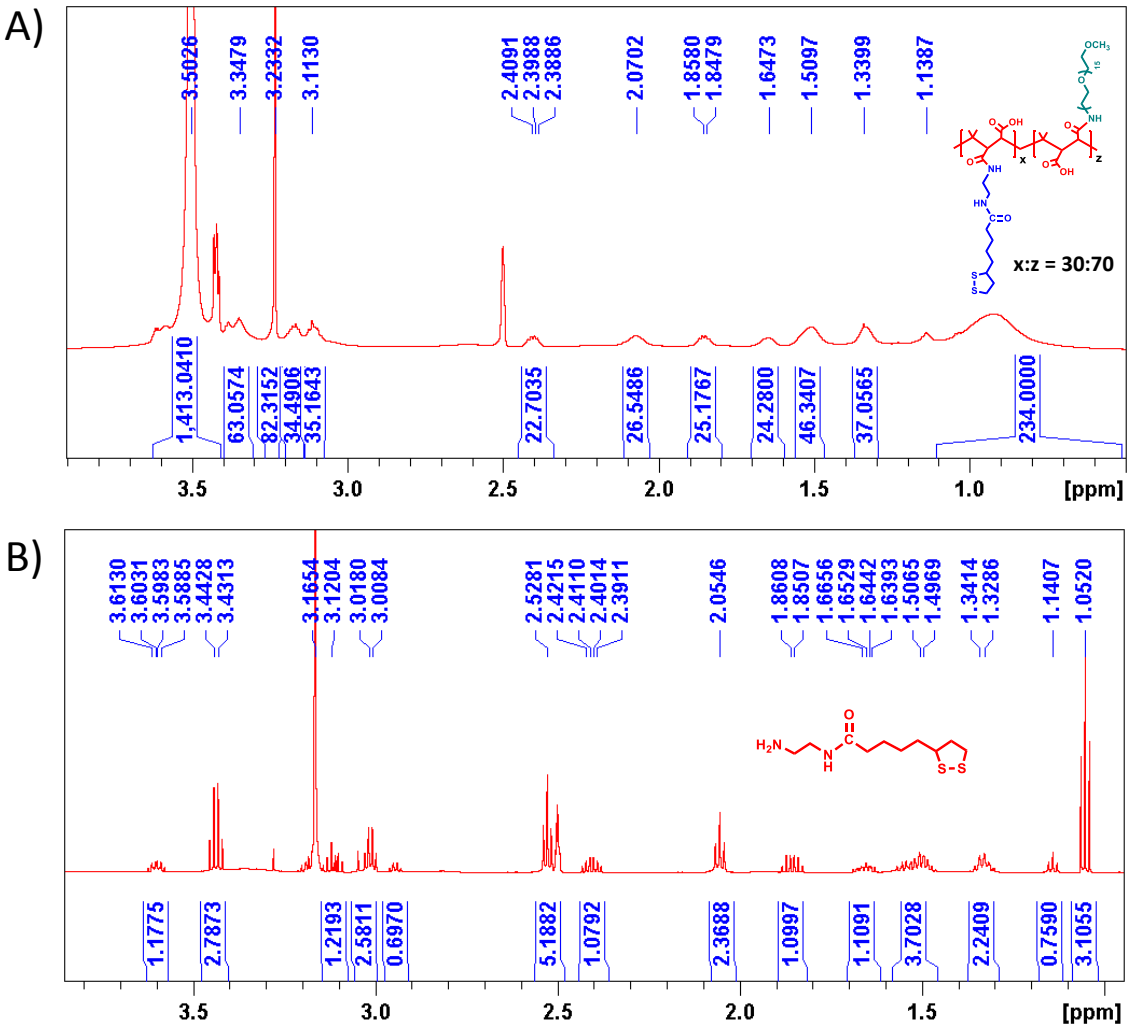


Figure S2. (A and B) ^{1}H NMR spectra collected from LA-PIMA-PEG polymer and LA-amine in DMSO-*d6*. The peaks at ~1.1-2.4 ppm and ~3.1 ppm are ascribed to the protons of the lipoic acid grafted on the PIMA backbone which are well matched with the peaks measured for the lipoic acid-amine (shown in B), while those at ~3.5 ppm and ~3.23 ppm are ascribed to the PEG and its terminal methoxy protons. A broad peak at ~0.9 ppm ascribed to the protons of the methyl groups in the polymer was also measured. The degree of grafting was estimated by comparing the integration values of the ^{1}H NMR peaks of LA (2 H, δ ~2.1 ppm) and PEG (3 H, δ ~3.2 ppm) to the two methyl repeat units of a single PIMA chain (~234 H, δ ~0.9 ppm). We deduced that there are ~13 lipoic acid groups (26 H) and ~27 PEG moieties (82 H) per LA-PIMA-PEG-OCH_3 chain. The peak at 3.16 ppm in spectrum (B) is due to the solvent and the peak at 1.05 ppm is due to impurities.

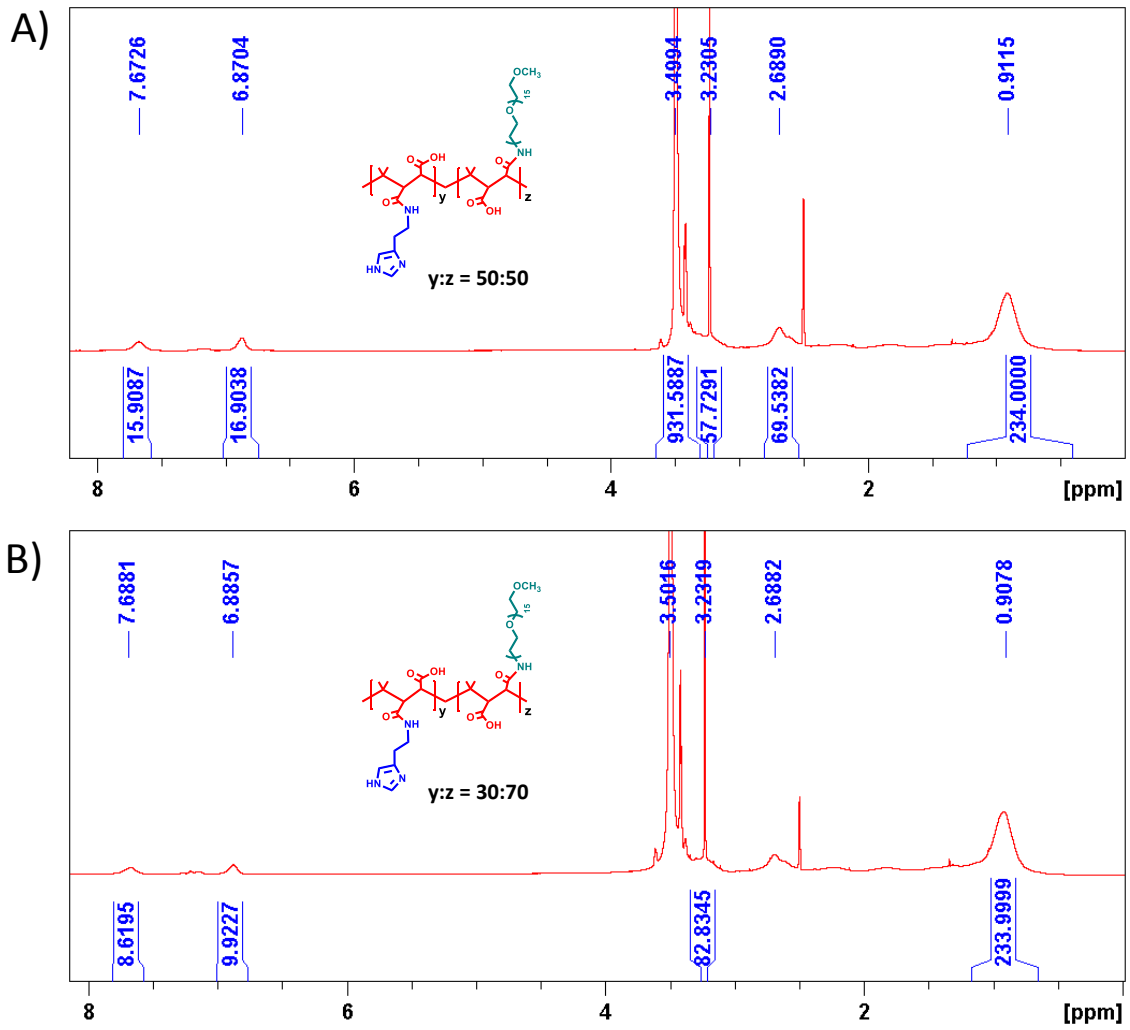


Figure S3. ^{1}H NMR spectra collected from two His-PIMA-PEG ligands in DMSO-*d6*: (A) $His_{50\%}$-$PIMA\text{-}PEG_{50\%}$ and (B) $His_{30\%}$-$PIMA\text{-}PEG_{70\%}$. The peaks at ~6.8 and ~7.6 ppm are attributed to the two protons on the imidazole rings. The degree of grafting was estimated by comparing the integration values of ^{1}H NMR peaks of the His (1 H, δ ~6.8 ppm), the terminal methoxy (3 H, δ ~3.2 ppm) to the two methyl repeat units of a single PIMA chain (~234 H, δ ~0.9 ppm). We deduced that there are ~16-17 histamines and ~19 PEG moieties per chain for $His_{50\%}$-$PIMA\text{-}PEG_{50\%}$, and ~9-10 histamines and ~27 PEG moieties per chain for $His_{30\%}$-$PIMA\text{-}PEG_{70\%}$.

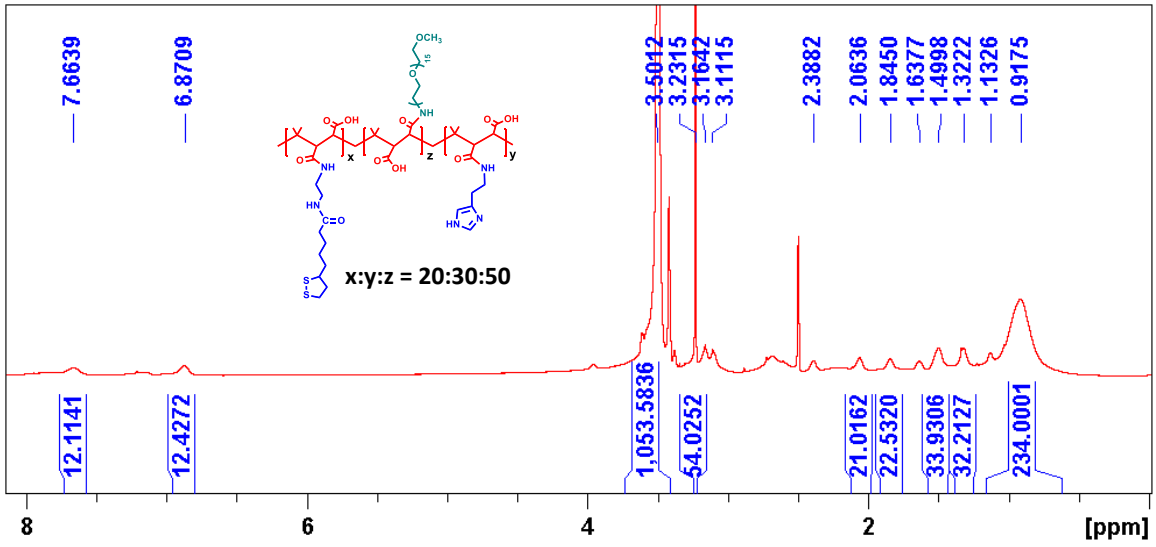


Figure S4. ^{1}H NMR spectrum collected from $LA_{20\%}/His_{30\%}$-PIMA-$PEG_{50\%}$ in DMSO-*d6*. The features of lipoic acid (1.1-2.4 ppm and ~3.1 ppm), histamine (~6.8 and ~7.6 ppm) and PEG moieties (~3.5 ppm and ~3.23 ppm) are well observed. The degree of grafting was estimated by comparing the integration values of ^{1}H NMR peaks of the LA (2 H, δ ~2.1 ppm), His (1 H, δ ~6.8 ppm), the terminal OCH_3 (3 H, δ ~3.2 ppm) to the two methyl repeat units of a single PIMA chain (~234 H, δ ~0.9 ppm). We estimate that ~10 LA groups (21 H), ~12 histamines (12 H) and ~18 PEG moieties (54 H) were coupled onto a PIMA chain.

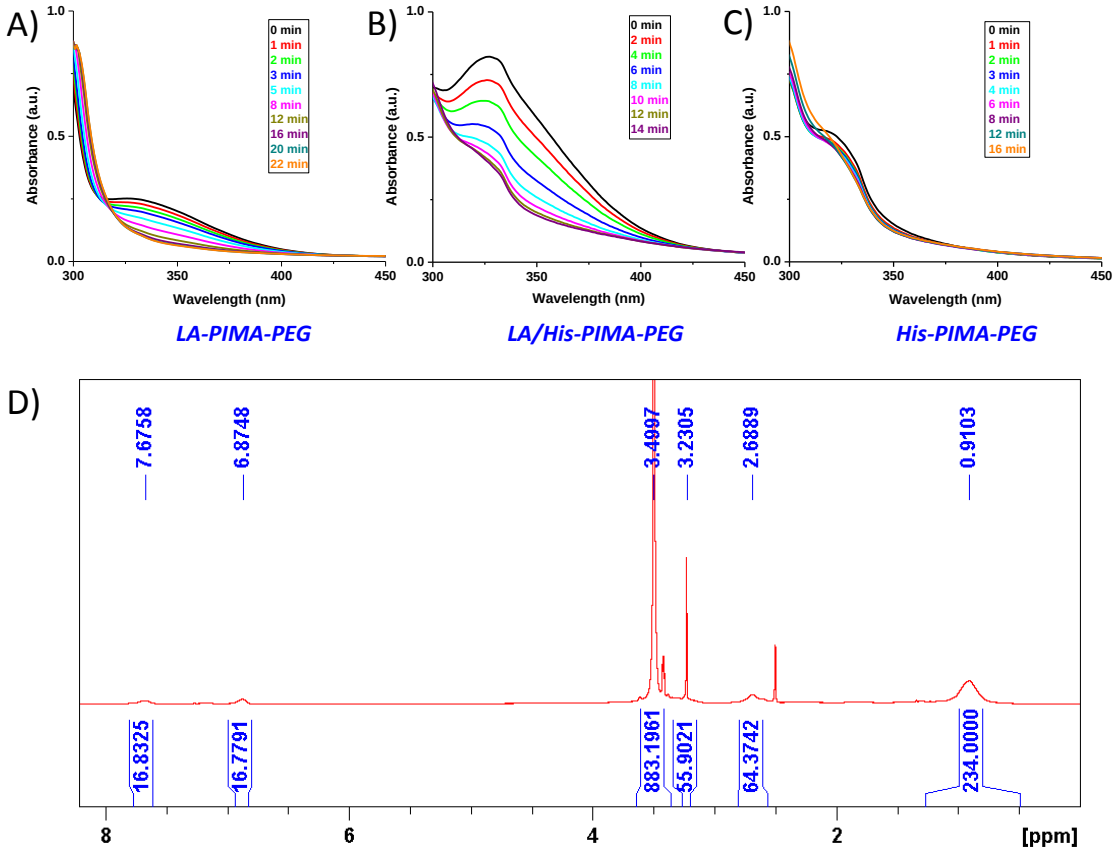


Figure S5. Progression of the UV absorption spectra of (A) LA-PIMA-PEG, (B) LA/His-PIMA-PEG, and (C) His-PIMA-PEG dissolved in THF collected following UV irradiation at different times; the ligand concentration was ~35 mg/mL. The absorption feature at 330 nm (associated with the lipoic aicd ring) underwent a progressive reduction in the spectra of LA-PIMA-PEG and LA/His-PIMA-PEG following UV irradiation for 20 min and 12 min, respectively. The absorption spectrum measured for His-PIMA-PEG ligands was essentially unchanged with UV irradiation. (D) ^{1}H NMR spectrum collected from His-PIMA-PEG subjected to UV irradiation for 10 min in DMSO-*d6*. The features and integration values of the spectrum are essentially unchanged in comparison to the spectrum shown in Figure S3A.

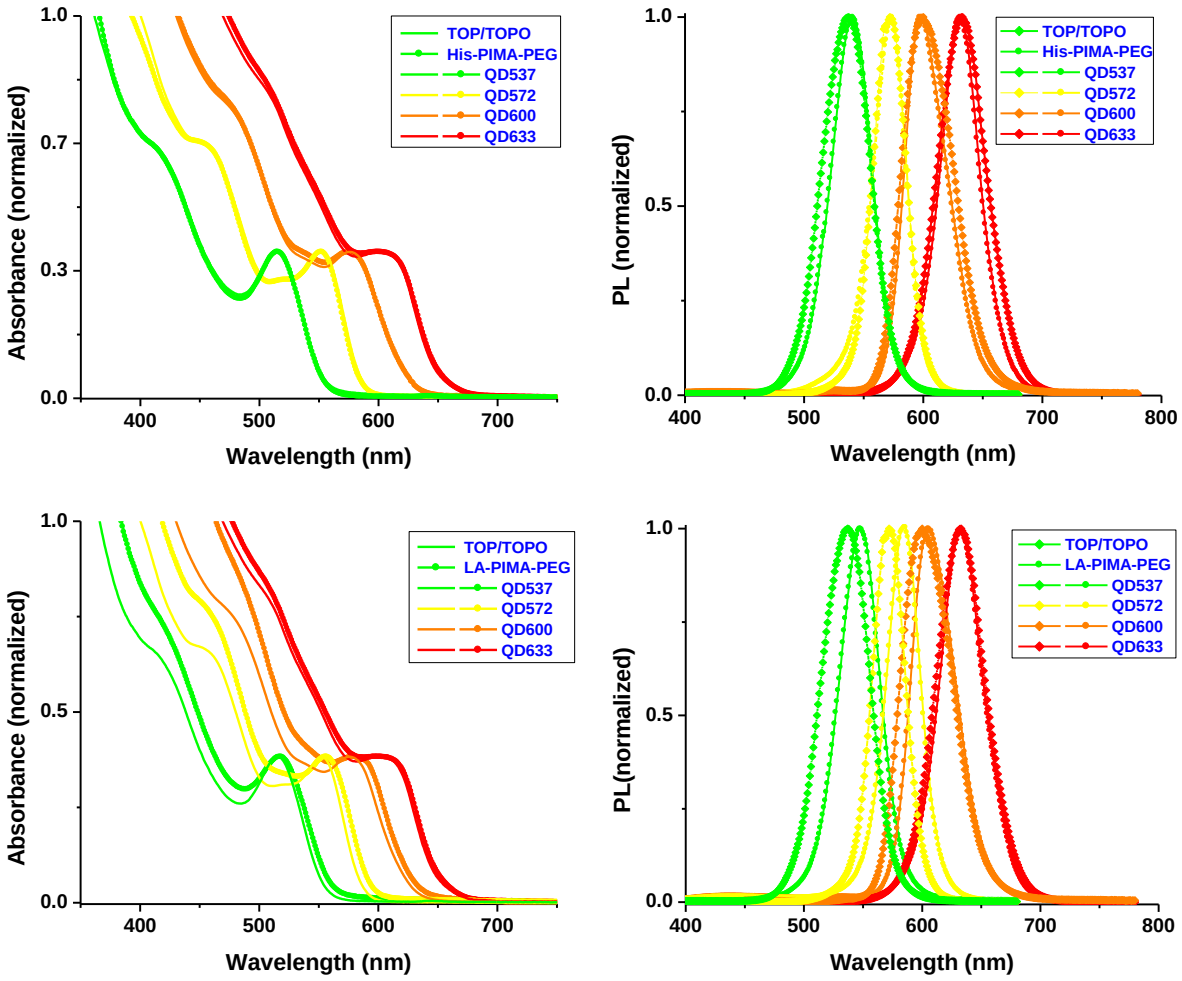


Figure S6. Normalized absorption and emission spectra collected from different size QDs (emission at 537, 572, 600, and 633 nm): (top) TOP/TOPO-capped in hexane side by side with His-PIMA-PEG-capped dispersed in DI water; (bottom) TOP/TOPO-QDs in hexane along with LA-PIMA-PEG-QDs in DI water.

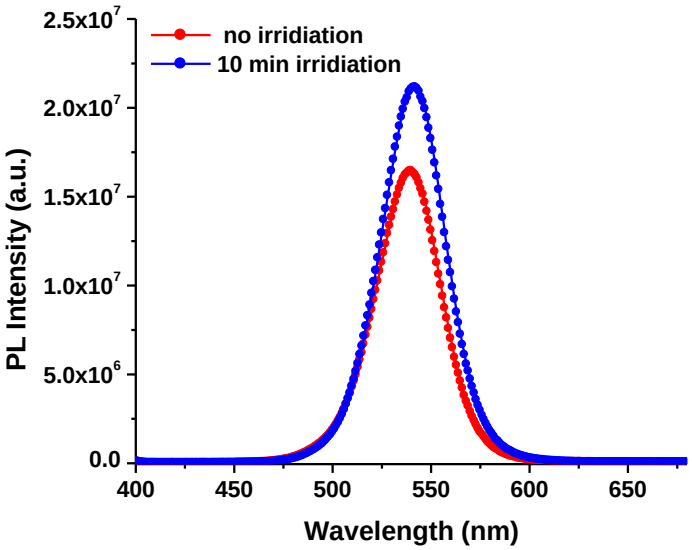


Figure S7. Emission spectra of His-PIMA-PEG-capped QDs before (red line) and after UV irradiation for 10 min (blue line); ~30% PL enhancement is measured following UV irradiation.

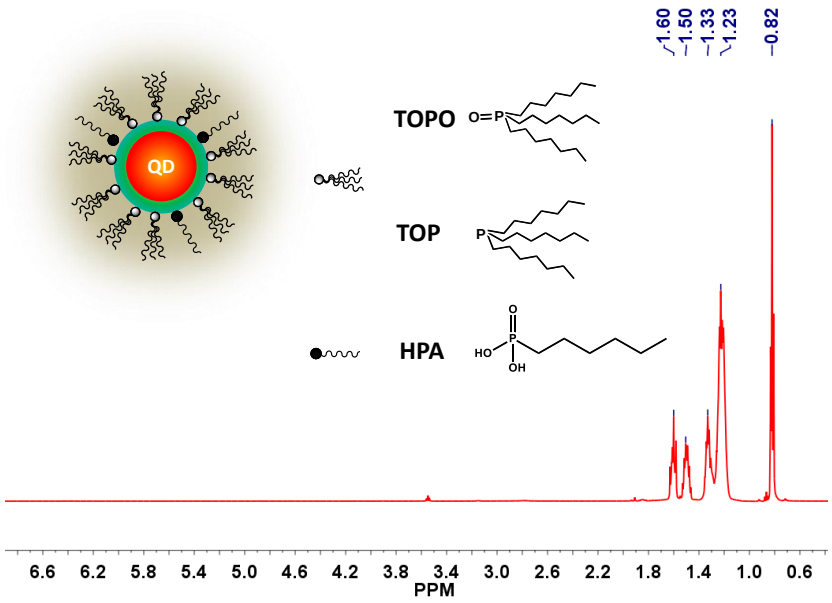


Figure S8. ^{1}H NMR spectrum collected from hydrophobic QDs capped with trioctylphosphine oxide (TOPO), trioctylphosphine (TOP) and hexylphosphonic acid (HPA) dispersed in *d*-$CDCl_3$.

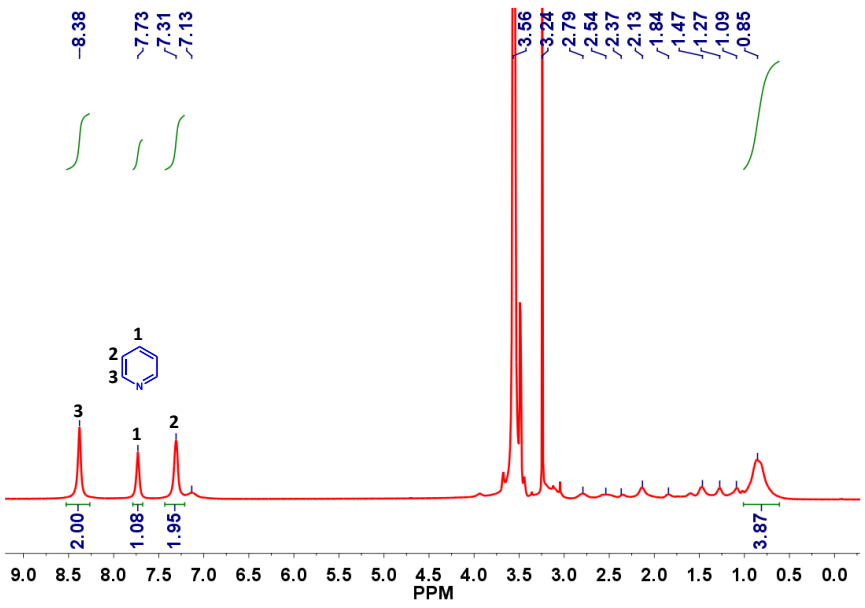


Figure 9. Surface ligand counting: ^{1}H NMR spectrum of the $LA_{20\%}$/$His_{30\%}$-PIMA-$PEG_{50\%}$-capped QDs calibrated with a fixed amount of pyridine (used as a control).

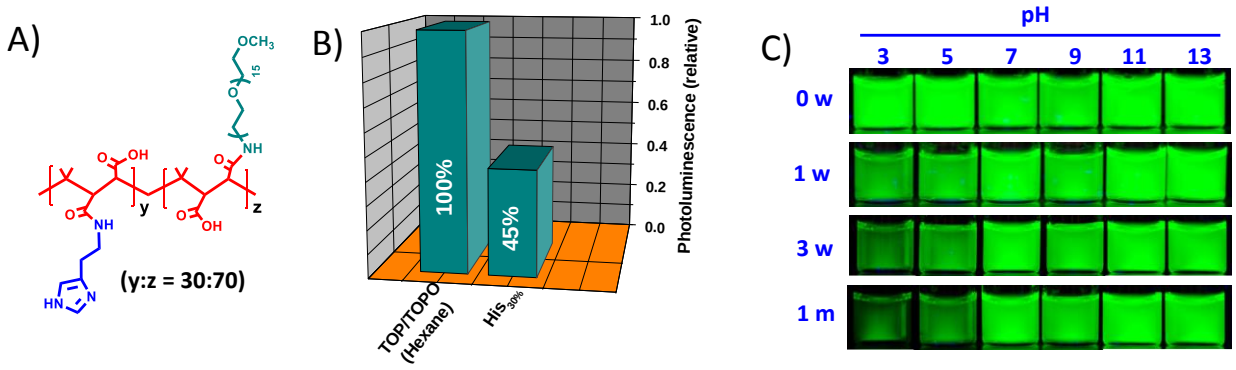


Figure S10. (A) Stuctre of $His_{30\%}$-PIMA-$PEG_{70\%}$ ligand. (B) Relative PL intensity measured from a dispersion of $His_{30\%}$-PIMA-$PEG_{70\%}$-QDs with repect to that of TOP/TOPO-capped QDs. This value is lower than those reported for QDs capped with other ligands (i.e., LA-PIMA-PEG, $His_{50\%}$-PIMA $PEG_{50\%}$, and LA/His-PIMA-PEG) shown in the main text. (C) Colloidal stability tests of QDs ligated with $His_{30\%}$-PIMA-$PEG_{70\%}$ (0.5 μM) dispersed in buffers at pH 3-13 at the indicate storage periods. The fluorescence of QD dispersion in buffers at pH 3 and 5 showed a measurable decrease in emission with storage, presumably due to protonation of the imidazole groups in acidic conditions.

FRET Analysis. The FRET (Föster Resonance Energy Transfer) efficiency E is measured experimentally using:[7]

$$E = \frac{F_D - F_{DA}}{F_D}$$

where F_D and F_{DA} are the fluorescence intensities collected from the donor alone and donor in the presence of the acceptor(s), respectively. The covalent coupling of dyes to QD provides conjugates with a centrosymmetric distribution of acceptors around the QD (one central donor and n acceptors), with a constant average center-to-center separation distance, *r*. If analyzed within the Förster dipole-dipole formalism, the energy-transfer efficiency can be fit to the expression:

$$E = \frac{nR_0^6}{nR_0^6 + r^6}$$

Where *n* is the average number of fluorescent dye per QD, which is estimated from the deconvoluted absorption spectra of QD-dye conjugates, and R_0 is the Förster separation distance corresponding to 50% energy-transfer efficiency; R_0 is expressed as:

$$R_0 = \left(\frac{[9000\times (ln10)]\times k_p^2}{128\,\pi^5 n_D^4 N_A} Q_D I\right)^{1/6}$$

or

$$R_0 = (9.78 \times 10^3)(n_D{}^{-4} k_p{}^2 Q_D I)^{1/6} \qquad (R\ in\ \text{Å})$$

where n_D is the refractive index of medium, Q_D is the QD donor PL quantum yield, and *I* designates the integral of the spectral overlap function, *J(λ)*, defined as:

$$I = \frac{\int F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int F_D(\lambda) d\lambda} = \int PL_{D-Corr}(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda,$$

with $\varepsilon_A(\lambda)$ being the acceptor extinction coefficient spectrum and $PL_{D\text{-}Corr}$ the normalized donor emission spectrum. A value of $\kappa_p^2 = 2/3$ for the dipole-dipole orientation factor was used for our QD-dye assembly. Table S1 shows the estimated number of acceptors along with the FRET efficiency, Q_D, overlap integral, R_0, and separation distance (*r*) for QD537-dye conjugates.

Table S1. Values for the various experimental parameters relevant to the FRET calculation for QD-Cy3 conjugates.

Donor-Acceptor Pair	QD537-Cy3
Number of Acceptors	2.8
FRET Efficiency (E)	33.4%
Quantum Yield	23%
Overlap Integral (I) (cm^3/M)	7.87×10^{-13}
R_0 (Å)	55.0
r (Å)	61.0

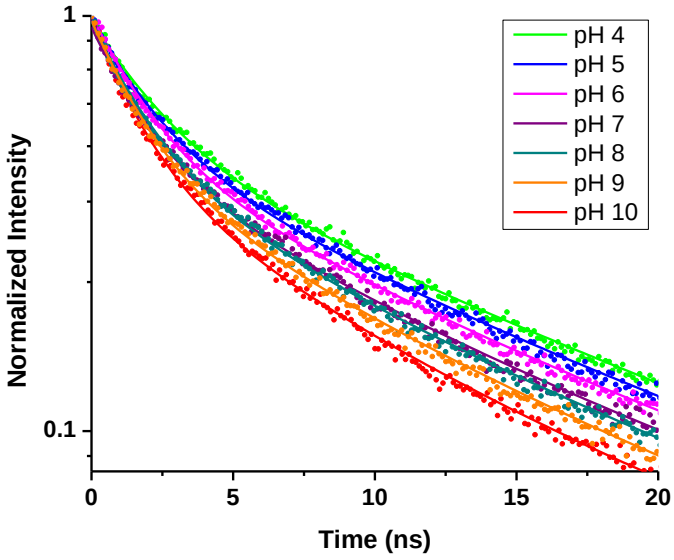


Figure S11. Time-resolved PL decay profiles collected from solutions of QD-dopamine conjugates in buffers ranging from pH 4 to 10.

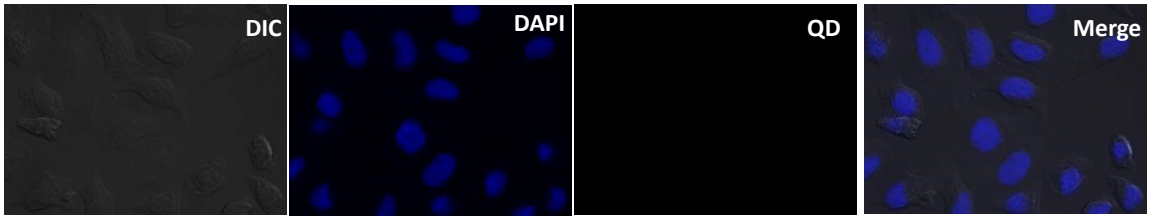


Figure S12. Epifluorescence images of HeLa cells incubated with 537 nm-emitting QDs without CPP (control) at 200 nM. No green fluorescence was detected in these images.

References

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