Compact Biocompatible Quantum Dots Functionalized for Cellular Imaging

Wenhao Liu[1], Mark Howarth[1,2], Andrew B. Greytak[1], Yi Zheng[1] , Daniel G. Nocera[*1], Alice Y. Ting[*1] and Moungi G. Bawendi[*1]

[1]Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge MA 02139-4307. [2]Department of Biochemistry, Oxford University, South Parks Road, Oxford, OX1 3QU, UK.

Email: mgb@mit.edu, ating@mit.edu, nocera@mit.edu

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Retention of QY Using Alloyed Shell CdSe/$Zn_xCd_{1-x}S$ QDs

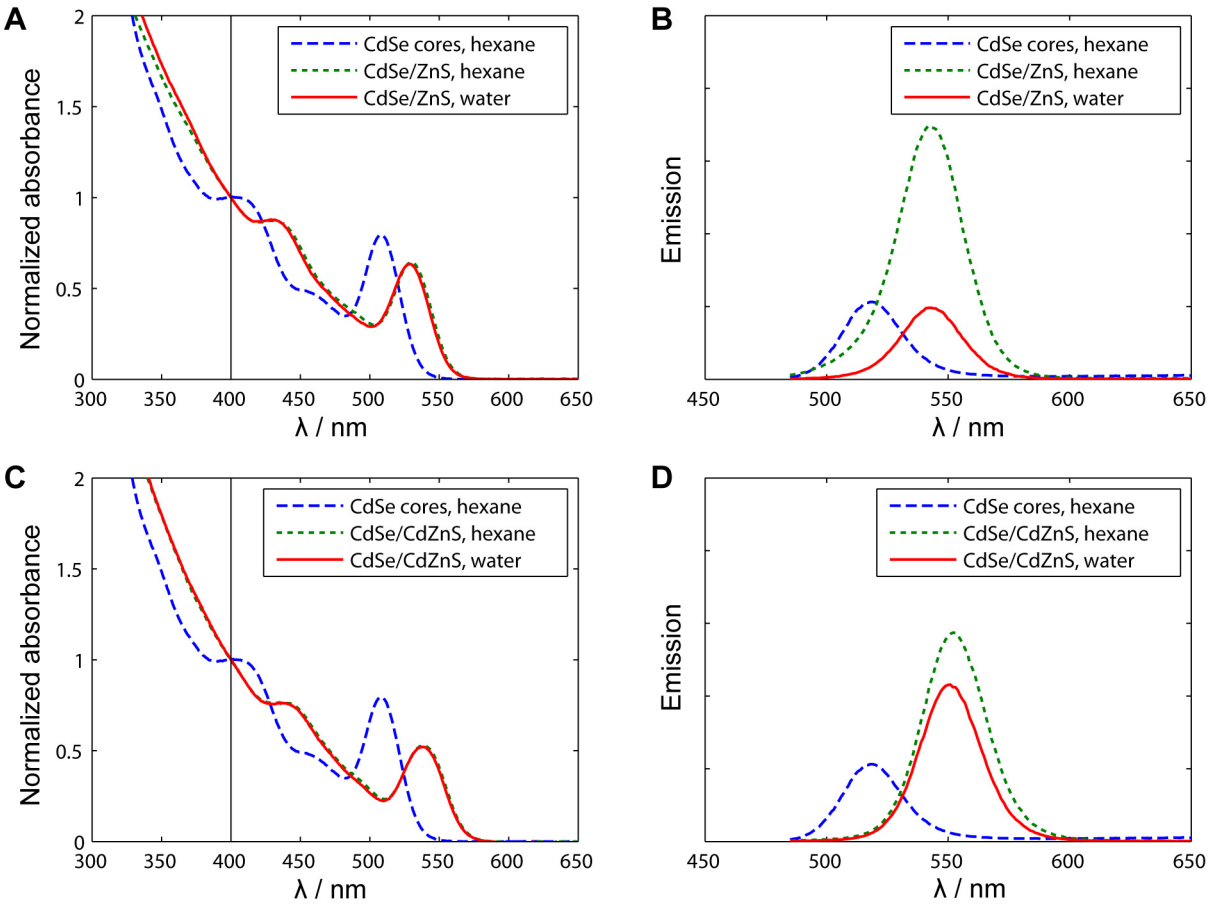


Figure S1. Absorption and emission spectra in hexane and in aqueous solution are shown for a representative batch of CdSe QD cores that was split and separately overcoated with either a pure ZnS shell or an alloyed $Zn_xCd_{1-x}S$ shell. (A) Absorption before (- - -) and after (—) growth of a pure ZnS shell (estimated thickness 3 monolayers), and after ligand exchange to make 20% aminoQDs (···). Spectra are normalized at 400 nm. (B) Emission spectra of QD samples in (A) under 400 nm excitation. Spectra are normalized according to their absorbance at 400 nm such that their intensities may be compared directly. (C) Absorption spectra, normalized as in (A), before and after growth of a $Zn_xCd_{1-x}S$ shell (x = 0.8) and using the same total equivalents of metal precursors as in (A), and after ligand exchange. (D) Emission spectra of QD samples in (C) under 400 nm excitation, normalized as in (B).

Dynamic Light Scattering

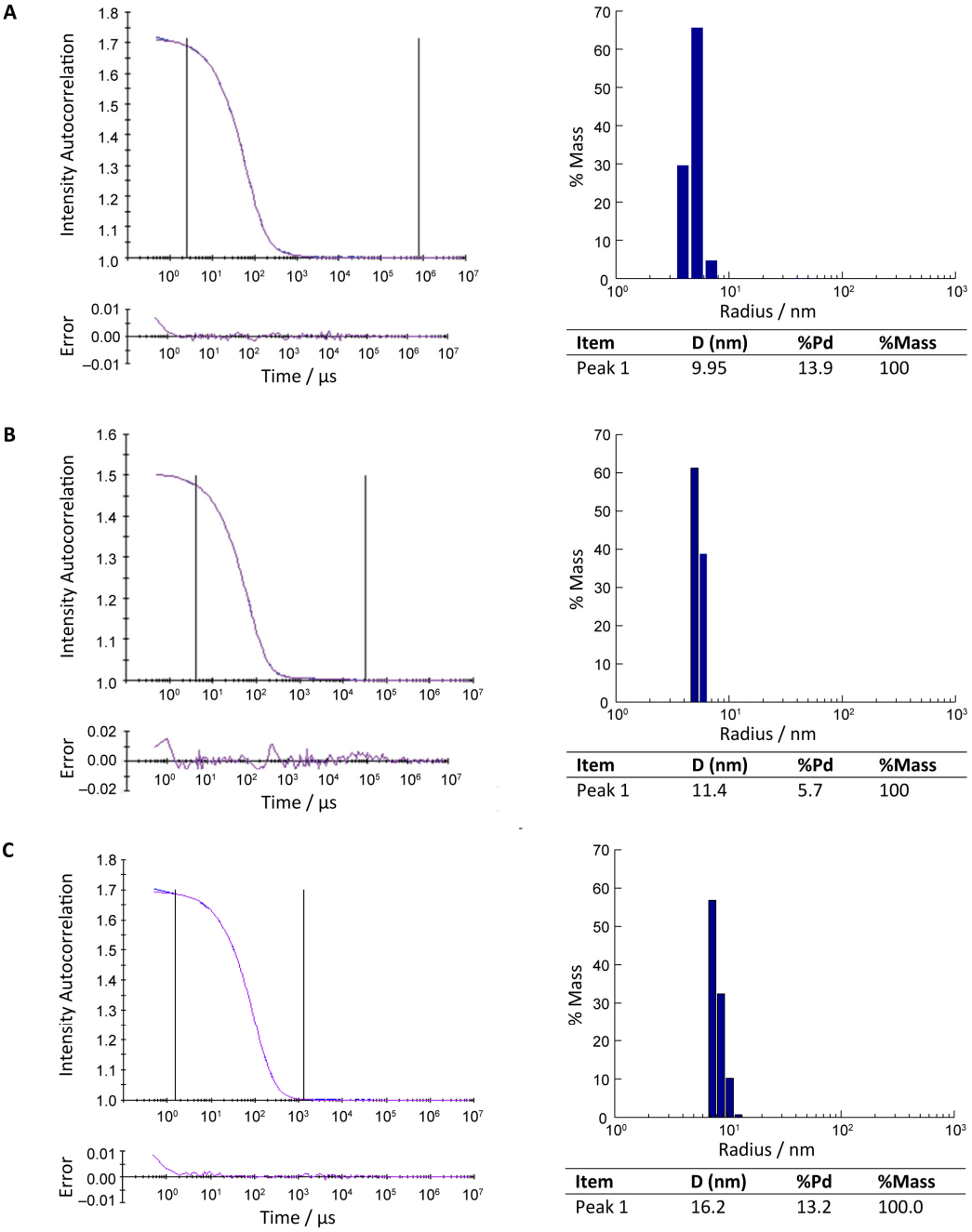


Figure S2. Size determination of QD HD by dynamic light scattering. (A) 565nm carboxyQDs. (B) 605 nm carboxyQDs. (C) Commercial amine functionalized, PEGylated QDs emitting at 525 nm (Invitrogen part no. Q22041, component A). Left: measured autocorrelation function (blue) with fit to theory (purple). Vertical lines indicate limits used for fitting. Right: histogram with table showing peak positions (D, diameter; %Pd, polydispersity, showing average of triplicate runs).

pH Stability of carboxyQDs and 20% aminoQDs

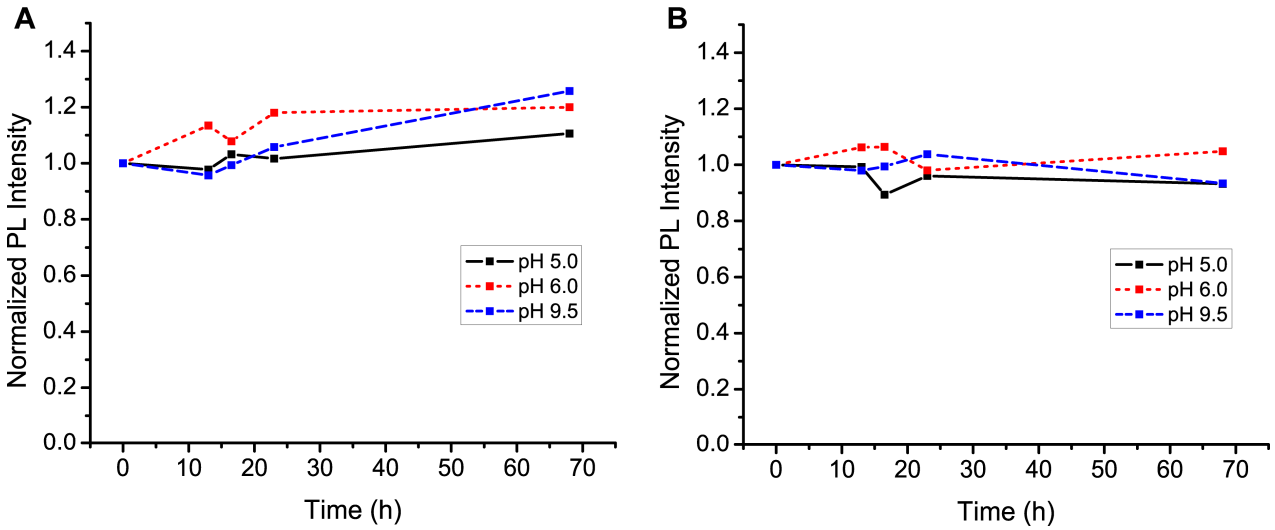


Figure S3. pH stability test of (A) carboxyQDs and (B) 20% aminoQDs (with 80% hydroxyPEG by mole ratio) at pH 5.0 (**—**), 6.0 (**···**), and 9.5 (**- - -**).

Fluorescamine-aminoQD Emission

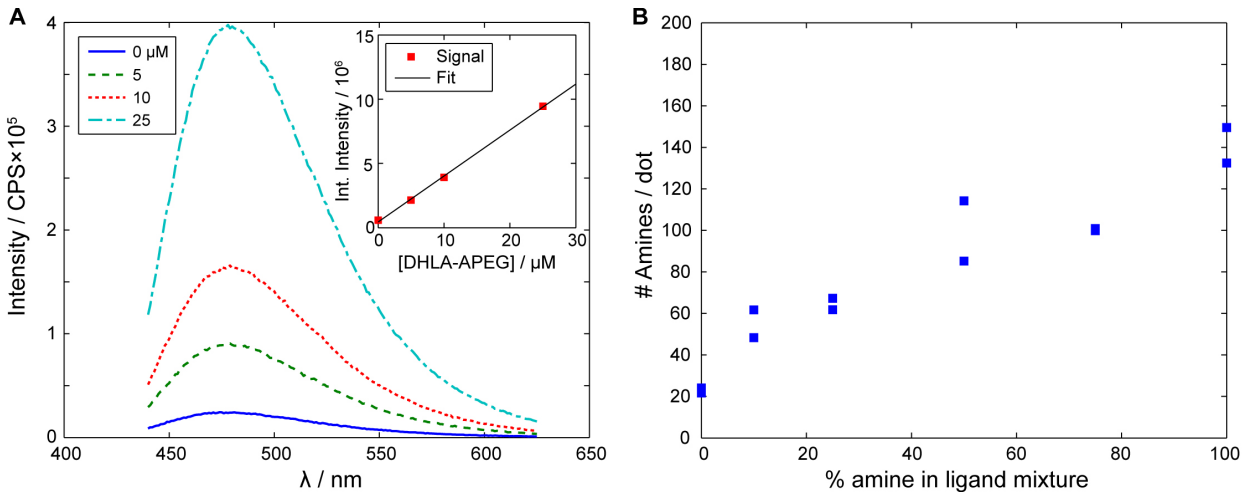


Figure S4. Measuring the number of aminoPEG per QD (558 nm emission). (A) Calibration: emission spectra of aminoPEG free ligand solutions in DMF after treatment with fluorescamine (380 nm excitation). Inset: integrated fluorescence intensity of these samples from 460-485 nm, along with a linear fit. (B) Ratio of detected amine concentration to QD concentration versus wt% of amine-terminated ligand in aminoPEG/hydroxyPEG ligand exchange mixtures. Samples were run in duplicate to improve accuracy (in some cases values overlap).

Spectroscopy of 20% aminoQD–carboxy-X-rhodamine conjugate

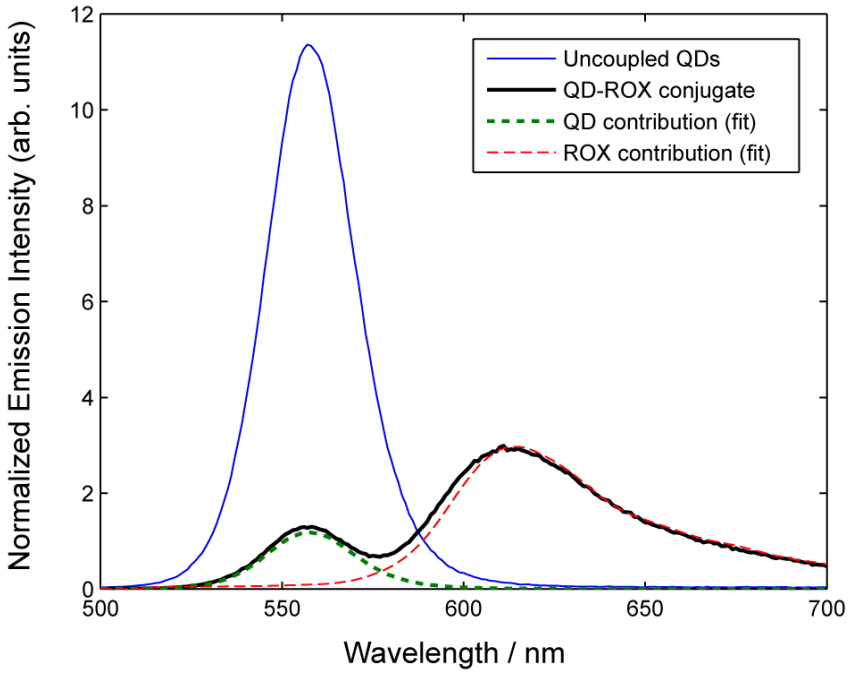


Figure S5. Emission spectra of purified QD-ROX conjugate described in the text and of a matching sample prepared with no dye. The spectra have been normalized according to the QD concentration in each sample so that the emission intensities may be directly compared. Fitting the emission spectrum of the conjugate as a linear combination of QD and dye components reveals a quench of the QD emission intensity by approximately 90% versus that in the absence of the ROX dye.

Conjugation of wild-type streptavidin to 20% aminoQDs

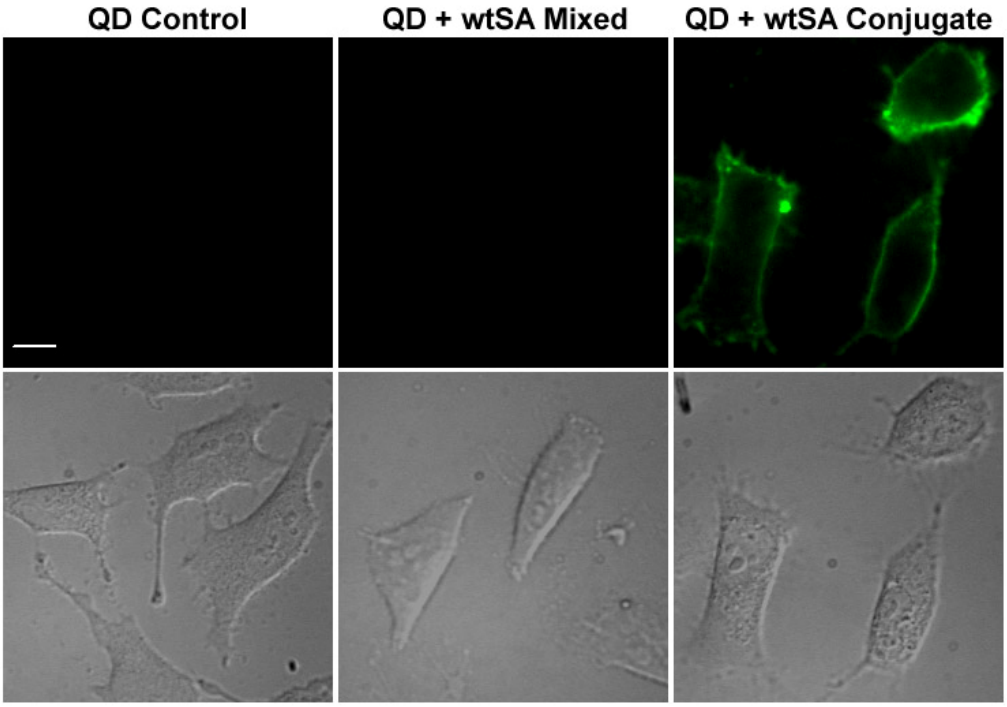


Figure S6. Testing for non-specific interactions between 20% aminoQDs and wtSA. HeLa cells were non-specifically biotinylated with biotin-sulfo-NHS and then incubated with 20% aminoQDs (left), 20% aminoQDs mixed with wtSA (middle), and 20% aminoQDs conjugated to wtSA using EDC/Sulfo-NHS (right). Top: fluorescence image at 565 nm. Bottom: DIC image. Scale bar, 5 μm.

Spectroscopy of 20% aminoQD–Alexa Fluor 568 conjugate

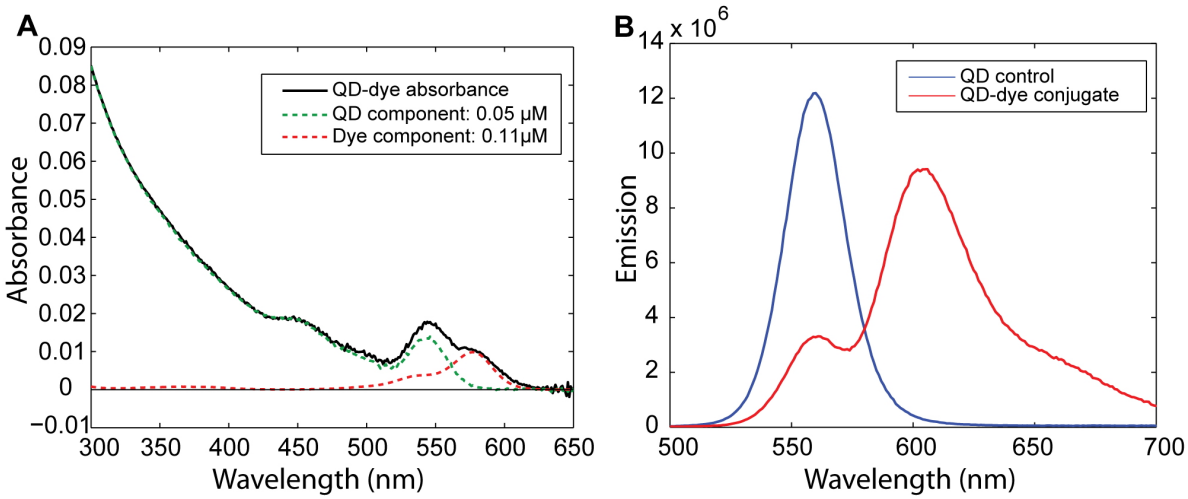


Figure S7. Spectral characterization for 20% aminoQDs-Alexa Fluor 568 conjugates. (A) Absorbance spectra for QD-dye conjugate fit as a linear combination of QD and dye components; Dye:QD ratio 2.3. (B) Emission spectrum of aminoQD and QD-dye conjugates.

QD–Dye Conjugate Targeting EGF Receptor

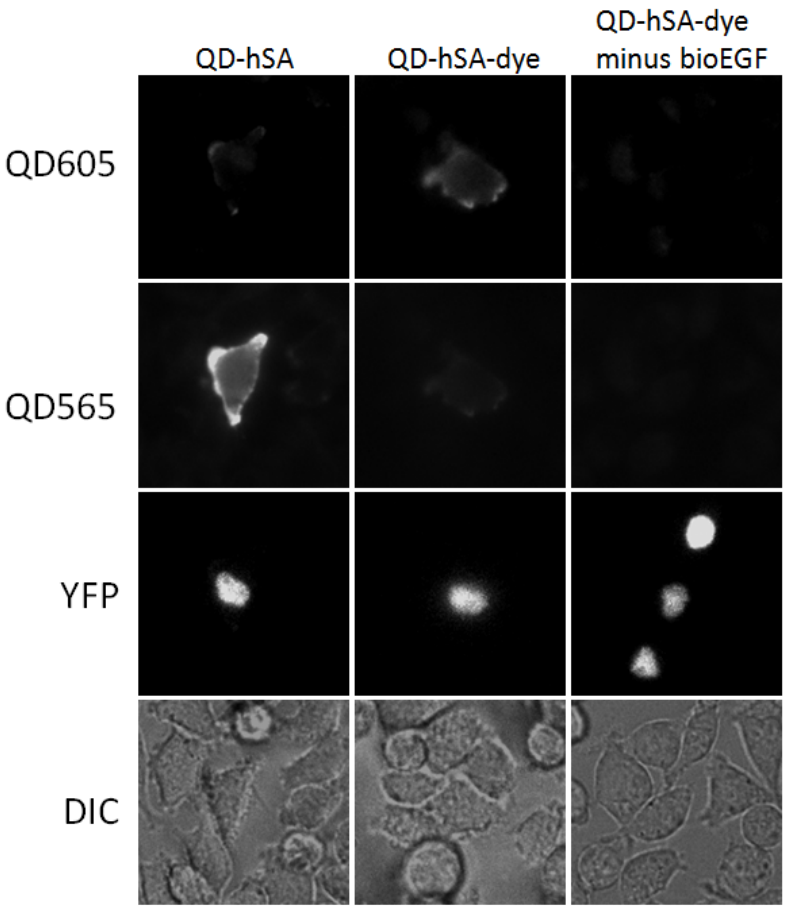


Figure S8. Targeting EGF receptor with 20% aminoQD-hSA-dye conjugate, showing individual channels: 605 nm (dye emission), 565 nm (QD emission), YFP (transfection marker), and differential interference contrast (DIC) image.

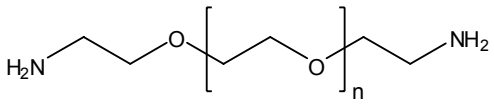
Receptor tracking with single QD conjugates

Movie S1. File name: SPT_EGF.avi

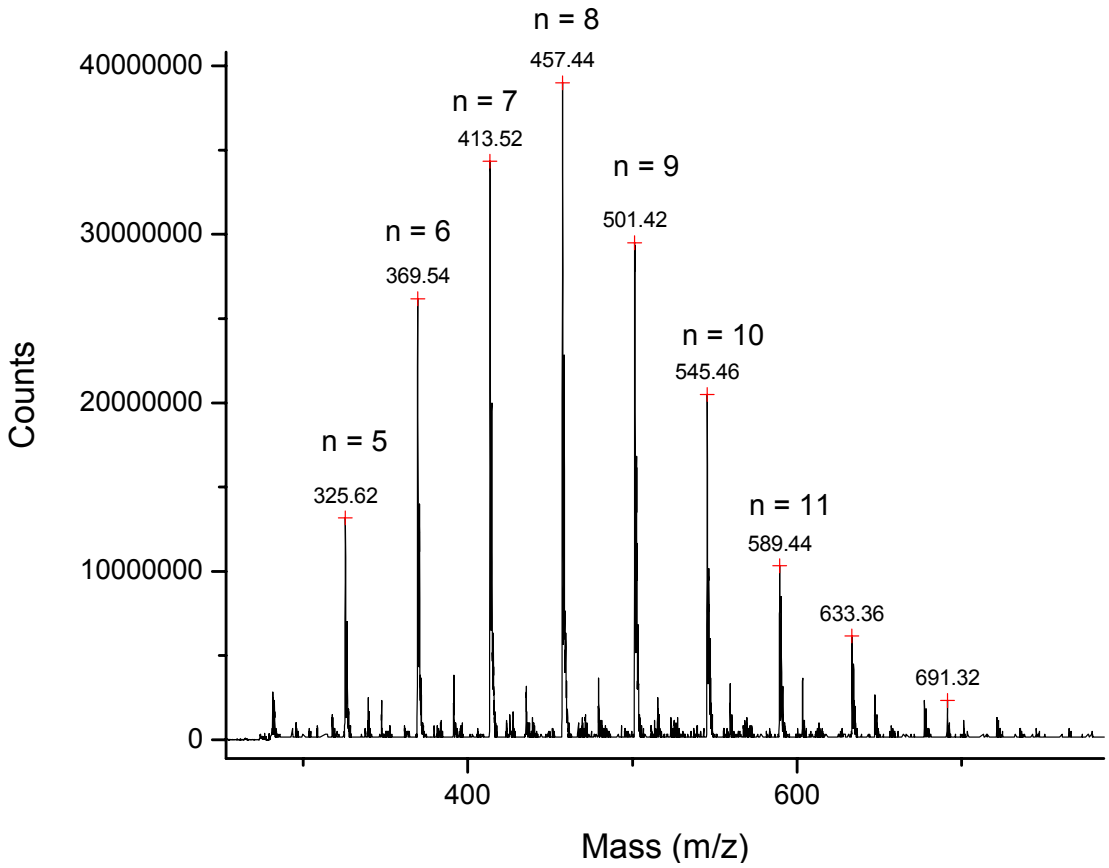
COS7 cells transfected with EGFR and BFP were labeled with biotinylated EGF and then 20% aminoQD-SA. QD mobility was imaged at 37 °C. The movie is sped up 5x. Individual QDs are seen, as indicated by their brightness and blinking kinetics. Brighter spots represent clusters of QDs. Some QDs undergo rapid movement in a consistent direction (Box 1 and 2), indicative of active transport of EGFR in vesicles inside the cell after internalization.

Diamino-PEG Spectra (Compound 1)

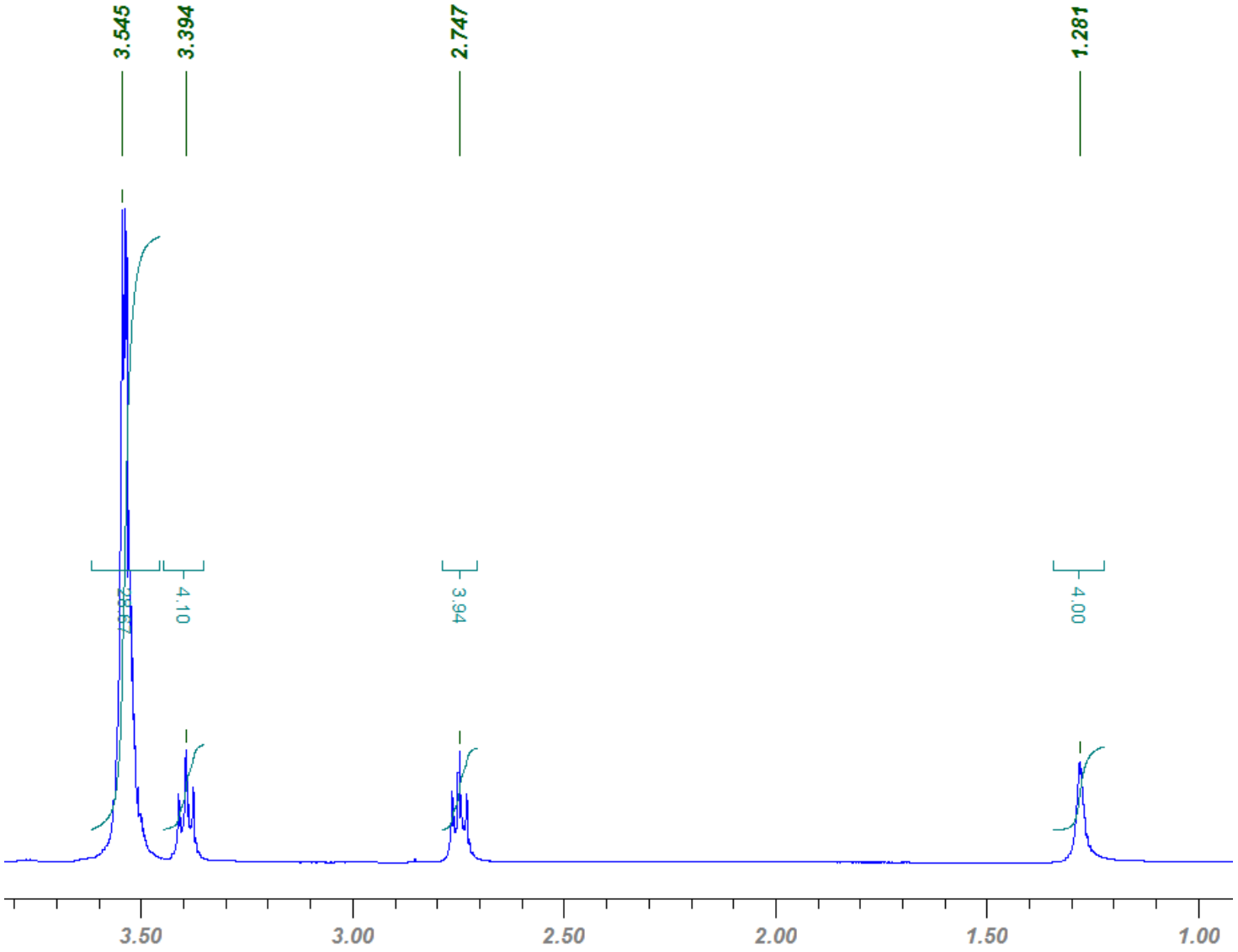
ESI-MS [M+H]+



Chemical Formula: $C_{20}H_{44}N_2O_9$
Molecular Weight (n = 8): 456.57

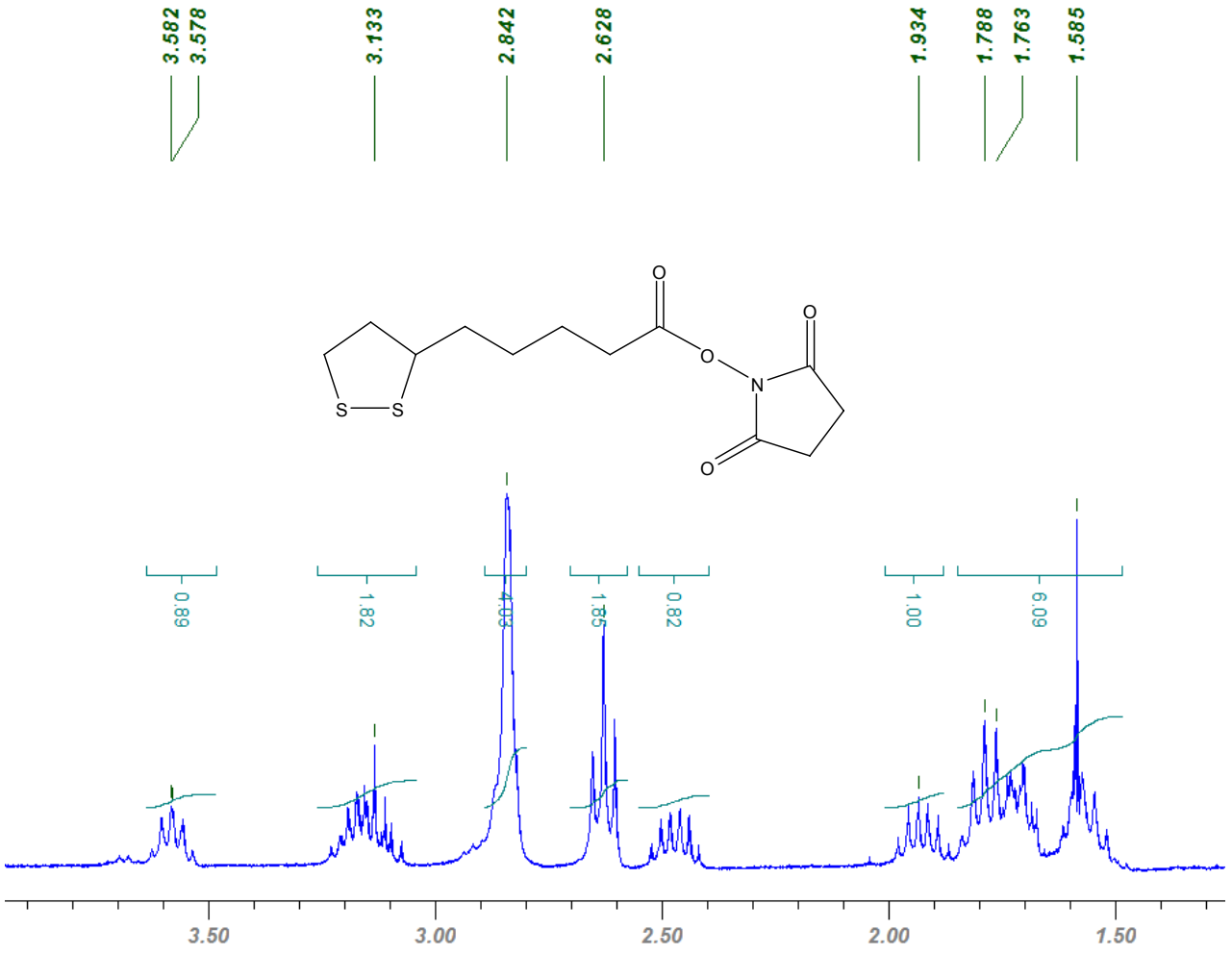


^{1}H-NMR (compound **1**)



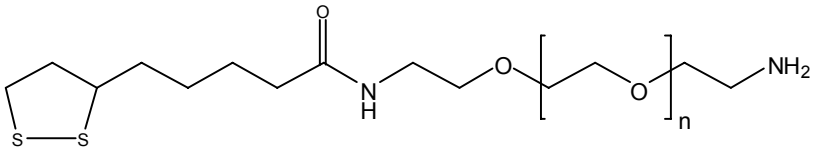
LA-NHS Spectra

^{1}H-NMR

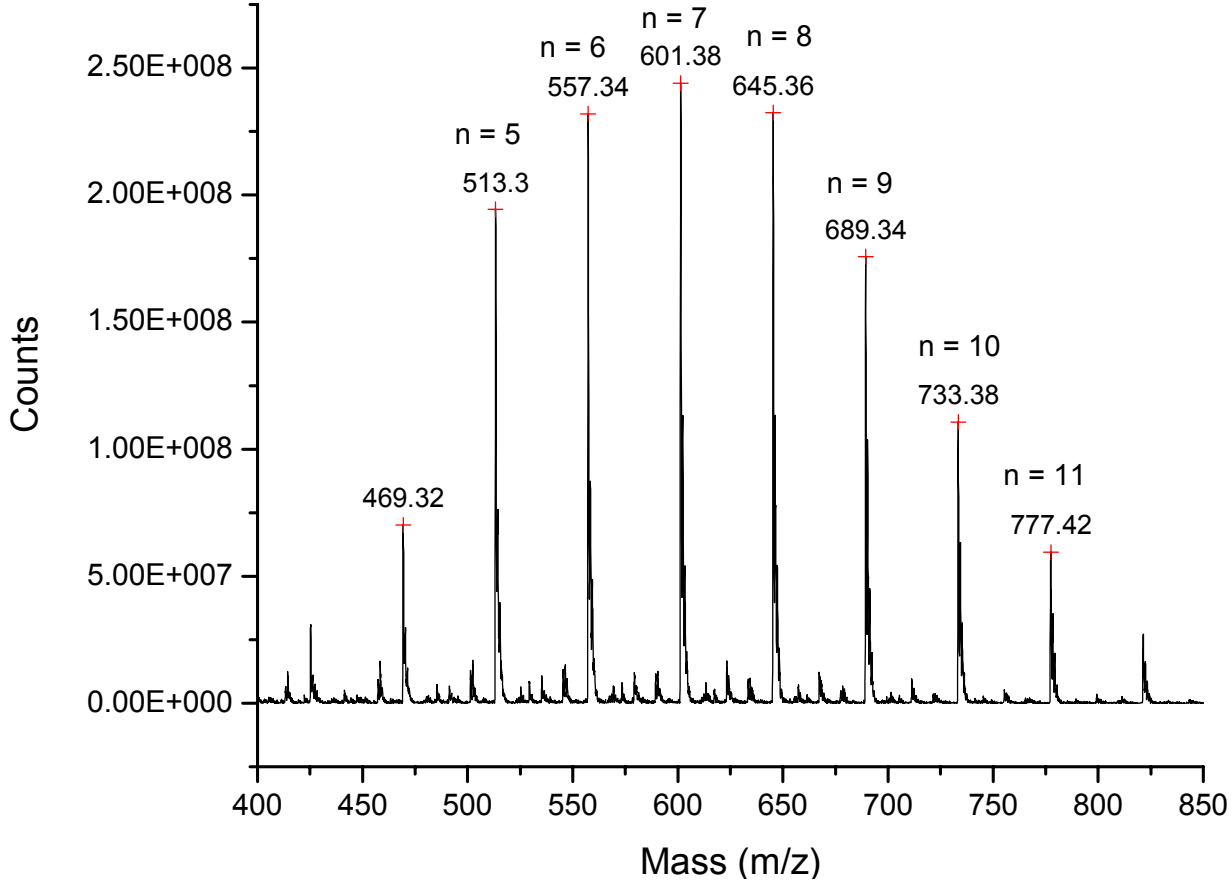


LA-PEG-NH_2 Spectra (Compound 2)

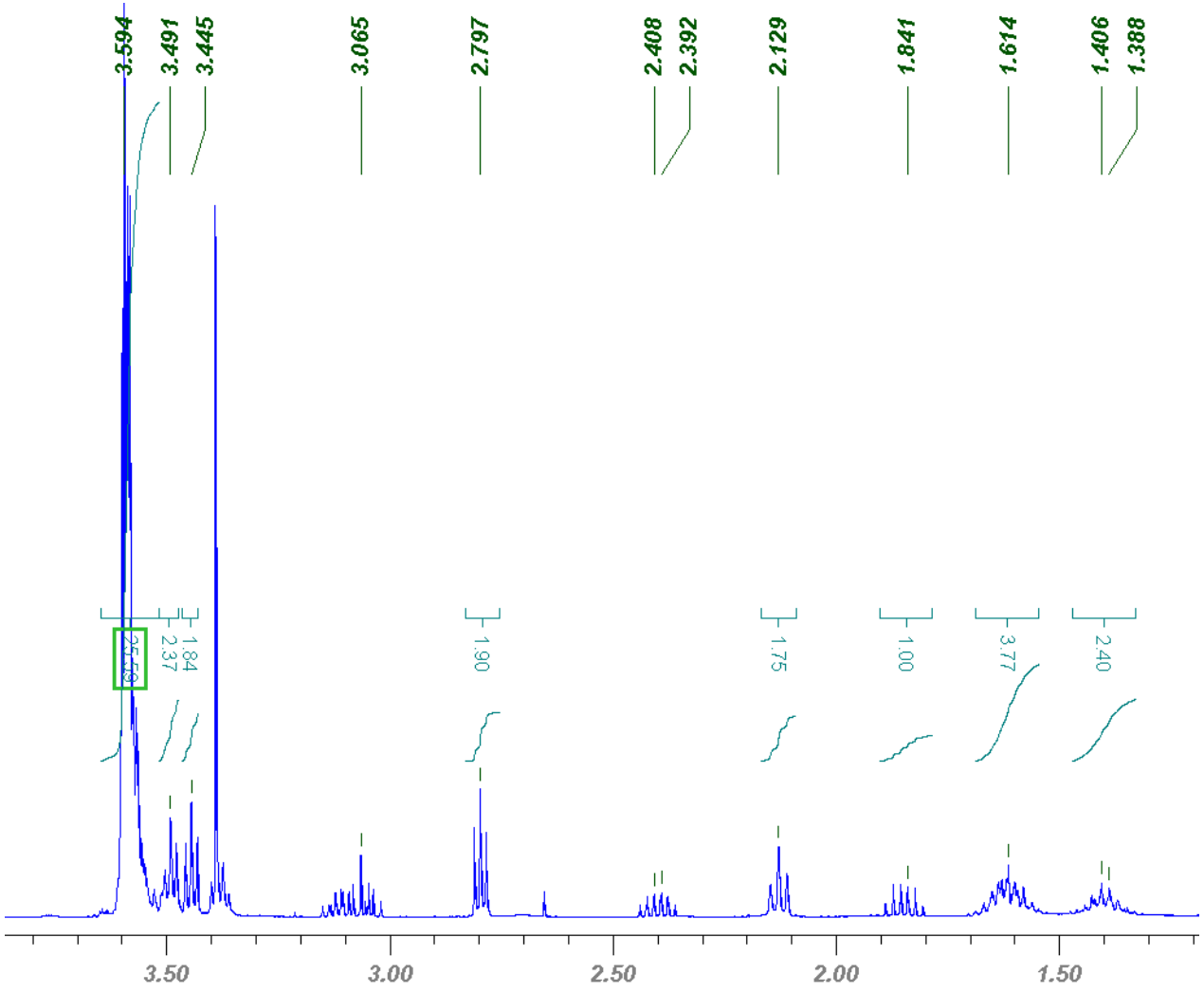
ESI-MS [M+H]+



Chemical Formula: $C_{28}H_{56}N_2O_{10}S_2$
Molecular Weight (n = 8): 644.88

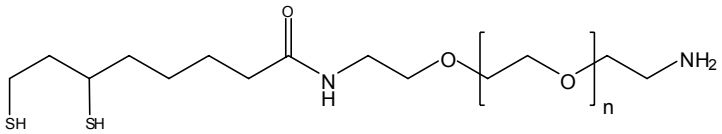


^{1}H-NMR (compound **2**)

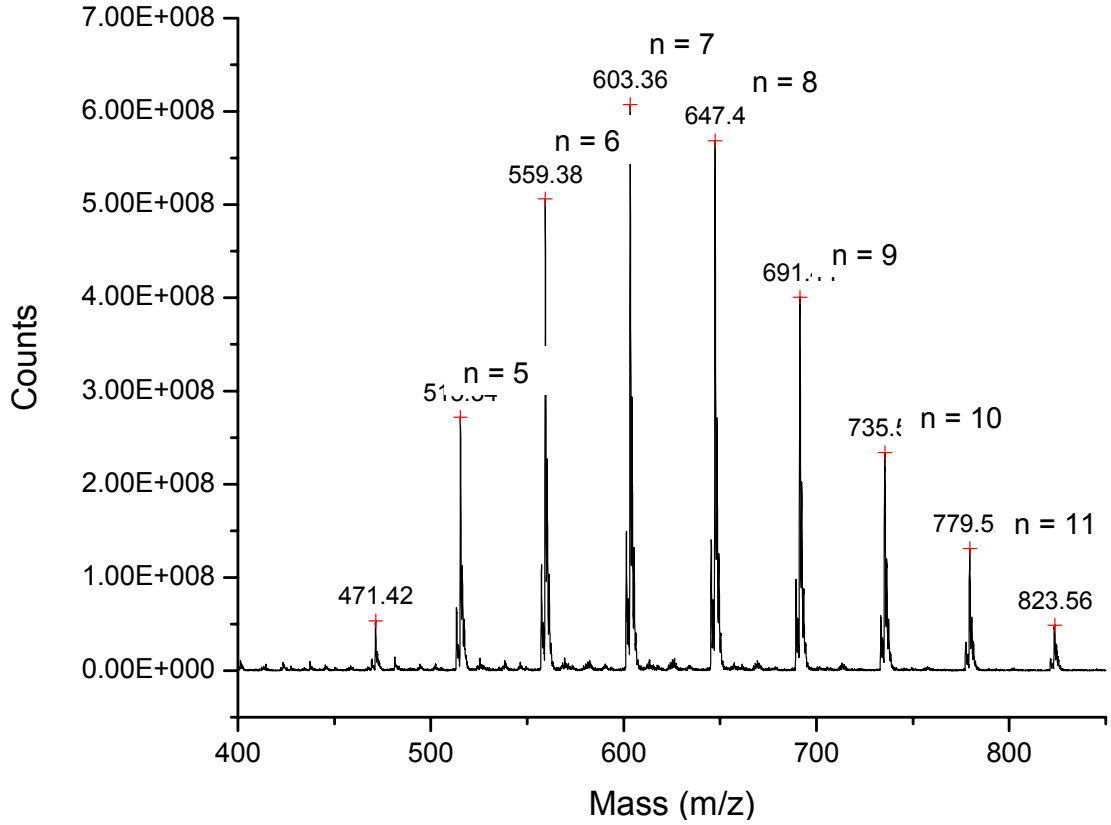


aminoPEG Spectra (Compound 3)

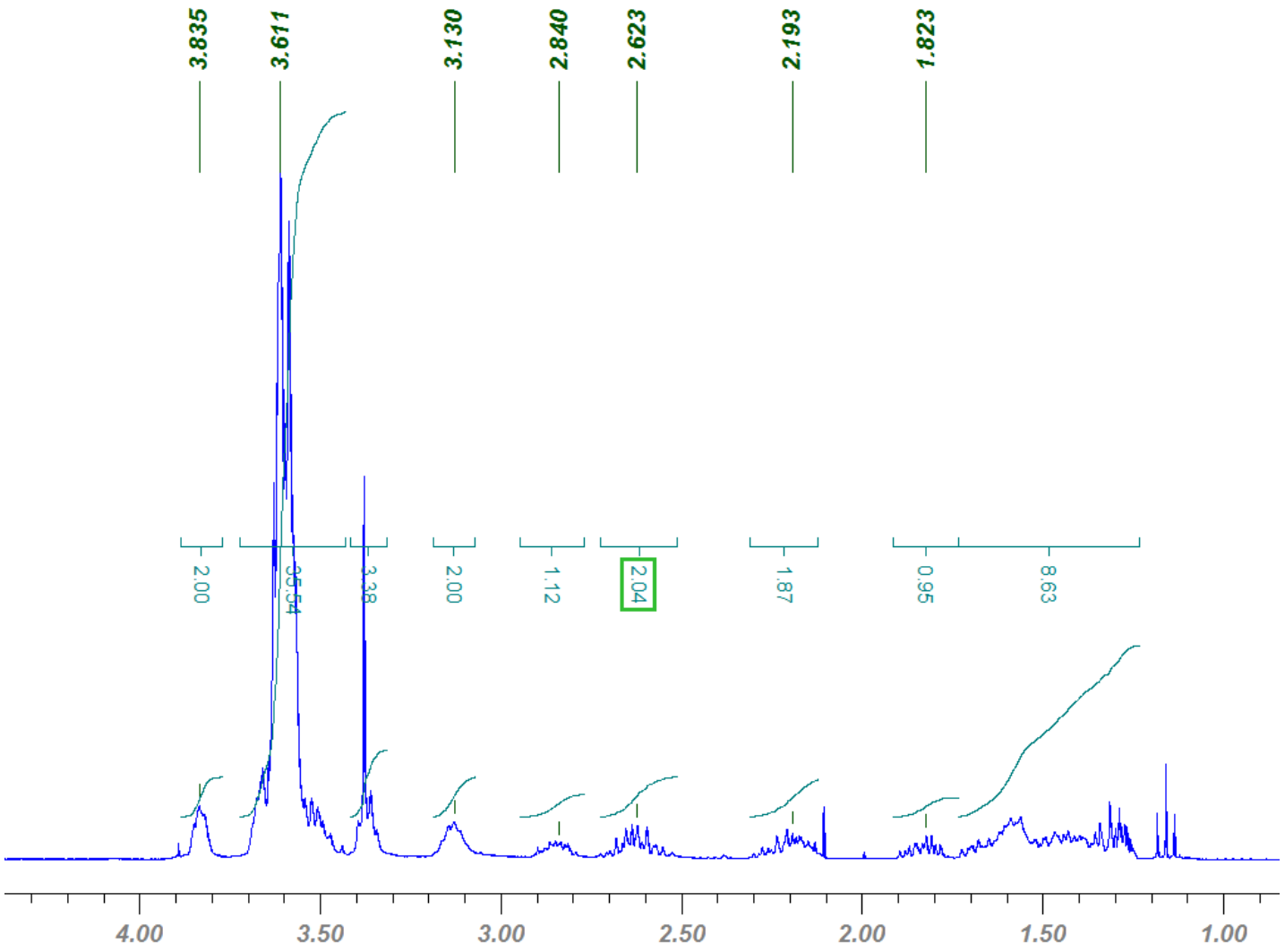
ESI-MS $[M+H]^+$



Chemical Formula: $C_{28}H_{58}N_2O_{10}S_2$
Molecular Weight (n = 8): 646.90

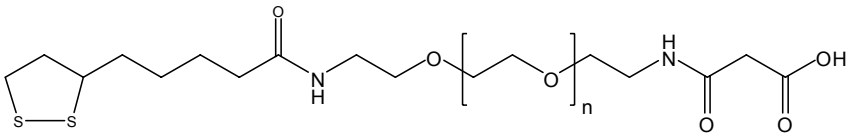


^{1}H-NMR (compound **3)**

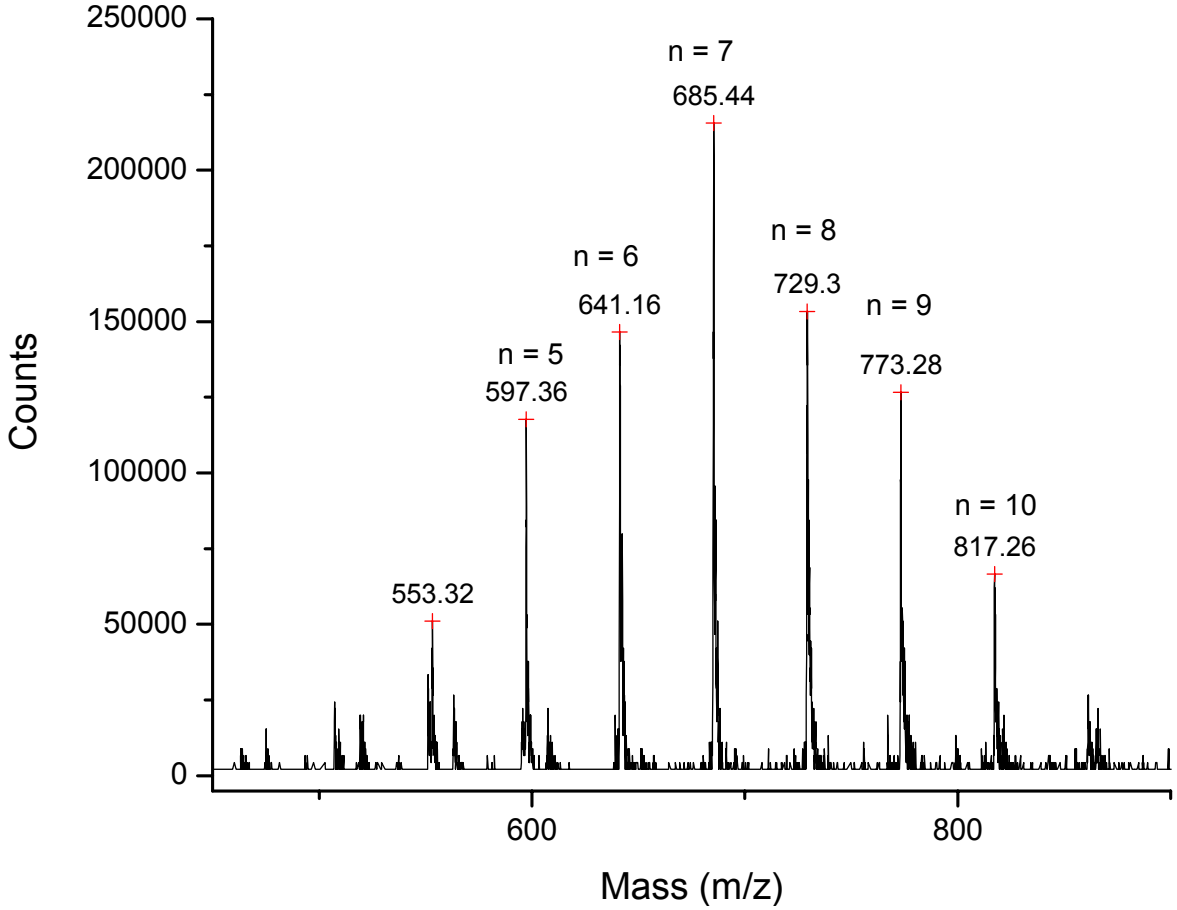


LA-PEG-CO_2H Spectra (Compound 5)

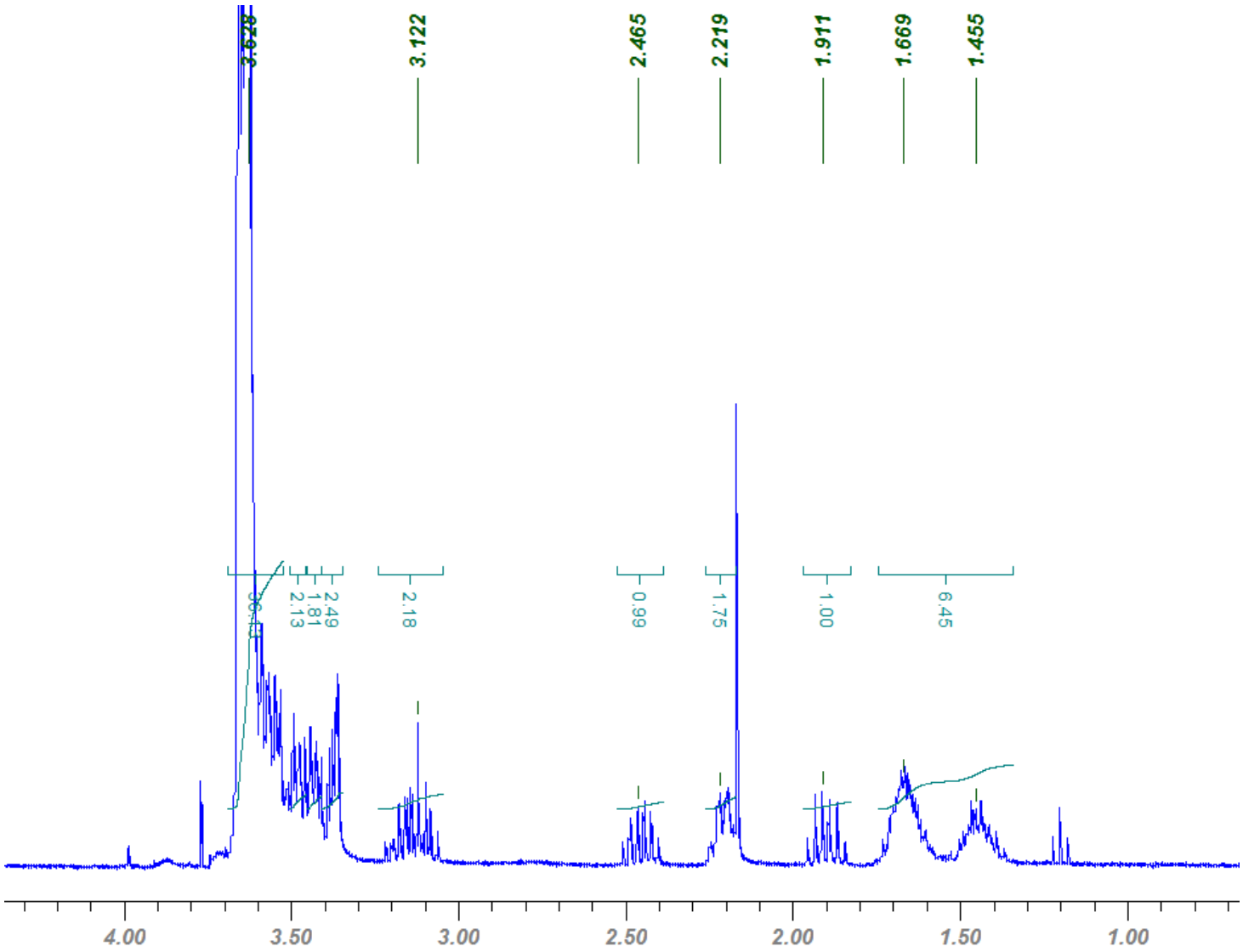
ESI-MS [M-H]⁻



Chemical Formula: $C_{31}H_{58}N_2O_{13}S_2$
Molecular Weight (n = 8): 730.93

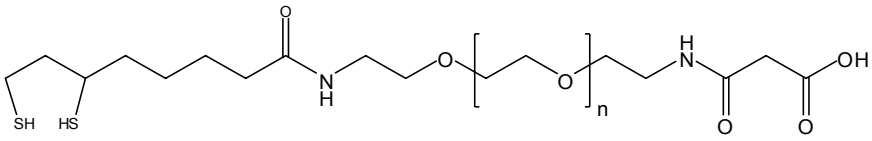


^{1}H-NMR (compound **5**)

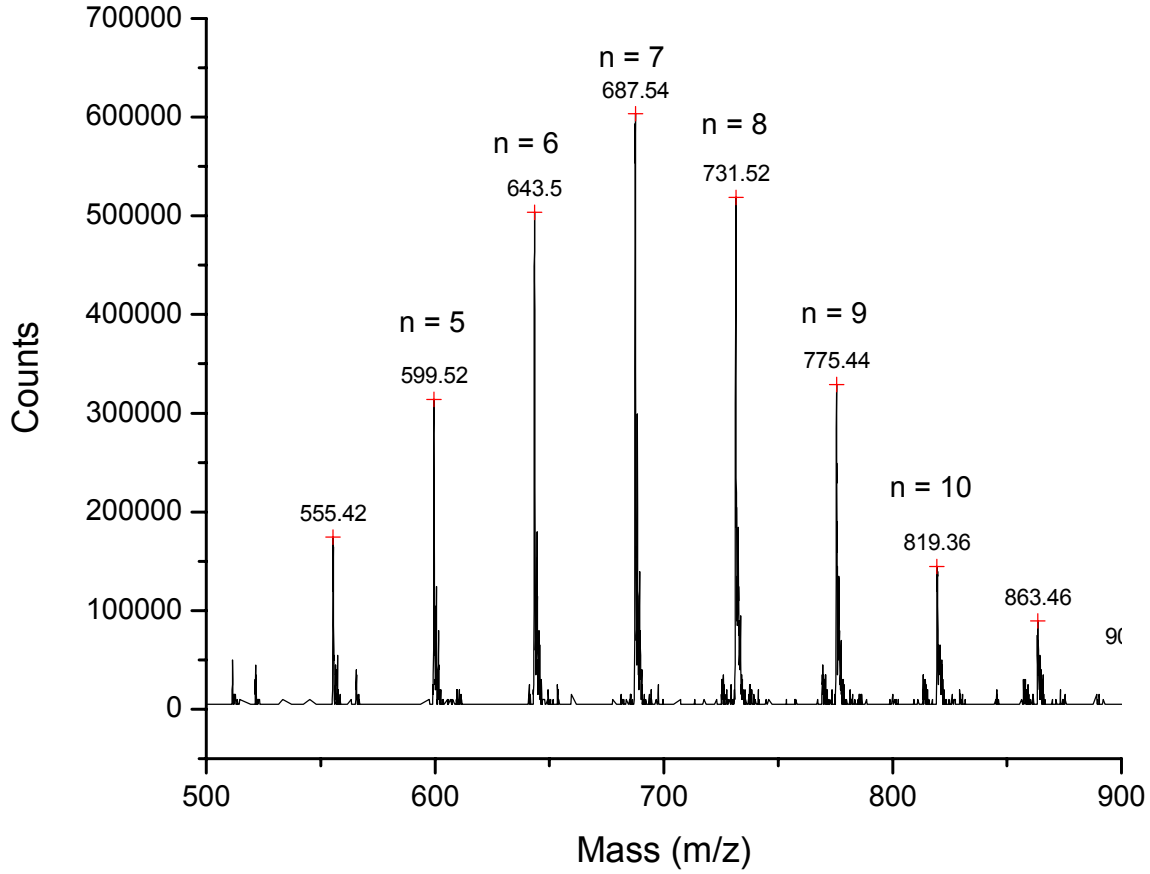


carboxyPEG Spectra (Compound 6)

ESI-MS $[M-H]^-$



Chemical Formula: $C_{31}H_{60}N_2O_{13}S_2$
Molecular Weight (n = 8): 732.94



^{1}H-NMR (compound **6**)