

Supporting Information:

Cetuximab-Ag₂S Quantum Dots for Fluorescent Imaging and Highly Effective Combination of ALA-based Photodynamic/Chemo-Therapy of Colorectal Cancer Cells

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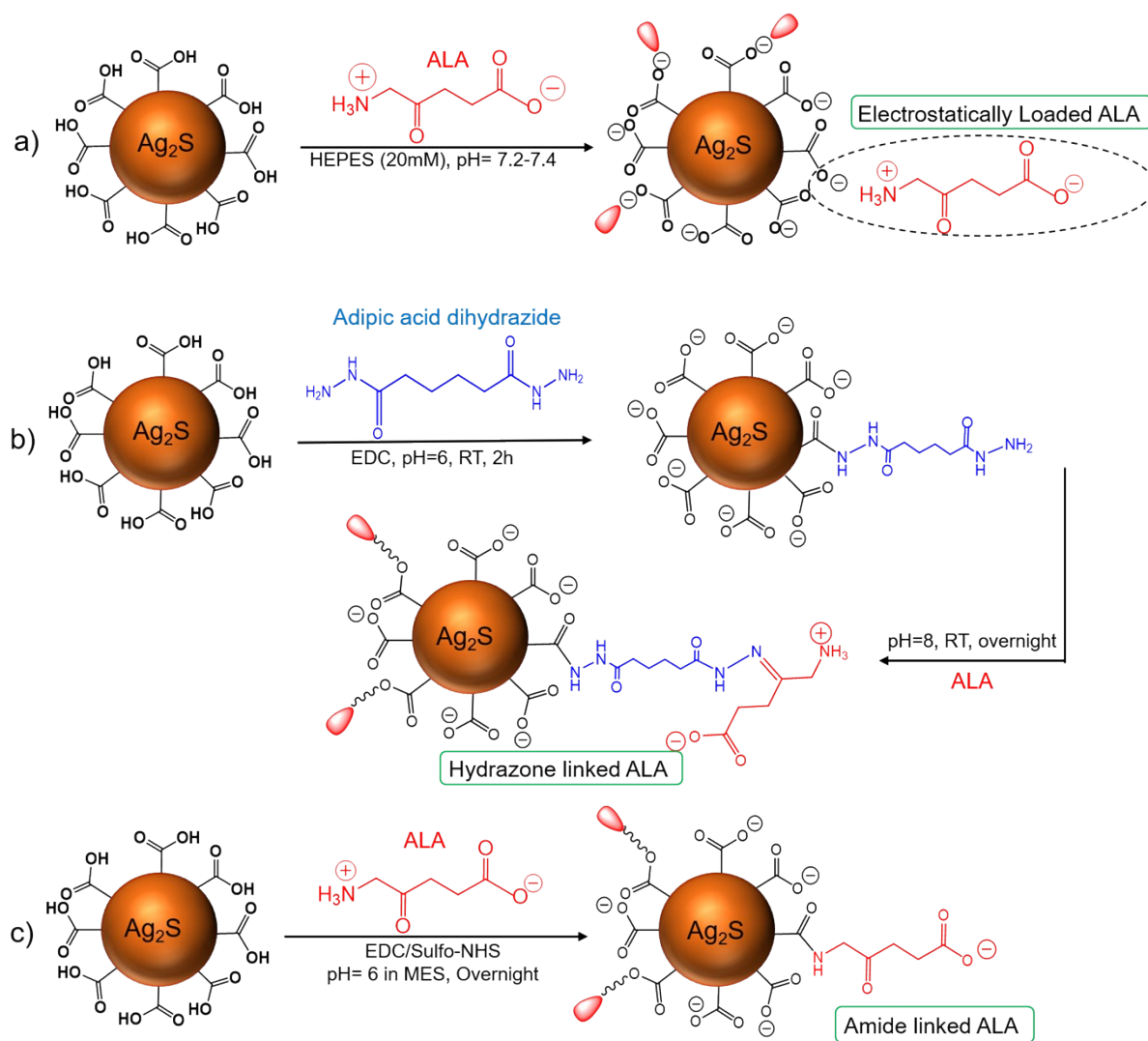
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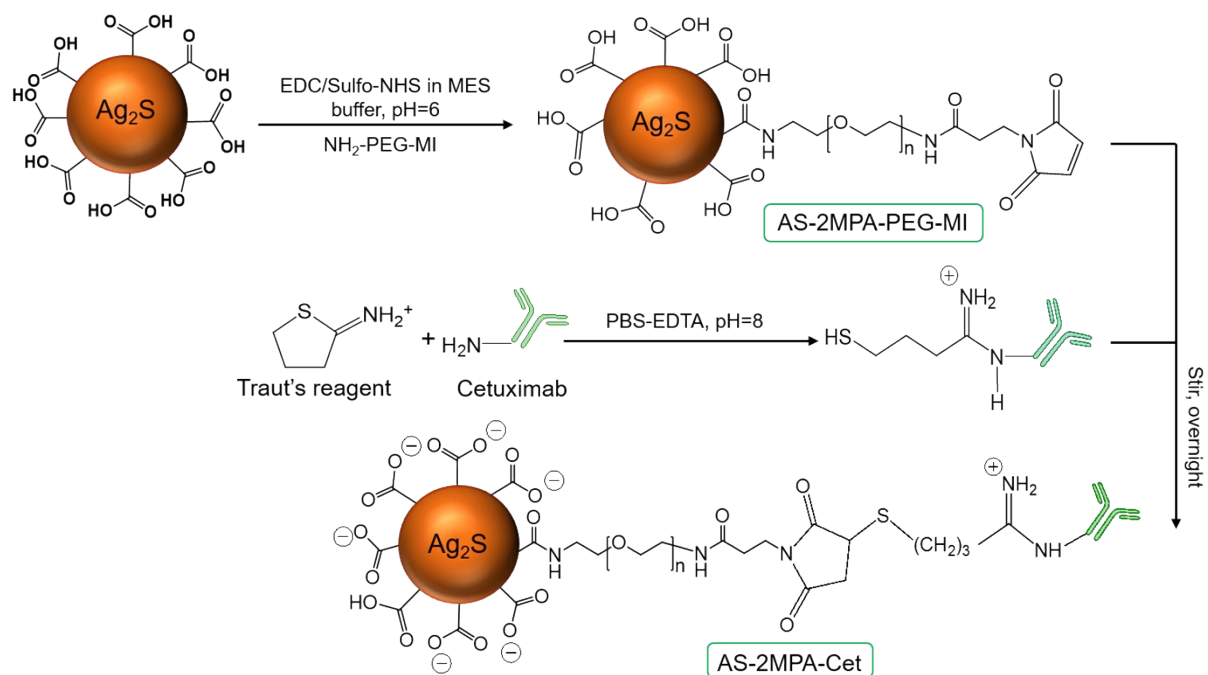
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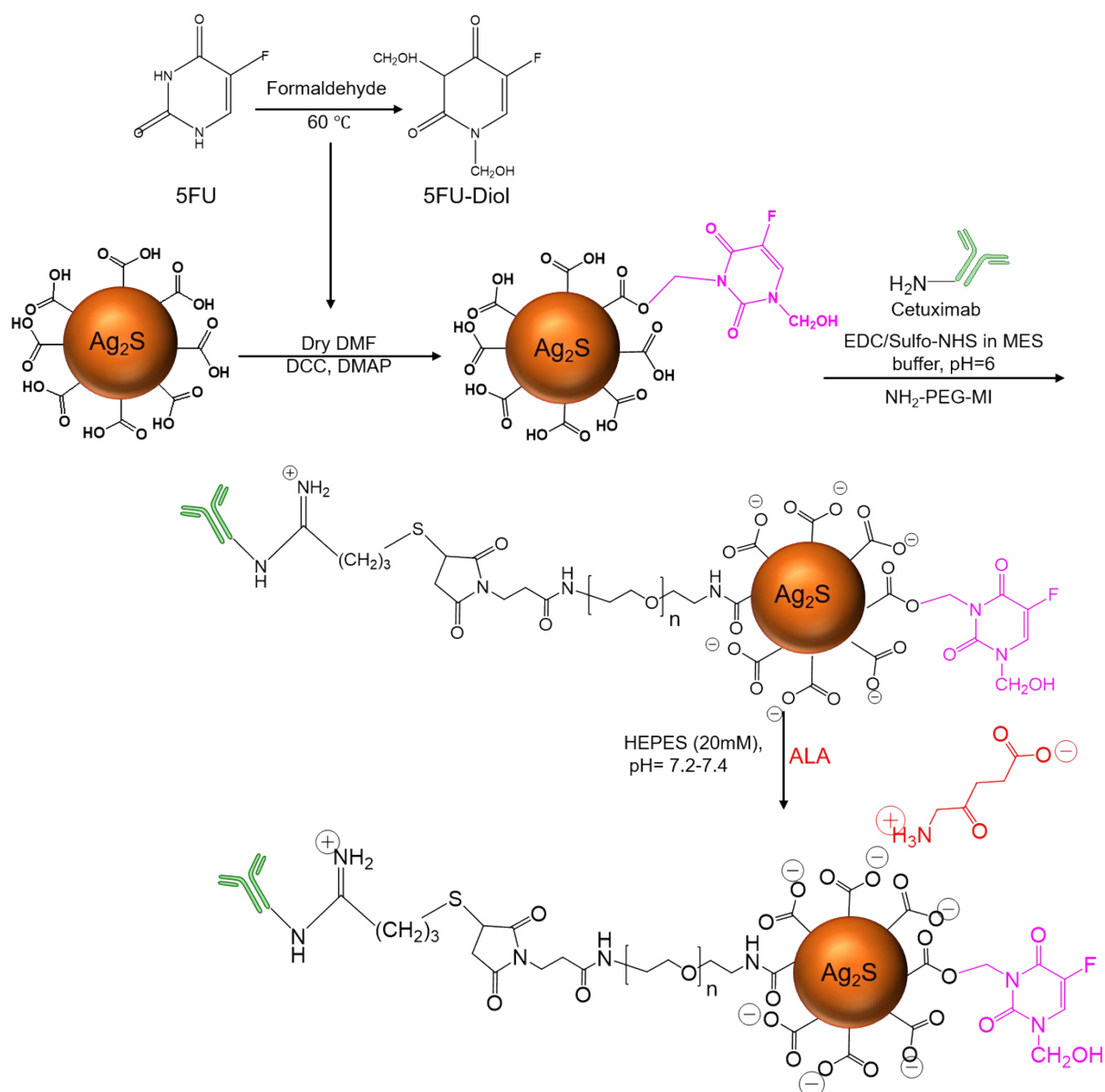
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Scheme S1: Synthesis of different methods of ALA conjugated AS-2MPA QDs.



Scheme S2: Synthesis of Cet conjugated AS-2MPA QDs.



Scheme S3: Synthesis of Cet and 5FU conjugated AS-2MPA electrostatically loaded with ALA (AS-2MPA-ALA-CET-5FU).

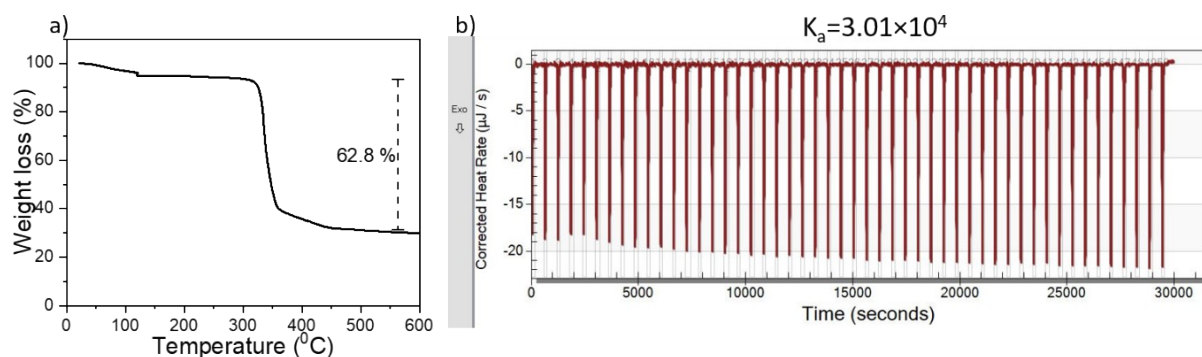


Figure S1: (a) TGA of AS-2MPA QDs, (b) Isothermal titration calorimetry (ITC) of AS-2MPA-ALA-Electrostatic QDs in HEPES at pH 7.4.

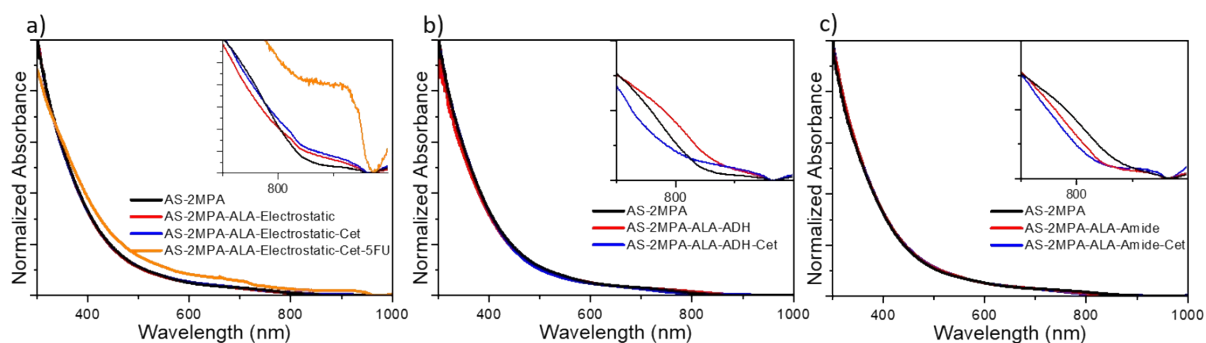


Figure S2: Absorbance spectra of Cet and ALA conjugate QDs via (a) electrostatic, (b) ADH, and (c) Amide bonds.

Table S1: optical properties of ALA conjugates QDs.

Name of samples	$\lambda_{\text{abs}}^{\text{a}}$ (nm)	Band gap (eV)	$\lambda_{\text{em}}^{\text{b}}$ (nm)
ALA in HEPES	263	-	-
AS-2MPA in HEPES	958	1.30	831.91
AS-2MPA in water	955	1.3	830.63
AS-2MPA in PBS	953	1.3	830.91
AS-2MPA-ALA-Electrostatic	957	1.3	830.62
AS-2MPA-ALA-ADH	960	1.29	851.17
AS-2MPA-ALA-Amide	962	1.29	840.59
AS-2MPA-ALA-Electrostatic-Cet	957	1.3	830.61
AS-2MPA-ALA-ADH-Cet	960	1.29	846.48
AS-2MPA-ALA-Amide-Cet	962	1.29	836.49
AS-2MPA-ALA-Electrostatic-Cet-5FU	973	1.27	836.30

^a Absorbance onset. ^b Emission maxima.

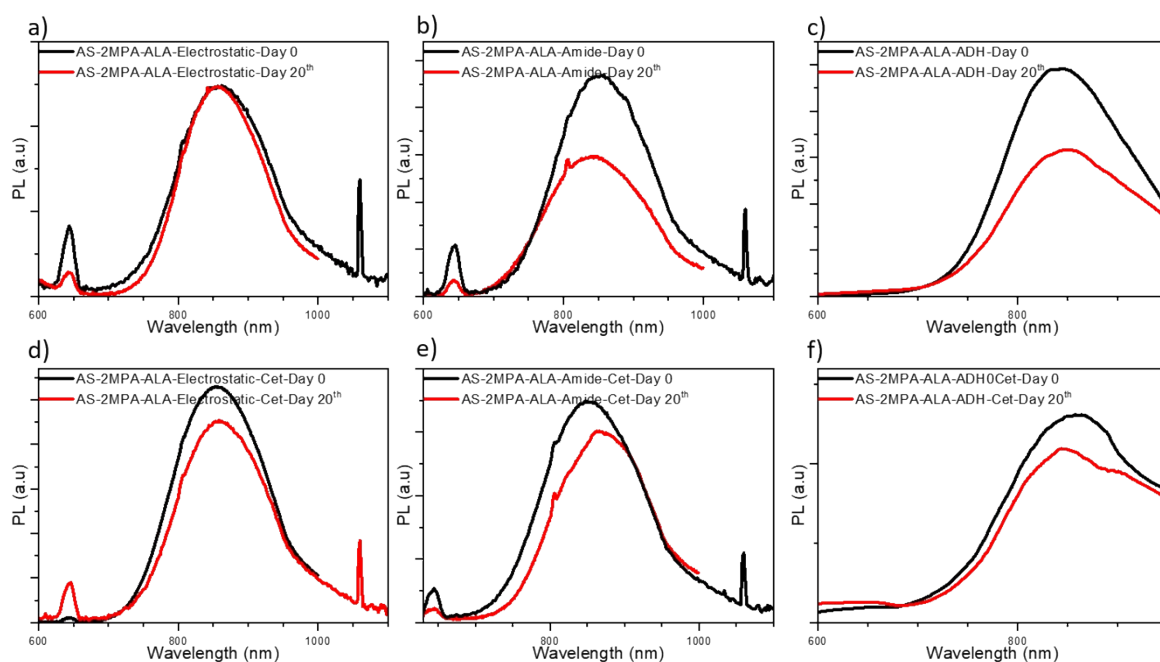


Figure S3: Photoluminescence spectra of AS-2MPA QDs loaded with ALA via (a) electrostatic loading, (b) ADH, and (c) amide bonds, (d-f) conjugated with Cet at day 0 and 20th.

Table S2: Hydrodynamic size, zeta potential of different QD compositions at day 0 and 20th.

Name of samples	PDI ^a	Dh-number ^b (nm)	Dh-intensity ^c (nm)	Zeta potential (mV)
AS-2MPA in water-Day 0	0.3	3.6	13.2	-39.1
AS-2MPA in water-Day 20 th	0.21	3.1	27.4	-35.3
AS-2MPA-ALA-Electrostatic-Day 0	0.27	3.5	19.5	-13.6
AS-2MPA-ALA-Electrostatic-Day 20 th	0.37	8.4	29.2	-16.7
AS-2MPA-ALA-Electrostatic-Cet- Day 0	0.34	5.9	49.8	-12.2
AS-2MPA-ALA-Electrostatic-Cet-Day 20 th	0.66	3.6	106.7	-4.5
AS-2MPA-ALA-ADH-day 0	0.33	4.8	118.5	-7.7
AS-2MPA-ALA-ADH- day 20 th	0.5	5.8	109.3	-7.4
AS-2MPA-ALA-ADH-Cet- Day 0	0.24	9.2	139.7	-14.3
AS-2MPA-ALA-ADH-Cet- day 20 th	0.3	86.3	170.1	-15.7
AS-2MPA-ALA-Amide day 0	0.41	3.9	112.2	-36.2
AS-2MPA-ALA-Amide- day 20 th	0.33	3.9	118.9	-36.3
AS-2MPA-ALA-Amide-Cet-day 0	0.27	6.4	147.1	-16.7
AS-2MPA-ALA-Amide-Cet-day 20 th	0.45	7.5	201.1	-30.1

^a Poly dispersity index, ^b Hydrodynamic diameter measured by DLS and reported as the number average. ^c Hydrodynamic diameter measured by DLS and reported as the intensity average.

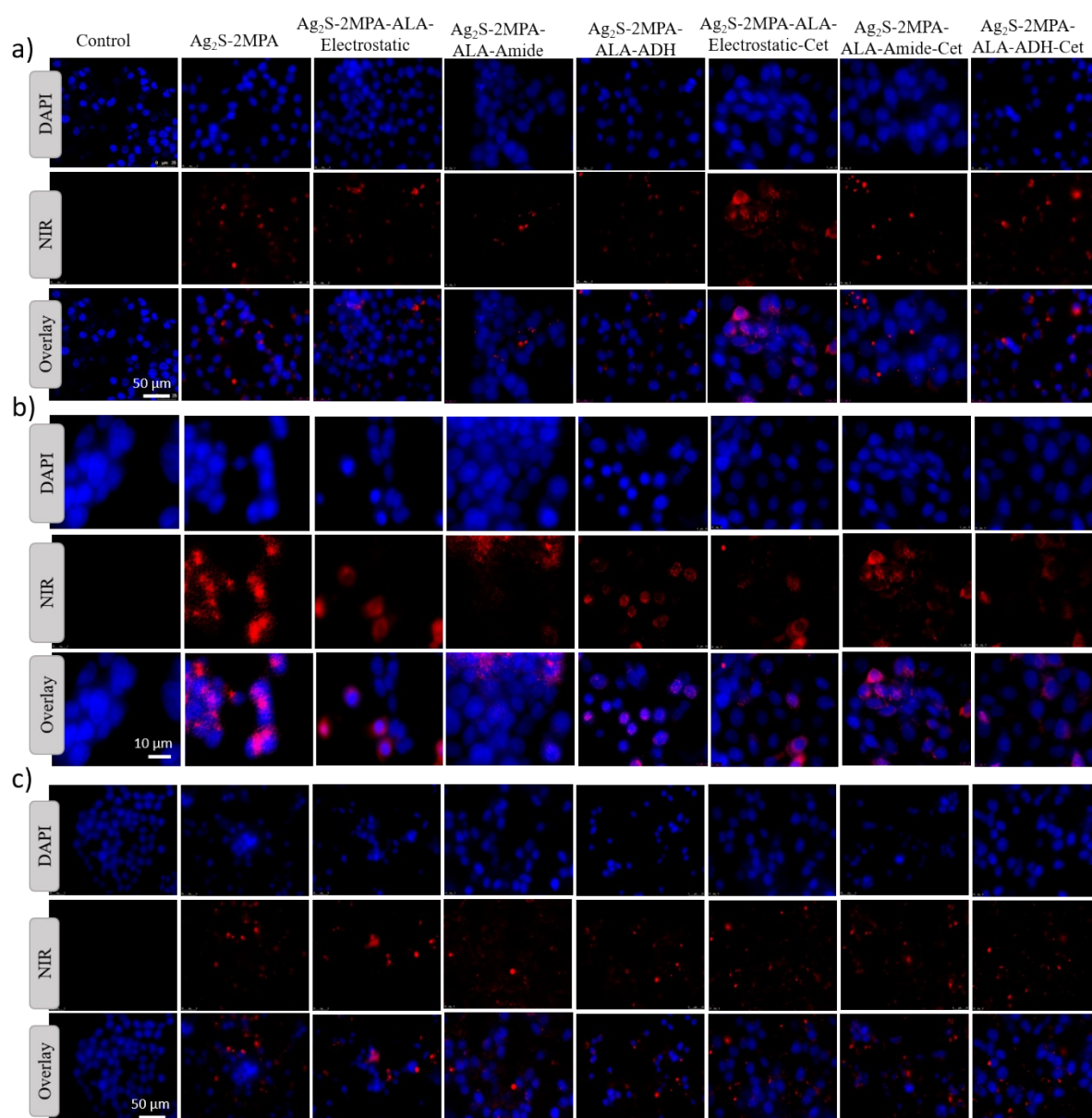


Figure S4: Fluorescence microscopy images of (a) HCT116, (b) SW480, and (c) HT29 cells incubated with different conjugates of AS-2MPA QDs for 24 h and control cells. Red: Luminescence from QDs (excitation/emission: 510-550/710 nm long pass filter), Blue: Nuclear stain (excitation/emission: 325-375/435-485 /nm). Scale bar is 50, 10, and 50 μm, respectively.

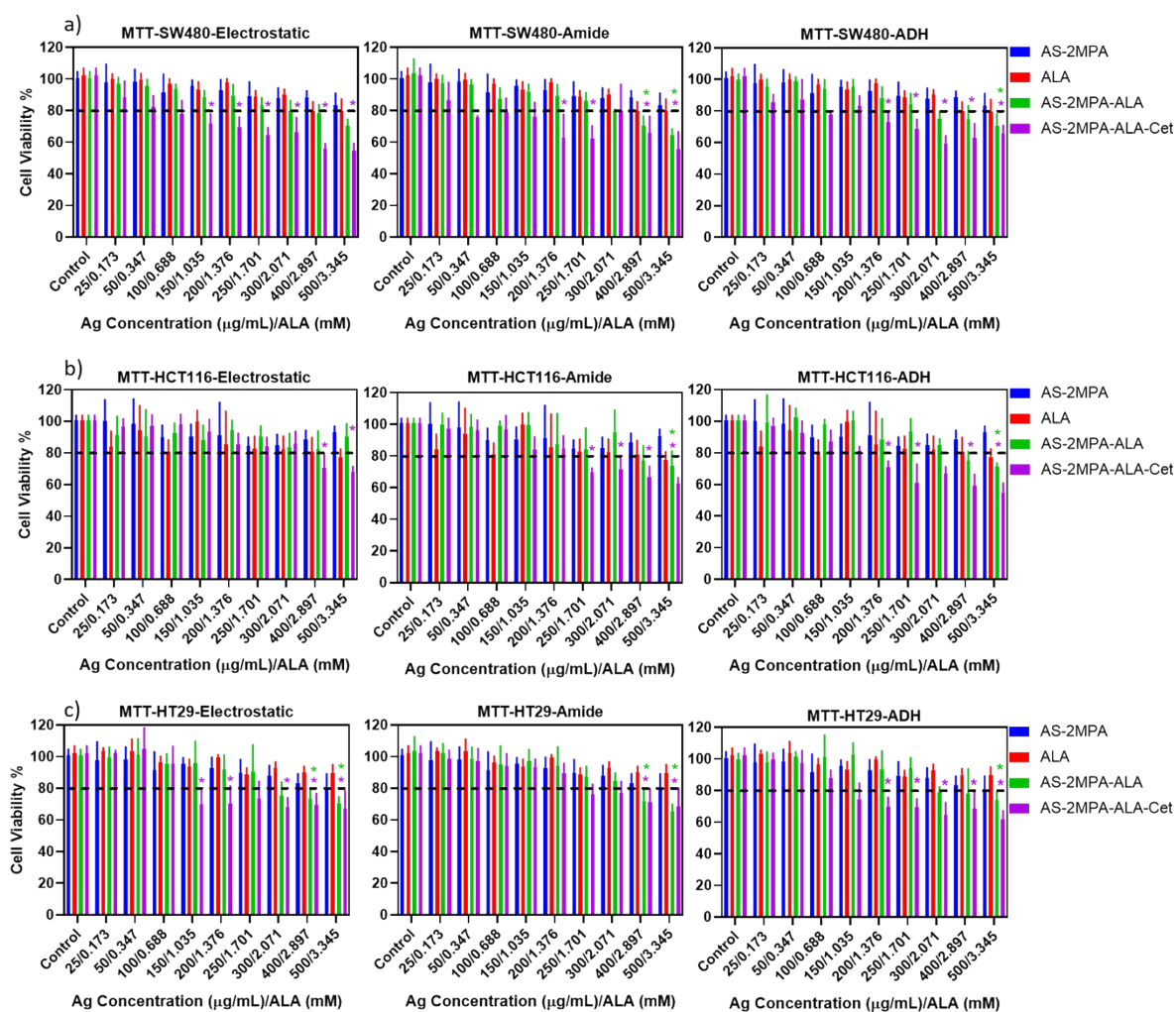


Figure S5: Viability of (a) HCT116, (b) SW480, and (c) HT29 cells treated with AS-2MPA QD conjugates at 25-500 $\mu\text{g/mL}$ [Ag] after 48 h incubation in 2D cell culture determined by MTT assay ($n=4$).

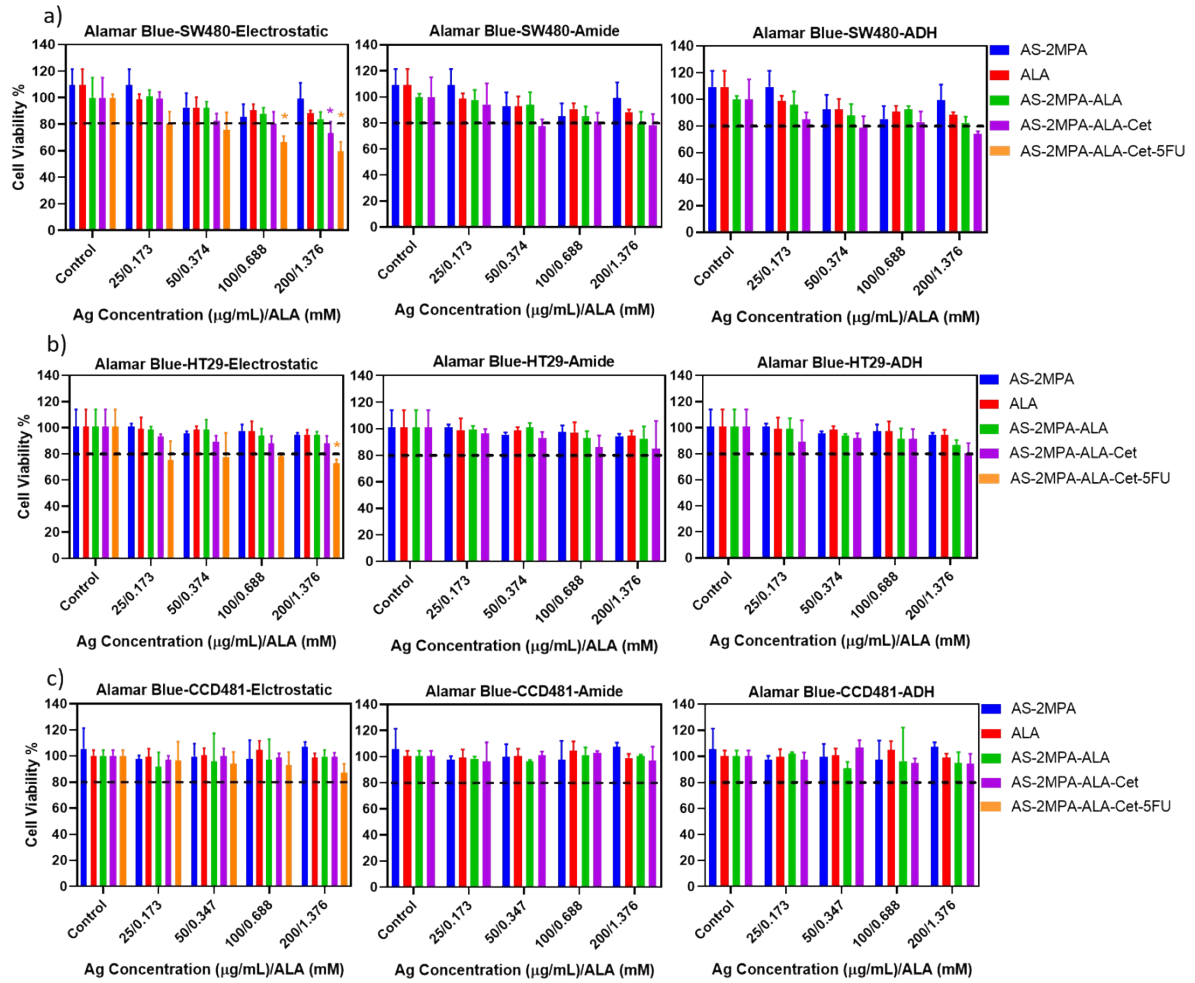


Figure S6: Viability of (a) SW480 and (b) HT29 cells treated with AS-2MPA QD conjugates at 25-200 $\mu\text{g/mL}$ [Ag] concentration after 48 h incubation, measured by Alamar blue assay (n=3). (c) Viability of CCD481 cells (healthy colorectal cells) treated with AS-2MPA QD conjugates at 25-200 $\mu\text{g/mL}$ [Ag] concentration after 48 h incubation, measured by Alamar blue assay (n=3).

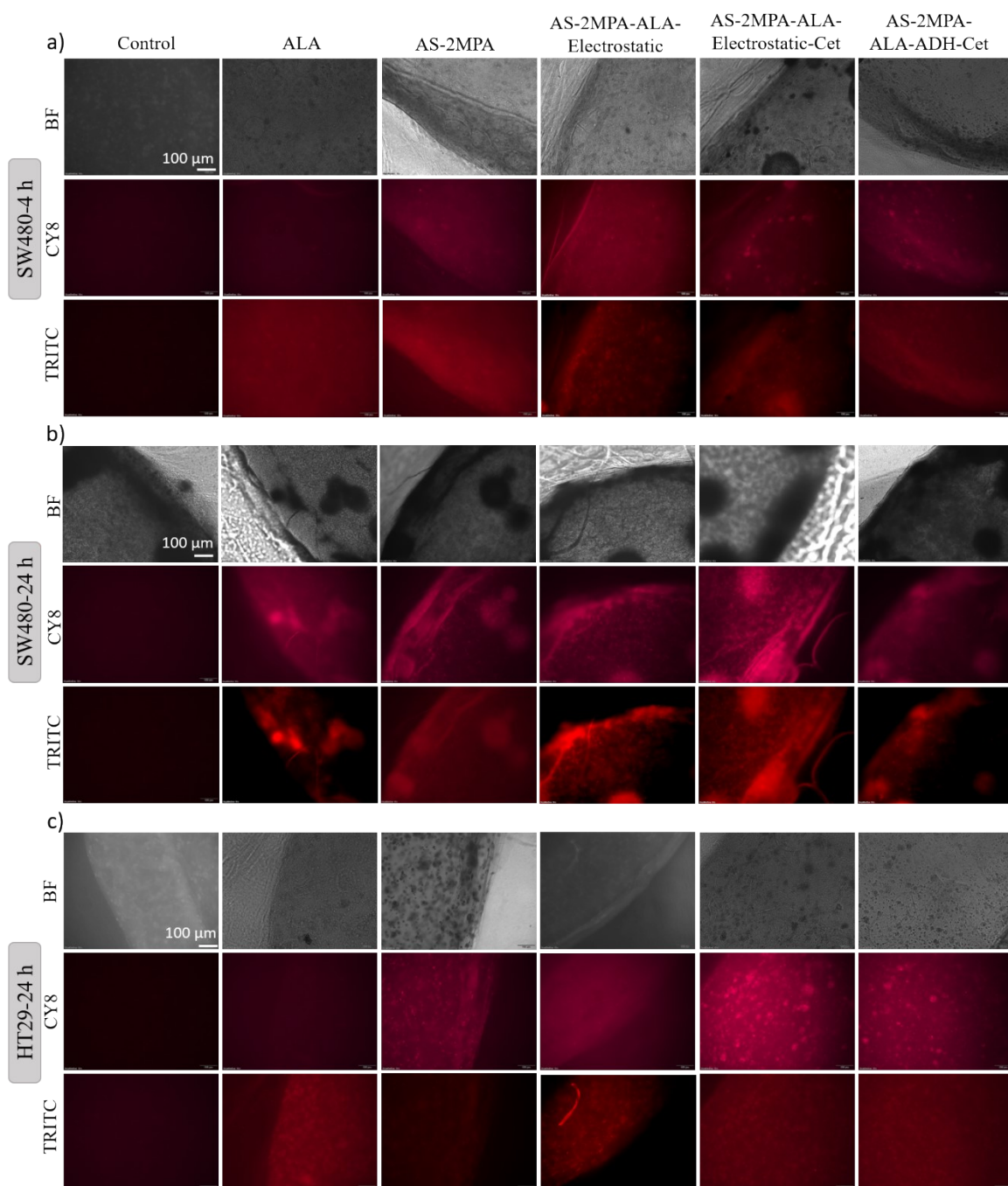


Figure S7: Intracellular quantification of QDs in SW480 after (a) 4h and (b) 24h, and (c) HT29 cells after 24h incubation of the NPs at 100 $\mu\text{g/mL}$ Ag concentration. CY8 (λ_{exc} : 480 nm and λ_{em} : 810 nm) and TRITC (λ_{exc} : 545 nm and λ_{em} : 580-650 nm (band pass filter)). The scale bar for all images is 100 μm .

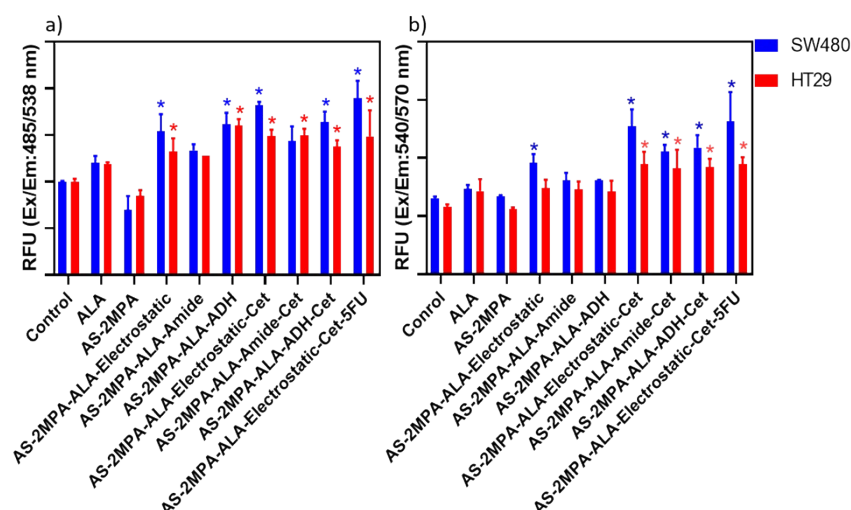


Figure S8: ROS production of SW480 and HT29 cells upon incubation with ALA, AS-2MPA, AS-2MPA-ALA, and AS-2MPA-ALA-Cet QDs at 100 $\mu\text{g/mL}$ [Ag] after 24 h incubation upon illumination of (a) blue lamp for 5 min and (b) 630 nm laser for 1 min. The data are expressed as mean $\pm$ S.D. ($n = 3$), ($p < 0.05$). All red and blue stars indicate significant difference from free ALA.

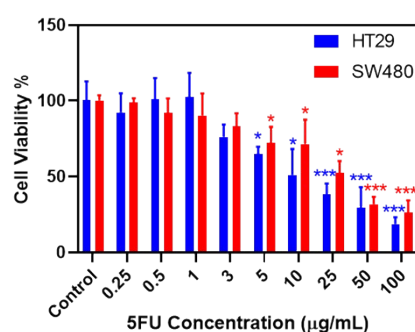


Figure S9: Viability of SW480 and HT29 cells treated with 5FU conjugates at after 48 h incubation measured by MTT assay ($n=3$), (* $p < 0.05$, *** $p < 0.001$).

Table S3: the half maximal effective inhibitory concentration (IC_{50}) of AS-2MPA, AS-2MPA-ALA, AS-2MPA-ALA-Cet, AS-2MPA-ALA-Cet, and AS-2MPA-ALA-Cet-5FU QDs after PDT at 420 and 640 nm in ALA concentration (mM).

Samples	SW480 cells, 4h		SW480 cells, 24h		HT29 cells, 24 h	
	420 nm	640 nm	420 nm	640 nm	420 nm	640 nm
ALA	-	-	-	-	-	-
AS-2MPA	-	-	-	-	-	-
AS-2MPA-ALA-Electrostatic	-	0.137	0.135	0.126	0.491	0.122
AS-2MPA-ALA-ADH	-	-	0.156	0.284	-	-
AS-2MPA-ALA-Amide	-	-	0.368	0.545	-	-
AS-2MPA-ALA-Electrostatic-Cet	0.359	0.129	0.106	0.104	0.504	0.098
AS-2MPA-ALA-ADH-Cet	-	0.257	0.120	0.178	-	0.345
AS-2MPA-ALA-Amide-Cet	-	0.347	0.233	0.178	-	0.368
AS-2MPA-ALA-Electrostatic-Cet-5FU	0.329	0.106	0.101	0.092	0.299	0.084