Supporting Information

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Hydrogel Nanoparticles with Covalently Linked Coomassie Blue for Brain Tumor Delineation Visible to the Surgeon

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Supporting Information

Targeted Hydrogel Nanoparticles with Covalently Linked Coomassie Blue for Brain Tumor Delineation Visible to the Surgeon's Eyes

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<Measurements>

Fourier-transformed nuclear magnetic resonance (NMR) spectroscopy was performed on a Varian INOVA-400 MHz and 500 MHz spectrometer. UV-vis absorption spectra were obtained on a Shimadzu UV-1601 spectrometer. Electrospray-ionization mass spectrometric (ESIMS) data were obtained on a Bruker Daltonics 7-T quadrupole FT-ICR and Waters Micromass LCT Time-of-Flight (TOF) mass spectrometer. Fourier-transformed Infrared (IR) spectroscopy was performed on a Perkin Elmer Spectrum BX spectrometer. The elemental analysis was performed by Galbraith laboratories, Inc (Knoxville, Tennessee). The elemental analysis of C, H, N, O and S were performed by CHN analyzer and oxygen analyzer after converting the sample elements to simple gases, CO_2, H_2O, N_2, and CO_2 respectively by combustion (C, H, N, S) or pyrolysis (O). The analysis of Na was performed by inductively coupled plasma optical emission spectrometry (ICP-OES) and Flame Atomic Absorption spectroscopy (FLAA). The analysis of P was performed by ICP-OES as well. The analysis of S was performed by ASTM D4239 Method B/ D1552. The quantitative amino acid assays was performed by Protein Chemistry Lab at Texas A&M University (College Station, Texas). The QAAA test was performed by AminoQuant Amino Acid Analyzer that analyzes peptides and proteins by pre-column derivatization of hydrolyzed samples with o-phthalaldehyde (OPA) and 9-fluoromethyl-chloroformate (FMOC). OPA reacts with primary amino acids and FMOC with secondary amino acids (proline). Both reagents react rapidly and quantitatively and give highly fluorescent and UV-absorbing isoindole derivatives. The derivatized amino acids are separated by reverse phase HPLC and detected by UV absorbance with a diode array detector or by fluorescence using an in-line fluorescence detector. Each sample was hydrolyzed at least twice (duplicates) and injected into the HPLC at least twice (replicates). Each series of samples also includes standard calibration amino acids, a blank control and a standard protein (generally, human serum albumin). The internal standards were added to each sample so as to control errors due to sample loss, injection variations and variability in preparing dilutions. The internal standards were also used to calculate the amount of each amino acid in the sample.

For scanning electron microscopy (SEM) studies, CB-loaded PAA NPs were dispersed in water, and the resultant solution was sonicated for 30 min to make a homogeneous solution without aggregates. A drop of the particle solution was placed on the SEM specimen mount and dried gradually at room temperature. The samples were then coated with a gold sputter and observed with a Philips XL30FEG

SEM to characterize the size and shape of the particles. For transmission electron microscopy (TEM) studies, CB-loaded PAA solution prepared as mentioned in SEM sampling was dropped on a TEM grid and dried gradually at room temperature. And then osmium tetroxide (OsO_4) solution was vaporized for staining the samples on the grid. The stained samples were observed with a Philips CM-100 to characterize the size and shape of the particles.
The size distribution of CB-loaded PAA NPs was measured by dynamic light scattering (DLS) with Delsa Nano (Beckman Coulter) to determine the size of nanoparticles in an aqueous solution and the presence of aggregates. The surface charge of the CB-loaded PAA NPs and their surface-modified NPs was also measured by zeta potential measurements in water with Delsa Nano.

<Characterization of CB (by NMR and mass spectrometry)>

To confirm each proton on the CB-APMA, we figured out the protons on commercial CB first. The ^{1}H NMR of CB is in accord with previously reported results. [K. Blinova, R. L. Levine, E. S. Boja, G. L. Griffiths, Z. D. Shi, B. Ruddy, R. S. Balaban, *Biochemistry* **2008**, *47*, 9636–9645.] There are 22 protons on the six benzene rings, their δ value is between 7.81-6.60 ppm. The protons of Ar-C$\underline{H}_2$-N show a singlet at 4.74 ppm. The protons of Ar-C$\underline{H}_3$ show a singlet, broad peak at 1.74 ppm. The protons of CH_3-C$\underline{H}_2$-O show a quartet at 3.95 ppm while their conjugated protons show a triplet at 1.30 ppm (See Figure S3, 2D ^{1}H NMR for their conjugation status). The protons of N-C$\underline{H}_2$-CH_3 show a quartet at 3.66 ppm and their conjugated protons of N-CH_2-C$\underline{H}_3$ show a triplet at 1.21 ppm.

CB ^{1}H NMR (CD_3OD, 500 MHz) δ 7.81-7.74 (4H, m, Ar-H), 7.44-7.10(7H, m, Ar-H), 7.04-6.86 (5H, m, Ar-H), 6.80-6.60(6H, m, Ar-H), 4.74(4H, s, Ar-CH_2), 3.95 (2H, q, J = 7.0 Hz, CH_3C$\underline{H}_2$-O), 3.66 (4H, q, J=7 Hz, CH_3C$\underline{H}_2$-N). 1.74 (6H, br, CH_3-Ar), 1.30 (3H, t, J=7.0 Hz, C$\underline{H}_3$$H_2$-O), 1.21 (6H, t, J=7.0 Hz, C$\underline{H}_3$$CH_2$-N). **MS** m/z cal.: 832.2[M-Na+H,], 854.3[M+H], 876.3[M+Na]; Found: ESI (m/z), 832.2 [M-Na+H]; 854.3 [M+H]; 876.2 [M+Na].

<Characterization of **CB-I** (by mass spectrometry)>
The mass spectrum of green CB-I (**Figure S1**) shows the peak at m/z=868.1 as shown in the CB-I structure in **Scheme 1**. CB-I: **MS** m/z cal: 868.2 ($C_{47}H_{48}{}^{35}Cl_2N_3O_5S_2$), 870.2($C_{47}H_{48}{}^{37}Cl_2N_3O_5S_2$,) found: ESI (m/z):868.1($C_{47}H_{48}{}^{35}Cl_2N_3O_5S_2$), 870.1($C_{47}H_{48}{}^{37}Cl_2N_3O_5S_2$).

<Characterization of CB-APMA (by NMR and IR spectroscopy, mass spectrometry, and elemental analysis)>

The protons of CB-APMA were confirmed by 1D and 2D 500 MHz ^{1}H NMR (CD_2Cl_2, CD_3OD). The proton assignments of the sites show in **Scheme 1, Figure S5**. The proton (#1) on sulfamide, and proton (#5) on amide were confirmed by 1D and 2D ^{1}H NMR (CD_2Cl_2) (**Figures S2** and **S5**). From 2D ^{1}H NMR of CB and CB-APMA (**Figures S3, S4**and **S5**), we can notice protons that are conjugated in CB-APMA. The proton of sulfamide (#1) appears at 7.04 ppm and their conjugated ones of SO_2NH-C$\underline{H}_2$ (#2) appear at 2.99-2.71 ppm. The proton of amide (#5) appears at 7.47 ppm and their conjugated protons of -CONH-C$\underline{H}_2$-(#4) at 3.49-3.41ppm. The protons in the middle of CH_2-C$\underline{H}_2$-CH_2 (#3) show at

1.70-1.54 ppm. The protons on double bond (#7) appear at 5.87-5.64 ppm and 5.31-5.10 ppm. Their conjugated protons from double bond in C<u>H</u>$_3$-C= (#6) show at 1.94-1.84 ppm. Mass spectra confirm CB-APMA at 1080.50. (**Figure S6**). IR spectra (KBr) show amide C=O at 1654, CO-N at 1605, C=CH at 1494 and SO_2-N at 1166 cm^{-1}.(**Figure S7**). The elemental analysis results show that the counter ions for CB-APMA are hydroxides and sulfates. We believe the sulfate counter ions come from the starting material CB.

CB-APMA ^{1}H NMR (CD_2Cl_2, 500 MHz) δ 7.91-7.69(4H, m, Ar-H), 7.47(2H, t, J=7.5 Hz, CONH), 7.43-7.34(3H, m, Ar-H),7.34-7.26(3H,m,Ar-H), 7.25-7.18(2H, m, Ar-H), 7.17-7.10(2H, m, Ar-H), 7.04(2H,t, J=6.5 Hz, SO_2-NH),6.97-6.74 (4H,m, Ar-H), 6.74-6.44 (4H,m,Ar-H),5.87-5.64(2H, m, CH_2=), 5.31-5.10(2H, m, CH_2=), 4.73(4H,S,Ar-CH_2), 4.02(2H, q, J=7 Hz, CH_3-C<u>H</u>$_2$-O), 3.85-3.49(4H, m, CH_3-C<u>H</u>$_2$-N), 3.49-3.41(4H, m, -CONH-C<u>H</u>$_2$-), 2.99-2.71(4H, m, SO_2NH-C<u>H</u>$_2$), 1.94-1.84(6H, m, CH_3-C=), 1.84-1.71(6H, br, CH_3-Ar). 1.70-1.54(4H, m, CH_2C<u>H</u>$_2$$CH_2$), 1.38(3H, t, J=7 Hz, C<u>H</u>$_3$-CH_2-O). 1.30(6H, t, J=7 Hz, C<u>H</u>$_3$-CH_2-N); **MS** m/z cal.: 1080.5 (M), 1081.5 (M+1), 1082.5 (M+2), Found: ESI (m/z):1080.5(M), 1081.5(M+1), 1082.5(M+2); **Elemental Anal**. Calcd. for $C_{61}H_{74}N_7O_7S_2^+(OH^-)_{0.6}$ $(SO_4^{2-})_{0.2}$: C, 64.04; H, 6.58; N, 8.58; O, 11.75; S, 6.17. Found: C, 64.01; H, 6.79; N, 8.42; O, 11.67; S, 6.23; **IR** νmax /cm^{-1} (KBr): 3420, 2925, 2857, 1654, 1605, 1583, 1494, 1451, 1384, 1340, 1243, 1166, 1049, 824.

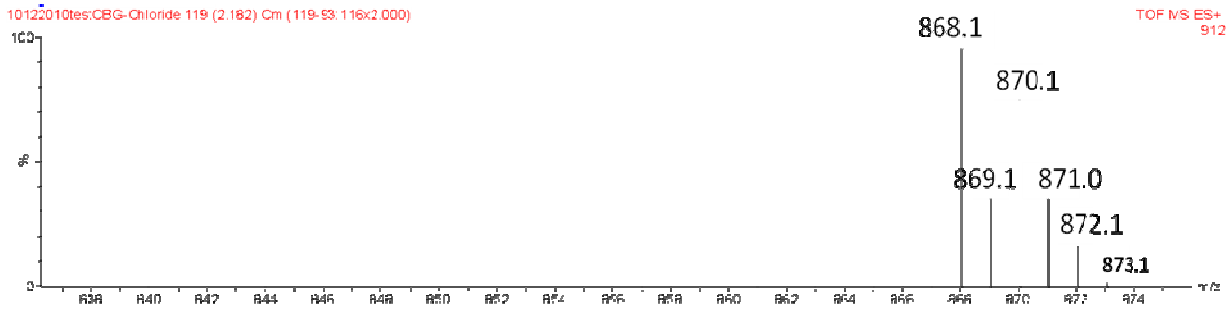


Figure S1. Mass spectrum of CB-I by Waters Micromass LCT mass spectrometer

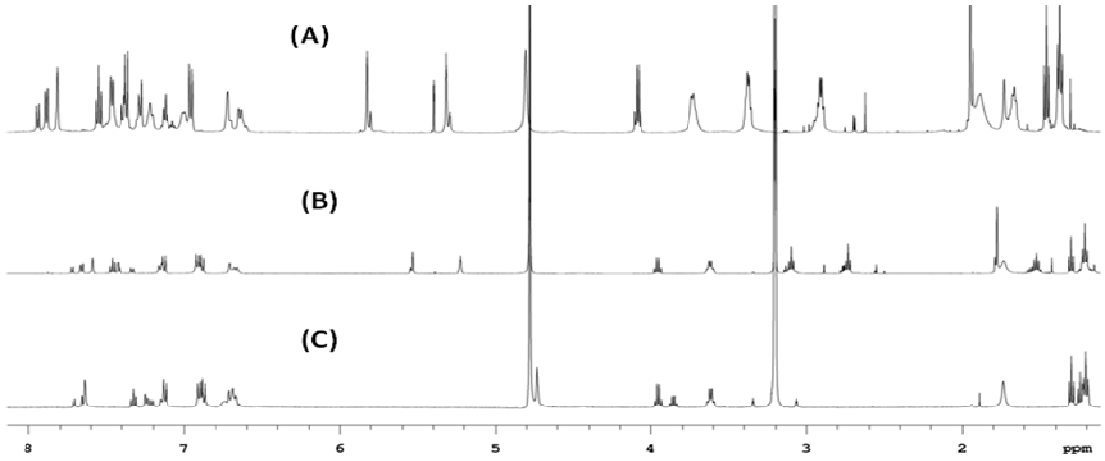


Figure S2. ^{1}H NMR spectra (500 MHz) of (A) CB-APMA in CD_2Cl_2 solution, (B) CB-APMA in CD_3OD solution, and (C) CB in CD_3OD solution.

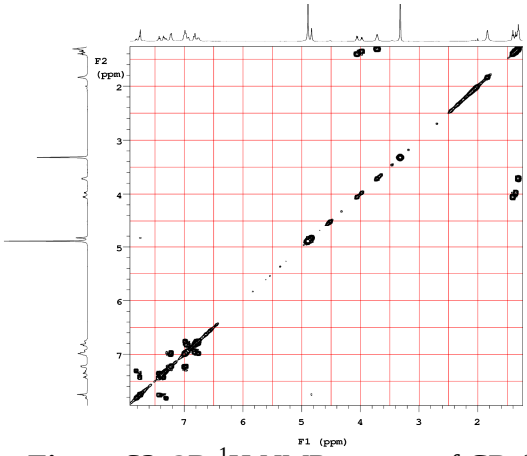


Figure S3. 2D ^{1}H NMR spectra of CB (CD_3OD)

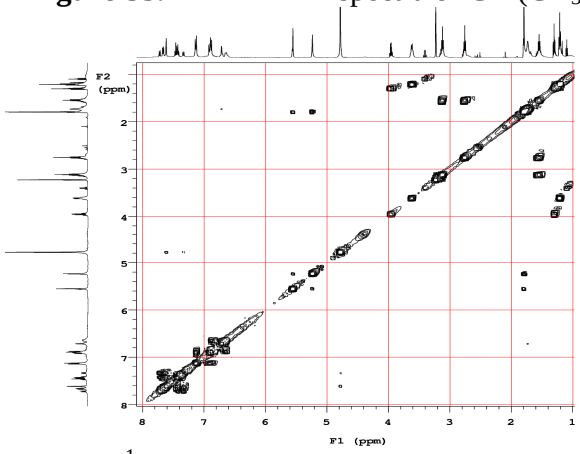


Figure S4. 2D ^{1}H NMR spectra of CB-APMA (CD_3OD)

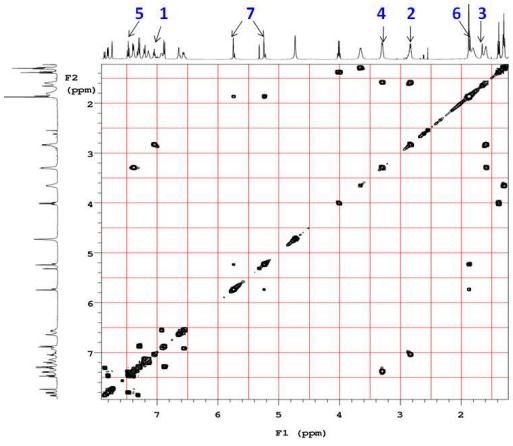


Figure S5. 2D ^{1}H NMR (500 MHz) spectra of CB-APMACl (CD_2Cl_2).
The numbers on top correspond to the numbers of protons in Scheme 1.

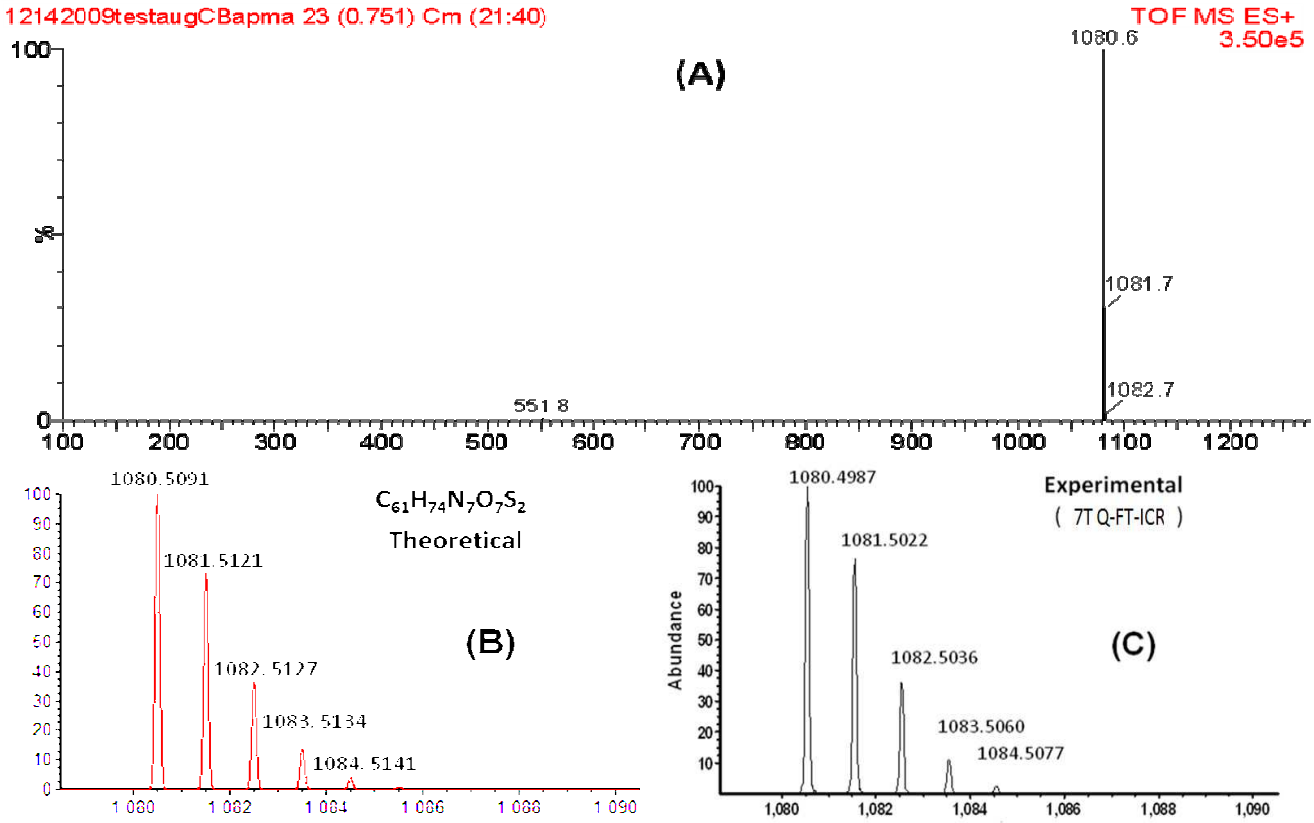


Figure S6. Mass spectra of CB-APMA: (A) mass spectrum by Waters Micromass LCT mass spectrometer, and (B) theoretically predicted spectra and (C) mass spectrum by Bruker Daltonics 7 Tesla ESI Q-FT-ICR mass spectrometer.

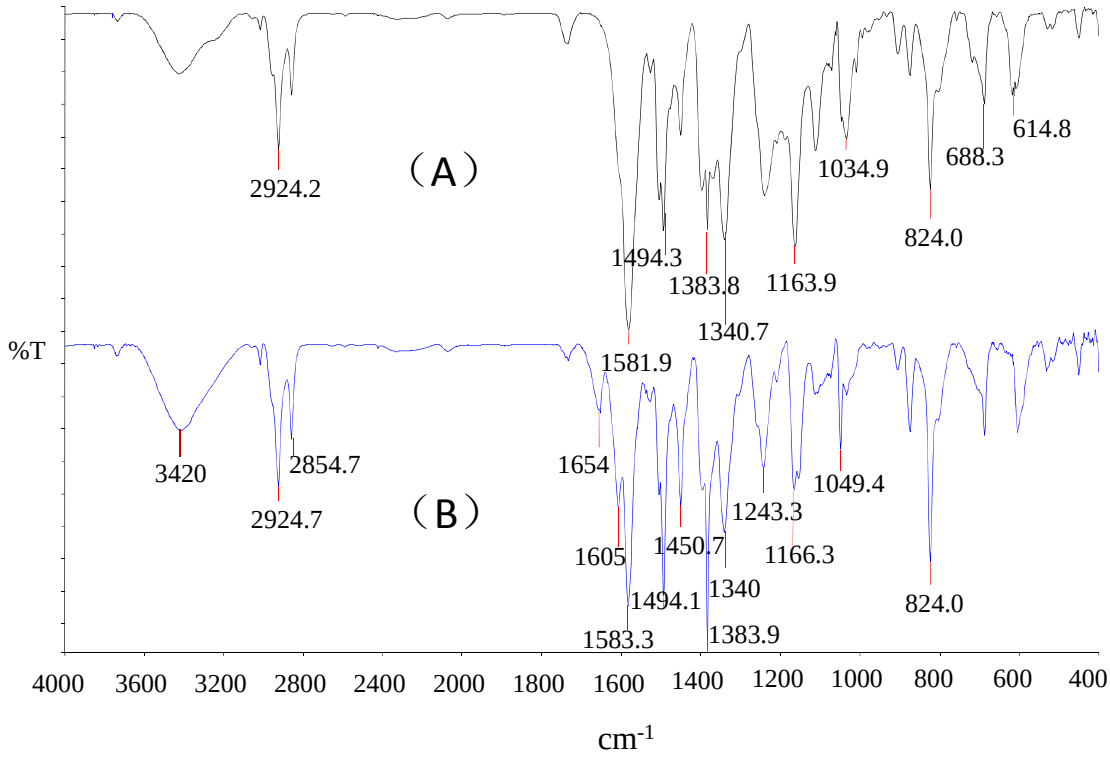


Figure S7. IR spectra of (A) CB (black) and (B) CB-APMA (blue)

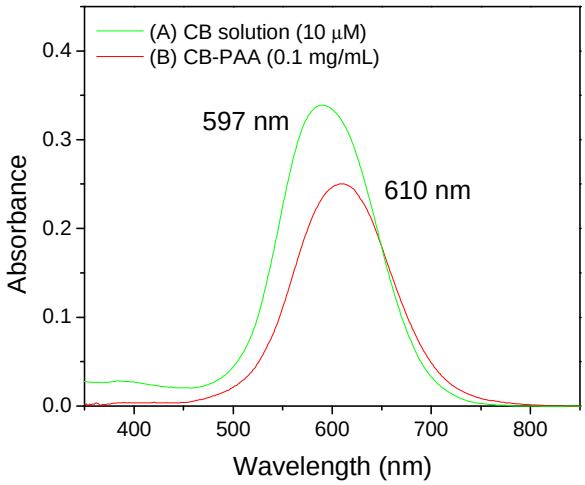
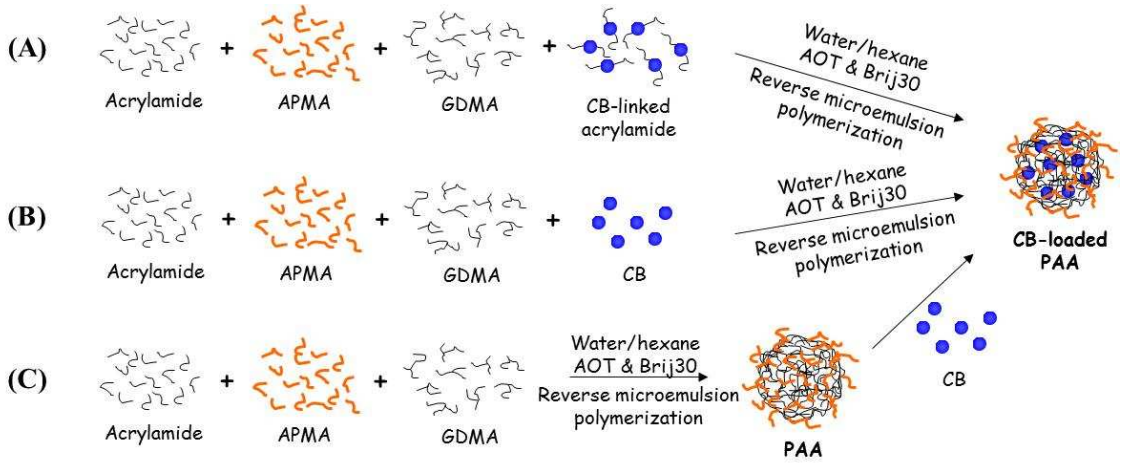
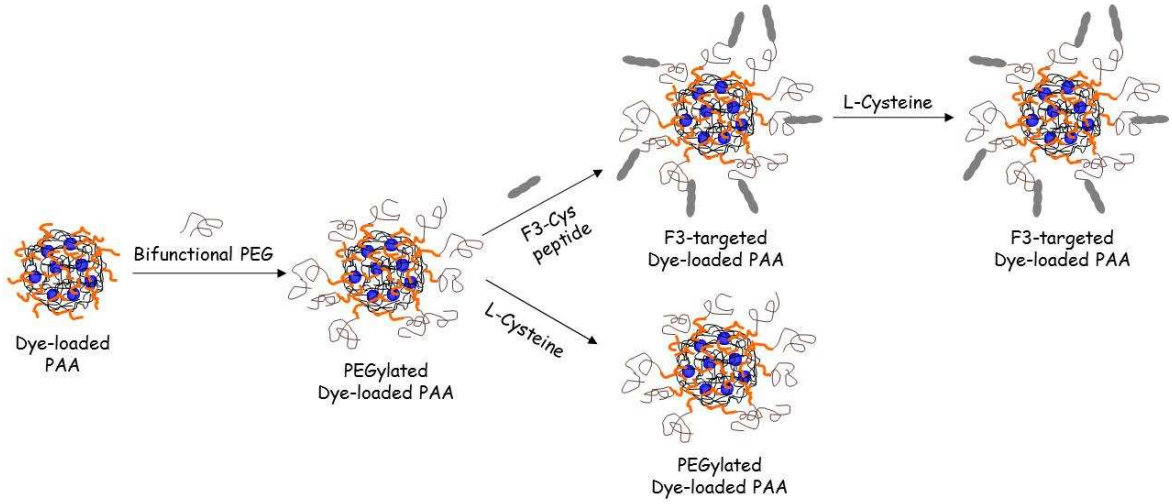


Figure S8. Absorption spectra of (A) CB solution (10 μM) and (B) CB covalently linked PAA NPs (0.1 mg/mL).



Scheme S1. Preparation of loaded PAA NPs by 3 different methods, (A) CB-linked PAA NPs, (B) CB-encapsulated PAA NPs, and (C) CB post-loaded PAA NPs.



Scheme S2. Surface modification and conjugation. First a polymeric cross linker, bifunctional PEG was conjugated to the NP surface by amine-succinimidyl ester reaction. Then, Cys terminated F3 peptide (F3-cys) was conjugated to the terminal of PEG linker by sulfhydryl-maleimidyl ester reaction. L-cys was conjugated instead of F3 peptide as a non-targeted control.