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Supporting Information

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Hydrophobic Functionalization of Poly Acrylic Acid as a Versatile Platform for the Development of Polymer Lipid Nanodiscs.

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Materials and Methods

Poly(acrylic acid) (PAA) average M_w 1800, (catalog 323667 Aldrich), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), diethyl ether (Ether), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), sodium chloride ($NaCl_2$), phosphoric acid (H_3PO_4) and potassium phosphate, were purchased from Sigma-Aldrich®. N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), Pentylamine, Butylamine, Hexylamine, Neopentylamine, Tetrahydrofuran (THF), Hydrochloric acid (HCl), Sodium hydroxide (NaOH) were purchased from Thermo Fisher Scientific®. 1,2- dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was purchased from Avanti Lipids Polar, Inc®. SMA-3000 was a kind gift from Polyscope. Precision Plus Protein™ Prestained Standards, Mini-PROTEAN® TGX™ Precast Protein Gels were obtained from BioRAD.

Synthesis of Akyl-PAA: All polymers were synthesized using the same PAA, Alkylamine, EDC, and solvent ratios. In general PAA (average M_w 1800, 25 acrylic acid groups per chain) was dissolved in THF. 2 eq EDC per acrylic acid group is added to solution and stirred. ddH_2O was added to the stirring solution until all the EDC was dissolved. 0.5 eq of alkyl amine per acrylic acid unit is added to the reaction mixture and the pH is adjusted to ~6.5 if needed. The mixture is diluted with THF and water to yield a final ratio of 70:30 THF:Water solution. The final solution is stirred overnight at room temperature. After completion, THF is removed using rotary evaporation and the cloudy mixture is diluted with an excess 1M NaOH and heated to 80 °C and stirred for 2 hours to hydrolyze any potential anhydrides formed during the reaction. The clear solution is precipitated using 1M HCl, centrifuged, washed 3 times with ddH_2O and lyophilized.

Example: 4 g PAA was dissolved in 160 mL of THF. 22 g EDC was added to the solution followed by 100 mL of ddH_2O and stirred until dissolved. 2.42 g (3.22 mL) of pentylamine was added to the solution and stirred (pH ~6.6). 40 mL of THF was added and the reaction was stirred overnight. The THF was removed using rotary evaporation at 40 °C. The cloudy mixture was diluted with 100 mL 1M NaOH and stirred for 2 hours at 80 °C. The solution was then cooled and precipitated with 1M HCl and centrifuged at 3500 g. The resulting precipitate was suspended in ddH_2O and centrifuged again (repeated 3 times). The final washed product was lyophilized until dry. The overall reaction yielded 4.5 g of final polymer.

Nanodiscs formation: DMPC (10 mg/mL) was suspended in 10 mM potassium phosphate buffer (pH 7.4) to form multilamellar vesicles (MLV). Alkyl-PAA stock solutions of 20 mg/mL were made by dissolving polymer with a minimum amount of 0.1M NaOH and diluting to the necessary concentration using potassium phosphate buffer. The resulting stock solutions where then added together at appropriate weight ratios and diluted to 1 mL using buffer. The resulting solutions were incubated at 35 °C overnight. Free polymer was removed either using SEC or centrifugation filtration using a 10 kDa filter.

^{1}H-NMR spectroscopy: 4 mg of dried polymer was dissolved in 600 µl of 0.1 M NaOD solution in D_2O. NMR spectra were acquired using an Inova 500 MHz NMR spectrometer. 40-168 scans were acquired with a recycle delay of 1s, processed and integrated using VNMRJ software.

Fourier-Transform Infrared (FT-IR) Spectroscopy: The FT-IR spectra from 4000 to 800 cm^{-1} were recorded using a Thermos scientific ATR-FTIR instrument. Lyophilized powder samples of polymers were used to record the spectra.

CP-MAS solid-state NMR experiments: Carbon-13 CPMAS experiments were carried out on a Bruker 500 MHz solid-state NMR spectrometer under 12 kHz MAS using a 3.2 mm triple-resonance MAS probe operating at 500.112 MHz and 125.721 MHz for ^{1}H and ^{13}C, respectively. Sample was loaded into a 3.2 mm zirconium oxide rotor. The reported ^{13}C CP-MAS spectra were acquired using the following experimental parameters: 3 µs 90° pulse, 2 ms CP contact time, 20 ms acquisition time, 3072 number of scans, 3 s recycle delay and a 58 kHz radio-frequency decoupling of protons during the acquisition of ^{13}C magnetization. ^{13}C

chemical shifts were calibrated by setting the chemical shift of CH_2 resonance of adamantane powder sample to 28.5 ppm.

Dynamic Light Scattering (DLS): All of the DLS experiments were performed using Wyatt Technology® DynaPro® NanoStar® using a 1 µL quartz MicroCuvette.

Transmission Electron Microscopy (TEM): All TEM micrographs were obtained using a Technai® T - 20® machine (FEI®, Netherlands) with a 80 kV operating voltage. A dilute solution was dropped on a carbon-coated copper grid and dried overnight at room temperature under vacuum before using in experiments.

Size exclusion chromatography (SEC): Polymer-lipid nanodiscs were purified by size exclusion chromatography (SEC), using self-packed Superdex 200 Increase 300/10 GL column operated on an AKTA purifier (GE Healthcare, Freiburg, Germany). Samples were monitored at 214 nm.

Static Light Scattering (SLS) solubilization experiments: The time dependent solubilization of DMPC MLVs was monitored by the intensity of scattered light at 90° angle. 1 mg/mL DMPC stock solutions were taken in a 4 mL cuvette (1 cm optical path) under continuous stirring at 20°C. Then the solution was equilibrated for 5 min before the addition of the Alkyl-PAA polymers. Excitation wavelength was set at 400 nm while the emission wavelength was set at 404 nm and the slit was set to 2 nm. All SLS experimental measurements were carried out using a FluoroMax-4® Spectrofluorometer from Horiba Scientific®.

SLS metal ion titrations: Nanodiscs stability was tested by titrating a 1 mg/mL solution of Alkyl-PAA:DMPC (1:1 w:w) nanodiscs in 10 mM HEPES buffer at pH = 7.4 with 2M $CaCl_2$ and 2M $MgCl_2$, and 4M NaCl. Results are shown below in Figures S3 and S4.

pH stability measurement using SLS experiments: To study pH stability of PAA based lipid nanodiscs, a solution made by 1 mg/mL Alkyl-PAA:DMPC (1:1 w:w) nanodiscs was titrated both with 1M HCl and 1M NaOH. Results are shown in Figure S5.

DSC experiments: NanoDSC (TA instruments) was used to determine the phase transition

temperature of the PAA based lipid nanodiscs. Nanodiscs stock solutions were made according to above procedure with an additional 3 times of freeze thaw cycles; free polymer was removed using centrifugation filtration using a 10 kDa filter and diluting with 10 mM potassium phosphate buffer. The stock solutions were then centrifuged at 11,000 g and filtered through a 0.2 micron filter. The samples were vacuum degassed before loading. 300 µl of sample was loaded into the sample cell and 10 mM potassium phosphate buffer was loaded into the reference cell. The temperature was cycled from 5 to 60 °C three times.

Phosphorous-31 NMR: Spectra were acquired using an Agilent/Varian 400 MHz solid-state NMR spectrometer and a 5 mm triple-resonance probe with ^{31}P and ^{1}H resonance frequencies of 161.974 MHz and 400.114 MHz, respectively. 200 µl of sample was inserted using a 3.2 mm glass tube. 5µs 90° pulse, 25 kHz ^{1}H continuous-wave decoupling, 1024 scans, and a 2 s recycle delay were used to acquire ^{31}P NMR spectra. ^{31}P chemical shifts were referenced by setting the ^{31}P chemical shift of 100 % H_3PO_4 sample to 0 ppm.

^{14}N-NMR Experiments Nitrogen-14 NMR spectra were acquired using an Agilent/Varian 400 MHz solid-state NMR spectrometer and a 5 mm double-resonance probe operating at the ^{14}N resonance frequency of 29.910 MHz. ^{14}N-NMR spectra were recorded using the quadrupole-echo pulse sequence with a 90° pulse length of 8 µs and an echo-delay of 80 µs. ^{14}N magnetization was acquired using 28.9 ms acquisition time (without ^{1}H decoupling), 20,000 scans and a recycle delay of 0.9 s.

Solubilization of native E. coli membranes: E. coli BL21(DE3) was obtained as described previously (Prade et al. *Angew. Chem. Int. Ed.* **2018**, *57*, 8458.). Cell pellets were re suspended in a 10-fold volume of ice-cold buffer (50 mM potassium phosphate buffer, 2 mM EDTA, 200 mM NaCl, pH 7.4) and subjected to ultrasonication. The cell lysate was subjected centrifugation for 1 h at 4 °C and 20,000 g and washed three times with buffer to remove soluble proteins. Membrane pellets were re-suspended in 50 mM potassium phosphate buffer, 200 mM NaCl, pH 7.4 to a final concentration of 50 mg/mL. Solubilization of the pellet was conducted by treating 500 µl of cell lysate with 250 µl of polymer solution (50 mg/mL). The samples were incubated overnight at room temperature while shaking. The resulting solutions were centrifuged at 11,000 g for 30 min. The supernatant was separated and analyzed using SDS PAGE gel. To remove the polymer and lipids, proteins were precipitated from polymer-containing samples with $CH_3OH/CHCl_3/H_2O$. 100 µL aliquot of ice-cold supernatant was

mixed with 400 µL ice-cold methanol, then 100 µL ice-cold chloroform was added, and the sample was mixed. 300 µL ice-cold water was then added and the sample was thoroughly mixed and centrifuged at 2,000 g for 5 mins and 11,000 g for 10 mins at 4 °C. The top aqueous layer was carefully removed and 400 µL of ice cold methanol was added and mixed thoroughly. The resulting precipitate was pelleted at 2,000 g for 5 mins and 11,000 g for 10 mins at 4 °C. The resulting pellet was washed two times with 800 µL of ice cold methanol. Resulting pellet was dried under N_2 for 10 minutes and then under high vacuum for 3 hrs. The dry pellet was suspended in SDS buffer, heated to 100 °C for 10 mins with shaking and subjected to a SDS-PAGE

SDS-PAGE: Samples from above were loaded onto a precast gel. A constant voltage of 170 V was applied for 40 min at 50 W. Gels were fixed in 10% (v/v) acetic acid and 40% (v/v) ethanol, stained with 0.025% (w/v) Coomassie brilliant blue in 10% (v/v) acetic acid, and de-stained with 10% (v/v) acetic acid. Finally, gels were photographed using slandered digital camera.

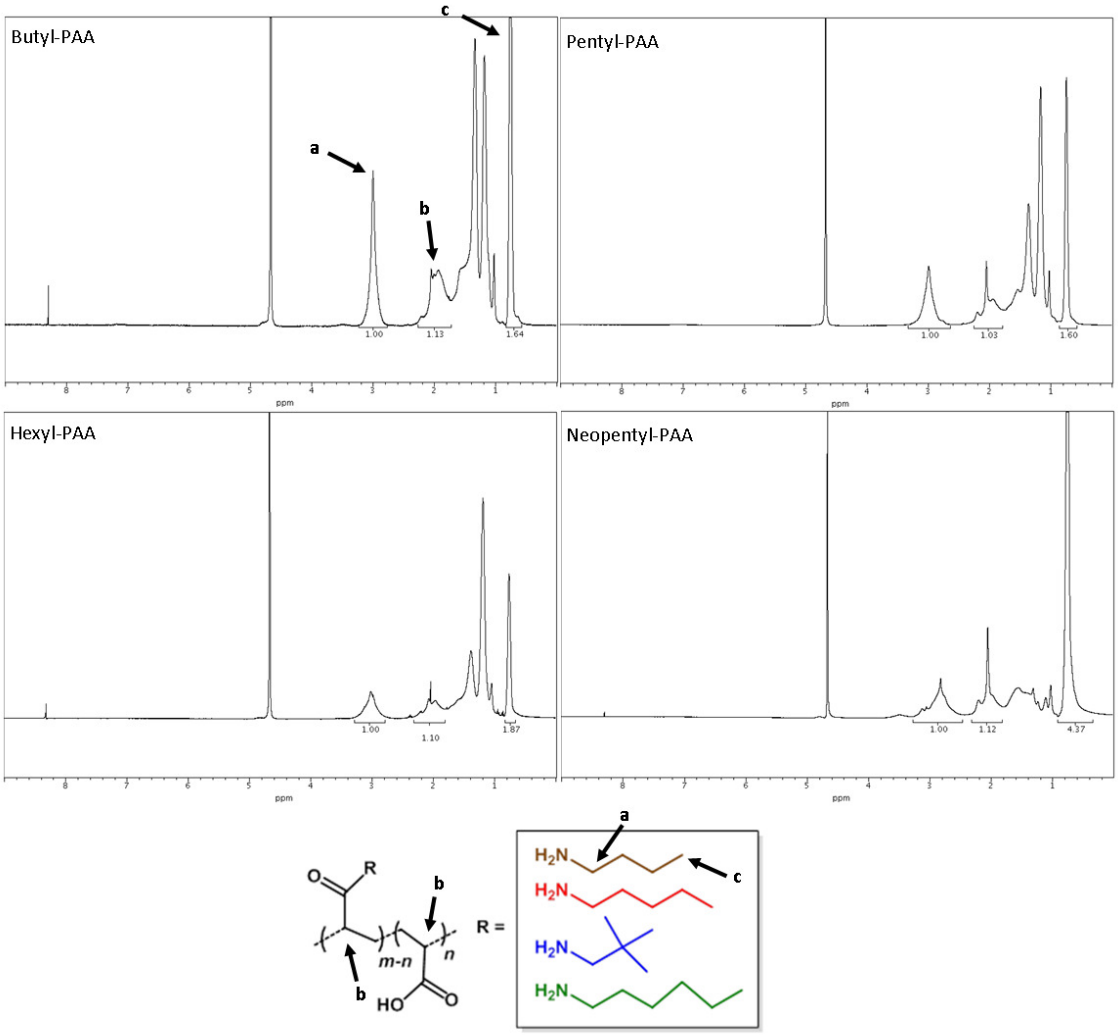


Figure S1. ^{1}H NMR spectra of alkyl-PAA polymers. Peaks labeled with a, b and c belong to alpha-CH_2, CH of the polymer backbone and CH_3 of the alkyl chain, respectively. Peak integration was done by setting the area of alpha CH_2 peak to 1.0.

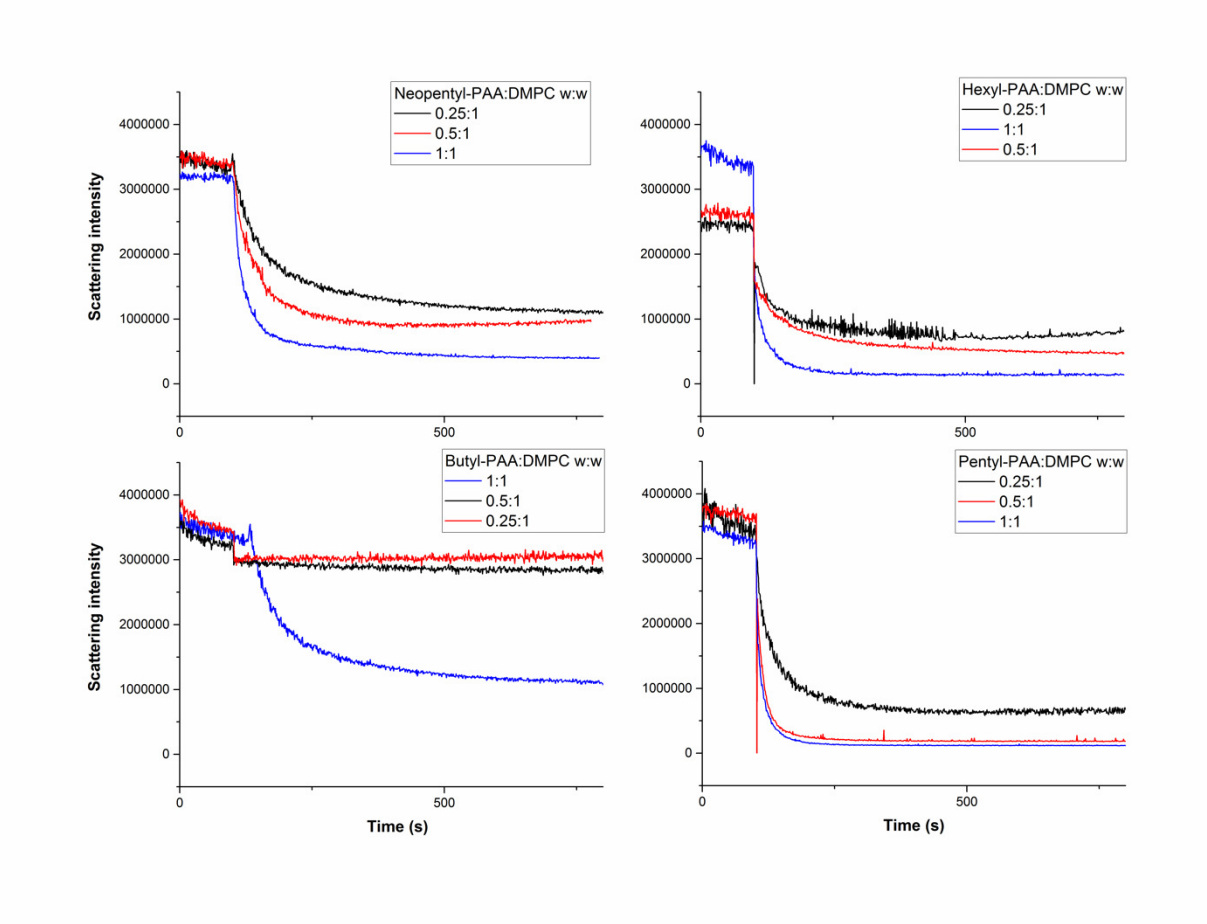


Figure S2. SLS profiles of nanodisc formation by the dissolution of DMPC multilamellar vesicles after the addition of alkyl-PAA polymers.

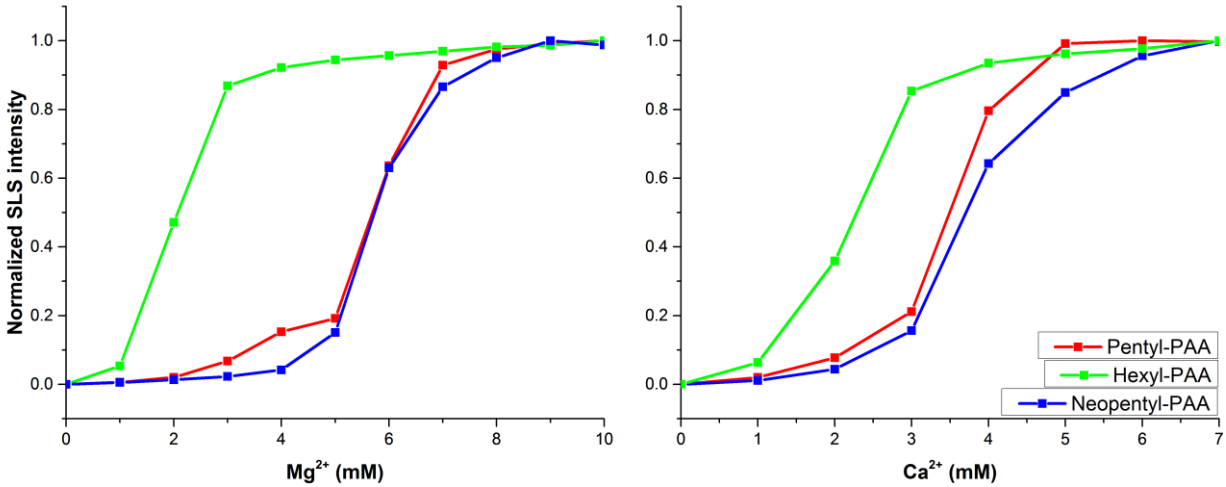


Figure S3. SLS profiles showing the stability of alkyl-PAA polymer DMPC-nanodiscs as a function of divalent metal ion concentration.

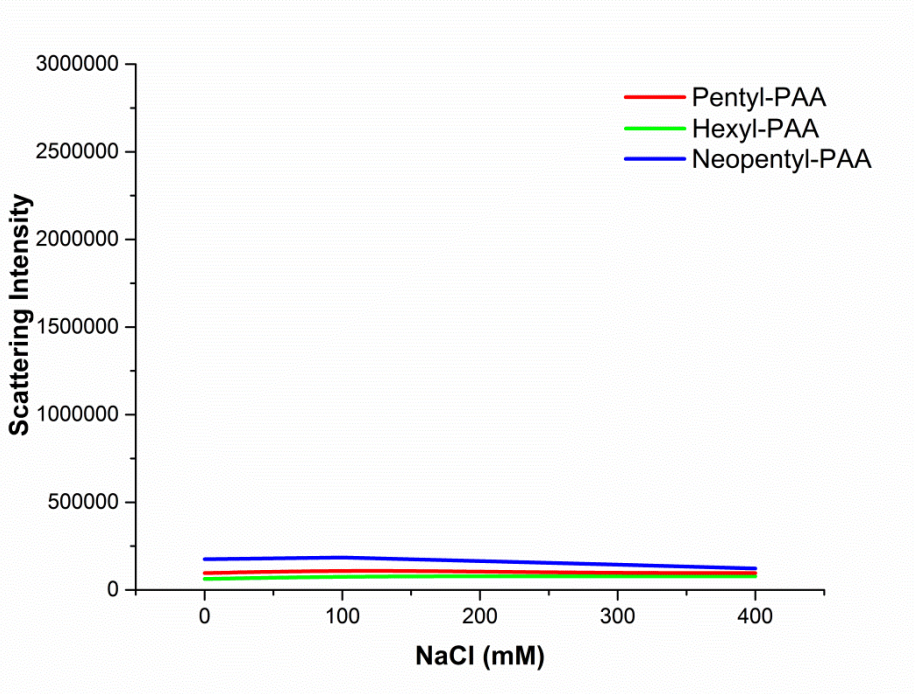


Figure S4. SLS profiles showing the stability of alkyl-PAA polymer DMPC-nanodiscs as a function of NaCl concentration.

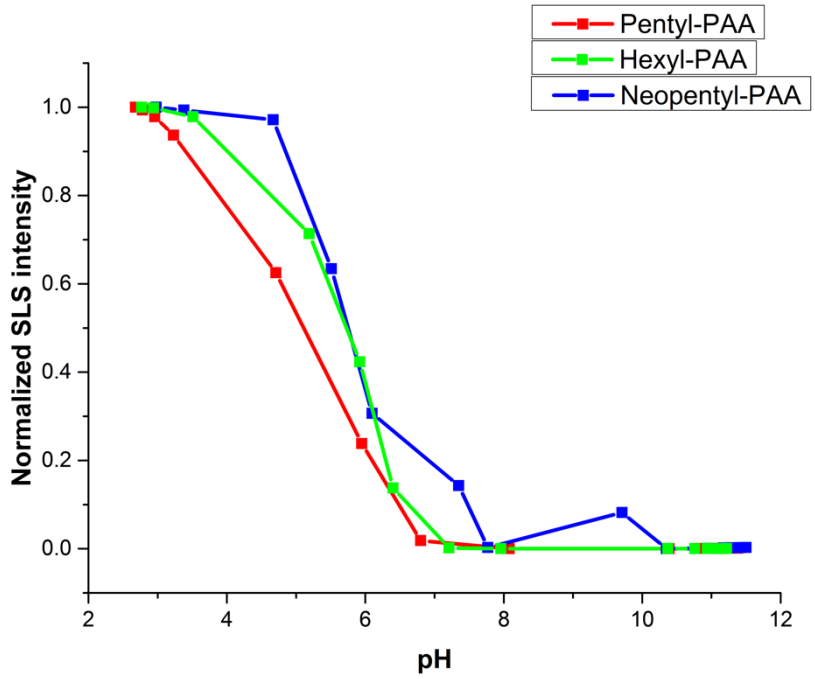


Figure S5. SLS profiles showing the stability of alkyl-PAA polymer DMPC-nanodiscs as a function of pH.

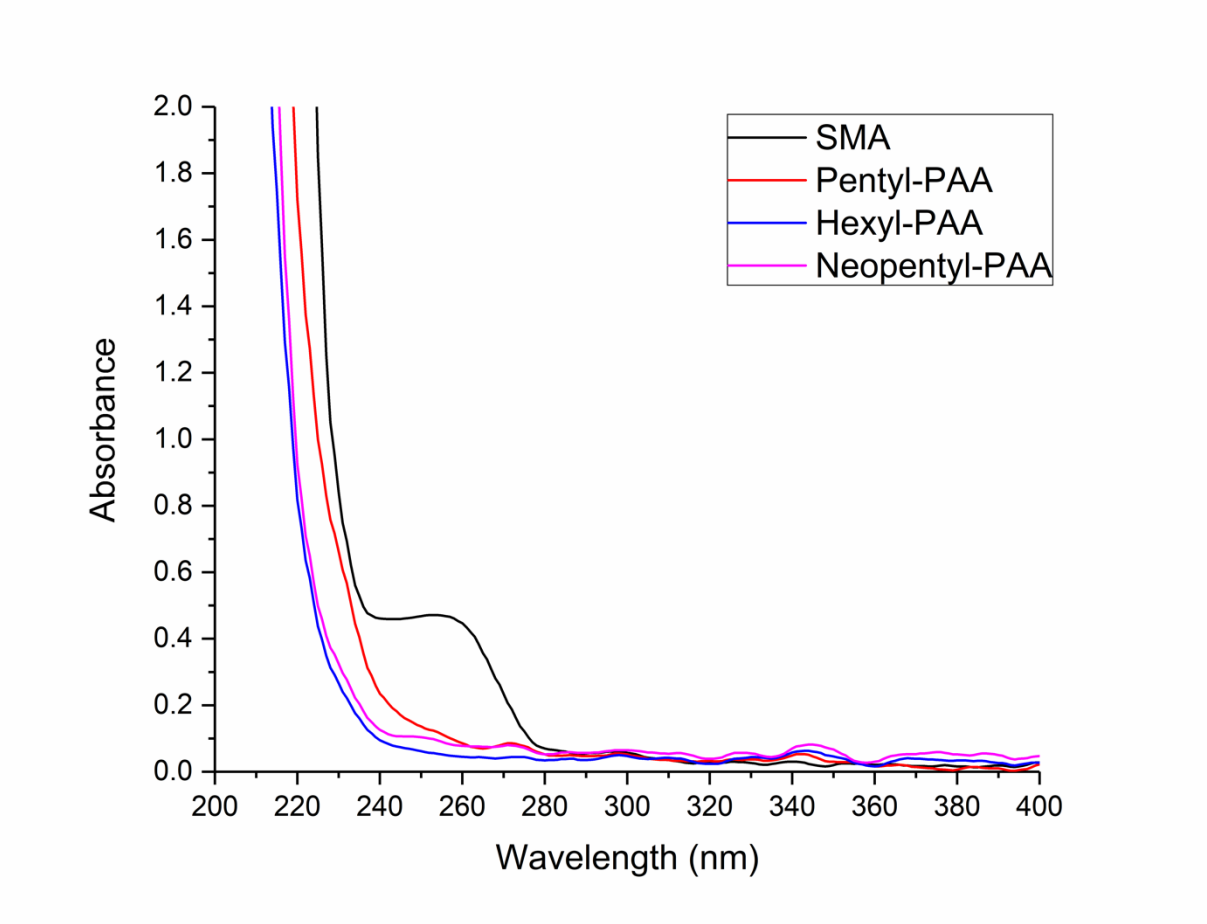
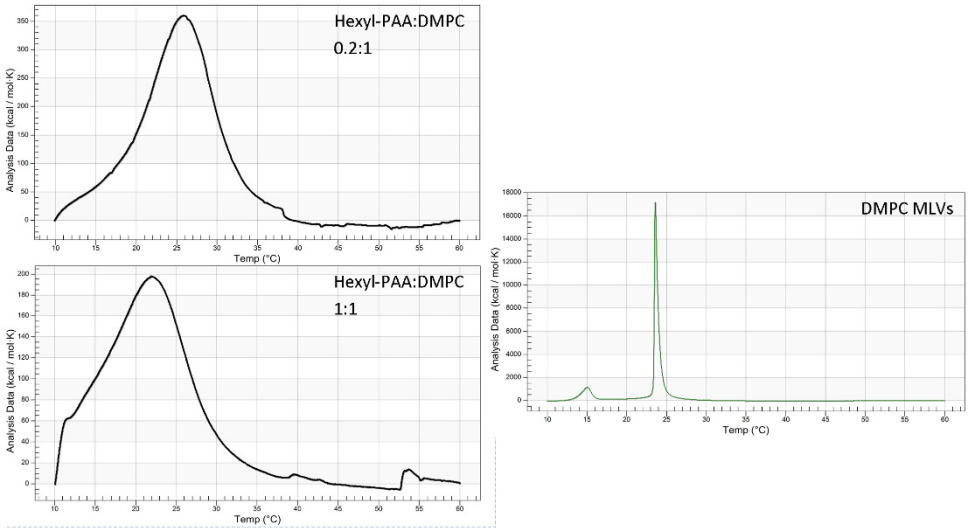


Figure S6. UV absorption spectra of alkyl-PAA and SMA polymers.



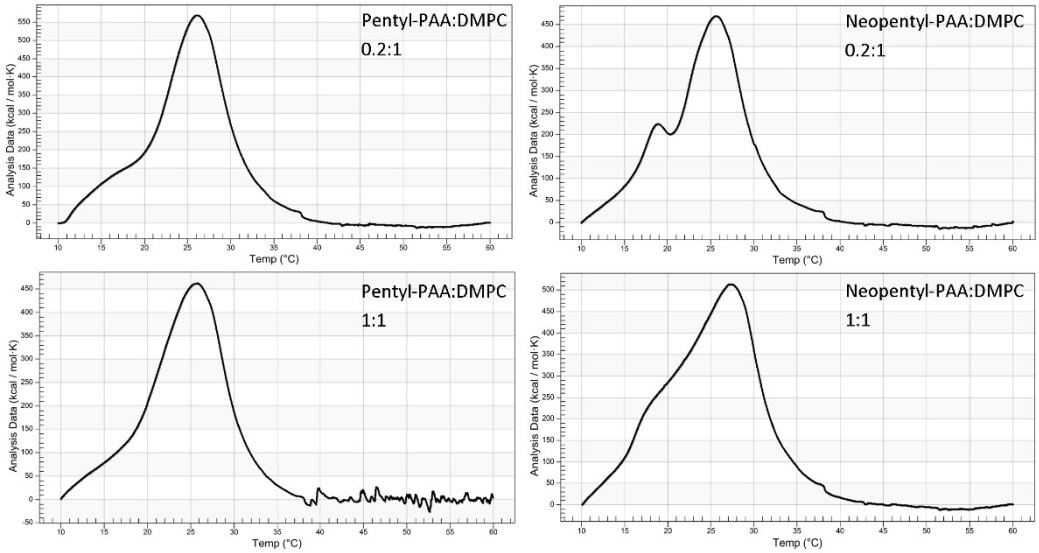


Figure S7. Gel-to-liquidcrystalline phase transition profiles obtained from differential scanning calorimetry (DSC) experiments on alkyl-PAA polymer DMPC nanodiscs for two different w:w ratios as indicated.

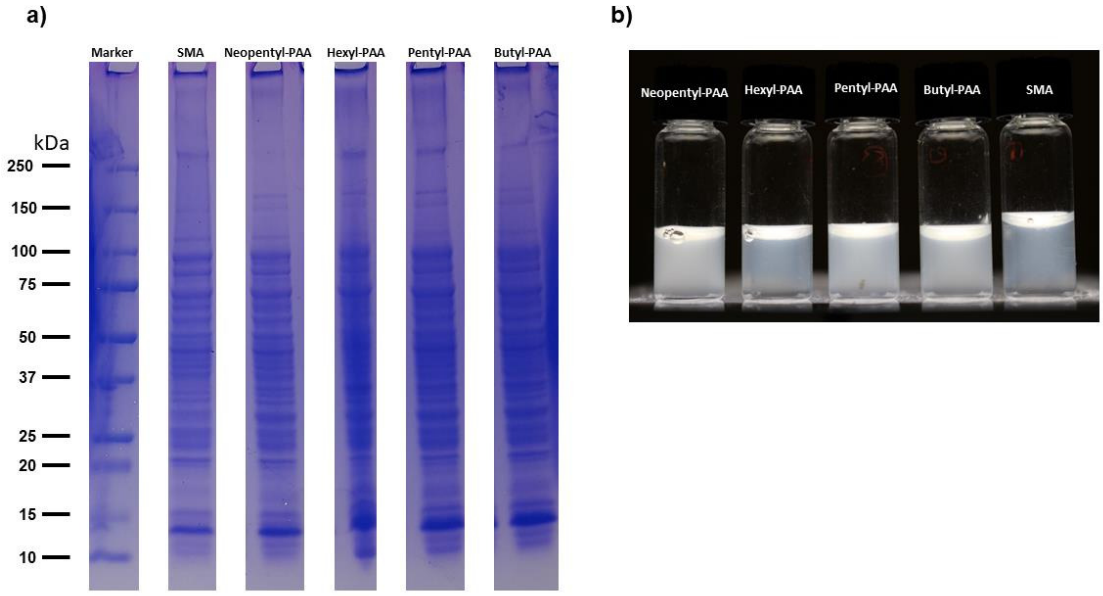


Figure S8. **Extraction of membrane proteins directly from E.coli lysate by polymers.** a) SDS-page gel showing membrane proteins extracted using different polymers. b) Photograph of the vials showing the cell lysate incubated with different polymers. The near-clear solution of a mixture of Hexyl-PAA and cell lysate indicates its excellent solubilizing potency, which is in good agreement with the least amount of polymer required to form nanodiscs.