Synthesis and Evaluation of a Library of Alternating Amphipathic Copolymers to Solubilize and Study Membrane Proteins

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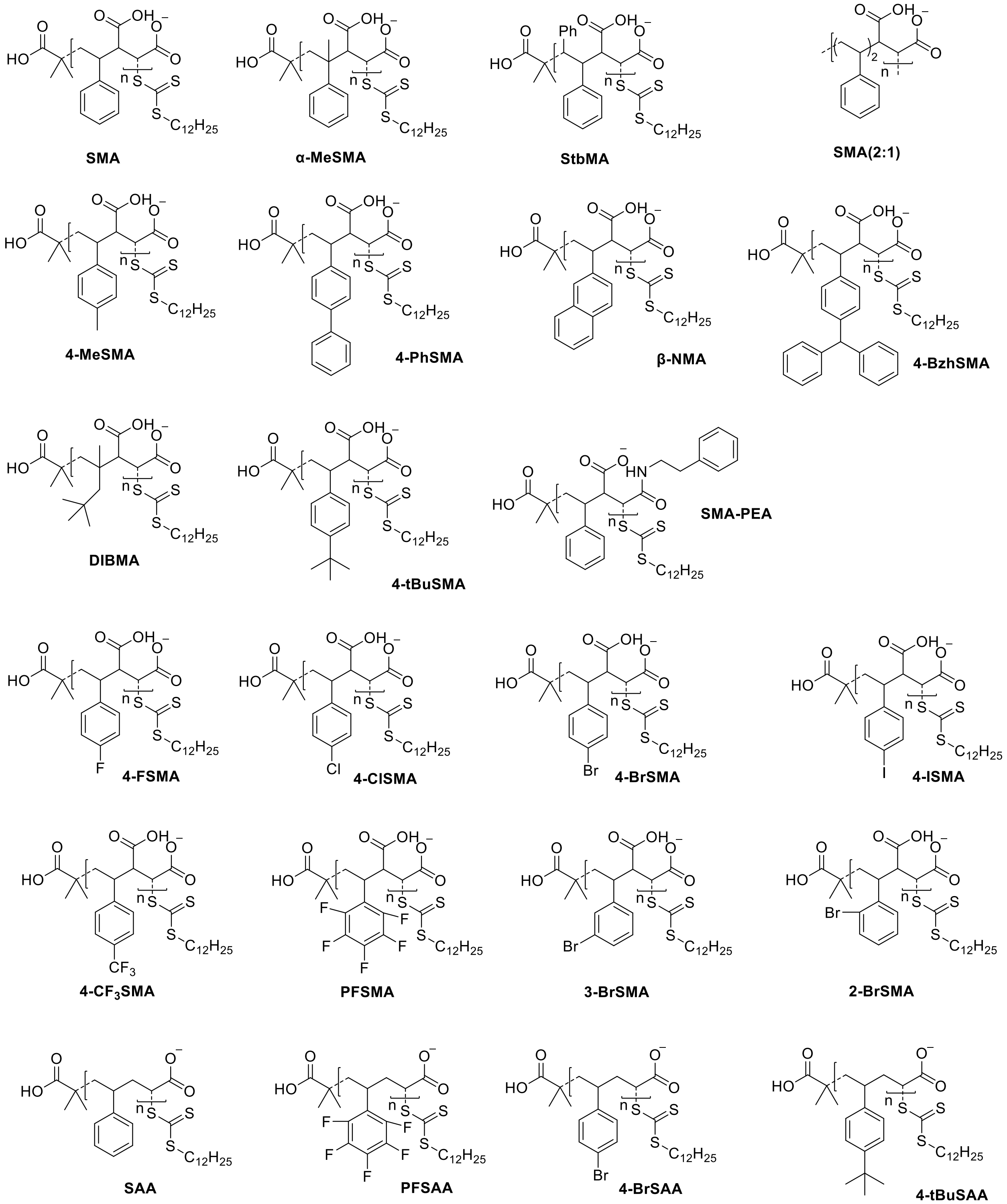
Supporting Information



SMA **α-MeSMA** **StbMA** **SMA(2:1)**

4-MeSMA **4-PhSMA** **β-NMA** **4-BzhSMA**

DIBMA **4-tBuSMA** **SMA-PEA**

4-FSMA **4-ClSMA** **4-BrSMA** **4-ISMA**

4-CF_3SMA **PFSMA** **3-BrSMA** **2-BrSMA**

SAA **PFSAA** **4-BrSAA** **4-tBuSAA**

Fig. S1. Full chemical structures of the library of RAFT(DDMAT)-synthesized alternating amphiphilic copolymers.

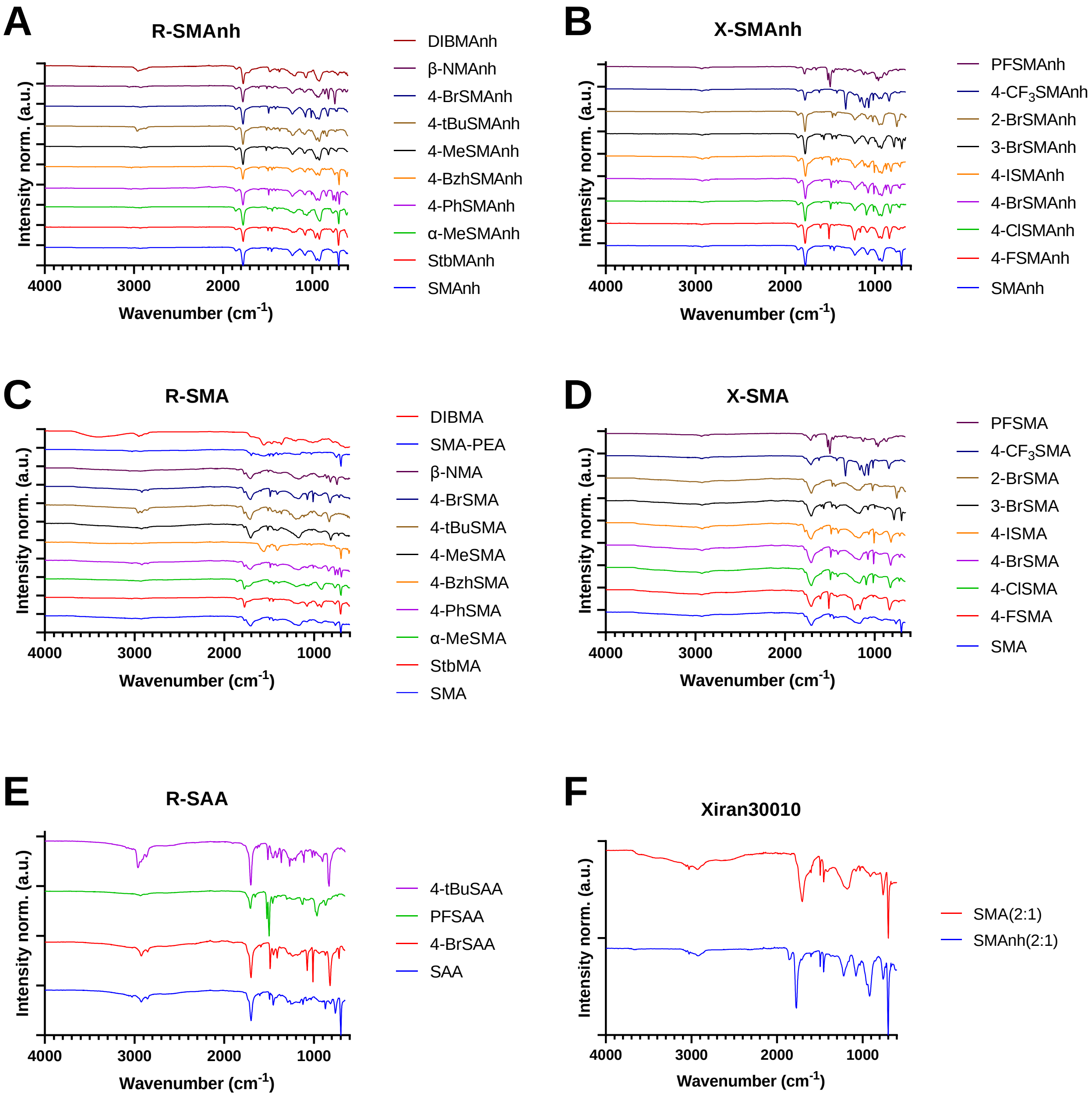


*Fig. S2. Fourier-transform infrared spectras of synthesized copolymers for the different classes: (**A**) R-SMAnh, (**B**) X-SMAnh, (**C**) R-SMA, (**D**) X-SMA, (**E**) R-SAA, and (**F**) control (SMA 2:1, Xiran30010). Maleic anhydride units are characterized by a strong absorbance band at ~1775 and ~1855 cm^{-1} (C=O anhydride stretching), whereas the hydrolysed copolymers with carboxylic acid groups have a characteristic absorbance band at ~1705 cm^{-1} (C=O acid stretching). Styrene subunits can be observed by strong peak(s) in the range of 700 – 900 cm^{-1} (C-H bending, exact position(s) depending on the number and nature of the substitutions on the aromatic ring).*

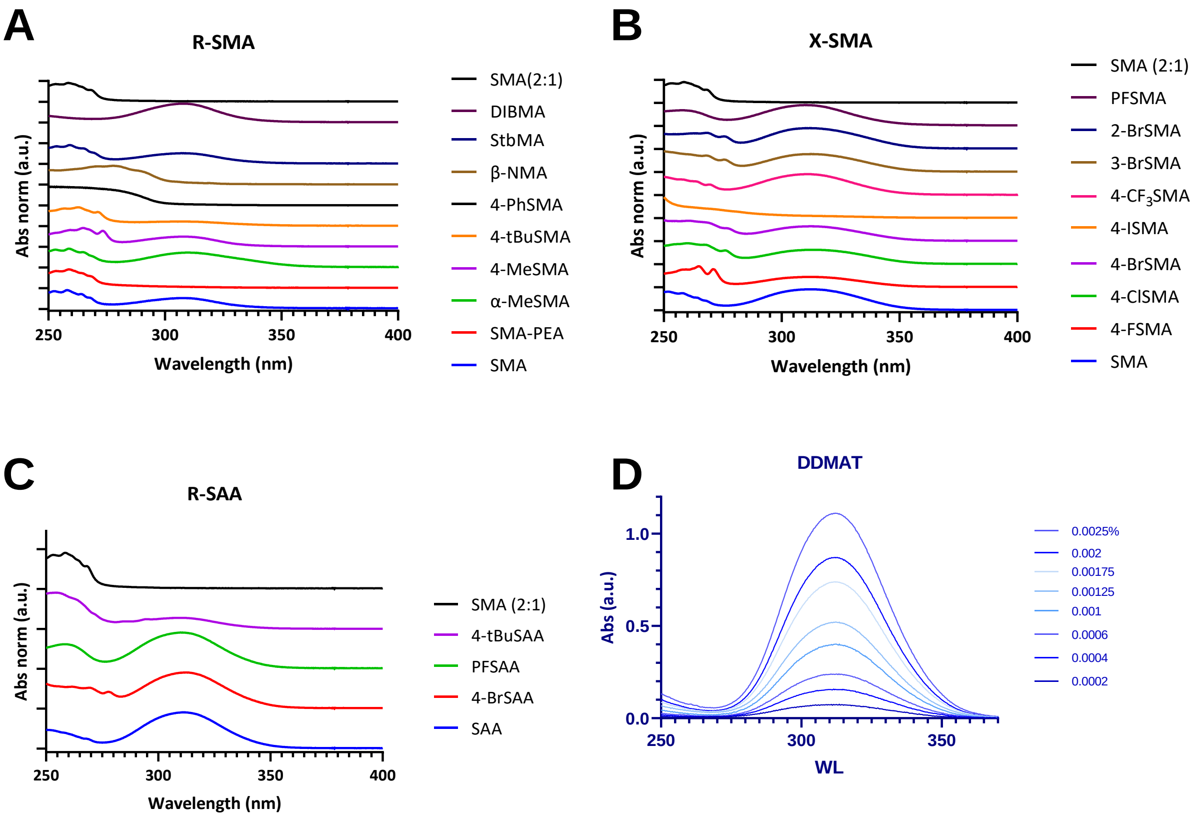


*Fig. S3. UV-vis wavelength scans of synthesized copolymers for the different sets: (**A**) R-SMA, (**B**) X-SMA, (**C**) R-SAA, and (**D**) DDMAT RAFT agent. Copolymers were measured in water at a concentration of 0.025% (w/v), DDMAT was dissolved in water at varying concentrations ranging from 0% - 0.0025% (w/v). The trithiocarbonate RAFT-end groups (DDMAT) have an absorbance peak at ~310 nm and the aromatic styrene analogues absorb in the range of 250 – 290 nm. The relative intensity at 310 nm can be used to infer information on the copolymer length. Furthermore, based on these results turbidimetry experiments were performed at 430 nm to avoid interference from the copolymer RAFT-end groups.*

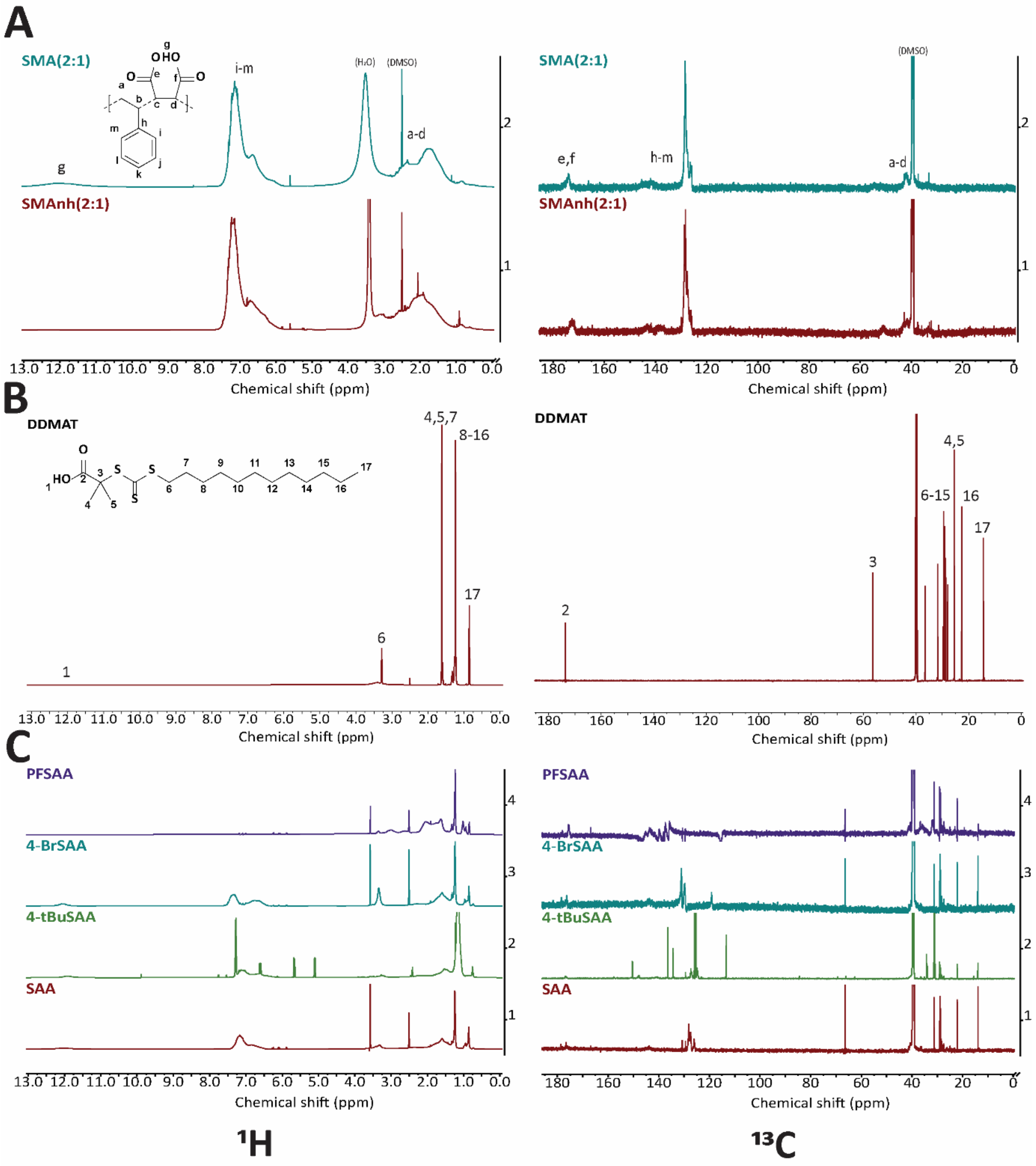


*Fig. S4. ^{1}H (left) and ^{13}C (right) NMR analysis of (**A**) SMA(2:1) copolymer in hydrolyzed form (top) and anhydride form (bottom), (**B**) DDMAT (RAFT agent), and (**C**) R-SAA copolymers. Artifacts from NMR solvent include a quintet at 2.5 ppm (DMSO-d_6) and a broad singlet at ~3.33 ppm (H_2O) for ^{1}H NMR spectra. In the ^{13}C NMR spectra DMSO-d_6 is found as a septet at 39.52 ppm and the reference standard TMS is found as a singlet at 0.0 ppm for both proton and carbon spectra. Impurities include residual solvent (dioxane) from the polymerization reaction as a singlet at 3.57 ppm (^{1}H NMR) and a singlet at 66.36 ppm (^{13}C NMR), as well as unreacted monomer as evident in the region 5–6 ppm (^{1}H NMR). The relative purity of the copolymers is estimated to be at least >95%, based on relative quantification of unreacted comonomer for the visibly most contaminated polymer (4-tBuSAA). Copolymeric material demonstrates characteristic broad peaks in both proton as well as carbon spectra. Assignment: acid group at 11-13 ppm (`$^1$H) and 170-180 ppm ($^{13}$C), aromatic units at 6-8 ppm (`^{1}H) and 110-160 ppm (^{13}C), aliphatic units at 1-4 ppm (`$^1$H) and 20-60 ppm ($^{13}$C), with the DDMAT terminal methyl ($-CH_3$) end group specifically at ~0.85 ppm (`^{1}H) and ~14 ppm (^{13}C). See also Fig. S5 – S8.*

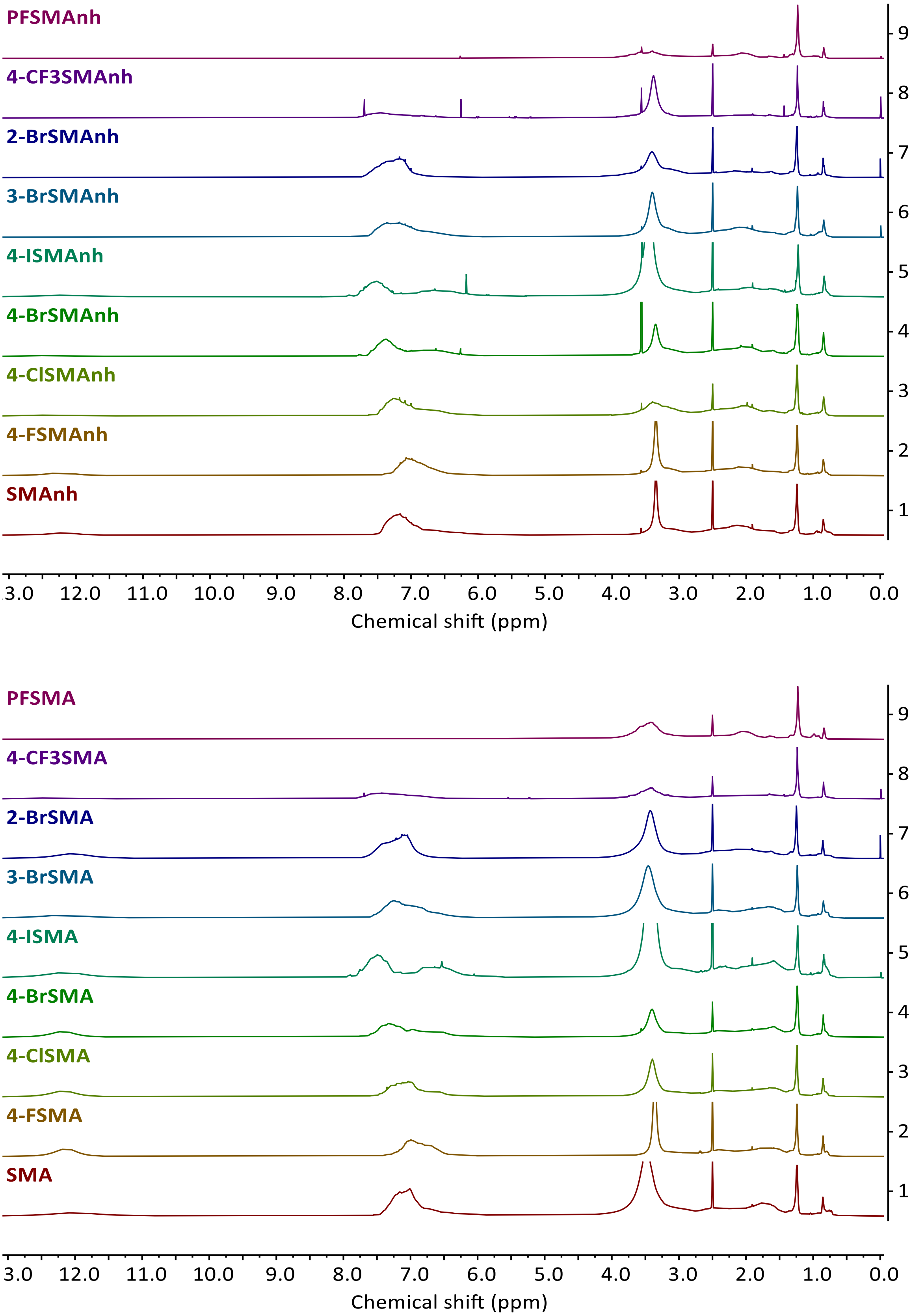
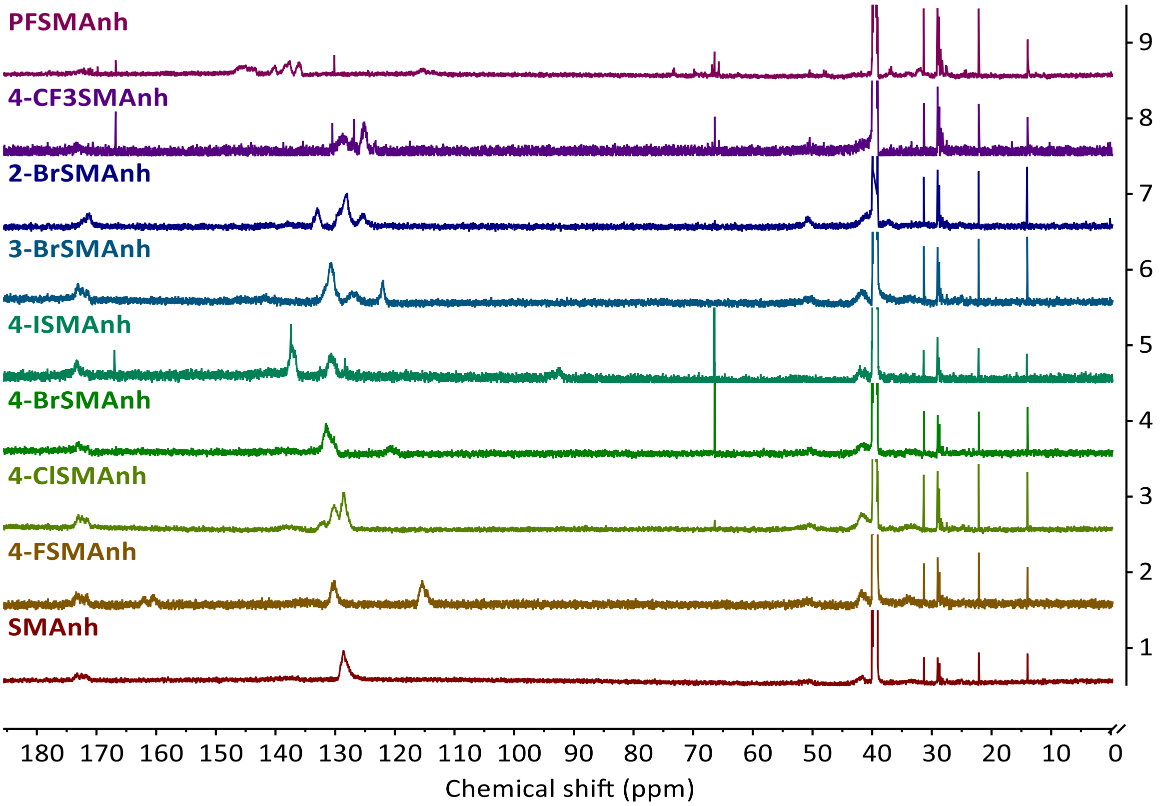


Fig. S5. ^{1}H NMR spectra of X-SMAnh (top) and hydrolyzed X-SMA (bottom) copolymers.



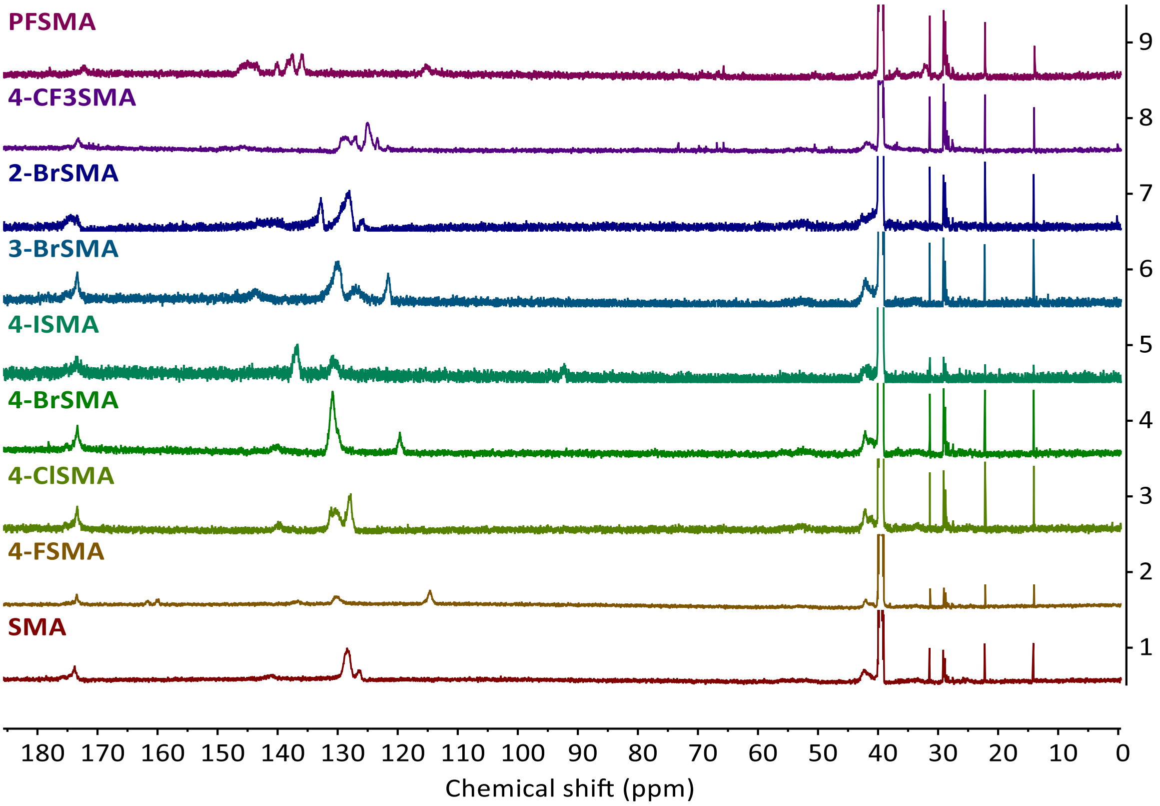


Fig. S6. ^{13}C NMR spectra of X-SMAnh (top) and hydrolyzed X-SMA (bottom) copolymers.

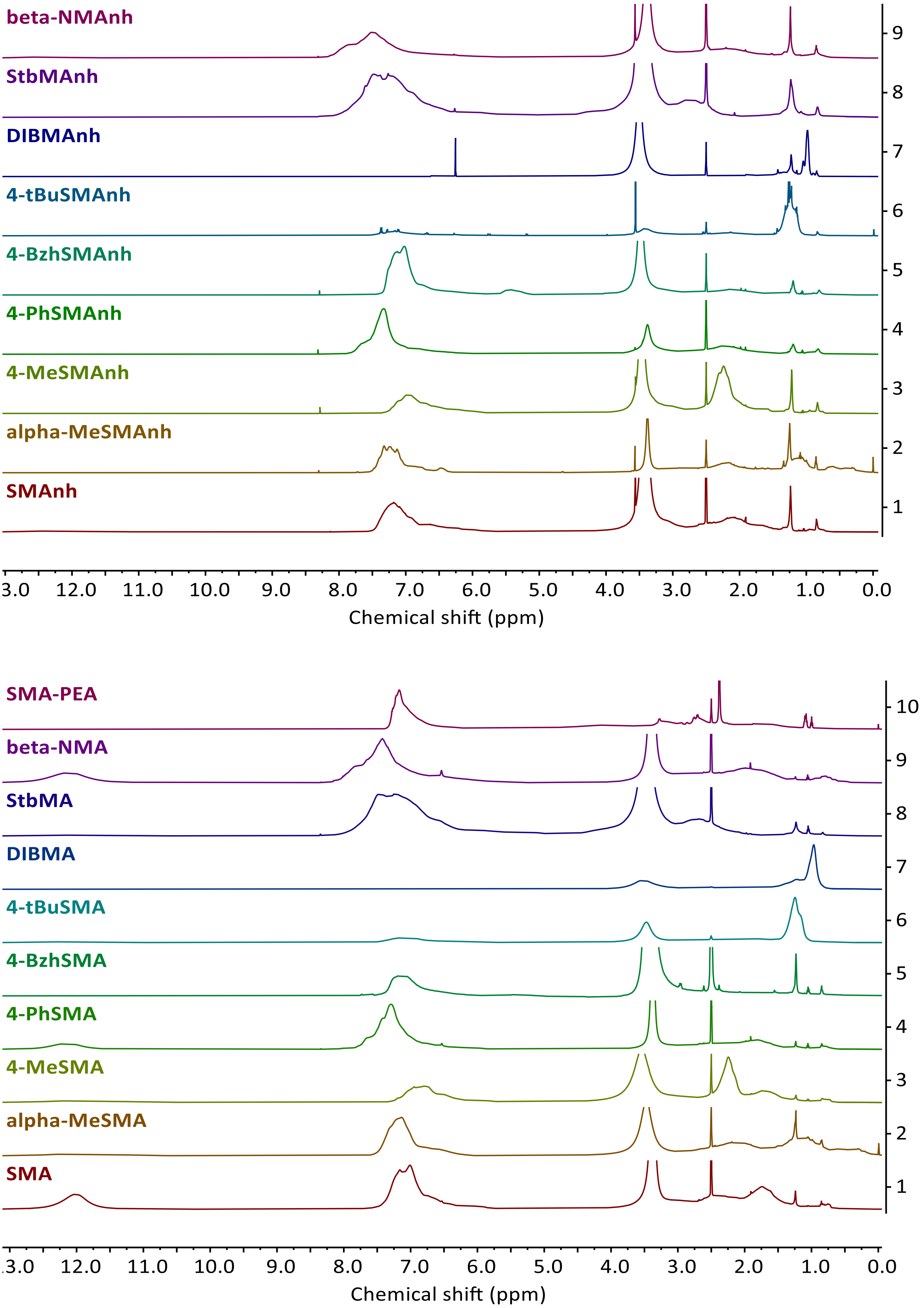
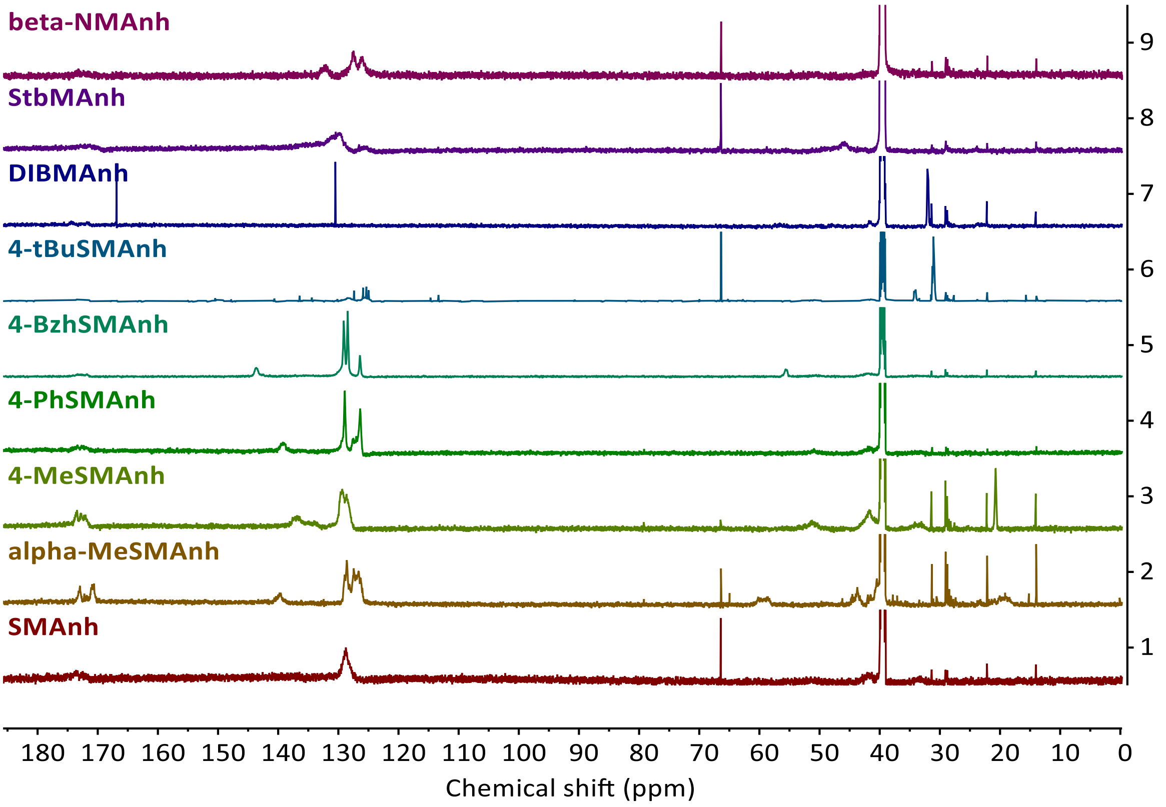


Fig. S7. ^{1}H NMR spectra of R-SMAnh (top) and hydrolyzed R-SMA (bottom) copolymers.



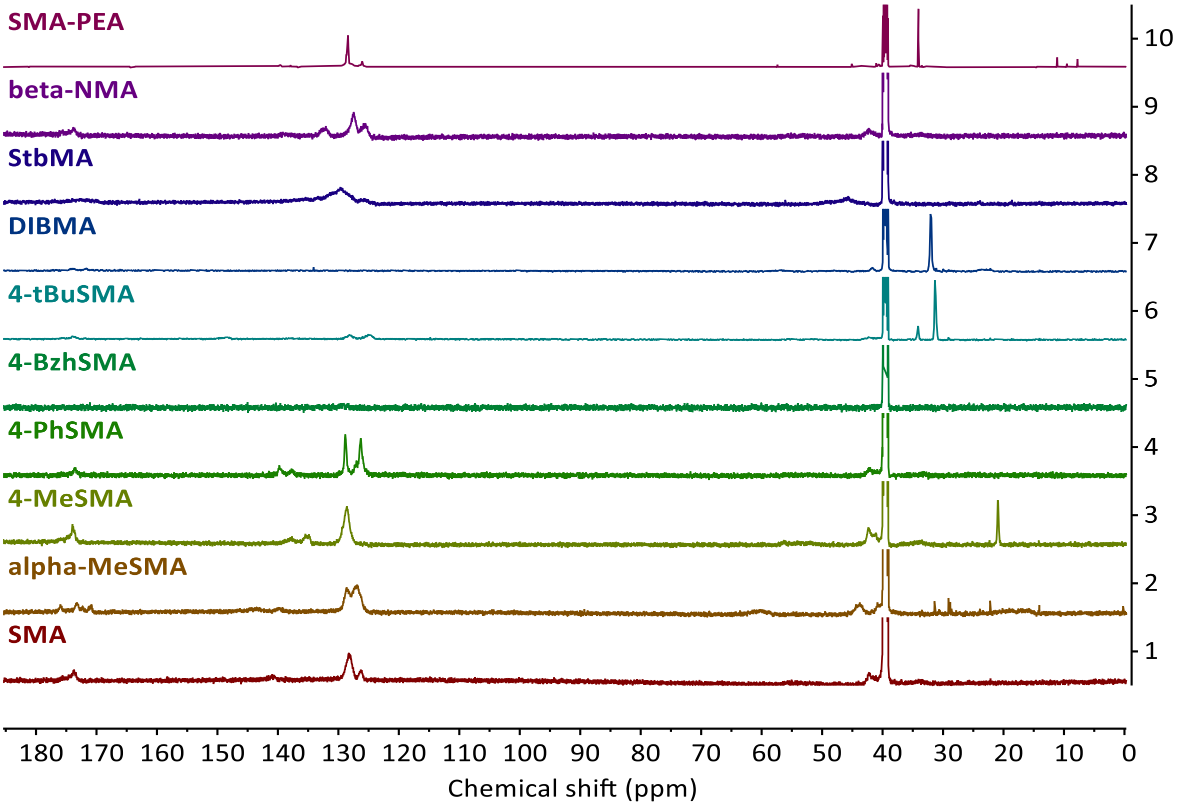


Fig. S8. ^{13}C NMR spectra of R-SMAnh (top) and hydrolyzed R-SMA (bottom) copolymers.

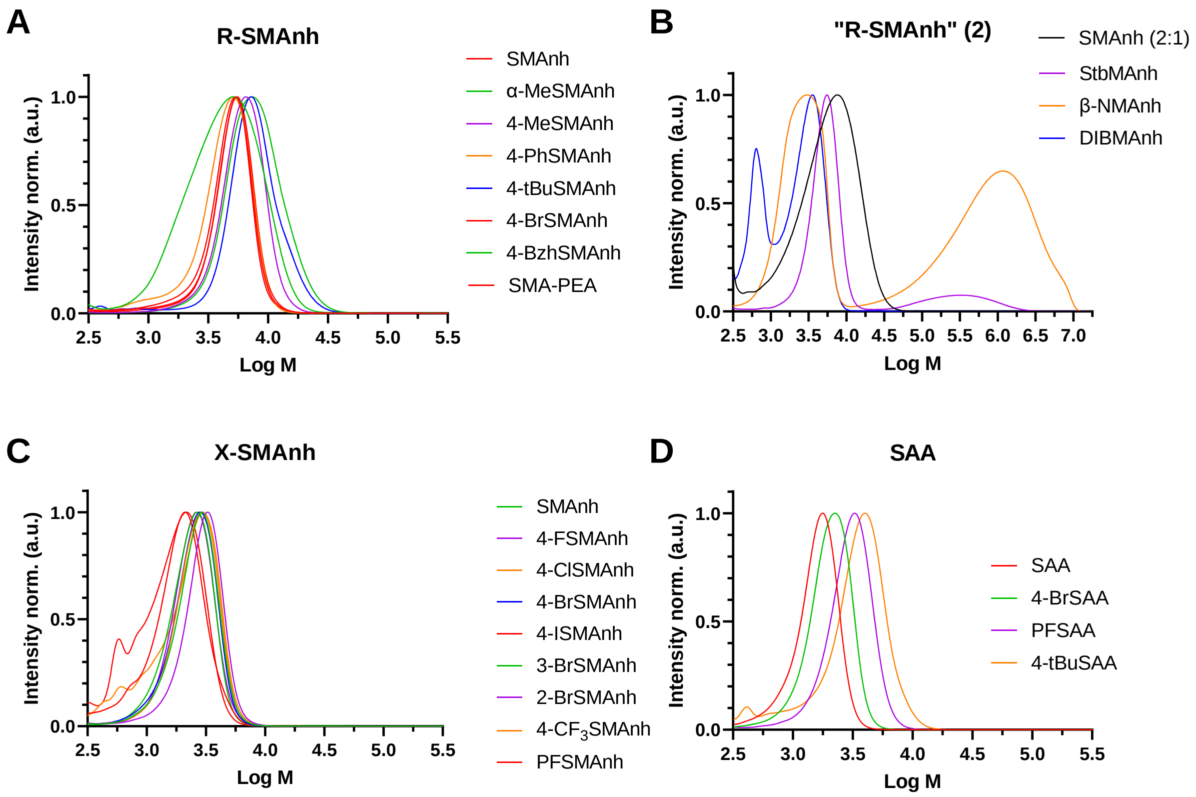


*Fig. S9. Gel permeation chromatography (GPC) traces of synthesized copolymers for the different sets: (**A**) R-SMAnh, (**B**) "R-SMAnh" (2), (**C**) X-SMAnh, and (**D**) R-SAA. Sizes were determined based on polystyrene standards in the range of 0.3 – 300 kDa. Bimodal size distributions can be observed in (**B**) for StbMAnh, β-NMAnh, and DIBMAnh.*

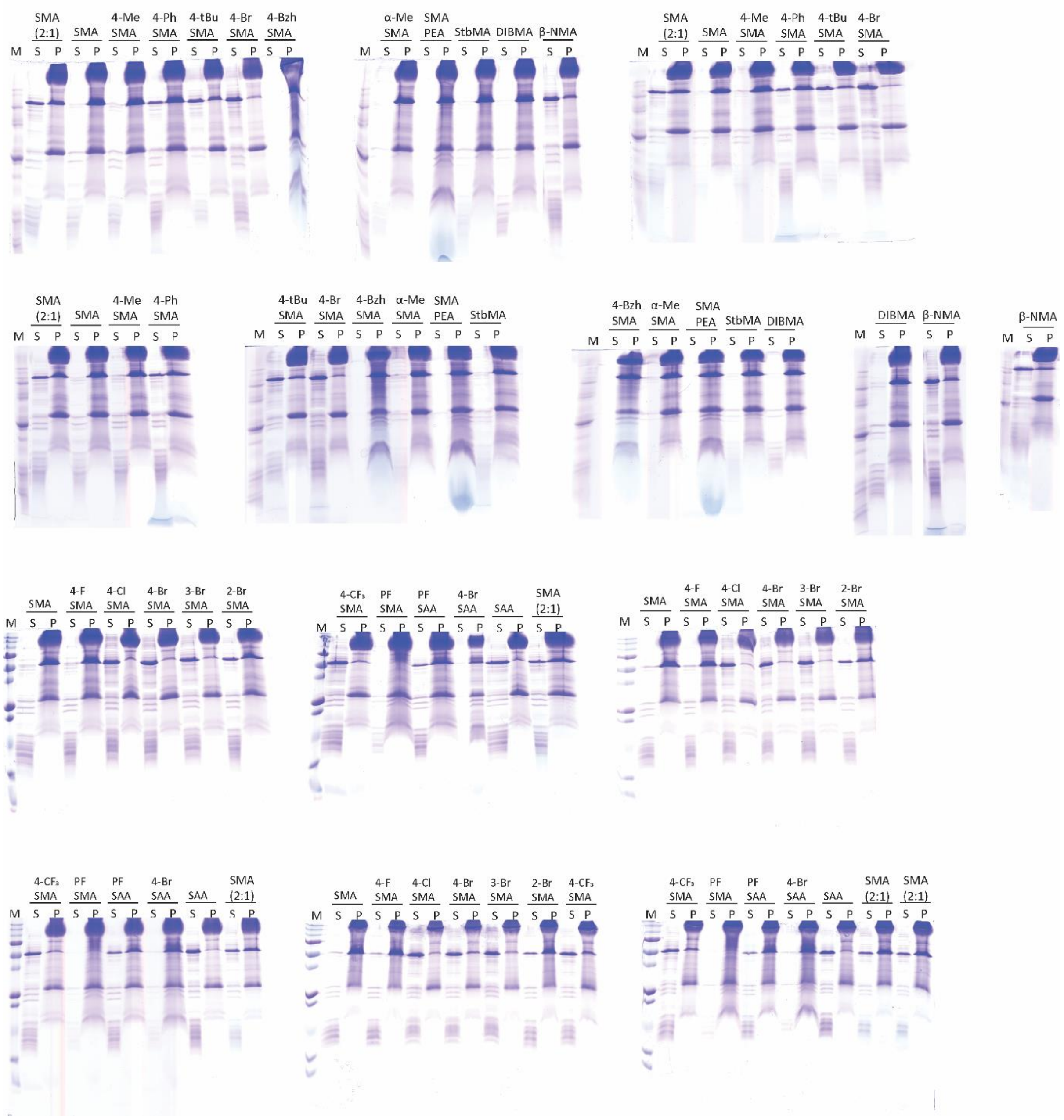


Fig. S10. SDS-PAGE analysis of KcsA solubilization from E.coli membranes. Solubilization by various copolymers with a polymer concentration of 0.25% (w/v) (polymer-to-lipid ratio of ~2.3:1 (w/w)), for 2 hours at 25°C. Gels were prepared using 13% acrylamide and stained with Coomassie. M, molecular weight marker; S, supernatant; P, pellet.

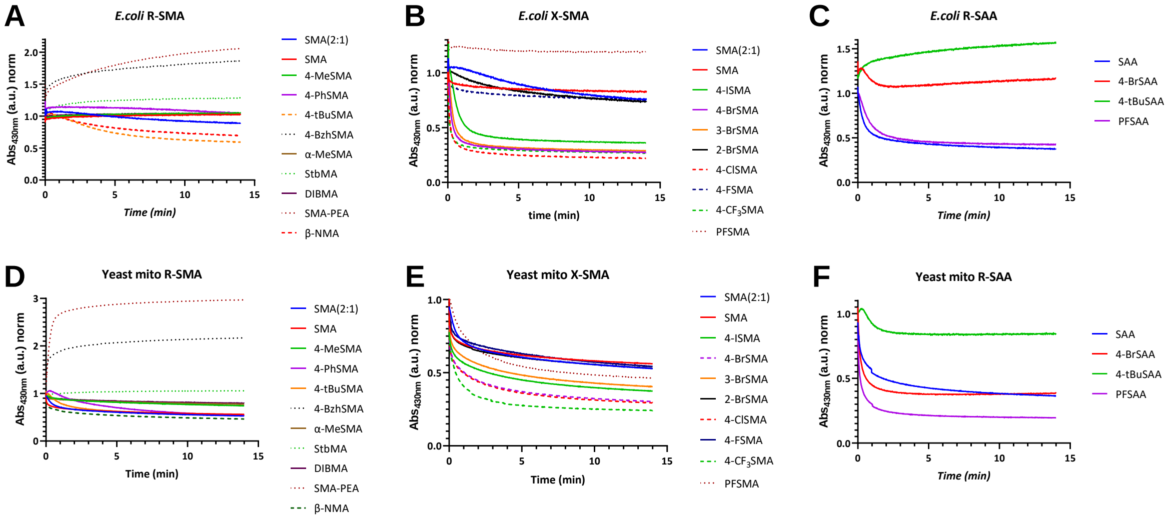


*Fig. S11. E.coli (top) and yeast mitochondria (bottom) membrane solubilization as based on turbidimetry for the three copolymer sets, R-SMA, (**A, D**) X-SMA, (**B, E**), and R-SAA (**C, F**).*

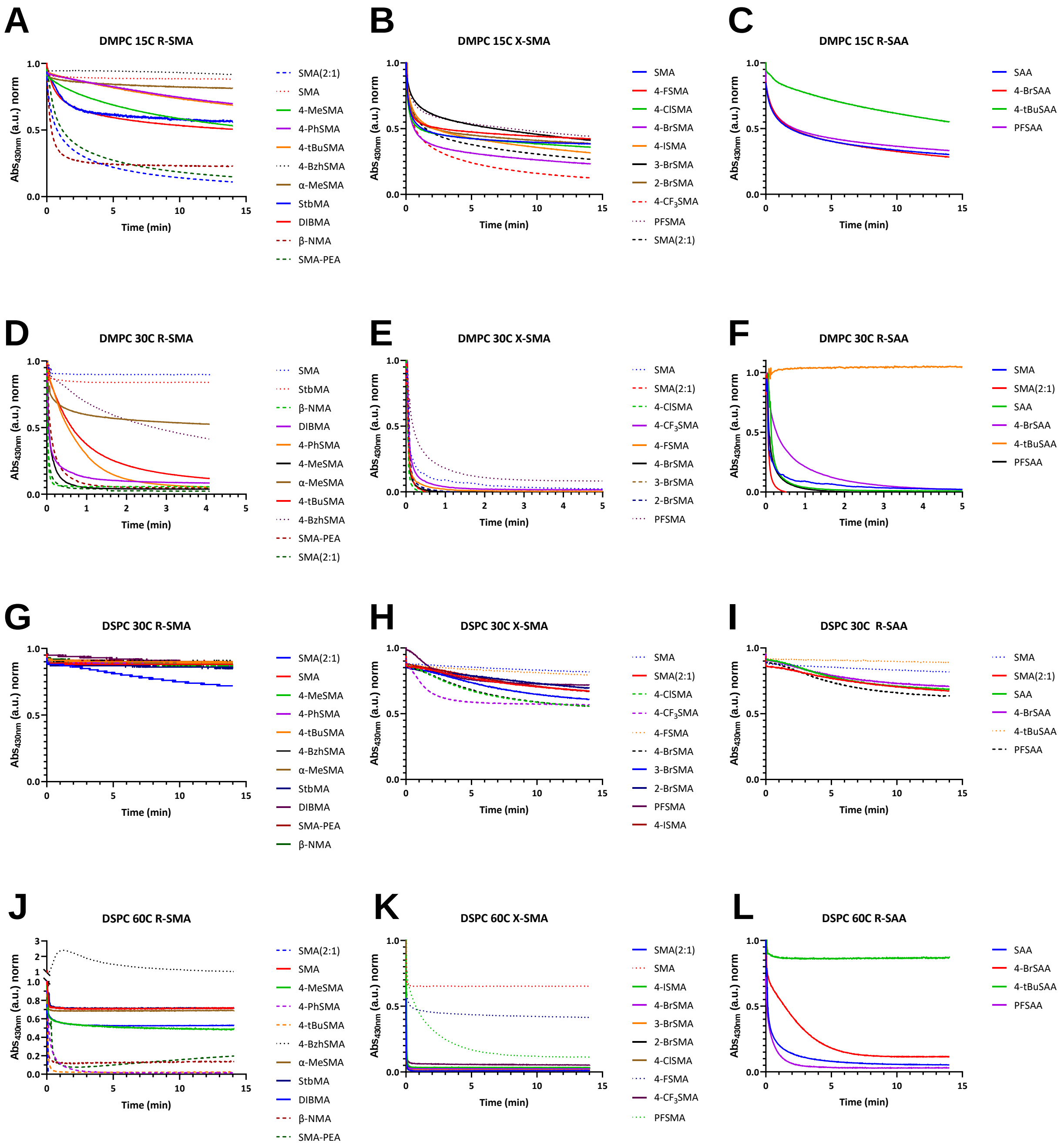


*Fig. S12. Turbidity data for vesicles of composed of DMPC (**A - F**) at 15°C (**A – C**) and 30°C (**D - F**), or DSPC (**G - L**), at 30°C (**G – I**) and 60°C (**J - L**), solubilized by different copolymer sets, namely R-SMA, (**A, D, G, J**) X-SMA, (**B, E, H, K**), and R-SAA (**C, F, I, L**). Data also shown as bar graphs in Fig S13.*

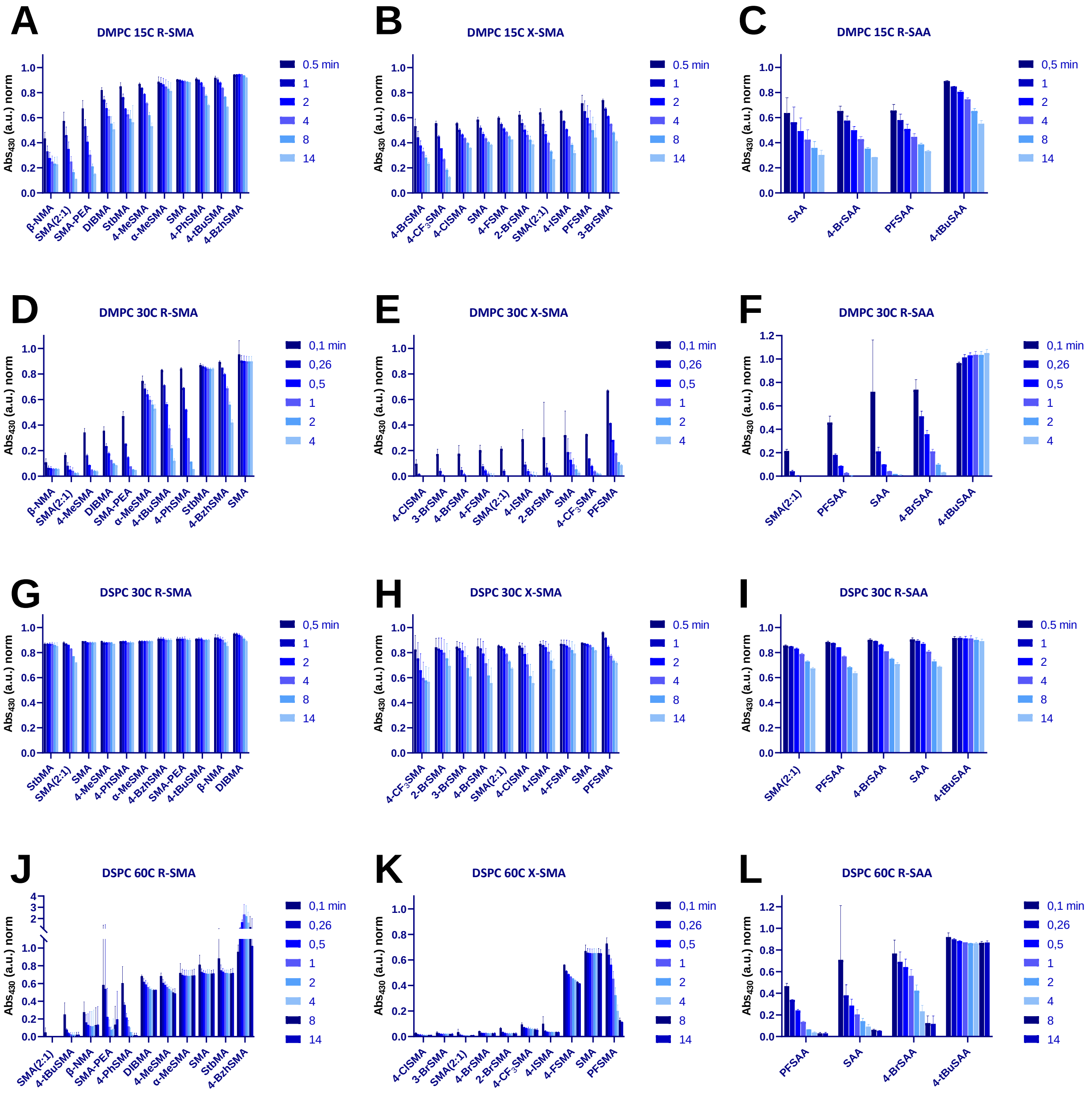


*Fig. S13. Turbidity data for vesicles of composed of DMPC (**A - F**) at 15°C (**A – C**) and 30°C (**D - F**), or DSPC (**G - L**), at 30°C (**G – I**) and 60°C (**J - L**), solubilized by different copolymer sets, namely R-SMA, (**A, D, G, J**) X-SMA, (**B, E, H, K**), and R-SAA (**C, F, I, L**). Plotted as bar-graphs showing the normalized absorbance at selected time points (0.1 – 14 min).*

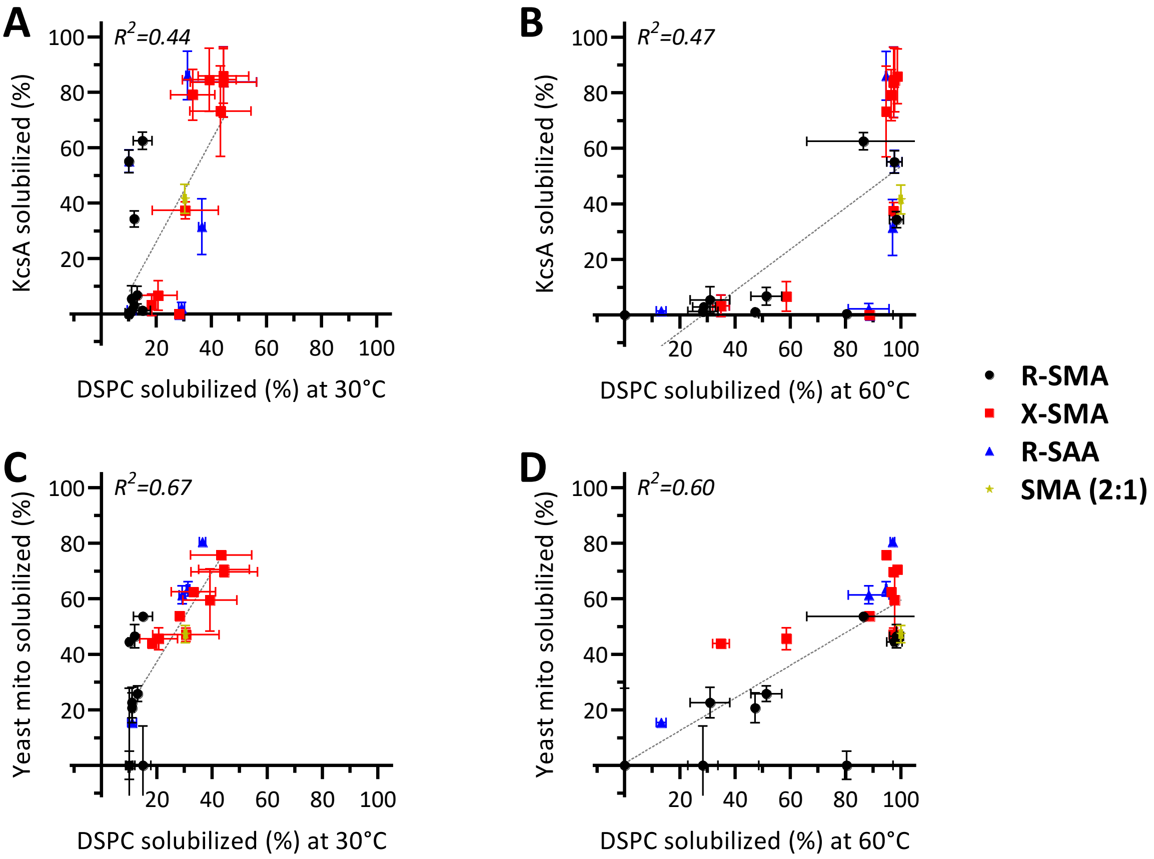


*Fig. S14. Comparison of the membrane protein, KcsA, solubilization (top) and yeast mitochondria solubilization (bottom) to model lipid-only vesicle solubilization. The data are shown for DSPC at 30°C (**A, C**) and 60°C (**B, D**). The different classes are depicted as follow: R-SMA, black circles; X-SMA, red squares; R-SAA, blue triangles. For systems $T < T_M$ the vesicle solubilization rate is derived after 14 min, and for $T > T_M$ time points are taken at 4 min. For the complete turbidity traces see Fig. S12 and Fig. S13. Dashed lines indicate the line of linear fit and corresponding correlation coefficients (R^2).*

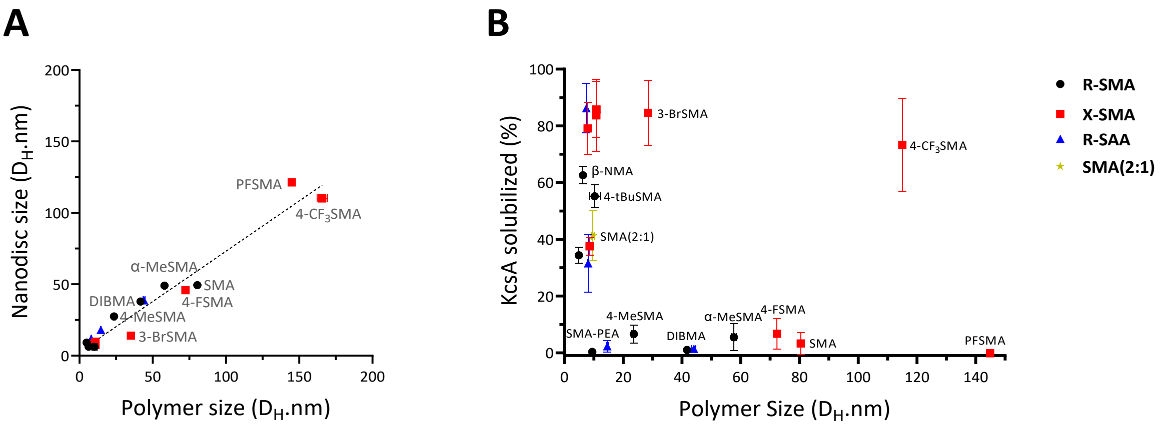


*Fig. S15. Nanoparticle sizes as determined by dynamic light scattering (DLS). Nanodisc (DMPC) Z-average sizes are directly correlated to copolymer Z-average sizes in (**A**), linear fitting provides a correlation coefficient (R^2) equal to 0.96. In panel (**B**) the solubilization efficacy of KcsA is compared to the polymer aggregate sizes in solution. R-SMA, black circles; X-SMA, red squares; R-SAA, blue triangles, and SMA(2:1) gold stars. For number-distribution plots see Fig. S16.*

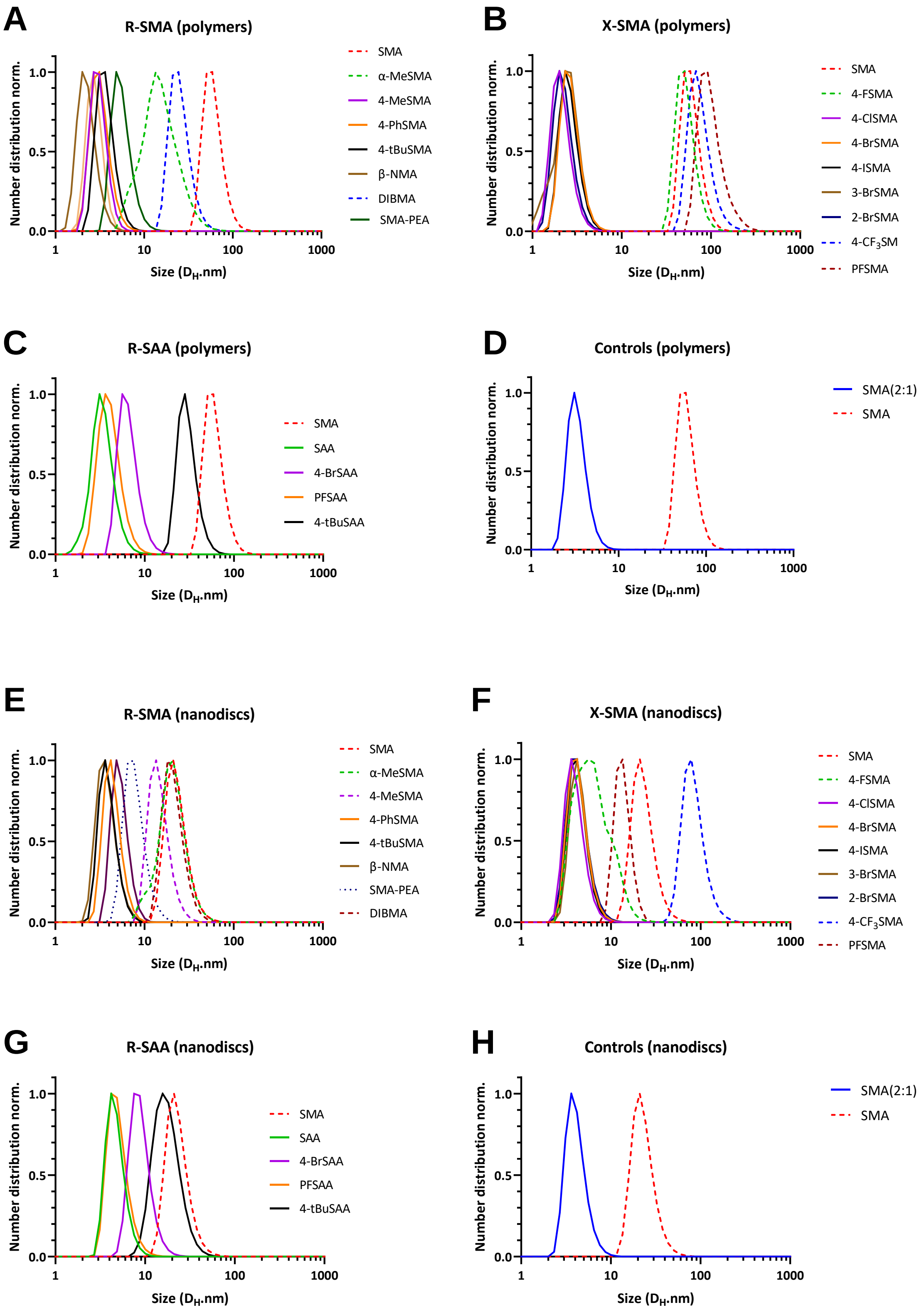


*Fig. S16. DLS analysis of synthesized copolymers (**A – D**) and DMPC nanodiscs (**E – F**) formed thereof. Separated into the different copolymer sets, namely R-SMA, (**A, E**) X-SMA, (**B, F**) R-SAA (**C, G**), and controls (**D, H**). All of the samples contain ~0.4% (w/v) polymer, nanodisc samples also contain ~2 mM lipid (polymer-to-lipid ratio of ~3:1 (w/w)).*

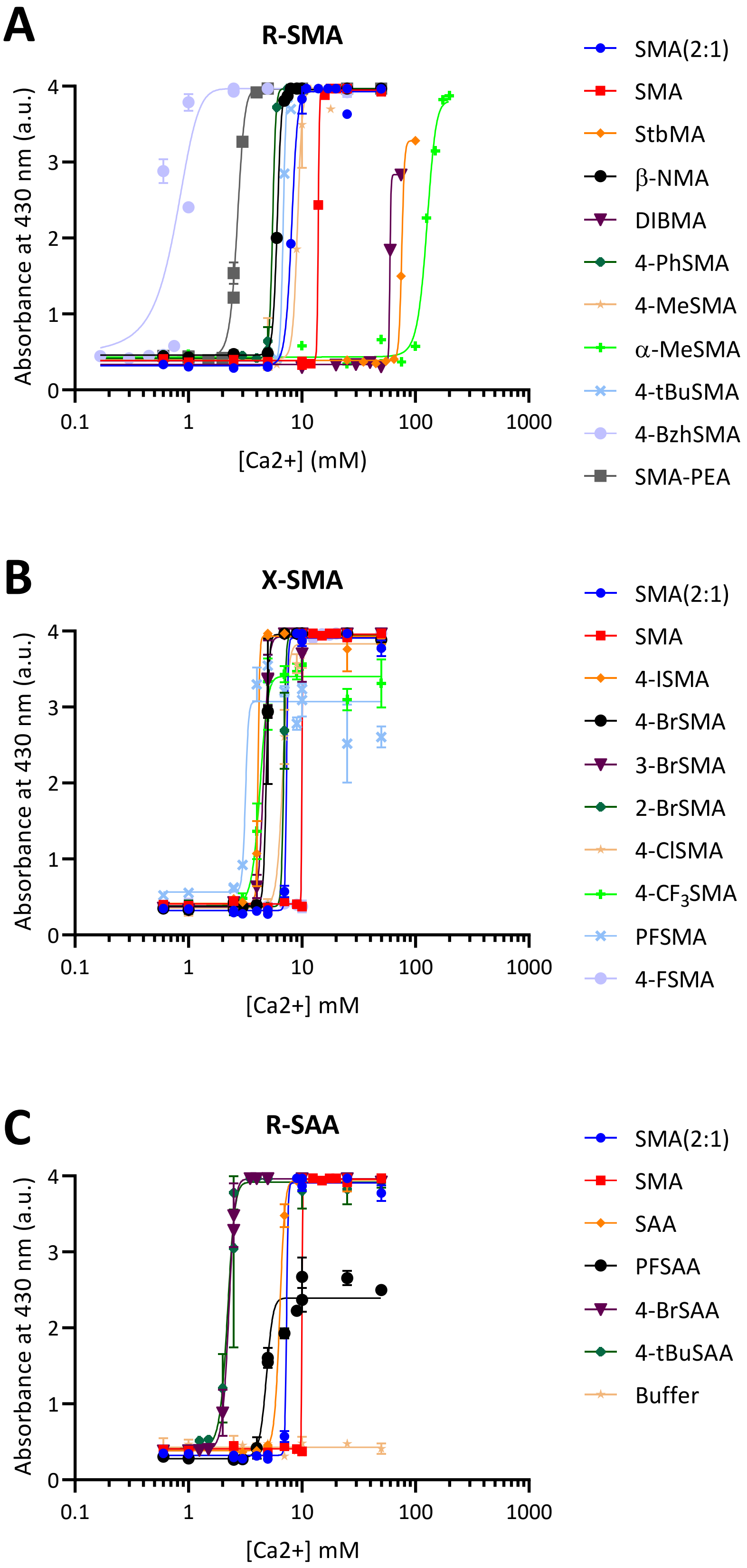


*Fig. S17. Sensitivity to divalent cations (Ca^{2+}) of the three copolymer sets (**A**) R-SMA, (**B**) X-SMA, and (**C**) R-SAA. Most of the copolymers precipitate and result in an increase of OD at a concentration of calcium ions in the range of 1 – 10 mM. The copolymers DIBMA, StbMA, and α-MeSMA in the R-SMA series (**A**) have a higher resistance to the Ca^{2+} (40 – 100 mM).*

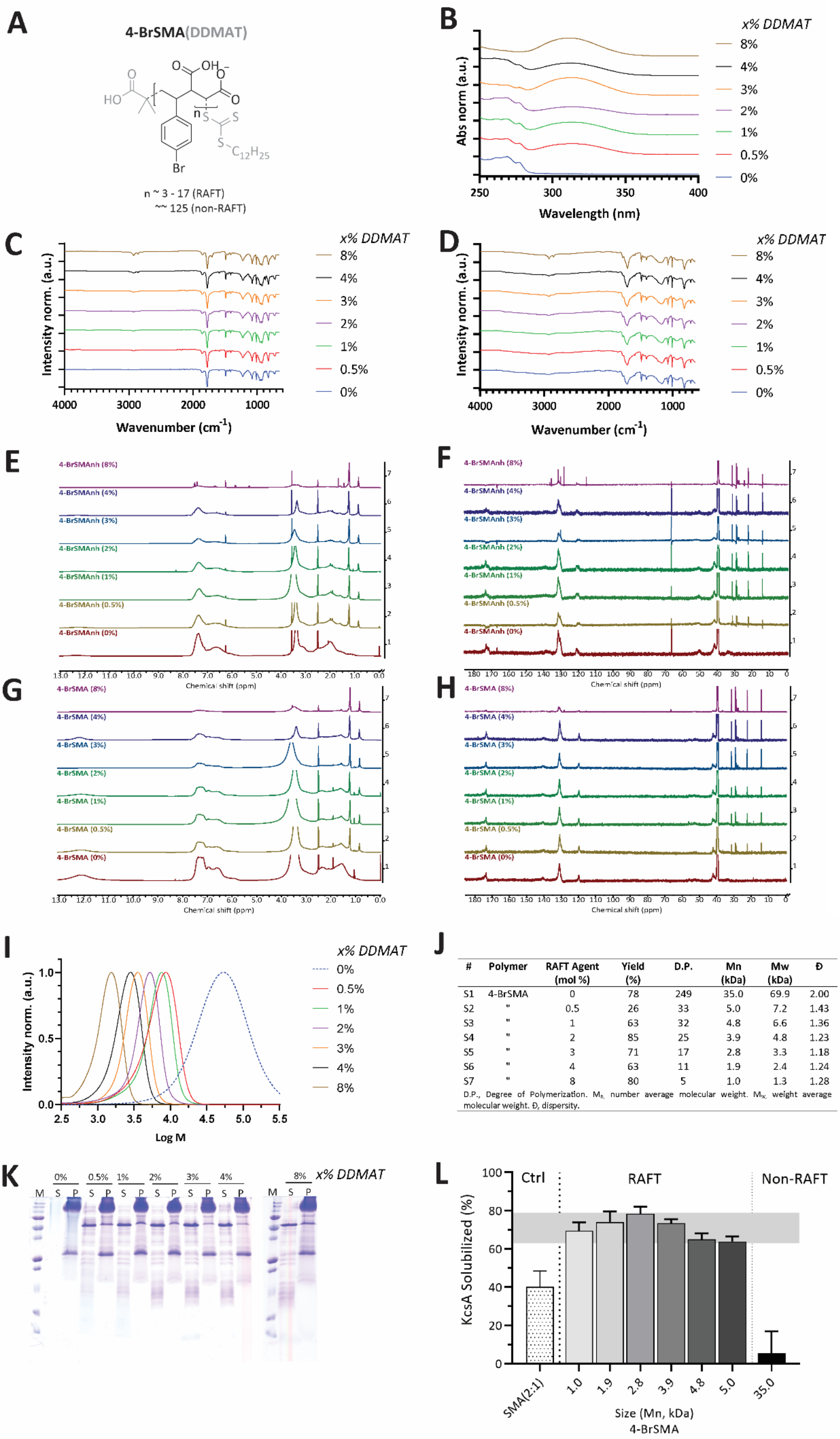
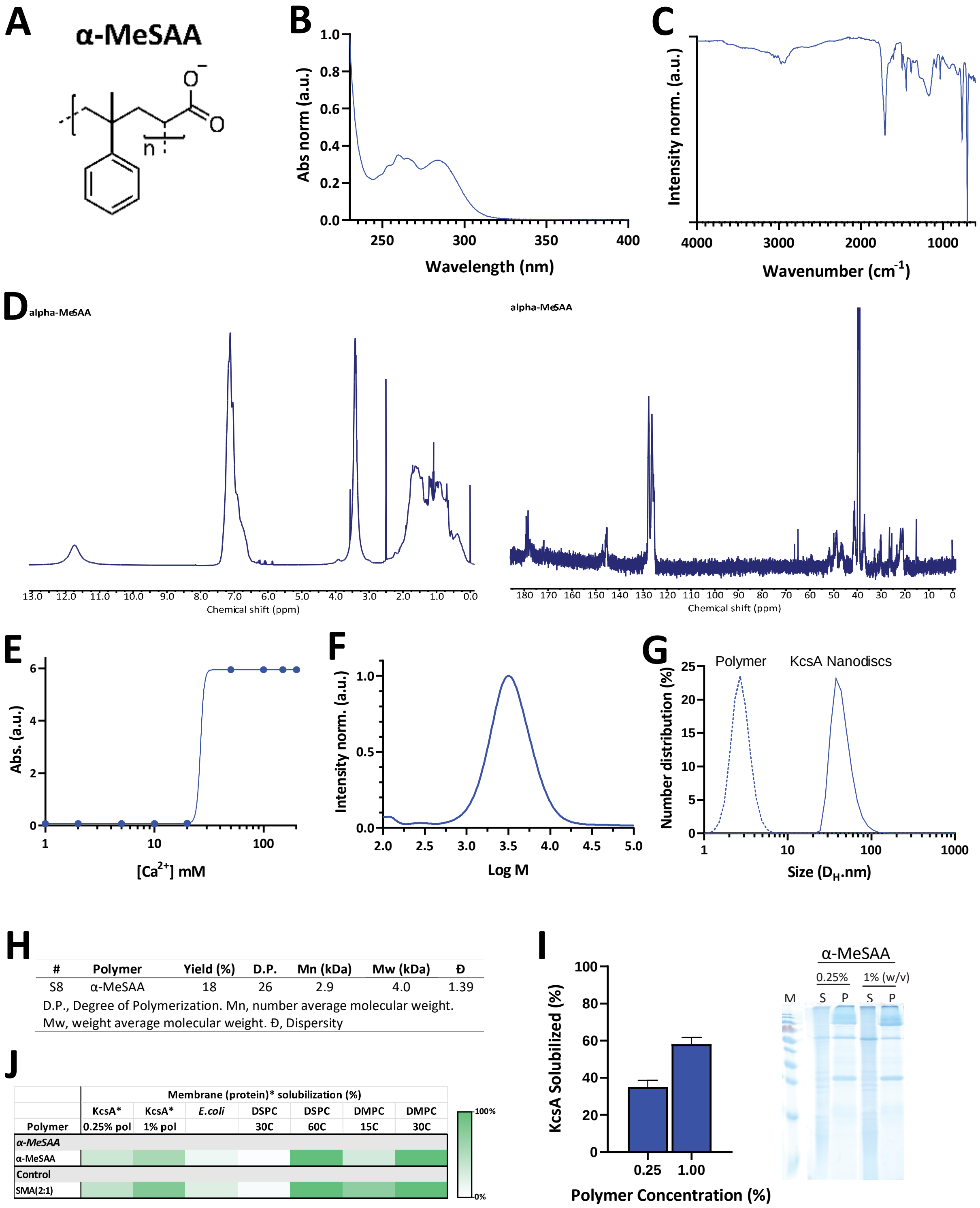


Fig. S18. 4-BrSMA copolymer size series prepared using various concentrations of RAFT agent (DDMAT) ranging from 0% (standard free-radical copolymerization as control) up to 8%. ***(A)*** *Chemical structure of 4-BrSMA(DDMAT) copolymers.* ***(B)*** *UV-vis wavelength scans.* ***(C)*** *FT-IR analysis of 4-BrSMAnh.* ***(D)*** *FT-IR analysis of 4-BrSMAnh.* ***(E)*** *^{1}H NMR spectra of 4-BrSMAnh.* ***(F)*** *^{13}C NMR spectra of 4-BrSMAnh.* ***(G)*** *^{1}H NMR spectra of 4-BrSMA.* ***(H)*** *^{13}C NMR spectra of 4-BrSMA.* ***(I)*** *GPC analysis.* ***(J)*** *Characterization 4-BrSMA copolymer size series.* ***(K)*** *Representative SDS-PAGE analysis of KcsA solubilization from E.coli membranes.* ***(L)*** *Quantification by densitometric analysis of KcsA solubilization by 4-BrSMA copolymer size series.*

#	Polymer	RAFT Agent (mol %)	Yield (%)	D.P.	Mn (kDa)	Mw (kDa)	Đ
S1	4-BrSMA	0	78	249	35.0	69.9	2.00
S2	"	0.5	26	33	5.0	7.2	1.43
S3	"	1	63	32	4.8	6.6	1.36
S4	"	2	85	25	3.9	4.8	1.23
S5	"	3	71	17	2.8	3.3	1.18
S6	"	4	63	11	1.9	2.4	1.24
S7	"	8	80	5	1.0	1.3	1.28

D.P., Degree of Polymerization. M_n, number average molecular weight. M_w, weight average molecular weight. Đ, dispersity.



#	Polymer	Yield (%)	D.P.	Mn (kDa)	Mw (kDa)	Đ
S8	α-MeSAA	18	26	2.9	4.0	1.39

D.P., Degree of Polymerization. Mn, number average molecular weight. Mw, weight average molecular weight. Đ, Dispersity

*Fig. S19. Characterization and evaluation of α-MeSAA copolymer. (**A**) Chemical structure of α-MeSAA (**B**) UV-vis wavelength scan. (**C**) FT-IR analysis. (**D**) ^{1}H (left) and ^{13}C (right) NMR spectra. (**E**) Investigation of divalent cation (Ca^{2+}) resistance. (**F**) GPC analysis. (**G**) DLS analysis of polymer in solution at a concentration of 0.4% and KcsA nanodiscs bounded by α-MeSAA copolymer. (**H**) Characterization of α-MeSAA. (**I**) Quantification by densitometric analysis of KcsA solubilization from E.coli membranes by α-MeSAA copolymer and representative SDS-PAGE. (**J**) Summary of solubilization of various membrane systems by α-MeSAA.*