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Supporting Information

Solubilization of Membrane Proteins into Functional Lipid-Bilayer Nanodiscs Using a Diisobutylene/Maleic Acid Copolymer

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Experimental Section

Materials

All chemicals were purchased in the highest purity available. DIBMA (trade name Sokalan CP9) and SMA(3:1) (trade name Xiran SL25010 S25) were kind gifts from BASF (Ludwigshafen, Germany) and Polyscope (Geleen, Netherlands), respectively. DLPC was purchased from Avanti Polar Lipids (Alabaster, USA) and Cayman Chemicals (Michigan, USA). DMPC and DPPC were from Lipoid (Ludwigshafen, Germany). D_2O, deuterated dimethylsulfoxide (DMSO, 99.5% isotopic purity), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid (EDTA), 85% (*w*/*v*) phosphoric acid in D_2O, and poly(ethylene oxide) dodecyl ether (Brij-35) were from Sigma–Aldrich (Steinheim, Germany). Benzonase was from Merck (Darmstadt, Germany), lauryldimethylamine *N*-oxide (LDAO) from Anatrace (Maumee, USA), 2-hexadecanoylthio-1-ethylphosphorylcholine (HEPC) from Biozol (Eching, Germany), isopropyl-*β*-D-1-thiogalactopyranoside (IPTG) from Carl Roth (Karlsruhe, Germany), NaCl from VWR (Darmstadt, Germany), tris(hydroxymethyl)aminomethane (Tris) from Carl Roth, and urea from Affymetrix (Santa Clara, California).

Preparation of copolymer stock solutions

Unless stated otherwise, all buffers contained 50 mM Tris, 200 mM NaCl and, for all experiments involving OmpLA, additionally 2 mM EDTA to prevent phospholipase activation by residual Ca^{2+}. ~3 mL of the commercial DIBMA or SMA(3:1) solution was dialyzed against 1 L buffer using a 5-mL QuixSep dialyzer (Membrane Filtration Products, Seguin, USA) and a Spectra/Por 3 dialysis membrane (Spectrum Laboratories, Rancho Dominguez, USA) with a nominal molar-mass cutoff of 3.5 kg mol^{-1}. Dialysis was carried out at room temperature for 24 h with buffer exchange after 12–16 h. Recovered polymers were filtered through 200-nm poly(vinylidene fluoride) (PVDF) filters (Carl Roth). Polymer stock concentrations were determined by refractometry on an Abbemat 500 instrument (Anton Paar, Graz, Austria). The molar concentration of SMA(3:1) was calculated using a molar refractive index increment, d*n*/d*c*, of 1.1176 M^{-1}.[1] The molar concentration of DIBMA was calculated in the same manner using a d*n*/d*c* value of 1.346 M^{-1} as determined below (cf. next section).

Characterization of DIBMA copolymer properties

Number- and mass-average molar masses of DIBMA were determined by SEC on an OmniSEC system (Malvern Instruments, Worcestershire, UK) equipped with a Superdex 200 Increase column (GE Healthcare, Freiburg, Germany) and coupled to an array of detectors measuring UV absorbance, light scattering intensity, and refractive index. The system was calibrated with a bovine serum albumin standard (Sigma–Aldrich). The column was equilibrated for 24 h with buffer (50 mM Tris, 200 mM NaCl, pH 7.4) at 20 °C and a steady flow rate of 0.5 mL min^{-1} before a 100-µL aliquot of 4.1, 7.5, or 15 mg mL^{-1} polymer was injected. Analysis of ten chromatograms with the software OmniSEC 5.02 yielded mean values and associated standard deviations of M_n = (8.4±0.4) kg mol^{-1}, M_w = (15.3±0.5) kg mol^{-1}, and M_w/M_n = 1.82±0.09 for the number-average molar mass, the mass-average molar mass, and the dispersity, respectively.

To determine the d*n*/d*c* value of DIBMA, the polymer was precipitated from the commercially available solution by addition of 4 M HCl, washed twice with triply distilled water, and resuspended in 0.5 M NaOH. The polymer was precipitated a second time with 4 M HCl and washed five times with water prior to lyophilization in an Alpha 2-4 LSCplus freeze–dryer (Martin Christ, Osterode am Harz, Germany). The polymer powder was resuspended in 100 mM NaOH, and the refractive indices of a dilution series were measured, yielding a d*n*/d*c* value of (1.346±0.066) M^{-1} after blank correction.

Production and purification of outer membrane phospholipase A

The *pldA* gene encoding OmpLA without signal sequence was amplified from genomic *E. coli* DNA by polymerase chain reaction and cloned into a pET-24a(+) expression vector (Merck, Darmstadt, Germany). Construct identity was confirmed by bidirectional sequencing (SEQ-IT, Kaiserslautern, Germany) using T7 promoter and terminator primers. Lysogeny broth medium was inoculated with transformed *E. coli* BL21(DE3) cells. To induce production of tag-free OmpLA, 0.4 mM IPTG was added to the inoculated medium, and the mixture was incubated for 4 h at 37 °C with constant agitation. Cells were harvested by centrifugation and washed twice with saline (154 mM NaCl). Cell pellets were suspended in a 10-fold volume of ice-cold lysis buffer (50 mM Tris, 2 mM $MgCl_2$, 40 mM EDTA, 25% (*w*/*v*) sucrose, 0.01% (*v*/*v*) Benzonase, pH 8.0). The suspension was sonicated with an S-250A sonifier (Branson Ultrasonics, Danbury, USA) in two runs of 10 min each. Brij-35 was added to a final concentration of 0.01% (*w*/*v*), and the lysate was centrifuged for 45 min at 4 °C and 4500 *g*. The recovered pellet containing inclusion bodies was washed with buffer (10 mM Tris, 1 mM EDTA, pH 8.0), centrifuged for 30 min at 4 °C and 4500 *g*, and stored at –80 °C.

OmpLA was recovered in unfolded form by solubilization of inclusion bodies in 8 M urea (20 mM Tris, 2 mM EDTA, 100 mM glycine, pH 8.3). The protein was refolded into LDAO micelles by drop dilution to reach final concentrations of 10.7 µM OmpLA, 0.87 M urea, and 12 mM LDAO (20 mM Tris, 2 mM EDTA, pH 8.3) and incubated for 16 h at 50 °C with stirring. Refolded OmpLA was purified on a diethylaminoethyl (DEAE) Sepharose ion-exchange column (GE Healthcare, Uppsala, Sweden), eluted in 35 mM LDAO (20 mM Tris, 2 mM EDTA, 1.5 M KCl, pH 9.5), and dialyzed against 12 mM LDAO (20 mM Tris, 2 mM EDTA, pH 8.3). OmpLA was concentrated on a HiTrap Q HP column and desalted on a PD-10 column (GE Healthcare). Protein concentration was determined by UV absorbance using an extinction coefficient of 82.3 mM^{-1} cm^{-1} at 280 nm (http://expasy.org/tools/protparam.html). Purified folded OmpLA was aliquoted and stored at –80 °C.

Preparation of liposomes and proteoliposomes

To prepare DMPC and DPPC liposomes, lipid powders were weighed on a high-precision XP Delta Range microbalance (Mettler Toledo, Greifensee, Switzerland) and suspended in buffer (50 mM Tris, 200 mM NaCl, pH 7.4). The dispersions were vortexed for 15 min followed by 35-fold extrusion through two stacked polycarbonate filters with a pore diameter of 100 nm using a Mini-Extruder (Avanti Polar Lipids) at 30 °C for DMPC and 45 °C for DPPC. Formation of LUVs was confirmed by DLS (see below), yielding hydrodynamic diameters of ~150 nm.

To prepare proteoliposomes, DLPC was suspended in buffer (50 mM Tris, 2 mM EDTA, 200 mM NaCl, pH 7.4), vortexed for 10 min, and extruded 35-fold through two stacked polycarbonate filters with a pore diameter of 100 nm using a LiposoFast extruder (Avestin, Mannheim, Germany) at room temperature, resulting in LUVs having a hydrodynamic diameter of ~140 nm, as confirmed by DLS. Under gentle agitation, 600 µL of a 100 µM OmpLA stock in 12 mM LDAO was added in 24 aliquots of 25 µL each, with time intervals of 5 min, to 1.4 mL of 60 mM DLPC LUVs to reach final concentrations of ~30 µM OmpLA, 42 mM DLPC, and 3.6 mM LDAO. Under these conditions, the LDAO/DLPC molar ratio was at least 10-fold lower than the saturation threshold determined for this system ($R_S^{b,SAT}$=1.39).[2] For comparison, protein-free DLPC liposomes were prepared in an analogous way using buffer that contained 12 mM LDAO but no OmpLA. After the last injection, both protein-free and proteoliposomes were incubated for ~1 h and then dialyzed for 24 h against a 1000-fold excess volume of buffer (50 mM Tris, 2 mM EDTA, 200 mM NaCl, pH 7.4) using a Spectra/Por 4 dialysis membrane (Spectrum Laboratories) with a molar-mass cutoff of 12–14 kg mol^{-1}. After dialysis, no residual LDAO was detected by thin-layer chromatography, and both types of liposomes retained their narrow, unimodal size distribution profiles.

UV–VIS spectroscopy

Absorbance spectra of 0–2.5 mM DIBMA or SMA(3:1) were recorded at 220–600 nm on a V-630 UV–VIS spectrophotometer (Jasco, Groß-Umstadt, Germany). Each sample in buffer (50 mM Tris, 200 mM NaCl, pH 7.4) was measured three times at room temperature in a 3 mm × 3 mm quartz glass cuvette (Hellma Analytics, Müllheim, Germany) using a scan rate of 200 nm min^{-1} and a bandwidth of 1.5 nm.

Kinetics of vesicle solubilization

Kinetic measurements were performed on an SF.3 stopped-flow apparatus (Applied Photophysics, Leatherhead, UK) equipped with a light-emitting diode (LED) operating at (572±13) nm and a photomultiplier detector mounted at a right angle. Temperature was maintained by an Alpha RA 8 water circulation bath (LAUDA, Königshofen, Germany). After 10 min of equilibration, equal volumes of 2 mM DMPC and 0.15 mM DIBMA in buffer (50 mM Tris, 200 mM NaCl, pH 7.4) were injected into the 2-mm flow cell, and the decay in light scattering intensity was monitored. Data were acquired using an integration time of 12.5 μs. Each measurement was repeated five times.

Dynamic light scattering

DLS was performed on a Zetasizer Nano S90 (Malvern Instruments) equipped with a 633-nm He–Ne laser and a right-angle photodetector. Measurements were carried out using a 45-μL quartz glass cuvette with a cross-section of 3 mm × 3 mm (Hellma Analytics). Attenuator settings were automatically optimized by the instrument software for determination of particle size distributions. The influence of buffer composition (50 mM Tris, 2 mM EDTA, 200 mM NaCl, pH 7.4 for DLPC; 50 mM Tris, 200 mM NaCl, pH 7.4 for all other samples) on sample viscosity and refractive index was accounted for during data analysis. To obtain intensity-weighted particle size distributions, experimentally determined autocorrelation functions were fitted with a non-negatively constrained least-squares function.[3,4] In addition to the *z*-average diameter, we estimated the size-distribution width by multiplying the *z*-average diameter with the square root of the corresponding polydispersity index (PDI).[5]

Transmission electron microscopy

TEM samples were prepared by loading 5 μL of SEC-purified nanodiscs onto a Cu grid coated with Formvar film (Plano, Wetzlar, Germany). Excess liquid was blotted off with a strip of filter paper after 30 s followed by staining with 5 μL 1% (*w*/*v*) aqueous uranyl acetate solution. Grids were examined on an EM 900 transmission electron microscope (Carl Zeiss, Oberkochen, Germany), and micrographs were taken with an SM-1k-120 slow-scan charge-coupled device (SSCCD) camera (TRS, Moorenweis, Germany).

^{31}P NMR spectroscopy

Series of 2.5, 5.0, 7.5, and 10 mM DMPC were treated with 0–2 mM DIBMA in buffer (50 mM Tris, 200 mM NaCl, pH 7.4) containing 10% D_2O as lock signal. Samples were incubated for 16 h at 30 °C prior to measurements at the same temperature. For DLPC, protein-free liposomes and proteoliposomes were incubated with polymer to give final concentrations of ~3 μM OmpLA, 5 mM DLPC, and 0–2 mM DIBMA in buffer (50 mM Tris, 2 mM EDTA, 200 mM NaCl, pH 7.4). Measurements were performed on an Avance 400 spectrometer (Bruker BioSpin, Rheinstetten, Germany) operating at a ^{31}P resonance frequency of 162 MHz. We acquired 256 scans per sample using a 5-mm broadband inverse probe, an inverse-gated ^{1}H decoupling pulse sequence, an acquisition time of 1.6 s, a sweep width of 9746 Hz, and a relaxation delay of 6 s. Chemical shifts were referenced to 85% (*w*/*v*) H_3PO_4 in D_2O as external standard at 0 ppm. Data were multiplied by an exponential function with a line-broadening factor of 10 Hz before Fourier transformation. Peak areas were obtained by numerical integration using the software Topspin 3.2 (Bruker BioSpin).

Raman scattering

Raman scattering experiments were performed on a Helix combined DLS/Raman system (Malvern Instruments) fitted with a 280-mW diode laser emitting at 785 nm. Spectra were acquired from 150 cm^{-1} to 1925 cm^{-1} at a resolution of 4 cm^{-1} in a 3 mm × 3 mm quartz glass cuvette (Hellma Analytics). Each sample was measured 20 times with an integration time of 20 s each. Fourier-transformed spectra were decomposed for contributions from quartz glass, buffer (50 mM Tris, 200 mM NaCl, pH 7.4), polymer, and lipid using the software HelixAnalyze (Malvern Instruments). Background-corrected lipid spectra were baseline-linearized and normalized to the C–N headgroup stretching band at 716 cm^{-1}.

Differential scanning calorimetry

A MicroCal VP-DSC (Malvern Instruments) was used to investigate thermotropic phase transitions of DMPC in LUVs, DIBMALPs, and SMALPs. 5 mM DMPC was titrated with increasing concentrations of DIBMA or SMA(3:1) in buffer of the same composition (50 mM Tris, 200 mM NaCl, pH 7.4) and incubated for 16 h at 30 °C. The sample and reference cells were filled with buffer and repeatedly heated and cooled at a rate of 30 °C h^{-1} before the buffer in the sample cell was replaced with sample. Apart from the first upscan, successive heating and cooling scans overlaid very closely. Data were averaged, blank-subtracted, and normalized against the concentration of DMPC in the sample using the software Origin 7.0 (OriginLab). The melting temperature, T_m, was taken as the temperature at which the excess molar isobaric heat capacity, ΔC_p, reached a maximum. The calorimetric enthalpy, ΔH_{cal}, was obtained by numerical integration of the peak area under the ΔC_p curve, while the van't Hoff enthalpy, ΔH_{vH}, was calculated as:[6]

$$\Delta H_{vH} = 4RT_m^2 \Delta C_p / \Delta H_{cal} \quad (1)$$

From these two enthalpies, the size of a "cooperative unit", n, was estimated as:[6]

$$n = \Delta H_{vH} / \Delta H_{cal} \quad (2)$$

Enzyme activity assay

The enzymatic activity of OmpLA was determined by monitoring the kinetics of HEPC hydrolysis.[7] The thiol group released upon HEPC hydrolysis rapidly and stoichiometrically reacts with DTNB to form 2-nitro-5-thiobenzoate (TNB^{2-}), which has a molar extinction coefficient of 13.6 mM^{-1} cm^{-1} at 412 nm. Typically, 0.3 μM OmpLA was incubated with 1 mM HEPC and 0.8 mM DTNB for 1 h before the mixture was transferred to a 3-mm quartz glass cuvette. Enzymatic activity was triggered by $CaCl_2$ addition, giving final concentrations of 0.64 μM OmpLA, 0.98 mM HEPC, 0.78 mM DTNB, and 19.6 mM $CaCl_2$ in buffer (50 mM Tris, 2 mM EDTA, 200 mM NaCl, pH 7.4).

Circular dichroism spectroscopy

For urea unfolding experiments, two stock solutions of OmpLA-containing DIBMALPs were incubated for 4 h either in the absence or in the presence of 8 M urea in buffer (50 mM Tris, 2 mM EDTA, 200 mM NaCl, pH 7.4). These stocks were mixed to reach final concentrations of 0–8 M urea, 3.8 μM OmpLA, 4.8 mM DLPC, and 1.0 mM DIBMA. Samples were transferred to a 96-deep-well plate and further incubated for 16 h at 20 °C prior to measurements. DIBMALPs containing no protein were prepared in the same way and used as blanks. Spectra were acquired at 210–280 nm using a Chirascan-plus automated CD spectrometer (Applied Photophysics, Leatherhead, UK) coupled to a robotic liquid-handling system (Tecan Systems, San Jose, USA) for automatic sampling and cleaning.[8] The instrument was equipped with a large-area head avalanche photodiode detector (Advanced Photonix, Ann Arbor, USA) and a 0.2-mm fused silica flow-through cell (Optiglass, Hainault, UK) having a probe volume of 40 μL.

For thermal unfolding experiments, CD spectra were acquired every 5 °C at 20–90 °C using a Chirascan-plus CD spectrometer (Applied Photophysics) equipped with a Peltier temperature control unit. Measurements were carried out in a 1-mm quartz glass cuvette. At each temperature, 20 scans were carried out over 190–280 nm using a bandwidth of 1 nm. Spectra were averaged, baseline-corrected by subtracting blank measurements, and offset-corrected by *y*-shifting each spectrum such that the average signal in the range of 260–280 nm vanished.

Protein chromatography

Proteoliposomes were mixed with DIBMA to give final concentrations of 13 μM OmpLA, 20 mM DLPC, and 2–5 mM DIBMA, incubated at 25 °C for 16 h, and centrifuged at 20,000 *g* for 20 min at 20 °C. Separation of protein-containing and protein-free DIBMALPs was carried out on an Äkta Purifier 10 system (GE Healthcare) fitted with a Sephacryl S-500 HR column equilibrated with buffer (50 mM Tris, 2 mM EDTA, 200 mM NaCl, pH 7.4) at 6 °C. ~500 μL of sample was injected, and the elution profile was monitored by UV absorbance at 260 nm and 280 nm at a flow rate of 0.3 mL min^{-1}. As a control, an elution profile of unsolubilized proteoliposomes (~3 μM OmpLA in 5 mM DLPC) was also recorded.

SDS-PAGE

The folding state of OmpLA and the protein-extraction profiles of polymers and detergents were determined by SDS-PAGE using a NuPAGE Bis–Tris system (Life Technologies, Carlsbad, USA) with a polyacrylamide gradient of 4–12%. Samples were diluted with SDS buffer (50 mM DTT, 106 mM Tris-HCl, 141 mM Tris, 2% (*w*/*v*) SDS, 10% (*w*/*v*) glycerol, 0.51 mM EDTA, 0.22 mM Coomassie brilliant blue G250, and 0.175 mM Phenol Red, pH 8.5). OmpLA samples were not boiled before SDS-PAGE. A constant voltage of 200 V was applied for 40 min at 50 W. Gels were fixed in 10% (*v*/*v*) ethanoic acid and 40% (*v*/*v*) ethanol, stained with 0.025% (*w*/*v*) Coomassie brilliant blue in 10% (*v*/*v*) ethanoic acid, and de-stained with 10% (*v*/*v*) ethanoic acid. Finally, gels were photographed, and protein bands were analyzed with the public-domain software ImageJ.[9]

Solubilization of native *E. coli* membranes

E. coli BL21(DE3) was transformed with an empty pET-24 vector and selected by kanamycin resistance. After incubation in 400 mL lysogeny broth overnight at 37 °C with constant agitation, cells were harvested by centrifugation and washed twice with saline (154 mM NaCl). Cell pellets were resuspended in a 10-fold volume of ice-cold buffer (50 mM Tris, 2 mM EDTA, 200 mM NaCl, pH 7.4) and subjected to ultrasonication in an S-250A sonifier (Branson Ultrasonics) in two runs of 10 min each. The lysate was further centrifuged for 30 min at 4 °C and 1000 *g*. The supernatant was subjected to ultracentrifugation (UC) for 1 h at 4 °C and 100,000 *g* and washed seven times with buffer to remove soluble proteins. Membrane pellets were resuspended in buffer to a final concentration of 42.5 mg mL^{-1} and treated with 10 mM DDM, 2.5 % (*w*/*v*) SMA(3:1), 2.5 % (*w*/*v*) DIBMA, or buffer. Samples were incubated for 16 h at 20 °C with gentle agitation and subsequently pelleted by UC for 1 h at 4 °C and 100,000 *g*. Solubilized fractions were analyzed for proteins by SDS-PAGE as described above. To avoid band smearing due to the presence of polymers,[10] proteins were precipitated from polymer-containing samples with $CH_3OH/CHCl_3/H_2O$.[11] Briefly, a 100-μL aliquot of ice-cold sample was mixed with 400 μL ice-cold CH_3OH by thorough vortexing. 100 μL ice-cold $CHCl_3$ was added, and the sample was vortexed again. Finally, 300 μL ice-cold water was added, and the sample was vortexed and centrifuged for 2 min at 4 °C and 14,000 *g*. After the top aqueous layer was removed, 400 μL CH_3OH was added, and the sample was vortexed. Precipitated proteins were pelleted for 1 min at 5000 *g* and 5 min at 20,000 *g*, both at 4 °C. CH_3OH was removed, the pellet was dried overnight, resuspended in SDS buffer, boiled for 10 min under agitation, and subjected to SDS-PAGE as described above.

Theoretical Background

Pseudophases in polymer/lipid mixtures

The pseudophase concept[12,13] yields the free energies accompanying the transfer of polymer and lipid from vesicular bilayers (b) into micelles (m),[13] which here are represented by DIBMALP nanodiscs. Briefly, the membrane-saturating (c_S^{SAT}) and solubilizing (c_S^{SOL}) polymer concentrations are linear functions of the lipid concentration (c_L), as reflected in:

$$c_S^{SAT} = c_S^{aq,o} + R_S^{b,SAT} c_L \tag{3}$$

$$c_S^{SOL} = c_S^{aq,o} + R_S^{m,SOL} c_L \tag{4}$$

Here, $c_S^{aq,o}$ is the concentration of free polymer in the aqueous phase, which is $c_S^{aq,o} \approx 0$ for DIBMA/lipid mixtures (cf. Figure 2c). The slopes of the SAT and SOL boundaries yield the polymer/lipid molar ratios at the onset ($R_S^{b,SAT}$) and completion ($R_S^{m,SOL}$) of solubilization, respectively. The corresponding critical mole fractions of polymer are obtained as:

$$X_S^{b,SAT} = \frac{R_S^{b,SAT}}{1+R_S^{b,SAT}} \tag{5}$$

$$X_S^{m,SOL} = \frac{R_S^{m,SOL}}{1+R_S^{m,SOL}} \tag{6}$$

Thus, partition coefficients characterizing the transfer of polymer ($K_S^{b\rightarrow m}$) and lipid ($K_L^{b\rightarrow m}$) from vesicular bilayers into nanodiscs are given by:

$$K_S^{b\rightarrow m} \equiv \frac{X_S^{m,SOL}}{X_S^{b,SAT}} = \frac{R_S^{m,SOL}(1+R_S^{b,SAT})}{R_S^{b,SAT}(1+R_S^{m,SOL})} \tag{7}$$

$$K_L^{b\rightarrow m} \equiv \frac{X_L^{m,SOL}}{X_L^{b,SAT}} = \frac{1-X_S^{m,SOL}}{1-X_S^{b,SAT}} = \frac{1+R_S^{b,SAT}}{1+R_S^{m,SOL}} \tag{8}$$

The corresponding vesicle-to-nanodisc transfer free energies are calculated as:

$$\Delta G_S^{b\rightarrow m,o} = -RT \ln K_S^{b\rightarrow m} = -RT \ln \left(\frac{R_S^{m,SOL}(1+R_S^{b,SAT})}{R_S^{b,SAT}(1+R_S^{m,SOL})} \right) \tag{9}$$

$$\Delta G_L^{b\rightarrow m,o} = -RT \ln K_L^{b\rightarrow m} = -RT \ln \left(\frac{1+R_S^{b,SAT}}{1+R_S^{m,SOL}} \right) \tag{10}$$

Analysis of ^{31}P NMR spectra

At given c_L, the area of the ^{31}P NMR peak is zero as long as c_S is below c_S^{SAT}, because there is no solubilized lipid. At the other extreme, all lipid molecules are solubilized in the presence of polymer concentrations greater than c_S^{SOL}, resulting in a sharp isotropic signal whose area is a function of c_L. Within the coexistence range, the peak area is proportional to the extent of solubilization.[1] These three ranges are quantitatively reflected in the following equations:

$$A(c_S \le c_S^{SAT}) = 0 \tag{11}$$

$$A(c_S \ge c_S^{SOL}) = f\, c_L \tag{12}$$

$$A(c_S^{SAT} \le c_S \le c_S^{SOL}) = f\, c_L (c_S - c_S^{SAT}) / (c_S^{SOL} - c_S^{SAT}) \tag{13}$$

The proportionality factor (f) depends on experimental conditions but has a constant value for a given NMR spectrometer operated using identical instrument settings and acquisition parameters. Nonlinear least-square fits in Excel[14] using Eqs. 11–13 yielded best-fit values and 95% confidence intervals of c_S^{SAT} and c_S^{SOL} at a given c_L.

Supporting Results

DIBMA characterization by SEC coupled to refractometry and light scattering

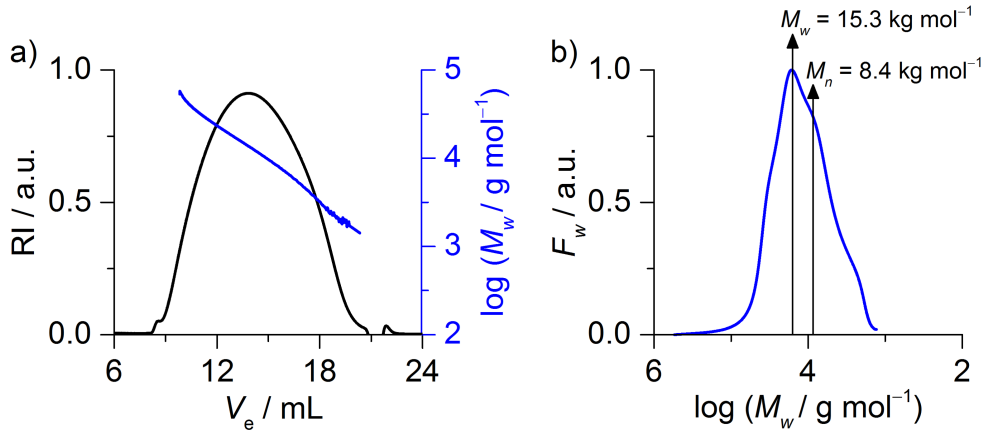

Figure S1. a) Refractive index, RI, and molar mass, M_w, of 7.5 mg mL^{-1} DIBMA as functions of elution volume, V_e. b) Differential molar-mass distribution function derived from data in panel a. Vertical arrows indicate mass-average and number-average molar masses, M_w and M_n, respectively. Experimental conditions were 50 mM Tris, 200 mM NaCl, pH 7.4, 20 °C, flow rate 0.5 mL min^{-1}.

Solubilization of DMPC vesicles by DIBMA

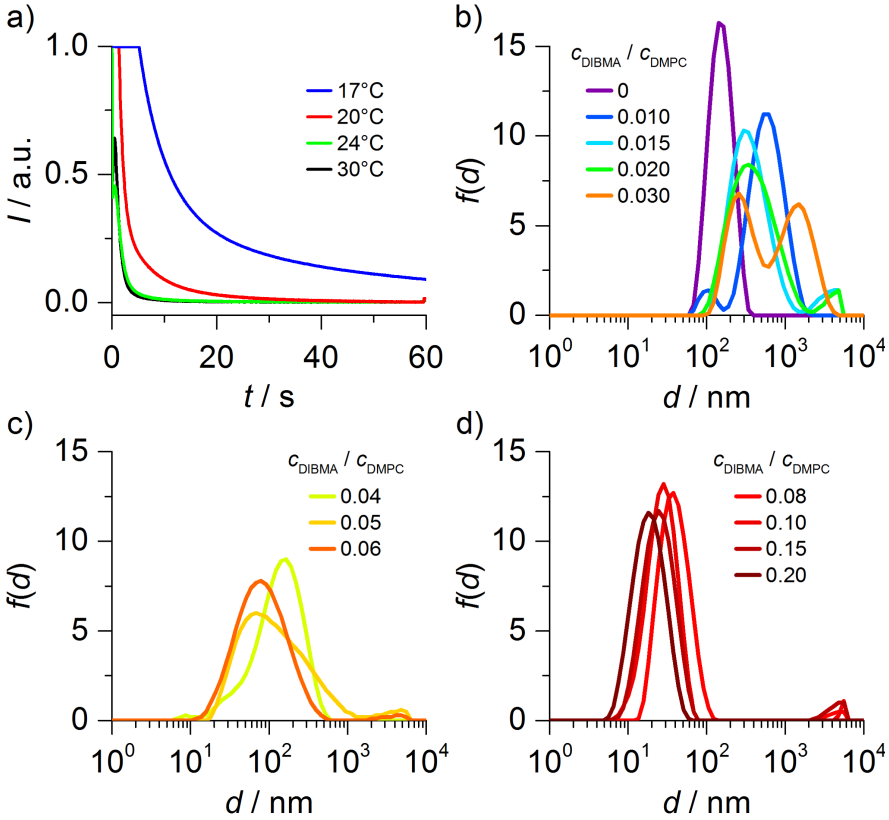

Figure S2. a) Decrease in normalized right-angle light scattering intensity, I, with time, t, as measured in a stopped-flow cell upon mixing equal volumes of 2 mM DMPC LUVs and 0.15 mM DIBMA at different temperatures. b–d) Intensity-weighted particle size distributions, $f(d)$, of 10 mM DMPC initially present in the form of LUVs upon titration with DIBMA as derived from DLS at 30 °C. Vesicle aggregates or agglomerates appeared below the SAT boundary (panel b), whereas nanodiscs formed between the SAT and SOL boundaries (panel c) and were the only particles left upon complete solubilization (panel d).

Solubilization of DPPC vesicles by DIBMA

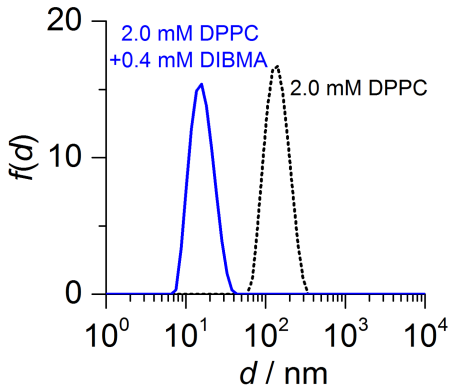

Figure S3. Intensity-weighted particle size distributions, $f(d)$, of fluid-phase DPPC LUVs before and after addition of DIBMA at 45°C.

Thermotropic phase transitions of DMPC in vesicles, DIBMALPs, and SMALPs

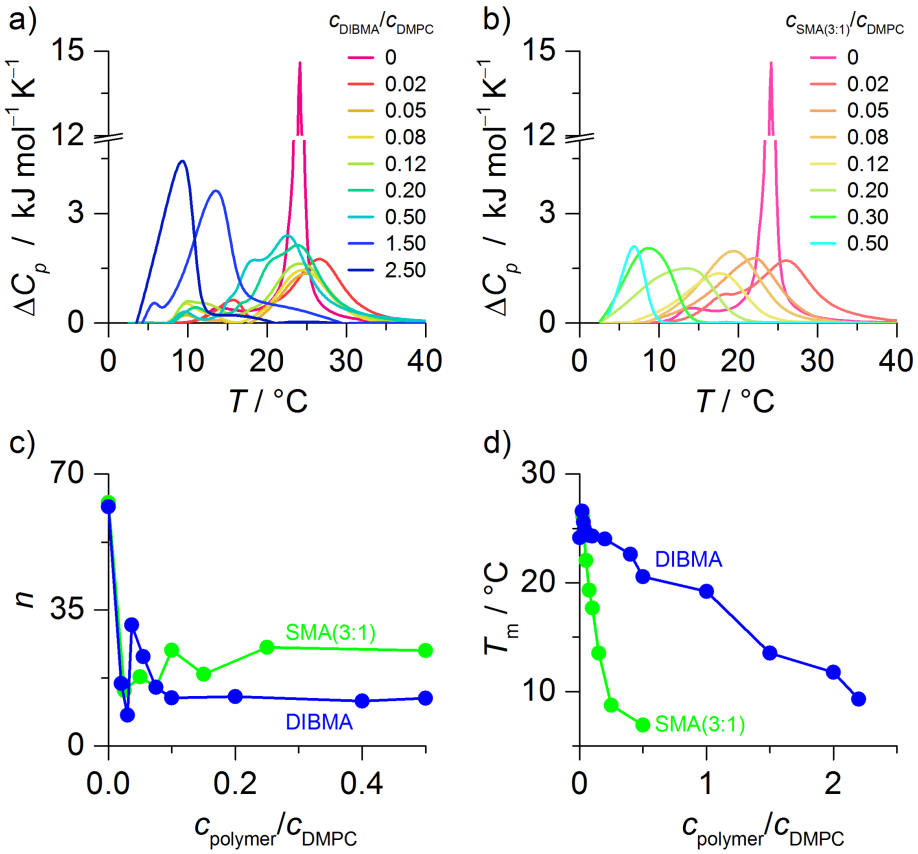

Figure S4. a,b) DSC thermograms of 5 mM DMPC LUVs solubilized by increasing concentrations of DIBMA (panel a) or SMA(3:1) (panel b). c,d) Size of the cooperative unit, n, (panel c) and main phase transition temperature, T_m, (panel d) as functions of the polymer/lipid molar ratio.

Impact of divalent cations on DIBMALPs and SMALPs

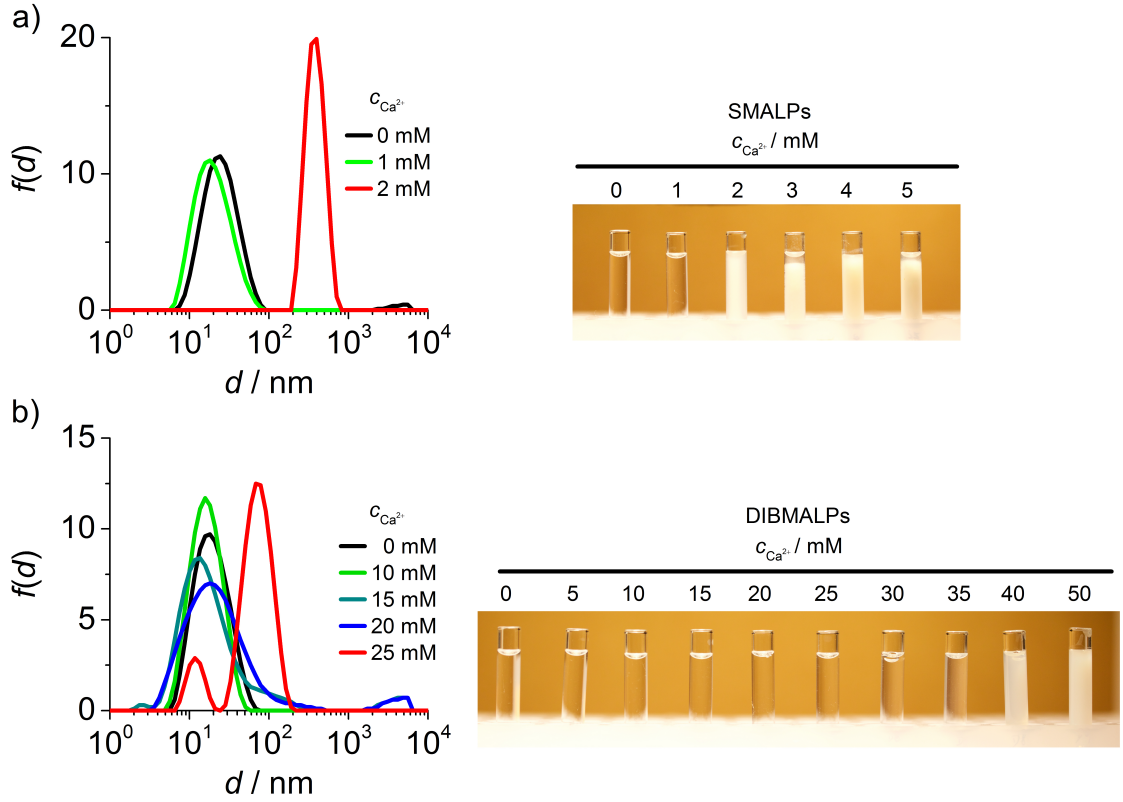

Figure S5. a) Intensity-weighted particle size distributions, $f(d)$, (left) and turbidity (right) of SMALPs (5 mM DMPC, 0.25 mM SMA(3:1) in the presence of increasing concentrations of $CaCl_2$. b) $f(d)$ (left) and turbidity (right) of DIBMALPs (5 mM DMPC, 0.20 mM DIBMA) in the presence of increasing concentrations of $CaCl_2$.

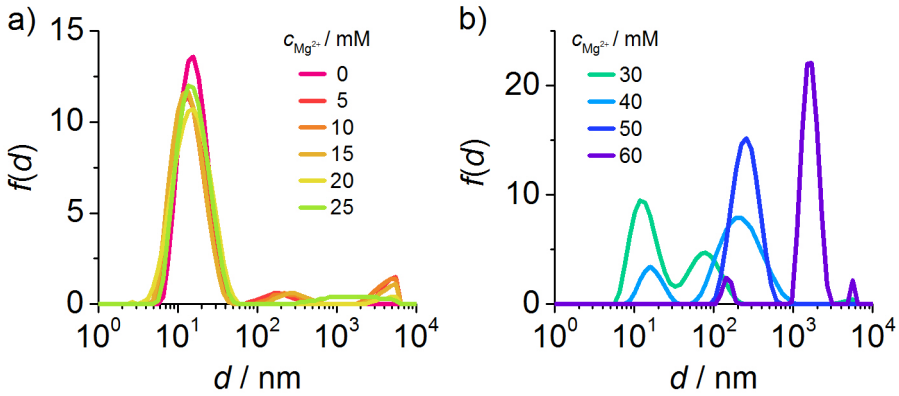

Figure S6. Intensity-weighted particle size distributions, $f(d)$, of DIBMALPs (5 mM DMPC, 0.2 mM DIBMA) in the presence of increasing concentrations of $MgCl_2$, indicating a high tolerance of DIBMALPs against Mg^{2+} concentrations of up to 25 mM (panel a) and precipitation only at higher Mg^{2+} concentrations (panel b).

Far-UV CD spectra of OmpLA in unpurified DIBMALPs and SMALPs

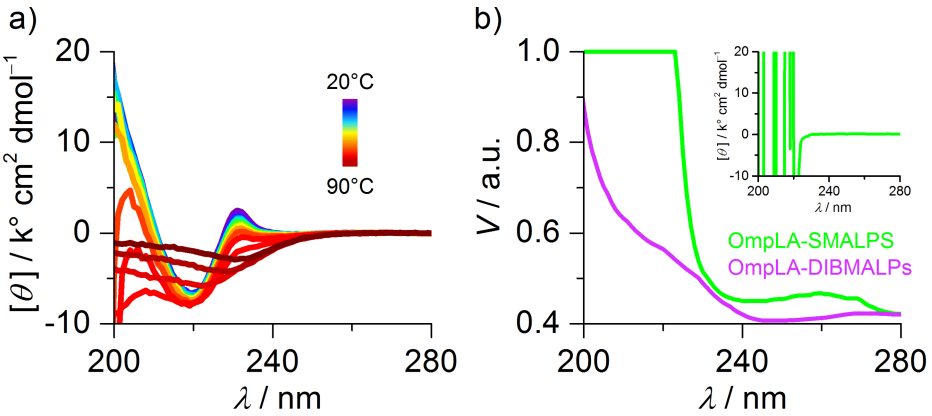

Figure S7. a) CD spectra of 3 µM OmpLA in the presence of excess DIBMALPs (5 mM DLPC, 1 mM DIBMA) demonstrating the compatibility of DIBMA with optical spectroscopy in the far-UV range and the high thermal stability of OmpLA in DIBMALPs. b) Normalized voltage of the CD detector (i.e., avalanche photodiode), *V*, as measured for OmpLA in DIBMALPs (5 mM DLPC, 1 mM DIBMA) or SMALPs (5 mM DLPC, 0.6 mM SMA(3:1)). In the latter case, the strong absorption of SMA(3:1) below 223 nm jeopardized the acquisition of far-UV CD spectra (inset).

Supporting References

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