Bilayer in small bicelles revealed by lipid-protein interactions using NMR spectroscopy

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Supporting Information

Sample preparation

OmpX was expressed, purified, and refolded in dihexanoyl phosphatidylcholine (DHPC) micelles as described in Fernández et al.[1]. The concentration of OmpX was approximately 3 mM. The concentration of DHPC (~300 mM) was verified by comparing the 1D spectrum with the spectrum from a sample containing 100 mM DHPC. A half molar ratio of dimyristoyl phosphatidylcholine (DMPC) (150 mM) was added to the NMR sample. It was vortexed briefly, heated to 42 ^{o}C and cooled in an ice bath. Heating and cooling was repeated until the sample was homogeneous and non-viscous.

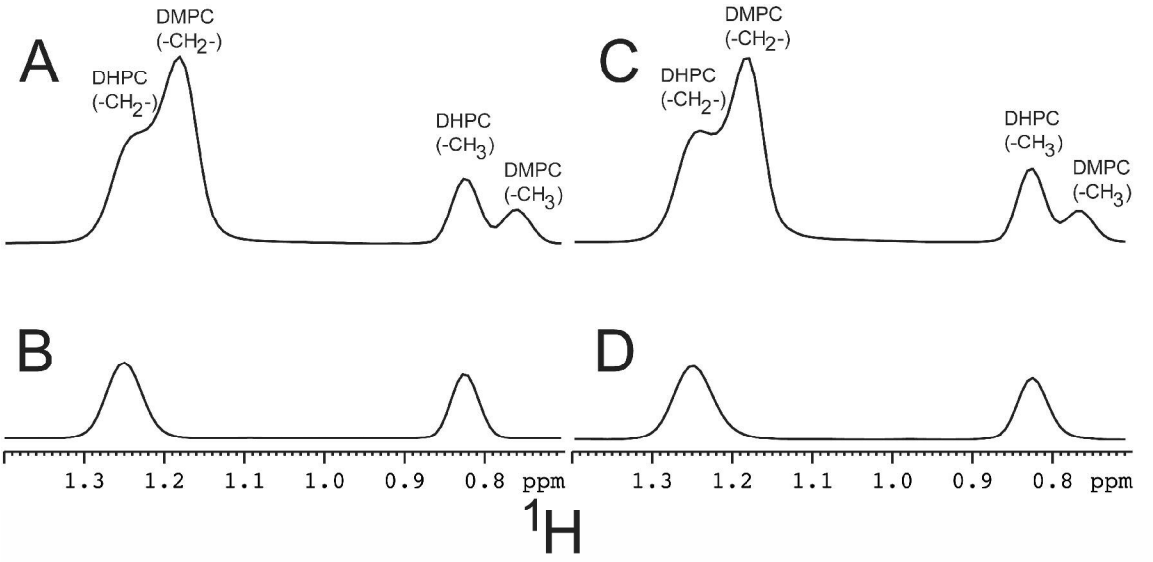

Figure S1. 1D ^{1}H spectra of small bicelles (A and C) and DHPC micelles (B and D) mixed with DHPC and DMPC at 30 ^{o}C (molar ratio [DMPC]/[DHPC]=0.5). The assignments are indicated on the top of spectra. The samples for the spectra of C and D contained also the integral outer membrane protein OmpX from *Escherichia coli* (*E. coli*). The spectra were recorded on a Bruker Avance 400 spectrometer equipped with a triple resonance probe head with an actively shielded z-gradient coil.

Supporting Information

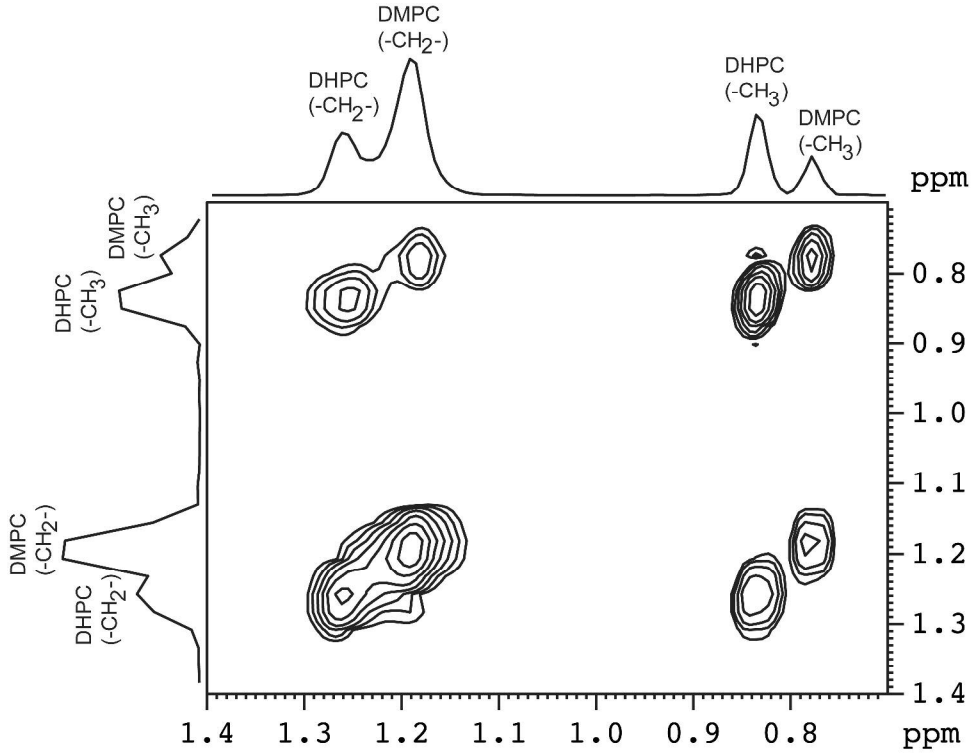

Figure S2. 2D [^{1}H,^{1}H]-TOCSY spectrum of small bicelles composed of DHPC and DMPC (molar ratio [DMPC]/[DHPC]=0.5), including OmpX from *E. coli* at 30 ^{o}C. With clean TOCSY mixing (80 ms) using the MLEV16 sequence,[2] 256 (t_1)*512 (t_2) complex points were accumulated yielding t_{1max}= 32.7 ms and t_{2max} = 65.4 ms. On the top and the left, projected 1D spectra as well as the assignment are shown. The spectrum was recorded on a Bruker AvanceIII 600 spectrometer equipped with a QXI probe head with an actively shielded z-gradient coil.

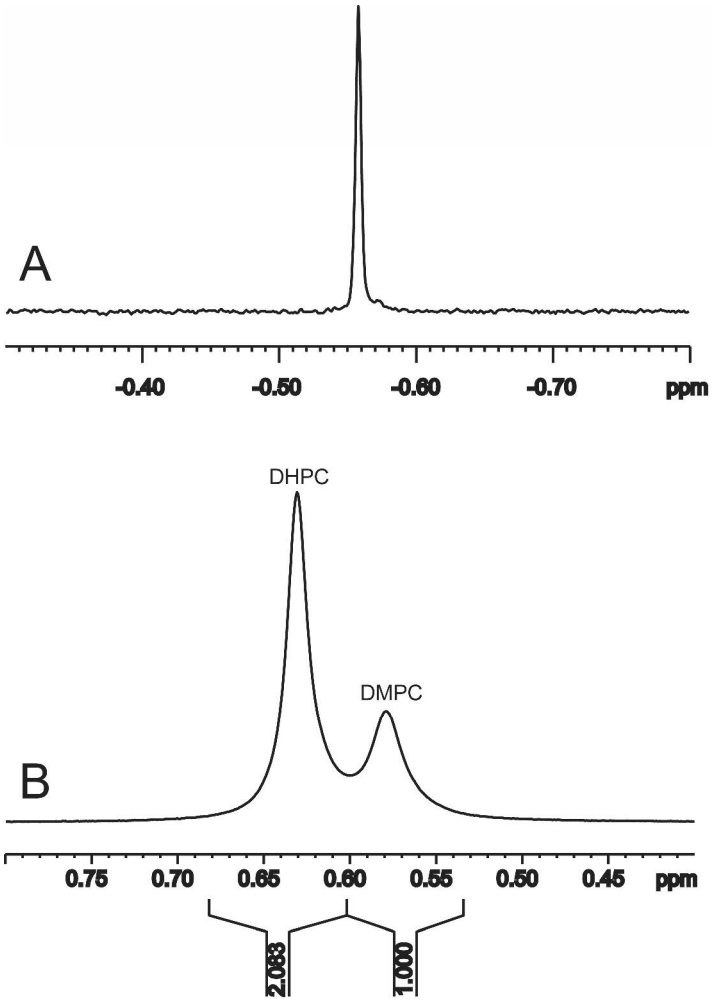

Figure S3. 1D ^{31}P spectrum of (A) mixture of DMPC and DHPC in methanol and (B) small bicelles mixed with DHPC and DMPC, including OmpX from *E. coli* at 30 ^{o}C (molar ratio [DMPC]/[DHPC]=0.5). On the bottom, integrals of the two peaks are shown. The assignment is indicated on the top of the spectrum[3]. The spectrum was recorded on a Bruker Avance DRX-600 spectrometer equipped with a QXI probe head with an actively shielded z-gradient coil.

Supporting information

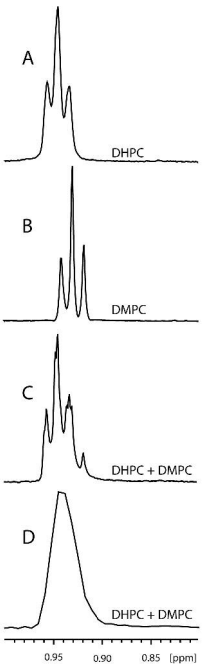

Figure S4. 1D ^{1}H spectra of DHPC (A), DMPC (B), mixtures of DHPC and DMPC (C and D) dissolved in methanol at 20 ^{o}C. Concentrations of DHPC and DMPC were approximately 6 and 2 mM, respectively (A and B). Then, DHPC and DMPC solutions were mixed (C and D). Spectra were recorded with t_{max} = 967 ms for A, B, and C. In order to compare chemical shifts of DHPC and DMPC in bicelles, D was recorded with t_{max} = 121 ms. The spectra were recorded on a Bruker Avance 600 spectrometer equipped with a cyroprobe head with an actively shielded z-gradient coil.

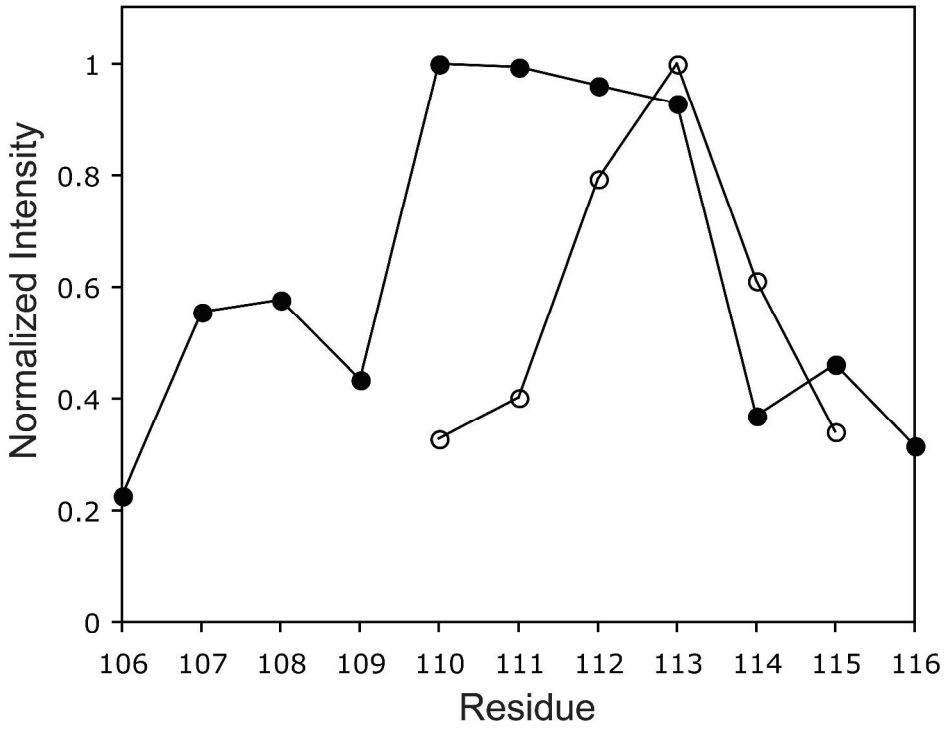

Figure S5. Plot of intermolecular NOE intensity versus residues 106-116. On the y-axis, normalized intensity, which was calculated by dividing the intensity with the maximum intensity, are shown. Open and close circles represent intermolecular NOEs from ω-methyl and methylene protons except α- and β-methylene protons of DMPC, respectively.

Supporting information

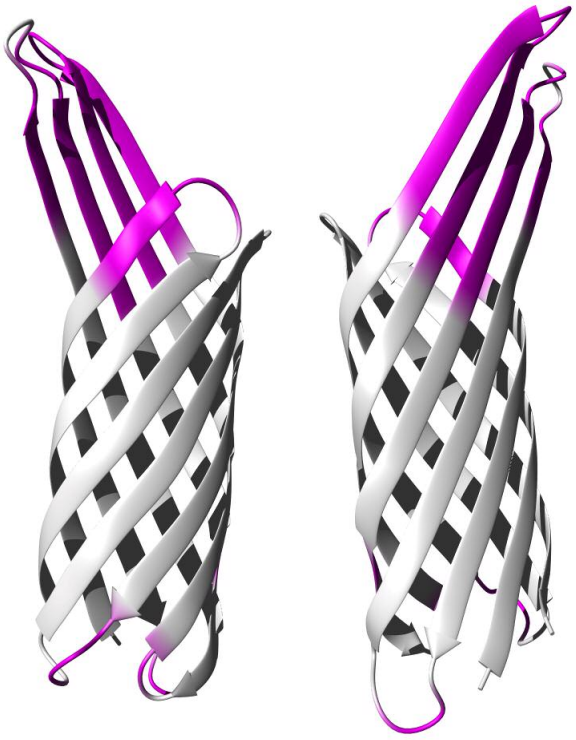

Figure S6. Ribbon drawing of OmpX (PDB access code: 1QJ8[4]) showing paramagnetic relaxation enhancement (PRE). Residues showing PRE higher than 6 mM^{-1} s^{-1} are color-coded in magenta. Similar to Hilty et al.[5], PRE was measured by titration of Gadolinium-diethylene triamine pentaacetic acid into the sample containing OmpX in bicelles using 2D [^{15}N,^{1}H]-TROSY on a 700 MHz spectrometer equipped with a triple-resonance probe head. The orientations of OmpX are the same as in Figure 2A from the publication.

Supporting information

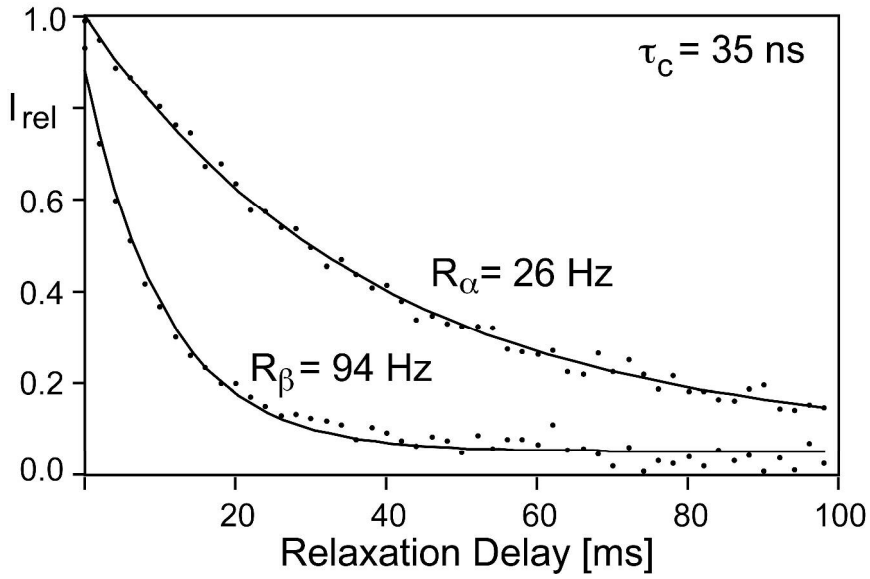

Figure S7. Decay curve of ^{15}N spins from 1D TRACT[6]. The relative intensity of the ^{1}H NMR signal, I_{rel}, was determined from integration of OmpX in the bicelles over the chemical shift range 6.5-10.5 ppm. The upper and lower curves correspond to the slowly relaxing α-spin state of ^{15}N and the more rapidly relaxing β-spin state, respectively. Exponential fits (solid lines) yielded relaxation rates of α- and β-spin states (R_α and R_β) as indicated. Using these rates, the overall rotational tumbling time (τ_c = 35 ns) was estimated.

Supporting information

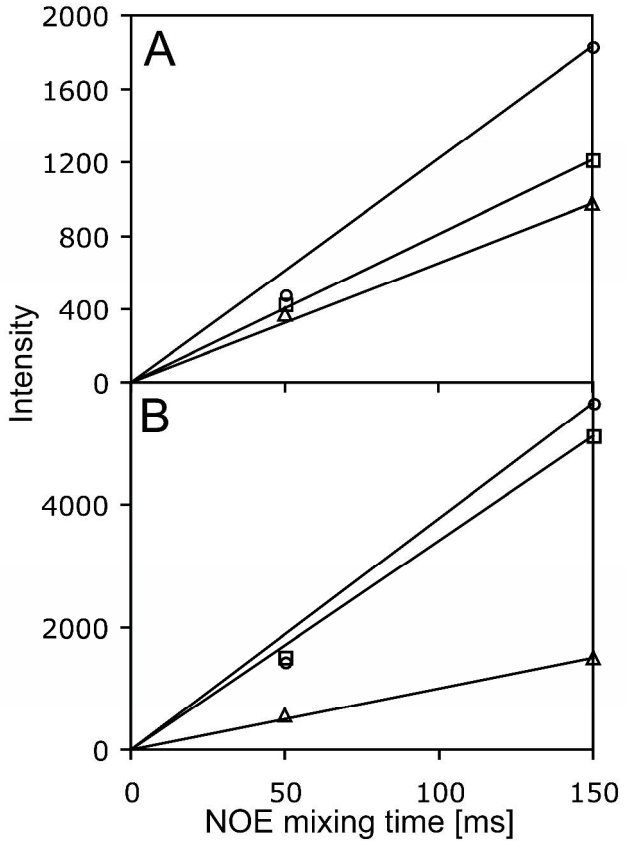

Figure S8. Build-up curves of intermolecular NOEs between the amide protons of OmpX and the hydrophobic tails of DMPC ((A) methyl protons and (B) methylene protons). Circles, squares, and triangles represent NOEs between residues 28, 67, and 114 of OmpX and DMPC. Other NOEs behave almost identical to the presented intermolecular NOEs. Intensity on y-axis is arbitrary unit. Ratios of intensities between methyl and methylene groups of residues 28, 67, and 114 are 0.32, 0.24, and 0.65, respectively.

Supporting information

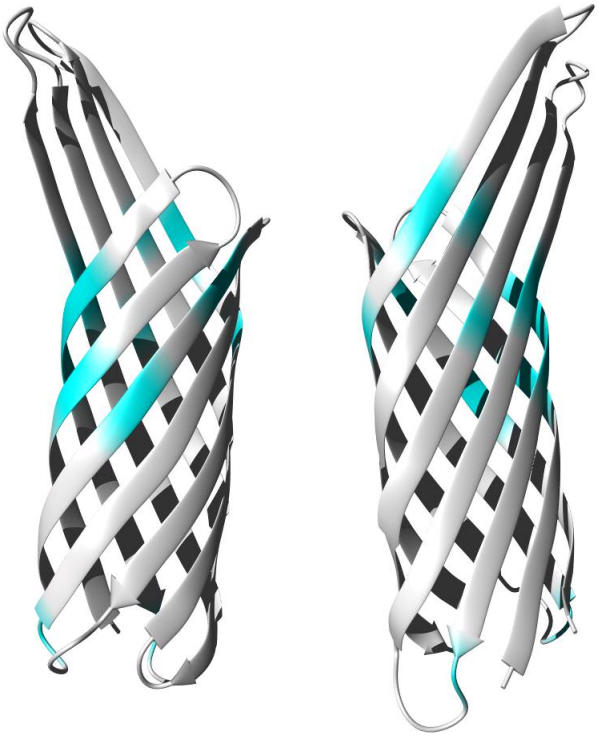

Figure S9. Ribbon drawing of OmpX (PDB access code: 1QJ8[4]). Residues showing intermolecular NOEs between α- and β-methylene protons of lipid and amide protons of OmpX in the ^{15}N-resolved ^{1}H,^{1}H NOESY spectrum with a mixing time of 150 ms are colored in cyan. The orientations of OmpX are the same as in Figure 2A from the publication.

Supporting information

Lipid aggregation number and model of protein-loaded bicelle

The overall rotational correlation time, (τ_c = 35 ns) of OmpX in bicelles was obtained using the 1D TRACT experiment. Comparison of τ_c's of OmpX in bicelles and in DHPC micelles (21 ns[6]) suggests that bicelles consist of approximately 90 and 45 molecules of DHPC and DMPC, respectively.

In order to access the shape information, we have considered the hemistroidal model from Vold and Prosser[7] (Figure S10). Assuming the total thickness of bilayer (2 r = 4.0 nm) and the radius of OmpX (Rp = 1.25 nm), volumes of bilayer and rim ($V_I = 2\ \pi\ r\ R\ (R+ 2\ Rp) = 12.57\ R\ (R+2.5)$ nm^3 and $V_{II} = \pi^2\ r^2\ (R+Rp) + (4\ \pi\ r^3)/3 = 39.48\ (R+1.25) + 33.51$ nm^3) were calculated. The ratio of V_I and V_{II} (V_I/V_{II}) should be linearly correlated with the ratio of individual volumes of DMPC and DHPC[8] (V_{DMPC}(=1.101 nm^3)/V_{DHPC}(=0.6452 nm^3) = 1.7064). Thus, $V_I/V_{II} = q\ V_{DMPC}/V_{DHPC}$ = 0.8532; q is a molar ratio between DMPC and DHPC of 0.5). From this ratio, the width of the bilayer (R = 2.46 nm) was obtained. Based on this width, approximately 140 and 280 molecules of DMPC and DHPC could be deduced to make a protein-loaded bicelle. However, this is unrealistic because the molecular weight of this model (18 (OmpX) + 140*0.678 (DMPC) + 280*0.482 (DHPC) = 248 kDa) does not fit with τ_c = 35 ns. Thus, we have slightly modified this hemstroidal model by replacing a half circle at the rim with a half ellipse (similar to prolate (aspect ratio = 2)) shown in Figure S11. From the modified model, we deduced widths of bilayer (1.0 nm) and rim (1.0 nm) and how many molecules of DMPC and DHPC a bicelle is composed of (approximately 46 and 92 molecules of DMPC and DHPC). This agrees well with τ_c=35 ns. Moreover, the total width of this modified disk (3.25 nm) fits very well with the radius (R_h=3.6 nm) obtained from the translational self-diffusion coefficient of OmpX-loaded bicelles ($D_0 = 5.95 \times 10^{-11}$ m^2/s) at 30 ^oC by comparison of $D_0^{lysozyme}$ of lysozyme (13.28×10^{-11} m^2/s) with the same buffer condition to OmpX in bicelles at 30 ^oC ($R_h = (D_0^{lysozyme}/D_0)\ R_{lysozyme}$; $R_{lysozyme}$=1.6 nm)[9].

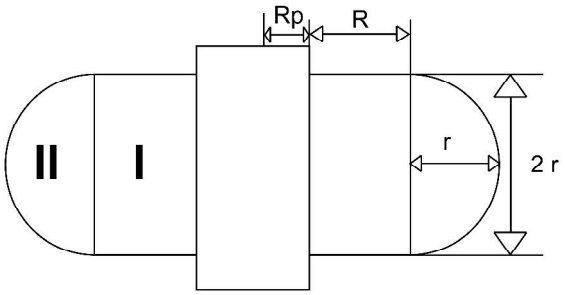

Figure S10. Hemistroidal model of protein-loaded bicelles[7]. The widths of protein, bilayer, and rim are marked as Rp, R, and r, respectively.

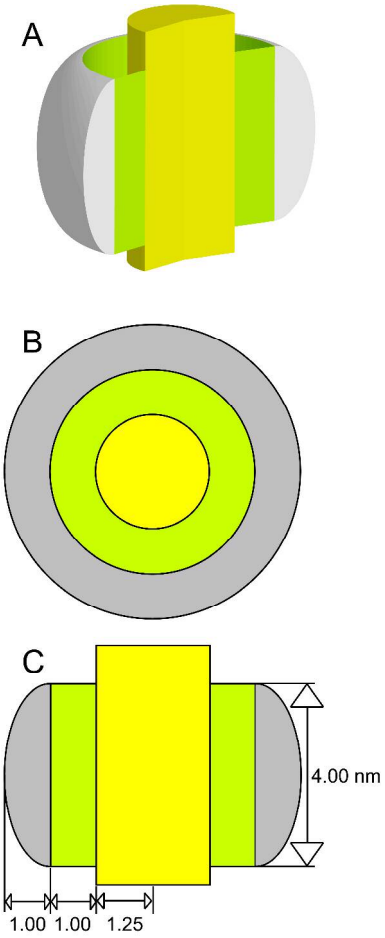

Figure S11. Model of OmpX-loaded bicelles with a molar ratio between DMPC and DHPC of 0.5. (A) 3D and 2D models viewed from (B) top and (C) side are shown. Protein, bilayer, and rim are color-coded in yellow, green, and grey, respectively. The widths of protein, bilayer, and rim are given in nm.

Reference

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