Supporting Information

Overcoming hysteresis to attain reversible equilibrium folding for outer membrane phospholipase A in phospholipid bilayers

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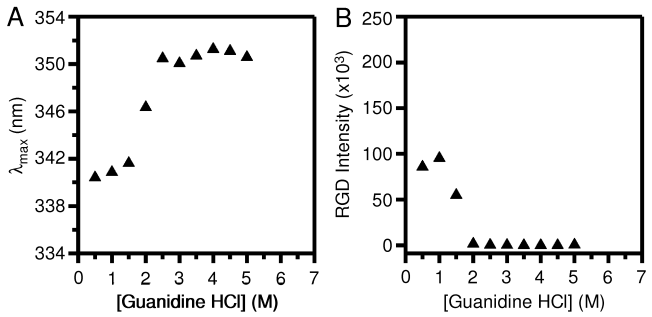

Fig. S1. Aggregates of OmpLA can be observed in the absence of LUVs. Shown are fluorescence emission results of an "aggregation" titration. The excitation wavelength for all protein fluorescence samples was 295 nm. (A) Values of λ_{max} (▲) for an "aggregation" titration of OmpLA in the absence of any lipid or detergent. Except for the lack of lipid or detergent, the titration was otherwise prepared according to the folding scheme in (Fig. 1A). (B) RGD light scattering at 295 nm for the same aggregation titration without LUVs shown in (A).

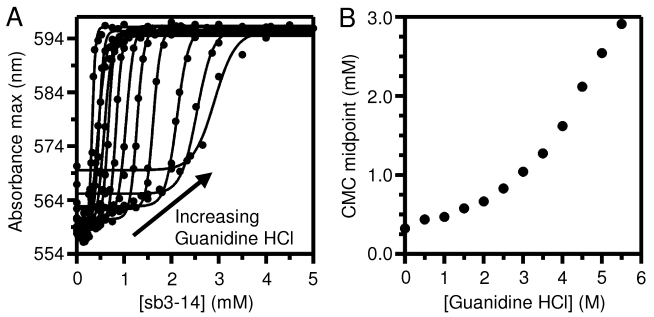

Fig. S2. Critical micelle concentration of SB3-14 increases with increasing concentrations of guanidine HCl. (A) Wavelength position of maximum absorbance intensity of Coomassie R-250 dye after it was added to titrations of SB3-14, where each titration was at a different final concentration of guanidine HCl. Solid lines represent fits of a sigmoid function to each titration's data set. (B) The concentration of SB3-14 at the midpoint of each sigmoid fit in (A) is plotted against the final concentration of guanidine HCl in the respective titration.

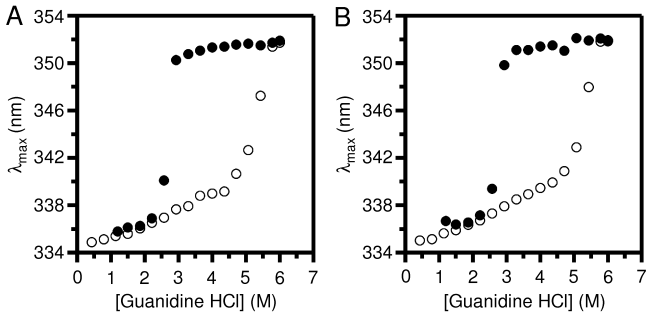

Fig. S3. Folding and unfolding of OmpLA remain irreversible, even when the lipids are prepared in different macromolecular structures. The excitation wavelength for all fluorescence samples was 295 nm. All titrations shown were prepared according to the scheme shown in Fig. 2A with incubation at 37° C and at pH 8.0 for 40 hours, except that no LUVs were used. (A) SUVs of DLPC were used instead of LUVs. (B) Bicelles of DLPC and diC_6PC (q = 3) were used instead of LUVs.

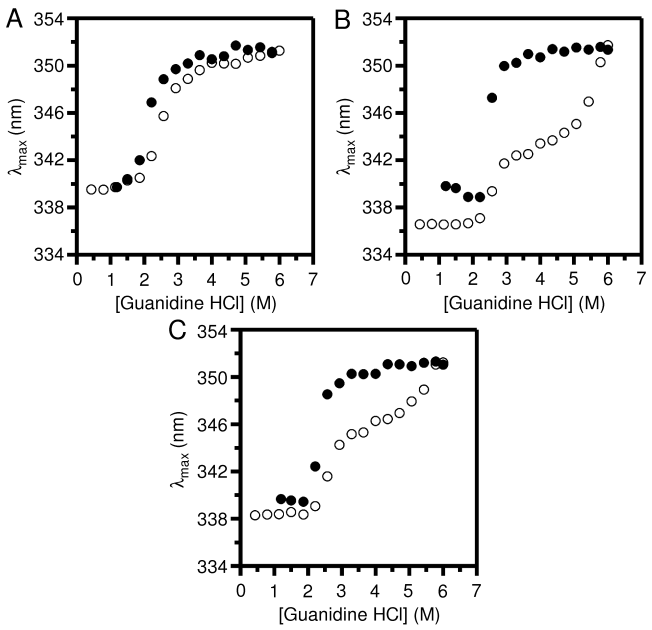

Fig. S4. Folding and unfolding of OmpLA remain irreversible, even with ionic lipid headgroups present in the LUVs. The excitation wavelength for all fluorescence samples was 295 nm. All titrations shown were prepared according to the scheme shown in Fig. 2A with incubation at 37° C and at pH 8.0 for 40 hours, except that 25% of the DLPC lipids were exchanged with a guest lipid having a different headgroup. (A) Guest lipid was the anionic DLPG. (B) Guest lipid was the anionic DLPS. (C) Guest lipid was the cationic 12:0 EPC.

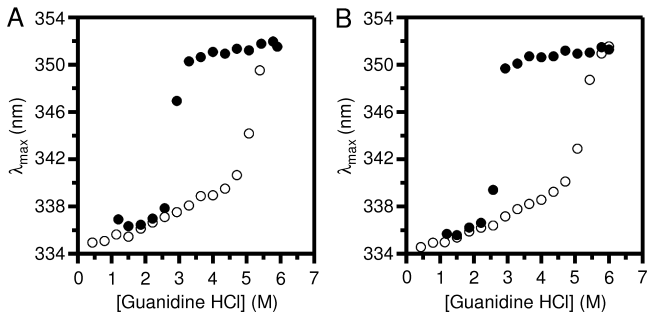

Fig. S5. Folding and unfolding of OmpLA remain irreversible, even when mixed with different salts. The excitation wavelength for all fluorescence samples was 295 nm. All titrations shown were prepared according to the scheme shown in Fig. 2A with incubation at 37° C and at pH 8.0 for 40 hours, except that different salts were also included in the buffer. (A) 400 mM KCl was included. (B) 20 mM $MgCl_2$ was included.

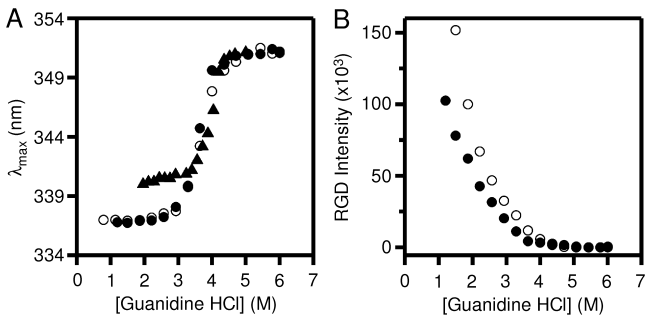

Fig. S6. At pH 3.8, there is remaining hysteresis between the folding and unfolding titrations of OmpLA. The excitation wavelength for all fluorescence samples was 295 nm. (A) Wavelength position of maximum fluorescence intensity (λ_{max}) for samples of OmpLA in folding (●) and unfolding (○) titrations with LUVs of DLPC at 37° C and at pH 3.8 after 40 hours. Values of λ_{max} (▲) for an "aggregation" titration of OmpLA in the absence of any lipid or detergent and at at 37° C and at pH 3.8 after 40 hours. Except for the lack of lipid or detergent, the titration was otherwise prepared according to the folding scheme in Fig. 2A. (B) Rayleigh-Gans-Debye (RGD) light scattering at 295 nm for the same folding and unfolding titrations shown in (A).

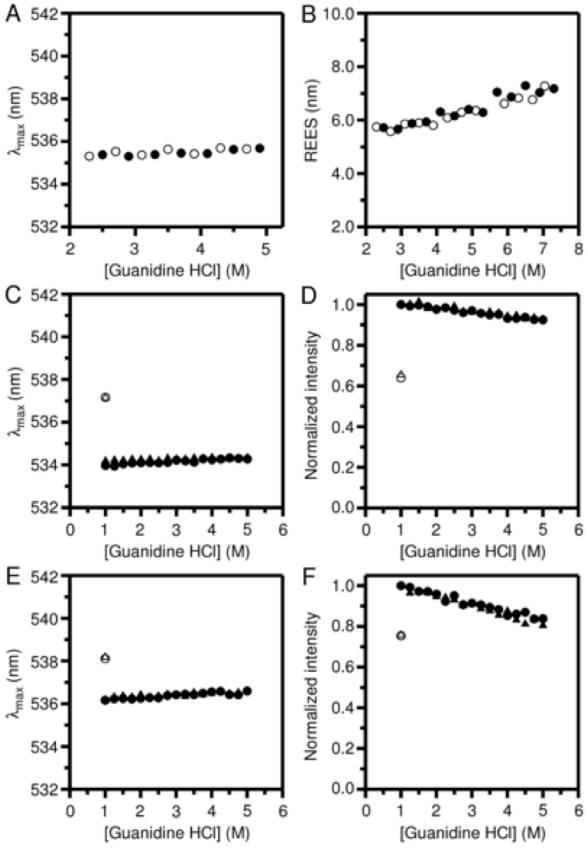

Fig. S7. LUVs are reversible with guanidine HCl and are not solubilized by the guanidine or the SB3-14 detergent when it is below its CMC. The environment around an NBD-label appeared reversible between folding and unfolding titrations, and it is not affected by SB3-14 at the low concentration used in our experiments with OmpLA. Fluorescence excitation wavelength was 465 nm. (A) Wavelength position of maximum fluorescence intensity (λ_{max}) for samples in folding (●) and unfolding (○) titrations that were prepared according to the scheme in Fig. 4A with incubation at 37° C and at pH 3.8 for 40 hours, except that no OmpLA was added and 0.1% of the DLPC was replaced with the guest lipid 14:0-12:0 NBD PC. (B) Red edge excitation shift (REES) for samples in folding"(●) and unfolding (○) titrations that were prepared according to the scheme in Fig. 4A, except that no OmpLA was added and 0.1% of the DLPC was replaced with the guest lipid 14:0-12:0 NBD PC. Fluorescence emission intensity was 520 nm. (C) Wavelength position of maximum fluorescence intensity (λ_{max}) for samples in two folding titrations that were prepared according to the scheme in Fig. 4A where one included 0.09 μM SB3-14 in each sample (▲) and the second did not have any SB3-14 added to any sample (●). For each titration, the sample at 1.0 M guanidine HCl was then mixed with enough SB3-14 to

reach a final concentration of 15 mM in order to act as a control for when the SB3-14 was above its CMC. These control samples are shown with open symbols: the open triangle (△) represents the sample initially having 0.09 μM SB3-14, and the open circle (○) represents the sample from the sample initially having no SB3-14. (D) Fluorescence emission intensity at 520 nm for the same samples in (C). (E) Same as (C), except the guest lipid was 14:0 NBD PE. (F) Fluorescence emission intensity at 520 nm for the same samples in (E).

Materials and Methods relevant to Supporting Information

Fluorescence using NBD-labeled lipids

For emission readings of the NBD-labeled lipids, we used the same protocols as for tryptophan emission except that the excitation and emission wavelengths were different. For full emission scans, the excitation wavelength was 465 nm and the emission range was 450 nm to 570 nm. For emission intensity measurements, the emission wavelength was 520 nm.

Measuring REES

The red edge excitation shift (REES) is defined as the shift in the peak fluorescence emission position (λ_{max}) of a fluorophore upon changing the excitation wavelength from a position near the peak of the fluorophore's absorbance spectra to a position on the red tail or edge of the fluorophore's absorbance spectra [S1]. For each sample of our NBD-labeled lipids, we measured the λ_{max} from an excitation wavelength of 465 and again form an excitation wavelength of 515 nm and then used the difference in those two λ_{max} values as the REES for the sample.

Coomassie dye binding assay

We used a version of the Coomassie dye binding assay described by Kleinschmidt *et al.* [S2] to measure the CMC of SB3-14 in various guanidine HCl concentrations. We prepared a concentrated stock of Coomassie by dissolving part of a solid tablet of Coomassie Brilliant Blue R-250 (Pierce) in water. We then added 10 μL of that Coomassie stock to 990 μL of an appropriate mixture of SB3-14 and guanidine HCl for each sample in our detergent titrations. We measured an absorbance for each sample using a Beckman DU 700 spectrometer from 500 nm to 650 nm and determined the absorbance peak. The spectrometer was blanked on the 990 μL sample of detergent and guanidine prior to the addition of the Coomassie.

Refined mechanics of mixing and dilution

The refined mechanics that we incorporated into the experiments shown in Fig. 4 compared to the experiments shown in previous figures included special attention to mixing and dilution. Specifically, we lowered the protein concentration in the initial dilution step with the detergent to 6.0 μM and then immediately diluted that sample three-fold into the LUVs. Since the dilution from the 6.0 μM protein to the folded sample at 2.0 μM was only three-fold, we also

reduced the SB3-14 concentration to 1.4 mM for the 6.0 μM protein sample in order to keep the detergent well below its CMC after dilution into the LUVs. Further, we again increased the guanidine HCl concentration in the initial 6.0 μM protein sample and the subsequent 2.0 μM folded protein sample with LUVs. We chose to use 2.5 M guanidine HCl as the highest concentration that would still promote efficient folding of OmpLA according to the results shown in Fig. S6A

We also made two other important changes to the mechanics of our experiments that we believe helped prevent protein aggregation. First, we emphasized fast and thorough mixing of the samples when we prepared dilutions. Slow or insufficient mixing could lead to locally high concentrations of species dwelling long enough to interact before they have a chance to diffuse away from each other. Concentrated stocks of protein, guanidine HCl, and LUVs are all quite viscous and they do not appear to mix quickly when pipetted by hand, especially when sample volumes exceed several milliliters. Therefore, we used mini magnetic stir bars and vortexing machines to support mixing. Our general strategy was to mix with a stir plate or vortex setting that was vigorous enough to dissipate any schlieren patterns in less than one second and yet not vigorous enough to cause bubbles to form at a sample's meniscus.

Our second change in mechanics was to slow down the dilution of the 6.0 μM protein sample into the LUVs. We took at least 20 minutes to drip the necessary volume of the 6.0 μM protein sample into the dilution buffer containing the LUVs. We paused after each drip to allow thorough mixing.

Supporting References

[S1] H. Raghuraman, S. Shrivastava, A. Chattopadhyay, Monitoring the looping up of acyl chain labeled NBD lipids in membranes as a function of membrane phase state, Biochimica et biophysica acta, 1768 (2007) 1258-1267.

[S2] J.H. Kleinschmidt, M.C. Wiener, L.K. Tamm, Outer membrane protein A of E. coli folds into detergent micelles, but not in the presence of monomeric detergent, Protein Sci, 8 (1999) 2065-2071.