Supplemental information for:

Negative charge neutralization in the loops and turns of outer membrane phospholipase A impacts folding hysteresis at a neutral pH

Sarah K. McDonald and Karen G. Fleming

Correspondence to Karen G. Fleming, Karen.Fleming@jhu.edu, 1-410-516-7256

Author affiliations

T.C. Jenkins Department of Biophysics

Johns Hopkins University

3400 North Charles Street

Baltimore, MD 21218

Supplementary Figures

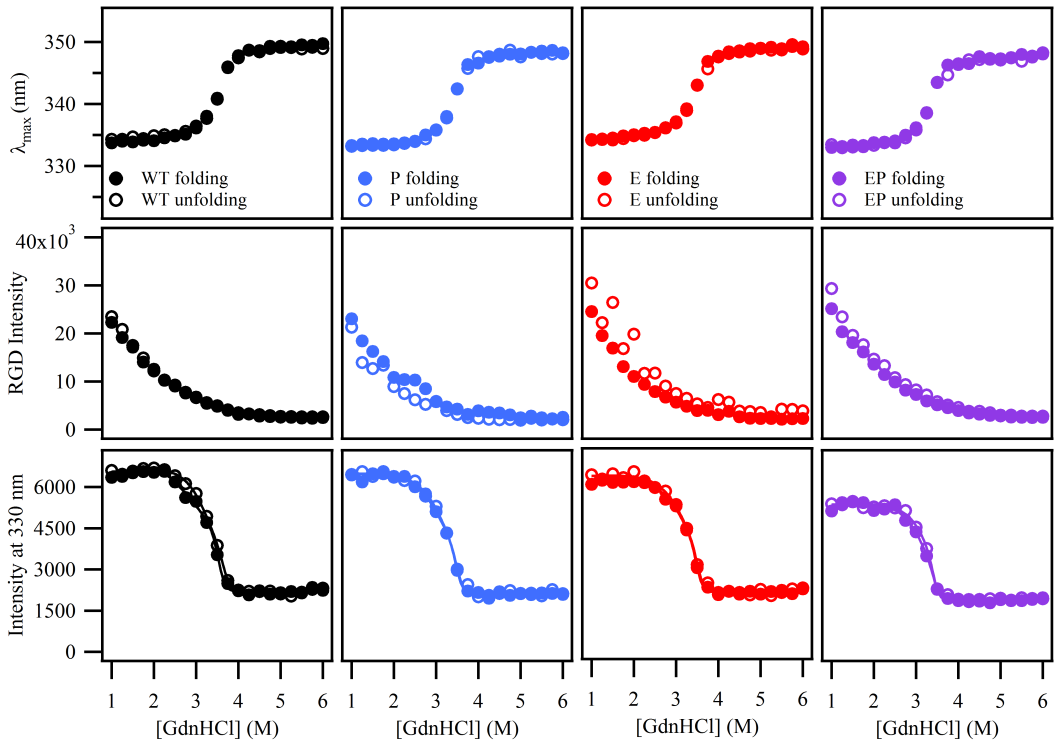

Fig. S1. All loop and turn variants fold reversibly at pH 3.8. Three parameters are shown for GdnHCl titrations; the top panels show the wavelength of maximum emission (λ_{max}) of tryptophan fluorescence emission spectra, the middle panels show RGD scattering, and the bottom panels show the intensity at 330 nm. Shown in black is WT as a reference, P is in blue, E in red, and EP in purple. Closed circles are folding titrations and open circles are unfolding titrations. Excellent agreement between all parameters is shown for these variants. Intensities at 330 nm for the two titration directions were fit to the linear extrapolation method modified to include an intermediate state as previously derived. [1] During fitting the m-values were held to previously determined values.[2] The folding free energies were found to be -31.5 ± 0.5, -32.0 ± 0.2, and -31.8 ± 0.4 kcal mol^{-1} for the P, E, and EP variants respectively.

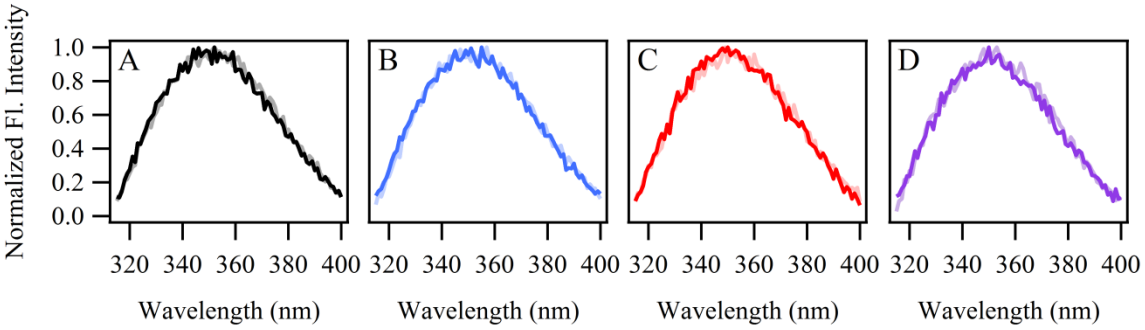

Fig. S2. Unfolded loop and turn variants are off the membrane. Shown are representative fluorescence spectra for unfolded variants at pH 8 in 6 M GdnHCl with (dark lines) and without (light lines) LUVs. Panels A, B, C and D are WT (black), P (blue), E (red), and EP (purple) variants respectively.

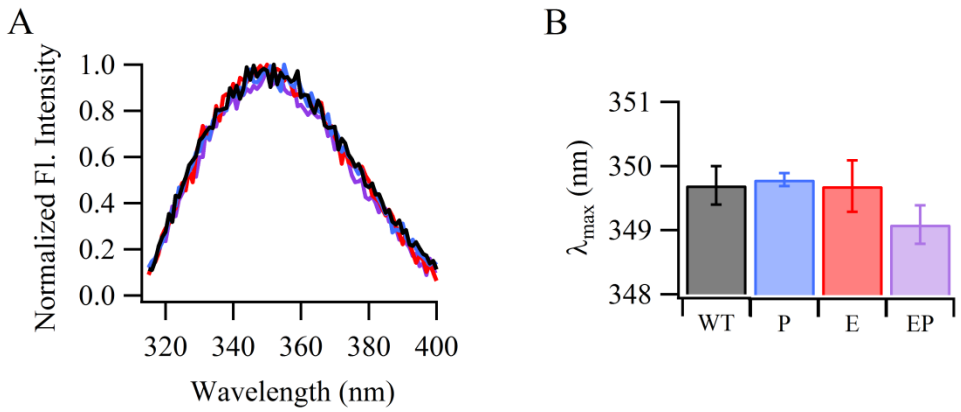

Fig. S3. Loop and turn variants display the same unfolded spectra as WT. Shown in panel A are representative unfolded fluorescence spectra at pH 8 in 6 M GdnHCl for WT (black), P (blue), E (red), and EP (purple) variants. Also shown in panel B are the average λ_{max} for unfolded variants at pH 8 (n=4). Error bars are the standard deviations from these measurements.

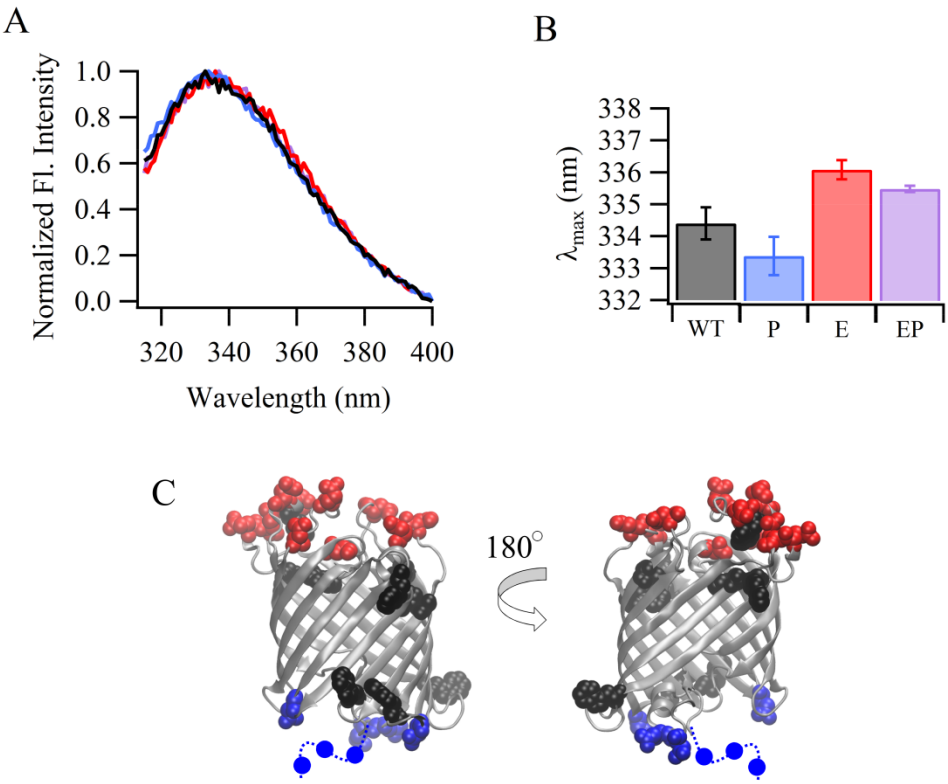

Fig. S4. Fluorescence spectra of OmpLA variants with mutations in the extracellular loops are shifted a few nm to higher wavelengths. Shown in panel A are representative fluorescence spectra of WT (black), P (blue), E (red), and EP (purple) variants folded at pH 8 in 1 M GdnHCl. Panel B shows the average λ_{max} for folded variants (n=4). Error bars are the standard deviation. In panel C the WT OmpLA crystal structure (1qd5) is shown with Trp (black) and Asp/Glu in extracellular loops (red) or periplasmic turns (blue) in sphere representation. This panel C was made with VMD [2].

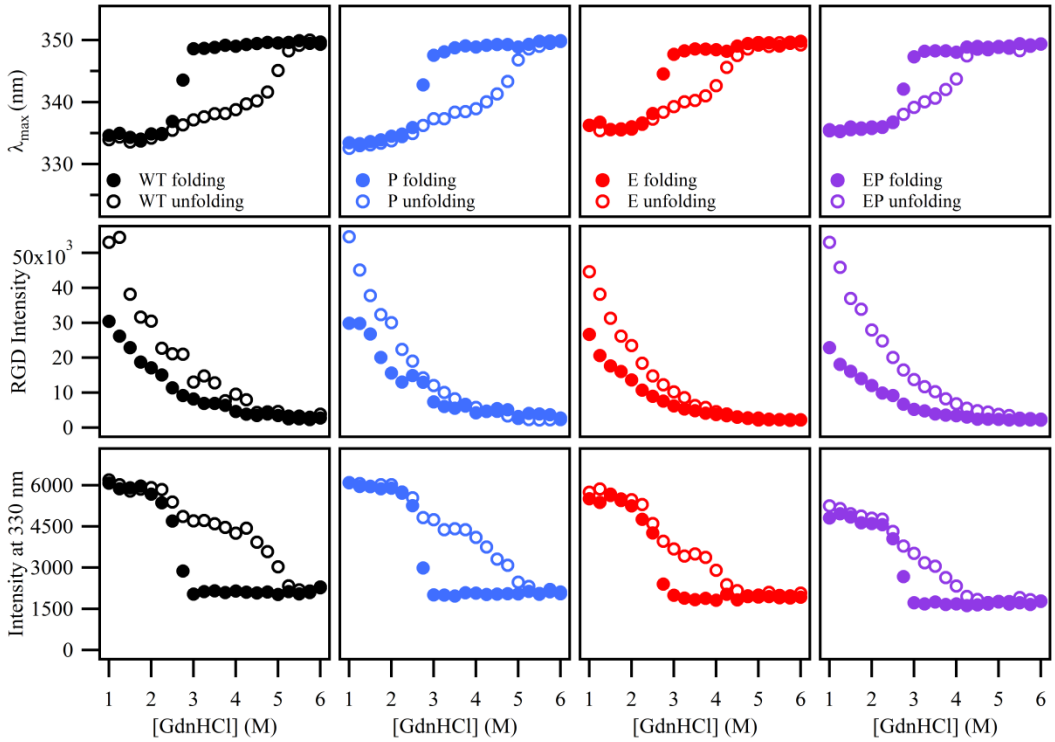

Fig. S5. Folding hysteresis is reduced is reduced in loop and turn variants relative to WT. Three parameters are shown for GdnHCl titrations; the top panels show the wavelength of maximum emission (λ_{max}) of tryptophan fluorescence emission spectra, the middle panels show RGD scattering, and the bottom panels show the intensity at 330 nm. Shown in black is WT as a reference, P is in blue, E in red, and EP in purple. Closed circles are folding titrations and open circles are unfolding titrations. Reduction in hysteresis is observed in both λ_{max} and intensity at 330 nm. Very similar RGD scattering is observed between all variants.

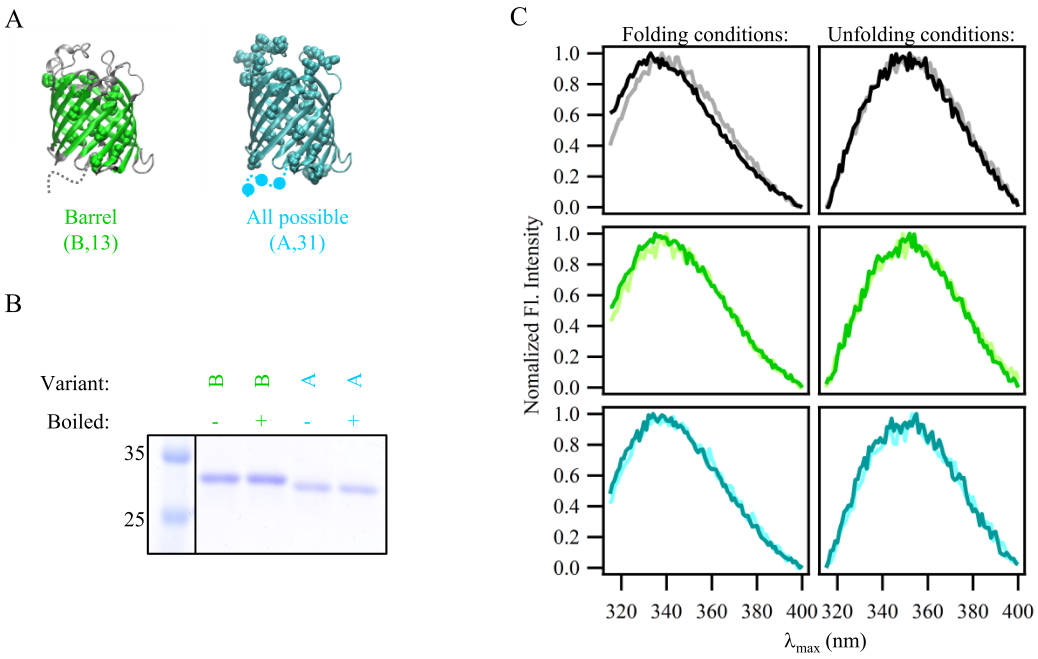

Fig. S6. OmpLA variants with charge mutations that point inside the barrel do not fold. (A) Two variants are shown that both have the same mutations in the barrel region shown in sphere representation. The A variant has the same mutations within the barrel as the B variant, but also the same mutations as the EP variant (Fig. 1). Because the EP variant can fold, this experiment demonstrates the negatively charged side chains that point inside the barrel are required for folding. Disordered regions in the crystal structure 1qd5 [3] are shown as dashed lines and mutations are shown as circles. These images were made with VMD [4]. (B) SDS-PAGE gel showing these variants do not adopt folded conformations without (-) boiling. All samples were subjected to folding conditions of 1 M GdnHCl, 20 mM TRIS (pH 8), 2 mM EDTA and at a 2000:1 DLPC to protein ratio. (C) Fluorescence spectra under folding (1 M GdnHCl, pH 8) and unfolding conditions (6 M GdnHCl, pH 8) B (green) and A (cyan) either with or without LUVs compared with WT (black). Spectra with LUVs are shown as the darker lines and without LUVs are shown as the lighter lines. This experiment shows that B and A variants are aggregating in titrations and not folding into LUVs.

Supplementary Tables

Table S1. Site numbers for charge neutralization mutations in OmpLA variants.

Variant	Asp→Asn residue #	Glu→Gln residue #	# total mutations
P	10, 27, 125, 205	2, 7, 25	7
E	47, 57, 149, 183, 184, 187, 251	51, 60, 104, 105	11
EP	10, 27, 47, 57, 149, 125, 183, 184, 187, 205, 251	2, 7, 25, 51, 60, 104, 105	18
B	36, 65, 135, 143, 267	66, 111, 115, 137, 165, 172, 224, 247	13
A	10, 27, 36, 47, 57, 65, 149, 125, 135, 143, 183, 184, 187, 205, 251, 267	2, 7, 25, 51, 60, 66, 104, 105, 111, 115, 137, 165, 172, 224, 247	31

Table S2. Amino acid sequences of OmpLA variants.

Variant	Sequence
P	MAQQATVKQVHNAPAVRGSIIANMLQQHNNPFTLYPYDTNYLIYTQTSDLNKEAI ASYDWAENARKDEVKFQLSLAFPLWRGILGPNSVLGASYTQKSWWQLSNSEESSPFR ETNYEPQLFLGFATNYRFAGWTLRDVEMGYNHDSNGRSDPTSRSWNRLYTRLMAE NGNWLVEVKPWYVVGNTDDNPDITKYMGYYQLKIGYHLGNAVLSAKGQYNWN TGYGGAELGLSYPITKHVRLYTQVYSGYGESLIDYNFNQTRVGVGVMLNDLF
E	MAQEATVKEVHDAPAVRGSIIANMLQEHDNPFTLYPYDTNYLIYTQTSNLNKQAIAS YNWAQNARKDEVKFQLSLAFPLWRGILGPNSVLGASYTQKSWWQLSNSQQSSPFR ETNYEPQLFLGFATDYRFAGWTLRDVEMGYNHDSNGRSNPTSRSWNRLYTRLMAE NGNWLVEVKPWYVVGNTNNNPNITKYMGYYQLKIGYHLGDAVLSAKGQYNWN TGYGGAELGLSYPITKHVRLYTQVYSGYGESLINYNFNQTRVGVGVMLNDLF
B	MAQEATVKEVHDAPAVRGSIIANMLQEHDNPFTLYPYNTNYLIYTQTSDLNKEAIAS YDWAENARKNQVKFQLSLAFPLWRGILGPNSVLGASYTQKSWWQLSNSEESSPFRQ TNYQPQLFLGFATDYRFAGWTLRNVQMGYNHNSNGRSDPTSRSWNRLYTRLMA QNGNWLVQVKPWYVVGNTDDNPDITKYMGYYQLKIGYHLGDAVLSAKGQYNW NTGYGGAQLGLSYPITKHVRLYTQVYSGYGQSLIDYNFNQTRVGVGVMLNNLF
EP	MAQQATVKQVHNAPAVRGSIIANMLQQHNNPFTLYPYDTNYLIYTQTSNLNKQAI ASYNWAQNARKDEVKFQLSLAFPLWRGILGPNSVLGASYTQKSWWQLSNSQQSSP FRETNYEPQLFLGFATNYRFAGWTLRDVEMGYNHDSNGRSNPTSRSWNRLYTRLM AENGNWLVEVKPWYVVGNTNNNPNITKYMGYYQLKIGYHLGNAVLSAKGQYNW NTGYGGAELGLSYPITKHVRLYTQVYSGYGESLINYNFNQTRVGVGVMLNDLF
A	MAQQATVKQVHNAPAVRGSIIANMLQQHNNPFTLYPYNTNYLIYTQTSNLNKQAI ASYNWAQNARKNQVKFQLSLAFPLWRGILGPNSVLGASYTQKSWWQLSNSQQSS PFRQTNYQPQLFLGFATNYRFAGWTLRNVQMGYNHNSNGRSNPTSRSWNRLYTR LMAQNGNWLVQVKPWYVVGNTNNNPNITKYMGYYQLKIGYHLGNAVLSAKGQ YNWNTGYGGAQLGLSYPITKHVRLYTQVYSGYGQSLINYNFNQTRVGVGVMLNN LF

Table S3. Percent hysteresis quantitated from the area between folding and unfolding titrations.

Variant	% Area normalized to WT	Standard error
WT	100	3.3
P	83	5.1
E	46	6.4
EP	39	7.4

Literature Cited in Supplementary Information

[1] McDonald SK, Fleming KG. Aromatic Side Chain Water-to-Lipid Transfer Free Energies Show a Depth Dependence across the Membrane Normal. J Am Chem Soc. 2016;138:7946-50.

[2] Moon CP, Fleming KG. Side-chain hydrophobicity scale derived from transmembrane protein folding into lipid bilayers. Proc Natl Acad Sci U S A. 2011;108:10174-7.

[3] Snijder HJ, Ubarretxena-Belandia I, Blaauw M, Kalk KH, Verheij HM, Egmond MR, et al. Structural evidence for dimerization-regulated activation of an integral membrane phospholipase. Nature. 1999;401:717-21.

[4] Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. J Mol Graph. 1996;14:33-8, 27-8.