Dramatic destabilization of transmembrane helix interactions by features of natural membrane environments

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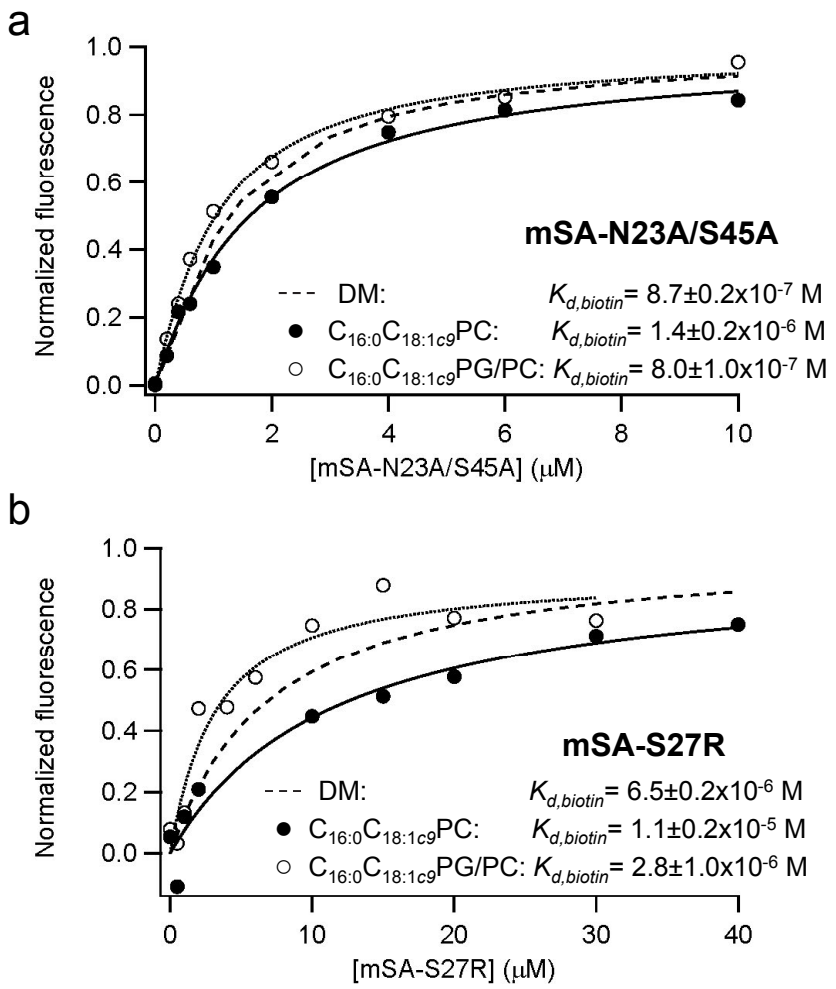
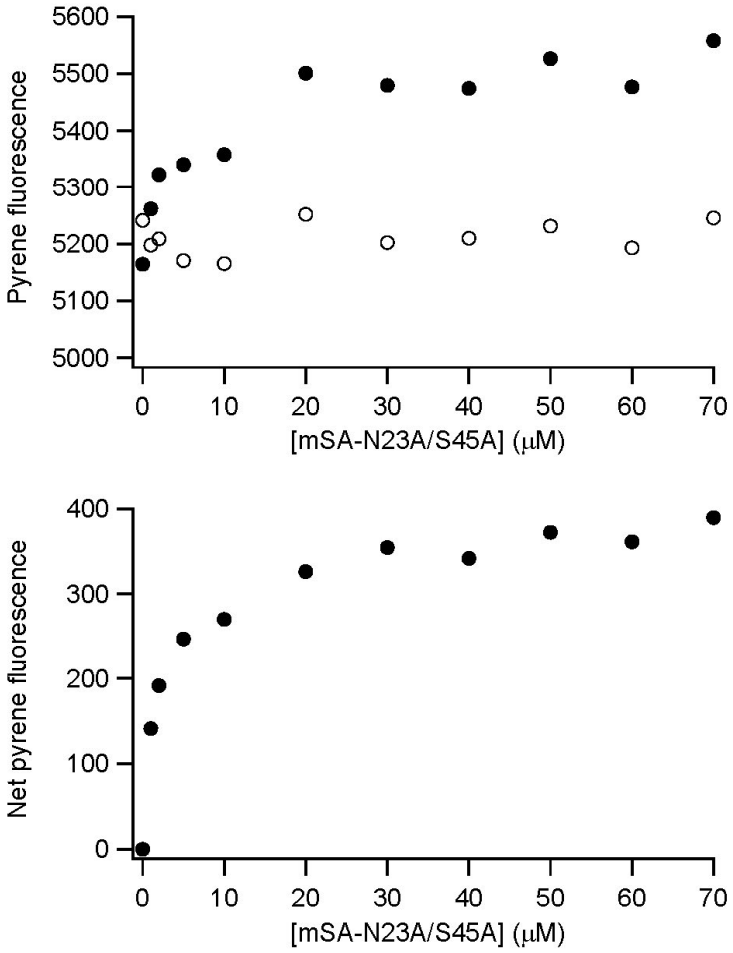
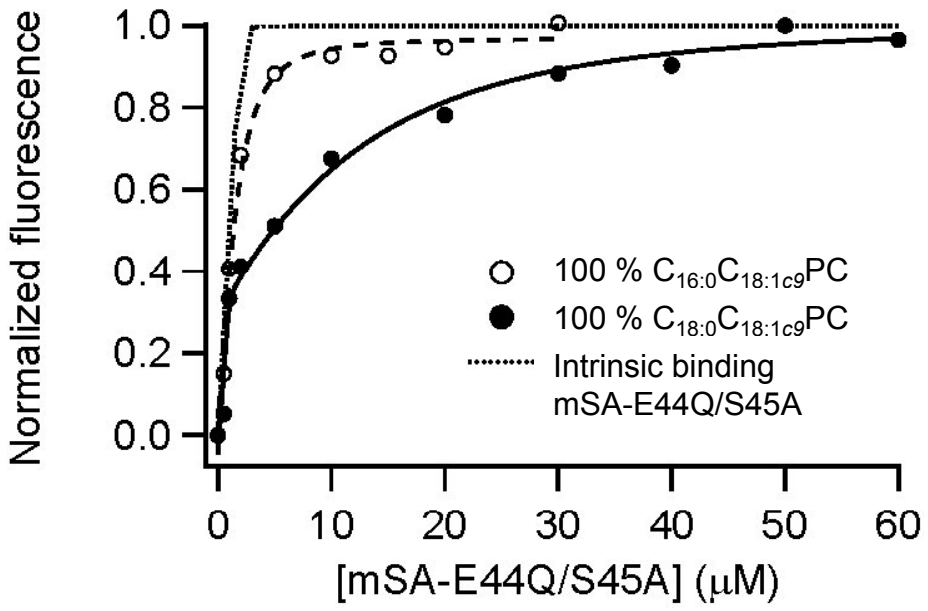


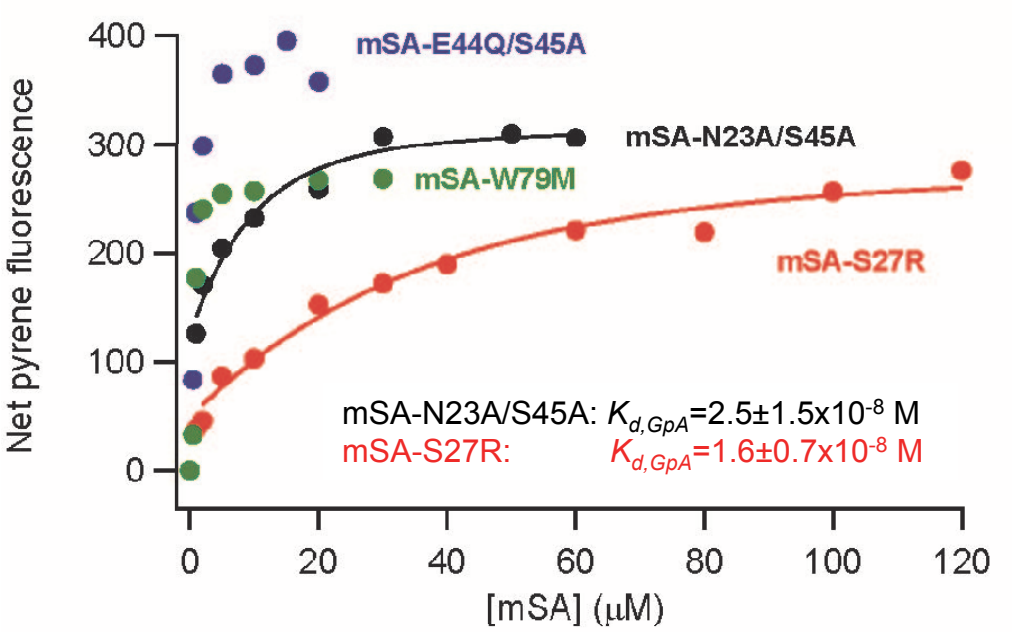
Supporting Figure 1. Effect of the negatively charged membrane on the intrinsic biotin binding affinity ($K_{d,biotin}$) of mSA mutants. To $K_{d,biotin}$ of mSA mutant, it is necessary to prepare for the GpATM in purely monomeric form. In the negatively charged membrane ($C_{16:0}C_{18:1c9}$PG/PC (20/80)), where GpATM is relatively unstable (see Figure 2 and 4), 800 nM of monomer-favoring GpATM-G83I mutant was reconstituted at high lipid concentration (*L/P*=3000) to ensure the exclusively monomeric GpATM. In neutral $C_{16:0}C_{18:1c9}$PC membranes, where GpATM G83I mutant is significantly stabilized ($K_{d,GpA}$=~10^{-9} M), ten-time molar excess of SNGpATM without biotin and pyrene labeles were added to disrupt the dimer formation at *L/P*=3000. But we expect that a small fraction of GpATM-G83I still exists in dimeric form. Thus, the apparent $K_{d,biotin}$'s measured for mSA-N23A/S45A and -S27R in $C_{16:0}C_{18:1c9}$PC bilayers are slightly larger than $K_{d,biotin}$'s obtained in high concentration of neutral DM micellar solution (dashed lines). Throughout this work, we took the $K_{d,biotin}$'s obtained from DM micelle condition for the $K_{d,biotin}$'s in neutral membrane systems. (a) $K_{d,biotin}$(8.0x10^{-7} M) of an isoelectric mutant mSA-N23A/S45A does not change in the negatively charged membrane ($C_{16:0}C_{18:1c9}$PG/PC (20/80)) relative to the neutral DM micelles ($K_{d,biotin}$=8.7x10^{-7} M). (b) $K_{d,biotin}$(2.8x10^{-6} M) of mSA-S27R, whose mutation increases the net charge of mSA, is reduced in $C_{16:0}C_{18:1c9}$PG/PC (20/80) membranes relative to the neutral environments ($K_{d,biotin}$=6.5x10^{-6} M).



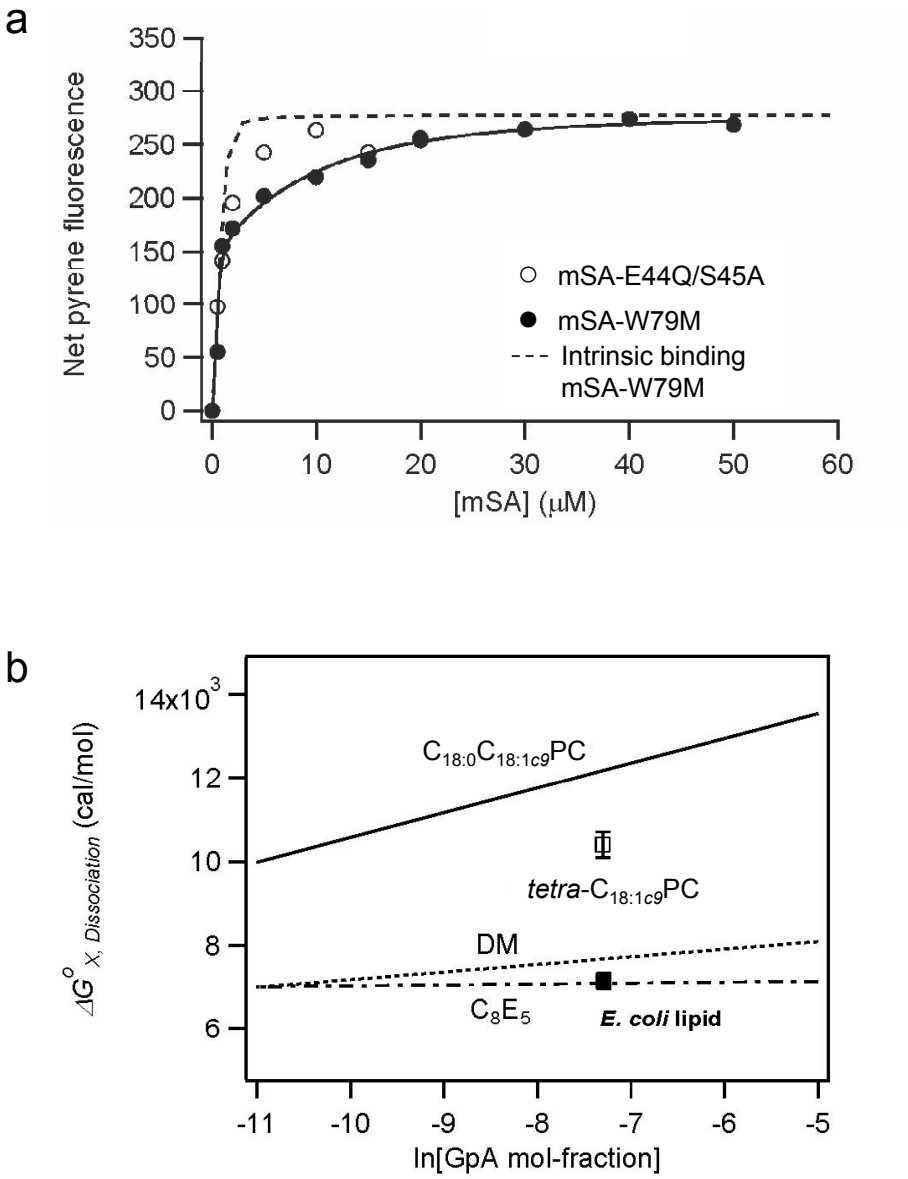
Supporting Figure 2. Construction of mSA-binding curves. Binding of mSA to biotinylated, pyrene-labeled SNGpATM was monitored by the increase of pyrene fluorescence. The raw intensity data (upper panel, filled circles) were subtracted by the background (upper panel, open circles), which was obtained by adding excess free biotin to a final concentration of 2 mM. The net increase of pyrene fluorescence was corrected by fixing the subtracted intensity at [mSA]=0 to zero and shifting the other intensity values likewise (lower panel). The excitation and emission wavlenlength were 330 nm and 390 nm, respectively.



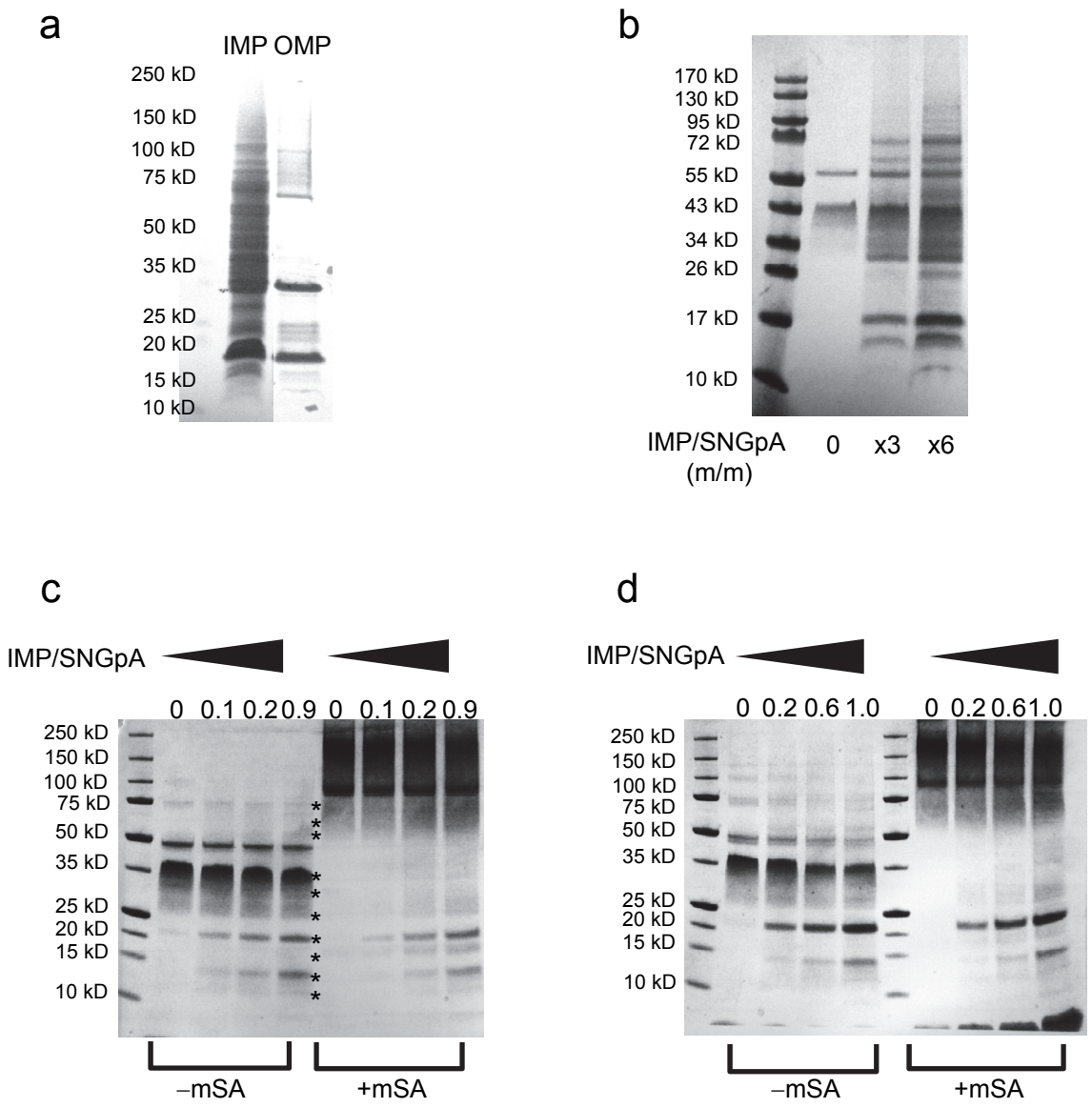
Supporting Figure 3. Binding curves of mSA-S45A to SNGpATM reconstituted in pure $C_{16:0}C_{18:1c9}$PC (*L/P*=1000) and $C_{18:0}C_{18:1c9}$PC (40/60, L/P=1000) membranes. The mSA-accessible GpATM concentrations were 1.6 μM and 2.7 μM in $C_{16:0}C_{18:1c9}$PC and $C_{16:0}C_{18:1c9}$PC lipid vesicles, respectively. The measurements were performed in 20 mM MOPS (pH 7.4) , 200 mM NaCl buffer solution.



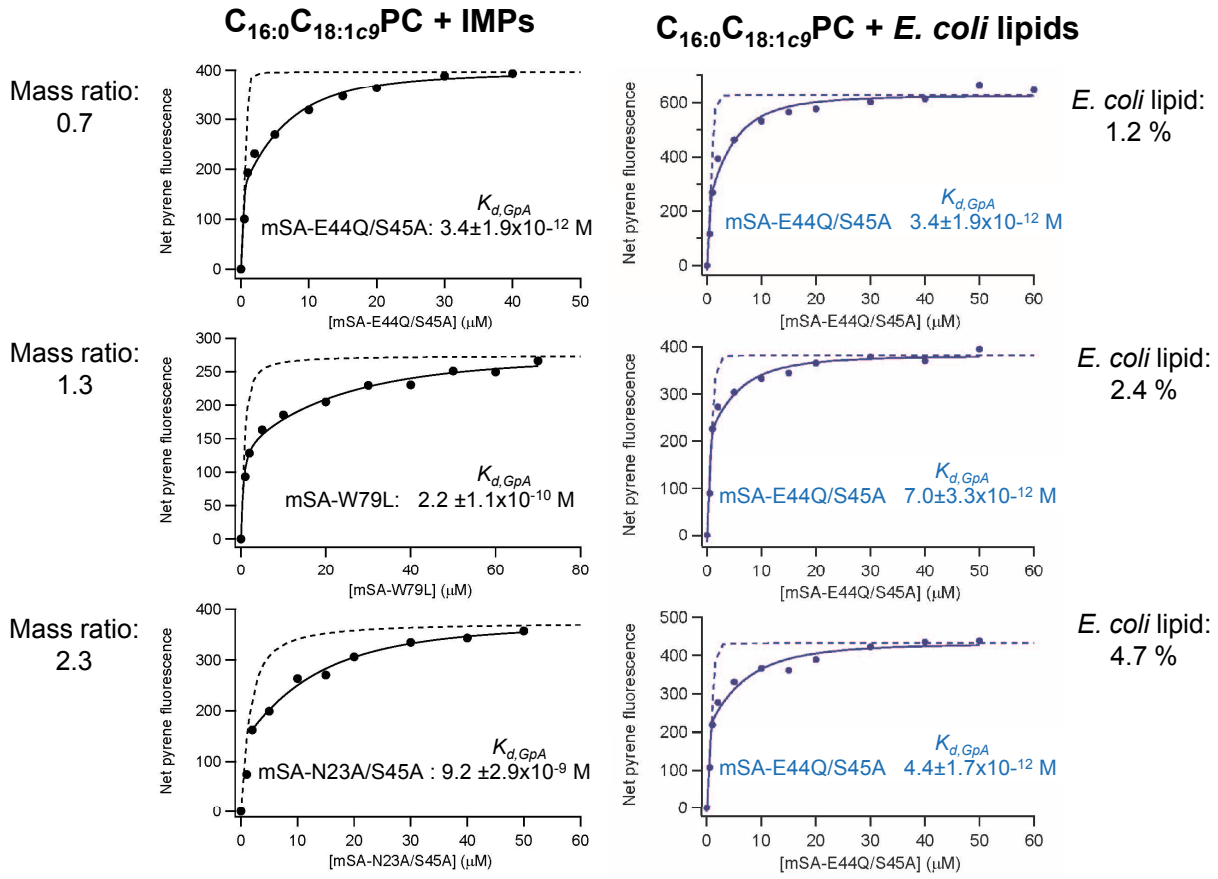
Supporting Figure 4. Selection of mSA-mutant with an optimal biotin binding affinity ($K_{d,biotin}$) to obtain the dissociation constant ($K_{d,GpA}$) of GpATM dimer in the negatively charged $C_{18:0}C_{18:1c9}$PS/PC (20/80, L/P=1800) membranes. mSA-N23A/S45A (black circles, $K_{d,biotin}$=8.7x10^{-7} M) and mSA-S27R (red circles, $K_{d,biotin}$=2.5x10^{-6} M) both yielded characteristic two-phase binding curves, from which similar $K_{d,GpA}$'s were obtained. The measurements were performed in 20 mM MOPS (pH 7.4) , 200 mM NaCl buffer solution with a mSA-accessible GpATM concentrations of 1.8 μM .



Supporting Figure 5. Effect of cardiolipin on the stability of GpATM dimer. (a) mSA-binding curves of GpATM in cardiolipin (*tetra*-$C_{18:1c9}$CL)/$C_{18:0}C_{18:1c9}$PC (5/95, *L/P*=1500) membranes. An optimal binding curve was obtained with mSA-W79M ($K_{d,biotin}$=4.5x10^{-8} M), which yielded a dimer dissociation constant of $K_{d,GpA}$=4.6 ±2.4x10^{-11} M. (b) 5 mol-% cardiolipin induced a moderate destabilization effect of ~2 kcal/mol relative to pure $C_{18:0}C_{18:1c9}$PC bilayers. The measurements were performed in 20 mM MOPS (pH 7.4) , 200 mM NaCl buffer solution with the mSA-accessible GpATM concentration of 1.6 μM .



Supporting Figure 6. (a) SDS-PAGE of *E. coli* total inner and outer membrane fractions (IMPs and OMPs, respectively) separated by sucrose gradient (see methods). (b) SDS-PAGE of mixture of the SNGpATM and the increasing amount of IMPs reconstituted in DM micelles. (c) SDS-PAGE of reconstituted SNGpATM and IMPs in *E. coli* lipid membranes. (d) SDS-PAGE of reconstituted SNGpATM and IMPs in $C_{16:0}C_{18:1c9}$PC lipid membranes. There are a number of reconsituted IMPs (asterics in (c)) visualized on SDS-PAGE.



Supporting Figure 7. Destabilization of GpATM dimer by IMPs in $C_{16:0}C_{18:1c9}$PC membranes. *Left* (top to bottom): mSA-binding curves with an increasing IMP/SNGpA mass ratio. As the relative IMP content increases, the mSA mutant with a lower intrinsic biotin binding affinity was required to obtain optimal mSA binding curves. The lipid-to-protein molar ratio (*L/P*) varied within a narrow range from 1400 to 1700. *Right*: mSA-binding curves with an increasing *E. coli* lipid content in $C_{16:0}C_{18:1c9}$PC membranes (*L/P*=1200~1400). These measurements were performed to take into account the effect of the intrinsic *E. coli* lipid contained in the detergent solubilized inner membrane fraction. The amounts of added *E. coli* lipids were determined based on the total phospholipid concentration of the detergent solubilized inner membrane strock solution (see methods). $K_{d,biotin}$(mSA-E44Q/S45A)=9.1x10^{-9} M, $K_{d,biotin}$(mSA-W79L)=3.5x10^{-7} M, and $K_{d,biotin}$(mSA-N23A/S45A)=8.7x10^{-7} M.