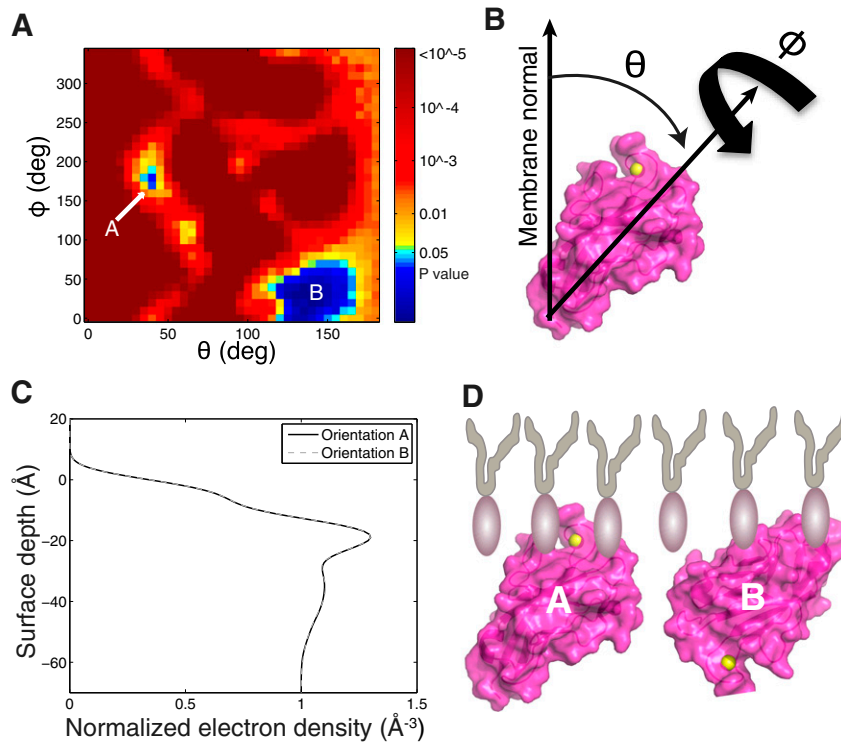# Supporting Information

Tietjen et al. 10.1073/pnas.1320174111

Fig. S1. Details of protein orientation fitting. (*A*) Reduced χ^2 map depicting two regions of best fit that are indistinguishable based on a *P* value < 0.05, as determined by a statistical *F* test comparing best-fit values between different protein orientation models. The characteristic electron density of Tim4 determines the width of the wells and how much the electron density changes under a given rotation. (*B*) Depiction of the θ and Φ rotational angles used to characterize different protein orientations. X-ray reflectivity is insensitive to in plane rotation (around the membrane normal), so these rotations are ignored. (*C*) Identical electron density profiles between orientations A and B. (*D*) Cartoon representations of orientations A and B, with calcium ion shown as yellow spheres.
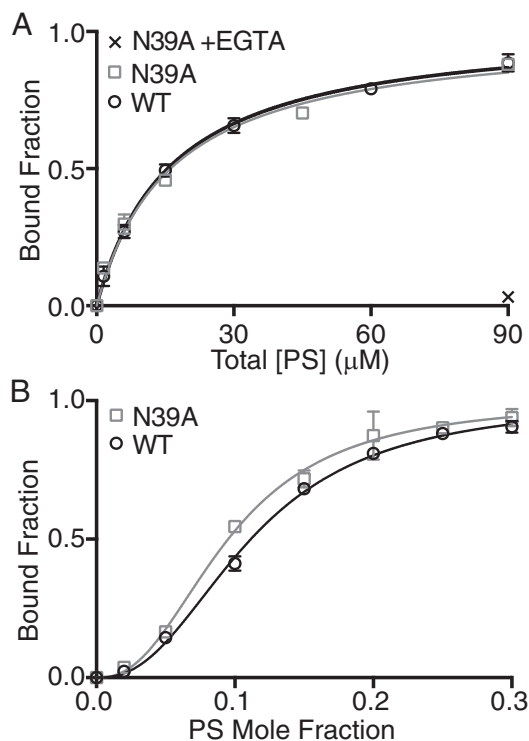
Fig. S2. N39 alanine mutant binding data. (*A*) Lipid vesicle titrations of 7:3 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC):1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) for N39A mutant (open gray square) relative to wild-type (open black circles). X corresponds to 90 μM [PS] + 10 mM EGTA. Lines through data are fits to a single-site binding model (N39-gray; WT-black). (*B*) PS mole fraction titrations at 600 μM total lipid with 170 nM N39A mutant (open gray squares) or wild-type (open black circles). Lines through data are fits to a Hill model (N39, gray; wild-type, black). All data points are mean of measurements done at least in triplicate, with error bars depicting 1 SD.

Table S1. Parameter values for Tim4 X-ray fitting

Parameter	7:3 SOPC:SOPS	+ 1 μM Tim4
θ, °	N/A	38 ± 8
Φ, °	N/A	186 ± 28
Coverage, %	N/A	39.2 ± 2.5
Protein position, Å	N/A	-11.3 ± 0.5
Lipid tail length, Å	13.4 ± 0.1	12.9 ± 0.1
Lipid head length, Å	10.5 ± 0.4	9.7 ± 0.3
Lipid tail density, $e^-/Å^{-3}$	0.231 ± 0.004	0.229 ± 0.003
Lipid head density, $e^-/Å^{-3}$	0.453 ± 0.004	0.444 ± 0.003

All errors represents the 95% confidence window for the given parameter with respect to the chosen fit model. SOPC, 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine; SOPS, 1-stearoyl-2-oleoyl-sn-glycero-3-phosphoserine.