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Supplemental Information

BamA POTRA Domain Interacts with a Native Lipid Membrane Surface

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FIGURES

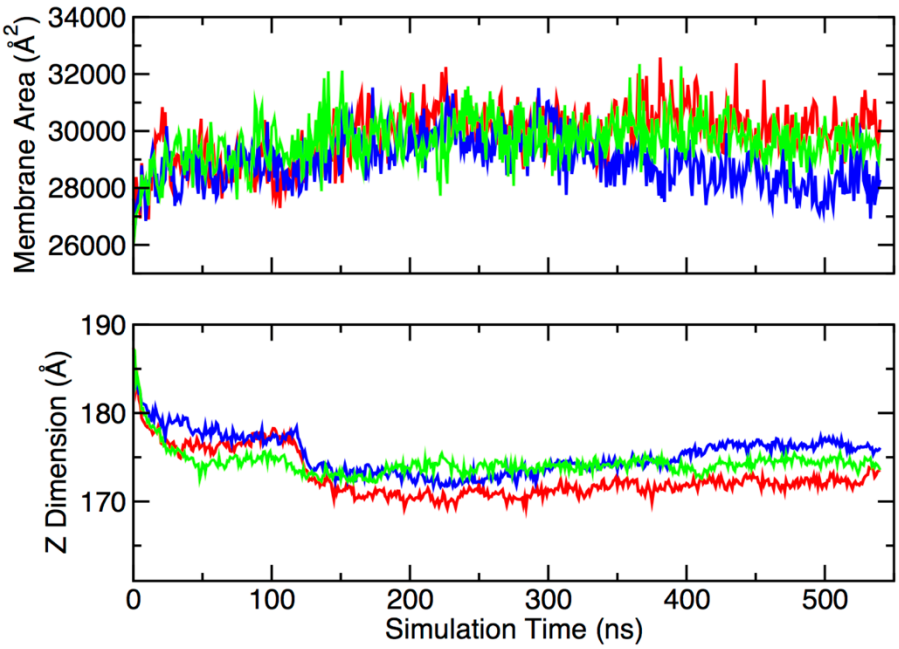

Figure S1. Time-series of the membrane surface areas and system *z* dimensions for three systems containing BamA in model *E. coli* membranes. Red, blue and green represent replicas 1, 2, 3, respectively.

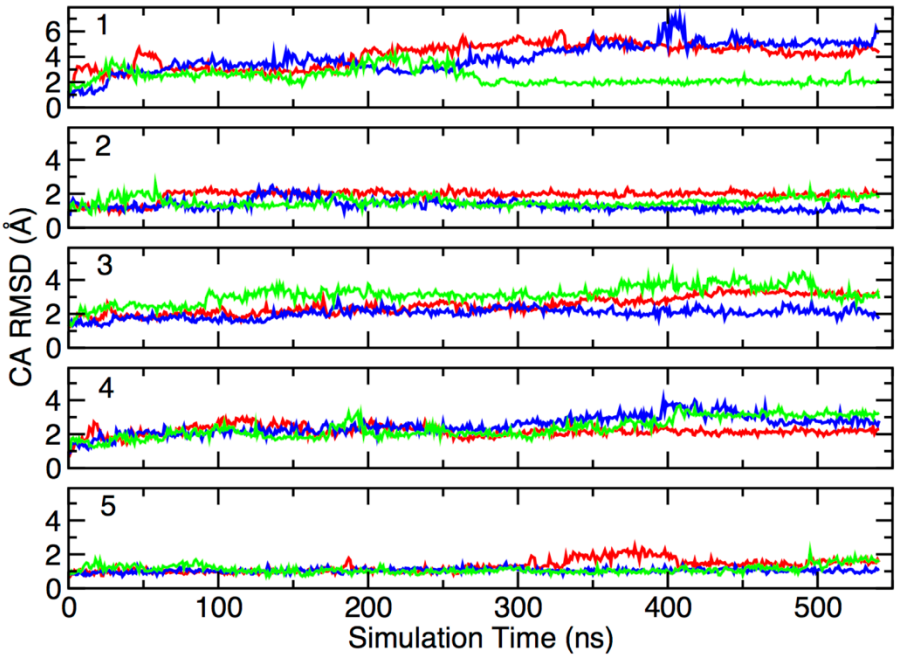

Figure S2. POTRA domains have variable backbone flexibility during MD simulation. The Cα RMSD compared to crystal structures for POTRA domains 1-5 are plotted. The colors red, blue and green indicate replicas 1, 2 and 3, respectively.

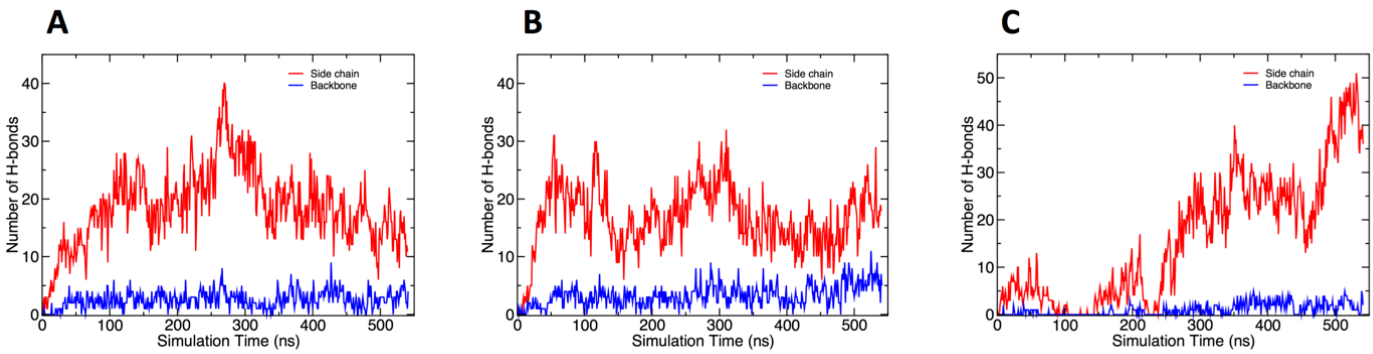

Figure S3. BamA POTRA domain forms many hydrogen bonds with phospholipid head groups. The total number of hydrogen bonds (heavy atom distance, 3.0 Å, maximum angle 45°) between POTRA1-4 and membrane phospholipids is plotted versus simulation time. Protein residue side chain (red) and backbone (blue) hydrogen bonds are plotted separately; data from replicas 1, 2, 3 are shown in plots A, B, C, respectively.

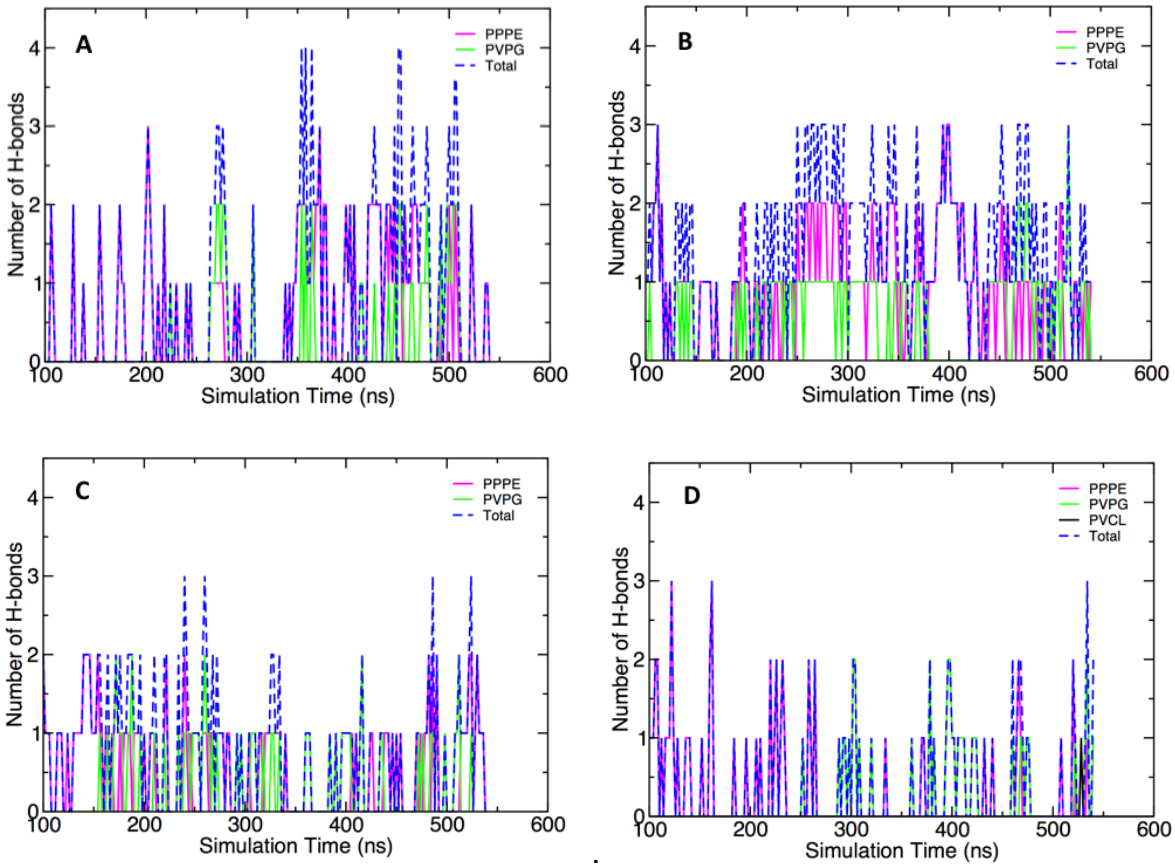

Figure S4. BamA hydrogen bonds to phospholipids with dynamic exchange of partners. Hydrogen bonding between amino acid side chains and phospholipids was measured using a distance cutoff of 3.0 Å, and maximum angle of 45° in full length BamA embedded in a model *E. coli* membrane. (A) BamA R64; (B) BamA K103; (C) BamA K106; (D) BamA R162. Data shown are for replica 1.

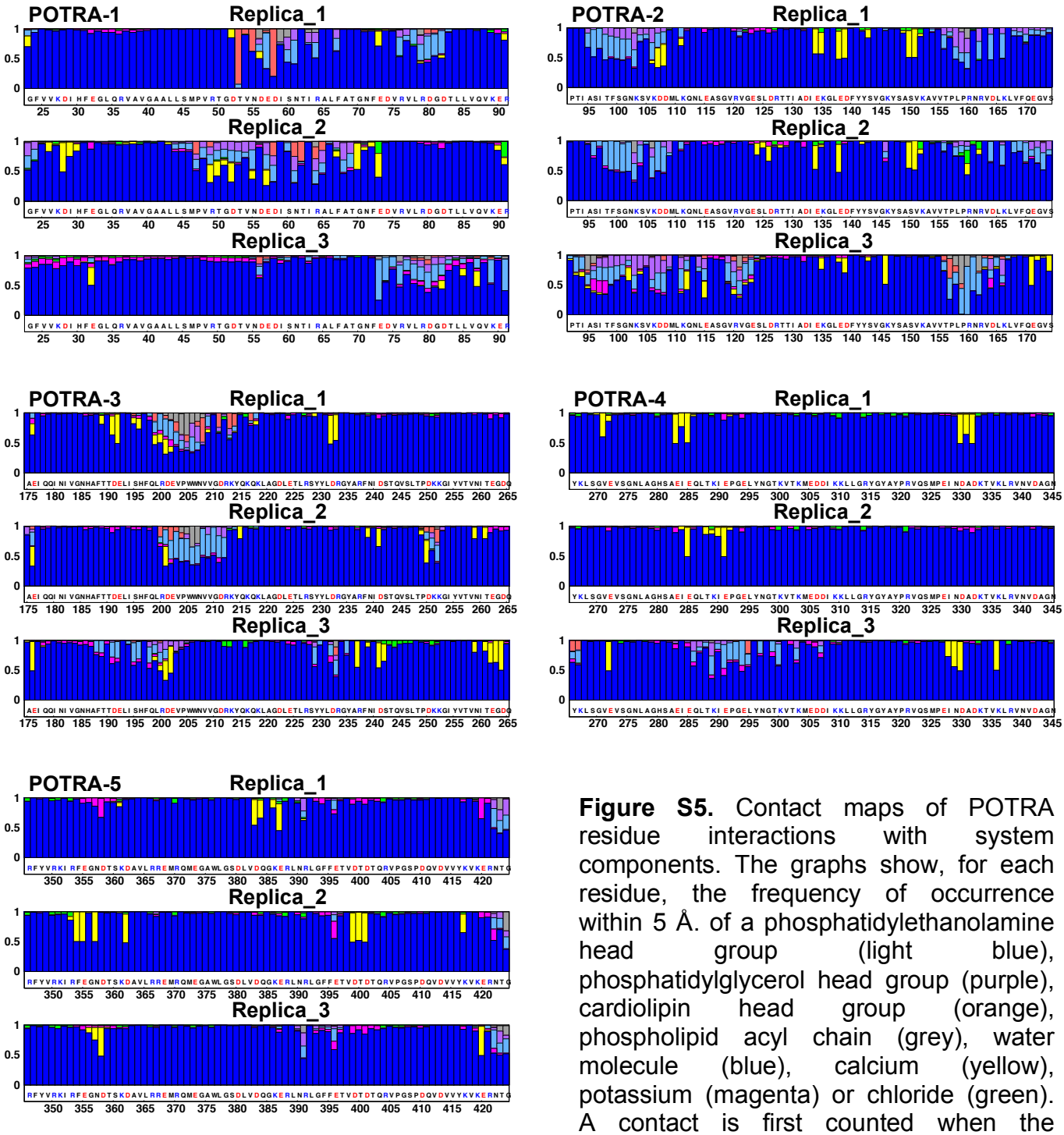

Figure S5. Contact maps of POTRA residue interactions with system components. The graphs show, for each residue, the frequency of occurrence within 5 Å. of a phosphatidylethanolamine head group (light blue), phosphatidylglycerol head group (purple), cardiolipin head group (orange), phospholipid acyl chain (grey), water molecule (blue), calcium (yellow), potassium (magenta) or chloride (green). A contact is first counted when the distance of between the heavy atoms of a residue and those of its interacting partner is <5 Å, and normalized for each interacting partner. The BamA amino acid sequence and residue number is below each pattern of interactions.

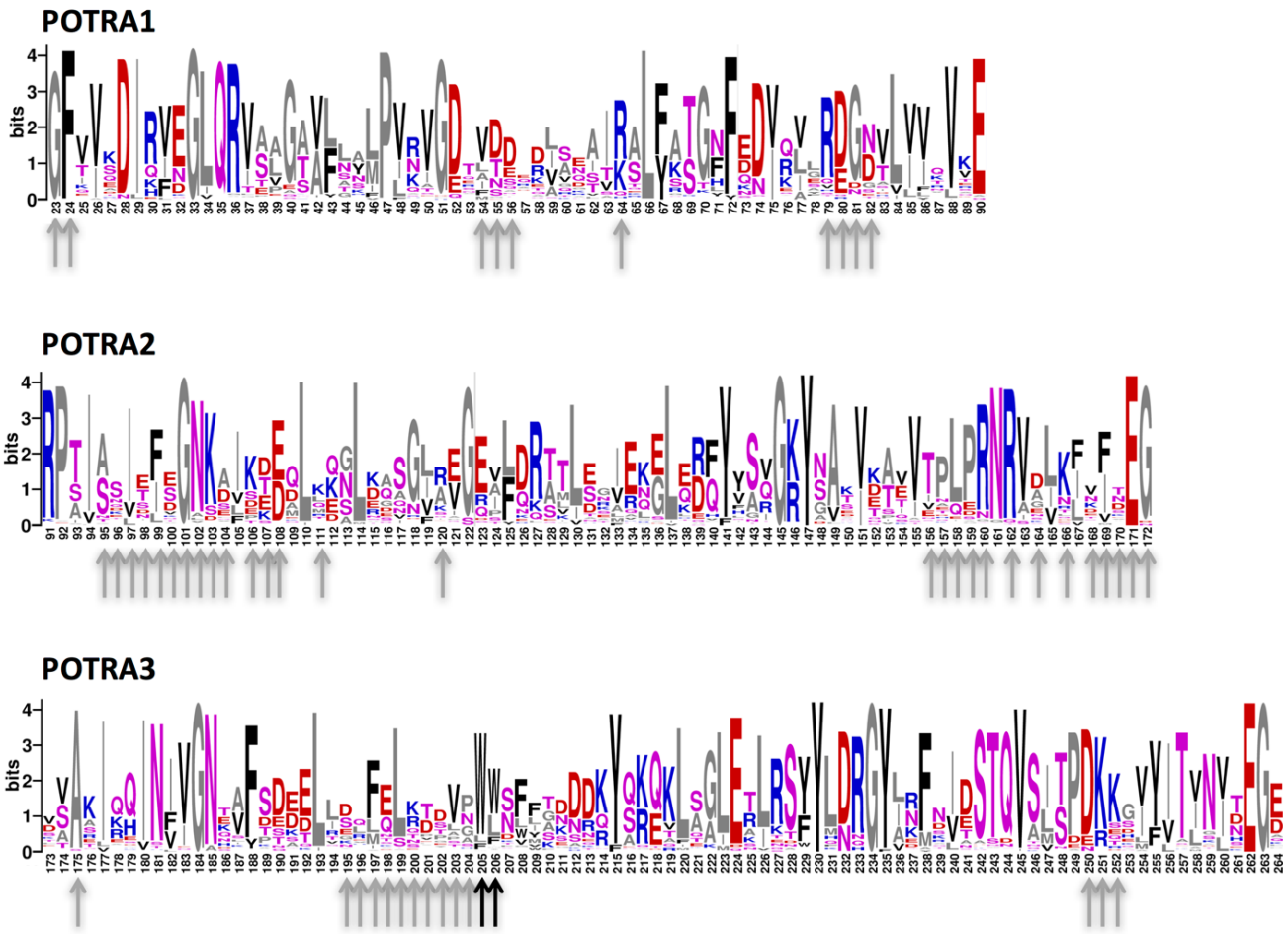

Figure S6. WebLogo diagram of POTRA1-3 consensus sequence. A multiple sequence alignment of 149 bacterial BamA homologs was used to generate the consensus sequence. POTRA1, 2 and 3 are shown in the top, middle, and bottom rows, respectively. Sites of significant interaction with the membrane are indicated with arrows (cf. Supp. Fig. S5); black arrows indicate conserved aromatic residues at positions 205 and 206 that insert into the membrane interfacial region.

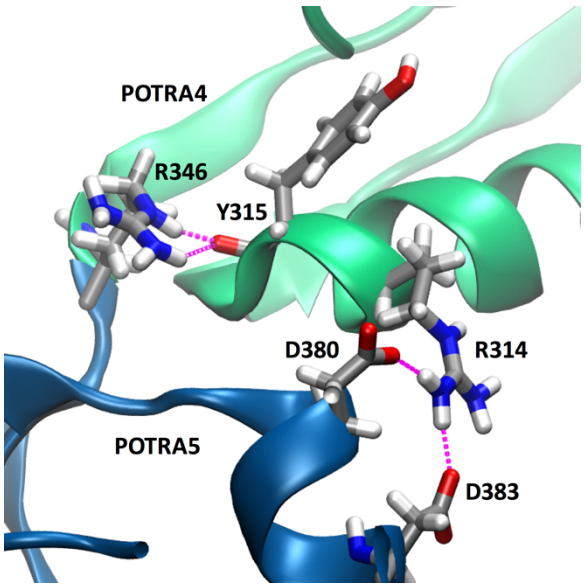

Figure S7. POTRA4-5 inter-motif orientation is stabilized during simulation by hydrogen bonds similar to those described for crystal structure 3OG5. Snapshot of POTRA3-4 linker region from replica 1 showing additional interaction between R314 and D380 that is occasionally observed. Compare to Figure 1 in [25].

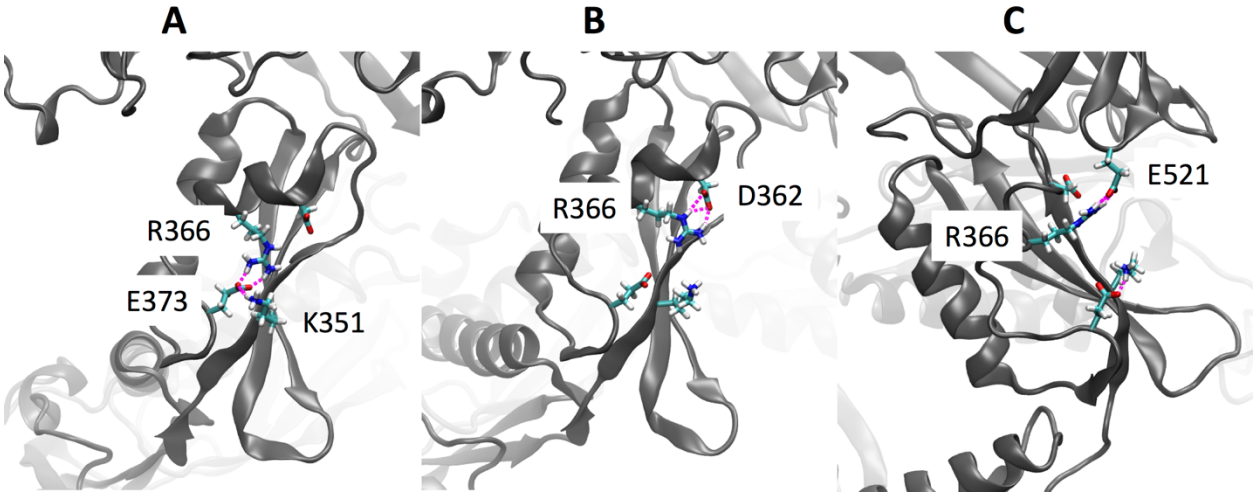

Figure S8. Residues in the POTRA5 core show conformational plasticity. Three separate snapshots (A,B,C) from trajectories of full length BamA embedded in a model *E. coli* membrane illustrating the dynamic electrostatic network between charged residues.

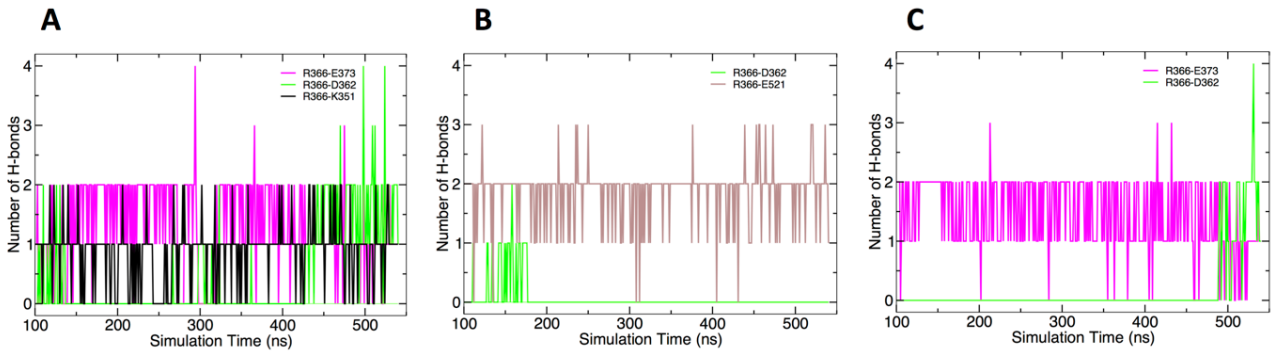

Figure S9. POTRA5 core residues exchange hydrogen bonding partners to create a dynamic electrostatic network. The number of, and partners for, R366 hydrogen bonding in full length BamA embedded in LPS/PL membranes are shown. (A), replica 1; (B), replica 2; (C), replica 3.

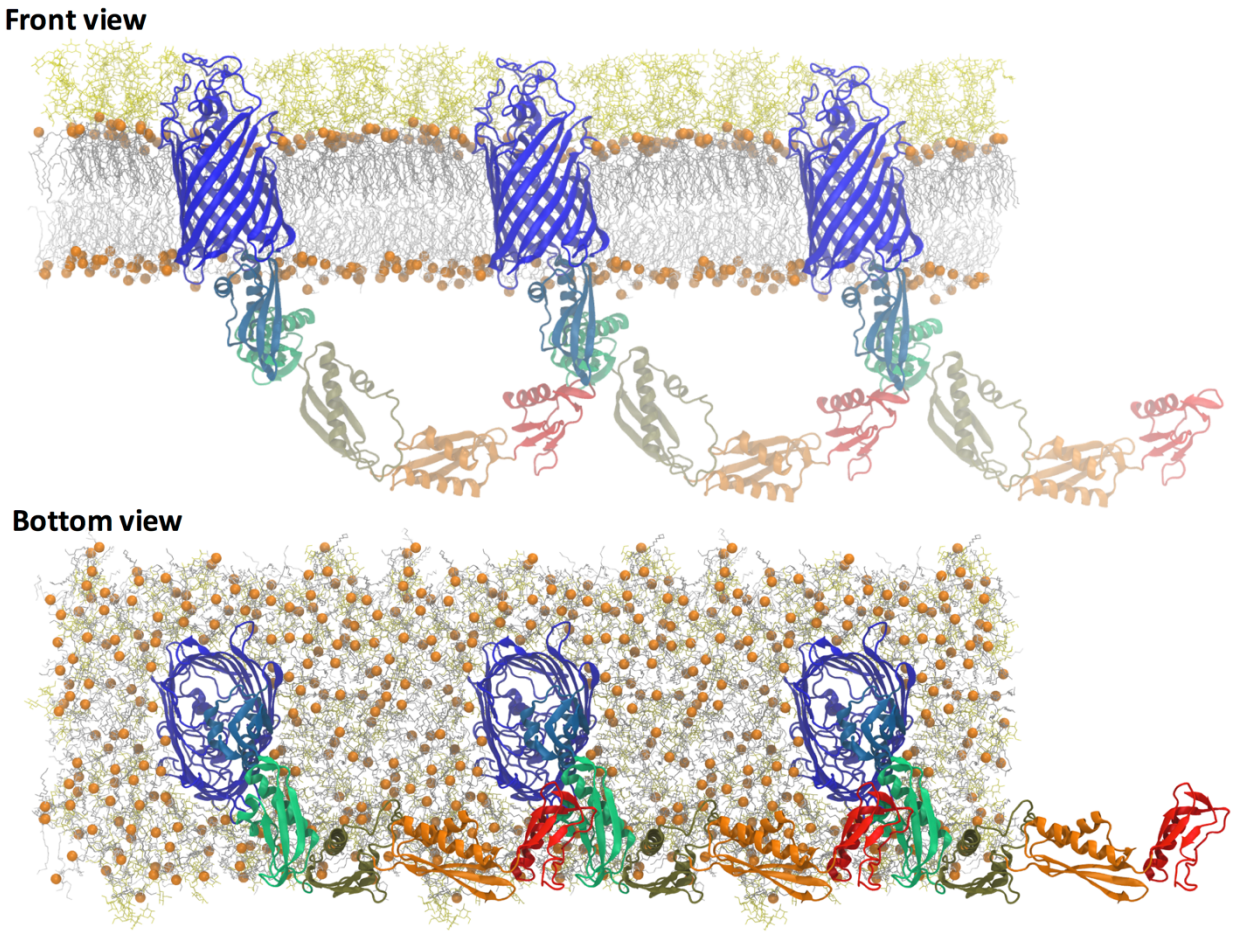

Figure S10. Periodic boundary image BamA contacts at small lipid to protein ratios. Two independent molecular systems with membrane surface dimensions of 90Å x 90Å (100 lower leaflet phospholipid:1 protein) were simulated with conventional MD. In both systems the periodic boundary protein neighbors made stable contact within 250 ns simulation time. Replica 2 shown.

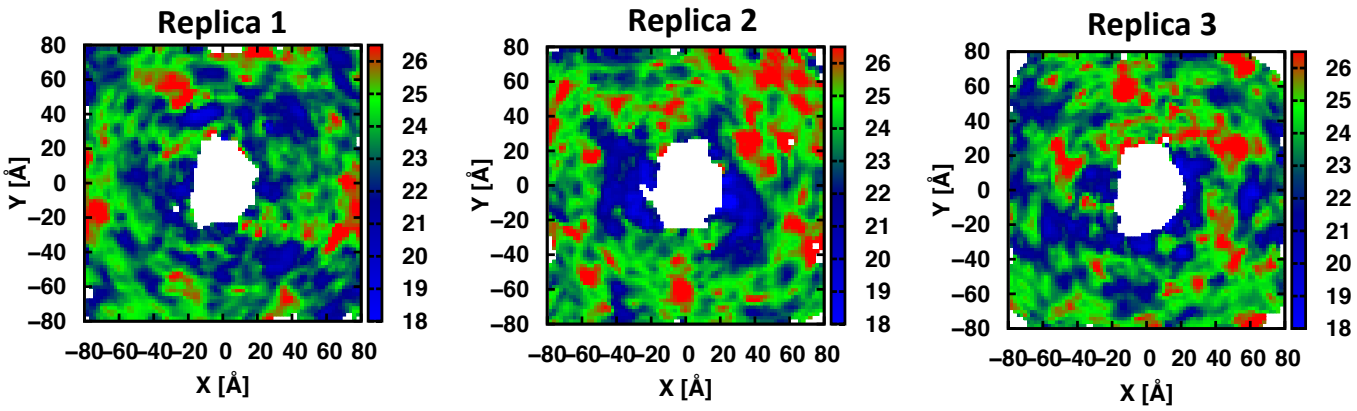

Figure S11. Two-dimensional z-thickness of the LPS/phospholipid membrane with embedded full-length BamA. Thickness is defined as the distance between the average of C2 and C4 atoms of lipid A (combined) and the average C2 positions of phospholipids within a 2 Å grid and represents the hydrophobic thickness. The relative orientation of the embedded BamA is the same as in Figure 6.

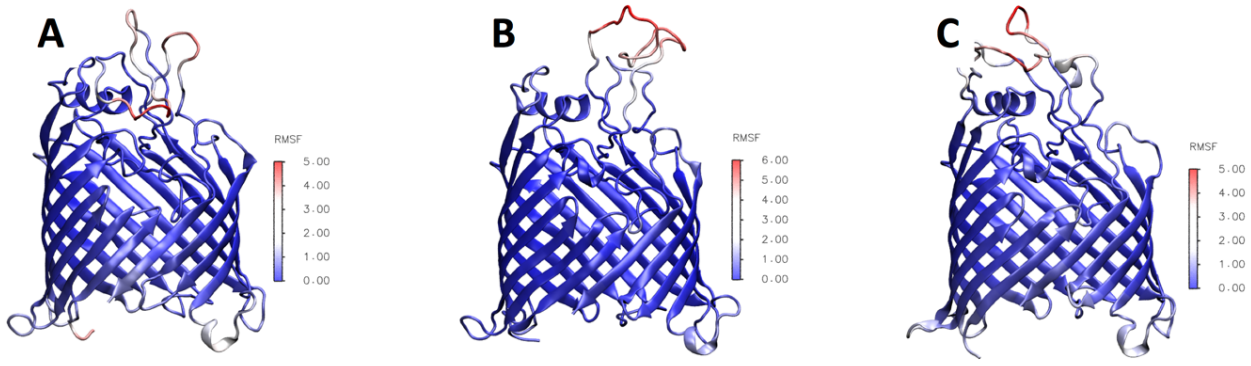

Figure S12. BamA β-barrel transmembrane strands have low RMSF in LPS/phospholipid membranes but extra-membranous loops and turns are variable. Ribbon diagrams of the β-barrel fragment are colored according to Cα RMSF calculated from 100 -540 ns. (A) Strand Cα RMSD to starting structure = 0.80 Å, total β-barrel Cα RMSD = 2.41 Å; (B) Strand Cα RMSD to starting structure = 0.93 Å, total β-barrel Cα RMSD = 3.75 Å. (C) Strand Cα RMSD to starting structure = 0.83 Å, total β-barrel Cα RMSD = 2.63 Å.

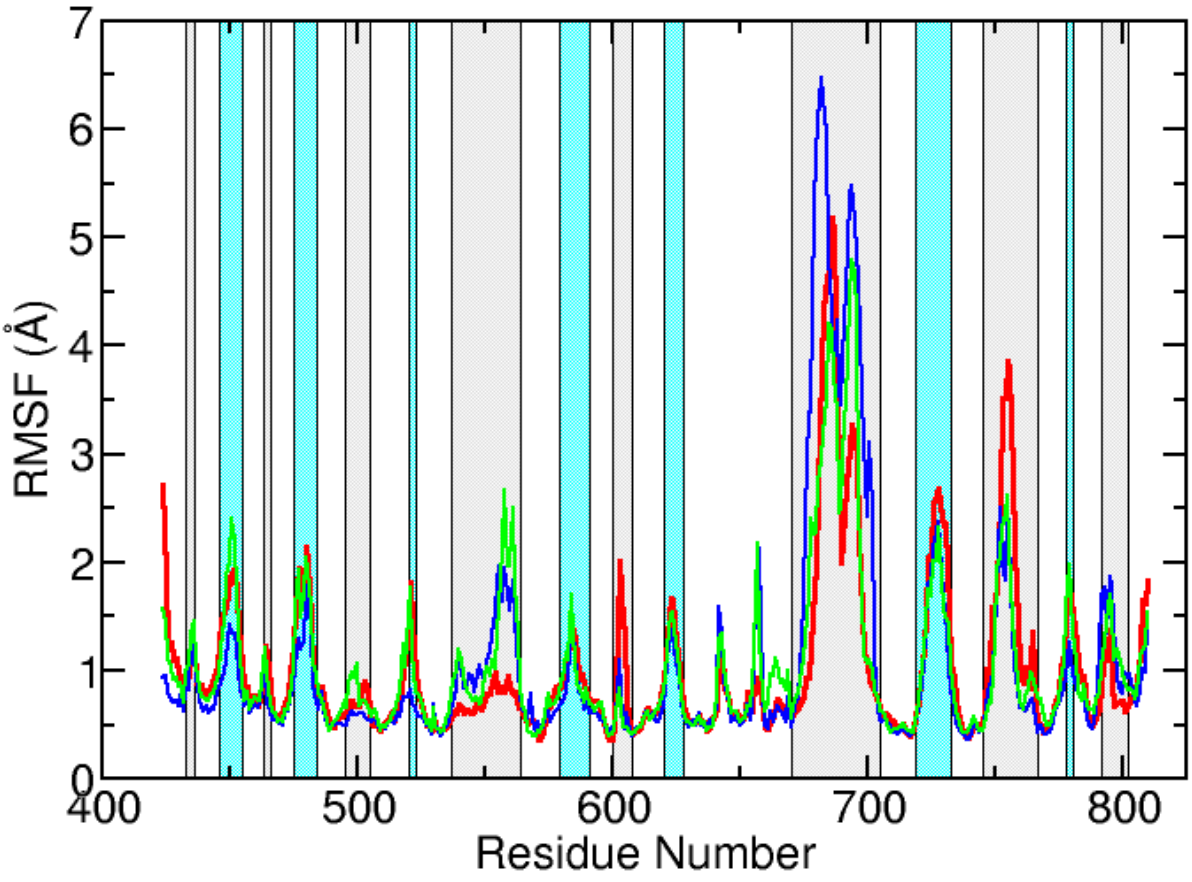

Figure S13. Root mean-square fluctuations (RMSF) of the BamA β-barrel Cα backbone atoms in replicas 1, 2, and 3 (red, blue, green curves) averaged over 540 ns. Protein structure is indicated by the colored bars: extracellular loops (grey) and periplasmic turns (cyan). The region between residues 640 and 709 includes the L6 loop that extends into the barrel cavity and continues into an external loop.

Movie Legends

Movie S1. Replica 1 simulation. Full length BamA embedded in an LPS/phospholipid membrane; simulation time shown is 1-540 ns. Red = POTRA1, orange = POTRA2, khaki = POTRA3, green = POTRA4, light blue = POTRA5, dark blue = β-barrel. Membrane phosphates delimiting the hydrophobic core are shown as orange spheres; polysaccharide as dark yellow lines and other lipid as grey lines.

Movie S2. Replica 2 simulation. Full length BamA embedded in an LPS/phospholipid membrane; simulation time shown is 1-540 ns. Red = POTRA1, orange = POTRA2, khaki = POTRA3, green = POTRA4, light blue = POTRA5, dark blue = β-barrel. Membrane phosphates delimiting the hydrophobic core are shown as orange spheres; polysaccharide as dark yellow lines and other lipid as grey lines.

Movie S3. Replica 3 simulation. Full length BamA embedded in an LPS/phospholipid membrane; simulation time shown is 1-540 ns. The POTRA domains contact the membrane at approximately 230 ns of simulation. Red = POTRA1, orange = POTRA2, khaki = POTRA3, green = POTRA4, light blue = POTRA5, dark blue = β-barrel. Membrane phosphates delimiting the hydrophobic core are shown as orange spheres; polysaccharide as dark yellow lines and other lipid as grey lines.

Movie S4. Replica 1 positional time evolution of POTRA domain center of mass for full length BamA embedded in LPS/PL membrane. The view is looking down the membrane normal toward the phospholipid surface of the membrane. The dark blue circle represents the center of the BamA β-barrel. Red = POTRA1, orange = POTRA2, khaki = POTRA3, light green = POTRA4, light blue = POTRA5. Note that the drift of POTRAs 1, 2 and 3 in this x, y plane (parallel to the membrane surface) is occasionally correlated whole POTRA domain drift is not due correlated with POTRA5 movement.

Movie S5. Replica 2 positional time evolution of POTRA domain center of mass for full length BamA embedded in LPS/PL membrane. The view is looking down the membrane normal toward the phospholipid surface of the membrane. The dark blue circle represents the center of the BamA β-barrel. Red = POTRA1, orange = POTRA2, khaki = POTRA3, light green = POTRA4, light blue = POTRA5. Note that the drift of POTRAs 1, 2 and 3 in this x, y plane (parallel to the membrane surface) is occasionally correlated whole POTRA domain drift is not due correlated with POTRA5 movement.

Movie S6. Replica 3 positional time evolution of POTRA domain center of mass for full length BamA embedded in LPS/PL membrane. The view is looking down the membrane normal toward the phospholipid surface of the membrane. The dark blue circle represents the center of the BamA β-barrel. Red = POTRA1, orange = POTRA2, khaki = POTRA3, light green = POTRA4, light blue = POTRA5. Note that the drift of POTRAs 1, 2 and 3 in this x, y plane (parallel to the membrane

surface) is occasionally correlated whole POTRA domain drift is not due correlated with POTRA5 movement.

Movie S7. POTRA5 relative motion is due to changes in principal axis angle not rotation around POTRA5 principal axis. The β-barrel and POTRA5 fragments from crystal structure 4K3B (translucent blue and orange, respectively) are aligned to the same from Replica 1 simulation at time zero (translucent blue and light blue, respectively). Simulation time shown is 1-540 ns.