Biophysical Journal, Volume *111*

Supplemental Information

Role of the Native Outer-Membrane Environment on the Transporter BtuB

Curtis Balusek and James C. Gumbart

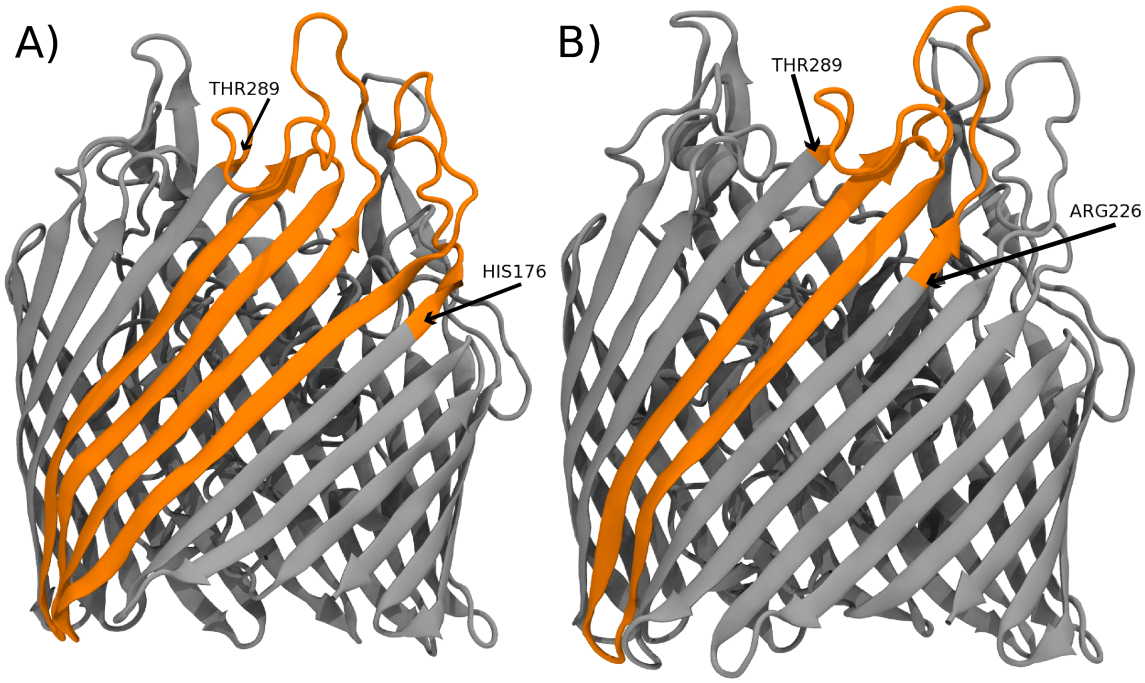
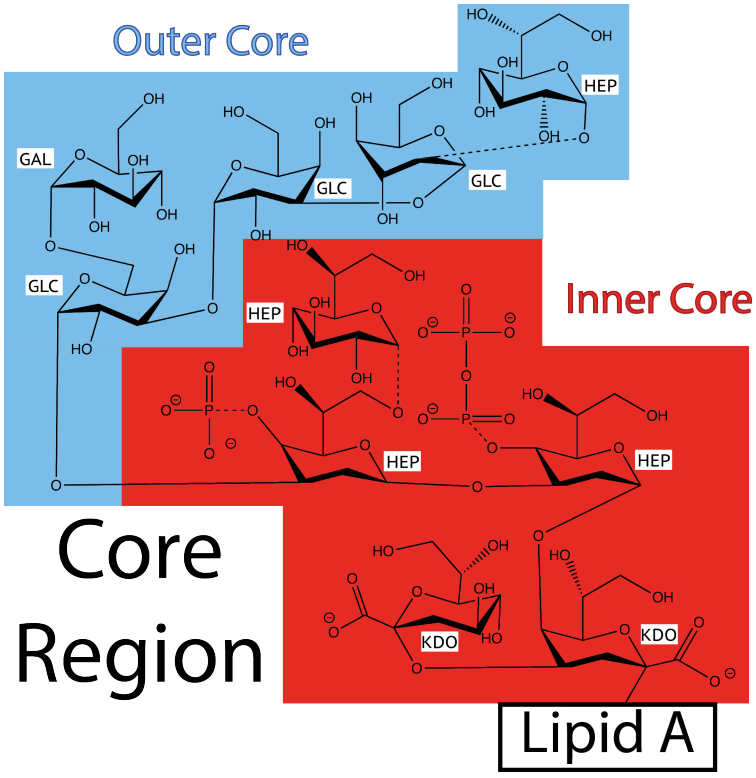
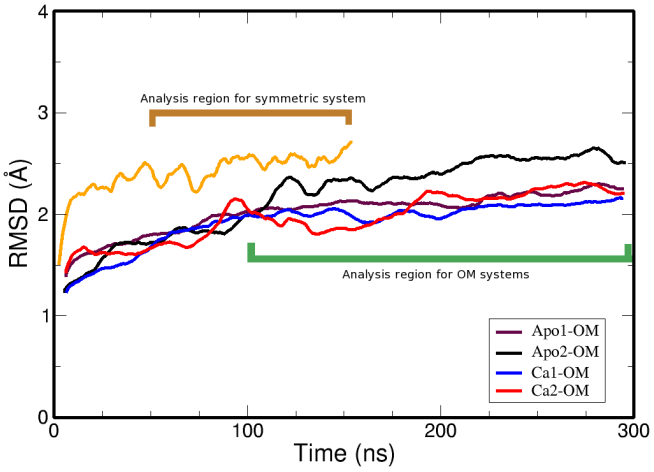


Figure S1: A graphical representation of the loop completions performed on the A) apo-state BtuB (PDB:1NQE) and the B) Ca^{2+}-bound BtuB. Loop completions, shown in orange, utilized the TonB-bound BtuB structure (PDB: 2GSK).



(A)

Figure S2: Chemical structure of the core oligosaccharide used in the model membrane simulations. Chemical structure is build in regards to the K12 *E. coli* oligosaccharide outlined in Nikaido 2003.



(A)

Figure S3: RMSD of the protein backbone for each simulated system compared to the CBL-bound crystal structure. Data regions used for analysis in each system has also been highlighted.

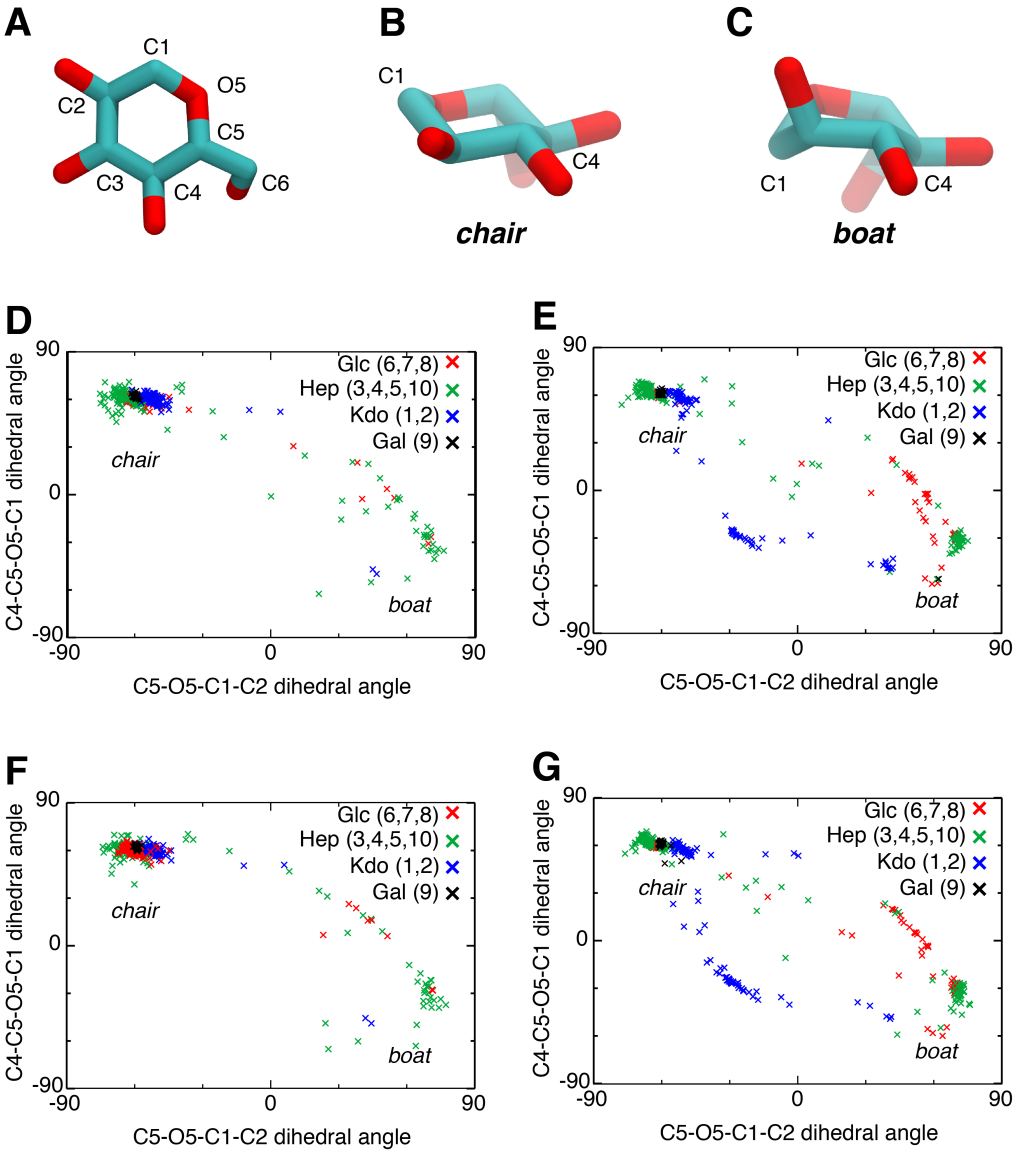
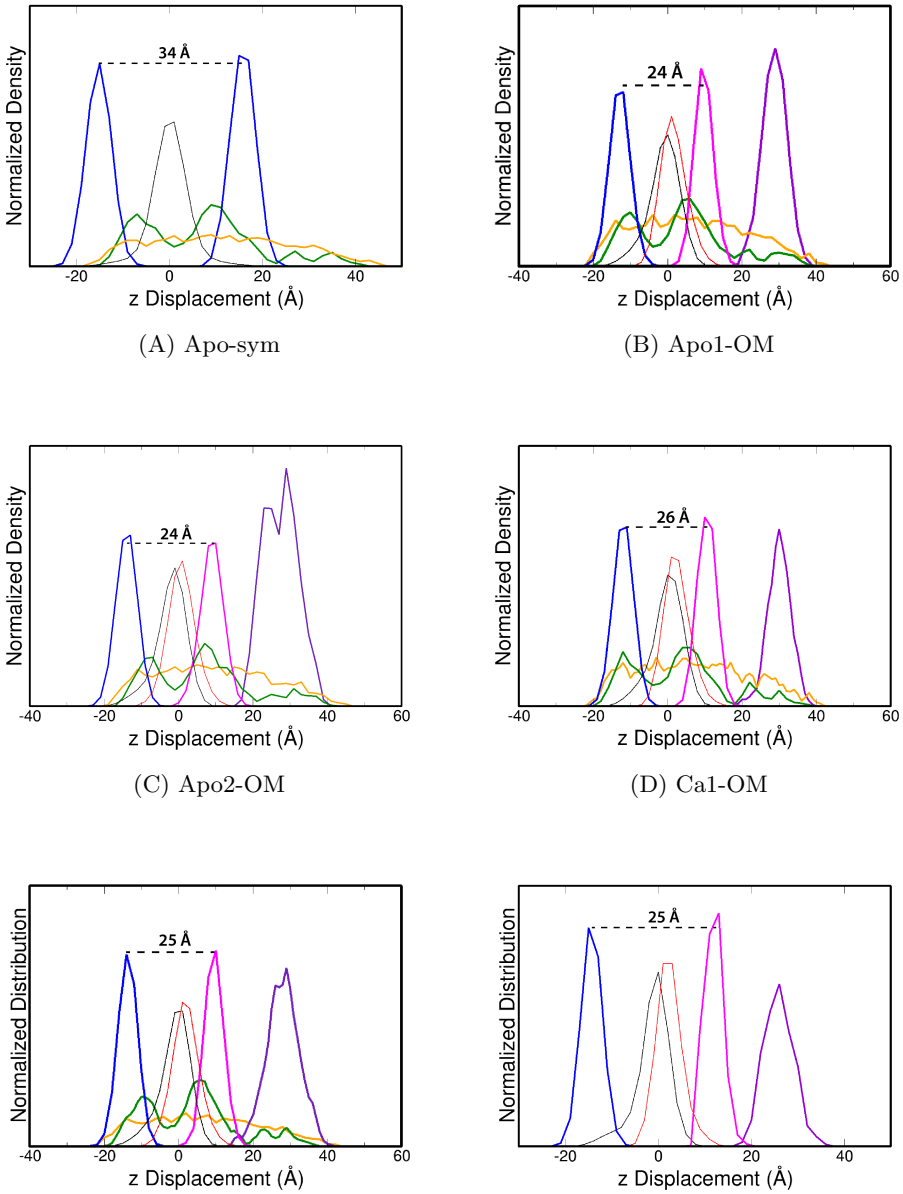


Figure S4: (A) Top down view of a pyranose ring, found in each of the sugars in the core oligosaccharide. The (B) chair conformation of a pyranose ring is the energetically favored conformation as compared to the (C) boat conformation. Determination of sugar conformation was done by measuring the dihedral angels on either side of the O5 oxygen. (D-G) include results from simulations of Apo1-OM, Apo2-OM, Ca1-OM, and Ca2-OM, respectively, as an average dihedral conformation throughout the trajectory.

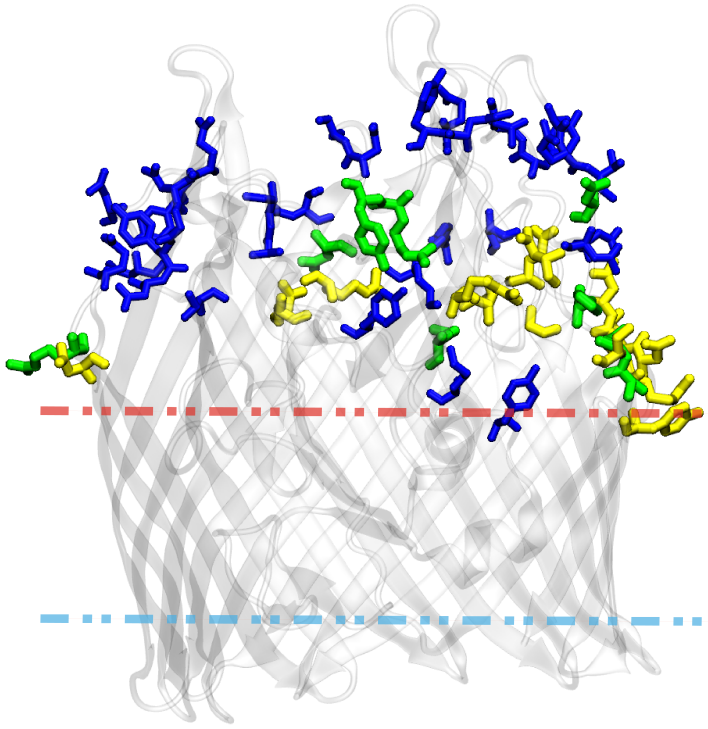


(A) Apo-sym (B) Apo1-OM

(C) Apo2-OM (D) Ca1-OM

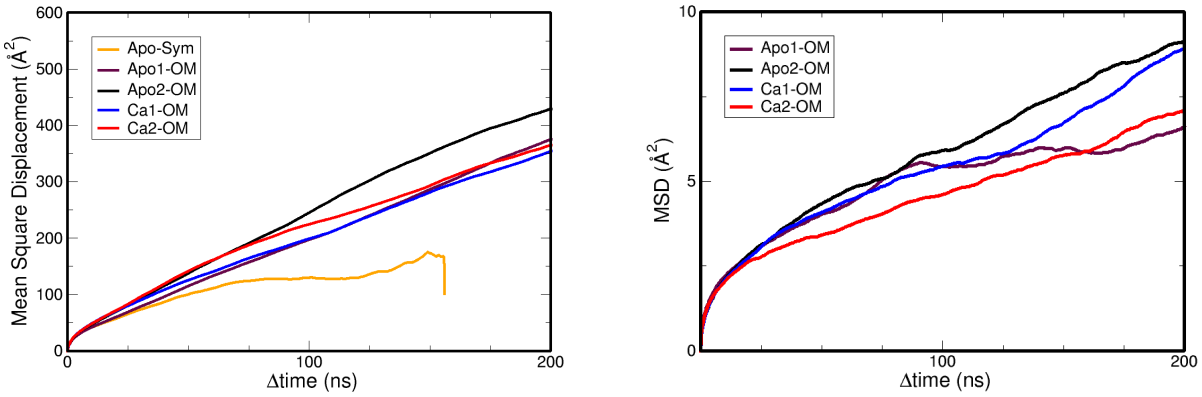
(E) Ca2-OM (F) LPS-mem

Figure S 5: Plot of the hydrophobic thickness profiles for the simulated systems. In each system, the protein density from carbon$_\alpha$ is shown in orange, lipid A acyl C2 and C4 atoms in magenta, the core oligosaccharides (LPS headgroup) in purple, POPE acyl C2 carbons in blue and the aromatic protein sidechains are in green. The number density of the last carbon atoms of the POPE acyl tails (black) and the last carbons on each of the lipid A tails (red) represent aliphatic tail interdigition at 0 z-displacement. The hydrophobic thickness is determined between the blue and magenta curves, as indicated by the dashed line.



(A)

Figure S6: Representation of the amino acids that hydrogen bond with LPS (blue), POPE (yellow) or both (green). The hydrophobic matching region as determined from aromatic side chains is represented as red (extracellular) and blue (periplasmic) dashed lines. Side chains that interact with LPS tend to be much farther up the EC loops than those interacting with POPE.



(A) Inner Leaflet of all systems

(B) Outer Leaflet of OM systems

Figure S7: Plots of Mean Square Displacement of (A) lipids in the inner phospholipid-containing leaflet and (B) outer LPS-containing leaflet. The diffusion coefficients for each leaflet are provided in Table S2.

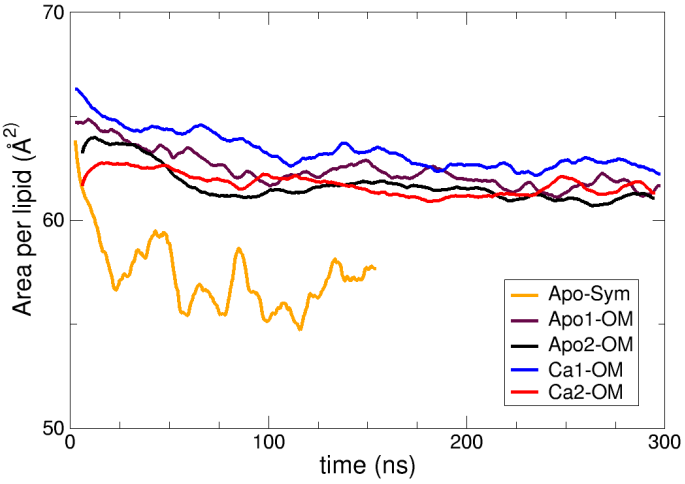
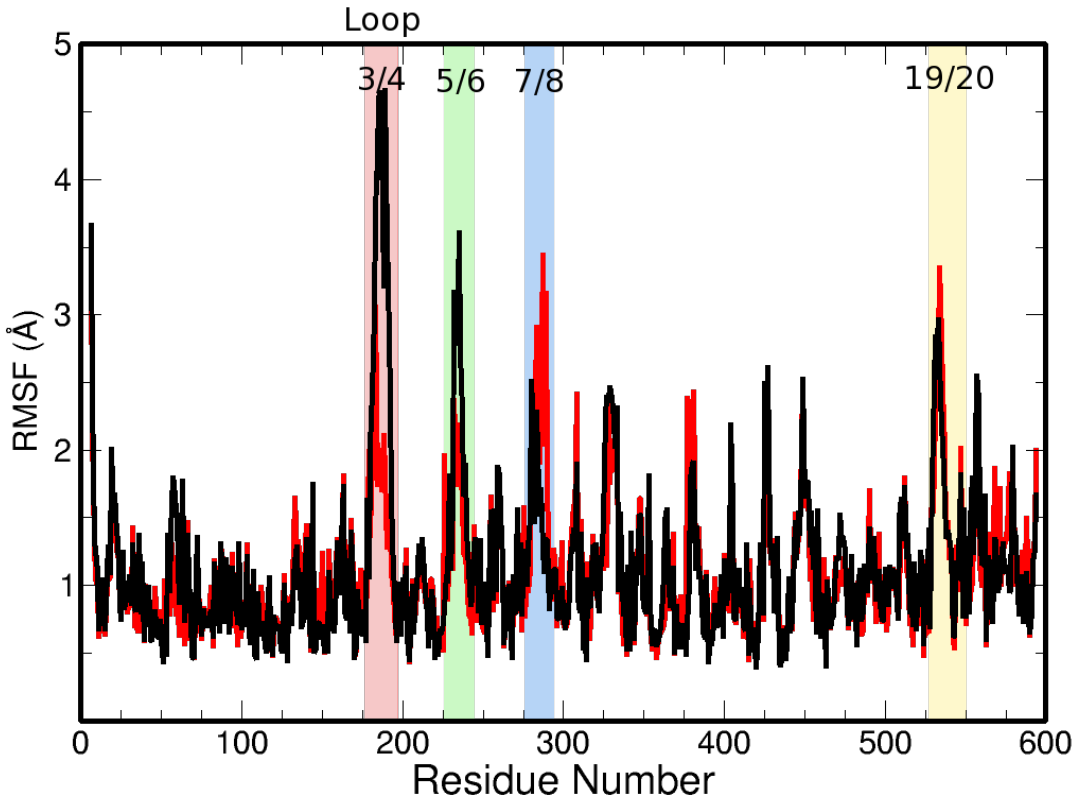
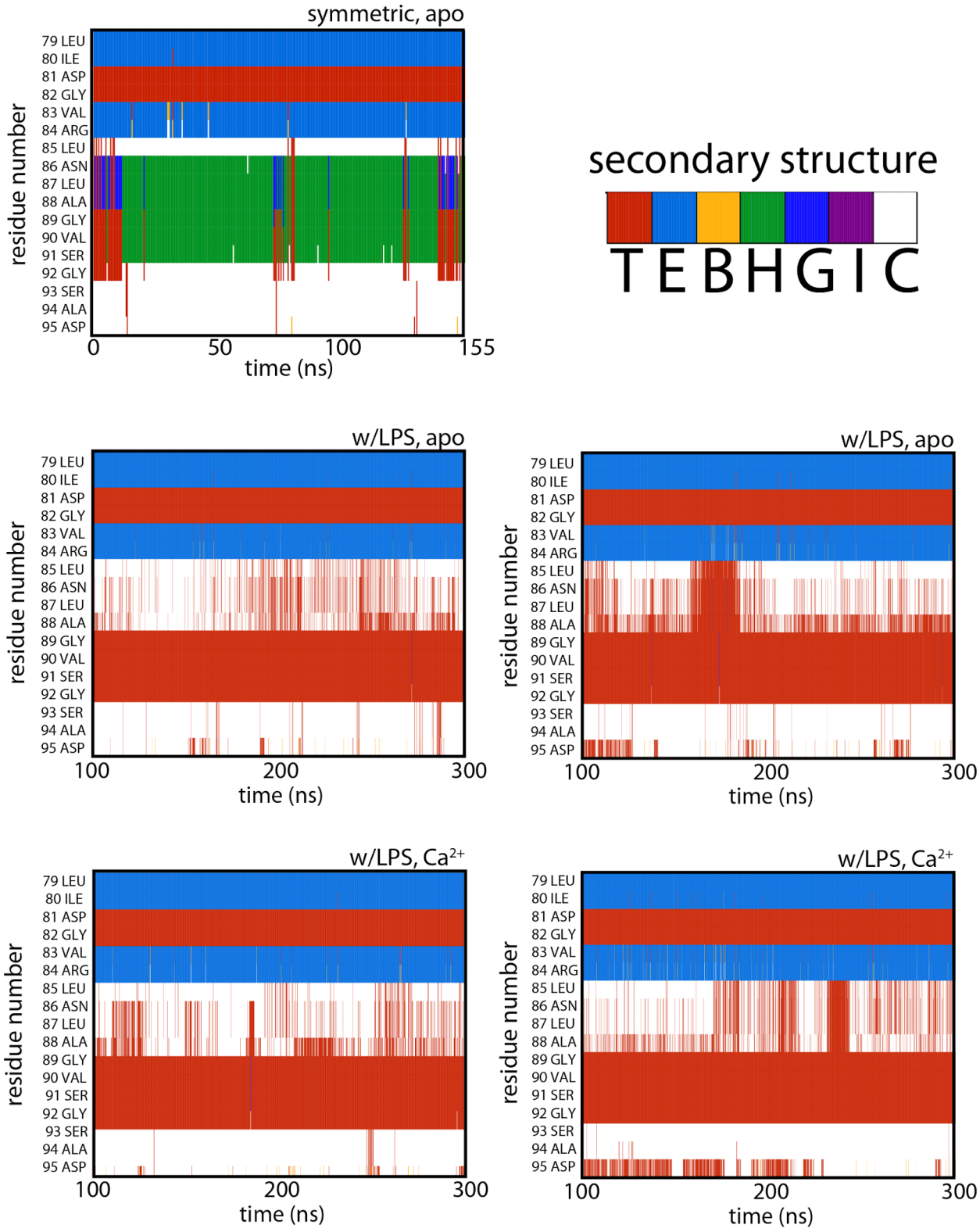


Figure S8: Plot of the area per lipid versus time for the inner phospholipid containing leaflet of each system. Each of the LPS-containing model membranes have an APL convergence around 63 Å^2.



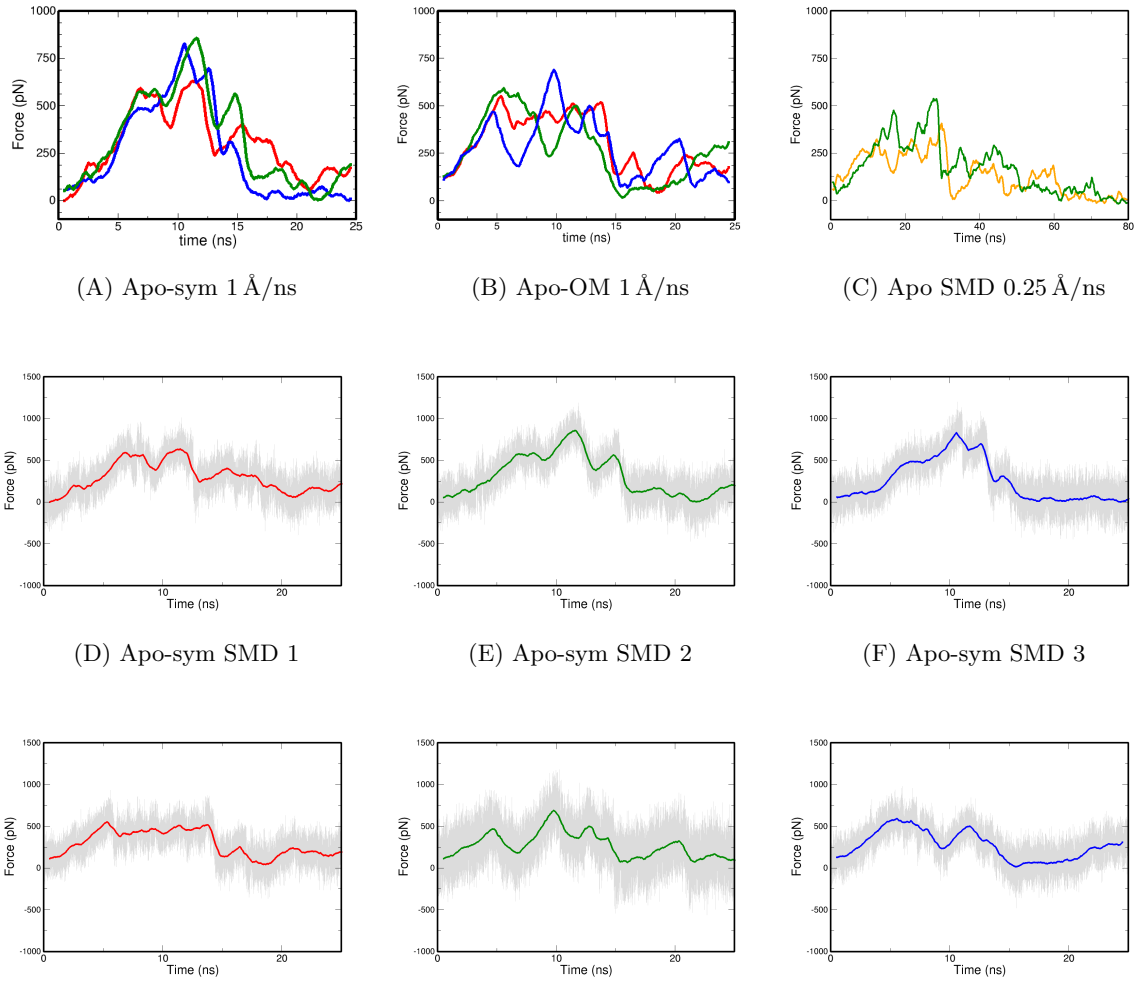
(A)

Figure S9: RMSF plots of Apo2-OM and Ca2-OM. Apo2-OM is shown in black and Ca2-OM is shown in red. Similar to the observations in Apo1- and Ca1-OM, there is a significant reduction in the fluctuation of loop 3/4 as well as a slight reduction in loop 5/6.



(A)

Figure S10: Timeline plots for the luminal domain region of Leu79 to Asp95. The $\alpha-$helix forms quickly in the Apo-sym simulation whereas the random coil is more prominent in the OM systems between residues Asn86 and Ser91. This random coil configuration permits Ser91 to be closer to the organization observed in the CBL-bound crystal structure.

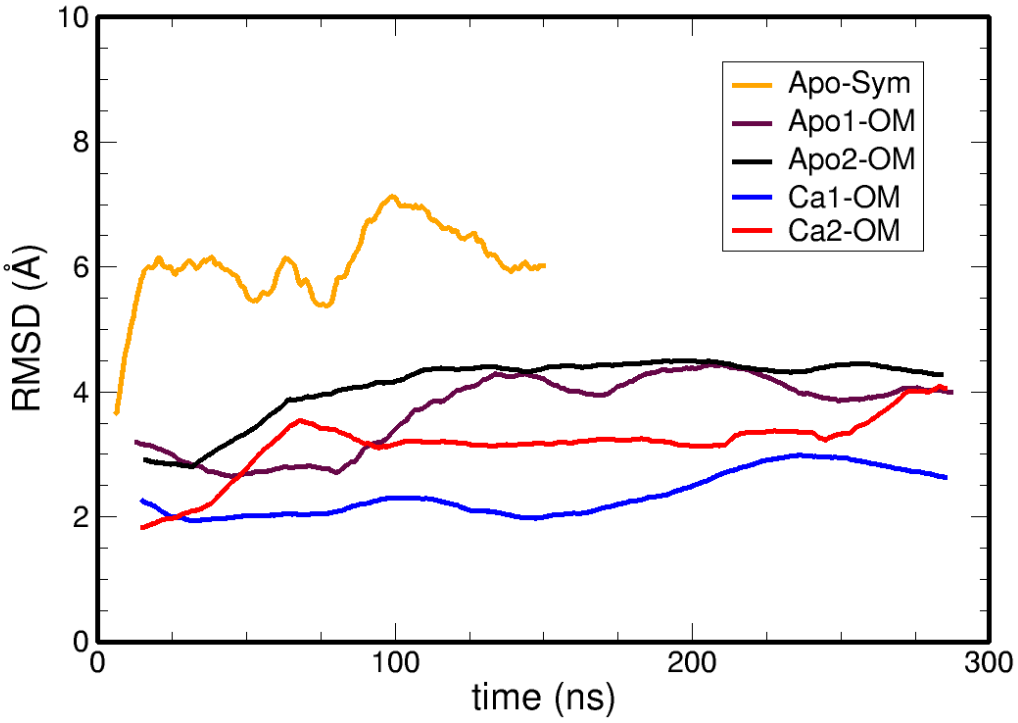


(A) Apo-sym 1 Å/ns (B) Apo-OM 1 Å/ns (C) Apo SMD 0.25 Å/ns

(D) Apo-sym SMD 1 (E) Apo-sym SMD 2 (F) Apo-sym SMD 3

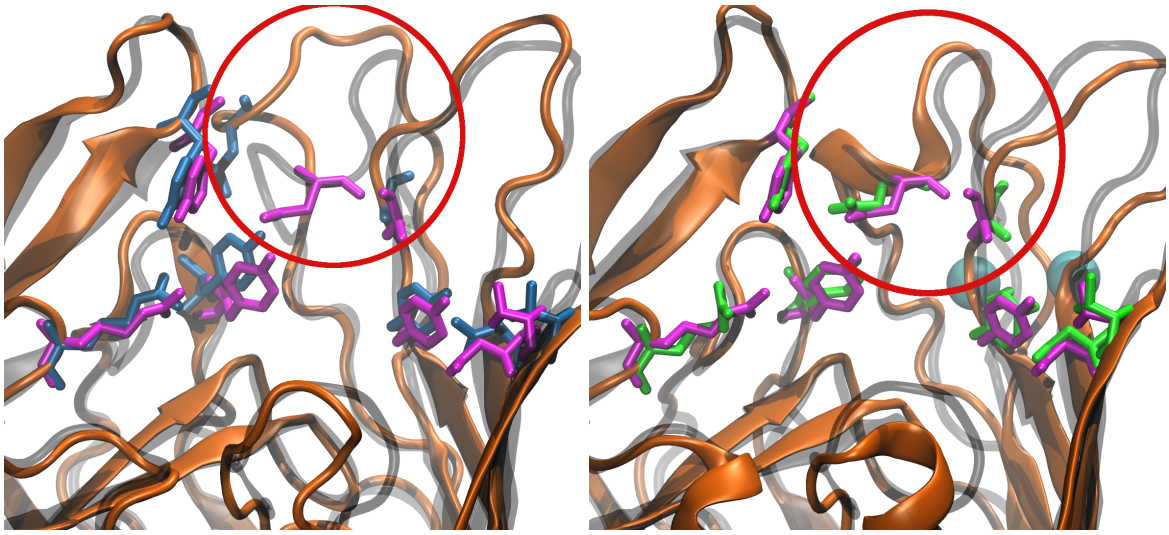
(G) Apo-OM SMD 1 (H) Apo-OM SMD 2 (I) Apo-OM SMD 3

Figure S11: Time averaged plots from the SMD extraction of the Ton box. (A) Three separate SMD simulations at 1 Å/ns of luminal domain extraction in the Apo-sym simulation. Start frames were seeded from 100 ns (red), 100 ns (blue), and 150 ns (green). (B) Three separate SMD simulations at 1 Å/ns of luminal extraction in the Apo1-OM (red, blue) and Apo2-OM (green) simulation. Start frames were seeded from Apo1-OM at 100 ns (red), 150 ns (blue), and Apo2-OM at 100 ns (green). (C) Force curve from constant SMD lumen extraction performed at 0.25 Å/ns. The Apo-OM system (orange) produces a smaller extraction force compared to the Apo-sym system (green). (D-I) Plots of time averaged curves shown in (A) and (B) with accompanying raw data.

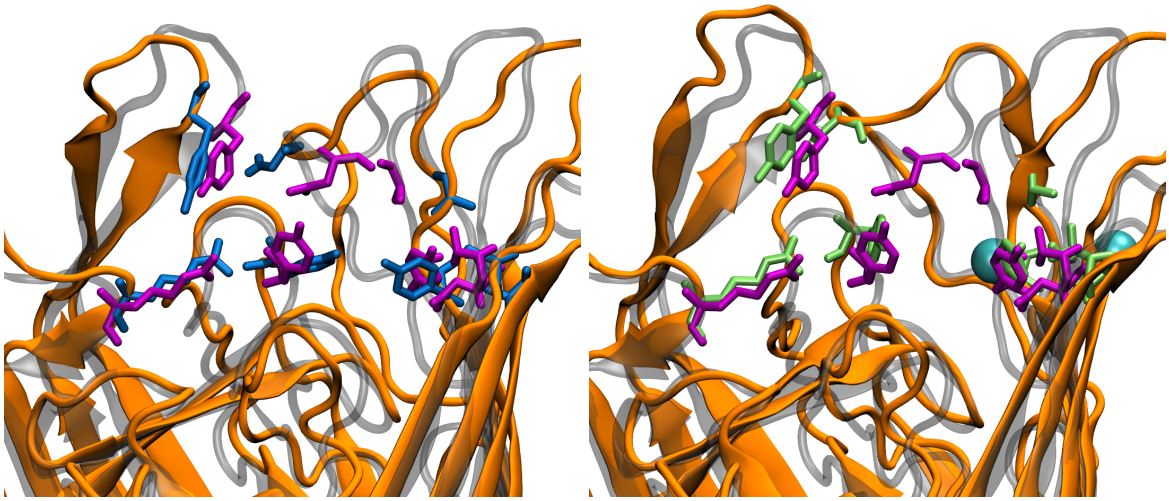


(A)

Figure S12: RMSD of Loop 3/4 in each of the simulated systems. RMSD was calculated for the sidechains and backbone of His176 to Lys200 in comparison to the CBL-bound crystal structure. Greatest amount of deviation ($>$ 8Å) is observed in the Apo-sym system with decreased values for the Apo1- and Apo2- as well as Ca1- and Ca2-OM.



(A) Apo1-OM (B) Ca1-OM



(C) Apo2-OM (D) Ca2-OM

Figure S13: Comparison of the (A) Apo- and (B) Ca^{2+}-bound states of BtuB in the second simulated OM systems. (A) Apo-state BtuB (orange ribbon) aligned to the CBL-bound crystal structure (transparent grey ribbon) from Chimento et al. [37]. Residues within 4 Å of CBL in the CBL-bound crystal structure are highlighted as blue (Apo2-OM) and purple (crystal structure) sticks. (B) Ca^{2+}-bound BtuB (orange ribbon) aligned to the CBL-bound crystal structure (transparent grey ribbon). Calcium ions are shown as cyan spheres and the green sticks represent the CBL binding residues in the Ca2-OM system.

Table S1: Summary of systems. Lipid counts are reported in Table S2.

System	Atoms	Water molecules	Ions
Apo-sym	69494	14823	99
Apo1-OM	137411	27019	452
Apo2-OM	137407	27019	448
Ca1-OM	137407	27019	448
Ca2-OM	137407	27019	448
OM only	71781	13068	207

Table S2: Summary of membrane results for each BtuB system.

System (time ns)	Bilayer composition (Top:Bottom)	Diffusion Coefficient (cm^2/s)	Area Per Lipid ($Å^2$/ Molecule) ± Std. Dev.	Hydrophobic Thickness (Å)
Apo-sym (150 ns)	66-POPE 61-POPE	2.3×10^{-8} 2.0×10^{-8}	58.8 ± 0.4 63.7 ± 1.3	34
Apo1-OM (300 ns)	50-LPS 157-POPE	7.2×10^{-10} 4.4×10^{-8}	194.6 ± 1.6 61.9 ± 0.5	24
Apo2-OM (300 ns)	50-LPS 157-POPE	6.2×10^{-10} 4.3×10^{-8}	192.6 ± 1.3 61.4 ± 0.4	24
Ca1-OM (300 ns)	50-LPS 157-POPE	9.8×10^{-10} 4.5×10^{-8}	197.3 ± 1.3 62.8 ± 0.4	26
Ca2-OM (300 ns)	50-LPS 157-POPE	6.0×10^{-10} 4.6×10^{-8}	193.1 ± 1.5 61.5 ± 0.5	25
OM only (172 ns)	36-LPS 102-POPE	8.1×10^{-10} 6.4×10^{-8}	178.9 ± 0.6 63.1 ± 1.4	25

Table S3: Summary of hydrogen bonds from the Apo1-OM and Apo2-OM systems. Occupancies over 100% are due to multiple hydrogen bonds forming simultaneously.

BtuB Residue	LPS component	Apo1-OM occ.	Apo2-OM occ.
Loop 1/2			
SER146	LipidA	91.75	-
ASN147	LipidA	96.30	-
Loop 3/4			
TYR172	LipidA	-	11.10
ASP179	Gal	11.10	-
ASP179	Gal	21.80	-
ASP179	Gal	38.55	-
ASP179	Glc	124.85	-
ASP179	KDO	-	13.95
TYR183	KDO	-	25.15
TYR183	KDO	-	10.50
GLY184	Hep	-	21.10
THR186	Hep	-	22.05

Continued on next page

Table S3 – continued from previous page

BtuB Residue	LPS component	Apo1-OM occ.	Apo2-OM occ.
THR188	Hep	-	11.45
THR192	Hep	17.00	-
ASP193	Hep	100.30	-
ASP193	Gal	20.80	15.15
ASP193	Glc	18.85	-
ASP193	Glc	15.30	-
ASN194	Hep	17.35	-
ASN194	Hep	24.10	-
ASN194	Gal	15.00	-
ASN194	Gal	19.20	-
LYS200	LipidA	-	73.05
Loop 5/6			
ASP241	Glc	23.70	-
THR242	Glc	10.70	-
LYS244	LipidA	-	33.30
TYR246	LipidA	-	53.35
GLN248	LipidA	29.40	-
TRP250	LipidA	-	10.70
Loop 7/8			
HSE280	Hep	11.00	-
TYR281	Hep	11.25	-
ARG283	KDO	-	11.15
ARG283	LipidA	-	14.95
TYR284	Hep	-	28.05
ASP285	Hep	218.50	35.25
ASP285	Hep	61.25	
SER286	Hep	19.65	-
SER286	Hep	44.65	-
SER287	Glc	-	11.00
SER287	Glc	-	12.80
Loop 9/10			
THR321	KDO	-	26.70
THR321	KDO	-	61.00
GLU330	Glc	16.35	-
GLU330	Gal	54.15	-
ASP331	Glc	35.15	85.40
ASP331	Glc	36.00	
ASP331	Hep	27.60	-
GLY332	Hep	66.10	-
GLY332	Gal	-	16.30
GLY332	Glc	-	38.20
TYR333	Glc	19.40	10.45
TYR333	Glc	24.30	
TYR333	Hep	-	23.95
TYR333	Hep	-	14.15
TYR333	Gal	-	11.20

Continued on next page

Table S3 – continued from previous page

BtuB Residue	LPS component	Apo1-OM occ.	Apo2-OM occ.
ASP334	Gal	-	86.10
Loop 11/12			
GLN364	Gal	24.05	24.70
ARG367	KDO	76.65	-
ARG367	LipidA	58.20	189.05
Loop 13/14			
TYR402	Gal	25.55	22.25
TYR402	Gal	11.55	
PRO408	Glc	-	13.40
ASN409	Gal	17.35	-
ASN409	Gal	10.75	-
ASN409	Glc	-	57.15
ASN409	Glc	-	50.15
ASP411	Hep	15.75	27.15
ASP411	Glc	65.75	-
Loop 15/16			
ASP442	Hep	117.45	-
GLU456	Gal	51.80	-
GLU456	Gal	22.75	-
ARG460	Hep	17.65	-
Loop 19/20			
THR525	LipidA	122.10	-
TYR527	KDO	51.70	-
TYR527	Hep	-	116.35
GLN537	Glc	10.70	13.20
GLN537	Glc	22.10	19.10
GLN537	Hep	29.15	-
THR538	Glc	37.45	-
LYS540	LipidA	105.15	-
LYS540	Glc	16.85	-
LYS540	KDO	36.85	-
LYS540	Hep	17.25	235.75
Loop 21/22			
LEU568	LipidA	-	19.20
ASP570	LipidA	-	146.15
LYS571	LipidA	34.50	191.10
TYR577	Hep	-	42.75
GLN580	Gal	10.65	-
GLN580	Gal	10.95	-
ARG584	LipidA	20.70	-
ARG584	KDO	-	327.05
TYR586	LipidA	28.10	-

Table S4: Summary of hydrogen bonds in the symmetric Apo-BtuB system.

Symmetric-Apo		
BtuB Residue	POPE component	Occupancy %
Loop 1/2		
SER146	PEhead	18.5
ASN147	PEhead	52.9
SER148	PEhead	40.3
Loop 3/4		
HSE176	PEhead	26.5
ASP193	PE-NH3	77.3
ASP195	PEhead	63.1
Loop 5/6		
ARG226	PEhead	83.7
Loop 7/8		
ARG283	PEhead	47.0
TYR284	PEhead	15.3
Loop 11/12		
ASP361	PE-NH3	20.6
ARG367	PEhead	45.5
Loop 15/16		
ARG460	PEhead	29.7
Loop 17/18		
ASP492	PE-NH3	82.5
ARG487	PEhead	19.9
LYS500	PEcore	28.9
Loop 19/20		
THR525	PE-NH3	97.8
GLY543	PEhead	35.8
Loop 21/22		
ASP570	PEhead	88.9
ASP570	PEcore	30.9
ASP570	PE-NH3	10.9
LYS571	PEcore	48.7
LYS571	PEhead	80.9
ASP572	PE-NH3	66.6
GLU574	PE-NH3	12.7
ARG584	PEhead	72.4
ARG584	PEcore	94.3
TYR586	PEcore	47.7

Table S5: Summary of hydrogen bonds from the Ca1-OM and Ca2-OM systems. Occupancies over 100% are due to multiple hydrogen bonds forming simultaneously.

BtuB Residue	LPS component	Ca1-OM occ.	Ca2-OM occ.
Loop 1/2			
SER146	LipidA	-	11.33
Loop 3/4			
HSE174	LipidA	-	102.20
TYR183	Gal	17.65	-
ALA190	Gal	39.40	-
GLN191	Hep	-	15.34
GLN191	Gal	12.70	-
THR192	Hep	36.50	10.94
THR192	Hep	12.55	
THR192	Gal	10.60	-
ASP193	Hep	-	93.06
ASP193	Gal	12.55	-
ASN194	Hep	18.95	-
ASN194	Hep	14.75	-
ASN194	Glc	17.00	-
ASN194	Glc	46.75	-
Loop 5/6			
ARG226	LipidA	-	148.38
TYR246	LipidA	20.95	18.51
GLN248	LipidA	23.70	13.70
GLN248	LipidA	12.15	
Loop 7/8			
ARG283	LipidA	157.75	-
TYR284	KDO	-	50.19
TYR284	KDO	-	15.48
TYR284	LipidA	16.85	-
TYR284	Hep	-	19.97
ASP285	Hep	66.20	-
ASP285	Glc	27.45	-
SER286	Hep	48.30	-
SER286	Hep	34.85	-
SER286	Glc	22.10	-
SER286	Glc	14.05	-
Loop 9/10			
GLU330	Hep	11.35	-
GLU330	Hep	12.05	-
ASP331	Glc	69.40	-
ASP331	Hep	-	11.15
ASP331	Hep	-	54.62
GLY332	Glc	19.65	-
TYR333	Glc	40.00	-
TYR333	Glc	29.55	-
TYR333	Gal	-	14.43

Continued on next page

Table S5 – continued from previous page

BtuB Residue	LPS component	Ca1-OM occ.	Ca2-OM occ.
ASP334	KDO	88.15	-
ASP334	Hep	41.55	-
ASP334	Hep	19.55	-
Loop 11/12			
GLN364	Gal	-	13.07
GLN364	Glc	-	19.66
PHE365	Hep	-	14.88
ARG367	KDO	25.70	66.71
ARG367	LipidA	177.50	107.88
Loop 13/14			
TYR402	Glc	19.70	-
TYR402	Glc	26.80	-
ASN409	Glc	-	17.15
ASN409	Glc	-	23.35
Loop 15/16			
SER441	Hep	-	37.05
ASP442	Hep	-	50.26
LYS458	Hep	85.50	84.00
ARG460	Hep	-	197.56
Loop 17/18			
ARG487	KDO	55.30	-
ARG487	Hep	12.95	125.13
ASP492	Hep	14.50	-
LYS500	LipidA	19.25	-
Loop 19/20			
TYR527	Glc	15.45	-
GLN537	Hep	14.25	19.52
THR538	Hep	15.60	51.03
THR538	Glc	13.15	-
LYS540	Hep	16.55	139.94
Loop 21/22			
ASP570	Hep	-	67.41
LYS571	LipidA	10.40	207.39
LYS571	KDO	16.00	-
TYR577	Hep	-	54.41
GLN580	Hep	10.60	11.99
GLN580	Hep	16.90	
THR581	Hep	-	11.89
ARG584	LipidA	167.90	84.07
TYR586	LipidA	30.90	56.54