Supplementary Table 1. Summary of data collection and refinement statistics for FadL mutants

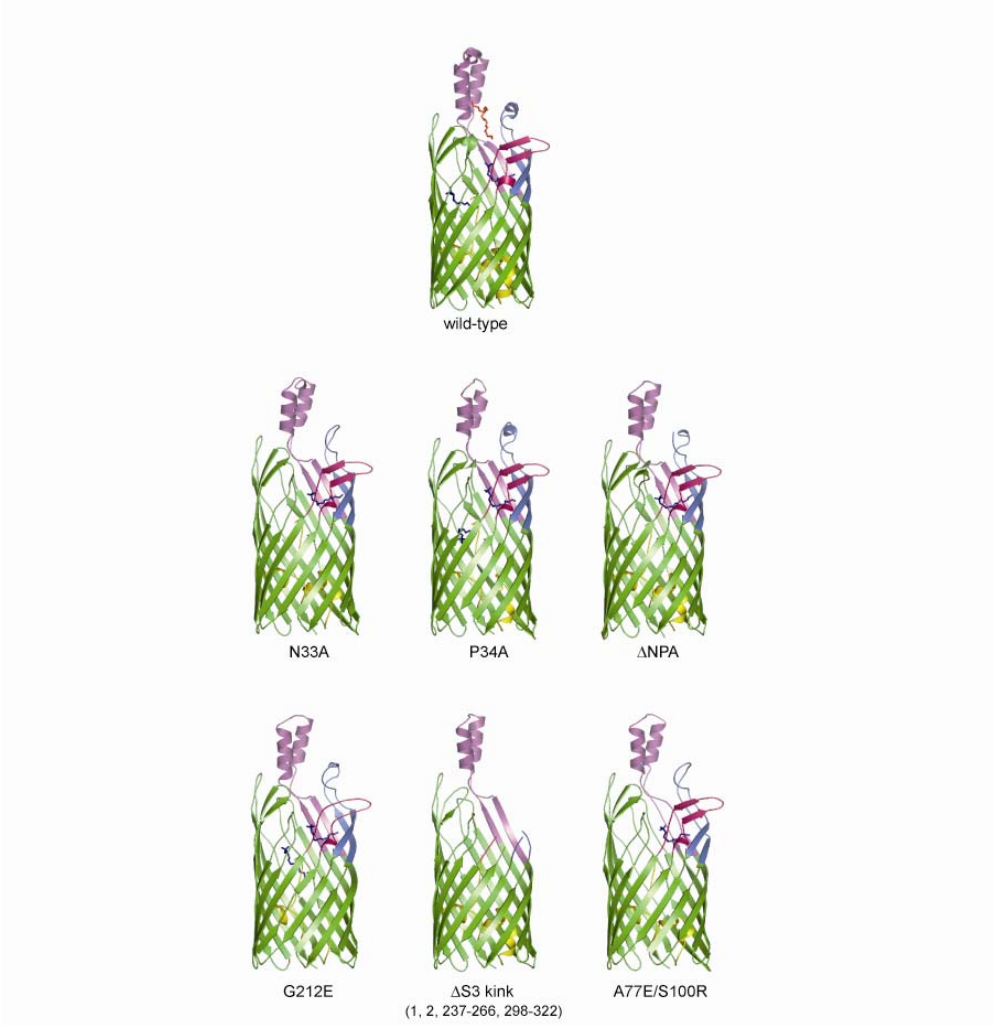
	N33A	**P34A**	**ΔNPA**	**G212E**	**ΔS3 kink**	**A77E/S100R**
Cell parameters						
Space group	$P2_1$	$C222_1$	$P2_12_12_1$	C2	$C222_1$	$P2_12_12_1$
a (Å)	70.2	112.8	63.1	232.5	91.3	63.1
b (Å)	40.0	167.0	146.6	70.0	91.4	147.0
c (Å)	175.0	197.4	151.2	84.2	267.3	152.0
α, β, γ (deg)	90, 90, 90	90, 90, 90	90, 90, 90	90, 101, 90	90, 90, 90	90, 90, 90
Data collection statistics						
Resolution (Å)	3.1	3.3	3.6	2.9	2.55	2.5
Unique reflections	15 295	25 992	16 885	29 131	33 965	48 715
Completeness (%)	85.1(76.9)	94.8(95.9)	98.7(97.0)	98.6(96.7)	91.9(84.2)	97.5(97.1)
Redundancy	2.9 (2.8)	5.2 (5.1)	4.4 (4.0)	3.7 (3.4)	8.9 (4.5)	5.1 (5.0)
R_{merge} (%)	12.6 (28.8)	16.3 (50.1)	28.7(59.3)	8.8 (44.9)	7.9 (56.3)	8.2 (47.5)
$I/\sigma I$	6.8 (3.8)	5.1 (2.7)	3.1 (2.2)	7.8 (2.3)	13.9 (2.4)	9.2 (3.0)
Refinement statistics						
Resolution range (Å)	10-3.2	10-3.3	10-3.6	10-2.9	10-2.6	10-2.5
Total no. atoms (non-hydrogen)	6524	9743	6552	6498	5628	6725
R_{work} (%)	23.5	23.4	24.3	26.7	26.2	23.9
R_{free} (%)	33.2	30.2	31.4	28.8	29.4	28.9
R.m.s.d., bonds (Å)	0.009	0.009	0.009	0.008	0.008	0.007
R.m.s.d., angles (deg)	1.60	1.60	1.55	1.40	1.40	1.46
Ramachandran plot						
Most favored and additionally allowed(%)	98.9	98.8	95.5	99.5	99.7	99.7
Disallowed regions (%)	0	0.2	0.4	0	0.3	0
Average B-factors ($Å^2$), main/side chain	21.4/23.7	23.3/25.0	8.6/18.5	41.2/42.1	48.5/51.1	36.5/39.5

Values in parentheses are for the highest resolution shell.
Each dataset was obtained from a single crystal.

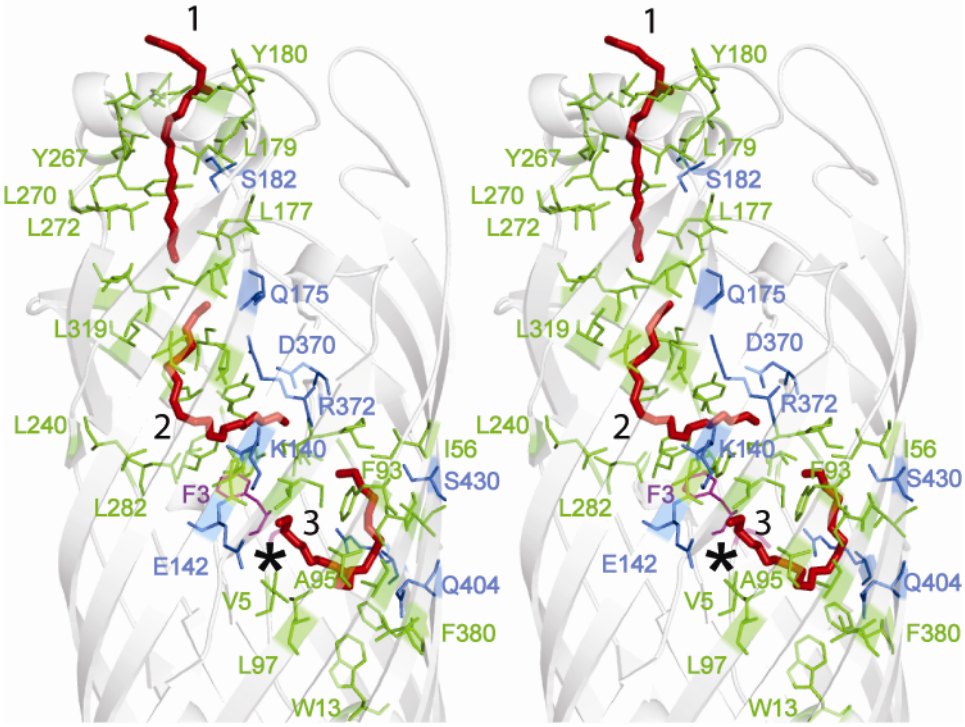
Supplementary Table 2. Summary of data collection, phasing statistics and refinement for PaFadL

	Native	**$KAuCl_4$**	**$OsCl_3$**
Cell parameters			
Space group	$C222_1$	$C222_1$	$C222_1$
a (Å)	56.8	56.5	56.5
b (Å)	233.1	233.7	233.6
c (Å)	81.9	81.7	81.9
α, β, γ (deg)	90, 90, 90	90, 90, 90	90, 90, 90
Data collection statistics and phasing			
Wavelength (Å)	0.9790	1.0375	1.1397
Resolution (Å)	50 - 2.1	50 – 2.2	50 – 2.5
Unique reflections	28897	28044	18437
Completeness (%)	89.7 (38.6)	99.9 (99.2)	99.9 (99.7)
Redundancy	5.6 (3.2)	7.2 (6.0)	8.9 (7.7)
R_{sym} (%)	7.3 (28.7)	10.5 (44.6)	12.5 (51.1)
$I/\sigma I$	10.0 (2.4)	8.2 (3.7)	6.7 (3.6)
Phasing statistics (20-2.5 Å)			
Phasing power isomorphous (centric/acentric)		0.75/0.82	0.34/0.66
Phasing power anomalous (acentric)		0.76	0.58
Number of sites		7	4
FOM (centric/acentric)		0.37/0.34	
Refinement statistics			
Resolution range (Å)	20-2.2		
Total no. of atoms (protein, C_8E_4, sulfate, water)	3386, 231, 5, 214		
R_{work} (%)	21.3		
R_{free} (%)	25.4		
R.m.s.d., bonds (Å)	0.0059		
R.m.s.d., angles (deg)	1.36		
Ramachandran plot			
Most favored (%)	99.5		
Disallowed regions (%)	0.0		
Average B-factors ($Å^2$), (protein, C_8E_4, sulfate, water)	32.3, 32.5, 52.6, 36.4		

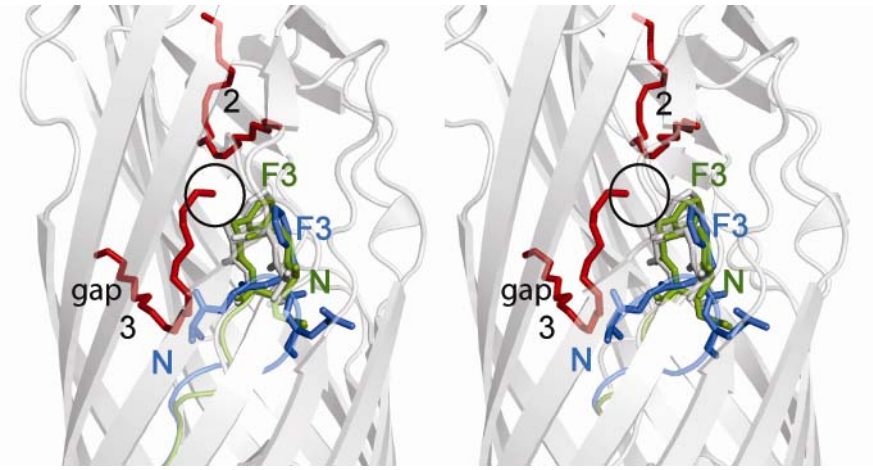
Values in parentheses are for the highest resolution shell.
Each diffraction data set was collected from one crystal.



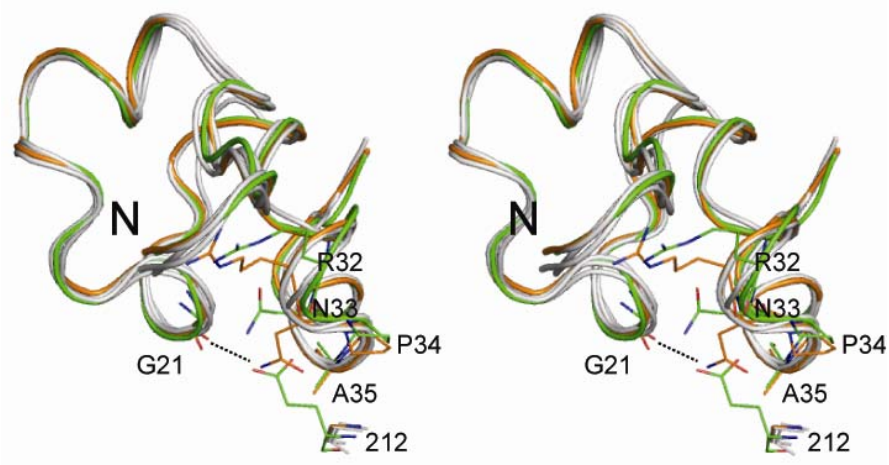
Supplementary Figure 1. Side view cartoon representations of the FadL mutants crystallized in this study, shown in identical orientations. Monoclinic wild-type FadL (PDB code 1T16) is shown for comparison. The proteins are colored as follows: hatch domain, yellow; loop L3, purple; loop L4, blue; loop L5, pink; LDAO molecules, dark blue; C_8E_4 molecules, red. The numbers of the amino acids not visible in the mutant structures are shown in parentheses.



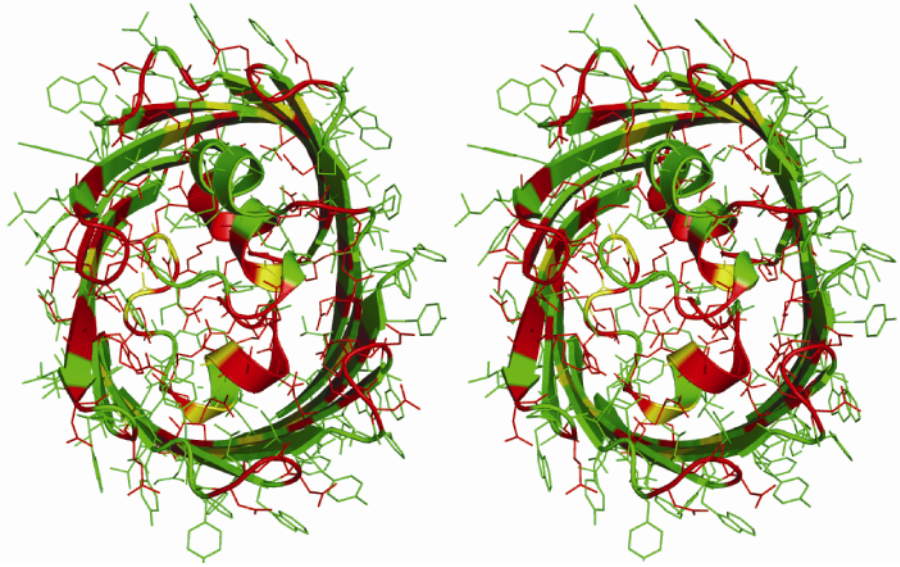
Supplementary Figure 2. The hydrophobic passageway in PaFadL. Stereo backbone view of PaFadL, with the three bound C_8E_4 detergent molecules (numbered 1-3) shown in red. Residues that are located within 4.5 Å distance of a detergent molecule are shown, with hydrophobic residues colored green (residue F3 is shown in magenta), and polar residues colored blue. For clarity, not all residues are labeled. The residues located close to the detergent molecules are (with their *E. coli* structural equivalents in parentheses, and polar residues underlined); **detergent 1**, L177(I197), L179(F247), Y180(T187), S182(-), M189(A266), M203(A189), L253(-), T262(A246), A266(Y250), Y267(L252), L270(-), L272(A257), I326(L314), V327(F315), L419(-); **detergent 2**, F3(F3), K140(D122), I141(L1123), V173(I155), Q175(R157), V205(L200), L240(F235), A280(L267), L282(L269), F314(F303), L317(L304), L319(A306), I329(K317), Y331(F321), D370(S358), R372(S360), F373(I361); **detergent 3**, V5(L5), W13(L13), A52(I52), I54(P54), I56(V56), F93(P75), A95(A77), L97(V79), M122(L104), E142(E124), R378(R366), F380(W368), Q404(M390), V406(G392), S430(G408), V434(L412). The proposed substrate exit site from the barrel, located close to one end of detergent molecule 3, is indicated with an asterisk.



Supplementary Figure 3. A conformational change as observed in the N-terminus of EcFadL would generate a continuous hydrophobic passageway. Stereo view of PaFadL in grey, with residues 1-10 of EcFadL superposed (green, blocked channel, PDB ID 1T16; blue, open channel, PDB ID 1T1L). The C_8E_4 detergent molecules 2 and 3 as present in PaFadL are shown in red. The N-terminus (N) and residue F3 are indicated, highlighting the conformational change of the N-terminus. The approximate location of the opening created by the conformational change, resulting in a continuous hydrophobic passageway, is indicated with a circle. The location of the lateral gap, forming the proposed exit site for substrate, is indicated.



Supplementary Figure 4. The hatch domain is rigid. Stereo backbone view of superpositions of the hatch domains of wild type FadL (orange) and the G212E mutant (green), showing the limited structural changes that occur as a result of mutations affecting the NPA sequence. The superposed structures of the mutants P34A, N33A and ΔNPA are shown in grey. The superpositions were generated for the entire protein molecules. Residues G21, R32, N33, P34, A35 and G/E212 are indicated for wild type FadL and G212E. The location of the N-terminus is shown as well. The hydrogen bond between the amide nitrogen of N33 and the carbonyl oxygen of G21 in wild type FadL is shown as a dashed line.



Supplementary Figure 5. The hatch domain has a mixed polar-apolar character, and is unlikely to form a hydrophobic channel. Stereoview from the periplasm of wild type *E. coli* FadL, with hydrophobic residues colored green and polar/charged residues colored red. Glycine residues are colored yellow.