Supplementary Information

The redesign of a plugged β-barrel membrane protein

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Running title: Redesign of an outer membrane protein

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Methods:

Circular dichroism spectroscopy (CD). All CD spectra were recorded on an Aviv 62DS circular dichroism spectropolarimeter (Aviv Instruments, Lakewood, NJ) equipped with a temperature control unit. Quartz cuvettes with a 1 mm-path length were employed. Measurements were carried out at 20 °C over an optical pathway of 1 mm, with a 1 nm step and a 10 s average time at protein concentration of 3.42 μM in 5 mM Tris, 100 mM NaCl, and 0.25% (w/v) DDM, pH 8.32. Deconvolution of the spectrum was accomplished using the CONTIN algorithm 1 (1) (http://dichroweb.cryst.bbk.ac.uk/html/home.shtml).

The prediction of protein structure. Energy minimization was performed using the INSIGHT II software package (MSI Scientific, San Diego, CA) with the modules View, Builder and Discover 3. The consistent valence force field (CVF) was used with a 10 Å cutoff, and 10000 minimization steps were run, 5000 iterations using the steepest descent gradient followed by 5000 iterations using conjugate gradients. For molecular dynamics (MD), a homology model for FhuA ΔC/Δ4L protein was constructed (Swiss Model). Then, it was used in a two-step molecular dynamics run. AMBER v11 was used in combination with the included force field and molecular simulations program. Minimization consisted of 100,000 steps with a 30 Å cutoff (50,000 steepest descent, which was followed by nearest neighbor algorithms). Molecular dynamics was then run under the following conditions: 30 Å cutoff, 250 ps run, 300°K, 2 fs time step, and solvated.

We also conducted standard MD simulations to determine which loops may fold back into the pore lumen upon deletion of the N-terminal cork domain. For our single and double deletion mutants, we utilized the crystals structures obtained from the pdb ids 1BY5 and 2FCP. We deleted the cork domain (residues 1-160) and utilized the resulting structure to give an indication of which loops were to be further modified. Our standard MD simulations did not place our protein under an electric field. We did not include KCl. Nonetheless, it provided us with key clues and assistance in the further rational design of the FhuA protein channel. Based on the MD simulations, four major loops exhibited high flexibility and a tendency to fold back into the cork-free pore lumen or to partially block access to it. These loops are L3, L4, L5 and L11.

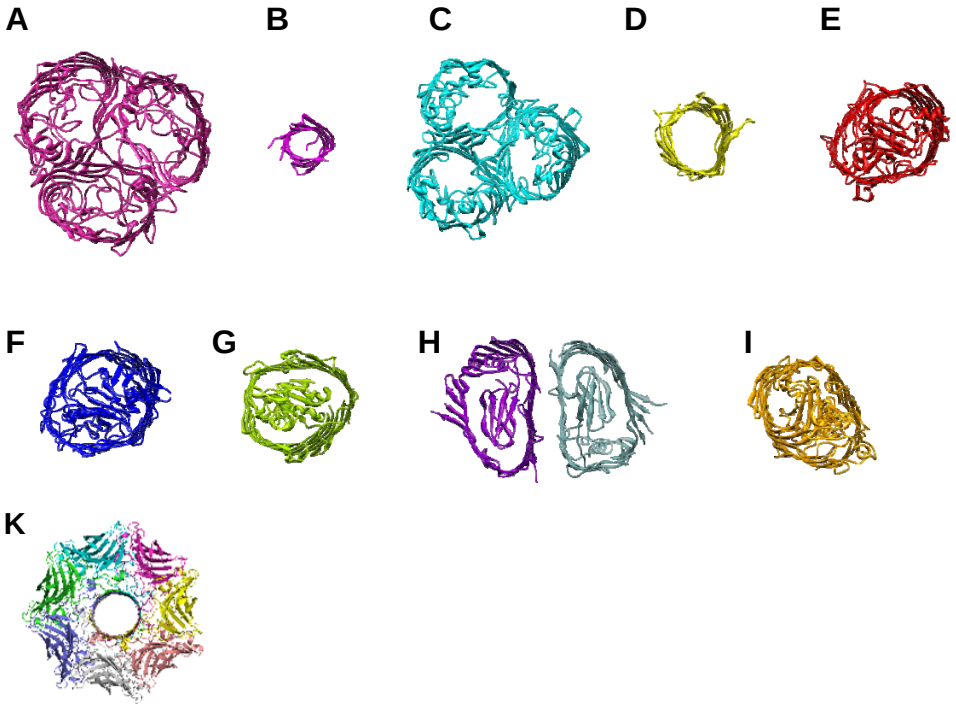

Figure S1. Cartoon presentations for crystal structures of β-barrel membrane protein channels, which have been studied by single-channel electrical recordings on planar lipid bilayers. (A) maltoporin (LamB) (2); (B) OmpA (3); (C) OmpF (4); (D) OmpG (5); (E) FecA (6); (F) FepA (7); (G) BtuB (8;9); (H) PapC (10); (I) FhuA (11;12), (J) αHL (13) (**Table 1**).

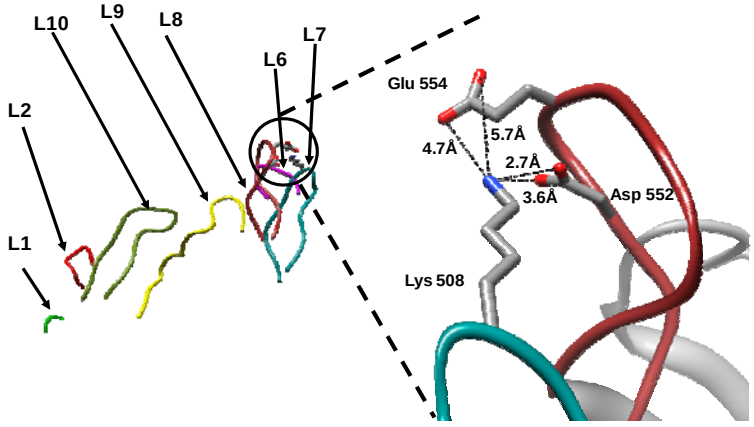

Figure S2. Unmodified extracellular loops and the network of ion-pair interactions in the wild-type FhuA (WT-FhuA) protein. Left, a cartoon presentation of the extracellular loops of the FhuA protein. The short loops L 1 (green), L2 (red) and L6 (magenta) were not modified by protein engineering. Loops L9 (yellow) and L10 (dark green) were altered by protein engineering (see main text). On the right side, we show an expanded region of the network of ion-pair interactions formed between L7 (dark cyan) and L8 (brown). The region was rotated for clarity. Lys508 from L7 forms a short-range ion-pair interaction with Asp552 in L8, whose N−O lengths are 2.7 and 3.6 Å. Also, Lys508 forms ion-pair interactions with Glu554 in L8, whose N−O lengths are 4.7 and 5.7 Å.

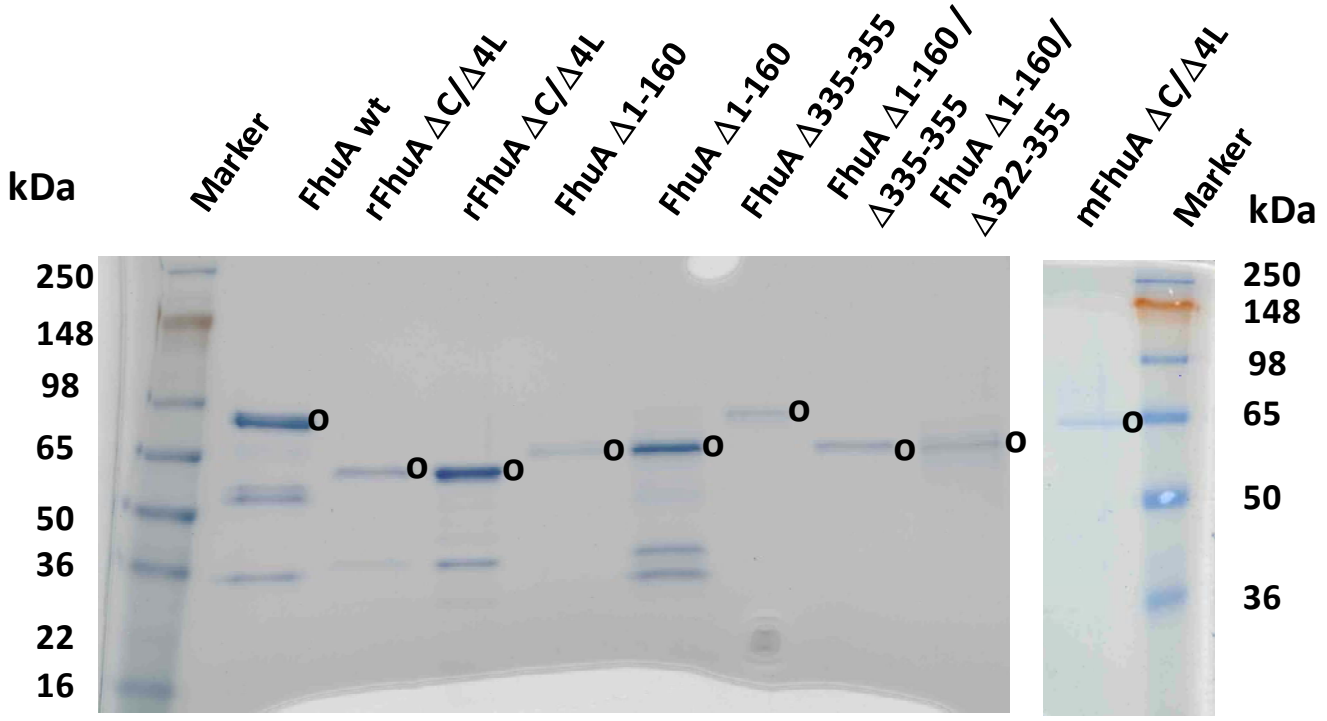

Figure S3. Assessment of the purified WT-FhuA protein and its deletion mutants. Proteins were loaded on 4-20% gradient SDS-PAGE (the left panel) and 12% SDS-PAGE (the right panel) (see the main text for the rFhuA ΔC/Δ4L and mFhuA ΔC/Δ4L abbreviations). In the left panel, two lanes of rFhuA ΔC/Δ4L represent two different refolding experiments. All samples were mixed with SDS loading buffer and heated at 95°C for five minutes prior to loading. Samples were run at constant voltage of 150 mV and stained with colloidal blue suspension. Open circles denote the purified proteins. Further, purity was assessed through His-tag specific staining, followed by visible inspection of the colloidal blue stained gel. The only bands present in the His-tag stained gel are those which are also seen in the colloidal blue gel, indicating the pure and desired product is obtained (data not shown). In some of the lanes, there are bands which are lower than the expected products. These were not due to contamination, but rather due to different conformations of the FhuA derived β-barrel constructs. A β-barrel protein migrates at a standard molecular weight that is different from its corresponding molecular weight. This phenomenon is known as heat modifiability (14).

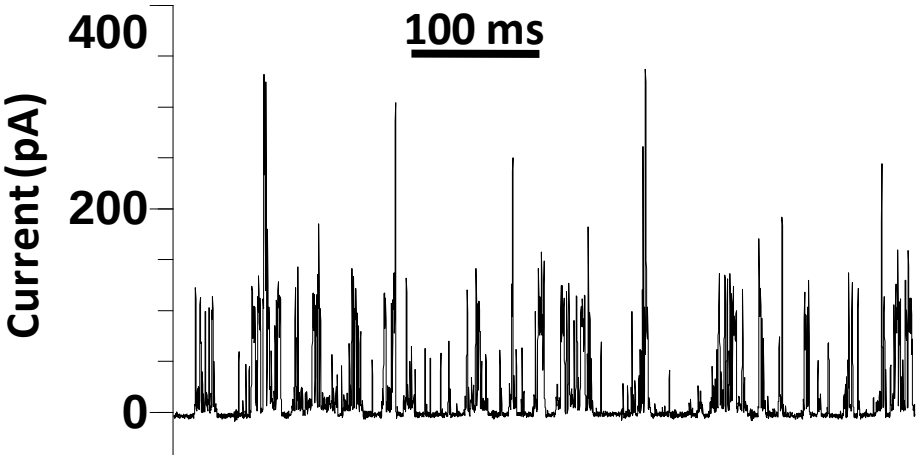

Figure S4. Single-channel electrical recording with the FhuA Δ1-160 protein. In some instances, there are events whose corresponding single-channel current amplitude is greater than 120 pA (see the main text). The transmembrane potential was +40 mV. The data was acquired in 1 M KCl, 10 mM potassium phosphate, pH 7.4. The single-channel trace was low-pass Bessel filtered at 2 kHz.

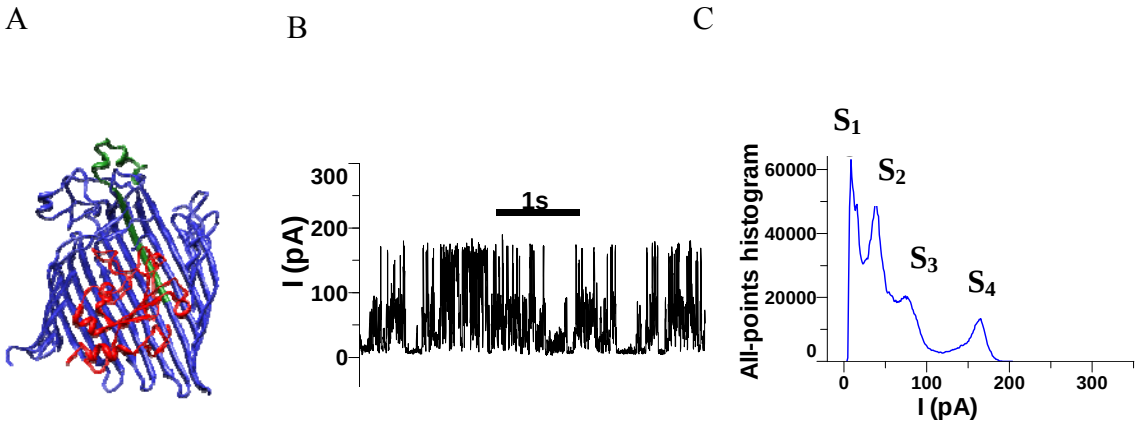

Figure S5. FhuA Δ322-355 has multiple channel sub-states. (A) Protein structure with cartoon presentation for the FhuA protein. Green part is the region that was deleted; (B) Representative single-channel electrical recordings with FhuA Δ322-355 in 1 M KCl, 10 mM potassium phosphate, pH 7.4. (C) All-point current amplitude histograms of (B). Protein was added to the *cis* chamber. The applied transmembrane potential was +40 mV.

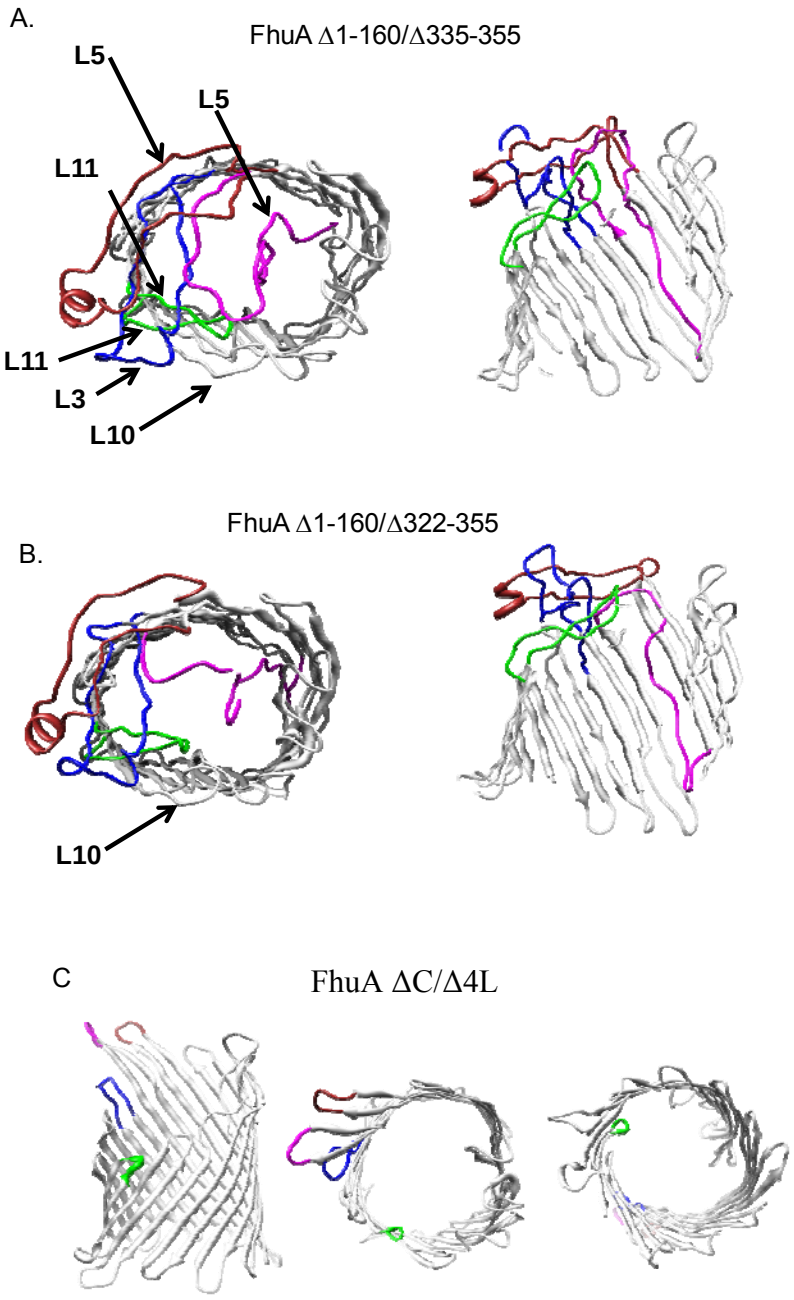

Figure S6. The predicted protein structures for FhuA Δ1-160/Δ335-355 (A), FhuA Δ1-160/Δ335-355 (B) and FhuA ΔC/Δ4L (C). Structures in (A) and (B) were obtained from energy minimization (A and B) and molecular dynamics (C). In (A) and (B), the left and right panels represent the extracellular and side views of the proteins, respectively (side views were sliced). In (C), side, extracellular and periplasmic views of FhuA ΔC/Δ4L are presented in the right, middle and left panel, respectively. The colors of the loops are as in **Fig. S2**, except for L10 (light grey). It should be noted that L10 maintains its conformation, as it did not fold back into the pore lumen in the mutants tested in our simulations.

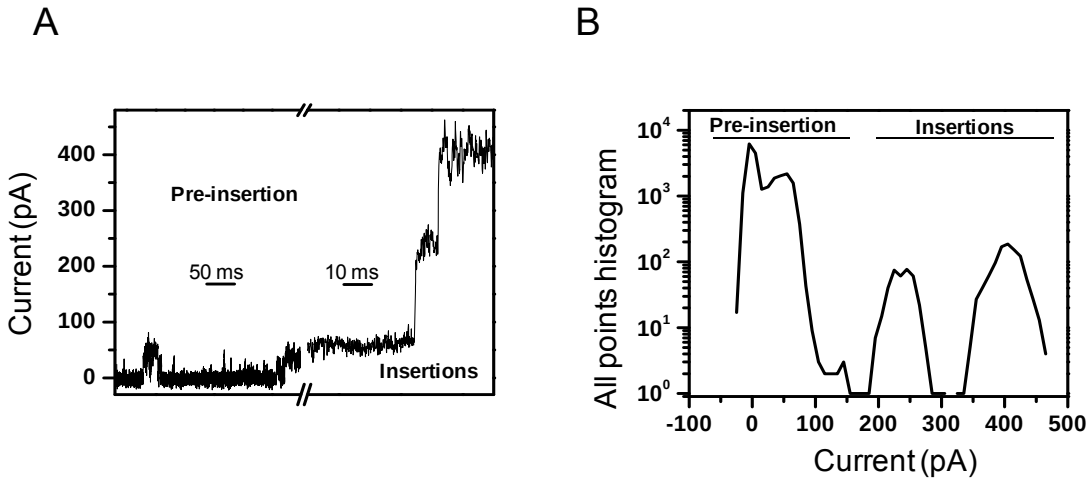

Figure S7. Single-channel electrical recordings of the membrane extracted FhuA ΔC/Δ4L protein (mFhuA ΔC/Δ4L). (A) A stepwise increase of the electrical current recordings showing pre-insertion activity of the mFhuA ΔC/Δ4L protein proceeded by single-channel insertions into the lipid bilayer. Protein was added to the *cis* side of the lipid bilayer. The transmembrane potential was +40 mV. Break was made in the X axis in panel (A) to compress the long trace, two different time scales are shown to the left and the right of the break. The pre-insertion conductance is 1.2 nS. The conductances of the first and second insertions are 5.4 and 4.2 nS, respectively. *(*B) All-points current amplitude Gaussian histograms that show the most probable current sub-states of the channel.

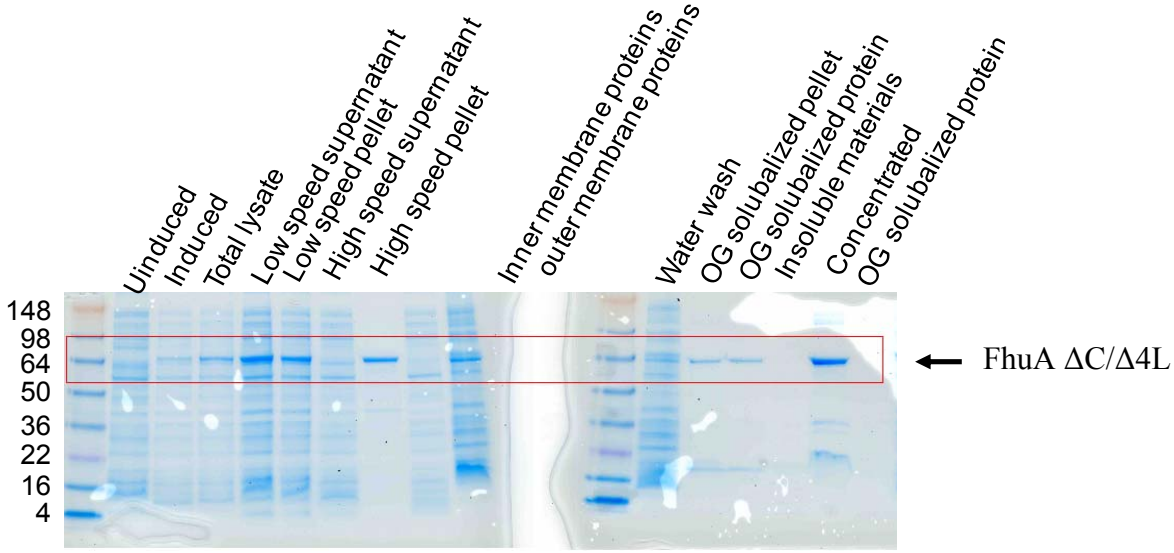

Figure S8. Purification scheme for the mFhuA ΔC/Δ4L protein. Selective fractionation and solubilization of expressed FhuA ΔC/Δ4L protein showed that some FhuA ΔC/Δ4L protein formed inclusion bodies, as the FhuA ΔC/Δ4L proteins sedimented at low-speed centrifugation. Proteins were run on 4-20% gradient SDS-PAGE and visualized as in **Fig. S3**.

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