Supporting Information:

The TonB-dependent Transporter FhuA in Planar Lipid Bilayers: partial exit of its plug from the barrel.

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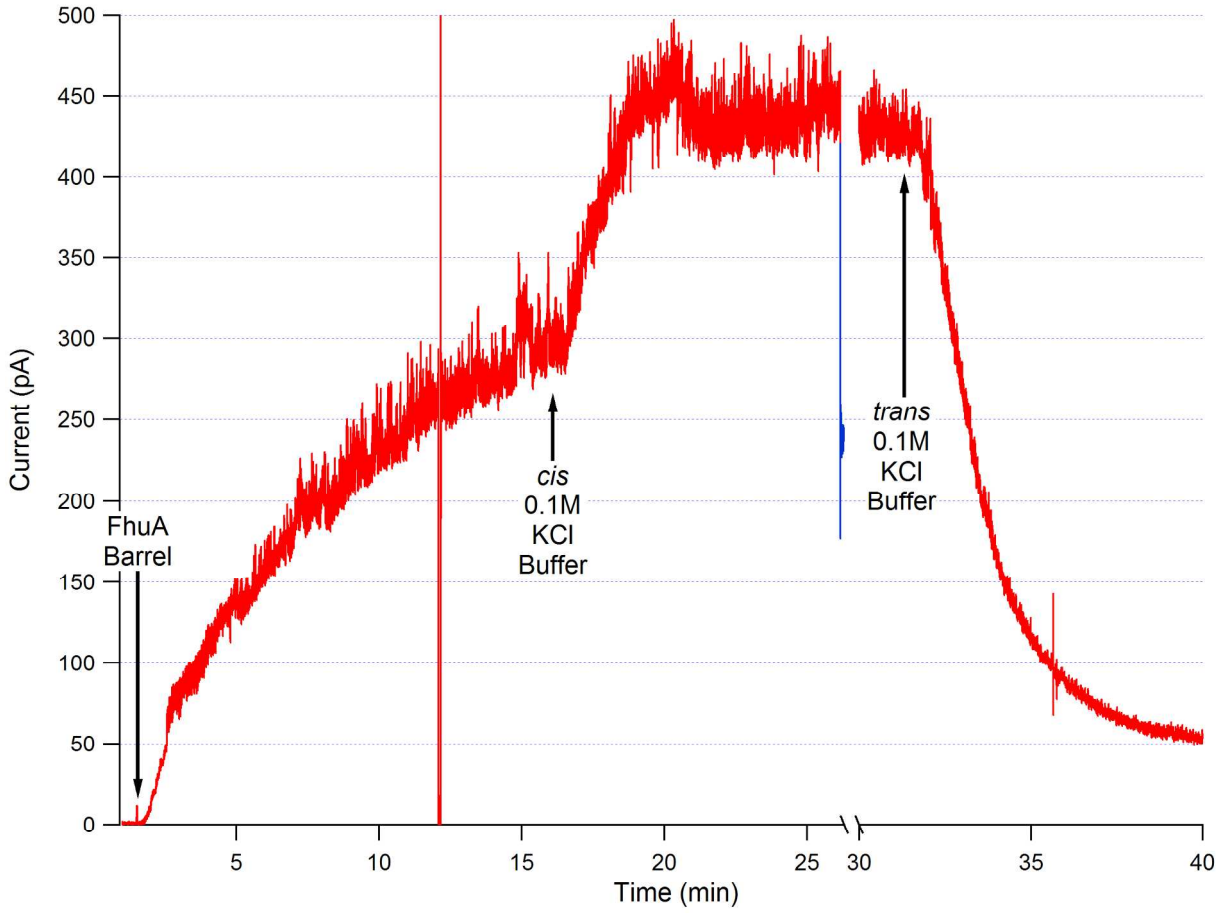
Figure S1: Current trace with the membrane in symmetric 1 M KCl. FhuA barrel (9 nM) was added to the *cis* compartment. The voltage was held at -20 mV throughout the experiment (red) except for when voltage was stepped to 0 mV to visualize the offset due to the salt gradient at the time (blue). Replacing the 1 M KCl buffers in the *cis* and then in the *trans* compartments with 0.1 M KCl resulted in the expected loss of current and demonstrates just how little conductance we get in the system with this protocol.



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Colicin M cloning and purification:

Plasmid pMLD170, containing the genes for colicin M and its immunity protein cloned in pTrc99A was a kind gift of Dominique Mengin-lecreulx (El Ghachi et al. 2006 JBC). For other purposes, we wanted to be able to express colicin M with a cleavable C-terminal histidine tag. pMLD170 was therefore used as the PCR template to first clone the colicin M gene between the NcoI and SacI sites of pET52-b (EMD Millipore) cut with the same two enzymes, to create pKSJ352. Splicing at the NcoI site created a frame shift in colicin M, so that full-length colicin protein was not synthesized, thus protecting host cells in the absence of the immunity protein. The colicin M immunity protein gene was subsequently amplified by PCR from pMLD170, with flanking AvrII sites, and cloned into the AvrII site of pKSJ352, such that the immunity protein is transcribed in the opposite direction from the colicin, and creating pKSJ353. Finally, pKSJ354 was created by site-directed mutagenesis to delete a G residue from the beginning of the colicin M sequence, in the NcoI site, to put the colicin gene back in the correct frame. pKSJ354 thus has the colicin M gene under IPTG-inducible control, with a thrombin cleavage site and His_{10}-tag at its C-terminus, followed by the colicin M immunity protein gene, whose expression is governed by its own promoter from pMLD170.

Colicin M was expressed and purified from pKSJ354 in BL21(DE3) cells in 500 ml of Overnight Express TB (EMD Millipore) essentially as described (Jakes and Finkelstein, 2010). The yield was over 100 mg of protein.

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Figure S2: The effect of colicin M. Trace begins with the membrane in *cis* 4 M urea to which 9 nM FhuA Δ6-160 (FhuA Barrel) was added. The *cis* compartment was then perfused with buffer removing the urea and FhuA barrel in solution. Addition of 78 nM colicin M to the *cis* compartment yielded no significant change in current, whereas its addition to the *trans* compartment yielded an increase in current across the membrane. This suggests the barrels may all be oriented extracellular-side-*trans*, unlike what was inferred from Figure 5. (Colicin M added to the *trans* compartment in the absence of *cis* colicin M also resulted in the same channel behavior.) Shown in blue is the fit of the effect of *trans* Colicin M on conductance using a single exponential. The observed time constants range from 4.4 – 8.9 minutes for 4 experiments. The voltage was held at -20 mV throughout the experiment.