Supporting Information

Inspection of the Engineered FhuA ΔC/Δ4L Protein Nanopore by Polymer Exclusion

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Running title: Sizing an engineered protein nanopore

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1. FhuA ΔC/Δ4L purification and refolding

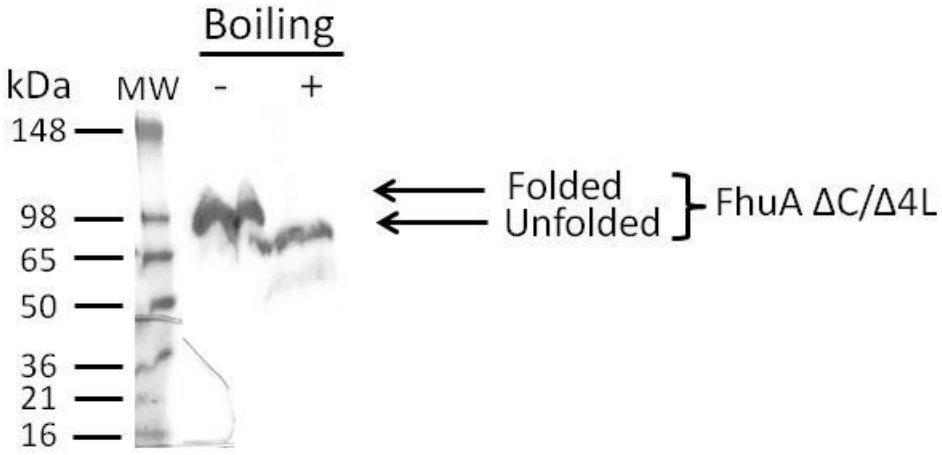

Figure S1: **Coomassie, blue-stained SDS-PAGE gel indicating the purified FhuA ΔC/Δ4L protein nanopore and its refolding.** 5 µg of protein samples were loaded on the gel without or after boiling for 5 minutes. Folded and unfolded FhuA ΔC/Δ4L proteins are indicated by arrows. MW stands for the molecular weight standard.

2. Selection criteria of nanopores

The FhuA ΔC/Δ4L nanopore inserts with a range of different conductances at a transmembrane potential of +40 mV. **Fig. S2** displays a histogram of the conductance of the nanopore at +40 mV. Only nanopores with conductance of 4.0 ± 0.2 nS were selected. The reasoning behind this choice is that the FhuA ΔC/Δ4L nanopores with closely similar conductance values are thought likely to have a similar structure.

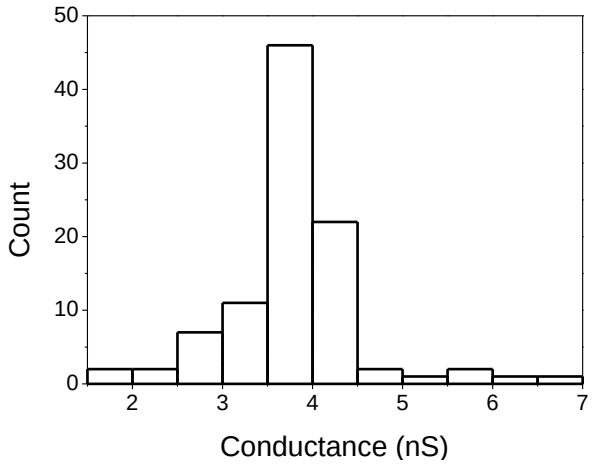

Figure S2: Histogram of different conductance values of the FhuA ΔC/Δ4L protein nanopore. The conductance values are taken at +40 mV in 1 M KCl, 10 mM potassium phosphate, pH 7.4. Histogram was constructed from a subset of data representing step-wise insertions of 98 different nanopores.

3. Purity of Reagents

PEG molecular mass ranges were the following: PEG 200 (M_r 190-210), PEG 300 (M_r 285-315), PEG 600 (M_r 570-630), PEG 1000 (M_r 950-1050), PEG 1500 (M_r 1400-1600), PEG 2000 (M_r 1900-2200), PEG 3000 (M_r 2700-3300), PEG 4000 (M_r 3500-4500), PEG 6000 (M_r 5000-7000), PEG 8000 (M_r 7000-9000), PEG 10000 (M_r 8500-11500), PEG 12000 (M_r 11000-15000).

4. Perfusion procedure of polymer solutions

The change of solution was accomplished by perfusion of 15% (w/w) PEG or dextran into the bottom of the chamber via a 0.5-mm diameter channel at a rate of 1 mL/min for 4 minutes. The steady perfusion of polymer solution from the bottom creates a fluid interface with the buffer solution in the chamber and displaces the buffer solution upward, where it is removed from the top of the chamber. After the desired experiments were performed, the resultant chamber solution was removed and its conductivity measured to confirm it matched the conductivity value corresponding to 15% (w/w) bulk PEG or dextran. This method consistently produced chamber solution conductivity values that matched the conductivity values obtained from the solution source.

5. Estimates of the bulk conductivity of the solutions used in this work

Table S1: Bulk conductivity of the PEG-containing solutions used in this work

Solution[a]	Conductivity (mS/cm)
PEG300	57.3
PEG600	56.6
PEG1000	56.1
PEG1500	57.0
PEG2000	56.6
PEG3000	57.6
PEG4000	57.6
PEG6000	57.2
PEG8000	57.2
PEG10000	56.6
PEG12000	56.8
Dextran	62.8

[a]Both PEG and dextran solutions were 15% (w/w) in 1 M KCl, 10 mM potassium phosphate, pH 7.4 . 1 M KCl solution was buffered with 10 mM potassium phosphate, pH 7.4 and had a conductivity of 98.6 mS/cm.

6. Single-channel experiments with dextran

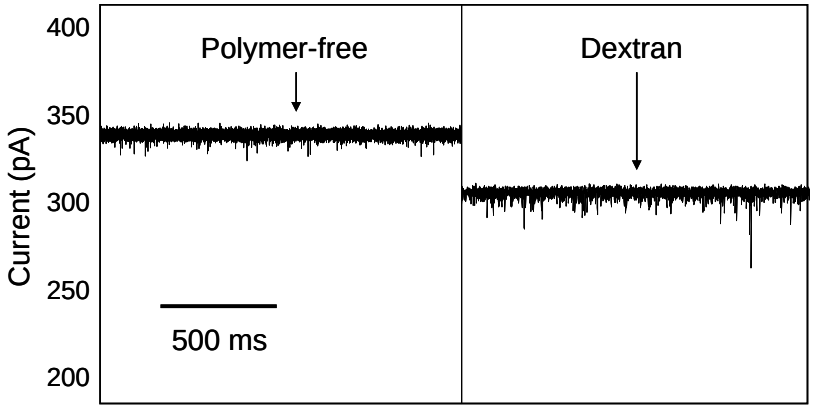

Figure S3: Effect of dextran on the single-channel current of a single FhuA ΔC/Δ4L nanopore. The single-channel electrical trace on the left side shows the data in 1M KCl, 10mM potassium phosphate, pH 7.4. The trace on the right side shows the data from the same nanopore after solution has been changed to 15% (w/w) 40000 Da dextran in 1 M KCl, 10 mM potassium phosphate, pH 7.4. The applied transmembrane potential was +80 mV. A reduction in the single-channel conductance of (8.8 ± 2.4)% was observed. Note the increased noise.

7. Calculations of expected conductance

A rough calculation of the expected single-channel conductance of the FhuA ΔC/Δ4L protein nanopore can be made assuming a conical geometry, which is derived from our PEG experiments, or an elliptical geometry, which approximates the crystal structure of the wild-type protein.

7.1. Calculation of the single-channel conductance of the FhuA ΔC/Δ4L nanopore assuming a conical geometry

Here, g, r_{eff} and χ denote the single-channel conductance of the nanopore, the effective radius of the nanopore and the conductivity of the bulk aqueous phase, respectively. l indicates the length of the nanopore. The conductance of the nanopore may be approximated by $g \approx \pi r_{eff}^2 \frac{\chi}{l}$. [1]

Let $r_{eff} = \left(\frac{\pi}{l} \int_0^l \frac{dz}{S(z)} \right)^{-1/2}$ where *S(z)* is the area of the cross-section of the nanopore at a point *z* along the axis, then

$$g \approx \frac{\pi \chi}{l \left(\frac{\pi}{l} \int_0^l \frac{dz}{S(z)} \right)},$$

Assume that the nanopore can be expressed as a simple symmetrical cone, where *a(z)* is the perpendicular distance of the cone from the z-axis at point *z*.

Then,

$$g = \frac{\chi}{\int_0^l \frac{dz}{\pi(a(z)^2)}}$$

$$g = \pi\chi\left(\int_0^l \frac{dz}{(a(z))^2}\right)^{-1}$$

$$g = \pi\chi\left(\int_0^l \frac{dz}{(a(0)-mz)^2}\right)^{-1}$$

Where *m* is a constant representing the slope of the cylinder given by $m = \frac{a(0)-a(l)}{l}$.

$$g = \pi\chi\left(\frac{1}{m(a(0)-mz)}|_0^l\right)^{-1}$$

$$g = \frac{\pi\chi a(0)a(l)}{l}$$

Relying on the PEG measurements and the bulk conductivity of 1M KCl, we may substitute in $\chi = 112\times10^{-1}$ S/m^2, $a(0) = 8\times10^{-10}$ m, $a(l) = 5\times10^{-10}$ m and $l = 50\times10^{-10}$ m,
and get *g* = 2.81 nS.

7.2. Calculation of the single-channel conductance of the FhuA ΔC/Δ4L nanopore using a smooth-walled ellipsoid geometry

Following the same equation as above, we may find the expected conductance of a smoothly changing ellipsoid.

$$g \approx \frac{\pi\chi}{l\left(\frac{\pi}{l}\int_0^l \frac{dz}{S(z)}\right)}$$

We will assume that the cross-sectional area may be expressed as an ellipse with major and minor axes given by *a(z)* and *b(z)*, respectively.

The above integral will now become

$$\int_0^l \frac{dz}{a(z)b(z)}$$

If we assume that *a(z)* and *b(z)* change with a constant slope, we have,

$$\int_0^l \frac{dz}{(a(0)-mz)(b(0)-\varsigma z)}$$

where m and ς denote the slopes of the ellipse axes, with respect to the z-axis.

From the crystal structure we may estimate values of $a(0)$ = 1.3x10^{-9} m, $b(0)$ = 1.95x10^{-9} m, l = 4.5x10^{-9} m, m =1/30, ς =3/10. Using these parameters, we find that the single-channel conductance of the nanopore is g = 3.4 nS.

Reference List

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