**ClyA expression and purification**

1. I used a pT7-SC1 plasmid containing the ClyA-AS gene
2. Cell cultures were induced using 0.5 mM isopropyl b-D-1-thiogalactopyranoside (IPTG), instead of 0.1 mM

**Recoding of single-channel current-voltage curves**

1. The lipid bilayers were not painted, I used the Langmuir-Blodgett method, most details are correct, but not *“by painting with 1,2-diphytanoyl-snglycero-3-phosphocholine (DPhPC, Avanti Polar Lipids, Alabaster, USA).”* (**see procedure below**)
2. The following chemicals were obtained from Carl Roth, Karlsruhe, Germany; not Sigma-Aldrich
   1. MOPS
   2. IPTG (isopropyl b-D-1-thiogalactopyranoside)
   3. Sodium chloride (NaCl)

5% hexadecane in pentane (Sigma-Aldrich) was applied over the aperture of the Teflon membrane and left to dry for 1 minute at 25 °C. The buffered electrolyte solution was added to both compartments, topped with 10 µl of 6.25 mg/ml 1,2-diphytanoyl-snglycero-3-phosphocholine (DPhPC, Avanti Polar Lipids) in pentane. The pentane was left to evaporate for 2 minutes at 25 °C. A lipid bilayer was formed by lowering and raising the buffer level over the aperture.

**Manuscript Typos and strange sentences:**

**Abstract:**

1. “…*the large atomic structure of biological nanopores makes the collection of statistically meaningful amount of data highly computationally expensive*.”
   1. this sentence seems strange.
2. *“…density, viscosity and relative permittivity of the solvent To demonstrate…*”
   1. missing period behind the sentence.
3. “*It also quantitatively describes many characteristics that are not directly experimentally accessible, including the ion selectivity, the distribution of ions and the electric potential inside the nanopore and, the magnitude of the electro-osmotic flow*”
   1. The magnitude of the electro-osmotic flow seems like an afterthought. The summation was complete after the electric potential inside the nanopore, perhaps put EOF in front?
4. “*simulations that are both qualitatively and quantitatively meaningful, into the*”
   1. Maybe put comma after “*simulations*”
5. Consider changing “*biological nanopore researchers*”
6. “*…nanopore experiments but may also…”*
   1. Consider a comma between “…*experiments*” and “*but may also…*”

**Manuscript**

1. “…*of biomarkers, to the fundamental…*”
   1. Twice “*to the*” in one sentence disturbs the flow
   2. Page 2
2. “*The ePNP-NS equations revert into the regular PNP-NS equations disabling the steric flux…”*
   1. To clarify, it says here that by reverting ePNP-NS to PNP-NS you disable the steric flux
   2. Page 15
3. “*In addition,* ***tThe*** *GHK equation…*”
   1. It says tThe, likely a mistake from previous corrections
   2. Page 20
4. *“…situation* ***on the on the*** *order of 10…”*
   1. Repeat of “*on the*”
   2. Page 21
5. “*We use the relative ion concentration averaged over the entire volume of the pore (hci=csip), as a function of bulk concentration, as a measure for global ionic conditions inside the pore*”
   1. It seems like something is repeated here, twice “*as a*”
   2. Page 22
6. *“…when* ***changes*** *the bias voltage…”*
   1. I think this should be “*…when* ***changing*** *the bias voltage…*”
   2. Page 22
7. *“The PB region encompasses a cylindrical volume at the* ***enter*** *of the pore up…”*
   1. I believe, “enter” should be “entrance”
   2. Page 23
8. *“values of -144 mV (-5.60 kBT/e)”*
   1. This sentence is not outlined
   2. Page 26
9. “*…at Vb = +150-150 mV…*”
   1. I think something went wrong here
   2. Page 27
10. *“a continuous ClyA,* ***will be rapidly move*** *towards the cis entry”*
    1. “…will be rapidly moving…”, or “…will rapidly move…“
    2. Page 29
11. “…such as αHL or FraC…”
    1. You did not define these abbreviations.
       1. On page 5, you mention aHL, however, there is no inclusion of this abbreviation
    2. Page 32
12. “For example, at Vb = -150 mV, Geo..”
    1. This sentence is not completely clear
    2. Page 33
13. “For all voltages magnitudes, ….”
    1. Is this correct?
    2. Page 33
14. “…magnitude of the pressures spots”
    1. Should this be “pressure spots”?, there are several spots with different pressure, or should it be “pressures spot”, one spot with different pressures.
    2. Page 34
15. “…force on particles translocation through…”
    1. Should this be “..force on particles translocating through” or “..force on particle translocation through…”?
    2. Page 34
16. “water through a biological nanopores,…”
    1. Should this be “water through biological nanopores” or “water through a biological nanopore”
    2. Page 34, conclusions
17. “…yielding a wealth of information that is both qualitative and quantitative accurate”
    1. Should this be “…yielding a wealth of information that is both qualitative and quantitatively accurate”
    2. Page 34, conclusions