Electro-Mechanical Conductance Modulation of a Nanopore Using a Removable Gate

Shidi Zhao[‡a], Laura Restrepo-Pérez[‡b], Misha Soskine [c], Giovanni Maglia [c], Chirlmin Joo [b], Cees Dekker [b] and Aleksei Aksimentiev [a]

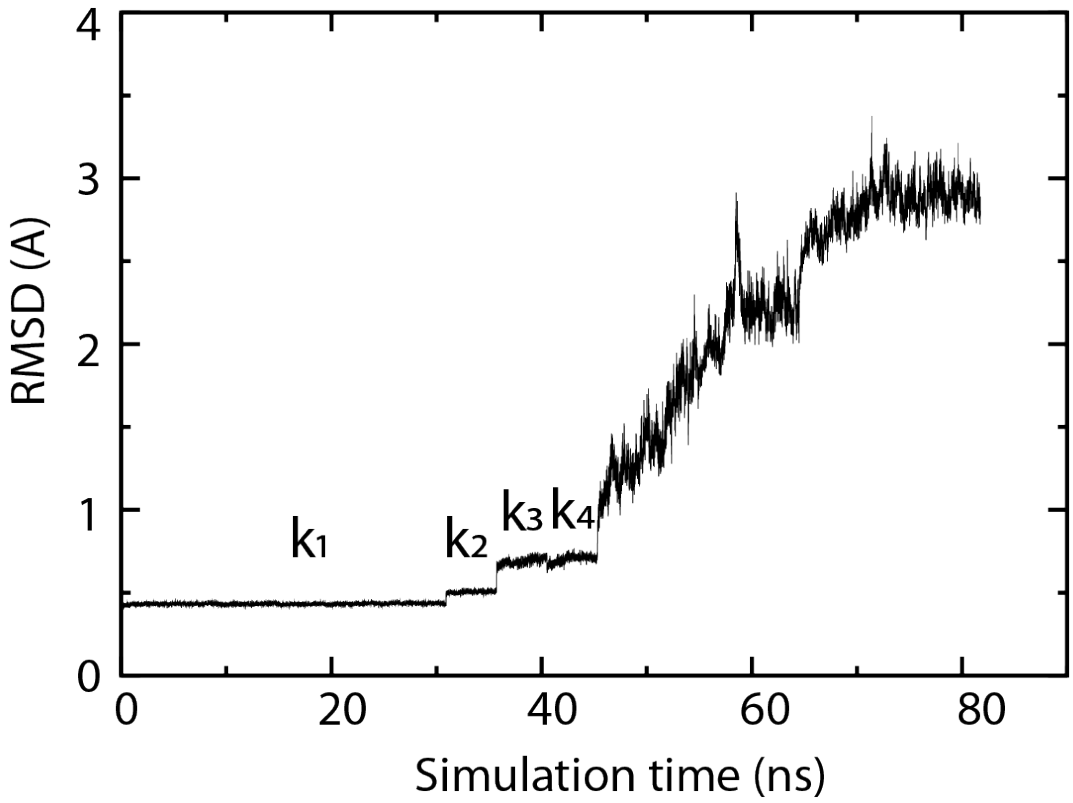
[a] Center for Biophysics and Quantitative Biology, Department of Physics and Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA.
E-mail: aksiment@illinois.edu

[b] Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, van der Maasweg 9, 2629 HZ Delft, The Netherlands.
E-mail: c.dekker@tudelft.nl, c.joo@tudelft.nl

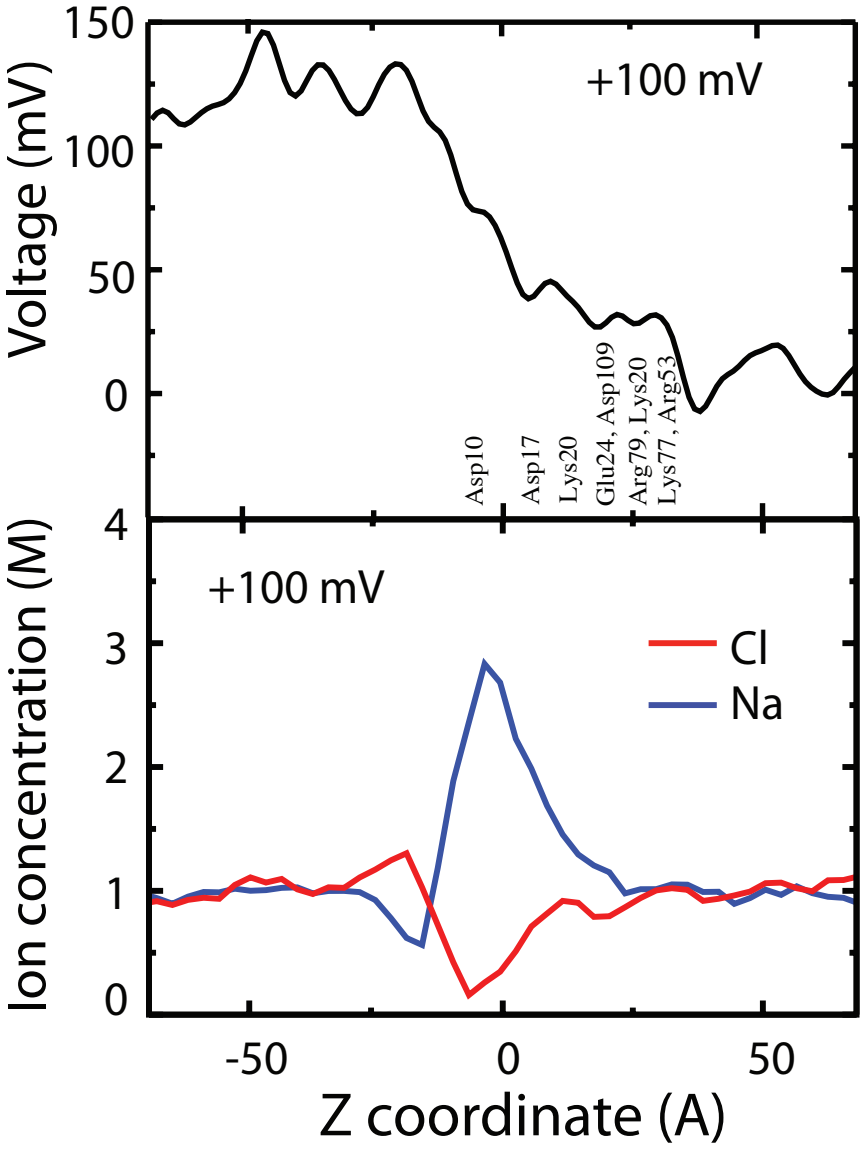
[c] Groningen Biomolecular Sciences & Biotechnology Institute, University of Groningen, 9747 AG Groningen, The Netherlands.

[‡]These authors contributed equally to this study.

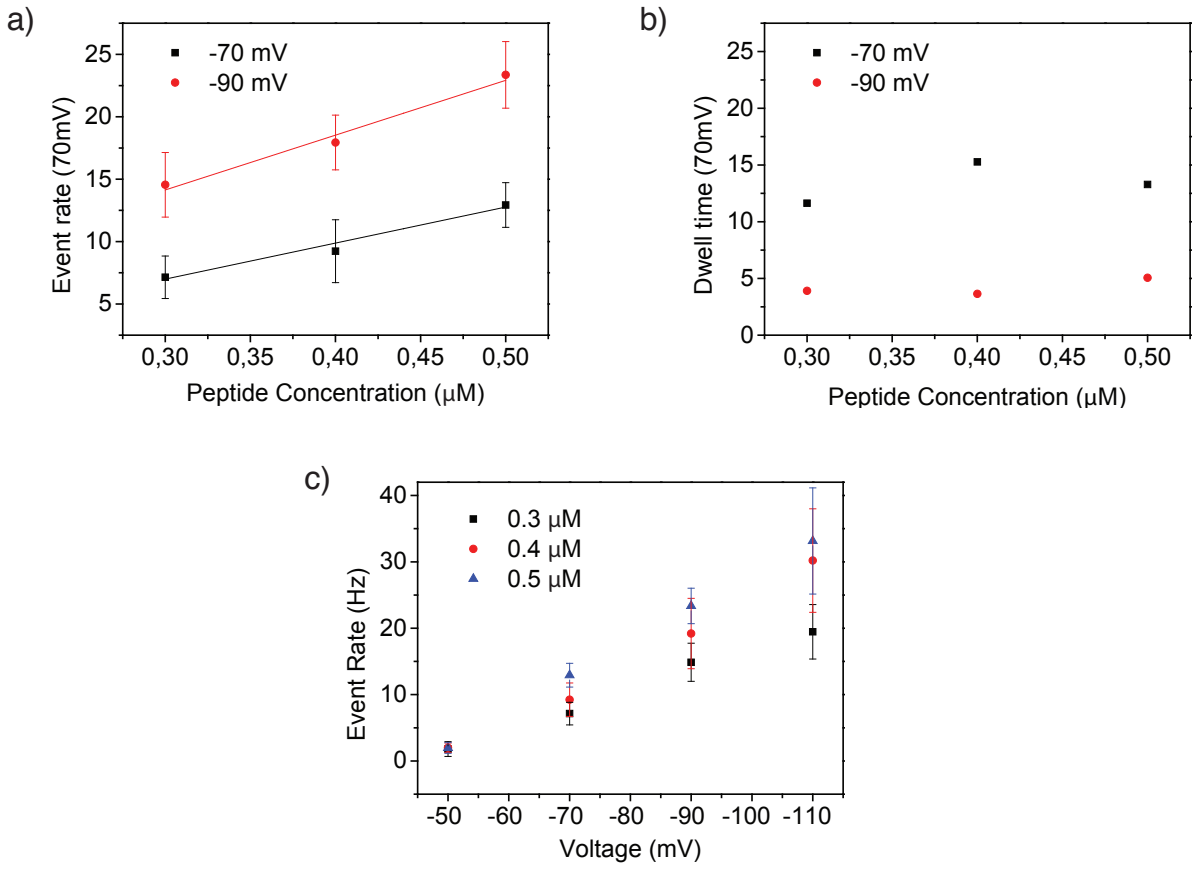
SUPPLEMENTARY MATERIAL



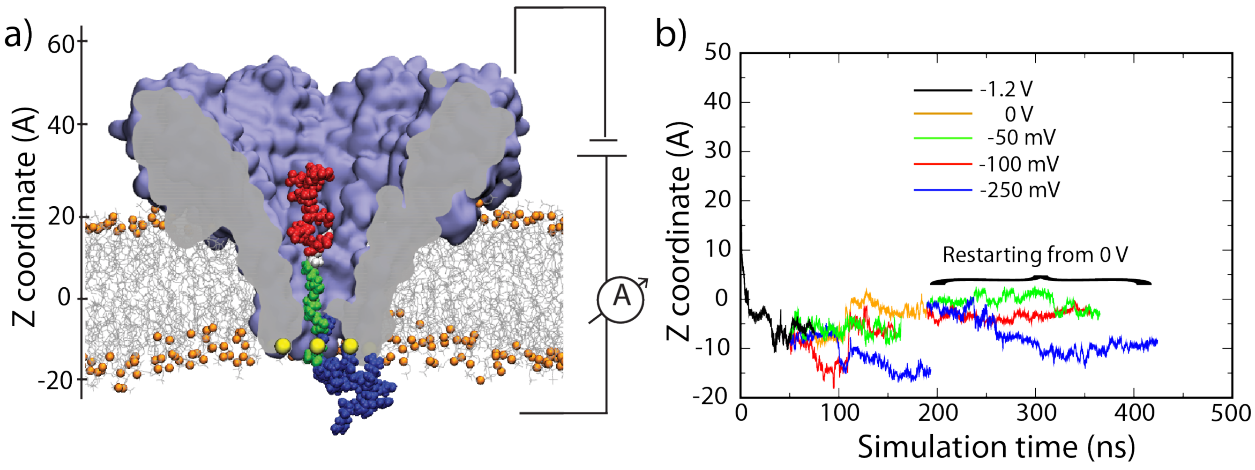
Supplementary Figure S1: Stability of a FraC nanopore in all-atom MD simulation. The root mean squared deviation (RMSD) of FraC's alpha carbon atoms from their crystallographic coordinates over the course of the equilibration trajectory. For the first 45 ns, the alpha-carbon atoms were restrained to their crystallographic coordinates using harmonic springs of the following magnitude: 1 (k_1), 0.8 (k_2), 0.5 (k_3), and 0.1 (k_4) kcal/(mol Å^2). The last 35 ns were performed in the absence of any restraints. RMSD values of less than 4 Å generally signify a stable structure. To reduce uncertainty of ionic current determination associated with fluctuations of the protein structure, all subsequent simulations were performed using the structure obtained at the end of the last restrained simulation (k_4), maintaining the same restraints.



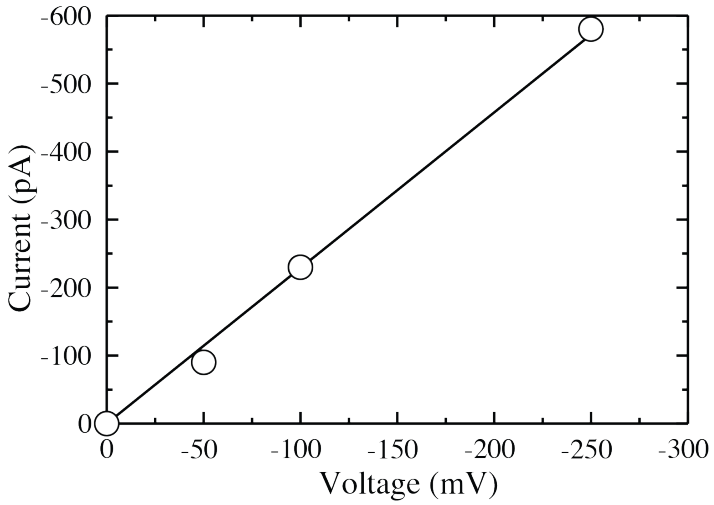
Supplementary Figure S2: Simulated profiles of the electric potential (top) and of the Na^+ and Cl^- concentrations (bottom) along the central axis of the FraC nanopore at a +100 mV bias. The data were obtained by averaging instantaneous configurations over a 48 ns MD trajectory. Approximate locations of the charged residues of the FraC nanopore are indicated at the middle horizontal axis.



Supplementary Figure S3: Characterization of peptide trapping experiments. (a) Event rate *versus* peptide concentration. The event rate increases linearly with peptide concentration indicating a simple bimolecular interaction between the peptide and the pore. (b) Dwell time *versus* peptide concentration. Dwell time remains constant at different peptide concentrations. (c) Event rate *versus* voltage magnitude. The event rate increases linearly with applied voltage, as expected.

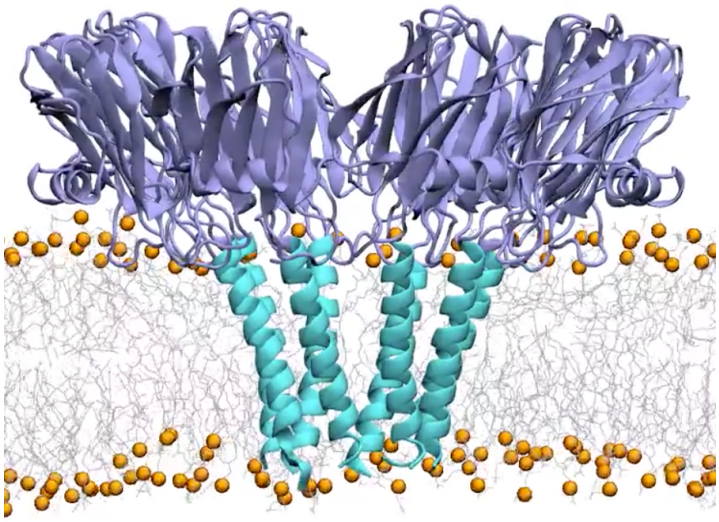


Supplementary Figure S4: MD simulation of peptide gate stretching by transmembrane voltage. (a) Representative configuration of the simulation system featuring a bipolar peptide that is lodged in the constriction of FraC. The amino acids of the bipolar peptide are colored according to their charge: negative, positive and neutral residues are shown in red, blue and green, respectively. (b) Center of mass coordinate of the "CGSGSGSKGS" segment (central part) of peptide *versus* simulation time under different biases condition. The *z* coordinate is defined in panel a. Using the conformation of the peptide observed in the second half of the peptide capture simulations under a -1.2V bias (Fig. 4 of the main text shows the first 20 ns of the 80 ns trajectory), four simulations were performed under voltage bias of -250 (blue), -100 (red), -50 (green), and 0 (orange) mV for approximately 120 ns each. The final state of the 0 V trajectory was used to initiate three additional simulations under -250 (blue), -100 (red), and -50 (green) mV, each lasting approximately 150 ns. There two sets of simulations were used determine the dependence of the water flux and ionic current on the degree of peptide stretching, main text Figure 5d-e.

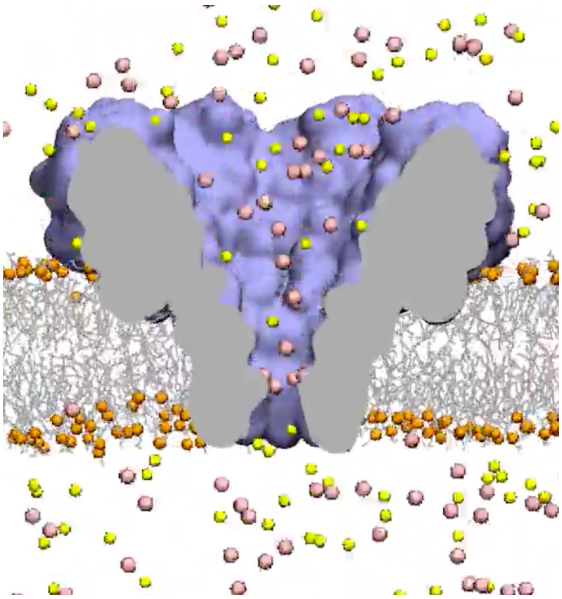


Supplementary Figure S5: Simulated current-voltage dependence of the FraC nanopore containing a fixed-conformation gate threaded through the FraC constriction. The gate conformation was taken from the last frame of the gate peptide capture simulation under -1.2 V. Each data point was obtained from a 50 ns MD trajectory.

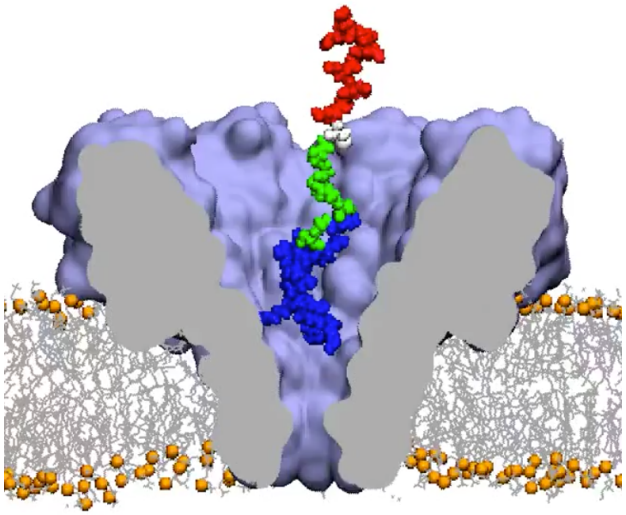
CAPTIONS TO AMINATIONS



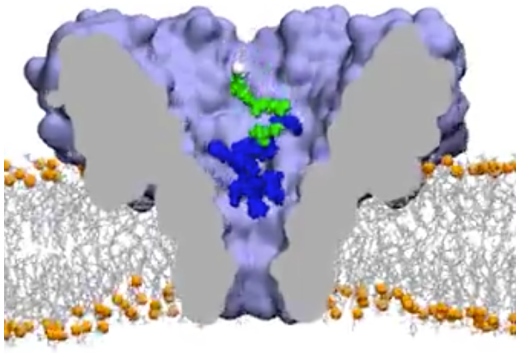
Supplementary Movie 1: Changes in the conformation of the FraC nanopore during the 80 ns equilibration simulation. The protein's alpha carbon atoms were constrained for the first 65 ns of the MD trajectory. The protein is shown using a cartoon representation; the phosphorous atoms of the lipid bilayer are shown as orange spheres, the lipid tails are shown in grey lines, the electrolyte solution is not shown.



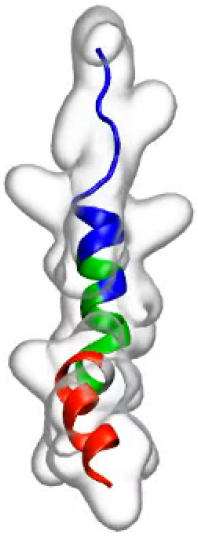
Supplementary Movie 2: MD simulation of an open FraC nanopore (blue cut-away surface) embedded in a DPhPC membrane (grey lines and orange spheres) and submerged in 1 M NaCl solution (pink and yellow spheres representing Na^+ and Cl^- ions, respectively), water not shown. A -100 mV bias was applied to produce current of ions through the FraC nanopore. The animation illustrates a 24 ns fragment of the MD trajectory.



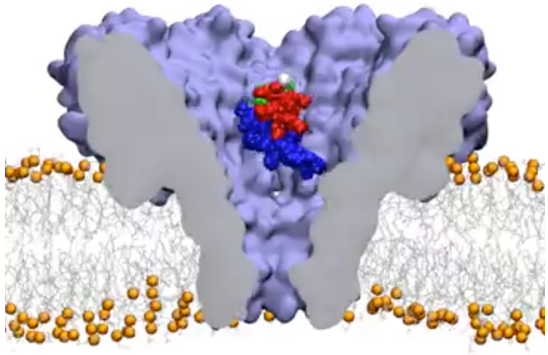
Supplementary Movie 3: MD simulation of the dipolar peptide gate capture under a -1.2V starting from a stretched conformation. The peptide has the following amino acid sequence: EEEEEEEEEECGSGSGSKGSRRRRRRRRRR. The movie illustrates a 12 ns fragment of the MD trajectory. The peptide is shown using spheres colored according to the amino acid charge: blue, red and green indicate positively charged, negatively charged, and neutral amino acids, respectively.



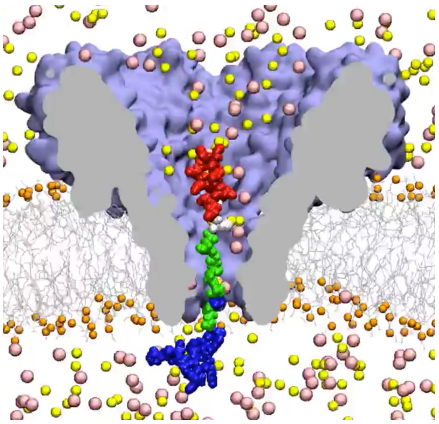
Supplementary Movie 4: MD simulation of the truncated peptide capture under a -1.2V. The peptide has the following amino acid sequence: CGSGSGSKGSRRRRRRRRRR. The movie illustrates a 12 ns MD trajectory. The peptide is shown using spheres colored according to the amino acid charge: blue and green indicate positively charged and neutral amino acids, respectively.



Supplementary Movie 5: Free equilibration of the dipolar peptide. The peptide was solvated in a $10\times10\times10$ nm^3 volume of 1 M NaCl solution. The movie illustrates a 50 ns MD trajectory. The conformation of the peptide was observed to change from extended, structure into an alpha-helical hairpin. The peptide is shown using both a semitransparent surface indicating its volume and a cartoon representation colored according to the amino acid charge: blue, red and green indicate positively charged, negatively charged and neutral amino acids, respectively.



Supplementary Movie 6: MD simulation of the dipolar peptide gate capture under a -1.2V starting from a hairpin conformation. The peptide has the following amino acid sequence: EEEEEEEEEECGSGSGSKGSRRRRRRRRRR. The movie illustrates a 12 ns fragment of the MD trajectory. The peptide is shown using spheres colored according to the amino acid charge: blue, red and green indicate positively charged, negatively charged, and neutral amino acids, respectively.



Supplementary Movie 7: MD simulation of the dipolar peptide stretching under a -100 mV. The movie illustrates a 72 ns MD trajectory. The peptide is shown using spheres colored according to the amino acid charge: blue, red and green indicate positively charged, negatively charged, and neutral amino acids, respectively. The FraC nanopore is shown as a blue, cut-away surface; the DPhPC membrane is shown in grey (lipid tails) and orange (head group); sodium and chloride ions are shown as pink and yellow spheres, water not shown.