

Supporting Information

Bilayer-Spanning DNA Nanopores with Voltage-Switching between Open and Closed State

Astrid Seifert^{1,#}, Kerstin Göpfrich^{2,#}, Jonathan R. Burns³, Niels Fertig¹, Ulrich F. Keyser^{2,},
Stefan Howorka^{3,*}*

¹ Nanion Technologies GmbH, D-80636 Munich, Germany

² Cavendish Laboratory, University of Cambridge, Cambridge CB3 0HE, United Kingdom

³ Department of Chemistry, Institute of Structural Molecular Biology, University College London, London WC1H 0AJ, United Kingdom

These authors contributed equally to the work.

* corresponding author: s.howorka@ucl.ac.uk, Tel 0044 20 7679 4702, ufk20@cam.ac.uk 0044 1223 337272

Table S1. Sequences of DNA oligonucleotides for assembling the porphyrin DNA-nanopore

ID	Sequence
A-TPP	TTATAAGGGATTTTGCCGATTT <u>P</u> GGAATTTTACAGGATTTTCGCCT GCTGGGGCAAACCAGCGTGGACCGCTTTTTTGGCTATTCTTTTGAT
B-TPP	GGCGCCAATACGCTTTTTCCCGCGCGTTGGCCGATTCATTAATGC AGCTGGCACGACATTTTTCTC <u>P</u> CTGGTGAAAAGAAAAACCACCT
C	TGTTCAAATAGCCAAGCGGTCCACGCTCCCTGAGGGGCGCC AGGGTGGGAATCGGACAAGAGTCCACTAAAATCCCCCAGCA
D	CATTAATTTTTCTCCTTCACCGCCTGGGGTTTGCTTATAAA TCAAAAGGTTTGGACCAACGCGGGGAGCGTATTAGAGTTG
E	CAACTCTCTCAGGGCCAGGCGGTGAAGGCAATC*A*G*C*T*G*TTGTTTTCAA* C*A*G*C*A*T*C*C*TGTTTC*C*G*A*A*A*TCGGCATTAAAG*A*C*CAGCTG
F	GGCGAA*A*T*GATTGCTTTCAC*C*A*G*T*G*AGATGT*C*G*T*G*A*C*G*T *GGATTTTCC*A*C*G*T*T*CTTTAATAGTGGACTCTTGTTCCAAACTGGAACA

The sequences start with the 5' terminus. p indicates a deoxy-uridine nucleotide carrying a tetra-phenyl porphyrin modification. * signifies a phosphorothioate (PPT) modification. This modification was included in the original nanopore design to be comparable with our previous nanopore work²⁸ and to be used an alternative method to generate nanopore insertions. In this eventuality, the PPT would have been chemically modified with an ethyl group to mask the negative charge.²⁸ The ethyl-groups would have formed a hydrophobic belt at the left end of the pore (Figure S5). However, this was not necessary as the native porphyrin-nanopore inserted at pH 8.0 (for consistency) where PPT is negatively charged and behaves as a regular phosphate group. Indeed, pores with non-alkylated PPT groups do not insert into lipid bilayers.²⁸ All experiments in this manuscript were carried out using a fully negatively charged porphyrin-nanopores in a buffer at pH 8.0.

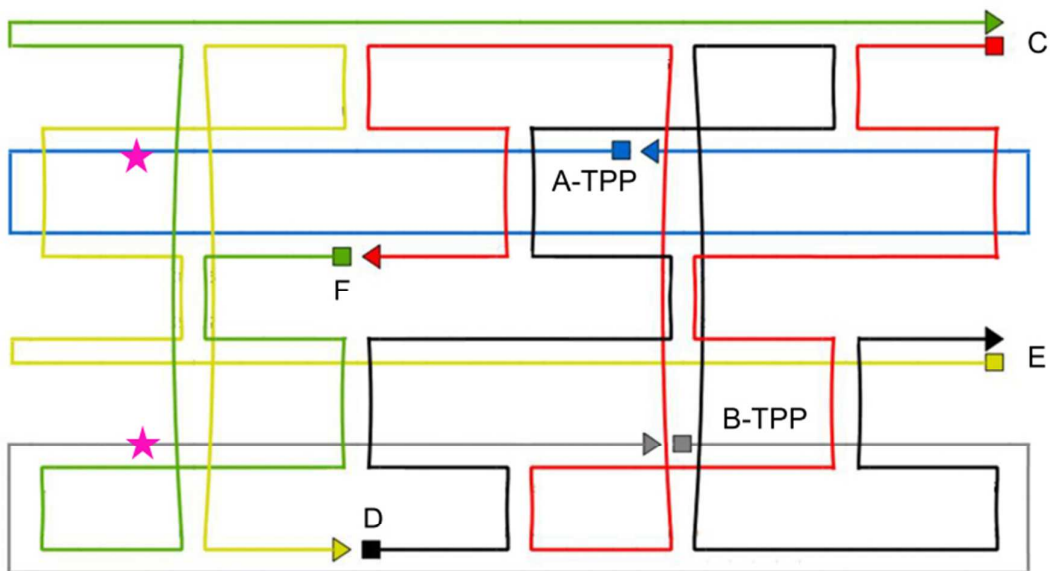


Figure S1. Map of the DNA nanobarrel composed of 6 DNA strands labeled by letters (Table S1). The pink stars indicate the base positions which carry a TPP porphyrin tag.

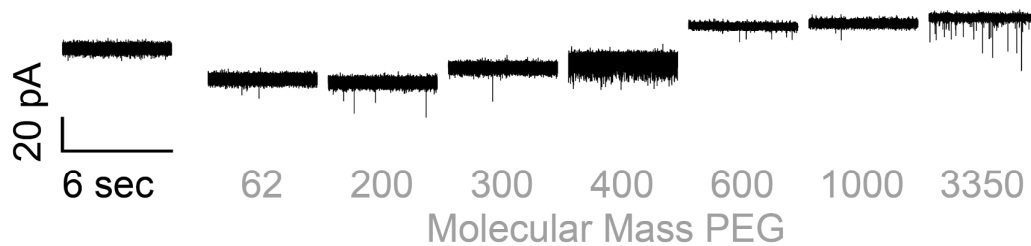


Figure S2. Current traces of individual pores in the absence and presence of PEG molecules of indicated molecular mass. The traces were recorded at + 40 mV.

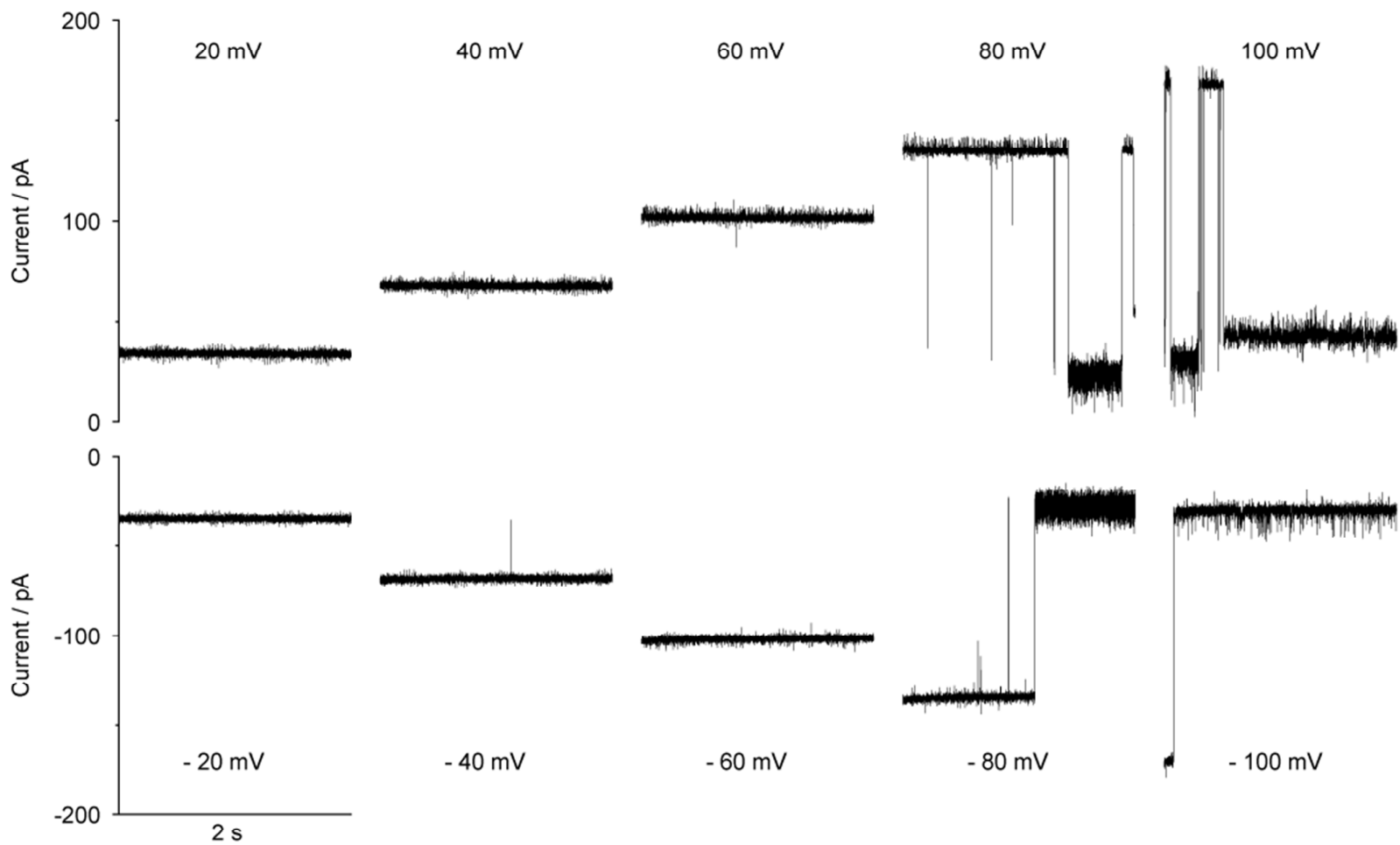


Figure S3. Representative single-channel current traces for a single DNA nanopore embedded in planar lipid bilayers recorded at the indicated voltages.

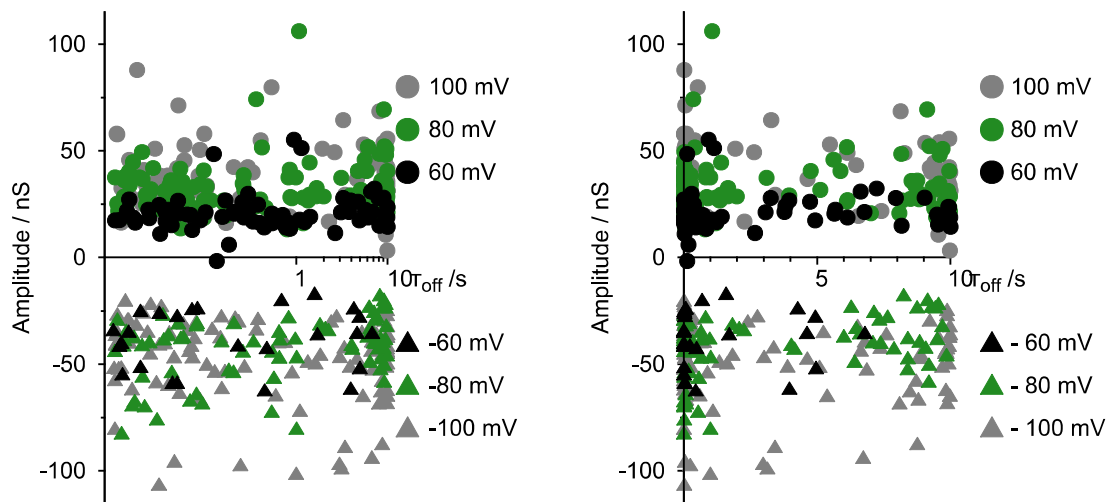


Figure S4. Scatter-plot analysis for amplitude A_b , and the dwell time τ_{off} for the low-conductance blockade events to voltages +60 mV, +80 mV, +100 mV, and -60 mV, -80 mV, -100 mV, represented in a hemi-logarithmic plot (left) and linear plot (right). A_b and τ_{off} are defined in Figure 4 of the main manuscript. The data are from current traces acquired with planar lipid bilayer recordings.

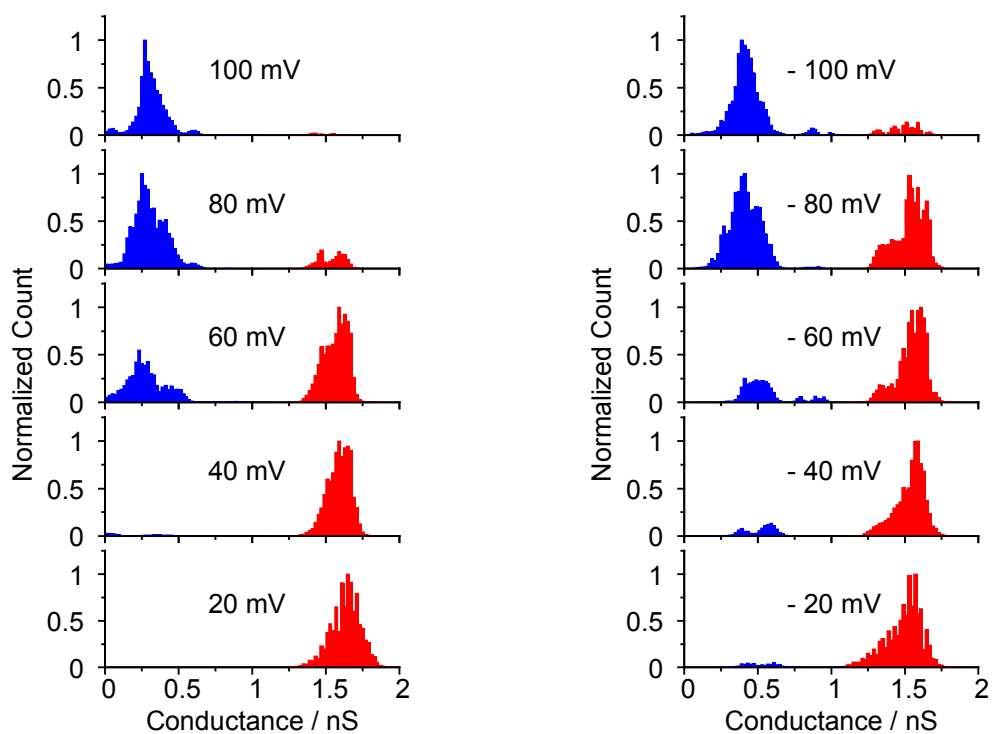


Figure S5. Cumulative all-point histogram of 38 single-channel current traces for negative potentials (left) and positive potentials (right). The data are from current traces acquired with planar lipid bilayer recordings.

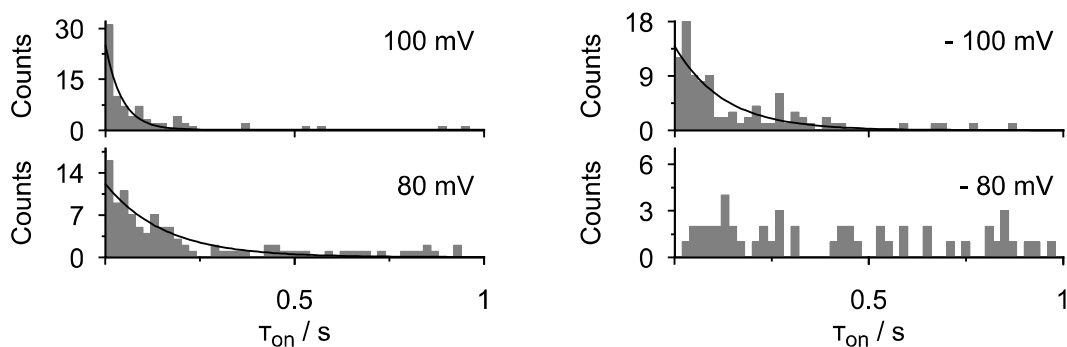


Figure S6. Histogram for inter-event intervals τ_{on} for voltages of +/- 80 and +/- 100 mV. The exponential fits for τ_{on} drop from 155 ms to 43.8 ms when increasing the voltage from +80 mV to +100 mV. The exponential fit for τ_{on} at -100 mV is 126 ms. The data are from current traces acquired with planar lipid bilayer recordings.

Table S2. The occurrence of the low-conductance state as a function of voltage for traces acquired with planar lipid bilayer recordings

Voltage [mV]	100	80	60	40	20	-20	-40	-60	-80	-100
Mean	112	414	2370	9750	-	-	9640	6940	1780	440
τ_{on} [ms] ^{1,2}										
Frequency [events/min] ¹	16.0	17.2	10.9	0.57	0	0	0.47	3.85	10.6	16.1
% of traces ≥ 1 low-cond. blockade ¹	100	100	83.3	7.1	0	0	7.1	53.8	88.1	100
% normalized proba. for low-cond. state ^{3,4}	98.1	86.3	39.4	3.01	0.03	5.12	11.5	23.8	54.2	88.4

¹The data were obtained from the analysis of 51 independently recorded single-channel DNA nanopore traces.

²The inter-event interval τ_{on} is defined in Figure 4. ³The probability for the occurrence of the low-conductance state was derived by normalizing the area of low-conductance state peak in all-point histogram to the sum of all other peaks. ⁴The data were obtained from the analysis of 38 independently recorded single-channel DNA nanopore traces which represent a subset of the 51 current traces.

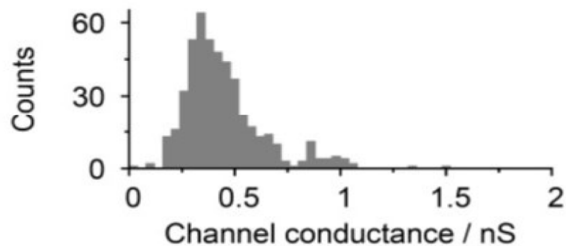


Figure S7. Histogram for low conductance state found for all voltages of all single-channel traces derived from all-point histogram analysis of individual traces obtained using planar lipid bilayer recordings.

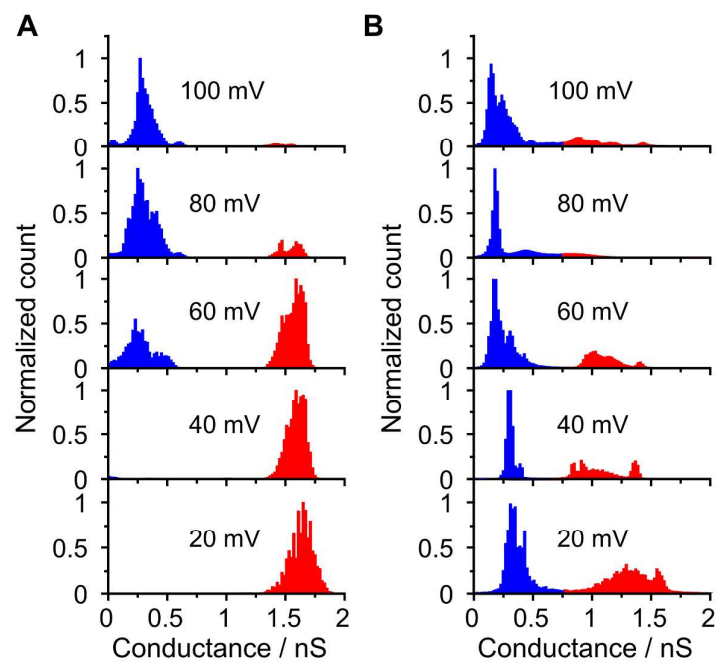


Figure S8. Cumulative all-point histograms for single-channel current traces acquired at the indicated voltages with (A) planar lipid bilayer recordings and (B) glass nanopipette-mounted bilayers.