

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Electrical recordings were performed using a patch-clamp amplifier (Axopatch 200B, Molecular Devices Inc., Sunnyvale, CA, USA)
Nanopore data were acquired and analyzed using pClamp 11 software (Molecular Devices Inc.).

Data analysis

We used Pymol 2.5.0, VMD 1.9.4, Coot 0.8.9.2, HOLE 2.2.005, cryoSPARC v3.2, and Phenix 1.18.2 for molecular visualizations. We used Clampfit 11.2, GraphPAD Prism 9, and Origin 2020b for statistical analysis of nanopore events.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Datasets can be provided by the corresponding authors upon reasonable requests. The source data underlying Figs. 1a, 1c, 1g, 2h, 3b, 3c, 3e, 3f, 4b, 4c, 5c, 5d, 5f, 5g and Supplementary Figs. 1, 2, 3-9, 11-12, 17-24 and Supplementary Table 1, 2, 3 are provided in the Source data file. PDB entries 6EL1 [<https://doi.org/10.2210/>

pdb6EL1/pdb], 1BXL [https://doi.org/10.2210/pdb1BXL/pdb], 2PPN [https://doi.org/10.2210/pdb2PPN/pdb] and 2MPS [https://doi.org/10.2210/pdb2MPS/pdb] were downloaded from the Protein Data Bank and used in this article for molecular visualizations. Negative-staining electron microscopy (EM) for pores generated in this study have been deposited in the Zenodo database under https://doi.org/10.5281/zenodo.7619077.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	No human research participant
Population characteristics	No human research participant.
Recruitment	No human research participant.
Ethics oversight	No human research participant.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Each experiment was repeated at least for three times. At least 100 events were used for statistical analysis of current blockades and dwell times. The sample size was determined by the reproducibility of the experimental observation, e.g. electrical signal pattern or the convergence of the cumulative mean of independent samples.
Data exclusions	All signal traces exhibiting the known behavior of the YaxAB pore were used indiscriminately.
Replication	Each specific data point were derived from at least three replications of independent experiments at the same condition.
Randomization	Randomization was not applicable for this work since the subject in this study was a specific protein under a specific experimental condition.
Blinding	Blinding was not applicable since the subject in this study was a specific protein.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging