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Reporting Summary

Nature Research 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 Research policies, see our Editorial Policies and the Editorial Policy Checklist.

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St	at	ict	100

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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> 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 <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated		
Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.		
Software and code		
Policy information about <u>availability of computer code</u>		
Data collection Topspin 3.6.0 (Bruker, AXS GmBH, DE) Spectra Manager™ 2.8 (JASCO Research Ltd. CA) HCImage 4.6.1 (Hamamatsu Photonics, JP)		
Data analysis NMRpipe 10.9 (Delaglio et al J. Biomol, NMR, 6, 277-293 (1995), University of Maryland, USA).		

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 Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:

Sparky 3.1 (Goddard TD & Kneller DG (2008) SPARKY 3. University of California San Francisco, USA)

 $\hbox{-} Accession codes, unique identifiers, or web links for publicly available datasets \\$

GraphPad Prism 9 (GraphPad Software, USA) ImageJ 1.52p (National Institute of Health, USA)

- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data supporting the findings of this study are available within the article and its Supplementary Information Files and from the corresponding author on request. Python processing scripts can be found at https://github.com/vrettasm/analysis_code_Man_et_al

Field-spe	ecific reporting
Please select the o	one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.
x 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
Life scie	nces study design
All studies must d	isclose on these points even when the disclosure is negative.
Sample size	The study presented in this manuscript does not comprise sample size and no statistical methods were used to predetermine the sample size. All molecules presented in the test tubes have been collectively probed in bulk measurements.
Data exclusions	No data were excluded from the study
Replication	The NMR data presented derive from a data collection based on one sample replicate and three experimental replicates. The CD data presented derive from a data collection based on one sample replicate and ten experimental replicates. CD experiments were repeated three times, using completely independent - freshly prepared - samples and resulting in reproducible results. The TIRF data presented derive from a data collection based on one sample replicate and three experimental replicates.
	For these techniques, in addition to the experimental replicates reported in the manuscript, additional sample replicates for CEST NMR, CD and TIRF imaging (one each) were prepared, providing extremely similar results to those reported in the article.
Randomization	Sample were not randomised for this study due to the small number of specialised samples that were prepared

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.

Sample blinding was not relevant given the ability of NMR to identify the sample from the spectrum and of TIRF to identify fluorescently

Materials & experimental system	ns Methods
n/a Involved in the study	n/a Involved in the study
X Antibodies	ChIP-seq
x Eukaryotic cell lines	Flow cytometry
Palaeontology and archaeology	MRI-based neuroimaging
🗶 🔲 Animals and other organisms	
Human research participants	
✓ Clinical data	
🗴 🔲 Dual use research of concern	

labelled vesicles from the images

Blinding