# nature portfolio

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## **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.

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

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| n/a | Cor | nfirmed  |
|-----|-----|--|
|     | ×   | 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.   |
| x   |     | 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) |
| x   |     | 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 <i>Give P values as exact values whenever suitable.</i>                        |
| x   |     | 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

SymphoTime 64 version 2.4 (PicoQuant) was used for single molecule data collection

Data analysis

Fretica, a custom add-on package for Mathematica v.12.3 (Wolfram Research) was used for the analysis of single-molecule fluorescence data and is available at https://github.com/SchulerLab.

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 <u>data availability statement</u>. 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  $\underline{\text{policy}}$

Source data are provided with this paper for the experimental measurements.

| Research involving human participants, their data, or biological material   |   |   |
|---|---|---|
|   |   | with <a documents="" href="https://www.news.news.news.news.news.news.news.n&lt;/td&gt;&lt;/tr&gt;&lt;tr&gt;&lt;td&gt;Reporting on sex a&lt;/td&gt;&lt;td&gt;ind gender&lt;/td&gt;&lt;td&gt;n/a&lt;/td&gt;&lt;/tr&gt;&lt;tr&gt;&lt;td&gt;Reporting on race, other socially relev&lt;/td&gt;&lt;td colspan=2&gt;race, ethnicity, or relevant groupings&lt;/td&gt;&lt;/tr&gt;&lt;tr&gt;&lt;td&gt;Population charact&lt;/td&gt;&lt;td&gt;teristics&lt;/td&gt;&lt;td&gt;n/a&lt;/td&gt;&lt;/tr&gt;&lt;tr&gt;&lt;td&gt;Recruitment&lt;/td&gt;&lt;td&gt;&lt;/td&gt;&lt;td&gt;n/a&lt;/td&gt;&lt;/tr&gt;&lt;tr&gt;&lt;td&gt;Ethics oversight&lt;/td&gt;&lt;td&gt;&lt;/td&gt;&lt;td&gt;n/a&lt;/td&gt;&lt;/tr&gt;&lt;tr&gt;&lt;td&gt;Note that full inform&lt;/td&gt;&lt;td&gt;ation on the appr&lt;/td&gt;&lt;td&gt;roval of the study protocol must also be provided in the manuscript.&lt;/td&gt;&lt;/tr&gt;&lt;tr&gt;&lt;td&gt;&lt;/td&gt;&lt;td&gt;&lt;/td&gt;&lt;td&gt;&lt;/td&gt;&lt;/tr&gt;&lt;tr&gt;&lt;td&gt;Field-spe&lt;/td&gt;&lt;td&gt;ecific re&lt;/td&gt;&lt;td&gt;porting&lt;/td&gt;&lt;/tr&gt;&lt;tr&gt;&lt;td&gt;Please select the o&lt;/td&gt;&lt;td&gt;ne below that i&lt;/td&gt;&lt;td&gt;s the best fit for your research. If you are not sure, read the appropriate sections before making your selection.&lt;/td&gt;&lt;/tr&gt;&lt;tr&gt;&lt;td&gt;Life sciences&lt;/td&gt;&lt;td&gt;&lt;/td&gt;&lt;td&gt;Behavioural &amp; social sciences&lt;/td&gt;&lt;/tr&gt;&lt;tr&gt;&lt;td&gt;For a reference copy of&lt;/td&gt;&lt;td&gt;the document with&lt;/td&gt;&lt;td&gt;all sections, see &lt;a href=" nature.com="" nr-reporting-summary-flat.pdf"="">nature.com/documents/nr-reporting-summary-flat.pdf</a> |
| Life sciences study design  |   |   |
| All studies must di   | sclose on these   | points even when the disclosure is negative.  |
| Sample size   | The sample size for all free diffusion lifetime measurements was always > 2000 freely diffusing molecules. The sample size for fluorescence correlation measurements was more than 100,000 freely diffusing molecules. The sample sizes were found to satisfactorily describe the conformational distributions and dynamics in the ensembles. |   |
| Data exclusions   | Bursts arising from freely diffusing molecules with only a single fluorophore and with < 50 photons were excluded from analysis. More details on Data exclusion can be found in the Methods section.  |   |
| Replication   | The overall reproducibility of the main experiments was confirmed by making multiple independent measurements.  |   |
| Randomization   | No randomization was used.  |   |
| Blinding  | Experiments were not blinded. No a priori knowledge could be assumed, thus no randomization was required.   |   |
|   |   |   |
| 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 Methods  |   |   |
| n/a Involved in th  | he study  | n/a Involved in the study   |

| n/a Involved in the study  x Antibodies  x ChIP-seq  x Eukaryotic cell lines  x Palaeontology and archaeology  x Animals and other organisms  x Clinical data  x Dual use research of concern  x Plants | Materials & experimental systems |                               | Methods |                        |
|---|----------------------------------|-------------------------------|---------|------------------------|
| Eukaryotic cell lines   X   Flow cytometry   X   Palaeontology and archaeology   X   MRI-based neuroimaging   X   Animals and other organisms   Clinical data   X   Dual use research of concern        | n/a                              | Involved in the study         | n/a     | Involved in the study  |
| X Palaeontology and archaeology   X MRI-based neuroimaging   X Animals and other organisms   X Clinical data   X Dual use research of concern   | ×                                | Antibodies                    | ×       | ChIP-seq               |
| Animals and other organisms     Clinical data     Dual use research of concern  | ×                                | Eukaryotic cell lines         | x       | Flow cytometry         |
| Clinical data      Dual use research of concern   | x                                | Palaeontology and archaeology | x       | MRI-based neuroimaging |
| Dual use research of concern  | x                                | Animals and other organisms   |         |                        |
|   | x                                | Clinical data                 |         |                        |
| X Plants  | ×                                | Dual use research of concern  |         |                        |
|   | x                                | Plants                        |         |                        |

## Plants

| Seed stocks           | n/a |
|-----------------------|-----|
|                       |     |
| Novel plant genotypes | n/a |
|                       |     |
|                       |     |
| Authentication        | n/a |
|                       |     |