



## **eLife's transparent reporting form**

We encourage authors to provide detailed information *within their submission* to facilitate the interpretation and replication of experiments. Authors can upload supporting documentation to indicate the use of appropriate reporting guidelines for health-related research (see [EQUATOR Network](#)), life science research (see the [BioSharing Information Resource](#)), or the [ARRIVE guidelines](#) for reporting work involving animal research. Where applicable, authors should refer to any relevant reporting standards documents in this form.

If you have any questions, please consult our Journal Policies and/or contact us: [editorial@elifesciences.org](mailto:editorial@elifesciences.org).

### **Sample-size estimation**

- You should state whether an appropriate sample size was computed when the study was being designed
- You should state the statistical method of sample size computation and any required assumptions
- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

Each of our experiments involves analysis of hundreds of single molecule binding events on hundreds of immobilized U1 molecules or U1 mimics. No estimations were required to interpret the data and reach conclusions discussed in the text.

### **Replicates**

- You should report how often each experiment was performed
- You should include a definition of biological versus technical replication
- The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

Each of our experiments involves analysis of hundreds of single molecule binding events on hundreds of immobilized molecules and were carried out in replicate. The number of events analyzed are reported in the figure legends or in the figure supplement tables as appropriate.



### Statistical reporting

- Statistical analysis methods should be described and justified
- Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's  $r$ , Cohen's  $d$ ))
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

Statistical methods are described in the methods sections. For each experiment the number of molecules or events analyzed (N) is reported in the appropriate position in the text, figure legend, or table. Error bars associated with fits to single molecule data are given where appropriate and identified in the figure legend and/or described in the materials and methods. For data shown in Figure 6, we additionally show the outputs of log-likelihood analysis of the goodness of fits in the figure supplemental table.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

### Group allocation

- Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
- Indicate if masking was used during group allocation, data collection and/or data analysis

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

Not applicable

### Additional data files ("source data")

- We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
- Where provided, these should be in the most useful format, and they can be uploaded as "Source data" files linked to a main figure or table
- Include model definition files including the full list of parameters used
- Include code used for data analysis (e.g., R, MatLab)
- Avoid stating that data files are "available upon request"

Please indicate the figures or tables for which source data files have been provided:



We have included numerical data that are represented in figure graphs as supplemental tables with the appropriate figure.

For figures containing gel images, we have uploaded a ZIP folder containing the raw, unprocessed gels and a corresponding annotation as Source Data (Figure 1-Supplemental Figure 2-Source Data 1 and 2). A legend for Source Data is now included in the text.

Raw microscopy images are housed on campus servers and can be downloaded by anyone using Globus and the link below.

[https://app.globus.org/file-manager?origin\\_id=2b62cfc8-0c02-42ca-bb75-1a257d7b4284&origin\\_path=%2F](https://app.globus.org/file-manager?origin_id=2b62cfc8-0c02-42ca-bb75-1a257d7b4284&origin_path=%2F)