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Sample-size estimation

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

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Does not apply. Sample size estimation was not performed in this work.

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
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- 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)

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The number of replicates performed for each experiment can be found in Supplementary File 2. We performed as many replicates as reasonably possible given time, cost, and reagent constraints. The only data exclusions in this work were in the analysis of the single-molecule FRET data (Figure 3 and Figure 4-figure supplement 3). As described in the materials and methods sections, smFRET trajectories were excluded from downstream analysis if they exhibited low signal-to-noise, multistep photobleaching, large fluctuations in fluorescence intensity not due to FRET, or if there was more than one peak present within a region of interest.

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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.

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Statistical reporting was carried out for the experiments shown in Figure 6B, Figure 2-figure supplement 1B, and Figure 4-figure supplement 3A. Details concerning the statistical test used, methods of multiple test correction, the exact p-values, the definition of center, and the measure of dispersion and precision can be found in the respective figure captions. Exact values of N can be found in Supplementary File 2.

(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
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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:



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The source data used to generate Figure 3G and Figure 4-figure supplement 3A (SR complex formation rate plots) and Figure 3D-F (histograms) are provided, and the MATLAB scripts used to analyze and produce those results are provided as well. Source data for the results shown in Figure 2-figure supplement 1B (bar graph of denaturing temperatures) are provided. The source data for the results in Figure 6B (bar graph of %GFP + cells) are provided.