***eLife’s* transparent reporting form**

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**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:

Sample size numbers are stated in figure legends and the methods section. The method of computing replicate number is not applicable, as performing biochemical experiments in biological triplicate is a widely-used replication standard.

**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:

The figure legends, source data files, and methods section explicitly state replicate numbers. We performed technical replicates by repeating the assays in independent reactions and did not perform biological replicates in this study, as all our assays reported here are biochemical *in vitro* experiments of bulk populations of at least 1x106 molecules. The method of computing replicate number is not applicable, as performing biochemical experiments in biological triplicate is a widely-used replication standard.

**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:

With the exception of Figure 3B, all error bars shown represent 1 SD, where values reported are the means and the replicate numbers (n) are provided in the figure legends, source data files, and methods section. For figure 3B, fraction crosslinked activity plotted represents the ratio of two means and error bars shown are the propagated error (formula reported in the source data file); values, replicate numbers, and propagated errors are provided in the figure legend, source data file, and methods section. Raw values of summary data are provided in the source data files. P-values were not calculated in this study.

(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:

Samples were not allocated into different experimental group, and sample size allocation was not required in this study. No cellular experiments were done, and proteins were purified from similar genetic backgrounds (NEB T7 Express)

**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
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* Avoid stating that data files are “available upon request”

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

Figure 2C, Figure 3A-D, and Figure 3-Supplement 1A-B have source data provided in supplemental tables.