***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:

The sample sizes are reported at the end of the caption of Figure 2. They comply with the general standard in the field. And the uncertainty added by the naturally finite sample size is accounted for in our uncertainty measures, the 95% confidence intervals (see Figure 3b, Figure 3-figure supplements). All values are also specified in Supplementary File 1 (Table 1).

**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)

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Our replicates should be considered as technical replications; the numbers are reported at the end of the caption of Figure 2. Standard smFRET trace selection criteria were applied, i.e.: constant total intensities (acceptor plus donor after donor excitation), constant directly excited acceptor intensity (using ALEX), single step bleaching. Except for these standard criteria, no outliers were excluded. No data was excluded.

**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:

We used SMACKS (single-molecule analysis of complex kinetic sequences), which was previously published, and is described in depth (including statistical tests and parameters) in "Schmid et al., BiophysJ, 2016" and "Schmid et al., JPC, 2018". The source code of SMACKS is available for download here: <https://www.singlemolecule.uni-freiburg.de/SMACKS>

All values of the quantified rates constants and 95% confidence intervals are provided in Supplementary Fila 1 (Table 1). A selection of raw data is depicted in Figure 2b and Figure 2-figure supplement 1. All smFRET trajectories are provided as source data.

(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:

All single-molecule FRET traces for each dataset are provided as source data in “smFRETdatasets” including a short description. The source code of SMACKS is available for download here: <https://www.singlemolecule.uni-freiburg.de/SMACKS>