***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

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**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:

**Figure 2.** (B) 3 replicates for calibration of the GIET curve. See also **Supplementary file 1 B,C**.

**Figure 3.** (D, E) 3 replicates for quantifying membrane association of HOPS complex. See also **Figure 3-figure supplement 2**.

**Figure 3-figure supplement 5** with3 replicates for FRAP experiments of each conditions.

**Supplementary file 2**. 5 replicates for fluorescence lifetime measurements of protein complexes.

**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:

**Figure 3.** (G) > 17 samples in each box chart for quantification the lifetime difference by two t-test with p-values.

**Figure 4.** (E) > 9 samples in each box chart for quantification the lifetime difference by two t-test with p-values. See also **Figure 3-figure supplement 4**B. (F) > 12 samples in each for determining the mean ± s.d of the lifetime ratio. See also **Figure 4-figure supplement 2**.

**Figure 5.** (A, B, C) Representatives of >100 single molecule intensity traces. See also **Figure 5-figure supplement 1**. (D, E) Pooled single molecule intensity distribution (D, n = 101615) (E, n = 84218). (G, H) Transition density plot. **Supplementary file 4** shows the sample size of transitions (from 34 to 534). **Figure 5-figure supplement 2** shows the sample size for calculating the mean value and ratios (n = 60498 and 33317).

**Table 1.** State occupancy based on observations of N = 101615 and N = 84218. Transition kinetics calculated from 34 to 534 transitions (see **Supplementary file 4**)

(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

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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”

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