

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	SEC-MALS: astra v5.0(Wyatt) smFRET data: SymPhoTime 64 5v2.4 b4874, PicoQuant HDX-MS data: ProteinLynx v3.0.1 (Waters), DynamX 3.0 (Waters)
Data analysis	Scripts to reproduce figures and analysis are made available on GitHub, both for HDX-MS (https://github.com/Jhsmiit/SecB_MBP_HDXMS) and smFRET (https://github.com/Jhsmiit/SecB_MBP_smFRET). Implementation of burst search and associated web server and user interface is made publicly available as a python package (PyPI) ('dont-fret': https://github.com/Jhsmiit/dont-fret ,). A library for symbolic model definition and fitting as developed and made available ('smitfit' (PyPI), https://github.com/Jhsmiit/smitfit).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

smFRET, SEC-MALS, and HDX-MS data have been deposited on Zenodo (Roussel, G., Smit, J.H., Karamanou, S. & Economou, A. smFRET/MALS/HDX-MS data for: How SecB maintains clients in a translocation competent state [doi:10.5281/zenodo.15847472]. Zenodo (2025).). Exported data from DynamX (.csv 'state' data files) are included in the GitHub repository (https://github.com/Jhsmit/SecB_MBP_HDXMS).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	smFRET: the burst search and thresholding were identical across all the experiential conditions and described in the Materials and Methods Section. The resulting list of accumulated number of events (total across the paper > 1.7 M) is reported in the Extended data Table 5.
Data exclusions	smFRET: Since the unfolded state of our client overlap with random coincidence events in smFRET datasets with more than 5% of random coincidence events were excluded from the analysis. Bursts with less than 50 photons identified with at less 35 neighboring photons in a 500 microseconds timescale were excluded; and when chaperones are added (SecB or SecA) additional filtering were applied to exclude files with spurious high-count rates (aggregates).
Replication	smFRET: Data collections was performed with at least three independently labeled proteins over different days. HDX-MS: three biological replicates, using different purified preparations; for each time point SEC-MALS: three independent replicates starting with independently unfolded proteins.
Randomization	n/a
Blinding	n/a

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involvement in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involvement in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a