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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 (n) 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
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ 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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ 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 d , Pearson's r), 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 MxCube2 was used for diffraction data collection

Data analysis XDS package (BUILT=20210205) for traditional crystallography data processing; CrystFEL (v 0.9.1) was used for time-resolved data processing; CCP4i program suite (v7.1.018) for structure solving; PHENIX program suite (v1.19.2-4158) for structure refinement; PyMol (v1.8.4.0) for structure visualization; Microsoft Excel (v16.58) for spectra visualization; Coot (v0.9.6); hmmer (v3.3.2) for bioinformatic analysis; iTol v6 online server for phylogenetic tree generation; Wolfram Mathematica (v11) for bioinformatic analysis; Jupyter Notebook (v5.2.2) for bioinformatic analysis; Python (v3.6.9) for bioinformatic analysis; refmac5 (v5.8.0267) for structure refinement; RADDOS (v4.0.1020) was used for calculation of the radiation dose per crystal.

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](#)

Atomic models built using X-ray crystallography data have been deposited in the RCSB Protein Data Bank with PDB codes 7ZMY (for the ground state at pH 8.2 at 100K), 7ZN3 (for the L state at pH 8.2 at 100K), 7ZN0 (for the M state at pH 8.2 at 100K), 7ZN8 (for the ground state at pH 7.0 at 100K), 7ZN9 (for the M state at pH 7.0 at 100K), 7ZNA (for the ground state at pH 5.2 at 100K), 7ZNB (for the M state at pH 5.2 at 100K), 7ZNC (for the ground state at pH 7.6 at 100K in the absence of sodium), 7ZND (for the M state at pH 7.6 at 100K in the absence of sodium), 7ZNE (for the ground state at pH 8.2 at 293K with 7.5-ms-long exposure), 7ZNI (for the 7.5-15-ms snapshot at pH 8.2 at 293K), 7ZNG (for the ground state at pH 8.2 at 293K with 500- μ s-long exposure), and 7ZNH (for the 250-750- μ s snapshot at pH 8.2 at 293K). Extrapolated structure factors and phases for the intermediate states of BcXer are available from Zenodo (10.5281/zenodo.7612803). Publicly available in RCSB Protein Data Bank structures of NsXer (PDB ID: 6EYU) and HsBR (PDB IDs: 5ZIL, 7ZOD, 7ZOE, 6RPH) were used for analysis.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

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

We did not use statistical methods to predetermine sample size. In order to guarantee reproducibility of the X-ray data the cryotrapping procedures were repeated 3-5 times with independent crystals on different days. The determined coordinates appeared identical; therefore larger sample size were not required.

Data exclusions

No data were excluded.

Replication

The cryotrapping procedures were repeated no less than 5 times for each of the intermediate states. At least two datasets were collected for each functional state of the BcXer photocycle and only the highest-resolution/highest quality data is presented in the manuscript. All attempts were successful and the resulting structures of all functional states of BcXer of all different attempts coincide. The functional tests of protein activity in E.coli cell suspension were repeated 3 times for each condition and showed similar results. Spectroscopy data (including the time-resolved data) were measured 2 times independently on different days and showed identical protein kinetics.

Randomization

The samples were not allocated into different groups, therefore the randomization was not required.

Blinding

No blinding was applied. Data collection and data processing and analysis were not done blinded, because the observed effects were apparent in the experiments and therefore revealed the conditions.

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	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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