Signal amplification and transduction in phytochrome photosensors

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Sensory proteins must relay structural signals from the sensory site over large distances to regulatory output domains. Phytochromes are a major family of red-light-sensing kinases that control diverse cellular functions in plants, bacteria and fungi1–7. Bacterial phytochromes consist of a photosensory core and a carboxy-terminal regulatory domain8–11. Structures of photosensory cores are reported in the resting state12–18 and conformational responses to light activation have been proposed in the vicinity of the chromophore19–23. However, the structure of the signalling state and the mechanism of downstream signal relay through the photosensory core remain elusive. Here we report crystal and solution structures of the resting and activated states of the photosensory core of the bacteriophytochrome from Deinococcus radiodurans. The structures show an open and closed form of the dimeric protein for the activated and resting states, respectively. This nanometre-scale rearrangement is controlled by refolding of an evolutionarily conserved ‘tongue’, which is in contact with the chromophore. The findings reveal an unusual mechanism in which atomic-scale conformational changes around the chromophore are first amplified into an ångstrom-scale distance change in the tongue, and further grow into a nanometre-scale conformational signal. The structural mechanism is a blueprint for understanding how phytochromes connect to the cellular signalling network.

The most common domain architecture of the photosensory core of phytochromes in bacteria, plants and fungi is PAS-GAF-PHY (Per/Arnt/Sim-cGMP phosphodiesterase/adenyl cyclase/FhlA-phytochrome specific)19. We investigate the PAS-GAF-PHY fragment of the bacteriophytochrome from D. radiodurans. Figure 1a shows that this protein fragment shares the prototypical response of phytochromes to red/far-red illumination. Its biliverdin chromophore relaxes into the Pr (red-absorbing) state when kept in the dark or after exposure to far-red light. The majority of molecules transform into the Pfr (far-red-absorbing) state after red-light exposure. The dark relaxation from Pfr to Pr occurs in a matter of days, and the optical response of PAS-GAF-PHY is very similar to the full-length protein24. For the PHY-less PAS-GAF construct, the absorption spectra (Fig. 1a) and dark reversion within minutes indicate an incomplete photocycle24.

Time-resolved solution X-ray scattering reports on structural changes in protein reactions25,26. We used it here to characterize the structural dynamics of the phytochrome photocycle. In a first experiment, the phytochrome fragments were repeatedly switched between the Pr and Pfr states using laser flashes of 10-ms duration at 671 nm (Pr → Pfr) and 750 nm (Pfr → Pr). The X-ray solution scattering was recorded in between the laser flashes for approximately 1 s (see Supplementary Information for details). The light-induced change in scattering, ΔS(q), encodes the structural change of the protein during the Pfr → Pfr transition. For the complete photosensory core (PAS-GAF-PHY) we find a large oscillating difference scattering signal at low angles, q < 2 nm−1 (Fig. 1b, red line), indicating large structural changes on the nanometre scale. Oscillations at higher q, which report on structural changes on smaller length scales, were also observed (Extended Data Fig. 1e), but are not discussed further here. The laser-induced ΔS of the PAS-GAF-PHY construct was reproduced by a standard small-angle X-ray scattering (SAXS) experiment with pre-illuminated samples (Fig. 1b, blue line). Time-resolved difference X-ray scattering data covering the micro- and millisecond time scales also reproduce these features when photoconversion is complete (delay time, 30 ms; Fig. 1b, black line). These data further establish that the discussed low-q features grow in with a time-constant of 4.3 ms (Fig. 1b and Extended Data Fig. 1d), which coincides with the formation of the Pfr state as measured by optical absorption of the chromophore (1.0 ms, Extended Data Fig. 1d).

When performing the same experiments on the PHY-less chromophore-binding (PAS-GAF), the oscillatory signal at low q is absent (Fig. 1c). The observed oscillations in the PAS-GAF-PHY domains are much smaller in amplitude and confined to higher q ranges (Fig. 1c and Extended Data Fig. 1e). These data show that quaternary structural changes occur only when the PHY domain is present. In the wild-type protein the PHY domain connects the chromophore binding domains PAS-GAF with the output kinase, making it likely that the detected rearrangement is functionally relevant.

To examine the nature of these conformational changes and to find out how they arise, we crystallized the photosensory core in the dark (referred to as dark) and under periodic illumination at 655 nm (referred to as illuminated), which transforms a large fraction of the protein molecules into the Pfr state (see Fig. 1a and Extended Data figures for details). Absorption spectra of the dark crystals indicate the Pr state, whereas the illuminated crystals are Pfr-enriched (Extended Data Fig. 3c). Omit maps indicate a change in the conformation of the chromophore between the two crystal forms (Extended Data Fig. 3b). Although the biliverdin conformation cannot be fit unambiguously to the electron density in the illuminated structure, the electron density of the rest of the protein is homogeneous (Extended Data Fig. 2b).

Our dark crystal structure was modelled from data up to 3.80 Å resolution (Fig. 2a). The observed domain arrangement is similar to previously reported structures of bacterial PAS-GAF and PAS-GAF-PHY protein fragments12–15,17, but the dimer arrangement differs from some of these structures12–17. The illuminated structure, modelled against data cut at 3.24 Å, shows marked differences compared to the dark structure (Fig. 2a). First, the dimer adopts an open Y-like shape of the PHY domains. This is in contrast to all reported resting state structures, where the dimer is closed (Fig. 2a)12,13,17,20. Second, the topology of the PHY domains (residues 446–477) appears as an α-helix and a loop in the illuminated model and is a β-sheet in the dark model (Fig. 2b and Extended Data Fig. 2). These two folds have been observed separately in non-canonical bacterial12,20 and prototypical cyanobacterial phytochromes13,17, respectively (Extended Data Fig. 4). Our structures establish that the refolding of the tongue occurs within the same bacterial phytochrome, and suggest that this refolding is associated with opening of the dimer. The change in fold causes the length of the tongue to vary between the

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dark and illuminated structures (Fig. 2b), which is key for understanding the role of the tongue in signal relay.

Before detailing this mechanism, it is important to test how well the crystal models represent the solution structures of the Pr and Pfr states. To this end, we performed structural refinement against the solution difference X-ray scattering data (Fig. 1), which is a sensitive indicator of conformational change. It has the advantage over absolute scattering profiles that experimental error and uncertainties arising from the choice of method for scattering calculations largely cancel out (Extended Data Fig. 5c).

We generated candidate solution structures by running two sets of molecular dynamics simulations starting from the dark and illuminated crystal structures (see Supplementary Information for details). These are referred to as Pr and Pfr trajectories as they aim to model solution scattering data representing these states. Snapshots were recorded every 50 ps and the solution X-ray scattering was calculated from each.

All pairs of Pr/Pfr candidate structures were then compared to the static difference X-ray scattering (Fig. 1b and Extended Data Fig. 7, see Supplementary Information for details). We selected the 100 Pr/Pfr pairs with best agreement to the data and consider the participating structures to be representative of the protein in solution. Considering all pairwise differences between these structures, a consistent set of 747 curves was generated and evaluated (Fig. 3a). The agreement of the model and experiment is excellent, and considerable improvement is made compared to the crystal structures.

To verify the choice of solution structures on the basis of difference scattering, these structures were cross-validated against absolute, population-corrected SAXS data as described in Supplementary Information (see also Extended Data Fig. 5). Figure 3b shows that the agreement with absolute SAXS data is strongly correlated with the separation of the PHY domains, and that the solution structures, proposed purely on

**Figure 1 | Time-resolved X-ray scattering of the PAS-GAF and PAS-GAF-PHY fragments from *D. radiodurans*.**

- **a**, Absorption (A) spectra of protein fragments after illumination with far-red (780 nm) and red (655 nm) light, labelled Pr and Pfr respectively. AU, arbitrary units.
- **b, c**, Solution X-ray scattering data from the PAS-GAF-PHY and PAS-GAF fragments shown on the same scale. Time-resolved data (black, BioCARS), direct static data collected by laser-induced population switching (red, cSAXS) and indirect static data from a standard SAXS experiment with pre-illuminated samples (blue, BM29) is shown. BioCARS, cSAXS and BM29 refer to synchrotron endstations (see Methods). \( \Delta S \) is the difference in scattered X-ray intensity caused by illumination at 671 nm. \( q = \frac{4\pi}{\lambda} \sin \theta \) at wavelength \( \lambda \) and scattering angle \( \theta \).

**Figure 2 | Dark and illuminated crystal structures of the PAS-GAF-PHY fragment from *D. radiodurans*.**

- **a**, Crystal structures of the PAS-GAF-PHY dimer in the dark and illuminated forms. The tongue of the PHY domain (green) changes fold and the dimer opens up in the illuminated state. The biliverdin chromophore is shown in orange.
- **b**, Fold and interactions of the PHY tongue. The \( \beta \)-sheet (dark) coordinates to Asp 207 and Tyr 263 via Arg 466, whereas the \( \alpha \)-helix (illuminated) coordinates via Ser 468, both of which are part of the conserved 465P-R-X-S-F469 motif. The named residues are shown as sticks. The \( \beta \)-sheet (dark) is further held by hydrogen-bonding interactions between the amide groups of Ala 450, Gly 452 and Arg 202. The change in PHY domain conformation leads to a shortening of the tongue by 2.5 Å as measured between GAF (Arg 202) and PHY (Tyr 479) domains (arrows). A backbone interaction close to the PHY domain between Leu 445 and Tyr 479, shared by both crystal structures, is also indicated. The green dashed lines indicate regions that are not modelled or not shown for clarity.
difference scattering, cluster in the low-error parts of these correlations. The agreement of the solution structures to the solution scattering is considerably improved compared to the crystal structures as can be seen in Fig. 3b. Representative structural models for Pr and Pfr states in solution are depicted in Fig. 4.

The solution-structural models show that the PHY domains of opposing monomers come fairly close to each other in the Pr state but move apart by ~3 nm during the Pr → Pfr transition (Fig. 4). The Y shape assumed by the solution structures in the Pfr state is in qualitative agreement with a low-resolution envelope of a *Rhodopseudomonas palustris* phytochrome determined by SAXS. Our crystal structures capture the essence of this quaternary conformational change, albeit with differences in the amplitude and details of the motion. These differences are probably caused by crystal contacts (Extended Data Fig. 6). Nevertheless, the overall agreement with the solution structures lends strong support to the idea that the refolding of the tongue observed in the crystal structures is intrinsic to the structural rearrangement between Pr and Pfr states.

From the crystal and solution structures the following mechanism for signal transduction through the photosensory core emerges. It is known that the rotation of the biliverdin D-ring causes atomic rearrangements in the chromophore binding pocket, including displacement of Asp 207 and Tyr 263 (refs 19–22). Our crystal structures suggest that this controls the fold of the tongue of the PHY domain. Importantly, the tongue is shortened as a result of the refolding and the distance between the GAF and PHY domain is reduced by 2.5 Å (Fig. 2b) upon red-light illumination. As a consequence of this, and demonstrated by our Pr and Pfr solution structures, the dimer opens up between the PHY domains by several nanometres (Fig. 4).

Three factors are essential for the proposed structural mechanism. First, the PAS, GAF and PHY domains, which are connected by the tongue, are known to be very rigid internally such that they cannot deform to absorb the length variation of the tongue. Second, the tongue and its junctions to PHY and GAF have to be rigid, especially in Pr, in which the PHY and GAF domains are pushed away from each other. In our dark structures the direction of the tongue is rigidly fixed with respect to the GAF domains by the conserved interaction Arg 466 to Asp 207 and Tyr 263 and additional backbone hydrogen bonding (Fig. 2b). In the illuminated structure the tongue binds to GAF through interaction of Ser 468 with Asp 207 and Tyr 263 (ref. 12), and through hydrophobic interactions, among others by Tyr 472 (Extended Data Fig. 4b).
tongue is also locked close to the PHY domain by additional backbone hydrogen bonding. Third, the PAS-GAF and PHY domains are connected by the unusually long scaffolding helix, which is necessary to redirect the shortening of the tongue into bending of the monomer. Indeed, our simulations reveal a hinge in the scaffolding helix (Fig. 4b). All phytochrome structures published to date support these three requirements12–15.

In bacterial and fungal phytochromes, signal output is through C-terminal histidine kinase domains that autophosphorylate and further phosphotransfer to a response regulator16. In plant phytochromes, two additional PAS domains are included in the C-terminal regulatory region and a more complex pattern of functions has to be controlled, such as serine/threonine kinase activity17, and affinity to interaction partners18,19.

In all cases, the output activity is probably controlled by a structural change in the photosensory core. The photosensory core and the key amino acid sequence in the tongue region 465P-R-X-S-F469 (where X denotes any residue) are highly conserved over the whole phytochrome superfamily (Extended Data Fig. 2c). Here we suggest a mechanism in which the fold of the PHY-tongue controls the bending of the monomer. For the isolated photosensory unit in this study, this results in marked opening of the dimer in Pfr (Figs 2 and 4). In full-length phytochromes, this could become accessible to interaction partners, or monomers could rearrange with respect to each other. It will be intriguing to see the emergence of these mechanisms, which become deductible with experimental approaches similar to the one presented here.

METHODS SUMMARY
The PAS-GAF-PHY and PAS-GAF fragments from D. radiodurans were expressed in the Escherichia coli strain BL21 (DE3) and purified by affinity and size-exclusion chromatography. Crystallographic data was collected at beamline ID23-1 of the European Synchrotron Radiation Facility (ESRF) (see Extended Data Table 1). Time-resolved X-ray scattering with millisecond time resolution were recorded at beamline CXSXS of the Swiss Light Source20. SAXS measurements were performed at beamline BM29 of the ESRF and analysed as summarised in Extended Data Table 2a. Time-resolved X-ray scattering data in the micro- and millisecond ranges were collected at beamline ID14-B, BioCARS, of the Advanced Photon Source at Argonne National Laboratory. All solution-scattering sample details are summarized in Extended Data Table 2b. Molecular dynamics simulations (GROMACS 4.5.5)21 were used to generate trial solution structures and theoretical scattering curves were evaluated using Zernike expansion as implemented in SASTBX22. The structures were scored against experimental scattering data as detailed in Supplementary Information.

Online Content
Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Butler, W. L., Norris, K. H., Siegelman, H. W. & Hendricks, S. B. Detection, assay, and preliminary purification of the pigment controlling photoresponsive development of plants. Proc. Natl Acad. Sci. USA 45, 1703–1708 (1959).

2. Kehoe, D. M. & Grossman, A. R. Similarity of a chromatic adaptation sensor to a photosensory unit of bacterial phytochrome. Proc. Natl Acad. Sci. USA 95, 13976–13981 (1998).

3. Yeh, K. C., Wu, S. H., Murphy, J. T. & Lagarias, J. C. A cyanobacterial phytochrome and ethylene receptors. Proc. Natl Acad. Sci. USA 77, 1833–1838 (1980).

4. Hughes, J. et al. A prokaryotic phytochrome. Nature 386, 663 (1997).

5. Jiang, Z. et al. Bacteriophytochrome. Proc. Natl Acad. Sci. USA 95, 13976–13981 (1998).

6. Blumenstein, A. et al. The Aspergillus nidulans phytochrome FphA represses sexual development in red light. Curt. Biol. 15, 1833–1838 (2005).

7. Ni, M., Tepperman, J. M. & Quail, P. H. Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. Nature 400, 781–784 (1999).

8. Rockwell, N. C., Su, Y. S. & Lagarias, J. C. Phytochrome structure and signaling mechanisms. Annu. Rev. Plant Biol. 57, 837–858 (2006).

9. Möglich, A., Yang, X. J., Ayers, R. A. & Moffat, K. Structure and function of plant photoreceptors. Annu. Rev. Plant Biol. 61, 21–47 (2010).

10. Yang, X., Kuik, J. & Moffat, K. Crystal structure of Pseudomonas aeruginosa bacteriophytochrome and signal transduction. Proc. Natl Acad. Sci. USA 105, 14715–14720 (2008).

11. Zernike expansion as implemented in SASTBX 30. The structures were

12. Anders, K., Daminelli-Widany, G., Mroginski, M. A., von Stetten, D. & Essen, L. O. Structure of the cyanobacterial phytochrome 2 photosensor implies a tryptophan switch for phytochrome signaling. J. Biol. Chem. 288, 35714–35725 (2013).

13. Narikawa, R. et al. Structures of cyanobacteriophytochromes from phototaxis regulators AnPivX and TePoul reveal general and specific photoversion mechanism. Proc. Natl Acad. Sci. USA 110, 918–923 (2013).

14. Yang, X., Kuik, J. & Moffat, K. Conformational differences between the Pfr and Pr states in Pseudomonas aeruginosa bacteriophytochrome. Proc. Natl Acad. Sci. USA 106, 15639–15644 (2009).

15. Yang, X., Ren, Z., Kuik, J. & Moffat, K. Temperature-scan cryocryocrystallography reveals structural intermediates in bacteriophytochrome. Nature 479, 428–432 (2011).

16. Song, C. et al. Two ground state isoforms and a chromophore D-ring phototip triggering extensive intramolecular changes in a canonical phytochrome. Proc. Natl Acad. Sci. USA 108, 3842–3847 (2011).

17. Burgie, E. S., Walker, J. M., Phillips, G. & Vierstra, R. D. A photo-labile thioether linkage to phytoivolinib binds the foundation for the blue/green photocycles in DXF-cyanobacteriophytochromes. Structure 21, 88–97 (2013).

18. Ulijasz, A. T. et al. Structural basis for the photoconversion of a phytochrome to the activated Pfr form. Nature 463, 250–254 (2010).

19. Yang, J. R. et al. Mutational analysis of Deinococcus radiodurans bacteriophytochrome reveals key amino acids necessary for the photochromicity and proton exchange cycle of phytochromes. J. Biol. Chem. 283, 12212–12226 (2008).

20. Cammarata, M. et al. Tracking the structural dynamics of proteins in solution using time-resolved wide-angle X-ray scattering. Nature Methods 5, 881–886 (2008).

21. Andersson, M. et al. Structural dynamics of light-driven proton pumps. Structure 17, 1265–1275 (2009).

22. Evans, K., Grossmann, J. G., Fordhorn-Skelton, A. P. & Papiz, M. Z. Small-angle X-ray scattering reveals the solution structure of a bacteriophytochrome in the catalytically active Pr state. J. Mol. Biol. 364, 655–666 (2006).

23. Westenhoff, S. et al. Rapid readout detector captures protein time-resolved WAXS. Nature Methods 7, 775–776 (2010).

24. Prosk, S. et al. GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. Bioinformatics 29, 845–854 (2013).

25. Liu, H. G., Hexemer, A. & Zwart, P. H. The Small Angle Scattering Toolbox (SASTBK): an open-source software for biomolecular small-angle scattering. J. Appl. Crystallogr. 45, 587–593 (2012).

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26. Evans, K., Grossmann, J. G., Fordhorn-Skelton, A. P. & Papiz, M. Z. Small-angle X-ray scattering reveals the solution structure of a bacteriophytochrome in the catalytically active Pr state. J. Mol. Biol. 364, 655–666 (2006).

27. Westenhoff, S. et al. Rapid readout detector captures protein time-resolved WAXS. Nature Methods 7, 775–776 (2010).

28. Prosk, S. et al. GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. Bioinformatics 29, 845–854 (2013).

29. Liu, H. G., Hexemer, A. & Zwart, P. H. The Small Angle Scattering Toolbox (SASTBK): an open-source software for biomolecular small-angle scattering. J. Appl. Crystallogr. 45, 587–593 (2012).

Supplementary Information is available in the online version of the paper.
31. Sievers, F. et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* 7 (2011).

32. Wagner, J. R., Zhang, J. R., Brunzelle, J. S., Vierstra, R. D. & Forest, K. T. High resolution structure of *Deinococcus* bacteriophytochrome yields new insights into phytochrome architecture and evolution. *J. Biol. Chem.* 282, 12298–12309 (2007).

33. Mailliet, J. et al. Spectroscopy and a high-resolution crystal structure of Tyr263 mutants of cyanobacterial phytochrome Cph1. *J. Mol. Biol.* 413, 115–127 (2011).

34. Konarev, P. V., Volkov, V. V., Sokolova, A. V., Koch, M. H. J. & Svergun, D. I. PRIMUS: a Windows PC-based system for small-angle scattering data analysis. *J. Appl. Crystallogr.* 36, 1277–1282 (2003).

35. Anders, K., Daminelli-Widany, G., Mroginski, M. A., Von Stetten, D. & Essen, L. O. Structure of the cyanobacterial phytochrome 2 photosensor implies a tryptophan switch for phytochrome signaling. *J. Biol. Chem.* 288, 35714–35725 (2013).

36. Gasteiger, E. et al. in *The Proteomics Protocols Handbook* (ed. Walker, J. M.) Ch. 52, 571–607 (Humana, 2005).
Extended Data Figure 1 | Difference scattering and kinetics.  a–c, Singular value decomposition ($\Delta S(q,t) = USV^T$, where $U$, $S$, and $V$ are matrix factors) of time-resolved solution scattering data from the PAS-GAF-PHY fragment. Two components ($n$) suffice to describe the data, the final product ($n = 1$) and a transient low-$q$ depression ($n = 2$). a, The first three basis spectra (first, second and third columns of $US$), and original X-ray scattering data (black) with reconstruction based on the first two singular values (all columns of $USV^T$, red) are shown. b, Relative amplitudes of the two first basis spectra (first and second columns of $V$). Lines are guides for the eye. c, Singular values (diagonal elements of $S$). d, The rise of the Pfr product state as measured by direct integration of difference scattering curves ($\langle |\Delta S(q)^2| \rangle_{125s \leq t \leq 1s}$) and by optical transient absorption at 754 nm after excitation with a 680 nm laser flash. Solid curve is the function $\left(1 - e^{-t/\tau}\right)$, where $t$ is time and $\tau$ a free fitting parameter. These data establish that the structural change occurs just after the Pfr state is formed in the chromophore. Note the positive signal in the absorption curve with very small amplitude at $3 \text{ ms}$, which appears to decay while the structural signal rises. This could be because the absorption properties depend weakly on the large-scale rearrangement. e, Direct static difference data from Fig. 1, amplified by $q^2$ to reveal wide-angle oscillations.
Extended Data Figure 2 | Light-induced changes in the secondary structure of the evolutionally conserved PHY tongue. a, Secondary structure and topology of the D. radiodurans PAS-GAF-PHY fragment. The structural elements in our crystal structures are very similar to other published phytochrome structures. The PHY tongue region (box), however, was found to refold upon illumination. The five-stranded β-sheet core of the GAF domain is extended by a small sixth β-strand (called 29) that interacts with the PHY tongue (see Fig. 2 and Extended Data Fig. 4). The mini-sheet structure at the knot region is not included in the graph. The figure is adapted from ref. 12. b, Omit map of the PHY tongue in the dark crystal form (top panel) and the illuminated crystal form (bottom panel). In the dark crystal form, the omit map density (blue) supports the built β-turn secondary structure (orange sticks), even though most of the side chains are poorly resolved. In the illuminated crystal form, the omit map (blue) clearly reveals the density of a helix with its bulky side chains (orange). The omit maps were calculated by repeating molecular replacement and a refinement step (see Supplementary Information) with a structure in which the PHY tongue was removed. All electron density maps are contoured at 3.0σ.

c, Sequence alignment of part of the GAF domain and of PHY loop region. The conserved 207D-I-P209 motif in the GAF domain and 465P-R-X-S-F469 motif in the PHY tongue are marked by asterisks. Five representatives from eubacterial (BphP), cyanobacterial (Cph), higher plant, fungi (Iph) and PAS-less phytochromes are shown. The full alignment is supplied as source data. Colour coding: dark grey, identical residues; light grey, similar residues. The amino acid sequences were aligned with Clustal Omega using the default settings. Sequences include Deinococcus radiodurans BphP, Pseudomonas syringae pv. tomatum T1 BphP, Rhodopseudomonas palustris TIE-1 BphP3, Pseudomonas aeruginosa PAO1 BphP, Agrobacterium fabrum str. C58 BphP1 (Agp1), Synechocystis sp. PCC6803 Syn-Cph1, Microcystis aeruginosa NIES-843, Nodularia spumigena CCY9414, Cyanobacte sp. PCC 7822, Anabaena variabilis ATCC 29413, Physcomitrella patens Phy1, Zea mays PhyB1, Populus trichocarpa PhyA, Selaginella martensi Phy1, Arabidopsis thaliana PhyA, Synechococcus OSA SyB-Cph1, Synechococcus OSA SyA-Cph1, Nostoc punctiforme PCC73102, Lyngbya sp. PCC 8106 and Anabaena variabilis ATCC 29413.
Extended Data Figure 3 | Biliverdin structure and spectra in crystals.

a, Photographs of the crystals under cryogenic conditions at the beamline ID23-1. b, Biliverdin omit maps of the dark (top left) and illuminated (top right) form support the existence of the modelled biliverdin conformations (yellow and orange). Comparison of the electron density around the biliverdin with published structures (bottom panels). In the dark form, the electron density indicates a conformation similar to the published Pr structures13,14,32,33, including a D. radiodurans structure (PDB accession 2O9C, cyan)32. Therefore we modelled the biliverdin as a 15Za isomer. In the illuminated form, however, the electron density supports neither the biliverdin as determined in the Pfr structure of PaBphP (PDB 3NHQ, red)20, nor as determined in the Pr structure of D. radiodurans (2O9C, cyan)32. Therefore the rotation of the biliverdin D-ring cannot be reliably determined and is modelled with both possibilities (15Za, and 15Ea, orange and yellow in top right panel). Omit maps were calculated as in Extended Data Fig. 2 and contoured at 3.0σ. c, Representative absorption spectra of the dark (black) and illuminated (grey) crystals, recorded at 123 K. Note that the terms ‘illuminated’ and ‘dark’ refer here to the crystallization conditions (see Supplementary Information for details). The dark crystal spectrum resembles the Pr spectrum in solution (Fig. 1a). Illumination with red light in the crystallization drops at ambient temperature led to a slight increase of far-red absorption and disintegration of the crystals (data not shown). Dark crystals were unaffected by far-red illumination. The spectrum of the illuminated crystals shows that a substantial proportion (>50%) of the proteins reside in Pfr state. A similar absorption was detected from solubilized crystals. The illuminated crystals could be switched to Pr-like absorption with far-red illumination. Reversely, the Pfr-like features could also be increased with red light (data not shown) with illumination at ambient temperature. Exposure with light increased the scattering background in the absorption measurements. The crystals seemed unaffected by the illumination when illuminated with red light in the crystallization drops. Although the spectral analyses of the illuminated crystals do not indicate a pure Pfr spectrum, and the biliverdin conformation cannot be fit unambiguously to the electron density, the remainder of the electron density is homogeneous (Extended Data Fig. 2b). Most importantly, the tongue region of the PHY domain adopts the conformation resembling the Pfr state of PaBphP (Extended Data Fig. 4b)12,19,20. The conformations of the four monomers in an asymmetric unit are practically identical and hence we conclude that biliverdin can co-exist in both Pr and Pfr states inside this crystal form and still the protein part represents the structural aspects of the Pfr state only.
Extended Data Figure 4 | Comparison of PHY tongue interactions with published structures. a, Comparison of the dark crystal form (green/dark grey) to cyanobacterial cph1 in Pr state (PDB 2VEA, orange/light grey)13. b, Comparison of the illuminated crystal form (green/dark grey) to PaBphP in Pfr state (PDB code 3NHQ10, orange/light grey). In both the Pr and Pfr forms, key interactions are conserved between the phycocyanins (black dashes), as well as the positions of three conserved tongue motives (see Extended Data Fig. 2c). The residues of these three motives are indicated as: 451W-(G/A)-G453, 465P-R-X-S-F469 and 472(W/F,Y)-X-E47413, with numbering from the D. radiodurans sequence. Trp 451 was not modelled in our illuminated crystal structure, and part of the PHY tongue has been removed for clarity. Small changes in relative orientations between the difference crystal structures are observed, for example, a slight tilt of helix of the Pfr tongue.
Extended Data Figure 5 | SAXS data and calculations. a, Experimental SAXS data of dark (‘Pr’) and pre-illuminated (‘Pfr’) samples. Here, \( I \) is scattered intensity, \( 2\theta \) is the scattering angle and \( q \) is the magnitude of the scattering vector. The data are merged from the concentration series (Extended Data Table 2b) and are normalized on \( 0.4 \text{ nm}^{-2} < q < 0.6 \text{ nm}^{-2} \). b, Guinier plot of the low-\( q \) region, shown for all concentrations. The curves converge at low concentration (\( C \)). Inset shows the radii of gyration (\( R_g \)) calculated from the curves in a according to the Guinier approximation. Dotted lines show the \( q \)-range used for linear regression (\( 0.15 \text{ nm}^{-2} < q^2 < 0.120 \text{ nm}^{-2} \)), which is such that \( qR_g < 1.3 \) as required. Arrows indicate increasing concentration. c, Average difference scattering signals calculated from the solution-structural models using three methods: Crysol (default settings), SASTBX with spherical harmonic expansion (SHE, default settings), and SASTBX with Zernike polynomial expansion as described in Supplementary Information. Different calculation methods result in very similar predicted difference X-ray scattering patterns. d, Determination of the relative Pr/Pfr populations represented by the BM29 data as described in Supplementary Information. SSE abs is the sum-squared error against absolute scattering data, SST is the sum of squares about the mean, and \( v \) is the Pfr fraction. We find that our Pr sample contained only Pr (top) whereas in the Pfr sample 64% of the protein molecules adopted the Pfr conformation (bottom). Notably, the static difference scattering patterns (Fig. 1) represent, up to a scaling factor, the relation between pure Pr and Pfr populations. This is in contrast to traditional SAXS which report on population mixtures, because the Pfr state cannot be easily produced with 100% population in solution.
Extended Data Figure 6 | Packing interactions of the crystal forms.
a, b, Crystal packing interactions of the dark (a) and illuminated (b) crystal forms. The dimer of an asymmetric unit is shown in red and the symmetry mates in grey. For the illuminated form, only chains A and B are shown in red. Interactions are shown from two orientations for clarity. In the dark form, crystal contacts are seen in the top regions of the PHY domains and therefore may cause artefacts in the long scaffolding helix and in the opening of the PHY domains. In the illuminated form contacts are such that the PHY domains may be pushed closer together, which is consistent with the larger separation of the PHY domains as refined from the solution X-ray scattering data. It is noteworthy that the relative orientation of the monomers in the dimer is different between all three known structures for PAS-GAF-PHY phytochromes. For P. aeruginosa the dimer is parallel with variations in between different copies of the dimer in the crystallographic unit cell, in two cyanobacterial phytochrome an antiparallel dimer is observed, and in our Pr structure, the monomer have an angle of approximately 45°. The rings highlight crystal contacts.
Extended Data Figure 7 | Solution-structural refinement. a, The distribution of PHY domain separations (R_{PP}) obtained from unbiased molecular dynamics simulations (production runs 1–3). The best-matching sets A and B of solution structures found from these conformations are indicated by crosses (with N = 100 best pairs chosen from M = 6 \times 10^6 possible combinations). Whereas the Pr structures cluster in a region of high sampling, the Pfr structures lie at the edges of the PHY-PHY distribution, suggesting inadequate sampling. To remedy this, we artificially scanned the PHY domain separation in separate simulations (production runs 4 and 5) to improve sampling. b, The new distribution of pulled PHY domain separations in the Pfr state. The final analysis and all solution-structural conclusions drawn in this study are based on the trajectories described in b. c–e, Consistency test of the structural refinement procedure. c, A cutoff parameter R_{cut} was introduced to reject all molecular dynamics frames R_{PP} > R_{cut}. The resulting average over R_{PP} of the best N = 100 pairs is plotted as a function of R_{cut}. It is found that R_{PP} = R_{cut}, which indicates that the best fit to the difference X-ray scattering data are always at the highest separations available in sampling range. d and e show the dependence of the total and average error (SSE_{tot} and SSE_{av}) as functions of R_{cut}. SST represents the overall sum of square about the mean. It is observed that the error decreases steeply for R_{cut} < 5 nm, and only marginally for R_{cut} > 5 nm. We therefore consider optimization in the latter range overfitting, and applied R_{cut} = 5.0 nm in the refinement for the solution structures. The refined structures (Fig. 4) should therefore be considered to represent lower limits on R_{PP}. f, Solution-structural refinement parameters and results.
## Extended Data Table 1 | Crystallographic data collection and refinement statistics

| Data collection                       | Dark form* | Illuminated form* |
|---------------------------------------|------------|------------------|
| Space group                           | P6₁         | P2₁2₁2₁          |
| Cell dimensions                       |             |                  |
| a, b, c (Å)                           | 150.28, 150.28, 145.95 | 86.90, 195.70, 225.00 |
| α, β, γ (°)                           | 90.0, 90.0, 120.0 | 90.0, 90.0, 90.0 |
| Resolution (Å)                        | 48.65-3.80 (3.90-3.80) | 49.22-3.24 (3.32-3.24) |
| R_{merge} (%)                         | 39.2 (451.3) | 24.1 (439.0)    |
| I/σ                                  | 4.72 (1.05) | 9.69 (0.83)     |
| Completeness (%)                      | 99.9 (100.0) | 99.9 (100.0)    |
| Redundancy                            | 11.0 (11.6) | 19.3 (20.3)     |

## Refinement

| Resolution (Å)                        | 48.65-3.80 (3.90-3.80) | 49.22-3.24 (3.32-3.24) |
| No. reflections                       | 17597 (1295)           | 58759 (4255)          |
| R_{work} / R_{free}                   | 25.3/26.6 (37.10/40.20) | 23.3/26.0 (39.7/39.7) |
| No. atoms                             |                         |                  |
| Protein                               | 7416                    | 14784             |
| Ligand/ion                            | 86                      | 172               |
| Water                                 | 0                       | 0                 |
| Average B-factor (Å²)                 | 210.24                  | 158.61            |
| Protein                               | 208.71                  | 156.92            |
| Ligand/ion                            | 170.21                  | 143.48            |
| Water                                 | -                       | -                 |
| Wilson B-factor (Å²)                  | 130.97                  | 118.65            |
| R.m.s deviations                      |                         |                  |
| Bond lengths (Å)                      | 0.006                   | 0.005             |
| Bond angles (°)                       | 1.112                   | 1.159             |

*Data from two crystals were used.

¹Highest resolution shell is shown in parenthesis.
Extended Data Table 2 | SAXS statistics and sample details

A

| PAS-GAF-PHY | Bovine serum albumin |
|-------------|----------------------|
| Dark        | Illuminated         |
| $R_g$ (nm)  | $R_g$ (nm)*         |
| 3.75 ± 0.02 | 3.78 ± 0.02         |
| $R_g$ (nm)* | 3.77                |
| $D_{max}$ (nm) | 11.5               |
| $D_{max}$ (nm) | 12.4               |
| $V_{Porod}$ (nm$^3$) | 173                |
| $l_0$       | $l_0$               |
| 97.3 ± 0.2  | 86.8 ± 0.3          |
| $MW_{exp}$ (kDa) | −100               |
| $MW_{exp}$ (kDa) | −90               |
| $MW_{exp}$ (kDa) | −66                |
| $MW_{calc}$ (kDa) | 113.0              |
| $MW_{calc}$ (kDa) | 113.0              |

* from manual analysis in Extended Data Figure 5

B

| Sample | Conc. [mg/ml] | Buffer | Salt | pH  |
|--------|---------------|--------|------|-----|
| cSAXS  |               |        |      |     |
| PAS-GAF| 18            | 20 mM Tris | 75 mM NaCl | 7.8 |
| PAS-GAF-PHY | 25            | 20 mM Tris | 150 mM NaCl | 7.0 |
| BioCARS|               |        |      |     |
| PAS-GAF| 28            | 30 mM Tris | 8.0  |
| PAS-GAF-PHY | 25            | 30 mM Tris | 8.0  |
| BM29   |               |        |      |     |
| PAS-GAF| 0.5-26*       | 30 mM Tris | 8.0  |
| PAS-GAF-PHY | 0.5-29*       | 20 mM Tris | 150 mM NaCl | 7.0 |

*see Extended Data Figure 5

a. Statistics of the static SAXS (BM29) data, including radii of gyration ($R_g$), maximum particle dimension ($D_{max}$), Porod volume ($V_{Porod}$), forward scattering ($l_0$). The molecular weights ($MW_{exp}$) were estimated from the $l_0$ of BSA using the formula $MW_{exp} = MW(BSA) * (l_0(sample)/l_0(BSA))$, where $MW(BSA) = 66\,kDa$. Theoretical molecular weights ($MW_{calc}$) were calculated from the protein sequence$^{36}$.

b. Details of the samples used for solution scattering measurements.