Supporting Information

Photocycle of Cyanobacteriochrome TePixJ

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EXPERIMENTAL PROCEDURES

SAMPLE PREPARATION

The gene encoding the GAF domain of TePixJ (UniprotID: Q8DLC7, encoding residues 437-588) was synthesized from GeneArt (Life Technologies) and subcloned into pET15b. The vector was then co-transformed along with pCola Duet vector containing pcyA and HO1 genes into BL21 E. coli cells. Cultures were grown at 30 °C, 190 rpm until an OD of 0.1 at 600 nm was achieved. The temperature was then dropped to 25 °C. Cultures were induced using 100 µM IPTG at an OD of between 0.4 and 0.6 at 600 nm, and grown overnight. Nickel-IDA resin was used as the first step purification. The resin was equilibrated with 5 column volumes of 'buffer A', 50 mM Tris pH 7.5, 300 mM NaCl, 5 mM imidazole, 1 % β -mercaptoethanol, and incubated with the protein for 3 to 4 hours. The protein flow through was collected and the resin washed with 4 column volumes of 'buffer B', 50 mM Tris pH 7.5 300 mM NaCl, 30 mM imidazole, 1 % β-mercaptoethanol. The protein was eluted with 'buffer C', 50 mM Tris pH 7.5, 300 mM NaCl, 250 mM imidazole, 1 % βmercaptoethanol. The purified protein was then desalted into 50 mM Tris pH 7.5, 300 mM NaCl (or 50 mM Tris pD 7.5, 300 mM NaCl for D₂O measurements), concentrated using a Sartorius Stedim Biotech vivaspin column with a 10 kDa cut-off, flash-frozen and stored at -80 °C. UV/Vis absorption spectroscopy was performed using a Cary 50 UV-Vis Spectrophotometer, Varian, Agilent Technologies. All spectra were recorded from 300 to 800 nm.

In all time resolved measurements the initial Pb or Pg/Pt states were generated by illumination with appropriately colored, 530 nm or 420 nm respectively, high power LED (Thorlabs)

VISIBLE TRANSIENT ABSORPTION

Data over the 0.2 ps – 3 ns time range were collected using a Helios (Ultrafast Systems LLC) spectrometer with an instrument response function of around 0.25 ps. The spectrometer was driven by a Ti:sapphire amplifier laser system (a Spectra Physics Solstice Ace). The amplifier

output, which has a wavelength of 800 nm, a 1 kHz repetition rate and ~100 fs pulse duration is split, and part of the output is used by an optical parametric amplifier (TOPAS Prime with NIRUVis unit) to generate the excitation beams of 0.6 μ J at 420 nm (for the Pb to Pg reaction) and 0.3 – 0.6 μ J at 530 nm (for the Pg to Pb reaction),. The probe beam consisted of a white light continuum generated in a CaF₂ crystal by a fraction of the amplifier output. Samples were flowed through a 2 mm pathlength quartz cuvette, where the sample reservoir was illuminated by the LED. The pre-excitation data were subtracted before the analysis and spectral chirp corrected for.

Measurements on µs to s timescales were carried out using a laser system consisting of an optical parametric oscillator (OPO) pumped by a Q-switched Nd: YAG laser (Brilliant B, Quantel). Excitation wavelengths of 420 and 532 nm were generated using the OPO pumped by the third harmonic of the laser. A quartz cuvette with a 2 mm path length for pump-excitation and 10 mm path length for probing of the was used. TA spectra were recorded using an LKS-60 Flash-Photolysis instrument (Applied Photophysics Ltd.). Kinetics were recorded in 5 nm increments across the visible spectral region.

INFRA-RED TRANSIENT ABSORPTION

Time-resolve infra-red spectroscopy was performed using the TRMPS set-up of the ULTRA-LIFEtime system at the Central Laser Facility, STFC, Rutherford Appleton Laboratory, UK. This uses a 100 kHz ultrafast laser based on a custom dual Yb:KGW system (Pharos, Light Conversion). Samples in D₂O buffer were contained between two CaF₂ windows, separated by a teflon spacer to give a pathlength of approximately 50 µm. Samples were flowed through the cell, and the sample holder was rastered to avoid sample damage. Excitation beams, at a 1kHz repetition rate were used at wavelengths of 425 nm, with 0.8 µJ pump energy, and 530 nm with 0.6 µJ energy. Data were collected for approximately 40 minutes per dataset. The polarization of the excitation beam was set at the magic angle with respect to the IR probe beam. Difference spectra were generated relative to the ground state at time delays ranging between 1 ps and 390 μ s. Pixel to wavenumber calibration was performed using a polystyrene standard. Infra-red pumpprobe transient absorption data from the TRMPS set-up were averaged 0.1 μ s on either side of each 10 μ s "TRMP" step after the first 10 μ s of data. Thus, 58 time points were spread over the first 10 μ s timerange, and thereafter timepoints were every 10 μ s up to 390 μ s (39 in total)

GLOBAL ANALYSIS

Global Analysis was performed using the open-source software Glotaran to obtain an evolution associated difference spectra (EADS).¹ This procedure reduces the matrix of change in absorbance as a function of time and wavelength, to a model of one or more exponentially decaying time components, each with a corresponding difference spectrum (EADS). All datasets were fitted to a sequential, unbranched model.

REFERENCES

[1] Snellenburg, J. J., Laptenok, S. P., Seger, R., Mullen, K. M., and van Stokkum, I. H. M. (2012)
 Glotaran: A Java-Based Graphical User Interface for the R Package TIMP, *J. Stat. Softw.* 49, 1-22.



Figure S1. UV/Vis absorption spectra of TePixJ A. The Pb and Pg states. B. Difference spectra of the blue to green conversion (black dots) fitted with Gaussian functions (lines) to identify peaks.
C. calculated absorption spectra derived from peaks fitted to the difference spectra shown in panel
B. D. spectra of non-reactive spectral components derived from subtraction of spectra in panel
C from those shown in panel A .



Figure S2. Time-resolved changes in the visible region from 0.5 ps to 3 ns after photoexcitation at 420 nm, after illumination with a 530 nm LED to generate the Pb state (in active samples). **A and B.** a primarily "non-reactive" sample; **C and D.** a primarily active sample in H₂O based buffer solution; **E and F.** a primarily active sample in D₂O based buffer solution. Kinetics compare the major bleach of the "non-reactive" component with the excited state absorption of the reactive component (wavelength chosen so non-reactive component has minimal contribution). The "non-

reactive" ground-state absorption at 575 nm indicates that illumination at 530 nm (which would be absorbed) has not changed the state of the species, confirming it as being non-reactive. The kinetics of the 575 nm bleach returning to the baseline within a nanosecond demonstrates the lack of any long-lived changes after photoexcitation.



Figure S3. Time-resolved changes in the visible region from 0.47 ps to 3.9 μ s after photoexcitation of the Pb state, in H₂O based buffer solution **A.** Contour plot of the raw data; **B.** and **C.** Difference spectra at selected time points; **D.** Kinetic traces at selected wavelengths (indicated by colored bars on panels B and C).



Figure S4. Time-resolved changes in the visible region from 0.30 ps to 3.6 μ s after photoexcitation of the Pb state, in D₂O based buffer solution **A.** Contour plot of the raw data; **B** and **C.** Difference spectra at selected time points; **D.** Kinetic traces at selected wavelengths (indicated by colored bars on panels B and C).



Figure S5. Time-resolved changes in the visible region after photoexcitation of the Pb state, in H_2O based buffer solution. **A** and **D**. Contour plots of the raw data; **B** and **E**. Difference spectra at selected time points; **C** and **F**. Kinetic traces at selected wavelengths (indicated by colored bars on panels B and E). Datasets recorded from 0.6 to 450 µs (**D** – **F**), and 0.9 to 994 ms (**A** – **C**) after photoexcitation.



Figure S6. Time-resolved changes in the infra-red region after photoexcitation of the Pb state, in D₂O based buffer solution **A.** Contour plot of the raw data; **B, C,** and **D.** Difference spectra at selected time points; **E.** Kinetic traces at selected wavelengths (indicated by colored bars on panel D).



Figure S7. Evolution Associated Difference Spectra resulting from global analysis of timeresolved changes in the visible region after photoexcitation of the Pb state. **A.** in H₂O based buffer solution from 0.47 ps to 3.9 μ s and **B.** D₂O based buffer solution from 0.30 ps to 3.6 μ s **C** – **E.** Comparison of EADS normalized to the largest negative feature in H₂O and D₂O. The feature at 570 nm in the D₂O datasets is due to an unusually high proportion of the non-reactive component in this sample (due to vagries of sample preparation/purification/storage etc.), in which the photoexcited state does not trigger reaction chemistry and protein structural changes, but merely relaxes back to the ground state within a few hundred picoseconds (see Figure S2).



Figure S8. Evolution Associated Difference Spectra (EADS) and corresponding lifetimes, with standard errors from the fitting, of the Pb to Pg conversion of TePixJ. From global analysis of visible datasets from 0.47 ps to 3.9 μ s (**A**), 0.6 to 450 μ s (**B**, see Figure S10 for more discussion of analysis), and 0.9 to 994 ms (**C**), and an infrared dataset from 1 ps to 400 μ s (**D**).



Figure S9. For the Pb to Pg conversion of TePixJ: data points and fitted curves at selected wavelengths/ wavenumbers (indicated in spectra in Figure S8) from global analysis of visible datasets from 0.47 ps to 3.9 μ s (**A**), 0.6 to 450 μ s (**B**, see Figure S10 for more discussion of analysis), and 0.9 to 994 ms (**C**), and an infrared dataset from 1 ps to 400 μ s (**D**).



Figure S10. Results of global analysis of visible datasets from 0.6 to 450 µs of the Pb to Pg conversion of TePixJ, (shown in Figure S5 D-F). EADS with corresponding lifetimes and fitting error and results of singular value decomposition of the resulting residual matrix. For the selected model of 3 components (two variable and one fixed) (**A-C**), and for the fit with one fewer variable component (**D-F**) The lack of obvious structure in the residuals of the 3-component fit, and the clear spectral and temporal feature in SV1 of the 2-component fit implies that 3 components are preferable.



Figure S11. Time-resolved changes in the visible region from 0.36 ps to 3.6 μ s after photoexcitation of the Pg state, in H₂O based buffer solution **A.** Contour plot of the raw data; **B.** and **C.** Difference spectra at selected time points; **D**. Kinetic traces at selected wavelengths (indicated by colored bars on panels B and C).



Figure S12. Time-resolved changes in the visible region from 0.33 ps to 3.6 μ s after photoexcitation of the Pg state, in D₂O based buffer solution **A.** Contour plot of the raw data; **B.** and **C.** Difference spectra at selected time points; **D**. Kinetic traces at selected wavelengths (indicated by colored bars on panels B and C).



Figure S13. Time-resolved changes in the visible region after photoexcitation of the Pg state, in H_2O based buffer solution **A** and **D**. Contour plots of the raw data; **B** and **E**. Difference spectra at selected time points; **C** and **F**. Kinetic traces at selected wavelengths (indicated by colored bars on panels B and E). Datasets recorded from 1 to 450 µs (**D** - **F**), and 0.1 to 45 ms (**A** - **C**) after photoexcitation.



Figure S14. Time-resolved changes in the infra-red region after photoexcitation of the Pg state, in D₂O based buffer solution **A.** Contour plot of the raw data; **B, C,** and **D.** Difference spectra at selected time points. **E.** Kinetic traces at selected wavelengths (indicated by colored bars on panel D).



Figure S15. Evolution Associated Difference Spectra resulting from global analysis of timeresolved changes in the visible region from 0.3ps to 3.6 μ s after photoexcitation of the Pg state. **A.** in H₂O and **B.** D₂O based buffer solution **C – G.** Comparison of EADS normalized to the largest negative feature in H₂O and D₂O.



Figure S16. Evolution Associated Difference Spectra (EADS) and corresponding lifetimes of the Pg to Pb conversion of TePixJ. From global analysis of visible datasets from 0.3 ps to 3.6 μs (**A**, **B**), 1 to 450 μs (**C**), and 0.1 to 45 ms (**D**, see Figure S18 for more discussion of analysis), and an infrared dataset from 1 ps to 400 μs (**E**,**F**).



Figure S17. For the Pg to Pb conversion of TePixJ: data points and fitted curves at selected wavelengths/ wavenumbers (indicated in spectra in Figure S16) from global analysis of visible datasets from 0.3 ps to 3.6 μ s (**A**), 1 to 450 μ s (**B**), and 0.1 to 45 ms (**C**, see Figure S18 for more discussion of analysis), and an infrared dataset from 1 ps to 400 μ s (**D**).



Figure S18. Results of global analysis of visible datasets from 0.1 to 45 ms of the Pg to Pb conversion of TePixJ, (shown in Figure S13 A-C). EADS with corresponding lifetimes and fitting error and results of singular value decomposition of the resulting residual matrix. For the selected model of 3 components (two variable and one fixed) (**A-C**), and for the fit with one fewer variable component (**D-F**) The lack of obvious structure in the residuals of the 3-component fit, and the clear spectral and temporal features in of the 2-component fit implies that 3 components are preferable.