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Femtosecond Structural Dynamics Drives the *Trans/Cis* Isomerization in Photoactive Yellow Protein

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Abstract

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M.S. prepared the proposal with input from J.J.vT., K.M., V.S., J.C.H.S., H.N.C., A.O. and P.F.; A.A., S.B., M.L., J.S.R. and J.E.K. operated the CXI instrument including the time-tool and the fs-laser; K.P., A.B., J.T., S.B., T.A.W., N.Z., O.Y. and T.D.G. analyzed the SFX data. C.D.M.H and J.J.vT. set up the FROG at the CXI instrument; G.G. and D.M. performed QM/MM calculations; J.T., J.B., D.O., P.L.X., C.G., C.K. and M.S. prepared protein and grew nano- and microcrystals; D.DeP., C.K., C.C., S.R-C., J.D.C., M.M., G. K., and U.W. provided and operated the injector system; M.F., R.F., M.S., J.T., P.F., D.O. and C.G. wrote the electronic log; M.F., M.S, J.T., J.S.R., J.J.vT. and K.M. discussed fs laser excitation; J.T., M.S, V.S, R.H, C.D.M.H. and J.J.vT. performed preliminary ultra-fast experiments on crystals; M.S. calculated and analyzed the difference maps; M.S., K.P., K.M., G.G., P.F. and J.J.vT. wrote the manuscript with improvements from all authors.

A variety of organisms have evolved mechanisms to detect and respond to light, in which the response is mediated by protein structural changes following photon absorption. The initial step is often the photo-isomerization of a conjugated chromophore. Isomerization occurs on ultrafast timescales, and is substantially influenced by the chromophore environment. Here we identify structural changes associated with the earliest steps in the *trans* to *cis* isomerization of the chromophore in photoactive yellow protein. Femtosecond, hard X-ray pulses emitted by the Linac Coherent Light Source were used to conduct time-resolved serial femtosecond crystallography on PYP microcrystals over the time range from 100 femtoseconds to 3 picoseconds to determine the structural dynamics of the photoisomerization reaction.

Trans-cis isomerization constitutes a major class of chemical reactions of critical importance to biology, where for example light-dependent isomerization of a retinal chromophore underlies vision (1). Since isomerization occurs on the femtosecond (fs) to picosecond (ps) time scale, ultrafast time-resolved methods are necessary to follow the reaction in real time. The spectral response after photon absorption reveals the dynamics of the molecules involved (2-5) but does not directly observe the associated structural changes, which have to be inferred by computational approaches (6). Until recently it has been impossible to directly determine the structure of molecules on ultrafast time scales. With the recent availability of hard X-ray pulses on the fs time scale emitted by free electron laser (FEL) sources such as the Linac Coherent Light Source (LCLS), the ultrafast fs to ps time scale has become experimentally accessible (7-11). Photochemical reactions (12) are initiated by photon absorption, which promotes electrons into the excited state. Thereafter, the nuclei experience - and the structure evolves on - the excited state potential energy surface (PES) (13, 14). The shape of the surface controls the subsequent nuclear dynamics. After returning to the ground state PES, the reaction continues and is driven thermally. Although structures of longer-lived excited state intermediates have been characterized with ~100 ps time resolution at synchrotrons (15-19), the fs structural dynamics of ultrafast photochemical reactions can only be investigated at an X-ray FEL (11). The photoactive yellow protein (PYP) is an ideal macromolecular system with which to investigate ultrafast *trans* to *cis* isomerization. Its chromophore, p-coumaric acid (pCA), can be photoexcited by absorbing a photon in the blue region of the spectrum. Upon photon absorption PYP enters a reversible photocycle involving numerous intermediates (Fig. 1A). The primary photochemical event that controls entry into the photocycle is isomerization of pCA about its C2=C3 double bond (see Fig. 1B for the pCA geometry). The pCA chromophore remains electronically excited for a few hundred fs (3, 5, 20). Excited state dynamics is thought to drive the configurational change from *trans* to *cis* (3, 21). The chromophore pocket within the PYP protein is sufficiently flexible to allow certain relatively large atomic displacements, but also imposes structural constraints that may affect the pathway and dynamics of isomerization (22, 23). In particular, the pCA chromophore is constrained by a covalent bond to the Cys69 side chain of PYP (Fig. 1B), by unusually short hydrogen bonds between its phenolate oxygen and nearby glutamate and tyrosine side chains (24), and by a hydrogen bond between the carbonyl oxygen of its tail and the main chain amide of Cys69.

Previously, we showed that time-resolved pump-probe serial femtosecond crystallography (TR-SFX) could be successfully carried out on PYP on the ns to microsecond (μs) time scales. Difference electron density (DED) maps of very high quality, which compare the structures before (dark) and after (light) absorption of a photon (25), were obtained to near-atomic (1.6 Å) resolution. These experiments used a nanosecond (ns) laser pulse to initiate isomerization and subsequent structural changes. An overall reaction yield as high as 40% (25) could be reached. However, achieving fs time resolution requires that a fs pump laser pulse be used, which restricts the reaction yield to the much lower value of the primary quantum yield (around 10%) and correspondingly reduces the structural signal. The energy of fs pulses i.e. the number of photons per pulse must also be limited to avoid damaging effects from their significantly higher peak power. Here, we present results of TR-SFX experiments covering the time range from 100 fs to 3 ps. We directly follow the *trans-cis* isomerization of the pCA chromophore and the concomitant structural changes in its protein environment in real time. Full details of the experiment and data analysis are provided in the Supplementary Materials (SM). Light-initiated structural changes in PYP were investigated at the Coherent X-ray Imaging (CXI) instrument of the LCLS (26). Electronic excitation was initiated in microcrystals of PYP by fs pump laser pulses (λ=450 nm). Permanent bleaching of the chromophore was avoided by limiting the laser pulse energy to 0.8 mJ/mm^2 (5.7 GW/mm^2). Laser pulse duration, spectral distribution and phase were characterized by ‘Second Harmonic Generation Frequency Resolved Optical Gating’ (SHG-FROG) (27). The pulse duration was 140±5 fs and had both positive group delay dispersion and third order dispersion to maximize the conversion to the excited state (28). Offline spectroscopic experiments on thin crushed crystals of PYP had established that photoexcitation with fs laser pulses under comparable conditions could be as high as 10% without inducing damage (SM). The structural changes induced by the laser pulse were probed with 40 fs X-ray FEL pulses at 9 keV (1.36 Å). Both the pump-probe and the reference X-ray diffraction data were collected at the full 120 Hz pulse repetition rate of the LCLS to a resolution of 1.6 Å and 1.5 Å, respectively. To address concerns that the detector response might be influenced by the stray light of the intense fs laser pulse, the reference data were collected as a negative time delay, where the fs laser pulse arrived 1 ps after the X-ray pulse.

To assess whether fs laser pulses excited a sufficiently large number of molecules under these experimental conditions, we first performed a positive control experiment with a 200 ns pump-probe time delay, where large structural differences between the light and dark states have been well characterized (25, 29). From the pump-probe TR-SFX data and the reference data, DED maps were calculated (SM). Fig. 1C shows that the fs laser pulses are able to initiate sufficient entry into the photocycle to produce strong, chemically meaningful features. The 200 ns DED map is essentially identical to maps determined earlier at both the LCLS (25) and at BioCARS (29) at a time delay of 1 µs, and can be interpreted with the same mixture of intermediates, pR_1 and pR_2. The extent of reaction initiation is 12.6 % as determined by fitting a calculated ‘pR_1&pR_2 minus pG’ difference map to the 200 ns DED map, a value which agrees with the maximum extent of excitation determined spectroscopically (7 – 10%). The fs time scale was explored by using nominal settings for the time delay of 300 fs and 600 fs. The timing jitter between the 140 fs laser pump and 40 fs X-ray probe pulses is ~280 fs (8). The jitter was measured for every X-ray pulse by a timing tool (30, 31), which was combined with adjustments that take longer-term experimental drift into account (see SM). Thus, each individual diffraction pattern was associated with a definite “time stamp”. However, due to the drift, the time stamps were non-uniformly distributed in time (Fig. S1). Since the quality of structure amplitudes and of the DED maps derived from them depends on the number of diffraction patterns, indexed, time-stamped diffraction patterns were binned into 8 different pump-probe delays with about the same number of patterns (40,000) in each bin , spanning the time range from 100 to 1000 fs (Tab. S1B). A set of diffraction patterns at a time delay of 3 ps was also collected. Since the jitter and drift are much smaller than the delay, time stamping was not necessary for the 3 ps or the 200 ns delays. The values of R-split for all datasets is 7.5 – 9.9% which indicates the high quality of the diffraction data, and results in DED maps of comparable, good quality for all delays. Maps at 7 time delays are shown in Fig. 2. Visual inspection of these maps reveals an important qualitative result. The features in all maps at delays less than 500 fs are similar (compare Fig. 2, A-C); and features in all maps at delays greater than 700 fs are also similar (compare Fig. 2, D-G), but differ from those in the first set. Consequently, there must be a structural transition between the 455 fs and 799 fs time delays that gives rise to the two distinct sets of features. To identify with more precision the time delay at which this transition occurs, the time-stamped diffraction patterns were re-binned into 16 narrower time bins with about 20,000 patterns in each bin (Tab. S1A). The resultant time series of 16 DED maps in the fs time range (together with the map for the 3 ps time delay) were subjected to singular value decomposition (SVD; Fig. S2B) (32). The volume occupied by the pCA chromophore, by the Cys69 sulfur and the Glu46 carboxyl was included in the analysis. When a time series exhibits a change, a corresponding change should be even more readily recognizable in the right singular vectors (rSVs). This change is evident in the magnitude of both the first and second rSVs around 550 fs (red arrow in Fig. S2B). The substantial increase in the magnitude of the first right singular vector after 155 fs (Fig. S2B) shows the earliest (fastest) evolution of the structure after excitation. We tentatively associate the structural transition at around 550 fs, qualitatively evident by inspection of the DED maps and more quantitatively in their SVD analysis, with the *trans* to *cis* isomerization of the pCA chromophore. The transition occurs within ~180 fs (Fig. S2B), but its exact duration needs to be further established. Rate kinetics would require that after a ~500 fs dwell time the transition time would be stretched beyond the bandwidth limited rate. Yet the observed transition time matches the experimental bandwidth of 3.15 THz. Therefore the ensemble phase relation imparted by the optical pulse appears to be maintained for the duration of the dwell time, which may be supported by coherent motion. Although no oscillatory motion was detected in the TR-SFX data (they may be masked by the non-uniform data sampling), the time delay is however within the vibrational dephasing time of the PYP S_1 state (3) and ground state modes in proteins (33). We further propose that at ~550 fs the system lies at or very close to a conical intersection (20) (Fig. S8), a branch point from which molecules either continue towards the *cis* configuration and enter the photocycle, or revert to the *trans* configuration and return to the resting (dark) state.

To identify the isomerization, refined structures before and after the transition are required. Initially, date in bins with 40,000 indexed diffraction patterns each were used, and preliminary PYP structures refined against these data. Refinement details are in the SM. The 3 bins with the shortest delays can be interpreted with chromophores in a twisted *trans* configuration (Fig. 2 A-C). After 700 fs the configuration is near *cis* (Fig. 2 D-E). The timecourse of the refined ϕ_{tail} torsional angles can be fit with a transition time identical to that observed in the second rSV (Fig. 3). We took advantage of the similarity of the DED maps for extended time ranges before and after the transition to further increase the accuracy of the refined structures. We combined the diffraction patterns into two bins: the fast time scale (100-400 fs with 81,237 patterns) and a slower time scale (800-1200 fs with 157,082 patterns) (Tab. S1C). We refined the structure denoted PYP_{fast} against the 100-400 fs data, and that denoted PYP_{slow} against the 800-1200 fs data. The refinement statistics are presented in Tab. S2. The DED maps are shown in Fig. 3, inserts (see also Fig. S9B,D), with the corresponding, refined structures of PYP_{fast} and PYP_{slow} in pink and light green, respectively. The 3ps DED map and the refined PYP_{3ps} structure are shown in Fig. 2G. We used as many diffraction patterns as possible to refine PYP_{slow} (Fig. S12 B,D) and PYP_{3ps} because at the transition, roughly 30% of the excited molecules return directly to the dark state, no longer contribute to the DED maps and reduce the signal. We emphasize that refinement of transient structures populated on an ultrafast timescale is challenging, since these structures are very far from equilibrium and likely to be highly strained. Restraints in standard libraries are derived from structures at equilibrium and are therefore not applicable. In order to provide restraints more appropriate for this refinement, we employed excited state quantum mechanics/molecular mechanics (QM/MM) calculations on PYP (20, 34) (SM). In addition, we employed an iterative procedure, in which improved difference phases $\phi^{\Delta F,calc}$ were obtained and used with observed difference structure factor amplitudes during refinement (SM). The structural results of the refinement are summarized in Tab. 1. For the shortest time delays (up to about 450 fs), the PYP chromophore tail adopts a highly strained, twisted *trans* configuration, in which the C_1-C_2=C_3-$C_{1'}$ torsional angle ϕ_{tail} (shown by the red line spanning these four atoms in Fig. 1B) is ~140°. The position of the C_2=C_3 double bond in PYP_{fast} is displaced by ~1Å behind the chromophore plane (loosely defined by the Cys69 sulfur, the tail carbonyl oxygen and the atoms of the phenyl ring; Fig. 2A-C). Hydrogen bonds to Glu46 and Tyr42, which are unusually short in the reference (dark) structure *(*24*),* are substantially elongated from 2.5 Å to 3.4 Å (Tab. 1). This structure is primed for the transition to *cis*. During the structural transition, substantial rotation about the double bond takes place. The head of the chromophore pivots about tail atom C_2 and thereby aligns the C_2=C_3 bond along the tail axis. Simultaneously, the head rotates about the C_3-$C_{1'}$ single bond. (The complex motions can be effectively illustrated by using an educator's stick model set, see Fig. S3). The phenolate oxygen (Fig. 1B, $O_{4'}$) moves even further away (3.6 Å, Tab. 1) from Glu46 (Fig. 2D-F and Fig. S9C-D), thereby breaking the hydrogen bond. At time delays longer than about 700 fs, ϕ_{tail} has decreased to ~50° (PYP_{slow}, Fig. 3), which is characteristic of a *cis* configuration. PYP_{slow} relaxes further towards the 3 ps structure (PYP_{3ps}), in which the hydroxyl oxygen of the head re-establishes its hydrogen bond with Glu46 (Fig. 2G). ϕ_{tail} changes slightly to ~35°. The PYP_{3ps} structure is already very similar to the early structures derived with 100 ps time resolution by independent, synchrotron-based approaches (Tab. 1; PDB entries 4I38 and 4B90) (22, 23), and has evolved only slightly from PYP_{slow} by establishing shorter hydrogen bonds to Tyr42 and Glu46.

The structures derived from the refinements confirm that the transition at around 550 fs is indeed associated with a *trans* to *cis* isomerization. Theoretical considerations (20) (Fig. S8) suggest that during isomerization the PYP chromophore relaxes through a conical intersection between the electronically excited state PES and the ground state PES. Accordingly, structures between 100 and 400 fs can be identified as electronically excited, whereas the structures at time delays > 700 fs can be identified with the electronic ground state. In both the excited and ground states, structural changes i.e. translation of atoms may also have occurred. Our experiments identify the ultrafast dynamics of both the excited state structures and the ground state structures (Figs. 2-3). Since we restricted our pump laser pulses to moderate power, we avoid damaging nonlinear effects (e.g. two photon absorption) and most excited molecules populate the excited state surface S_1 (5). Part of the stored energy is used to rapidly displace the chromophore by about 0.7 Å within the crowded molecular environment in the interior of PYP (Fig. 2A, Tab. 1). If this initial displacement is complete after 250fs the chromophore must have experienced an acceleration of ~2×10^{15} m/s^2 and attains a final velocity of 500 m/s (SM). Fig. 1B shows that 9 carbon atoms, two oxygens and 7 hydrogen atoms (molecular mass = 147 g/mol) are displaced. During the first few hundred fs the force on the chromophore is ~500 pN which is enormous compared to forces in single molecules at thermal equilibrium which are usually only a few pN (35). The origin of the force is due to the change of the potential energy surface when the chromophore is excited to the electronic excited state which affects the intra and intermolecular interactions of the chromophore as also inferred from ultrafast Raman spectroscopy(3). The energy required to displace the chromophore is ~0.2 eV which is ~10% of the blue photon energy (2.76 eV) that starts the reaction. It appears that by rapidly evolving down the excited state PES, part of the photon energy is initially converted into kinetic energy which is then released by collision of the chromophore atoms with the surrounding protein atoms comprising the chromophore pocket. The excited chromophore loses 0.12 eV energy by intramolecular vibrational energy redistribution on the sub-100 fs time scale (39) which can be roughly estimated from the Stokes shift by comparing absorption and fluorescence spectra(3). Accordingly,~85% of the photon energy remains stored as strain and electronic excitation in the chromophore before isomerization occurs. On passing through the conical intersection (20), the molecules either revert towards the initial dark state (30% of the excited molecules, Tab. 1, see also Tab. S3) or continue relaxing towards the *cis* isomer (70%), gradually releasing the excess energy as heat. Because the chromophore pocket tightly restricts the chromophore head displacements, further structural changes must be volume-conserving i.e. they minimize the volume swept out by the atoms as they move. Accordingly, the chromophore performs the complex motions described above (Fig. S3). Although the energy stored in the chromophore is sufficient to break the hydrogen bonds (~0.1 eV), the spatial constraints imposed by the chromophore pocket direct the reformation of the hydrogen bonding network at longer time delays (Tab. 1). This is a 'macromolecular cage effect' reminiscent of the 'solvent cage effect' in liquid chemical dynamics (36). The 'macromolecular cage' in PYP, however, is soft enough to allow certain specific, relatively large (up to 1.3 Å, Tab. 1) structural changes. This contrasts with crystals of small molecules, where the stronger crystal lattice constraints usually do not allow such large displacements. Hence, biological macromolecular crystallography aimed at elucidating biological function may also provide insight into the reaction mechanisms of small molecules.

To assess global conformational changes of PYP on the fs time scale, we calculated the radius of gyration R_g from each refined structure (SM). R_g fluctuates by only 0.2% in all structures from 200 fs to 200 ns (Tab. 1). An increase of R_g by up to 1 Å determined by others using X-ray scattering in solution upon photo-dissociation of CO from CO-myoglobin in solution (9) is not observed in our PYP crystals. Concomitant, systematic large volume changes are also not apparent in PYP crystals over the first 3 ps that our data span. Our data show no evidence for a protein quake (9, 10, 37), characterized by an ultrafast and large change in R_g that occurs significantly before a large volume change. The reason for this is unclear and will require further experiments.

Ultrafast fluorescence and transient absorption spectroscopy of PYP has shown that excited state decay is multi-phasic (3, 5, 38). The fast (sub-ps) time constants are significantly more productive in creating the *cis*-like photoproduct than the slow (ps) time constants; the long-lived excited state population primarily decays back to the ground state (5, 39). With excitation at 450 nm, at least 50% of the total isomerization yield is generated with a dominant ~600 fs time constant (5), which agrees with our observation of a transition at ~550 fs. It should be noted that a 'ground state intermediate' with a 3-6 ps life time has been proposed by ultrafast spectroscopy (39). However under the conditions employed here, the peak concentration of this intermediate is expected to be small (5). In contrast to spectroscopic techniques that reported vibrational coherence with 50 cm^{-1} and 150 cm^{-1} frequency (3, 40), we could not unambiguously detect oscillations in our data (see above). Intense femtosecond optical pumping of PYP crystals generates both excited state and ground state vibrational coherences within the 3.15 THz experimental bandwidth(41). It will be an important goal of future experiments to structurally characterize these coherences using fs TR-SFX. Nevertheless, our data show that before 400 fs there are large distortions corresponding to a Franck-Condon (FC) excited state (42). The nuclear dynamics of the FC excited state at 100-200 fs agrees with the conclusions from ultra-fast spectroscopy (3, 42-45) that also suggest a distortion of the C_2=C_3 double bond on similar timescales, as in the PYP_{fast} structure. The isomerization at 550 fs through the conical intersection between the excited state and ground state PES is in reasonable agreement with the timescales for isomerization reported by others (3, 5, 42, 46). After passing through the conical intersection, the chromophore is *cis*-like and still highly strained. The transiently-broken hydrogen bond is reestablished quickly as the structure relaxes, exemplified by the PYP_{3ps} structure (Fig. 3). Further relaxation on the ground state PES completes the initial phase of the isomerization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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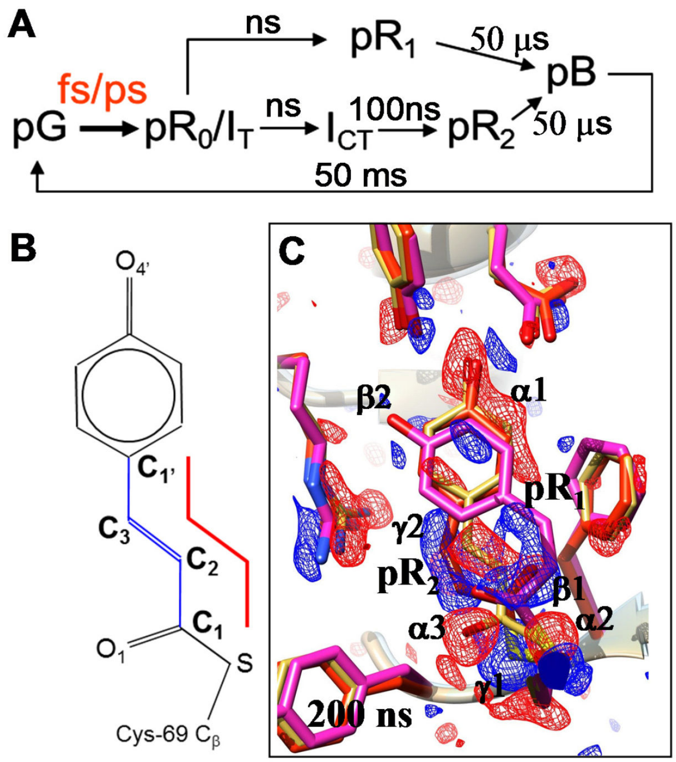

Figure 1.

A. The PYP photocycle from the perspective of a time-resolved crystallographer. Approximate time scales are given. The fs/ps time scale (in red) is structurally charted in this paper. B. The chemical structure of the pCA chromophore. The red line marks the four atoms that define the torsional angle ϕ_{tail} about the C2=C3 bond. C. Results of the positive control experiment at a 200 ns time delay. Reaction initiated by fs laser pulses. Negative (red) and positive (blue) DED features on the $-3\sigma/3\sigma$ level. A mixture of the pR_1 (magenta) and pR_2 (red) structures is present. Main signature of pR1: features β1 and β2. Main signature for pR_2: features γ1 and γ2. Structure of PYP_{ref} (dark) in

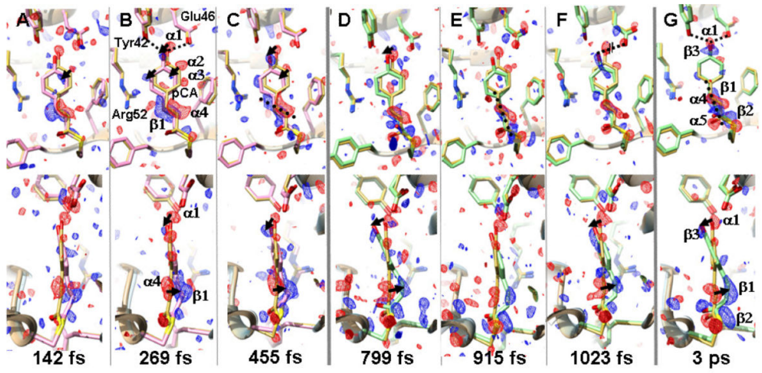

Figure 2.

Trans to *Cis* isomerization in PYP. Weighted DED maps in red (−3 σ) and blue (3σ); front (upper) and side view (lower). Each map is prepared from about the same number of diffraction patterns, except the 3 ps map (see Tab. S1 B-C). The reference, dark structure is shown in yellow throughout; structures before the transition and still on the electronic excited state PES are shown in pink; structures after the transition and on the electronic ground state PES are shown in light green. Important negative difference density features are denoted α, positive features as β in panels B and G. Pronounced structural changes are marked by arrows. A-C: time-delays before the transition. A. Twisted *trans* at 142 fs, ϕ_{tail} 154°. B. Twisted *trans* at 269 fs, ϕ_{tail} 140° some important residues are marked; dotted lines in B: hydrogen bond of the ring hydroxyl to Glu46 and Tyr42. C. Twisted *trans* at 455 fs, ϕ_{tail} 144°; dotted line in C: direction of C_2=C_3 double bond. D-G: time delays and chromophore configuration after the transition. D. Early *cis* at 799 fs, ϕ_{tail} 50°. E. Early *cis* at 915 fs; dotted line in E: direction of C_2=C_3 double bond. F. Early *cis* at 1023 fs; for E and F ϕ_{tail} ~65°. G. 3 ps delay; dashed line: direction of C_2=C_3 double bond, feature β1; ϕ_{tail} is 35°.

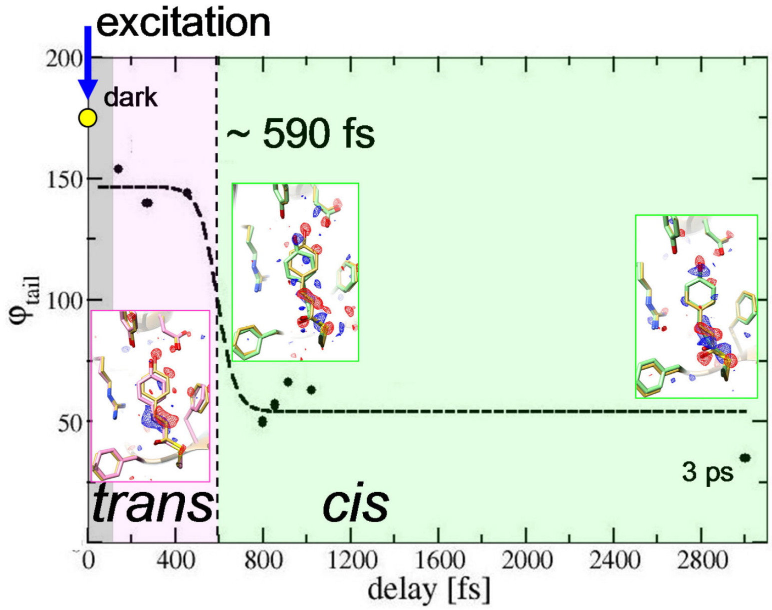

Figure 3.

Trans to *cis* isomerization in PYP. Pink: twisted *trans* on excited state PES; light green: *cis* on ground state PES. Torsional angle ϕ_{tail} (solid spheres) from structural refinement at various delays (see also Tab. S3). Gray region: not time-resolved. Dashed line: fit with eqn. S2, with a transition time of about 590 fs (see also Fig. S2). Inserts: structures of PYP_{fast} (pink), PYP_{slow} and PYP_{3ps} (light green), and dark state structure PYP_{ref} in yellow. Difference electron density in red (−3σ) and blue (3σ).

Table 1

Geometry of PYP structures. The PYP_{fast} structure was refined using a data bin spanning 100-400 fs with 81327 snapshots, and the PYP_{slow} structure from a bin spanning 800 – 1200 fs with 157082 snapshots (Tab. S1b). Structures of I_T, pR_0 and pB_1 from Protein Data Bank, code listed in brackets (22, 23, 47). Uncertainties of the torsional angles can be estimated to be +/− 20° by displacing the 4 atoms that define the angle with the coordinate error (0.2 Å).

	PYP_{ref} (dark)	**PYP_{fast}**	**PYP_{slow}**	**PYP_{3ps}**	**PYP_{200ns} (fs-laser) pR1/pR2**	**I_T (4I38)**	**pR0 (4B90)**	**pB_1 (1TS0)**
Time Delay	**0**	**100-400 fs**	**800 - 1200 fs**	**3 ps**	**200 ns**	**100 ps**	**100 ps**	**ms**
	Torsional Angles [°]							
C1-C2=C3-C1’ (ϕ_{tail})	172	136	53	35	3/−8	90	33	−27
O1-C1-C2=C3	−15	−21	28	30	12/−6	11	29	−10
CB-S-C1-C2	−185	−171	−164	−137	163/−165	−136	−123	180
	Hydrogen bonds [Å]							
pCA-$O_{4'}$ - Glu46-O_e	2.50	3.40	3.60	2.94	4.97/2.88	2.73	2.73	8.03
pCA-$O_{4'}$ - Tyr42-O_η	2.54	2.92	2.63	2.88	2.97/2.66	2.57	2.59	5.19
pCA-O_1 – Cys69-N	2.77	3.11	2.50	3.12	3.37/4.29	3.04	3.05	2.88
	others							
<pCA>[a] [Å]	0	0.66	0.78	0.60	1.55/0.81	0.67	0.68	2.39
<global>[b] [Å]	0	0.20	0.19	0.24	0.13	0.13	0.19	0.17
Radius of gyration [c] [Å]	13.32	13.33	13.30	13.34	13.29	- nd -	- nd -	- nd-
Volume [$Å^3$]	17831	17856	17833	17838	17672	17830	17683	17807
ΔV to dark [$Å^3$]	0	25	2	7	−159	−1	−148	−24
Photoactivation Yield [%][d]	- na -	15.2	9.6	10.1	12.5[c]	(5%)[e]	(10%)[e]	(10%)[e]

[a]Mean displacement of equivalent chromophore atoms relative to dark (SM).

[b]Mean displacement of equivalent cα atoms relative to dark (SM).

[c]See SM for the calculation.

[d]Determined by by fitting calculated DED maps to the experimental DED maps in the chromophore region.

[e]Estimate