Supporting Methods

Molecular Dynamics (MD) Simulations. All simulations were carried out with the GROMACS 3.2.1 simulation suite (1). The simulations were started from the 1.3-Å crystal structure of wt asFP595 described in this article (PDB code 2A50). Protonation states of the standard amino acids were calculated by using the program WHATIF (2) and its interface to DELPHI (3). Because of its close proximity to MYG, the protonation state of H197 is crucial and was predicted to be cationic. To discriminate between the possible chromophore protonation states, simulations of the neutral state A (imidazolinone-N, Tyr-OH), the anionic state B (imidazolinone-N, Tyr-O^-), and the zwitterionic state C (imidazolinone-NH^+, Tyr-O^-) were performed, and the protein–chromophore interactions were monitored. A and B both showed disintegration of the chromophore-protein hydrogen bond network, with ruptures of the M63-C62, Y64-E215, and Y64-S158 hydrogen bonds within <100 ps. The only stable H-bond for A and B was the one to R92. In sharp contrast, all these four hydrogen bonds stayed intact in case of the zwitterionic protonation state C, which is also reflected by a low deviation from the wild-type crystal structure (rms deviation = 1.0 Å). These findings pinpoint the zwitterion to be the correct chromophore protonation state in the asFP595 "off" state. Thus, all simulations were carried out with this state.

The OPLS force field (4) was applied. A monomer of the protein was solvated in a 8.9 × 7.9 × 7.9 nm^3 box of TIP4P water molecules. Twenty-five sodium and 28 chloride ions were added to the simulation system to compensate for the overall positive charge of the protein and to mimic physiological conditions, yielding a total system size of 71,734 atoms. Simulations were carried out with periodic boundary conditions. Application of the Lincs (5) and Settle (6) methods allowed for an integration time step of 2 fs. Electrostatic and Lennard–Jones interactions were calculated within a cutoff of 1 nm, and the neighbor list was updated every 10 steps. For the long-range electrostatic interactions, the Particle-Mesh-Ewald (PME) method (7) was used. An N, p, T ensemble was simulated, with separate coupling of the protein, solvent, and ions to a 300 K heat bath (τ = 0.1) (8). The system was isotropically coupled to a 1-bar pressure bath (τ = 1.0) (8).

Initially, the system was energy minimized (steepest descent, 1,000 steps) before equilibrating the solvent for 200 ps with positional restraints on protein heavy atoms. Then, the whole system was equilibrated (0.1 ns at 300 K). As expected, within this timescale the chromophore stayed in its trans conformation.

In subsequent simulations, trans–cis isomerization of the chromophore was induced by means of force probe MD simulations (9). For this purpose, the free energy perturbation algorithm of the GROMACS simulation package was used. Simulation details were as described above, apart from the calculation of nonbonded interactions. Here, no PME, but a cutoff of 1.5 nm and 1.2 nm for electrostatical and Lennard–Jones interactions, respectively, was used. The R mechanism along the top or bottom path was enforced by shifting the minimum of the τ dihedral potential by 180° backward or forward, respectively. The minima of both dihedral potentials τ and φ were shifted simultaneously in equal measure to enforce the HT mechanism. To induce the isomerization at a 20 ps timescale (see below), a Ryckaert-Bellemans constant of 140 kJ/mol for τ (R mechanism) was found necessary. To yield forces along the isomerization pathway that can be compared with each other, we required from geometric considerations that

$$\frac{1}{\sqrt{2}}\left(\frac{dE_{HT}(\tau)}{d\tau}+\frac{dE_{HT}(\varphi)}{d\varphi}\right)=\frac{dE_{R}(\tau)}{d\tau},$$

with E the respective dihedral potential. Accordingly, an RB constant of $140/\sqrt{2}=100$ kJ/mol for τ and φ (HT mechanism) were applied. Dihedral potentials with the same central atoms as the perturbed ones were set to zero. Partial charges calculated by using a configuarion interaction singles (CIS) wavefunction (see below) were used to mimic the excited trans state and were smoothly converted into their cis ground state values during the simulations. This prohibited the usage of PME (see above). The force was recorded at each integration step. The values shown in Fig. 5 were calculated with respect to a semicircular coordinate with a radius of 0.5 nm, described by the idealized rotation of the center of the chromophore phenyl moiety upon isomerization. For the corresponding

simulations in water, the chromophore model obtained from the quantum mechanical calculations (see Fig. 5*b Inset*) was used, with the full M63 residue included.

Because the asFP595 off state is nonfluorescent, photoisomerization has to be significantly faster than fluorescence and, therefore, is an intrinsically nonequilibrium process. Because typical fluorescence lifetimes are in the order of nanoseconds, we conducted the force probe MD simulations within a significantly faster timescale (20 ps). This timescale is comparable with the isomerization timescale of related chromophores, e.g., in the photoactive yellow protein (10). Isomerization timescales were controlled by the dihedral force constants. Additional simulations on a longer timescale (200 ps) yielded similar mechanistic and energetic results (data not shown). After the isomerization, the system was further equilibrated for 1.0 ns with the chromophore in its cis conformation.

To monitor spontaneous isomerization starting from a trans conformation in the excited state, free MD simulations have been carried out. The applied simulation protocol was similar to the one used for equilibration (see above), apart from the chromophore charges and dihedral potentials. To mimic the excited state, CIS charges were applied. The dihedral potentials for τ and φ were taken from CASPT2/CASSCF calculations on the GFP chromophore (11), with energy barriers of 8.4 kJ/mol and 0 kJ/mol, respectively (Fig. 10). This semiquantitative description models the transition from an excited-state potential through a conical intersection back to the ground state (12). Because MYG and the GFP chromophore are not identical (in particular their protonation patterns differ), their S1 surfaces cannot be assumed to be identical. To elucidate their effect on the mechanism, simulations with varying barrier heights were therefore performed. No change in the mechanism, but only in the isomerization timescale, was found. For barrier heights significantly exceeding the kinetic energy of the system, no isomerization was seen. Additional kinetic energy (21 kJ/mol) of the chromophore due to relaxation from the Franck-Condon region on the S1 surface was accounted for by increasing the MYG atomic velocities correspondingly (11).

The protonation state with an anionic chromophore and a protonated E215, i.e., the same hydrogen bond network as the zwitterionic state C, did yield highly similar spontaneous HT^{bot} isomerization trajectories.

Chromophore Force Field. Quantum mechanical calculations were carried out with the GAUSSIAN03 program (13). In the model chromophore used for these gas-phase calculations, the M63 side chain and G65 were replaced by methyl groups, respectively. The chromophore was considered to be in its zwitterionic form (see above). The atomic partial charges for the ground state (optimized at the B3LYP/6-31+G* level) were estimated by fitting to the molecular electrostatic potential of the chromophore *in vacuo* according to the CHELPG scheme (14), both for the cis and trans conformations of the chromophore. In a similar manner, the excited-state partial charges were obtained from a configuration interaction calculation (CIS). Heavy-atom bond lengths and angles were taken from the x-ray structure, those involving hydrogen atoms from the QM-optimized structures. For the force constants and Lennard-Jones parameters, OPLS force field parameters of residues with similar chemical nature were adopted (H, Y, M, styrene), and are summarized in Table 2. Except for partial charges, the same parameters were used for the trans and cis state.

1. Lindahl, E., Hess, B. & van der Spoel, D. (2001) *J. Mol. Model.* **7,** 306–317.

2. Vriend, G. (1990) *J. Mol. Graphics.* **8,** 52–56.

3. Klapper, I., Hagstrom, R., Fine, R., Sharp, K. & Honig, B. (1986) *Proteins* **1,** 47–59.

4. Jorgensen, W. L. & Tirado-Rives, J. (1988) *J. Am. Chem. Soc.* **110,** 1657–1666.

5. Hess, B., Bekker, H., Berendsen, H. J. C. & Fraaije, J. G. E. M. (1997) *J. Comput. Chem.* **18,** 1463–1472.

6. Miyamoto, S. & Kollman, P. A. (1992) *J. Comput. Chem.* **13,** 952–962.

7. Darden, T., York, D. & Pedersen, L. (1993) *J. Chem. Phys.* **98,** 10089–10092.

8. Berendsen, H .J. C., Postma, J. P. M., van Gunsteren, W. F., Dinola, A. & Haak, J .R. (1984) *J. Chem. Phys.* **81,** 3684–3690.

9. Grubmüller, H., Heymann, B. & Tavan, P. (1996) *Science* **271,** 997–999.

10. Groenhof, G., Bouxin-Cademartory, M., Hess, B., De Visser, S. P., Berendsen, H. J., Olivucci, M., Mark, A. E. & Robb, M. A. (2004) *J. Am. Chem. Soc.* **126,** 4228–4233.

11. Martin, M. E., Negri, F. & Olivucci, M. (2004) *J. Am. Chem. Soc.* **126,** 5452–5464.

12. Saam, J., Tajkhorshid, E., Hayashi, S. & Schulten, K. (2002) *Biophys. J.* **83,** 3097–3112.

13. Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Montgomery, J. A., Jr., Vreven, T., Kudin, K. N., Burant, J. C., *et al.* (2003) *Gaussian 03 Software Suite* (Gaussian, Pittsburgh).

14. Breneman, C. M. & Wiberg, K. B. (1990) *J. Comput. Chem.* **11,** 361–373.