Supporting Information

Palczewska et al. 10.1073/pnas.1410162111

SI Methods

Bleaching of Rhodopsin Crystals. Trigonal crystals of ground-state bovine rhodopsin were grown as previously described (1, 2). A single crystal was transferred to an ~2-μL drop of paraffin oil located in a glass-bottomed 35-mm dish (MatTek Corp.). The crystal was placed on the 2PO microscope stage, and about half of it was irradiated with a 1,000-nm laser beam (15 mW, 20-μm slices, 20 frames, two line averages, 2.3 ms/line). The crystal was placed under a bright-field microscope and immediately photographed (to reveal bleaching of the section irradiated with the 1,000-nm laser), and then the whole crystal was exposed to intense white light for 2 min (to bleach the entire crystal).

Bleaching of Rhodopsin. ROS (2, 3) were solubilized with lauryl maltoside neopentyl glycol/cholesteryl hemisuccinate (LMNG/CHS) to achieve a final concentration of ~100 μM rhodopsin in 5 mM LMNG, 0.5 mM CHS, 25 mM Tris/HCl, pH 7.4, 140 mM NaCl, and 3 mM KCl. The sample was centrifuged for 15 min at 15,000 × g to remove unsolubilized material. Next, 3 μL of sample were pipetted into a 1.1-mm-diameter capillary tube, and the sample was bleached with the 2PO microscope 1,000-nm, 75-fs laser (40 mW, 20-μm slices, 20 frames, two line averages, 2.3 ms/line). After bleaching, the sample was diluted with 1.2 mL of buffer (50 mM Hepes, pH 7.4, containing 0.15 M NaCl, 0.25 mM LMNG, and 25 μM CHS) to a final rhodopsin concentration of ~0.2 μM, and its absorbance spectrum was measured. Then 11-cis-retinal in DMSO stock solution was added to a final concentration of ~0.3 μM at room temperature, and the spectrum was measured again. Finally, the sample was incubated overnight on ice, and the absorbance spectrum was measured once more. All procedures were carried out under dim red light illumination.

Bleaching of Green and Blue Cone Opsins. A human green cone opsin construct with WT-like spectral properties was engineered and expressed in insect sf9 cells in a manner similar to the β2-adrenergic receptor-T4 lysozyme (T4L) chimera (4). The only purification tag carried by this construct was the C-terminal se-

**Fig. S1.** 2PO excited emission spectrum from fresh WT mouse retina. (A) 2PO images of mouse photoreceptors with excitation wavelengths indicated in each image. (B) Emission spectra from photoreceptors: excitation wavelengths were 1,000 (black) and 730 nm (red). Dashed green line indicates the position of the expected SHG signal (not present) after 1,000-nm excitation.

**Fig. S2.** Bleaching of detergent-solubilized bovine rhodopsin with white light. The spectrum of unbleached rhodopsin is shown in black, and the spectrum of rhodopsin after bleaching with white light is shown in red. The indigo arrow indicates the loss in 500-nm absorbance.
Fig. S3. Bleaching of cone pigments. (A) Spectra from green cone pigment. The unbleached control is shown in green; bleached with 1,000-nm light in red; bleached with 1,000-nm light immediately after adding 9-cis-retinal in dotted gray; and after overnight incubation with 9-cis-retinal in solid gray. The control bleached with white light is shown in black. The indigo arrow indicates the loss of absorbance at 500 nm after exposure to 1,000-nm light. The hypsochromic shift of about 30–40 nm is caused by the use of 9-cis-retinal instead of 11-cis-retinal. (B) Spectra from blue cone pigment. The unbleached control is shown in blue; bleached with 850-nm light in red; and control bleached with white light in black.

Fig. S4. Normalized 2PO excited emission spectra of a bleached rhodopsin crystal. Excitation wavelengths and their color coding are indicated in the plot.
Fig. S5. Products of full length 11-cis-retinal-propylamine Schiff base photoisomerization. (A) HPLC chromatogram of products after exposure to white light. (B) HPLC chromatogram of unbleached control sample. (C) HPLC chromatogram of products after exposure to 880-nm femtosecond laser light. Indicated isomers in each chromatogram are as follows: 11-cis, 11-cis-retinal oxime; 13-cis, 13-cis-retinal oxime; 9-cis, 9-cis-retinal oxime; all-trans, all-trans-retinal oxime. Total amounts of retinoids in each sample were comparable indicating that degradation or loss of retinoids during the experiment were negligible. Additionally, no obvious products of retinoid oxidation were observed. However, spontaneous isomerization of the 11-cis-retinylidene Schiff base in the dark was observed even at pH 13, and its isomerization pattern was similar to that of 11-cis-retinal incubated with trace amounts of acetic acid (1). This outcome suggests that the Schiff base facilitated the protonation of retinoids, which in turn, catalyzed their isomerization (2).

Fig. S6. Structural model of rhodopsin used for QM/MM simulations. A cartoon of the protein structure (PDB ID code 1F88) (1) is shown. The chromophore region, treated at the QM level in these calculations, is represented by orange spheres. The embedding POPE bilayer is represented with thin lines. To preserve clarity, solvating water molecules are omitted.