Supplemental Data

The magnitude of the light-induced conformational change in different rhodopsins correlates with their ability to activate G proteins.

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Supplemental Text

TrIQ-bimane studies using mBBr support helical movement model shown in Fig. 5.

The movement proposed in Fig. 5 predicts that position 250 moves nearer to position 226 during activation. We attempted to test this proposal by carrying out Trp-bimane quenching experiments as were done for the bimane at position 251. Unfortunately, parapinopsin mutant 250C was not efficiently labeled with PDT-bimane. Thus, we labeled position 250 using mBBr, and carried out similar Trp-bimane quenching studies on bovine rhodopsin and parapinopsin $250B_1$ mutants with/without the Trp-226 and Trp-227 residues. The results are in agreement that for bovine rhodopsin, photoactivation moves the bimane label at position 250 closer to Trp-226 (supplemental Fig. S3, A and B), consistent with the model proposed in Fig. 5. In contrast, such a large displacement of residues at positions 226 and 250 was not detected in parapinopsin, since the change of the fluorescence intensity upon photoactivation of parapinopsin $250B_1$ mutant was not affected by the presence of Trp-226 (supplemental Fig. S3, D and E).

When analyzing these studies, it is necessary to account for the possibility of efficient energy transfer of bimane fluorescence to the retinal chromophore. The more spectral overlap that exists between the retinal absorbance and the bimane emission, the greater will be the amount of the energy transfer, which will result in lower observed fluorescence. Thus, one sees increase in fluorescence intensity for bimane labeled bovine rhodopsin mutants upon photoactivation, due to reduced energy transfer from bimane to the retinal, caused by the decreased spectral overlap of bimane emission and rhodopsin absorption (supplemental Fig. S2, A and B). The opposite pattern of energy transfer is expected for the bimane labeled parapinopsin mutants: no overlap of bimane emission with retinal absorbance in dark-state parapinopsin, whereas the amount of overlap increases upon photoactivation, and thus one sees reduced bimane fluorescence (see supplemental Fig. S2, C and D).

However, the expected fluorescence increase of $250B_1$ did not occur in the presence of Trp-226, in contrast to mutant $250B_1$ in the presence of Trp-227 (supplemental Fig. S3, A - C). These results qualitatively suggest that for bovine rhodopsin, photoactivation moves the bimane label at position 250 closer to Trp-226, consistent with the model proposed in Fig. 5. In addition, unlike the case of bovine

rhodopsin, changes of fluorescence intensity of $250B_1$ mutant upon photoactivation of parapinopsin were not affected by the presence of Trp-226 or Trp-227 (supplemental Fig. S3, D - F).

Supplemental figure legends

Supplemental Fig. S1

Spectral and biochemical properties of parapinopsin background mutant (V138F/C140A/C316A/C322A).

(A) Absorption spectra of parapinopsin WT. (B) Absorption spectra of parapinopsin "background" mutant V138F/C140A/C316A/C322A. Black and red lines indicate absorption spectra in the dark and after photoactivation, respectively. The irradiation converted more than 90 % of the pigments to the active form (photoproduct) having all-*trans*-retinal based on HPLC analyses (data not shown). (C) Gi activation of parapinopsin WT. (D) Gi activation of parapinopsin mutant V138F/C140A/C316A/C322A. The closed and open circles indicated amount of GTPγS bound to Gi in the dark state and after photoactivation, respectively.

Supplemental Fig. S2

The spectral overlap of bimane emission with bovine rhodopsin and parapinopsin absorbance changes in the dark and active states.

Black lines indicate absorption spectra of bovine rhodopsin in the dark (A), after photoactivation (B), parapinopsin in the dark (C) and after photoactivation (D). Red lines indicate typical fluorescence emission spectra of bimane. Absorption maximum of bovine rhodopsin before and after photoactivation is at visible and UV regions, respectively. In contrast, absorption spectra of parapinopsin before and after photoactivation are at UV and visible regions, respectively. As can be seen, the amount of spectral overlap (pale blue) between bimane emission and the retinal absorbance increases upon photoactivation of parapinopsin and decreases upon photoactivation of bovine rhodopsin. This increase and decrease of spectral overlap changes the amount of bimane fluorescence energy transfer to retinal, and thus make fluorescence lifetime shorter and longer, respectively (see supplemental Table S1).

Supplemental Fig. S3

Changes in the Trp-induced quenching of bimane emission for $250B_1$ mutants support helical movement model shown in Fig. 5.

Spectra of mBBr labeled bovine rhodopsin (A - C) and parapinopsin (D - F) 250C mutants in the dark (black) and after photoactivation (red). The fluorescence intensity of the dark state of each mutant was normalized to 1.0. In the $226W/250B_1$ and $227W/250B_1$ mutants, a Trp residue is additionally introduced at positions 226 and 227 to $250B_1$, respectively. Dotted red lines indicate fluorescence

intensities of 226W/250B_1 mutants after photoactivation.

Supplemental Fig. S4

Differences in polarity cannot explain the large difference observed for 227W quenching of 251B2 rhodopsin and parapinopsin mutants.

(Left panel) Effect of polarity on Trp-bimane quenching efficiency, as assessed by Stern-Volmer plots of free bimane (bimane labeled L-cysteine) in solutions of different apparent polarity / dielectric constant. The plots report the ratio of fluorescence intensity in the absence (Fo) and presence (Fw) of free tryptophan (N-acetyl-L-tryptophan-amide) as a function of free tryptophan concentration. The solutions of different polarity were obtained by mixing dioxane and water together at different ratios. To generate the Stern-Volmer plots, mBBr reacted with L-cysteine was used as it is more stable than PDT-bimane reacted with L-cysteine under various solvent conditions. The apparent polarity surrounding the bimane probes attached to the protein was estimated by comparing the emission λmax to calibration curves generated for free bimane in different polar solutions, as previously described (1,2). Using this approach, we estimate that after photoactivation, the apparent polarity around the bimane for the 227B_1 mutants of bovine rhodopsin and parapinopsin is ~23 and ~30, respectively. Similarly, the apparent polarity around the bimane at position 251 for both rhodopsins is ~ 40. (Right panel) Re-plotting of the data shown in Fig. 4, comparing the quenching efficiency of the Trp at position 227 on the bimane at position 251 for both rhodopsins in their photoactivated state. Notice the large difference between the fluorescence intensity ratios in the absence (Fo) and presence (Fw) of tryptophan at position 227, compared to the small changes in the Stern Volmer at this Trp quenching efficiency under different polarities. These data clearly indicate the large difference in quenching efficiency between 227W/251B_2 mutants of rhodopsin and parapinopsin cannot be caused by the small difference in the apparent polarities surround the bimane probe and Trp residue at these sites.

Supplemental Fig. S5

Fluorescence emission spectra of the 244B_1 mutants of bovine rhodopsin and parapinopsin between pH 6 and 8.

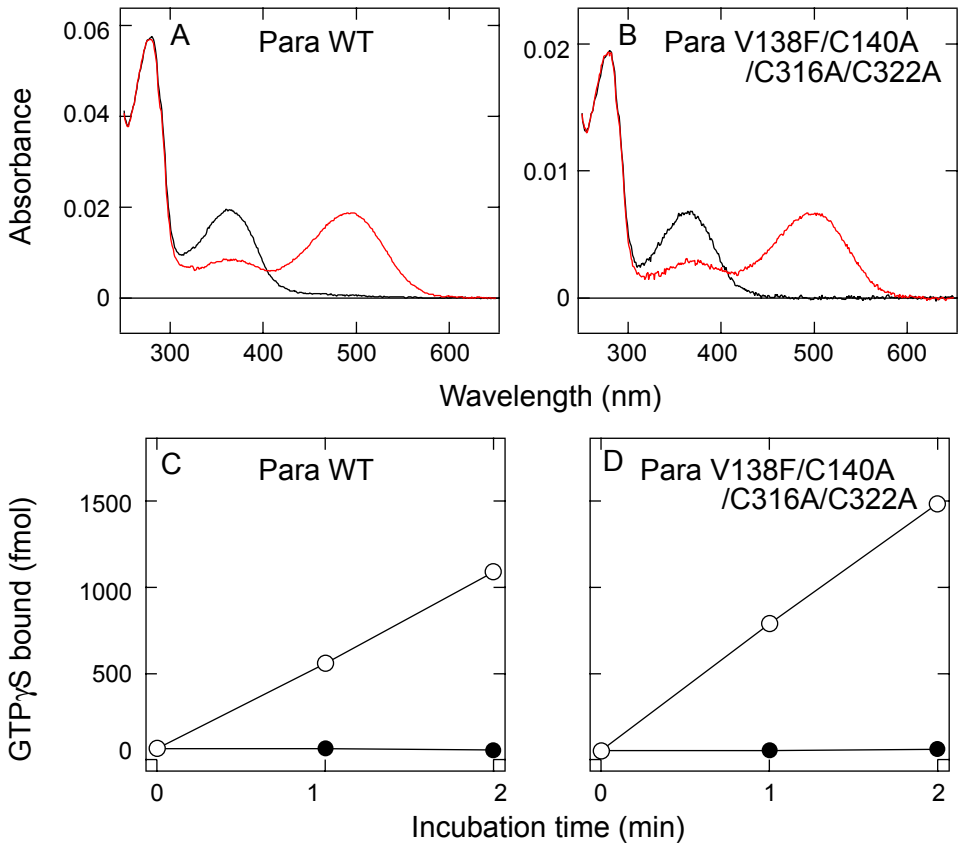
Emission spectra of mBBr labeled bovine rhodopsin 244C mutant at pH 6 (A) or 8 (B) and spectra of mBBr labeled parapinopsin 244C mutant at pH 6 (C) or 8 (D) are shown. Spectra are normalized by maximal fluorescence intensity as 1.0. Black and red lines indicate spectra before and after photoactivation, respectively. The spectral changes in 244B_1 mutants upon photoactivation of bovine rhodopsin and parapinopsin were not changed between pH 6 and 8, suggesting that differences of conformational changes between the two rhodopsins are not due to differences in pH effects.

Supplemental References

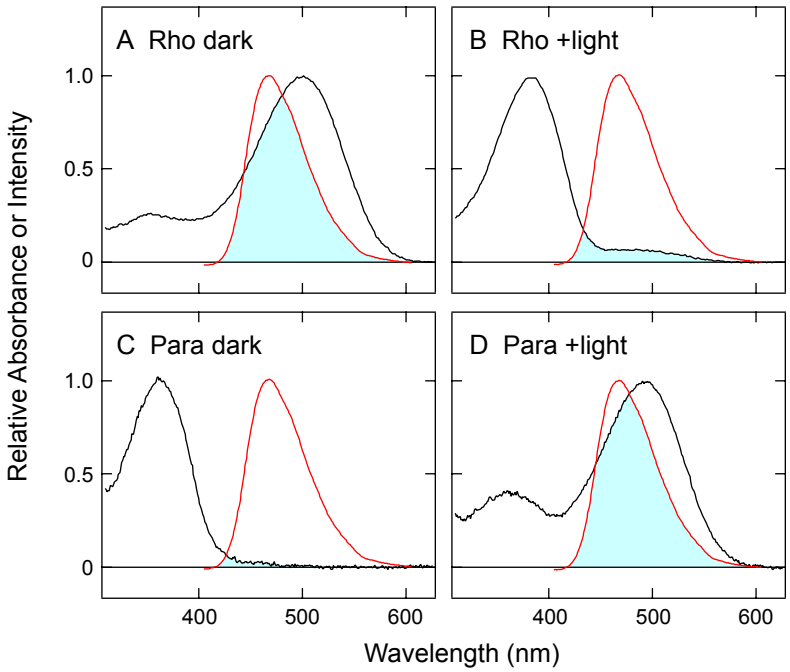
1. Mansoor, S. E., McHaourab, H. S., and Farrens, D. L. (1999) *Biochemistry* **38**, 16383-16393
2. Mansoor, S. E., and Farrens, D. L. (2004) *Biochemistry* **43**, 9426-9438

Supplemental Table S1 Parameters for calculation of *kq* values of bovine rhodopsin and parapinopsin mutants

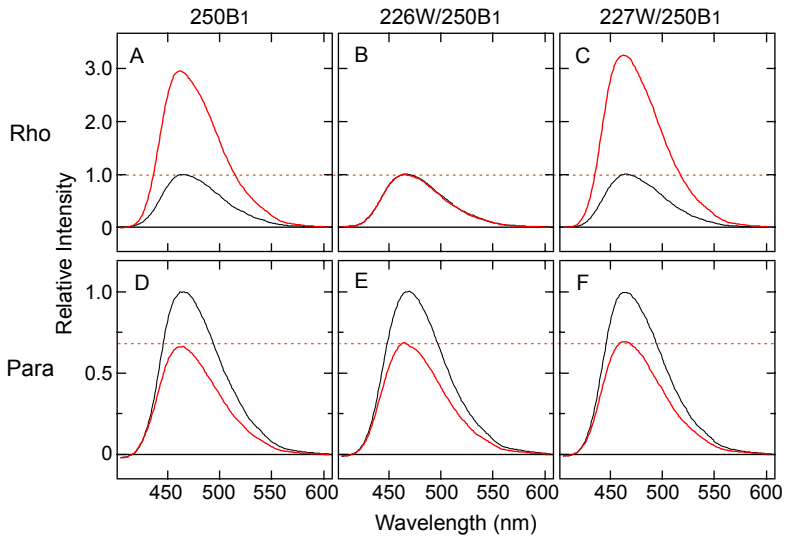
	mutant		Ksv (1/M)	<τ> (ns)	kq ($*10^9$)	kq_{light}/kq_{dark}
Bovine rhodopsin	$227B_1$	dark	12.3±0.6	8.2±1.8	1.5±0.3	0.35±0.1
		light	6.4±0.6	12.3±1.0	0.52±0.07	
	$250B_1$	dark	11.6±1.8	6.8±1.5	1.7±0.5	2.6±0.8
		light	64.5±3.9	14.0±2.2	4.5±0.7	
	$251B_1$	dark	15.3±1.5	7.8±1.3	2.0±0.3	0.85±0.2
		light	24.7±1.6	14.8±2.0	1.7±0.3	
Parapinopsin	$227B_1$	dark	22.9±0.7	11.0±1.8	2.1±0.3	0.9±0.2
		light	11.5±0.4	6.5±1.2	1.8±0.3	
	$250B_1$	dark	43.3±6.2	11.2±1.8	3.9±0.8	1.3±0.4
		light	35.8±5.0	7.3±1.2	4.9±1.1	
	$251B_1$	dark	19.3±2.2	15.7±2.3	1.2±0.2	1.3±0.3
		light	14.8±1.1	9.4±1.5	1.6±0.3	



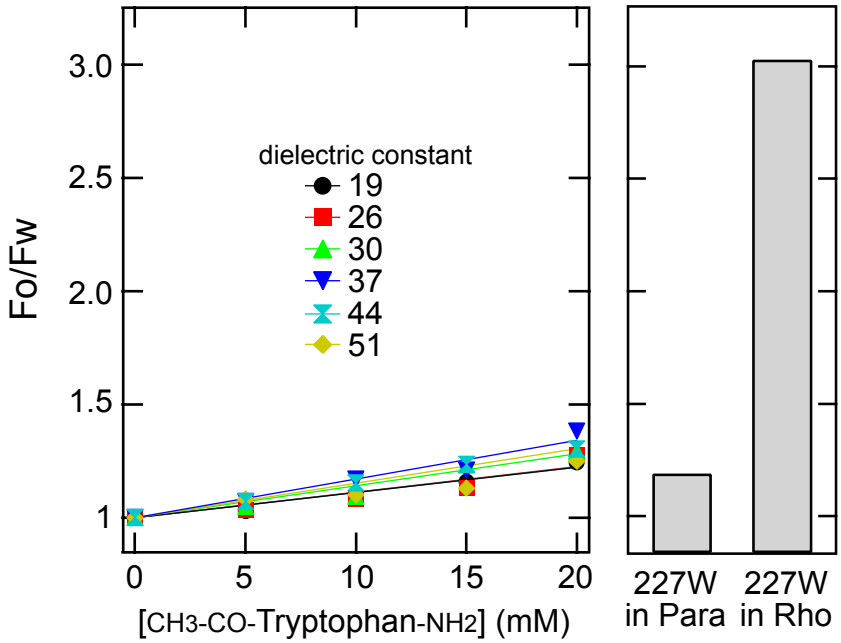
Supplemental Fig. S1 Tsukamoto et al.



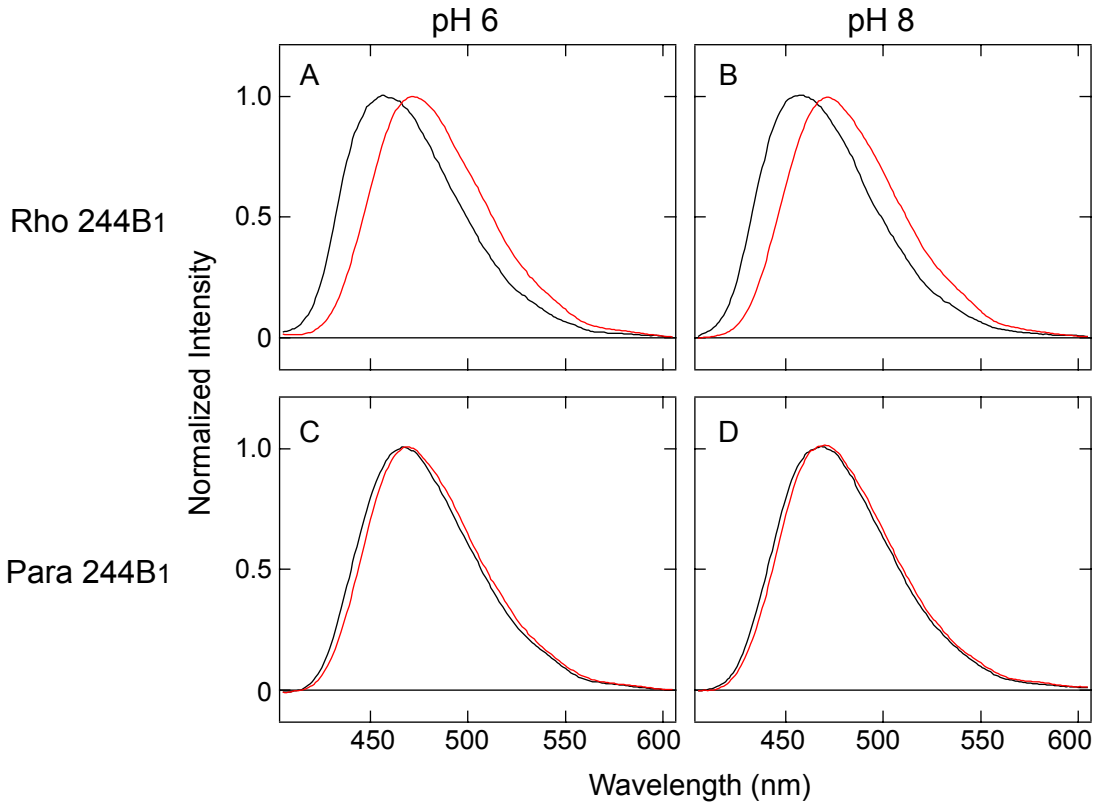
Supplemental Fig. S2 Tsukamoto et al.



Supplemental Fig. S3 Tsukamoto et al.



Supplemental Fig. S4 Tsukamoto et al.



Supplemental Fig. S5 Tsukamoto et al.