Supplementary Information

H-bond network around retinal regulates the evolution of ultraviolet and violet vision

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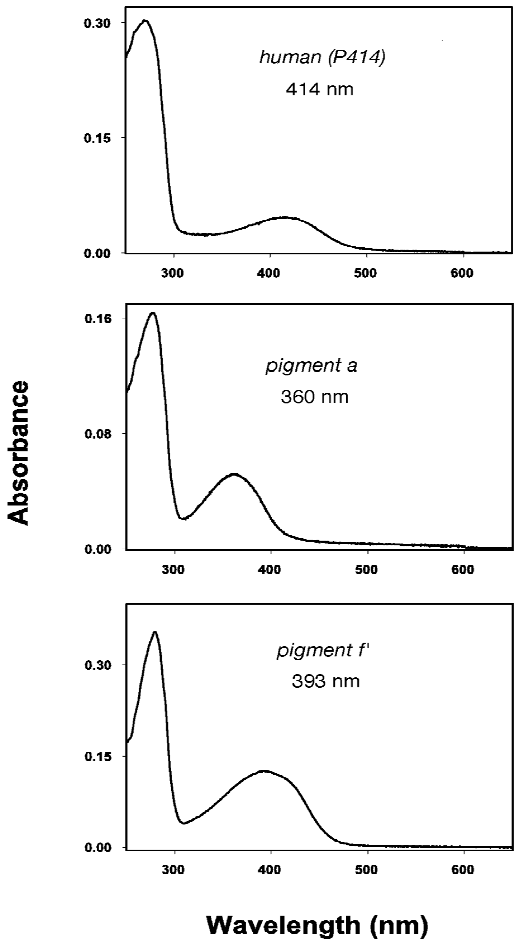
Supplementary Table 1. Critical H-bond distances (in Å) in the SWS1 pigments.[a]

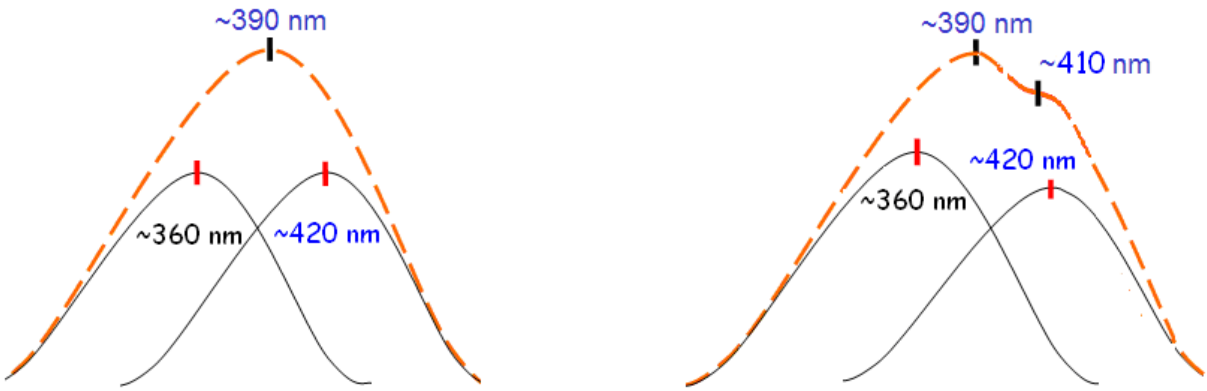
Pigment	mutation	d_1 (PSBR) NH···O	d_1 (SBR) N···HO	d_2 (PSBR) O···HO/HS	d_2 (SBR) O···HO/HS	d_3 (PSBR) O···HO/HS	d_3 (SBR) O···HO/HS
a		2.11	1.99	1.88	1.95	–	–
	Phe86	2.21		–	–	–	–
	deletion		1.99				
b		2.18	1.94	1.61	1.74	–	–
	Phe86	2.15		–	–	–	–
	deletion		2.22				
d		2.35	1.83	1.73	1.71	–	–
	d-7m	1.89	1.95	1.64	1.86	–	–
f		2.08	1.93	1.61	1.85	–	–
f'		1.79	1.88	1.54	1.76	1.70	1.71
	S86C	1.79	1.87	1.55	1.77	3.47	2.03
	S90C	1.77	1.86	1.78	1.93	1.79	1.83
	S86C/S90C	1.77	1.87	1.76	1.89	1.96	2.12
h		2.16	2.09	1.64	1.79	-	–
	F86S/L116V	1.81	2.02	1.55	1.72	1.67	1.66
	F86Y	1.93	2.23	1.59	1.81	1.92	1.87
	h-7m	1.80	2.06	1.57	1.71	–	–

[a]The minus (–) sign indicates that there is no H-bond involving d_2 and/or d_3 distance either with side-chain rotation of Ser90 in Phe86 deletion analogs of *pigments a* and *b* or from the absence of H-bond donor at position 86.

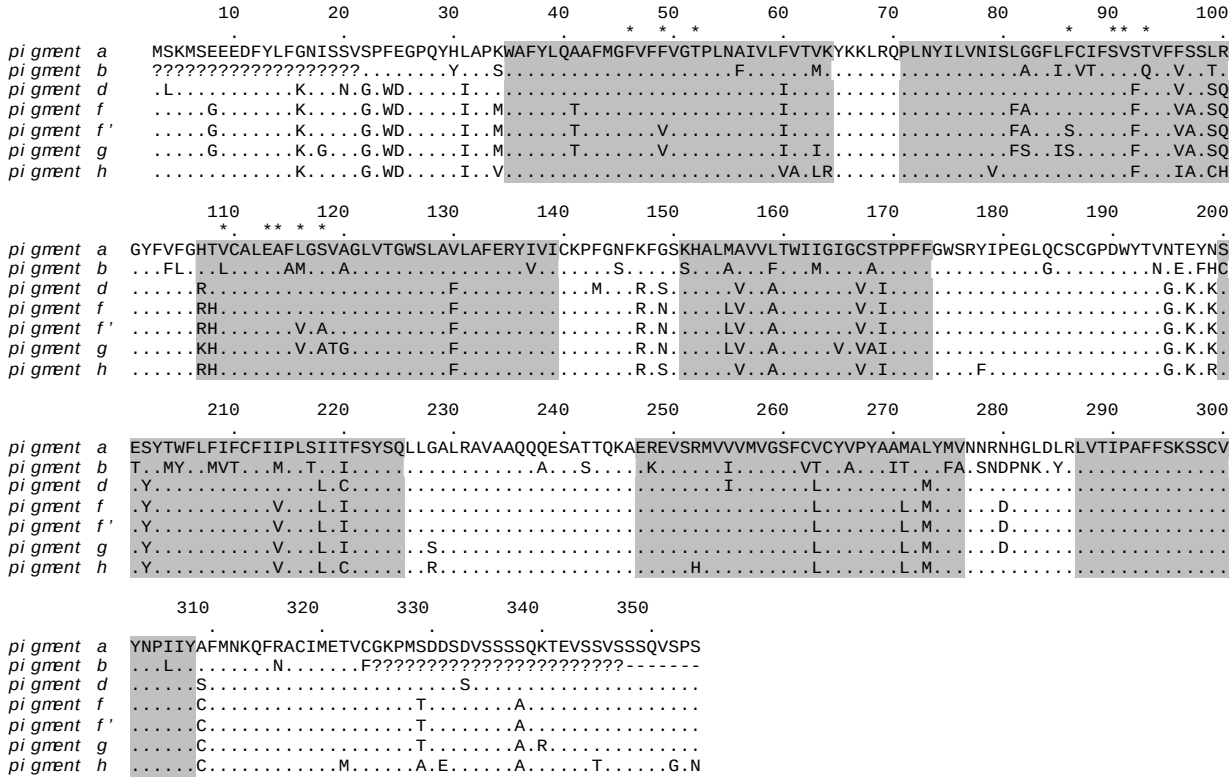


Supplementary Figure 1. The absorbance spectra of the representative pigments.

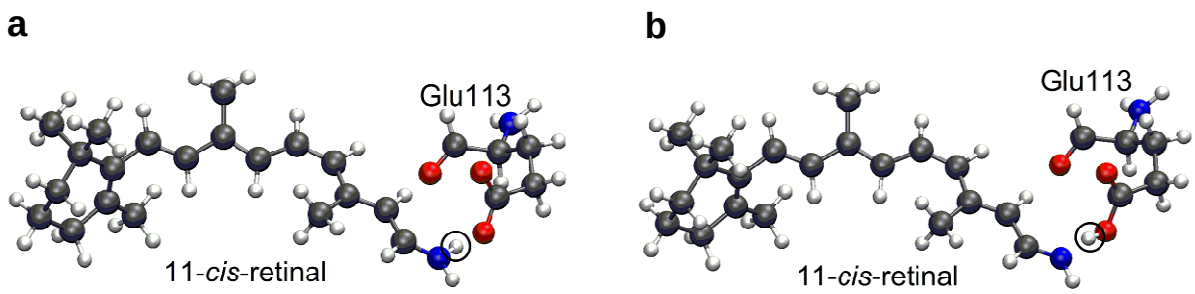
a **b**



Supplementary Figure 2. Schematic representation of superposition of two normal curves with identical line widths. The superposition of the absorption curves (i.e., the curves with broken red lines) of a visual pigment with (a) equal population of its SBR and PSBR analogs and (b) its slightly more populated SBR analog.



Supplementary Figure 3. Aligned amino acid sequences of ancestral SWS1 pigments. Dots indicate identical amino acids with those of *pigment a* and dashes indicate the deletion of amino acid sites. Question marks indicate that amino acids have not been inferred. Seven shaded segments indicate TMI-VII helices. The positions of twelve critical amino acid sites, 46, 49, 52, 86, 90, 91, 93, 109, 113, 114, 116, and 118, are marked by asterisks. *Pigment f* and *pigment f'* differ at sites 49, 86, 116, and 118. The amino acid site numbers are those of bovine rhodopsin.



Supplementary Figure 4. The QM model used in ONIOM calculations. The circled H is bonded to (a) the SB nitrogen (PSBR) or (b) the carboxylic oxygen of Glu113 that results in SBR. Black, blue, red and white colors represent carbon, nitrogen, oxygen and hydrogen atoms, respectively.

Supplementary Methods

QM/MM energy decomposition analysis. In electronic embedding scheme of QM/MM calculations, the charges of each QM atom are affected by the fixed charges of each protein atom. QM/MM interaction energies are calculated by using these two types of charges. Therefore, QM/MM energy decomposition analysis with sophisticated electronic embedding scheme is not as easy as that with gas-phase calculations and with mechanical embedding scheme. However, contributions of certain parts of the protein to the QM/MM energy can fortunately be estimated by setting the point charges of each atom in these protein moieties to zero, which is standard procedure in QM/MM studies (supplementary refs. 1–6). The difference in QM/MM energies before and after setting the point charges to zero gives their contributions to the QM/MM energy. Hence, estimation of an amino acid to ΔE involves calculation of four QM/MM energies belonging to PSBR and SBR analogs of the pigments before and after setting the charges to zero. Since δE is the difference in ΔE of ancestral and mutated pigments, estimation of an amino acid to δE thus requires evaluating eight QM/MM energies; four for the ancestral pigment and four for the mutated pigment.

Accuracy of the Structures and Numerical Values: Visual pigments have characteristic seven helical structures. During MD studies, the special helical structure (i.e., backbone structure) does not change (Supporting Information of supplementary ref. 7). Its change already means nonfunctioning pigments. Therefore, the only structural concern is the sampling of side chains. Based on this fact, taking initial homology-modeled structures, we performed an extensive conformational study to

sample side chain orientations rather than performing molecular dynamics simulations. Then, we performed energy minimizations that already respond well the conformational changes around mutated residues. However, it should be pointed out that all energy values and geometry parameters reported in this study should be judged qualitatively due to the uncertainties in the quality of the initial homology-modeled structures and the limited sampling of the conformations away from the mutation zones.

Supplementary References

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