SUPPORTING MATERIAL

Large deformation of helix F during the photoreaction cycle of *pharaonis* halorhodopsin in complex with azide

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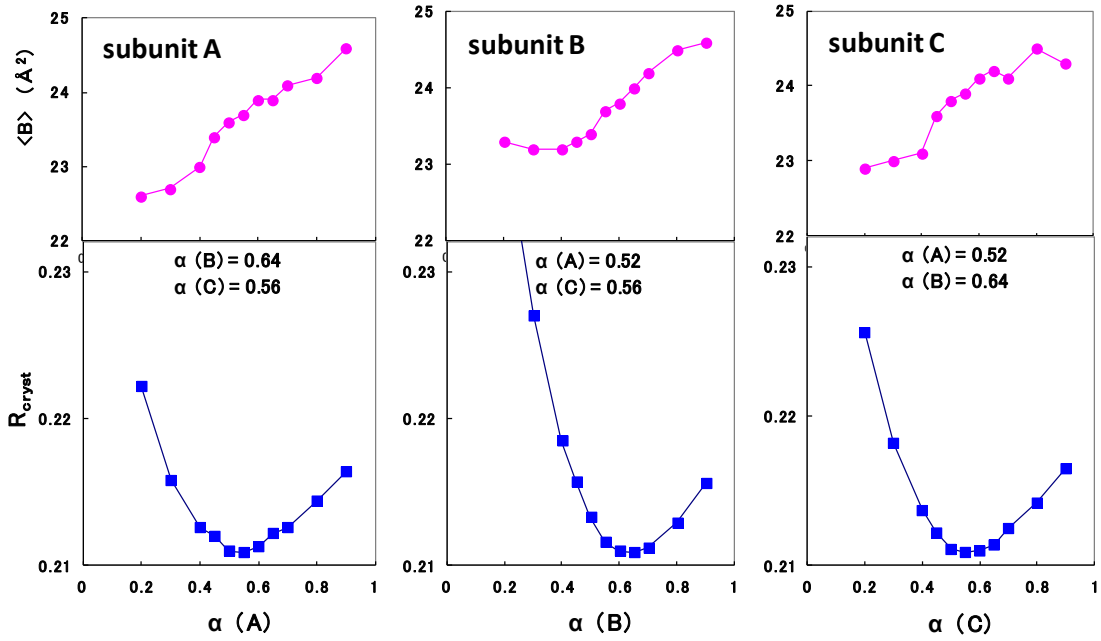

Figure S1. Estimation of the occupancy α_i of a reaction state trapped in the i-th subunit (subunit A, B or C) within the asymmetric unit of the *C2* crystal of the pHR-azide complex that was flash-cooled under illumination with orange light. Lower panels: The R_{cryst} value (= Σ_{hkl} ($|F_{obs}| - |F_{calc}|$) / Σ_{hkl} $|F_{obs}|$) is plotted as a function of the occupancy α_I (i=1, 2, 3). Upper panels: The averaged B-factor of residues in the reaction state is plotted as a function of α_i.

Note: The structural analysis was performed on the approximation that two conformers (a reaction state and the unphotolyzed state) were contained in each subunit and the calculated amplitude F_{obs} was evaluated as follows: $|F_{calc}| = \Sigma_i \{\alpha_i \cdot |F_{i\_React}| + (1-\alpha_i) \cdot |F_{i\_Ground}|\}$, where F_{i_React} and F_{i_Ground} are the structure factors of the reaction state and the unphotolyzed state, respectively, in the *i*-th subunit. At each value of α_i, the structure of the reaction state in the i-th subunit was refined by the simulated annealing method, while the structure of the second conformer in each subunit was assumed to be identical to that of the unphotolyzed state in a crystal that was flash cooled in dim light; subsequently the B-factor refinement was performed for the reaction state alone. In the initial search of the optimal α_i values, the B factors of residues in the second conformer were set identical to those that were evaluated using diffraction data from a crystal that was flash-cooled in dim light. (In a late stage of refinement, the B-factors of residues in the second conformer were refined using the optimal α_j values.) In a new cycle of search for the optimal α_i values, the structure refinement of the reaction state in the i-th subunit was performed using the previously determined optimal α_j ($j \neq i$) values of the reaction states in the other subunits. Such refinement cycle was repeated until no large decrease in the R_{cryst} value (at the optimal α_j values) was observed.

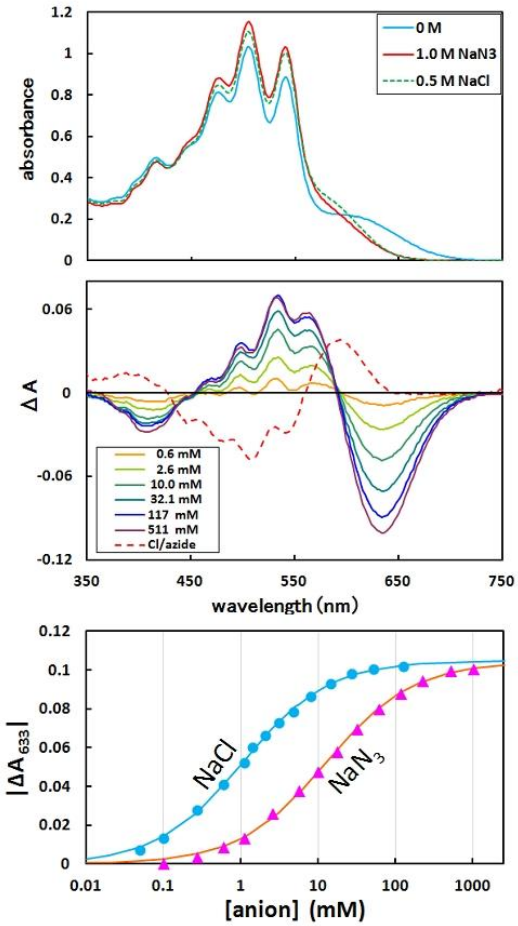

Figure S2. a) Absorption spectrum of claret membrane at pH 7 in 1.0 M sodium azide (red line), 0.5 M NaCl (broken green line), and 0 M azide/chloride (blue line). The spectra were corrected for light scattering, whose contribution was assumed to be proportional to $\lambda^{-2.4}$. b) Spectroscopic titration of claret membrane with NaN_3. The solid lines show difference absorption spectra derived by subtracting the absorption spectrum of the anion-depleted form of *p*HR from those observed at various concentrations of sodium azide. The broken line represents the difference absorption spectrum between the chloride-bound purple form and the azide-bound purple form. c) Anion-induced absorption change at 632 nm is plotted against the concentration of NaN_3 or NaCl. Experimental data (closed circles) are fitted with theoretical curves (solid lines) in which the absorption changes are assumed to be described by the equation: $\Delta A = \Delta A_{max}/(1 + ([anion]/K_d)^n)$. The K_d and *n* value were evaluated to be 1.06 mM and 0.76 for the chloride titration and 12.7 mM and 0.78 for the azide titration.

Note: The positive peaks at 498, 532 and 568 nm seen in the difference spectra are due to a small red-shift of the absorption band of the second chromophore bacterioruberin, whose vibronic bands occur at 476, 504 and 540 nm in the anion-depleted-form. A similar red-shift of bacteriruberin's absorption band is induced upon addition of sulfate ion, which does not bind to the primary chloride binding site (site I). It appears that the absorption spectrum of bacterioruberin that binds to the crevice between neighboring subunits in the trimeric assembly of pHR is sensitive to the charge distribution or the electric field in the second anion binding site, which is located at the cytoplasmic membrane surface and near one terminal end of bacterioruberin.

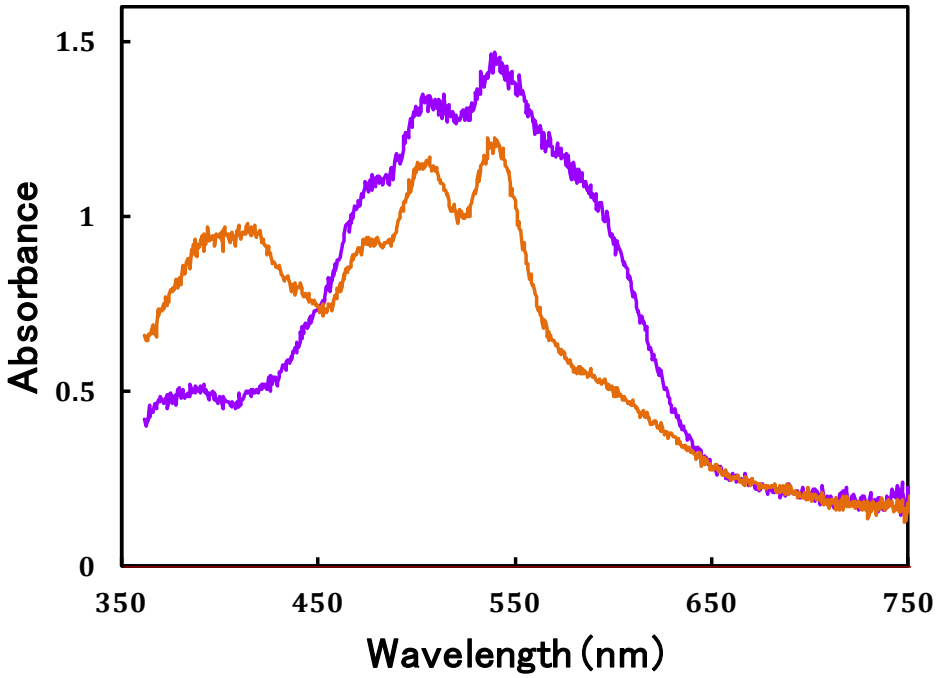

Figure S3. Absorption spectra of un-illuminated (purple line) and illuminated (orange line) states of the *C2* crystal of the pHR-azide complex, recorded at 100 K. A reaction state(s) with λ_{max} at 410 nm was efficiently trapped when the *C2* crystal was flash-cooled under illumination with orange light (~100 mW/cm^2). The spectra were recorded using a thin rectangular crystal that was grown on the wall of a lower glass of the crystallization kit; the lower glass was broken mechanically and a small broken piece to which the crystal was adhered was soaked in a solution containing 0.18 M NaN_3, 2.7 M ammonium sulfate, 0.9 M glycine (pH 9) and 35 % trehalose for ~20 minutes and then cooled to 100 K under a stream of cold nitrogen gas. The crystal was mounted in a sample stage of a micro-spectrophotometer, in which the measuring light from a double-monochromator was passed through a pin hole with a diameter of 50 μm and, using an achromatic UV lens, focused on a central part of the C*2* crystal, and the transmitted light was passed through another pinhole that was set before a photomultiplier tube.

Note: The *b*-axis of the *C2* crystal (i.e., the absorption dipole moment of bacterioruberin bound to the trimeric assembly of *p*HR) was tilted largely from the optical pass of the (non-polarized) measuring light. In this case, the absorbance is given by: $A = -\log_{10}((T^{/\!/} + T^{\perp})/2)$, where $T^{/\!/}$ and $T^{\perp}$ represent transmittances that would be measured when the polarization plane of polarized light is parallel and perpendicular, respectively, to the longest axis of the *C*2 crystal.

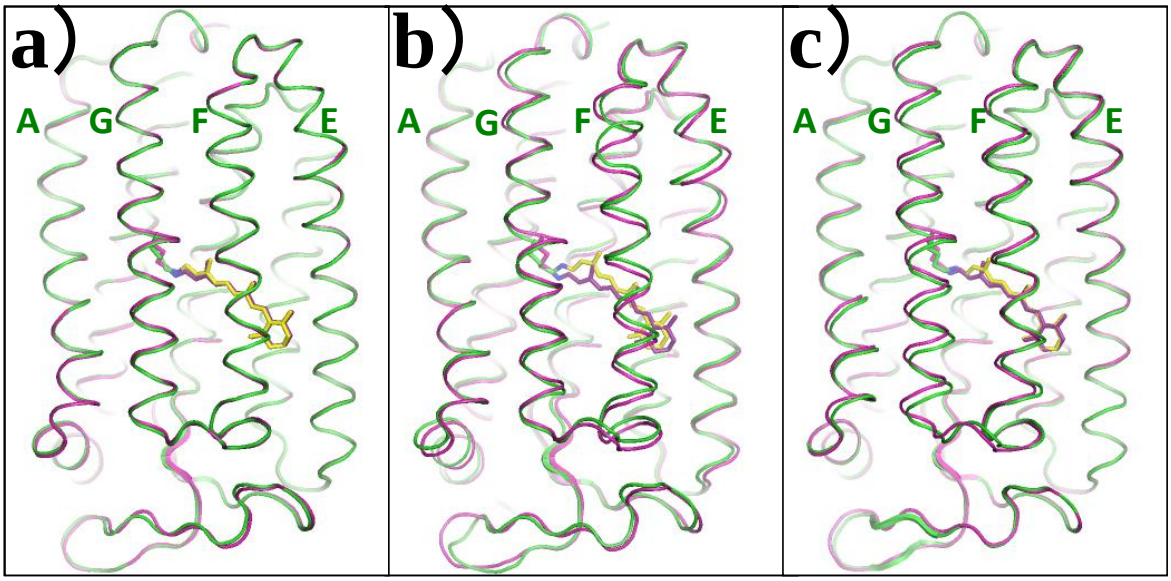

Figure S4. Light-induced changes in the main-chain structure of subunit A (a), subunit B (b), and subunit C (c). The reaction state and the unphotolyzed state are drawn in green and magenta, respectively.

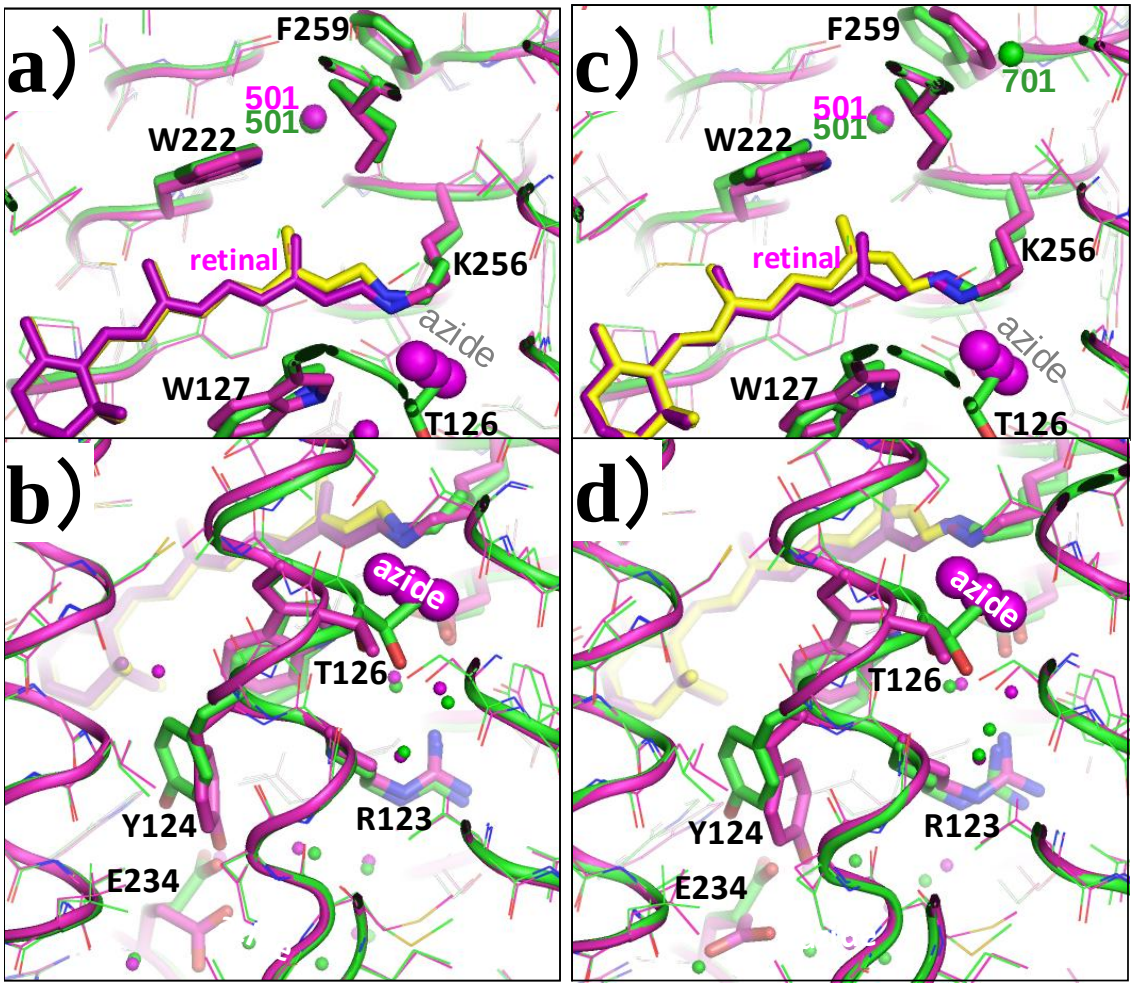

Figure S5. Light-induced structural changes in subunit A (a, b) and subunit C (c, d). Carbon atoms, water molecules and azide ion in the reaction state and the unphotolyzed state are drawn in green and magenta, respectively.

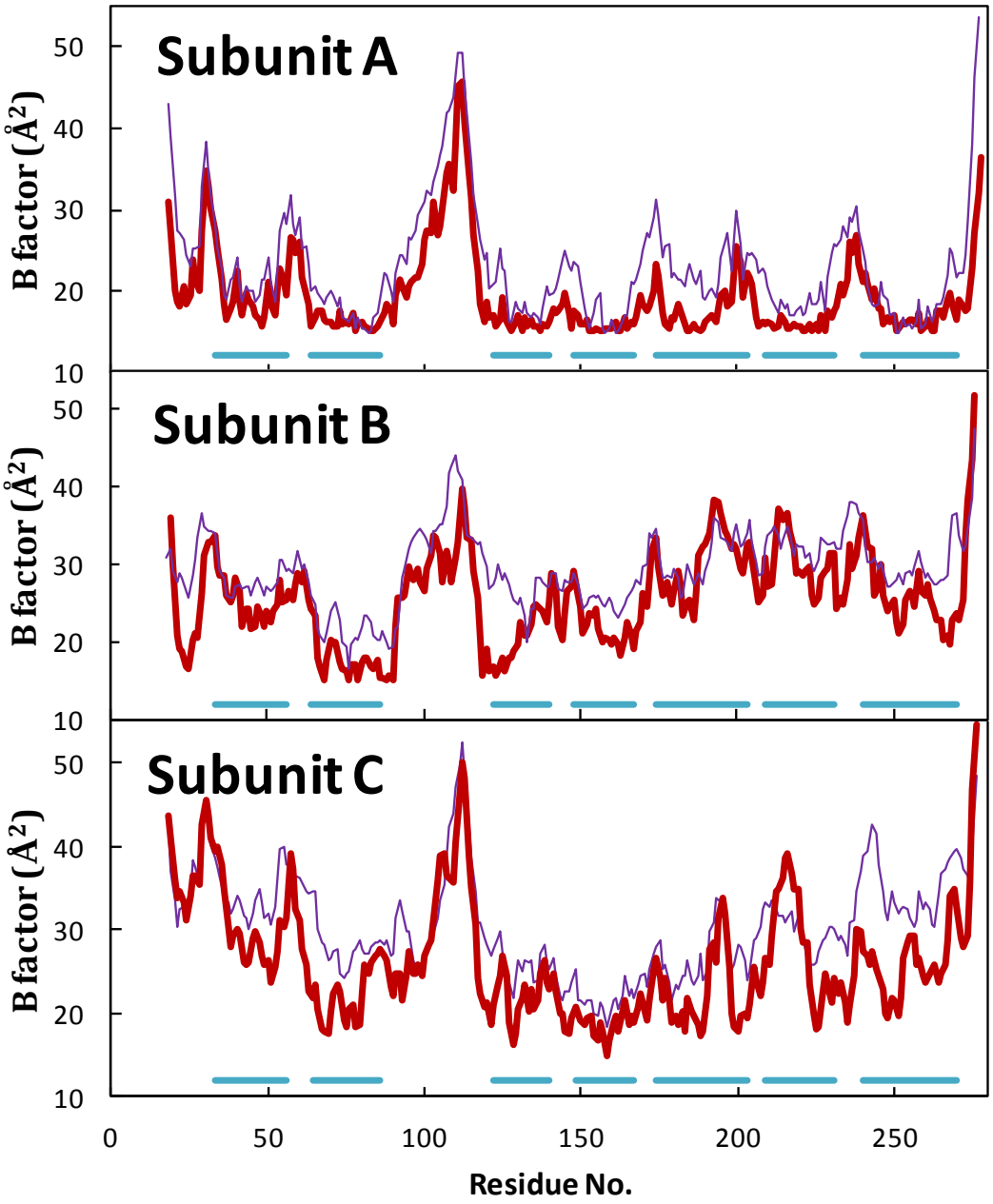

Figure S6. The B factor of alpha carbon in the unphotolyzed state (thin purple line) and the reaction state (thick brown line) trapped in each subunit (subunit A, B, and C) is plotted against the residue number. The blue bars represent the transmembrane region (helices A through G).

Table S1. Data collection and final refinement statistics.

Crystals				
Soaking solution				
Azide	0.2 M NaN_3	0.2 M NaN_3	0.2 M NaN_3	None
pH*	7	9	9	10
Illumination †	Off	Off	On	Off
Data collection				
Resolution	50.1-1.9	50.0-2.3	50.0-2.3	45.1-2.1
Space group	*C*2	*C*2	*C*2	*C*2
Unit cell (Å) a	152.09	152.75	153.85	153.95
Unit cell (Å) b	98.79	98.09	97.90	97.77
Unit cell (Å) c	100.14	100.48	101.02	100.65
Unit cell (°) β	127.85	128.34	128.53	128.68
Data completion (%)	87.9	96.2	92.5	99.2
No. unique reflections	80687	53007	48009	67414
Multiplicity	3.1	2.7	3.6	3.1
Rsym‡ (%) (highest resolution)	6.5 (50.2)	10.2 (50.7)	9.7 (34.8)	10.2 (50.7)
I/σ (highest resolution)	11.4 (2.4)	8.4 (2.5)	9.6 (3.5)	10.0 (2.7)
Refinement				
Resolution limit (Å)	15.0-1.9	15.0-2.3	15.0-2.3	15.0-2.1
Protein residues	18-277	18-277	(18-277) × 2	18-277
No. lipids+NG	10+4	11+4	11+4	11+4
No. anion	3	3	0 +3	0
Number of water	127	126	155 +123	217
Rcryst § (%)	24.2	23.3	21.0	23.1
Rfree (%)	26.2	26.9	24.4	26.1
Rmsd				
Bond length (Å)	0.0063	0.0062	0.0065	0.0063
Bond angle	1.11	1.11	1.22	1.11
% bond angles within the acceptable Ramachandran region	100	100	100	100
B factor (Å^2)				
Residues	29.7	32.3	25.7	28.1
Azide ion	32.4	32.9	22.7	-
water	34.0	39.6	25.2	36.2

*, The pH of the soaking solution was adjusted by 0.1 M HEPES (pH 7.0) or 0.1 M glycine (pH 9.0 and pH 10).

† The *C*2 crystal was flash-cooled in dim light or under illumination with orange light (>540 nm, ~100 mW/cm^2).

‡ $R_{sym} = \Sigma_{hkl}\Sigma_i |I_i - \langle I \rangle| / \Sigma_{hkl}\Sigma_i I_i$, where I_i is the intensity of an individual reflection and <I> is the mean intensity obtained from multiple observations of symmetry related reflections.

, § $R_{cryst} = \Sigma_{hkl} (|F_{obs}| - |F_{calc}|) / \Sigma_{hkl} |F_{obs}|$ (5 % randomly omitted reflections were used for R_{free}).