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Supporting information for article:

Structural basis for slow photocycle and late proton release in *Acetabularia* rhodopsin I from the marine plant *Acetabularia acetabulum*

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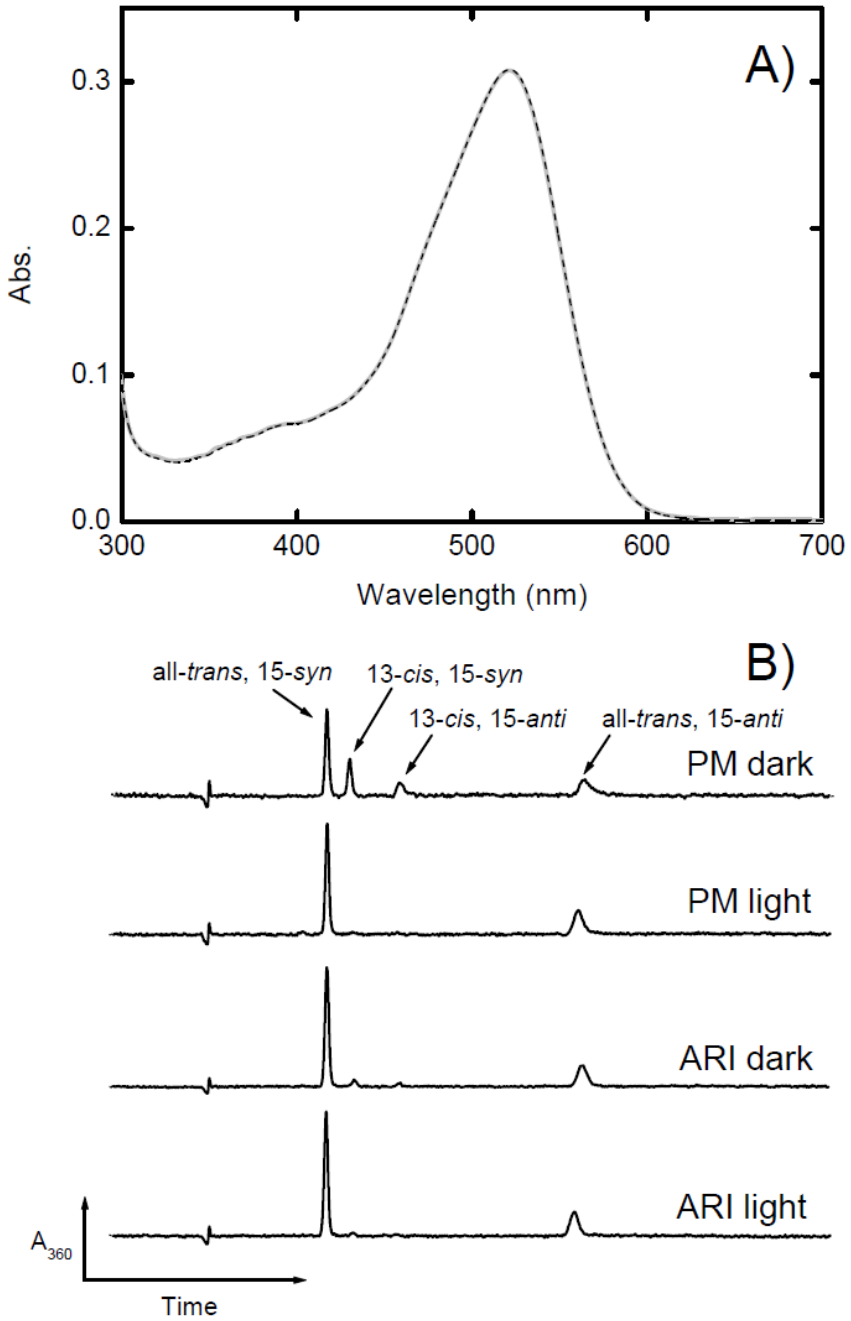

Figure S1. The absorbance spectra and the retinal configurations of the light- and dark-adapted ARI. A) Absorbance spectra of dark-adapted and light-adapted ARI are shown in grey continuous and black dashed lines, respectively. Measurements were performed in a solution containing 400 mM NaCl, 0.05% DDM and 10 mM MOPS (pH 7.0). B) HPLC analyses for the retinal isomer composition. The light- and dark-adapted purple membranes (PM) expressing numerous BR proteins were used as a reference. The retinal isomer composition in the extract was investigated with the same apparatus and procedure described previously (Wada et al., 2011). See also Figure 1 and Figure 4.

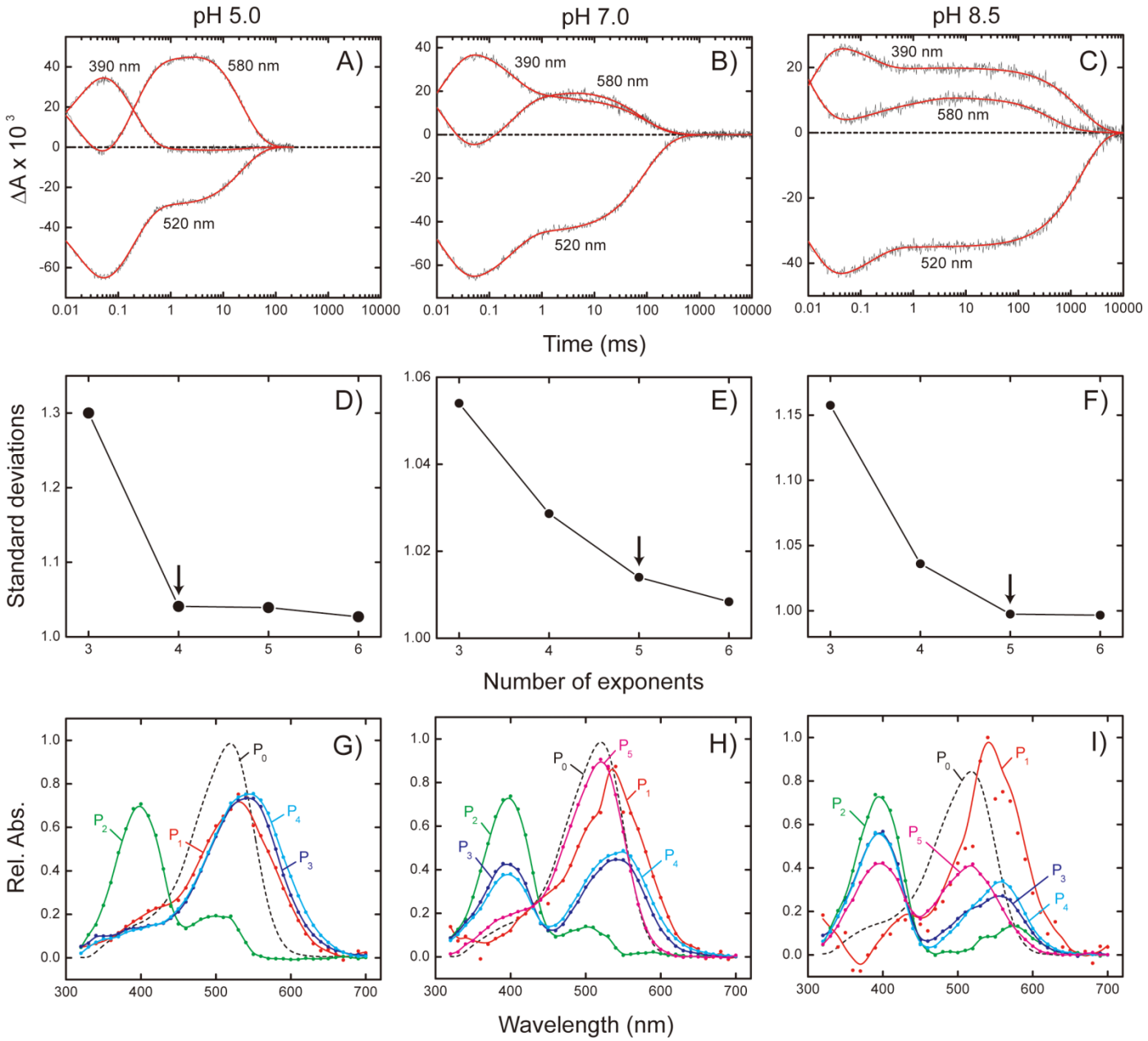

Figure S2. Global fitting analysis for the flash photolysis data at three different pH values. The upper three panels show the time-dependent absorbance changes at 390, 520 and 580 nm, at pH 5.0 (A), pH 7.0 (B) and pH 8.5 (C). The observed data and the fitting curves by the multi-exponential function are shown by the noisy black and smooth red lines, respectively. The middle three panels represent the relationships between the standard deviations of the weighted residuals and the number of exponents used for the global fitting at pH 5.0 (D), pH 7.0 (E) and pH 8.5 (F). These plots reveal the numbers of exponents required to let the standard deviations between the observed and fitting values become saturated. The data at pH 5.0 are sufficiently fitted by the function with four exponential terms, whereas the fittings for the data at pH 7.0 and pH 8.5 require five exponential terms (shown by the black arrows). The lower three panels show the relative absorbance spectra of the photochemically defined photoproducts at pH 5.0 (G), pH 7.0 (H) and pH 8.5 (I).

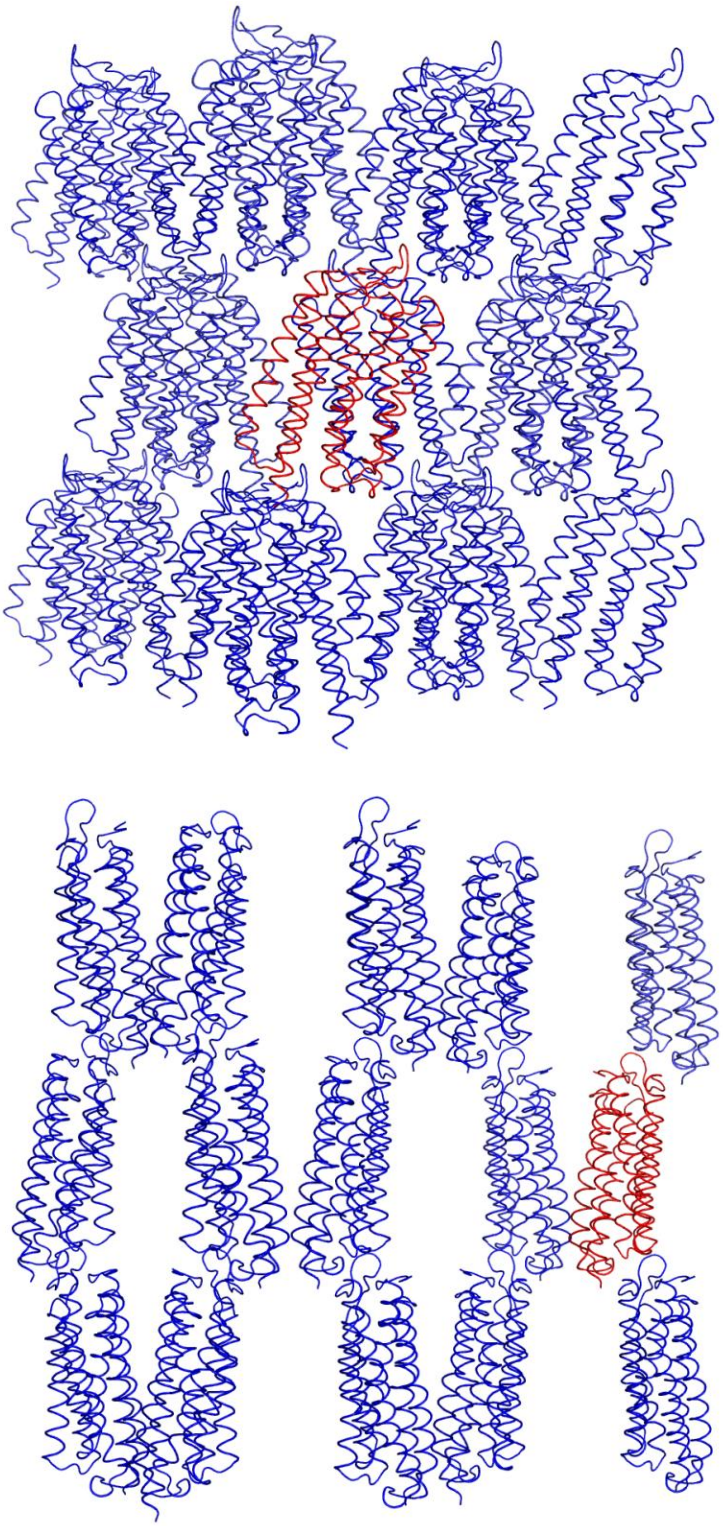

Figure S3. ARI crystal packing. An ARI monomer is shown in red and the other ARI monomers are shown in blue. See also Figure 3.

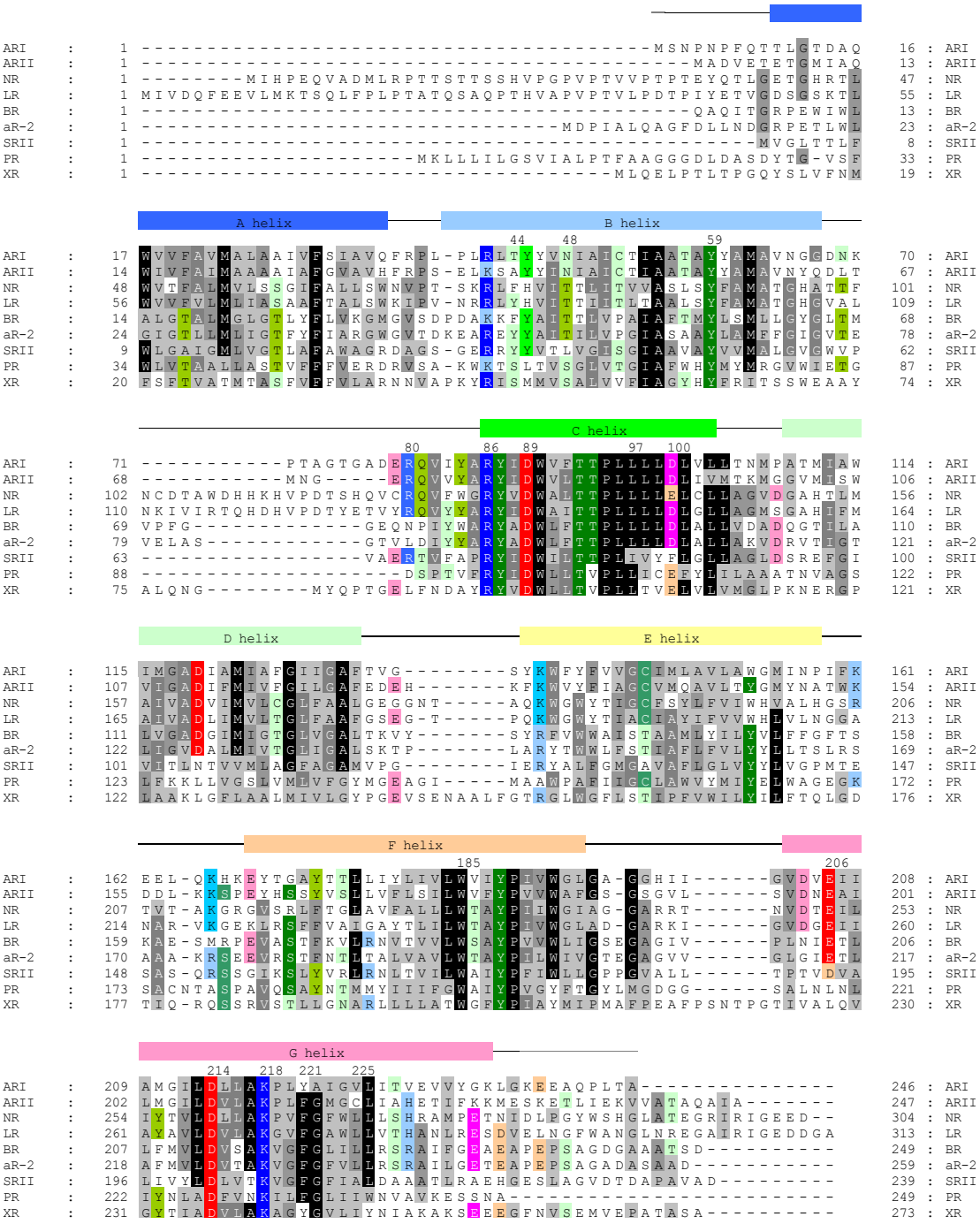

Figure S4 (Continues on the next page). Amino-acid alignment of representative microbial rhodopsins. The denoted rhodopsins act as a light-driven proton pumps. The SRII without its transducer acts as a pump, rather than a sensor. The sequences of ARI and ARII from *Acetabularia acetabulum* (Uniprot, G3CEP6, G3CEP7, respectively), NR from *Neurospora crassa* ATCC 24698 (Q9UW81), LR from *Leptosphaeria maculans* (Q9HGT7), bacteriorhodpsin (BR) from *Halobacterium salinarum* NRC-1 (P02945), archaerhodopsin 2 (aR-2) from *Halobacterium* sp. aus-2 (P29563), sensory rhodopsin II (SRII) from *Natronomonas pharaonis* (P42196), green-absorbing PR (PR) from γ-proteobacteria EBAC31A08 (Q9F7P4) and xanthorhodopsin (XR) from *Salinibacter ruber* DSM 13855 (Q2S2F8) were used for comparison.

(**Figure S4**. Continues from previous page.) The numbers shown in the top row represent the amino acid residues numbering in ARI. The amino acid residues with maximum identical numbers (>33%) in each position are highlighted in blue (positively charged), red (negatively charged), green (hydrophilic) and black or gray (hydrophobic). Residues are depicted as colored filled boxes from dark to light as more identical. The residues that are similar to these colored residues are shown in the lightest colors. The sequence identities and similarities of ARI to the others are following: ARII (identity, 52% / similarity, 68%), NR (24% / 39%), LR (29% / 40%), BR (26% / 43%), aR-2 (27% / 45%), SRII (27% / 42%), PR (21% / 39%) and XR (22% / 35%). The α-helices in the ARI structure are depicted as colored filled boxes, and the dotted line represents the missing residues in the determined structure. See also Figure 3 and Figure 7.

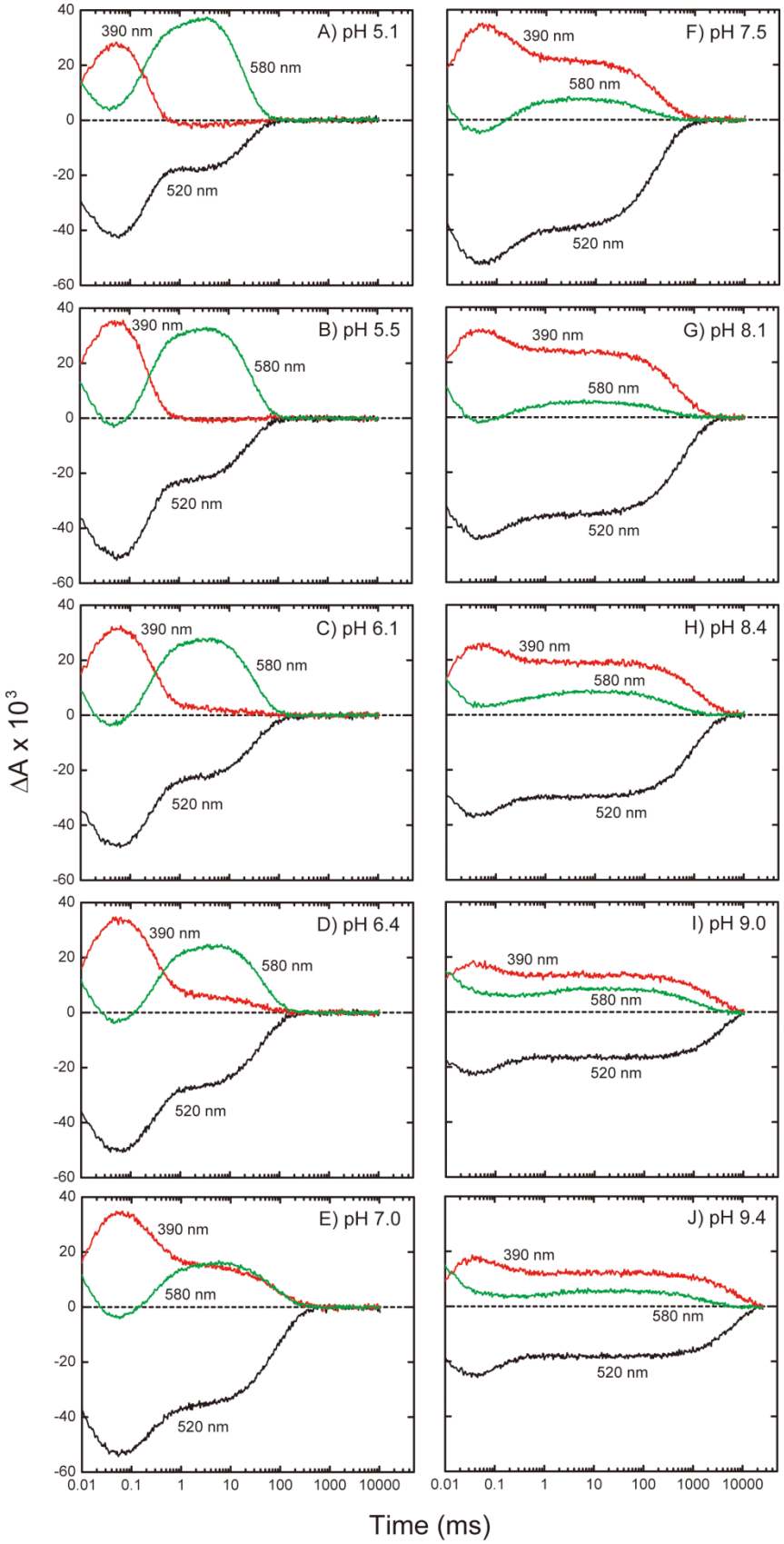

Figure S5. Flash-induced absorbance changes at 390, 520 and 580 nm at various pH values. The time-dependent absorbance changes in ARI (WT) at pH 5.1 (A), pH 5.5 (B), pH 6.1 (C), pH 6.4 (D), pH 7.0 (E), pH 7.5 (F), pH 8.1 (G), pH 8.4 (H), pH 9.0 (I) and pH 9.4 (J) are shown.

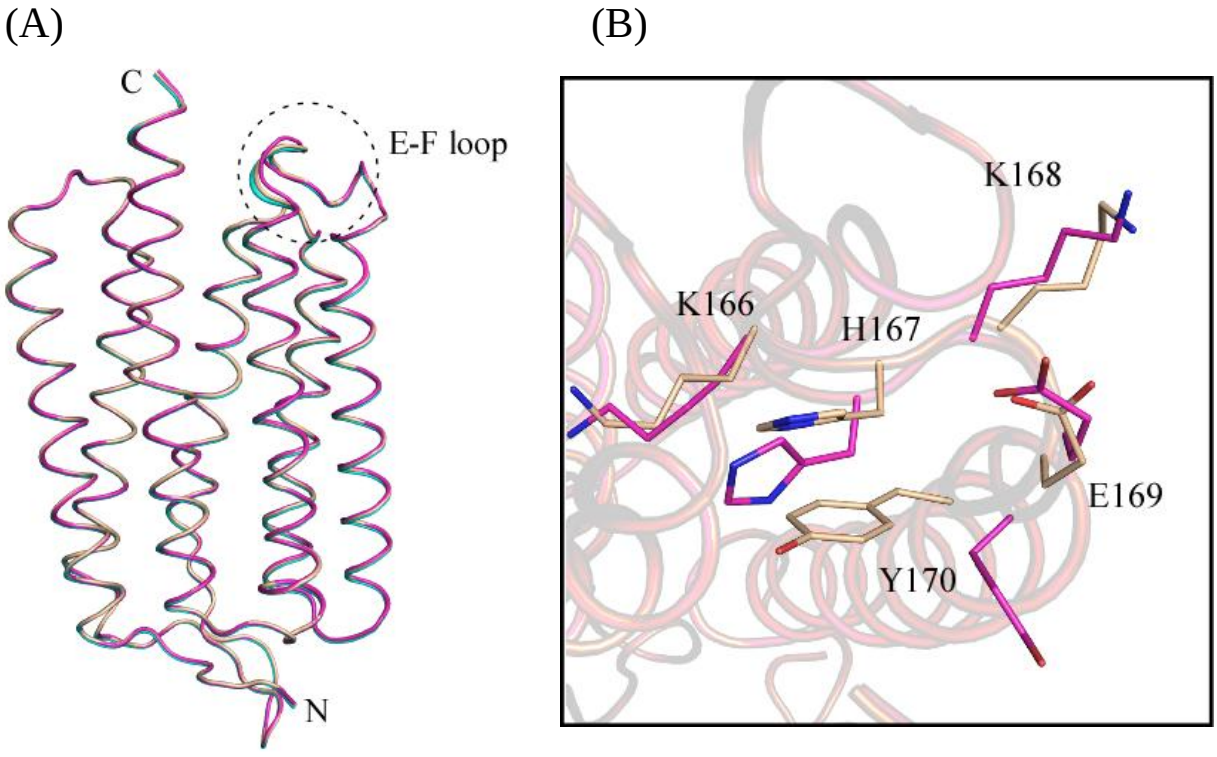

Figure S6. (A) Superimposition diagram of three monomers at pH 6.5, pH 7.0 and pH 8.0, colored wheat, cyan and pink, respectively. (B) Close-up view of the superimposition of the E-F loop. The diagrams at pH 6.5 and pH 8.0 are colored wheat and pink, respectively. See also Figure 8.

Table S1 B-factors of amino acids and water molecules in Figure 6 (A).

molecule	B factor
Thr 44	20.5
Asn 48	17.1
Leu 97	15.4
Asp 100	19.9
Trp 185	16.2
Asp 214	16.5
Lys 218	17.5
Tyr 221	18.3
water 501	27.4
water 503	21.5
water 505	16.7