Supporting Information

Freier et al. 10.1073/pnas.1104735108

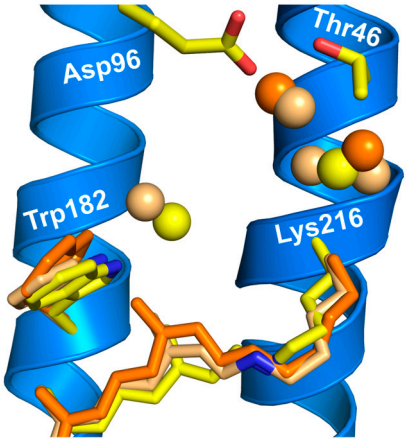

Fig. S1. *Water molecule positions and retinal movements in different structures.* Comparison of retinal movements and water position in the cytoplasmic half of bacteriorhodopsin (bR) as compared to WT [yellow, helices in blue, 1C3W (1)]. For comparison, the M intermediate crystal structure of mutant E204Q [1F4Z (2)] is shown in light orange and a late M of mutant D96N is shown in dark orange [1C8S (3)]. Movement of the C_{13} methyl group of the retinal is most pronounced in late M, as is the resulting movement of the indole ring of Trp182 and Lys216. Numbers and positions of water molecules differ, but in the M intermediate additional water molecules are moved from Wat501 into the vicinity of Asp96. None of these crystal structures show a chain of water molecules spanning Asp96 to Schiff base (SB).

1. Luecke H, Schobert B, Richter HT, Cartailler JP, Lanyi JK (1999) Structure of bacteriorhodopsin at 1.55 angstrom resolution *J Mol Biol* 291:899–911.
2. Luecke H, et al. (2000) Coupling photoisomerization of retinal to directional transport in bacteriorhodopsin *J Mol Biol* 300:1237–1255.
3. Luecke H, Schobert B, Richter HT, Cartailler JP, Lanyi JK (1999) Structural changes in bacteriorhodopsin during ion transport at 2 angstrom resolution *Science* 286:255–261.

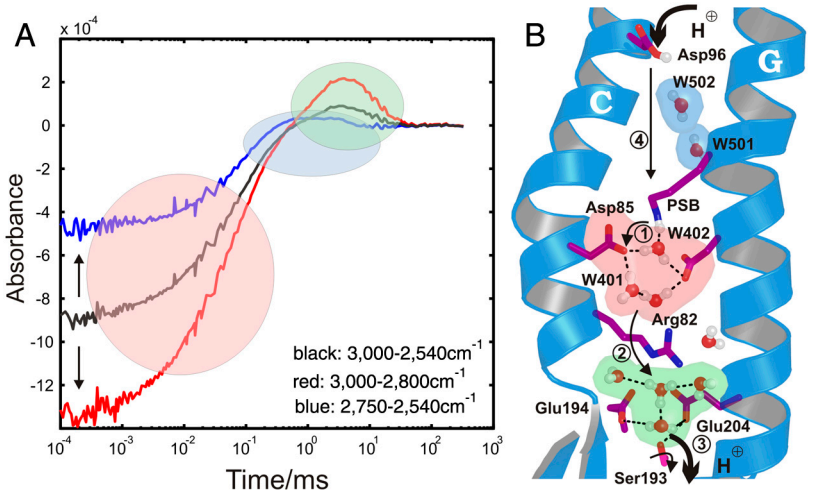

Fig. S2. *Decomposition of IR absorbance changes due to strong H-bonded water molecules.* Time-resolved absorbance changes of bR WT from 3,000 to 2,540 cm^{-1}, (*A*, black line) show the (not time-resolved) rupture of the strong H bond of water 402 (*B*, red) to SB due to isomerization, and the appearance of strong H bonds in the M intermediate at the release site (1) (*A* and *B*, green). Dividing this spectral range into smaller domains reveals different absorbance changes. The domain from 3,000 to 2,800 cm^{-1} (*A*, red line) shows the H-bond breakage of Wat402 (A, B, red) and the appearance of a strong H-bond at the release site (1) even more pronounced (*A* and *B*, green). The domain from 2,750 to 2,540 cm^{-1} (*A*, blue line) shows an overlaid positive contribution already in the apparent L-to-M transition (marked in blue in *A*), which we attribute now to a transient water chain (*B*, blue), responsible for the reprotonation of SB by Asp96 in step 4.

1. Garczarek F, Gerwert K (2006) Functional waters in intraprotein proton transfer monitored by FTIR difference spectroscopy *Nature* 439:109–112.

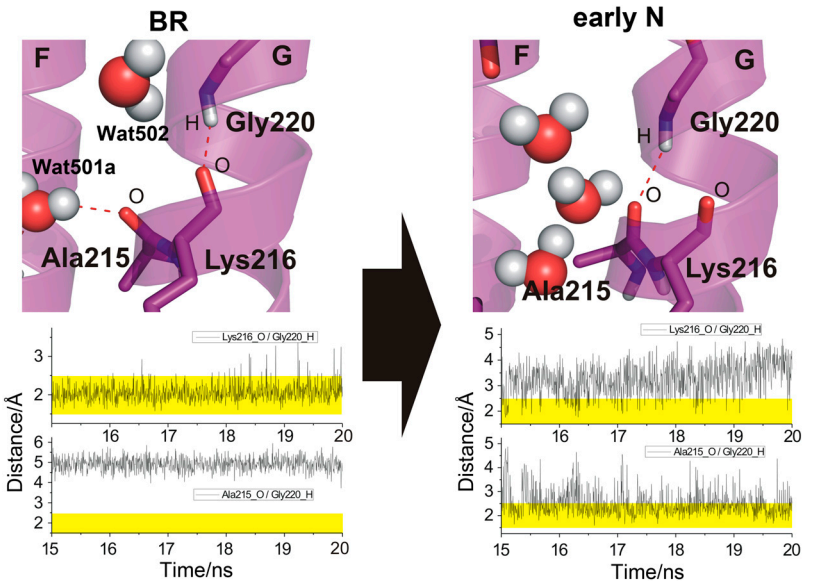

Fig. S3. *The π-bulge during molecular dynamics (MD) simulations*. The π-bulge during MD simulations in the bR ground state and the simulated N structure. Hydrogen bond distances are indicated in yellow. In the simulations of WT bR (1), Lys216 and Gly220 form a stable helical hydrogen bond. Ala215 is oriented out of the helix and forms hydrogen bonds to Wat501a. In the simulated N structure, the π-bulge has switched: Ala215 forms a hydrogen bond to Gly220, whereas the carbonyl group of Lys216 is oriented out of the helix. However, the Ala215/Gly220 hydrogen bond in the simulated N structure is less stable than the Lys216/Gly220 hydrogen bond in the bR ground state, and Lys216 can transiently form a hydrogen bond with Gly220. This suggests that the switch of the π-bulge defect in N applies additional conformational stress to helix G and may transiently store energy from the retinal twist relaxation in the backbone. This may drive the back isomerization to *all-trans* retinal after reprotonation in N.

1. Wolf S, Freier E, Gerwert K (2008) How does a membrane protein achieve a vectorial proton transfer via water molecules? *Chemphyschem* 9:2772–2778.

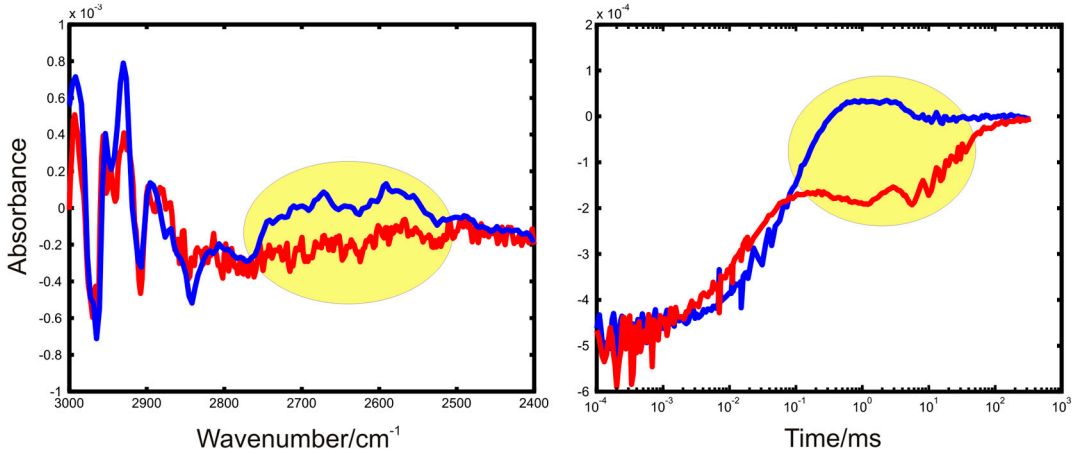

Fig. S4. *Spectrally and kinetically resolved absorbance changes of strong H-bonded water molecules*. M intermediate spectra are shown between 3,000 and 2,400 cm^{-1} (*A*) for WT (blue) and T89A (red), respectively. There is a clear additional band in the WT from 2,750 to 2,540 cm^{-1} as indicated in yellow (*A*). The time-dependent absorbance change between 2,750 and 2,540 cm^{-1} is shown in *B*. The additional band in the WT between 2,750 and 2,540 cm^{-1} disappears for T89A, and V49A (Fig. 1*B*). Its disappearance is better resolved by kinetics (*B*). An absorbance change in M is assigned to a linear water chain between Asp96 and SB.