Supplemental Information

The arrestin-1 finger loop interacts with two distinct conformations of active rhodopsin

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Calculation of peptide binding spectra (PBS)

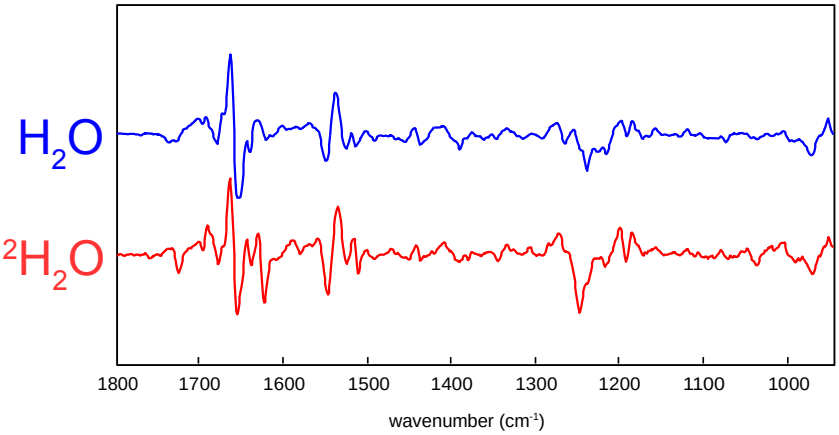
Peptide binding spectra (PBS) were determined as following: Firstly, difference spectra in the absence and presence of 20 mM ArrFL peptide were recorded. In a second step the two difference spectra were subtracted (w/ peptide minus w/o peptide) to obtain the PBS. To calculate this double difference spectrum both difference spectra have to be normalized, we chose the 1050-970 cm^{-1} region where mostly chromophore vibrations absorb. This second step is slightly more difficult for the PBS of the R*·ArrFL-1 complex, since at high pH a significant amount of inactive R (Meta I) is present in the presence and absence of ArrFL-1. To determine the amount of R (Meta I) to be subtracted, we minimized the following difference spectrum:

$$\Delta(\text{w/ ArrFL-1}) - (A*\Delta(\text{w/o ArrFL-1}) + B*\Delta(\text{Meta I}))$$

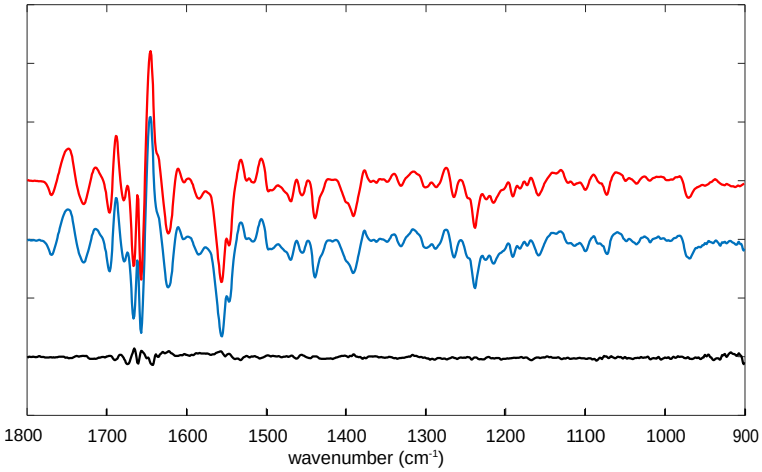
The pre-factors A and B were determined by a least squares fit in the region 1010-945 cm^{-1}, where Meta I exhibits a distinct positive band (cf. SI Figure 3).

In case of the R3.50L mutant pigment R (Meta I) correction was unnecessary, as the equilibrium or R and R* states becomes completely pH insensitive (3).

(1) Sommer, M. E.; Elgeti, M.; Hildebrand, P. W.; Szczepek, M.; Hofmann, K. P.; Scheerer, P. In Meth Enzymol; 2015; pp. 563–608.
(2) Elgeti, M.; Rose, A. S.; Bartl, F. J.; Hildebrand, P. W.; Hofmann, K.-P.; Heck, M. J. Am. Chem. Soc. 2013, 135, 12305.
(3) Vogel, R.; Mahalingam, M.; Lüdeke, S.; Huber, T.; Siebert, F.; Sakmar, T. P. J. Mol. Biol. 2008, 380, 648.



SI Figure 1. Difference spectra of the inactive R (Meta I) conformation stabilized at pH/p^{2}H 8.5, -14°C. Even for the spectra in the presence of 20 mM ArrFL-1, residual amounts of (R) were determined by a least-squares fit of the high pH spectrum to a mix of low pH spectrum and the spectrum of inactive (R). This fit was performed in the 1010-945 cm^{-1} region, where the inactive (R) conformation exhibits unique features due to specific ligand-protein interactions. The scaled spectra were then subtracted from the high pH (or p^{2}H) difference spectra to obtain pure deprotonated R* difference spectra.



SI Figure 2. Assessment of experimental signal to noise ratio. FTIR difference spectra of WT rhodopsin in egg-PC vesicles, pH 5.5, 30°C (R*H$^+$). Two separately prepared samples. Noise level is best observed in the double difference (black), certain regions exhibit higher noise due to strong absorption (H_2O or protein, 1700-1600 cm^{-1}), inhomogeneous IR source intensity or lower MCT detector sensitivity (below 950 cm^{-1}).