Supporting Information

Primary Photoinduced Protein Response in Bacteriorhodopsin and Sensory Rhodopsin II

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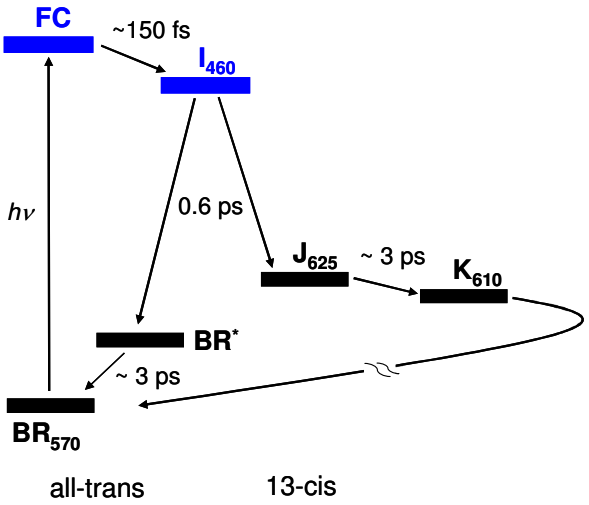

Fig. SI 1: Simplified scheme of the BR primary reaction. This scheme was suggested on the grounds of femtosecond time resolved IR spectroscopy[1]. It is in accordance with schemes derived earlier from UV/VIS transient absorption spectroscopy[2-4].

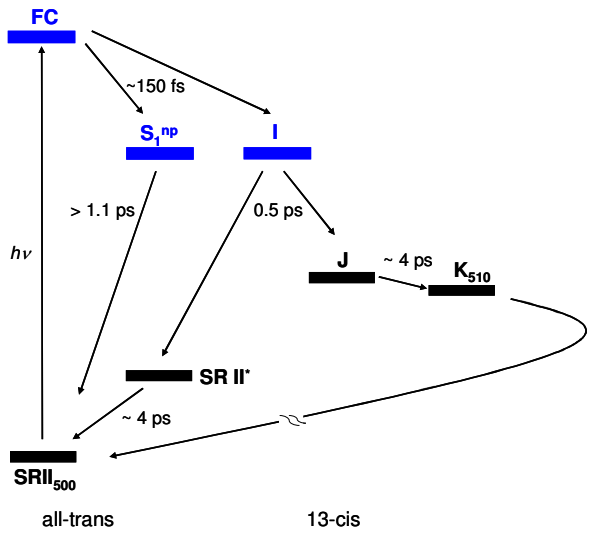

Fig. SI 2: Simplified reaction scheme of SRII as derived from this work. It combines almost identical features from the BR reaction scheme (Fig. SI.1) with a weak additional decay channel from an excited electronic state. The biphasic excited state decay is suggested by the global fit of the data from the fingerprint (FP) region (Fig. SI 3). At 1220 cm^{-1} positive signals appear with the system-response time and must be assigned to excited electronic state vibrational modes. Both, $A_{1,FP}$-SRII and $A_{2,FP}$-SRII, exhibit positive amplitudes at this position, indicating their biphasic decay.

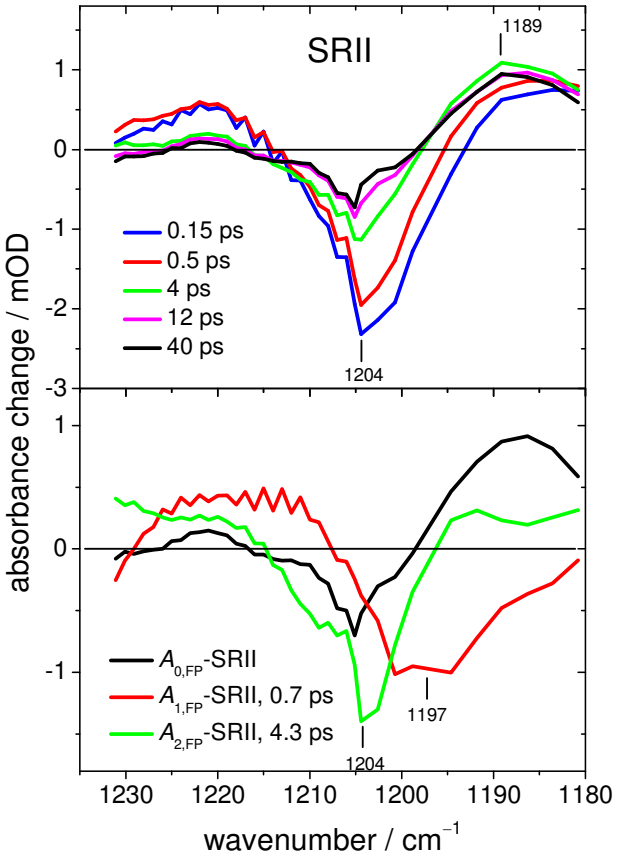

Fig. SI 3: Transient absorption (top) data and global fit (bottom) of the fingerprint region of SRII. The spectra presented here are extended versions of those published earlier[5] and reproduce the essential features. The negative band at 1204 cm^{-1} and the positive band at 1190 (top) correspond to the chromophore C_{14}-C_{15} stretching mode in the all-trans SRII ground state and in the 13-cis K-state, respectively[6]. In analogy to BR this band is a marker band for the chromophore configuration[7,8]. Accordingly, the DAS (bottom) display the formation of the 13-cis configuration with the short time constant (0.7 ps) only (neg. amplitude of $A_{1,FP}$-SRII around 1190 cm^{-1}) and the biphasic partial recovery of the all-trans configuration (neg. amplitude of $A_{2,FP}$-SRII and of $A_{1,FP}$-SRII at 1204 cm^{-1}).

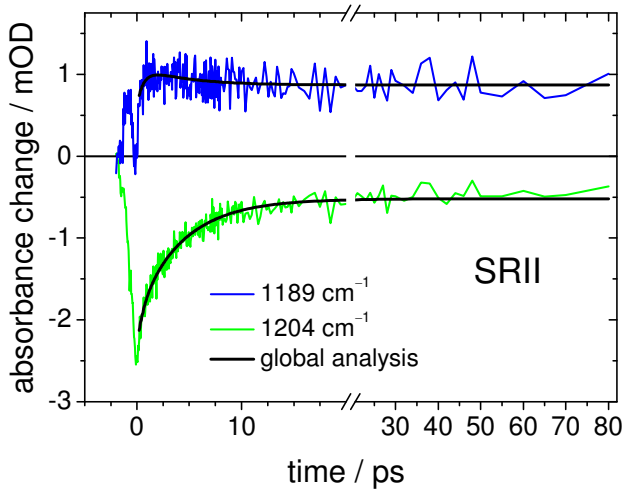

Fig. SI 4: Absorbance transients of SRII in the fingerprint region with the result of a global fit of this region (cp. Fig. SI 3) They display the fast (0.7 ps) rise of the 13-cis marker band (chromophore C_{14}-C_{15} stretching mode) at 1189 cm^{-1} and the slower partial recovery of the all-trans analogue at 1204 cm^{-1}.

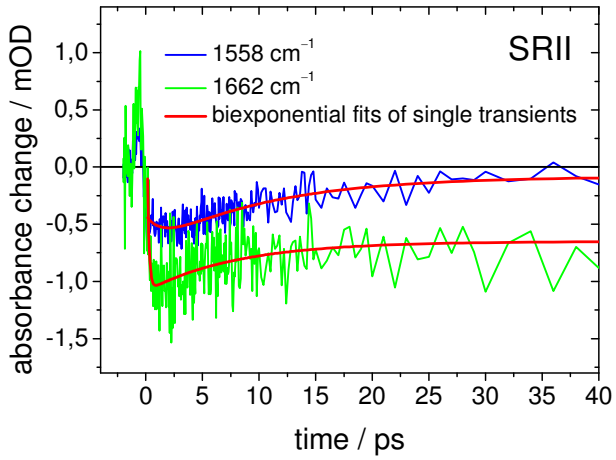

Fig. SI 5: Absorbance transients of SRII in the amide I and the amide II region with the result of a biexponential fit. Both time traces reflect the fast rise (ca. 1 ps) of the putative protein bands as well as their slow (ca. 11 ps) partial decay.

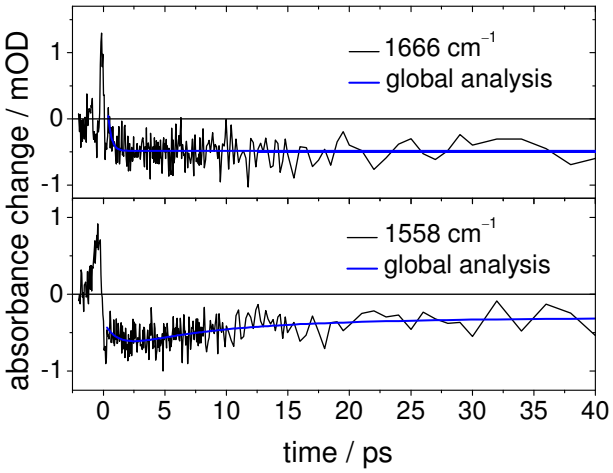

Fig. SI 6: Absorbance transients of SRII-D_2O in the amide I and the amide II region together with the results of global fits. Both time traces reflect the fast rise (ca. 1 ps) of the putative protein bands but in contrast to the band around 1558 cm^{-1} the band around 1666 cm^{-1} does not exhibit a slow component as the band at the similar position (1662 cm^{-1}) in SRII-H_2O (Fig. SI 5).

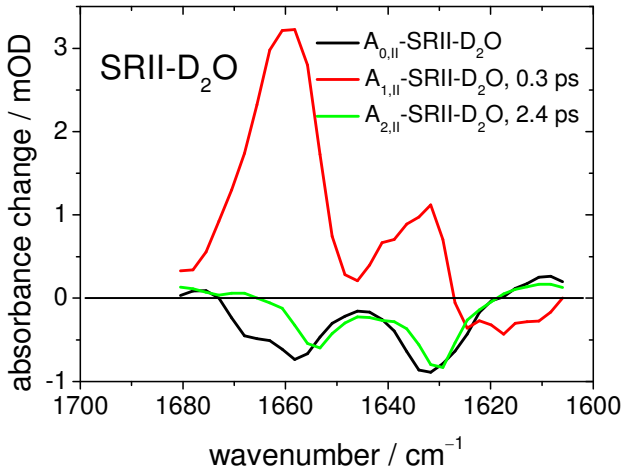

Fig. SI 7: Decay associated spectra of the amide I region of SRII in D_2O buffer obtained by a biexponential global analysis.

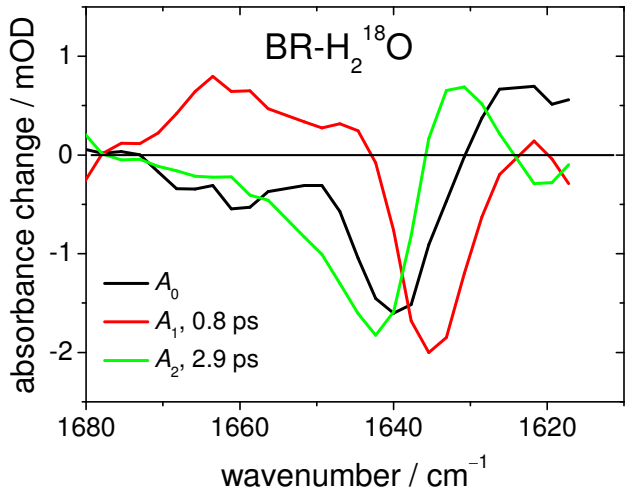

Fig. SI 8: Decay associated spectra (A_i=A_i-BR-$H_2{}^{18}O$) of the amide I region of BR in $H_2{}^{18}O$ obtained by a bixponential global analysis.

Estimate of the expected $H_2{}^{18}O$-effect in the IR spectra: The molar extinction coefficient of the stretching and bending vibrations of H_2O in liquid water is about 60 and 20 M^{-1} cm^{-1}, respectively[9]. Comparing low temperature FTIR spectra of BR[10] with our spectra and assuming 100% conversion of the initial BR to the K-state in the FTIR experiment and ca. 10% conversion in our experiment, the estimated absorbance difference for the bending vibration of one water molecule in our experiment is ca. 0.03 mOD. This is currently beyond the detectable limit of our experiment in this spectral region which is limited by the small IR transmittance (due to the high amide I absorbance background) and the finite degree of the homogeneity of the protein film.

References

(1) Herbst, J.; Heyne, K.; Diller, R. *Science* **2002**, *297*, 822-825.

(2) Dobler, J.; Zinth, W.; Kaiser, W.; Oesterhelt, D. *Chemical Physics Letters* **1988**, *144*, 215-220.

(3) Mathies, R. A.; Brito Cruz, C. H.; Pollard, W. T.; Shank, C. V. *Science* **1988**, *240*, 777-779.

(4) Doig, S. J.; Reid, P. J.; Mathies, R. A. *The Journal of Physical Chemistry* **1991**, *95*, 6372-6379.

(5) Diller, R.; Jakober, R.; Schumann, C.; Peters, F.; Klare, J. P.; Engelhard, M. *Biopolymers* **2006**, *82*, 358-362.

(6) Hein, M.; Wegener, A. A.; Engelhard, M.; Siebert, F. *Biophysical Journal* **2003**, *84*, 1208-1217.

(7) Gerwert, K.; Siebert, F. *EMBO Journal* **1986**, *5*, 805-811.

(8) Braiman, M.; Mathies, R. *Proceedings of the National Academy of Sciences-Biological Sciences* **1982**, *79*, 403-407.

(9) Bayly, J. G.; Kartha, V. B.; Stevens, W. H. *Infrared Physics* **1963**, *3*, 211-222.

(10) Kandori, H.; Kinoshita, N.; Shichida, Y.; Maeda, A. *Journal of Physical Chemistry B* **1998**, *102*, 7899-7905.