# Ultrafast Protein Response in Channelrhodopsin-2 Studied by Time-Resolved Infrared Spectroscopy

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## S1 Methods

### Vis-pump-IR-probe experiments

Samples of ChR2 were prepared in $D_2O$ as described before.[1] The vis-pump/IR-probe measurements were performed using a solubilized ChR-2 sample in HEPES buffer solution (20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 100 mM NaCl, 0.1 % n-decyl-β-D-maltopyranoside (DM)) in $D_2O$ at pD = 7.4. The concentration was adjusted in a $CaF_2$ window with 50 μm path length to an optical density of 0.12 at 450 nm. For transient IR experiments, we used a Clark MXR-CPA-2001 laser system (Horiba Jobin Yvon GmbH, Bensheim, Germany) with a central wavelength of 775 nm, repetition rate of 1 kHz and pulse duration of 170 fs. The 480 nm excitation pulse with ~300 nJ pulse energy was generated using a non-collinear optical parametric amplifier (NOPA).[2] The IR probe-pulse generation as well as the general experimental setup is described by Neumann et al.[3]

### Data analysis

Data analysis of the transient absorption was performed using the program z20, based on IDL 6.0 as described before.[4] The transient IR data were used from 0.4 ps on due to the perturbed free induction decay (PFID)[5,6] and the cross-phase modulation[7] at negative time delays and around time zero. The remaining data were fitted with a sum of n = 3 exponential decay functions in a global fit analysis. The time- and wavelength-dependent absorbance change is given by

$$\Delta A(\lambda, t) = \sum_{i=1}^{n} A_i(\lambda) exp\left(\frac{t_c^2}{4\tau_i^2} - \frac{t}{\tau_i}\right)\left[\frac{1}{2} erf\left(\frac{t}{t_c} - \frac{t_c}{2\tau_i}\right) + \frac{1}{2}\right]$$

with the cross-correlation width $t_c$, the wavelength-dependent fit amplitude $A_i$ and the time constants $\tau_i$.

## S2 References


(1) Bamann, C.; Gueta, R.; Kleinlogel, S.; Nagel, G.; Bamberg, E. Structural Guidance of the Photocycle of Channelrhodopsin-2 by an Interhelical Hydrogen Bond. *Biochemistry* **2010**, *49*, 267–278.

(2) Riedle, E.; Beutter, M.; Lochbrunner, S.; Piel, J.; Schenkl, S.; Spörlein, S.; Zinth, W. Generation of 10 to 50 Fs Pulses Tunable through All of the Visible and the NIR. *Appl. Phys. B* **2000**, *71*, 457–465.

(3) Neumann, K.; Verhoefen, M.-K.; Weber, I.; Glaubitz, C.; Wachtveitl, J. Initial Reaction Dynamics of Proteorhodopsin Observed by Femtosecond Infrared and Visible Spectroscopy. *Biophys. J.* **2008**, *94*, 4796–4807.

(4) Neumann-Verhoefen, M.-K.; Neumann, K.; Bamann, C.; Radu, I.; Heberle, J.; Bamberg, E.; Wachtveitl, J. Ultrafast Infrared Spectroscopy on Channelrhodopsin - 2 Reveals Efficient Energy Transfer from the Retinal Chromophore to the Protein. *J. Am. Chem. Soc.* **2013**, *135*, 6968–6976.

(5) Hamm, P. Coherent Effects in Femtosecond Infrared Spectroscopy. *Chem. Phys.* **1995**, *200*, 415–429.

(6) Wynne, K.; Hochstrasser, R. M. The Theory of Ultrafast Vibrational Spectroscopy. *Chem. Phys.* **1995**, *193*, 211–236.

(7) Ekvall, K.; van der Meulen, P.; Dhollande, C.; Berg, L.-E.; Pommeret, S.; Naskrecki, R.; Mialocq, J.-C. Cross Phase Modulation Artifact in Liquid Phase Transient Absorption Spectroscopy. *J. Appl. Phys.* **2000**, *87*, 2340–2352.