Electronic Supplementary Information for

A quantum cascade laser setup for studying irreversible photoreactions in H₂O with nanosecond resolution and microlitre consumption

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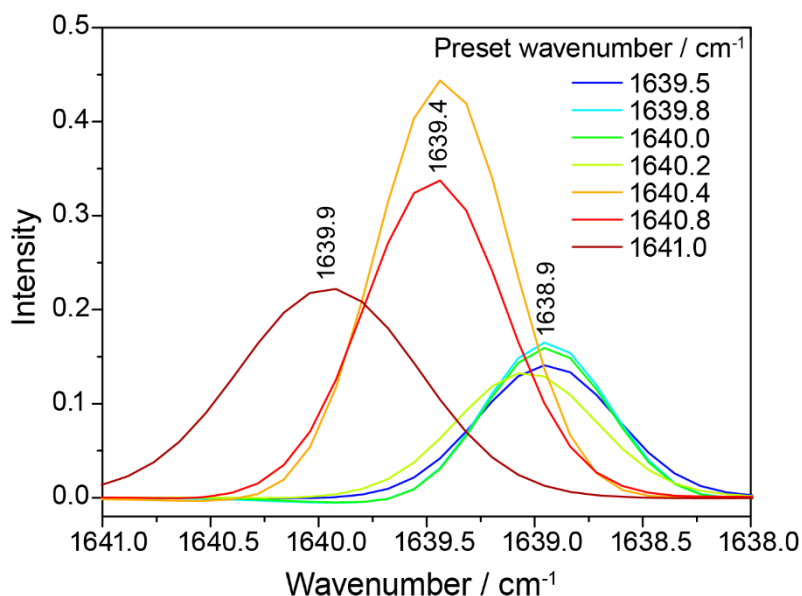
Fig. S1 Determination of the QCL wavenumber by coupling a QCL beam as the light source into an FTIR spectrometer. The entire spectral tuning range of the laser was scanned stepwise and a linear calibration function was calculated. Moreover, the selected region of the spectrum shows that only discrete wavenumbers corresponding to QCL modes are accessible. Taken together, a deviation of ±0.5 cm^{-1} was determined for the entire spectral tuning range of the laser. It should be noted that the real bandwidth of the QCL is much smaller, but is folded with the experimental resolution of 0.5 cm^{-1}. Experiments were performed on an IFS 66v FTIR spectrometer (Bruker, Ettlingen, Germany) using a focused QCL beam.
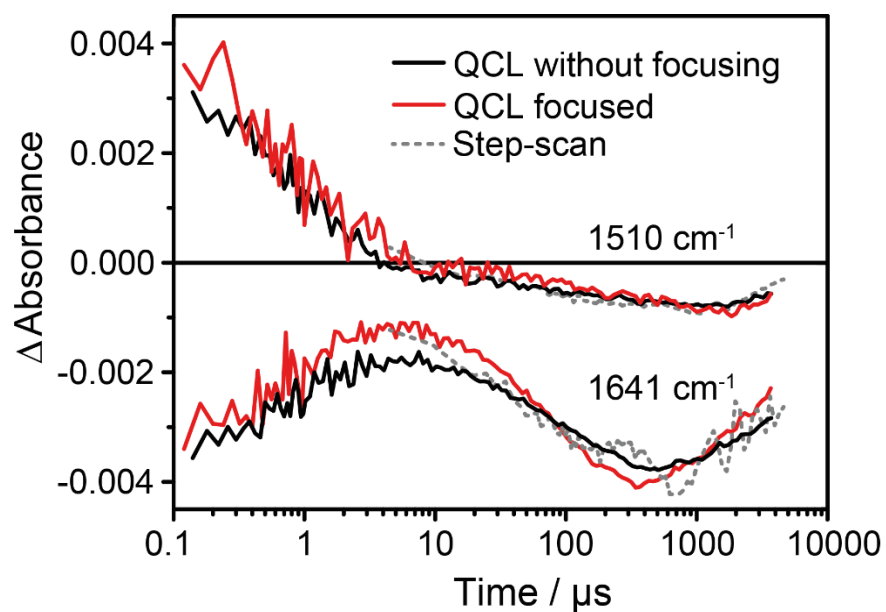
Fig. S2 Investigation of heating effects caused by focusing of the QCL beam. The proton pump bacteriorhodopsin (BR) was used as a model system.[1] Fragments of purple membrane were studied as a well-hydrated film at pH 7.2, sandwiched between two BaF_2 windows. The temperature was kept constant at 17 °C by a circulating water bath and the sample was excited by a Nd:YAG laser at 532 nm with a repetition rate of 0.25 Hz. The kinetics at two selected wavenumbers with 100 averages each are in agreement with step-scan FTIR measurements under the same experimental conditions except for a higher repetition rate of 2 Hz. Moreover, kinetics recorded with focusing of the QCL beam show no deviation from experiments without focusing.
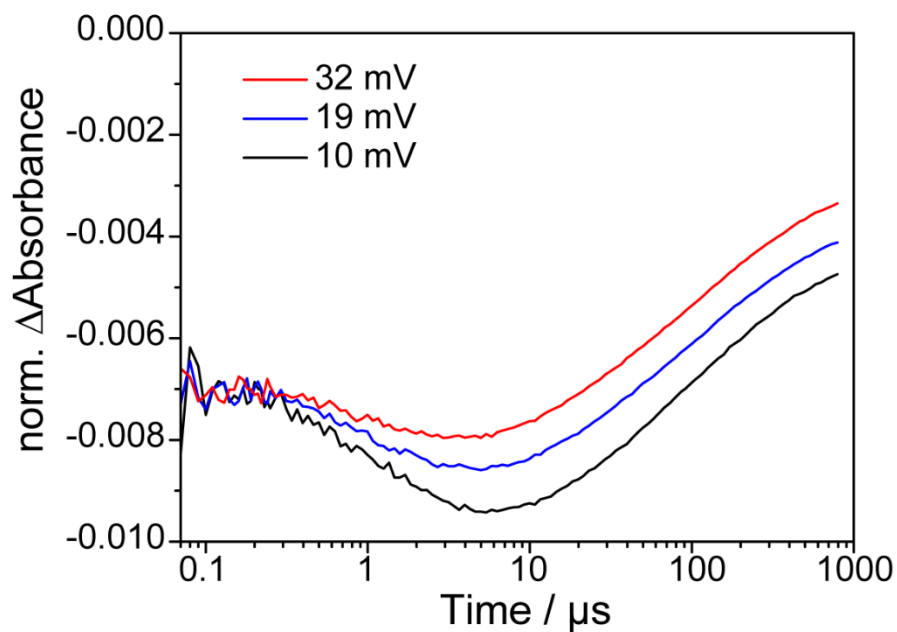
Fig. S3 Dependence of the reaction rate on the concentration of photoexcited flavin. The excitation energy of the Nd:YAG laser was increased to generate different concentrations of photoexcited molecules. The kinetic traces were scaled to the difference absorbance at 100 ns of the lowest excitation energy (black line). The reaction rate in the time range of 10 μs to 1 ms increased with higher anion radical concentration, which excludes a first order reaction but is consistent with a bimolecular disproportionation of the anion radicals. Kinetics were measured at 1550 cm^{-1} on 20 mM FMN, 70 mM EDTA, 50% (w/w) sucrose in 50 mM phosphate buffer, pH 9.0, with 60 averages each and a flow rate of 100 nL / min without using the micrometre stage.
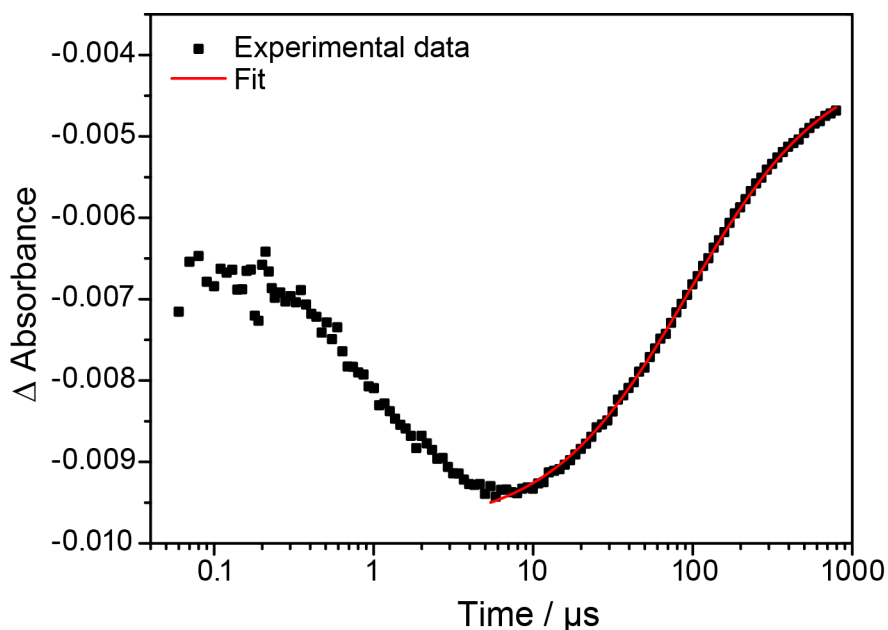
Fig. S4 Fit of the recovery of oxidized FMN at 1550 cm^{-1} in the time range later than 5 μs. Under the assumption that the anion radical does not absorb at this wavenumber,[2] product formation is directly observed. The increase in difference absorbance of oxidized FMN is then defined by the integrated second-order rate law[3]

$$\Delta A(t) = \frac{\varepsilon d}{2}\left(C_0 - \frac{1}{C_0^{-1} + kt} \right) + y_0$$

with C_0 being the starting concentration of anion radical. An offset y_0 is introduced to compensate for the negative difference absorbance at the start of the reaction. The extinction coefficient of oxidized FMN at 1550 cm^{-1} has been determined to ε = 2200 L mol^{-1} cm^{-1} [4] and the path length of the flow cell to d = 8 μm. The fit yielded a rate constant of k_{1550} = 1.6 x 10^6 s^{-1} M^{-1} and a starting concentration of the flavin anion radical of C_0 = 0.0066 M.

Supplementary References

1   J. K. Lanyi, *Annu. Rev. Physiol.*, 2004, **66**, 665-688.
2   C. B. Martin, M. L. Tsao, C. M. Hadad and M. S. Platz, *J. Am. Chem. Soc.*, 2002, **124**, 7226-7234.
3   J. H. Espenson, *Chemical kinetics and reaction mechanisms*, 1995, **2nd edition**, 70-100.
4   M. Spexard, D. Immeln, C. Thöing and T. Kottke, *Vib. Spectrosc.*, 2011, **57**, 282-287.