Rapid Reaction Monitoring of Proteins with Dual-Comb Spectroscopy

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Scope of this work

Time-resolved infrared spectroscopy is a powerful tool to acquire information useful for elucidating structural changes and protonation dynamics in proteins. While step-scan FT-IR or scanning techniques require several excitations to acquire spectro-temporal information, dual comb spectroscopy (DCS) provides access to time-resolved IR spectra even with single shot experiments [1][2]. Therefore DCS based on quantum cascade lasers (QCL) provides an opportunity to investigate even irreversible reactions. Here we present the first DCS data in the Amide I region of the well studied proton pump bacteriorhodopsin (bR) and provide a comparison to our homebuilt tunable QCL based setup[3].

External Cavity QCL

Time-Resolved IR Spectroscopy Based on **Tunable External Cavity Quantum Cascade Lasers (EC-QCL)**

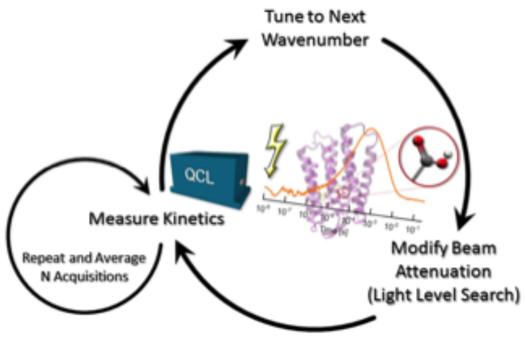

Fig.1: Principle of time-resolved infrared spectroscopy using EC-QCLs. After tuning the QCL to a specific wavenumber the beam attenuation is regulated to maximize the signal-to-noise ratio. Multiple acquisitions can be averaged before tuning the QCL to the next wavenumber and repeating the process. Measurements for different wavenumbers are independent from each other. Central image adapted from [3].

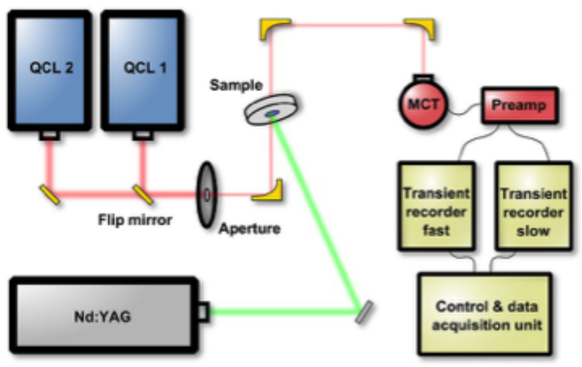

Fig.2: Experimental setup for monitoring transient infrared absorbance changes after laser excitation using cw EC-QCL emission as measuring light, as shown in [3]. The signal measured by the MCT (HgCdTe) detector is recorded with two oscilloscopes operating at different sampling rates to get information on a wide time range with a single acquisition. The reference intensity is determined from the measured pre-trigger values.

Key Facts:

- Tunable Emission (150-200 cm^{-1} nominal tuning range for each laser cavity)
- Wavelength Accuracy ≤ 1 cm^{-1}
- Time Resolution 20 ns (50 MHz bandwidth)

Introducing IRis-F1

Dual-Comb Spectroscopy Based on Quantum Cascade Laser (QCL) **Frequency Combs**

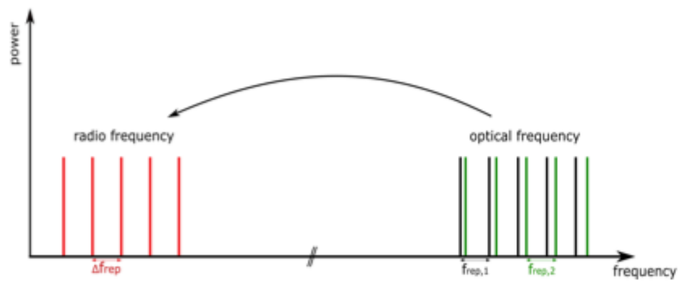

Fig.3: Principle of dual-comb spectroscopy, adapted from [1]. Two frequency combs with slightly different repetition rates (f_{rep}) create a multiheterodyne beat in the radio frequency (rf) range. Thereby an absorption feature in the optical frequency range is mapped onto the rf range.

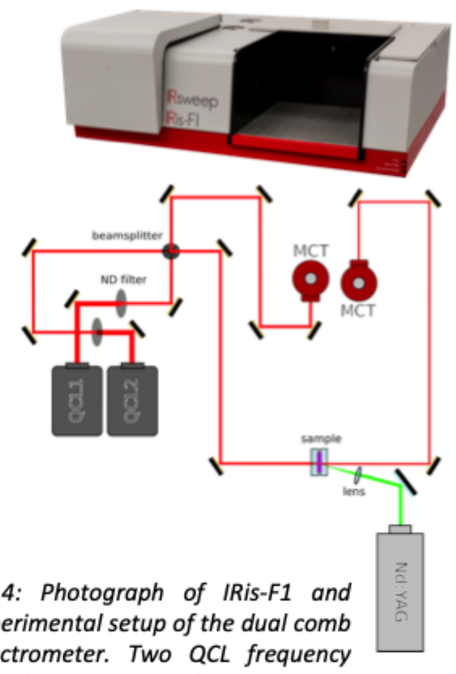

Fig.4: Photograph of IRis-F1 and experimental setup of the dual comb spectrometer. Two QCL frequency combs are combined at a beamsplitter after attentuation by neutral density filters (ND filter). One combined beam is passed through the sample chamber, the other one is used as reference. Both beams are detected by two separate MCT (HgCdTe) detectors. The sample is excited by a Nd:YAG laser. Adapted from [1].

Key Facts:

- 'broadband' emission (ca. 50 cm^{-1} bandwith / laser module)
- spectral resolution up to 3 x 10^{-4} cm^{-1} (laser linewidth)
- spectral sampling ca. 0.3 cm^{-1}
- time resolution ca. 1 µs (sub-microsecond possible)
- single-shot experiments possible[2]

Bacteriorhodopsin: EC-QCL vs DCS

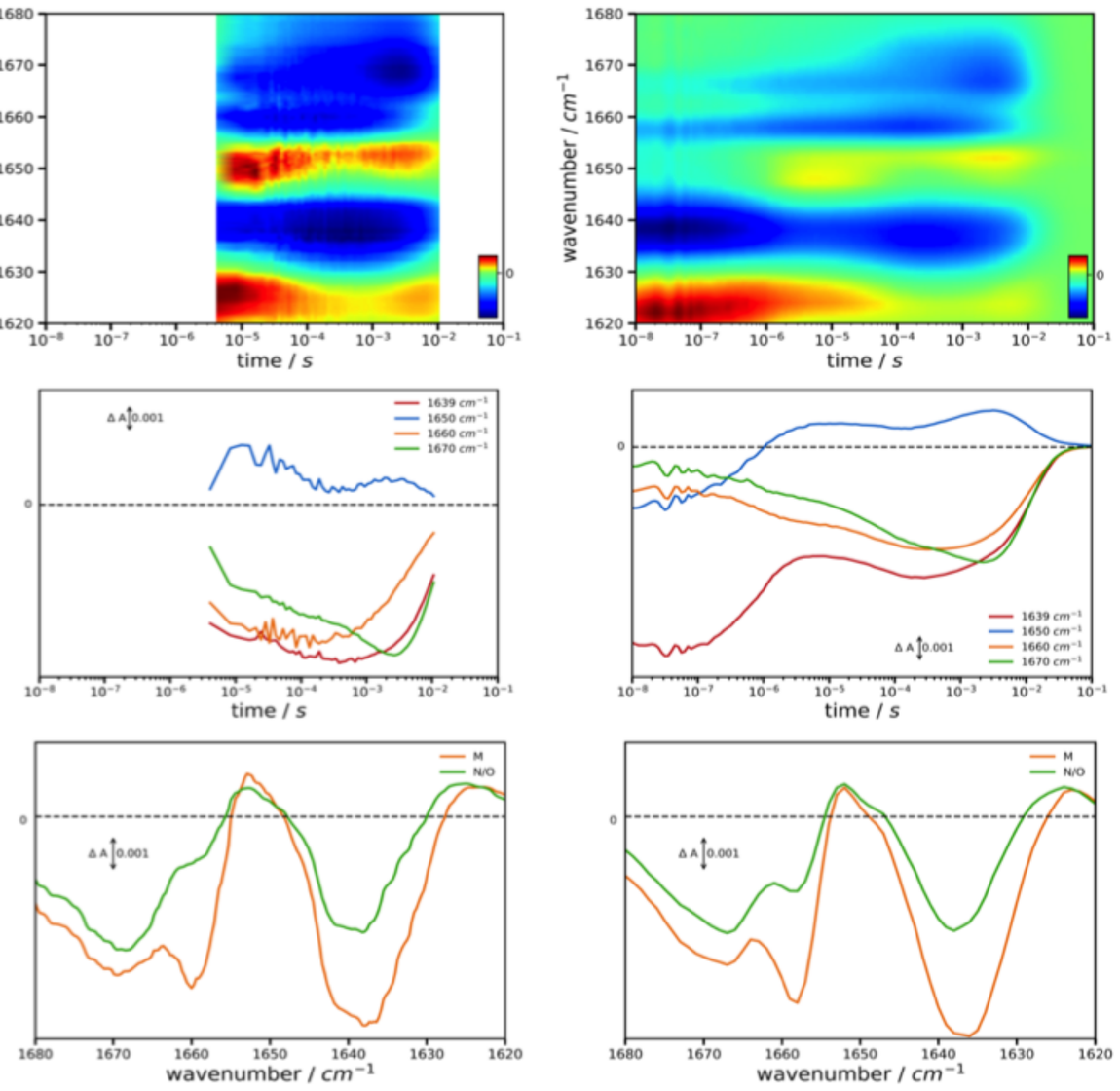

Fig.5: Comparison of time-resolved IR absorption data of bacteriorhdopsin acquired by a tunable QCL-setup (right) and DCS (left). Top: Contour plot covering the frequency range from 1620 – 1680 cm^{-1} . Middle: Kinetic traces of the most prominent bands: 1639 cm^{-1} (-) Schiff-base vibration, 1650 cm^{-1} (+) and 1660 cm^{-1} (-) and 1670 cm^{-1} (-) amide I vibrations as already reported in [4,5]. Bottom: Spectra 299 µs (orange) and 8.5 ms (green) after light-excitation at 4.5 cm^{-1} spectral resolution, representative for the M- and N/O-intermediate. Both data sets were acquired by averaging over 3000 laser excitations.

Approaching single-shot experiments

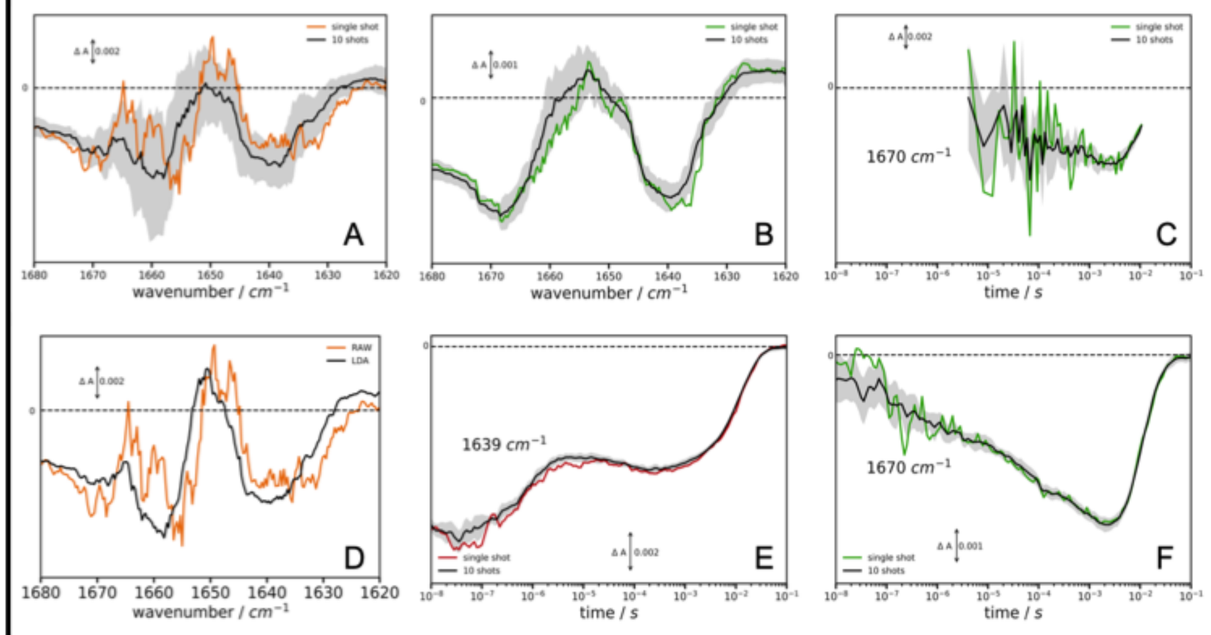

Fig.6: comparison of single-shot experiments using the DCS and EC-QCL setup. DCS (A-D) single shot spectra at 299 µs (A), 8.5 ms (B), 1670 cm^{-1} kinetic (C). In all plots except for (D) the black trace is the average of 10 single-shots with their standard deviation as grey shading. (D) shows the same single shot spectra as in (A) but with a LDA fitted spectra as black trace. (E) and (F) show the 1639 cm^{-1} and 1670 cm^{-1} kinetic respectively acquired by the EC-QCL setup.

Observing protonation dynamics

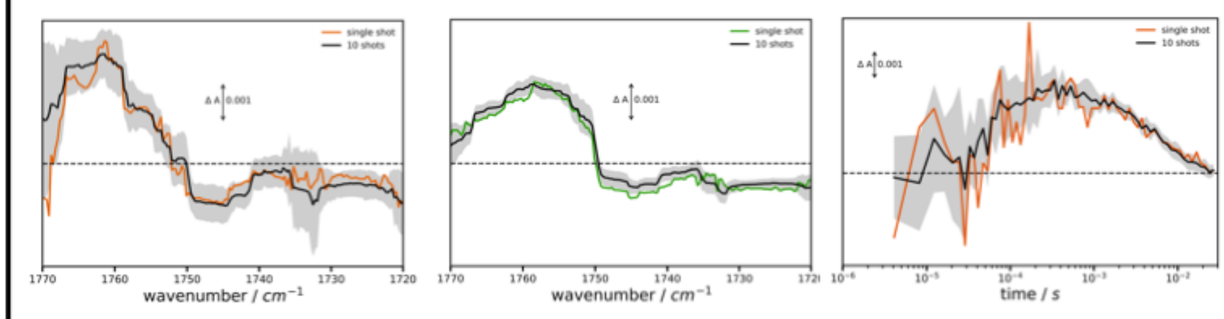

Fig.7: Protonation dynamics of bR. Left: M-state (orange, 300 µs) and left N/O state (green, 3.5 ms) spectra acquired by averaging 10 single-shots (black). Right: Kinetic trace of the band at 1762 cm^{-1}. This band is assigned to the C=O stretching vibration of the primary proton acceptor D85 [6].

References

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