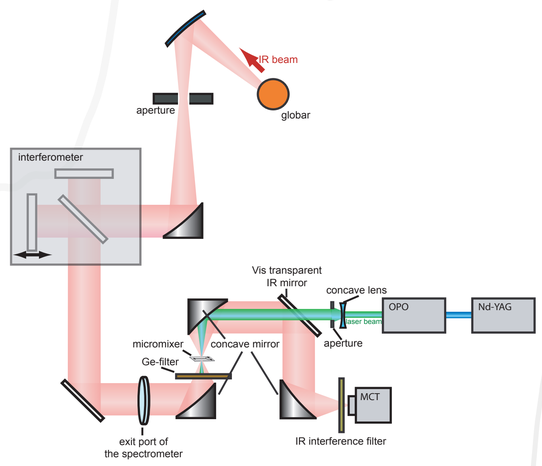
Light induced time resolved Flow-Flash FTIR investigation in micro mixing cells

Christoph Wagner[1], Michael Schleeger[2], Michiel Vellekoop[3], Joachim Heberle[2], Bernhard Lendl[1]
[1]Inst. of Chemical Technologies and Analytics, Vienna University of Technology, Austria
http://www.cta.tuwien.ac.at/cavs
[2]Biophysical Chemistry (PCIII), Bielefeld University, Germany
[3]Inst. of Sensor and Actuator Systems, Vienna University of Technology, Austria

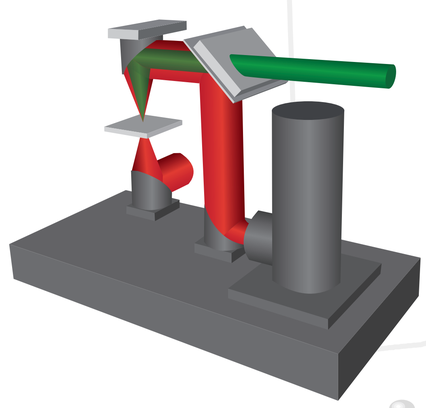
Abstract

In this work we present our latest developments for step scan FTIR measurements to widen its applicability to noncyclic reactions. With our approach the reaction between two chemicals can be monitored as well with a time resolution in the µs time range.
We are using a micro mixing device [1, 2] to pump the sample solution through the measurement spot of a specially designed focusing unit. The reaction in the mixer is triggered by a laser flash and the spectrometer starts measuring the respective timeslice shortly before the laser flash hits the sample. While the measurement takes place the sample is continously flowing. The flow rate is set in such a way that only a small portion of the sample volume is exchanged during the measurement. After the measurement of the timeslice the whole volume is purged and the next laser pulse is fired onto a completley fresh sample in order to measure the next timeslice. A typical step-scan experiment consumes only a few 10 µl of a mM sample solution, rendering it useful especially for biological samples.
We applied our technique to the photodissociation of the CO-myoglobin complex and followed the rebinding of CO including changes in the protein.

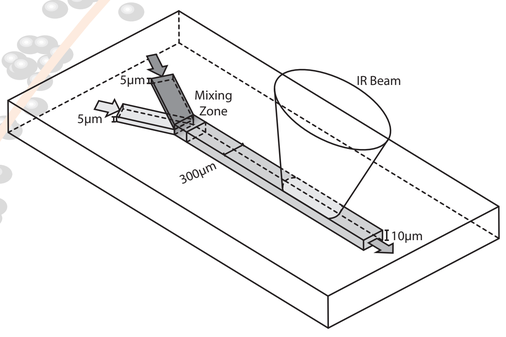
Experimental Setup



The IR radiation of a Bruker IFS66 v/S was focused onto the microchip from beneath with our focusing device, as shown above. The laser flash initiating the photolysis of the sample was focused onto the mixer from above and was blocked beneath it with an IR transparent Ge-filter. The laser wavelength was adjusted to 540 nm by an OPO which was driven by the third harmonic of a Nd:YAG laser.

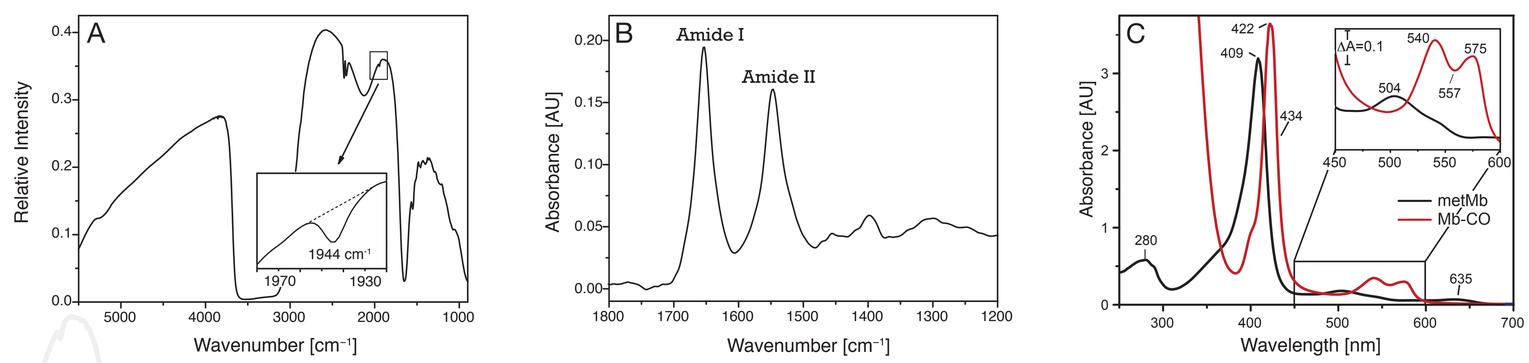


To focus the light beams onto the chip the focusing unit shown above was used. The laser and the IR beam are combined via a dichroic mirror from Kevley Technology which is highly reflective to infrared and transmissive to visible laser radiation. To focus the light beams onto the mixer and the MCT detector three parabolic mirrors were used.
The experiments were carried out in two spectral regions by using interference filters reducing the interferogram points during the S^2 - FTIR experiment to a minimum.



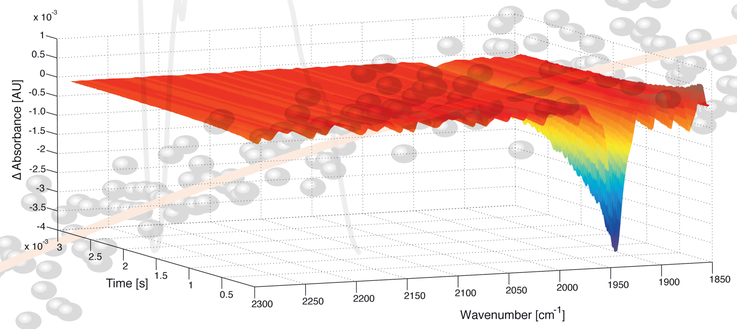
The micro fluiding cell used consists of two 1 mm thick CaF_2 windows sealing the micro structures on the top and bottom. The structures themselves are built through a photostructuring process in SU8 resin. Two 5 µm thick laminar flow sheets are combined forming the 10 µm thick and 300 µm broad observation channel.

Sample Preparation

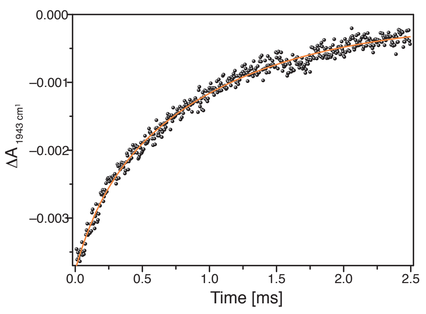


A 4 mM myoglobin solution was chemically reduced by adding $Na_2S_2O_4$ under anaerobic conditions. The gas phase above the sample solution was replaced by carbon monoxide and the solution stirred for 30 minutes.
Figure A and B show IR spectra of the Mb-CO complex in the mixing cell. Figure A shows a single channel spectrum where the band of the bound CO at 1943 cm^{-1} is clearly visible whereas figure B shows an absorption spectrum of the Amide region. Due to the small pathlength of 10 µm even the Amide I band can by clearly detected in an aqueous medium.
Figure C shows UV/VIS spectra of myoglobin and the Mb-CO complex. The strong absorption of the β–band of CO-bound heme at 540 nm was selected for the laser excition wavelength.

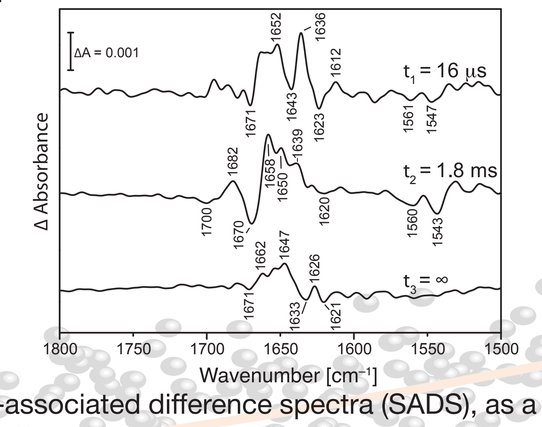
S^2-FTIR Measurement[3]



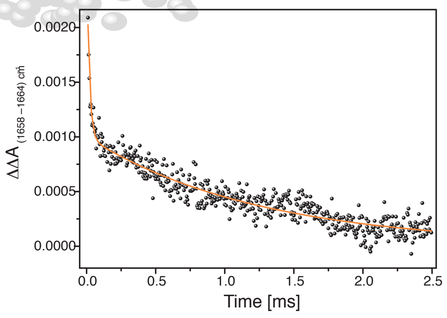
The first 36 spectra, before the laser hits the sample, were averaged and used as the background spectrum. The figure above shows the CO rebinding at 1943 cm^{-1} after reducing the noise level by applying a SVD analysis.



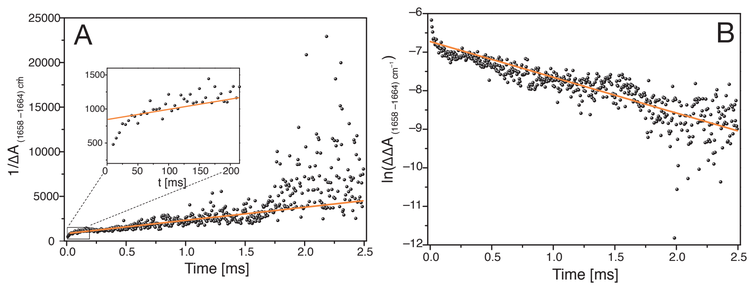
A bi-exponential fit applied to the CO stretching vibration (shown in orange) resulted in time constants of τ_1 = 185 µs and τ_2 = 1.0 ms. A kinetic analysis revealed second order kinetics at the beginning and pseudo first order kinetics for t > 1 ms.



Species-associated difference spectra (SADS), as a result of a global fitting analysis, are shown above with the latter one not decaying over the recording range of 2.5 ms.



A time trace to a band in the Amide region is plotted above. Baseline drifts were eliminated by taking the difference to an isobestic point. The early changes (t < 1 ms) exhibit a second order kinetics (Fig. A below), being in good agreement with the CO rebinding.



The first few data points (5 to 30 µs) show a significant deviation from the linear behaviour implying that these changes are not correlated to the CO rebinding. This could be explained with an ongoing relaxation of the Mb-CO conformation to deoxy-Mb after photolysis. Plotting the reaction time against the natural logarithm of the absorption reveals a first-order behaviour for 1 to 2.5 ms.

Conclusion

We could demonstrate the coupling of a flow-flash experiment with IR step-scan measurements for the first time.

This widens the applicability of the step-scan technique to monitor slow cyclic reactions such as the reaction of the retinal protein with light or even to the reaction of cytochrome c oxidase with oxygen.

Our method is particularly interesting for samples which are only available in small quantities, such as biological samples, due to its small sample consumption.

[1]N. Kaun, S. Kulka, J. Frank, U. Schade, M.J. Vellekoop, M. Harasek, B. Lendl, Analyst, 131 (2006), 489-494
[2]P. Hinsmann, J. Frank, P. Svasek, M. Harasek, B. Lendl, Lab Chip, 1 (2001), 16-21
[3]M. Schleeger, C. Wagner, M.J. Vellekoop, B. Lendl, J. Heberle, ABC, 2009, DOI 10.1007/s00216-009-2871-0

Financial support from Carinthian Tech Research AG and the COMET Competence Center Program of the Austrian Government is gratefully acknowledged.