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A Stop-Flow Sample Delivery System for Transient Spectroscopy

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A stop-flow sample delivery system for transient spectroscopy is presented, which is in particular suited for laser based instruments (quantum cascade lasers or amplified femto-second lasers) with excitation pulse repetition rates in the range 10-100 Hz. Two pulsing micro-valves are mounted onto a flow-cuvette designed for transient IR spectroscopy, which is integrated into a flow cycle driven by a peristaltic pump. The performance of the system is demonstrated with transient IR experiments of the *trans*-to-*cis* photoisomerization of a water-soluble azobenzene derivative. The sample stands still when the micro-valves are closed, and is pushed out from the probe beam focus on a 1 ms time-scale when opening the micro-valves. The setup is extremely sample efficient. It needs only small sample volumes and at the same time enables excitation of a large fraction of molecules in solution.

I. INTRODUCTION

In pump-probe spectroscopy, or more traditionally in flash photolysis, a sample is excited with an actinic light pulse and then probed as a function of time after this event. Probing can be either with *cw*-light,¹⁻⁹ a properly delayed probe pulse,¹⁰⁻¹³ or more recently with a sequence of probe pulses derived from a high frequency (100 kHz) laser source¹⁴⁻¹⁶ or a LED.¹⁷ The experiments we have in mind here are on photo-active proteins with photoreactions that cover a wide range of time-scales from picosecond for the initial photochemical reaction of the chromophore to milliseconds or even longer for the response of the protein.¹⁸

In order to achieve a good signal-to-noise ratio, the data very often have to be averaged over many excitation events. If the sample does not regenerate into its resting state fast enough, the sample needs to be exchanged between subsequent excitation pulses. The sample exchange is commonly achieved by continuous flow of the sample,¹⁹ sometimes in connection with a translation of the sample cell,^{8,14,15} or by scanning the measurement beams.²⁰ In either case, the dead time between the usable time range after one excitation pulse and the subsequent excitation event is at least a factor of 10. That is, one has to adjust the flow rate and/or the translation speed such that the excited sample is essentially stationary in the probed volume during the probed time, but exchanged with fresh sample at the time of the next excitation pulse. For slow overall reaction cycles, the repetition rate of the excitation pulses is not limiting. A dead time of a factor of 10 then prolongs the total measurement time by the same amount, which is very unsatisfying and sometimes prohibitive.

This situation calls for a stop-flow setup, in which the sample stands still for the time-range of the measurement and then is pushed out quickly from the probed region in the sample cuvette just before the next excitation pulse arrives. For laser-based instruments, where the beam foci in the sample are typically small ($\sim 100 \mu\text{m}$), the

the sample needs to stand still on a few μm scale.

In a recent paper,¹⁷ we have addressed this issue with a stop-flow setup based on a syringe pump driven with a pulsed stepper motor. However, when transferring this setup from the transient fluorescence spectrometer in Ref. 17 to a transient IR spectrometer,¹⁶ it no longer worked. The only difference of the sample delivery system has been the thinner sample thickness needed for transient IR spectroscopy ($50 \mu\text{m}$), compared to $200 \mu\text{m}$ for the transient fluorescence experiment. The flow resistance through the rectangular flow channel of the measurement cuvette scales inversely with the 3rd-power of its thickness.²¹ The $4^3=64$ larger flow resistance, together with stretchability of the teflon tubing between syringe pump and measurement cuvette, rendered the flow through the cuvette essentially continuous despite the pulsed driving of the syringe pump. While a more rigid stainless steel tubing might improve on that, we present here a concept with pulsing micro-valves directly mounted onto the sample cuvette, with no tubing in between.

II. STOP-FLOW SETUP

Fig. 1a shows the overall layout of the stop-flow setup and Fig. 1b,c the measurement cuvette with two micro-valves attached. The measurement cuvette is constructed as described in Ref. 19 with two CaF_2 windows (dia 25 mm, thickness 2 mm, fabricated by Crystan, UK) separated by a $50 \mu\text{m}$ teflon spacer, into which the 1 mm wide flow channel is cut (see position 8 in Fig. 1c). One of the two CaF_2 windows has two 1 mm dia holes for the in- and outlet (position 7). The two micro-valves (position 3, SMLD 300G H J0.1 T1 M F M6x0.75, Fritz Gyger AG, Switzerland), which have a plane surface at their outlet, are pressed onto CaF_2 window with a O-ring (position 5), sealing the connection. Each of the micro-valves is driven with a solenoid, and has a specified switching time of $\lesssim 0.5$ ms. We pulse the micro-valves for typically 1 ms,

using the driver shown in Appendix A.

The total sample volume in the flow cycle is ~ 1 ml.

The flow cycle allows for a continuous measurement, in contrast to the setup of Ref. 17, where the measurement needed to be interrupted for pulling back the syringe pump.

III. DEMONSTRATION EXPERIMENT

To demonstrate the performance of the stop-flow setup, we carried out transient IR experiments of the light-induced *trans*-to-*cis* isomerization of an azobenzene derivative. Since the solvent viscosity is an important parameter for the flow characteristics, the experiments were performed in water (D₂O), in anticipation of future experiments on proteins. We therefore selected the water-soluble azobenzene derivative 3,3'-bis(sulfonato)-4,4'-bis(chloroacetamido)azobenzene for these demonstration experiments.²²

In the dark, the molecule adopts the *trans*-state, which is the starting point of the experiment. Optical excitation at 438 nm induces an isomerization of the molecules into the *cis*-state with a quantum yield of $\sim 10\%$.²³ The photoreaction occurs within a few picoseconds.²⁴ The molecules then stays in the *cis*-state on a minute time-scale,²² without any further kinetic process during the time window of the measurement. The overall length of the flowcycle provides enough time for the molecules to

FIG. 2. Transient IR response of the azobenzene derivative at 1489 cm^{-1} , demonstrating the performance of the stop-flow setup. The red data show the result with the micro-valves open all time, revealing a continuous flow through the measurement cuvette, the blue data by applying a 1 ms pulse to the valves 45 ms after the excitation pulse, and the black data when randomizing the time of the valve by ± 5 ms.

thermally back-isomerize into the *trans*-state before re-entering the sample cuvette another time.

The transient IR spectrometer used for this experiment has been in essence the same instrument as for Ref. 16. In brief, a 100 kHz Yb-doped fiber laser/amplifier system (short-pulse Tangerine, Amplitude, France) together with an OPA (Twin STARZZ, Fastlite, France) and subsequent frequency mixing stage has been used as a source of femtosecond IR pulses. Excitation pulses at 438 nm (width 40 ns) have been obtained from a diode-pumped solid state laser (CrystaLaser). Excitation and probe beams had a focus size in the sample of $150\text{ }\mu\text{m}$ and $100\text{ }\mu\text{m}$, respectively. The probe pulses were dispersed with a spectrometer and the component at 1489 cm^{-1} was detected with one pixel of an MCT array equipped with customized measurement electronics.²⁵

Data have been taken in a single-VIS-pump-multiple-IR probe fashion,^{14–16} where the repetition rate of the laser system defines the time-resolution ($10\text{ }\mu\text{s}$). The time-separation between subsequent excitation pulses has been 65 ms, determining the overall repetition frequency of the experiment. The timings of the VIS excitation laser, the IR probe laser, as well as the micro-valves were controlled with a programable delay generator (T560, Highland Technology).

We probed an isolated vibrational mode of the molecule at 1489 cm^{-1} , which bleaches upon *trans*-to-*cis* isomerization. Fig. 2 shows an instantaneous drop of the absorbance upon photo-excitation at time-zero due to the bleach of this band. When the micro-valves are kept open all time (red data), the response relaxes back to zero on a 10 ms time-scale due to the continues flow of the sample through the measurement cuvette and the resulting loss of overlap of the photoexcited sample with the probe beam. The signal drop would be acceptable only for the first few milliseconds, illustrating the factor 10 of dead time discussed in the Introduction.

When, in contrast, the valves were switched 45 ms af-

ter the excitation pulse (1 ms opening time), the blue data are obtained. Apart from noise, the signal stays constant between 0 ms and 45 ms, indicating that the sample does not move at all during that period. Upon pulsing the micro-valve, the sample is quickly pushed out and the signal decays to zero. Afterwards, some ringing is observed with various frequencies between $\sim 200\text{ Hz}$ to $\sim 1.6\text{ kHz}$, which we attribute to mechanical vibrations of the measurement cuvette. The ringing decays on a $\sim 20\text{ ms}$ timescale, but some part of it survives and enters the data around laser pumping (to that end, one must keep in mind that the data in Fig. 2 are periodic). The effect can be suppressed by randomizing the time-points at which the micro-valves are pulsed by ± 5 ms (black data).

It turned out that a second micro-valve at the outlet of the cuvette is indeed needed. In a first realization of the setup, that second valve was not present, in which case the sample drifted after switching to an extent that the pump-probe overlap was affected. The second valve efficiently stabilized the sample in the measurement cuvette during the measurement periods.

IV. CONCLUSION

We have presented a stop-flow sample delivery system that is particularly suited for laser-based transient spectroscopy with repetition rates of the excitation pulses between 1-100 Hz, where the upper limit is dictated by the time scale needed to suppress the ringing upon valve switching. Studying the complete reaction cycle of photoactive proteins has long been the realm of step-scan FTIR spectroscopy,^{1,2} but is now taken over by laser-based techniques, either quantum-cascade lasers^{3–7} or femtosecond lasers.^{10–16} In either case, the foci in the sample cuvette can be small (in the order of $100\text{ }\mu\text{m}$). In connection with the stop-flow sample delivery described here, the instrument can be extremely sample efficient; i.e., only a small volume is needed for the measurement and at the same time, a large fraction of proteins in the sample solution is in fact utilized. The setup will be particularly important for transient 2D IR spectroscopy,^{26,27} where signals are very small and a dead time of a factor of 10 would be prohibitive. One might also imagine a combination of rapid mixing with transient IR spectroscopy, by adding an additional micro-valve for a second inlet and a mixing chamber between the CaF_2 windows.

In the context of serial crystallography, sample holders have been developed that are rastered in a step-like manner.^{28,29} In that case, the even smaller focus of x-ray light helps, requiring correspondingly smaller steps of the sample. Nonetheless, since the mass that needs to be moved is significantly lower, and since no mechanical movement of any optical component is needed that otherwise might displace an optical beam, the present approach is advantageous for liquid samples. At very low repetition rates of the experiment ($\lesssim 1\text{ Hz}$), and when

only small overall sample amounts are available, a rastering setup might be considered as well for optical spectroscopy.

Data Availability Statement: The data that support the findings of this study are openly available on Zenodo (<https://doi.org/10.5281/zenodo.5582650>)

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Appendix A: Driving the Micro-Valves

Each solenoid has a resistance of $6\ \Omega$, and requires a switching current of $\sim 1\text{ A}$ for $\sim 500\ \mu\text{s}$ and a holding current of $\sim 200\text{ mA}$. That is achieved with the simple

circuit shown in Fig. 3

FIG. 3. Circuit used to drive the two solenoids, which are connected in parallel.

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