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ABSTRACT

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 femtosecond 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 timescale when opening the micro-valves. The setup is extremely sample efficient. It needs only small sample volumes, and at the same time, it enables excitation of a large fraction of molecules in solution.

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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 source^{14–16} or a LED.¹⁷ The experiments we have in mind here are on photoactive proteins with photoreactions that cover a wide range of timescales 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 a 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 a 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 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 μm), compared to 200 μm for the transient fluorescence experiment. The flow resistance through the rectangular flow channel of the measurement cuvette scales inversely with the third power of its thickness.²¹ The $4^3 = 64$ larger flow resistance, together with the stretchability of the Teflon tubing between the syringe pump and the 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

Figure 1(a) shows the overall layout of the stop-flow setup, and Figs. 1(b) and 1(c) show the measurement cuvette with two micro-valves attached. The measurement cuvette is constructed as described in Ref. 19 with two CaF_2 windows (diameter = 25 mm, thickness = 2 mm, fabricated by Crystan, UK) separated by a 50 μm Teflon spacer into which the 1 mm wide flow channel is cut [see position 8 in Fig. 1(c)]. One of the two CaF_2 windows has two 1 mm diameter holes for the inlet and outlet (position 7). The two micro-valves (position 3, SMLD 300G H J0.1 T1 M F M6 \times 0.75, Fritz Gyger AG, Switzerland), which have a plane surface at their outlets, are pressed onto the CaF_2 window with an 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 the Appendix.

Figure 1(a) shows how the measurement cuvette is integrated into a flow cycle driven by a peristaltic pump (ISMATEC IPC Model

935C, Wertheim, Germany, ACIDFLEX tubing in the peristaltic pump with an inner diameter of 0.89 mm). The speed of the peristaltic pump is very low (2 rpm) and is adjusted such that sample exchange would just be completed with open valves for a given repetition rate of the excitation laser (which is typically in the range of 10–100 Hz). To reduce the strong pulsation of the peristaltic pump, a dead end tubing is included into the cycle as a “pressure reservoir.” The air inside the pressure reservoir is compressed by the liquid sample. The inner diameter of the Teflon tubing between the peristaltic pump and the pressure reservoir is thin [100 μm , indicated in Fig. 1(a)] to increase its flow resistance, which scales inversely with the fourth power of the diameter.²¹ Together with the pressure reservoir (300 μm diameter), it acts as a low-pass filter, and an essentially constant pressure of ~ 2 bars builds up in front of the first micro-valve (the pressure can be estimated from the amount of the sample entering the pressure reservoir). All other tubings are thick (300 μm) to minimize flow resistance. In addition, the outlet from the measurement cuvette to the sample reservoir is kept as short as possible to release the sample as quickly as possible.

The total sample volume in the flow cycle is ~ 1 ml. The flow cycle allows for a continuous measurement, in contrast to the setup

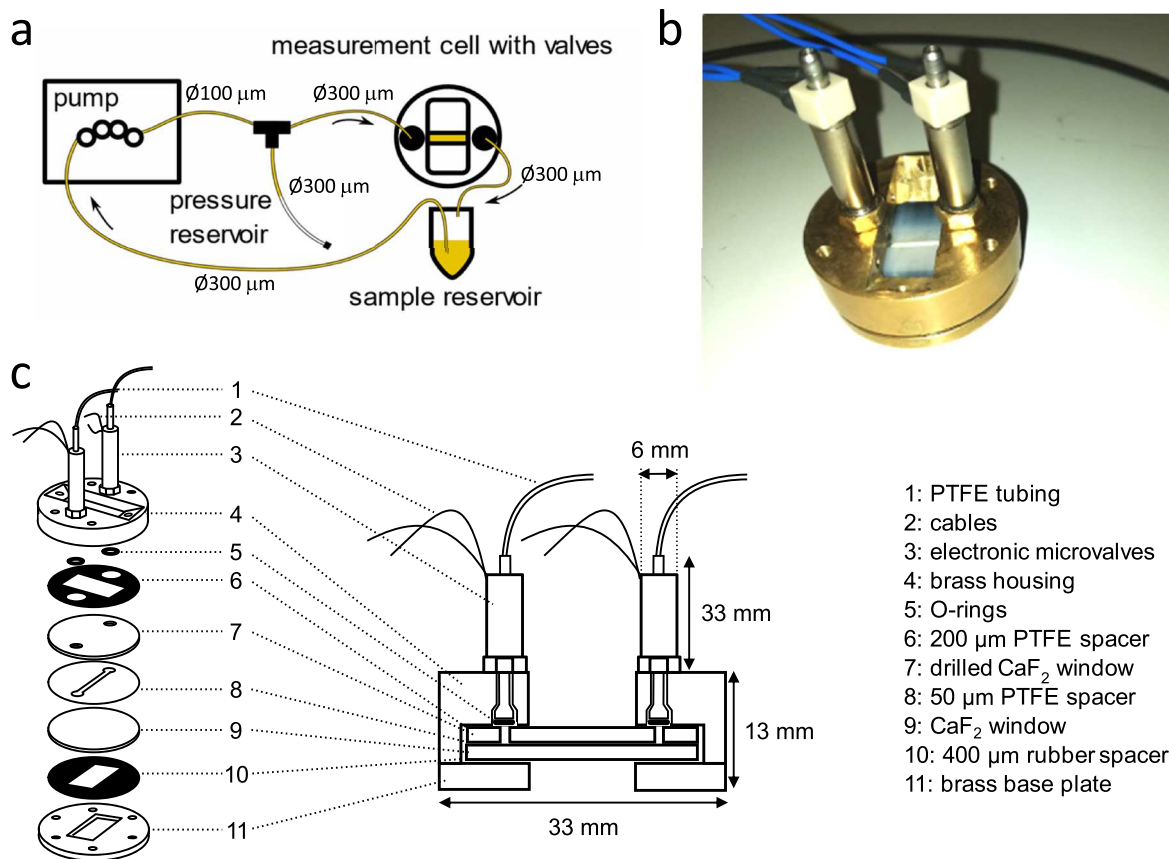


FIG. 1. (a) Overall stop-flow setup. (b) Photograph and (c) detailed drawing of the measurement cuvette with two micro-valves. The diameters of the tubes are indicated in panel (a).

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 (D_2O), 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 stay in the *cis*-state on a minute timescale,²² without any further kinetic process during the time window of the measurement. The overall length of the flow cycle provides enough time for the molecules to 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 optical parametric amplifier (OPA) (Twin STARZZ, Fastlite, France) and a 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). In the sample, excitation and probe beams had a focus size of 150 and 100 μm , 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 μs). 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, and the micro-valves were controlled with a programmable 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. Figure 2 shows an instantaneous drop in the absorbance upon photoexcitation at time zero due to the bleach of this band. When the micro-valves are kept open all the time (red curve), the response relaxes back to zero on a 10 ms timescale due to the continuous 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 after the excitation pulse (1 ms opening time), the blue curve was obtained. Apart from noise, the signal stays constant between 0 and 45 ms, indicating that the sample does not move at all during that period.

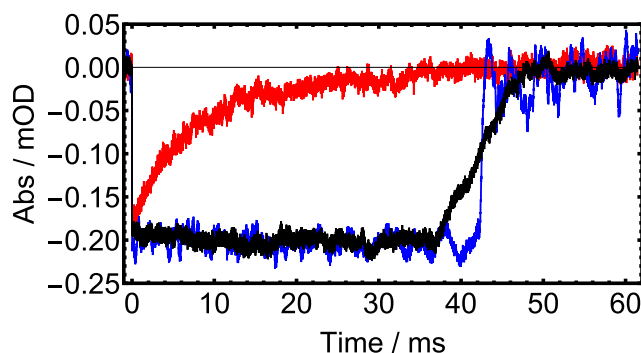


FIG. 2. Transient IR response of the azobenzene derivative at 1489 cm^{-1} , demonstrating the performance of the stop-flow setup. The red curve shows the result with the micro-valves open all the time, revealing a continuous flow through the measurement cuvette, the blue curve, by applying a 1 ms pulse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

Upon pulsing the micro-valve, the sample is quickly pushed out and the signal decays to zero. Afterward, some ringing is observed with various frequencies between $\sim 200\text{ Hz}$ and $\sim 1.6\text{ kHz}$, which we attribute to mechanical vibrations of the measurement cuvette. The ringing decays on an $\sim 20\text{ ms}$ 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 $\pm 5\text{ ms}$ (black curve).

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 and 100 Hz, where the upper limit is dictated by the timescale 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 lasers³⁻⁷ or femtosecond lasers.¹⁰⁻¹⁶ In either case, the foci in the sample cuvette can be small (in the order of 100 μm). 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 (≤ 1 Hz) and when only small overall sample amounts are available, a rastering setup might be considered as well for optical spectroscopy.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors declare no competing financial interest.

Author Contributions

D.B. and J.R. contributed equally to this work.

DATA AVAILABILITY

The data that support the findings of this study are openly available in Zenodo at <https://doi.org/10.5281/zenodo.5582650>.

APPENDIX: DRIVING THE MICRO-VALVES

Each solenoid has a resistance of $6\ \Omega$ and requires a switching current of ~ 1 A for $\sim 500\ \mu\text{s}$ and a holding current of

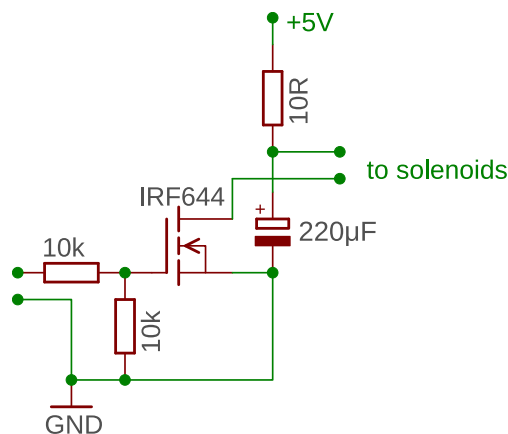


FIG. 3. Circuit used to drive the two solenoids, which are connected in parallel.

~ 200 mA. This is achieved with the simple circuit shown in Fig. 3.

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