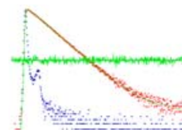


Stopped flow time-resolved fluorescence study of serum albumin – curcuminoid binding

***Rapid mixing accessories to perform stopped flow measurements have found application in characterising interactions and reactions occurring in solution. Reactants are expelled from syringes, mixed and injected into a flowcell. The flow is then stopped with the ensuing reaction / interaction monitored. If one of the reactants is fluorescent this phenomenon maybe used to follow the interaction. However, intensity based approaches can be influenced by fluctuations in the detected signal, eg. caused by photobleaching. This can be avoided by making use of the fluorescence lifetime, but data collection times have limited its usage. The recent introduction of very low deadtime electronics coupled with high repetition rate excitation sources have now allowed efficient and fast measurement of fluorescence decays. Here we monitor the interaction of a fluorescent curcuminoid mixture with serum albumin using a stopped flow accessory on a DeltaFlex system with DeltaDiode excitation at 100MHz*.**

Stopped flow – kinetic TCSPC measurements

A typical stopped flow accessory is shown, schematically, in Fig. 1. Driving the reactant syringes, either manually or mechanically, expels the reactants with are rapidly mixed and enter the flowcell, replacing the existing liquid volume. There is a stop that limits the volume entering the syringe and can be used to provide an electronic signal to start data collection, if configured.

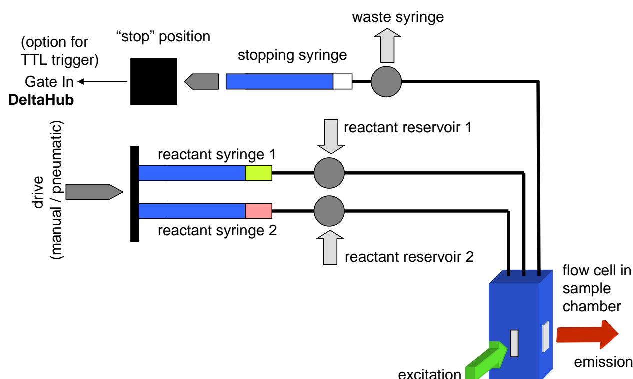


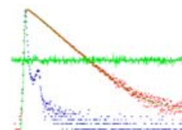
Fig. 1. Schematic representation of a stopped flow set up

Mixing reactions that occur on the millisecond timescale can be monitored using this technique and, if using fluorescence, this has previously involved an intensity based approach. However, these kinds of measurements can be influenced by fluctuations in excitation source intensity and sample photobleaching. The fluorescence lifetime is advantageous since, as well as its inherent sensitivity, it is an absolute measurement (not relative as in the case of steady state fluorescence) and the lifetime is not affected by dilution as the reactant liquids mix or if the sample photobleaches. Until recently instrumentation was not efficient enough to collect sufficient data on these timescales. However, with the introduction of very low deadtime electronics, coupled with high repetition rate excitation sources this has now become possible.

The kinetic TCSPC measurement mode, available in both *DeltaFlex* and *DeltaPro* systems, enables up to 10,000 time-resolved fluorescence decays to be obtained; with each histogram seamlessly collected in as little as 1ms. The 100MHz capability of the *DeltaDiode*, is well matched to the very low dead time of the *DeltaHub* (10ns). When short data collection times are employed, this efficiency is required in order to obtain sufficient data quality. The stopped flow accessory fits into the standard cuvette (10mm pathlength) holder of the *DeltaFlex* (Fig. 2) via a modified sample chamber lid.

Fig. 2. *DeltaFlex* with stopped flow accessory entering via a "split" sample chamber lid.

Using the *DataStation* control software, data collection can be started either using an external TTL signal from the stopped flow accessory (if configured) or manually, within the software, in the kinetic TCSPC measurement mode.



Curcuminoid - serum albumin binding

The study of the interaction of curcuminoids with proteins is of interest as these compounds are being promoted as having potential health benefits. The role of serum albumin in the blood makes it an ideal choice for a model protein with which to observe this interaction. In the experiment performed here a solution of curcuminoid in DMSO / buffer was placed in one syringe, with the other reactant syringe filled with serum albumin in buffer. In this solvent mixture the curcuminoids are weakly fluorescent and exhibit a short fluorescence lifetime. Upon interaction with the protein both the fluorescence quantum yield and lifetime increase. In order to obtain as many photons as possible a repetition rate of 100MHz was used and a 2ms collection time per decay employed. The data acquisition was started in *DataStation* to provide a "background" and the reactants expelled manually. After analysis using *DAS6* software the change in average lifetime during the course of the experiment was plotted and this is displayed in Fig. 3. From this plot it is possible to determine kinetic rates, if required.

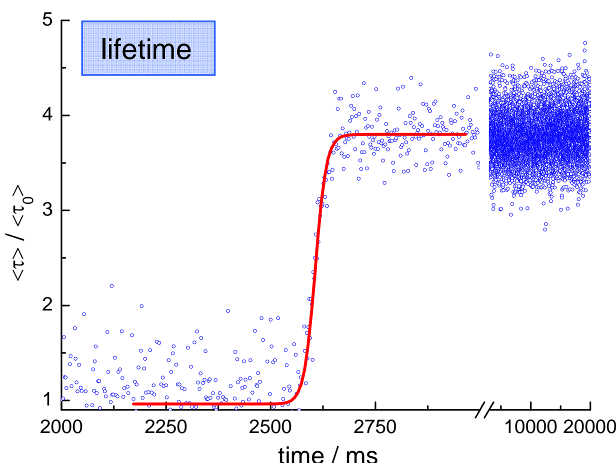


Fig. 3. Change in average fluorescence lifetime with time. The excitation source was a DD-395L operating at 100MHz with decay histograms collected every 2ms. Stopped flow accessory operated manually.

There can be a problem when using these compounds in a small volume flowcell with a high intensity (and repetition rate) excitation source. As they are not very photostable, photobleaching can be seen to occur. Although the fluorescence lifetime is unaffected, a measurement of the intensity (obtained from the TCSPC measurement) will exhibit a decrease with time. This is demonstrated in a second measurement (performed under similar conditions), but using 5ms per decay data collection time for the acquisition of the 10,000 time-resolved fluorescence decay curves. This is shown in Fig. 4.

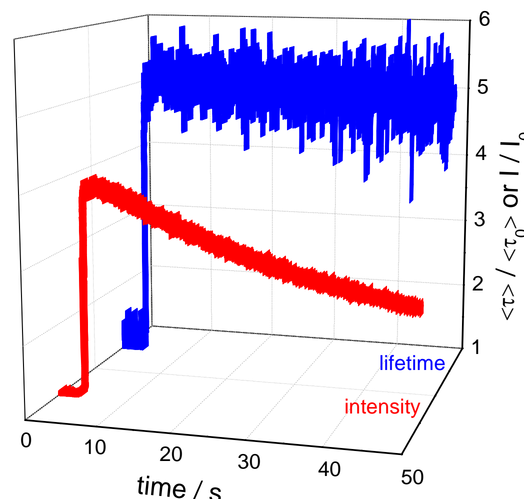


Fig. 4. Comparison between intensity and lifetime data obtained from a stopped flow measurement with data collected every 5ms.

From this figure it can be clearly seen that the fluorescence intensity decreases with time after the initial rise relating to the curcuminoids binding to the serum albumin. This is attributed to photobleaching. However it is noteworthy that the fluorescence lifetime remains stable. The influence of photobleaching obviously has implications if the analysis of this kinetic process were to be performed using the intensity based measurement.

Summary

The ability to monitor kinetic processes occurring on the millisecond timescale has been demonstrated by the simple addition of a stopped flow accessory to the *DeltaFlex* system. This was operated in the kinetic TCSPC measurement mode and was aided by the use of a high repetition rate excitation source coupled with very low dead time electronics to efficiency collect the data. The data obtained clearly shows the advantage of using the fluorescence lifetime in this case, to obtain kinetics unaffected by sample photobleaching.

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