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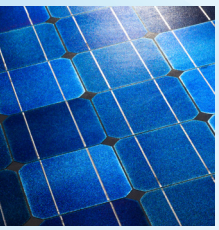

Dye–protein binding monitored in a microliter volume using time–resolved fluorescence

TRFA-10

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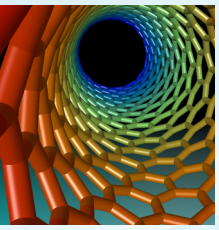
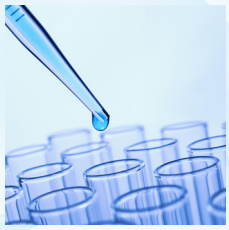
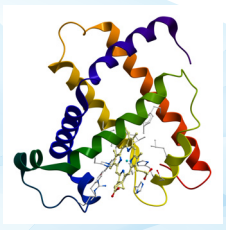


Rapid time-resolved fluorescence of microliter volumes

Introduction

The potential health benefits stemming from the antioxidant activity of curcumin, commonly found in turmeric (*Curcuma longa L*), has attracted the interest of several research groups. To fully investigate curcumin's potential there is an obvious need to elucidate its interaction with proteins and this has already resulted in several published works. Fluorescence is a powerful technique by which to uncover molecular interactions, principally because of its sensitivity. Time-resolved fluorescence adds an extra dimension and has the major advantage that the fluorescence lifetime is largely independent of concentration and illumination intensity, thus returning an absolute measurement. Curcuminoids can be weakly fluorescent in aqueous solution, but their quantum yield increases upon interaction with a protein, such as human serum albumin (Fig. 1). This interaction is addressable by fluorescence measurement techniques.

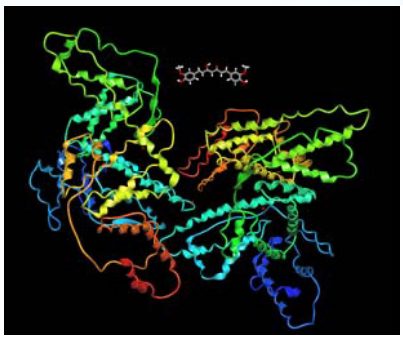

Figure 1: Human serum albumin interacting with a curcuminoid.

Experiment

To be able to measure fluorescence from a volume less than 5 µL, a Microsense cuvette (inset in Fig. 1) was employed. The cuvette, which has similar external dimensions to a standard 10 mm path-length fluorescence cell, was placed inside the sample chamber of a HORIBA Scientific DeltaFlex fluorescence lifetime spectrometer (Fig. 2). The DeltaFlex was operated in

Figure 2: DeltaFlex fluorescence lifetime system. Inset: Microsense cuvette.

Kinetic TCSPC mode. This allows for the collection of up to 10 000 sequential fluorescence decays, from data-acquisition times ranging from 1 ms to 1 min. The collection time is determined by the quantum yield of the sample (how many photons can be collected), the timescale of the kinetic process to be observed, and fluorophore lifetime. In this case a data-collection time of 10 ms was used and 10 000 decays were collected, along with the instrumental response in order to analyze the data using reconvolution analysis. This was performed in a batch mode using DAS6 analysis software. The excitation source was a DeltaDiode-425L operating at a repetition rate of 100 MHz. The DeltaHub timing electronics in the system process a low (<10 ns) dead time, which enables it to collect photons efficiently at this high excitation rate. Detection was at 530 nm using a PPD-650 detection module.

The experiment was performed by placing 2 µL of curcuminoid solution (DMSO/phosphate-buffered saline,

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pH 7.4) onto the Microsense cuvette and then adding 3 µL human serum albumin in phosphate-buffered saline during the kinetic TCSPC run. The instrumental response was measured using the same cell, but with 5 µL of Ludox® used as a scattering solution.

Results

From the analysis of the 10 000 individual fluorescence decays, each acquired sequentially for 10 ms, the average lifetime was obtained. This was returned using a bi-exponential decay model and a plot of average lifetime versus time is shown below (Fig. 3). Note that the log scale after the time axis breaks, and for clarity not all data points are plotted.

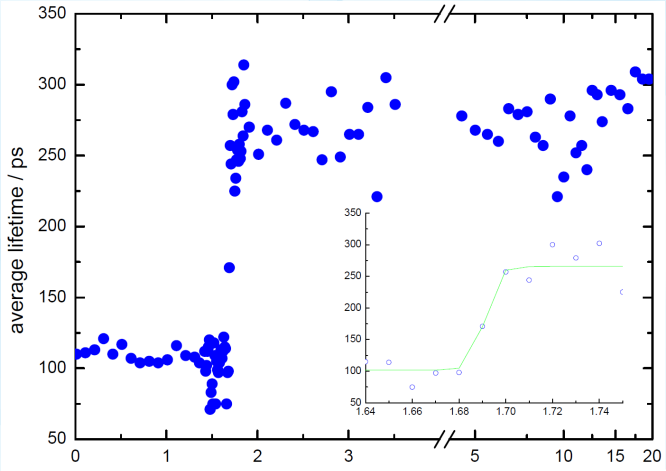

Figure 3: Lifetime vs time. Note logarithmic scale after break in time-axis.

Two representative fluorescence decays showing shorter and longer average lifetimes are presented in Fig. 4. There is a clear increase in the average decay time, which is indicative of the curcuminoid interacting with the protein. The use of the fluorescence lifetime is advantageous, because it is not affected by the dilution of the probe volume on addition of the larger volume associated with the addition of the protein. In a steady-state measurement, this would influence the measured intensity as, in effect, the probe concentration would be diluted.

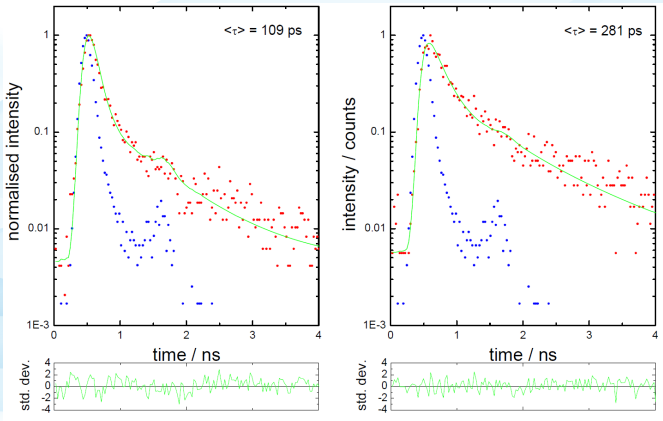

Figure 4: Fluorescence decays with shorter and longer lifetimes.

Conclusions

The DeltaFlex system, with the Microsense cuvette, can perform time-resolved fluorescence to obtain kinetic information on processes occurring on the millisecond time scale in microliter volumes. In this case the interaction of a dye binding to a protein was monitored. This was achieved by the rapid and efficient data-acquisition enabled by the 100 MHz excitation rate, coupled with the low dead time of the timing electronics. The use of time-resolved fluorescence enables a fuller picture to be obtained, by providing a further fluorescence parameter (the fluorescence lifetime) with the advantage that this is also independent of concentration.

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