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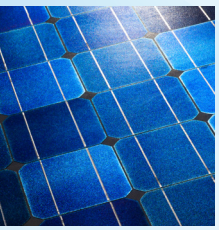
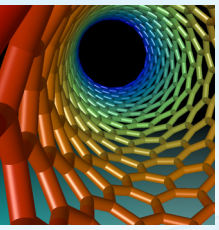
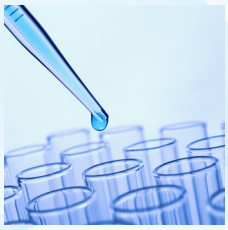


Effect of temperature on HSA structure inferred using time-resolved room-temperature phosphorescence

TRFA-15

ELEMENTAL ANALYSIS
FLUORESCENCE
GRATINGS & OEM SPECTROMETERS
OPTICAL COMPONENTS
FORENSICS
PARTICLE CHARACTERIZATION
RAMAN
SPECTROSCOPIC ELLIPSOMETRY
SPR IMAGING

Study of temperature-induced conformational changes in proteins

Introduction

Although the fluorescence time-scale is well-suited to follow many biological processes, often there is a requirement for processes to be monitored over longer time-scales. To access intrinsic amino acids, such as tryptophan, as probes, the UV excitation wavelengths for pulsed phosphorescence measurements have long been the preserve of low-repetition-rate gas-filled lamps or larger laser systems. Recent developments have enabled the use of interchangeable semiconductor diodes, with their inherent ease of use. SpectraLEDs (Fig. 1) provide spectral coverage from the UV to the near-IR. They are adept for this type of measurement and have been previously used for the study of lanthanides and singlet oxygen (see Application Notes TRFA-4, -6, -11, and -12). These sources do not exhibit a "tail" present with xenon flashlamps, and are able to run at higher repetition rates (several kHz).

Figure 1: Selection of SpectraLED pulsed excitation sources.

These sources can attach directly to the DeltaHub or FluoroHub timing electronics and their repetition rate automatically adjusts to the time-range selected using DataStation measurement software. This software can also adjust the duty cycle and temporal position of their output as needed to suit sample conditions.

Experiment

Measurements were performed using a DeltaFlex, equipped with either a SpectraLED S-295 or DeltaDiode DD-295 excitation source for phosphorescence (44 ms range) and fluorescence lifetime (100 ns range) measurements respectively. Data were analyzed using DAS6 as the sum of exponentials, with either the relative amplitudes (or fractionals, f_i) or normalized pre-exponentials (α_i) presented. See below for the main governing equations:

Sum of exponentials

$$I(t) = \sum_{i=1}^{n} \alpha_i \exp\left(-t/\tau_i\right),$$

The contribution of each emitting species can either be represented as a normalized pre-exponential (α) or relative amplitude (or fractional, f).

$$\alpha_i = \frac{\alpha_i}{\sum_{i=1}^{n} \alpha_i} \qquad f = \frac{\alpha_i \tau_i}{\sum \alpha_i \tau_i}$$

Here, the average lifetime can be represented:

$$\tau_{ave} = \sum a_i \tau_i$$

Treated data were plotted using Origin-based FluorEssence software.

The phosphorescence emission is observed at longer wavelengths than the fluorescence, although excitation is via the singlet state. In order to observe RT phosphorescence from the tryptophan, potassium iodide and sodium sulfite, as a deoxygenator, were added to the HSA solution.

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HSA fluorescence with increasing temperature

Studies have shown that HSA undergoes several reversible structural changes prior to an irreversible denaturation over ~75 °C. To see if the time-resolved tryptophan decay was influenced by this, a temperature study was performed, monitoring its fluorescence. The decay, as expected, required the sum of three exponentials to fit the data. Results are shown in Fig. 2.

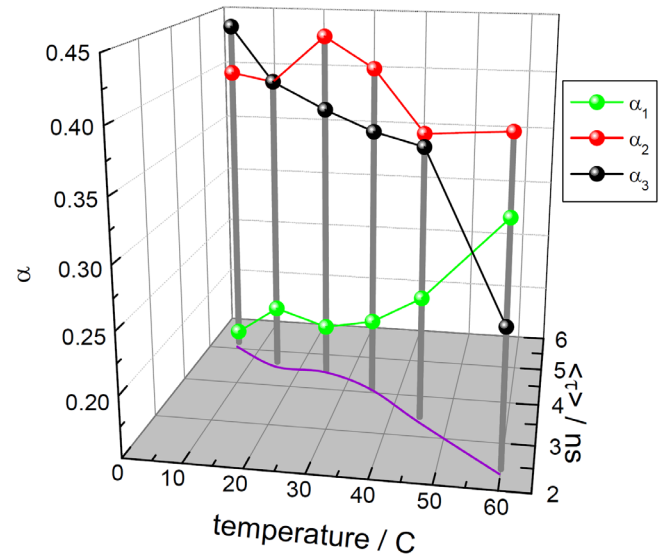

Figure 2: Effect of temperature on the time-resolved decay of tryptophan in HSA, with exponential analysis showing normalized pre-exponentials (α_i, 1 relates to shortest-lived, 3 to longest) and average lifetime, $\langle\tau\rangle$.

This form of analysis shows that a change in the fluorescence is evident with increasing temperature. Overall the lifetime decreases and a change in contribution from each of the lifetimes to the emission occurred. The actual choice of analysis selected will, in practice, depend on the system under study and the process to be monitored.

Time-resolved phosphorescence

The wavelength of the phosphorescence emission is red-shifted in relation to the fluorescence. Here the phosphorescence decays, collected at 450 nm, were measured over a similar temperature range; the outcome from these measurements is presented in Fig. 3.

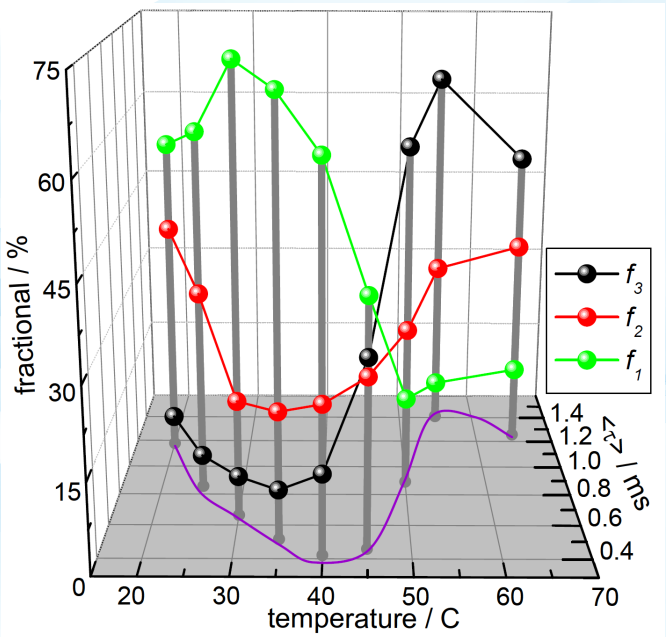

Figure 3: Time-resolved phosphorescence data, showing the fractional contributions (f_i, 1 shortest-lived, 3 longest-lived) and the average lifetime.

These results show the evidence for the presence of different emitting species, whose average lifetime and relative contribution vary with temperature. The trend in the average lifetime reflects other work showing transitions relating thermally-induced conformational changes.

Conclusions

The measurements presented show the ability of the DeltaFlex equipped with different excitation sources to monitor the fluorescence and phosphorescence of tryptophan in human serum albumin. The influence of temperature on the tryptophan decay kinetics can be seen in the analysis, and appears most marked in the phosphorescence data, showing the potential of this form of measurement with this protein.

The measurements presented here are based on those by K. Sagoo, *et al. Spectrochim. Acta A*, **124** (2014) 611–617.

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