FRET with a HORIBA Jobin Yvon Phosphorimeter

Introduction

A phosphorimeter integral to the FluoroMax®-P spectrofluorometer or as the FL-1042 accessory on the Fluorolog® system can uncover important information on various systems of chemical and biochemical interest by revealing luminescence data normally masked by intense but rapid fluorescence.

This Technical Note describes an example of Förster resonance energy-transfer (FRET) from a peptide-terbium-complex donor to a fluorescein acceptor, using the HORIBA Jobin Yvon phosphorimeter. The peptide in the complex absorbs light at 280 nm, and the terbium phosphoresces at 485 nm, where fluorescein dye absorbs. The fluorescence of fluorescein can be observed when the sample is excited at 280 nm, using our phosphorimeter accessory.

Experimental procedure

Peptide-terbium complex was dissolved in aqueous solution, along with fluorescein in some samples. The measurements were taken on a Fluorolog®-3-22 spectrofluorometer, including double-grating excitation and emission monochromators. Fluorescence measurements used a 450-W CW xenon lamp as the photon-source, and phosphorescence measurements used a xenon flash lamp as the photon-source. An R928 photomultiplier tube, operated at 950 V in the photon-counting mode, was the detector. The FL-1042 phosphorimeter accessory includes a dual-lamp housing (containing both CW and pulsed Xe lamps), and all control electronics. A light-pulse excites the sample, and variable delays control when the detection window opens, and for how long. Sample excitation was at 280 nm, with 100 flashes measured. For luminescence spectra, the integration time was 0.2 s, except as noted. The scans were taken under ambient room conditions.

Results and discussion

Fig. 1 compares luminescence from the peptide-Tb complex with no delay and a 50-µs delay following the excitation light pulse. With the delay, the fluorescence at 363 nm from the peptide disappears, isolating the Tb phosphorescence at 486 and 540 nm. Fig. 2 (next page) shows fluorescence from a solution of peptide + fluorescein dye, also with and without delay. With a 50-µs delay, all fluorescence from the peptide + fluorescein vanishes. Taken together, Figs. 1 and 2 show that the phosphorescent species is Tb.

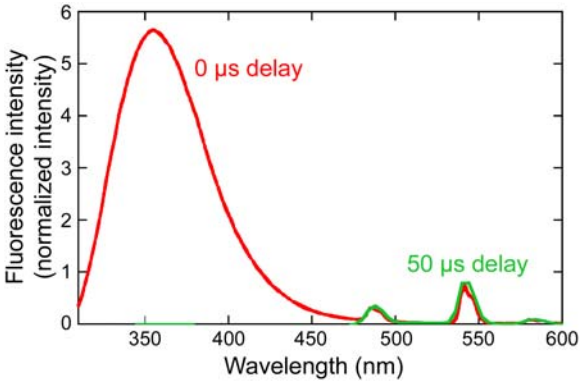

Fig. 1. *Luminescence spectra of peptide + terbium with (green) and without (red) a 50-µs delay. λ_{exc} = 285 nm, and bandpass = 2 nm for both excitation and emission.*

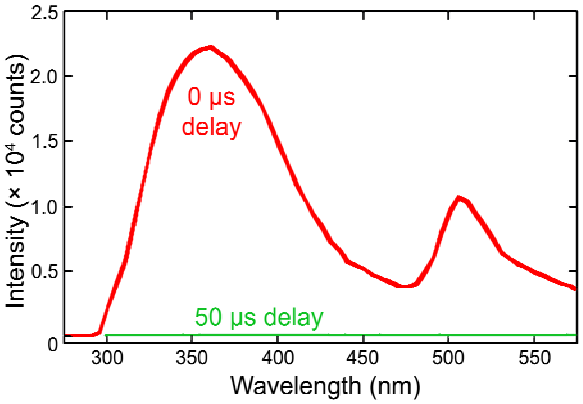

Fig. 2. *Luminescence spectra of peptide + fluorescein with (green) and without (red) a 50-µs delay. All bandpasses = 5 nm.*

Fig. 3 combines all three species, in a plot of peptide-terbium complex with and without 0.67-µ*M* fluorescein, without and with a 50-µs delay between excitation with a xenon flash lamp. Again, the spurious fluorescence at 363 nm is removed by delaying the sample window for 50 µs. The blue and green curves directly compare fluorescein-containing and fluorescein-lacking solutions, showing fluorescein phosphorescence at 511 nm from energy-transfer from complex to fluorescein.

Fig. 4 shows phosphorescence-decay curves of peptide with terbium ion alone (τ = 3.8 ms), and in the presence of fluorescein (τ = 3.0 ms). The faster phosphorescence decay in the presence of fluorescein (blue curve in Fig. 4) confirms energy-transfer from the peptide-Tb donor to the fluorescein acceptor.

Similar experiments can be performed with the FluoroMax®-P spectrofluorometer, which contains an integrated phosphorimeter along with CW lamp.

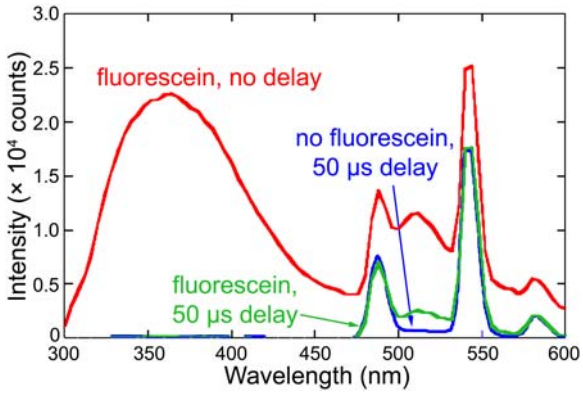

Fig. 3. *Luminescence spectra of complex, with (red) 0.67-µ*M *fluorescein and no delay after excitation, (green) 0.67-µ*M *fluorescein and a 50-µs delay after excitation, and (blue) no fluorescein and a 50-µs delay after excitation. Bandpass = 5 nm for excitation and emission.*

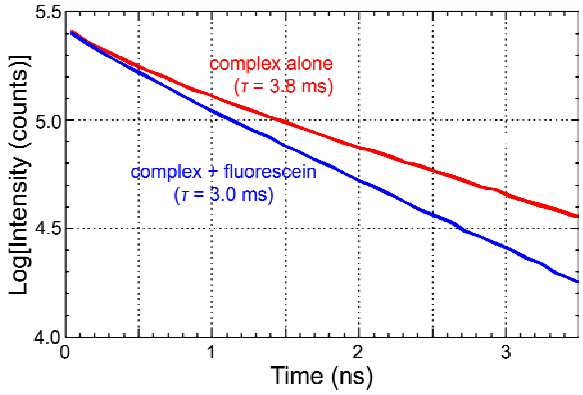

Fig. 4. *Phosphorescence decay curve from an aqueous solution of: (red) complex alone, and (blue) complex + fluorescein. Integration time = 0.4 s; bandpass = 5 nm on excitation (280 nm) and emission (540 nm).*

Conclusions

Use of a HORIBA Jobin Yvon phosphorimeter, including gated delay of signal-acquisition, can reveal extra information, such as FRET, about the physical and chemical properties of materials.

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