Performance of the TemPro Fluorescence Lifetime System

Introduction

The TemPro lifetime system is for cost-conscious research or a teaching laboratory interested in fluorescence lifetimes and phosphorimetry. Using Time-Correlated Single-Photon Counting (TCSPC), the TemPro is reliable, intuitive, and sensitive. Filter-based, it can measure the complete range of lifetimes from 100 picoseconds to 1 second and beyond, with our NanoLED and SpectraLED solid-state pulsed excitation sources from UV to near-IR. Applications for the TemPro include Förster Resonance Energy Transfer, Stern-Volmer quenching, photosynthetic efficiency, deconvolution of mixing processes, anisotropy-decay, protein denaturation, phosphorescence, and coral fluorescent lifetimes. This *Technical Note* highlights major performance characteristics of the TemPro.

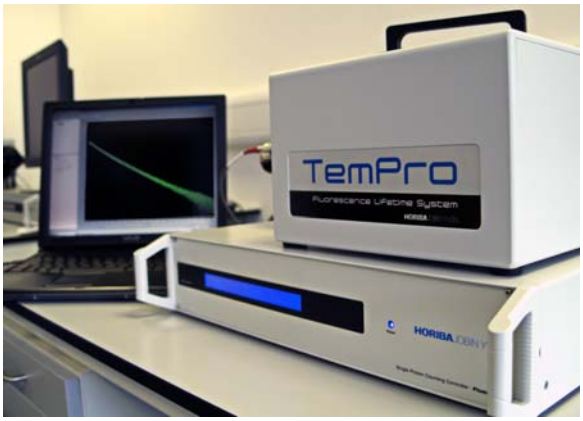

Fig. 1. *TemPro lifetime system.*

Fast lifetimes

The TemPro can measure lifetimes < 100 ps with appropriate sources and detectors. An example is the decay of aqueous erythrosin B, a short-lifetime standard. Our NanoLED N-488L excited the sample at 488 nm. A long-pass filter removed emissions < 550 nm. In Fig. 2, the recovered lifetime was 85 ± 4 ps.

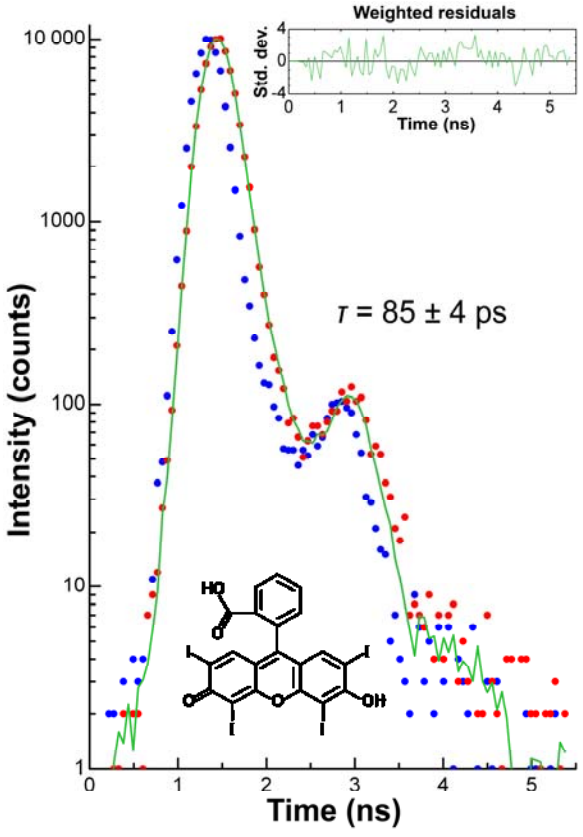

Fig. 2. *Fluorescence decay of 10^{-5}* M *erythrosin B in water, giving a lifetime of 85 ps. Excitation = 488 nm; emission > 550 nm. Blue points are the instrumental response; red are the fluorescence data. Green is the fitted lifetime decay. The structure of erythrosin B is shown inset.*

Fluorescence anisotropy

With optional polarizers, the TemPro can record fluorescence-anisotropy. Fig. 3 shows data from diphenylhexatriene (DPH) dissolved in the mineral oil Kaydol®, with excitation at 373 nm, and a long-pass filter for emission > 418 nm. Anisotropy showed free rotation with correlation time = 4.9 ns.

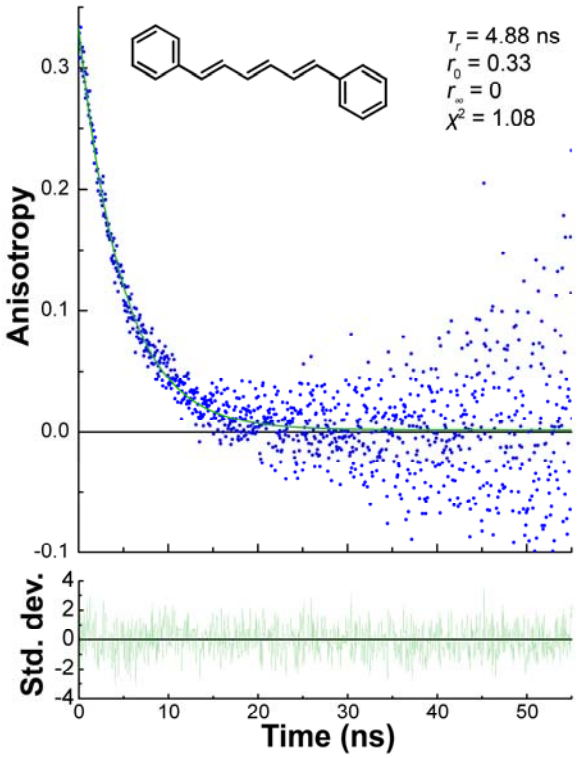

Fig. 3. *Anisotropy decay of 10^{-5}* M *DPH in Kaydol®, recorded with the TemPro. Excitation = 373 nm; emission > 418 nm. The initial anisotropy of 0.33 drops to zero within ~ 30 ns. The structure of DPH is given above the data.*

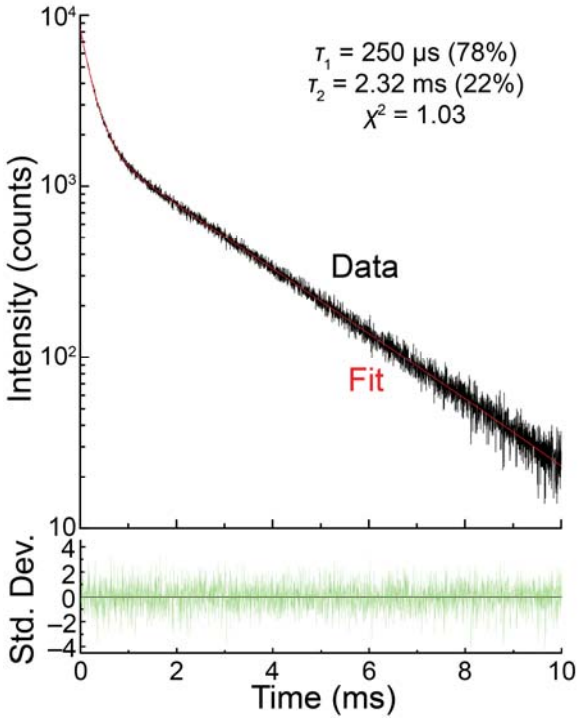

Fig. 4. *Luminescence decay from EuPOM. The upper plot is the decay (black) with bi-exponential fit (red; χ^2 = 1.03) for τ_1 = 250 µs (78%) and τ_2 = 2.32 ms (22%). Below are the residuals.*

Phosphorescence decay analysis

Long lifetimes are also accessible with the TemPro. For the biological label europium polyoxometalate (EuPOM), a SpectraLED at 395 nm was the excitation source, and a long-pass filter (λ > 550 nm) filtered the emission. An excellent fit (χ^2 = 1.03) to the decay data (Fig. 4) resulted with two lifetimes, 250 µs (0.78) and 2.32 ms (0.22). The two components may stem from monomers (shorter τ) and EuPOM–EuPOM interaction because of the concentration (longer τ).

When [EuPOM] in PBS was varied from 0.029–1.18 m*M* (Fig. 5), the average lifetime rose from 124 to 626 µs, because of growth on a longer-lived component related to POM–POM interactions. Global analysis on the addition of bovine serum albumin (BSA) uncovered a three-component system (140 ± 7 µs, 395 ± 4 µs, 2.3 ± 0.1 ms, χ^2 = 1.11) (Fig. 6). With increasing [BSA],[1] the longer-lived decay (2.3 ms) emerged at the expense of the 395-µs component.

[1] Hungerford, *et al.*, "Luminescence enhancement of a europium containing polyoxometalate on interaction with bovine serum albumin," *Photochem. Photobiol. Sci.* **7** (2008), 734–737.

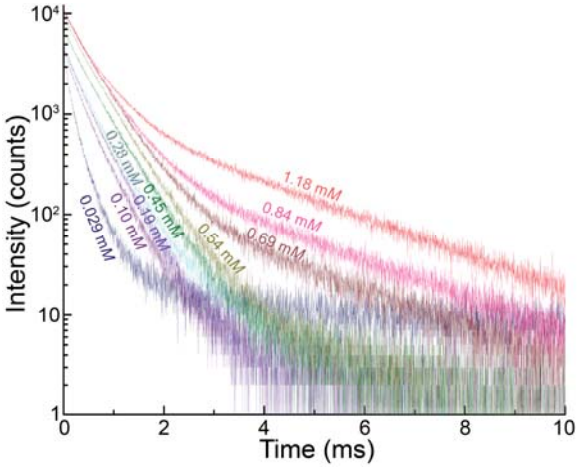

Fig. 5. *[POM] vs. decay time. As concentration increased, the lifetime rose from 124 to 626 µs.*

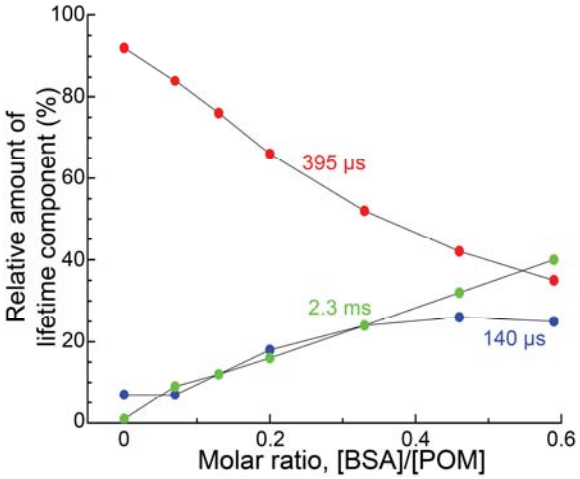

Fig. 6. *Global analysis of lifetime decays as BSA is added to EuPOM. Average lifetime increased as the [BSA] rose. Simultaneously the longest lifetime-component increased, while the 395-µs lifetime decreased.*

Complex analysis of fluorescence

DASPMI (compound 2; Fig. 7) is more hydrophilic than 4,4′-difluoro-4-bora-3a,4a-diaza-s-indacene (compound 1). Thus 1 and 2 ought to act as complementary probes for hydrogels of an aqueous solution of Gelrite® gellan gum (compound 3). Gelrite® exhibits ~100-fold drop in viscosity from 20–85°C. The photophysics of 1 and 2 are largely ruled by bond-rotation, plus, in 2, stabilization of intramolecular charge-transfer states—which is dependent on the probe's local viscosity.

Our TBX-04 detector and N-488L excitation source were used. A KV 550 cut-off filter selected emission. Temperature was maintained with our F-1001 recirculating bath.

For both probes, the decay kinetics were complex (Fig. 7), needing three exponents (χ^2 < 1.2), relating to monomer aggregates for 1, and charge-transfer and bond-twisting states in 2.

Simplifying the tri-exponential fit to an average lifetime, a plot of lifetime vs. temperature showed a decrease of ~ 70% in average lifetime (Fig. 8). (The probes monitor local viscosity instead of bulk, so decreases are larger than if purely thermally induced non-radiative pathways were the sole mechanism.) From previous work on 1, a viscosity decrease from ~ 2000 to 30 cP can be estimated. For 2, the main influence is the proportions of the fluorescing states—the longer-lived state decreasing with temperature—which cause a decrease in average lifetime.

The viscosity of these hydrogels using complementary fluorescent molecular rotors was studied with respect to [Mg^{2+}] (Fig. 9). The hydrophobic compound 1 likely associates with polysaccharide chains (and aggregates), while 2 probes the aqueous solution. Compound 1 shows an initial decrease in lifetime on addition of Mg^{2+}, after which it remains constant. Compound 2 shows

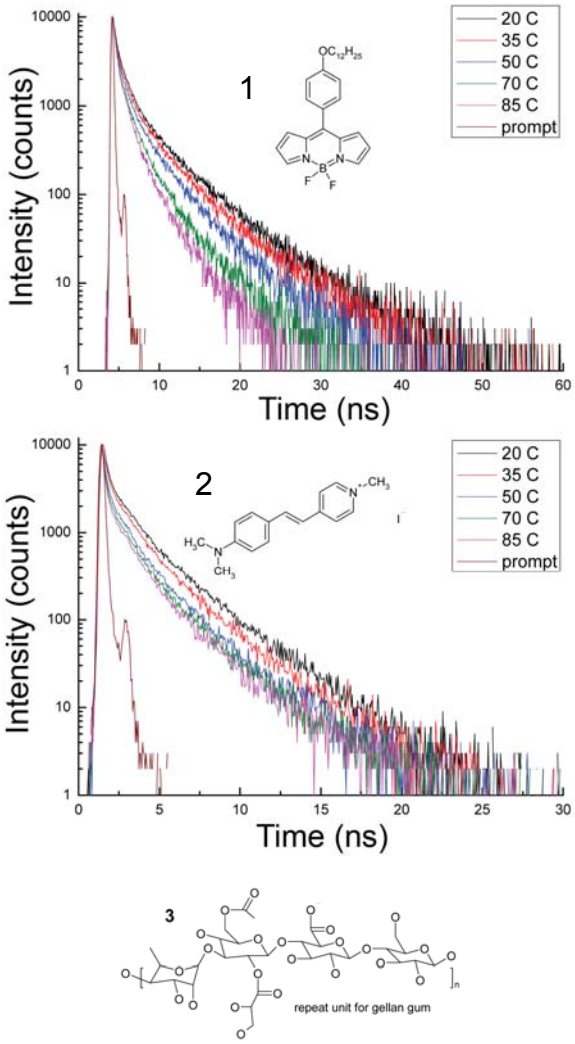

Fig. 7. *Fluorescence decay of 1 (top) and 2 (middle) in Gelrite® (3, bottom structure) at various temperatures.*

a steady increase in average lifetime, indicating an increase in viscosity.

These complementary probes reveal the microheterogeneous nature of gels, not seen in measurements on the bulk phase alone.

Conclusions

The TemPro is a versatile instrument for investigating a variety of chemical, physical, and biochemical processes for the budget-conscious researcher.

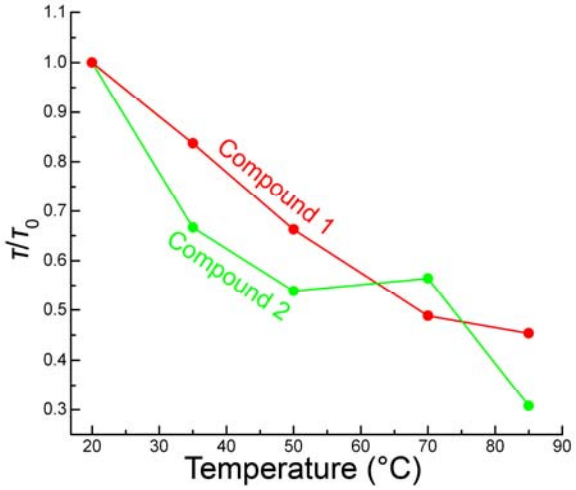

Fig. 8. *Change in average lifetimes of compounds 1 and 2 in Gelrite® upon heating with respect to the initial lifetimes at 20°C.*

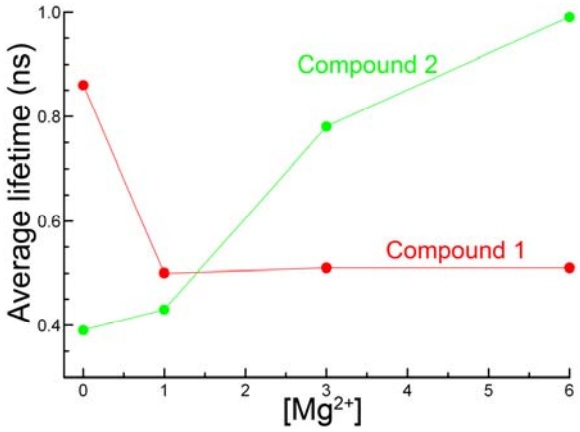

Fig. 9. *Change in average lifetimes of compounds 1 and 2 in Gelrite® as Mg^{2+} is added.*

Acknowledgements

We thank Marina Kuimova, Gokhan Yahioglu (Chem. Dept., Imperial Coll., London), and Klaus Suhling (Phys. Dept., King's Coll., London) for 4,4′-difluoro-4-bora-3a,4a-diaza-s-indacene. Mark Green (Phys. Dept., King's Coll., London) provided the EuPOM.