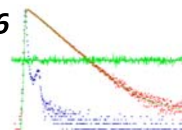


Polyoxometalate-protein interactions monitored by time-resolved luminescence

Polyoxometalates (POMs) are useful in a wide range of biological applications and rare-earth based POMs provide a potentially new biological optical label. The fact that the decay of lanthanide luminescence occurs on a timescale slower than that of molecular fluorescence, typically micro- to milliseconds allows for discrimination from cellular autofluorescence. It also permits the probing of cellular processes that occur on these longer timescales and not accessible via fluorescence techniques. The use of lanthanides is also advantageous as they are not as prone to photobleaching as organic dyes.

Lanthanide containing polyoxometalates

Polyoxometalates (POMs) are a class of inorganic metal-oxygen compounds that are finding increased application, especially in the biological field. Their relatively small size, potentially low toxicity and high stability are amongst the factors influencing their use. There are several structural types of these anionic molecules, usually incorporating transition metal ions linked to oxygen atoms. More recently there has been an interest in the inclusion of lanthanides, since the POM coupled with the luminescence emission characteristics make this class of compound promising for biological imaging. Examples of compounds used in this work are given below (Fig. 1) and incorporate either europium or terbium as the optically emissive species (see reference for details).

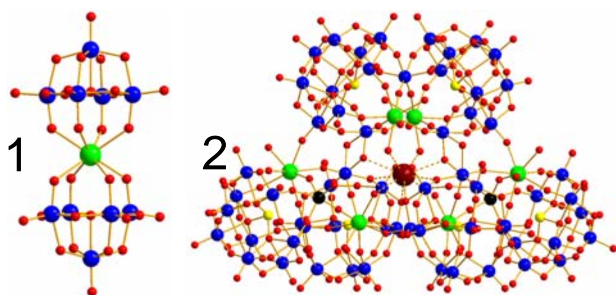


Fig. 1. POM structures. Left - containing a central europium atom. Right – the central atom can be either europium or terbium. See reference.

POM-Protein interactions viewed by time-resolved luminescence

To ascertain the possible interaction between the POMs and protein, serum albumin was chosen. The time-resolved emission from the incorporated lanthanides is well suited to be measured using a *HORIBA Scientific TemPro* (shown in Fig. 2). This can be equipped with either *NanoLED* or *SpectraLED* excitation, which allows lifetimes from 100ps to 1 second to be determined.

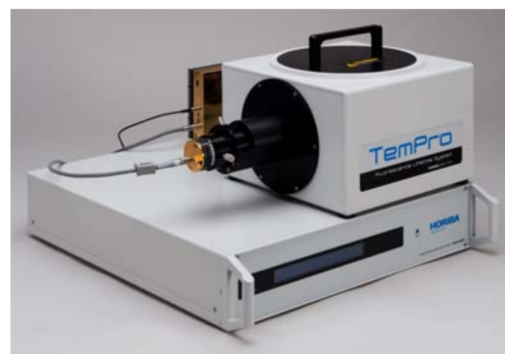


Fig. 2. TemPro compact fluorescence lifetime system.

Making use of this luminescence lifetime range allowed the emission from the lanthanide to be measured (micro- to millisecond time scale), as well as the intrinsic protein fluorescence of serum albumin, which originates from tryptophan (nanosecond time scale).

The emission from the incorporated lanthanides is shown in Fig. 3 and shows their relatively narrow emission bands. Another advantage is the wavelength separation between the excitation wavelength (370nm for Tb, 390nm for Eu) and the emission.

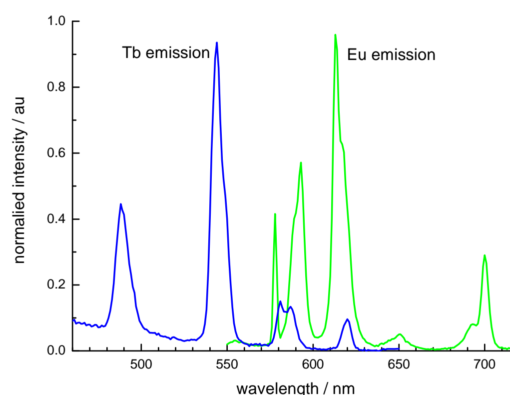
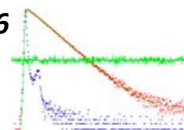


Fig. 3. Emission spectra for the selected lanthanides



The europium III ion is known to be quenched by the presence of water, so interaction with a protein may result in luminescence enhancement. This can be seen in Fig. 4 for POM **1**, where it can visually determined that there is an increase in the decay time.

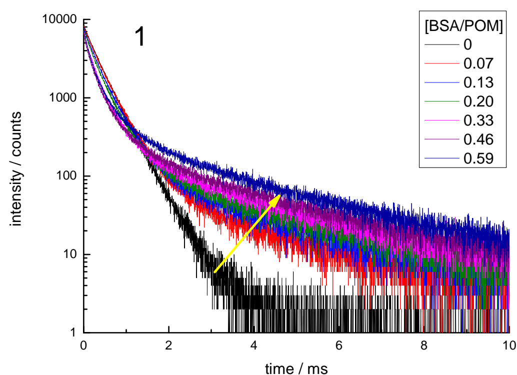


Fig. 4. Decay of POM **1** upon addition of bovine serum albumin, with SpectraLED-390 excitation.

Analysis of these decay kinetic was performed by globally linking the decay times using DAS6 software and allowed the following plot (Fig. 5) to be obtained.

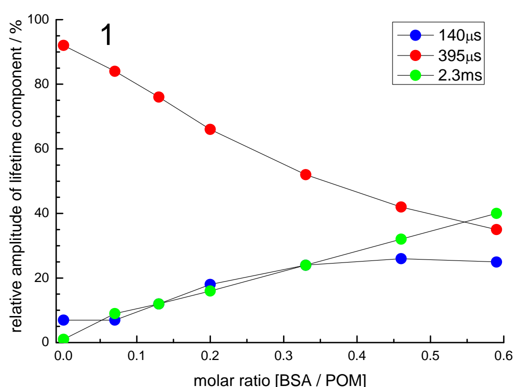


Fig. 5. Global analysis of the decay of POM **1** upon addition of bovine serum albumin.

This shows that the overall lengthening of decay time is related to an increase in a species with a 2.3ms decay time and a concomitant decrease in a species with a 395µs decay time. This behaviour is attributed to association of the POM with the protein.

The influence of the effect of the POMs on the fluorescence of the tryptophan residue (this time in human serum albumin) is ascertained by excitation of this amino acid at 295nm. On increasing POM concentration a quenching in tryptophan emission occurs, see Fig. 6, and treatment of these results helps to elucidate the POM-protein interaction.

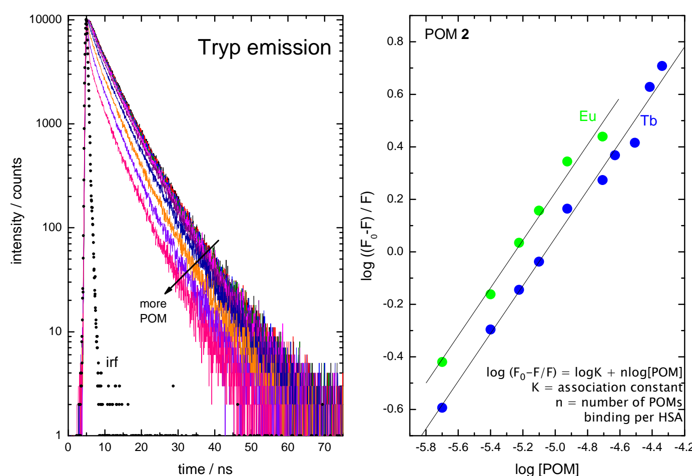


Fig. 6. Quenching of HSA tryptophan emission upon increasing POM concentration and subsequent analysis – see reference for details.

Summary

It is possible using one time-resolved instrument to monitor luminescence on several timescales to help characterise the interaction between lanthanide containing POMs and protein (serum albumin).

Reference

G. Hungerford, F. Hussain, G.R. Patzke and M. Green, **2010**. *The photophysics of europium and terbium polyoxometalates and their interaction with serum albumin: a time-resolved luminescence study*. Phys. Chem. Chem. Phys. 12, 7266-7275.

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