# Synergistic Effects on Europium Luminescence in Aqueous Media

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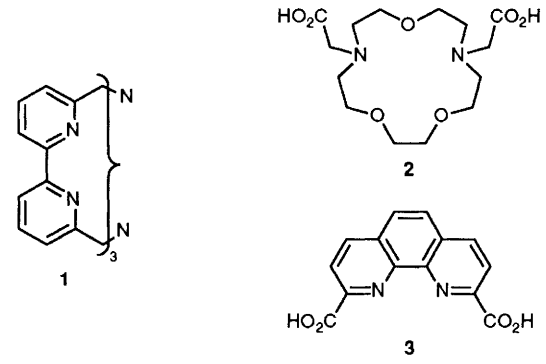
A new approach is described for the design of cooperative ligands that enhance europium luminescence in aqueous solution.

The unique luminescence characteristics of certain lanthanide ions, notably europium(III), have been exploited in at least two commercially available immunoassay systems. In the first[1] (the DELFIA system) europium is attached to a target by a strong chelating agent and the luminescence signal measured by dissociating and assaying the ions. In the second approach[2] (the Cyberfluor method), a photosensitiser is attached to an antibody and is detected by saturation with europium and direct sensitisation of the europium luminescence, a process involving ligand to metal energy transfer.[3] In aqueous solution, free europium ions do not luminesce efficiently since any excited-state species are relaxed by vibronic energy transfer to the aqueous solvent shell.[4] In order to observe luminescence such relaxation mechanisms have to be plugged and this can be achieved either by removing the water or by replacing the solvent shell by other ligands. Typically luminescence is enhanced by use of micelle-forming reagents and water-exclusion ligands such as trioctylphosphine oxide.[5]

Recent synthetic efforts to enhance the efficiency of europium luminescence have aimed at designing highly caged ligands ('sarcophagates') incorporating photosensitising groups in order to shield the ion from water ligands, such as the complex **1**.[6] Herein, we report a new method for enhancing europium luminescence using cooperative ligands, *viz.* a three-component system comprised of the europium ion, a sensitizing ligand and a separate, non-sensitising ligand that acts as a shield to prevent the ingress of water to the complex (Scheme 1); recently Toner has briefly mentioned this effect.[7]

Our approach has been to try to understand how to prepare such tertiary complexes. Since the europium ion behaves as a highly charged sphere, we concentrated on exploiting electrostatic field effects. Thus, choosing ligands that do not completely saturate the positive charge on an europium ion allows the initial 1:1 complex to retain a positive charge so that a second negatively charged ligand can be attracted into its coordination sphere.

Of known ligands that bind to the lanthanides the bis-acetic acid derivative of 1,7-diaza-4,10,13-trioxacyclopentadecane ('[2.2.1]') **2** forms tight complexes with europium ions ($K_{ass} > 10^{11}$)[8] and molecular models show that this is achieved whilst leaving a cleft suitable for occupation by either water




$[Eu{\cdot}PDA]^{+} + [2.2.1]^{2-} \rightleftharpoons Eu^{3+} + PDA^{2-} + [2.2.1]^{2-} \rightleftharpoons [Eu{\cdot}2.2.1]^{+} + PDA^{2-} \rightleftharpoons [Eu{\cdot}2.2.1{\cdot}PDA]^{-}$

**Scheme 1**

molecules *or another ligand*, such as the known photosensitiser, 1,10-phenanthroline dicarboxylate (PDA) **3**.[9] Ratios of [2.2.1] to europium are kept at 1:1 in order to avoid 2:1 complexes involving these species.

As expected, the $Eu^{3+}$·[2.2.1] complex shows no luminescence when irradiated at 300 nm [Fig. 1(*a*)]. However, as soon as the PDA **3** is added, luminescence is observed [Fig. 1(*c*)]. As predicted, in the absence of the shielding [2.2.1] ligand, the PDA·$Eu^{3+}$ complex ($K_{ass}$ *ca.* $10^7$) only shows weak luminescence [Fig. 1(*b*)].

The luminescence curves show an interesting pH dependence. At high pH the europium complexes decompose by hydroxide attack, formation of europium oxides being observed. At lower pH protonation of the carboxylate groups occurs, thus lowering their affinity for the metal ion. Furthermore, the luminescence peak for the tertiary complex [Fig. 1(*c*)] occurs at a different point (pH 8.0) from that of the binary complex [Fig. 1(*b*)] (pH 9.2): over a sixfold enhancement in luminescence is observed at pH 8.0.

Another observation was also of interest. The formation of both the 1:1 PDA·$Eu^{3+}$ complex and the 1:1:1 tertiary complex occurs rapidly upon addition of the PDA to the other components, as reflected by the rapid onset of the observed luminescence. In contrast, addition of [2.2.1] **2** to the PDA·$Eu^{3+}$ complex results only in a slow increase in luminescence to the full value (halflife *ca.* 3 h). We explain this by the fact that the organisation of the more mobile [2.2.1] complex about the europium ions is a far more demanding process, in terms of conformational reorganisation and displacement of water,[10] than that of, for example, PDA **3** with europium. The reverse is also true and, once formed, unravelling of the [2.2.1] ligand (total dissociation) from the europium ion is also slow.

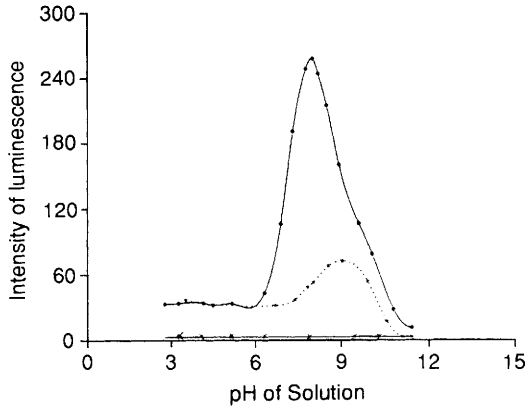



**Fig. 1** Variation of luminescence with changing pH (*a*) —×— $Eu^{3+}$ ($10^{-4}$), [2.2.1] ($10^{-4}$); (*b*) ···×·· $Eu^{3+}$ ($10^{-4}$), PDA ($10^{-6}$); (*c*) —●— $Eu^{3+}$ ($10^{-4}$), [2.2.1] ($10^{-4}$), PDA ($10^{-6}$ mol $dm^{-3}$). Aqueous solutions; pH measured immediately prior to luminescence measurements; graphs are average of triplicates; $\lambda_{em}$ 615 nm.

We are currently trying to exploit these observations in the development of new assay systems. We thank Cambridge Research Biochemicals Ltd. for financial support of this work.

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