Effect of lipophilicity in oxyanions responsive Eu(III) complexes

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Abstract: Responsive lanthanide(III) complexes—europium(III) complexes in particular—has been the focus of studies for several decades. The response, in the form of changes of photophysical and electronic properties of the lanthanide(III) ion, arises through supramolecular interactions between a guest or analyte molecule, and the lanthanide(III) complex which acts as host. While responsive lanthanide complexes have been reported and investigated frequently, the supramolecular aspects and linear free-energy relationship have had less attention. Here, we are revising five europium(III) complexes and investigating their binding to nine different guests, all with a primary interaction between europium(III) ion and a bidentate carboxylate anion. The media effect was investigated, and by eliminating the impact of hydrophobic effects, we can show that selectivity in these host-guest systems can be tuned by the secondary lipophilic interactions.

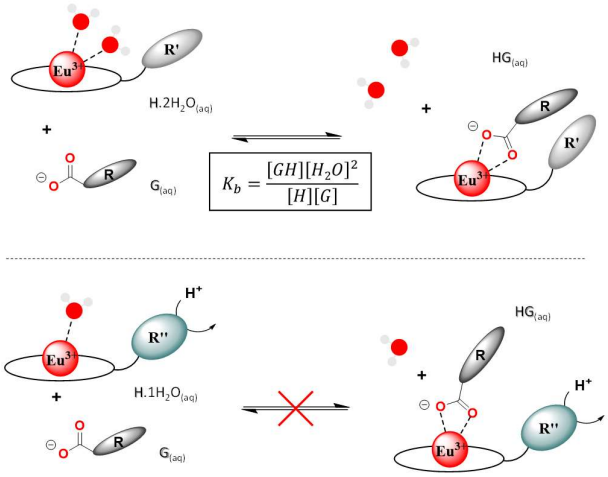
Introduction

Lanthanide luminescence has the unique photophysical combination of a millisecond luminescence lifetime and a high photoluminescence quantum yield.[1] This gives rise to unique applications in bioassay and bioimaging.[2–11] Furthermore the luminescence lifetime depends on quenching by water,[12–14] and the spectral shape can be changed by modulation of the ligand field.[15–19] This can be—and has been—used extensively to create lanthanide(III) complexes with a response specific to an analyte.[7,20–29] The basis of the response is a lanthanide(III) centred interaction, which we are not able to describe to an adequate degree. Here, we aim to lay the foundation for linear free energy relationships for lanthanide(III) centred interactions as we know them from supramolecular chemistry.[30]

We have previously worked on quantifying interactions in lanthanide based supramolecular chemistry,[31–36] where quantification required highly alkaline, non-aqueous media. In general, there is a significant focus on media and salt effects in supramolecular chemistry,[37–40] and in this work we seek an aqueous solvent system that allow us to mitigate the effects of salinity, pH and any non-specific hydrophobic interactions of guest and host molecules. Our starting point is a recent paper by our group, where luminescent response to bicarbonate of the europium(III) complex of 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A) was thoroughly studied in various aqueous media.[41] Competition with other oxyanions of interest, such as monohydrogenophosphate, *L*-lactate and citrate, showed that, at relevant biological concentrations, the optical response was not influenced by these species despite a stronger binding to the europium(III) centre. Changes in concentrations of these anions cannot be selectively reported by Eu.DO3A, yet this is an excellent starting point for a supramolecular analysis of these binding events. In particular, as there exists a large literature on the synthesis of various derivatives of lanthanides azamacrocyclic complexes that bind to oxyanions.[23,27,32,42–51]

The mechanism of binding between a lanthanide(III) host and an oxyanion guest is most readily described in the scenario where the host is neutral and ion pairing effects can be ignored. Figure 1 shows this case, the equilibria involved, and the potential competitive binding events that change as a function of pH and media composition. Figure 1 includes two scenarios: a europium(III) complex formed from a 7-dentate ligand that create ternary

complexes with bidentate anions following water displacement.[20,23,45] And an example of an europium(III) complex formed from a 8-dentate ligand, where the lack of oxyanion binding can be exploited for pH and oxygen sensing.[23,52–54]



$$K_b = \frac{[GH][H_2O]^2}{[H][G]}$$

Figure 1. Mechanism of binding of a bidentate carboxylate (G = guest) to a 7-coordinated Eu(III) centre (H = host) with displacement of two water molecules (top). Absence of water displacement by the oxyanion (G) on a 8-coordinated Eu(III) complex (H) (bottom). R' and R'' represents the fourth pendant arm of the DO3A scaffold. Here, R'' is illustrated as pH-responsive.

Here, we wish to determine the effect of a change in structure of the binding pocket on the binding event. To do so we must first discriminate between effects due to non-specific interactions or changes in media.[55] In other words, we must ensure that we are seeing a binding event. When this is established, we wish to probe the effect of the lipophilicity of the fourth appended arm on the DO3A scaffold. A position that is readily available for functionalisation. Chart 1 shows the host molecules we chose to work with alongside the oxyanion guest that were tested. We start by validating the lack of binding in the europium(III) complex with 8-dentate ligands, which also reports the error on the experimental method we selected to use. That is the magnitude of changes that must be surpassed to claim a lanthanide(III) centred interaction. Then we move on to show that the effect of pH is negligible, after having considered the effect of medium in general. After moving to a medium capable of dissolving guest, host, and the ternary complex, we are able to show a direct effect of the nature of the appendant fourth arm on the guest binding in these systems. In total we have worked with five different europium(III) complexes and ten different oxyanion guest molecules (Chart 1). The guest was chosen from three groups. 1) Biological buffers: carbonate,[41,42,56–60] and phosphate.[47,61] 2) Catabolites: *L*-lactate, *L*-malate, and citrate.[48,62–64] And 3) model guests: acetate, propionate, succinate, butyrate, and benzoate.

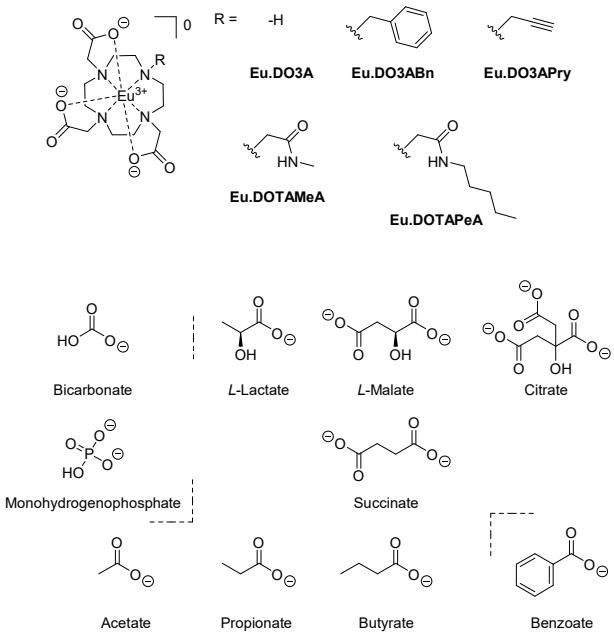


Chart 1. Eu(III) complexes (hosts) and various analytes (guests) discussed in this work.

Results and Discussion

Response upon binding at Eu(III) centres.

The five europium(III) host complexes (Chart 1) were synthesized as previously described.[15] Three different types of heptadentate 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A) complexes functionalised on the fourth nitrogen with: nothing **Eu.DO3A**, a benzyl group **Eu.DO3ABn**, and a terminal alkyne **Eu.DO3APry**, were used; alongside with two 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra acetic acid (DOTA) monoamides complexes: one with *N*-methyl group **Eu.DOTAMeA** and one with *N*-pentyl chain **Eu.DOTAPeA**. The longer carbon chain was used to mimic the lipophilic effect of a spacer. Each host was considered as one molecular entity in this work, despite the knowledge of the variety of forms present in solution.[15,65]

The spectra of the five europium(III) complexes are shown in Figure 2.A, free and in a medium high in bi-carbonate. The changes on the spectra show the effect of forming a ternary complex and the difference between complexes formed from 7- and 8-dentate ligands. Note that only complexes from 7-dentate ligands form ternary complexes, and the differences in the spectra of the complexes from 8-dentate ligands shows the magnitude of medium effects. Only very limited effects in band shapes and the number of lines observed is seen upon formation of the ternary complex. This is in stark contrast to what is observed for binding of ligands to free europium(III) ions, but is readily explained by the minimal change in crystal field between the hydrated and the ternary complex, as illustrated on Chart 2.[18,19] Thus, our main tool for analysing the formation of ternary complexes is the change in luminescence lifetime.

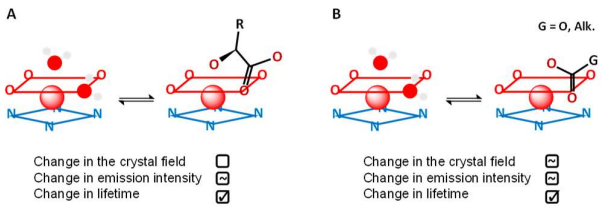


Chart 2. Overview of the expected experimental changes in Eu(III) luminescence upon introduction of an analyte in the coordination sphere.
A: coordination of an *α*-hydroxycarboxylate; B: coordination of a carboxylate.

To qualify these assumptions, titrations were performed with four of the analytes: bicarbonate, monohydrogenophosphate, *L*-lactate and citrate. At each point, excitation and emission spectra and time-resolved emission decay curves were recorded, see the SI Figures S1-8 for details. We noticed an instability in the response (Figures S1-S2), which we attribute to instability of the stock solutions in HEPES buffer. It is clearly seen as a significant decrease in total intensity. As example, over a 4h period the intensity of the hypersensitive ΔJ = 2 transition at ~615 nm decreases of about 30 a.u. following excitation at 395 nm between first point in the HCO_3^- titration (recorded first) and the citrate titration (recorded last). Thus, selected titrations were performed again after a three-week period. While the total intensity changes, overlays of lifetimes and intensity ratios ΔJ = 2 /ΔJ = 0 (I_{616}/I_{580}) isotherms from the two titrations of HCO_3^- and HPO_4^{2-} show no significant differences (Figure S9). While changes clearly occur over both shorter (hours) and longer (weeks) timescales, it does not interfere the complex ability to monitor changes in carbonate and phosphate concentrations using the ratiometric readouts.

The emission spectra of **Eu.DO3A**, **Eu.DO3ABn** and **Eu.DO3APry** change in a similar pattern upon addition of either one of the studied analytes (Figures S4, S5 and S6 respectively). Coordination of the analyte to the europium(III) centre generate a slight red-shift in spectra for all the transition bands due to minute changes in the crystal field. The effect is greatest for the ΔJ = 1 transition at ~590 nm and ΔJ = 2 transition at ~615 nm. The displacement of water leads to the increase in intensity of ΔJ = 1, ΔJ = 2 and ΔJ = 4 (at ~700 nm) transitions, as pathways of non-radiative deactivation is removed.[12–14] However, it has to be noted that the monitored increase in emission intensity for phosphate titration is at least 3 times smaller than for bicarbonate. The ΔJ = 0 transition at 580 nm, on the other hand, decrease as the crystal field change: most importantly for bicarbonate and phosphate but discreetly for *L*-lactate and citrate. As expected,[23] no variations in the spectra were observed for the titrations performed with 8-coordinated **Eu.DOTAMeA** and **Eu.DOTAPeA** (Figures S7 and S8 respectively).

Throughout the following analysis, we only use the ratiometric responses. These are independent of the Eu(III) complex concentration, and the change in time described above. An intensity ratiometric isotherm can be plotted by dividing the luminescence intensity of the hypersensitive ΔJ = 2 transition at 615 nm by the ΔJ = 0 transition at 580 nm (

Figure **3**). Luminescence lifetimes isotherms are intrinsically ratiometric and display similar profiles, see Figure S10. In HEPES buffer, lifetimes for **Eu.DO3A** ternary complexes formed with analytes range from 0.3 to 0.4 ms. Lifetimes for ternary assemblies with **Eu.DO3APry** and **Eu.DO3ABn** start at 0.4 ms and show more steep slopes than their **Eu.DO3A** counterpart. While lifetimes of **Eu.DOTAMeA** and **Eu.DOTAPeA** remain constant at 0.6 ms.

Figure **3** shows that the presence of the coordinating amide function prevents monitoring of any of the four bidentate analytes tested in this part of the study, despite the presence of a water molecule available to be displaced. Those results are in accordance with previous experiments where $[Eu.DOTA]^-$ was tested as a possible bicarbonate sensor.[41] Repulsive electrostatic effects cannot alone to explain the lack of responsiveness towards oxy-anions, as both **Eu.DOTAMeA** and **Eu.DOTAPeA** are neutrally charged. Rather, we—supported by vast literature evidence—conclude that two available water molecules are necessary to form a ternary complexes between bidentate oxy-anions and europium(III) complexes.[23,45,66–70] In addition, the absence of differences between titrations performed with either the methylamide or the pentylamide complexes excludes that lipophilic effects alone accounts an increase in europium emission. The events responsible for the optical response in the ratiometric isotherms are therefore ascribed to a coordination event occurring at the europium(III) centre.

If we turn to the response displayed in

Figure **3**, it is evident that all four oxy-anions bind, and the response induced by addition of bicarbonate is the strongest. Both bicarbonate and monohydrogenophosphate are bidentate and replace two water molecules by forming a four-membered ring. The alpha-hydroxy acid are also bidentate, but are assumed to form five-membered rings upon water displacement.[45,48]

Contrasting the three europium(III) complexes formed from 7-dentate ligands, the lipophilicity of the fourth arms of the **Eu.DO3ABn** and **Eu.DO3APry** complexes leads to higher binding affinities for all four analytes. Considering the slope of the isotherms in

Figure **3**, the strongest binding event is observed between **Eu.DO3ABn** and **Eu.DO3APry** and *L*-lactate, suggesting that tuning the ligand lipophilicity can help reach selectivity for this particular analyte.

We thus have a means of analysing the formation of ternary complexes and design hypothesis. As propargyl is a fragile function, the hyphothesis was tested by comparing **Eu.DO3ABn** to **Eu.DO3A**.

Solubility issues.

It is well known in literature that media plays a major effect on the recognition event in a host-guest systems.[39,40] It can either be competitive or not, and no general conclusion should be drawnbased on data from a unique media.[33–35] We noted that europium(III) complexes and analytes were not fully soluble in mixture of MeOH and water, and that the ternary complexes were not fully soluble in pure buffer. Thus, we turned to a mixture of 10 % DMSO in water where guests, hosts, and ternary complexes were soluble. Optical responses of **Eu.DO3A** and **Eu.DO3ABn** were compared with previous experiments. Emission spectra are recorded using the same parameters (Figures S11, S13, S15 and S17). When comparing spectra before titrations in the different media (Figure S19), one can observe that emission intensity of the hypersensitive band increases once going from HEPES to 10 % DMSO for **Eu.DO3ABn** but remains similar for **Eu.DO3A**. This is attributed to better solubility of **Eu.DO3ABn** in the latter media. Band behaviours for the ΔJ = 0, 1 and 2 transitions are plotted against analyte concentrations (Figures S12, S14, S16 and S18). Binding events occur in DMSO-containing solutions for the four analytes: HCO_3^-, HPO_4^{2-}, *L*-lactate and citrate with both **Eu.DO3A** and **Eu.DO3ABn**. Therefore, DMSO is not competing with the coordination of those analytes at the Eu centre.

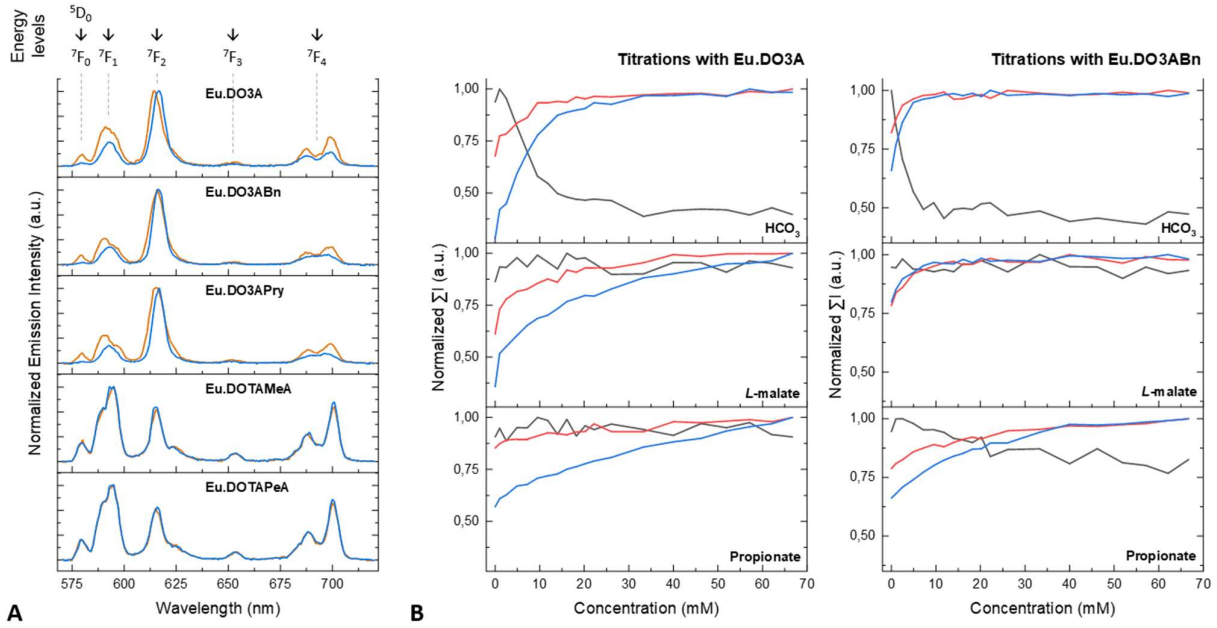


Figure 2. A. Normalised emission spectra (λ_{ex} = 395nm) of the five Eu(III) complexes in HEPES buffer, both alone (brown) or with bicarbonate (66.7 mM, blue).B. Illustration of expected changes from

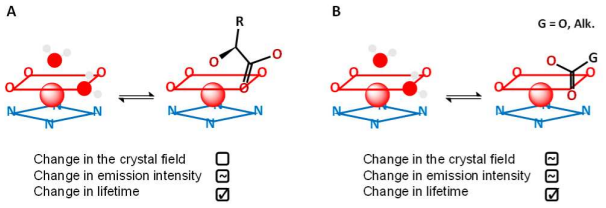


Chart **2** by plotting the band behaviours of ΔJ = 0, 1 and 2 transitions (grey, red and blue respectively) for *L*-malate, propionate and carbonate titrations in 10%DMSO/H_2O.

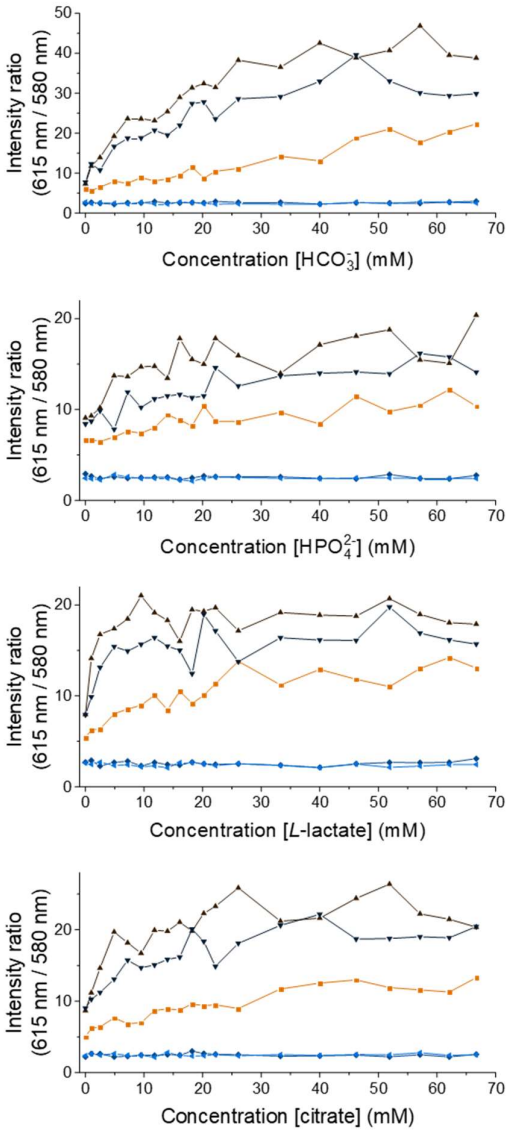


Figure 3. Intensity ratios (615 nm / 580 nm) for the detection of bicarbonate, phosphate, *L*-lactate and citrate in 1 mM solution of Eu.DO3A (■), Eu.DO3ABn (▼), Eu.DO3APry (▲), Eu.DOTAMeA (♦) and Eu.DOTAPeA (◄) in HEPES buffer (100 mM, pH 7.4).

The ratiometric responses remain a good analytical tool in the mixed solvent system. Note that the solvent system is not buffered, which we will consider in detail below.
In this solvent system we can be certain that a change in the ratiometric response of a europium(III) complex is due to a binding event and not induced by low solubility.

Screening of analytes.
Ten different guests were titrated into solutions containing the two host complexes, **Eu.DO3A** and **Eu.DO3ABn**. Experiments were performed with concentration of Eu(III) complexes at 1 mM and with concentration of the analytes up to 100 mM. Of the two ratiometric isotherms, the most straightforward analysis of the results are obtained from the luminescence lifetimes isotherms. The first panel of Figure 4 show that the response recorded from the **Eu.DO3A** complex give three groups of isotherms. The strongest response is recorded by the two

bidentate anions, an intermediate response is seen for the *α*-hydroxycarboxylates that form 5-membered rings, while a weaker response is seen for the bidentate carboxylates.

Further distinction can be made according to the observed band behaviours (Figure 2.B). HCO_3^- and HPO_4^{2-} clearly bind with both change in lifetime and crystal field (see also Chart 2.B, Figures S23-S24). For the *α*-hydroxycarboxylates, no clear trend can be made based on the spectral shape. That is, no crystal field changes are seen. This is most likely due to the perfect fit of the analyte that upon coordination to the europium centre, take the exact same position as the water molecules (Chart 2.A). Indeed, the ΔJ = 0 transition remain unchanged for *L*-lactate, *L*-malate and citrate when titrated with both **Eu.DO3A** and **Eu.DO3ABn** (

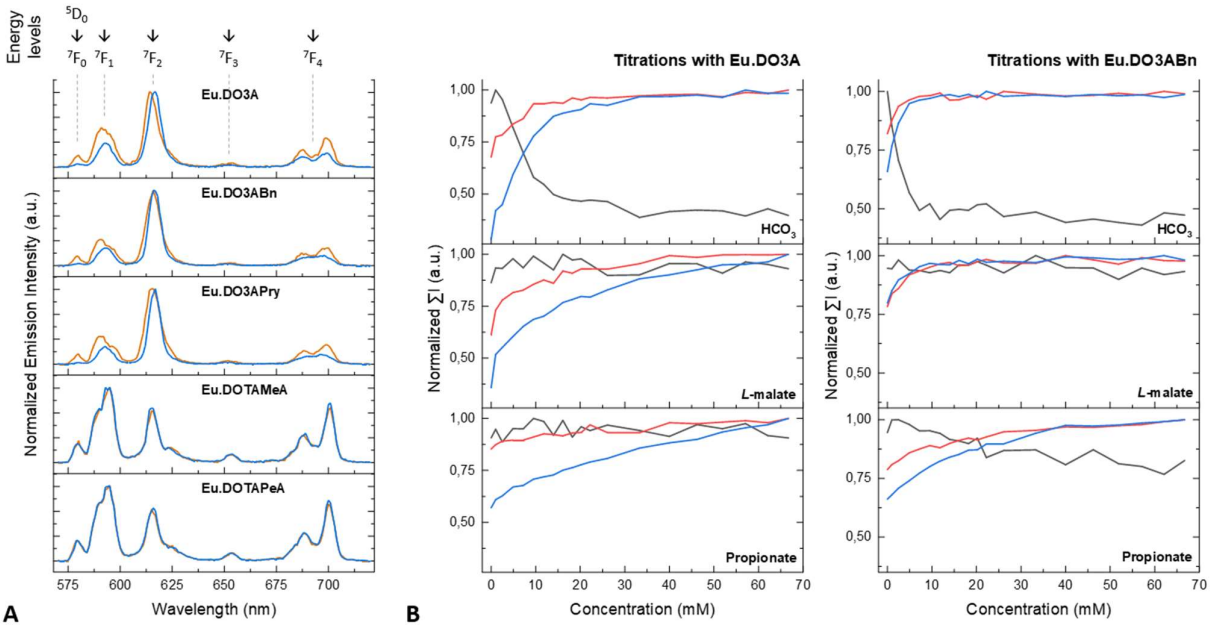


Figure 2.B, see also Figures S25-27). On the other hand, the ΔJ = 2 transition, which is mainly modulated according to solvent environment, clearly indicates that water molecules are being displaced. Strongly in the case of **Eu.DO3A** but weaker for **Eu.DO3ABn**, where the assembly have more to do with association in the outer sphere rather than clear binding.

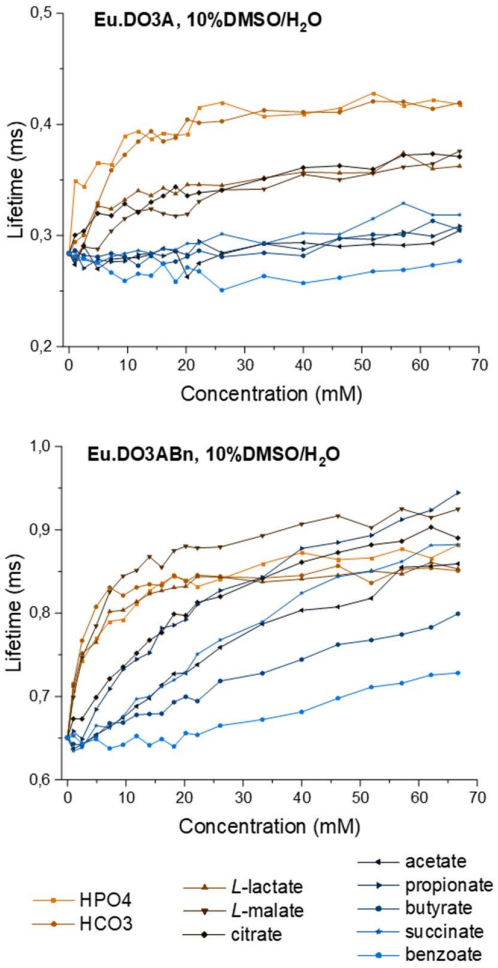


Figure 4. Luminescence lifetimes isotherms of Eu.DO3A (top) and Eu.DO3ABn (bottom) in 10%DMSO/H_2O upon coordination of an analyte. Isotherms are normalised to have the same starting point for easier comparison (Starting point references: bicarbonate titration for Eu.DO3A; citrate titration for Eu.DO3ABn). Non-corrected isotherms are available in the SI Figure S22.

When it comes to the carboxylates, it can be argued that they do not bind to **Eu.DO3A** in the concentration range studied here. There is very limited change in luminescence lifetime and no change in the emission spectra. This is in stark contrast to what is observed with the more lipophilic host system.

The second panel of Figure 4 show the luminescence lifetime isotherms for **Eu.DO3ABn** and the presence of the lipophilic binding pocket has a clear influence. The band behaviours and emission spectra for the model guests are available in the ESI (Figures S28-32), but the lifetimes isotherms are representative of the positive effect of the lipophilic binding pocket created by the insertion of the benzyl group on the DO3A scaffold.

The *L*-lactate and *L*-malate now induces a very strong response along with bicarbonate and monohydrogenophosphate. Citrate and propionate show a strong response, while acetate and succinate gives rise to an intermediate response. And while all the carboxylates act as innocent anions—inducing no lifetimes response—for **Eu.DO3A**, also butyrate and benzoate induces a response in **Eu.DO3ABn**. So all oxyanions coordinate to **Eu.DO3ABn** although the larger carboxylates do so without inducing a change in the crystal field.

The observations are summarised in Chart 3. By eliminating effects of poor solubility, we are able to see the effect of adding a hydrophobic auxiliary on the selectivity of ternary complex formation. While we, in the simple systems studied here, increase the binding of all guests and thus reduce the selectivity, these data opens an avenue for host design based on lipophilic effects. We note that the selectivity of *L*-lactate over citrate is significantly increased, which promises selectivity for smaller oxy-monoanions over the prevalent oxy-polyanions.

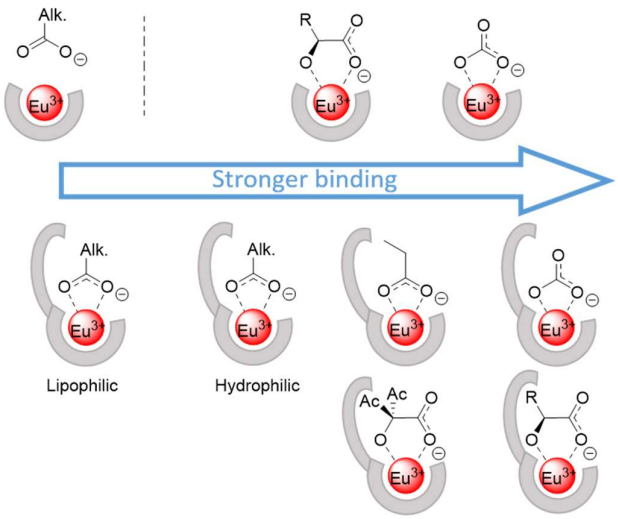


Chart 3. Binding strength and structure of ternary complexes

Two words on pH

By scrutinising the emission spectra at start and end-points of the titrations in 10 % DMSO, we could see that **Eu.DO3ABn** is quite sensitive to ambient carbon dioxide absorption (Figure S20). As the start and end-points of titrations also vary in pH, we had to document whether pH has to be considered a main factor in the observed response. To quantify the effect of pH, titrations with hydroxide anions were performed. The hydroxide anion was treated as any other guest in this study. The highest recorded pH in the stock solutions of ternary assemblies was measured at roughly 10, which is equivalent to $[OH^-] = 0.1$ mM. Thus, titrations were performed with **Eu.DO3A** and **Eu.DO3ABn** starting from 0.1 mM hydroxide stock solutions (Figure S38), and pH was monitored simultaneously (Figure S37). Results are displayed in

Figure **5**, top panel. Cursory inspection of the isotherms shows a clear lack of response to the hydroxide anion.

To assess the limit where hydroxide anions start interacting with the Eu complexes, the pH was further increased until observable changes could be monitored. **Eu.DO3A** tolerates roughly 0.3 mM of NaOH before the hydroxide anions induce lifetime and emission changes. **Eu.DO3ABn** is more sensitive as increase in lifetime is observed starting at 0.05 mM of NaOH (

Figure **5**, bottom panel; see also Figure S39).

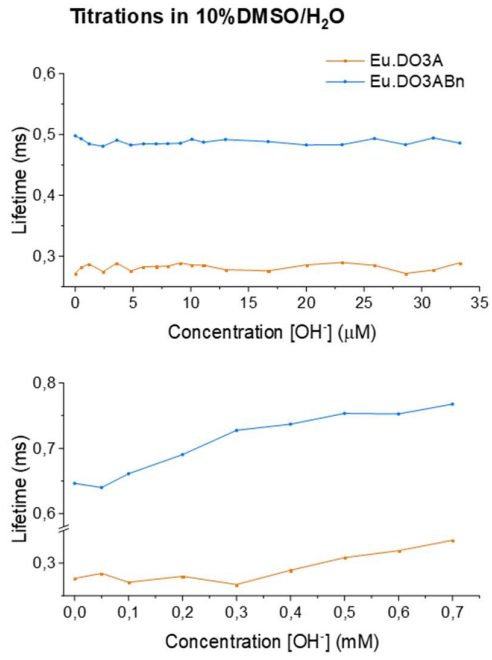


Figure 5. Emission lifetimes according to hydroxide concentrations.

While pH is not a concern, the observation that solution of **Eu.DO3ABn** shows response to absorption of ambient carbon dioxide should be taken into account. Thus, further studies should consider using a buffered DMSO containing solution with a pH below the pKa of carbonic acid.

Conclusion

Media effects and non-specific interactions often hinder the ability to obtain a comprehensive overview of more specific interactions in supramolecular chemistry. Here, by discriminating for these former effects, we were able to investigate further the influence of structure on the binding event between a Eu(III)-based host and an analyte guest.

In the first part, focus was given to the outcomes of a change of the fourth arm on the DO3A scaffold. The presence of a lipophilic arm, such as propargyl or benzyl, turned out to enhance the binding affinities of all analytes tested, *L*-lactate in particular.

Thus, in the second part, investigations were performed in an appropriate medium to ensure solubility of all parts of the ternary complexes to study the effect of lipophilicity in the formation of ternary complexes. A series of ten analytes were titrated with the two hosts **Eu.DO3A** and **Eu.DO3ABn** in a 10 % DMSO/H_2O solvent system. The results clearly revealed that lipophilic effects, despite not being responsible for the detection event itself, play a role in binding affinities and therefore on the possible tuning of the selectivity of responsive complexes for a specific analyte.

Experimental Section

Synthesis.
Complexes shown on Chart 1 were synthesised as described in a recent paper by our group,[15] and were adapted from already reported procedures.[65,71,72]

Spectroscopic studies.
Sample preparations and titrations were performed following similar methodologies described in previous work.[35,41] Single media stock solutions were prepared and used to prepare all other solutions to ensure uniform background composition. The HEPES buffer solution was prepared at a concentration of 100 mM; the pH was controlled and adjusted to 7.40 with $NaOH_{(s)}$. The 10 % DMSO/H_2O solution was prepared using freshly-opened HPLC-grade DMSO. Water from a MilliQ purification system was used (deionised with resistivity > 18.2 MΩ·cm; microfiltered with total organic carbon (TOC) < 5 ppb). Solutions were prepared in volumetric flasks or with volume displacement micropipettes, and compounds mass was determined to 2 significant digit at the mg scale.
For each Eu(III) complex studied, a stock solution of said complex was prepared at 1 mM in the appropriate media and used to prepare the concentrated analytes solutions. This method ensure constant and uniform concentration of the emitting Eu(III) host during titrations. Each analyte solutions were prepared at a concentration of 200 mM using the sodium salt (mono-, di- or tri-) of the targeted analyte. The use of the salt form is necessary to avoid potential absence of binding at the europium center due to protonated carboxylate functions.
Titrations were performed directly in the cuvette (1.000 cm, 4 mL quarts fluorescence cuvettes from Starna) from an initial volume of 2 mL of the Eu(III) complex stock solution to a total volume of 3 mL. For each titration point, a fixed volume of the concentrated analyte solution (prepared from the matching Eu(III) complex stock solution) was added into the cuvette, then emission spectrum and lifetime were measured. 20 points per titration were recorded, resulting to “in-cuvette” concentration of analyte ranging from 0 to 66.7 mM.
Time resolved excitation and emission spectra, and time-resolved emission decay profiles were recorded using a Cary Eclipse Fluorescence Spectrophotometer with the associated software from Agilent Technologies (Scan & Lifetimes applications; Version 1.2). Excitation and emission spectra were recorded with the “Phosphorescence” data mode to accommodate the long-lived luminescence of Europium. Total decay time was set at 5 ms, the delay time at 0.1 ms and the gate time at 0.3 ms. PMT voltage was set at the maximum, *i.e.* 950 V, to recover enough intensity signal and compensate for the low concentration of Eu(III) complexes in the cuvette (1 mM). Excitation spectra are reported from 300 to 500 nm with the following parameters: λ_{em} = 615 nm, excitation and emission slits set at 5 and 20 nm respectively. Emission spectra are reported from 570 to 720 nm with the following parameters: λ_{ex} = 395 nm, excitation and emission slits set at 20 and 5 nm respectively. Scan rate was set at

333.33 nm/s, with automatic excitation and emission light filters. Lifetimes were recorded with a total decay time of 30.0 ms, λ_{ex} = 395 nm, λ_{em} = 615 nm, and both excitation and emission slits were set at 20 nm.
Intensity ratios were calculated using the intensity values at 615 nm and 580 nm, which are the maximums of the ΔJ = 2 and ΔJ =0 transitions respectively. Band behaviours were evaluated by adding together the intensity values across each band. As the spectrum is slightly redshifted throughout the titrations, working with total intensities gives a more truthful representation of the modulation of the transition bands. Lifetimes were calculated *via* Origin software, using an exponential decay fit: $y = A \times e^{-x/t} + B$, where ***t*** is the lifetime.

pH titrations.
100 mM and 0.1 mM NaOH solutions in Milli-Q water were used to titrate 2 mL of 1 mM **Eu.DO3A** or **Eu.DO3ABn** stock solutions in 10 % DMSO/H_2O. The total added volume for these titrations did not exceed 20 µL in order to keep the dilution factor of the emitting Eu(III) complex non-significant.
pH titrations were performed in duplicate simultaneously; pH was measured on one set while spectroscopic data were recorded on the other. pH measurements were performed with a SevenEasy pH-meter from Mettler Toledo, calibrated with InLab™ buffer solutions (pH 7.00, 4.01 and 10.00) from the same brand.

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Keywords: Europium • DO3A • Oxyanions • Media effect • Lipophilicity

References

1. Hänninen, P. & Härmä, H. *Lanthanide Luminescence*. *Springer Series on Fluorescence, Vol. 7* vol. 7 (Springer Berlin Heidelberg, 2011).
2. Zwier, J. M. & Hildebrandt, N. Time-Gated FRET Detection for Multiplexed Biosensing. in *Reviews in Fluorescence 2016* (ed. Geddes, Chris, D.) 17–43 (2017). doi:10.1007/978-3-319-48260-6_3.
3. Delbianco, M. *et al.* Bright, Highly Water-Soluble Triazacyclononane Europium Complexes To Detect Ligand Binding with Time-Resolved FRET Microscopy. *Angew. Chemie Int. Ed.* **53**, 10718–10722 (2014).
4. Soulié, M. *et al.* Comparative Analysis of Conjugated Alkynyl Chromophore-Triazacyclononane Ligands for Sensitized Emission of Europium and Terbium. *Chem. - A Eur. J.* **20**, 8636–8646 (2014).
5. Moore, E. G., Samuel, A. P. S. & Raymond, K. N. From Antenna to Assay: Lessons Learned in Lanthanide Luminescence. *Acc. Chem. Res.* **42**, 542–552 (2009).
6. Xu, J. *et al.* Octadentate Cages of Tb(III) 2-Hydroxyisophthalamides: A New Standard for Luminescent Lanthanide Labels. *J. Am. Chem. Soc.* **133**, 19900–19910 (2011).
7. Montgomery, C. P., Murray, B. S., New, E. J., Pal, R. & Parker, D. Cell-Penetrating Metal Complex Optical Probes: Targeted and Responsive Systems Based on Lanthanide Luminescence. *Acc. Chem. Res.* **42**, 925–937 (2009).
8. Picot, A. *et al.* Long-Lived Two-Photon Excited Luminescence of Water-Soluble Europium Complex: Applications in Biological Imaging Using Two-Photon Scanning Microscopy. *J. Am. Chem. Soc.* **130**, 1532–1533 (2008).
9. Martinić, I., Eliseeva, S. V., Nguyen, T. N., Pecoraro, V. L. & Petoud, S. Near-Infrared Optical Imaging of Necrotic Cells by Photostable Lanthanide-Based Metallacrowns. *J. Am. Chem. Soc.* **139**, 8388–8391 (2017).
10. Vereb, G., Jares-Erijman, E., Selvin, P. R. & Jovin, T. M. Temporally and Spectrally Resolved Imaging Microscopy of Lanthanide Chelates. *Biophys. J.* **74**, 2210–2222 (1998).
11. Hemmilä, I., Dakubu, S., Mukkala, V.-M., Siitari, H. & Lövgren, T. Europium as a label in time-resolved immunofluorometric assays. *Anal. Biochem.* **137**, 335–343 (1984).
12. Horrocks, W. D. & Sudnick, D. R. Lanthanide ion probes of structure in biology. Laser-induced luminescence decay constants provide a direct measure of the number of metal-coordinated water molecules. *J. Am. Chem. Soc.* **101**, 334–340 (1979).
13. Beeby, A. *et al.* Non-radiative deactivation of the excited states of europium, terbium and ytterbium complexes

by proximate energy-matched OH, NH and CH oscillators: an improved luminescence method for establishing solution hydration states. *J. Chem. Soc. Perkin Trans. 2* **2**, 493–504 (1999).
14. Tropiano, M. *et al.* Exploring the effect of remote substituents and solution structure on the luminescence of three lanthanide complexes. *J. Lumin.* **167**, 296–304 (2015).
15. Nielsen, L. G. & Sørensen, T. J. Including and Declaring Structural Fluctuations in the Study of Lanthanide(III) Coordination Chemistry in Solution. *Inorg. Chem.* **59**, 94–105 (2020).
16. Nawrocki, P. R., Kofod, N., Juelsholt, M., Jensen, K. M. Ø. & Sørensen, T. J. The effect of weighted averages when determining the speciation and structure–property relationships of europium(iii) dipicolinate complexes. *Phys. Chem. Chem. Phys.* **22**, 12794–12805 (2020).
17. Kofod, N., Nawrocki, P., Platas-Iglesias, C. & Sørensen, T. J. Electronic Structure of Ytterbium(III) Solvates—a Combined Spectroscopic and Theoretical Study. *Inorg. Chem.* **60**, 7453–7464 (2021).
18. Blackburn, O. A. *et al.* Fluoride Binding and Crystal-Field Analysis of Lanthanide Complexes of Tetrapicolyl-Appended Cyclen. *Chem. - A Eur. J.* **22**, 8929–8936 (2016).
19. Blackburn, O. A. *et al.* Substituent effects on fluoride binding by lanthanide complexes of DOTA-tetraamides. *Dalt. Trans.* **45**, 3070–3077 (2016).
20. Heffern, M. C., Matosziuk, L. M. & Meade, T. J. Lanthanide Probes for Bioresponsive Imaging. *Chem. Rev.* **114**, 4496–4539 (2014).
21. Pershagen, E. & Borbas, K. E. Designing reactivity-based responsive lanthanide probes for multicolor detection in biological systems. *Coord. Chem. Rev.* **273**–**274**, 30–46 (2014).
22. Shuvaev, S., Starck, M. & Parker, D. Responsive, Water-Soluble Europium(III) Luminescent Probes. *Chem. - A Eur. J.* **23**, 9974–9989 (2017).
23. Parker, D. Luminescent lanthanide sensors for pH, pO2 and selected anions. *Coord. Chem. Rev.* **205**, 109–130 (2000).
24. New, E. J., Parker, D., Smith, D. G. & Walton, J. W. Development of responsive lanthanide probes for cellular applications. *Curr. Opin. Chem. Biol.* **14**, 238–246 (2010).
25. Routledge, J. D. *et al.* Lanthanide Complexes that Respond to Changes in Cyanide Concentration in Water. *Angew. Chemie Int. Ed.* **56**, 7783–7786 (2017).
26. Martins, A. F. *et al.* Imaging Insulin Secretion from Mouse Pancreas by MRI Is Improved by Use of a Zinc-Responsive MRI Sensor with Lower Affinity for Zn 2+ Ions. *J. Am. Chem. Soc.* **140**, 17456–17464 (2018).
27. Trokowski, R., Zhang, S. & Sherry, A. D. Cyclen-Based Phenylboronate Ligands and Their Eu 3+ Complexes for Sensing Glucose by MRI. *Bioconjug. Chem.* **15**, 1431–1440 (2004).
28. Nielsen, L. G., Junker, A. K. R. & Sørensen, T. J. Composed in the f-block: solution structure and function of kinetically inert lanthanide(iii) complexes. *Dalt. Trans.* **47**, 10360–10376 (2018).
29. Cosby, A. G. *et al.* Accessing lanthanide-based, in situ illuminated optical turn-on probes by modulation of the antenna triplet state energy. *Chem. Sci.* **12**, 9442–9451 (2021).
30. Schneider, H.-J. Binding Mechanisms in Supramolecular Complexes. *Angew. Chemie Int. Ed.* **48**, 3924–3977 (2009).
31. Tilney, J. A., Sørensen, T. J., Burton-Pye, B. P. & Faulkner, S. Self-assembly between dicarboxylate ions and a binuclear europium complex: formation of stable adducts and heterometallic lanthanide complexes. *Dalt. Trans.* **40**, 12063 (2011).
32. Tropiano, M. *et al.* Using Remote Substituents to Control Solution Structure and Anion Binding in Lanthanide Complexes. *Chem. – A Eur. J.* **19**, 16566–16571 (2013).
33. Sørensen, T. J. *et al.* Self-Assembly between Dicarboxylate Ions and Dinuclear Lanthanide Complexes: A Surprisingly Complicated Problem. *Eur. J. Inorg. Chem.* **2014**, 2520–2528 (2014).
34. Hill, L. R. *et al.* Self-assembly between dicarboxylate ions and binuclear europium complexes: moving to water—pH dependence and effects of buffers. *Dalt. Trans.* **42**, 67–70 (2013).
35. Sørensen, T. J., Hill, L. R. & Faulkner, S. Thermodynamics of Self-Assembly of Dicarboxylate Ions with Binuclear Lanthanide Complexes. *ChemistryOpen* **4**, 509–515 (2015).
36. Blackburn, O. A., Tropiano, M., Natrajan, L. S., Kenwright, A. M. & Faulkner, S. Luminescence of a binuclear europium complex bearing a 4-nitrophenolate chromophore: a different way of seeing pH dependence. *Chem. Commun.* **52**, 6111–6114 (2016).
37. Marcus, Y. & Hefter, G. Ion Pairing. *Chem. Rev.* **106**, 4585–4621 (2006).

38. Gasa, T. B. *et al.* Complexation between Methyl Viologen (Paraquat) Bis(Hexafluorophosphate) and Dibenzo[24]Crown-8 Revisited. *Chem. - A Eur. J.* **15**, 106–116 (2009).
39. Jordan, J. H., Ashbaugh, H. S., Mague, J. T. & Gibb, B. C. Buffer and Salt Effects in Aqueous Host–Guest Systems: Screening, Competitive Binding, or Both? *J. Am. Chem. Soc.* jacs.1c08457 (2021) doi:10.1021/jacs.1c08457.
40. Mandal, P., Kretzschmar, J. & Drobot, B. Not just a background: pH buffers do interact with lanthanide ions—a Europium(III) case study. *JBIC J. Biol. Inorg. Chem.* (2022) doi:10.1007/s00775-022-01930-x.
41. Bridou, L., Nielsen, L. G. & Sørensen, T. J. Using europium(III) complex of 1,4,7,10-tetraazacyclododecane-1,4,7-triacedic acid Eu.DO3A as a luminescent sensor for bicarbonate. *J. Rare Earths* **38**, 498–505 (2020).
42. Bruce, J. I. *et al.* The Selectivity of Reversible Oxy-Anion Binding in Aqueous Solution at a Chiral Europium and Terbium Center: Signaling of Carbonate Chelation by Changes in the Form and Circular Polarization of Luminescence Emission. *J. Am. Chem. Soc.* **122**, 9674–9684 (2000).
43. Carr, R. *et al.* A chiral probe for the acute phase proteins alpha-1-acid glycoprotein and alpha-1-antitrypsin based on europium luminescence. *Dalt. Trans.* **41**, 13154 (2012).
44. Ren, J., Trokowski, R., Zhang, S., Malloy, C. R. & Sherry, A. D. Imaging the tissue distribution of glucose in livers using a PARACEST sensor. *Magn. Reson. Med.* **60**, 1047–1055 (2008).
45. Butler, S. J. & Parker, D. Anion binding in water at lanthanide centres: from structure and selectivity to signalling and sensing. *Chem. Soc. Rev.* **42**, 1652–1666 (2013).
46. Hewitt, S. H. *et al.* Tuning the anion binding properties of lanthanide receptors to discriminate nucleoside phosphates in a sensing array. *Chem. Sci.* **11**, 3619–3628 (2020).
47. Hammell, J., Buttarazzi, L., Huang, C.-H. & Morrow, J. R. Eu(III) Complexes as Anion-Responsive Luminescent Sensors and Paramagnetic Chemical Exchange Saturation Transfer Agents. *Inorg. Chem.* **50**, 4857–4867 (2011).
48. Dickins, R. S. *et al.* Structural, Luminescence, and NMR Studies of the Reversible Binding of Acetate, Lactate, Citrate, and Selected Amino Acids to Chiral Diaqua Ytterbium, Gadolinium, and Europium Complexes. *J. Am. Chem. Soc.* **124**, 12697–12705 (2002).
49. Gempf, K. L., Butler, S. J., Funk, A. M. & Parker, D. Direct and selective tagging of cysteine residues in peptides and proteins with 4-nitropyridyl lanthanide complexes. *Chem. Commun.* **49**, 9104 (2013).
50. Mailhot, R., Traviss-Pollard, T., Pal, R. & Butler, S. J. Cationic Europium Complexes for Visualizing Fluctuations in Mitochondrial ATP Levels in Living Cells. *Chem. - A Eur. J.* **24**, 10745–10755 (2018).
51. Smrčka, F., Lubal, P. & Šídlo, M. The urea biosensor based on luminescence of Eu(III) ternary complex of DO3A ligand. *Monatshefte für Chemie - Chem. Mon.* **148**, 1945–1952 (2017).
52. Law, G.-L., Pal, R., Palsson, L. O., Parker, D. & Wong, K.-L. Responsive and reactive terbium complexes with an azaxanthone sensitiser and one naphthyl group: applications in ratiometric oxygen sensing in vitro and in regioselective cell killing. *Chem. Commun.* 7321 (2009) doi:10.1039/b920222f.
53. Carr, R., Evans, N. H. & Parker, D. Lanthanide complexes as chiral probes exploiting circularly polarized luminescence. *Chem. Soc. Rev.* **41**, 7673 (2012).
54. Pal, R. & Parker, D. A single component ratiometric pH probe with long wavelength excitation of europium emission. *Chem. Commun.* 474–476 (2007) doi:10.1039/B616665B.
55. Kofod, N., Thomsen, M. S., Nawrocki, P. & Sørensen, T. J. Revisiting the Assignment of Innocent and Non-Innocent Counter ions in Lanthanide(III) Solution Chemistry. *ChemRxiv* (2022).
56. Supkowski, R. M. & Horrocks, W. D. Displacement of Inner-Sphere Water Molecules from Eu 3+ Analogues of Gd 3+ MRI Contrast Agents by Carbonate and Phosphate Anions: Dissociation Constants from Luminescence Data in the Rapid-Exchange Limit. *Inorg. Chem.* **38**, 5616–5619 (1999).
57. Bretonnière, Y., Cann, M. J., Parker, D. & Slater, R. Ratiometric probes for hydrogencarbonate analysis in intracellular or extracellular environments using europium luminescence. *Chem. Commun.* 1930–1931 (2002) doi:10.1039/B206286K.
58. Smith, D. G. *et al.* Evidence for the optical signalling of changes in bicarbonate concentration within the mitochondrial region of living cells. *Chem. Commun.* **47**, 7347 (2011).
59. Vaněk, J., Lubal, P., Hermann, P. & Anzenbacher, P. Luminescent Sensor for Carbonate Ion Based on Lanthanide(III) Complexes of 1,4,7,10-Tetraazacyclododecane-1,4,7-Triacetic Acid (DO3A). *J. Fluoresc.* **23**, 57–69 (2013).

60. Vaněk, J., Smrčka, F., Lubal, P., Třísková, I. & Trnková, L. Dual carbonate sensor based on Eu(III) complex of DO3A ligand. *Monatshefte für Chemie - Chem. Mon.* **147**, 925–934 (2016).
61. Wilharm, R. K., Huang, S.-Y., Gugger, I. J. & Pierre, V. C. A Walk Across the Lanthanide Series: Trend in Affinity for Phosphate and Stability of Lanthanide Receptors from La(III) to Lu(III). *Inorg. Chem.* **60**, 15808–15817 (2021).
62. Aime, S., Delli Castelli, D., Fedeli, F. & Terreno, E. A Paramagnetic MRI-CEST Agent Responsive to Lactate Concentration. *J. Am. Chem. Soc.* **124**, 9364–9365 (2002).
63. Pal, R., Parker, D. & Costello, L. C. A europium luminescence assay of lactate and citrate in biological fluids. *Org. Biomol. Chem.* **7**, 1525 (2009).
64. Parker, D. & Yu, J. A pH-insensitive, ratiometric chemosensor for citrate using europium luminescence. *Chem. Commun.* **7**, 3141 (2005).
65. Regueiro-Figueroa, M., Esteban-Gómez, D., de Blas, A., Rodríguez-Blas, T. & Platas-Iglesias, C. Structure and Dynamics of Lanthanide(III) Complexes with an N-Alkylated do3a Ligand (H3do3a = 1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic Acid): A Combined Experimental and DFT Study. *Eur. J. Inorg. Chem.* **2010**, 3586–3595 (2010).
66. Parker, D. Excitement in f block : structure, dynamics and function of nine-coordinate chiral lanthanide complexes in aqueous mediaBased on a 2003/4 Tilden Lectureship of the Royal Society of Chemistry first delivered in Strasbourg, 5th December 2003. *Chem. Soc. Rev.* **33**, 156 (2004).
67. Parker, D., Dickins, R. S., Puschmann, H., Crossland, C. & Howard, J. A. K. Being excited by lanthanide coordination complexes: Aqua species, chirality, excited-state chemistry, and exchange dynamics. *Chem. Rev.* **102**, 1977–2010 (2002).
68. Pope, S. J. A. *et al.* Self-Assembly of Heterobimetallic d−f Hybrid Complexes: Sensitization of Lanthanide Luminescence by d-Block Metal-to-Ligand Charge-Transfer Excited States. *J. Am. Chem. Soc.* **126**, 9490–9491 (2004).
69. Faulkner, S. & Burton-Pye, B. P. pH Dependent self-assembly of dimetallic lanthanide complexes. *Chem. Commun.* 259 (2005) doi:10.1039/b412329h.
70. Lehr, J. *et al.* Reversible Recruitment and Emission of DO3A-Derived Lanthanide Complexes at Ligating Molecular Films on Gold. *Langmuir* **29**, 1475–1482 (2013).
71. Junker, A. K. R., Tropiano, M., Faulkner, S. & Sørensen, T. J. Kinetically Inert Lanthanide Complexes as Reporter Groups for Binding of Potassium by 18-crown-6. *Inorg. Chem.* **55**, 12299–12308 (2016).
72. Barge, A. *et al.* Bifunctional ligands based on the DOTA-monoamide cage. *Org. Biomol. Chem.* **6**, 1176 (2008).

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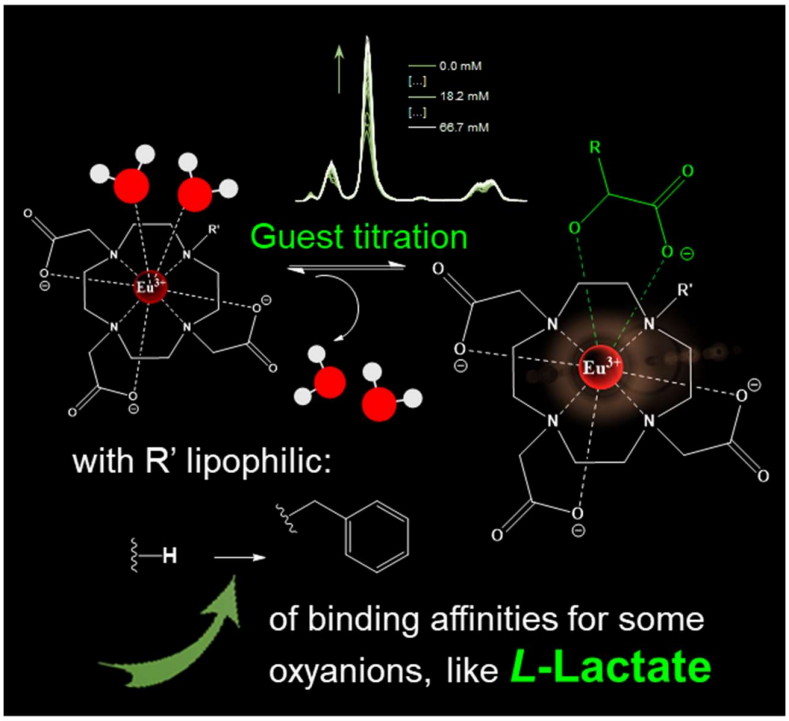

Supramolecular interactions between Eu(III) complexes and oxyanions are investigated by variating both the structure of the responsive complex and the nature of the targeted analyte ≡ guest. Focus are given to the lipophilicity of the binding pocket and lipophilicity that is found to strongly influence the strength of binding affinities.