

Kinetically Inert Lanthanide Complexes as Reporter Groups for Binding of Potassium by 18-crown-6

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Abstract

The barcode-like spectrum of lanthanide centered emission has been used in imaging and to make responsive luminescent reporters. The intensities and the shapes of each line in the luminescence spectrum can also report on the coordination environment of the lanthanide ion. Here, we have used lanthanide centered emission to report on the binding of potassium in an 18-crown-6 binding pocket. The responsive systems were made by linking a crown ether to a kinetically inert lanthanide binding pocket using a molecular building block approach. Specifically, an alkyne appended Ln.DO3A was used as a building block in a copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) “click” reaction with azide-functionalized crown ethers. The resulting complexes were investigated using NMR and optical methods. Titrations with potassium chloride in methanol following the sensitized europium and terbium centered emission were used to investigate the response of the systems. The molecular reporters based on aliphatic crown ethers were found to have strongly inhibited binding of potassium, while the benzo-18-crown-6 derived systems had the same association constants as the native crown ethers. The shape of the lanthanide emission spectra was shown to be unperturbed by the binding of potassium, while the binding was reported by an overall increased intensity of the lanthanide centered emission. This observation was contrasted to the change in spectral shape between propargyl-Ln.DO3A and the triazolyl-Ln.DO3A complexes. The solution structure of the lanthanide complexes was found to be determining for the observed physical chemical properties of these systems.

Introduction

The solution chemistry of the lanthanide ions are of ever increasing importance,¹ as these elements are of widespread use in modern technology. Specifically, lanthanide ions are used every day in the clinic as MRI contrast agents,²⁻⁵ in optical amplifiers,⁶ and in high contents screening.⁷⁻⁹ While MRI contrast agents rely on the magnetic properties of the lanthanide ions—gadolinium in particular, the two other applications use the unique optical properties of lanthanide ions to enhance optical signals in telecommunication and to provide contrast in luminescent experiments. The unique optical properties of the lanthanide ions have also found use in bioimaging,¹⁰⁻¹² where the long luminescence lifetime and sharp emission lines have been used in time-gated experiments and as donors in FRET assays.¹³⁻¹⁸ Lanthanide centered luminescence have also been used to follow the binding of analytes and chemical transformations in responsive systems.¹⁹⁻³²

While lanthanide luminescence is unique due to the narrow emission lines from the *f-f* transitions and the long lifetime resulting from the fact that these transitions are formally forbidden,³³ the spectra also carry an additional layer of information. The line shape and branching ratio in the emission spectrum of a lanthanide ion in solution changes according to the symmetry and donor set of the coordination environment, as well as with changes in the surrounding solution.³⁴⁻³⁹ Thus, the solution structure of a lanthanide containing system can be probed by luminescence spectroscopy,^{24, 40} and the solution composition may be investigated by observing changes in the lanthanide centered luminescence.⁴¹⁻⁴²

A pre-requisite for using lanthanide complexes as contrast agents is that the toxic lanthanide ions are not released and subsequently accumulated in the patient.^{2, 43} In the laboratory, a similar requirement is necessary to use a lanthanide complex as a molecular building block.⁴⁴ Therefore, lanthanide binding pockets that are able to form kinetically inert complexes must be used.⁴³ In some cases kinetical stability may be more important than thermodynamic stability,⁴⁵ while both thermodynamic and kinetic stability is to be preferred. While other kinetically inert lanthanide complexes have also been developed, kinetic inertness is readily achieved using cyclen (1,4,7,10-tetraazacyclododecane) derived ligands with monodentate pendant arms such as DOTA and 1,4,9-triazacyclononane (TACN) derived ligands with bidentate arms.^{2, 46-50}

Here, we are exploring the luminescent signature of binding a potassium ion in a 18-crown-6 binding pocket in molecules where the crown ether is covalently attached to a kinetically stable lanthanide complex, see chart 1. Crown ether appended lanthanide complexes have previously been reported, and it is well known that the luminescence intensity of a lanthanide center can be modulated by binding an alkali ion in such responsive systems.^{26-27, 29-32, 51-52} Thus, these systems are ideally suited to investigate the structural change that occurs upon binding an alkali ion in a crown ether as reported by the luminescence of an appended lanthanide complex.

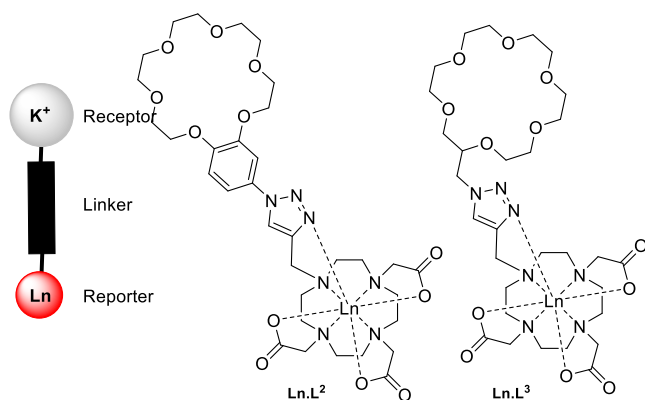


Chart 1. Design concept and molecular structures for potassium binding kinetically inert lanthanide complexes.

Complexes of three different lanthanide ions were prepared, and coupled to two different crown ethers in a copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) “click” reaction. This reaction has proven highly efficient for preparing larger architectures using kinetically stable lanthanide complexes as molecular building blocks.^{20, 37, 44, 53-58} Amino-functionalized 2-methyl-18-crown-6 and 18-benzocrown-6 binding pockets were readily converted to azides using an azide transfer reagent⁵⁹⁻⁶⁰ The azides were subsequently coupled to lanthanide complexes of 1-propargyl-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (Ln.propargoyl-DO3A, Ln.L¹) in a CuAAC “click” reaction. Three different lanthanide complexes— with europium(III), terbium(III)

and ytterbium(III)—were coupled to the benzocrown (Ln.L^2), while only the europium and terbium complex were prepared from the aliphatic crown ether (Ln.L^3).

We investigated the effect of binding potassium to the crown ether by performing titrations in methanol following the lanthanide centered luminescence. No change in the spectral shape was found to occur upon binding of potassium to the benzocrown ether, although a change in the total intensity of the emission was observed. We found that the responsive molecule (Ln.L^2) retained the binding properties of the free 18-benzocrown-6. In contrast, the systems based on the aliphatic crown ether (Ln.L^3) were not responsive, as the resulting molecular solution structure inhibited the binding of potassium to a high degree. We conclude that the lack of a luminescent signature upon binding of potassium is due to the fact that the rigid triazolyl-DO3A binding pocket inhibits the transmission of structural information from the crown ether. That structural changes are reported by the luminescence spectrum can clearly be seen by comparing of Ln.L^1 and Ln.L^2 , allowing the conclusion that systems studied here in particular, and possibly Ln.triazolyl-DO3A complexes in general, are not ideal to study the transmission of structural information in solution.

Results and Discussion

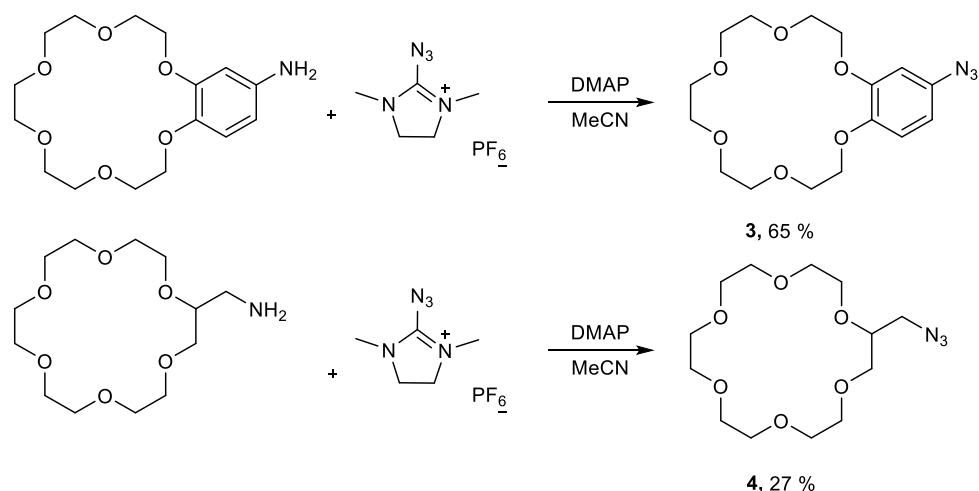
Synthesis

The target molecules (chart 1) were synthesized using a copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) “click” reaction between an alkyne appended kinetically inert lanthanide complex (or Ln.L^1) and azide appended crown ethers **3** and **4**. The synthetic route to the lanthanide complexes, Ln.propagyl-DO3A or Ln.L^1 , is shown in the supporting information. Complexes of europium and terbium were obtained following literature procedures.^{25, 53, 57-58} In brief, the synthesis required three steps. First, alkylation of the triester **1** using propagyl bromide and isolation of the protected pre-ligand **2**, followed by deprotection of the three *t*-butyl esters with trifluoroacetic acid (TFA) in a second synthetic step yielding the ligand L^1 . The third step is a complexation with the appropriate lanthanide triflate ($\text{Ln}(\text{OTf})_3$) that upon purification gave the alkyne appended lanthanide complexes (Ln.L^1 , Ln = Eu, Tb or Yb).^{53, 56-58}

The organic azides of 18-benzocrown-6 and 2-methyl-18-crown-6 were prepared by a diazo-transfer reaction of commercial derivatives sporting primary amines, see Scheme 1. We selected to use 2-azido-1,3-dimethylimidazolium hexafluorophosphate (ADMP) as an diazo-transfer reagent, as it has been shown to be efficient in various nucleophilic transfer reactions.⁵⁹⁻⁶⁰ Furthermore it is stable in its crystalline form. The diazo-transfer reaction using ADMP has been shown to work well in both acetonitrile (MeCN) and dichloromethane (DCM).⁵⁹⁻⁶⁰ Here, we performed the reactions in acetonitrile to avoid formation of diazomethane (an explosive gas) from the side reaction of ADMP reaction with dichloromethane. The diazo-transfer is best facilitated by base-catalysis, where the use of either 4-dimethylaminopyridine (DMAP) or trimethylamine (Et_3N) is considered standard conditions. DMAP was chosen as it was previously proven to be a very effective catalyst for the formation reaction between ADMP and primary aryl amines.⁶⁰

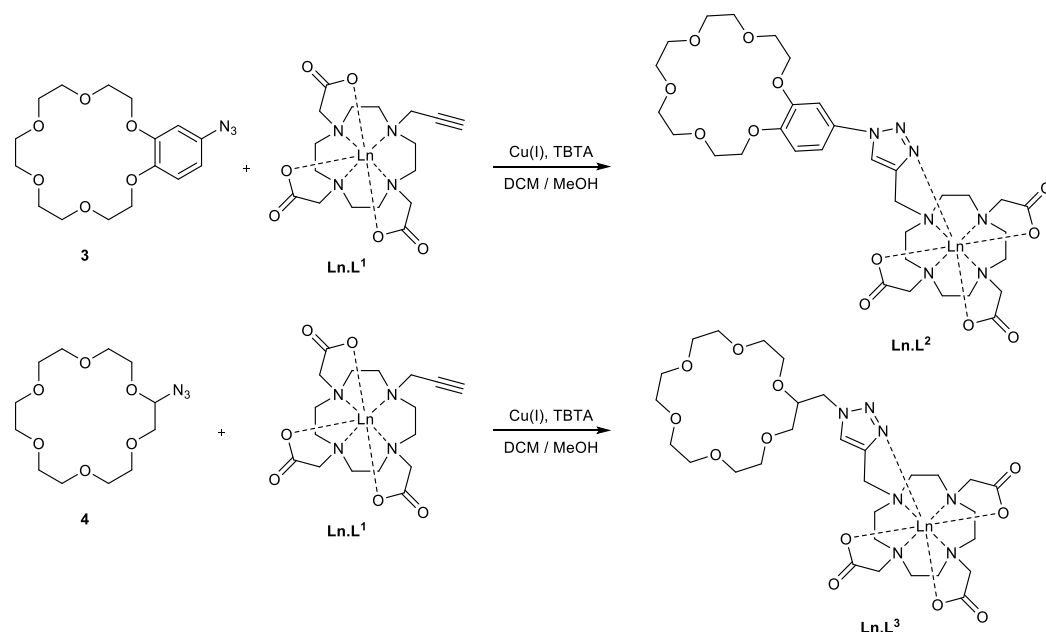
In our hands the diazo-transfer reaction worked well with 4'-aminobenzo-18-crown-6, where the product 4'-azidobenzo-18-crown-6 **3** was isolated in a good yield of 65 % on a milligram scale. In the formation of **3** very little side-product formation was observed (Scheme 1). In contrast, when we applied the same reaction conditions to the reaction between 2-aminomethyl-18-crown-6 and ADMP (Scheme 1) a significant amount of side-products were isolated. NMR and mass spectrometry suggest that the dominating side-product is a result of

imine formation between the primary amine and the imidazole of the ADMP reagent resulting in a guanidine like structure.⁶⁰ Attempts to optimize the reaction conditions by varying time, temperature, and the base catalyst were unsuccessful, and the target molecule 2-azidomethyl-18-crown-6 **4** was isolated in an acceptable yield of 27 % on a milligram scale. The purity of the products **3** and **4** was controlled using HPLC, and was determined to be 94 % or greater with residual starting material present as the main impurity, see supporting information.



Scheme 1. Azide-transfer reactions using ADMP to make 4'-Azidobenzo-18-crown-6 **3** and 2-Azidomethyl-18-crown-6 **4**

The responsive lanthanide complexes derived from 4'-azidobenzo-18-crown-6 Ln.L^2 and 2-azidomethyl-18-crown-6 Ln.L^3 were prepared by a copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) “click” reaction between the Ln.L^1 and **3** or **4** respectively, see Scheme 2. The reaction conditions used for the formation of the five responsive lanthanide complexes deviate from the standard conditions where copper sulfate and sodium ascorbate are used. Here, copper sulfate was replaced with tetrakis(acetonitrile)copper(I) hexafluorophosphate ($\text{Cu(I)(MeCN)}_4\text{PF}_6$), and tris(benzyltriazolylmethyl)amine (TBTA) is used instead of sodium ascorbate. These reaction conditions have been shown to work well for large organic azides, and proved to work well here. Ln.L^2 and Ln.L^3 were isolated after work-up that have shown to effectively remove the reagents and byproducts from the CuAAC reaction.³⁷ Ln.L^2 was prepared from the europium, terbium and ytterbium complexes of Ln.L^1 , while Ln.L^3 was prepared exclusively with europium and terbium.



Scheme 2. CuAAC “Click” reaction between the azide **3/4** and Ln.L^1 complex forming Ln.L^2 and Ln.L^3 (DMAP = 4-Dimethylaminopyridine, TBTA = Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine).

NMR

The new organic molecules **3** and **4** were characterized as appropriate for organic molecules, while the lanthanide containing complexes Ln.L^1 , Ln.L^2 and Ln.L^3 were characterized by paramagnetic ^1H NMR, mass spectrometry, HPLC and luminescence spectroscopy (see supporting information for details). The ^1H NMR spectrum of Eu.L^1 is consistent with that observed for 7-coordinated unsymmetrically cyclen derivatives, where all protons on the cyclen ring are non-equivalent, see figure 1.^{53, 58} The four axial protons of the cyclen ring are assigned to the most shifted resonance in the down field region of the spectrum (around 23 ppm), while the three acetate protons are assigned to the most shifted resonance in the up field region of the spectrum (around -18 ppm).⁶¹ For the europium complex of propagoyl-DO3A the four axial protons give rise to only a single resonance at the conditions used here (25°C, pH = 10). We assume that this is due to a fast exchange between multiple forms of the complex in solution, where molecular conformation and the number and nature of capping ligands may vary significantly. The spectral properties of the terbium complex Tb.L^1 (not shown), are similar, though the form of the spectrum is reversed as a consequence of the difference in sign in the magnetic anisotropy.³⁴

Eu.L^2 and Eu.L^3 exhibit a much more rigid complex, with octadentate binding pockets formed by the coordination of one of the nitrogen atoms on the triazole ring and the seven donor atoms of the original DO3A ligand. An 8-coordinated Eu-complex typically shows a broad range of resonances from -18 – 33.5 ppm.⁵⁸ The ^1H NMR spectrum of Eu.L^2 is given in figure 1, and shows resonances in the range from -20 – 35 ppm, indicating that the triazole ring is indeed coordinating to the europium(III) center of the DO3A ligand. The transition from a flexible 7-coordinated (Eu.L^1) to a locked 8-coordinated complex (Eu.L^2) results in the resolution of the axial ring protons of cyclen indicating that the fast exchange between the different forms of the complex is prohibited. The formation of the triazole ring greatly reduces the number of possible forms of the

complex in solution. In Eu.L^2 , only one capping ligand may be present at the lanthanide centre and the conformational space of the 8-coordinate binding pocket is greatly reduced. When comparing the ^1H NMR spectra of Eu.L^1 and Eu.L^2 in figure 1, this is particularly pronounced for the four axial resonances, which split up into three separate resonances and shifts further down field in the spectrum. The same effect can be seen for the acetate protons around -18 ppm that split into two distinct set of resonances. The ^1H NMR spectra of Ln.L^2 suggest that the complexes are predominantly in a square antiprismatic (SAP) geometry for europium and terbium, the NMR spectra of Yb.L^2 suggest a mixture of conformers are present, see supporting information. While no resonances corresponding to the twisted square antiprismatic (TSAP) geometry could be observed in the NMR spectra of Ln.L^2 , these could be seen as small resonances in both Eu.L^3 and Tb.L^3 .

Optical Spectroscopy

The observations from paramagnetic ^1H NMR regarding the solution structure of the three investigated lanthanide complexes were compared to the observations done using optical spectroscopy. The absorption spectra (see supporting information) clearly indicate the formation of triazole chromophores. In contrast, no strong absorption is seen above 250 nm for the alkyne appended lanthanide complexes (Ln.L^1). The formation of the triazole chromophore is accompanied by an absorption band at 250 nm with a weak shoulder extending to almost 400 nm. In the phenyl-triazole of Ln.L^2 the band at 250 nm is accompanied by a second band absorbing at 300 nm, while the red shoulder completely disappears. The lanthanide centered absorption is not resolved in these experiments.

While the broad ligand centered absorption documents the constitution of the molecules, the data does not give additional information regarding the solution structure of the complexes. Instead, the narrow emission bands resulting from lanthanide centered emission carry information about the local symmetry, and can thus be used to probe the solution structure of a lanthanide complex.^{40, 62} This is particularly pronounced in lanthanides that have so-called hypersensitive transitions, although all lanthanide centered transitions directly report on the local symmetry.⁶³ The f - f transitions occur between J -states, for each J -state the number and energy of m_J states is determined by the local ligand field. Fluctuations in the ligand field will lead to broad transitions, while changes in symmetry introduce different numbers of lines in the spectra. For instance, the $\Delta J = 4$ transition around 700 nm in europium can be used to distinguish between europium complexes in SAP and TSAP geometries of $[\text{Eu.DOTA}]$, as the geometries give rise to two distinct set of emission lines that can be counted if the spectral resolution is sufficient. Figure 2 show the europium centered emission followed excitation at 290 nm from solution of Eu.L^1 , Eu.L^2 , and Eu.L^3 in methanol. The lack of fine structure in the emission from Eu.L^1 indicates a structural flexibility of the complex in solution, while the spectra of Eu.L^2 and Eu.L^3 show the fine structure expected of a DOTA-like lanthanide complex. The narrow lines of the $\Delta J = 4$ transition in Eu.L^2 supports the observation that only one isomer is present in solution, similarly the less well defined $\Delta J = 4$ transition in Eu.L^3 is consistent with the observation of a minor isomer by ^1H -NMR. The relatively strong $\Delta J = 0$ peak at 580 nm shows that all complexes have broken symmetry, while the differences in the ratio of intensity of the hypersensitive $\Delta J = 2$ transition and the $\Delta J = 1$ transition confirms that the local structure around the lanthanide is different in the complexes. This is particularly evident when comparing Eu.L^1 to Eu.L^2 and Eu.L^3 . Similar observations regarding the terbium centered emission (Figure 2) can be made, although the amount of information that can be gained of the terbium centered emission is significantly smaller. Here, the fine structure that can be seen in the emission spectra of Tb.L^2 , and Tb.L^3 suggest that the solution structures of these complexes are more defined than that of Tb.L^1 .

The time-gated excitation spectra monitoring lanthanide centered emission show a moderately efficient energy transfer from the triazolyl ligands to the lanthanide in Ln.L² and Ln.L³, while direct excitation is pronounced in Ln.L¹, see supporting information for details. Time-resolved emission decay profiles were recorded in water and D₂O and the luminescence lifetimes of the lanthanide centered emission was determined for europium and terbium complexes, see table 1. The lifetime of the lanthanide excited state is greatly reduced by energy transfer to high energy oscillators in the solvent. This can be used to determine the number of inner sphere solvent molecules q using the modified Horrocks' equation.^{40, 64-65} The europium and terbium excited state lifetimes are shorter in Ln.L¹ than in Ln.L² and Ln.L³, and thus we can conclude that this is due to a higher solvation of the hepta-dentate complex as corroborated by the value of q . Upon formation of an octa-dentate complex only one solvent molecule is coordinated to the lanthanide center, giving rise to a longer excited state lifetime and lower value of q .

Table 1. Luminescence lifetimes of Ln.L¹, Ln.L² and Ln.L³ in water and D₂O, and in methanol and methanol-d₄ with and without potassium. The modified Horrocks' equation was used to calculate the number of inner sphere solvent molecules q .

Lifetimes	H ₂ O (ms)	D ₂ O (ms)	q^a	MeO H (ms)	MeOD (ms)	q	MeOH/ KCl (ms)	MeOD/ KCl (ms)	q
Eu.L¹	0.42	1.34	1.7	0.95	-	-	-	-	-
Tb.L¹	1.01	2.03	2.2	1.69	-	-	-	-	-
Eu.L²	0.60	1.97	1.1	0.93	2.34	1.0	0.95	2.13	0.8
Tb.L²	1.48	2.32	0.9	1.94	1.63	-1.6	2.10	1.98	-0.9
Eu.L³	0.57	1.78	1.1	0.82	2.39	1.3	0.94	1.87	0.7
Tb.L³	1.69	2.44	0.6	1.83	2.95	1.5	1.74	2.75	1.5

^a q , the number of inner sphere solvent molecules coordinated to the lanthanide centre, was calculated using the equation:^{40, 65} $q = A(\tau_{\text{H}_2\text{O}}^{-1} - \tau_{\text{D}_2\text{O}}^{-1} - B)$; for terbium $A = 5.0$ ms and $B = 0.06$ ms⁻¹, while for europium $A = 1.2$ ms and $B = 0.25$ ms⁻¹.

The spectroscopic data clearly demonstrate the formation of Ln.L² and Ln.L³, and show that their solution structure is rigid around the lanthanide center. Ln.L² was found to be present in just one form, while Ln.L³ showed a more dynamic structural behavior.

Response to potassium

We went on to investigate the response of Ln.L² and Ln.L³ upon addition of potassium chloride. It is well known that 18-crown-6 and 18-benzocrown-6 binds potassium ions in water and alcohols.⁶⁶⁻⁶⁸ The association constants between the crown ethers and potassium in water are $\log K[\text{crown}] = 2.0$ and $\log K[\text{benzocrown}] = 1.7$, which increase to $\log K[\text{crown}] = 6.1$ and $\log K[\text{benzocrown}] = 5.3$ in methanol. We investigated the association between Ln.L² and Ln.L³ and potassium in water and methanol, but we only observed a response of our systems in methanol (see SI). Figure 3 shows a titration following the europium centered luminescence. As the responsive system binds potassium the total intensity increases, while the band shape of the five depicted transitions remain constant. This observation bears out the conclusion that no structural rearrangement occur at the lanthanide center upon binding of potassium. Evidently, the triazolyl-DO3A binding pocket has a well-

defined geometry, which is difficult to perturb using remote effects in responsive architectures as exemplified by Eu.L^2 .

Plotting the total intensity and the intensity of the individual transitions affords a binding isotherm, see figure 4. The isotherm was fitted using the Dynafit software package.⁶⁹⁻⁷⁰ The binding constant was determined to be $\log K[\text{Eu.L}^2] = 5.3$, which is within error identical to the literature value. The determined binding affinities are compiled in table 2. cursory inspection of the data shows that the benzocrown derived complexes (Ln.L^2) binds potassium to the same extent as the free crown ether, while the aliphatic crown ether derived complexes (Ln.L^3) does not bind potassium efficiently. It seems reasonable to conclude that the flexible Ln.L^3 complex must have a solution structure that inhibits binding.

Table 2. Affinity constants K (M^{-1}) of for potassium in methanol.

	Log(K)	Confidence interval
Eu.L^2	5.3 ± 0.02	[5.25-5.36]
Tb.L^2	5.4 ± 0.05	-
Eu.L^3	2 ± 0.5	[1.00-2.00]
Tb.L^3	n.d.	-

Even though the Ln.L^3 complexes do not show affinity for potassium, a spectral change is observed upon the addition of potassium to a solution of the complexes in methanol. As for Ln.L^2 , the spectral shape remains constant, while the total emission intensity increases upon the addition of potassium, see figure 5. This once again highlight the necessity of performing titration in controlled experiments, as a two point experiment would lead to the false conclusion that all four systems bind potassium. Here we stipulate that secondary effects—such as unspecific binding and ionic strength—drastically alter the photophysical properties of the Ln.L^3 complexes upon addition of an excess of potassium chloride.⁷¹⁻⁷² Note that the increase in intensity of emission from Ln.L^3 greatly exceeds the changes in intensity in Ln.L^2 upon binding of potassium.

Time-resolved emission decay profiles were recorded at the start and end points of the titrations in methanol and in methanol-d4. Thus q could be determined and the values are reported in table 1. The q -values for Eu.L^2 support the spectral data, as they are within error before and after binding of potassium thus confirming that the binding does not influence the lanthanide binding pocket. For Tb.L^2 , the negative calculated values of q present a puzzle. While the observed luminescence lifetimes in MeOH are comparable to those of the other systems studied, the lifetimes in deuterated methanol are consistently shorter than those in protic media. This is suggestive of involvement of the solvent structure in energy transfer and quenching processes, and merits further investigation, but cannot readily be assigned to a single phenomenon. In the case of Ln.L^3 the q -values suggest that the terbium complex does not undergo any structural change, while average solution structure of the europium complex upon addition of potassium changes and now includes a pronounced amount of uncapped complexes. The spectra of Eu.L^3 (Figure 5) show that the change in q must take place without changing the symmetry of the lanthanide complex as the spectral shape is the same for the complex with and without potassium present and that the addition of potassium ion might play a role in the energy transfer cascade resulting in a higher antenna-to-lanthanide energy transfer yield.

As the lifetimes of the lanthanide centers are only moderately affected by the addition of potassium chloride (table 1), the significant changes in the observed total emission intensity (figure 5) must be due to changes in the photophysics of the sensitizer chromophore. For Ln.L² we suggest that the intensity increase is due to a diminished degree of quenching of the chromophore first excited state by the crown ether oxygens. These are able to partake in photoinduced electron transfer quenching, when not bound to potassium. We speculate that the same is true in Ln.L³, where PET quenching of the triazole first excited singlet state by crown ether oxygens is blocked upon addition of an excess of potassium.

Conclusion

Here, we have synthesized and investigated lanthanide complexes of three different DO3A-based ligands: the alkyne-appended starting material and two responsive complexes derived from crown ethers. The complex derived from 18-benzocrown-6 was found to bind potassium with an affinity identical to that of the free crown ether. The binding was detected as an increase of the total emission intensity. The complex derived from 18-crown-6 did not show an affinity for potassium. Evidently, the solution structure of this complex prohibits binding of potassium by the crown ether. Despite the fact that weak binding (Eu) or a complete lack of a binding isotherm (Tb) was determined, a dramatic increase in the total emission intensity was observed upon addition of potassium to the solutions containing the complexes. This we ascribe to a combination of non-specific solvent effects,^{38, 40, 73} and—for terbium in particular—binding of potassium at the lanthanide centre.⁷⁴⁻⁷⁵ These observations highlight the complicated nature of the structure of lanthanide complexes in solution.

We set out to demonstrate that structural changes that occur upon binding an analyte at a remote site in a lanthanide complex can be reported in the spectral shape of lanthanide centered emission. We found that the rigid triazole-DO3A lanthanide binding pocket created by the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) “click” chemistry we used to synthesize the responsive lanthanide complexes prohibited all transfer of structural information to the lanthanide center. Clearly, more flexible lanthanide binding pockets must be used for this mode of response to be feasible.

Methods and materials

Compounds and solvents. All chemicals and solvents were used as received. All solvents used for spectroscopic experiments were of HPLC grade and used as received. Where dry solvents were used, they were degassed with nitrogen, dried by passing through an MBraun MPSP-800 column and then used immediately. Water was deionised and microfiltered using a Milli-Q Millipore machine. Tetrabutylammonium (TBA) salts were stored under vacuum in a desiccator. Chromatographic purification was performed on silica gel (SiO₂) with pore-size 60Å and particle size of 40-63 µm. Low-resolution mass spectra were recorded on a Waters Micromass LCT Premier XE spectrometer. Accurate masses were determined to four decimal places using Bruker µTOF and Micromass GCT spectrometers at the Chemistry Research Laboratory of The University of Oxford, and on an ESP-MALDI-FT-ICR instrument equipped with a 7 Tesla magnet (the instrument was calibrated using sodium trifluoroacetate cluster ions prior to acquiring the spectrum) at the Department of Chemistry of The University of Copenhagen. For these measurements dithranol was used as matrix. HPLC analysis was performed on a Dionex Ultimate 3000 system coupled to an Ultimate 200 variable wavelength detector. The reported separation was achieved using a Dionex Acclaim RSLC PolarAdvantage II (PA2) 2.2µM 120Å 2.1x100mm and the column

maintained at 30°C. The mobile phase solution was prepared by 0.1% formic acid in H₂O and 0.1% formic acid in methanol or 1% trifluoroacetic acid in H₂O and 1 % trifluoroacetic acid in acetonitrile. The water used as eluent was purified by a Millipore system. ¹H NMR spectra were recorded on a Bruker 400 or 500 MHz instrument. ¹³C NMR spectra were recorded on a Bruker 126 MHz instrument equipped with a (non-inverse) cryoprobe. All chemical shift (δ) are given in parts per million. Compounds **1**, **2**, **L**¹ and Ln.**L**¹ were synthesized as previously reported^{25, 53, 56-58}. Compounds **3**, **4**, Ln.**L**² and Ln.**L**³ are new compounds and their synthesis and characterization are included in the Supporting Information.

Spectroscopy. Absorption spectra, luminescence spectra, and luminescence lifetimes were measured and determined using standard procedures. Typically settings for the time-gated emission and excitation spectra were gate times of 1 ms and a window of 3 ms. Absorption spectra were measured with a Cary 300 UV-Vis spectrometer from Agilent Technologies using the pure solvent as baseline. Luminescence spectra and lifetimes were measured on a Horiba Jobin Yvon FluoroLog-3 equipped with a Hamamatsu R928 PMT detector and a double-grating emission monochromator. In the case of the ytterbium complex, the sample was excited using a pulsed nitrogen laser (PTI-3301-337nm. Light emitted at right angles to the excitation beam was focused onto the slits of the monochromator (PTI120), which was used to select the appropriate wavelength. Time-gated emission spectra and lifetimes were recorded on a Cary Eclipse fluorescence spectrometer with PMT (photo multiplier tube) from Agilent Technologies. For all luminescence measurements, the absorbance at the excitation wavelength and at longer wavelengths was kept below 0.1 to avoid inner filter effects and intermolecular interactions. All luminescence experiments at ambient temperatures were performed in 10.00 mm Hellma quartz fluorescence cuvettes. Luminescence decays were analyzed and fitted to exponential decay functions using the Origin software package. All solvents used in the experiments were of HPLC grade and used as received, and water was purified using a MilliQ apparatus. For the potassium binding experiments a solution of potassium chloride, used as received, in methanol was prepared, see supporting information for details.

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Supporting Information.

Synthesis and characterization, optical spectra, titration isotherms with fits, and time-resolved emission profiles with fits are included as supporting information. This material is available free of charge via the Internet at <http://pubs.acs.org>

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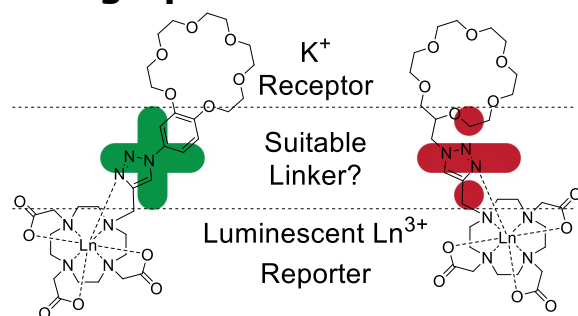
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TOC synopsis

Lanthanide luminescence can be used as a direct reporter of coordination geometry. Combined with the extremely low detection limits of time-gated detection luminescence, this can be exploited to make highly sensitive optical sensors. The linchpin of an optical sensor is a responsive luminophore. Here, we have investigated the effect of the lanthanide binding pocket and linker type on the response of two potassium responsive lanthanide complexes. The key findings reported pertains to the highly complex nature of lanthanide solution structure, which was found to inhibit potassium binding in one of the systems.

TOC graphics



Figures

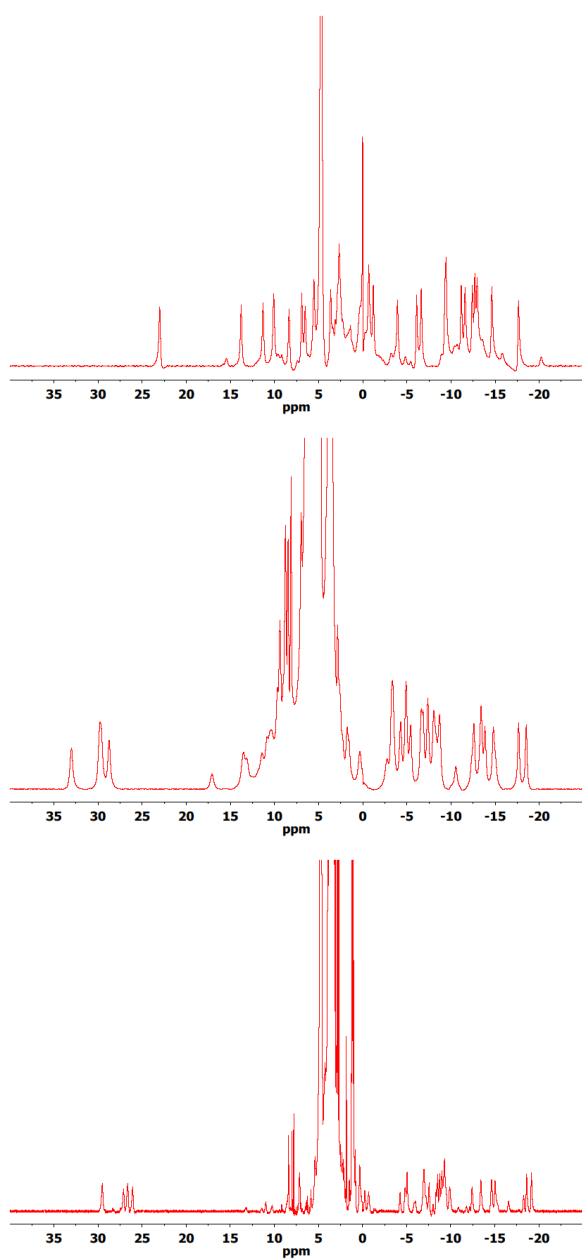


Figure 1. ^1H NMR spectra (500 MHz, 296 K) of Eu.L¹ (Top), Eu.L² (middle). Eu.L³ (Bottom).

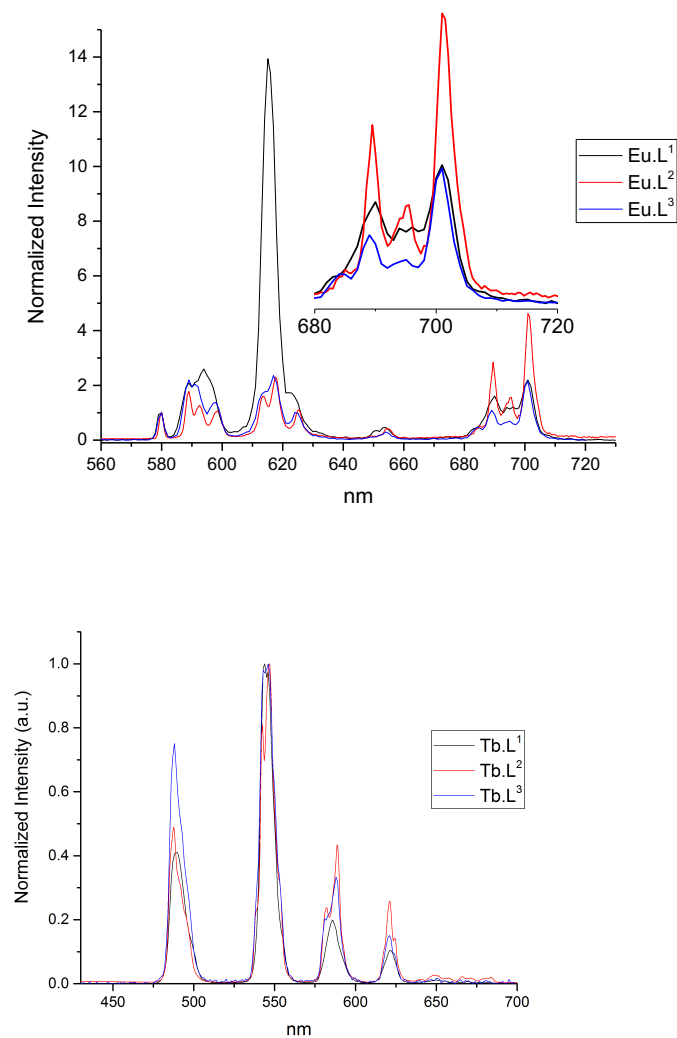


Figure 2. Normalized time-gated emission spectra of Ln.L^1 [$1.1 \cdot 10^{-5}$ M], Ln.L^2 [$1.8 \cdot 10^{-5}$ M] and Ln.L^3 [$1.2 \cdot 10^{-5}$ M] (europium, top; terbium, bottom) following excitation at 290 nm in methanol (delay = 1 ms, window = 3 ms). The spectra show a pronounced change in going from the Ln.L^1 structure to the clicked structures in Ln.L^2 and Ln.L^3

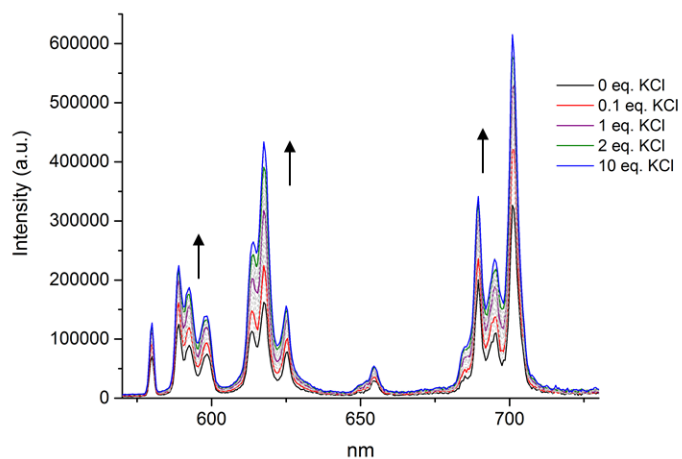


Figure 3. Luminescence spectrum of Eu.L^2 [$1.8 \cdot 10^{-5}$ M] in MeOH (Ex. 290 nm) showing the observed increase of intensity following addition of KCl.

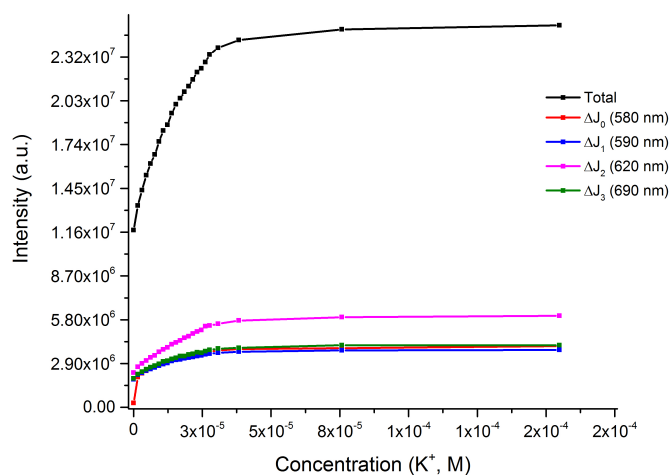


Figure 4. The change in europium emission of Eu.L^2 [$1.8 \cdot 10^{-5}$ M] as a function of the potassium concentration, the titration isotherm is plotted using the total emission intensity and each emission band (indicated by the ΔJ).

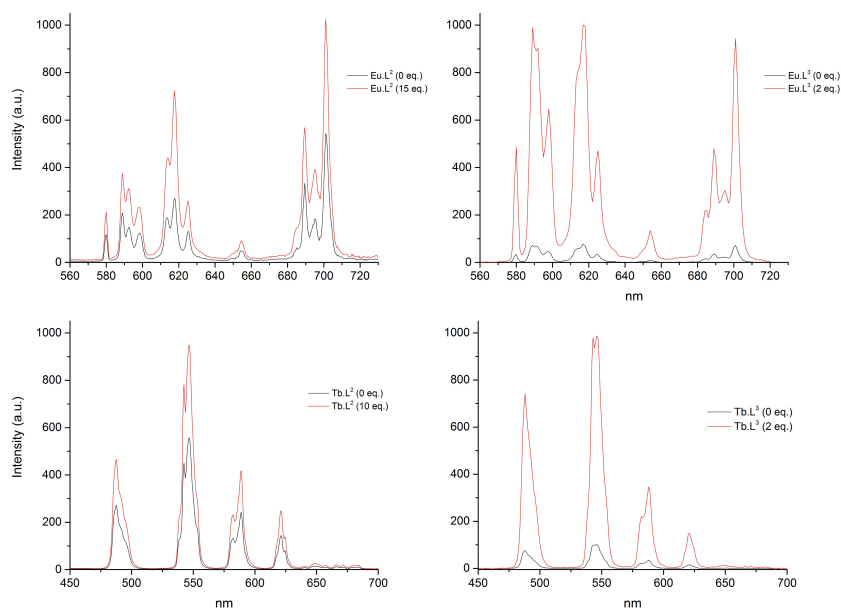


Figure 5. Time-gated luminescence emission spectra of Eu.L^2 [$1.8 \cdot 10^{-5}$ M] (top left) and Tb.L^2 [$1.8 \cdot 10^{-5}$ M] (bottom left) following excitation at 290 nm, and Eu.L^3 [$1.2 \cdot 10^{-5}$ M] (top right) and Tb.L^3 [$1.2 \cdot 10^{-5}$ M] following excitation at 250 nm (bottom right) with (red) and without (black) addition of potassium.