Luminescent Lanthanide Probes for Inorganic and Organic Phosphates

Thibaut L. M. Martinon and Valérie C. Pierre

[Review]

doi.org/10.1002/asia.202200495

Chem Asian J. 2022, 17, e202200495 (1 of 23) © 2022 The Authors. Chemistry – An Asian Journal published by Wiley-VCH GmbH
Abstract: Inorganic and organic phosphates—including orthophosphate, nucleotides, and DNA—are some of the most important biological anions. Inorganic phosphates, present as a near 1:1 ratio of $\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$ at neutral pH, buffers intra and extracellular media and is a key component of hard tissues such as bones and teeth. Nucleotides such ATP and GTP are small organic phosphates with crucial roles in energy metabolism, as well as allosteric regulators, physiological mediators, activated intermediates and components of co-enzymes. Nucleotides are also the precursors of nucleic acids, both DNA and RNA, which store and express genetic information. Given their fundamental anions in cellular biology, regulating numerous processes of both medical and environmental significance. The characteristic long lifetimes of emitting lanthanides, including the brighter europium(III) and terbium(III), make them ideally suited for the development of molecular probes for the detection of phosphates directly in complex aqueous media. Moreover, given their high oxophilicity and the exquisite sensitivity of their quantum yields to their hydration number, those luminescent lanthanides are perfect for the detection of phosphates. Herein we discuss the principles that have guided the recent developments of molecular probes selective for inorganic or organic phosphates and how these lanthanide complexes facilitate the study of numerous biological processes.

1. Introduction

Inorganic and organic phosphates are among the most important biological anions. Inorganic phosphates, present as a near 1:1 ratio of $\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$ at neutral pH, buffers intra and extracellular media and is a key component of hard tissues such as bones and teeth. Nucleotides such ATP and GTP are small organic phosphates with crucial roles in energy metabolism, as well as allosteric regulators, physiological mediators, activated intermediates and components of co-enzymes. Nucleotides are also the precursors of nucleic acids, both DNA and RNA, which store and express genetic information. Given their fundamental anions in cellular biology, regulating numerous processes of both medical and environmental significance.

Understanding and exploiting the intricate roles of both inorganic and organic phosphates, including screening of molecules targeting phosphate-dependent enzymes, is achieved more readily with luminescent probes. The accuracy of these assays require probes that are stable, function in water at neutral pH within the concentration range of the targeted phosphates and with high selectivity over competing anions. Phosphates are, however, highly hydrophilic. The high hydration energy of inorganic phosphate ($\Delta_{\text{hydration}} \text{H}_2\text{PO}_4^- = -465 \text{ kJ mol}^{-1}$) positions it among the highest anions in the Hofmeister series. In water, this hydration outcompetes with hydrogen-bonding and electrostatic interactions even if they are predispositioned into tetrahedral symmetry, as is commonly featured in organic receptors. Most organic receptors, therefore, do not function in water.

Lanthanides are well recognized for their strong oxophilic character; a property that, unlike for early transition metals, is not only a result of their hardness but also arises from the low ionization potential of their 4f-valence electrons that contribute to their overall poor electronegativity. Moreover, the ionic nature of lanthanide coordination favors the more basic anions. In the absence of steric hindrance, the affinity oftripodal Gd$^{3+}$ complexes for anions was determined to follow directly not with the hardness but with the basicity of the anion. These properties advantageously confer lanthanide complexes with an intrinsic preference for phosphate over bicarbonate and fluoride as well as other anions. Together with their lability that enables rapid coordination of anions, the characteristic coordination chemistry of lanthanides makes them particularly well-suited for the recognition of phosphates in water, the only solvent relevant to biological studies.

It is these characteristics that are increasingly exploited in the design of lanthanide-based luminescent probes for inorganic and organic phosphates that we review herein with a focus on the more recent developments over the last decade. While outside the scope of this contribution, recent progress on lanthanide-based receptors for inorganic phosphate involving luminescent lanthanide MOFs, nanopores, and coordination polymers should also be noted.

1.1. Principles of Sensitized Lanthanide Luminescence

The field of luminescent lanthanide probes has grown dynamically over the last several decades and has been the subject of thorough reviews including on their design principles, applications to anion recognition, enzyme activity assays, and DNA probes. The following discussion will therefore focus on the key parameters of lanthanide luminescence as it concerns the recognition and sensing of inorganic and organic phosphates.

Central to the applications of luminescent lanthanide probes for analyte sensing and high throughput screening of enzymes and receptors is the Laporte-forbidden nature of the $f\rightarrow f$ transitions. Because of this forbidden nature, lanthanide-centered luminescence is characterized by narrow emission bands occurring at fixed wavelength, very large Stokes shifts, and extremely long luminescence lifetimes (Figure 1a). The ms lifetimes of Eu$^{3+}$ and Tb$^{3+}$ phosphorescence, the most emissive lanthanides, facilitates time-delayed spectroscopy, allowing for the signal to be accurately quantified without interference from the fast-decaying autofluorescence of the sample via the incorporation of a gate time (Figure 1b).

The Laporte forbidden nature of the $f\rightarrow f$ transitions also dictates that lanthanide ions have a negligible absorption

[a] T. L. M. Martinon, Prof. V. C. Pierre
Department of Chemistry
University of Minnesota
207 Pleasant Street SE,
Minneapolis MN 55455 (USA)
E-mail: pierre@umn.edu

This manuscript is part of a special collection on Responsive Probes and Molecular Bioimaging.

© 2022 The Authors. Chemistry – An Asian Journal published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
This problem is circumvented by indirect excitation via a sensitizer or “antennae” incorporated in the ligand (Figure 1c and 1d). These chromophores absorb UV-visible radiation and sensitize the lanthanide either via Dexter electron exchange,\[12\] as is the case for Eu\(^{III}\)-1,2-HOPO complexes (Figure 3),\[13\] or via Förster resonance energy transfer (FRET)\[14\] mechanisms, as typical for probes where the antennae does not coordinate the lanthanide ion. The quantum yield of the probe (\(F_{Ln}\)), which defines the efficiency by which photons absorbed by the antennae are emitted by the lanthanide ion, is dependent on, among other parameters, the efficiency of energy transfer (ET) and intersystem crossing (ISC), and the energy difference between the triplet energy state (\(T_1\)) and the excited state of the lanthanide ion (\(5D_n\)). If this difference is too large, ET to the lanthanide is inefficient; if it is too small, back energy transfer (BET) to the antennae triplet state occurs, thereby reducing lanthanide emission. Probes that function via a Förster mechanism are highly dependent on the distance separating the antennae from the lanthanide ion, a limitation that has been exploited in the design of responsive probes. Another key parameter influencing the brightness of lanthanide complexes is the fourth overtone of nearby O–H and N–H oscillators, which are slightly lower in energy than the excited \(5D_n\) states of both Eu\(^{III}\) and Tb\(^{III}\). As such, any water molecules directly coordinated to the lanthanide will partially quench lanthanide emission.

**Figure 1.** Fundamentals of sensitized lanthanide luminescence: a) Representative absorbance and emission spectra of sensitized Eu\(^{III}\) and Tb\(^{III}\) demonstrating the characteristic large Stokes shift between absorption of the antenna (blue) and narrow lanthanide-centered emission bands of europium(III) (red) and terbium(III) (green). b) Time-gated spectroscopy exploits the long luminescent lifetimes of lanthanide complexes to remove the interfering autofluorescence of complex biological media via the incorporation of a time delay. c) Excitation pathways within a coordinated lanthanide complex. The antenna absorbs light at \(h\nu_1\); ISC and ET to the lanthanide enables lanthanide-centered phosphorescence at \(h\nu_2\) concomitant with ligand-centered fluorescence at \(h\nu_3\). Coordinated water molecule(s) partially quench lanthanide-centered emission. d) Simplified Jablonski diagram of sensitized luminescence for Eu\(^{III}\) and Tb\(^{III}\).
emission and reduce their lifetimes. This dependence is key to the design of numerous probes for phosphates that function by direct coordination to the metal via displacement of water molecules.

Almost all luminescent lanthanide probes employ either Eu$^{3+}$, with a characteristic red emission centered at 615 nm, or Tb$^{3+}$, with a primary emission band at 545 nm, due to the large energy gap between their ground state and their lowest-lying excited state from which luminescence arise (Figure 1a and 1d). Other lanthanides, including Sm$^{3+}$ ($^{4}G_{5/2} \rightarrow ^{6}H_{5/2}$) and Dy$^{3+}$ ($^{4}F_{5/2} \rightarrow ^{4}I_{5/2}$), have smaller energy gaps that favor non-radiative decay to peripheral high-energy vibrators, which leads to poor quantum yields and are therefore rarely used.

Although not yet used for the detection of phosphates, fluorescing lanthanides such as Er$^{3+}$ ($^{4}I_{13/2} \rightarrow ^{4}I_{15/2}$), Nd$^{3+}$ ($^{4}F_{3/2} \rightarrow ^{4}I_{15/2}$), and Yb$^{3+}$ ($^{2}F_{5/2} \rightarrow ^{4}I_{15/2}$) do offer promising characteristics, particularly since their near infra-red (NIR) emission facilitates imaging in biological tissues.

1.2. Design of Receptors for Phosphate

The dependence of lanthanide-based luminescence on the parameters described above opens the door to numerous
approaches for the design of responsive probes that are unique to F-block elements and have been described in prior reviews. A few of these are particularly well-suited for the detection of inorganic and organic phosphates and will be detailed here.

The most common approach to detecting phosphates exploits the oxopholicity of lanthanide ions which favors coordination of phosphate over water, and the propensity of bound water molecules to quench EuIII and TbIV phosphorescence. In these cases, the presence of one or more inner-sphere water molecules in the “off” state decreases substantially both the quantum yield and the luminescence lifetime of the emitting lanthanide. If the complex LnIII is stable, phosphate coordination occurs via displacement of the quenching water molecules, resulting in an increase in both Φ and τ (Figure 2a). Since lanthanide ions are labile, this process is reversible, which enables direct monitoring of enzymatic reactions that affect the position of this equilibrium. Advantageously, this mechanism is easily probed by determining the number of inner-sphere water molecules, q, via the isotope effect on the luminescence lifetimes of EuIII or TbIV complexes as follows: \[ q_{\text{Eu}} = 1.2 \left( \frac{1}{\tau_{\text{H}_2\text{O}}} - \frac{1}{\tau_{\text{D}_2\text{O}}} \right) - 0.25 - 0.0075x \] \[ q_{\text{Tb}} = 5 \left( \frac{1}{\tau_{\text{H}_2\text{O}}} - \frac{1}{\tau_{\text{D}_2\text{O}}} \right) - 0.06x \] where q is the number of inner-sphere water molecule, τH2O and τD2O are the luminescence lifetimes of the probe in H2O and D2O, respectively, and x is the number of nearby N–H or O–H oscillators arising from the ligand. If coordination of phosphate occurs only via displacement of inner-sphere water molecules, a clear decrease in q will be observed. Of note, similar relationships have been established for NIR fluorescing lanthanides.

Indicator displacement assays are a modification of this mechanism whereby phosphate does not displace inner-sphere water molecules but a weakly-coordinated indicator. If this indicator is also the lanthanide sensitizer, its displacement will result in a decrease in lanthanide-centered emission (Figure 2b). If, on the other hand, the indicator is a quencher, its displacement by phosphate will result in an increase in intensity.

As will be detailed later, these two mechanisms require stable LnIII complexes. Unstable ones will undergo ligand exchange where L decomposes the LnIII, resulting in the formation of insoluble LnIII phosphate salts (Figure 2c). Because of its heterogeneous nature, such equilibria are poorly controllable and reversible and are not appropriate to the study of complex biological or environmental samples.

Recognition of phosphates does not necessarily require direct coordination to the lanthanide ion. Outer-sphere binding is also an efficient strategy for the recognition and detection of nucleotides and nucleic acids. In these cases, weak interaction between the organic phosphate and the antenna is sufficient to favor photoelectron transfer between the nucleobase and the antenna, which in turn decreases lanthanide sensitization (Figure 2d). This approach is favored for the detection of both nucleotides and nucleic acids.

2. Luminescent Probes for Inorganic Phosphate

Inorganic phosphate (Pi) is present in water at neutral pH as a near-equal ratio of H3PO4− and HPO42−. For healthy patients, its concentration in blood ranges from 0.8 – 1.45 mM, while much higher concentrations are present within cells (up to 100 mM). Given the higher concentration of HCO3− (23-30 mM) and Cl− (98-106 mM) in blood, biomedical applications require highly selective probes. Environmental applications have even stringier requirements since the low ppm levels of phosphate present in even polluted surface water (0.1–1 μM) require probes that have a substantially higher affinity for phosphate. Nonetheless, although not all luminescent lanthanide probes fulfill each of these requirements, more recent ones have been developed with sufficient sensitivity, selectivity, and efficacy in water, to be successfully deployed. All function via direct coordination of the oxoanion to the lanthanide ion, although some also employ indicators. Do note that the requirements in term of sensitivity (anion affinity) and selectivity are dictated by the intended application. Luminescent probe for sensing inorganic phosphate in blood or environmental water samples do not need to be selective over nucleotides because there is no nucleotide present in either blood or surface or wastewater samples. For these applications, selectivity over bicarbonate and other endogenous anions are more relevant. The properties and molecular composition of these complexes are summarized in the following Table 1 and Figures 3–6.

2.1. Recognition of Phosphate via Formation of Ternary Complexes

The majority of lanthanide coordination complexes used in biomedical imaging employ polyaminocarboxylate ligands due to their high-water solubility, high stability, and ease of synthesis. In the absence of steric hindrance, most neutrally or positively charged lanthanide complexes of polyaminocarboxylate ligands with one or more open coordination site will bind hard anions such as phosphate. As was first demonstrated by Dickins with Ln-1 and Ln-2 (Figure 3), such complexes have nearly no selectivity and also bind bicarbonate, acetate and fluoride with very similar affinity. More recently, Butler developed brighter derivatives employing pyridine-type antennae that permeated cells more effectively due to their hydrophobic nature, distributing primarily in lysosomes. Although brighter than Ln-1, the receptors Eu3 to Eu7 also demonstrated very poor selectivity, binding not only phosphate but also phosphorylated amino acids, bicarbonate, citrate, and lactate.

Some selectivity can be achieved by increasing steric hindrance at the open coordination site. As demonstrated by Morrow, the PARACEST agent EuIII(S-THP) (Eu-8) similarly cannot differentiate between organophosphates, inorganic phosphate, carbonate, or carboxylates. Addition of a pendant chromophore near the open coordination site resolves some of the selectivity problem. The derivative EuIII(THPC) (Eu-9), for in-
Table 1. Affinity and selectivity in select solvent of lanthanide probes for inorganic phosphate.

| Complex | Selectivity | Solvent | Binding Affinity | Ref. |
|---------|-------------|---------|------------------|------|
| Yb-1    | P<sub>i</sub> | H<sub>2</sub>O | n.d. | 18 |
| F<sup>a</sup> | H<sub>2</sub>O | n.d. | |
| acetate | H<sub>2</sub>O | n.d. | |
| oxalate | H<sub>2</sub>O | n.d. | |
| lactate | H<sub>2</sub>O | n.d. | |
| Eu-3    | HCO<sub>3</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 3.47 | 19 |
| lactate | H<sub>2</sub>O | log K<sub>i</sub> = 3.51 | |
| citrate | H<sub>2</sub>O | log K<sub>i</sub> = 3.54 | |
| benzoate | H<sub>2</sub>O | log K<sub>i</sub> = 3.73 | |
| P<sub>i</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 3.78 | |
| O—P—Tyr | H<sub>2</sub>O | log K<sub>i</sub> = 3.20 | |
| O—P—Ser | H<sub>2</sub>O | log K<sub>i</sub> = 3.30 | |
| O—P—Thr | H<sub>2</sub>O | log K<sub>i</sub> = 3.35 | |
| Eu-4    | HCO<sub>3</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 3.28 | 19 |
| lactate | H<sub>2</sub>O | log K<sub>i</sub> = 4.14 | |
| citrate | H<sub>2</sub>O | log K<sub>i</sub> = 3.72 | |
| HCO<sub>3</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 3.40 | |
| Eu-5    | lactate | H<sub>2</sub>O | log K<sub>i</sub> = 2.70 | 19 |
| citrate | H<sub>2</sub>O | log K<sub>i</sub> = 3.91 | |
| HCO<sub>3</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 3.52 | |
| Eu-6    | lactate | H<sub>2</sub>O | log K<sub>i</sub> = 3.37 | 19 |
| citrate | H<sub>2</sub>O | log K<sub>i</sub> = 3.71 | |
| P<sub>i</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 2.71 | |
| O—P—Tyr | H<sub>2</sub>O | log K<sub>i</sub> = 3.00 | |
| O—P—Ser | H<sub>2</sub>O | log K<sub>i</sub> = 3.90 | |
| O—P—Thr | H<sub>2</sub>O | log K<sub>i</sub> = 3.70 | |
| Eu-7    | n.d. | H<sub>2</sub>O | K<sub>i</sub> = 3.0, 3.0 mM | 19 |
| Eu-8    | acetate | H<sub>2</sub>O | K<sub>i</sub> = 3.14 mM | |
| lactate | H<sub>2</sub>O | K<sub>i</sub> = 3.16 mM | |
| lactate | H<sub>2</sub>O | K<sub>i</sub> = 3.33 mM | |
| Yb-8    | HCO<sub>3</sub> | H<sub>2</sub>O | K<sub>i</sub> = 0.07, 9.8 mM | 19 |
| MeP<sub>i</sub> | H<sub>2</sub>O | K<sub>i</sub> = 1.9, 19 | 63 |
| DEP | H<sub>2</sub>O | K<sub>i</sub> = 2.8, 63 | |
| Eu-9    | P<sub>i</sub> | H<sub>2</sub>O | K<sub>i</sub> = 4.2 mM | 20 |
| acetate | H<sub>2</sub>O | K<sub>i</sub> = 0.31 mM | |
| Eu-11   | P<sub>i</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 5.4 | 21 |
| NO<sub>3</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 2.9 | |
| ATP | H<sub>2</sub>O | n.d. | |
| AMP | H<sub>2</sub>O | n.d. | |
| Tb-11   | P<sub>i</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 5.5 | 21 |
| NO<sub>3</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 4.6 | |
| ATP | H<sub>2</sub>O | n.d. | |
| Tb-12   | P<sub>i</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 4.0 | 45 |
| Sm-12   | P<sub>i</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 4.0 | 45 |
| Gd-13   | HCO<sub>3</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 4.0 | 25 |
| Gd-15   | F<sup>a</sup> | H<sub>2</sub>O | log K<sub>i</sub> = 4.0 | 11 |
| HCO<sub>3</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 4.0 | 11 |

Table 1. continued

| Complex | Selectivity | Solvent | Binding Affinity | Ref. |
|---------|-------------|---------|------------------|------|
| Gd-16   | HCO<sub>3</sub> | F<sup>a</sup> | log K<sub>i</sub> = 3.0 | 20 |
| Gd-17   | P<sub>i</sub> | H<sub>2</sub>O | log K<sub>i</sub> = 29 | 4 |
| Tb-18   | HCO<sub>3</sub> | ATP | log K<sub>i</sub> = 3.42 | 24 |
| Dy-18   | HCO<sub>3</sub> | ADP | log K<sub>i</sub> = 3.59 | |
| Eu-19   | P<sub>i</sub> | H<sub>2</sub>O | K<sub>i</sub> = 3.0 | 20 |
| Pr-19   | HCO<sub>3</sub> | ATP | log K<sub>i</sub> = 3.47 | 24 |
| Nd-18   | HCO<sub>3</sub> | ADP | log K<sub>i</sub> = 3.59 | |
| Sm-18   | HCO<sub>3</sub> | AMP | log K<sub>i</sub> = 4.7 | |
| Gd-19   | HCO<sub>3</sub> | CN<sup>−</sup> | log K<sub>i</sub> = 8.14 | 28 |
| Dy-19   | HCO<sub>3</sub> | NO<sub>3</sub> | log K<sub>i</sub> = 6.8 | 30 |
| Tb/Lu-18 | HCO<sub>3</sub> | CH<sub>3</sub>OH | log K<sub>i</sub> = 6.8 | 30 |
| Ln-27   | HCO<sub>3</sub> | CH<sub>3</sub>OH | log K<sub>i</sub> = 6.8 | 30 |
| Tb-29   | HCO<sub>3</sub> | ATP | log K<sub>i</sub> = 3.47 | |
| Eu-30   | HCO<sub>3</sub> | ATP | log K<sub>i</sub> = 3.47 | 33 |
| Eu-31   | P<sub>i</sub> | H<sub>2</sub>O | K<sub>i</sub> = 4.7 | 29 |
| Tb-32   | P<sub>i</sub> | H<sub>2</sub>O | K<sub>i</sub> = 4.7 | |

Phenanthroline-base complexes such as Ln<sup>−</sup>-10 and Ln<sup>−</sup>-11 investigated by Albrecht employ a similar mechanism for staining phospholipids in cells. It should be noted that in the absence of stability studies (vide infra, section 2.3), the quenching of Eu<sup>3+</sup> and Tb<sup>3+</sup> phosphorescence observed upon phosphate coordination could also be attributed to ligand exchange and displacement of the antenna by the phospholipids instead of the formation of a ternary complex postulated by the authors. While it is reasonably selective for most anions other than nitrate, these receptors are unlikely to be selective for inorganic phosphates over their organic counterparts. More recently, Jena reported a similar phenanthroline macrocycle, only binds phosphate and citrate, albeit with a 10-fold lower affinity (Table 1).
replacing the amides by weaker coordinating amines (Ln-12), a change that likely destabilizes further the complex.\(^{[22]}\) The probe is somewhat selective for phosphate, binding carbonate only weakly. Coordination of a first phosphate anion to Eu-12 initially results in an increase in luminescence via displacement of an inner-sphere water molecule. Further addition of phosphate quenches the terbium luminescence via a mechanism postulated by the authors to involve photoelectron transfer in a manner similar to that observed by Anslyn for the detection of 2,3-bisphosphoglycerate,\(^{[23]}\) although phosphate-based decomplexation has not been ruled out by the authors. It should be noted that “off-on-off” responses to analytes, such as those observed with Ln-12 have limited practicality.

Importantly, lanthanide receptors can be rationally designed to be highly selective for inorganic phosphate over other anions in water.\(^{[24]}\) As we demonstrated with Gd-13 to Gd-17 (Figure 4), tripodal lanthanide complexes with 2 inner-sphere water molecules and no steric hindrance at the open coordination sites can bind phosphate and other oxyanions only if the complex Gd\(^{III}\)L is not too stable, which occurs when the coordinating ligand L is not too basic.\(^{[4]}\) In those cases, the selectivity of the selectivity of the Gd\(^{III}\) complex always trends in the order phosphate > arsenate > carbonate > fluoride, an order that follows the basicity (not the hardness) of the anion. The more stable the Gd\(^{III}\) complex, the lower its affinity for anion. The more stable, the 2,3-hydroxypyridinonate-based Gd-14, has
no affinity for any anions whatsoever. Instead of forming ternary complexes, the least stable complexes undergo ligand exchange whereby the anion removes the lanthanide ion, resulting in the formation of insoluble salts. This observation underlines that phosphate “extrusion” of the metal, as opposed to formation of ternary Ln³⁺-L-Pi, likely occurs with many poorly stable lanthanide receptors published. Gd³⁺-TREN-MAM (Gd-13), which employs a ligand of intermediate basicity, binds phosphate with high affinity in water but not perfect selectivity over bicarbonate. Importantly, this first-generation complex demonstrates the recyclability of this class of receptor and its ability to catch-and-release phosphate upon changes in pH, an important property for applications of phosphate remediation (Figure 4) Indeed, the acidic form of a ligand (i.e. the protonated H₂PO₄⁻) has limited affinity for a metal ion. It is only the basic form (i.e. the deprotonated form) that does. The pH dependency observed for phosphate binding thus follows the general trend of lower stability of coordination complexes at lower pH. Further studies on the mechanism of phosphate recognition demonstrated the highly flexible nature of the receptors, underlining the fact that selective anion recognition can be achieved without rigid predisposition of hydrogen-bonding and electrostatic interactions.[26]

Complete selectivity for phosphate over other oxyanions can be achieved by slightly destabilizing Ln³⁺-TREN-HOPO (Ln-14, Figure 3) via slight modification of the ligand cap.[23] The complex Eu³⁺-2,2-Li-HOPO (Eu-18) and Eu³⁺-3,3-Gly-HOPO (Eu-19) both bind phosphate with very high affinity in water at neutral pH with complete selectivity over bicarbonate, carboxylates, fluorides and other competing anions (Table 1). An important conclusion from this study is that there is no relationship between the number of inner-sphere water molecules on the lanthanide ion and the affinity of the complex for phosphate. Some complexes, such as Eu-14, Eu-20, and Eu-21, which also have two inner-sphere water molecules, as well as Eu-22, which has a q = 3, have no affinity for any anions. Only those complexes where the ligand destabilizes one of the 1,2-HOPO chelation bind phosphate. This same destabilization also decreases the quantum yield of the complex. Importantly, selectivity of any lanthanide probe that function via direct coordination of the anion is highly pH dependent. This dependency was demonstrated with Eu-23, a probe that is exquisitely selective for phosphate in water at neutral pH, but binds the more basic cyanide at basic pH.[28]

Advantageously, the affinity of these receptors can be tuned at will via the incorporation of charged or hydrogen-bonding moieties in close proximity to the open coordination site.[29] The addition of a neutral hydrogen-bonding alcohol of Eu-24 increases the affinity of the probe for phosphate by 20-fold, whereas a single ammonium in Eu-23 increases 200-fold. On the other hand, the presence of a single negatively charged group, such as a sulfonate (Eu-25) or a carboxylate (Eu-26) is sufficient to block any anion recognition. These observations highlight the stringent dependency of lanthanide-based receptors on nearby electrostatic charges, a dependency that results from the purely ionic nature of lanthanide coordination. The same ionic dependence on anion coordination enables one to tune the affinity of the receptor by changing the lanthanide ion.[30] The later, and smaller, lanthanides have higher affinity for phosphate than the earlier, larger ones. For flexible complexes such as the tripodal Ln-18 and Ln-19, the affinity for phosphate can vary by more than 1,000-fold between La³⁺ and Lu³⁺.

2.2. Lanthanide-Based Indicator Displacement Assays

The probes discussed so-far function by the intrinsic effect that displacement of inner-sphere water molecules by coordination anions has on the lanthanide’s luminescence. A response can also be induced not by displacing water molecules but by displacing an indicator. An indicator is a small anion that coordinates to the lanthanide more weakly than phosphate such that it can be displaced by the harder oxyanion. If the indicator is also the antenna for Eu³⁺ or Tb³⁺, than its displacement results in a decrease of lanthanide-centered emission. This approach was first demonstrated by Faulkner and Pope with
3. Luminescent Probes for Nucleotides

One of the earliest and still most widespread commercial translation of luminescent lanthanide probes is in high throughput screening (HTS) for drug discovery and development. The long luminescent lifetimes of EuIII and TbIII complexes and the facile implementation of time-resolved spectroscopy are ideal for HTS assays since they readily discriminate competing autofluorescence arising from either the enzyme, the substrate(s) or the drug candidate. One of the largest classes of druggable targets with particular significance in oncology are kinases.[37] Kinases are enzymes that catalyzes the transfer of a phosphate group from ATP to a specific substrate with implications in cell signaling, among others. Luminescent lanthanide probes that could thus distinguish between ATP, ADP, AMP, and phosphate thus offers the alluring capability to monitor kinase inhibition and kinetics easily, without the use of antibody or modified substrate. The most relevant probes will be able to differentiate nucleotides at their cellular concentration. Healthy cells have ATP concentrations in the range of 1 to 10 mM and a normal ATP:ADP ratio of 1000:1.[19] Hence, kinase studies that are more relevant to cellular conditions require probes that have low affinity for the nucleotides, with $K_a$ between $10^{-2}$ and $10^{-3}$. The properties and molecular composition of these complexes are summarized in the following Table 2 and Figures 7–9.

3.1. Recognition of Nucleotides via Direct Coordination

As discussed in the previous section, the high oxophilicity and hardness of lanthanides favours coordination of hard and highly basic oxoanions such as HPO$_4^{2-}$.[1] By extension, nucleotides that contain either one, two, or three phosphates (such as AMP, ADP and ATP, respectively) also have a high affinity for lanthanide complexes with open coordination that are not sterically hindered. In most cases, coordination of the phosphoester to the lanthanide ion via displacement of inner-sphere water molecule(s) will also increase the intensity of lanthanide-centered emission by displacing the quenching water molecules (Figure 2a).

Interestingly, the first luminescent lanthanide probe for ATP, developed by Charbonnière and Ziesler, is not a turn-on probe.[39] Instead, coordination of ATP to EuIII (Figure 7) quenches 80% of EuIII’s emission. This significant, but not complete, quenching is not due to transmetallation. Indeed, this is one of the few studies in which formation of the ternary complex was clearly established by a combination of solution studies. The probe is unique in its near-complete selectivity for ATP over ADP and AMP. Unfortunately, the probe cannot differentiate between inorganic phosphate and ATP, an observation that is common to most probes of this class.

In order to avoid transmetallation, most modern probes for nucleotides employ thermodynamically stable and kinetically inert DOTA-type macrocycles. These include the library of probes developed by Butler. The C$_2$-symmetric cationic complexes Eu-34 to Eu-37 can bind reversibly to ATP via displace-
| Complex | Selectivity | Solvent | Binding Affinity | Ref. |
|---------|-------------|---------|-----------------|------|
| Eu-33   | P<sub>i</sub> | H<sub>2</sub>O | log K<sub>i</sub> 4.0 | 38   |
|         | ATP         |         | log K<sub>i</sub> 4.2 |      |
| Tb-33   | P<sub>i</sub> |         | log K<sub>i</sub> 4.5 |      |
|         | ATP         |         | log K<sub>i</sub> 4.6 |      |
| Eu-34   | ATP/ADP    | H<sub>2</sub>O | log K<sub>i</sub> 4.4 | 39   |
|         | ATP/GDP    |         | log K<sub>i</sub> 4.6 |      |
|         | AMP/GMP    |         | log K<sub>i</sub> 3.4 |      |
|         | P<sub>i</sub> |         | log K<sub>i</sub> 3.5 |      |
|         | UTP        |         | log K<sub>i</sub> 3.6 |      |
|         | UDP        |         | log K<sub>i</sub> 4.2 |      |
|         | UMP        |         | log K<sub>i</sub> 2.7 |      |
|         | HCO<sub>3</sub> |       | log K<sub>i</sub> 2.1 |      |
|         | ATP–Mg     |         | log K<sub>i</sub> 3.0 |      |
| Eu-35   | ATP        | H<sub>2</sub>O | log K<sub>i</sub> 3.0 | 39   |
|         | ADP        |         | log K<sub>i</sub> 3.3 |      |
|         | AMP        |         | log K<sub>i</sub> 3.3 |      |
|         | P<sub>i</sub> |       | log K<sub>i</sub> 2.4 |      |
| Eu-36   | ATP        | H<sub>2</sub>O | log K<sub>i</sub> 5.8 | 39   |
|         | ADP        |         | log K<sub>i</sub> 5.7 |      |
|         | AMP        |         | log K<sub>i</sub> 4.8 |      |
|         | P<sub>i</sub> |       | log K<sub>i</sub> 4.7 |      |
|         | GTP        |         | log K<sub>i</sub> 5.3 |      |
|         | GDP        |         | log K<sub>i</sub> 5.2 |      |
|         | GMP        |         | log K<sub>i</sub> 3.9 |      |
|         | UTP        |         | log K<sub>i</sub> 4.9 |      |
|         | UDP        |         | log K<sub>i</sub> 5.4 |      |
|         | UMP        |         | log K<sub>i</sub> 4.3 |      |
|         | P<sub>i</sub> |       | log K<sub>i</sub> 2.7 |      |
|         | HCO<sub>3</sub> |      | log K<sub>i</sub> 3.0 |      |
| Eu-37   | ATP–Mg     |         | log K<sub>i</sub> 4.6 | 40   |
|         | ADP–Mg     |         | log K<sub>i</sub> 3.8 |      |
|         | AMP–Mg     |         | log K<sub>i</sub> 3.8 |      |
| Eu-38   | ATP        | H<sub>2</sub>O | log K<sub>i</sub> 3.8 | 39   |
|         | ADP        |         | log K<sub>i</sub> 3.8 |      |
|         | AMP        |         | log K<sub>i</sub> 3.8 |      |
|         | P<sub>i</sub> |       | log K<sub>i</sub> 3.5 |      |
| Eu-39   | ATP        | H<sub>2</sub>O | log K<sub>i</sub> 3.6 | 41   |
|         | ADP        |         | log K<sub>i</sub> 3.3 |      |
|         | AMP        |         | log K<sub>i</sub> 2.77 |     |
|         | ATP–Mg     |         | log K<sub>i</sub> 3.26 |     |
|         | ADP–Mg     |         | log K<sub>i</sub> 2.93 |     |
| Eu-40   | P<sub>i</sub> | H<sub>2</sub>O | log K<sub>i</sub> 3.6 | 42   |
|         | PAPS       |         | log K<sub>i</sub> 4.0 |      |
| Eu-41   | PAP        | H<sub>2</sub>O | log K<sub>i</sub> 3.7 | 42   |
|         | PAPS       |         | log K<sub>i</sub> 4.0 |      |
| Eu-42   | PAP        | H<sub>2</sub>O | log K<sub>i</sub> 3.5 | 42   |
|         | PAPS       |         | log K<sub>i</sub> 4.0 |      |
|         | 3‘-AMP     |         | log K<sub>i</sub> 4.0 |      |
|         | 5‘-AMP     |         | log K<sub>i</sub> 4.0 |      |
|         | APS        |         | log K<sub>i</sub> 4.0 |      |
| Eu-43   | AMP        | H<sub>2</sub>O | log K<sub>i</sub> 3.83 | 44   |
|         | P<sub>i</sub> |       | log K<sub>i</sub> 4.15 | 45   |
| Eu-44   | P<sub>i</sub> | H<sub>2</sub>O | log K<sub>i</sub> 4.15 | 45   |
| Eu-45   | P<sub>i</sub> | H<sub>2</sub>O | log K<sub>i</sub> 4.40 | 45   |
ment of the inner-sphere water molecule concomitant with second-sphere hydrogen-bonding interactions between the quinolone antennas and the nucleobase.[46] Water displacement leads to the telltale increase in both luminescence intensity and the lifetime of europium(III). Key to achieving selectivity for ATP over ADP and AMP is the incorporation of amides on the quinolines of Eu-34 and Eu-36 that hydrogen bond to the nucleobase. This study demonstrated that conjugation to a lanthanide ion alone is often insufficient to distinguish between organic phosphates. Although only 3-fold selective for ATP over inorganic phosphate, Eu-36 can distinguish between ATP, ADP, and AMP at concentrations that are relevant physiologically (1-5 mM ATP) even in the presence of Mg$^2+$. The addition of magnesium is indeed crucial to the ability of these probes to successfully monitor kinase kinetics since in cells ATP is stabilized via coordination to Mg$^2+$. In more complex assay conditions, coordination of ATP to Eu$^{III}$ therefore requires displacement of Mg$^2+$.

In-depth solution studies by the Butler group[42] expanded the functionality of their probes and enabled further study into their mechanism of action. The Tb$^{III}$ analog Tb-34 is more sensitive to quenching by dissolved oxygen. The cis-configuration of quinolone arms in Eu-38 favors ligand exchange and decomplexation upon ATP coordination, likely because of steric hindrance. Replacement of amide groups by oxymethyl groups that cannot hydrogen bond to the nucleobase in Eu-39 decreased affinity for the nucleotides as well as their selectivity. In the presence of Mg$^2+$, the apparent association constants decrease by one order of magnitude since the alkali earth competes with the rare-earth for phosphate coordination. Advantageously, principal component analysis (PCA) using all four complexes can distinguish between all nucleotide polyphosphate (NPP) species. Derivative complexes containing distal benzyl groups (Eu-40 to Eu-42) were shown to be able to distinguish between adenosin-3’,5’-diphosphate (PAP) and adenosin-3’-phosphate-5’-phosphosulfate (PAPS) due to unique turn-on intensities, even though they were not particularly selective between NPPs.[43] This unusual selectivity enabled ratiometric screening of sulfotransferase activity. More recently, Butler was able to switch the preference of this class of probes from ATP to AMP, which is uncommon in that the chelate effect usually favors coordination of the triphosphate.[44] This was previously achieved by Albrecht with the lanthanide helicase Eu-43.[45] The helicate is indeed perfectly preorganized to bind the phosphate moiety on one end and the purine on the other end, with the length of the helicate favoring AMP (Figure 7). It would be interesting if this approach could be extended to the detection of other nucleotides.

In the absence of second-sphere interactions directed to the nucleobase, almost all lanthanide probes that bind phosphate will also bind ATP with similar affinity. The phenanthroline Ln-44 and Ln-45, which are minor derivatives of the phosphate probes Ln-10 and Ln-11, as well as Ln-12 also respond to ATP similarly as to phosphate, and likely via the same mechanism.[46] Predictably, the greater the number of open coordination sites on a lanthanide complex, the higher its affinity for phosphate species. Schäferling demonstrated this with the polyaminocarboxylate Eu-46 to Eu-51.[47] All complexes demonstrated higher affinity for ATP and pyrophosphate (PPI) over AMP due to the higher denticity of the former anions. An apparent higher affinity for phosphate species is observed with europium complexes with greater number of open coordination sites. Not surprisingly, the complexes also respond to other coordinating oxyanions such as oxalate and citrate. The mechanism of action, however, is likely not a true formation of a well-defined ternary complex as postulated. Ligands of low denticity are well-known to form poorly stable complexes and are highly susceptible to ligand exchange in the presence of strongly coordinating oxyanions such as phosphate.[48] Moreover, often underappreciated, adenosine is a very good antenna for Eu$^{III}$. Indeed, europium(III) complexes of ATP are quite luminescent.[49] Increase in luminescence intensity is thus not necessarily solely a result of displacement of water molecules and formation of a ternary complex. Determination the nature of the complex formed requires more in-depth solution studies besides luminescence intensity.

### Table 2. continued

| Complex | Selectivity | Solvent Binding Affinity | Ref. |
|---------|-------------|--------------------------|------|
| Eu-60   | PP, ATP, ADP, Citrate, Tartrate | CPB Micellar solution | K$^+$ 13.47, K$^+$ 12.26, K$^+$ 11.36, K$^+$ 11.42, K$^+$ 7.32, K$^+$ 3.33 | 57 |
| Eu-61   | PP, ATP, ADP, Citrate, Tartrate | CPB Micellar solution | K$^+$ 13.43, K$^+$ 12.28, K$^+$ 11.32, K$^+$ 11.44, K$^+$ 7.44, K$^+$ 5.26 | 57 |
| Eu-62   | PP, ATP, ADP, Citrate, Tartrate | CPB Micellar solution | K$^+$ 13.49, K$^+$ 12.21, K$^+$ 11.37, K$^+$ 11.40, K$^+$ 7.29, K$^+$ 5.42 | 57 |
| Eu-63   | PP, ATP, ADP, Citrate, Tartrate | CPB Micellar solution | K$^+$ 13.47, K$^+$ 12.21, K$^+$ 11.37, K$^+$ 11.40, K$^+$ 7.29, K$^+$ 5.42 | 57 |
| Eu-64   | ATP, ADP, AMP, Pi, P, PP, ATP, ADP, AMP, Pi, P, PP | H$_2$O | K$^+$ 6.0, K$^+$ 4.7 | 58 |
| Eu-65   | ATP, ADP, AMP, Pi, P, PP, ATP, ADP, AMP, Pi, P, PP | H$_2$O | K$^+$ 6.6, K$^+$ 6.1, K$^+$ 5.8, K$^+$ 6.0, K$^+$ 6.3, K$^+$ 6.8, K$^+$ 6.6 | 58 |
| Eu-66   | ATP, ADP, AMP, Pi, P, PP, ATP, ADP, AMP, Pi, P, PP | H$_2$O | K$^+$ 6.6, K$^+$ < 5.0, K$^+$ 6.5, K$^+$ 6.9 | 58 |
3.2. Recognition of Nucleotides via Second Sphere Interactions

Differentiating nucleotides while minimizing the risks of both ligand exchange and hydrolysis of polyphosphates can be accomplished by preventing coordination of the phosphate moiety to the lanthanide ion, instead relying solely on outer-sphere interactions. This approach enables the use of kinetically inert and thermodynamically stable complexes such as those formed with macrocyclic DOTA-type ligands. Selective recognition of organic phosphate is achieved with the traditional toolbox of organic supramolecular chemistry. Electrostatic and π-stacking interactions are particularly well suited for nucleotides such as ATP and GTP.

This is the approach that we have employed with the ATP-selective receptor Tb-52 (Figure 8). π-π Interactions between the nucleobase and the phenanthridine enable photoelectron transfer from the purine to the antenna, which prevents sensitization of TbIII resulting in a substantial decrease in the lanthanide’s centered emission. Photoelectron transfer and π-stacking is favored for purines over pyrimidines, so the probe is only responsive to adenosine and guanosine nucleotides. Electrostatic interactions with the positively charged Tb-52 favors the more negatively charged ATP over ADP and AMP. The importance of the electrostatic interactions in water is further supported with studies with the neutral analog Eu-53, which does not distinguish between the triphosphate, diphosphate, and monophosphate purine nucleotides. The advant-
age of relying on second-sphere interactions is that, since they are weaker, they confer the probe’s weak affinity for their substrate. This, in turn, enables measuring nucleotide concentrations in the mM range that are more relevant to cellular conditions. Indeed, concomitant use of the responsive Tb-52 and unresponsive Eu-53 probes enabled the first study of kinase kinetics by ratiometric time-gated luminescence spectroscopy. This approach has several advantages over commercial kits: it does not require antibodies, it uses natural substrates that are neither modified nor labeled, and it enables continuous monitoring of the reaction and its inhibition, thereby facilitating kinetic studies of drugs.

Tang subsequently developed the analog Eu-54 that uses instead a terpyridine antenna and functions similarly. The authors propose that the first equivalent of ATP partially displaces the inner-sphere water molecule causing an increase in luminescence intensity. A second equivalent of ATP causes a decrease in luminescence, likely via PeT. It should be noted that bell-shaped curve responses have limited practicality. More recently, the Patra group reported a similar bis(amido-phenylterpyridine) complex with a DTPA backbone and comparable response.13

Peripheral or outer-sphere interactions need not be limited to those between organic moieties. The binuclear TbIII–ZnII complexes Eu-55 to Eu-57 developed by Parker exploited the ability of the terminal phosphate of both ADP and ATP to bridges the two metal ions to induce a luminescence response.14 Uniquely, since the two adduct have strong induced circularly polarized emission but of opposite sign, conversion between the two nucleotides can be monitored by measuring the probe’s emission dissymmetry factor (Figure 8). The complex Tb-58 developed by Tuck,15 also exploits coordi-

Figure 8. Responsive luminescent lanthanide probes for nucleotides functioning via peripheral interactions.
nucleotides. The trinuclear Tb⋯bisZn is more selective and has higher affinity for GMP via the formation of a 1:1 assembly. Interestingly, this complex is also more selective for acyclic GMP over its cyclic cGMP derivative, likely due to the higher negative charge of the former. This is a rare example of a molecular probe that can monitor catalytic conversion of cGMP to GMP via a phosphodiesterase enzyme.

Bimetallic complexes, particularly ones employing metals of different hardness, are well suited for distinguishing between nucleotides by enabling coordination to both phosphate and the nucleobase. As discussed throughout this review, lanthanides are highly oxophilic and thus have high affinity for phosphate. On the other hand, softer purines, and in particular guanine, have high affinity for softer metal ions such as platinum(II). The bimetallic Tb\(^{III}\)–Pt\(^{II}\) developed by Guo, Tb-59, is the only example that exploits this duality in metal coordination by nucleotides to favor recognition of GTP.\(^{[66]}\) Whereas the phosphate moiety of the nucleotide coordinates Tb\(^{III}\) thereby displacing the inner-sphere water molecule, the guanine binds the Pt\(^{II}\) by displacing the weaker Cl\(^–\) ligand (Figure 8). Unfortunately, coordination of guanosine to Pt\(^{II}\) is irreversible, so such bimetallic probes, while sensitive, cannot monitor enzymatic reactions.

3.3. Luminescence Response via Ligand Exchange

As discussed above, weakly coordinating ligands that can also sensitize either Eu\(^{III}\) or Tb\(^{III}\) are all intrinsically phosphate probes since phosphate, a strong ligand for lanthanides, easily displaces weak ones. Ligand exchange always results in a decrease in lanthanide-centered luminescence intensity. Due to the chelate effect, ATP has higher affinity for lanthanides than ADP, AMP, and orthophosphate, so some selective response can be observed under the right conditions. Schaferling first demonstrated the potential of this approach with the weak Eu\(^{III}\)-tetracycline complex.\(^{[57]}\) While such metal-exclusion assays are simple, they are poorly selective between phosphate species and toward carboxylates. The formation of insoluble lanthanide phosphate salts, which practically preclude the reversibility of binding, make them ill-suited for monitoring enzymatic reactions. This is demonstrated, for instance, with the β-diketonate europium(III) complexes Eu-60 to 63 (Figure 9) developed by the Yuan group, which show low selectivity between orthophosphate, pyrophosphate, ATP, and ADP.\(^{[58]}\)

They are, however, quite sensitive, with limits of detection for pyrophosphate as low as 0.02 \(\mu\)M for Eu-60.

More recently, Anzenbacher modified this approach by monitoring the luminescence of the weakly coordinating chromophores as opposed to that of the lanthanide ion.\(^{[59]}\) Since europium(III) quenches the luminescence of the carbamate quinolone, its displacement by phosphate returns the fluorescence of the organic chromophore. Like any displacement assay, the selectivity of Eu-64 to 66 between phosphate species remains poor.

4. Luminescent Probes for Organic Phosphates

Probes for organophosphates other than nucleotides and nucleic acids are less developed. Yet, the same principles can be applied to their development. Interest in this field focuses primarily on chemical warfare agents and certain pesticides that contain phosphate moieties for which facile detection is the first step in remediating contaminated areas. Markers of biochemical processes, some of which are over expressed in disease tissues, are also of interest. Successful translation hinges on achieving very high selectivity, particularly over orthophosphate. The properties and molecular composition of these complexes are summarized in the following Table 3 and Figures 10–11.

4.1. Recognition of Organophosphates via Direct Coordination

Organophosphates, like nucleotides and inorganic phosphates, have some affinity for many lanthanide complexes with at least one open coordination site. This was first demonstrated by Bretonnière and Parker with the complexes Eu-67 to 69 (Figure 10), which all bind various phosphorylated species via displacement of the inner-sphere water molecule resulting in the characteristic increase in luminescence intensity.\(^{[60]}\) Interestingly, Eu-68 has higher affinity for phosphorylated tyrosine than phosphorylated serine. The probe is chemoselective. Given a hexapeptide containing both phosphorylated Tyr and Ser residues, it will only bind the phosphorylated tyrosine. Further studies with the analogs Ln-1 and Ln-70 demonstrated that the
receptor does not bind the protein via its N-terminus, preferring instead monodentate phosphate coordination. This first study demonstrated the role that the ligand can play in selecting for a desired organophosphate.

As demonstrated by Parker with the complexes Ln-71 to Ln-74, chiral O-phosphono amino acids can also be probed by circular polarized luminescence (CPL). An ammonium group on the meta position of the benzyl differentiates the two sets of ligands. It is this ammonium that directs coordination of the O-phosphono amino acids and model phosphorylated peptide to the lanthanide ion via hydrogen-bonding. The luminescent and water-soluble probe Eu-72 showed no preference between O-phosphono amino acids. However, O-P-Ser and O-P-Tyr residues could be distinguished by CPL spectral changes. This probe also

| Complex | Selectivity | Solvent | Binding Affinity | Ref. |
|---------|-------------|---------|------------------|------|
| Eu-67   | O-P-Ser     | H₂O     | n.d.             | 59   |
|         | O-P-Tyr     | H₂O     | n.d.             |      |
|         | O-P-Thr     | H₂O     | n.d.             |      |
|         | AMP         | Gluc-6-P | n.d.             |      |
| Eu-68   | O-P-Ser     | H₂O     | Log Kᵦ 2.7       | 59   |
|         | O-P-Tyr     | H₂O     | Log Kᵦ 4.2       |      |
| Eu-69   | O-P-Ser     | H₂O     | n.d.             | 59   |
|         | O-P-Tyr     | H₂O     | n.d.             |      |
| Ln-70   | O-P-Ser     | H₂O     | n.d.             | 59   |
|         | O-P-Tyr     | H₂O     | n.d.             |      |
|         | O-P-Thr     | H₂O     | n.d.             |      |
|         | AMP         | Gluc-6-P | n.d.             |      |
| Eu-72   | Lactate     | 1:1 H₂O/CH₃OH | Log Kᵦ 4.37   | 60   |
| P       |             |          | Log Kᵦ 4.20     |      |
|         | O-P-Ser     | H₂O     | Log Kᵦ 4.80     |      |
|         | O-P-Tyr     | H₂O     | Log Kᵦ 4.80     |      |
|         | O-P-Thr     | H₂O     | Log Kᵦ 4.80     |      |
| Eu-74   | Lactate     | 1:1 CH₃OH | Log Kᵦ 3.15    | 60   |
| P       |             |          | Log Kᵦ 5.25     |      |
| Eu-75   | Glycophosate | H₂O     | Log Kᵦ 5.36     | 61   |
|         | N-methyl gly- |          | Log Kᵦ 4.35     |      |
|         | phosphate    |          | Log Kᵦ 3.93     |      |
| Eu-76   | AMPA        | H₂O     | Log Kᵦ 3.30     | 62   |
| Eu-77   | GB          | H₂O     | log Kₑ 3.56      | 64   |
|         | DCP         | H₂O     | log Kₑ 3.66      |      |
|         | DECP        | H₂O     | log Kₑ 3.70      |      |
| Tb-77   | GB          | H₂O     | log Kₑ 5.0       |      |
|         | DCP         | H₂O     | log Kₑ 5.1       |      |
|         | DECP        | H₂O     | log Kₑ 4.5       |      |
| Eu-78   | R/S pybox   | CH₃CN   | n.d.             | 65   |
| Eu-79   | DCP         | H₂O     | n.d.             | 66   |
| Eu-80   | DCP         | H₂O     | n.d.             | 66   |

Figure 10. Responsive luminescent lanthanide probes for organophosphates functioning by direct coordination of the anion.
enabled sensitive detection of the ovarian cancer marker oleoyl-L-α-lysophosphatidic acid.

The same motifs that are used for the selective detection of nucleotides can also be employed with organophosphates. Parker, for instance, tuned his bimetallic Eu\textsuperscript{III}-Zn\textsuperscript{II} complexes initially developed for nucleotide sensing for the detection of glyphosate, a problematic herbicide.\textsuperscript{62} The zinc(II) ions of Eu-55 bind the phosphate moiety, thereby assisting its concomitant coordination of the lanthanide ion. In the absence of zinc(II) (Eu-75), hydrogen bonding between the phosphate and the pendant tertiary amine substantially increases the affinity for glyphosate, particularly if measurements are done above its pK\textsubscript{a} at pH 5.9. Under these conditions, the presence of a hydrogen-bonding motif bestows the probe with selectivity for phosphate diesters with two terminal oxygens and two identical ester groups. Similar analytes such as phosphorothioic acid, O,O-diethylester, ethyl methylphosphonate, O-(4-nitrophenylphosphoryl)choline, and cyclic 3,5-adenosine monophosphate do not yield a response. As with our work with nucleotides, Morrow’s contribution to anion recognition by lanthanide probes demonstrates that binding does not necessitate coordination to the metal center and that exquisite selectivity can be achieved with the use of outer-sphere supramolecular recognition motifs.

4.2. Outer-Sphere Recognition of Organophosphates

Organophosphates can also be recognized solely via outer-sphere interactions and without coordination to the lanthanide ion. Morrow followed this approach to detect phosphate diesters with identical ester groups such as diethylphosphate and (bis(4-nitrophenyl) phosphate) with the PARACEST (paramagnetic chemical exchange saturation transfer) MRI agent Ln-8 (Figure 3).\textsuperscript{63} Since recognition occurs via outer-sphere hydrogen-bond interactions to the organophosphate rather than by displacing the bound water molecule, the probe is selective for phosphate diesters with two terminal oxygens and two identical ester groups. Similar analytes such as phosphorothioic acid, O,O-diethylester, ethyl methylphosphonate, O-(4-nitrophenylphosphoryl)choline, and cyclic 3,5-adenosine monophosphate do not yield a response. As with our work with nucleotides, Morrow’s contribution to anion recognition by lanthanide probes demonstrates that binding does not necessitate coordination to the metal center and that exquisite selectivity can be achieved with the use of outer-sphere supramolecular recognition motifs.

4.3. Luminescence Response via Ligand Exchange

Weak lanthanide complexes readily undergo ligand exchange with strong chelating ligands such as phosphates and phosphonates. Ligand displacement enables sensitive but neither selective nor reversible detection of inorganic phosphate, nucleotides and therefore, by extension, other organophosphates. The phenanthrolines of the very weak Ln-77 complex (Figure 11), for instance, are readily displaced by V-series organophosphate chemical warfare agents (CWA).\textsuperscript{64} The less basic G-series agents have lower affinity for coordinating Eu\textsuperscript{III}. Similarly, omethoate readily displaces the triazole-pyridine ligands of the bis-tridentate assembly of Sun, Eu-78.\textsuperscript{66} Decomplexation results in complete quenching of the Eu\textsuperscript{III}-centered emission. Likewise, the luminescent but unstable Eu-79 and Eu-80 complexes of Patra are displaced by diethyl chlorophosphate, a nerve agent, also resulting in complete loss of phosphorescence.\textsuperscript{67} Despite their sensitivity, it is likely that all of these probes also fall apart in the presence of inorganic phosphates or basic organic phosphates, which limits their practicality.
5. Luminescent Probes for Nucleic Acids

While the above discussion has focused on the design of molecular probes that target small molecular weight phosphates, such as orthophosphate and nucleotides, lanthanide complexes have also been designed for macromolecular phosphates. These include dsDNA, aptamers—single-stranded oligonucleotides that selectively recognize targeted analytes due to their unique three-dimensional conformations—and DNAzymes that cleave upon complexation of a lanthanide ion. The molecular composition of these complexes is summarized in the following Figures 12–15.

5.1. Lanthanide Probes for dsDNA

There are two general classes of lanthanide-based molecular probes for dsDNA: metallointercalators and molecular beacons. Metallointercalators intercalate between two base pairs of dsDNA, resulting in a doubling of the rise and, most of the time, a change in the lanthanide-centered luminescence. These form supramolecular assemblies with dsDNA and thus have, for the most part, poor selectivity for a specific sequence, albeit higher sensitivity. Instead, sequence selectivity can be achieved either with molecular beacons or with responsive lanthanide probes covalently conjugated to a complementary strand.

The first lanthanide complexes designed to bind dsDNA were developed by Parker. In most cases, the lanthanide-centered emission is quenched upon interaction with guanines due to the formation of a charge-transfer complex with the excited state of the antenna. Two more recent examples include the complexes Tb-81 and Tb-82 (Figure 12), the luminescence of both of which is decreased upon interaction with electron-rich GC base pairs. Of note, in this case, the luminescence of the europium(III) analogs was not affected by guanines. The response of both probes to dsDNA binding was used to monitor changes in nucleic acid in dividing cells.

In the absence of crystal structures of the Ln complex-DNA adduct, true intercalation can be difficult to prove, although it can be inferred by changes in the excitation spectra of the complex. A recent example of a true intercalator is the heterotrimetallic Eu-83 developed by Pikramenou. The two square planar terpyridyl platinum(II) moieties intercalate in dsDNA, thereby solidly anchoring the central europium(III) in a hairpin fashion. Unlike other lanthanide-based intercalators, the europium(III) centered luminescence of Eu-83 increases upon dsDNA binding, likely due to the effect of the electron-rich and hydrophobic environment of the intercalated tpy ligands on the efficacy of ligand-to-ligand charge transfer (LLCT).

In all other cases, intercalation of a lanthanide complex in dsDNA result in significant reduction of the lanthanide-centered emission. This is the case for the two complexes Tb-52 and Eu-
previously (Figure 8) reported by our group for nucleotide sensing. These two complexes can intercalate in three-dimensional DNA nanostructure such as Turberfield’s tetrahedron with as much as one metallointercalator for every two base pairs.

The positively charged Tb-

52 has substantially higher affinity for dsDNA than the neutral Eu-

53. Advantageously, the supramolecular assembly increases significantly the cell-uptake of the lanthanide complexes in the presence of lipofectamine.

Metallointercalators-DNA self-assemblies are thus a unique and efficacious strategy for carrying metalprobes and drugs that have otherwise poor uptake inside cells.

Supramolecular assemblies can also be achieved by conjugating the terbium(III) complex onto iron oxide nanoparticles via a polyethylene glycol linker. The incorporation of superparamagnetic nanoparticles bestows the construct with a second modality to detect dsDNA. Not only is the Tb emission quenched, but the formation of three-dimensional nanoparticle-DNA assemblies gives a magnetic response with significant changes in both the longitudinal and transverse relaxivities. Advantageously, since the metallointercalators have poor affinity for denatured DNA, the magnetic assemblies offer a unique strategy for purifying DNA by magnetic catch-and-release.

A metallointercalator can become selective for a specific oligonucleotide sequence if it is covalently conjugated to its complementary strand. One such example is the luminescent lanthanide complexes Tb-

84 and Eu-

85 reported by Matsumoto. The use of a nonadentate ligand ensures that the europium(III) complex is highly stable, and that it has no open coordination site for phosphate coordination. This ensures that the complex intercalates in the π-stack of the dsDNA and prevents coordination by the sugar phosphate backbone, which might result in displacement of the ligand. Intercalation favors energy transfer between the antenna of Eu-

85 and the purines of the dsDNA, and consequently significantly decreases the luminescence intensity of the emitting lanthanides.

In the absence of a strong, octa- or nonadentate ligand, the complex is much less stable in solution. Strongly coordinating phosphates-including the phosphates of the backbone of nucleic acids-can thus displace weak mono- or bidentate

53 previously (Figure 8) reported by our group for nucleotide sensing. These two complexes can intercalate in three-dimensional DNA nanostructure such as Turberfield’s tetrahedron with as much as one metallointercalator for every two base pairs.

Figure 13. Responsive luminescent lanthanide-based aptamers.

Figure 14. Mechanism of lanthanide ion-catalyzed RNA cleavage in RNAzymes.
ligands with much lower basicity. Such complexes will display apparent high affinity for dsDNA, but they will not necessarily stay intact and will behave essentially as indicator displacement assays (section 2c). This is likely the mechanism of action of the probes Ln-86 and Yb-87 developed by Patra. Of note, poorly stable probes can still present biological activity. Both Ln-86 and Yb-87 cleave DNA hydrolytically, as does a bimetallic complex comprising tolfenamic acid. These studies serve as a reminder not to overestimate the stability of lanthanide complexes and not underestimate their ability to undergo rapid ligand exchange in solution with strong phosphate or even carboxylate ligands. Unless a well-studied, stable and inert ligand structure is used, such as one based on the macrocyclic polyaminocarboxylate DOTA ligand, a mechanism of action should not be asserted without solution studies confirming the stability of the probe and its resistivity to anion-based decomplexation.

5.2. Lanthanide-Based Luminescent Aptamer Probes

Aptamers are short, single-stranded nucleic acids—either RNA or DNA—that bind selectively to a specific target. The target can be small, such as a single ion or small molecule, or large, such as a protein or even a live cell. They are, essentially, nucleic acid versions of antibodies. As such, any luminescent lanthanide probe for nucleic acid can be modified to detect, in principle, any analyte simply by conjugating the lanthanide complex to the corresponding aptamer. (In practice, the number of aptamers reported remain limited.) Indeed, the phenanthridine-based complexes Tb-52 and Eu-53 (Figure 8), developed by our group, can monitor enzymatic reactions by monitoring the ratio of ATP/ADP or GTP/GDP, the same complexes self-assemble in three-dimensional dsDNA nanostructure and one-dimensional dsDNA as seen in Eu-88 (Figure 13). Extension of the same principles enable detection of Hg\(^{2+}\), a soft-metal, simply by conjugating either complex to an aptamer for Hg\(^{2+}\). In the absence of mercury, the phenanthridine intercalates in the dsDNA bound to D1, and the europium’s luminescence is weak. Coordination of Hg\(^{2+}\) by the thymines releases the complementary oligonucleotide, which prevents intercalation of the phenanthridine and thus restores the luminescence of the Eu\(^{3+}\).

These approaches can be flipped: instead of using a luminescence lanthanide complex to detect an analyte that is not luminescent, one or two organic fluorophores can be used to detect the presence of a lanthanide ion. Edogun demonstrated this methodology with the lanthanide aptamer Gd-89. The aptamer strand D2 was optimized to selectively coordinate gadolinium(III). Lanthanide coordination releases the quenching fluorophore strand resulting in an increase in the luminescence of the fluorescein. This mode of action should not be confounded with chemosensors, in which free metal ions exhibit unique luminescence responses due to sensitization from nucleotide bases.

Lanthanide-labeled aptamers easily enable detection of analytes by luminescence; this can be further coupled with other modalities such as ICP-MS. Many of these modalities, however, suffer from poor sensitivity so that some method of signal amplification must be incorporated. Often, such amplifications are performed by polymerase chain reaction (PCR) which in turn requires heat-stable lanthanide complexes. This is the case with the probe Tb-90 developed by Linscheid. High sensitivity was achieved using two tagged probes, one tagged with the luminescent terbium(III) complex (D3-D5) and the other capture probe-labeled with biotin (D6-D8). The biotin moiety has high affinity to streptavidin-coated on nanoparticles, which enables amplification of the target sequence D9 with each PCR cycle. This approach enables detection of the target down to 0.4 fM.
5.3. Lanthanide-Based RNAzymes

RNAzymes, also called RNA enzymes or catalytic RNA, undergo a specific catalytic reaction, behaving essentially as artificial ribozymes. Lanthanide ions, in particular, are good Lewis acids with propensity to cleave the backbones of RNA, and to a lesser extent those of DNA, according to a general mechanism depicted in Figure 14. The lanthanide ion(s) triggers a nucleophilic attack from the alcohol moiety to the phosphate, which cleaves the phosphoester bond in the \( “rA” \) (ribo-adenosine) nucleotide. This reactivity enables differentiation of lanthanide ions through cleavage efficiency that can be monitored by luminescence response when a fluorophore is liberated upon release of the targeted \( rA \) nucleotide.\(^8\) In a manner similar to that used to obtain aptamers, RNA sequences were conservatively selected for reactivity with desired lanthanide ions, which ultimately enabled the differentiation of rare-earths.

The first generation of lanthanide-selective RNAzymes developed by the Liu group used the lanthanide ion(s) as the cofactor.\(^9\) Although these initial artificial enzymes did not distinguish between all the trivalent lanthanide ions on their own, it was found \( Ln-91 \) (Figure 15) was selective for \( Ce^{III} \) over \( Ce^{IV} \). Further studies with \( Ln-91 \) demonstrated that lanthanide coordination was not the rate-limiting step and that guanine had the highest affinity for the lanthanides.\(^10\) Interestingly, this complex had the strongest affinity for \( Nd^{III} \) and \( Ho^{III} \), which share three unpaired \( f \) electrons. The importance of the size of the lanthanide ion in achieving selectivity was established with \( Ln-92 \), with which the smaller late lanthanides have the highest reactivity.\(^11\) More RNAzyme families were developed in subsequent studies to enhance the selectivity for heavy lanthanides, including \( Ln-93 \) and \( Ln-94 \).\(^12\) \( Ln-95 \), for instance, is more selective for lighter trivalent lanthanide ions and selectively cleaves 2’-5’ RNA.\(^13\) \( Ln-96 \) is selective for lanthanides in the middle of the series.\(^14\) Impressively, when combined together and with \( Ln-97 \) (another middle series selective RNAzyme), the above RNAzymes can differentiate the lanthanide ions with high accuracy, utilizing relative cleaving efficiency.\(^15\) The potential of this unique selective affinity could be functionalized further for lanthanide separation, establishing unique pre-organization strategies dependent on conservatively selected sequences.

5.4. An Overlooked Flaw: Phosphodiesterase Activity of Lanthanides

The ability of lanthanide ions to cleave DNA and RNA and function in DNAzymes and RNAzymes arises from their unique properties. They are excellent Lewis acids, with high charge density, large ionic radii, and high oxophilicity favoring phosphate coordination. The combination of these factors also make them excellent catalysts for cleaving phosphoesters. Indeed, several lanthanide complexes with open coordination sites behave as excellent phosphodiesterase mimics. This behavior should be kept in mind when designing probes for polyprophosphates such as ATP, since some complexes could be capable of not just binding ATP, but also cleaving it.

The ability of lanthanide complexes to behave as phosphodiesterases was studied primarily by Morrow. The complex \( Eu-98 \) (Figure 16) efficiently cleaves ApUp and oligomers of adenylic acid at \( 37°C \).\(^16\) Importantly, because it is both thermodynamically stable and kinetically inert, it does not undergo ligand exchange with phosphates. Further studies with macrocyclic complexes established guidelines for predicting lanthanide phosphodiesterase activity.\(^17\) The tetraamide La-\(99\) and the tetra-hydroxypropyl Eu-\(8\) (Figure 3) are both catalytically inactive. On the other hand, the complex Eu-\(100\), which also contains four hydroxyl groups, does display good phosphodiesterase activity. Interestingly, it preferentially cleaves \( \alpha \)- phosphates on the G residue of the monoribonucleotide \( m7GpppG \) than on the \( m7G \) one, possibly due to electropositive charge of the latter. The complex Eu-101, which contains a single amide arm, has similar catalytic activity as its tetrahydroxylethyl analog Eu-100. That Eu-100 displays phosphodiesterase activity whereas its analog Eu-8 does not is likely due to the increased steric hindrance at the coordination site arising from the four methyl groups of Eu-8. Zinc(Ill), another excellent Lewis acid, enhances the phosphodiesterase activity of lanthanide complexes, likely by facilitating the initial nucleophilic attack.

Phosphodiesterase activity extends to macrocyclic triamide structures similar to many molecular probes developed for nucleotide sensing.\(^18\) Both monooester and diester phosphates coordinate the lanthanide ion of Eu-102 to 105 via displacement of the inner-sphere water molecule. Phosphate monoester bridges the two lanthanides of the dinuclear Eu-102 and Eu-103. All four complexes promote cleavage of 2-hydroxypropyl-4-nitrophenyl phosphate (HpPNP), an RNA analogue, and uridylyl-3’,5’-uridine (UpU), a dinucleoside, with a simple linear dependence of reactivity on \( pH \). Further mechanistic studies demonstrated that the catalytic activity results from stabilization of the developing negative charge on the phosphorane transition state by the lanthanide ion. Unlike \( Zn^{II} \) and \( Cu^{II} \), which hydrolyze at neutral \( pH \) to form hydroxido species, the higher \( pK_a \) of lanthanide ions favors the formation of an aqua complex that is more reactive.

The phosphodiesterase mimics Eu-99 to Eu-105 are quite similar to some of the luminescent lanthanide probes for nucleotide sensing that function by direct coordination to the lanthanide ion. Both classes contain pendant aromatic groups, both have macrocyclic ligands with amide arms, both enable phosphate coordination. This similarity raises the specter that some probes for ATP and GTP could not just bind the nucleotide but also catalyze its hydrolysis. Because such reactivity would undoubtedly affect the ability of the probes to monitor kinase or adipose activity, it should be kept in mind in studying the kinetics of nucleotide binding and determining their mode of action.
6. Conclusion and Outlook

The above discussion has focused on the varied principles that have been exploited successfully over the last decade in the design of lanthanide complexes with unique selectivity for specific inorganic or organic phosphates. The field of lanthanide-based molecular probes for phosphates is now well rounded. Selectivity is almost never complete, but is often sufficient to monitor a variety of enzyme assays or other biological processes with ease. Efficient and bright complexes that are selective for a variety of substrates such as orthophosphate, ATP, or AMP enable their detection directly in water at neutral pH. These should fulfill almost all needs for bioassays in drug discovery, among other applications. Nonetheless, certain areas remain underdeveloped, including the development of sensitive and selective probes for nerve agents and pesticides and sequence-specific lanthanide metallointercalators. Modifications that allow for two-photon excitation and/or near-infrared emission would further empower this class of molecular probes.

The review of the literature has brought up a few points that we advise the reader not to overlook when designing and evaluating lanthanide-based molecular probes. Selectivity toward orthophosphate should always be determined when evaluating probes targeting organic phosphates and nucleic acids. Selectivity toward other anions should be investigated at relevant conditions; depending on the intended applications, this is often at either the intracellular or blood levels. The mechanism of action, and in particular the nature of the probe-phosphate product, should not be assumed solely based on a change in luminescence intensity. More thorough solution studies, including luminescence lifetime and NMR, are needed to truly assess the formation of the adduct. The luminescent properties of each probe, including quantum yields and extinction coefficient, which determine brightness, are important parameters that also affect the translational potential of probes. Yet, those values are seldom reported. We thus encourage the reader to consider and optimize them in the design of future probes.

Phosphates are excellent chelators for lanthanides, readily capable of displacing a weakly coordinating ligand. As such, the stability of the complex should always be taken into consideration in the design of such probes. Weak ligands of low denticity are ill-advised as they are almost always displaced. On the same note, obtaining a crystal structure of a complex, often from organic solutions, does not necessarily guarantee that the complex will stay intact in solution. Kinetics of phosphate binding are rarely reported, but they offer much insight into the behavior of such probes. We have found, for instance, that phosphate coordination to Eu\(^{2+}\) can take as long as 15 min to reach equilibrium and form a Eu\(^{2+}\)-Pi\(_3\) adduct. Those kinetic studies should be performed before titrations so as to ensure that the latter are measured at thermodynamic equilibrium. It is

Figure 16. Lanthanide complexes as phosphodiesterase mimics.

Chem Asian J. 2022, 17, e202200495 (21 of 23) © 2022 The Authors. Chemistry – An Asian Journal published by Wiley-VCH GmbH
always preferable to achieve statistical significance by repeating each experiment at least three times. Taking the above points into consideration will help the investigator establish a more accurate picture of the true potential and mode of action of their probes.

**Acknowledgements**

The authors thank the support of the National Institutes of Health provided by R01 DK124333-01A1 and National Science Foundation provided by CHE-2203624.

**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords:** lanthanide · phosphate · luminescent · ATP · DNA

[1] Y. Marcus, J. Chem. Soc. Faraday Trans. 1991, 97, 2995–2999.
[2] a) Y. Liu, A. Sengupta, K. Raghavachari, A. H. Flood, Chem. 2017, 3, 411–427; b) P. S. Cremer, A. H. Flood, C. B. Gibson, D. L. Mobley, Nat. Chem. 2018, 10, 8–16; c) A. E. Hargrove, S. Nieto, T. Zang, J. L. Sessler, E. V. Anslyn, Chem. Rev. 2013, 113, 6603–6682.
[3] K. P. Kepp, Inorg. Chem. 2016, 55, 9461–9470.
[4] M. V. Ramakrishnan Raju, R. K. Wilharm, M. J. Dresel, M. E. McGreel, J. P. Mansergh, S. T. Martin, J. D. Goodpasture, V. C. Pierre, Inorg. Chem. 2019, 58, 15189–15201.
[5] a) C.-X. Zhao, X.-P. Zhang, Y. Shu, J.-H. Wang, ACS Appl. Mater. Interfaces 2012, 4, 22593–22600; b) T. Gori, W. Schmitt, T. Gunnlaugsson, Dalton Trans. 2021, 50, 770–784; c) P. Chandra Rao, S. Mandal, Inorg. Chem. 2018, 57, 11855–11858; d) P. Zhao, Y. Liu, C. He, C. Duan, Inorg. Chem. 2022, 61, 3132–3140.
[6] a) J. Masse, S. J. Quinn, T. Gunnlaugsson, J. Am. Chem. Soc. 2008, 130, 6900–6901; b) W. Miao, L. Wang, Q. Liu, S. Guo, L. Zhao, J. Peng, Asian J. Chem. 2021, 16, 247–251; c) S. Comby, E. M. Sureneder, O. Kotova, L. K. Truman, J. K. Molloy, T. Gunnlaugsson, Inorg. Chem. 2014, 53, 1867–1879; d) H.-H. Zeng, F. Liu, Z.-Q. Peng, K. Yu, L.-Q. Rong, Y. Wang, P. Wu, R.-P. Liang, J.-D. Qiu, ACS Appl. Nano Mater. 2020, 3, 2336–2345.
[7] a) M. Wang, Z. Liu, X. Zhou, H. Xiao, Y. You, W. Huang, Inorg. Chem. 2020, 59, 18027–18034; b) Y. Kitagawa, P. P. Ferreira da Rosa, Y. Hasegawa, Dalton Trans. 2021, 50, 14978–14984.
[8] a) J. Sahoo, C. Krishnani, J. Sun, B. B. Panda, P. S. Subramanian, H. S. Jena, Coord. Chem. Rev. 2022, 466, 214583; b) J.-C. G. Bünzli, Chem. Soc. Rev. 2005, 34, 1048–1077; c) J.-C. G. Bünzli, Coord. Chem. Rev. 2014, 53, 1867–1879; d) H.-H. Zeng, F. Liu, Z.-Q. Peng, K. Yu, L.-Q. Rong, Y. Wang, P. Wu, R.-P. Liang, J.-D. Qiu, ACS Appl. Nano Mater. 2020, 3, 2336–2345.
[9] a) M. Wang, Z. Liu, X. Zhou, H. Xiao, Y. You, W. Huang, Inorg. Chem. 2020, 59, 18027–18034; b) Y. Kitagawa, P. P. Ferreira da Rosa, Y. Hasegawa, Dalton Trans. 2021, 50, 14978–14984.
[10] a) C.-X. Zhao, X.-P. Zhang, Y. Shu, J.-H. Wang, ACS Appl. Mater. Interfaces 2012, 4, 22593–22600; b) T. Gori, W. Schmitt, T. Gunnlaugsson, Dalton Trans. 2021, 50, 770–784; c) P. Chandra Rao, S. Mandal, Inorg. Chem. 2018, 57, 11855–11858; d) P. Zhao, Y. Liu, C. He, C. Duan, Inorg. Chem. 2022, 61, 3132–3140.
[11] a) J. Masse, S. J. Quinn, T. Gunnlaugsson, J. Am. Chem. Soc. 2008, 130, 6900–6901; b) W. Miao, L. Wang, Q. Liu, S. Guo, L. Zhao, J. Peng, Asian J. Chem. 2021, 16, 247–251; c) S. Comby, E. M. Sureneder, O. Kotova, L. K. Truman, J. K. Molloy, T. Gunnlaugsson, Inorg. Chem. 2014, 53, 1867–1879; d) H.-H. Zeng, F. Liu, Z.-Q. Peng, K. Yu, L.-Q. Rong, Y. Wang, P. Wu, R.-P. Liang, J.-D. Qiu, ACS Appl. Nano Mater. 2020, 3, 2336–2345.
[12] D. L. Dexter, J. Chem. Phys. 1953, 21, 836–850.
[13] M. W. Mara, D. S. Tatum, A.-M. March, G. Doumy, E. G. Moore, K. N. Raymond, J. Am. Chem. Soc. 2019, 141, 11071–11081.
[14] T. Förster, Chem. Phys. Lett. 1971, 12, 422–424.
