Supporting Information

**Americium preferred: Lanmodulin, a natural lanthanide-binding protein favors an actinide over lanthanides**

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**Abstract:** The separation and recycling of lanthanides is an active area of research with a growing demand that calls for more environmentally friendly lanthanide sources. Likewise, the efficient and industrial separation of lanthanides from the minor actinides (Np, Am - Fm) is one of the key questions for closing the nuclear fuel cycle; reducing costs and increasing safety. With the advent of the field of lanthanide-dependent bacterial metabolism, bio-inspired applications are in reach. Here, we utilize the natural lanthanide chelator Lanmodulin and the luminescent probes Eu³⁺ and Cm³⁺ to investigate the competitive binding behavior of all lanthanides (except Pr) and four actinides (Np, Pu, Am, Cm) to Lanmodulin. Using time-resolved laser-induced fluorescence spectroscopy we show that Lanmodulin has the highest relative binding affinity to Nd³⁺ and Eu³⁺ among the lanthanide series. When equimolar mixtures of Cm³⁺ and Am³⁺ are added to Lanmodulin, Lanmodulin preferentially binds to Am³⁺ over Cm³⁺ whilst Nd³⁺ and Cm³⁺ bind with similar relative affinity. The results presented show that a natural lanthanide-binding protein can bind various actinides with high relative affinity, paving the way to bio-inspired separation applications. In addition, an easy and versatile method was developed, using the fluorescence properties of only two elements, Eu and Cm, for inter-metal competition studies regarding lanthanides and selected actinides and their binding to biological molecules.
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1. Experimental Procedures

1.1. Molecular cloning, expression and protein purification of LanM

A synthetic gene coding for LanM from *M. extorquens* without its N-terminal signal sequence (amino acids 1-21) was ordered from TWIST Bioscience (USA) and subcloned into a modified pET29b expression vector coding for a C-terminal Strep-tag. This tag was used to avoid possible metal binding, as it would be the case for a His-tag. The amino acid sequence, molecular weight MW, and extinction coefficient ε280 of the construct are listed below:

\[
\text{MW} = 13.1 \text{ kDa}, \text{ε}_{280} = 6990 \text{ M}^{-1} \text{ cm}^{-1}
\]

The protein was produced in *E. coli* BL21 Gold in an overnight expression at 18 °C after induction with 0.5 mM IPTG. Cells were harvested by centrifugation and lysed by sonication in a lysis buffer containing 1.0 mg/ml lysozyme. Cleared cell lysate was loaded on a Strep-Tactin® affinity column (IBA, Germany). Protein purification was performed in the presence of EDTA following the standard protocol of the supplier: wash buffer (40 mM HEPES pH 8.0, 150 mM NaCl, 1 mM EDTA), elution buffer (40 mM HEPES pH 8.0, 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin). The elution fraction was dialyzed twice against sample buffer (10 mM Tris/HCl pH 6.8, 100 mM KCl). The protein was concentrated in an Amicon® centrifugal device to a final volume of 9.5 mL.

The elution buffer (40 mM HEPES pH 8.0, 150 mM NaCl, 1 mM EDTA) was used in all affinity chromatography steps. A 6 mM Pu(IV) stock solution (without Am) in 0.1 M HCl was used. Pu(IV) was converted from HClO₄ to HCl by electrochemical reduction. The potential was set to −0.6 V for Pu(IV) production. After approximately 120 minutes, all Pu(IV) was reduced to Pu(III). The pure Pu(III) solution was obtained as a result. The vial with the solution was wrapped with parafilm and stored inside an inert gas glovebox for one week before commencing with the preparation of a Pu(III) stock solution at pH 4.275 mL of this solution was diluted with 6.75 mL of MilliQ water to a total volume of 9.5 mL. The initial concentration of the Pu(III) stock solution for titration to a pH of approximately 4 was therefore 3.6 mM. The final pH adjustment to pH 3.95 take place by the addition of aliquots of NaOH under a constant voltage of 0.6 V for Pu(III) production. After approximately 120 minutes, all Pu(IV) was reduced to Pu(III). The pure Pu(III) solution was obtained as a result. The vial with the solution was wrapped with parafilm and stored inside an inert gas glovebox for one week before commencing with the preparation of a Pu(III) stock solution at pH 4.275 mL of this solution was diluted with 6.75 mL of MilliQ water to a total volume of 9.5 mL. The initial concentration of the Pu(III) stock solution for titration to a pH of approximately 4 was therefore 3.6 mM. The final pH adjustment to pH 3.95 take place by the addition of aliquots of NaOH under a constant voltage of 0.6 V for Pu(IV) production. After approximately 120 minutes, all Pu(IV) was reduced to Pu(III). The pure Pu(III) solution was obtained as a result. The vial with the solution was wrapped with parafilm and stored inside an inert gas glovebox for one week before commencing with the preparation of a Pu(III) stock solution at pH 4.275 mL of this solution was diluted with 6.75 mL of MilliQ water to a total volume of 9.5 mL. The initial concentration of the Pu(III) stock solution for titration to a pH of approximately 4 was therefore 3.6 mM. The final pH adjustment to pH 3.95 take place by the addition of aliquots of NaOH under a constant voltage of 0.6 V for Pu(IV) production. After approximately 120 minutes, all Pu(IV) was reduced to Pu(III). The pure Pu(III) solution was obtained as a result.

1.2. Preparation of An and Ln working solutions

1.2.1. Actinide solutions

\[ \text{^{237}Np} \]

\[ 5.0 \text{ mg NpCl}_4(\text{dme})_2 \text{ (dme = 1,2-dimethoxyethane)} \text{ was dissolved in 1 mL tetrahydrofuran. The solution was added to an excess (i.e. 10 mg) of potassium graphite (KClO}_4 \text{ and stirred for 30 minutes showing a color change from pink to yellow-green. The potassium graphite was separated from the solution by centrifugation and the supernatant was evaporated until dryness. The residue was dissolved in 1 mL dimethyl sulfoxide (DMSO) yielding a greenish solution. This stock solution (c = 9mM) was hence diluted (first: 1:100 v/v, second 1:3 v/v) to yield a solution of c = 30μM Np}^{3+} \text{ in DMSO.} \]

\[ \text{^{242}Pu (92%)} / \text{^{243}Pu (8%)} \]

A 6 mM Pu(IV) stock solution (without Am) in 0.1 M HCl was used to produce a Pu(III) by electrochemical reduction. The potential was set to −0.6 V for Pu(III) production. After approximately 120 minutes, all Pu(IV) was reduced to Pu(III). The pure Pu(III) solution was obtained as a result. The vial with the solution was wrapped with parafilm and stored inside an inert gas glovebox for one week before commencing with the preparation of a Pu(III) stock solution at pH 4.275 mL of this solution was diluted with 6.75 mL of MilliQ water to a total volume of 9.5 mL. The initial concentration of the Pu(III) stock solution for titration to a pH of approximately 4 was therefore 3.6 mM. The final pH adjustment to pH 3.95 take place by the addition of aliquots of NaOH under a constant voltage of -0.4 V to prevent the formation of Pu(V) polymers or colloids during the NaOH addition process. The final concentration of the Pu(III) solution was analyzed with liquid scintillation counting (LSC) and determined with 3.4 mM.

\[ \text{^{243}Am (100%)} \]

Am(III) stock solution (26 μM) in 0.001 M HCl was used.

\[ \text{^{246}Cm (97.3%)} / \text{^{246}Cm (2.6%)} / \text{^{247}Cm (0.04%)} / \text{^{247}Cm (0.02%)} / \text{^{248}Cm (0.009%)} \]

A 7.3 mM stock solution of the long-lived curium isotopes in 1.0 M HClO₄ was used. In a first step the background electrolyte was converted from HClO₄ to HCl. To 500 μl of the perchlorate solution were added 1.5 ml 5 M NaOH raise the pH clearly in the alkaline region. Afterwards the solution was centrifuged (10 min with 13000 rpm) and the supernatant was castaway. The solid residual was dissolved in 500 μl 1 M HCl and the conversion will be repeat four times. The final concentration of the Cm(III) solution in 1 M HCl was analyzed with LSC and determined with 7 mM.

1.2.2. Lanthanide solutions

MPTTTTVKDIADPDKDGTDKLKEALAAQSAAFDKLDLPDKGTDKLDAKGRVSEADLKKLDNDGTIDARELASPAGSLVNLIRGSAWSHPQFEK

Molecular cloning, expression and protein purification of LanM
All lanthanides Ln$^{3+}$ were available as LnCl$_3$ hydrates. 10 mM stocks were prepared in MilliQ water and stored at – 20 °C until they were used.

1.3. Binding studies with TRLFS

Important: When working with actinides, special safety precautions need to be considered. All measurements were carried out in a radioactively controlled environment in compliance with regulations.

To study the binding of different lanthanides and/or actinides, fluorescence properties of Eu$^{3+}$ and Cm$^{3+}$ were used. All measurements were performed in a 2 mL quartz glass cuvette, and temperature was adjusted to 25 °C in the temperature-controlled cuvette holder. For sufficient and fast mixing, stirring speed of 1200 rpm was set. An excitation wavelength of 394 nm was chosen for binding studies regarding Eu$^{3+}$, for Cm$^{3+}$ a wavelength of 396 nm was applied (Ekspla, NT230, ~5 ns pulse). The cuvette holder was connected to the spectrograph (Andor, SR-303i-A) via a light guide. Spectra were recorded with an ICCD (Andor (Star), DH320T-18U-63). Data deconvolution was performed with PARAFAC (N-way toolbox for Matlab)$^{[2]}$ and is summarized in Figure S1.

All experiments were performed in a TRIS-KCl buffer (10 mM TRIS, 100 mM KCl, pH 6.7). This pH was chosen to mimic physiological conditions. With higher pH, most probably the amount of available Ln aquo ions might decrease due to hydrolysis. Ln solutions were available in 10 mM stocks in MilliQ water and were diluted to 1 mM in the buffer if required. Available An stock solutions (in detail described above) were adjusted to 30 µM with the buffer. For Cm$^{3+}$ binding studies, a 5 µM solution of LanM in buffer was prepared while for studies with Eu$^{3+}$ the frozen stocks with concentration of 181 µM were used. When working with An, a final concentration of 100 nM of Cm$^{3+}$ was not exceeded which was found to be a good compromise between sensitivity and radioactivity. For fluorescence studies with Eu$^{3+}$, an amount of 5.5 µM was used during the measurements.

Different experiments were carried out to shed light on various issues and will be described in the following. For competitive binding studies, different combinations of Ln and An were chosen.

Ln series: La$^{3+}$/Eu$^{3+}$, Ce$^{3+}$/Eu$^{3+}$, Pr$^{3+}$/Eu$^{3+}$, Nd$^{3+}$/Eu$^{3+}$, Sm$^{3+}$/Eu$^{3+}$, Gd$^{3+}$/Eu$^{3+}$, Tb$^{3+}$/Eu$^{3+}$, Dy$^{3+}$/Eu$^{3+}$, Ho$^{3+}$/Eu$^{3+}$, Er$^{3+}$/Eu$^{3+}$, Tb$^{3+}$/Eu$^{3+}$, Lu$^{3+}$/Eu$^{3+}$

An series: Np/Cm$^{3+}$, Pu/Cm$^{3+}$, Am$^{3+}$/Cm$^{3+}$, Nd$^{3+}$/Cm$^{3+}$

![Figure S1. The emission spectrum of Eu$^{3+}$ consists of several electronic transitions. Its main transitions are to the F levels of the excited term D$_0$ with j=0,…,6. Often transitions to the F$_2$ and F$_3$ transitions are not observable. Therefore, the graph shows only transitions up to j=4. The F$_j$ level can split into a set of crystal field levels. The maximum number of crystal field induced split levels is given by 2j+1 and depends on the symmetry of the crystal field. This splitting is responsible for the unsymmetrical shape of individual D$_0$ → F$_j$ transitions.](image)

1.3.1. Eu$^{3+}$/Cm$^{3+}$ titration to LanM

To see if the purified LanM is able to bind Eu$^{3+}$ or Cm$^{3+}$, initial binding studies were performed to determine the exact amount of Eu$^{3+}$ or Cm$^{3+}$ which could be bound by LanM.

The protein LanM was diluted to a concentration of 1 µM in the buffer in a total volume of 1000 µL. A 1 mM Eu$^{3+}$ solution was added in 0.5 and 1 µL steps until a concentration of 10 µM was reached. TRLFS parameters for the measurement were exponential step size 0.5 µM, initial delay 275 µs, gate width 100 µs, accumulations 200, kinetic series length 25, gain 4000, 600 µm slit width, grating 300 l/mm.

A 100 nM solution of Cm$^{3+}$ in buffer. In total, 20 titration steps were performed to reach a concentration of 57 nM LanM. TRLFS parameters for the measurement were linear increasing step size 0.05 µM, initial delay 250 µs, gate width 100 µs, accumulations 200, kinetic series length 25, gain 4000, 600 µm slit width, grating 600 l/mm.

1.3.2. A to Eu$^{3+}$-LanM or Cm$^{3+}$-LanM – Ln/An addition

In addition, it was tested if different subsequently added Lns or An$^{3+}$ have an impact on LanM which has prior bound to Eu$^{3+}$ (Ln series) or Cm$^{3+}$ (An series).
For experiments regarding the Ln series, 1 µM LanM was incubated with 5.5 µM Eu³⁺ for 10 minutes at room temperature with constant stirring. Then, 5.5 µM of Ln was added and immediately after the addition the data acquisition was started. The measurements were initially carried out in short intervals, with ongoing time the intervals were increased. Spectra were collected until no more changes occurred. TRLFS parameters for the measurement were exponential step size 5·e⁻¹º, initial delay 288 µs, gate width 200 µs, accumulations 200, kinetic series length 5, gain 400, 100 µm slit width, grating 300 l/mm.

The procedure for the An series was carried out in the same way but different concentration of An or Nd³⁺ and protein was used. 100 nM Cm³⁺ and the additional An or Nd was mixed in buffer. Shortly after addition of 18 nM LanM, the spectra collection was started. TRLFS parameters for the measurement were exponential step size 20·e⁻⁰·²⁷º, initial delay 275 µs, gate width 600 µs, accumulations 200, kinetic series length 5, gain 4000, 600 µm slit width, grating 1200 l/mm.

1.3.3. A/Eu³⁺ to LanM or A/Cm³⁺ to LanM – Ln/An competition

Focusing the differences of preferred Ln binding of LanM, 5.5. µM of Eu³⁺ and 5.5 µM of Ln (see Ln series) were added to the cuvette in buffer. Then 1 µM of LanM was added, and the mix was incubated for 24 hours at room temperature. Spectra were collected with parameters of linear increasing step size 3·e⁻¹º, initial delay 288 µs, gate width 500 µS, accumulations 200, kinetic series length 25, gain 4000, input slit 100 µM, grating 300l/mm. Spectra were collected before addition of LanM, shortly after addition of the protein, and after 24 hours incubation period.

For experiments regarding the An series or Ln/An series, 100 nM of Cm³⁺ and 100 nM of Ans or Nd³⁺ (see An series) in the cuvette with buffer. Subsequently, 18 nM of LanM were added and it was incubated for 24 hours at room temperature. TRLFS parameters for the measurement were linear increasing step size 3·e⁻¹º, initial delay 275 µs, gate width 600 µs, accumulations 200, kinetic series length 25, gain 400, 600 µm slit width, grating 1200 l/mm. Spectra were collected before addition of LanM, shortly after addition of the protein, and after 24 hours incubation period.

1.4. Binding studies with CD spectroscopy

CD spectra were collected using a JASCO J-810 CD and ORD spectropolarimeter with thermostat controlled cell holder in combination with software Spectra Manager Version 2.06.00.

Experiments to study a possible conformational change of apo-LanM and Eu³⁺were carried out with frozen LanM stocks without further purification or washing procedure in a macro cuvette (QS) with 1 mm pathlength at 25 °C. For the stoichiometric titration with Eu³⁺, 20 µM of apo-LanM were diluted in TRIS-KCl buffer (10 mM TRIS, 100 mM KCl, pH 6.7) to a final volume of 200 µL. The Eu³⁺ stock solution (10 mM) was prepared as described in 1.2.2.. Each equivalent of metal corresponded to a volume of 0.4 µL of the Eu³⁺ stock. Collection of spectra was started 2 minutes after metal addition. Spectra were scanned from 180 – 300 nm with 1 nm bandwidth, 0.5 nm data pitch, 50 nm/min scan rate, D.I.T. 4 seconds. Three accumulations were acquired, and spectra were baseline corrected with a previously collected buffer sample. Due to small volume changes, no correction of concentration was applied.

![Figure S1. SDS-PAGE of the purified protein LanM.](image-url)
2. Results and Discussion

Figure S2. Previously reported affinity constants for Ln and An of Ln-binding systems LBT[3] and LaMP1[4] or proteins which were described to bind Lns LanM,[5] TFD-EE N6W,[6] Siderocalin,[7] Transferrin,[8] Measurements regarding the LBT were performed with fluorescence spectroscopy at pH 7.0 in 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer and 100 mM NaCl. K_d of LaMP1 was obtained by FRET ratios with EDDS-buffered metal solutions at pH 7.2 in 30 mM MOPS and 100 mM KCl. For LanM, K_d was determined with UV-Vis spectrophotometry at pH 5 in 100 mM KCl/K-acetate buffer. Studies regarding Siderocalin were done with fluorescence quenching analysis at pH 7.4 in 100 mM HEPES buffer. Pu was provided for the experiments in oxidation state +IV. Binding constants of Transferrin were obtained with HPLC measurements in NH4HCO3 buffer at pH 7.4. TFD-EE N6W was studied with EGTA-buffered solutions in 25 mM HEPES and 100 mM NaCl at pH 7.5 in combination with sensitized luminescence.

Figure S3. CD-spectra of 20 µM apo-LanM with addition of different equivalents of Eu^{3+}. 
Table S1. Intensities of Eu$^{3+}$-LanM and Cm$^{3+}$-LanM signal of figure 4 and 6 and calculated time constants of figure 3C and 5C.

| Element | Eu$^{3+}$-LanM signal (%) | Cm$^{3+}$-LanM signal (%) | Time constants (s) |
|---------|---------------------------|---------------------------|-------------------|
| La$^{3+}$ | 69.71                     | -                         | 1222.00           |
| Ce$^{3+}$ | 62.43                     | -                         | 1094.30           |
| Pr$^{3+}$ | 58.92                     | -                         | 811.48            |
| Nd$^{3+}$ | 49.73                     | 51.77                     | 986.60 (Eu) / 1334.80 (Cm) |
| Pm$^{3+}$ | -                         | -                         | -                 |
| Sm$^{3+}$ | 60.38                     | -                         | 767.52            |
| Gd$^{3+}$ | 67.35                     | -                         | 648.95            |
| Tb$^{3+}$ | 68.31                     | -                         | 692.92            |
| Dy$^{3+}$ | 78.16                     | -                         | 647.12            |
| Ho$^{3+}$ | 76.49                     | -                         | 483.83            |
| Er$^{3+}$ | 80.93                     | -                         | 482.49            |
| Tm$^{3+}$ | 81.19                     | -                         | 378.40            |
| Yb$^{3+}$ | 82.14                     | -                         | 453.53            |
| Lu$^{3+}$ | 89.51                     | -                         | 167.80            |
| Np     | -                         | 76.50                     | 426.15            |
| Pu     | -                         | 69.02                     | 416.87            |
| Am$^{3+}$ | -                         | 42.10                     | 1461.80           |

Table S2. Ionic radii of Ln and An and their oxidation states$^{[b]}$

| Element | Ionic radius (pm)$^{[a]}$ | Oxidation states$^{[b]}$ |
|---------|---------------------------|--------------------------|
| La      | 103.2                     | + III                    |
| Ce      | 101.0                     | + III, + IV              |
| Pr      | 99.0                      | + III, + IV              |
| Nd      | 98.3                      | + III                    |
| Pm      | 97.0                      | + III                    |
| Sm      | 95.8                      | + II, + III              |
| Eu      | 94.7                      | + II, + III              |
| Gd      | 93.8                      | + III                    |
| Tb      | 92.3                      | + III, + IV              |
| Dy      | 91.2                      | + III                    |
| Ho      | 90.1                      | + III                    |
| Er      | 89.0                      | + III                    |
| Tm      | 88.0                      | + III                    |
| Yb      | 86.8                      | + II, + III              |
| Lu      | 86.1                      | + III                    |
| Np      | 101.1                     | + III, + IV, +V, +VI, +VII |
| Pu      | 99.5                      | + III, + IV, +V, +VI, +VII |
| Am      | 98.0                      | + III, + IV, +V, +VI     |
| Cm      | 97.0                      | + III, + IV              |

[a] Ionic radii for +III oxidation state [b] in bold the most prevalent one, not well characterized or solid-state oxidation states were not included
The redox chemistry of Ans differs completely from the Lns. While Am and Cm only reveal a few oxidation states, Np and Pu have multitudes which also coexist. Factors like pH or the presence of reducing or oxidizing reagents as well as complexing agents and the concentration of the element itself play an important role. For a better overview, relevant notes on the oxidations important for the discussion are given.

### Table S3. Notes on the versatile redox chemistry of the actinides\(^{[10]}\)

| Element | Oxidation state | Ionic radius (pm)\(^{[a]}\) | Note |
|---------|----------------|-----------------------------|------|
| Np      | III            | 101.1                       | Np\(^{3+}\); stable in aqueous acidic solution but gets easily oxidized in the presence of air to Np\(^{5+}\) and NpO\(^{2+}\) |
| Np      | IV             | 87.4                        | Np\(^{4+}\); stable in aqueous solution but slow oxidation to NpO\(^{2+}\) was observed with air, in low acidic environment also hydrolysis was described |
| Np      | V              | n.a.                        | Dioxo species NpO\(^{2+}\); most stable form in aqueous solution, disproportionation 2 NpO\(^{2+}\) + 4 H\(^{+}\) = Np\(^{4+}\) + NpO\(^{4+}\) + 2 H\(_2\)O only described at high concentrations or at strongly acidic environment, formation of hydroxides in neutral and basic environment |
| Np      | VI             | n.a.                        | Dioxo species NpO\(^{2+}\); mainly present at acid pH but reduction to NpO\(^{2+}\) was described, formation of hydroxides in neutral and basic environment |
| Pu      | III            | 99.5                        | Pu\(^{3+}\); stable in aqueous acidic solution and on air but is easily oxidized to Pu\(^{4+}\) |
| Pu      | IV             | 85.9                        | Pu\(^{4+}\); only stable in highly acidic environment, with lower acidic conditions a disproportionation to Pu\(^{3+}\) and PuO\(^{2+}\) was described as well as the formation of colloidal hydrolysis products |
| Pu      | V              | n.a.                        | Dioxo species PuO\(^{2+}\), is unstable and disproportion to Pu\(^{4+}\) and PuO\(^{2+}\) was described, in aqueous solutions the formation of Pu\(^{3+}\) and PuO\(^{2+}\) was observed |
| Pu      | VI             | n.a.                        | Dioxo species PuO\(^{2+}\); reduction to Pu\(^{4+}\) in acidic medium was described |
| Am      | III            | 98.0                        | Stable and not easy to oxidize |
| Am      | IV             | 84.8                        | Fluorides or carbonates in aqueous solution |
| Cm      | III            | 97.0                        | Stable |
| Cm      | IV             | 84.1                        | Fluorides CmF\(_4\) in aqueous solution, CmO\(_2\); ion stable in aqueous solutions with highly complexing agents |

\(^{[a]}\) n.a. = not available

### Table S4. Published dissociation constants \(K_d\) for Ln and LanM\(^{[1, 5a, 6b]}\)

Please note that the different \(K_d\) were determined with various methods which explains the strong deviations. Also, intrinsic and apparent \(K_d\) are listed here.

| Average intrinsic \(K_d^{[5a]}\) | Apparent \(K_d^{[3, 4]}\) | Apparent \(K_d^{[4]}\) |
|-------------------------------|------------------------|----------------------|
| 3 La\(^{3+}\) + LanM = La\(_3\)LanM | n.d.\(^{[3]}\) | n.d. |
| 3 Pr\(^{3+}\) + LanM = Pr\(_3\)LanM | 11 ± 1 pM | 70 ± 10 pM |
| 3 Nd\(^{3+}\) + LanM = Nd\(_3\)LanM | 22 ± 2 pM | n.d. |
| 3 Sm\(^{3+}\) + LanM = Sm\(_3\)LanM | 24 ± 6 pM | n.d. |
| 3 Gd\(^{3+}\) + LanM = Gd\(_3\)LanM | n.d. | 100 ± 10 pM |
| 3 Tb\(^{3+}\) + LanM = Tb\(_3\)LanM | n.d. | n.d. |
| 3 Dy\(^{3+}\) + LanM = Dy\(_3\)LanM | 15 ± 4 pM | 200 ± 50 pM |
| 3 Ho\(^{3+}\) + LanM = Ho\(_3\)LanM | 8.6 ± 1.4 pM | 260 ± 60 pM |
| 3 Er\(^{3+}\) + LanM = Er\(_3\)LanM | 1.4 ± 0.2 pM | n.d. |
| 3 Tm\(^{3+}\) + LanM = Tm\(_3\)LanM | 0.41 ± 0.13 pM | n.d. |

\(^{[a]}\) n.d. = not determined \(^{[b]}\) determined with UV-Vis spectrophotometry at pH 5 in 100 mM KCl/K-acetate buffer \(^{[c]}\) determined with CD spectroscopy in combination with chelator-buffered solutions at pH 5, buffer with 20 mM acetate and 100 mM KCl \(^{[d]}\) determined with CD spectroscopy at pH 7.0 in buffer 20 mM MOPS, 20 mM acetate and 100 mM KCl
Supporting References

[1] J. A. Cotruvo, E. R. Featherston, J. A. Mattocks, J. V. Ho, T. N. Laremore, J. Am. Chem. Soc. 2018, 140, 15056-15061.

[2] C. Andersson, R. Bro, Chemom. Intell. Lab. Syst. 2000, 52, 1-4.

[3] M. Nitz, M. Sherawat, K. J. Franz, E. Pelsach, K. N. Allen, B. Imperiali, Angew. Chem. Int. Ed. 2004, 43, 3682-3685.

[4] J. A. Mattocks, J. V. Ho, J. A. Cotruvo, J. Am. Chem. Soc. 2019, 141, 2857-2861.

[5] a) G. J. P. Deblonde, J. A. Mattocks, D. M. Park, D. W. Reed, J. A. Cotruvo, Y. Jiao, Inorg. Chem. 2020, 59, 11855–11867; b) G. Deblonde, J. A. Mattocks, Z. Dong, P. T. Wooddy, J. A. J. Cotruvo, M. Zavarin, ChemRxiv 2021, DOI 10.26434/chemrxiv.14763426.v1. This content is a preprint and has not been peer-reviewed.

[6] a) S. J. Caldwell, I. C. Haydon, N. Piperidou, P.-S. Huang, M. J. Bick, H. S. Sjöström, D. Hilvert, D. Baker, C. Zeymer, Proc. Natl. Acad. Sci. U.S.A. 2020, 117, 30362; b) J. A. Mattocks, J. L. Tirsch, J. A. Cotruvo, in Methods Enzymol., Vol. 651 (Ed.: J. A. Cotruvo), Academic Press, 2021, 23-61.

[7] B. E. Allred, P. B. Rupert, S. S. Gauny, D. D. An, C. Y. Ralston, M. Sturzbecher-Hoehne, R. K. Strong, R. J. Abergel, Proc. Natl. Acad. Sci. U.S.A. 2015, 112, 10342-10347.

[8] G. J. P. Deblonde, M. Sturzbecher-Hoehne, A. B. Mason, R. J. Abergel, Metallomics 2013, 5, 619-626.

[9] G. T. Seaborg, D. E. Hobart, Summary of the properties of the lanthanide and actinide elements, Indian Association of Nuclear Chemists and Allied Scientists, India, 1996.

[10] L. R. Morss, N. M. Edelstein, J. Fuger, J. J. Katz, L. Morss, The chemistry of the actinide and transactinide elements, Vol. 1, Springer, 2006.