Cation–π Interactions

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Lanthanide Luminescence Modulation by Cation–π Interaction in a Bioinspired Scaffold: Selective Detection of Copper(I)

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Abstract: *A prototype luminescent turn-on probe for Cu^+ (and Ag^+) is described, harnessing a selective binding site ($\log K_{ass}$ = 9.4 and 7.3 for Cu^+ and Ag^+, respectively) based on the coordinating environment of the bacterial metallo-chaperone CusF, integrated with a terbium-ion-signaling moiety. Cation–π interactions were shown to enhance tryptophan triplet population, which subsequently sensitized, on the microsecond timescale, the long-lived terbium emission, offering a novel approach in bioinspired chemosensor design.*

Copper is an essential element for life.[1] It is required for various biological processes and its homeostasis is finely regulated in living organisms.[2] Misregulation of copper can lead to various diseases (e.g., Menkes, Wilson, and Parkinson diseases).[3] To better understand the biology of copper, techniques are required to detect and quantify it, knowing that extracellular copper is in the +II oxidation state, whereas mobile copper is in the reduced +I state in cells. Generally, fluorescence detection is considered to be one of the cheapest and easiest techniques.[4] However, the design of fluorescent probes for Cu^+ is more challenging than many other cations, such as Ca^{2+} or Zn^{2+}, because Cu^+ is an effective quencher of fluorescence through charge transfer and intersystem crossing (ISC) mechanisms.[5] As turn-on emission is preferred for detecting an analyte, Cu^+-selective fluorescent probes were designed in which the fluorophore is spatially disconnected from the chelate.[5,6] These probes rely on a photoinduced electron transfer (PET) mechanism in which the chelator, in its unbound form only, acts as an electron donor to the excited state of the fluorophore and quenches its emission.[5,6] In this communication, we report a new type of turn-on Cu^+-responsive probe based on a lanthanide ion (Ln^{3+}) emitter, that has a long luminescence lifetime (in the millisecond range) compared to classical organic fluorophores (nanosecond range) and that allows time-gated detection to suppress background fluorescence contributions.[7–10]

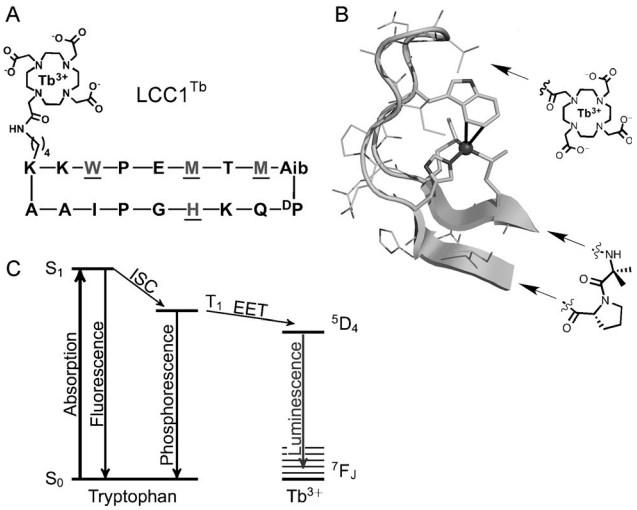

Figure 1. A) Amino acid sequence of $LCC1^{Tb}$, chelating moieties are underlined. B) Principle of the probe design based on the X-ray structure of the Cu^+ binding loop of CusF.[14] C) Simplified Jablonski–Perrin diagram of $LCC1^{Tb}$ probe and pertinent photophysical processes.

Our probe structure (Figure 1A) is inspired by the metal binding site of the metallo-chaperone CusF,[11] which is part of the CusCFBA system responsible for copper or silver detoxification in gram-negative bacteria.[12] CusF binds either Cu^+ or Ag^+ by the side chains of four amino acids: two methionines (M), a histidine (H), and a tryptophan (W) as shown in Figure 1B (right).[13,14] Indeed, the indole ring of the tryptophan establishes a cation–π interaction with the metal ion that red-shifts the π–π* transition of the indole and fully quenches its fluorescence.[14] Metal cation–π interactions are known to efficiently enhance ISC and increase the population of the excited triplet state of a fluorophore, thereby quenching the fluorescence.[15]

Ln^{3+} ions have desirable luminescence properties that make them prime candidates for biological applications.[8,9,16,17] Direct lanthanide excitation is inefficient because 4f–4f transitions are Laporte forbidden. However, indirect excitation of Ln^{3+} ions is possible in complexes incorporating a chromophore that, once excited, transfers its energy to the lanthanide (this photosensitization process has been deemed an antenna effect).[18] One of the main pathways for lanthanide sensitization involves electronic energy transfer (EET) from the excited triplet state of the antenna to the

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emissive Ln^{3+} ion (Figure 1C).[7,18] Among natural amino acids, tryptophan is an efficient antenna for Tb^{3+} sensitization.[19] Therefore, we designed a probe based, on the one hand, on a peptide mimicking the Cu^{+} binding site of CusF providing high affinity and selectivity and, on the other hand, on a Tb^{3+} complex as signaling unit. We reasoned that we could benefit from an ISC enhancement due to a cation–π interaction between Cu^{+} and the tryptophan to increase the population of the tryptophan excited triplet state and, subsequently, increase also the population of Tb^{3+} excited states to transduce the copper-binding event into an increased Tb^{3+} emission.

The peptidic probe, namely $LCC1^{Tb}$ (Figure 1A and B), comprises 1) the 16-amino-acid sequence of the Cu^{+} binding loop of CusF, which includes the four metal binding amino acids (see above), 2) an Aib-^{D}Pro dipeptide[20] to cyclize the loop and preorganize it, and 3) a DOTA macrocycle grafted on the amine side chain of a lysine to bind a Tb^{3+} ion. $LCC1^{Tb}$ was synthesized by a combination of solid-phase and solution reactions (Supporting Information, SI). The metal-binding properties of $LCC1^{Tb}$ were investigated under argon by circular dichroism (CD) spectroscopy (Figure 2). The titration of $LCC1^{Tb}$ in phosphate buffer (10 mM, pH 7.5) by Cu^{+}, generated in situ by reduction of Cu^{2+} by NH_2OH, shows a linear evolution of the CD signal which reaches a plateau in the presence of 1.0 equiv Cu^{+}, indicating the formation of a 1:1 complex, $Cu^{I}{\cdot}LCC1^{Tb}$, which was confirmed by ESI-MS analysis (SI). The same behavior is observed with Ag^{+} due to the similarity between these two ions. $LCC1^{Tb}$ is not able to bind any of the other physiologically relevant metal ions [Na^{+}, K^{+} (100 mM), Ca^{2+}, Mg^{2+} (10 mM), Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} (30 μM)] as demonstrated by the absence of change in the CD spectrum (Figure 2B). It is noteworthy that $LCC1^{Tb}$ can bind Cu^{+} but not Cu^{2+}.

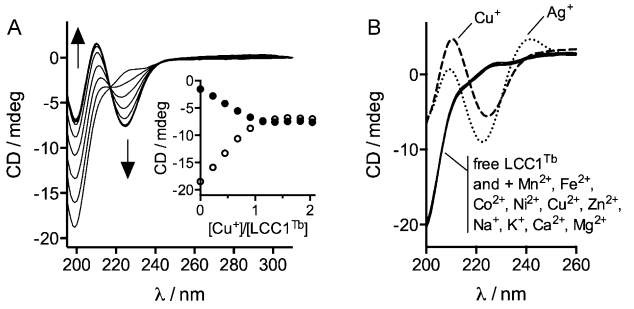

Figure 2. A) CD titration of $LCC1^{Tb}$ (16 μM) in phosphate buffer (10 mM, pH 7.5) by Cu^{+} generated in situ by reduction of $CuSO_4$ by NH_2OH (2 mM). The inset shows the evolution of the CD signal at 200 nm (○) and 225 nm (●). B) CD spectra of $LCC1^{Tb}$ (18 μM) before and after addition of various metal ions.

The coordination of Cu^{+} or Ag^{+} was further investigated by electronic absorption spectroscopy and photoluminescence to gain further insight into the establishment and effect of a cation–π interaction. Concerning the UV/Vis absorption and the fluorescence of tryptophan, the binding of Cu^{+} or Ag^{+} is associated with a red-shift of the indole π–π* transition absorption band (Figure 3A) and a partial quenching of its fluorescence (Figure 3D). This suggests the presence of a cation–π interaction in $Cu^{I}{\cdot}LCC1^{Tb}$ and $Ag^{I}{\cdot}LCC1^{Tb}$ as observed for CusF.

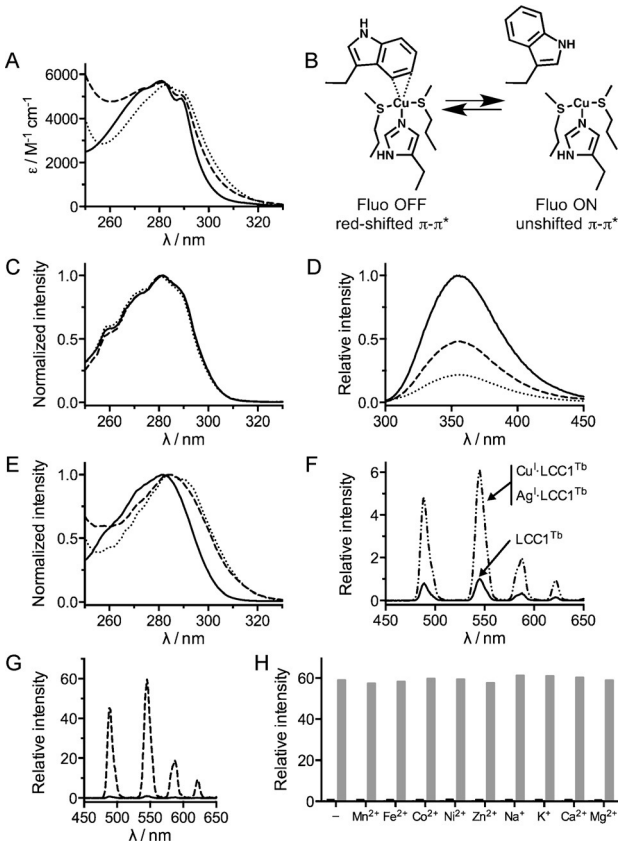

Figure 3. Steady-state spectroscopic characterization of $LCC1^{Tb}$ (solid line), $Cu^{I}{\cdot}LCC1^{Tb}$ (dashed line), and $Ag^{I}{\cdot}LCC1^{Tb}$ (dotted line). A) Electronic absorption spectra. B) Representation of possible fluorescent and non-fluorescent forms of tryptophan in $Cu^{I}{\cdot}LCC1^{Tb}$. C,D) Tryptophan fluorescence excitation (C, $\lambda_{em}=355$ nm) and emission (D, $\lambda_{ex}=280$ nm) spectra. E,F) Time-gated Tb^{3+} luminescence excitation (E, $\lambda_{em}=545$ nm) and emission (F, $\lambda_{ex}=280$ nm) spectra. G) Time-gated emission spectra with excitation at 310 nm. H) Selectivity diagram showing the time-gated Tb^{3+} emission at 545 nm ($\lambda_{ex}=310$ nm) of $LCC1^{Tb}$ (5 μM) before (black) and after (grey) addition of 1.5 equiv Cu^{+} in the presence of various cations (from left to right: none, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+} (10 μM), Na^{+}, K^{+} (100 mM), Ca^{2+}, and Mg^{2+} (10 mM)). Spectra were recorded in HEPES buffer (10 mM, pH 7.5) under argon.

The Tb^{3+} luminescence properties were investigated by exciting the tryptophan antenna at 280 nm, which corresponds to the maximum absorption of the tryptophan indole π–π* transition in $LCC1^{Tb}$. Titrations of $LCC1^{Tb}$ by Cu^{+} or Ag^{+} show that the formation of $Cu^{I}{\cdot}LCC1^{Tb}$ and $Ag^{I}{\cdot}LCC1^{Tb}$ is associated with an increase of the Tb^{3+} emission. The Tb^{3+} luminescence excitation spectra of $LCC1^{Tb}$, $Cu^{I}{\cdot}LCC1^{Tb}$, and $Ag^{I}{\cdot}LCC1^{Tb}$ (Figure 3E) correspond to the π–π* transition observed in the electronic absorption spectra, indicating that the tryptophan acts as an antenna for Tb^{3+} in $LCC1^{Tb}$ and its Cu^{+} or Ag^{+} complexes. Interestingly, the Tb^{3+} excitation spectra ($\lambda_{em}=545$ nm) of $Cu^{I}{\cdot}LCC1^{Tb}$ and $Ag^{I}{\cdot}LCC1^{Tb}$ are red-shifted compared to $LCC1^{Tb}$ (Figure 3E), but the tryptophan fluorescence excitation spectra ($\lambda_{em}=355$ nm) are not

(Figure 3C). This is consistent with two kinds of tryptophan indole that are present in solution when Cu^+ or Ag^+ are bound to $LCC1^{Tb}$: one corresponding to an indole that is fluorescent and has an unshifted π–π* transition and the other one corresponding to a non-fluorescent indole with a red-shifted π–π* transition and a higher Tb^{3+} luminescence. As the cation–π interaction in CusF totally quenches the tryptophan fluorescence, we can propose that two forms of the 1:1 complex co-exist in solution, one with the tryptophan indole establishing a cation–π interaction and the other not (Figure 3B). Figure 3F compares the time-gated Tb^{3+} emission spectra of $LCC1^{Tb}$, $Cu^{I}{\cdot}LCC1^{Tb}$, and $Ag^{I}{\cdot}LCC1^{Tb}$ with excitation at 280 nm. Cu^+ and Ag^+ enhance the Tb^{3+} emission six times with respect to $LCC1^{Tb}$ and thus, $LCC1^{Tb}$ acts as a turn-on luminescent probe for these cations. Moreover, the red-shift of the indole π–π* transition can be used to increase the contrast of the probe: Tb^{3+} luminescence enhancement factors of 58 and 52 were obtained for Cu^+ and Ag^+, respectively, by exciting the probe at 310 nm (see SI for rationalization of this wavelength choice) instead of 280 nm (Figure 3G). Furthermore, the Tb^{3+} emission of $LCC1^{Tb}$ and $Cu^{I}{\cdot}LCC1^{Tb}$ is not affected by the presence of physiological cations (Figure 3H). Overall, $LCC1^{Tb}$ is a high contrast turn-on luminescent probe for the time-gated detection of Cu^+ among physiological cations. It is also able to detect Ag^+. The binding constants for Cu^+ and Ag^+, determined by competition experiments with imidazole are $10^{9.4}\,M^{-1}$ and $10^{7.3}\,M^{-1}$, respectively (SI). The K_M for other physiological cations is estimated to be below $10^3\,M^{-1}$.

The enhancement of Tb^{3+} luminescence upon Cu^+ or Ag^+ binding may originate from 1) a reduction of the number of water molecules bound to Tb^{3+}, 2) a change in photophysical processes caused by the cation–π interaction, or 3) a conformational change, that is, a shortening of the distance between the antenna and the Tb^{3+} ion and/or a change in the orientation of the antenna with respect to Tb^{3+}. Concerning the latter point, changes in CD upon Cu^+ or Ag^+ binding may arise from conformational changes but also from the contribution of ligand–metal charge transfer transitions. The NMR spectra of $LCC1^{La}$, the diamagnetic homologous probe in which the Tb^{3+} ion is replaced by a La^{3+} ion, and of its Cu^+ or Ag^+ complexes display broad resonances that preclude any structural analysis, unfortunately. To elucidate the mechanism of the Tb^{3+} luminescence enhancement and quantify fast processes, the emission of the probe was characterized in detail. Regarding Tb^{3+} emission, Cu^+ or Ag^+ binding has almost no effect on the luminescence lifetime ($\tau \approx 1.9$ ms). Measurements of luminescence lifetime values in H_2O and D_2O additionally showed that only one water molecule is coordinated to the Tb^{3+} ion in $LCC1^{Tb}$ and its Cu^+ and Ag^+ complexes (SI).[7,18,21] Therefore, the enhancement of Tb^{3+} emission is not due to a change in the Tb^{3+} primary coordination sphere. Emission was further investigated at the ns and μs timescale by time-resolved emission spectroscopy with streak-camera detection. The fluorescence of $LCC1^{Tb}$ is characterized by a bi-exponential decay ($\tau_1 = 0.9$ ns and $\tau_2 = 4.8$ ns, Table 1), which is common for tryptophan.[22] The lifetimes of the fluorescence of $Cu^{I}{\cdot}LCC1^{Tb}$ and $Ag^{I}{\cdot}LCC1^{Tb}$, which accounts for the species with the indole not involved in a cation–π interaction, are similar. Emission on the μs timescale was investigated in a time-gated mode to eliminate the tryptophan fluorescence signal (SI).

Table 1: Decay lifetimes of tryptophan emission and rise time of Tb^{3+} emission for $LCC1^{Tb}$, $Cu^{I}{\cdot}LCC1^{Tb}$, and $Ag^{I}{\cdot}LCC1^{Tb}$. Error on τ values is estimated at 10%.

Compound	Tryptophan fluorescence decay (ns)	Tryptophan phosphorescence decay (μs)	Tb^{3+} luminescence rise (μs)
$LCC1^{Tb}$	0.9 (13%), 4.8 (87%)	not detected	23
$Cu^{I}{\cdot}LCC1^{Tb}$	0.7 (16%), 3.9 (84%)	16	16
$Ag^{I}{\cdot}LCC1^{Tb}$	0.9 (17%), 4.2 (83%)	19	18
$Cu^{I}{\cdot}LCC1^{La}$	–	18	–
$Ag^{I}{\cdot}LCC1^{La}$	–	20	–

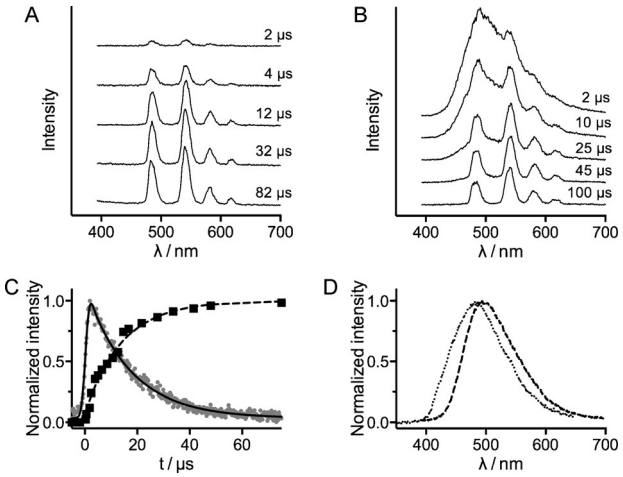

Figure 4. Time-resolved emission spectroscopy in degassed buffer solutions. Spectra were recorded in time-gated mode using a streak-camera ($\lambda_{ex} = 266$ nm). Time-resolved emission spectra of A) $LCC1^{Tb}$ and B) $Cu^{I}{\cdot}LCC1^{Tb}$ recorded several μs after the laser pulse (2 μs integration time). C) Evolution of the tryptophan phosphorescence emission at 440 nm (dots) and of the Tb^{3+} emission at 545 nm (square; the tryptophan phosphorescence has been subtracted) for $Cu^{I}{\cdot}LCC1^{Tb}$. The solid and dashed lines correspond to the respective fits which yielded $\tau = 16 \pm 2$ μs for both phosphorescence decay and Tb^{3+} emission grow-in. D) Phosphorescence emission spectra of $Cu^{I}{\cdot}LCC1^{La}$ (dashed line) and $Ag^{I}{\cdot}LCC1^{La}$ (dotted line).

Figures 4A and 4B compare the emission spectra of $LCC1^{Tb}$ and $Cu^{I}{\cdot}LCC1^{Tb}$ recorded several μs after the laser pulse ($\lambda_{ex} = 266$ nm, 2 μs integration time). For $LCC1^{Tb}$, the rise of Tb^{3+} luminescence is the only observed emission with a rise time of 23 μs. This rise time on the μs scale is in agreement with a sensitization of the Tb^{3+} taking place by energy transfer from the triplet state of the tryptophan. However, tryptophan triplet emission could not be detected for $LCC1^{Tb}$ or for $LCC1^{La}$, the homologous probe with the non-luminescent La^{3+} ion. Conversely, for $Cu^{I}{\cdot}LCC1^{Tb}$, the growing Tb^{3+} emission overlaps with a broad emission band that decays with a lifetime of 16 μs, which is synchronous with

the rise of Tb^{3+} emission ($\tau = 16$ μs, Figure 4C). The broad decaying emission band is clearly seen with the Cu^+ complex of $LCC1^{La}$ (Figure 4D). Due to its lifetime in the μs scale and spectrum, this band can be attributed to the triplet emission of the tryptophan. This confirms that Tb^{3+} sensitization occurs through a tryptophan(T_1) to $Tb^{3+}(^5D_4)$ energy transfer. $Ag^I{\cdot}LCC1^{Ln}$ (Ln = Tb or La) behaves in the same way as $Cu^I{\cdot}LCC1^{Ln}$ but with blue-shifted tryptophan phosphorescence emission compared to the analogous copper complex (Figure 4D). The above results show that both Cu^+ and Ag^+ binding to $LCC1^{Tb}$ increase tryptophan triplet state emission as well as Tb^{3+} emission. Together with the loss of tryptophan fluorescence for the 1:1 complex conformer that establishes a cation–π interaction, this is compatible with an ISC enhancement promoted by the cation–π interaction.[15] Therefore, the binding of Cu^+ or Ag^+ to $LCC1^{Tb}$ through a cation–π interaction favors ISC and increases the population of the excited triplet state of the tryptophan. Hence, more energy can be transferred to the $Tb^{3+}\,{}^5D_4$ excited state, which in turn emits more. Although it cannot be excluded that conformational changes may be, in part, responsible for Tb^{3+} luminescence enhancement, the spectroscopic data presented here point to a major role of the cation–π interaction that is established between the metal ion and the tryptophan indole. In addition to the global ISC enhancement, the cation–π interaction with Cu^+ and Ag^+ shifts the tryptophan triplet excited state emission but to a different extent. Indeed, comparison of the room temperature phosphorescence spectra of $Cu^I{\cdot}LCC1^{La}$ and $Ag^I{\cdot}LCC1^{La}$ (SI) with those reported in the literature for proteins[23,24] show that Cu^+ and Ag^+ lower the energy of the excited triplet state of tryptophan by ca. 2300 cm^{-1} and 500 cm^{-1}, respectively.

Here we describe a new luminescent probe for selective Cu^+ detection among physiological cations. This probe is characterized by a high contrast and long-lived emission of its Tb^{3+} ion, which allows time-gated detection. Additionally, detailed spectroscopic characterization shows that the cation–π interaction established between the metal ion and the tryptophan indole plays a major role in modulating the Tb^{3+} luminescence in this prototype by modulation of the photophysical properties of the tryptophan antenna. As cation–π interactions may be formed with several cations (e.g., Cu^+, Ag^+, Cd^{2+}, Hg^{2+}, and Pb^{2+}), this work paves the way for the design of lanthanide-based luminescent probes for Cu^+ or toxic cations with desirable emission properties relying on a mechanism other than metal-induced PET quenching.

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[1] S. J. Lippard, J. M. Berg, *Principles of Bioinorganic Chemistry*, University Science Books, Mill Valley, CA, **1994**.
[2] B.-E. Kim, T. Nevitt, D. J. Thiele, *Nat. Chem. Biol.* **2008**, *4*, 176–185.
[3] P. Delangle, E. Mintz, *Dalton Trans.* **2012**, *41*, 6359–6370.
[4] R. McRae, P. Bagchi, S. Sumalekshmy, C. J. Fahrni, *Chem. Rev.* **2009**, *109*, 4780–4827.
[5] C. J. Fahrni, *Curr. Opin. Chem. Biol.* **2013**, *17*, 656–662.
[6] J. A. J. Cotruvo, A. T. Aron, K. M. Ramos-Torres, C. J. Chang, *Chem. Soc. Rev.* **2015**, 44, 4400–4414.
[7] J. C. G. Bünzli, C. Piguet, *Chem. Soc. Rev.* **2005**, *34*, 1048–1077.
[8] J.-C. G. Bünzli, *Chem. Rev.* **2010**, *110*, 2729–2755.
[9] A. Thibon, V. C. Pierre, *Anal. Bioanal. Chem.* **2009**, *394*, 107–120.
[10] H. J. Tanke in *Lanthanide Luminescence* (Eds.: P. Hänninen, H. Härmä), Springer, Berlin, **2011**, pp. 313–328.
[11] E.-H. Kim, C. Rensing, M. M. McEvoy, *Nat. Prod. Rep.* **2010**, *27*, 711–719.
[12] J. A. Delmar, C.-C. Su, E. W. Yu, *Biometals* **2013**, *26*, 593–607.
[13] I. R. Loftin, S. Franke, N. J. Blackburn, M. M. McEvoy, *Protein Sci.* **2007**, *16*, 2287–2293.
[14] Y. Xue, A. V. Davis, G. Balakrishnan, J. P. Stasser, B. M. Staehlin, P. Focia, T. G. Spiro, J. E. Penner-Hahn, T. V. O'Halloran, *Nat. Chem. Biol.* **2008**, *4*, 107–109.
[15] H. Masuhara, H. Shioyama, T. Saito, K. Hamada, S. Yasoshima, N. Mataga, *J. Phys. Chem.* **1984**, *88*, 5868–5873.
[16] S. V. Eliseeva, J.-C. G. Bünzli, *Chem. Soc. Rev.* **2010**, *39*, 189–227.
[17] M. C. Heffern, L. M. Matosziuk, T. J. Meade, *Chem. Rev.* **2014**, *114*, 4496–4539.
[18] J.-C. G. Bünzli, S. V. Eliseeva in *Lanthanide Luminescence* (Eds.: P. Hänninen, H. Härmä), Springer, Berlin, **2011**, pp. 1–45.
[19] W. Horrocks, B. Holmquist, B. Vallee, *Proc. Natl. Acad. Sci. USA* **1975**, *72*, 4764–4768.
[20] U. S. Raghavender, S. Aravinda, R. Rai, N. Shamala, P. Balaram, *Org. Biomol. Chem.* **2010**, *8*, 3133–3135.
[21] A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams, M. Woods, *J. Chem. Soc. Perkin Trans. 2* **1999**, 493–504.
[22] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, **2006**.
[23] Y. Kai, K. Imakubo, *Photochem. Photobiol.* **1979**, *29*, 261–265.
[24] V. M. Mazhul', A. V. Timoshenko, E. M. Zaitseva, S. G. Loznikova, I. V. Halets, T. S. Chernovets, *Reviews in Fluorescence*, Springer, Dordrecht, **2008**, pp. 37–67.

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