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

Manon Isaac, Sergey A. Denisov, Amandine Roux, Daniel Imbert, Gediminas Jonusauskas, Nathan Mc Clenaghan, Olivier Snque

► To cite this version:

Manon Isaac, Sergey A. Denisov, Amandine Roux, Daniel Imbert, Gediminas Jonusauskas, et al.. Lanthanide Luminescence Modulation by Cation– π Interaction in a Bioinspired Scaffold: Selective Detection of Copper(I). *Angewandte Chemie International Edition*, 2015, 54 (39), pp.11453-11456. 10.1002/anie.201505733 . hal-01211550

HAL Id: hal-01211550

<https://hal.science/hal-01211550>

Submitted on 20 Feb 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destine au dpt et la diffusion de documents scientifiques de niveau recherche, publis ou non, manant des tablissements d'enseignement et de recherche franais ou trangers, des laboratoires publics ou privs.

Distributed under a Creative Commons Attribution - ShareAlike| 4.0 International License

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

Manon Isaac, Sergey A. Denisov, Amandine Roux, Daniel Imbert, Gediminas Jonusauskas, Nathan D. McClenaghan,* and Olivier Snque*

Abstract: A prototype luminescent turn on probe for Cu⁺ (and Ag⁺) is described, harnessing a selective binding site ($\log K_{\text{ass}} = 9.4$ 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 Ca²⁺ or Zn²⁺, 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 (Ln³⁺) emitter, that has a long luminescence lifetime (in the millisecond range) compared to classical organic fluorophores (nano

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.

second range) and that allows time gated detection to suppress background fluorescence contributions.^[7–10]

Our probe structure (Figure 1 A) 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 1 B (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]

Ln³⁺ 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 Ln³⁺ 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

[*] M. Isaac, Dr. A. Roux, Dr. O. Snque
Univ. Grenoble Alpes, LCBM/PMB, and CEA, IRTSV/CBM/PMB
and CNRS, LCBM UMR 5249, PMB
38000 Grenoble (France)
E mail: olivier.seneque@cea.fr
Dr. S. A. Denisov, Dr. G. Jonusauskas, Dr. N. D. McClenaghan
Univ. Bordeaux and CNRS, ISM (UMR 5255) and LOMA (UMR 5798)
33405 Talence (France)
E mail: nathan.mcclenaghan@u-bordeaux.fr
Dr. D. Imbert
Univ. Grenoble Alpes and CEA, INAC/SCIB/RICC
38000 Grenoble (France)

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^DPro 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 titra-

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 ($\circ$) and 225 nm ($\bullet$). B) CD spectra of LCC1^{Tb} (18 μM) before and after addition of various metal ions.

tion 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, $\text{Cu}^{\text{I}}\text{LCC1}^{\text{Tb}}$, 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+} .

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

Figure 3. Steady state spectroscopic characterization of LCC1^{Tb} (solid line), $\text{Cu}^{\text{I}}\text{LCC1}^{\text{Tb}}$ (dashed line), and $\text{Ag}^{\text{I}}\text{LCC1}^{\text{Tb}}$ (dotted line). A) Electronic absorption spectra. B) Representation of possible fluorescent and non fluorescent forms of tryptophan in $\text{Cu}^{\text{I}}\text{LCC1}^{\text{Tb}}$. C,D) Tryptophan fluorescence excitation (C, λ_{em} 355 nm) and emission (D, λ_{ex} 280 nm) spectra. E,F) Time gated Tb^{3+} luminescence excitation (E, λ_{em} 545 nm) and emission (F, λ_{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 (λ_{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.

fluorescence (Figure 3D). This suggests the presence of a cation π interaction in $\text{Cu}^{\text{I}}\text{LCC1}^{\text{Tb}}$ and $\text{Ag}^{\text{I}}\text{LCC1}^{\text{Tb}}$ as observed for CusF.

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 $\text{Cu}^{\text{I}}\text{LCC1}^{\text{Tb}}$ and $\text{Ag}^{\text{I}}\text{LCC1}^{\text{Tb}}$ is associated with an increase of the Tb^{3+} emission. The Tb^{3+} luminescence excitation spectra of LCC1^{Tb} , $\text{Cu}^{\text{I}}\text{LCC1}^{\text{Tb}}$, and $\text{Ag}^{\text{I}}\text{LCC1}^{\text{Tb}}$ (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 (λ_{em} = 545 nm) of $\text{Cu}^{\text{I}}\text{LCC1}^{\text{Tb}}$ and $\text{Ag}^{\text{I}}\text{LCC1}^{\text{Tb}}$ are red shifted compared to LCC1^{Tb} (Figure 3E), but the tryptophan fluorescence excitation spectra (λ_{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 $\pi \pi^*$ transition and the other one corresponding to a non fluorescent indole with a red shifted $\pi \pi^*$ 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} , $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$, and $\text{Ag}^{\text{I}}\text{-LCC1}^{\text{Tb}}$ 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 $\pi \pi^*$ 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 $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$ 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 $10^{9.4}\text{M}^{-1}$ and $10^{7.3}\text{M}^{-1}$, respectively (SI). The K_{M} for other physiological cations is estimated to be below 10^3M^{-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 ($\tau \approx 1.9\text{ms}$). 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 ($\tau_1 = 0.9\text{ns}$ and $\tau_2 = 4.8\text{ns}$, Table 1), which is common for tryptophan.^[22] The lifetimes of the fluorescence of $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$ and $\text{Ag}^{\text{I}}\text{-LCC1}^{\text{Tb}}$, which accounts for the species with the indole

Table 1: Decay lifetimes of tryptophan emission and rise time of Tb^{3+} emission for LCC1^{Tb} , $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$, and $\text{Ag}^{\text{I}}\text{-LCC1}^{\text{Tb}}$. 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
$\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$	0.7 (16%), 3.9 (84%)	16	16
$\text{Ag}^{\text{I}}\text{-LCC1}^{\text{Tb}}$	0.9 (17%), 4.2 (83%)	19	18
$\text{Cu}^{\text{I}}\text{-LCC1}^{\text{La}}$		18	
$\text{Ag}^{\text{I}}\text{-LCC1}^{\text{La}}$		20

Figure 4. Time resolved emission spectroscopy in degassed buffer solutions. Spectra were recorded in time gated mode using a streak camera ($\lambda_{\text{ex}} = 266\text{nm}$). Time resolved emission spectra of A) LCC1^{Tb} and B) $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$ 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 $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$. The solid and dashed lines correspond to the respective fits which yielded $\tau = 16 \pm 2\text{ms}$ for both phosphorescence decay and Tb^{3+} emission grow in. D) Phosphorescence emission spectra of $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{La}}$ (dashed line) and $\text{Ag}^{\text{I}}\text{-LCC1}^{\text{La}}$ (dotted line).

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).

Figures 4A and 4B compare the emission spectra of LCC1^{Tb} and $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$ recorded several μs after the laser pulse ($\lambda_{\text{ex}} = 266\text{nm}$, 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 $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$, 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 ($\tau = 16 \mu\text{s}$, 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 $\text{Tb}^{3+}({}^5\text{D}_4)$ energy transfer. $\text{Ag}^+\text{LCC1}^{\text{Ln}}$ ($\text{Ln} = \text{Tb}$ or La) behaves in the same way as $\text{Cu}^+\text{LCC1}^{\text{Ln}}$ 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 $\text{Tb}^{3+}({}^5\text{D}_4)$ 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 $\text{Cu}^+\text{LCC1}^{\text{La}}$ and $\text{Ag}^+\text{LCC1}^{\text{La}}$ (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 photo physical 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.

Acknowledgements

O.S. acknowledges the support of the Agence Nationale de la Recherche (ANR 12 BS07 0012 01) and Labex ARCANÉ (ANR 11 LABX 0003 01).

Keywords: cation π interactions · copper · luminescence · peptides · terbium

- [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, *Biomaterials* **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. Dickens, 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.