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# A bioinspired luminescent europium-based probe capable of discrimination between Ag<sup>+</sup> and Cu<sup>+</sup>

Manon Isaac,<sup>a</sup> Sergey A. Denisov,<sup>b</sup> Nathan D. McClenaghan,<sup>\*b</sup> Olivier Sénèque<sup>\*a</sup>

<sup>a</sup> Univ. Grenoble Alpes, CNRS, CEA, IRIG, LCBM (UMR 5249), 38000 Grenoble, France.

<sup>b</sup> Univ. Bordeaux, CNRS, ISM (UMR 5255), 33405 Talence, France.

Email: *olivier.seneque@cea.fr* and *nathan.mcclenaghan@u-bordeaux.fr*

## Abstract

Due to their similar coordination properties, discrimination of Cu<sup>+</sup> and Ag<sup>+</sup> by water-soluble luminescent probes is challenging. We have synthesized LCC4<sup>Eu</sup>, an 18 amino acid cyclic peptide bearing a europium complex, that is able to bind one Cu<sup>+</sup> or Ag<sup>+</sup> ion by the side chains of two methionines, a histidine and a 3-(1-naphthyl)-L-alanine. In this system, the naphthyl moiety establishes a cation- $\pi$  interaction with these cations. It also acts as an antenna for the sensitization of Eu<sup>3+</sup> luminescence. Interestingly, when excited at 280 nm behaves as a turn-on probe for Ag<sup>+</sup> (+150 % Eu emission) and as a turn-off probe for Cu<sup>+</sup> (-50% Eu<sup>3+</sup> emission). Shifting the excitation wavelength to 305 nm makes the probe responsive to Ag<sup>+</sup> (+380% Eu<sup>3+</sup> emission) but not to Cu<sup>+</sup> or other physiological cations. Thus, LCC4<sup>Eu</sup> is uniquely capable of discriminating Ag<sup>+</sup> from Cu<sup>+</sup>. A detailed spectroscopic characterization based on steady state and time-resolved measurements clearly demonstrates that Eu<sup>3+</sup> sensitization relies on electronic energy transfer from the naphthalene triplet state to the Eu<sup>3+</sup> excited states and that the cation- $\pi$  interaction lowers the energy of this triplet state by 700 cm<sup>-1</sup> and 2400 cm<sup>-1</sup> for Ag<sup>+</sup> and Cu<sup>+</sup>, respectively. Spectroscopic data point to a modulation of the efficiency of the electronic energy transfer caused by the differential red-shift of the naphthalene triplet, deciphering the differential luminescence response of LCC4<sup>Eu</sup> toward Ag<sup>+</sup> and Cu<sup>+</sup>.

## Keywords

Peptide – Lanthanide – Silver – Copper – Luminescence

## Introduction

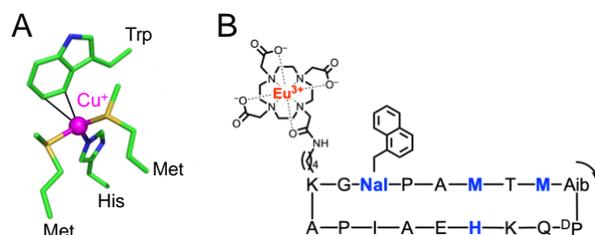
Copper is a group 11 element that is an essential micronutrient for living organisms, where it exists in the +I and +II oxidation states.<sup>1,2</sup> It is an important catalytic cofactor for many enzymes involved in electron transfer or oxidative processes. In the oxidizing extracellular environment, copper is in the +II state whereas it is mainly in the +I state in the intracellular environment.<sup>3,4</sup> Since both deficiency and excesses of copper are toxic, its homeostasis is highly regulated in living systems. In order to prevent mobile cellular copper, i.e. copper that is not a cofactor of redox enzymes, from reacting with oxygen and producing reactive oxygen species that are deleterious to the cell, copper(I) is always taken in charge by proteins that bind it tightly and maintain it in the +I state. These proteins generally bind  $\text{Cu}^+$  through a set of cysteine, methionine and/or histidine side chains.<sup>5</sup> In particular, the soft S donors of cysteines and methionines favor binding copper in its reduced state.

Silver, which is also a group 11 element, has long been identified as toxic for living organisms. However, being considered as more toxic for bacteria than for humans, it has been used as an antibacterial agent.<sup>6</sup> Conversely to copper, silver exists in biological environments in the +I oxidation state but not the +II one.  $\text{Cu}^+$  and  $\text{Ag}^+$ , with their  $[\text{Ar}]3d^{10}$  and  $[\text{Kr}]4d^{10}$  electronic configurations, respectively, share similar coordination properties, especially with respect to protein binding. As a consequence,  $\text{Ag}^+$  can replace  $\text{Cu}^+$  in proteins.<sup>7-10</sup> This may be detrimental as far as redox proteins are concerned but this can be turned into an advantage when Nature uses very similar proteins in the bacterial CusCFBA or SilCFBA efflux systems to detoxify  $\text{Cu}^+$ ,  $\text{Ag}^+$ , or both.<sup>11-13</sup>

The toxicity of  $\text{Ag}^+$  has elicited the development of molecular  $\text{Ag}^+$ -responsive fluorescent probes to decipher its impact in biology. Several probes have been described in the literature, but a majority are soluble in organic solvents or water-organic solvent mixtures,<sup>14,15</sup> which limits their use in biological studies. Indeed, only a few probes are soluble in purely aqueous solutions.<sup>16-23</sup> Another issue when developing a  $\text{Ag}^+$ -responsive probe for biological application is the selectivity with respect to  $\text{Cu}^+$ . The similarity in coordination properties of these two cations may cause a similar response for both. Interestingly, this issue is rarely considered. While selectivity toward  $\text{Cu}^{2+}$  is often examined for  $\text{Ag}^+$ -responsive probes, their behavior towards  $\text{Cu}^+$  has only been reported in a few instances for water-soluble probes.<sup>17,24</sup> The propensity of free  $\text{Cu}^+$  to disproportionation in aqueous solutions or to oxidation in aerated solutions necessitate strict anaerobic conditions in the presence of a protective reductant such as ascorbate or hydroxylamine<sup>25</sup> and/or a suitable chelating environment (1-3% acetonitrile)<sup>26</sup> to ensure stabilization of Cu in the +I state in water at the micromolar or sub-micromolar concentration range investigated in these studies. Such conditions were not mentioned in these studies, casting doubt on the real  $\text{Ag}^+ / \text{Cu}^+$  selectivity of these probes. It is also interesting to note that the  $\text{Cu}^+ / \text{Ag}^+$  selectivity issue is generally not discussed for  $\text{Cu}^+$ -responsive probes for biological applications, although the selectivity is evaluated for other toxic cations (e.g.  $\text{Cd}^{2+}$ ,  $\text{Hg}^{2+}$  or  $\text{Pb}^{2+}$ ).<sup>27-30</sup> Therefore, it appears that discrimination between  $\text{Ag}^+$  and  $\text{Cu}^+$  by responsive fluorescent probes remains a challenge.

We have recently described LCC1<sup>Tb</sup>, a bioinspired  $\text{Cu}^+$ -responsive Tb<sup>3+</sup>-based luminescent probe.<sup>31,32</sup> This probe comprises (i) an 18-amino acid cyclic peptide inspired by the copper-binding loop of the protein CusF,<sup>33,34</sup> a metallochaperone involved in  $\text{Cu}^+$  trafficking in the periplasm of Gram negative bacteria,<sup>11,12</sup> and (ii) a DOTA[Tb] complex grafted on a lysine side chain of the peptide. LCC1<sup>Tb</sup> binds one  $\text{Cu}^+$  by the side chains of two methionines (Met/M), one histidine (His/H) and a tryptophan (Trp/W). The indole ring of the latter forms a cation- $\pi$  interaction with  $\text{Cu}^+$  as observed in CusF (Figure 1A).<sup>34</sup> Besides being a  $\text{Cu}^+$  ligand, the Trp residue serves as an antenna to sensitize Tb<sup>3+</sup> luminescence: upon excitation of the Trp in its  $\pi$ - $\pi^*$  transition at ca. 280 nm, Tb<sup>3+</sup> emission is observed.<sup>35,36</sup> Formation of the  $\text{Cu}^+$ -LCC1<sup>Tb</sup> complex results in a 6-fold increase of the Tb<sup>3+</sup> emission. Besides  $\text{Cu}^+$ , LCC1<sup>Tb</sup> responds to  $\text{Ag}^+$  in a very similar fashion, but it does not respond to any other metal cation.

We have synthesized several Tb<sup>3+</sup>-based probes derived from LCC1<sup>Tb</sup> with varied peptide sequences but all of them behaved similarly with respect to Cu<sup>+</sup> and Ag<sup>+</sup>.<sup>32</sup> They constitute a perfect example of the difficulty of discriminating between Cu<sup>+</sup> and Ag<sup>+</sup>. In this article, we describe a novel probe, LCC4<sup>Eu</sup>, which harnesses Eu<sup>3+</sup> and a naphthalene antenna instead of Tb<sup>3+</sup> and tryptophan (Figure 1B). The luminescence of this probe is turned on by Ag<sup>+</sup> but not by Cu<sup>+</sup>, providing a clear example of Ag<sup>+</sup> / Cu<sup>+</sup> discrimination in aqueous solution.



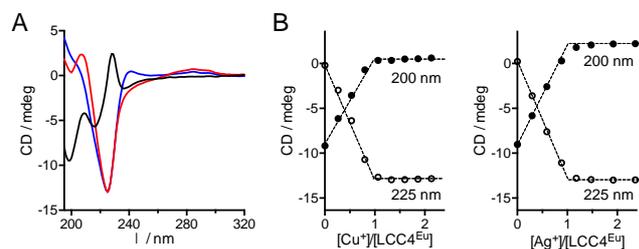
**Figure 1.** (A) Cu<sup>+</sup> binding site of CusF (pdb 2VB2<sup>34</sup>). (B) Amino acid sequence of LCC4<sup>Eu</sup>, Cu/Ag-chelating amino acids in blue (Nal = 3-(1-naphthyl)-L-alanine; Aib = 2-aminoisobutyric acid; <sup>D</sup>P = D-proline). The arrow indicates de N-to-C direction within the cyclic peptide.

## Results and discussion

**Probe design.** LCC1<sup>Tb</sup> displays a turn-on response to the presence of Cu<sup>+</sup> or Ag<sup>+</sup>. A detailed photophysical study of LCC1<sup>Tb</sup> has shown that the cation- $\pi$  interaction that is established between Cu<sup>+</sup> or Ag<sup>+</sup> and the indole ring of the tryptophan upon metal binding to the peptide enhances intersystem crossing (ISC) and thus increases the population of the tryptophan excited triplet state, which is the energy transferring state in the Tb<sup>3+</sup> sensitization process.<sup>31</sup> Therefore, the population of the Tb(<sup>5</sup>D<sub>4</sub>) excited state increases as well as Tb<sup>3+</sup> emission, *in fine*. In order to develop probes that emit in the red or the near-infrared using other lanthanides, we decided to focus firstly on Eu-based probes first. Since tryptophan cannot sensitize Eu<sup>3+</sup> luminescence,<sup>37</sup> naphthalene was chosen as an antenna,<sup>38-40</sup> which features a triplet excited state at ca. 21 300 cm<sup>-1</sup>.<sup>41</sup> The amino acid sequence of LCC4<sup>Eu</sup> (Figure 1B) corresponds to the sequence of the parent probe LCC2<sup>Tb</sup> but with naphthalene and Eu<sup>3+</sup> replacing tryptophan and Tb<sup>3+</sup>, respectively.<sup>32</sup> Compared to LCC1<sup>Tb</sup>, this sequence change has almost no consequence on the luminescence properties in response to Cu<sup>+</sup> and Ag<sup>+</sup> but confers higher binding constants (ca. one order of magnitude higher).<sup>32</sup> The synthesis of LCC4<sup>Eu</sup> is described in the Supporting Information (SI).

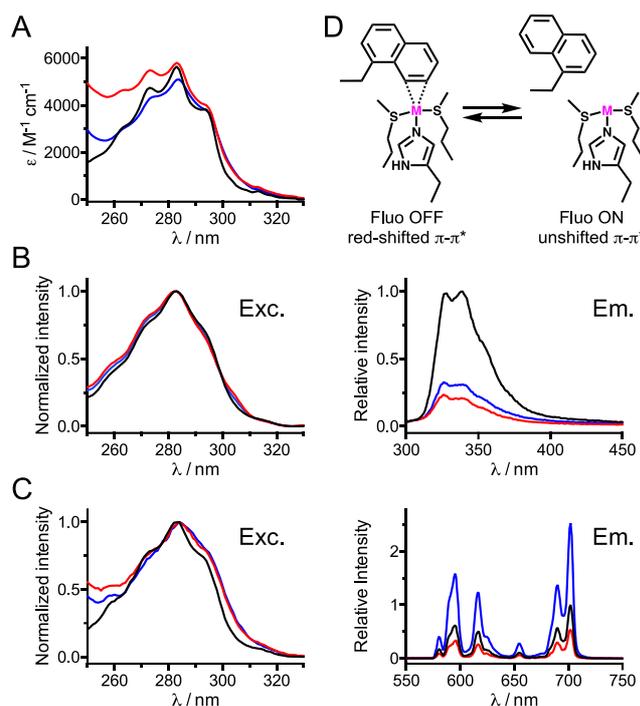
**Spectroscopic characterization of LCC4<sup>Eu</sup>.** LCC4<sup>Eu</sup> was studied in water buffered with HEPES (10 mM, pH 7.5) except for circular dichroism (CD) measurements for which a phosphate buffer (10 mM, pH 7.0) was used. All solutions were prepared in a glovebox in order to prevent oxidation of Cu<sup>+</sup> by O<sub>2</sub>. For comparison purposes, anaerobic conditions were also used for Ag<sup>+</sup>-containing samples. For Cu<sup>+</sup>-containing samples, NH<sub>2</sub>OH (2 mM) was added to the buffer to generate Cu<sup>+</sup> *in situ* by reduction of Cu<sup>2+</sup> added as a CuSO<sub>4</sub> solution. Firstly, titrations of LCC4<sup>Eu</sup> by Cu<sup>+</sup> and Ag<sup>+</sup> were monitored by CD. The CD spectrum of LCC4<sup>Eu</sup> (Figure 2A) displays several bands at 198 (-), 216 (-), 228 (+) and 236 nm (-) and is more structured than that of LCC2<sup>Tb</sup>. Upon addition of Cu<sup>+</sup> or Ag<sup>+</sup>, a linear evolution of the spectrum is observed up to 1.0 eq. of the metal cation while the spectrum remains unchanged above this value (Figure 2B). This indicates the formation of 1:1 Cu-LCC4<sup>Eu</sup> and Ag-LCC4<sup>Eu</sup> complexes. The CD spectra of the Cu<sup>+</sup> and Ag<sup>+</sup> complexes are very similar. They both display a strong negative signal at 225 nm but with an additional small positive one at 240 nm for Ag<sup>+</sup>. These features were already observed with the Cu<sup>+</sup> and Ag<sup>+</sup> complexes of LCC1<sup>Tb</sup> and LCC2<sup>Tb</sup>.<sup>31,32</sup> Interestingly, a positive CD band appears around 280 nm upon complexation, corresponding to the naphthalene  $\pi$ - $\pi^*$  transition. This may be attributed to restrictions

in the accessible conformations of the 3-(1-naphthyl)alanine side chain that renders the naphthalene moiety CD-active because of a well-defined chiral environment.



**Figure 2.** (A) CD spectra of LCC4<sup>Eu</sup> (black), Cu-LCC4<sup>Eu</sup> (red) and Ag-LCC4<sup>Eu</sup> (blue) in phosphate buffer (10 mM, pH 7.0). (B) Evolution of the CD signal at 200 and 225 nm during titrations with Cu<sup>+</sup> (left) and Ag<sup>+</sup> (right).

Complexation of Cu<sup>+</sup> and Ag<sup>+</sup> was then investigated by titrations, monitored by electronic absorption and luminescence spectroscopy, which further confirmed the 1:1 binding stoichiometry (Figure S2 in SI). Upon metal binding, a slight shift (1-2 nm) of the  $\pi$ - $\pi^*$  transition is observed in the absorption spectrum (Figure 3A). A stronger effect is observed for naphthalene fluorescence, with an 80 % and a 70 % quench caused by Cu<sup>+</sup> and Ag<sup>+</sup>, respectively (Figure 3B, right). The red-shifted absorption and fluorescence quench are indicative of the formation of the cation- $\pi$  interaction between Cu<sup>+</sup> or Ag<sup>+</sup> and naphthalene, represented in Figure 3D, as observed for CuSF<sup>34</sup> or LCC1<sup>Tb</sup>.<sup>31</sup> Excitation of the naphthalene at 283 nm results in Eu<sup>3+</sup> emission with peaks at 580, 595, 616, 654 and 702 nm corresponding to the  $^5D_0 \rightarrow ^7F_J$  ( $J = 0,1,2,3,4$ ) transitions (Figure 3C, right). The shape of the Eu<sup>3+</sup> emission spectrum remains unchanged upon Cu<sup>+</sup> and Ag<sup>+</sup> binding as well as the Eu<sup>3+</sup> emission decay lifetime ( $\tau_{Eu} \approx 0.63$  ms, Table 1). Such a lifetime value is typical of a mono-hydrated ( $q = 1$ ) Eu<sup>3+</sup> ion bound to a DOTA-monoamide chelate<sup>42-44</sup> as confirmed by the experimental determination of  $q$  for LCC4<sup>Eu</sup> and Ag-LCC4<sup>Eu</sup> (Figure S3 and Table S1 in SI). Interestingly, Ag<sup>+</sup> binding induces a 2.5-fold enhancement of intensity of the sensitized Eu<sup>3+</sup> emission ( $\lambda_{ex} = 283$  nm) while Cu<sup>+</sup> decreases it by 50 % (Figure 3C, right). The corresponding Eu<sup>3+</sup> emission quantum yields ( $\Phi_{Eu}^{Nap}$ ) were determined to be 0.018, 0.009 and 0.044 for LCC4<sup>Eu</sup>, Cu-LCC4<sup>Eu</sup> and Ag-LCC4<sup>Eu</sup>, respectively (Table 1). For Cu-LCC4<sup>Eu</sup> and Ag-LCC4<sup>Eu</sup> a 2 nm red-shift of the Eu<sup>3+</sup> luminescence excitation spectrum is observed while naphthalene fluorescence excitation spectra are unshifted. As for LCC1<sup>Tb</sup>, this can be rationalized by an equilibrium between two forms for the complex: one with the naphthalene bound to Cu<sup>+</sup> or Ag<sup>+</sup> through a cation- $\pi$  interaction and another one with an unbound naphthalene. The former is non-fluorescent and displays a red-shifted  $\pi$ - $\pi^*$  transition in contrast to the latter (Figure 3B). Therefore, the behavior of LCC4<sup>Eu</sup> is very similar to the one of LCC1<sup>Tb</sup> except that Cu<sup>+</sup> decreases the Eu<sup>3+</sup> emission ( $\times 0.5$ ) and Ag<sup>+</sup> increases it ( $\times 2.5$ ), while in the case of LCC1<sup>Tb</sup> both induce an increase in Tb<sup>3+</sup> emission ( $\times 6$ ). Hence, LCC4<sup>Eu</sup> is able to discriminate between Cu<sup>+</sup> and Ag<sup>+</sup>, whereas LCC1<sup>Tb</sup> is not.



**Figure 3.** (A) Electronic absorption spectra, (B) fluorescence spectra and (C) time-gated luminescence (delay = 100  $\mu$ s) spectra of LCC4<sup>Eu</sup> (black), Cu·LCC4<sup>Eu</sup> (red) and Ag·LCC4<sup>Eu</sup> (blue). In B and C, the emission spectrum is shown on the right (B:  $\lambda_{\text{ex}} = 290$  nm, C:  $\lambda_{\text{ex}} = 283$  nm) and the excitation spectrum is shown on the left (B:  $\lambda_{\text{em}} = 350$  nm, C:  $\lambda_{\text{em}} = 595$  nm, excitation slit = 5 nm). (D) Equilibrium between the two forms of the M·LCC4<sup>Eu</sup> complex (M = Ag or Cu), with or without metal-bound naphthalene. Solutions were prepared in HEPES buffer (10 mM, pH 7.5).

**Table 1.** Photophysical data for LCC4<sup>Ln</sup>, Cu·LCC4<sup>Ln</sup> and Ag·LCC4<sup>Ln</sup> (Ln = Eu or La). Error on  $\tau_{\text{Nap}}^{\text{F}}$ ,  $\tau_{\text{Nap}}^{\text{P}}$ ,  $\tau_{\text{Eu}}^{\text{rise}}$  values is estimated at 10 %. Error on  $\tau_{\text{Eu}}^{\text{dec}}$  is estimated 0.02 ms. Error on  $\Phi_{\text{Eu}}^{\text{Nap}}$  is estimated at 10 %. n.d. = not detected.

Compound	Naphthalene		Europium				$\eta_{\text{sens}}$	$\Phi_{\text{Eu}}^{\text{Eu}}$	$\Phi_{\text{Eu}}^{\text{Nap}}$
	S <sub>1</sub> decay	T <sub>1</sub> decay	<sup>5</sup> D <sub>0</sub> rise	<sup>5</sup> D <sub>0</sub> decay	$\tau_{\text{R}}$ / ms	$\tau_{\text{Nap}}^{\text{F}}$ / ns			
LCC4 <sup>Eu</sup>	2.8 (33%), 17.5 (67%)	n.d.	40	0.63	6.78	0.19	0.093	0.018	
Cu·LCC4 <sup>Eu</sup>	2.5 (38%) 15.2 (62%)	24	25	0.61	6.78	0.10	0.090	0.009	
Ag·LCC4 <sup>Eu</sup>	2.5 (44%) 14.1 (56%)	110	105	0.65	6.78	0.47	0.095	0.044	
LCC4 <sup>La</sup>	2.3 (8%) 17.9 (92%)	41	–						
Cu·LCC4 <sup>La</sup>	2.0 (33%) 17.9 (66%)	32	–						
Ag·LCC4 <sup>La</sup>	2.0 (13%) 17.0 (87%)	53 (10%) 312 (90%)	–						

**Mechanistic insight into the Ag<sup>+</sup> / Cu<sup>+</sup> discrimination.** The overall quantum yield of sensitized lanthanide luminescence,  $\Phi_{\text{Ln}}^{\text{antenna}}$ , is the product of the sensitization efficiency,  $\eta_{\text{sens}}$ , and of the intrinsic metal-centered luminescence quantum yield,  $\Phi_{\text{Ln}}^{\text{Ln}}$ .<sup>36</sup> In the present case, this translates into Equation 1.

$$\Phi_{\text{Eu}}^{\text{Nap}} = \eta_{\text{sens}} \times \Phi_{\text{Eu}}^{\text{Eu}} \quad (1)$$

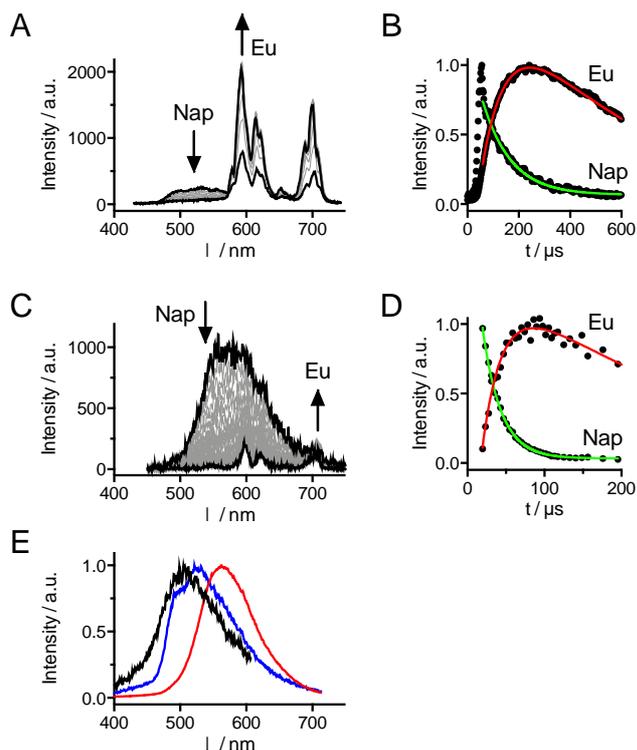
$\eta_{\text{sens}}$  quantifies the efficiency of the population of the  $\text{Eu}^{3+}$  emissive state ( $^5\text{D}_0$ ) starting from photons absorbed by the antenna and  $\Phi_{\text{Eu}}^{\text{Eu}}$  quantifies the efficiency of  $\text{Eu}^{3+}$  emission from its  $^5\text{D}_0$  excited state.  $\Phi_{\text{Eu}}^{\text{Eu}}$  can be determined using Equation 2 from the measured emission lifetime ( $\tau_{\text{Eu}}^{\text{dec}}$ ) and the radiative lifetime of  $\text{Eu}^{3+}$  ( $\tau_{\text{R}}$ ) determined from the emission spectrum by Equation 3.<sup>45</sup>

$$\Phi_{\text{Eu}}^{\text{Eu}} = \tau_{\text{Eu}}^{\text{dec}} / \tau_{\text{R}} \quad (2)$$

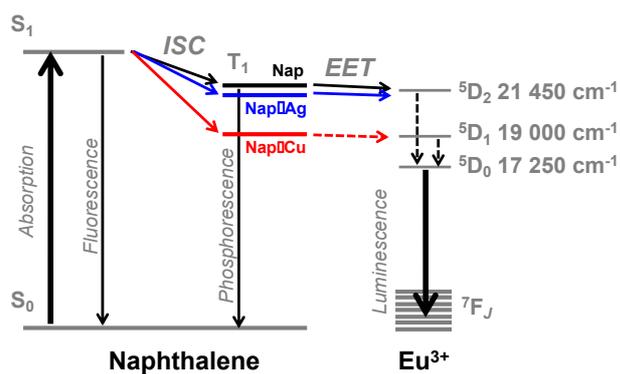
$$\tau_{\text{R}}^{-1} = A_{\text{MD},0} \times n^3 \times (I_{\text{tot}} / I_{01}) \quad (3)$$

$A_{\text{MD},0}$  is the spontaneous emission probability for the  $^5\text{D}_0 \rightarrow ^7\text{F}_1$  transition and it is calculated to be  $14.65 \text{ s}^{-1}$ ,  $n$  is the refractive index of the medium and  $I_{\text{tot}}$  and  $I_{01}$  are the total area of the corrected  $\text{Eu}^{3+}$  emission spectrum and the area of the  $^5\text{D}_0 \rightarrow ^7\text{F}_1$  transition band, respectively. As the  $\text{Eu}^{3+}$  emission spectrum of  $\text{LCC4}^{\text{Eu}}$  remains unchanged upon  $\text{Cu}^+$  or  $\text{Ag}^+$  binding, the value of  $\tau_{\text{R}}$  equals 6.78 ms for the free probe and the  $\text{Cu}^+$  and  $\text{Ag}^+$  complexes. As  $\tau_{\text{Eu}}^{\text{dec}}$  is similar for the three compounds,  $\Phi_{\text{Eu}}^{\text{Eu}}$  is almost the same, ca. 0.093 (Table 1). Therefore, the observed differences in  $\Phi_{\text{Eu}}^{\text{Nap}}$  are only due to differences in  $\eta_{\text{sens}}$  (Table 1). Notably, the sensitization process is ca. 5 times more efficient for  $\text{Ag} \cdot \text{LCC4}^{\text{Eu}}$  than for  $\text{Cu} \cdot \text{LCC4}^{\text{Eu}}$  (0.47 vs 0.10).

In order to determine the sensitization pathway, time-resolved emission measurements on the nanosecond and microsecond timescale were performed using streak-camera detection on  $\text{LCC4}^{\text{Eu}}$ ,  $\text{Ag} \cdot \text{LCC4}^{\text{Eu}}$  and  $\text{Cu} \cdot \text{LCC4}^{\text{Eu}}$ , as well as their analogues with  $\text{La}^{3+}$  in place of  $\text{Eu}^{3+}$ , namely  $\text{LCC4}^{\text{La}}$ ,  $\text{Ag} \cdot \text{LCC4}^{\text{La}}$  and  $\text{Cu} \cdot \text{LCC4}^{\text{La}}$ . Unlike  $\text{Eu}^{3+}$ ,  $\text{La}^{3+}$  is non-luminescent and cannot accept energy from the naphthalene excited states due to empty  $f$  orbitals. On the nanosecond timescale, for  $\text{LCC4}^{\text{Eu}}$ , naphthalene fluorescence is characterized by a bi-exponential decay with a short lifetime of 2.8 ns and a longer one of 17.5 ns. In the case of the  $\text{Ag}^+$  and  $\text{Cu}^+$  complexes, naphthalene fluorescence, emanating from the species where the naphthalene is not engaged in a cation- $\pi$  interaction, shows similar lifetimes, as do all three lanthanum analogues. On the microsecond time scale, for  $\text{LCC4}^{\text{Eu}}$ , only bands characteristic of  $\text{Eu}^{3+}$  emission from the  $^5\text{D}_0$  level are detected, with a rise time of  $42 \pm 6 \mu\text{s}$ . On the contrary, for  $\text{Ag} \cdot \text{LCC4}^{\text{Eu}}$  on this timescale, besides the rise and decay of  $\text{Eu}^{3+}$  luminescence, an additional broad decaying emission is observed above 480 nm (Figure 4A), which was attributed to naphthalene triplet emission on the basis of its spectrum and decay time in the  $\mu\text{s}$  scale ( $110 \pm 15 \mu\text{s}$ , Table 1). Interestingly, naphthalene triplet emission decay is synchronous with  $\text{Eu}^{3+}$  luminescence rise (Figure 4B and Table 1). Similar behavior was observed for  $\text{Cu} \cdot \text{LCC4}^{\text{Eu}}$  (Figure 4B) but with a red-shifted naphthalene phosphorescence (above 500 nm) and shorter (but still synchronous) triplet emission decay and  $\text{Eu}^{3+}$  luminescence rise (ca.  $25 \mu\text{s}$ , Figure 4D). The broad naphthalene triplet emission could be detected for  $\text{Ag} \cdot \text{LCC4}^{\text{La}}$  and  $\text{Cu} \cdot \text{LCC4}^{\text{La}}$  (Figure 4E) but decay times are longer than those of the europium counterparts (Table 1). It was also detected for metal-free  $\text{LCC4}^{\text{La}}$  (Figure 4E, black). The clear correlation between naphthalene triplet emission decay and  $\text{Eu}^{3+}$  luminescence rise and the shorter characteristic times in the case of the  $\text{Eu}^{3+}$  compounds compared to  $\text{La}^{3+}$  indicate that the naphthalene( $T_1$ ) is transferring energy to  $\text{Eu}^{3+}$ . The energy of naphthalene( $T_1$ ) was estimated to be 21 500, 20 800 and 19 100  $\text{cm}^{-1}$  for  $\text{LCC4}^{\text{La}}$ ,  $\text{Ag} \cdot \text{LCC4}^{\text{La}}$  and  $\text{Cu} \cdot \text{LCC4}^{\text{La}}$ , respectively, by taking the wavelength of half-maximum. The value obtained for  $\text{LCC4}^{\text{La}}$  is in good agreement with the one (21 300  $\text{cm}^{-1}$ ) tabulated for 1-methylnaphthalene.<sup>41</sup> Importantly, both  $\text{Ag}^+$  and  $\text{Cu}^+$  lower the  $T_1$  state of the antenna engaged in a cation- $\pi$  interaction by 700 and 2400  $\text{cm}^{-1}$ , respectively (Figure 5). These values are similar to those measured in the case of  $\text{LCC1}^{\text{Tb}}$  (600 and 2300  $\text{cm}^{-1}$ , respectively).<sup>31</sup>



**Figure 4.** Time-resolved spectroscopy with streak-camera detection ( $\lambda_{\text{ex}} = 266$  nm). (A) Time-resolved emission spectra of Ag-LCC4<sup>Eu</sup>. (B) Evolution of the naphthalene triplet emission (integrated from 495 to 535 nm) and europium  $^5D_0 \rightarrow ^7F_1$  emission (595 nm). The solid lines correspond to the fit that yielded  $\tau_{\text{Nap}}^p = 110$   $\mu\text{s}$  and  $\tau_{\text{Eu}}^{\text{rise}} = 105$   $\mu\text{s}$ . (C) Time-resolved emission spectra of Cu-LCC4<sup>Eu</sup>. (D) Evolution of the naphthalene triplet emission (integrated from 500 to 550 nm) and of the deconvoluted europium  $^5D_0 \rightarrow ^7F_4$  emission (680-710 nm). The solid lines correspond to the fit that yielded  $\tau_{\text{Nap}}^p = 24$   $\mu\text{s}$  and  $\tau_{\text{Eu}}^{\text{rise}} = 25$   $\mu\text{s}$ . (E) Naphthalene phosphorescence emission spectra of LCC4<sup>La</sup> (black), Cu-LCC4<sup>La</sup> (red) and Ag-LCC4<sup>La</sup> (blue). Solutions were prepared in HEPES buffer (10 mM, pH 7.5).



**Figure 5.** Jablonski-Perrin diagram of LCC4<sup>Eu</sup> showing pertinent photophysical processes ( $ISC$  = intersystem crossing,  $EET$  = electronic energy transfer) and influence of  $\text{Ag}^+$  (blue) and  $\text{Cu}^+$  (red).

As naphthalene( $T_1$ ) is the energy donor state in the sensitization process, the sensitization efficiency  $\eta_{\text{sens}}$  can be expressed as the product of the triplet quantum yield  $\Phi_T$  (also called the intersystem crossing yield) and of the energy transfer efficiency  $\eta_{\text{ET}}$  (Equation 3).

$$\eta_{\text{sens}} = \Phi_T \times \eta_{\text{ET}} \quad (3)$$

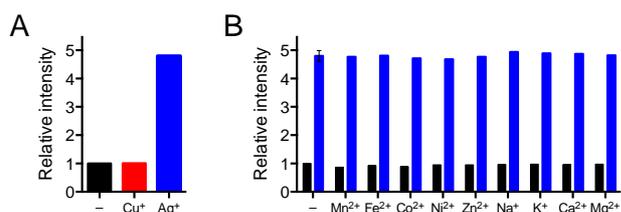
The difference in  $\eta_{\text{sens}}$  observed between Cu·LCC4<sup>Eu</sup> and Ag·LCC4<sup>Eu</sup> may thus be the result of a difference in intersystem crossing efficiency or energy transfer efficiency or both. Naphthalene derivatives have rather high  $\Phi_T$ . For instance, for naphthalene and 1-methylnaphthalene the value of  $\Phi_T$  is 0.75 and 0.58, respectively. A similar value is expected for LCC4<sup>Eu</sup>. As the cation- $\pi$  interaction can enhance ISC,<sup>46</sup>  $\Phi_T$  might be even higher for its Cu<sup>+</sup> and Ag<sup>+</sup> complexes. Considering the values of  $\eta_{\text{sens}}$  determined above (Table 1) and assuming  $\Phi_T$  values varying between 0.6 and 1, the value of  $\eta_{\text{ET}}$  falls in the range 0.47–0.78 for Ag·LCC4<sup>Eu</sup> but drops to 0.10–0.16 for Cu·LCC4<sup>Eu</sup>. This means that the energy transfer is significantly less efficient for Cu than for Ag. Considering the energy transfer, a collisional / electron exchange (Dexter) mechanism is the most widely-evoked pathway in antenna-to-lanthanide transfer.<sup>36,47–49</sup> A prerequisite for such a pathway would be orbital contact between the naphthalene donor and the europium acceptor, either through preorganization or close approach via dynamic excited state folding. For structural reasons this transfer may be anticipated to be disfavored in the current case. As such, an alternative non-radiative pathway, which we can tentatively consider would be a dipole-dipole (Förster) mechanism. It is often proposed as an alternative mechanism to Dexter one for Ln sensitization. Some examples of Förster resonance energy transfer involving triplet states have been recently reported.<sup>50,51</sup> Equation (4) links  $\eta_{\text{ET}}$  to the lifetime of the donor state in the presence ( $\tau_{\text{DA}}$ ) and absence ( $\tau_{\text{D}}$ ) of the acceptor in the case of a resonant energy transfer.<sup>52</sup>

$$\eta_{\text{ET}} = 1 - \tau_{\text{DA}} / \tau_{\text{D}} \quad (4)$$

Values of  $\eta_{\text{ET}}$  calculated from  $\tau_{\text{Nap}}^{\text{P}}$  values of Eu (for  $\tau_{\text{DA}}$ ) and La (for  $\tau_{\text{D}}$ ) compounds in Table 1 are  $0.64 \pm 0.10$  (considering the 10 % error margin on  $\tau$  values) and  $0.25 \pm 0.14$  for Ag and Cu, respectively (the averaged lifetime  $\langle \tau_{\text{Nap}}^{\text{P}} \rangle$  was used as  $\tau_{\text{D}}$  for Ag·LCC4<sup>La</sup>). This is in good agreement with the range of  $\eta_{\text{ET}}$  determined above. A good overlap between donor emission and acceptor absorption is required for efficient energy transfer. Eu<sup>3+</sup> has three possible accepting excited states whose population from the <sup>7</sup>F<sub>0</sub> ground state match the energy range of naphthalene(T<sub>1</sub>) emission (14 000 – 22 000 cm<sup>-1</sup>): <sup>5</sup>D<sub>0</sub> (17 250 cm<sup>-1</sup>), <sup>5</sup>D<sub>1</sub> (19 000 cm<sup>-1</sup>) and <sup>5</sup>D<sub>2</sub> (21 450 cm<sup>-1</sup>). However, from the selection rules, <sup>7</sup>F<sub>0</sub> → <sup>5</sup>D<sub>0</sub> transition is formally not allowed ( $\Delta J = 0$ ) and <sup>7</sup>F<sub>0</sub> → <sup>5</sup>D<sub>1</sub> transition is only Dexter-allowed ( $\Delta J = 1$ ). On the contrary, <sup>7</sup>F<sub>0</sub> → <sup>5</sup>D<sub>2</sub> transition is Förster-allowed ( $\Delta J = 2$ ). Therefore, naphthalene(T<sub>1</sub>) → Eu(<sup>5</sup>D<sub>2</sub>) energy transfer appears to be the most favored pathway for LCC4<sup>Eu</sup> and Ag·LCC4<sup>Eu</sup>, as naphthalene(T<sub>1</sub>) is almost resonant with Eu(<sup>5</sup>D<sub>2</sub>). In the case of Cu·LCC4<sup>Eu</sup>, for which naphthalene(T<sub>1</sub>) is 2 300 cm<sup>-1</sup> below Eu(<sup>5</sup>D<sub>2</sub>), energy transfer to Eu(<sup>5</sup>D<sub>2</sub>) is unlikely and the accepting state should rather be <sup>5</sup>D<sub>0</sub> or <sup>5</sup>D<sub>1</sub>. This is possible because selection rules can be relaxed due to thermal population of <sup>7</sup>F<sub>1</sub> (ca. 30 % at room temperature)<sup>36,53</sup> and  $J$ -mixing of <sup>7</sup>F<sub>0</sub> and <sup>7</sup>F<sub>2</sub>. However, a less efficient energy transfer is expected in this case. Therefore, it seems that the differential modulation of the naphthalene(T<sub>1</sub>) energy that occurs upon establishment of the cation- $\pi$  interaction can play a major role in the Ag<sup>+</sup> / Cu<sup>+</sup> discrimination observed with this Eu<sup>3+</sup>-based probe. Figure 5 summarizes the proposed sensitization pathways.

**Practical insight into Ag<sup>+</sup> detection with LCC4<sup>Eu</sup>.** With LCC1<sup>Tb</sup>, we noticed that the red-shift of the antenna  $\pi$ - $\pi^*$  transition could be used advantageously to magnify the probe response.<sup>31</sup> For instance, while absorption of LCC1<sup>Tb</sup> and its Cu<sup>+</sup> and Ag<sup>+</sup> complexes are similar at 280 nm, which corresponds to the band maximum, the absorption of LCC1<sup>Tb</sup> was much lower than that of the complexes at 310 nm. Therefore, upon excitation at 310 nm, due to the combination of higher absorption and enhanced ISC, Cu<sup>+</sup> and Ag<sup>+</sup> caused a ca. 55-fold increase of Tb<sup>3+</sup> emission compared to 6-fold when excitation was at 280 nm.<sup>31</sup> In the case of LCC4<sup>Eu</sup>, shifting the excitation to 305 nm (5 nm excitation slit) resulted in a 4.8-fold enhancement of Eu<sup>3+</sup> emission upon Ag<sup>+</sup> binding to LCC4<sup>Eu</sup> whereas Cu<sup>+</sup> did not induce any change in the intensity of the Eu<sup>3+</sup> emission (Figure 6A). Hence, under 305 nm excitation, LCC4<sup>Eu</sup> responds to Ag<sup>+</sup> but not to Cu<sup>+</sup>. In order to assess the metal preference

of the probe, we determined its  $\text{Ag}^+$  and  $\text{Cu}^+$ -binding constants,  $K_{\text{Ag}}$  and  $K_{\text{Cu}}$ , respectively, by competitive titrations with imidazole as previously described (see SI).<sup>31,42</sup> The binding of  $\text{Cu}^+$  proved to be much tighter than  $\text{Ag}^+$  ( $K_{\text{Cu}} = 10^{11.0}$  and  $K_{\text{Ag}} = 10^{8.1}$ ). As a consequence,  $\text{Ag}^+$  is not able to displace  $\text{Cu}^+$  from the  $\text{Cu}\cdot\text{LCC4}^{\text{Eu}}$  complex but  $\text{Cu}^+$  is able to displace  $\text{Ag}^+$ . This was confirmed by successive addition of  $\text{Ag}^+$  and  $\text{Cu}^+$  addition to  $\text{LCC4}^{\text{Eu}}$  (and *vice versa*): under 305 nm excitation, addition of excess  $\text{Cu}^+$  to a solution of  $\text{Ag}\cdot\text{LCC4}^{\text{Eu}}$  brings the emission level of  $\text{LCC4}^{\text{Eu}}$ , while addition of excess  $\text{Ag}^+$  to a solution of  $\text{Cu}\cdot\text{LCC4}^{\text{Eu}}$  does not induce any increase of Eu emission. The influence of other physiological cations was also investigated: among  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , none of them affected the  $\text{Ag}^+$  response (Figure 6B). Therefore, with  $\text{LCC4}^{\text{Eu}}$ , selective detection of  $\text{Ag}^+$  is possible in the presence of  $\text{Cu}^+$ , provided that the concentration of the probe is higher than the total concentration of these two cations. At 10  $\mu\text{M}$   $\text{LCC4}^{\text{Eu}}$ , a limit of detection ( $3\sigma$ ) of 9 nM was determined for  $\text{Ag}^+$ .



**Figure 6.** (A) Compared time-gated  $\text{Eu}^{3+}$  emission (delay = 100  $\mu\text{s}$ ) of  $\text{LCC4}^{\text{Eu}}$  (black),  $\text{Cu}\cdot\text{LCC4}^{\text{Eu}}$  (red) and  $\text{Ag}\cdot\text{LCC4}^{\text{Eu}}$  (blue) under 305 nm excitation. (B) Selectivity diagram showing the integrated time-gated  $\text{Eu}^{3+}$  emission ( $\lambda_{\text{ex}} = 305$  nm) of  $\text{LCC4}^{\text{Eu}}$  (10  $\mu\text{M}$ ) before (black) and after (blue) addition of 1.5 eq.  $\text{Ag}^+$  in the presence of various cations (from left to right: none,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  (10  $\mu\text{M}$ ),  $\text{Na}^+$ ,  $\text{K}^+$  (1 mM),  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (1 mM)). Solutions were prepared in HEPES buffer (10 mM, pH 7.5).

## Conclusion

$\text{LCC4}^{\text{Eu}}$ , a novel member of the LCC family of luminescent probes inspired by the copper chaperone CusF, is able to bind  $\text{Cu}^+$  and  $\text{Ag}^+$  but respond spectroscopically to  $\text{Ag}^+$  only when excited at 305 nm. As for other members of the family based on a  $\text{Tb}^{3+}$  emitter and on a tryptophan antenna,  $\text{LCC4}^{\text{Eu}}$  is able to establish a cation- $\pi$  interaction between the bound  $\text{Ag}^+/\text{Cu}^+$  cation and the naphthalene antenna, which modifies the properties of the antenna. In particular, the energy of the triplet excited state is lowered by the cation- $\pi$  interaction and to a larger extent for  $\text{Cu}^+$  than for  $\text{Ag}^+$ . A detailed time-resolved spectroscopic study has elucidated the sensitization pathway, which involves electronic energy transfer from the naphthalene triplet excited state to europium excited states. It has also revealed a significant difference in the energy transfer efficiency for these two ions. Indeed, we propose that the more efficient energy transfer observed for  $\text{Ag}^+$  may arise from a good match between the energy of the donor triplet state and the energy on the europium  $^5\text{D}_2$  state (allowed process), whereas for  $\text{Cu}^+$  electronic energy transfer to  $^5\text{D}_1$  or  $^5\text{D}_0$  excited state could occur (non-allowed process). This is to our knowledge, the first time that the ladder-like arrangement of energy levels of lanthanides is used to create a selective responsive luminescent probe.

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