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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 Séneque*

Abstract: A prototype luminescent turn on probe for Cu⁺ (and Ag⁺) is described, harnessing a selective binding site (log Kass = 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. It is required for various biological processes and its homeostasis is finely regulated in living organisms. Misregulation of copper can lead to various diseases (e.g., Menkes, Wilson, and Parkinson diseases). 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. 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. 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. 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. 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 second range) and that allows time gated detection to suppress background fluorescence contributions.

Our probe structure (Figure 1A) is inspired by the metal binding site of the metallo chaperone CusF which is part of the CusCFBA system responsible for copper or silver detoxification in gram negative bacteria. 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). Indeed, the indole ring of the tryptophan establishes a cation π interaction with the metal ion that red shifts the π* transition of the indole and fully quenches its fluorescence. 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.

Ln³⁺ ions have desirable luminescence properties that make them prime candidates for biological applications. 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). One of the main pathways for lanthanide sensitization involves electronic energy transfer (EET) from the excited triplet state of the antenna to the...
emissive Ln³⁺ ion (Figure 1C).⁷,⁸ Among natural amino acids, tryptophan is an efficient antenna for Tb³⁺ sensitization.⁹ Therefore, we designed a probe based, on the one hand, on a peptide mimicking the Cu²⁺ binding site of CusF providing high affinity and selectivity and, on the other hand, on a Tb³⁺ complex as signaling unit. We reasoned that we could benefit from an ISC enhancement due to a cation π interaction between Cu²⁺ and the tryptophan to increase the population of the tryptophan excited triplet state and, subsequently, increase also the population of Tb³⁺ excited states to transduce the copper binding event into an increased Tb³⁺ emission.

The peptidic probe, namely LCC1³⁺ (Figure 1A and B), comprises 1) the 16 amino acid sequence of the Cu²⁺ binding loop of CusF, which includes the four metal binding amino acids (see above), 2) an Aib-Pro dipeptide to cyclize the loop and reorganize it, and 3) a DOTA macrocycle grafted on the amine side chain of a lysine to bind a Tb³⁺ ion. LCC1³⁺ was synthesized by a combination of solid phase and solution reactions (Supporting Information, SI). The metal binding properties of LCC1³⁺ were investigated under argon by circular dichroism (CD) spectroscopy (Figure 2). The titration of LCC1³⁺ in phosphate buffer (10 mM, pH 7.5) by Cu²⁺, generated in situ by reduction of CuSO₄ by NH₄OH, shows a linear evolution of the CD signal which reaches a plateau in the presence of 1.0 equiv Cu²⁺, indicating the formation of a 1:1 complex, Cu²⁺LCC1³⁺, which was confirmed by ESI MS analysis (SI). The same behavior is observed with Ag⁺ due to the similarity between these two ions. LCC1³⁺ is not able to bind any of the other physiologically relevant metal ions [Na⁺, K⁺ (100 mM), Ca²⁺, Mg²⁺ (10 mM), Mn²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, and Zn²⁺ (30 μM)] as demonstrated by the absence of change in the CD spectrum (Figure 2B). It is noteworthy that LCC1³⁺ can bind Cu²⁺ but not Cu²⁺.

The coordination of Cu²⁺ 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 Cu²⁺ or Ag⁺ is associated with a red shift of the indole ππ* transition absorption band (Figure 3A) and a partial quenching of its fluorescence (Figure 3D). This suggests the presence of a cation π interaction in Cu²⁺LCC1³⁺ and Ag⁺LCC1³⁺ as observed for CusF.

The Tb³⁺ luminescence properties were investigated by exciting the tryptophan antenna at 280 nm, which corresponds to the maximum absorption of the tryptophan indole ππ* transition in LCC1³⁺. Titrations of LCC1³⁺ by Cu²⁺ or Ag⁺ show that the formation of Cu²⁺LCC1³⁺ and Ag⁺LCC1³⁺ is associated with an increase of the Tb³⁺ emission. The Tb³⁺ luminescence excitation spectra of LCC1³⁺, Cu²⁺LCC1³⁺, and Ag⁺LCC1³⁺ (Figure 3E) correspond to the ππ* transition observed in the electronic absorption spectra, indicating that the tryptophan acts as an antenna for Tb³⁺ in LCC1³⁺ and its Cu²⁺ or Ag⁺ complexes. Interestingly, the Tb³⁺ excitation spectra (λem = 545 nm) of Cu²⁺LCC1³⁺ and Ag⁺LCC1³⁺ are red shifted compared to LCC1³⁺ (Figure 3E), but the tryptophan fluorescence excitation spectra (λem = 355 nm) are not

**Figure 2.** A) CD titration of LCC1³⁺ (16 μM) in phosphate buffer (10 mM, pH 7.5) by Cu²⁺ generated in situ by reduction of CuSO₄ by NH₄OH (2 mM). The inset shows the evolution of the CD signal at 200 nm (○) and 225 nm (●). B) CD spectra of LCC1³⁺ (18 μM) before and after addition of various metal ions.
(Figure 3C). This is consistent with two kinds of tryptophan indole that are present in solution when CuI or AgI are bound to LCC1Tb, 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 TbI\( ^{3+} \) luminescence. As the cation π interaction in CuI\( ^{3+} \) 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 TbI\( ^{3+} \) emission spectra of LCC1Tb, Cu\( I \)-LCC1Tb, and Ag\( I \)-LCC1Tb with excitation at 280 nm. Cu\( I \) and Ag\( I \) enhance the TbI\( ^{3+} \) emission six times with respect to LCC1Tb and thus, LCC1Tb acts as a turn on luminescence probe for these cations. Moreover, the red shift of the indole \( \pi \pi^* \) transition can be used to increase the contrast of the probe: TbI\( ^{3+} \) luminescence enhancement factors of 58 and 52 were obtained for Cu\( I \) and Ag\( I \), respectively, by exciting the probe at 310 nm (see SI for rationalization of this wavelength choice) instead of 280 nm (Figure 3G). Furthermore, the TbI\( ^{3+} \) emission of LCC1Tb and Cu\( I \)-LCC1Tb is not affected by the presence of physiological cations (Figure 3H). Overall, LCC1Tb is a high contrast turn on luminescence probe for the time gated detection of Cu\( I \) among physiological cations. It is also able to detect Ag\( I \). The binding constants for Cu\( I \) and Ag\( I \), determined by competition experiments with imidazole are \( 10^{9} \text{M}^{-1} \) and \( 10^{3.3} \text{M}^{-1} \), respectively (SI). The \( K_M \) for other physiological cations is estimated to be below \( 10 \text{M}^{-1} \).

The enhancement of TbI\( ^{3+} \) luminescence upon Cu\( I \) or Ag\( I \) binding may originate from 1) a reduction of the number of water molecules bound to TbI\( ^{3+} \), 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 TbI\( ^{3+} \) ion and/or a change in the orientation of the antenna with respect to TbI\( ^{3+} \). Concerning the latter point, changes in CD upon Cu\( I \) or Ag\( I \) binding may arise from conformational changes but also from the contribution of ligand metal charge transfer transitions. The NMR spectra of LCC1\( ^{14} \), the diamagnetic homologous probe in which the TbI\( ^{3+} \) ion is replaced by a LaI\( ^{3+} \) ion, and of its Cu\( I \) or Ag\( I \) complexes display broad resonances that preclude any structural analysis, unfortunately. To elucidate the mechanism of the TbI\( ^{3+} \) luminescence enhancement and quantify fast processes, the emission of the probe was characterized in detail. Regarding TbI\( ^{3+} \) emission, Cu\( I \) or Ag\( I \) binding has almost no effect on the luminescence lifetime (\( \approx 1.9 \text{ ms} \)). Measurements of luminescence lifetime values in H\( _2 \)O and D\( _2 \)O additionally showed that only one water molecule is coordinated to the TbI\( ^{3+} \) ion in LCC1Tb and its Cu\( I \) and Ag\( I \) complexes (SI).\( ^{7,19,21} \) Therefore, the enhancement of TbI\( ^{3+} \) emission is not due to a change in the TbI\( ^{3+} \) 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 LCC1Tb 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 Cu\( I \)-LCC1Tb and Ag\( I \)-LCC1Tb, 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).

Figures 4A and 4B compare the emission spectra of LCC1Tb and Cu\( I \)-LCC1Tb 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 TbI\( ^{3+} \) emission at 545 nm (square; the tryptophan phosphorescence has been subtracted) for Cu\( I \)-LCC1Tb. The solid and dashed lines correspond to the respective fits which yielded \( \tau = 16 \pm 2 \text{ µs} \) for both phosphorescence decay and TbI\( ^{3+} \) emission grow in. D) Phosphorescence emission spectra of Cu\( I \)-LCC1\( ^{14} \) (dashed line) and Ag\( I \)-LCC1\( ^{14} \) (dotted line).

**Table 1:** Decay lifetimes of tryptophan emission and rise time of TbI\( ^{3+} \) emission for LCC1Tb, Cu\( I \)-LCC1Tb, and Ag\( I \)-LCC1Tb. Error on \( \tau \) values is estimated at 10%.

| Compound       | Tryptophan fluorescence decay (ns) | Tryptophan phosphorescence decay (µs) | TbI\( ^{3+} \) luminescence rise (µs) |
|----------------|-----------------------------------|--------------------------------------|--------------------------------------|
| LCC1Tb         | 0.9 (13%)                         | not detected                         | 23                                   |
| Cu\( I \)-LCC1Tb | 0.7 (16%)                         | 16                                   | 16                                   |
| Ag\( I \)-LCC1Tb | 0.9 (17%)                         | 19                                   | 18                                   |

The solid and dashed lines correspond to the respective fits which yielded \( \tau = 16 \pm 2 \text{ µs} \) for both phosphorescence decay and TbI\( ^{3+} \) emission grow in.
the rise of Tb\(^{3+}\) emission (\(\tau = 16\ \mu s\), Figure 4C). The broad decaying emission band is clearly seen with the Cu\(^{2+}\) complex of LCC1\(^{1+}\) (Figure 4D). Due to its lifetime in the \(\mu s\) 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\(_t\)) to Tb\(^{3+}\)(T\(_D\)) energy transfer. Ag\(^{+}\)-LCC1\(^{1-}\) (Ln = Tb or La) behaves in the same way as Cu\(^{2+}\)-LCC1\(^{1-}\) but with blue shifted tryptophan phosphorescence emission compared to the analogous copper complex (Figure 4D). The above results show that both Cu\(^{2+}\) and Ag\(^{+}\) binding to LCC1\(^{1-}\) 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 \(\pi\) interaction, this is compatible with an ISC enhancement promoted by the cation \(\pi\) interaction.\(^{[15]}\) Therefore, the binding of Cu\(^{2+}\) or Ag\(^{+}\) to LCC1\(^{1-}\) through a cation \(\pi\) interaction favors ISC and increases the population of the excited triplet state of the tryptophan. Hence, more energy can be transferred to the Tb\(^{3+}\)-T\(_D\) excited state, which in turn emits more. Although it cannot be excluded that conforma
tional changes may be, in part, responsible for Tb\(^{3+}\) luminescence enhancement, the spectroscopic data presented here point to a major role of the cation \(\pi\) interaction that is established between the metal ion and the tryptophan indole. In addition to the global ISC enhancement, the cation \(\pi\) interaction with Cu\(^{2+}\) and Ag\(^{+}\) shifts the tryptophan triplet excited state emission but to a different extent. Indeed, comparison of the room temperature phosphorescence spectra of Cu\(^{2+}\)-LCC1\(^{1-}\) and Ag\(^{+}\)-LCC1\(^{1-}\) (SI) with those reported in the literature for proteins\(^{[23,24]}\) show that Cu\(^{2+}\) 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\(^{2+}\) 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 \(\pi\) 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 \(\pi\) interactions may be formed with several cations (e.g., Cu\(^{2+}\), Ag\(^{+}\), Cu\(^{2+}\), Hg\(^{2+}\), and Pt\(^{2+}\)), this work paves the way for the design of lanthanide based luminescent probes for Cu\(^{2+}\) or toxic cations with desirable emission properties relying on a mechanism other than metal induced PET quenching.

**Keywords:** cation \(\pi\) interactions · copper · luminescence · peptides · terbium

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