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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 S  n  que*

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 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 (nano

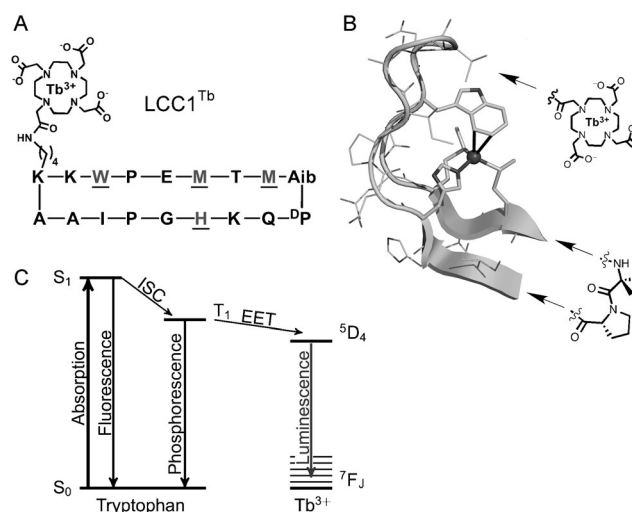


Figure 1. A) Amino acid sequence of LCC1^{Tb}, chelating moieties are underlined. B) Principle of the probe design based on the X ray structure of the Cu^+ binding loop of CusF.^[14] C) Simplified Jablonski Perrin diagram of LCC1^{Tb} probe and pertinent photophysical processes.

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]

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

[*] M. Isaac, Dr. A. Roux, Dr. O. S  n  que
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-

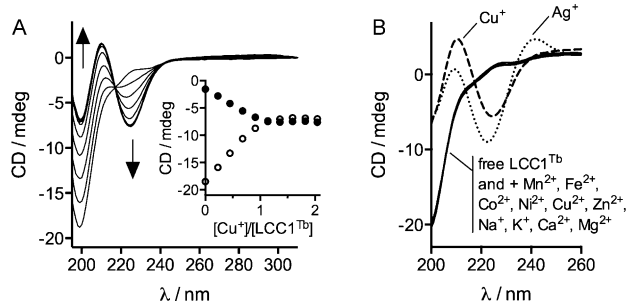


Figure 2. A) CD titration of LCC1^{Tb} (16 μM) in phosphate buffer (10 mM, pH 7.5) by Cu^+ generated in situ by reduction of CuSO_4 by NH_2OH (2 mM). The inset shows the evolution of the CD signal at 200 nm (\circ) and 225 nm (\bullet). B) CD spectra of LCC1^{Tb} (18 μM) before and after addition of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

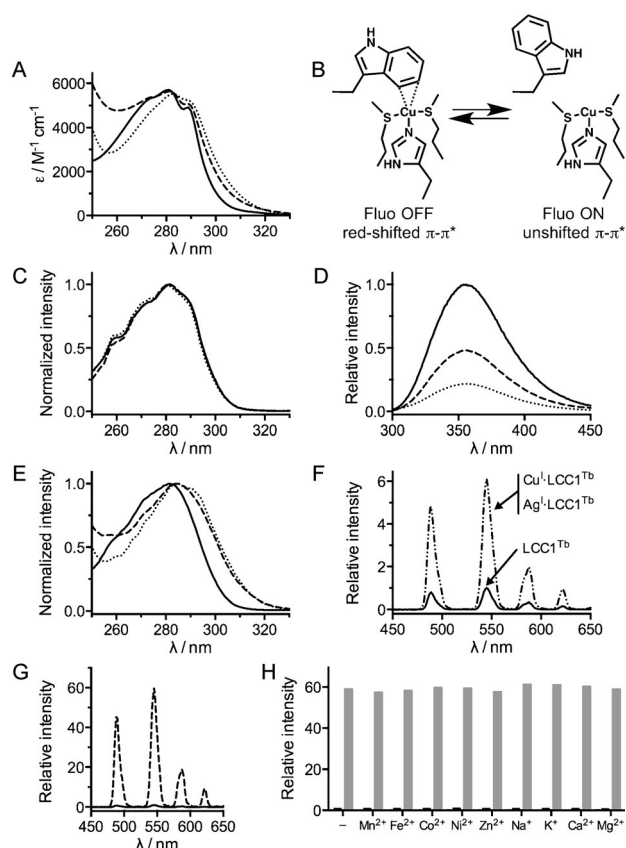


Figure 3. Steady state spectroscopic characterization of LCC1^{Tb} (solid line), $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$ (dashed line), and $\text{Ag}^{\text{I}}\text{-LCC1}^{\text{Tb}}$ (dotted line). A) Electronic absorption spectra. B) Representation of possible fluorescent and non fluorescent 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 ($\lambda_{\text{em}} = 545$ 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 ($\lambda_{\text{em}} = 355$ 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 ($\tau \approx 1.9$ 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 $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$ 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	

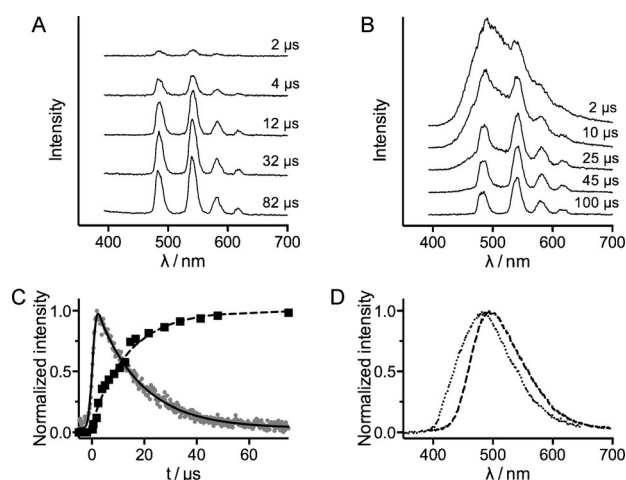


Figure 4. Time resolved emission spectroscopy in degassed buffer solutions. Spectra were recorded in time gated mode using a streak camera ($\lambda_{\text{ex}} = 266$ nm). Time resolved emission spectra of A) LCC1^{Tb} and B) $\text{Cu}^{\text{I}}\text{-LCC1}^{\text{Tb}}$ recorded several μs after the 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 $\tau = 16 \pm 2$ μs 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 ($\lambda_{\text{ex}} = 266$ 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 Tb³⁺ 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 Tb³⁺ sensitization occurs through a tryptophan(T₁) to Tb³⁺(⁵D₄) energy transfer. Ag⁺-LCC1^{Ln} (Ln = Tb or La) behaves in the same way as Cu⁺-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 Tb³⁺ 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 Tb³⁺-⁵D₄ excited state, which in turn emits more. Although it cannot be excluded that conformational changes may be, in part, responsible for Tb³⁺ 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⁺-LCC1^{La} and Ag⁺-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 Tb³⁺ 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 Tb³⁺ 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⁺, Cd²⁺, Hg²⁺, and Pb²⁺), 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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Keywords: cation π interactions · copper · luminescence · peptides · terbium

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