Lanthanide 8-hydroxyquinoline-based podates with efficient emission in the NIR range

Daniel Imbert,* Steve Comby, Anne-Sophie Chauvin and Jean-Claude G. Bünzli

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The novel hydroxyquinoline-containing tetrapodal ligand forms water soluble and stable chelates and is a good sensitizer of the NIR luminescence of its Nd^{III} and Yb^{III} complexes; its easy synthesis opens the way for potential biomedical applications.

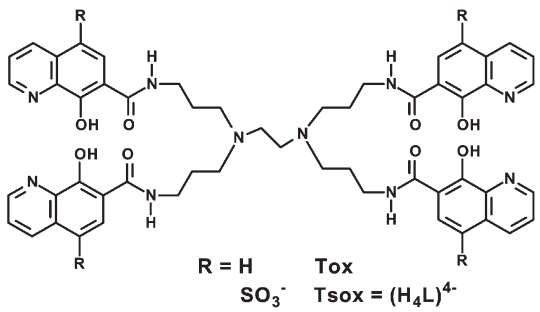
Besides being major components of diode lasers and optical fibers for telecommunication,[1] near-infrared lanthanide-based emitters (900–1600 nm) are presently attracting considerable interest in biomedical analysis[2] in view of their potential for non-invasive *in vivo* imaging. Recently, there have been attempts to use NIR tomography to examine deep tissues, with the idea of developing highly sensitive methods for early detection of cancer.[3] In this respect, receptor-targeted optical imaging of tumours is gaining in interest because haemoglobin has low absorption coefficients above 650 nm while the absorption of water, a major component of biological tissues, diminishes drastically below 900 nm.[4] Sensitivity of the luminescent probes can be enhanced in two ways. Firstly by using quenched precursors that can be activated *in vivo* by a suitable biochemical reaction.[4] Secondly, by taking advantage of time-resolved luminescence measurements (TRL)[5] to separate the probe luminescence from the unwanted background luminescence. Lanthanide ions which feature very typical, narrow-band emission spectra and often long-lived excited states are ideal probes for TRL experiments, especially when inserted into chelates when they also display a large Stokes shift upon ligand excitation.[6] Immunoassays, often based on the highly luminescent Eu^{III} and Tb^{III} ions,[7] are now common analytical assays in hospital and medical laboratories.[5] On the other hand, NIR-luminescent Ln^{III} ions such as Nd^{III} (emitting in three distinct spectral ranges: 870–920, 1060–1090, and 1320–1390 nm) or Yb^{III} (980–1030 nm), although potentially interesting in view of their emission wavelengths, have two intrinsic drawbacks: (i) low sensitization of the metal-centered luminescence due to a small energy gap between their excited and ground-state levels, favoring efficient non-radiative processes, and (ii) relatively short lifetimes (ns to μs) which limit the efficiency of time-resolved detection. Recently, we have shown how the latter limitation can be overcome by introducing the Ln^{III} ion into a 3d–4f bimetallic edifice in which population of the Ln^{III} excited state is controlled by a long-lived emitting Cr^{III} ion.[8] Here we address the first problem, namely the design of a chelating agent able to efficiently transfer its energy onto the metal ion.

*daniel.imbert@epfl.ch

The overall quantum yield of a luminescent Ln^{III} chelate, upon ligand excitation, is given by the following equation:

$$Q_{Ln}^{L} = \eta_{sens} \cdot Q_{Ln}^{Ln} = \eta_{isc} \cdot \eta_{et} \cdot Q_{Ln}^{Ln} \quad (1)$$

where η_{sens} is the efficacy of the ligand to sensitize the metal-centered luminescence and Q_{Ln}^{Ln} the intrinsic quantum yield of the lanthanide ion (*i.e.* upon direct excitation); η_{isc} and η_{et} are the yields for intersystem crossing and ligand-to-metal transfer, respectively. The intrinsic quantum yield is much influenced by the presence of high energy vibrations in the inner coordination sphere (and also in the neighbourhood of the ion); since biomedical analyses are performed in water, it is essential that the targeted chelating agent minimizes the presence of these groups and, therefore offers a good protection against solvent interaction. With ions such as Nd^{III} and Yb^{III}, the reported quantum yields in water are usually smaller than 0.1%,[2] with, to our knowledge, the only exception of a bimetallic Yb^{III} complex with 1,10-phenanthroline substituted in the 2,9 positions by benzo-azacyclic moieties and having $Q_{Ln}^{L} = 0.53\%$ in water.[9]



Our ligand design relies on 1,2-diamino-ethane fitted with four chromophoric 8-hydroxyquinoline chelating arms. It was obtained by coupling 1,1,2,2-tetraaminopropyl-1,2-diaminoethane with 7-carboxy-8-hydroxyquinoline to produce Tox (yield: 32%) which was subsequently sulfonated with oleum to give Tsox (yield: 83%; thereafter $(H_4L)^{4-}$).† The 10 pK_as of $(H_4L)^{4-}$, ranging from 1.82 to 12.05, have been determined by a combination of potentiometric and UV-vis titrations, as well as the stability constants of the resulting chelates with Eu^{III}. In the pH range 7–9, the major species is the 1 : 1 $[Eu(H_2L)]^{3-}$ species (> 95% at 10^{-5} M); its conditional stability constant at physiological pH and $I = 0.1$ M (KCl) amounts to $\log \beta_{11} = 8.1 \pm 0.1$. This translates into a pEu value of 15.6, as compared to 19.6 for diethylenetriamine pentaacetic acid (dtpa) (computed from the stability constants).[10] Provided the kinetic stability is large enough (tests are in progress),

chelates based on Tsox therefore possess sufficient stability in water for potential *in vivo* applications.

The photophysical properties of Tsox and its 1 : 1 chelates with Nd^{III} and Yb^{III} are illustrated in Fig. 1. Both the free and complexed forms of $(H_2L)^{6-}$ (as measured on the non-luminescent La^{III} and Lu^{III} complexes) present a broad singlet state emission in the range 400–500 nm, while a long-lived triplet state emission is seen at low temperature as a broad and structured band extending from 500 to 650 nm (lifetime: 42 $\pm$ 1 ms). Energy of the $^3\pi\pi^*$ 0-phonon transition is around 19 000 cm^{-1}, leading to a rather weak sensitization of the Eu^{III} luminescence (Q^L_{Eu} = 0.02%, as determined with respect to tris(dipicolinate) $[Eu(dpa)_3]^{3-}$[11]).

However, a sizeable metal-centered NIR luminescence is seen in the Nd^{III}- and Yb^{III}-containing aqueous solutions (Fig. 1) while the ligand luminescence almost disappears, with only a faint emission from the singlet state (<10% compared with the free ligand). At room temperature and upon broad band excitation through the ligand levels, the luminescence spectrum of the Nd–Tsox complex displays three bands in the spectral ranges 845–947, 1000–1163 and 1274–1408 nm. They are assigned to transitions from the $^4F_{3/2}$ level to the $^4I_{9/2}$, $^4I_{11/2}$ and $^4I_{13/2}$ sublevels, respectively. Under the same conditions, the Yb–Tsox complex displays the typical Yb^{III} fluorescence with a sharp band at 976 nm assigned to the $^2F_{5/2} \rightarrow {}^2F_{7/2}$ transition and a broader vibronic component[12] at longer wavelength. The excitation spectra of the Nd^{III} and Yb^{III} chelates clearly demonstrate the antenna effect of the ligand with, for instance, the component at 364 nm matching the low-energy absorption band of the electronic spectra.

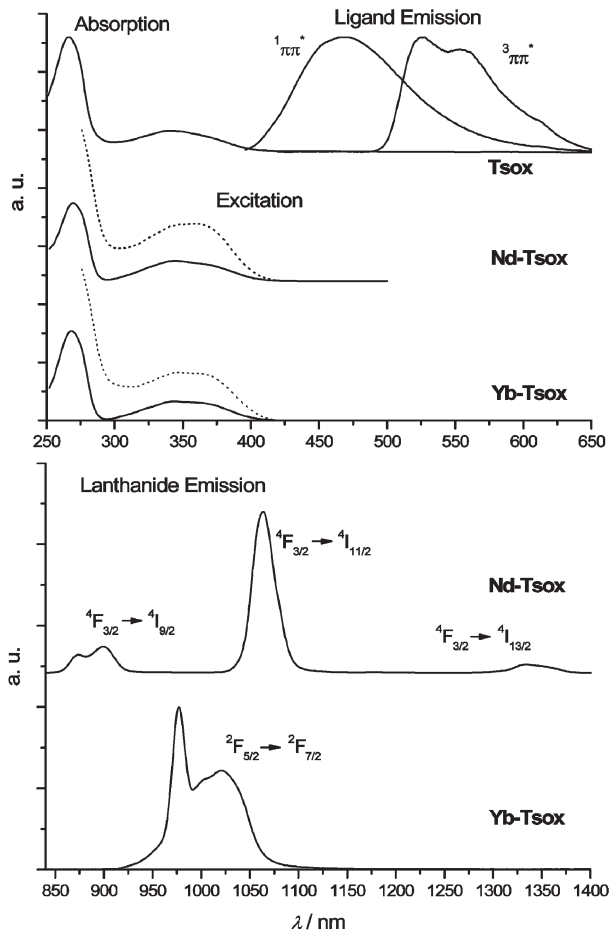


Fig. 1 (Top): Normalized absorption, emission (λ_{ex} = 344 nm) and excitation spectra (dotted line, λ_{an} = 1063 (Nd) and 976 nm (Yb)); (bottom): emission spectra in the NIR region (λ_{ex} = 344 nm) of the 1 : 1 chelates of Nd^{III} and Yb^{III}. All solutions in water at pH 7.4 and room temperature.

To quantify the capacity of the chromophoric subunits of Tsox to sensitize the luminescent properties of the NIR-emitting lanthanides, the absolute quantum yields of the chelates have been determined upon ligand excitation with respect to $Yb(TTA)_3$ in toluene (Q^L_{Yb} = 0.35%; TTA = thenoyltrifluoroacetylacetonate).[13] The absolute quantum yields of 6.2 $\times$ 10^{-5} M aqueous solutions amount to 0.02 and 0.18% for Nd and Yb, respectively. In deuterated water, the corresponding figures are more than five times larger (Nd: 0.1%; Yb: 0.81%). These quantum yields are sizeable with respect to published literature data (*vide supra*) particularly for Yb^{III} and for aqueous solutions in which the presence of proximate OH vibrations (and/or possibly coordinated water molecules(s)) induces a large quenching effect for the ions for which the energy gap is small.

Evaluation of the efficiency with which the ligand transfers energy to the metal ions, η_{sens} (eqn. 1), requires knowledge of the intrinsic quantum yield $Q^{Ln}_{Ln} = \tau_{obs}/\tau_{rad}$.[14] We have therefore determined the lifetimes of the $Nd(^4F_{3/2})$ and $Yb(^2F_{5/2})$ excited states upon excitation through the ligand levels (355 nm): they amount, in water, to 0.13(1) and 2.21(1) μs for Nd and Yb, respectively, while the corresponding figures in deuterated water are 0.58(2) and 10.0(1) μs, respectively. These lifetimes are in line with those published for polycarboxylate complexes derived from dtpa.[14] Moreover, calculation of the number of bound water molecules (q)[15] yields q = 0.1–0.2, pointing to the complete coordination of the four pendant arms of the podand which acts as an octadentate host. Literature data for τ_{rad} vary widely (by more than a factor 2), depending on the Ln^{III} host, so that only a rough estimate could been made for η_{sens} (> 50%). Given the quasi absence of ligand luminescence and the sizeable absolute quantum yields, Tsox appears to be both a very good sensitizer of the Yb^{III} luminescence in water, and a reasonable one of the Nd^{III} emission, as well as a highly protective host.

The overall efficiency of a luminescent probe is given by the factor $\varepsilon \times Q$, which takes into account the amount of light that can be harvested by the ligand. With molar absorption coefficients of 92 200 and 19 400 M^{-1} cm^{-1} at 267 and 344 nm, respectively, $\varepsilon \times Q$ = 166 and 35 M^{-1} cm^{-1} for the Yb–Tsox chelate, which compares favourably with the bimetallic complex cited above (113 and 87 M^{-1} cm^{-1} at 245 and 280 nm, respectively)[9] and with the Yb–dtpa chelate (116 M^{-1} cm^{-1} at 500 nm).[14]

In conclusion, we have shown that Tsox forms soluble and stable 1 : 1 chelates in water at physiological pH and that NIR luminescence from Yb^{III} (and Nd^{III}) is substantially sensitized. In addition, we were able to detect luminescence from the Er^{III} chelate in water. This opens astounding perspectives for the development of bio-probes based on these systems, since the synthesis of Tsox can be undertaken on a large scale effortlessly and derivatization of this chelating agent is also easily at hand.

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Daniel Imbert,* Steve Comby, Anne-Sophie Chauvin and Jean-Claude G. Bünzli
Laboratory of Lanthanide Supramolecular Chemistry, École Polytechnique Fédérale de Lausanne (EPFL), BCH 1405, 1015 Lausanne, Switzerland. E-mail: daniel.imbert@epfl.ch

Notes and references

† Characterization: Anal. calcd. for $C_{54}H_{56}N_{10}O_{20}S_4{\cdot}8H_2O$: C, 45.06; H, 5.05; N, 9.74. Found: C, 44.93; H, 4.75; N, 9.52. ^{1}H-NMR (400 MHz in DMSO-d_6): δ 1.99 (8H, m, CH_2), 3.26 (8H, m, CH_2), 3.46 (8H, m, CH_2), 3.52 (4H, m, CH_2), 7.85 (4H, dd, ArH), 8.44 (4H, s, ArH), 8.96 (4H, d, ArH), 9.31 (4H, t, NH), 9.45 (4H, d, ArH). IR (ATR): 3500–3100 cm^{-1} (O–H), (N–H); 1637 cm^{-1} (C=O), 1599, 1539 cm^{-1} (C=C), (C=N); ESI-TOF MS: 322.17 $[H_4L]^{4-}$ (calcd. 322.06), 663.27 $[H_6L + 2H_2O]^{2-}$ (calcd. 663.12).

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