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Water-Soluble Mitochondria-Specific Ytterbium Complex with Impressive NIR Emission

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Supporting Information

ABSTRACT: A water-soluble porphyrinato ytterbium complex linked with rhodamine B (Yb-**2**) showed mitochondria-specific subcellular localization and strong two-photon-induced NIR emissions (λ_{em} = 650 nm, porphyrinate ligand $\pi \rightarrow \pi^*$ transition; λ_{em} = 1060 nm, Yb(III) $^5F_{5/2} \rightarrow {}^5F_{7/2}$ transitions; σ_2 = 375 GM in DMSO) with an impressive Yb(III) NIR emission quantum yield (1% at λ_{ex} = 340 nm; 2.5% at λ_{ex} = 430 nm) in aqueous solution.

Luminescent lanthanide complexes have attracted increasing interest recently, particularly in the field of molecular imaging.[1] For cell and tissue imaging, there are several serious problems associated with most of the commercially available molecular imaging probes: toxic UV excitation (damages specimens and limits experimental duration), non-tissue-penetrating fluorescence signal, and autofluorescence.[2] Lanthanide ions are attractive alternatives to the more commonly used organic fluorophores because they exhibit long-lived luminescence, thus allowing their potential application as time-resolved imaging agents,[3] and their emission is hypersensitive to their coordination environments, making real-time responsive imaging possible.[4] Numerous studies have shown that time-resolved microscopic techniques using long-lived emissive lanthanide probes coupled with delayed signal acquisition can be a solution to the autofluorescence problem in in vitro imaging.[5] Recently, several terbium/europium complexes showing long-lived lanthanide ion luminescence sensitized by multidentate antenna ligands following single or multiphoton excitation have been reported.[6] Their visible emissions at λ_{em} < 650 nm, however, are not ideal in terms of tissue penetration for development as in vitro (or possibly in vivo) imaging agents.

Ytterbium ion, despite its sharp, highly characteristic, long-lived (microsecond range) emission in the tissue-transparent near-IR (NIR) region,[7] has not found as wide application as Eu and Tb in bioimaging and bioanalysis. This is mainly due to its low NIR-emission quantum yield,[8] with the highest Yb emission quantum yield (reported by Korovin et al.[9a] in 2002) being ca. 0.5% in water.[9] The NIR emission lifetimes of Yb complexes are always less than 5 μs in water.[2,9a] Furthermore, the stability and biocompatibility of lanthanide complexes in aqueous media have often been questioned. For bioimaging, it is critical that these complexes do not dissociate at low concentration, resulting in the release of free lanthanide cations. Herein we report the synthesis of the two porphyrin-based Yb(III) complexes **Yb-1** and **Yb-2** (Figure 1) together with their linear and two- and three-photon-induced photophysical properties in water and dimethyl sulfoxide (DMSO) (Table 1). **Yb-2** was found to give a substantially stronger NIR emission ($^5F_{5/2} \rightarrow {}^2F_{7/2}$) in water, with quantum yields of ca. 1% at λ_{ex} = 300 nm and ca. 2.5% at λ_{ex} = 430 nm. The aqueous stability of **Yb-2** in the presence of various biomolecules, including citrate and human serum albumin (HSA), and its cytotoxicity and subcellular localization were examined as well.

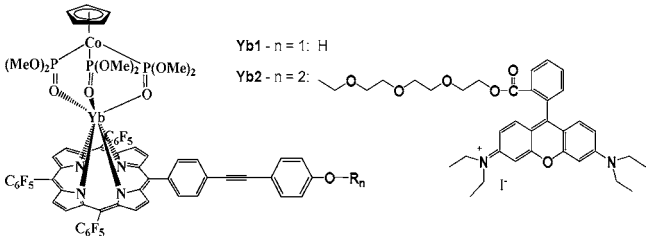

Figure 1. Molecular structures of the porphyrin-based Yb complexes **Yb-1** and **Yb-2**.

Table 1. Luminescence Lifetimes (τ), Quantum Yields (Φ), and Two-Photon Absorption Cross Sections (σ_2) of Yb-1 and Yb-2 in DMSO and Water

	τ (μs)[a]		Φ (%)			
	DMSO	H_2O	DMSO[b]	H_2O[b]	H_2O[c]	σ_2 (GM)[d]
Yb-**1**	17.5	–[e]	2	–[e]	–[e]	319
Yb-**2**	20.2	18.1	3.5	2.5	1	375

[a]Determined from the emission decay curve monitored at λ_{em} = 1060 nm ($^5F_{5/2} \rightarrow {}^2F_{7/2}$) with λ_{ex} = 430 nm. [b]λ_{ex} = 430 nm. [c]λ_{ex} = 340 nm. [d]Two-photon absorption cross section in DMSO measured by *Z*-scan[10] with λ_{ex} = 860 nm (1 GM = 10^{-50} cm^4 s photon^{-1} molecule^{-1}). [e]Yb-**1** is not soluble in water.

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Upon photoexcitation of the porphyrin Soret absorption band at ~430 nm (log ε = 5.84; Figure S7 in the Supporting Information), the two Yb(III) porphyrinate complexes **Yb-1** and **Yb-2** exhibited a strong red emission (550−700 nm) corresponding to the $\pi \rightarrow \pi^*$ transition of porphyrin and an NIR emission corresponding to the $^5F_{5/2} \rightarrow {}^2F_{7/2}$ transitions of Yb(III) ion (Figure 2). The emission intensities and the ratios

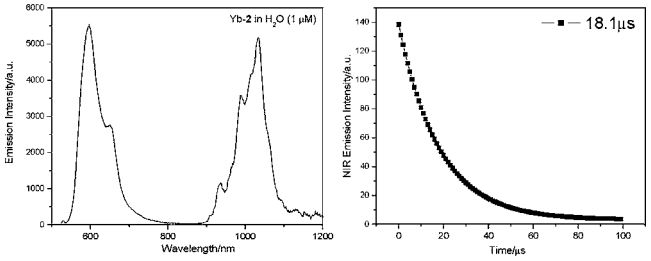

Figure 2. (left) Emission spectrum and (right) NIR emission decay of Yb-**2** obtained using the vis−NIR detector (λ_{ex} = 430 nm, 1 μM in H_2O, 298 K).

of the sublevels of the Yb(III) $^5F_{5/2} \rightarrow {}^2F_{7/2}$ transitions for the two Yb complexes are similar, suggesting a similar chemical environment for their Yb(III) ions, and are indicative of a low-symmetry environment of the emitting center. Porphyrin was found to be a better antenna (with an appropriate singlet state for energy transfer to the $^5F_{5/2}$ state for Yb NIR emission) than rhodamine B (Rh B) for Yb NIR emission on the basis of the higher $^5F_{5/2} \rightarrow {}^2F_{7/2}$ emission intensities of **Yb-1** and **Yb-2** upon excitation of the porphyrin moiety (430 nm) as compared to excitation of the Rh B moiety (560 nm) in DMSO (Figure S8). The relative quantum yields of the Yb emission (λ_{ex} = 430 nm) from the two Yb complexes in DMSO and H_2O were obtained by comparison with the reported values for $[Yb(tta)_3H_2O]$, $[Yb(tta)_2(phen)]$, and Yb(TPP)(Tp) [tta = thenoyltrifluoroacetonate; TPP = 5,10,15,20-tetraphenylporphyrinate; Tp = hydridotris(1-pyrazolyl)borate][11] (Figures S4−S6, λ_{ex} = 340 nm/430 nm) as well as with the absolute ligand visible-emission quantum yield measured by a wide-range (300−1700 nm) vis−NIR detector (with correction spectrum; Figure S14) using an integrating sphere. The Yb(III) NIR emission quantum yields of **Yb-2** were determined to be ca. 1% at λ_{ex} = 300 nm and ca. 2.5% λ_{ex} = 430 nm, with an emission lifetime of 18.1 μs (λ_{ex} = 430 nm) in water at 1 μM.

To develop biologically useful Yb imaging probes, the porphyrinato Yb(III) ion was capped with a tripodal monoanion $[(\eta^5\text{-}C_5H_5)Co\{(MeO)_2P{=}O\}_3]$ to prevent solvolysis of Yb(III). The aqueous stability of **Yb-2** in the presence of various biomolecules (e.g., citrate, bicarbonate, phosphate, and HSA) and biologically important metal ions [e.g., Zn(II) and Cu(II)] (Figure S12) and at different pH (Figure S13) was studied by monitoring its Yb(III) NIR emission. No significant change in its NIR luminescence was observed under these various conditions, thus indicating its potential application as a bioimaging agent.

Ligation of Rh B in **Yb-2** helps improve the solubility of the capped Yb(III) porphyrinate complex and functions as a target for mitochondria. The emission maxima, band shapes, and bandwidths for **Yb-1** and **Yb-2** in DMSO upon linear excitation (Figure S8) were quite similar to those observed for NIR excitation (i.e., via two- or three-photon absorption; Figure 3). The two-photon-induced (λ_{ex} = 860 nm) and three-photon-induced

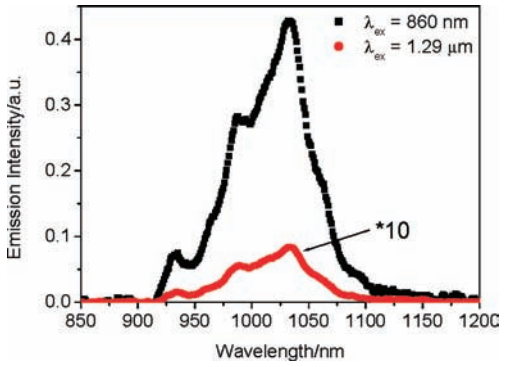

Figure 3. Two-photon-induced (λ_{ex} = 860 nm) and three-photon-induced (λ_{ex} = 1.29 μm) Yb NIR emission spectra of Yb-**2** (1 μM, DMSO).

(λ_{ex} = 1.29 μm) NIR emission spectra of **Yb-2** are shown in Figure 3. Power-dependence experiments confirmed the number of photons involved in the NIR emission band under excitation at 860 nm and 1.29 μm. The output intensity of the two- and three-photon-excited fluorescence was linearly dependent on the quadratic and cubic powers, respectively, of the input laser intensity (Figure S9). The two-photon absorption cross sections (σ_2) of **Yb-1** and **Yb-2**, determined using the open-aperture *Z*-scan method[10] in DMSO, were 319 and 375 GM, respectively. There are few Yb complexes exhibiting two-photon absorption, not to mention in water.[9] Thus, **Yb-2** is a notable example.

Multiphoton confocal laser scanning microscopy offers excellent resolution for three-dimensional imaging of fluorescently labeled live-cell samples with pinpoint excitation in micrometer dimensions, thus making possible fluorescence imaging of organelles, subcellular structures, and subcellular distributions of molecules labeled with appropriate fluorophores. The results of in vitro linear and two-photon confocal microscopic imaging of **Yb-1** (in 5% aqueous DMSO) and **Yb-2** performed on HeLa cells are shown in Figure 4. Both **Yb-1** and **Yb-2** showed substantial emission upon single-photon (430 nm) and two-photon (860 nm) excitation of the porphyrin moiety. Furthermore, **Yb-2** appeared to show preferential localization in mitochondria (95% of the cells examined), which was confirmed by a costaining experiment using a mitochondria-specific dye (Figure 5). This is presumably due to the mitochondria-targeting property of the linked Rh B. **Yb-1**, however, showed no organelle-specific localization, with emission emanating mainly from the cytoplasm. This observation is consistent with the lack of any organelle-specific targeting moiety in this complex.

To assess the potential application of these Yb(III) complexes as NIR bioimaging probes, the cytotoxic properties of **Yb-1** and **Yb-2** were evaluated using an MTT assay after 24 h of incubation with HeLa cells, and the results are shown in Figure S15. Both complexes appeared to be quite nontoxic, with IC_{50} values in the millimolar range, a level far above the normal working concentration of a bioprobe.

In conclusion, **Yb-2**, a new water-soluble porphyrinato Yb(III) complex capped with a tripodal $[(\eta^5\text{-}C_5H_5)Co\{(MeO)_2P{=}O\}_3]^-$ anion, has been synthesized. The complex exhibited a moderate two-photon absorption cross-section of 375 GM in DMSO and an impressive Yb(III) NIR quantum yield of 2.5% in water. The conjugation with Rh B enhances the Yb(III) porphyrinate water solubility as well as its mitochondria-specific

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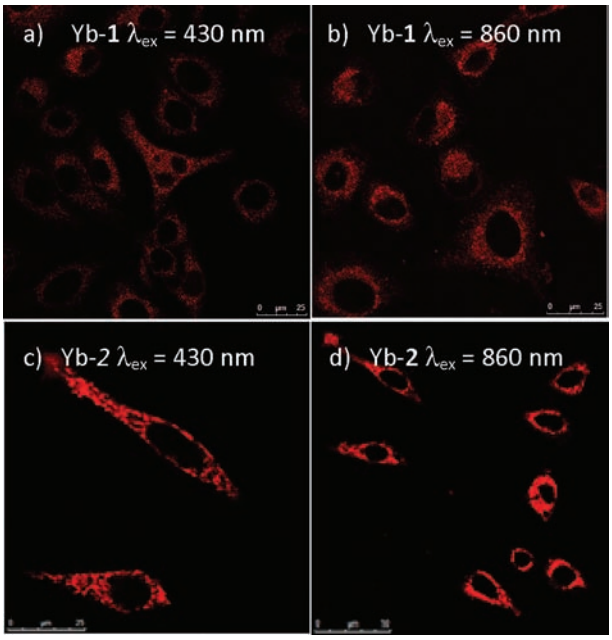

Figure 4. Linear (λ_{ex} = 430 nm)- and two-photon (λ_{ex} = 860 nm)-excitation-induced in vitro imaging of (a, b) 10 μM **Yb-1** (3% aqueous DMSO) and (c, d) **Yb-2** (H_2O) in HeLa cells (40× magnification, λ_{em} = 500–800 nm).

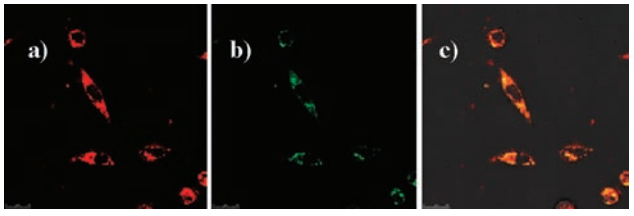

Figure 5. (a) Linear confocal microscopy images of the red in vitro emission from Yb-**2** (10 μM, λ_{ex} = 430 nm) after 30 min exposure in HeLa cells. (b) Green mitochondria marker (Invitrogen M7514, 1 μM; λ_{ex}= 430 nm, 3 min exposure) in HeLa cells. (c) Merged image.

localization in cells. These properties of **Yb-2**, together with its minimal cytotoxicity (IC_{50} = 1.2 mM) suggest its potential as a new time-resolved imaging probe that can be excited and imaged in the tissue-transparent (i.e., NIR) window.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic routes for **Ln-1** and Ln-**2** (Ln = Gd, Yb); ^{1}H, ^{13}C, and ^{31}P NMR spectra of Yb-**1**, Yb-**2**, and their intermediates; spectroscopic data; fluorescence and phosphorescence emission spectra of Gd-**1** and Gd-**2**; emission spectra of Yb-**2** from the titration of various biological small molecules and pH; power dependence curve for two-photon-induced f–f emission of Yb-**2**; and the calibration curve for the vis–NIR detector. This material is available free of charge via the Internet at http://pubs.acs.org.

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