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Efficient and selective singlet oxygen sensitized NIR luminescence of a neodymium(III) complex and its application in biological imaging

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ABSTRACT

A responsive neodymium NIR emission ($^4F_{3/2} \rightarrow {}^4I_{11/2,\ 9/2}$) was recorded upon binding with singlet oxygen ($K_B = 1.79 \times 10^6\ M^{-1}$) *via* the anthracene moiety. The motif ytterbium analog served as a negative control with no significant NIR enhancement/quenching with the addition of the same amount of singlet oxygen. Our complex was also found to react with $^1O_2$ generated by a known photosensitizer TMPyP inside HeLa cells without inducing cell death and display no significant cytotoxicity.

1. Introduction

Singlet oxygen ($^1O_2$) has been an intriguing rendezvous for chemists and biologists due to its ability to oxidize different entities in the environment and other biological systems. Regarding the latter, its ability to destroy malignant cells is utilized and further developed in photodynamic therapy (PDT) [1]. However, various kinds of biological molecules such as proteins [2] and DNA [3] could also be oxidized by $^1O_2$ and such cytotoxicity *in vivo* could not be ignored. Furthermore, it is believed that $^1O_2$ could aid divulging the secrets of eukaryotic gene expression [4] and bactericidal response of certain antibodies [5]. Monitoring $^1O_2$ hence becomes vital in opening new doors for various researches. Direct monitoring of the weak phosphorescence at 1270 nm [6], although non-invasive, is difficult for quantitative detection and is not a popular way for biological monitoring since the lifetime of the emission in aqueous media is in the scale of microseconds or less [7].

In this communication, our group presents a lanthanide-based near infra-red (NIR) emissive sensor for $^1O_2$ with concrete potential in biological applications. A number of excited tripositive lanthanide ions are known to relax via characteristic radiative transitions from with lifetimes of the order of milliseconds, so that their emissions can be easily differentiated from autofluorescence of biological entities [8]. Although Eu(III) and Tb(III) ions (with red and green emissions respectively) have been intensively studied and developed as emissive probes [9], visible light remains intrinsically inferior to wavelengths in the NIR region, where light achieves maximum penetration through biological tissues [10]. The ability to be excited and emit at NIR wavelengths are desired properties for efficient and effective *in vivo* optical probes, and recent demonstration of utilizing Nd(III) as NIR emitting systems for deep tissue imaging showed promising potential [11], thus neodymium (Nd) was chosen as the NIR emissive center for investigation.

2. Results and discussion

The 2,4-bis(3,5-dimethylpyrazol-1-yl)-1,3,5-triazine scaffold has been known as a strong and reliable scaffold for lanthanide-thenoyltrifluoroacetonate complexes as well as for its ability to impart two-photon excitation potential [12] onto the complex and possible cell localization [13]. Thus this scaffold was chosen and an anthracene moiety was incorporated into the ligand system of the organo-lanthanide complex to make use of the specific reaction between singlet oxygen and anthracene to form an endoperoxide [14] as the major propellant behind the working principle of our probe. Like most other organo-lanthanide complexes, the characteristic emission from the Nd(III) center is sensitized efficiently by a chelated chromophore, *i.e.* the antenna effect [15]; provided that the energy level of the antenna triplet state is slightly higher than that of the emissive state of the Nd(III) center. Originally, the excited triplet state

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of the antenna was unsuitable to sensitize the emission center; yet upon formation of the endoperoxide, the energy level of the antenna is perturbed to an extent that sensitization is achieved.

The UV/vis absorption behavior of the Nd and Yb complexes with the above ligand (Nd-1 and Yb-2 respectively) is shown in Supporting information. The three absorption bands observed for Nd-1 and Yb-2 (see the arrows in Figs. S3 and S4 *ca.* 35714 cm^{-1}, *ca.* 28571 cm^{-1}, and *ca.* 23047 cm^{-1}) are similar to the peaks observed in the UV/vis spectrum of the ligand. By comparison of the UV/vis spectra for Nd-1 and Yb-2 with that of the ligand L, the bands can be attributed to benzyl π–π* transitions that were red-shifted to approximately 1000 cm^{-1} upon complexation. The high degree of similarity between the UV/vis absorption bands of Nd-1 and Yb-1 implies that they are likely to have similar or the same ligand–metal coordination modes, and hence similar or the same coordination structure. The UV absorption properties of these two lanthanide complexes were monitored with the addition of $^1O_2$ (Figs. S3, S4). The absorption spectrum of Yb-1 remained unchanged throughout the addition whereas that of Nd-1 increased and the three bands gradually merged together with its maximum at around 275 nm and a shoulder at around 300 nm.

The luminescence properties of the ligands and the lanthanide complexes were measured in aqueous buffer at room temperature. The intense broad emission band (λ_{ex}=350 nm) at *ca.* 517 nm was attributed to the benzyl π–π* relaxation ($S_1 \rightarrow S_0$). The two structured, narrow NIR emission bands at 900 and 1075 nm for Nd-1 and those at 1100 nm for Yb-2 were assigned to an electronic energy transition between the $^3F_4 \rightarrow {}^4I_J$ (J=11/2 and 9/2) and $^2F_{5/2} \rightarrow {}^2F_{7/2}$ states respectively. There was weak NIR emission intensity in the Nd and Yb complexes in the original. The matching of the triplet states in these lanthanide systems is an important criteria for the energy transfer from the ligand to the lanthanide.

When the complexes were titrated against singlet oxygen in aqueous buffer, significant enhancement in the luminescence signals was observed in Nd-1 with UV excitation (λ_{ex}=350 nm, Fig. 1), indicating the occurrence of reaction between the Nd-1 and $^1O_2$ to form an endoperoxide moiety. After binding with $^1O_2$, the emission intensity of Nd-1 increased by more than five times. The binding ratio (1:1) and binding constant (1.79×10^6 M^{-1}) between Nd-1 and $^1O_2$ had been determined by the f–f emission intensity ($^4F_{3/2} \rightarrow {}^4I_{11/2,\ 9/2}$) at various concentrations of $^1O_2$ (Fig. 1 and Supporting information).

However the Yb analog Yb-2 showed no significant enhancement under the same experimental conditions. The Yb-2 complex thus acted as a perfect control experiment for the responsive NIR emission enhancement with singlet oxygen binding. The large enhancement in emission (Nd-1) and reduction in emission (Yb-2) could be ascribed to the formation of an endoperoxide from the polyaromatic anthracene moiety with $^1O_2$. Such formation induced perturbation of the π-conjugation along the system from the anthracene along the alkyl group to the chelating triazine scaffold. Hence electronically, the conjugation could not extend to the endoperoxide part or the two unaffected benzenes and geometrically the planarity was also disturbed, both resulting in a less efficient energy transfer to the lanthanide center (Scheme 1).

The phosphorescence of the Gd complexes was measured under cold conditions (77 K) in solution state (see Fig. S10). Since the lowest excited state energy level of Gd^{3+} ($^6P_{7/2}$) is much higher than energy levels of the ligands, it is therefore assumed that no energy transitions take place between the ligand and lanthanide center and such phosphorescence could then be inferred as ligand phosphorescence.

The luminescence lifetimes were recorded to verify the fluorescence and phosphorescence at 77 K. The emission lifetimes at *ca.*517 nm were on a nanosecond scale ($S_1 \rightarrow S_0$) and those at *ca.* 545 nm were on a microsecond scale ($T_1 \rightarrow S_0$) form the ligand. The

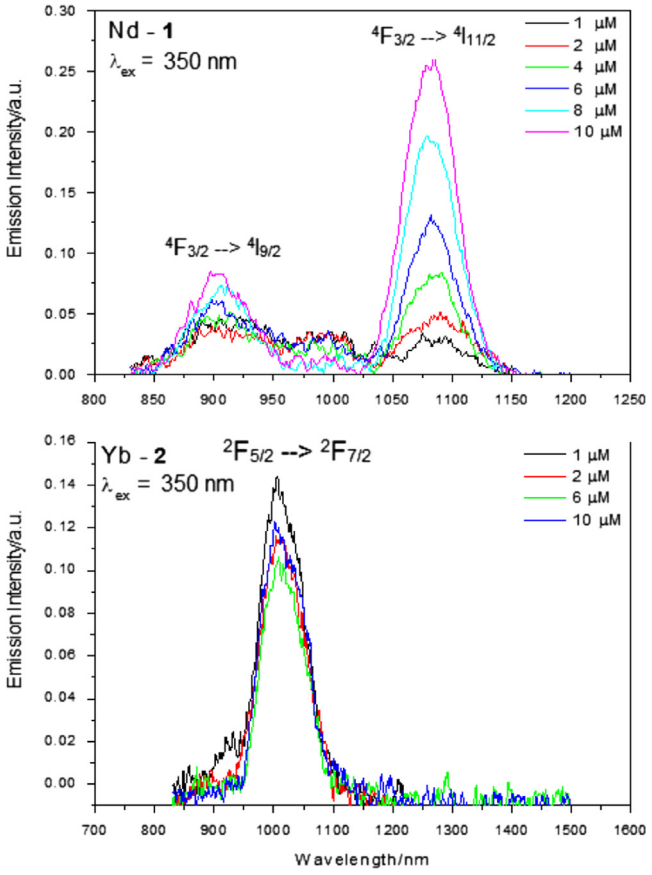
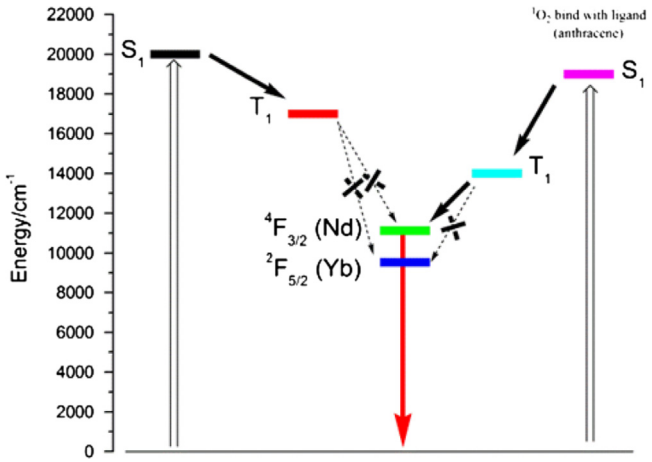


Fig. 1. The NIR emission change of Nd-1 (upper-enhancement) and Yb-2 (lower – quenching as a control experiment) with the addition of $^1O_2$ (λ_{ex}=350 nm).



Scheme 1. Schematic diagram defining the relative energies of the excited states of the chromophore before (black and red) and after (magenta and aqua) addition of $^1O_2$ and the salient lanthanide (Nd and Yb) excited states as well as the proposed energy transfer pathway.

schematic energy level diagram, which includes the triplet state energy levels of the Gd complexes, and the energy transfer process are shown in Scheme 1. Given that the energy level $^2F_{5/2}$ for Yb is *ca.* 9800 cm^{-1} and $^4F_{3/2}$ for Nd is *ca.* 11000 cm^{-1} the energy gap between the ligand triplet state and the metal center is greater than 5000 cm^{-1} in Yb/Nd complexes of ligand L, a gap that is too high to allow effective energy back transfer. In contrast, the triplet state energy levels in the Gd complexes after addition of $^1O_2$ are