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Impressive Europium Red Emission Induced by Two-Photon Excitation for Biological Applications

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Supporting Information

ABSTRACT: Three triazine-based europium(III) complexes were synthesized that demonstrated strong two-photon induced europium emission with a high two-photon absorption cross-section. The modified triazine ligand of complex **3** initiated over 100% enhancement of the two-photon absorption cross-section (σ_2: 320 GM) when compared with complex **1** (σ_2: 128 GM) in a solution of DMSO. Europium complex **3** is also stable *in vitro*, and power-dependence curves were obtained *in vitro* to confirm the two-photon-induced f−f emission in HeLa cells.

There are two main problems with most of the commercially available molecular imaging probes—requiring excitation by a UV source and autofluorescence[1−3]—which limit the quality of *in vitro* imaging.[4,5] Lanthanide materials, with their distinctive features, such as long-lived luminescence lifetimes, large Stokes shifts, and sharp emission peaks, for molecular imaging, diagnosis, or therapy, offer a solution to the latter problem.[5−7] The development of time gating technology combined with the characteristic microsecond lifetimes of lanthanide complexes help to eliminate nanosecond autofluorescence in order to achieve better quality imaging *in vitro*/*in vivo*.[8] For these reasons, lanthanide materials have developed rapidly in the past two decades.[9]

Lanthanide materials can provide long lifetime emissions for time-resolved imaging, but the source of excitation used is a major obstacle.[6,10−12] The f−f transitions of lanthanide ions are forbidden by selection rules, and thus the resulting emissions from direct excitation of the metal are very weak.[7] The use of organic ligands as suitable antennae allows energy from the excited antennae to be transferred to the lanthanide ions first by intersystem crossing (singlet to triplet state) of the ligand and then to the excited states of the lanthanide ions.[13,14] This energy transfer pathway remarkably enhances emission. In general, most organic ligands are only effectively excited in the UV region.[15] In aiming to develop nonphototoxic time-resolved imaging tools, lanthanide chemists have been urged to look for organic chromophores that can absorb numerous near-infrared photons and efficiently transmit to lanthanide ions to induce f−f emissions.

However, even though the energy transfer pathway between the organic antenna and lanthanide by linear and two-photon absorption is the same, it is inevitable that energy is lost during the transfer from the ligand (antenna) to the excited state of the lanthanide ion. Therefore, when compared to the emission quantum efficiency to organic or organic-transition metal complexes, the cross-sections of lanthanide materials should be relatively diminished. Also, the two-photon absorption cross-sections in this manuscript have been measured by a reference standard method (with rhodamine 6G) which is based on the f−f emission.[12,16,17] Notable exceptions are europium complexes with dipicolinic acid derivatives reported by Maury et al., which have two-photon absorption cross-sections up to 775 GM in dichloromethane.[18,19] The demand for different two-photon lanthanide materials for further development as time-resolved *in vitro* probes is still very high; thus we hereby present three biological compatible europium complexes that are triazine-based ligands with great potential as candidate probes. Complex **3**, coordinated with (2-(*N*,*N*-diethylanilin-4-yl)-4,6-bis-(pyrazol-1-yl)-1,3,5-triazine) and 2-thenoyl-trifluoroacetone, has the strongest two-photon absorption cross-section for triazine-based molecular europium complexes in DMSO (320 GM). The stability of these europium complexes has been examined, and they have demonstrated cell permeability and low cytotoxicity and are potential candidates for time-resolved molecular probes when conjugated with appropriate vectors such as peptides or specific antibodies.

In our previous studies, the triazine-based europium complex **1** showed promising two-photon-induced f−f emission.[16] The modification of the triazine-based ligand in complex **1** here shows an increased two-photon absorption of the triazine-based europium materials from ∼100 GM to 320 GM. Complexes **1**−**3** (Figure 1) were synthesized by reacting $Eu(tta)_3$ with the respective ligands ($\mathbf{L_2}$ and $\mathbf{L_3}$ are newly synthesized): 2-(*N*,*N*-diethylanilin-4-yl)-4,6-bis(3,5-dimethyl-pyrazol-1-yl)-1,3,5-triazine ($\mathbf{L_1}$), 2-(*N*,*N*-diethylanilin-4-yl)-4,6-bis(indazole-1-yl)-1,3,5-triazine ($\mathbf{L_2}$), and 2-(*N*,*N*-diethylanilin-4-yl)-4,6-bis(pyrazol-1-yl)-1,3,5-triazine ($\mathbf{L_3}$). The newly synthesized complexes **2** and **3** are characterized by conventional methods (see Supporting Information).

The solution state electronic excitation spectra of the europium complexes display band shapes similar to their absorption spectra in a solution of DMSO (Figures S9, Supporting Information). The excitation bands of complexes **1**−**3** have maxima at ∼400 and 330 nm (attributed to the intraligand charge transfer and $\pi \rightarrow \pi^*$ transitions of ligands $\mathbf{L_1}$−$\mathbf{L_3}$ in complexes **1**−**3**) and ∼350 nm

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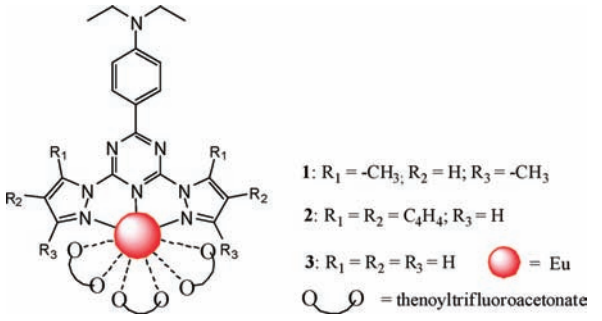

Figure 1. The molecular structures of europium complexes (**1**−**3**) with triazine-based ligands.

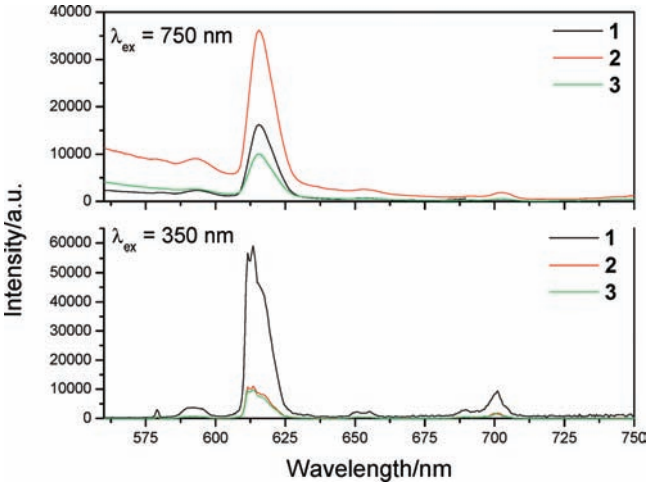

Figure 2. Two-photon (upper, λ_{ex} = 750 nm) and linear (lower, λ_{ex} = 350 nm) induced europium emission in a solution of DMSO.

Table 1. Quantum Yields, Emission Lifetimes, and Two-Photon Absorption Cross Sections of 1−3 in DMSO

	Φ^a	τ (ms)^b	σ_2 (GM)^c
1	0.48	1.34	128
2	0.33	1.12	98
3	0.32	1.09	320

^{a} λ_{em} = 560 − 700 nm, λ_{ex} = 350 nm. ^{b} Decay curve monitored at 620 nm ($^5D_0 \rightarrow {}^7F_2$, λ_{ex} = 350 nm). ^{c} Two-photon absorption cross-section (GM = 10^{-50} cm^4 s photon^{-1} molecule^{-1}, λ_{em} = 560 − 700 nm, λ_{ex} = 750 nm).

(attributed to $\pi \rightarrow \pi^*$ transitions of thenoyltrifluoroacetonate). The room-temperature emission spectra of complexes **1**−**3** under 350 nm laser excitation in the solution state (DMSO) are displayed in Figure 2 (lower), which show characteristic $^5D_0 \rightarrow {}^7F_J$ (J = 0−4) transitions of Eu^{3+}. As expected, the emission spectra of complexes **1**−**3** are very similar, particularly with regard to the band energies (Figure 2 upper).

In our previous studies, we defined complex **1** as having undergone a direct energy transfer from the ligand singlet to $^5D_0$ and $^5D_1$ of the europium ion.[16] The two-photon induced photophysical properties of triazine−europium complexes were examined in a solution of DMSO with femtosecond excitation at 750 nm. The two-photon absorption cross-section and absolute

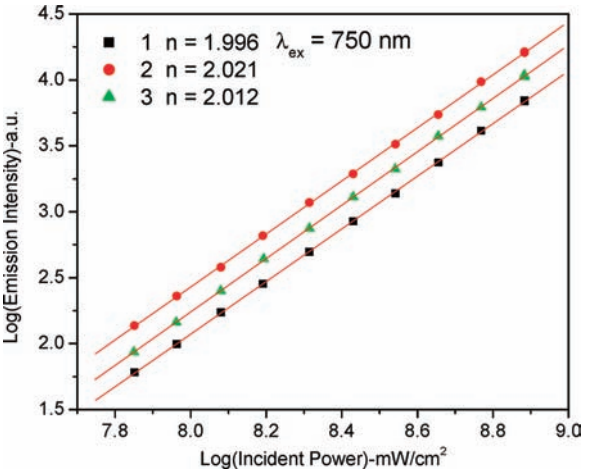

Figure 3. Results of the power-dependence experiments showing emission intensity and incident power on log scales.

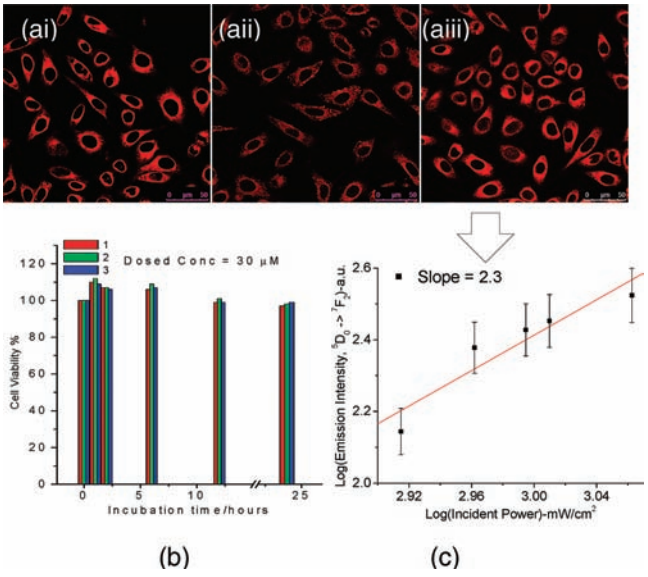

Figure 4. (a) Two-photon induced *in vitro* imaging of HeLa cells incubated with europium complexes **1** (ai), **2** (aii), and **3** (aiii) for 1 h. (b) The MTT assay results of complexes **1**−**3** for 24 h at 10 times the dosed concentration used for the molecular imaging in a. (c) The *in situ* power dependence curve (europium emission and incident power on log scales) of complex **3**; *in situ* emission spectra for the power dependence curve were obtained via lambda scan from aiii—BP filter 550−650 nm, λ_{ex} = 750 nm.

quantum yield (measured using an integrating sphere) of the europium complexes were monitored between 560 and 700 nm. The triazine−europium complex **3**, without the electron donating group or electron withdrawing groups borne by $\mathbf{L_1}$ and $\mathbf{L_2}$, featured 1.5-fold larger two-photon-induced f−f emissions and a 2.5-fold greater two-photon absorption cross-section (Table 1). Power-dependence experiments have been carried out to confirm that the energy of two near-infrared femtosecond photons are absorbed by the triazine ligands, then transferred to the europium excited state for their emission (Figure 3). No significant cytotoxicity was observed for the three europium complexes (dosed concentration = 30 μM, 24 h; Figure 4b), and

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the IC_{50} values for HeLa cells were found to be around 150 μM. All three europium complexes do not have any specific localization profile, and strong two-photon induced f−f emissions in the *in vitro* emission spectra can be observed (Figure 4). There are numerous studies on two-/three-photon-induced f−f *in vitro* imaging with lanthanide materials, but *in situ* power dependence experiments are rare. The *in situ* power dependence experiments with lanthanide complexes were conducted initially on cells with complex **3**. Only complex **3** showed an impressively high two-photon absorption cross-section among europium complexes in the power dependence experiments (Figure 4c). Variations in laser power were recorded with a power meter for the coherent (II) laser output from a confocal microscope, and the emission intensities were monitored after adjusting the laser power for 5 min. Complex **3** demonstrated an *in situ* power dependence curve which confirms that the europium signal originated from within the HeLa cells (Figure 4aiii) was induced by a two-photon process (slope = ∼2.3) rather than scattering or the SHG of the laser line. The stability of the three europium complexes *in vitro* was examined via titration of various biologically meaningful small molecules, such as citrate, bicarbonates, and HSA (Figure S12 and S13, Supporting Information). Slight significant luminescent enhancement/quenching was observed.

In conclusion, a new motif of triazine-based europium complex **3** has been synthesized, and it demonstrates a strong two-photon absorption cross-section (320 GM). The dramatic change of two-photon photophysical properties of these three europium complexes has been induced by slight modification of the triazine ligand. Europium complex **3** has demonstrated low cytotoxicity, cell permeability, and fast cellular uptake without a specific localization profile, and therefore it has the potential to become a new time-resolved imaging probe that is excitable at a wavelength within the "biological window"—in which light penetration through biological tissue is maximal.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details of syntheses, photophysical and in vitro studies. ^{1}H and ^{13}C NMR spectra of ligands and complexes. UV−vis absorption and *in vitro* emission spectra of complex **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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