

Supramolecular self-assembly enhanced europium(III) luminescence under visible light

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In this paper, we report on the luminescence of europium by directly exciting europium ions with visible light in aqueous medium. Upon replacing all the water molecules that coordinate around a central europium ion with a ditopic ligand 1,11-bis(2,6-dicarboxypyridin-4-yloxy)-3,6,9-trioxaundecane (L₂EO₄), the quenching from water molecules is efficiently eliminated, offering considerable europium emission. By stoichiometrically mixing with a positively charged block polyelectrolyte, the negatively charged L₂EO₄-Eu coordinating complex can be transformed into a coordination 'polymer', which simultaneously forms electrostatic micelles with further enhanced europium fluorescence emission, owing to the increased fraction of L₂EO₄-coordinated Eu(III) as revealed by the fluorescence lifetime measurements. This approach avoids the use of the antenna effect that often utilizes UV light as the irradiation source. We further use those micelles for bio-imaging, and for the first time demonstrate the use of directly excited Eu-containing nano-probes for *in vivo* fluorescence imaging in small animals under visible excitation. Although literature results have shown that the direct excitation of europium ions in water may lead to emissions in the presence of coordinating ligands, those emissions were too weak to be applied due to the remaining water molecules in the coordination sphere. Our work points out that the direct excitation of europium can generate considerable europium emission given that all the water molecules in the coordination sphere are excluded, which does not only greatly reduce tedious lab work in synthesizing antenna molecules, but also facilitates the application of europium in aqueous medium under visible light.

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Introduction

The luminescence of trivalent europium ions (Eu³⁺) is of great interest in biotechnology because of their unique luminescence properties, such as high color purity, long lifetimes, and high resistance to photo-bleaching.¹⁻⁴ These unusual spectral properties make them well suited for use as luminescent reporter groups, with many currently developing applications in bio-imaging and biolabeling.⁵⁻⁸ Unfortunately, as a consequence of the parity (Laporte) forbidden nature of the 4f transitions, the direct absorption of Eu(III) cations is very weak, and hence has rather low molar absorption coefficients (typically less than 10 M⁻¹ cm⁻¹) which limits their practical applications.¹ In order to circumvent this problem, the Eu(III) ion can be chelated to a chromophore-containing group which functions as an 'antenna',

absorbing incident light and then transferring this excitation to the Eu(III) ion, which can then get deactivated by undergoing its typical luminescence emission.⁹⁻¹² However, the commonly employed excitation wavelength for luminescent europium complexes is usually limited to <360 nm, owing to the energetic constraints of the 'antenna effect'.⁵ Because these short-wavelength UV radiation can damage living biological systems,^{5,13} it is highly desirable to develop luminescent Eu complexes that can be sensitized by visible light. So far, considerable efforts have been devoted to the synthesis of complicated 'antenna' molecules, which either increase the size of light absorbing groups,¹⁴⁻¹⁷ or change the emission from one photon to two photon sensitization,¹⁸⁻²³ to extend the excitation wavelength of Eu complexes to the visible region. As a consequence of the structural complexity, the resultant lanthanide complexes are often insoluble in water,¹⁵⁻²¹ and thus cannot be directly used as biolabels. To date, it is still an on-going challenge to develop water soluble lanthanide coordinating complexes with easy chemistry approaches and which can be sensitized by visible light.

Herein, we attempt to achieve considerable Eu(III) luminescence under visible light by excluding the coordinating water facilitated by a supramolecular approach. It has long been known that Eu(III) ions have weak absorptions at 395 and 465 nm.¹ In principle, these weak absorptions may produce

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emissions as well, because the long lifetimes of these emissions may counterbalance the shortcoming of low absorbance. Unfortunately, in the presence of water, the absorbed energy is mostly dissipated by water molecules coordinated to its first coordination sphere *via* O–H vibrations.²⁴ Therefore the emissions from native Eu(III) ions in water are usually drastically quenched. As a result, it has been considered, for decades, that the direct excitation of Eu(III) in water cannot lead to efficient emissions. Many efforts have thus been focused on the study of Eu(III) emissions in organic media or nanoparticles which is in virtue of the antenna effect.^{2,25–29} The study of emissions obtained by direct excitation of Eu(III) ions while excluding the quenching from water has long been neglected. Although some preliminary studies have shown that direct excitation of Eu(III) may produce emissions when part of the water molecules were replaced by ligands^{30–32} such as nicotinate, acac, isonicotinate, terpyridyl, EDTA,²⁴ and CF₃SO₃,³¹ no report has figured out how strong this direct emission is, especially whether it is strong enough to be detected in bioimaging. Actually, due to the significant quenching from the remaining water molecules in the coordination sphere, the emissions are still too weak when only part of the coordinating sites are taken by ligands. However, we anticipate that if suitable ligands that can take all the 9 coordinating sites of an Eu(III) ion are employed to exclude all the water molecules from the first coordination sphere, the direct excited Eu(III) emissions may be greatly enhanced so that it might be applicable in bioanalysis.

In this study, we show that it is indeed possible to achieve considerable Eu(III) emissions by stepwise exclusion of water molecules from the first coordination sphere. A water soluble ditopic ligand L₂EO₄ with two dipicolinate (DPA) heads is employed. DPA is well-known to strongly chelate with various metal ions using 3 chelating sites.^{33–37} Upon complexation with Eu(III) ions at various molar ratios in water, characteristic Eu(III) emissions can be obtained under visible light, but only those complexes for which L₂EO₄/Eu > 3/1 have the strongest emissions, suggesting that water molecules have to be effectively excluded from the first coordination sphere of Eu(III). Alternatively, the partly coordinated L₂EO₄/Eu = 3/2 complexes can also be used to produce applicable emissions upon formation of polyion micelles. The rationale is that the ditopic nature of the L₂EO₄ ligand allows chain extension at this molar ratio at high enough concentrations.³⁸ Since electrostatic interactions can enhance the local concentration of L₂EO₄/Eu = 3/2 complexes,³⁸ chain extension occurs simultaneously upon the addition of an oppositely charged block polyelectrolyte, which finally leads to polyion micelles. In this way, the emission of Eu(III) can be enhanced to the level of L₂EO₄/Eu > 3/1 systems. The emission under this visible excitation can even be applied in *in vivo* bioimaging.

Experimental section

Materials

The bisligand 1,11-bis (2,6-dicarboxypyridin-4-yloxy)-3,6,9-triox-aundecane (L₂EO₄) and diblock polyelectrolyte poly(*N*-methyl-2-vinylpyridinium iodide)-*b*-poly(ethylene oxide) (PMVP₄₁-*b*-PEO₂₀₅,

Mw = 18.5 K, PDI = 1.05, about 90% quaternized) used in this work were prepared according to previously reported procedures.^{38,39} Diblock polymer poly (2-vinylpyridine)-*b*-poly (ethylene oxide) (P2VP₄₁-*b*-PEO₂₀₅, Mw = 13.3 K, PDI = 1.05) were obtained from Polymer Science. Eu(NO₃)₃·6H₂O (99.99%) was obtained from Sigma. D₂O was obtained from J&K. Ultra-pure water was used and no extra salt was added. Stock solutions of P2VP₄₁-*b*-PEO₂₀₅, L₂EO₄, and Eu(NO₃)₃ were prepared at appropriate concentrations. To prepare the Eu coordination complexes, 20 mM L₂EO₄ solution and 50 mM Eu(NO₃)₃ solution were mixed at desired molar ratios. The coordination complexes were added in stoichiometric amounts to a P2VP₄₁-*b*-PEO₂₀₅ aqueous solution ([P2VP] = 2 mM, [L₂EO₄] = 1 mM, [Eu³⁺] = 0.67 mM). HCl and KOH were used to control the pH.

Luminescence spectrometer measurements

A lifetime and steady state spectrometer FLS920 was used to measure the luminescence emission and lifetimes of europium(III)-containing solutions. The excitation wavelength was set at 395 and 465 nm to directly excite the europium ions. Emission spectra were recorded in the range of 500–750 nm. Excitation spectra were recorded in the range of 250–500 nm. The lifetimes were measured under 395 nm since the excitation wavelength does not affect the lifetime. A Nanolog FL3-2iHR integrating sphere was used to measure the quantum yield of europium(III)-containing solutions.

Light scattering measurements

Dynamic light scattering measurements were carried out using a laser light scattering spectrometer ALV/DLS/SLS-5022F of standard design (ALV-5000/E/WIN Multiple Tau Digital Correlator) with a 22 mW He–Ne laser (wavelength: 632.8 nm). The scattering angle was 90° and the CONTIN method was used to analyze the distribution of the radii of micelles. Unweighted data were recorded for all experiments.

Transmission electron microscopy (TEM)

A JEOL 2100F TEM was employed to observe the morphology of the micelles. Drops of samples were put onto 230 mesh copper grids coated with a carbon film. Excess water was removed by filter paper, and samples were then allowed to dry in ambient air at room temperature, before TEM observation.

Animal experiments and *in vivo* imaging

Balb/c mice (~20 g) experiment (Suzhou Belda Bio-Pharmaceutical Co.) was performed under protocols approved by Soochow University Laboratory Animal Center.

For *in vivo* fluorescence imaging, the Balb/c mouse with hair removed was subcutaneously injected with 50 μL corresponding samples at its back after being anesthetized. Then, the injected mouse was imaged according to the same procedure as that used for *in vitro* solution fluorescence imaging apart from an exposure time of 400 ms. (Background was removed by using the spectral unmixing software.)

Aqueous solutions of $L_2EO_4/Eu = 3/1$ and Eu-micelles ($[Eu^{3+}] = 3.33 \text{ mM}$, $L_2EO_4/Eu = 3/2$) were imaged using a Maestro EX *in vivo* spectral imaging system under the excitation of a 455 nm laser. A 500 nm shortpass emission filter was used to prevent the disturbance of excitation light with the charge coupled device (CCD) camera. The *in vitro* solution fluorescence imaging from 500 nm to 720 nm (in 10 nm steps) was conducted with an exposure time of 6000 ms. Background was removed by using the spectral unmixing software.

Isothermal titration microcalorimetry

All measurements were performed using a TAM 2277-201 microcalorimetric system (Thermometric AB, Järfälla, Sweden) with a stainless steel sample cell of 1 mL. The sample cell was initially loaded with 0.7 mL of H_2O or L_2EO_4 solution. The $Eu(NO_3)_3$ solution was injected into the sample cell *via* a 500 μL Hamilton syringe controlled by a 612 Thermometric Lund pump. A series of injections were made until the desired concentration range had been covered. The system was stirred at 60 rpm with a gold propeller. The observed enthalpy (ΔH_{obs}) was obtained by integration over the peak for each injection in the plot of heat flow P against time t . The dilution heats of $Eu(NO_3)_3$ solution were subtracted from the heats of binding. The data fitting was performed by using the Origin software (supplied by Microcal Inc.). By fitting the observed enthalpy curves plotted against the molar ratio of $Eu(NO_3)_3$ to L_2EO_4 , the binding stoichiometry (n), binding constant (K) and the binding enthalpy (ΔH) were derived. All of the measurements were conducted at $298.15 \pm 0.01 \text{ K}$.

Results and discussions

Emissions of L_2EO_4 -Eu excited with visible light

The emissions for the aqueous solution of $Eu(NO_3)_3$ are negligible. However, upon mixing Eu^{3+} ions with L_2EO_4 at various molar ratios, detectable red emissions are observed under UV light. The pH (= 6.9) was chosen high enough to favor metal complexation over protonation of the ligands and low enough to prevent the formation of insoluble metal hydroxide. The excitation spectra reveal that the DPA 'antenna' produces the strongest excitation at 285 nm, whereas the direct absorbance by Eu(III) ions leads to two weak excitations at 395 and 465 nm, corresponding to characteristic transitions of $Eu^7F_0-^5L_6$, and $^7F_0-^5D_2$, respectively.⁴⁰ Because the f-f transitions are forbidden, the low strength of these excitations is as expected. However, it should be noticed that the strength of excitation at 395 nm is still about one tenth of that at 285 nm. Since the excitation at 285 nm is extremely strong, one tenth of this excitation can produce remarkable emissions. In Fig. 1A we demonstrate that the $L_2EO_4/Eu = 3/1$ system shows characteristic europium luminescence at 595, 614, and 694 nm under 395 nm, corresponding to $^5D_0-^7F_1$, $^5D_0-^7F_2$, and $^5D_0-^7F_4$ transitions of europium, respectively.⁴¹ The emission reaches a plateau at $L_2EO_4/Eu > 3/1$ (Fig. 1B), suggesting one europium ion maximally coordinates with 3 L_2EO_4 . Since the number of coordinating sites for one Eu^{3+} is 9 and each head of L_2EO_4 contributes

3 chelating sites (Scheme 1B), this means L_2EO_4 uses one head to coordinate with one Eu^{3+} ion. It is worth noting that as the L_2EO_4/Eu ratio increases from 1 to 3, the plateau luminescence at 614 nm has been enhanced by a factor of 16. The steep increase of the emission intensity with increasing the L_2EO_4/Eu ratio clearly demonstrates how significant the quenching effect of water can be for Eu(III) coordinating complexes. This unambiguously implies that excluding water from the coordination sphere may indeed activate considerable emissions.

To examine whether this emission is applicable in bio-imaging, the $L_2EO_4/Eu = 5/1$ coordination complexes were injected into the back of nude mice, and then imaging was done using the Maestro EX *in vivo* spectral system with the 455 nm laser as the excitation light. A distinctive red color was observed at the corresponding injection site (Fig. 1C and D) after spectral mixing, suggesting that visible light may indeed excite applicable emissions for Eu(III) ions in aqueous media when the water molecules are effectively excluded from the first coordinating sphere.

Visible light sensitized Eu(III) luminescence in electrostatic micelles

Although Scheme 1B indicates that one L_2EO_4 uses only one head to bind with europium ions to reach the maximum luminescence in water, ITC experiments suggest that both the heads of L_2EO_4 contribute to the binding equilibrium. In Fig. 2 we show the enthalpy change during the calorimetric titration of L_2EO_4 with $Eu(NO_3)_3$. The dramatic change of the enthalpy occurs at the $Eu^{3+}/L_2EO_4 \approx 0.7$, with the equilibrium constant $K = 1.9 \times 10^5$ and $\Delta H = -53.5 \text{ kJ mol}^{-1}$. This suggests that in the sense of chemical equilibrium, the binding stoichiometry between L_2EO_4 and Eu^{3+} is about 3 : 2, which is in good agreement with the previous result for the binding of rare earth metal ions with L_2EO_4 . The free energy changes (ΔG) were calculated from $\Delta G = -RT \ln K$, and the entropy changes were from $\Delta S = (\Delta H - \Delta G)/T$. The calculated ΔG is $-32.6 \text{ kJ mol}^{-1}$ and ΔS is $-70.1 \text{ J mol}^{-1} \text{ K}$. Hence, the coordination between the europium ion and the ligand is an enthalpy driven process.

The binding stoichiometry between L_2EO_4 and Eu being 3/2 implies an open network structure may form at higher concentrations. Namely, the L_2EO_4/Eu system with the molar ratio of 3/2 allows chain extension. We have reported that chain growth occurred readily in the presence of a positively charged-*b*-neutral block polyelectrolyte PMVP₄₁-*b*-PEO₂₀₅, as a result of the enhancement of local concentration.^{38,39,42} This simultaneously led to the formation of polyion micelles (Scheme 2C). In order to exclude the interference of iodide on the europium luminescence, we did not use the methyl iodide quaternized PMVP₄₁-*b*-PEO₂₀₅ here as in our previous studies.^{38,39,42} Positive charges were triggered by protonating the primitive P2VP-*b*-PEO₂₀₅ at pH 4.0 (Scheme 2B). In Fig. 3 the dynamic light scattering (DLS) measurements suggest that the average hydrodynamic radius of the micelles is about 20 nm, which is in good agreement with other micelles reported in our previous study.^{38,43,44}

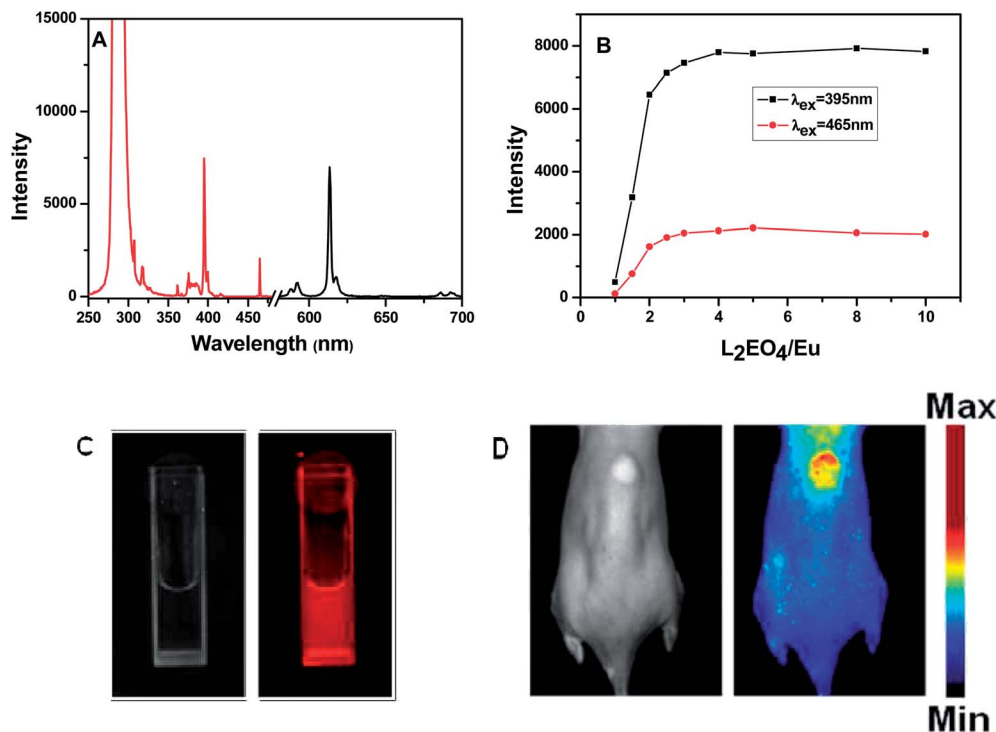
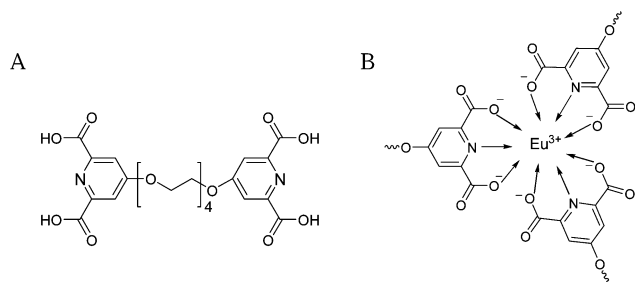


Fig. 1 (A) The excitation (left) and emission (right) spectra of L₂EO₄/Eu = 3/1 complexes ([Eu³⁺] = 0.67 mM, pH = 6.9) under 614 nm and 395 nm respectively. (B) The emission intensity at 614 nm of L₂EO₄-Eu complexes formed at various molar ratios ([Eu³⁺] = 0.67 mM, pH = 6.9) under 395 and 465 nm irradiation, respectively. (C and D) *In vitro* and *in vivo* fluorescence imaging: (C) a bright field image (left) and an *in vitro* solution fluorescence image (right) of L₂EO₄/Eu = 5/1 complexes ([Eu³⁺] = 3.33 mM) in a quartz cell imaged by the Maestro *in vivo* imaging system (CRI, Inc.); (D) a bright field image (left) and an *in vivo* fluorescence image (right) of a mouse subcutaneously injected with 50 μL L₂EO₄/Eu = 5/1 solution (after spectral unmixing).



Scheme 1 (A) Structure of L₂EO₄. (B) Demonstration of coordination between the head of L₂EO₄ and Eu³⁺.

The luminescence intensity and lifetimes were measured to get information about the luminescence properties of the micelles. For a doubly exponentially decayed emission, the decay I_t can be expressed by eqn (1):⁴⁵

$$I_t = I_1 e^{-t/\tau_1} + I_2 e^{-t/\tau_2} \quad (1)$$

$$\alpha_i = \frac{I_i}{I_1 + I_2} \quad (2)$$

where I_t is the emission intensity at time t , I_i ($i = 1, 2$) is the emission intensity of luminophores in different environments at time 0, τ_i ($i = 1, 2$) is the lifetime of luminescence for luminophores in different environments, whereas the pre-

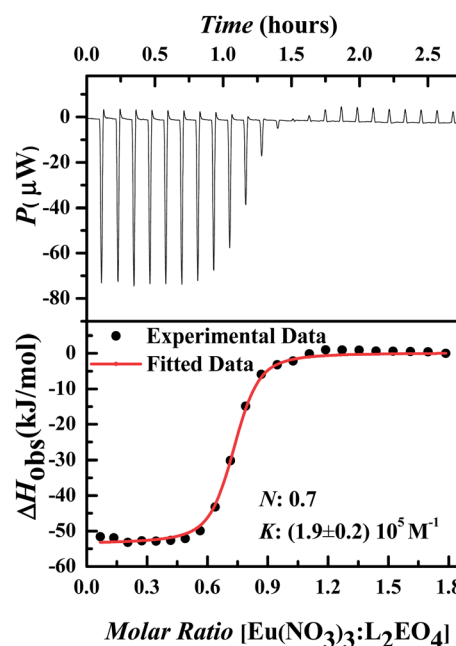
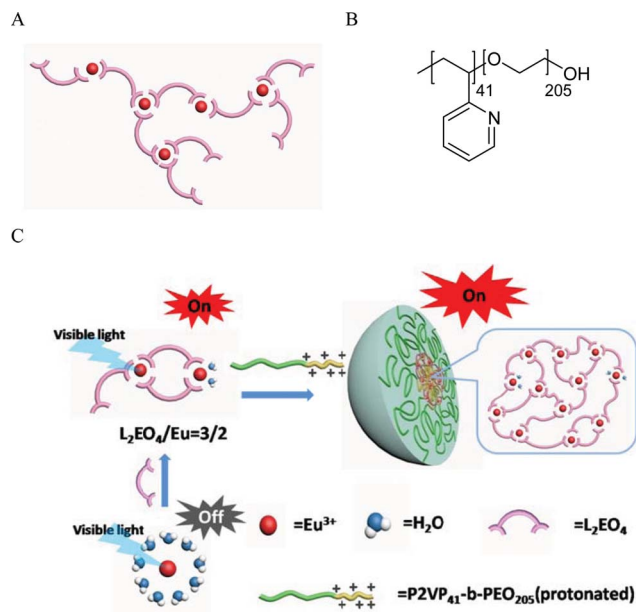


Fig. 2 Calorimetric titration of L₂EO₄ (1.5 mM) with Eu(NO₃)₃ (7 mM) in H₂O at 298 K: raw data (upper panel) of heat evolution with injection of Eu(NO₃)₃. Fitted binding isotherms (lower panel) as a function of [Eu(NO₃)₃]/[L₂EO₄] molar ratio.



Scheme 2 (A) Demonstration of the linear structure formed between Eu and L₂EO₄ ligand in the L₂EO₄/Eu = 3/2 system. (B) Structure of P2VP₄₁-b-PEO₂₀₅. (C) Illustration of the mechanism of the enhancement of luminescence emission of L₂EO₄-Eu coordination complexes with and without P2VP₄₁-b-PEO₂₀₅. The size of the stars demonstrates the strength of the luminescence emissions.

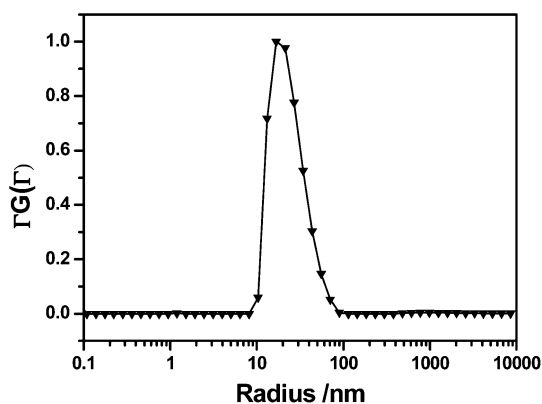


Fig. 3 DLS size distribution of P2VP₄₁-b-PEO₂₀₅/L₂EO₄-Eu (3/2) micelles at pH = 4.0. ([Eu³⁺] = 0.67 mM).

exponential factors α_i ($i = 1, 2$) represent their fraction. In this study, the luminophores are europium ions. The lifetime of Eu(III) is closely related to the amount of water in its coordinating sphere. The more water molecules coordinate with the Eu(III), the shorter the lifetime is.⁴⁶

Two lifetimes are noticeable in the L₂EO₄/Eu = 3/2 solution, which indicates that the europium ions are in two different coordinating states.^{12,47} These two states can be ascribed to the europium ions fully and partly coordinated with L₂EO₄, as illustrated in Scheme 2A. The long lifetimes τ_2 in Table 1, corresponding to europium ions that are fully coordinated with L₂EO₄, account for 47%. It is remarkable that upon micellization, the luminescence decay rate in the micellar system is even

smaller than that in the L₂EO₄/Eu > 3/1 system, suggesting that micellization can produce a better coordination environment. The fraction of long lifetime is estimated to be enhanced to 83% from Fig. 4B (Table 1) upon micellization. In line with this, the luminescence is enhanced 6 times (Fig. 4A), suggesting the luminescence intensity is positively related to the fraction of fully coordinated Eu(III). We also measured the quantum yield of micelle solution using an integrating sphere based on the method described by Lin.⁴⁸ The quantum yield for the 6 mM micelles at pH = 4.0 under 395 nm excitation is 8.16%, and the absorption cross section is 1.63×10^{-20} cm². Although these values are only about one third of some often used luminescent probes,^{49,50} prolongation of the irradiation time may lead to stronger signals in virtue of the long lifetime and anti-bleaching nature of the europium luminescence.

Control experiments in deuterium oxide (D₂O) were made to further examine the average number of water molecules coordinating to one europium ion. Because D₂O does not quench the emission of europium,²⁴ only one lifetime was obtained for all the L₂EO₄-Eu complexes (Table 1), and the lifetimes with and without the presence of polymers are almost the same. The number of water molecules in the first coordination sphere of europium ions can be calculated using eqn (3):⁴⁶

$$q = 1.2 \times (k_{\text{H}_2\text{O}} - k_{\text{D}_2\text{O}} - 0.25) \quad (3)$$

Here, $k_{\text{H}_2\text{O}}$ and $k_{\text{D}_2\text{O}}$ represent the rate constants of luminescence decay, which are measured in H₂O and D₂O, respectively. The q values for L₂EO₄/Eu = 3/2, 3/1 complexes are 0.20 and 0.07, respectively, confirming that water molecules are giving place to ligands with increasing the L₂EO₄/Eu molar ratio. It is remarkable that decreasing pH drastically weakens the coordination between L₂EO₄ and Eu(III), which is evidenced by the increased q values as the pH is decreased. However, upon micellization, the number of water molecules in the first coordination sphere of Eu(III) is reduced to 0, indicating that micellization has greatly enhanced the coordination between L₂EO₄ and Eu(III). We have envisaged this in our previous work,^{38,43} however, by then we could not provide more details for the structure of coordination polymers in the micelles. Obviously, the results in Table 1 have for the first time presented a clear picture of the coordination status of europium under various conditions.

In vivo application of the Eu(III) luminescence sensitized by visible light

The high luminescence of europium sensitized by visible light holds great promise from the application point of view, so we demonstrate the feasibility of *in vivo* luminescence imaging with luminescent europium coordination complexes and Eu-micelles. For practical considerations, the micelles were made with a permanently quaternized PMVP₄₁-b-PEO₂₀₅, as described in our previous work.^{38,43} Two solutions containing L₂EO₄/Eu = 3/1 coordination complexes and Eu-micelles were subcutaneously injected into the back of nude mice. Here, a distinguished red color is observed at the

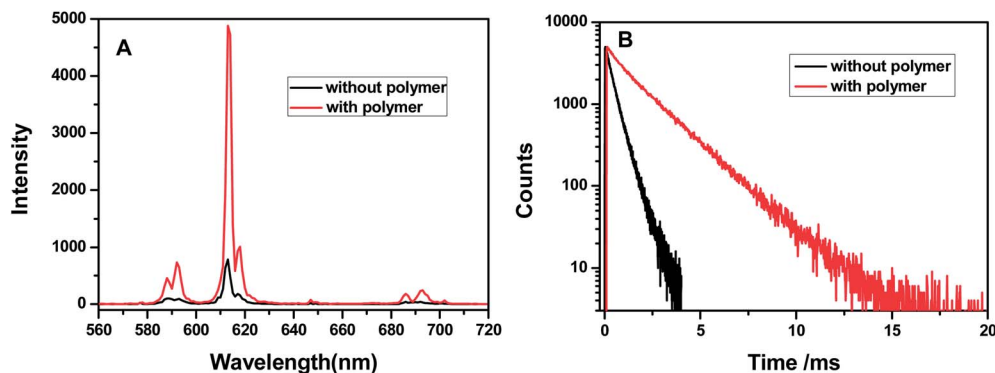


Fig. 4 (A) Emission spectra of micelles and L_2EO_4 -Eu at 3/2. (B) Decay curves of luminescence of micelles and L_2EO_4 -Eu complexes at 3/2 ($[Eu^{3+}] = 0.67$ mM, pH = 4.0).

corresponding injection site (Fig. 5A and B) only for injections containing micelles, and the luminescence intensity for the $L_2EO_4/Eu = 3/1$ is not strong enough to show a clear contrast. As we have shown in Fig. 1 applicable emissions could be obtained as the L_2EO_4/Eu ratio was increased to 5. It should be noticed that the signals were obtained with the spectral unmixing technique upon extending the irradiation time by virtue of the long lifetime of rare earth metals and their anti-photo bleaching nature. The occurrence of emission signals in the micelles, where L_2EO_4/Eu ratio is only 1.5, clearly revealed the advantage of Eu-micelles than the L_2EO_4 -Eu coordinating complex in *in vivo* imaging.

In conclusion, we have for the first time demonstrated that direct excitation of Eu(III) ions can lead to considerable luminescence in aqueous medium if all the coordinated water molecules were excluded from the first coordination sphere using a suitable ligand. This can be achieved by coordinating the Eu(III) ions to a ligand with three chelating sites at a ligand to Eu(III) ratio >3 . In the case of using a ditopic ligand, say L_2EO_4 in this study, a polymeric network may be formed at a ligand to Eu(III) ratio of 1.5 in the presence of an oppositely charged block polyelectrolyte, which simultaneously leads to the formation of electrostatic micelles. *In vivo* fluorescence imaging in mice using those micelles was realized, suggesting that the resultant luminescence is very promising for practical applications. Our strategy avoids using the 'antenna effect' or 'up-conversion luminescence', thus greatly simplifying the fabrication of luminescent Eu(III) probes for use in bioimaging. This novel

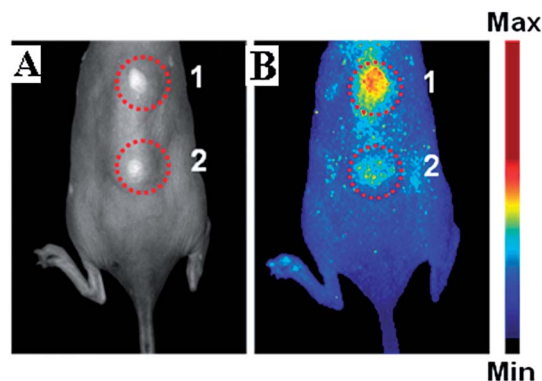


Fig. 5 *In vivo* fluorescence imaging made with the Maestro *in vivo* imaging system (CRI, Inc.): (A) a bright field image and an *in vivo* fluorescence image (B) of a mouse subcutaneously injected with 50 μ L samples (after spectral unmixing). Sample 1 is imaged with micelles formed with $L_2EO_4/Eu = 3/2$ (with $[Eu^{3+}] = 0.67$ mM) and charge balancing polymer PMVP₄₁-b-PEO₂₀₅, whereas 2 with $L_2EO_4/Eu = 3/1$ complex ($[Eu^{3+}] = 0.67$ mM).

path significantly lowers the barriers in developing water soluble europium nanoprobe and opens up a new vista in the design and fabrication of a new generation of bioimaging reagents. We expect that with the guidance of the principles reported in our work, some bio-compatible labels that are based on the direct irradiation of Eu(III) can be achieved in the near future simply by a suitable choice of the chelating ligands.

Table 1 Summary of lifetimes of L_2EO_4 -Eu complexes with and without diblock copolymer in H_2O and in D_2O ($\lambda_{ex} = 395$ nm, $[Eu^{3+}] = 0.67$ mM)

Systems	pH	H_2O			D_2O τ /ms	q
		τ_1 /ms	τ_2 /ms	α_2		
$L_2EO_4/Eu = 3/1$	6.9	—	1.43	1	2.57	0.07
$L_2EO_4/Eu = 3/2$	6.9	0.59 ± 0.02	1.25 ± 0.02	0.47	2.69	0.21
$L_2EO_4/Eu = 3/2$	4.0	0.35 ± 0.01	0.62 ± 0.03	0.32	2.86	1.22
P2VP ₄₁ -b-PEO ₂₀₅ / L_2EO_4 -Eu (3/2)	4.0	0.46 ± 0.03	1.98 ± 0.01	0.83	2.95	0

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