Energy transfer in ternary TbEDTA chelates with a series of dipicolinic acid derivatives

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Abstract

The energy transfer in lanthanoid chelates was studied using the ternary TbEDTA (EDTA = ethylenediaminetetraacetic acid) chelates with several dipicolinic acid derivatives (pyridine-2,6-dicarboxylic acid (L1, dpa), 4-(9H-fluoren-3-yl)pyridine-2,6-dicarboxylic acid (L2), 4-(dibenzo[b,d]furan-2-yl)pyridine-2,6-dicarboxylic acid (L3), 4-(dibenzo[b,d]thiophen-2-yl)pyridine-2,6-dicarboxylic acid (L4) and 4-(9H-carbazol-3-yl)pyridine-2,6-dicarboxylic acid (L5)) and spectroscopic methods (absorption and luminescence spectroscopy and the effect of argon treatment and temperature on luminescence lifetime(s)). The results revealed that the ILCT (intra-ligand charge transfer) state is inefficient in exciting the Tb(III) ion and the emissive states of the ligands, as well as the triplet states, act as quenching states by receiving energy back transfer from the Tb(III) ion. The stability constants for these ternary complexes were also determined.

Keywords: lanthanoid, luminescence, dipicolinic acid, energy transfer

1. Introduction

The luminescent lanthanoid chelates have a wide application area including bioassays[1], bioimaging[2,3] and light emitting diodes (LEDs)[4,5] due to their special photophysical properties. These include narrow emission lines and long luminescence lifetimes, and they are based on the existence of shielded f-orbitals in the lanthanoid(III) ions and the f-f transitions forbidden by the Laporte rule. The latter property increases the luminescence lifetimes and necessitates the use of ligands for excitation because of the very low molar absorption coefficients of the Ln(III) ions. The ligands harvest light energy and transfer it to the central ion. In principle, the transfer may occur directly from the ligand singlet state or via its triplet state, or may involve intra-ligand (IL) or ligand-to-metal (LM) charge transfer (CT) states. Generally, the energy transfer route mediated by triplet state has been regarded as dominant.[6-10] However, the CT states have been shown to play a role in the energy transfer in the Eu(III) complexes.[11-15] This can be related to the easily reducible Eu(III) ($E^{\ominus}_{Eu^{3+}(aq)/Eu^{2+}(aq)} = -0.35$ V vs. NHE) [16], which suggests that the energy transfer is enhanced by the Horrocks' step-by-step mechanism[17]. The presence of a conjugated structure and electron donating and accepting groups is a prerequisite for an ILCT state.[18]

Most applications of Ln(III) chelates are based on Eu(III) and Tb(III) ions, for which several efficient chelates have been developed.[1-5] However, chelates with improved properties are still needed even for these ions and, especially, Sm(III) and Dy(III) ions, which are used in applications demanding bio-harmless excitation wavelengths.[19,20] The basic photophysical properties of lanthanoid(III) complexes are well-known[21] but the energy transfer phenomena are not fully understood. A better understanding of the fundamental physics of the luminescence of lanthanoid complexes could significantly help the development of chelates with tuned properties. This study tries to shed light on the energy

transfer in terbium(III)-EDTA complexes with pyridine-2,6-dicarboxylic acid (L1), 4-(9H-fluoren-3-yl)pyridine-2,6-dicarboxylic acid (L2), 4-(dibenzo[b,d]furan-2-yl)pyridine-2,6-dicarboxylic acid (L3), 4-(dibenzo[b,d]thiophen-2-yl)pyridine-2,6-dicarboxylic acid (L4) and 4-(9H-carbazol-3-yl)pyridine-2,6-dicarboxylic acid (L5) (Scheme 1). In the following, ligands L2 – L5 are collectively called fluorenyl dipicolinic acids (fdpa). We compare the previously studied ternary EDTA (ethylenediaminetetraacetic acid) - dipicolinic acid derivatives complexes with Eu(III) [22] with the corresponding Tb(III) complexes and determine the temperature dependence of luminescence in these TbEDTA-fdpa complexes. As Tb(III) is not easily reducible ($E^{\ominus}_{Tb^{3+}(aq)/Tb^{2+}(aq)} = -3.5$ V vs. NHE)[16] the Tb(III) complexes cannot have a LMCT state and transfer energy by the Horrocks' mechanism. This also excludes the possibility of the photoinduced electron transfer (PET) process in Tb(III) chelates, with the exception of PET between the organic moieties[23]. In addition, Tb(III) has only one, long-lived energy state within 19000 – 26000 cm^{-1} (E(^{5}D$_4$) = 20545 cm^{-1}), and so the energy back-transfer to ligand is significant in many Tb(III) complexes.[24,25] For this study, a series of (f)dpa(s) (L1 – L5), was selected. The electron donating ability of the group directly conjugated to the 4-position of dipicolinic acid varies in the series (see Table 3 below), giving different ILCT properties to the ligands. For Eu(III), the published quantum yields in this series seem to follow the electron donating ability of the *p*-substituent.[22]

In addition to attempt to clarify the energy transfer phenomenon in Ln(III) chelates, the ternary Tb(III) complexes have been studied with aim to find a new, more efficient Tb(III) chelate for DNA-hybridization assay[26]. TbEDTA based ternary complexes are also used in other bio-assays [27,28], and our TbEDTA-(f)dpa complexes can act as model systems for these applications. To our best knowledge, such TbEDTA-(f)dpa complexes have not been previously studied, and also the excitation of Tb(III) via the ILCT state has received little

attention. However, O. Maury *et al*. have described twisted antenna ligands which can be used to excite Tb(III) luminescence through the ILCT state.[29]

## 2. Experimental

2.1. Materials

Reverse osmosis water was distilled twice in quartz vessels before use. Tris(hydroxy-methyl)aminomethane) (TRIS, p.a.), sodium acetate, ethylenediaminetetraacetic acid (EDTA, Titriplex III, p.a.) and xylenol orange were purchased from Merck. Hydrochloric acid (HCl, 37 % AR) was from VWR and DPA ($\geq$ 99.5 %) and $TbCl_3 \cdot 6H_2O$ (99.9 %) from Aldrich. Scheme 1 shows the studied ligands (their synthesis is described ref. [22]).
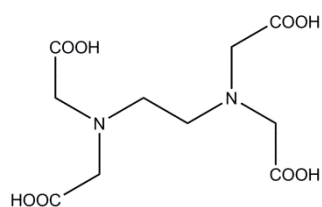
H4Y (EDTA)
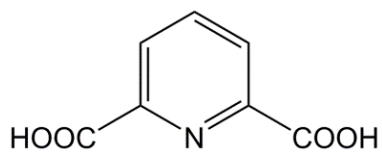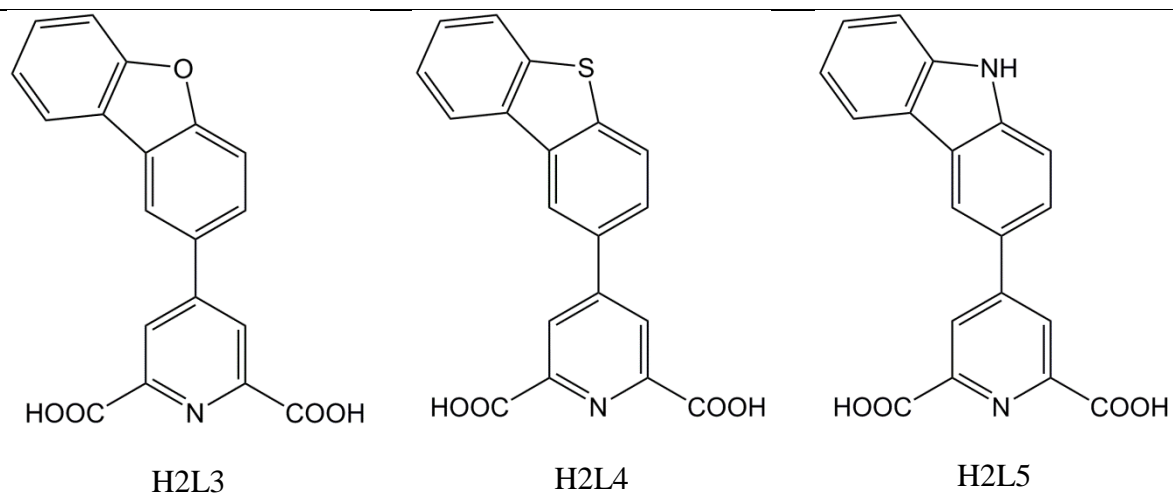
H2L1 (dipicolinic acid, dpa)
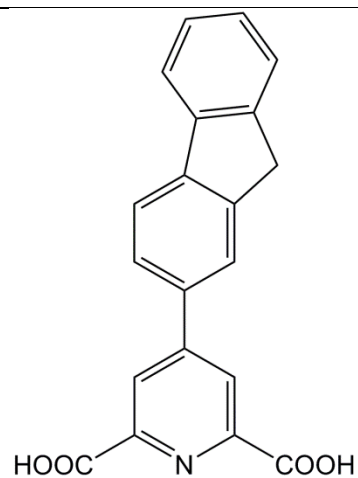
H2L2

Scheme 1. Studied ligands.

The 500 µM TbY stock solution in TRIS buffer was made by mixing 1.022 mM EDTA (in water) and 1.01 mM Tb(III) (in a TRIS buffer). Sample solutions TbY-Lx (x = 1 – 5) in TRIS buffers (C_{TRIS} = 50 mM, pH = 7.75) were prepared using the stock solutions of the ligands Lx (C ≈ 100 µM in TRIS; C_{TRIS} = 50 mM, pH = 7.75) and TbY. The concentration of the Tb(III) solution was determined by titrating it with EDTA and using xylenol orange as the indicator. [30]

2.2. Methods

The excitation and emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrometer. The luminescence lifetimes of the terbium complexes were determined by the laboratory-built frequency-domain equipment with a UV-LED (UVTOP285, Roithner Lasertechnik GmbH, Vienna) for excitation.[31] Data treatment was done as described previously.[31,32] The luminescence measurement devices were equipped with Peltier temperature controllers. UV-vis absorption spectra were recorded on an Agilent Technologies Cary 60 spectrometer. The 2 mm cuvette was used in the spectroscopic measurements and, unless otherwise mentioned, the measurements have been done at room temperature. The

Gaussian deconvolutions of the absorption and emission spectra were done on the energy scale with Origin 2015 software (OriginLab, U.S.A.).

3. Result and discussion

3.1. Stability constants

The comparison of the luminescence properties of different lanthanoid chelates requires the knowledge of the stability constants (K, for the reaction $LnY + Lx \rightleftharpoons LnY\text{-}Lx$) of the complexes. They can be conveniently obtained by measuring the total intensity (I_{tot}) of the complex-based luminescence as a function of the total concentration C and fitting the data to equation

$$I_{tot} = a\frac{2KC + 1 - \sqrt{4KC + 1}}{2K} \tag{1}$$

where a is a proportionality constant that comprises all instrumental factors. We have previously determined the stability constants of Eu(III) chelates using the frequency domain (FD) technique.[22]. We tested the method also using the conventional pulse excitation technique with EuY-L1 and obtained a value of $\log \beta = 5.30 \pm 0.12$, very close to the value $\log \beta = 5.38 \pm 0.16$ previously measured using the FD technique.[22]. Therefore, the method is applicable with both excitation techniques. The stability constant of the ternary complex of Tb(III)Y with ligand L1 was also determined by the pulse method. The stability constants of these Tb(III) and Eu(III) complexes can be assumed to be similar, and the fit of data to eq. (1) yielded a value of $\log \beta = 5.40 \pm 0.34$ (Fig. 1).
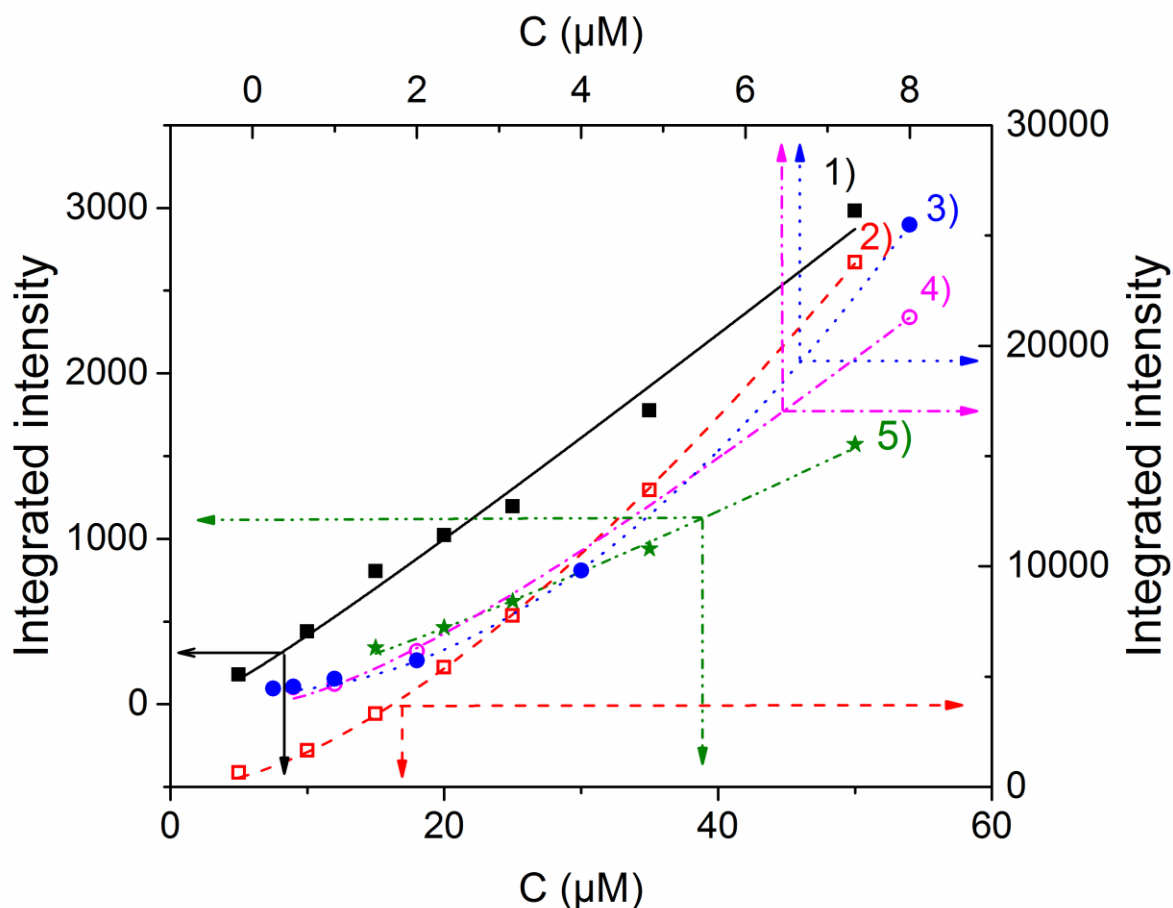
Figure 1. Luminescence intensity of TbY-L1 (1, black filled squares and solid line), TbY-L2 (2, red open squares and dashed line), TbY-L3 (3, blue solid circles and dotted line), TbY-L4 (4, purple open circles and dash-dotted line) and TbY-L5 (5, green filled stars and dash-dot-dotted line) vs. concentration. Corresponding axis are marked with arrows. Measured with pulse excitation luminometer in TRIS-buffer (pH = 7.75). Excitation wavelength 285 nm, emission wavelength 545 nm and delay 100 µs.

Table 1 shows the obtained stability constants for all the ligands in the studied series (fits shown in Figure 1), fraction of ligands bound to ternary complexes in 25 µM solutions and substituent constant values (σ_p^+). It is worth mentioning that significant amount of ligands are uncomplexed, especially in the cases of ligands L2, L3 and L5. The Ln(III) complexes are

based mainly on electrostatic interactions, which suggests that the stability constants would increase with the increase of the electron donating ability of the *p*-substituent and the negative charge of the chelating groups of fdpas. However, this prediction is not fulfilled (See Table 1), which can be attributed to a combination of electronic repulsion between the negatively charged ligands and electronic attraction between the negatively charged donor groups and the positively charged Tb(III) ion.

Table 1. Substituent constant values (σ_p^+), stability constant (log β) for reaction TbY + Lx → TbY-Lx (x = 1 – 5) and calculated fraction of complexed ligand in 25 µM solutions.

TbY + Lx → TbY-Lx	log β	Complexed fraction in 25 µM solutions	σ_p^{+a} (value for)
L1	5.40 ± 0.34	0.68 ± 0.12	-
L2	4.10 ± 0.03	0.20 ± 0.01	-0.18 (Ph)
L3	3.85 ± 0.27	0.15 ± 0.07	-0.50 (OPh)
L4	5.55 ± 0.24	0.70 ± 0.06	-0.55 (SPh)
L5	4.83 ± 0.15	0.46 ± 0.04	-1.40 (NPh)

[a] From ref.[33];

3.2. Absorption and luminescence spectra

Figure 2 shows the absorption spectra of 25 µM solutions of ligands L1 – L5 and the ternary complexes TbY-L1 – TbY-L5 and their Gaussian deconvolution. The spectra show that the complexation does not affect the absorption spectrum of L1 but reduces the apparent molar absorptivity of the other ligands. The detailed reason for this phenomenon is not clear at the moment. The absorption bands in the spectra of TbY-L2 – TbY-L5 display shoulders, and the spectra have been deconvoluted using Gaussian peaks on the energy scale. For these ligands, resonance structures can be drawn with the heteroatom or substituent group (O, S, N, or Ph) is positively charged and the pyridine ring carrying a negative charge. This implies that these ligands possess an ILCT state, and we assigned the high wavelength absorption shoulders to

CT bands, similarly as in other studies.[29,34] Therefore, two identified transitions in the spectra of complexes L2 – L5 below and above 300 nm were assigned to π-π* and ILCT transitions, respectively. The complexation seems to lower the energy of the ILCT states because the shoulders are red-shifted in complexes, and the shift is the most prominent for L5, which also has the lowest-lying ILCT state. We tentatively assign the red-shifts to the electron withdrawing nature of the highly positively charged Tb(III) ion. A similar shift was also observed in the corresponding Eu(III) complexes (see the absorption spectra in ref. [22]), which indicates that the absorption band was an inter-ligand band and not a ligand-to-metal band. The absorption of L2 extends to higher wavelengths, as compared to the other ligands in the series, because its geometry allows a more extensive conjugation. The terminal benzene ring of L2 is in *p*-position with respect to the central benzene and pyridine rings, thus forming a well-conjugated system.
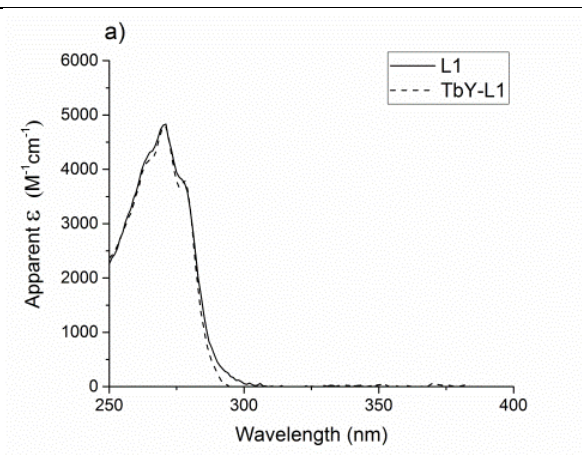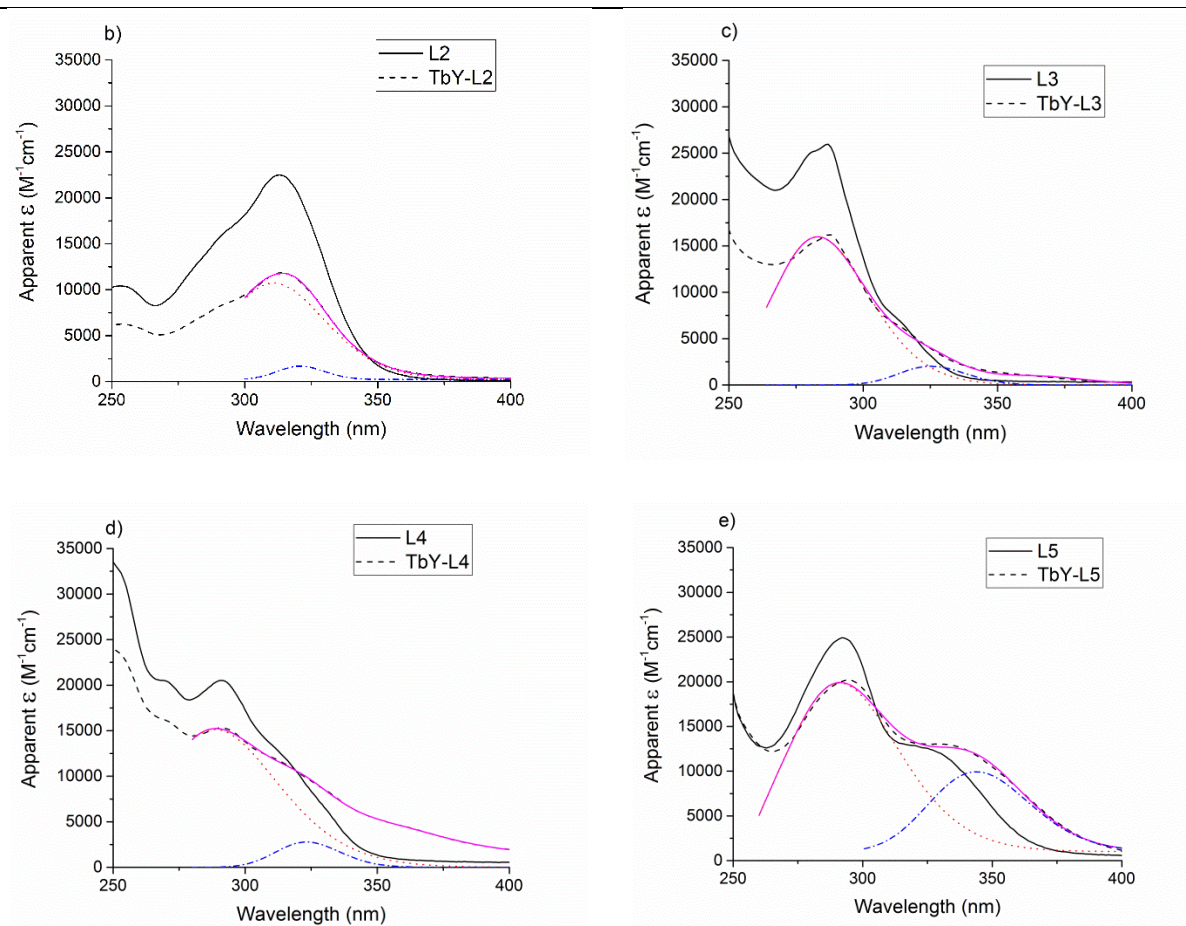
Figure 2. Absorption spectra of 25 µM L1 and TbY-L1 (a), L2 and TbY-L2 (b), L3 and TbY-L3 (c), L4 and TbY-L4 (d), and L5 and TbY-L5 (e) in TRIS (pH = 7,75) and deconvoluted spectra of the complexes TbY-L2 – TbY-L5. Black solid lines, Lx; black dashed lines, TbY-Lx; purple solid lines, cumulative fitted curve; red and blue dotted/dashed lines, deconvoluted components. Note the different scale in (a).

Figure 3 shows the excitation and emission spectra of 25 µM solutions of the ternary complexes TbY-L1 – TbY-L5, measured using either 0 – 40 µs or 0.1 – 100 ms integration time but under otherwise identical conditions, and the convoluted ligand emission spectra of the complexes TbY-L2 – TbY-L4. In line with the absorption spectra the deconvoluted

emission bands at lower and higher wavelength were tentatively assigned to luminescence

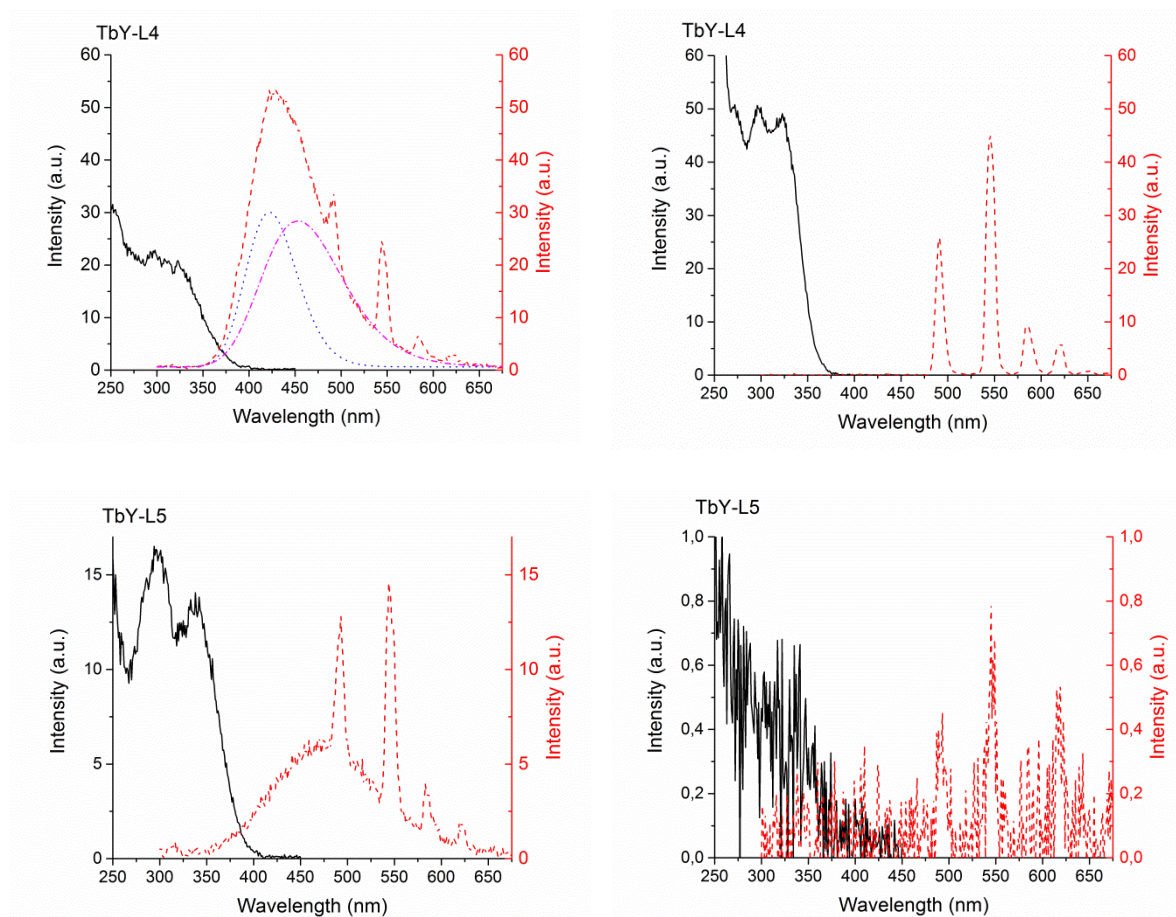from the singlet and ILCT state, respectively.
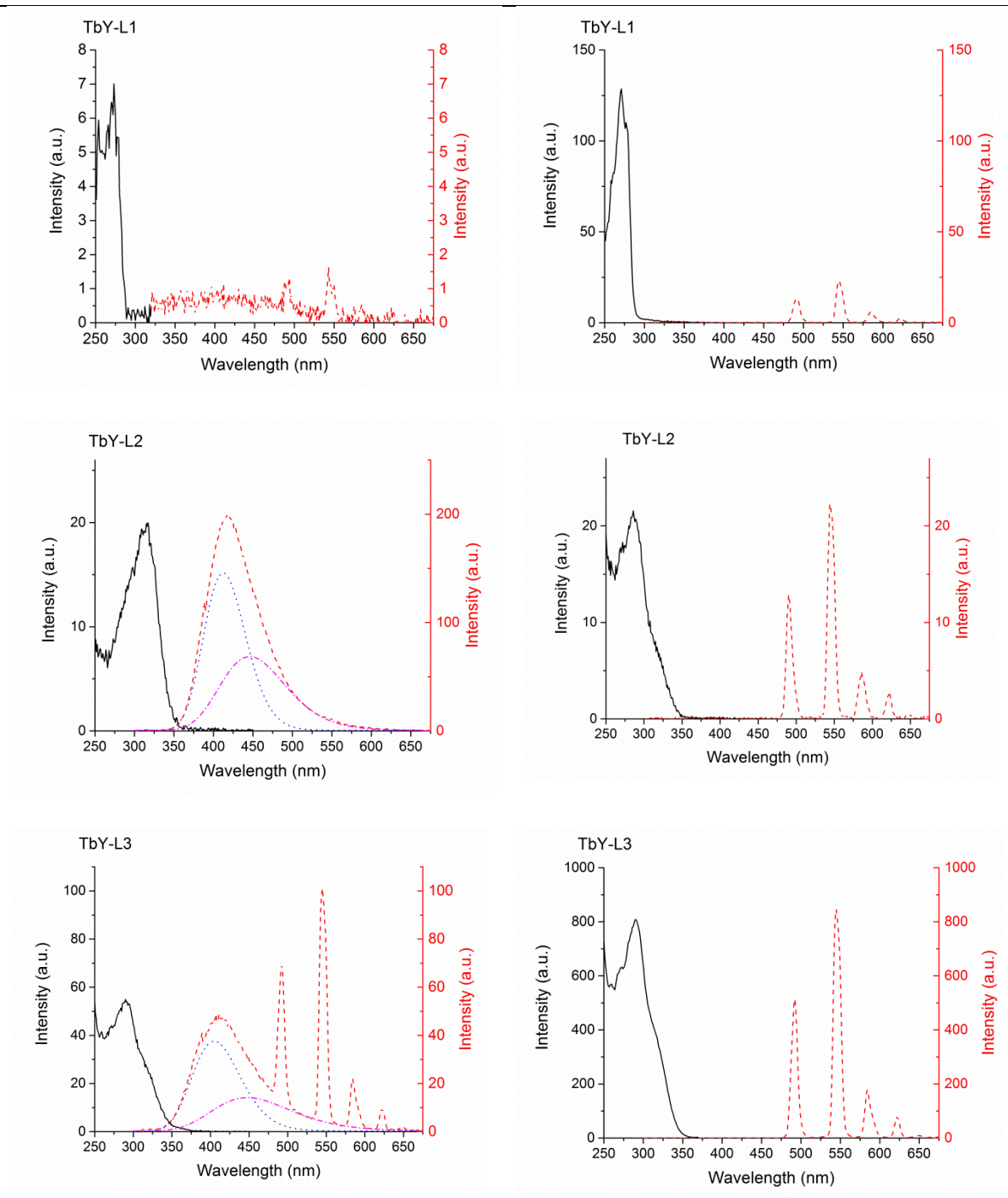
Figure 3. Excitation (solid black lines, left axis) and emission (dashed red lines, right axis) spectra and convoluted ligand emission spectra (complexes TbY-L2 – TbY-L4) of 25 µM TbY-Lx (x = 1 – 5) in a TRIS buffer (pH = 7.75) with integration time 0 – 40 µs (left column) and 0.1 – 10 ms (right column). Emission wavelengths for excitation measurements 545 nm and excitation wavelength for emission measurements 285 nm, and filters, slits, scan rate and PMT voltage same for every measurements. Note the differing of the emission scale from the excitation scale in the TbY-L2 spectra measured with 0 – 40 µs integration window.

The studied solutions are a mixture of ternary Tb(III) complexes and free ligand, and both emission bands, measured using a 0 – 40 µs integration time, contain contribution from both species. The spectrum of TbY-L2 measured with the delay of 0 µs differs from the other

measured ones because the high fluorescence from the ligand L2 completely masks emission from Tb(III). This can be attributed to the low stability constant of the ternary complex (only 20% of L2 is complexed, see Table 1), the high fluorescence efficiency of L2 and the low luminescence efficiency of Tb(III) because of a low-lying ligand triplet state (*v.i.*). Comparison with the absorption spectra (Fig. 2) shows that the ILCT shoulders were seen also in the excitation spectra. As the emission at 545 nm is dominated by the emission from Tb(III) in most cases, it is possible that the Tb(III) is efficiently excited via the ILCT state, and the effect of the excitation wavelength on the emission spectra of TbY-L3 (at 545 nm) was tested to clarify this assumption. The ratio of the TbY-L3 total ligand fluorescence peak intensity to that of the Tb(III) 545 nm peak increased by 55 % when the excitation wavelength was changed from 290 nm to 320 nm. This implies that the radiative decay of the ligand ILCT state is more efficient than the internal conversion, or that Tb(III) is only poorly excited via the ILCT state. However, the Tb(III) ion can probably be excited from the ILCT state because the metal emission peaks were still observed when the complex was excited at 320 nm even though the singlet absorption band also extends to this region. The Tb(III) emission of TbY-L5 can be excited at 375 nm. Whether the energy is transferred from the ILCT state to the Tb(III) ion directly or via the triplet state cannot be unambiguously determined.

Table 2 shows the absorption and emission maxima and Stokes shifts of the deconvoluted singlet and ILCT bands. The maxima of the singlet states follow the conjugation length of the structures and the Stokes shift seems to follow the electron donating ability of *p*-substituent (see Table 3) but the Stokes shift related to the ILCT states is practically constant.

Table 2. Deconvoluted absorption and emission maxima: $\lambda_{S\ abs}^{max}$ = absorption maximum of singlet state, $\lambda_{S\ em}^{max}$ = emission maximum of singlet state, S Stokes shift = Stokes shift between singlet absorption and emission, $\lambda_{ILCT\ abs}^{max}$ = absorption maximum of ILCT state, $\lambda_{ILCT\ em}^{max}$ = emission maximum of ILCT state, ILCT Stokes shift = Stokes shift between ILCT absorption and emission.

(TbY-)Lx	$\lambda_{S\ abs}^{max}$ (nm)	$\lambda_{S\ em}^{max}$ (nm)	S Stokes shift (nm)	$\lambda_{ILCT\ abs}^{max}$ (nm)	$\lambda_{ILCT\ em}^{max}$ (nm)	ILCT Stokes shift (nm)
L2	311±1	412±1	103	321±1	446±9	127
L3	283±0	405±8	122	318±1	448±23	130
L4	288±0	422±1	134	323±1	453±0	130
L5	291±0	-	-	344±0	471	127

Values from Figures 2 and 3.

Table 3. Singlet (E_S), ILCT (E_{ILCT}) and triplet state (E_T) energies of the ligands Lx and I/A_{285nm} values for TbY-Lx.

(TbY-)Lx	E_S (cm^{-1})[a]	E_{ILCT} (cm^{-1})[a]	E_T (cm^{-1})[b]	I/A_{285nm}[c]
L1	-	-	25300	1.9
L2	27700	26100	20000	0.16
L3	30000	26500	22200	42
L4	29200	26500	21300	0.30
L5	-	25200	21000	0.030

[a] From convoluted spectra; [b] From ref.[22]; [c] Calculated from the absorbance values at 285 nm (A_{285nm}, Figure 1) and emission intensities at 545 nm (I, from FD curves) (The absorbance values were multiplied with the fraction of complexed ligand (Table 1)); - Missing value.

The energies of the singlet (E_S) and ILCT (E_{ILCT}) states shown in Table 3 were estimated from the absorption and emission spectra. The absorption usually occurs from the lowest vibrational level in the ground state to a higher vibrational level of the excited state and the emission from the lowest vibrational level of the excited state to a higher vibrational level of the ground state.[35] Thus, the average of the corresponding deconvoluted absorption and emission band maxima (Figures 2 and 3) were taken as a rough approximation of the first excited singlet (S_1^*) and ILCT state energies. The ligand L5 in TbY-L5 has only one emission peak which is understandable due to the strong charge separation. The E_{ILCT} has an approximate linear dependence on the substituent constant σ_p^+ (see Supporting Information).

Table 3 shows the values of the ratio I/A_{285nm} for the complexes TbY-Lx (x = 1 – 5). This ratio can be regarded as a crude approximation of the relative quantum efficiency of the fluorescence within this series of complexes, and is used here only to clarify the differences in the luminescence properties. It can be seen that the I/A_{285nm} values of complexes TbY-Lx, with x = 2, 3, or 4, follow the triplet state energy but TbY-L5 exhibits an anomalously low value. In the corresponding Eu(III) complex series the quantum efficiency followed the ILCT energy order and increased with increasing ILCT energy, except for the carbazol compound (EuY-L5). [20] This implies that the ILCT state excites the Eu(III) ion much more efficiently than the Tb(III) ion, as already discussed above. This different behavior can be attributed to the suggested Horrocks' step-by-step energy transfer mechanism in the easily reducible Eu(III) complexes.[17] In addition, the very high value of the I/A_{285nm} ratio for the complex TbY-L3 implies a high quantum yield. In fact, this complex has markedly higher relative quantum efficiency (I/A_{285nm}) than TbY-L1 (when excited via the singlet state of ligand). It is noteworthy that Tb(L1)$_3$ has, according to Latva *et al.*[10], the quantum yield which is about half of the quantum yield of the corresponding complex of the ligand currently used in the DNA hybridization assay.[26]

3.3. Luminescence lifetimes

Table 4 shows the lifetimes of luminescence (at 545 nm) of the ternary TbY-Lx complexes before and after argon treatment (solutions were passed 20 min with argon flow). The luminescence of Ln(III) complexes usually proceeds via a triplet state, and argon treatment would be expected to increase the luminescence lifetimes because O_2 is an efficient triplet quencher.[35] The complex TbY-L1 exhibited only one emission process, and its lifetime

decreased but its amplitude increased slightly as a result of the argon treatment. This is consistent with the general fact that oxygen does not quench the luminescence of $Tb(L1)_{n(=1-3)}^{+3-2n}$.[36] It suggests that the energy is either directly transferred from the singlet state of L1 to Tb(III), or that the lifetime of the L1 triplet state in the complex is too short for O_2 quenching. If the triplet state lifetime of the ligand in Tb(III) complexes is several microseconds oxygen decreases the luminescence lifetime of complexes.[37] Attempts to show a direct singlet energy transfer within $Tb(L1)_{n(=1-3)}^{+3-2n}$ have been made [36,38] but the observations can be explained also differently. The rate constant of the ISC (inter system crossing) is of the order of 10^{10} s^{-1}.[35] According to de Sá et al.[39], the energy transfer between the $^7F_6$ and $^5G_2$ states of Tb(III) (ΔE ($^7F_6 - ^5G_2$) = 29600 cm^{-1}) can be induced by the E (S_1^*) of dpa (estimated to be 30000 cm^{-1}) via the multipolar mechanism. The rate of the resonance energy transfer (RET) can be expressed by equation $k_T(r) = (1/\tau_D)(R_0/r)^6$, where r is the distance between the donor and acceptor, R_0 is a Förster distance and τ_D is the lifetime of donor in the absence of the energy transfer.[33] The fluorescence lifetime of dipicolinic acid is reported to be 5.3 ns in a polymer film[40]. The distance between the Tb(III) ion and the nitrogen of dpa was reported to be 2.49 Å[41], and we may use this value to approximate the donor-acceptor distance in Tb(dpa)$_3$. Because the value of R_0 can be estimated to be > 5 Å, the singlet energy transfer rate constant between dpa and Tb(III) can be approximated to be of the order of $k > 10^{10}$ s^{-1}. Therefore, the direct singlet energy transfer and the triplet mediated route may compete with each other in the absence of other factors.

The determined luminescence lifetime of L1 in the ternary complex deviates from the previously reported value (2250 µs), measured with Tb(L1)$_3$ (Latva). This is probably due to the symmetry effect of different coordinative environments, additional C-H quenching groups in the vicinity of Tb(III) or fast ligand exchange. [42-44] The observation of only one

luminescence lifetime for TbY-L1supports the latter because the corresponding Eu(III) complex exhibited two lifetimes. If the fully and partially complexed forms are in rapid equilibrium with each other in the time scale of the luminescence decay only a single average lifetime is seen.[43]

The complex TbY-L2 exhibited both the luminescence decay and rise times (negative amplitude in the FD method can be interpreted to represent a rise time). Because the ligand triplet state is below the emitting $^5D_4$ state of Tb(III) the lifetime must represent either the ILCT excitation or the temperature driven upstream energy flow from the triplet state, or both. However, in the latter case, the deaeration should lengthen and intensify the luminescence lifetime. On the contrary, the observed lifetime shortening implies that the excitation must, at least partially, take place from the ILCT state. On the other hand, we assign the observed rise time to the upward energy flow from the triplet state, and suggest that the luminescence of Tb(III) in the TbY-L2 complex is mediated by both the ILCT and triplet state. Because the rise time represents the upward energy flow from the triplet state to the Tb(III) $^5D_4$ state, it is the lifetime of the triplet state of L2 in TbY-L2. An interesting question is why the lifetime (τ_1) is so long as compared to the other series (TbY-L3 – L5) although the triplet state of L2 is beneath the $^5D_4$ state of Tb(III) but the reason cannot be derived from these results.

Two observed luminescence lifetimes in the TbY-L3 – TbY-L5 complexes are assigned to the ternary TbY-Lx complex (longer τ) and to the intermediates introduced earlier in the case of EuY-Lx complexes (shorter τ)[22]. The lengthening of the luminescence lifetimes of TbY-L4 and TbY-L5 after argon treatment suggests that the energy transfer in these complexes occurs mainly via the triplet state, and the triplet state of ligands is long-lived enough for the O_2 quenching. On the contrary, oxygen increases the lifetimes in TbY-L2 and TbY-L3 but, at

the moment, we have no plausible explanation for this observation. Based on Latva's results[10], the energy back-transfer is not significant in TbY-L3 because the triplet state of L3 lies 1700 cm^{-1} above the $^5D_4$ state of Tb(III), and the limit for the energy back transfer is ca. 1500 cm^{-1} (at room temperature). However, minor back-transfer may occur because the τ_1 of the TbY-L3 complex is somewhat smaller than that of the TbY-L2 complex. The luminescence lifetimes of the TbY-L4 and TbY-L5 complexes were shorter than that of the TbY-L3 complex. The shorter luminescence lifetime (τ_1) of the TbY-L4 complex can be explained by a low-lying triplet state (800 cm^{-1} above the $^5D_4$ state of Tb(III)) but it is not clear if a decrease of 300 cm^{-1} in the triplet state energy can alone cause the observed large decrease in the luminescence lifetime between complexes TbY-L4 and TbY-L5 (*v.i.*).

Table 4. Luminescence lifetimes of 25 µM TbY-Lx:s in TRIS (pH = 7,75) before and after argon treatment (solutions passed 20 min with argon flow). The values in brackets are relative intensities. Excitation wavelength was 285 nm and emission wavelength 545 nm. Fittings are shown in Supplementary Information.

Complex	In the presence of O_2		Deaerated	
	τ_1 (µs) (I)	τ_2 (µs) (I)	τ_1 (µs) (I)	τ_2 (µs) (I)
TbY-L1	1878±7 (86)	-	1724±4 (116)	-
TbY-L2	1449±11 (165)	4.4 (-43)	1150±7 (225)	3.9 (-24)
TbY-L3	1359±13 (341)	765±11 (209)	982±5 (59)	262±8 (9)
TbY-L4	358±13 (213)	25±19 (19)	885±15 (203)	233±16 (64)
TbY-L5	17±1 (3)	6.64±0.05 (25)	37±3 (4)	11.1±0.1 (24)

3.4. Temperature dependence

In order to assess the possibility of an energy back transfer process during the emission the temperature dependence of the luminescence life times in the studied ligand series was measured and with the energy back transfer equation (2) [45]

$$\tau^{-1} = k = k_0 + k'\exp(-\Delta E/k_B T) \qquad (2)$$

Here k_0 is the decay rate constant at the low temperature limit, k' is the pre-exponential factor, ΔE the energy difference between the feeding and receiving states, and k_B is the Boltzmann's constant (0.69503 cm^{-1}/K). Figure 4 shows the results for one observed life time of the complex TbY-L1, which yielded a value of 1731 ± 141 cm^{-1} for ΔE. At this energy level ($E(^5D_4$ of Tb(III)) $+ \Delta E = 22300$ cm^{-1}), there are no energy states in dipicolinic acid (triplet state energy $E_T = 25300$ cm^{-1} and the possible ILCT state should be still higher in energy) or Tb(III) (the energy of the state $^5D_3$ is 26300 cm^{-1}), which leaves the question of the quenching mechanism open. In addition, the temperature dependence is quite small compared to other complexes. A vibrational quenching mechanism could be anticipated but the fitting of the vibrational quenching equation[21] to the data was unsuccessful.
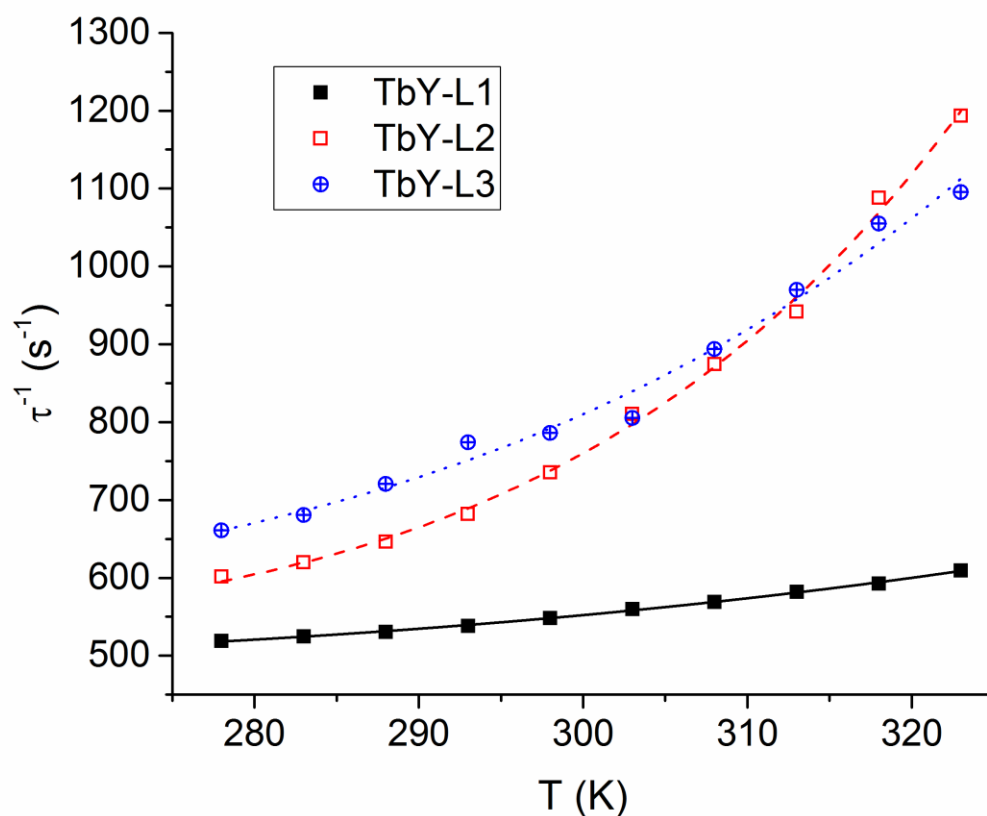
Figure 4. Temperature dependencies of luminescence lifetimes. Black squares, TbY-L1 (τ); red squares, TbY-L2 (τ_1); blue crossed circles, TbY-L3 (τ_1) (all in 25 µM in TRIS at pH 7.75). Lines are the fits to the energy back-transfer equation (2).

Figure 4 shows also the dependence of the longest life time τ_1 of the complex TbY-L2 on temperature. In this case, a value of $\Delta E = 3046 \pm 246$ cm^{-1} is obtained. Added to the energy of the Tb(III) $^5D_4$ state gives 23590 cm^{-1}, which is close to the energy corresponding to the ILCT fluorescence emission maximum of ligand L2 (deconvoluted $E_{ILCT\ em.max}$ for L2 in TbY-L2 is $\lambda_{max,em} = 446$ nm $\triangleq 22420$ cm^{-1}). This suggests that, in the ternary complex, energy can be transferred back to the emissive state of the ligand L2, as illustrated in Figure 5a. This emissive state represents the ILCT emission. This must mean that the energy from the excited Tb(III) ion back-transfers to the ligand faster than the thermal relaxation occurs from the higher vibrational levels of the ground electronic state of the ligand (to which ligand is ended up after the energy transfer to Tb(III)) to the zero vibrational level of the ground state. The energy back-transfer must be faster because otherwise the energy back-transfer would demand that the vibrational level 4480 cm^{-1} (E_{ILCT} - $E_{ILCT\ em.max}$ = 26900 cm^{-1} - 22420 cm^{-1} = 4480 cm^{-1}) above the lowest state should be populated at room temperature, which is impossible. This energy back-transfer also explains the very short luminescence lifetimes (see Table 3) and weak Tb(III) emission intensity of TbY-L5 because the fluorescence emission maximum of L5 is only slightly above the $^5D_4$ emission (485 nm).
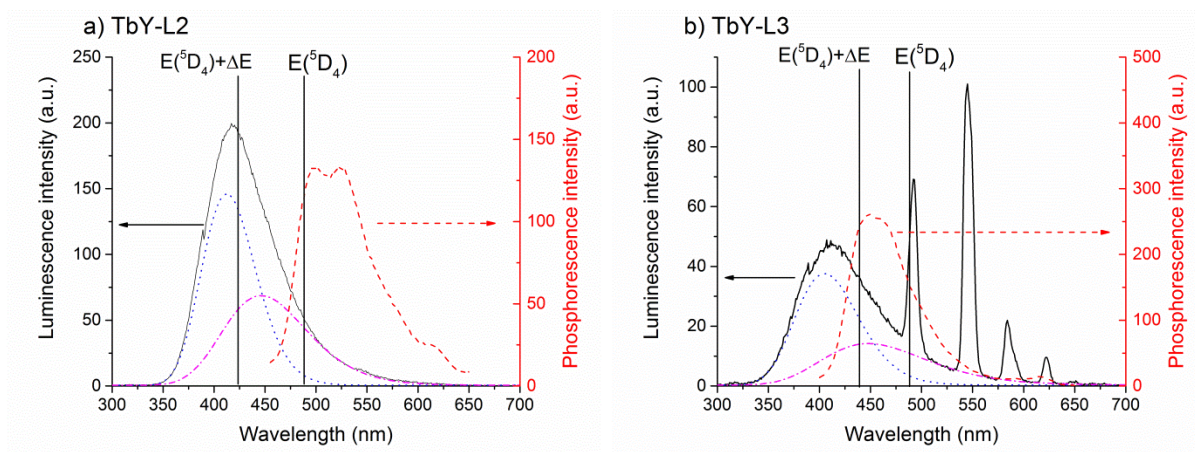
Figure 5. Luminescence spectrum (with convolution; singlet emission with blue dashed line and ILCT emission with purple dashed-dotted line) of TbY-L2 (black solid line) and phosphorescence spectrum of Gd(L2)$_3$ (red dashed line) (a), and those of TbY-L3 and Gd(L3)$_3$ (b). The energy level of the Tb(III) $^5D_4$ state and sum of $^5D_4$ and ΔE from energy back transfer equation (2) are shown for both complexes.

The more complicated life time distribution of the complexes TbY-L3 – TbY-L5 decreases the reliability of the fit in these cases (see Supporting Information). However, for TbY-L3 a value of $\Delta E = 2267 \pm 500$ cm^{-1} was obtained (Fig. 4). The sum of this value and the energy of the $^5D_4$ state of Tb(III) is 22820 cm^{-1}, which is relatively close to the triplet state energy of the L3 ligand (22200 cm^{-1}, see Figure 5b) but, however, also to that of the ILCT emissive state. This implies that energy can be thermally transferred to the ligand triplet state in the complexes if there are no states between it and the emissive state of the lanthanoid ion. The FD-curves of TbY-L4 as a function temperature are shown in Supplementary Information as an example of the behavior of these complexes.

3.5. Energy diagram

Scheme 2 shows the energy level diagram of the studied complexes, based on this work and literature data. The values of the energy of the emissive states (E) are taken from the ILCT emission maxima. The energy transfer arrows have not been drawn for the sake of clarity. The scheme implies that with complex TbY-L3 energy is probably transferred via the ligand triplet state. It also suggests that the energy back transfer from Tb(III) to the triplet states of ligands Lx can occur mainly with TbY-L4 and TbY-L5. In principle, the ILCT states would allow to extend the excitation wavelength towards the longer wavelengths but, unfortunately, the lowering of the ILCT state decreases also the energy of the quenching emissive state because the ligand emission occurs, probably at least partly, from the ILCT states. These results emphasize the significance of the small ligand Stokes shift in the extending of the excitation wavelength towards the visible region.

Generally, the ILCT (and emissive) states lay between the singlet and triplet states, and the use of the role of the charge transfer states in the ligand development for various applications is challenging. However, L.-M. Fu et al.[14,46] have shown that even if the triplet state energy is close to the lowest excited state of Ln(III) ($^5D_0$ state of Eu(III) in their study) the ligand can efficiently excite europium from a CT state unless the triplet state is below the emissive state of Ln(III) (as in the case of Tb(III) in their study). Generally, the ILCT mediated energy transfer may be more efficient than the triplet mediated one, and may enable the shift of the excitation towards longer wavelengths in Eu(III) complexes.[14,47-51] However, this is hardly true for the Tb(III) complexes as the Tb(III) ion is poorly excited via this route, at least with these complexes.
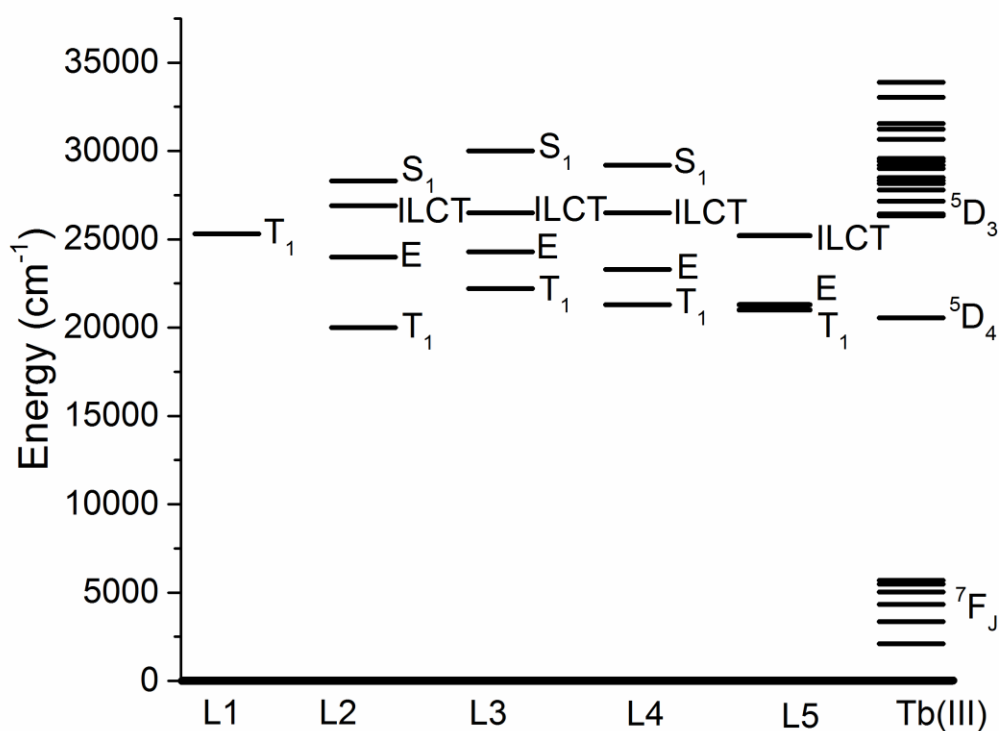
Scheme 2. Energy diagram of Tb(III) ion and ligands L1 – L5. S_1 = the first excited singlet state, ILCT = intra-ligand charge transfer state, E = the emissive state and T_1 = the lowest triplet state. The triplet state energies of ligands from ref.[20] and the calculated energy levels of Tb(III)(aq) from ref.[52].

4. Conclusions

The energy migration in lanthanoid complexes is a complicated phenomenon. A direct energy transfer from the singlet state to the lanthanoid ion exists probably whenever it is fast enough to compete with the heavy and paramagnetic atom assisted ISC, and is involved, *e.g.*, in the dipicolinic acid complexes. These results confirm that the energy transfer involves the triplet state in every case where it is possible and, in addition, the triplet state can be a quenching

state if it lies near or below the emissive state of the lanthanoid ion and the excitation of Ln(III) occurs via the ILCT(/LMCT) states. The ILCT route is less efficient in the Tb(III) complexes than in the Eu(III) complexes. This work shows that the emissive state of the ligand can also quench the Ln(III) ions (at least Tb(III) ion), which hampers the possibility of using the ILCT state as a means to lower the excitation wavelength as the emission takes place, at least partially, from the ILCT state. Based on the results in this work, ligand L3 may be a good candidate for the DNA-hybridization assays.

Acknowledgements

References

[1] I. Hemmilä, V. Mukkala, Time-Resolution in Fluorometry Technologies, Labels, and Applications in Bioanalytical Assays, Crit. Rev. Clin. Lab. Sci. 38 (2001) 441 – 519.

[2] Z. Li, H. Zhang, J. Shen, Synthesis of ultrastable Eu-complex/polystyrene composite luminescent nanoparticles using a solvent swelling method, Polymer Composites 32 (2011) 1712 – 1717.

[3] L. Zhang, L. Tian, Z. Ye, B. Song, J. Yuan, Preparation of visible-light-excited europium biolabels for time-resolved luminescence cell imaging application, Talanta 108 (2013) 143 – 149.

[4] Y. Luo, Q. Yan, Z. Zhang, X. Yu, W. Wu, W. Su, Z. Qijin, White LED based on poly(N-vinylcarbazole) and lanthanide complexes ternary co-doping system, J. Photochem. Photobiol. A: Chem. 206 (2009) 102 – 108.

[5] H. Wang, P. He, H. Yan, M. Gong, Synthesis, characteristics and luminescent properties of a new europium(III) organic complex applied in near UV LED, Sens. Act. B: Chem. 156 (2011) 6 – 11.

[6] G. Crosby, M. Kasha, Intramolecular energy transfer in ytterbium organic chelates, Spectrochimica Acta 10 (1958) 377 – 382.

[7] G. Crosby, R. Whan, R. Alive, Intramolecular Energy Transfer in Rare Earth Chelates. Role of the Triplet State, J. Chem. Phys. 34 (1961) 743 – 748.

[8] G. Crosby, R. Whan, J. Freeman, Energy transfer in collisions of rare gas atoms with CS_2: Translational excitation of internal degrees of freedom, J. Phys. Chem. 66 (1962) 2493 – 2499.

[9] S. Sato, M. Wada, Relations between Intramolecular Energy Transfer Efficiencies and Triplet State Energies in Rare Earth β-diketone Chelates, Bull. Chem. Soc. Jpn. 43 (1970) 1955 – 1962.

[10] M. Latva, H. Takalo, V. Mukkala, C. Matachescu, J.C. Rodríguez-Ubis, J. Kankare, Correlation between the lowest triplet state energy level of the ligand and lanthanide(III) luminescence quantum yield, J. Lumin. 75 (1997) 149 – 169.

[11] A. D'Aléo, A. Picot, A. Beeby, J. Gareth Williams, B. Le Guennic, C. Andraud, O. Maury, Efficient Sensitization of Europium, Ytterbium, and Neodymium Functionalized Tris-Dipicolinate Lanthanide Complexes through Tunable Charge-Transfer Excited States, Inorg. Chem. 47 (2008) 10258 – 10268.

[12] A. D'Aléo, F. Pointillart, L. Ouahab, C. Andraud, O. Maury, Charge transfer excited states sensitization of lanthanide emitting from the visible to the near-infra-red, Coord. Chem. Rev. 256 (2012) 1604 – 1620.

[13] W. Faustino, O. Malta, G. de Sa, Intramolecular energy transfer through charge transfer state in lanthanide compounds: A theoretical approach, J. Chem. Phys. 122 (2005) 054109 – 10.

[14] L. Fu, X. Ai, M. Li, X. Wen, R. Hao, Y. Wu, Y. Wang, J. Zhang, Role of Ligand-to-Metal Charge Transfer State in Nontriplet Photosensitization of Luminescent Europium Complex, J. Phys. Chem. A 114 (2010) 4494 – 4500.

[15] Y. Kim, N. Baek, H. Kim, Sensitized Emission of Luminescent Lanthanide Complexes Based on 4-Naphthalen-1-yl-Benzoic Acid Derivatives by a Charge-Transfer Process, ChemPhysChem 7 (2006) 213 – 221.

[16] L. Morss, Thermochemical properties of yttrium, lanthanum, and the lanthanide elements and ions, Chem. Rev. 76 (1976) 827 – 841.

[17] R. Supkowski, J. Bolender, W. Smith, L. Reynolds, W. Horrocks Jr, Lanthanide ions as redox probes of long-range electron transfer in proteins, Coord. Chem. Rev. 185–186 (1999) 307 – 319.

[18] Z. Grabowski, K. Rotkiewicz, W. Rettig, Structural Changes Accompanying Intramolecular Electron Transfer: Focus on Twisted Intramolecular Charge-Transfer States and Structures, Chem. Rev. 103 (2003) 3899 – 4031.

[19] M. Delbianco, V. Sadovnikova, E. Bourrier, G. Mathis, L. Lamarque, J.M. Zwier, D. Parker, Bright, Highly Water-Soluble Triazacyclononane Europium Complexes To Detect

Ligand Binding with Time-Resolved FRET Microscopy, Angew. Chem. Int. Ed. 53 (2014) 10718-10722.

[20] L. Zhang, L. Tian, B. Song, J. Yuan, Preparation of visible-light-excited europium biolabels for time-resolved luminescence cell imaging application, Talanta 108 (2013) 143 – 149.

[21] J. Bunzli, S. Eliseeva, in: Hänninen P., Härmä H. (Eds.), Lanthanide Luminescence: Photophysical, Analytical and Biological Aspects, vol. 7, Springer, Berlin, 2011.

[22] M. Räsänen, J. Rosenberg, J. Lukkari, K. Haapakka, J. Kankare, H. Takalo, Study on luminescent ternary EuEDTA complexes with a set of substituted 4-phenylethynyl and 4-aryl pyridine-2,6-dicarboxylic acids, J. Lumin. 187 (2017) 471 – 478.

[23] T. Terai, K. Kikuchi, S. Iwasawa, T. Kawabe, Y. Hirata, Y. Urano, T. Nagano, Modulation of Luminescence Intensity of Lanthanide Complexes by Photoinduced Electron Transfer and Its Application to a Long-Lived Protease Probe, J. Am. Chem. Soc. 128 (2006) 6938 – 6946.

[24] A. Beeby, S. Faulkner, D. Parker, J. Williams, Sensitised luminescence from phenanthridine appended lanthanide complexes: analysis of triplet mediated energy transfer processes in terbium, europium and neodymium complexes, J. Chem. Soc. Perkin Trans. 2 (2001) 1268 – 1273.

[25] D. Parker, P. Senanayake, J. Williams, Luminescent sensors for pH, pO$_2$, halide and hydroxide ions using phenanthridine as a photosensitiser in macrocyclic europium and terbium complexes, J. Chem. Soc. Perkin Trans. 2, (1998) 2129 – 2139

[26] A. Lehmusvuori, A. Tapio, P. Mäki-Teeri, K. Rantakokko-Jalava, Q. Wang, H. Takalo, T. Soukka, Homogeneous duplex polymerase chain reaction assay using switchable lanthanide fluorescence probes, Anal. Biochem. 436 (2013) 16 – 21.

[27] M. Walash, M. Rizk, M. Eid, M. Fathy, Spectrofluorimetric determination of ciclopirox olamine *via* ternary complex with Tb(III) and EDTA, Acta Pharm. 56 (2006) 431 – 444.

[28] P. Ioannou, E. Lianidou, D. Konstantianos, Simple, rapid and sensitive spectrofluorimetric determination of diflunisal in serum and urine based on its ternary complex with terbium and EDTA, Anal. Chim. Acta 300 (1995) 237 – 241.

[29] A. Bui, A. Roux, A. Grichine, A. Duperray, C. Andraud, O. Maury, Twisted Charge-Transfer Antennae for Ultra-Bright Terbium(III) and Dysprosium(III) Bioprobes, Chem. Eur. J. 24 (2018) 3408-3412.

[30] S. Hung, S. Liang, Consecutive Chelatometric Micro-Determination of Scandium and the Sum of Other Rare Earths, Scientia Sinica 8 (1964) 1619 – 1923.

[31] I. Hyppänen, T. Soukka, J. Kankare, Frequency-Domain Measurement of Luminescent Lanthanide Chelates, J. Phys. Chem. A 114 (2010) 7856 – 7867.

[32] J. Kankare, I. Hyppänen, in: Hänninen P., Härmä H. (Eds.), Lanthanide Luminescence: Photophysical, Analytical and biological Aspect, vol. 7, Springer, Berlin, 2011.

[33] C. Hansch, A. Leo, R. Taft, A survey of Hammett substituent constants and resonance and field parameters, Chem. Rev. 91 (1991) 165–195.

[34] A. D'Aléo, A. Picot, P.L. Baldeck, C. Andraud, O. Maury, Design of Dipicolinic Acid Ligands for the Two-Photon Sensitized Luminescence of Europium Complexes with Optimized Cross-Sections, Inorg. Chem. 47 (2008) 10269 – 10279.

[35] J. Lakowicz, Principles of Fluorescence Spectroscopy, Third ed., Springer, New York, 2006.

[36] F. Prendergast, J. Lu, P. Callahan, Oxygen quenching of sensitized terbium luminescence in complexes of terbium with small organic ligands and proteins, J. Biol. Chem. 258 (1983) 4075 – 4078.

[37] H. Lemmetyinen, E. Vuorimaa, A. Jutila, V. Mukkala, H. Takalo, J. Kankare, A time-resolved study of the mechanism of the energy transfer from a ligand to the lanthanide(III) ion in solutions and solid films, Lumin. 15 (2000) 341 – 350.

[38] J. Lamture, Z. Zhou, A. Kumar, T. Wensel, Luminescence Properties of Terbium(III) Complexes with 4-Substituted Dipicolinic Acid Analogs, Inorg. Chem. 34 (1995) 864 – 869.

[39] G. de Sá, O. Malta, C. de Mello Donegá, A. Simas, R. Longo, P. Santa-Cruz, E. da Silva Jr., Spectroscopic properties and design of highly luminescent lanthanide coordination complexes, Coord. Chem. Rev. 196 (2000) 165 – 195.

[40] J. Anderson, J. Nelson, C. Reynolds, D. Ringelberg, G. Tepper, D. Pestov, Steady-State and Frequency-Domain Lifetime Measurements of an Activated Molecular Imprinted Polymer Imprinted to Dipicolinic Acid, J. Fluoresc. 14 (2004) 269 – 274.

[41] L. Van Meervelt, K. Binnemans, K. Van Herck, C. Görller-Walrand, , Bull. Soc. Chim. Belges, X-ray crystal structure analysis of the terbium dipicolinate complex $Na_3[Tb(C_7H_3NO_4)_3].NaClO_4 \cdot 6H_2O$, 106 (1997) 25 – 27.

[42] N. Lima, S. conçalves, S. Júnior, A. Simas, A Comprehensive Strategy to Boost the Quantum Yield of Luminescence of Europium Complexes, Scien. Rep. 3 (2013) 2395 – 2407.

[43] D. Rosen, S. Niles, Chelation Number of Terbium Dipicolinate: Effects on Photoluminescence Lifetime and Intensity, App. Spectr. 55 (2001) 208 – 216.

[44] G. Blasse, N. Sabbatini, The quenching of rare-earth ion luminescence in molecular and non-molecular solids, Mat. Chem. Phys. 16 (1987) 237 – 252.

[45] G. Liebsch, I. Klimant, O. Wolfbeis, Luminescence Lifetime Temperature Sensing Based on Sol-Gels and Poly(acrylonitrile)s Dyed with Ruthenium Metal–Ligand Complexes, Adv. Mater. 11 (1999) 1296 – 1299.

[46] C. Yang, L. Fu, Y. Wang, J. Zhang, W. Wong, X. Ai, Y. Qiao, B. Zou, L. qui, A Highly Luminescent Europium Complex Showing Visible-Light-Sensitized Red Emission: Direct Observation of the Singlet Pathway, Angew. Chem. Int. Ed. 43 (2004) 5010 – 5013.

[47] M. Werts, M. Duin, J. Hofstraat, J. Verhoeven, Bathochromicity of Michler's ketone upon coordination with lanthanide(III) β-diketonates enables efficient sensitisation of Eu^{3+} for luminescence under visible light excitation, Chem. Commun. (1999) 799 – 800.

[48] F. Xue, Y. Ma, L. Fu, R. Hao, G. Shao, M. Tang, J. Zhang Y. Wang, A europium complex with enhanced long-wavelength sensitized luminescent properties, Phys. Chem. Chem. Phys. 12 (2010) 3195 – 3202.

[49] P. Kadjane, L. Charbonnière, F. Camerel, P. Lainé, R. Ziessel, Improving Visible Light Sensitization of Luminescent Europium Complexes, J. Fluoresc. 18 (2008) 119 – 129.

[50] S. Borisov, I. Klimant, Blue LED Excitable Temperature Sensors Based on a New Europium(III) Chelate, J. Fluoresc. 18 (2008) 581 – 589.

[51] M. Räsänen, H. Takalo, T. Soukka, K. Haapakka, J. Kankare, Photophysical study of blue-light excitable ternary Eu(III) complexes and their encapsulation into polystyrene nanoparticles, J. Lumin. 160 (2015) 128 – 133.

[52] W. Carnall, P. Fields, K. Rajnak, Electronic Energy Levels of the Trivalent Lanthanide Aquo Ions. III. Tb^{3+}, J. Chem. Phys. 49 (1968) 4447 – 4449.