Luminescent Lanthanide Complexes: Visible Light Sensitised Red and Near-infrared Luminescence.
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Chapter 5

Efficient visible light sensitisation of water soluble near-infrared lanthanide complexes

5.1 Introduction

Europium(III) and terbium(III) complexes that luminesce in the visible and contain organic chromophores as photosensitisers are already applied as labels for marking molecules of biological interest, e.g. in fluoroimmunoassays\cite{1-5} and in fluorescence microscopy.\cite{6,7} The incentive for the use of these labels instead of "conventional" fluorophores is that their long luminescence lifetimes (milliseconds) can be used to discriminate the label emission from the short-lived (nanoseconds) autofluorescence of the biological matrix. Using time-gated detection, i.e. exciting the sample with a short light pulse and only detecting the photons emitted at times later than a few microseconds, the autofluorescence and other interferences are effectively suppressed leaving only the lanthanide labels to be detected. This has been convincingly demonstrated by De Haas et al. who combined biochemical (peroxidase mediated) signal amplification with time-delayed detection of Eu$^{3+}$-chelate labelled streptavidin and were able to observe the label luminescence by eye through a modified fluorescence microscope using delayed detection.\cite{7}

In typical luminescent lanthanide complexes, population of the luminescent (lanthanide centered) state is achieved by energy transfer from a triplet excited organic chromophore.\cite{8,9} This indirect path, which involves the efficient excitation of the chromophore into its singlet state followed by intersystem crossing, is necessary since direct excitation of the lanthanide ion is difficult. Its intraconfigurational f-f transitions are only weakly allowed,\cite{10} which on one hand gives rise to the long luminescence lifetimes, but on the other introduces the need for excitation via organic "antenna" chromophores. Here lies also the cause of one of the drawbacks of Eu$^{3+}$ and Tb$^{3+}$ based labels. The luminescence of these ions being in the visible, sensitisation of that luminescence through organic chromophores can only be effective if these have their lowest singlet transitions in the near-UV,\cite{11} although in Chapter 7 we will demonstrate that efficient sensitisation of Eu$^{3+}$ luminescence for blue light excitation is possible under certain conditions.

One might expect that if lanthanide ions with lower lying luminescent states are used, the absorption bands of the antenna chromophores can be pushed more towards the red. In the previous Chapter, we described complexes of neodymium(III), erbium(III) and ytterbium(III) containing fluorescein and eosin as sensitising chromophores (Ln(AMFLU-DTPA) and Ln(AMEO-DTPA), see Figure 5-1). These chelates show sensitised near-
infrared luminescence from all three lanthanide ions upon excitation of the chromophore with visible light, and were the first near-infrared luminescent complexes in which ion and sensitiser had been brought together in a well-defined one-on-one way. In fact, the complexes of AMEO-DTPA and AMFLU-DTPA with Er\(^{3+}\) were the first examples of their kind to show Er\(^{3+}\) luminescence in solution. However, our study made it clear that in these complexes the energy transfer from the chromophores to the ions is not rapid enough. Dissolved molecular oxygen can compete well with the lanthanide ion as an alternative acceptor of the triplet energy of the chromophore, judging from the presence of \(^{1}\text{O}_2 (^{1}\Delta_g \rightarrow ^{3}\Sigma_g)\) phosphorescence at 1276 nm and the increase of lanthanide luminescence quantum yield upon deoxygenation. Moreover, a considerable amount of the excitation energy is lost due to fluorescence of the antenna chromophore, especially in the fluorescein containing complexes, as a result of incomplete intersystem crossing.

![Molecular structures of the Ln(AMFLU-DTPA) and Ln(AMEO-DTPA) complexes studied in the previous chapter.](image)

The actual chromophore of fluorescein, eosin and other xanthene dyes is the annelated three-ring system. The phenyl ring has been calculated to be almost perpendicular to this system.\(^{[12]}\) In fact, dyes lacking this group have recently been synthesised and show virtually the same photophysical parameters as the original dyes.\(^{[13, 14]}\) Looking again at the molecular structures of the AMEO-DTPA and AMFLU-DTPA, we concluded that the phenyl ring might act as a spacer between the chromophore and the lanthanide ion, increasing the distance between the two and thereby reducing two interactions crucial to the efficiency of sensitisation. Firstly, the paramagnetic and heavy atom effects\(^{[15]}\) of the ion on the chromophore are diminished. These effects enhance intersystem crossing, favouring
generation of sensitising triplets over emission of antenna fluorescence. Secondly, the exchange interaction between the electronic system of chromophore and that of the lanthanide ion is weakened, which is detrimental to the efficiency of (Dexter type) excitation energy transfer.

We therefore decided to investigate complexes of Yb$^{3+}$, Nd$^{3+}$ and Er$^{3+}$ with 4',5'-bis[N, N-bis(carboxymethyl)aminomethyl]fluorescein or fluorexon (Figure 5-2).* The binding site of the lanthanide ion in these complexes should be much closer to the chromophore than in the AMFLU-DTPA and AMEO-DTPA complexes studied earlier. It is anticipated that if effective complexation with the lanthanide ion occurs the sensitisation efficiency will be higher.

![Molecular structure of the Ln(fx) complex](image)

Figure 5-2. Molecular structure of the Ln(fx) complex

A preliminary experiment, of which the result is shown in Figure 5-3, shows that fluorexon indeed yields a water-soluble luminescent Yb$^{3+}$ complex that has a (much) higher near-infrared luminescence quantum yield than Yb(AMFLU-DTPA). Note that the measurements were done on aerated aqueous solutions, the emission spectrum of Yb(AMFLU-DTPA) in the previous Chapter (Figure 4-2) was that of a deoxygenated D$_2$O solution. In Figure 5-3, the lanthanide luminescence of Yb(AMFLU-DTPA) can hardly be recognised, especially because the fluorescence from the sensitising fluorescein chromophore itself is so intense. A solution of 1:1 Yb$^{3+}$/fluorexon does not suffer from such problems, and is in aerated aqueous solution - approximately 100 times as efficient in producing photosensitised Yb$^{3+}$ luminescence as Yb(AMFLU-DTPA).

In this Chapter, we will demonstrate that fluorexon (fx) forms water-soluble 1:1 complexes with lanthanide ions, having a stability comparable to that of lanthanide ion-EDTA complexes. In complexes of fx with Nd$^{3+}$, Er$^{3+}$ and Yb$^{3+}$, highly efficient population of the lanthanide ions' excited states is achieved via excitation of the fx chromophore. We will show that this is a result of both enhanced intersystem crossing in the antenna chromophore and rapid intracomplex energy transfer.

* We refer here to the pure 4',5' isomer instead of the "indicator grade" fluorexon/calcein which is a mixture of isomers whose iminodiacetic acid groups are attached to the fluorescein chromophore at different positions.
Figure 5-3. Emission spectra ($\lambda_{\text{exc}} = 490$ nm, CCD detector) of Yb(fx) (dashed line) and Yb(AMFLU-DTPA) (solid line) in aerated TRIS buffer (pH 8). Both solutions are of equal optical density. The inset shows the signals after subtraction of the contribution of antenna fluorescence, and multiplication of the Yb(AMFLU-DTPA) signal by 20.

5.2 Experimental

The spectroscopic equipment has been described in Chapter 2.

5.2.1 Chemicals and solutions

Fluorexon (4',5'-bis[N,N-bis(carboxymethyl)aminomethyl]fluorescein) was obtained from both Molecular Probes Europe (Leyden, The Netherlands) and Fluka. Ethylenediaminetetraacetic acid (EDTA), ordinary and perdeuterated Tris (tris(hydroxymethyl)aminomethane), deuterium oxide, deuterium chloride (37% wt. in D$_2$O) and all lanthanide chlorides (as the hexahydrates) were purchased from Aldrich. Stock solutions ($10^{-3} \ldots 10^{-4}$ M) of fluorexon (in 0.1 M Tris-HCl buffer, pH 8.3) and lanthanide chlorides (in 0.5 M HCl) were freshly prepared before each experiment and diluted where appropriate. Unless otherwise indicated measurements were done on complexes in aerated 0.1 M Tris-HCl buffer (pH 8.3). The complexes were prepared by mixing equal amounts of equimolar solutions ($10^{-5} \ldots 10^{-6}$ M) of ligand and lanthanide ion. The resulting solutions were heated briefly and then allowed to equilibrate for three hours at room temperature.

5.2.2 Fluorimetric titrations

For the determination of the preferred ion-ligand stoichiometry, the equimolar solutions were mixed in different ratios and submitted to the same equilibration procedure as mentioned above. The samples were excited at 480 nm, and the spectrum of the main luminescent transition of the respective lanthanide ion was recorded: the region around 980 nm for Yb$^{3+}$, around 1530 nm for Er$^{3+}$ and around 1060 nm for Nd$^{3+}$. The emission bands were
integrated to yield the total luminescence intensity. Also the antenna fluorescence band (500-700 nm) was recorded and integrated. The spectrograph/CCD combination was particularly convenient in this respect, since -with the proper optical filtering- it enables simultaneous recording of the tail of the antenna fluorescence band and the Nd$^{3+}$ and Yb$^{3+}$ emission bands.

5.2.3 Near-infrared luminescence quantum yield measurements

The near-infrared quantum yields of the Nd$^{3+}$ and Yb$^{3+}$ complexes were measured relative to Ru(bpy)$_3^{2+}$ in deoxygenated water\cite{16} ($\eta_{\text{lum}} = 0.042$ at 293 K) using the spectrograph/CCD combination following well-known procedures\cite{17, 18}. The emission spectra were corrected using factors obtained by measuring the emission of a calibrated lamp (EG&G Gamma Scientific RS10A lamp with RS3 power supply). The validity of the correction factors was checked by comparing the corrected recorded spectrum and the measured quantum yield of cresyl violet with the data published by Magde et al.\cite{19} All values agreed within 5%. The quantum yield of the Er$^{3+}$ complex was determined relative to Yb(fx) and Nd(fx) using the corrected emission spectra of the complexes recorded using the Ge-detector.

5.3 Results and Discussion

5.3.1 Nature of the complex

Addition of Yb$^{3+}$ ions to a solution of fx changes the absorption spectrum and quenches the fluorescence of fx. Together with the observation of Yb$^{3+}$ luminescence ($\lambda = 980$ nm) upon excitation of fx, these effects clearly indicate the formation of luminescent complexes between Yb$^{3+}$ and fx. Before undertaking a study of the photophysical properties of such complexes, it is necessary to find out what kind of complexes will be dealt with. The EDTA-like structure of the chelating part of the molecule, combined with simple electrostatic considerations suggest that a 1:1 complex is most likely, but other types of complexes cannot be ruled out beforehand. Varying both the total added concentration of Yb$^{3+}$ ($c_{\text{Yb}}$) and that of fluorexon ($c_{\text{fx}}$), the complex formation between the two was investigated.

Insight in the preferred stoichiometry is provided by an experiment in which the total concentration of Yb$^{3+}$ and fx is held fixed ($c_{\text{Yb}} + c_{\text{fx}} = 1.1 \times 10^{-5}$ M), varying the ratio of the components ($c_{\text{Yb}} / c_{\text{fx}}$). This is achieved by mixing equimolar solutions ($1.1 \times 10^{-5}$ M) of the components, heating the solutions briefly using a hot air gun, and letting them cool down to room temperature. At $c_{\text{Yb}} / c_{\text{fx}} = 1$ the luminescence intensity (upon excitation of the ligand at 470 nm) is maximised (Figure 5-4). In the region $c_{\text{Yb}} / c_{\text{fx}} \leq 1$ this intensity is linearly correlated with the concentration of Yb$^{3+}$, and so is the quenching of the fluorexon fluorescence (Figure 5-4). Evidently, under these conditions 1:1 complex formation takes place. The linearity is lost when $c_{\text{Yb}} / c_{\text{fx}} > 1$, indicating the formation of other species.

Time-resolved measurements of the near-infrared Yb$^{3+}$ luminescence yield monoexponential decays for the samples with $c_{\text{Yb}} / c_{\text{fx}} \leq 1$ (τ = 1.9 ms, Figure 5-5 left), strengthening...
Figure 5-4. Job plot of the complex formation of Yb\(^{3+}\) with fluorexon, monitored with luminescence spectroscopy. \(c_{\text{Yb}} + c_{fX}\) was held at \(1.1 \times 10^{-5}\) M in 0.1 M Tris-HCl (pH 8.3). The sample was excited at 480 nm.

our idea that under these conditions only one luminescent species, the 1:1 complex, is present. The formation of multiple species in the case of excess Yb\(^{3+}\) is apparent from the multi-exponential behaviour of the luminescence decays observed from such samples.

Figure 5-5. NIR luminescence decay curves of solutions of Yb\(^{3+}\) and fx in Tris-HCl buffer monitored at 980 nm under excitation with 337 nm pulses. Left: Overlaid decays of the samples having \(c_{\text{Yb}}/c_{fX} \leq 1\) (Yb\(^{3+}\)/fx ratios 1:4, 1:2, 2:3 and 1:1). Right: Decays of the samples having \(c_{\text{Yb}}/c_{fX} \geq 1\). Yb\(^{3+}\)/fx ratios (A) 1:1, (B) 3:2, (C) 2:1 and (D) 4:1.

The 1:1 complex is detectable with electrospray mass spectrometry. Under the condition that excess fx is present, the typical isotope pattern for a mononuclear Yb\(^{3+}\) complex is found at \(m/z\) 792 (Yb(fx) - H\(^+\)). With excess Yb\(^{3+}\) this pattern disappeared, but unfortunately no other types of complexes could be identified in these solutions under the experimental conditions, preventing a more precise characterisation of the type of ‘aggregates’ formed.
Based on the mass spectrometric data and the spectroscopic titration, it can be concluded that stable 1:1 complexes between fx and Yb\(^{3+}\) are formed provided no excess of lanthanide ions is present. All complexes for the photophysical measurements were therefore made by mixing equal amounts of equimolar solutions of ion and ligand. Formation of 1:1 complexes was then checked by measuring the luminescence decay curves: these should be monoexponential.

5.3.2 Complex stability

A way to find out about the stability of Yb(fx) is taking a solution of this complex and adding a second ligand, L, to it that also forms 1:1 complexes with Yb\(^{3+}\). Now fx and L have to compete for the Yb\(^{3+}\) ions. The following equilibria exist:

\[
Yb^{3+} + fx \rightleftharpoons Yb(fx) \quad (K_1)
\]

\[
Yb^{3+} + L \rightleftharpoons Yb(L) \quad (K_2)
\]

If ligand L forms complexes that do not luminesce upon excitation with visible light, the success of fx in the ‘struggle’ for Yb\(^{3+}\) is directly related to the sensitised Yb\(^{3+}\) photoluminescence intensity of the solution. In the case of an optically dilute solution, this intensity is in fact linearly proportional to the concentration of Yb(fx).

Let \(c_{Yb}\), \(c_{fx}\), and \(c_{L}\) be the total concentrations of Yb\(^{3+}\), fluorexon and non-sensitising competing ligand, respectively. We have equal total concentrations of Yb\(^{3+}\) and fx:

\[
c_{Yb} = c_{fx} = c \quad (5-1)
\]

The relative luminescence intensity of the solution, \(I\), is given by

\[
I = \left[ \frac{[Yb(fx)]}{c} \right] \quad (5-2)
\]

so that it equals 1 before L is added (provided of course that \(c \gg 1/K_1\)). After addition of L and equilibration of the solution, some Yb(fx) will have been replaced by Yb(L), resulting in a decrease of the luminescence. It can be shown that the relative stability of Yb(fx) is related to \(I\) in the following way:

\[
\frac{K_1}{K_2} = \frac{I^2 + I \left( \frac{c_L}{c} - 1 \right)}{I^2 - 2I + 1} \quad (5-3)
\]
Figure 5-6 contains the result of a typical competition experiment in which at a certain time ("A", after 755 s), 5 equivalents of EDTA are added to a solution of Yb(fx) in Tris-HCl buffer. One sees that after the addition the Yb$^{3+}$ luminescence intensity gradually decreases (monoeXponentially with a time constant of $7.1 \times 10^{-4} \text{s}^{-1}$) and settles at a value 12% of that of the initial value. The rate determining step in the equilibration is probably the dissociation of Yb(fx), which is apparently quite slow indicating a high kinetic stability of the complex. The thermodynamic stability is also rather high. It is of the same order of magnitude as that of the Yb(EDTA) complex. The ratio of their equilibrium constants was calculated using equation 5-3, and amounts to 0.7.

![Figure 5-6](image_url)

**Figure 5-6.** NIR luminescence (excited at 480 nm, monitored at 980 nm) of a solution of Yb(fx) (1.1 $\times$ 10$^{-5}$ M) in water. At "A" (t = 755 s), five molar equivalents of EDTA were added to the solution.

### 5.3.3 Photophysical processes and properties

Having established that stable 1:1 complexes of fx with lanthanide ions can be prepared, the photophysical properties of these complexes were studied. The main photophysical processes are depicted in Figure 5-7. The antenna function of the chromophore relies on it having a large absorption cross-section (extinction coefficient) compared to the lanthanide ion. $e_{\text{antenna}}/e_{\text{ion}}$ might very well exceed 10000. Then, the antenna should waste none of the absorbed energy in a process like (antenna) fluorescence ($k_f$), but rather transfer its singlet energy directly to the lanthanide ion or undergo intersystem crossing to the triplet state ($k_{\text{ISC}}$). The process of singlet energy transfer has never been observed experimentally, and is expected to be inefficient when it has to compete with other processes. Thus, intersystem crossing is also a necessary process. Next, the triplet energy should be transferred to the lanthanide ion as quickly as possible ($k_{\text{ET}}$), to prevent other deactivating processes such as phosphorescence ($k_p$) and non-radiative decay ($k_{\text{np}}$, including quenching by oxygen) from competing. Finally, the lanthanide ion should be efficient in emitting photons ($k_e$) instead of releasing its energy to vibrational modes in the surrounding ligand and solvent molecules ($k_{\text{nr}}$). We will not focus on the latter aspect, but it is known that the near-infrared
emissions of Nd$^{3+}$, Er$^{3+}$ and Yb$^{3+}$ are easily quenched by most common organic vibrations, such as O-H, N-H and C-H bonds,\textsuperscript{[20-24]} so one should expect the lanthanide emission step to limit the overall luminescence quantum yield.

\[ (S_1; \text{Ln}^{3+}) \rightarrow k_{isc} \rightarrow (T_1; \text{Ln}^{3+}) \rightarrow k_{ET} \rightarrow (S_0; \text{Ln}^{3+}) \]

\[ S_0 \rightarrow k_f \rightarrow k_{nf} \rightarrow k_p \rightarrow k_{np} \rightarrow k_{r} \rightarrow k_{nr} \]

\textbf{Figure 5-7.} Simple photophysical diagram, showing the main processes in luminescent lanthanide complexes. The electronic states of the complex are indicated in the form (antenna; ion), where \textit{antenna} denotes the state of the antenna chromophore and \textit{ion} denotes the state of the lanthanide ion. \( k_{nf}, k_f \): rates of non-radiative and radiative deactivation of the antenna singlet; \( k_{isc} \): intersystem crossing rate; \( k_{np}, k_p \): rates of non-radiative and radiative deactivation of the antenna singlet; \( k_{ET} \): rate of energy transfer; \( k_{nr} \): non-radiative and radiative rates of depopulation of the lanthanide's excited state.

\textbf{Absorption properties.} Interestingly, we discovered that the absorption spectra of the complexes are different for each lanthanide ion. Therefore, not only the spectra of the Nd$^{3+}$, Yb$^{3+}$ and Er$^{3+}$ complexes were recorded but also those of Gd$^{3+}$, Eu$^{3+}$ and Pr$^{3+}$ (Figure 5-8). There is a correlation between the shape of the spectrum and the size of the lanthanide ion. On going from the "big" Nd$^{3+}$ to the "small" Er$^{3+}$ and Yb$^{3+}$ ions, the absorption band becomes sharper with a higher maximum extinction coefficient. This behaviour contrasts with that of the AMFLU-DTPA ligand, which shows no significant changes in its absorption spectrum upon complexation with a lanthanide ion. The chromophore in fluorexon obviously senses the presence of the ion in its vicinity, indicating that the chromophore-ion distance is considerably smaller than in the corresponding AMFLU-DTPA complexes. Compared to 'free' fx, the lanthanide complexes have larger absorption cross-sections and have their maximum at slightly longer wavelengths.

\textbf{Antenna fluorescence.} Unlike in the AMFLU-DTPA/lanthanide complexes,\textsuperscript{[25]} the antenna fluorescence is effectively quenched in the fluorexon complex. The free ligand has a fluorescence quantum yield of 0.85, in the Yb$^{3+}$ complex this is less than 0.01\textsuperscript{*}. This is not only the case in Yb(fx), but also in Nd(fx), Er(fx), and Gd(fx). Three mechanisms may be invoked to explain the quenching: energy transfer from the singlet state of the chromo-
Figure 5-8. Absorption spectra of fluorexon (dotted line) and its 1:1 lanthanide complexes (solid lines) in 0.1M Tris-HCl buffer (pH 8.3)

phore, electron transfer and intersystem crossing. Singlet energy transfer can be ruled out: Gd\(^{3+}\) still quenches the fluorescence to the same extent as the three other ions, but has no energy levels that can receive energy from singlet excited fluorexon.

Electron transfer might only occur in the case of Yb(fx), since Yb\(^{3+}\) is quite easily reduced and xanthene dyes such as fluorexon are able to act as electron donors in their excited states.\(^{[26]}\) It has been found that in contrast to the other (electrochemically inert) lanthanide ions, Yb\(^{3+}\) and Eu\(^{3+}\) quench the fluorescence of fluorophores with high singlet energies, such as tryptophan.\(^{[27, 28]}\) Horrocks et al.\(^{[29]}\) claim that such redox processes may even lead to photosensitised Yb\(^{3+}\), when the energy released in subsequent charge recombination is enough to excite Yb\(^{3+}\). The fluorexon fluorescence, however, is also quenched by Nd\(^{3+}\), Gd\(^{3+}\) and Er\(^{3+}\). Therefore, we expect that electron transfer quenching does not play a role. This expectation is supported by the fact that neither Yb\(^{3+}\) nor Eu\(^{3+}\) quenches the fluorescence of the chromophore in AMFLU-DTPA to a larger extent than other lanthanide ions do.\(^{[25, 30]}\)

Thus, enhancement of the intersystem crossing due to the paramagnetic and heavy atom effects is the most likely cause of the fluorescence quenching. Compared to AMFLU-DTPA complexes the effect in the fluorexon systems is much more pronounced, again a result of the smaller distance between chromophore and lanthanide ion. On basis of the complete quenching of antenna fluorescence, \(k_{\text{isc}}\) can be estimated to be \(> 10^{10} \text{s}^{-1}\).** Triplet state.** Also in Gd(fx) the fluorexon fluorescence is almost totally quenched, which can only be due to enhancement of intersystem crossing. Gd\(^{3+}\) is electrochemically inactive and has no energy levels below 32200 cm\(^{-1}\), preventing it to quench the singlet state of fluorexon by electron transfer or singlet energy transfer. Moreover, the lack of accepting levels makes the Gd\(^{3+}\) complex suitable for having a look at the triplet state of the antenna.

* The excitation spectrum of the residual fluorescence reveals that it probably stems from a minor impurity, fluorescein.
chromophore. In deoxygenated solution, even at room temperature, phosphorescence of Gd(fx) is readily observed by using gated detection: equipping the fluorimeter with a rotating drum to introduce a delay between excitation and detection yields the phosphorescence spectrum shown in Figure 5-9.

Figure 5-9. Phosphorescence of Gd(fx) in water at room-temperature, excited at 500 nm and detected using delayed detection.

Also transient absorbance measurements at room temperature reveal efficient formation of a triplet state. Whereas free ligand fluoresxon and Yb(fx), Nd(fx), Er(fx) show no long-lived (> 20 ns) excited state absorptions under the experimental conditions, Gd(fx) displays the transient absorption spectrum in the left of Figure 5-10. The right part of Figure 5-10 shows the corresponding single-wavelength decays at 530 nm, in deoxygenated and aerated solution. In aerated solution the triplet lives 3.1 µs, this lifetime increases to 21 µs in deoxygenated buffer.

The heavy atom induced formation of fluoresxon triplet states implies that Gd(fx) is an able sensitiser of singlet oxygen. Interaction of triplet molecular oxygen with triplet states produces singlet oxygen, which we monitor by its phosphorescence at 1276 nm. In buffered air-equilibrated D₂O the formation of singlet oxygen by excited Gd(fx) is almost as efficient as that observed with eosin (which under those conditions has a singlet oxygen quantum yield of 0.58⁴¹,²). The Yb³⁺, Nd³⁺, Er³⁺ complexes of fluoresxon show no singlet oxygen signal at all, whereas the corresponding AMEO-DTPA complexes (Chapter 4) produce fair amounts of singlet oxygen upon excitation.

Luminescence. Excitation of the corresponding complexes of fluoresxon leads to near-infrared luminescence of Yb³⁺ (²F₇/₂ → ²F₅/₂, 980 nm), Nd³⁺ (main band is at 1060 nm, ⁴F₅/₂ → ⁴I₁₁/₂) or Er³⁺ (⁴I₁₃/₂ → ⁴I₁₁/₂, 1530 nm) (Figure 5-11). The structure on the single emission lines of the lanthanide ions is due to crystal field splitting of the degenerate J levels.

The excitation spectra of lanthanide luminescence match the absorption spectra. Interestingly, the overall quantum yield of this luminescence is virtually independent of the
oxygen concentration. In contrast to our earlier observations for NIR emitting lanthanide complexes of AMFLU-DTPA oxygenation did not decrease the luminescence intensity. Therefore not only intersystem crossing is fast and efficient, but also the transfer of triplet energy to the lanthanide ion, since oxygen cannot compete as an alternative acceptor.

This is also apparent from time-resolved luminescence measurements (Table 5-1). Whereas the luminescence of Yb(AMFLU-DTPA) and that of Er(AMFLU-DTPA) in deoxygenated D$_2$O show biexponential behaviour having both a rising and a decaying component on the microsecond timescale due to slow energy transfer, all fx complexes show monoexponential decay. Slow energy transfer would result in a rising component in the time-resolved luminescence, but clearly the formation of the lanthanide excited state in
the Ln(fx) complexes (which includes the energy transfer) occurs within the time resolution of the measurement (≤ 100 ns).

Table 5-1. Observed luminescence lifetimes, $\tau_{\text{obs}}$, and estimates for the quantum yield of the lanthanide photoluminescence step, $\Phi_{\text{Ln}}^{\text{est}}$.

<table>
<thead>
<tr>
<th>Ln(fx)</th>
<th>$\tau_{\text{obs}} / \mu$s</th>
<th>$\Phi_{\text{Ln}}^{\text{est}}$</th>
<th>$\tau_{\text{obs}} / \mu$s</th>
<th>$\Phi_{\text{Ln}}^{\text{est}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yb$^{3+}$</td>
<td>1.91</td>
<td>$1 \times 10^{-3}$</td>
<td>10.4</td>
<td>$5 \times 10^{-3}$</td>
</tr>
<tr>
<td>Nd$^{3+}$</td>
<td>0.25</td>
<td>$3 \times 10^{-4}$</td>
<td>0.58</td>
<td>$7 \times 10^{-4}$</td>
</tr>
<tr>
<td>Er$^{3+}$</td>
<td>-</td>
<td>-</td>
<td>1.46</td>
<td>$2 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

The luminescence lifetimes are in line with what is usually observed for the three near-infrared emitting ions encapsulated in organic ligands. Nonradiative deactivation of the luminescent state is efficient, since it can be effectively mediated by the ubiquitous molecular vibrations, as already mentioned.

**Overall luminescence quantum yield.** That non-radiative deactivation of the luminescent state is indeed efficient is seen from the quantum yields in Table 5-2. Each of these "overall" luminescence quantum yields ($\Phi_{\text{tot}}$) is the product of the yields of the three steps involved in producing photoluminescence: intersystem crossing ($\Phi_{\text{ISC}}$), energy transfer ($\Phi_{\text{ET}}$) and lanthanide luminescence ($\Phi_{\text{Ln}}$).

$$\Phi_{\text{tot}} = \Phi_{\text{ISC}} \Phi_{\text{ET}} \Phi_{\text{Ln}} \quad (5-4)$$

The quantum yield of the lanthanide luminescence step can be calculated from the observed luminescence lifetime ($\tau_{\text{obs}}$), if the radiative (or "natural") lifetime $\tau_{\text{rad}}$ is known.

$$\Phi_{\text{Ln}} = \frac{\tau_{\text{obs}}}{\tau_{\text{rad}}} \quad (5-5)$$

Estimates of $\tau_{\text{rad}}$ in organic systems are 8 ms, 2 ms and 0.8 ms, for Er$^{3+}$, Yb$^{3+}$ and Nd$^{3+}$, respectively. The values of $\Phi_{\text{Ln}}$ calculated on basis of equation 5-5 and these estimates have been tabulated in Table 5-1. These values of $\Phi_{\text{Ln}}$ enable us to estimate the sensitisation efficiency, $\eta_{\text{sens}}$, given by

$$\eta_{\text{sens}} = \Phi_{\text{ISC}} \Phi_{\text{ET}} \quad (5-6)$$
The calculated efficiencies collected in Table 5-2 demonstrate that sensitisation of NIR lanthanide photoluminescence in the fluorexon complexes is indeed an efficient process.

### Table 5-2. Observed overall NIR luminescence quantum yields upon ligand excitation, $\Phi_{tot}$, and estimated sensitisation efficiencies, $\eta_{sens}^{est}$.

<table>
<thead>
<tr>
<th>Ln(fx)</th>
<th>$\Phi_{tot}$</th>
<th>$\eta_{sens}^{est}$</th>
<th>$\Phi_{tot}$</th>
<th>$\eta_{sens}^{est}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>Yb$^{3+}$</td>
<td>8.9 x $10^{-4}$</td>
<td>0.9</td>
<td>4.5 x $10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>Nd$^{3+}$</td>
<td>1.7 x $10^{-4}$</td>
<td>0.5</td>
<td>3.8 x $10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Er$^{3+}$</td>
<td>a</td>
<td></td>
<td>1.9 x $10^{-4}$</td>
</tr>
</tbody>
</table>

a. Er$^{3+}$ shows no detectable luminescence in H$_2$O, because H$_2$O has strong absorptions in the 1500 nm range.

### 5.4 Conclusion

Fluorexon forms water-soluble 1:1 complexes with lanthanide ions. These complexes have a high kinetic stability and a thermodynamic stability that is approximately equal to the corresponding EDTA complexes. Enhanced sensitisation of Yb$^{3+}$ (980 nm), Nd$^{3+}$ (main transition at 1060 nm) and Er$^{3+}$ (1530 nm) has been achieved compared to the former “generation” of complexes. The sensitisation efficiency is determined by $\Phi_{ISC}$ and $\Phi_{ET}$, and both these factors benefit from the shortened distance in the present fluorexon/lanthanide ion system.

This result once again points out the superiority of complexes that have the antenna chromophore and lanthanide binding site integrated over complexes in which these have been introduced as separate units. An example are the triphenylenes which are much less effective antenna chromophores when attached to a calixarene ionophore$^{11}$ than when being the complexing moiety themselves.$^{35}$

Another fact demonstrated by the fluorexon/near-infrared emitting lanthanide ion system is that a fluorescent chromophore is not necessarily a bad antenna for lanthanide ions. High radiative rates for fluorescence imply large absorption cross-sections and high fluorescence quantum yields indicate the absence of non-radiative deactivation. If the lanthanide ion is close enough to enhance intersystem crossing in a chromophore by heavy atom and paramagnetic effects, an initially highly fluorescent chromophore may become a powerful sensitiser of lanthanide luminescence.

The ability of Gd$^{3+}$ to increase intersystem crossing and the radiative rate of phosphorescence of fluorexon is demonstrated by the presence of long-lived triplet-triplet absorption, generation of singlet oxygen and room-temperature phosphorescence. This appears to be a relatively unexplored and unexploited area of the photophysical properties of lantha-
nide complexes containing organic chromophores. Enhanced singlet oxygen generation by chromophores in Gd\(^{3+}\) complexes may have medical use, e.g. in photodynamic therapy.

The fluorexon ligand might be fitted with a reactive group so that it can be attached to biological molecules (proteins, nucleotides). It is then possible to construct near-infrared luminescent probes for fluoroimmunoassays and fluorescence microscopy. In these applications, the advantages of long-lived, near-infrared luminescence excited with visible light may very well outweigh the low luminescence quantum yields.

References

