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Mixed f−d Coordination Complexes as Dual Visible- and Near-Infrared-Emitting Probes for Targeting DNA

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A new family of mixed-lanthanide (Yb^{III} and Nd^{III}) transition-metal (f−d) cyclen-$Ru^{II}(phen)_3$ (phen = 1,10-phenanthroline) complexes were synthesized as dual visible- and near-infrared (NIR)-emitting DNA probes/sensors. Significant changes were seen in both the Ru^{II} visible and the Yb^{III}-centered NIR emission, which was switched off upon binding to DNA at pH 7.4. In contrast, no changes were seen in the Nd^{III} emission of the analogue f−d conjugate.

The recognition and sensing of ions and (bio)molecules, such as DNA, are of great current interest.[1] Lanthanide complexes are particularly attractive for luminescent sensing and biological imaging.[2] While few examples of DNA-targeting lanthanide complexes, or probes, have been developed to date, these were mostly based on the use of visibly emitting lanthanide ions.[3] While those based on Eu^{III} emit in the red, the use of near-infrared (NIR)-emitting lanthanides such as Nd^{III} and Yb^{III} is particularly attractive for probing biological interactions[4] because biological tissues are transparent in this spectral range. Furthermore, the excited states of these lanthanides can be sensitized using visible-absorbing organic antennae. Such NIR sensitizers can also be achieved using d-block transition-metal complexes as antennae.[5−7] However, only a few such f−d complexes, based on palladium porphyrin[8] and platinum hairpin,[9] have been used for targeting DNA. Classically, ruthenium(II) polypyridyl (bpy, phen, or dppz) metal complexes have been developed as DNA-targeting complexes.[1,10] We have recently reported the synthesis and photophysical analysis of both Nd^{III}- and Yb^{III}-cyclen-appended phen-based complexes, which we used to form mixed f−d complexes with $[Ru(bpy)_2]^{2+}$.[11] While these f−d complexes gave rise to both Yb- and Nd-centered NIR emission, upon excitation of the $[Ru^{II}phen(bpy)_2]^{2+}$ triplet metal-to-ligand charge-transfer (^{3}MLCT) band, neither the MLCT nor the NIR emissions were modulated in the presence of DNA. Herein, we show that replacing the ancillary Ru^{II} moiety with the stronger DNA-binding complex $[Ru(phen)_3]^{2+}$ (yielding **Ln.1.Ru.phen**, Ln = Yb or Nd) gives rise to significant changes in the MLCT and NIR emissions upon

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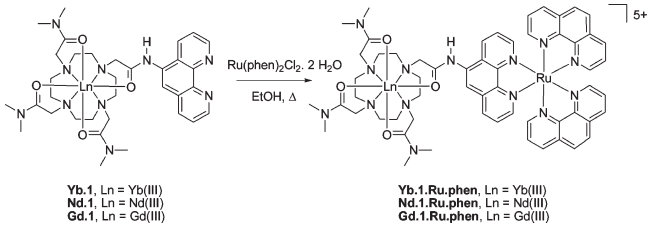
Scheme 1. Synthesis of the Mixed f−d Conjugates **Yb.1.Ru.phen**, **Nd.1.Ru.phen**, and **Gd.1.Ru.phen** from the **Ln.1** Complexes



titration with DNA. To the best of our knowledge, these f−d complexes are the first examples of such cyclen-based, dual-emitting, DNA-targeting probes.

The synthesis of **Yb.1.Ru.phen**, **Nd.1.Ru.phen**, and **Gd.1.Ru.phen** (see the Supporting Information, SI) was achieved in one step, Scheme 1, from the corresponding lanthanide complexes (which were formed from **1**[12]) by refluxing **Yb.1**,[11] **Nd.1**,[11] and **Gd.1** in ethanol overnight with Ru(phen)$_2$Cl$_2$·2H$_2$O. The desired products were obtained in ca. 40% yields as red solids after purification by size-exclusion column chromatography on a Sephadex LH20. The ^{1}H NMR spectra (400 MHz, D_2O) of the Yb^{III} and Nd^{III} complexes were characteristic of the presence of the paramagnetic lanthanide ions (Figures S1 and S2 in the SI),[13] while MALDI-TOF MS experiments showed the correct isotopic distribution patterns (Figures S3−S5 in the SI).

All of the mixed f−d complexes were water-soluble, and their photophysical properties were investigated in 10 mM (OD ∼ 0.1) phosphate-buffered aqueous solutions at pH 7.4. The characteristic absorbance spectra, together with the excitation and emission spectra of all three, are shown in Figure 1. Here, the absorption spectra of all three were centered at ca. 262 nm, characteristic of phen unit, with a MLCT band at 448 nm corresponding to the Ru^{II} center (log ε = 4.10). Exciting the complexes at either of these wavelengths gave rise to a Ru^{II}-based MLCT emission, with λ_{max} at 605 nm. Their excitation spectra (λ_{em} = 605 nm; Figure 1) were also recorded and showed that, while the these were structurally identical with the absorption spectra, their relative intensities differ greatly, reflecting that seen in the emission spectra in Figure 1. Furthermore, the quantum yields for the Ru^{II}-based MLCT emission (Φ_{MLCT}) were measured as 0.027, 0.019, and 0.008 for **Gd.1.Ru.phen**, **Yb.1.Ru.phen**, and **Nd.1.Ru.phen**, respectively, using an air-equilibrated solution of Ru(bpy)$_3$, which has a Φ_{MLCT} of 0.028.[14] The decay of the MLCT emission was best fitted to a biexponential decay, which could possibly be due to the presence of several diastereomers in solution, with a major lifetime (∼99%) of τ = 766 ns and a minor component of τ = 96.6 ns for **Gd.1.Ru.phen**. Similarly, for **Yb.1.Ru.phen**, τ = 567 and 92.7 ns were recorded, while **Nd.1.Ru.phen** gave τ = 526 and 100.7 ns (see Figures S6−S8 in the SI). These lifetimes also reflected the ability of the Ru^{II} complex to sensitize the excited states of the Yb^{III} ($^2F_{5/2}$) and Nd^{III} ($^4F_{3/2}$) ions. Because Gd^{III} ($^6P_{7/2}$) is too high in energy to be sensitized, we were able to determine the rate of the intramolecular intermetallic Ru−Ln energy transfer (ET; Table S2 in the SI). The results demonstrate that the ET is significantly faster in the case of **Nd.1.Ru.phen** than in the case of **Yb.1.Ru.phen**.

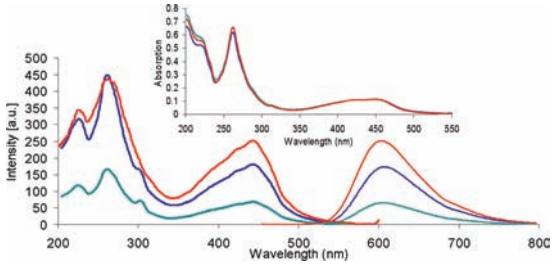


Figure 1. Excitation and emission spectra of **Yb.1.Ru.phen** (blue), **Nd.1.Ru.phen** (green), and **Nd.1.Ru.phen** (red) (at 9.0 μM) when recorded in a 10 mM phosphate buffer at pH 7.4 in the absence of DNA. Inset: Corresponding absorption spectra of the three complexes.

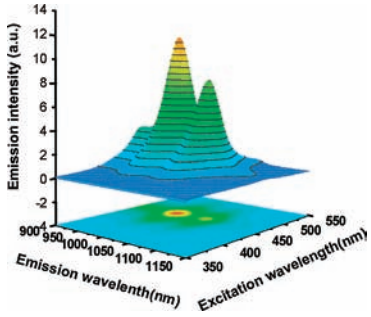


Figure 2. Excitation−emission profile of **Yb.1.Ru.phen** (11.7 μM) in a 10 mM phosphate buffer at pH 7.4, showing that effective sensitization of the $^2F_{5/2}$ exctied state of Yb^{III} was observed upon excitation at λ_{max} of the MLCT band, using a 580 nm filter.

The NIR lanthanide luminescence was also recorded, upon excitation at the MLCT band. For **Yb.1.Ru.phen**, a sharp emission band centered at 978 nm was observed for the $^2F_{5/2} \rightarrow {}^2F_{7/2}$ transition, with a broader vibronic component at longer wavelengths. The excitation−emission profile of **Yb.1.Ru.phen** is shown in Figure 2 and clearly demonstrates that effective sensitization was achieved upon excitation at λ_{max} of the MLCT band. Similarly, the Nd^{III} complex displayed an emission band centered at 1062 nm ($^4F_{3/2} \rightarrow {}^4I_{11/2}$), with two other bands occurring between 857 and 870 nm ($^4F_{3/2} \rightarrow {}^4I_{9/2}$) and between 1280 and 1456 nm ($^4F_{3/2} \rightarrow {}^4I_{13/2}$) (see the SI).

As discussed above, ruthenium(II) polypyridyl complexes can interact with DNA, often through both hydrophobic interactions at the minor groove and intercalation into the double helix at the major groove.[1,10,15−17] The ability of the three **Ln.1.Ru.phen** complexes to interact with DNA was investigated by carrying out thermal denaturation studies. The results (Figures S9 and S10 in the SI) clearly demonstrate that all three f−d complexes stabilized both salmon sperm (*ss*) and calf thymus (*ct*)-DNA at medium loading (P/D = 30), in a 10 mM phosphate buffer at pH 7.4, with a >7 °C increase in T_m of DNA. A similar pattern

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was observed for the reference compound $[Ru(phen)_3]^{2+}$, therefore suggesting a similar binding behavior.

The ability of all three complexes to bind to DNA was further investigated by observing the changes in their absorption spectra (at 12 μM), as well as in the MLCT emission in a 10 mM phosphate buffer at pH 7.4. Furthermore, in the cases of **Yb.1.Ru.phen** and **Nd.1.Ru.phen**, their NIR emission was also monitored upon binding to DNA. Upon titration of **Gd.1.Ru.phen**, with *ss*-DNA, only minor changes were initially observed in the absorption spectra (Figure S11 in the SI), where the MLCT band was hyperchromically shifted by ca. 3% at P/D = 0−2.5. This was followed by a decrease of 12% between P/D = 2.5 and 5. We attribute this to biphasic binding interactions, as previously observed for cationic porphyrins[18] and 1,8-naphthalimide ruthenium conjugates.[1] Moreover, the observed 12% hypochromic effect is in agreement with the that previously reported for $[Ru(phen)_3]^{2+}$.[19] Similarly, changes were also observed in the ^{3}MLCT emission spectra of **Gd.1.Ru.phen** (Figure S12 in the SI) where the band was blue-shifted with an isosbestic point at 616 nm. Similar effects were also observed with *ct*-DNA (Figures S13 and S14 in the SI). The changes in the absorption spectra of both **Yb.1.Ru.phen** and **Nd.1.Ru.phen** were similar to those observed above (Figures S15−S22 in the SI). However, the changes in the emission spectra were significantly different from that observed for **Gd.1.Ru.phen**. Figure 3 shows the changes observed for **Yb.1.Ru.phen**, where MLCT emission was blue-shifted by ca. 13 nm, with concomitant 31% enhancement. Similarly, for **Nd.1.Ru.phen**, a 11 nm blue shift and 27% enhancement was observed. Moreover, the lifetimes of the MLCT luminescent decay were on both occasions significantly longer in the presence of DNA (and best-fitted to biexponential decay), with τ_1 = 401 ns (~16%) and τ_2 = 1181 ns (~84%) for **Yb.1.Ru.phen-DNA** (Figures S23−S25 in the SI). From these results, the intrinsic binding constants (K, expressed as log K) were determined using the model of McGhee and von Hippel (see the SI) as log K = 5.83, 5.76, and 5.55 for **Gd.1.Ru.phen**, **Yb.1.Ru.phen**, and **Nd.1.Ru.phen**, respectively (Figures S26−S28 in the SI).[20,21] Also, **Gd.1.Ru.phen** was found to effectively displace ethidium bromide from bound DNA, from which a binding constant of 2.5×10^7 M^{-1} was determined (Figures S29 and S30 in the SI).[22]

In contrast to the above changes in the MLCT emission, dramatic changes were observed in the Yb^{III} emission of **Yb.1.Ru.phen** (45.5 μM) upon the addition of *ss*-DNA (Figure 4), where the emission was almost completely "switched off", clearly indicating that the ET from the ^{3}MLCT to the $^2F_{5/2}$ excited state was inhibited upon binding to DNA. The changes observed at 978 nm are shown as the inset as a function of P/D. From these changes, the intrinsic binding constant (Figure S31 in the SI) was also able to be determined as log K = 5.98(6), which correlates very well with that determined from the MLCT emission above. This clearly demonstrates that both Ru^{II}- and Yb^{II}-based emissions can be employed in determining the affinity of **Yb.1.Ru.phen** for DNA. Hence, **Yb.1.Ru.phen** functions as a

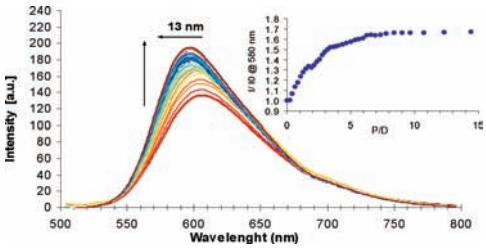

Figure 3. Changes in the ruthenium emission spectrum (λ_{ex} = 448 nm) of **Yb.1.Ru.phen** (11.3 μM) in a 10 mM phosphate buffer, pH 7.4, with increasing concentration of *ss*-DNA (0−335.5 μM). Inset: Changes in the MLCT emission as a function of P/D.

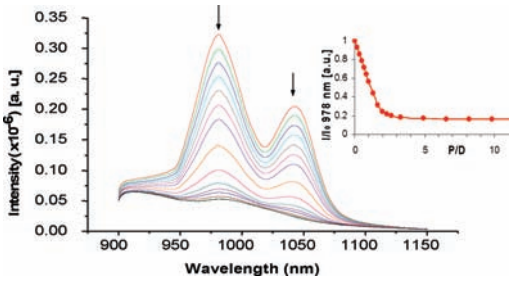

Figure 4. Changes in the Yb^{III} emission spectrum (λ_{ex} = 466 nm) of **Yb.1.Ru.phen** (45.5 μM) in a 10 mM phosphate buffer, pH 7.4, with increasing concentration of *ss*-DNA (0−3.6 mM). Inset: Changes at 978 nm as a function of P/D.

dual emitting probe for DNA in the visible as well as NIR regions. In contrast to these results, no significant changes were observed in the Nd^{III} emission of **Nd.1.Ru.phen** (46.7 μM) upon excitation at 466 nm and titration with *ss*-DNA (Figures S32 and S33 in the SI). This indicates that the Nd^{III} unit acts as a luminescent "reporter" group, which does not reflect the binding of the Ru^{II} moiety to DNA. As before, we determined the rate of ET in **Yb.1.Ru.phen** and **Nd.1.Ru.phen** in the presence of 10 equiv of DNA (Table S2 in the SI). The results demonstrate that the rate of ET is reduced for both, but in particular for **Yb.1.Ru.phen**. This could be the main contributor to the quenching observed in Figure 4. We are in the process of further investigating the nature of this quenching.

In summary, we have developed a novel family of mixed f−d complexes possessing a visibly absorbing $Ru^{II}(phen)_3$ antenna for sensitizing the NIR emission of both Nd^{III} and Yb^{III} and demonstrated that the Yb^{III} emission was significantly affected upon binding of **Yb.1.Ru.phen** to DNA, giving rise to the formation of dual visible- and NIR-emitting probes for DNA, from which the affinity of **Yb.1.Ru.phen** could be determined (see also Figure S34 in the SI). We are currently investigating these and other mixed f−d complexes in greater detail.

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Supporting Information Available: Synthesis and characterization of the complexes, Figures S1−S34, and Tables S1 and S2.This material is available free of charge via the Internet at http://pubs.acs.org.

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