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Communication

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Binding of Eu^{III} to 1,2-Hydroxypyridinone-Modified Peptide Nucleic Acids

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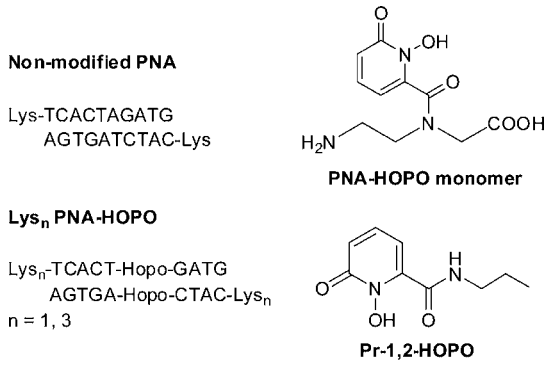
Supporting Information

ABSTRACT: Substitution of a nucleobase pair with a pair of 1,2-hydroxypyridinone (**1,2-HOPO**) ligands in the center of a 10-base-pair peptide nucleic acid (PNA) duplex provides a strong binding site for Eu^{III} as evidenced by UV thermal melting curves, UV titrations, and luminescence spectroscopy. Eu^{III} excitation spectra and luminescence lifetime data are consistent with Eu^{III} bound to both **1,2 HOPO** ligands in a **PNA-HOPO** duplex as the major species present in solution.

The self-assembly and molecular recognition properties of nucleic acids and of their synthetic analogues, such as peptide nucleic acids (PNAs), facilitate the design of new functional supramolecular structures that have applications as sensors for small molecules or nanomachines for molecular computing or drug delivery. Inorganic chemists have been at the forefront of this research by capitalizing on the interesting spectroscopic, redox, and magnetic properties of metal ions. These ions have been incorporated in both nonmodified and modified nucleic acids to form hybrid inorganic−nucleic acid molecules. A strategy for the incorporation of metal ions into nucleic acids is to use ligand-modified nucleic acid monomers instead of nucleobases.[1] PNAs are uniquely suited for the incorporation of ligands for binding of metal ions because the ligands can be readily connected to the PNA backbone through a peptide linker. Nucleobase-like ligands such as bipyridine or 8-hydroxyquinoline have been incorporated pairwise into the two strands of PNA duplexes.[1b] Most studies of metal-containing, ligand-modified nucleic acids have involved transition-metal ions that form four-coordinate complexes (it is possible that the metal weakly coordinates two more ligands, e.g., nucleobases, to achieve six-coordination). To the best of our knowledge, large metal ions such as trivalent lanthanides have not been incorporated into metal complexes within PNA, although a DNA with a non-nucleoside linker was shown to bind Eu^{III} in a bulgelike structure.[2] Here we report the first example of a lanthanide ion incorporated into PNA by the substitution of nucleobases with bidentate ligands. The rich luminescence, magnetic, and catalytic properties of the lanthanide ions together with the specific and high affinity binding of PNA to DNA and RNA make compounds such as the one we report here interesting for the design of new classes of optical and MRI sensors.

1,2-Hydroxypyridinone (**1,2-HOPO**) is a ligand with a high affinity for lanthanides and can sensitize Eu^{III} luminescence.[3] We have incorporated this ligand into a PNA monomer and used the monomer to create a binding site for Eu^{III} in a PNA duplex. The 1,2-HOPOBn acid was synthesized according to a slightly modified procedure[4] and was coupled to Boc-protected *tert*-butylaminoethyl glycinate to obtain the *tert*-butyl ester of the **PNA-HOPO** monomer,[5] which was hydrolyzed using NaOH (Schemes 1 and S1 in the Supporting Information, SI). The

Scheme 1



PNA-HOPO monomer was incorporated into two complementary PNA oligomers with the sequence shown in Scheme 1 by solid-phase peptide synthesis.[6] The bidentate ligand **Pr-1,2-HOPO** (Scheme 1) was also synthesized (Scheme S2 in the SI).[7] To improve the PNA solubility, we included at the C end of each oligomer one or three lysine residues (**Lys PNA-HOPO** or **Lys_3 PNA-HOPO**, respectively). The solubility, melting, and optical properties of the PNA duplexes with different numbers of lysines are similar.

Variable-temperature UV−vis spectroscopy was used to evaluate the thermal stability of the nonmodified and HOPO-containing PNA duplexes (Figure 1). The UV melting curves showed that the **Lys_3 PNA-HOPO** duplex has a T_m of ∼47 °C, which is 21 °C lower than the T_m of the nonmodified **PNA** duplex and is comparable to that of PNA duplexes with the same sequence that had a central pair of bipyridine ligands.[8] The melting temperature of either of the two **PNA-HOPO** duplexes measured in the presence of Eu^{III} was higher by several degrees than that measured in the absence of Eu^{III} (Table 1), which suggests that the Eu^{III}−**1,2-HOPO** coordination bonds contribute to the duplex stability. This suggestion is supported by the

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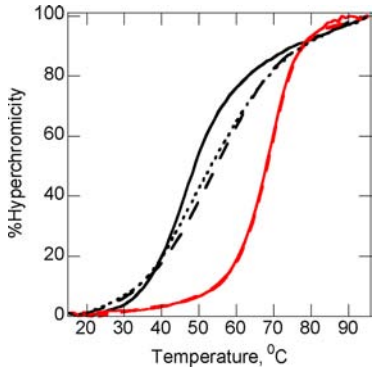

Figure 1. UV melting curves measured at 260 nm for 5 μM **Lys$_3$ PNA-HOPO** duplex (black) and a nonmodified PNA duplex (red) in the absence (solid line) and presence of 0.50 equiv (dotted line) and 1.0 equiv (dashed line) of Eu^{III} per duplex in pH 6.5, 20 mM MES, and 0.10 M NaCl buffer.

Table 1. Melting Temperature of PNA (T_m/°C) in the Absence and Presence of Eu^{III}

Eu^{III}/ds PNA	ds PNA	ds Lys HOPO	ds Lys$_3$ HOPO
0	68	48	47
0.5	68	52	52
1.0	68	52	54

fact that the thermal melting temperatures of nonmodified PNA in the absence and presence of 1 equiv of Eu^{III} are the same (Figure 1).

UV titration of **Lys$_3$ PNA-HOPO** with Eu^{III} is consistent with Eu^{III} coordination to the **1,2-HOPO** ligands (Figures 2 and S2 in the SI). Upon the addition of Eu^{III}, the UV spectrum of the duplex shows changes in the absorption at 290−400 nm, which corresponds to n−π* transitions of HOPO (Figure 2). In contrast, titration of Eu^{III} into a solution of nonmodified PNA gives rise to a very small decrease in absorbance at 260 nm (Figure S3 in the SI). These spectral changes are indicative of the coordination of Eu^{III} to the **1,2-HOPO** ligands of the **Lys$_3$ PNA-HOPO** duplex. A decrease in the absorbance of the 260 nm band of the nucleobases is also observed. This decrease is indicative of changes in the nucleobase stacking caused by Eu^{III} interaction with **1,2-HOPO** (Figure 2). An increase in the absorbance at 312 nm as a function of the Eu^{III}/**Lys$_3$ PNA-HOPO** ratio can be fitted to the formation of Eu^{III} complexes with two to four **1,2-HOPO** ligands from **Lys$_3$ PNA-HOPO** duplexes (see below).

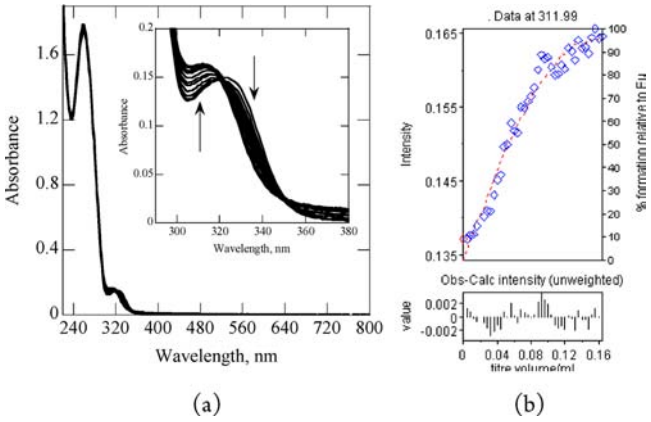

Figure 2. (a) UV titration spectra for a 10 μM solution of the **Lys$_3$ PNA HOPO** duplex in pH 6.5, 20 mM MES, 0.10 M NaCl buffer with Eu^{III}, measured 10 min after each addition of Eu^{III}. The final concentration of Eu^{III} was 15 μM. Each addition was of 4 μL of a 100 μM solution of Eu^{III} solution. The inset shows an expanded view of the spectra. (b) Fit of the absorbance at 312 nm as a function of the volume of Eu^{III} to a binding isotherm using the equilibria listed in Table S4 in the SI.

The circular dichroism (CD) spectrum of the **Lys PNA-HOPO** duplex is consistent with the presence in solution of a left-handed duplex; the preferred handedness is induced by L-Lys at the C end of the PNA oligomers (Figure S4 in the SI).[9] The CD amplitude decreased in the presence of Eu^{III}. Considered together with the cooperative melting curve for the **Lys PNA-HOPO** duplex in the presence of Eu^{III}, this observation indicates a decrease in the chiral induction effect exerted by L-Lys in the Eu^{III}-containing PNA, and it suggests that Eu^{III} coordination to the **1,2-HOPO** ligands in the **Lys PNA-HOPO** duplex causes structural changes in the duplex.

Luminescence spectroscopy experiments support binding of Eu^{III} to the **1,2-HOPO** ligands of the **Lys PNA-HOPO** duplex. Excitation at λ = 318 nm of the HOPO moiety of **Lys PNA-HOPO** in solutions that contain 1 equiv of Eu^{III} (with respect to the duplex) gives rise to a typical Eu^{III} emission spectrum that has a pronounced $^5D_0 \rightarrow {}^7F_2$ emission peak at 614 nm (Figure S5 in the SI). These spectral data show that **1,2-HOPO** sensitizes Eu^{III} luminescence through the formation of a coordination complex. More information is obtained from direct excitation spectroscopy of the $^7F_0 \rightarrow {}^5D_0$ transition of Eu^{III} in which luminescence is monitored at the $^5D_0 \rightarrow {}^7F_2$ emission through a bandpass filter (628 ± 27 nm). The excitation spectra of a solution containing Eu^{III} at varying concentrations of the **Lys PNA-HOPO** duplex are shown in Figures 3 and S6 in the SI. A peak-fitting analysis of the excitation spectrum at either a 1:1 or 1:2 ratio of Eu^{III}/**Lys PNA-HOPO** duplex shows two major peaks centered at 579.21 and 579.72 nm (Figure 3). For comparison, solutions containing **Pr-1,2-HOPO**[4b] and Eu^{III} in a 1:4 ratio give excitation spectra with peaks at 579.53 and 579.90 nm (Figures S7 and S8 in the SI). The red-shifted excitation peaks for Eu^{III} bound to **Lys PNA-HOPO** or **Pr-1,2-HOPO** are consistent with the addition of an anionic donor group to the Eu^{III} coordination.[10] In contrast, titration of nonmodified PNA with Eu^{III} leads to a slight

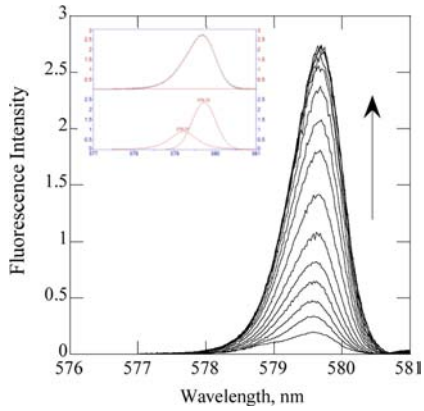

Figure 3. (a) $^7F_0 \rightarrow {}^5D_0$ excitation spectrum of a 20 μM Eu^{III} solution in pH 6.50, 0.020 M MES, and 0.100 M NaCl buffer (λ_{em} = 628 ± 27 nm) in the presence of an increasing amount of **Lys PNA-HOPO** from 3.18 μM to 53.4 μM. Top of the inset: excitation spectrum of the solution containing 42.2 μM **Lys PNA-HOPO** and 20 μM Eu^{III}, 0.10 M NaCl, and 0.020 M MES at pH 6.5. Bottom of the inset: peak-fit analysis of the spectrum showing two bands at 579.21 and 579.72 nm.

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quenching of the free Eu^{III} excitation peak but no further shift of the excitation peaks (Figure S9 in the SI).

Luminescence lifetime data give further information about the Eu^{III} species in solution. Excitation at several wavelengths across the excitation peak in solutions containing either 1:1 or 1:2 Eu^{III}/**Lys PNA-HOPO** at 20 μM Eu^{III} gives luminescence lifetimes consistent with 4 or 5 bound water molecules (q number), respectively (Table S1 and eq S1 in the SI). This is consistent with the displacement of four out of nine water ligands of Eu^{III} upon binding to two **1,2-HOPO** moieties in a single **Lys PNA-HOPO** duplex. That similar luminescence lifetimes are recorded suggests that the two peaks represent two different isomers of Eu^{III} bound to **Lys PNA-HOPO** with the same number of bound water molecules. Alternately, interconversion of two species each with different numbers of water ligands that is rapid on the luminescence lifetime time scale may give rise to an average q number. Solutions with Eu^{III} and nonmodified PNA under similar conditions contain fully hydrated Eu^{III} (Table S2 in the SI). For solutions containing **Pr-1,2-HOPO** and Eu^{III} in a 1:1 ratio, luminescence lifetime data are indicative of binding to Eu^{III} of a single bidentate ligand and seven water ligands (q = 7; Table S3 in the SI). Again, the similar lifetimes obtained upon excitation at the two peaks suggest that the two species are isomers with the same number of bound water molecules or, alternatively, are interconverting. Higher ratios of **Pr-1,2-HOPO** to Eu^{III} (i.e., 4:1) give rise to a third excitation peak and q numbers that are consistent with two bound **Pr-1,2-HOPO** ligands (Table S3 in the SI).

Binding curves derived from both UV−vis titrations and Eu^{III} excitation luminescence spectra as a function of the Eu^{III} or **Lys PNA-HOPO** concentration, respectively, were used to determine the binding constants of Eu^{III} complexes with the **PNA-HOPO** duplexes (Figures S10 and S11 in the SI). The unusual shape of the binding curve at the three excitation wavelengths suggests the coexistence in solution of more than one Eu^{III} species. Fitting of the data using *HyperSpec* required three species including Eu_2L, EuL, and EuL_2, where L is a **Lys PNA-HOPO** duplex. The majority species under most conditions (10−20 μM Eu^{III} and 1−2 equiv of the **Lys PNA-HOPO** duplex) is the 1:1 EuL complex. However, when the **Lys PNA-HOPO** duplex is in excess, the data are consistent with the formation of a EuL_2 species, presumably involving two **Lys PNA-HOPO** duplexes binding to one Eu^{III}. Conversely, at excess Eu^{III} to **Lys PNA-HOPO**, Eu_2L is formed. Detailed information about the coordination sphere of Eu^{III} in Eu_2L or EuL_2 is difficult to obtain because these species are not present in solution in sufficiently large concentrations to distinguish their spectroscopic signatures from those of the EuL complex. The binding constants for these EuL_2, Eu_L, EuL_2 species are given in Table S4 in the SI. Of these constants, the most important is that for the EuL complex, log K = 9.4. By comparison, titration of **Pr-1,2-HOPO** with Eu^{III} (Figure S12 in the SI) gave a binding isotherm that was fit to the formation of mono-, di-, and triadducts of Eu^{III} with the ligand. The binding constant for Eu(**Pr-1,2-HOPO**)$_2$, log β = 5.4, is *4 orders of magnitude weaker* than that for the complex formed between Eu^{III} and the **Lys PNA-HOPO** duplex. We attribute this difference in the binding constants to a supramolecular chelate effect exerted by the duplex on the complex of Eu^{III} with **1,2-HOPO**. This effect was previously observed for complexes formed by Cu^{II} with PNA duplexes containing 8-hydroxyquinoline as well as for metal complexes within ligand-modified ds DNA.[1b,11]

The results of this study establish that it is possible to coordinate Eu^{III} to PNA duplexes modified with ligands for lanthanides. Our data suggest that Eu^{III} can bind to both **HOPO** moieties of the duplex and retains five coordinated water molecules. This approach leads to PNA duplexes with binding sites for Eu^{III} that retain bound waters required for MRI applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The synthesis of the HOPO-modified PNA monomer, **Pr-1,2-HOPO**, and PNA oligomers, excitation spectra and simulations, melting curves and UV spectra of the **Lys PNA-HOPO** duplex, luminescence lifetimes and q numbers for Eu^{III} complexes with **Pr-1,2-HOPO** and **Lys PNA-HOPO**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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