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Side-Chain Effects on the MRI and Optical Properties of Bimodal Imaging Agents

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Introduction

- In the management of cancer, distinguishing healthy from diseased tissue can make surgical removal of tumors challenging.¹
- This difficulty can lead to insufficient removal of cancerous cells or excision of healthy tissue.¹
- Bimodal imaging agents that have both magnetic resonance imaging (MRI) and optical imaging properties have the potential to improve visualization of tumors, ensuring complete removal (**Figure 1**).²

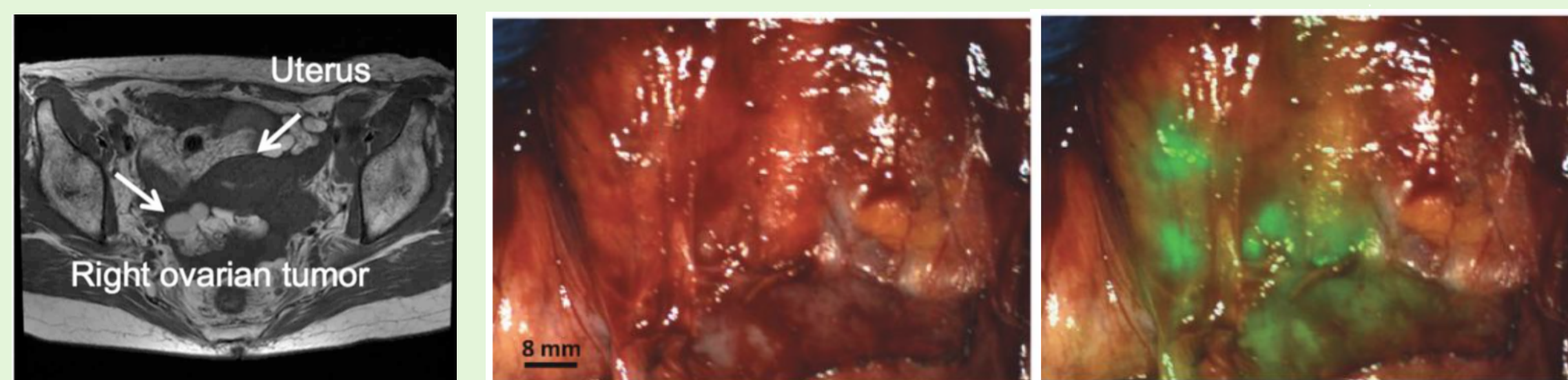


Figure 1: Preoperative MRI used to locate an ovarian tumor (left). Intraoperative images without (middle) and with (right) use of a fluorescent dye.^{3,4}

- Paramagnetic Chemical Exchange Saturation Transfer (PARACEST) agents are lanthanide complexes that darken an image to produce MRI contrast through slow-to-intermediate exchange of paramagnetically-shifted protons

- Lanthanides such as Europium and Terbium have been shown to have long-lived luminescence lifetimes.⁵
- When chelated with a ligand containing a chromophore such as pyridine, these lanthanide complexes have been shown to have MRI properties as well as optical imaging capabilities.

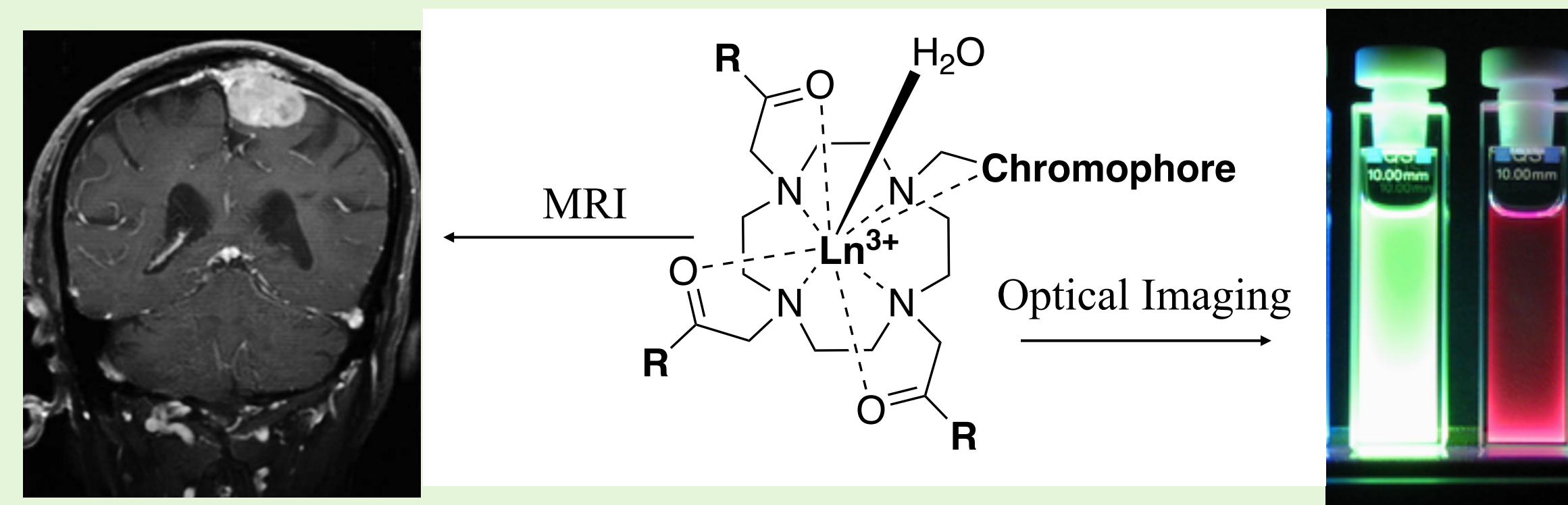
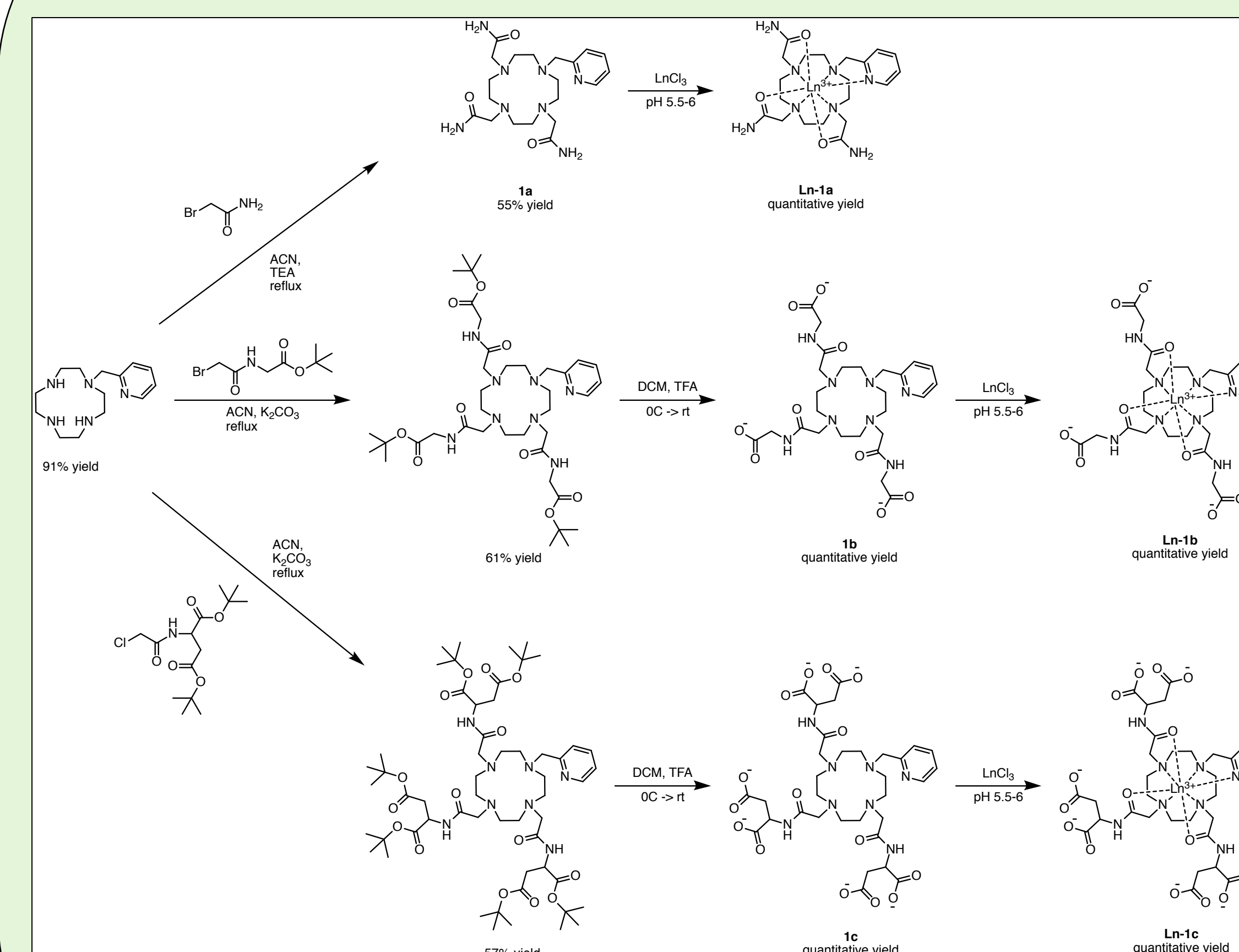


Figure 2: A sample octadentate lanthanide complex (middle) with bimodal properties. Identity of the ligand side-chains (R-groups) can vary. Upon excitation of the chromophore,^{6,7}

Project Goal:

- The goal of this project is to develop a library of bimodal imaging agents with an appended pyridine chromophore. We hypothesize that the identity of the remaining three ligand side-chains will affect the MRI and optical imaging properties of the metal complexes.

Methods and Materials



Scheme 1: Synthetic scheme of **Ln-1a**, **Ln-1b**, and **Ln-1c**. Lanthanides used in this study are Tb³⁺ and Eu³⁺.

Ligand Synthesis

All reagents and solvents were purchased from commercial vendors. Ligands **1a-1c** were synthesized according to **Scheme 1**. Monoalkylation of cyclen was achieved with 2-picolylchloride hydrochloride. This was followed by trialkylation with the appropriate bromo- or chloroacetamides under standard conditions. The resulting ligands were filtered or purified by Flash Chromatography. The tert-butyl groups were removed with TFA in DCM. All compounds were characterized by ¹H and ¹³C NMR spectroscopy.

Preparation of Lanthanide Complexes

Complexation was performed at pH 5.5-6 using equimolar amounts of LnCl₃ and ligand. After 24 h, the pH of the solution was raised to 10 to precipitate excess metal. The absence of free metal was confirmed using a xylenol orange test and the concentration of metal in solution was determined through ICP analysis.

Results and Discussion

Absorption and Excitation Spectra

- Absorption spectra of Eu³⁺ and Tb³⁺ compounds are shown in **Figure 3**.
- Three maxima can be seen at 261 nm, 266 nm, and 272 nm. Absorption at these wavelengths is most likely due to $\pi-\pi^*$ electronic transitions.⁴
- λ_{max} in the excitation spectra was consistently found to be between 271 and 274 nm for all complexes. This can also be attributed to $\pi-\pi^*$ electronic transitions observed in the absorption spectra.

Emission Spectra

- The pyridine-sensitized emission spectra are shown in **Figure 4**.
- Eu³⁺ complexes show characteristic emission bands with $^5D_0 \rightarrow ^7F_J$ ($J = 0-5$) bands for Eu³⁺ and the Tb³⁺ compounds show characteristic emission bands with $^5D_4 \rightarrow ^7F_J$ ($J = 2-6$).⁵
- The overall emission intensities for 1b and 1c complexes are greater than those of 1a complexes. This observation is probably due to the luminescence quenching by the three additional closely oscillating NH groups in the 1a compounds.⁶

PARACEST MRI Studies

- PARACEST spectra of the Eu³⁺ complexes acquired from 100 to -100 ppm at 298 K and 310 K are shown in **Figure 5**.
- The two main peaks at 0 ppm and ~40 ppm can be attributed to the bulk water and bound water of the SAP isomer, respectively.
- For all complexes, a minor exchange peak can also be observed at ~55 ppm. This is likely due to a minor SAP isomer.
- The breadth of the CEST exchange peaks for Eu-1a compared to Eu-1b and Eu-1c indicates faster bound water exchange kinetics.
- Although the minor exchange peak for Eu-1a disappeared at 310 K, it was still observable for Eu-1b and Eu-1c.
- The increase in the magnitude of the CEST effect exhibited by Eu-1c indicates that this complex has the most favorable CEST properties at higher temperatures.

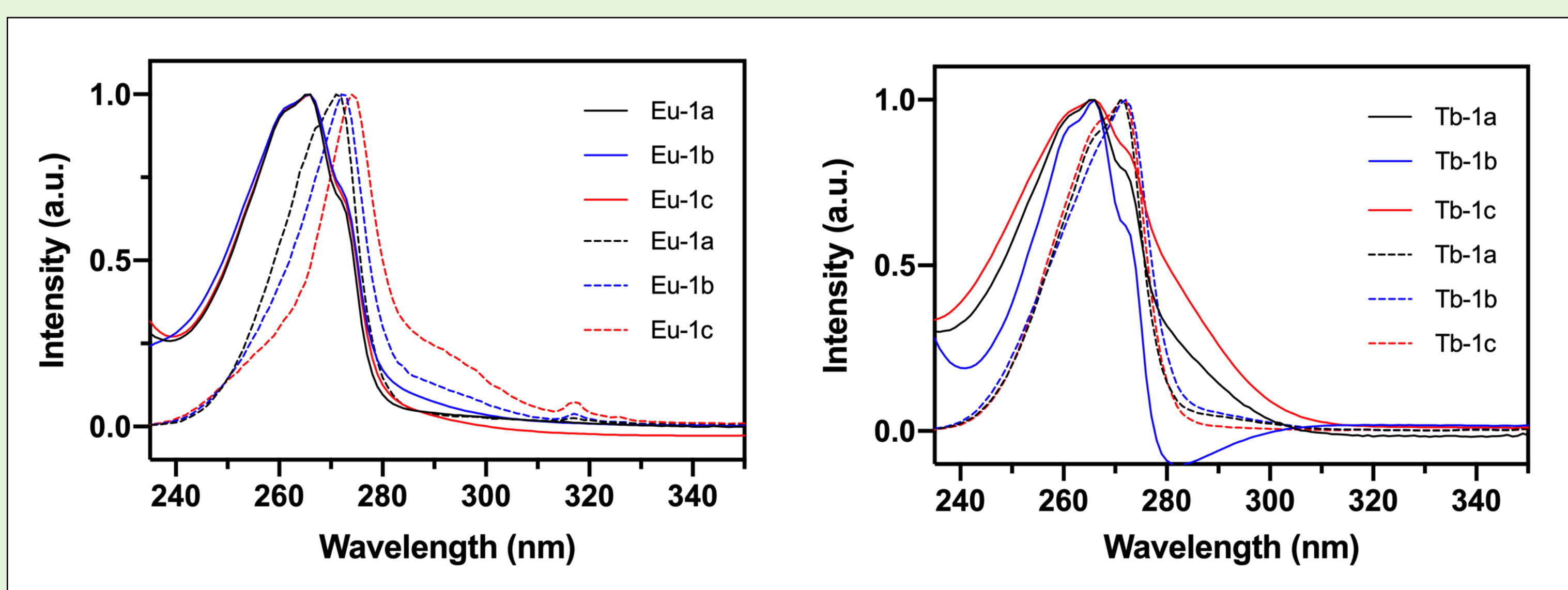


Figure 3: Normalized absorption spectra (solid lines) and excitation spectra (dashed lines) of Eu³⁺ (left) and Tb³⁺ (right) complexes in 0.1 M HEPES buffer (pH 7.4). Excitation spectra of Eu³⁺ and Tb³⁺ were acquired with $\lambda_{\text{em}} = 613$ nm and 564 nm using 2 nm slit widths. Absorption and excitation spectra were acquired at concentrations of 6.25×10^{-5} M and 1×10^{-4} M respectively.

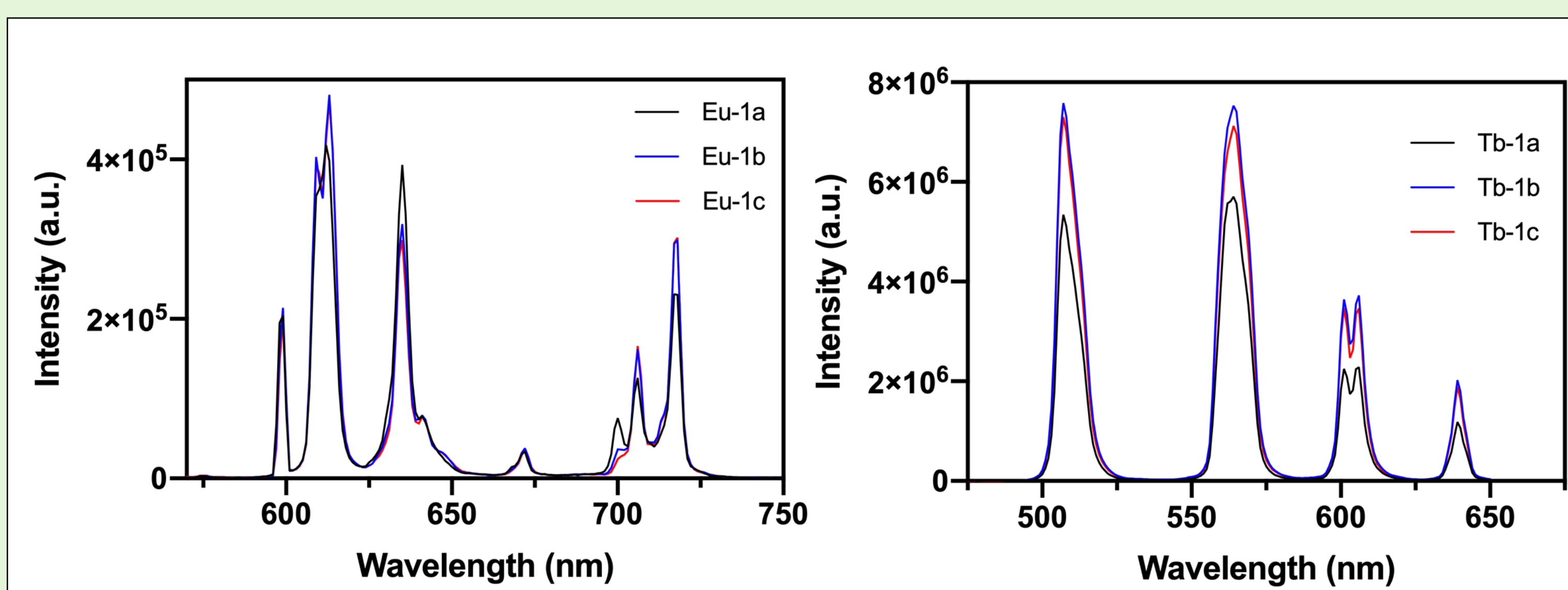


Figure 4: Emission spectra of 1×10^{-4} M Eu³⁺ (left) and Tb³⁺ (right) complexes in 0.1 M HEPES buffer (pH 7.4). Emission spectra of Eu³⁺ and Tb³⁺ were acquired upon excitation at 272 nm and 271 nm respectively using 2 nm slit widths.

Conclusion

- Side-chain identity has little influence on absorption and excitation properties.
- Emission intensity and MRI properties are affected by side-chain identity
- 1a complexes have the lowest emission intensity.
- Eu-1a has faster exchange kinetics while Eu-1c has more optimum qualities at physiological temperature.

Future Work

- Further evaluation of the MRI properties of the library of complexes.

Acknowledgements

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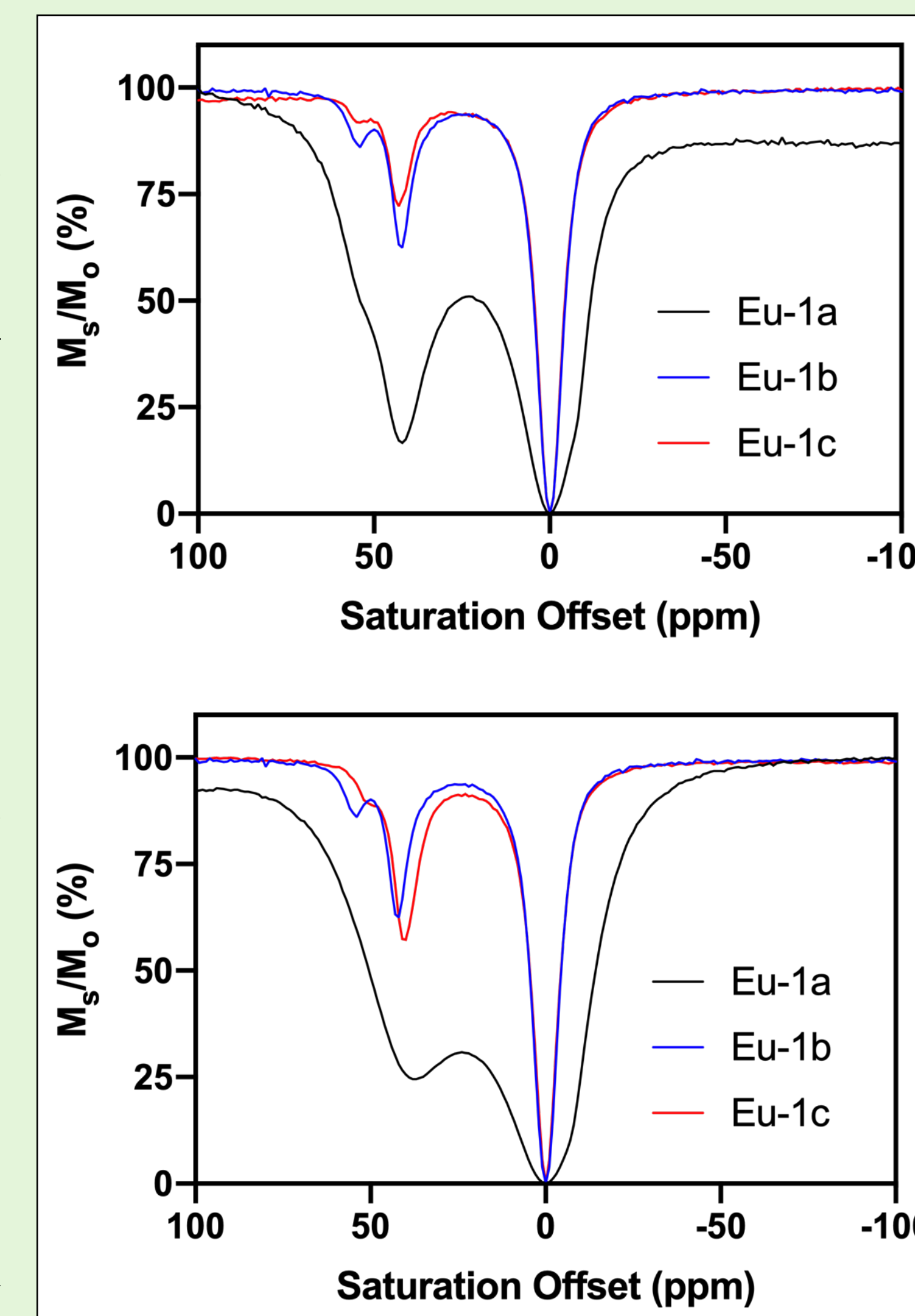


Figure 5: PARACEST spectra of Eu³⁺ (10 mM) acquired at 9.4 T, 298 K (top) and 310 K (bottom). pH = 7, $B_1 = 18.8 \mu\text{T}$, and irradiation time = 5 s.