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Introduction

Copper(II) is important for the biological processes of living organisms and there are many known Cu(II)-containing enzymes that catalyze redox processes. However, chronic Cu(II) exposure at high enough levels has been associated with multiple neurodegenerative diseases in humans¹, as well as acute toxicity to several fish species²⁻⁴. In fact, the toxic concentrations of Cu(II) to fish can be as low as 0.05 mg/L which constitutes 1/20 of the accepted standard concentration in drinking water⁵. Cu(II) even at such low concentrations is capable of damaging the gills and other tissues of various fish species, while also degrading their immune system^{1, 4, 6, 7}.



The effects of Cu(II) exposure on fish gills: the skin darkens, thickens and appears fragile and ulcerated, in particular around the mouth. Excessive mucus is secreted, gill function and resistance against parasite and bacteria are compromised^{4,6,7}.

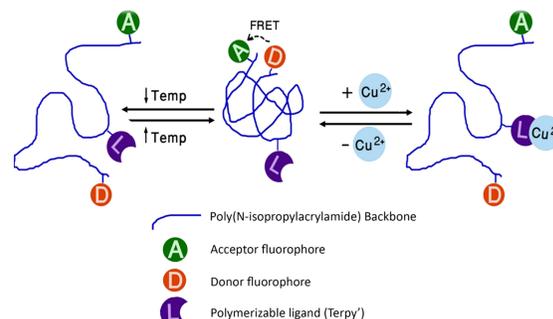
It has been found that Cu(II) toxicity in aquatic organisms does not correlate with total Cu(II) in water, but rather the free form which is in stoichiometric excess of the dissolved organic matter^{3,4,8}. For this reason, designing a sensor that only measures the free form of Cu(II) is of great interest. One of the most common and convenient methods used for accurate measurement of metal ions is fluorescence. However, fluorescence-based Cu(II) sensors face challenges such as the difficulty of distinguishing free and total Cu(II), difficulty to design the favorable turn-on sensor, quenching of fluorescence signals due to the paramagnetic nature of Cu(II), and interference of other metals that may be present in a water sample.

Goals & Design

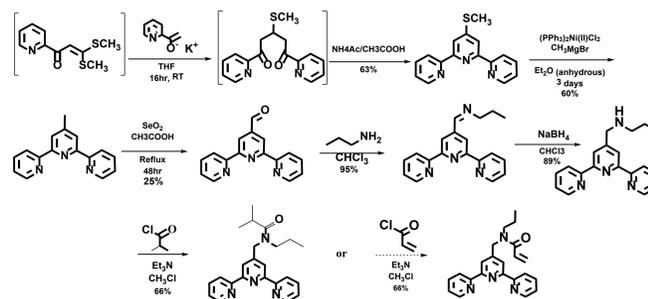
We have designed a polymer-based Cu(II) sensor that utilizes Förster Resonance Energy Transfer (FRET) to measure low concentrations of Cu(II). The sensor is based on the phase transition of poly(N-isopropylacrylamide) (PNIPAM) induced by changes in the hydrophilicity of the polymer backbone. It consists of PNIPAM copolymerized with small amounts of donor and acceptor fluorophores, and bifunctional Cu(II) and Zn(II) ligands. The design of this sensor allows the detection method to: 1) detect low concentrations, be turn-on and ratiometric; 2) overcome the problem of signal quenching; and 3) minimize the interference of other metals which maybe be present in a given sample such as Zn(II). We focus on Zn(II) because it is likely to compete with Cu(II) over the Cu(II) ligand, despite the relatively high affinity of the ligand towards Cu(II). This is because Zn(II) is often present in much greater concentrations than Cu(II) in various types of water and it is similar to Cu(II) in size and hardness⁹⁻¹². Terpyridine has a high log K_{Cu} of 12.3 and terpyH⁺ has a desirable low pK_a of 4.7. We herein describe the synthesis of a new polymerizable Cu(II) ligand (terpy') to be used as the Cu(II) binding site in a ratiometric Cu(II) sensor. Fluorescence and UV-Vis spectral behavior of the Cu(II) and Zn(II) complexes in solution is discussed to shine light on the extent of interference, and single crystal X-ray data are presented.

Mechanism

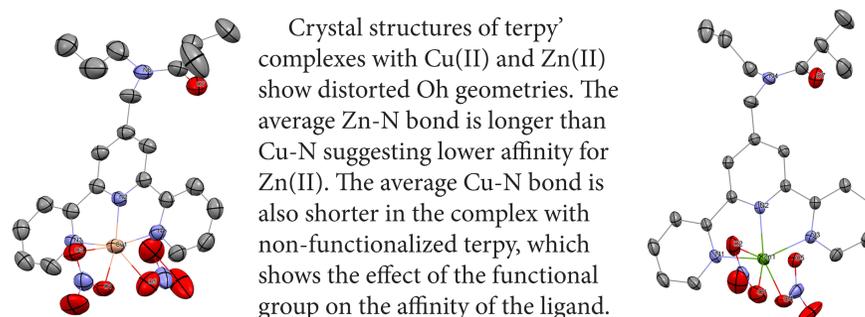
When the Cu(II) ligands are neutral and the temperature is above the lower critical solution temperature (LCST) of PNIPAM, the polymer collapses and FRET occurs. However, when Cu(II) binds to the ligands, the polymer expands due to the increased hydrophilic interactions caused by the cationic character introduced through Cu(II) and the imposed hydration sphere around Cu(II). As a result of this expansion, the fluorophores are forced apart from one another and FRET decreases.



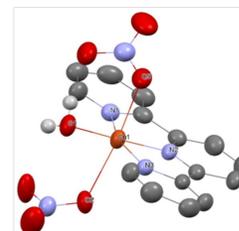
Synthesis of Bifunctional Cu(II) Ligand (terpy')



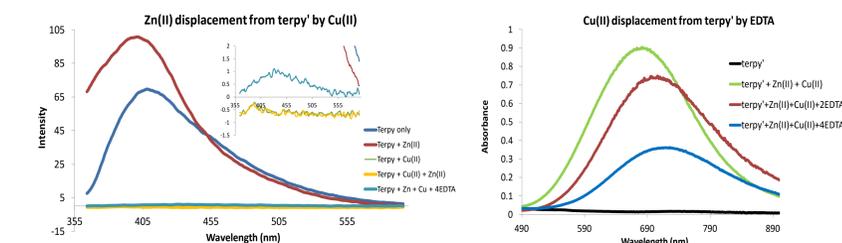
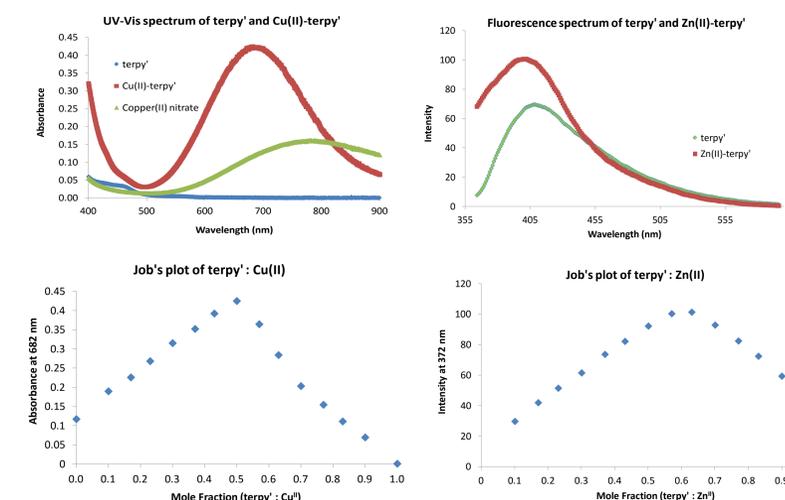
Crystal Structures



Coordination Sphere Bond Distances (Å)					
	Terpy'-Cu(II)		Terpy-Cu(II)		Terpy'-Zn(II)
Cu-N1	2.032(3)	Cu-N1	2.021(3)	Zn-N1	2.129(2)
Cu-N2	1.938(2)	Cu-N2	1.933(2)	Zn-N2	2.069(1)
Cu-N3	2.020(3)	Cu-N3	2.017(3)	Zn-N3	2.138(2)
Cu-O1	1.978(3)	Cu-O1	1.946(2)	Zn-O1	2.145(2)
Cu-O3	2.648(3)	Cu-O2	2.476(2)	Zn-O2	2.369(2)
Cu-O5	2.25(1)	Cu-O5	2.410(2)	Zn-O3	2.042(1)



Fluorescence and UV-Vis Spectra of Terpy' complexes with Cu(II) and Zn(II)



Conclusion

- We have developed a ratiometric turn-on sensor to measure free Cu(II) concentration in the environment
- A bifunctional Cu(II) ligand based on terpyridine was synthesized and spectral behavior of its metal complexes in solution was investigated
- Crystal structures of Cu(II)-terpy' and Zn(II)-terpy were reported and they show stronger bonds formed between the ligand and Cu(II) compared to Zn(II).

Acknowledgements

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