

Investigating the toxicities of metals and identifying cadmium centers in metallothioneins

1. Use the following questions to help predict whether Cr(VI) or Cr(III) is more toxic:
 - a. How many d-electrons does each species have?

 - b. Which do you expect to be the stronger oxidant?

 - c. Which do you expect to be the stronger reductant?

 - d. Cr(III) tends to form hydroxide complexes. Propose four chemical formulas for Cr(III) hydroxide complexes. Hint: Cr is the central atom and can be bound to 1-4 hydroxides.

 - e. Using only four oxygen atoms, up to two hydrogen atoms, and a central Cr atom, propose three structures for Cr(VI) complexes.

 - f. In order for Cr to be toxic, it has to be able to enter the cell. What biologically relevant polyatomic ions are your answers to the previous question structurally similar to? Do you think the complexes from part d or part e will more easily enter the cell?

- g. Using hard-soft acid-base (HSAB) theory discuss what types of ligands/atoms are likely to preferentially bind Cr(VI) over Cr(III).
- h. Metallothioneins are intracellular proteins that are often involved in biological regulation of toxic heavy metals. Given the following amino acid sequence for a metallothionein (PDB code 5ML1):
- [Insert figure and key for amino acid sequence from PDB website <http://rscb.org> for the PDB code 5ML1]**
- i. How many cysteine residues are there?
- ii. How many metal centers could this metallothionein potentially sequester?
- iii. Use arrows to propose which cysteines on the protein sequence diagram could potentially form each metal binding site.

4. It is also possible to conduct a ^{113}Cd COSY experiment. What information can be learned from the $[^{113}\text{Cd}-^{113}\text{Cd}]$ -COSY experiment?

5. The following spectrum is the $[^{113}\text{Cd}-^{113}\text{Cd}]$ -COSY from metallothionein.

[Insert figure S3: $[^{113}\text{Cd}-^{113}\text{Cd}]$ -COSY spectrum from supporting information.]

- a. Identify each cadmium center on the $[^{113}\text{Cd}-^{113}\text{Cd}]$ -COSY using the shifts from the previous ^{113}Cd NMR spectrum. Use numbers to label each Cd.
- b. Propose which cadmium centers are in close proximity to one another. Include drawings for your proposals.

6. Finally, a [^{113}Cd - ^1H]-HMQC experiment can be conducted and the spectrum is shown:

[Insert figure S4: [^{113}Cd , ^1H]-HMQC-TOCSY spectrum from supporting information.]

- a. How many protons are interacting with each cadmium center through 2-3 bond connections? Use the previous ^{113}Cd NMR spectrum to help identify each cadmium center. List each separately.

- b. The following are chemical shift ranges for cysteine protons: H^α (5-6 ppm), $\text{H}^{\beta\text{a}}$ (3-4 ppm), $\text{H}^{\beta\text{b}}$ (2-3 ppm), NH (7-9 ppm). Where might the proton signals in the NMR spectrum be coming from? Be specific.

- c. What does the HMQC tell you about the coordination environment around each cadmium center?

Created by S. Chantal E. Stieber (California State Polytechnic University, Pomona – sestieber@cpp.edu) and posted on VIPER on June 3, 2017. Copyright S. Chantal E. Stieber, 2017. This work is licensed under the Creative Commons Attribution Non-commercial Share Alike License. To view a copy of this license visit <http://creativecommons.org/about/license/>.

- d. Based on data from all three NMR spectra, use pictures to propose coordination environments, including ligands, for each cadmium center.

References:

1. Zerbe, O.; et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 4617.
2. Jacob, S. T.; et al. *J. Biol. Chem.* **2003**, *278*, 26216.
3. Fidalgo, F.; et al. *Food Energy Secur.* **2013**, *2*, 130.