Metal Binding and Interdomain Thermodynamics of Mammalian Metallothionein-3: Enthalpically Favoured Cu+ Supplants Entropically Favoured Zn2+ to form Cu+4 Clusters Under Physiological Conditions, by Matthew R. Mehlenbacher,a Rahma Elsiesy,b Rabina Lakha,b Rhiza Lyne E. Villones,c Marina Orman,b Christina L. Vizcarra,b\* Gabriele Meloni,c\* Dean E. Wilcox,a\* Rachel N. Austinb\*

This paper reports efforts to quantify the thermodynamics of Cu(I) and Zn(II) binding to several members of the metallothionein protein family in an effort to understand the structural basis of metal ion speciation. While the paper might initially seem insurmountably biochemical for an inorganic student, this learning objective is designed to help students read past background material that may not be familiar to focus on inorganic chemistry ideas initially introduced in general chemistry.

1. Cysteine (shown below) is an amino acid that binds metal ions through the thiol (SH) group. Two cysteines can be oxidized to form a disulfide bond, releasing two electrons and two protons. Draw that reaction and explain how the oxidation of cysteine would hamper metal ion binding to this residue.



1. In this work, the authors titrated Zn(II)-replete MT-3 with DTPA. Zn binds to DTPA with a log K of 18.2. In general terms, how does this experiment enable them to determine the binding constant of Zn(II) to MT-3?



1. The authors used a similar approach to determine the binding constant of Cu(I) to MT-3 by titrating Zn(II)-replete MT-3 with Cu(I) in the presence of excess glutathione (GST).
* A Cu(I) stabilizing ligand is necessary because Cu(I) disproportionates. Write the chemical equation describing the Cu(I) disproportionation reaction. Describe the thermodynamic conditions that must exist for this kind of reaction to occur.
* What does “pZn2+free= 10.6” mean and what is the significance to the overall chemistry that is occurring in this titration by being carried out in a large excess of GSH?
1. Titrations involve reactions of known stoichiometry that typically take place between a titrant of known concentration with an analyte of known volume. The volume of titrant required to reach the end point is determined experimentally, enabling one to calculate the concentration of analyte. In the titrations described in this paper, the concentration of protein and the number of metal ions bound to the protein, need to be known as well so that the enthalpy associated with chemical reactions can be measured. All experimental techniques have limitations. Explain how the use of ICP-MS and luminescence spectroscopy helped limit the uncertainty of the conclusions drawn from the ITC measurements.
2. Calorimetry measures the heat released or absorbed in the course of the chemical reaction. Explain why simply reporting the heat released or absorbed for any of the chemical reactions described in this paper is of limited value. (Hint: review Scheme S1 and Scheme S2 in the supplemental material.)
3. What is “desolvation” of a metal ion and how does it contribute to the binding free energy when a metal ion binds to a protein or a ligand?
4. The key findings for this paper are reported in table 6. Write the equations that show how the enthalpy and entropy of a reaction contribute to its free energy and equilibrium constant. Explain how Zn(II) binding to a protein can be entropically favorable. Summarize the paper’s finding in your own words.

**Here are some additional questions that dig a bit deeper into the experimental methods and thermodynamic analyses that were done in the work and that more explicitly connect the work to a biochemical context, which might be appropriate to include depending on the course (e.g. for a biophysics course).**

1. Why was only part of the experimental data (ITC binding isotherms) fit with the binding model?

1. Why was further (ad hoc) analysis of the experimental values undertaken?

1. The fundamental concept of Hess’s Law is used to analyze the net binding enthalpy (DH(ITC)) to determine the binding enthalpy of interest (DH(M-P)). What are the individual equilibria that are included in this analysis?
2. Biochemists typically quantify equilibria with a value of Kd (ML $⇌$ M + L), while chemists typically report, and calorimeters measure, K (M + L $⇌$ ML), where Kd = 1/K. What are the average MT3 affinities for Zn2+ and Cu+ in values of Kd, which are often given units of concentration.
3. Why is the change in entropy reported as -T(DS) at the experimental temperature?
4. The net binding enthalpy is predominantly due to the sum of the enthalpies of the bonds that are broken and bonds that are made. What are these bonds when Zn2+ binds to MT3?
5. What is enthalpy-entropy compensation and what does it indicate about the net binding thermodynamics?
6. MT consists of two tethered domains that may or may not bind metal ions independently. How was the presence or absence of this inter-domain influence (interaction) determined and quantified? Hemoglobin consists of a cluster of four subunits, (a)2(b)2. How could you investigate whether or not there is any subunit-subunit influence (interaction) on the binding of O2 by the subunits of hemoglobin?
7. What are the individual equilibria that are involved when Cu+ in 5 mM GSH is titrated into Zn7MT3 in 5 mM GSH?