

## Background Information

- Methanotropic bacteria use methanobactin (mb) as a copper sequestering, reducing, and transporting enzyme (Kim et al. 2004)
- This class of bacteria needs copper for use in an enzyme that oxidizes methane to methanol
- Therefore, methanol is a waste product of metabolism, which has many practical uses in industry and consumer energy supply
- Kim et al. (2004) determined that the optimal ratio of copper:mb for homeostatic living of the bacteria is 1:1
- Previous work at UWEC by Shafe et al. (2006) found that mb specifically binds to and reduces copper (II) to copper (I)
- However, little is yet known about the actual mechanism in which mb binds to and reduces copper
- Additionally, not much work has been done to see if mb will bind to other transition metals, such as silver or gold.
- To help elucidate the mechanism of how mb binds to copper, this study used drastically higher ratios of Cu:mb, as well as silver, than previously found in the literature
- Quantitative analysis of mass spectroscopy data allowed us to determine how many ions mb reduced, but not necessarily bound to the molecule

# Transition Metal Ion Reduction Characterization of Methanobactin

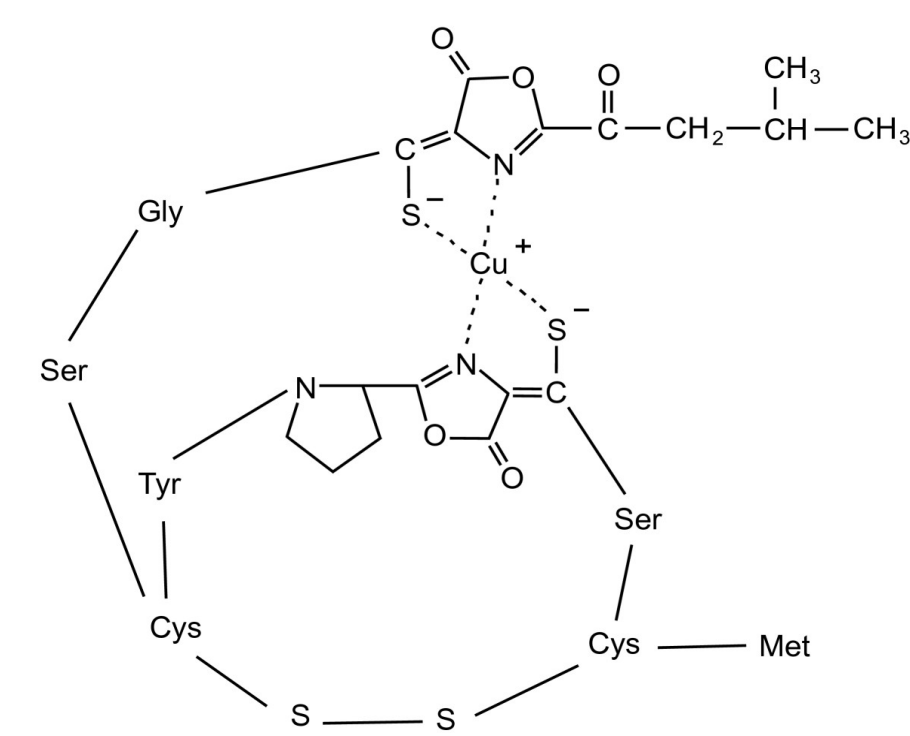


Figure 1. Proposed structure of Cu bound mb, courtesy of Pesch et al. (2011)

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## Methodology

### Standards Preparation:

- For both Cu and Ag the same method of standard prep was used, only the ion solution changed
- The exception was for Cu complexed with EDTA, in all EDTA trials the concentration of EDTA was 0.005M
- All standards had a total volume of 1mL, the ion solution was added first and the rest of the volume was filled by the buffer solution
- Concentrations of metal ions in the standards were 0.000M (Blank), 0.00015M, 0.0003M, 0.0006M, 0.0009M, 0.0015M, 0.0025M, and 0.005M

### Sample Preparation:

- Each sample had a total volume of 1mL
- The buffer solution was added first to stabilize the pH; the amount varied dependant on the amount needed to bring the total volume to 1mL
- Next, the ion solution was added in final concentrations of 0.0003M, 0.0009M, 0.0015M, 0.0021M, and 0.0024M
- The concentration of mb in each sample was 0.003M
- Only in the Cu trials that were made on 1/24/11, EDTA was added to make a final concentration of EDTA of 0.005M

### Chromatography/Mass Spectrometry:

- Liquid chromatography was used to separate the components of the standards and samples
- The ionic components of the mixtures eluted first due to their affinity of the polar mobile phase
- An electrospray mass spectrometer was used to obtain the masses of the ions
- The mass spectrometer was run in the positive ion mode
- The mass spectrometer operated in a vacuum and nitrogen gas was used as a drying agent

### Analysis:

- The Analyst software program was used for computing the area of intensities of analytes
- For silver, the areas under the intensity peaks for the mass to charge ratios of 106.9085m/z and 108.9041m/z were recorded; corresponding to the free silver ions
- For copper not complexed with EDTA (samples made on 1/10/11), the areas under the intensity peaks for the mass to charge ratios of 107.9644m/z and 109.9617m/z were recorded; corresponding to the free copper (II) ions complexed with 9 - NH<sub>3</sub> molecules
- For copper complexed with EDTA, the areas under the intensity peaks for the mass to charge ratios of 354.0196m/z and 356.0177m/z were recorded; corresponding to the copper (II)/EDTA complex
- Microsoft Excel was used to generate a standard curve and calculate the number of ions reduced; outliers were omitted from the analysis
- The TI-84 plus Silver Edition Calculator's logistic fit function was used to generate the Logistic fits of the graphs of Ions Reduced per mb vs the Ratio of Ion to mb

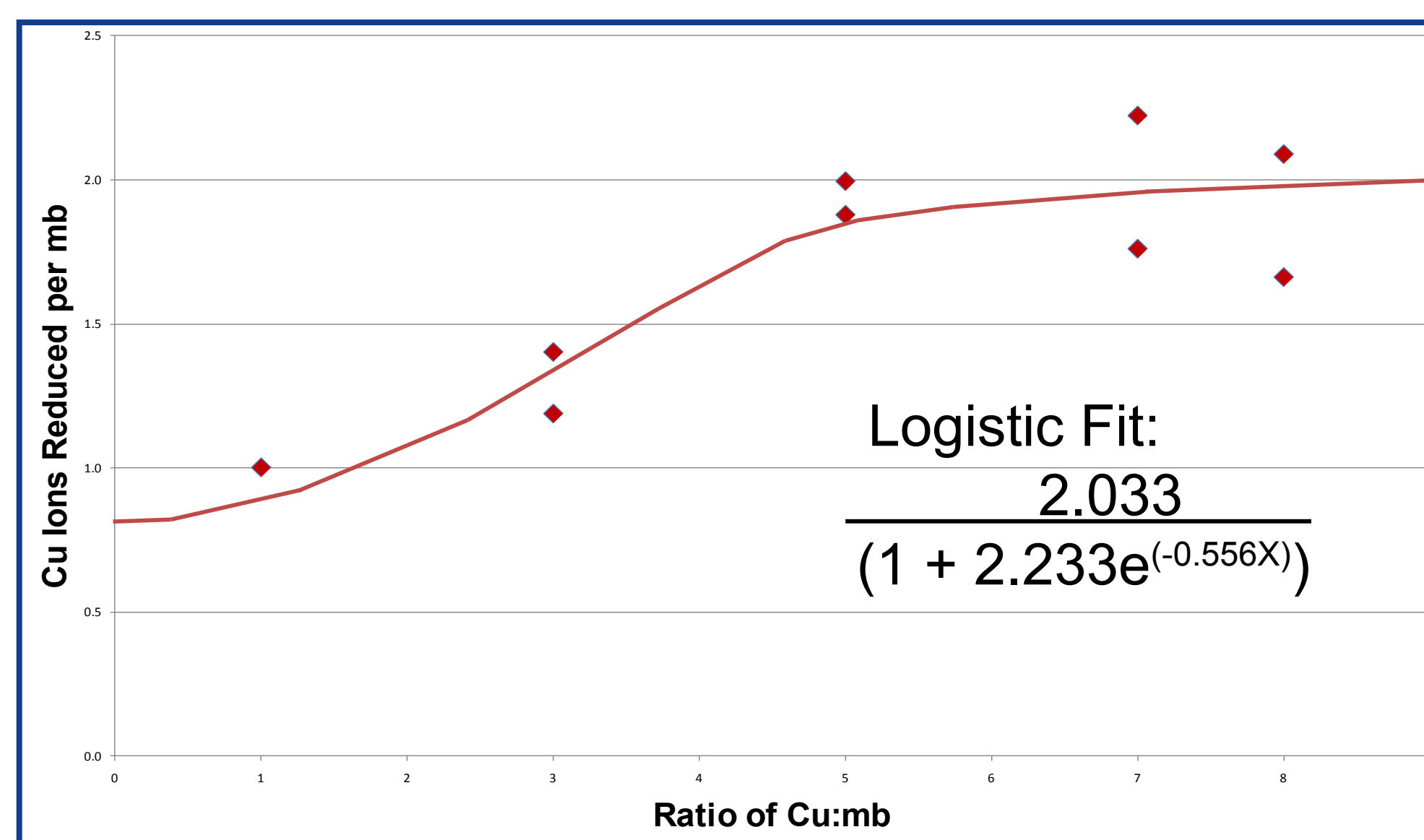


Figure 2. Graph of number of Cu (II) ions reduced to Cu (I) per molecule of mb. Contains data from copper samples made on both 1/10/11 and 1/24/11. Logistic fit was produced on a TI-84 Calculator. Line representation in graph was manually drawn. See Figure 4. for list of values.

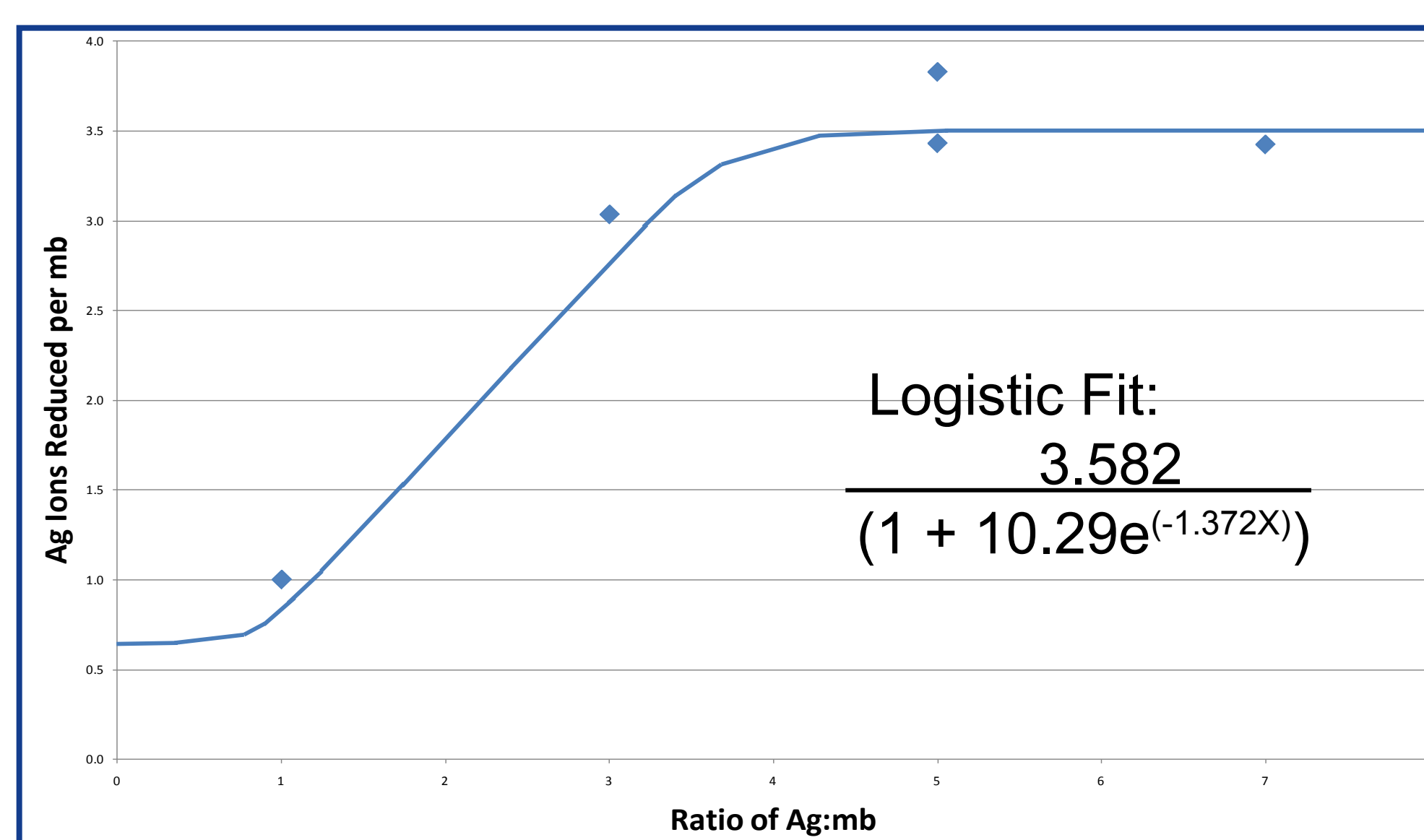


Figure 3. Graph of number of Ag ions reduced to elemental Ag per molecule of mb. Contains data from silver samples made on both 1/5/11 and 1/6/11. Logistic fit was produced on a TI-84 Calculator. Line representation in graph was manually drawn. See Figure 4. for list of values.

Sample Name (Date)	Number of Ions reduced
Ag1:1 (1/6)	1*
Ag3:1 (1/6)	3.04
Ag5:1 (1/5)	3.83
Ag5:1 (1/6)	3.43
Ag7:1 (1/5)	3.43
Cu1:1 (1/24)	1*
Cu3:1 (1/10)	1.40
Cu3:1 (1/24)	1.19
Cu5:1 (1/10)	2.00
Cu5:1 (1/24)	1.88
Cu7:1 (1/10)	2.22
Cu7:1 (1/24)	1.76
Cu8:1 (1/10)	2.09
Cu8:1 (1/24)	1.66

Figure 4. Table of ions reduced per molecule of mb.

\*These samples were on the lower end of our detection limit and produced a result of more ions reduced than were present, thus the maximum value of "1" was entered.

## Results

- mb reduces **2.03 Copper (II) ions per molecule**, thus 2.03 electrons from mb are available to reduce Copper (II)
- mb reduces **3.58 Silver ions per molecule**, thus 3.58 electrons from mb are available to reduce Silver
- The differing amounts of electrons available to reduce metal ions suggest that a different source of electrons within mb is used for each ion or that mb is not the source for the electrons

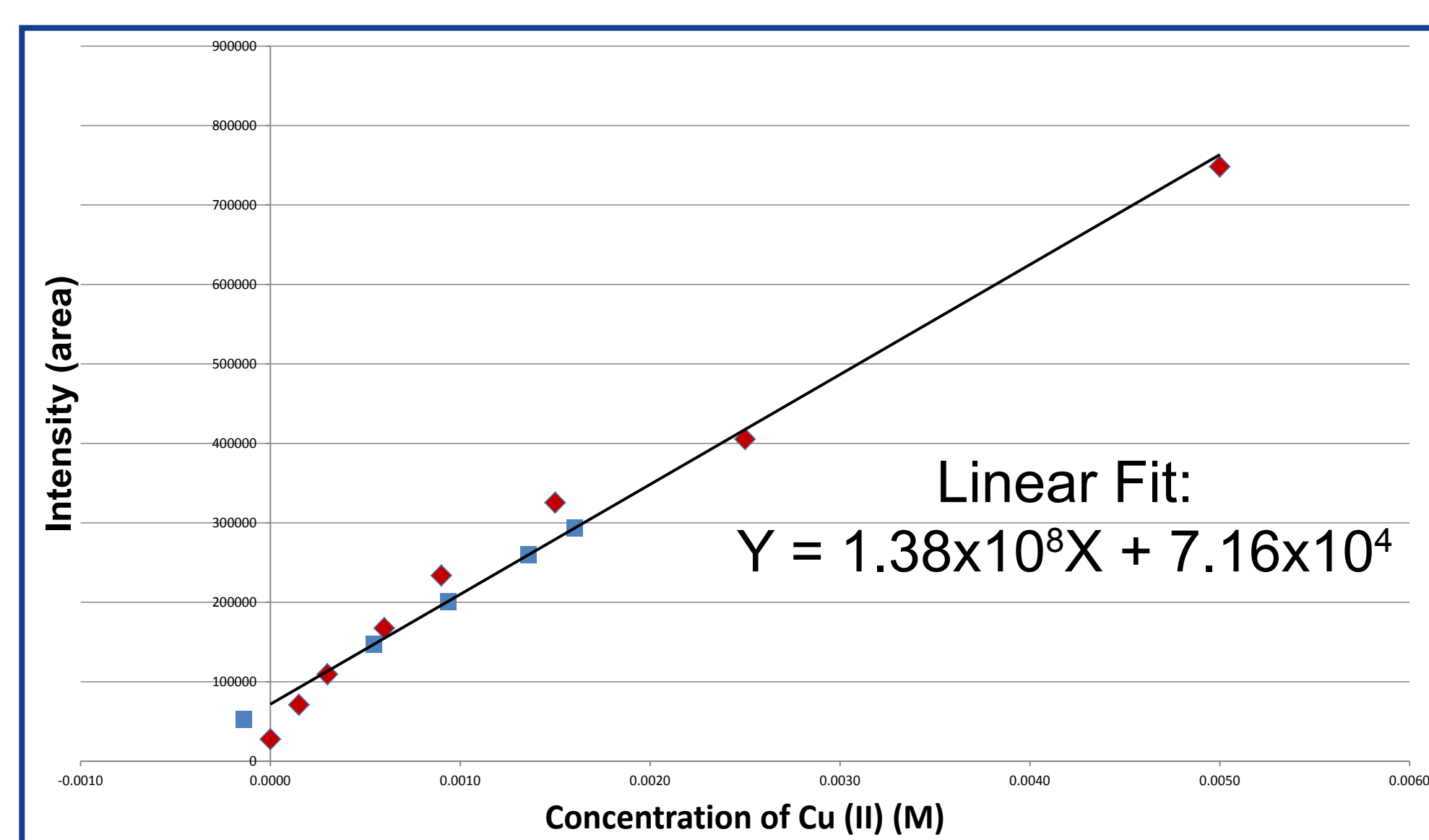


Figure 5. Sample Standard curve for the Copper trials made on 1/24/11. Linear fit was calculated on Microsoft Excel the R<sup>2</sup> value of the linear fit was 0.9831. See Figure 6. for list of values.

Sample Name	Mass/Charge (m/z)	Intensity (area)	Total Intensity	Initial Conc of Cu (M)	Observed Conc of Cu (M)
Buffer	354.02	12131	27664	0.0000	N/A
	356.02	15533			
Cu 15	354.02	42710	70970	0.00015	N/A
	356.02	28260			
Cu 30	354.02	70513	109380	0.00030	N/A
	356.02	38867			
Cu 60	354.02	109300	167484	0.00060	N/A
	356.02	58184			
Cu 90	354.02	154290	233753	0.00090	N/A
	356.02	79463			
Cu 150	354.02	218070	325250	0.00150	N/A
	356.02	107180			
Cu 250	354.02	270590	405280	0.00250	N/A
	356.02	134690			
Cu 500	354.02	507150	748100	0.00500	N/A
	356.02	240950			
Cu 1:1 (1/24)	354.02	32826	52424	0.00030	-0.00014
	356.02	19598			
Cu 3:1 (1/24)	354.02	97476	146877	0.00090	0.000544
	356.02	49401			
Cu 5:1 (1/24)	354.02	135500	201196	0.00150	0.000937
	356.02	65696			
Cu 7:1 (1/24)	354.02	173690	259879	0.00210	0.001361
	356.02	86189			
Cu 8:1 (1/24)	354.02	197550	293309	0.00240	0.001602
	356.02	95759			

Figure 6. Table of standard curve and sample intensities.

## Conclusion

Our analysis of the amount of unreduced ions in the mass spectrometer has given a clearer picture of how methanobactin works. From the data we were able to infer how many electrons mb is actually capable of contributing to the reduction of various transition metals. The data suggest that each molecule of mb can reduce 2.03 copper (II) ions to copper (I) ions and 3.58 silver ions to elemental silver. These numbers also correspond to how many electrons are needed for this reaction. However, a clear mechanism for how the electrons are transferred to the ions is still unknown.

There are a few possibilities for where the electrons are coming from. The most likely answers are that the electrons could be coming from the molecule itself or the catalytic oxidation of water. If the extra electrons given to silver are coming from mb, that could lead to a shift in its geometry, which could be analyzed in a future project. Conversely, if the electrons are coming from water, excess oxygen gas must be created and could be detected in a future project. Whatever the case, the strength of the ions to be oxidizing agents seems to play a role in how mb behaves.

This project is currently investigating the number of gold ions reduced by mb, however, there have been procedural problems that need to be worked out due to the characteristics of gold ions.

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### - Collaboration

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## References

- H. Kim, D. Graham, A. DiSpirito, M. Alterman, N. Galeva, C. Larive, D. Asunskis, P. M. A. Sherwood. (2004) Methanobactin, A Copper-Acquisition Compound From Methane-Oxidizing Bacteria. *Science* 305, 1612
- M. Pesch, I. Christl, K. Barmettler, S. Kremer, R. Kretzschmar. (2011) Isolation and purification of Cu-free methanobactin from *Methylosinus trichosporium* OB3b. *Geochemical Transactions* 12:2
- P. H. Shafe, M. T. McEllistrem, D. W. Choi, and A. D. DiSpirito. (2006) Copper(II) binding to and reduction by methanobactin. University of Wisconsin – Material Science Program

There is a closely related project, which gave insight to this project, displayed by Benjamin Krentz and Ashley Van Galen on the *Isolation and Characterization of a New Form of Methanobactin Isolated from Methylocystis Strain SB2*

