**Proposal ID#: 19834**
**Title: Correlation of Structure and Function of Zinc Metalloproteins Via a Combined NMR/Molecular Theory Approach**

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**Progress Report**

This report of our progress is for the first 9-month block of this project and will cover that everything to date and what we expect to accomplish in the coming year.

The first progress is on the Zn-S coordination systems. We have a recently published paper in J. Am. Chem. Soc. on zinc-sulfur model systems (Lipton, A. S.; Ellis, P. D. J. Am. Chem. Soc.; (Article); 2007; 129(29); 9192-9200. DOI: 10.1021/ja071430t). This paper presents the measurements on $Zn[SC(NH_2)_2]_4(NO_3)_2$,[1] $Zn[SPh]_4(Me_4N)_2$,[2, 3] $Zn[SPhMe]_4(Me_4N)_2$,[4] $Zn[S_2CN(CH_3)_2]_2$ [5] as well as $[Tm^{Ph}]ZnSPh$.[6] Also discussed in the paper is a survey of methods of *ab initio* molecular orbital calculations predicting the electric field gradients (EFG) for each system. We find that the local density approximation (LDA) functional of a density functional theory (DFT) calculation with a triple-ζ basis set does a good job or predicting the EFG, and therefore Cq, when sufficient pieces (such as counter ions) are included in the calculation. A second paper is in preparation discussing the method of data collection and interpretation for zinc rubredoxin, a structural site with 4 cysteines coordinating the metal. The lineshape was determined to have a sizeable shielding component as well as quadrupole interaction. This was borne out with calculations as the predicted shieldings for the models mentioned above were in line with observations and the increased magnitude is predicted by theory. The calculations utilized a combined Quantum Mechanics and Molecular Mechanics or QM/MM method.[7-10] The follow up paper to this work is also progressing as the NMR data collection has been completed on a series of 4 cysteine proteins with a mix of structural and catalytic sites. We are in the process of several molecular orbital calculations to see if we can predict the trend observed experimentally.

For the hydrolytic proteins, hCA II, LpxC, and AP we have worked out methodoloies and some key initial observations this past year. A series of NMR experiments on LpxC have been performed at multiple pH's and at multiple fields. This data is being analyzed to determine the pH dependence of the lineshape and perhaps the pKa of the water bound to the metal. Preliminary results suggested a pKa of 7.5, which is in the range expected for a histidine that is located next to the active site. We have run a series of NMR experiments on a mutant form of LpxC that does not contain an ionizable residue in this position and are in the process of analyzing that data as well. We have also begun QM/MM calculations on LpxC to model this protein and interpret the NMR results. Similar calculations have begun on hCA II and AP.

**Request for Extension**:
In the coming year, we expect to publish a paper on both the LpxC and AP work. The planned NMR experiments in the coming year involve finishing any AP experiments that are outstanding. We also plan on adding the native substrates to these enzymes to attempt to trap the reaction intermediates as

well as preparing several new Ada protein samples to follow its reaction. We also plan to continue exploring model compounds at room temperature on the 900 MHz NMR (as well as at 10 K on lower fields) to develop new methodologies to measure distances from dipolar interactions, such as Zn-N. Therefore we request the following for instrument time in the coming year;

*Instrumentation:* 20 wks/year 11.7 T (Shasta – 500 MHz WB), 20 wks/year 18.8 T (800 MHz), and 10 wks/year 21.15 T (900 MHz).

*Special needs:* All experiments on the 18.8 T and 11.7 T magnets are to be run at 10 K and require liquid helium.

*Support:* NIH

## References


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