INTRODUCTION TO OUR RESEARCH

WHAT ARE MOLYBDOENZYMES? These enzymes contain the pyranopterin molybdenum cofactor (Moco), and are found in numerous important enzymes that are critical to life processes, planetary ecosystems, and global carbon, nitrogen and sulfur cycles. The canonical pyranopterin Mo enzymes include sulfite oxidase (SO), xanthine oxidase (XO), and dimethyl sulfoxide reductase (DMSO reductase). The names of these three enzymes also represent the names of the three pyranopterin enzyme families. These three families are broadly distinguished from one another by the nature of the reactions they catalyze, their protein fold, and their active site structure. The related pyranopterin tungsten enzymes most closely resemble DMSO reductase family enzymes and, in some cases, the two metals can be interchanged in the active sites. The SO and DMSO reductase family enzymes typically catalyze oxygen atom transfer (OAT) reactions. Here, the oxygen atom being transferred either derives from, or is converted into, a water molecule. The XO family enzymes catalyze a more complicated hydroxylation reaction. The unique nature of this hydroxylation reaction generates, rather than consumes, reducing equivalents and activates H_2O instead of O_2 as the source of the oxygen atom formally inserted into a substrate C-H bond. Importantly, different types of transformations are catalyzed by some of the non-canonical members of these three enzyme families. Although two-electron chemistry dominates in the catalytic cycles of these enzymes, one-electron chemistry has been observed in the generation of NO from nitrite.

Mo and W are the only second and third row transition metal ions that are utilized in biological systems. Pyranopterin Mo enzymes are essential to human health and catalyze important chemical transformations that occur in the metabolic pathways of carbon, nitrogen, and sulfur compounds. A properly synthesized molybdenum cofactor (Moco) is essential in humans, since Moco deficiency results in severe neurological disorders and infant mortality. XO family enzymes catalyze important oxidative transformations of N-heterocycles and aldehydes, and are involved in xenobiotic detoxification, catabolic processes, prodrug activation and conversion, drug metabolism, oxidative stress leading to postischemic reperfusion injury, and NO synthase activity. The enzyme carbon monoxide dehydrogenase (CODH) is a unique XO family enzyme THAT possesses a Mo-Cu active site and catalyzes the oxidation of CO to CO2. Vertebrate SO catalyzes the physiologically important oxidation of sulfite to sulfate, a reaction that represents the terminal step in the oxidative degradation of cysteine and methionine. Isolated sulfite oxidase deficiency derives from specific mutations in the SO gene, and leads to severe neurological abnormalities, dislocation of the ocular lens, and attenuated brain growth. The bacterial methionine sulfoxide reductase is an SO family enzyme that functions to repair oxidized surface methionines that have been damaged by antimicrobials. The DMSO reductase family enzymes are the most structurally and catalytically diverse pyranopterin Mo enzymes. Bacterial DMSO reductase and trimethylamine-N-oxide reductase (TMAO reductase) are of increasing environmental and health importance. TMA/TMAO regulation is of human health importance since TMA can be converted to TMAO by human gut microbiota and TMAO has been implicated in cardiovascular disease, glucose and lipid homeostasis, and reverse cholesterol transport.

WHAT ARE WE DOING? We are filling fundamental gaps in the knowledge base regarding pyranopterin molybdenum enzyme function and mechanism. In these studies, we are use a combination of molecular biology and protein biochemistry, multicomponent spectroscopy,

reaction coordinate computations, and structural probes (XAS and EXAFS). Ongoing projects in the lab currently focus on the final steps of molybdenum cofactor (Moco) biosynthesis, Moco transport, and Moco sulfuration, how the Mo methionine sulfoxide reductase utilizes the pyranopterin dithiolene component (MPT) of the cofactor in catalysis, and the role of non-MPT ligands in the catalytic cycles of dimethylsulfoxide reductase family enzymes. Our long-term goal is to understand geometric and electronic structure contributions to pyranopterin molybdenum enzyme reactivity and function in order to provide a positive impact on the quality of human health. The primary objectives of this research are to determine critical Moco maturation, transport and sulfuration steps, define the mechanism of MsrP and the role of the MPT in Msr mediated catalysis, and understand the electronic structure of key paramagnetic intermediates in DMSOR family enzymes using a combined spectroscopic approach augmented by detailed bonding, spectroscopic, and reaction coordinate calculations. We explore in detail how specific geometric and electronic structure modifications of protein-bound Moco define the unique reactions catalyzed by the enzymes. Our rationale for this research focuses on how the development of a comprehensive understanding of Moco maturation and transport, the complex interplay between Mo the MPT in Msr catalysis, and the nature of paramagnetic DMSOR family intermediates will provide new insights into disease states and have a positive impact on human health. Our current research efforts are significant, and will impact and advance our understanding of molybdate insertion and post-translational sulfuration processes, sulfur and Moco trafficking, molybdoenzyme mediated rescue of oxidatively damaged proteins, and the roles of amino acid and pyranopterin dithiolene ligands in molybdoenzyme catalysis.