SMALL MOLECULE ANALOG STUDIES OF PARAMAGNETIC DMSO REDUCTASE ENZYME FAMILY INTERMEDIATES

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SMALL MOLECULE ANALOG STUDIES OF PARAMAGNETIC
DMSO REDUCTASE ENZYME FAMILY INTERMEDIATES

by

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BSc. Chemistry, Tribhuvan University, Nepal, 2000
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DISSERTATION

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Requirements for the Degree of

Doctor of Philosophy
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DEDICATION

To my late father, Thakur Prasad Khatri who did not wait one more year to see this achievement.
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ABSTRACT
Pyranopterin molybdenum and tungsten enzymes are expressed in numerous organisms from all domains of life. These enzymes catalyze a variety of atom, hydride, and electron transfer reactions. The enzymes are of great importance in the biogeochemical cycles of nitrogen, carbon, and sulfur. In recent years, chemists have investigated applications for some of these enzymes as industrial biocatalysts. The dimethylsulfoxide reductase (DMSOr) enzyme family is the broadest family of pyranopterin Mo enzymes. The first coordination sphere of the Mo or W-containing active site for each member of this family is made up of 4 sulfur donors from the two pyranopterin dithiolenes (PDTs); either a SCys, SeSec or OSer donor from the polypeptide; and for the oxidized form of the enzyme a terminal oxo or sulfido ligand. Although numerous spectroscopic studies have contributed to our understanding of the geometric and electronic structures of reduced (Mo (IV)) and oxidized (Mo (VI)) forms of these enzymes, studies on paramagnetic (Mo
(V)) intermediates of the DMSOr family are limited. Remarkably, there is a dearth of detailed studies on synthetic analogs for EPR active forms of DMSOR enzymes. We have now synthesized and characterized new compounds as models of paramagnetic DMSOr enzyme intermediates. Through interpretation of their XAS, EPR, and electronic absorption spectra, in addition to computational results for these model compounds, we have added new insight into the electronic and geometric structures of the respective enzymes. Understanding their geometric and electronic structures also provides insight into the mechanistic details of the corresponding enzymes.
Contents

Glossary ........................................................................................................................................... xi
List of figures ....................................................................................................................................... xii
List of tables ....................................................................................................................................... xvi
Chapter 1......................................................................................................................................... 1
Molybdenum enzymes ....................................................................................................................... 1
1.1 Introduction to molybdenum enzymes ....................................................................................... 1
1.2 Spectroscopic tools .................................................................................................................... 7
   1.2.1 Electronic absorption spectroscopy ................................................................................... 7
   1.2.3 X-ray absorption spectroscopy (XAS) ............................................................................. 11
1.3 References .................................................................................................................................. 16
Chapter 2......................................................................................................................................... 20
Geometric and electronic structure studies of EPR active models for Fdh and Nap catalytic
intermediates .................................................................................................................................. 20
2.1 Introduction to formate dehydrogenase (Fdh) and periplasmic nitrate
reductase (Nap) enzymes ............................................................................................................... 20
   2.1.1 Fdh and Nap crystal structures ......................................................................................... 21
   2.1.2 EPR spectroscopic studies of Fdh and Nap ................................................................. 22
   2.1.3 Proposed Mechanisms for Fdh and Nap enzymes .......................................................... 24
2.2 Specific challenges associated with the study of paramagnetic enzyme forms
......................................................................................................................................................... 28
2.3 Synthesis of EPR active analogs for Fdh and Nap enzymes ......................... 30
2.4 Characterization of the Fdh and Nap enzyme models ................................. 33
  2.4.1 Mass spectrometry ............................................................................. 33
  2.4.2 Elemental Analysis ........................................................................... 35
  2.4.3 EXAFS ................................................................................................. 35
  2.4.4 EPR spectroscopy of New Fdh and Nap Models ................................. 40
2.5 Magneto-Structural correlations .................................................................. 45
2.6 Optical spectroscopy and electronic structure ............................................ 50
  2.6.1 Electronic absorption spectroscopy ..................................................... 50
  2.6.2 Electronic absorption data analysis ..................................................... 51
2.7 Conclusions ................................................................................................. 57
2.8 References ................................................................................................. 58

Chapter 3 ............................................................................................................ 64
New insights into the geometric and electronic structure of the DMSO reductase
high-g split catalytic intermediate .................................................................. 64
3.1 Introduction to the DMSO reductase (DMSOr) enzyme subfamily .......... 64
  3.1.1. Roles of DMSOr in the global biogeochemical cycles ....................... 64
  3.1.2 Proposed catalytic mechanism of DMSOr ........................................... 65
  3.1.3 DMSOr enzyme structure ................................................................. 66
3.2 Spectroscopic studies on the high-g split DMSOr catalytic intermediate ..... 71
3.2.1. Electronic absorption spectroscopy .......................................................... 71

3.2.2 EPR spectroscopic studies on the DMSO reductase intermediate (I_{Mo(V)}) ........ 72

3.3 Synthesis of new paramagnetic models for the “high-g split” intermediate ... 76

3.3.1 Synthesis of [Mo(V)(cydt)_{2}Cat]^{1-} (1): ................................................... 76

3.3.2 Synthesis of [(Mo(V)(pdt)_{2}Cat)]^{1-} (2): ................................................... 77

3.4. Characterizations of DMSO reductase enzyme models 1 and 2 ............... 78

3.4.1 Mass spectrometry .................................................................................... 79

3.4.2 EPR spectroscopy .................................................................................... 80

3.5. XAS and EXAFS experiments on model compounds 1 and 2 .............. 83

3.5.1. Experimental ......................................................................................... 83

3.5.2 Data observation and analysis ................................................................. 84

3.5.3. Bond valence sum (BVS) analysis ......................................................... 87

3.6 EPR Spectroscopy ....................................................................................... 88

3.6.1 Experimental ......................................................................................... 88

3.6.2 Data collection and analysis ................................................................. 89

3.7 Mechanistic significance of the “high-g split” intermediate .................... 94

3.8 Electronic absorption spectroscopy .............................................................. 98

3.8.1 Experimental ......................................................................................... 98

3.8.2 Electronic absorption spectra and band assignments ......................... 98

3.8.3 Electron density difference maps (EDDMs) .......................................... 100
Spin densities of model compounds .............................................................. 147
Appendix C .................................................................................................. 149
EPR spin-Hamiltonian parameters ................................................................ 149
Appendix D .................................................................................................. 153
EXAFS fitting parameters ............................................................................ 153
Appendix E .................................................................................................. 154
Electronic absorption spectra and EDDMs .................................................... 154
Appendix F .................................................................................................. 160
Relaxed and non-relaxed Mo-O-ser bond scans in DMSO(V) computational
models ........................................................................................................ 160
Appendix G .................................................................................................. 161
S K-edge spectra and associated tables ......................................................... 161
References .................................................................................................. 166

Glossary

PDT = Pyranopterin dithiolene
pdt = 1,2 diphenyl-ethenedithiolate
cydt = 1,2 cyclohexenedithiolate
XO = Xanthine oxidase
SO = Sulfite oxidase
DMSO\textsubscript{r} = Dimethyl sulfoxide reductase

AO = Aldehyde oxidase

AOR = Aldehyde oxidoreductase

CODHs = Carbon monoxide dehydrogenase

Nar = Respiratory nitrate reductase

TMAO\textsubscript{r} = Trimethyl-N-O reductase

MsrP = Methionine sulfoxide reductase protein

EPR = Electron para magnetic resonance

MCD = Magnetic circular dichroism

SORCI = Spectroscopic oriented configurational interactional

XAS = X-ray absorption spectroscopy

XANES = X-ray absorption near edge structure

EXAFS = Extended X-ray absorption fine structure

SSRL = Stanford Synchrotron Radiation Light Source

**List of figures**

Figure 1. 1: PDT structures ................................................................. 1

Figure 1. 2: XO active site ................................................................. 3

Figure 1. 3: SO active site ................................................................. 3

Figure 1. 4: DMSO\textsubscript{r} active site .............................................. 4

Figure 1. 5: W-Fdh active site ........................................................... 5

Figure 1. 6: Nitrogenase active site .................................................... 6

Figure 1. 7: EPR absorption ............................................................. 9
Figure 1. 8: BL 4-3 SSRL instrumentation (schematic diagram).......................... 12
Figure 1. 9. Examples of XAS spectra................................................................. 15

Figure 2. 1: Active sites of Fdh and Nap enzymes................................................. 20
Figure 2. 2: Crystal structures of oxidized Fdh and Nap ....................................... 22
Figure 2. 3: Proposed structures for Mo(V) very high-g and high-g Fdh and Nap enzymes ........................................................................................................................ 23
Figure 2. 4: Fdh mechanism 1; direct binding of substrate to Mo .......................... 24
Figure 2. 5: Fdh mechanism 2; protein derived Cys/Sec detaching for incoming substrate................................................................................................................................. 25
Figure 2. 6: The S-shift Fdh mechanism 3.............................................................. 25
Figure 2. 7: Fdh mechanism 4; H⁻ binding to metal ............................................... 26
Figure 2. 8: Fdh mechanism 5; The H⁻ transfer mechanism .................................... 26
Figure 2. 9: Nap inner-sphere and outer sphere mechanisms.................................. 27
Figure 2. 10: Formation of EPR active Fdh and Nap enzyme active sites............. 29
Figure 2. 11: Mass spectra (with Mo isotope patterns) of Fdh and Nap model complexes .......................................................................................................................... 34
Figure 2. 12: EXAFS.............................................................................................. 36
Figure 2. 13: XANES ........................................................................................... 39
Figure 2. 14: X-band EPR spectra for complexes.................................................. 42
Figure 2. 15: Orientation of the g-tensor and the A-tensors.................................... 44
Figure 2. 16: g-anisotropy (g₁-g₃) correlations with g-average for enzymes XO, SO and DMSOr................................................................. 45
Figure 2. 17: $g_{\text{aniso}}$ vs $g$ values plot for very high-g and high-g Fdh and Nap enzymes and model complexes ................................................................. 47
Figure 2. 18: Computational models of Fdh and Nap enzymes ......................... 48
Figure 2. 19: Electronic absorption spectra of complexes .................................. 51
Figure 2. 20: Electronic absorption with TDDFT of models ............................... 52
Figure 2. 21: Inverted bonding schemes for complexes ..................................... 53
Figure 2. 22: Spin-unrestricted representations of the orbitals involved in charge
transfer bands .................................................................................................. 53
Figure 2. 23: MO diagram of complex 1 ............................................................. 55
Figure 2. 24: MO diagram of complex 2 ............................................................. 56
Figure 2. 25: MO diagram of complex 3 ............................................................. 56

Figure 3. 1: DMSOr crystal structure-1EU1 ...................................................... 67
Figure 3. 2: Proposed structures of DMSOr from EXAFS study by George ..... 68
Figure 3. 3: Proposed DMSOr structures from EXAFS study by Baugh ......... 69
Figure 3. 4: UV-Vis with MCD and molecular orbital diagram (right) for DMSOr. 72
Figure 3. 5: Low temperature X-band EPR spectrum for DMSOr(V) .......... 73
Figure 3. 6: $g$ values correlations with $g_{\text{aniso}}(g_{1}-g_{3})$. Nar, DMSOr, 1-TMAO .. 74
Figure 3. 7: Proposed computational model from Leimkuhler’s TMAO enzyme. 75
Figure 3. 8: Model framework for the DMSOr catalytic intermediate, lMo(V) ..... 75
Figure 3. 9: Mass spectra of complexes .......................................................... 79
Figure 3.10: Optimized structures of synthetic and computational models .... 81
Figure 3. 11: Mo-Kedge XAS spectra of complexes ......................................... 83
Figure 3.12: EXAFS (Fourier transformed) six- vs five-coordinate fits. .......... 86
Figure 3.13: Δ-twist (left) and λ-twist (right) models 2 and 1, respectively. .... 89
Figure 3.14: Orientations of A-g tensors along with respective β-LUMO.......... 90
Figure 3.15: EPR spectra for complexes. .................................................. 91
Figure 3.16: Possible mechanistic pathways............................................. 95
Figure 3.17: β-LUMO of 2 (left) and model [Mo(V)(pyran-dt)2(Oser)(OH)]+ .... 96
Figure 3.18: 6-coordinate (a) and 5-coordinate DMSO high-g split model. .... 97
Figure 3.19: Electronic absorption spectra for the DMSO high-g split
intermediate and model complexes ......................................................... 98
Figure 3.20: Spin-unrestricted representations of the orbitals involed in charge
transfer bands in complex 1........................................................................ 99
Figure 3.21: Spin-unrestricted representations of the orbitals involed in charge
transfer bands 2 in complex 2................................................................. 100
Figure 3.22: EDDMs for electronic transitions . ......................................... 101
Figure 3.23: Spin-unrestricted DFT computed valence molecular orbitals (MOs)
.................................................................................................................. 102

Figure 4.1: Oxidized active site structure of Fdh (left), Nap (middle) and DMSO
.................................................................................................................. 112
Figure 4.2: Schematic diagram of BL-4-3 at SSRL..................................... 116
Figure 4.3: XANES spectra for model complexes........................................ 118
Figure 4.4: Experimental and TDDFT XANES spectra of complexes........... 119
Figure 4. 5: Demonstrating the DFT computed energy gap between upper lying LUMOs and low lying LUMOs for complex 1 and 2 ................................................. 121

Figure 4. 6: Pre-edge energy in the first derivative of S Kedge spectra for complexes ................................................................. 122

Figure 4. 7: Twist angles in the complexes.................................................. 123

Figure 4. 8: The first derivative of the rising edge........................................ 124

Figure 4. 9: Optimized structures (truncated) of complexes ....................... 125

List of tables

Table 2. 1: Fdh and Nap enzymes g-values ................................................. 23

Table 2. 2: EXAFS fitting results .................................................................. 37

Table 2. 3: EPR spin Hamiltonians parameters for complexes ................... 43

Table 3. 1: EPR spin-Hamiltonian parameters of 2 and computational models.. 82

Table 3. 2: Bond lengths and BVS for 2 and computational models............ 82

Table 3. 3: EXAFS fitting (R-space) parameters for 1 ................................. 85

Table 3. 4: EXAFS fitting (R-space) parameters for 2 ................................. 85

Table 3. 5: EPR spin-Hamiltonian parameters of complexes 1 and 2 .......... 90

Table 3. 6: EPR spin-Hamiltonian parameters for 6- and 5-coordinate DMSOr enzyme model .................................................................. 97

Table 4. 1: Mo-S covalency (%S in 3p) and energy of peaks under pre edges for DMSOr model complexes.................................................. 120
Table 4. 2: Twist angles in degree of the complexes........................................ 123

Table 4. 3: Bond lengths (Mo-S and Mo-O) in the complexes .................... 126
Chapter 1
Molybdenum enzymes

1.1 Introduction to molybdenum enzymes

Molybdenum and Tungsten are rare, heavy, non-toxic elements. More than 50 enzymes containing Mo and W have been discovered. Molybdenum and tungsten containing enzymes are found in most organisms, including both prokaryotes and eukaryotes. In addition to their roles in biogeochemical C, N, and S cycles, among others, the enzymes have important physiological roles in the organisms in which they are expressed. A properly synthesized molybdenum cofactor (Moco) is essential for function, and in humans the deficiency of Moco results several neurological disorders and infant mortality. Sulfite oxidase (SO) and xanthine oxidase (XO) family enzymes are important for various xenobiotic detoxifications. The bacterial DMSO family enzyme triethylamine-N-oxide (TMAO) reductase found in human gut microbiota converts TMAO (a cause of cardiovascular disease) into trimethylamine (TMA).

Figure 1.1: PDT structures (‘a’ is the pyran ring, ‘b’ is the piperazine and ‘c’ is the pyrimidine ring); (i) tetrahydro PDT and (ii) 10-10a dihydro PDT.
All molybdenum enzymes have one or two coordinated pyranopterin dithiolene (PDTs) ligands, as shown in Figure 1.1, with the sole exception of nitrogenase, which does not possess a PDT. The structure of the PDT shows three distinct rings that are fused together. The dithiolene chelate is connected to the pyran ring, which is fused to a piperazine ring that is connected to the pyrimidine. The fused piperazine and pyrimidine rings comprise a pterin ring system. From an analysis of PDT distortions, two different kinds of PDT structures have been identified. The more distorted PDT is possesses a structure similar to tetrahydro PDTs, while the other less distorted PDT is structurally similar to a 10-10a dihydro PDT. However, there remains no direct evidence for an oxidized PDT in an active enzyme. One role for the PDT in the catalytic cycle of the enzymes is to tune the redox potential of the Mo ion. Most of the pyranopterin Mo and W enzymes possess an ancillary donor ligand that derives from the polypeptide. Additional ligands coordinated to the Mo ion may include terminal oxo (hydroxide, aqua) and sulfido (sulfhydryl) donors. The role of the protein derived ligand in catalysis is not completely understood at present but is likely to fine-tune the redox potential during the course of catalysis. Based on the Mo/W active site coordination geometry, Mo and W enzymes have been grouped into five different families: (i) Xanthine oxidase (XO), (ii) Sulfite oxidase (SO), (iii) Dimethyl sulfoxide reductase (DMSOr), (iv) Tungsten enzymes and (v) Nitrogenase.

(I) **Xanthine oxidase (XO) family enzymes.** Oxidized XO family enzymes have a square pyramidal coordination geometry with an apical oxo donor, an equatorial dithiolene chelate, a terminal sulfido, and a hydroxide donor (Figure
Xanthine oxidase/dehydrogenase is the canonical member of this enzyme family. Other XO family enzymes include aldehyde oxidase (AO), aldehyde oxidoreductase (AOR), xanthine oxidoreductase (XOR), and carbon monoxide dehydrogenase (CODH). The basic function of these enzymes is to activate substrate C-H bonds in hydroxylation transformations (Scheme 1.1), with CODH functioning to convert CO to CO₂.

Scheme 1.1: \[ \text{E-Mo}^{VI} + \text{R-H} + \text{H}_2\text{O} \rightarrow \text{E-Mo}^{IV} + \text{R-OH} + 2\text{H}^+ + 2\text{e}^- \]

(II) **Sulfite oxidase (SO) family enzymes.** SO family enzymes are found in both prokaryotic and eukaryotic organisms and, in their oxidized states, possess a square pyramidal coordination geometry with an apical oxo, a dithiolene chelate deriving from the PDT, a cysteine thiolate, and an equatorial oxo ligand (Figure 1.3). In reduced enzyme forms, the equatorial oxo exists as a protonated hydroxido or aqua ligand. Sulfite oxidase functions to convert toxic sulfite \( (\text{SO}_3^{2-}) \)

Scheme 1.2: \[ \text{E-Mo}^{VI} + \text{SO}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{E-Mo}^{IV} + \text{SO}_4^{2-} + 2\text{H}^+ \]
to sulfate, as indicated in Scheme 1.2. In addition to the sulfite oxidizing enzymes, this family possess the eukaryotic nitrate reductases, which reduces nitrate to nitrite, the moonlighting enzyme mARC, and molybdenum dependent methionine sulfoxide reductase (MsrP, previously called YedY) which catalyzes the reduction of oxidized methionine.

(III) *Dimethyl sulfoxide reductase (DMSOr) family enzymes.* DMSOr is the broadest family of mononuclear molybdenum enzymes. Two PDTs are bound to the Mo ion in this enzyme family, and in most of the enzymes either a terminal oxo or terminal sulfido is found bound to Mo in the oxidized state. The sixth ligand derives from serine, aspartate, cysteine, or selenocysteine residues, or from solvent water.12 13, 14 The dimethyl sulfoxide reductase enzyme is the eponymous member of this family, and functions to reduce dimethyl sulfoxide (DMSO) to dimethyl sulfide (DMS), as shown in Scheme 1.4. DMS is an important contributor to the Earth’s albedo.15 The DMSOr enzyme family can be further divided into three subfamilies based on the identity of the coordinated protein residue (Figure 1.4) and the reaction they catalyze. Thus, the Type-I enzymes possess a coordinated cysteine or selenocysteine and include periplasmic nitrate reductase (Nap) and

![DMSO active site: oxidized (left) and reduced (right)](image)

**Figure 1.4:** DMSOr active site: oxidized (left) and reduced (right)

Scheme 1.4: \( E-\text{Mo}^{IV} + (\text{CH}_3)_2\text{SO} \rightarrow E-\text{Mo}^{VI}=\text{O} + (\text{CH}_3)_2\text{S} \)
formate dehydrogenase (Fdh). Type-II enzymes possess a coordinated aspartate and include the hydroxylating enzyme ethyl benzene dehydrogenase (EBDH) and the respiratory nitrate reductases (Nar). In addition to the coordinated aspartate, EBDH is also ligated by a water derived ligand (i.e., aqua, hydroxido, oxido). The non-PDT ligands found in Nar derive from aspartate and H₂O. Nar functions to reduce nitrate to nitrite whereas EBDH hydroxylates benzylic C-H bonds.

Finally, the Type-III enzymes are coordinated by a serinate oxygen and include the aforementioned dimethyl sulfoxide reductase (DMSOr) and trimethyl amine N-oxide reductase (TMAOr). We will discuss the Type-I enzymes in Chapter 2, and the Type-III enzymes will be discussed in chapter 3.

(IV) Pyranopterin tungsten enzymes. Compared to the molybdenum-containing enzymes, the tungsten enzymes are limited to two families: formate dehydrogenase and aldehyde ferredoxin reductase. The W-Fdh is very similar to Mo-Fdh in terms of structure and function. W-Fdh also contains four S derived donors from two PDTs, a Scys or SeSec donor, and a coordinated sulfido/sulphydryl coordinating to ‘W’ as shown in Figure 1.5. W-Fdh oxidizes formate to CO₂. The reverse reaction i.e. reduction of CO₂ to formate by W-Fdh is kinetically faster than for Mo-Fdh. The W-ferrodoxin oxidoreductase is found in hyperthermophilic
archaea and plays functions as an oxo transfer catalyst. The reduced form of the enzyme has distorted square pyramidal structure.  

$(V)$ Nitrogenase. Nitrogenase is a complex enzyme that contains a unique MoFe$\text{S}_9\text{C}$ cluster (M-cluster or FeMoco) with a novel interstitial carbon in the core as illustrated in Figure 1.6. 

![Figure 1.6: Nitrogenase active site](image)

Nitrogenase is different than the oxotransferase pyranopterin molybdenum enzymes. It reduces atmospheric $\text{N}_2$ to bioavailable $\text{NH}_3$ as shown in Scheme 1.5. Thus, it functions to fix nitrogen on the planet. The presence of the interstitial carbon was unclear in the first crystal structure of nitrogenase as determined by Einsle and coworkers. Interestingly, the oxidation state of the Mo in the nitrogenase has been assigned as being +3, in contrast to the pyranopterin molybdenum enzymes where it occurs in either the +4, +5, or +6 states.

\[
\text{Scheme 1.5: } \text{N}_2 + 8\text{e}^- + 8\text{H}^+ \rightarrow 2\text{NH}_3 + \text{H}_2
\]
1.2 Spectroscopic tools

Three important spectroscopic tools have been employed in my research. These tools are electronic absorption, electron paramagnetic resonance, and X-ray absorption spectroscopies, and they are briefly discussed in the following sections.

1.2.1 Electronic absorption spectroscopy

In electronic absorption spectroscopy, UV-Vis-NIR light is passed through a sample. Some of the light is absorbed by the sample and some of it is transmitted. The percentage of transmitted light (T) is measured and given as the negative log of T. The transmitted light depends on the path length and concentration of the absorbing sample. The transmitted light, T, exponentially decreases as a function of the path length and concentration of the sample. So, 

\[ T = 10^{-\varepsilon lc} \]

where \( \varepsilon \) is the molar absorption coefficient, a constant. The absorbance,

\[ \text{ABS} = -\log T = \varepsilon lc \]

The molar absorption coefficient (\( \varepsilon \)) is a characteristic property of a molecule. The peak energy position and the molar absorption coefficient (\( \varepsilon \)) of molecular transitions depend on the symmetry and electronic structure of the molecule. The magnitude of ABS depends on the strength of electric dipole integral for the transition, and dipole selection rules correlate with the triple product representation of the dipole integral, \( <\psi_e|\mu_d|\psi_g> \), where \( \psi_g \) is the ground state wave function, \( \psi_e \) is the excited state wave functions, and \( \mu_d \) is the transition dipole operator. If the triple product of the transition dipole integral does not transform as the totally symmetric representation of the molecular point group,
then the transition is not dipole allowed.\textsuperscript{23} Electronic transitions in transition metal complexes can be categorized based on the nature of the donor and acceptor orbitals, and these include ligand to metal charge transfer (LMCT), metal to ligand charge transfer (LMCT), metal to metal charge transfer (MMCT) and ligand to ligand charge transfer (LLCT) transitions. LMCT and MLCT transitions are more commonly occurring transitions in transition metal complexes. For high symmetry molecules (e.g. D\textsubscript{4h} or O\textsubscript{h}), which inversion symmetry, g \rightarrow g or u \rightarrow u transitions are dipole-forbidden (Laporte's rule),\textsuperscript{23} and are said to be Laporte-forbidden. In the low-symmetry Fdh, Nap and DMSOr model complexes, the most intense transitions are LMCT in nature, and the details will be discussed in successive chapters. There are also d-d transitions, which are also known as ligand field transitions, that most often occur with very low intensity when compared to charge transfer transitions. Electronic absorption spectroscopy can reveal a considerable degree of information regarding the nature of the active site geometric and electronic structure for molybdoenzymes, and we will discuss this in greater detail in the following chapters.

1.2.2 Electron paramagnetic resonance (EPR) spectroscopy

In EPR, the absorption of a microwave photon allows for transitions from one energy state (e.g. m\textsubscript{s} = -1/2) to another (e.g. m\textsubscript{s} = +1/2) as illustrated in Figure 1.7. Before applying the magnetic field, B, the energy of two electron spins (m\textsubscript{s} states) are the same. In the cw-EPR experiment, we scan B at a constant incident microwave energy (hv) and once energy gap between two electron spin states
matches the microwave energy (hv) the EPR transition occurs. This occurs at what is known as the resonance frequency (energy). Thus, at resonance energy difference between the two ms spin states is equivalent to the applied microwave energy. This resonance condition is given by equation 1.1, and the selection rules

\[ B = \frac{hv}{\beta g} = \frac{0.07145 \nu (GHz)}{g} \quad 1.1 \]

are \( \Delta m_s = \pm 1/2 \). The proportionality constant, or g-factor, provides considerable information about the geometric and electronic structure of the metalloenzyme or model complex. The g-tensor is represented as a 3X3 matrix. Another important spin-Hamiltonian parameter is the hyperfine term (\( A^n \)) as shown in equation 1.2.

The hyperfine term arises from the interaction between spin magnetic moments and nuclear magnetic moments. The hyperfine term has three contributions: (1) the Fermi contact term, which depends on s-orbital character in the singly occupied molecular orbital (SOMO); (2) the traceless anisotropic spin dipolar term, which depends on the nature and orientation of the SOMO, and (3) an indirect dipolar contribution that is proportional to the g-tensor anisotropy. Like the g-tensor, the hyperfine term is also a tensor represented by a 3X3 matrix. We

![Figure 1. 7: EPR absorption.](image-url)
can obtain both qualitative and quantitative information about the metal-ligand bonding scheme from an analysis of EPR spectra that yields the spin-Hamiltonian parameters \( g_i \) and \( A_i \). Additionally, we also obtain information about the geometric structure and the nature of the SOMO wavefunction. The magnitude and relative orientations of the \( g \)- and \( A \)-tensor components depend on the degree of metal-ligand covalency, the energy of charge transfer states, and the metal and ligand spin-orbit coupling constants.\(^{26-29}\) The relative orientation of the \( g \)- and \( A \)-tensors results from the cumulative effect of configuration interaction, excited state mixing into the ground state wave function, and ligand spin-orbit coupling constants.\(^{27, 28, 30}\)

In the absence of a single crystal EPR study, it can be difficult to quantitatively determine the magnitude and the orientations of the individual tensor components and the diagonal components of the \( g \)- and \( A \)-tensors. However, the determination of the relative orientation of the \( A \) and \( g \)-tensors from spectral simulations of high resolution EPR spectra yield considerable information regarding to geometric and electronic structure of the molecule or enzyme active site.\(^{30}\)

In the research, all EPR data of the model complexes were collected using a Bruker EMX X-band EPR spectrometer. The resonance filed was typically \(~3375\text{G}\) for an \(~9.4\text{GHz}\) microwave frequency. Most of the RT and 77K EPR spectra were collected at 30dB attenuation and using 100 kHz field modulation. The amplitude modulation was adjusted from 2 to 6 G. Low temperature EPR data were simulated in MATLAB using the ‘pepper’ function found in the EasySpin program.\(^{31}\) The ‘garlic’ function was used to simulate the room temperature data.
The relative orientation of the A- and g-tensor principle components have been plotted using a combination of VMD, ORCA, and MATLAB programs.

1.2.3 X-ray absorption spectroscopy (XAS)

In X-ray absorption spectroscopy, very high-energy synchrotron light is generated from accelerated electrons produced from the electron gun, which works based on thermionic principles.\textsuperscript{32} In this process, once a metal is heated, electrons are ejected and enter a linear accelerator (LINAC), where the electron velocity is increased to as much as 99% of the velocity of light in a high voltage field. The LINAC at the Stanford Synchrotron Radiation, remarkably, is two miles long and passes beneath US Interstate 280. The electron stream is accelerated in the LINAC is then allowed circulate around a booster ring that consists of bending magnets. The energy of the electrons is further increased in the high magnetic field of the booster ring. The synchrotron light thus produced goes to a storage ring consisting of clusters of opposing magnetic poles arranged vertically in two layers. These are wigglers or undulators. At SSRL, all beam line light passes through the wigglers. The basic principle in both wigglers and undulators is same; when light goes to the different magnetic poles, it oscillates and propagate in one direction. The energy of the synchrotron light can be tuned by changing the number of poles or by changing the space between the magnetic layers, similar to EPR Q-band and X-band wave guides. In EPR, the microwave radiation going through a narrow wave guide (e.g. at Q band) has a higher energy, $\sim35$ GHz, than at X-band $\sim9$ GHz. SSRL beam lines 4-3 (BL 4-3) and beam line 7-3 (BL 7-3) were used for
our XAS experiments. Both beamlines have 20 magnetic poles at 2T magnetic field. Energy at BL 4-3 is 2-5 keV and for that for BL7-3 is 5-33 keV. Inside the hutch (radiation shielding enclosures), a liquid N₂-cooled, Si double-beam monochromator (shown in Figure 1.8) is used for energy selection and is calibrated using Mo-foil (K-edge energy ~20000eV). The temperature is maintained near ~11K using a liquid He cryostat. Model compound data were collected in fluorescence mode using a Lytle detector.

Figure 1. 8: BL 4-3 SSRL instrumentation (schematic diagram)

In the XAS experiment, when the synchrotron light interacts with sample the intensity of transmitted light (Iₜ) is given by the Beer-Lambert law, Iₜ = I₀ e⁻¹μ(E)ₓ, where I₀ is intensity of the incident light and x is the thickness of the sample, which has unit length. The absorption coefficient is a function of the energy (μ(E)), and in absorption mode it is given by equation 1.3. The absorption coefficient in fluorescence mode is given by equation 1.4. In XAS spectra there are two main

\[ \ln \frac{I₀}{Iₜ} = \mu(E) \]  

\[ \frac{Iₜ}{I₀} = \mu(E) \]
energy regions: the X-ray absorption near edge structure (XANES) region, and the extended X-ray absorption fine structure (EXAFS) region. The XANES region is comprised of the near edge and rising edge. The near edge peak results from quadrupole allowed transitions when the transition is from the K-shell (1s orbital) to (n)d orbitals. The pre-edge intensity may be increased when the symmetry of the site is such that metal d-orbitals can be mixed with metal p-orbitals. From the pre-edge peak, we can obtain information regarding the number of ligands bound to the metal, and its geometric and electronic structure. The rising edge is electric dipole allowed and intense, revealing the amount of energy required to excite a core electron into virtual orbitals (e.g. 1s → np), and typically shifts to higher energy and the oxidation state of the excited atom increases. Thus, the XANES region gives us the information about the effective nuclear charge on the metal, and the geometric and electronic structure of the site. The highest peak after the rising edge is called the white line. At energies greater than the white line there are oscillations of the photo ejected photoelectrons within the XANES region. EXAFS region starts at ~36 eV above the threshold energy. The oscillations observed in the EXAFS region (up to 1000eV) arise from the constructive and destructive interferences of the ongoing photoelectrons and backscattering photoelectrons from atomic scatterers near the excited atom. The EXAFS function $\chi(E)$ is given by equation 1.5. EXAFS oscillations result from the wave behavior of photoejected electrons in the absorption process. The oscillation function is expressed in terms of a wave vector $k$, $\chi(k)$. the wave vector $(k)$ is related to the energy as described

$$
\chi(E) = \frac{\mu(E) - \mu_0(E)}{\Delta\mu_0(E)}
$$

1.5
in the equation 1.6. Also, \( \chi(k) \) is the fractional modulation of the X-ray absorption coefficient as shown in equation 1.7. The EXAFS region of the X-ray absorption spectrum gives information about the number, type, and distance of ligand atoms from the absorbers in addition to bond length variations. Details regarding our use of EXAFS are described in the following chapters.

The Athena program can be used to plot XAS data as the absorption coefficient vs the incident energy. Since the higher energy radiation is more penetrating, the absorption intensity decreases as the energy increases. The EXAFS region contains oscillations due to constructive and destructive scattering processes. The x-axis in the post-XANES region is typically expressed in units of \( k \), the wave vector, as shown in Figure 1.9. The relationship between \( k \) and energy is given by equation 1.8.

\[
\chi(k) = \frac{\mu(E) - \mu_0(E)}{\Delta \mu_0(E)} \quad 1.7
\]

\[
k = 0.512 \sqrt{E - E_0} \quad 1.8
\]
Figure 1. 9. Examples of XAS spectra. The full XAS spectrum showing (a) the XANES and EXAFS regions, (b) the expanded XANES region, and (c) and EXAFS region in k-space (c).
1.3 References


31. EasySpin 5.2.28.


2.1 Introduction to formate dehydrogenase (Fdh) and periplasmic nitrate reductase (Nap) enzymes

Dissimilatory periplasmic nitrate reductase (Nap) and formate dehydrogenase (Fdh) are Class-I DMSOr family enzymes found in archaea and bacteria. In contrast, the sulfite oxidase (SO) family assimilatory nitrate reductases are found in all kinds of organisms.\textsuperscript{1,2} Like many other pyranopterin Mo enzymes, Nap catalyzes oxygen atom transfer (OAT) reactions. However, Fdh catalyzes hydride (H\textsuperscript{−}) transfer reactions that are distinct from the formal hydride transfer step in the catalytic cycles of xanthine oxidase and aldehyde oxidase. Both Nap and Fdh enzymes are important enzymes in the metabolic pathways of nitrogen and carbon, respectively. Fdh and Nap contain two pyranopterin dithiolene (PDT) ligands bound to the Mo ion as shown in Figure 2.1. A \text{S\text{Cys}} is also coordinated to the metal, which may be a \text{Se\text{Sec}} in certain Fdh enzymes. Recent X-ray
crystallographic and EXAFS studies of oxidized Class-I enzymes reveal the presence of an exogenous ligand as sulfur donor.\textsuperscript{3-6} The roles of the protein derived (X) and exogenous (Y) ligands are believed to tune redox properties, reactivity, and substrate specificity. It has been hypothesized that one of the PDTs functions as an electron transfer conduit in the catalytic cycle.\textsuperscript{7-9} Although structurally similar, the Fdh and Nap enzymes differ with respect to the reactions they catalyze, as shown in Scheme 2.1.

\begin{align*}
\text{NO}_3^- \text{ reduction} & \quad \text{E-Mo}^{\text{IV}} + \text{NO}_3^- + 2\text{H}^+ \rightarrow \text{E-Mo}^{\text{VI}} + \text{NO}_2^- + \text{H}_2\text{O} \\
\text{HCOO}^- \text{ oxidation} & \quad \text{E-Mo}^{\text{VI}} + \text{HCOO}^- \rightleftharpoons \text{E-Mo}^{\text{IV}} + \text{CO}_2 + \text{H}^+ + 2\text{e}^- 
\end{align*}

The H\textsuperscript{+} transfer reactions catalyzed by Fdh enzymes are reversible providing the potential for the enzyme to catalyze the reduction of CO\textsubscript{2} to formate, which can be used as biofuel.\textsuperscript{10} Thus, there is interest in developing Fdh as a biocatalyst to exploit the reversibility of this reaction.\textsuperscript{11} It has been shown that reduction of CO\textsubscript{2} by W-Fdh is more efficient than by Mo-Fdh,\textsuperscript{11} and this is likely a function of the different redox potentials for Mo vs. W sites. Our work is discussed in the context of prior results from X-ray crystallography, EPR spectroscopy, and computational probes of proposed catalytic mechanisms.

\subsection*{2.1.1 Fdh and Nap crystal structures}

The Fdh and Nap have very similar active site structures despite their different functions. For both enzymes, there are four sulfur donors from the two PDT ligands that are coordinated to the metal center. The Se\textsubscript{Sec} found in Fdh enzymes\textsuperscript{12} or S\textsubscript{Cys} in other Fdh enzymes and in Nap\textsuperscript{13} is the protein derived ligand.
(X) shown in Figure 2.2. A fundamental structural difference between Fdh and Nap enzymes is the nature of the protein residue that resides above the active site. In Fdh this is a histidine, and Nap this residue is a methionine. From earlier crystal structure studies on these enzymes by Boyington et al., and Dias et al., the ‘Y’ ligand present in the oxidized enzyme form has been suggested to be a terminal oxo. However, later crystal structure studies on W-Fdh, Mo-Nap, and an EXAFS study on Mo-Fdh by George, have suggested that the exogenous ligand ‘Y’ in oxidized Nap is a sulfido that possesses partial persulfide character through its interaction with the coordinated cysteine residue.

![Crystal structures of oxidized Fdh-1fdo and Nap-3ml enzymes](image)

Figure 2.2: Crystal structures of oxidized Fdh-1fdo (left) and Nap-3ml (right) enzymes

### 2.1.2 EPR spectroscopic studies of Fdh and Nap

EPR studies on Fdh and Nap enzymes indicate the presence of a flexible coordination geometry. Depending on the nature of the reaction and the substrate used, EPR active species have been labeled as very high-g and high-g types, and this is detailed in Figure 2.3. The EPR spin-Hamiltonian g-values of some Nap and Fdh enzymes are shown in Table 2.1. Interested readers are encouraged to read
the prior literature\textsuperscript{1, 5, 14-23} for details regarding the EPR spectroscopy of these enzymes. Based on the g-values shown in Table 2.1, Guigliarelli and coworkers have correlated the EPR g-values $g_1$, $g_2$, and $g_3$, and the g-anisotropy ($g_1 - g_3$) to postulated structures for these enzymes.\textsuperscript{24, 25} The very high-g type spectra for Nap/Fdh enzymes are obtained by reducing the oxidized form with inhibitors or by oxidizing the reduced form in air. The high-g type spectra for Nap/Fdh enzymes are obtained under turnover conditions with added substrate. Further analysis of

Table 2. 1: Fdh and Nap enzymes g-values

<table>
<thead>
<tr>
<th>Enzyme / species</th>
<th>$g_1$</th>
<th>$g_2$</th>
<th>$g_3$</th>
<th>$g_1-g_3$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Very high-g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Nap-as / A. vinelandii</td>
<td>2.023</td>
<td>1.998</td>
<td>1.993</td>
<td>0.030</td>
<td>Ganeswaran (1993)</td>
</tr>
<tr>
<td>3. Fdh / M. formicicum</td>
<td>2.020</td>
<td>2.006</td>
<td>1.997</td>
<td>0.023</td>
<td>Barber (1986)</td>
</tr>
<tr>
<td><strong>High-g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Nap-as / A. vinelandii</td>
<td>1.998</td>
<td>1.999</td>
<td>1.981</td>
<td>0.017</td>
<td>Ganeswaran (1993)</td>
</tr>
<tr>
<td>10. Nap / P. pantotropha</td>
<td>1.999</td>
<td>1.991</td>
<td>1.981</td>
<td>0.018</td>
<td>Bennett (1994)</td>
</tr>
<tr>
<td>15. Fdh / D. desulfuricans</td>
<td>2.012</td>
<td>1.996</td>
<td>1.985</td>
<td>0.027</td>
<td>Riva (2007)</td>
</tr>
</tbody>
</table>
the proposed structures for very high-g and high-g type Nap/Fdh enzymes have been performed using both experimental and computational EPR active models and this work is detailed in the following sections.

2.1.3 Proposed Mechanisms for Fdh and Nap enzymes

Despite the similar structures of Fdh and Nap, these enzymes catalyze markedly different reactions. Additionally, the catalytic cycle starts with oxidized enzyme for Fdh and reduced enzyme for Nap. At least five different catalytic mechanisms have been proposed for Fdh based on a combination of data from crystal structures, EPR spectroscopy, and computational studies. The proposed reaction mechanisms \(^{26, 27}\) of these enzymes are detailed below.

Mechanism 1 (Figure 2.4) is based on the crystal structure\(^ {12}\) of Fdh-F from \textit{E. coli}. The oxidized form of this enzyme is observed to be distorted trigonal prismatic with four sulfur donors from the two chelating PDTs, one Se\textit{Sec}, and one terminal oxo ligand coordinated to the Mo ion. The reduced form of the enzyme has a \textit{des}-oxo square pyramidal structure. In the catalytic cycle, the oxo group is lost to solvent by becoming protonated to form labile water. The loss of this ligand provides an empty coordination site for the incoming substrate (formate), which binds to the metal prior to being oxidized to CO\textsubscript{2}. Substrate oxidation leads to the

Figure 2. 4: Fdh mechanism 1; direct binding of substrate to Mo
two-electron reduction of the metal to the Mo (IV) form. Interestingly, a crystal structure reinterpretation by Raaijmakers and Romao suggested that the Se$_{\text{Sec}}$ was detached and located 12 Å away from the Mo center in the reduced form of the enzyme. Thus, ligand dissociation results in an open coordination site that can accommodate the binding of the incoming substrate, formate, as shown in mechanism 2 (Figure 2.5). It has been suggested that nitrate reduction catalyzed by Fdh proceeds when Se$_{\text{Sec}}$ is detached from the metal center under turnover conditions. However, both EXAFS$^6$, $^{30}$ and X-ray crystallographic data$^4$ for these enzymes suggest that the sixth ligand is a sulfur donor, with partial “side-on” persulfide (S/Se-S) character. In the catalytic cycle, the ligand shift leads to “end-on” coordination as shown in mechanism 3 (Figure 2.6). This provides an empty coordination position for the incoming substrate. The details of mechanism 3 have been supported by a computational study.$^{31-33}$ Tiberti et al. conducted a theoretical

Figure 2. 5: Fdh mechanism 2; protein derived Cys/Sec detaching for incoming substrate$^{28}$

Figure 2. 6: The S-shift Fdh mechanism 3$^4$
study and suggested that Se\textsubscript{Sec} detaches from the metal, and then hydride is cleaved from formate and then binds the Mo-sulfido in the reduced form as illustrated in Figure 2.7.\textsuperscript{34}

![Figure 2. 7: Fdh mechanism 4; H\textsuperscript{+} binding to metal\textsuperscript{34}](image)

However, an EPR study using \textsuperscript{77}Se labeling on \textit{E. coli} FdhH by Khangulov and coworkers\textsuperscript{35} suggests that Se\textsubscript{(Sec)} is still attached to the Mo center. The computational study by Dong\textsuperscript{27} and experimental studies by Maia and Hille\textsuperscript{10, 36} show that the catalytic process occurs through a hydride (H\textsuperscript{-}) transfer as illustrated in mechanism 5 (Figure 2.8). In this mechanism the hydride transfer occurs in the second coordination sphere. Once Fdh is reduced by 1e\textsuperscript{-}, this yields a six-coordinate EPR active species.\textsuperscript{36}

![Figure 2. 8: Fdh mechanism 5; The H\textsuperscript{-} transfer mechanism\textsuperscript{36}](image)

As observed in mechanism 1 for Fdh (Figure 2.4), for the dissimilatory Nap from \textit{Desulfovibrio desulfuricans} the oxo ligand is replaced by the substrate.\textsuperscript{13} However, both the crystal structure of the NapA from \textit{Desulfovibrio desulfuricans}\textsuperscript{5} and the crystal structure of NapAB from \textit{Cupriavidus necator}\textsuperscript{4}, revealed the
exogenous ligand ‘Y’ bound to Mo to be a sulfur donor. Coelho’s observation also suggested there is some degree of $S_{\text{Cys}}$-S persulfide character present. Based on this newly defined crystal structure and a corresponding EPR study, a new mechanism was proposed for Nap that was later intensively studied by a number of investigators using a variety of computational approaches. The computational study of Xie and coworkers on a Nap computational model shows direct binding of nitrate to the Mo center to yield a 7-coordinate complex, as shown in Figure 2.9, which results in a low energy barrier of 1.7 kcal mol$^{-1}$. In contrast, direct binding of substrate to Mo after $S_{\text{Cys}}$ detachment requires 19.8 kcal mol$^{-1}$, and binding to sulfur requires a very large amount of energy (56.8 kcal mol$^{-1}$). A QM/MM study by Dong et al. on an FdhN structure with $S_{\text{Cys}}$ coordinated to Mo instead of $S_{\text{Sec}}$ in the calculations, showed that the direct binding of formate to Mo is not favorable, and the detached $S_{\text{Cys}}$ always ends up binding again to the metal center. They argue that the detachment of $S_{\text{Cys}}$/Se$_{\text{Sec}}$ protein residues changes the protein conformation and this leads to very high energy barriers. Due

![Diagram of Nap inner-sphere and outer sphere mechanisms](image)
to the proposed structural similarity of Fdh and Nap enzymes, the detachment of $\text{SCys}$ and $\text{SeSec}$ is anticipated to be unfavorable for both enzymes.

### 2.2 Specific challenges associated with the study of paramagnetic enzyme forms

Many chemists are studying the implications of the crystal structures for Fdh and Nap enzymes. Although the presence of additional protein chromophores like Fe-S clusters and flavin effectively prevent the use of optical spectroscopies (electronic absorption, Raman, etc.) for the study of these enzymes, EPR spectroscopy has been used to gain additional insight into the structure of paramagnetic enzyme forms and catalytic intermediates. For most of these enzymes, EPR spectroscopy has been the only plausible spectroscopic technique for obtaining information about the geometric and electronic structure of the active site. There is currently considerable debate regarding the identity of the exogenous ‘$Y$’ ligand in these systems and more generally regarding the roles of the ancillary ligands (i.e. the protein derived (X) and exogenous Y donors) in the catalytic process. Metal ion coordination flexibility in the enzymes has been suggested from EPR spectroscopy.$^{16, 17, 22, 25, 35}$

If the protein derived ligand ‘X’ bound to Mo in Fdh and Nap is an oxygen donor, the reaction mechanism is likely quite straightforward as observed in mechanism 1 (Figure 2.4). In this case, a labile $\text{H}_2\text{O}$ molecule leaves the Mo first coordination sphere, creating an open site for the incoming substrate (formate for Fdh and nitrate for Nap) to bind to the metal. The substrate transformation
component of the catalytic cycle starts with Mo being in different oxidation states for Fdh and Nap. Being an oxidizing enzyme, the Fdh cycle begins with Mo(VI), and nitrate reductase starts the cycle in the reduced Mo (IV) form. As mentioned previously, recent X-ray crystal structures and EXAFS studies indicate that the exogeneous ligand ‘Y’ is likely to be a sulfur donor in Nap and Fdh enzymes. Considering the thiophilic nature of Mo, the sulfur donor ligand is not anticipated to be very labile and readily leave the first coordination sphere to accommodate the incoming substrate. Either a Se or S shift to the terminal sulfido, or the detachment of the protein-derived ligand is also questionable from data obtained for E. coli Fdh via labeled EPR spectroscopy, and the observation of a nonexchangeable proton by EPR in C. nector Nap. Also, the α-hydrogen of formate (pKa -23) is not acidic enough to be abstracted by a histidine residue, as suggested in the amino acid decoordination and shifting mechanism (Mechanism 3 from figure 3.6) for Fdh. Recently, Dong et al. used a computational approach to show that Se/Scys detachment from Mo is not energetically favorable. More

Figure 2. 10: Formation of EPR active Fdh and Nap enzyme active sites
recent computational and experimental studies directed toward understanding the Fdh reaction mechanism indicate that a second coordination sphere mechanism is may be applicable for both Nap (Figure 2.9, right middle) and Fdh, even though direct binding of nitrate to the Mo center after detachment of S<sub>Cys</sub> is energetically favorable.<sup>37</sup> In these plausible mechanisms the EPR active species in both types of enzymes would be a six coordinate species as shown in Figure 2.8.<sup>5, 39</sup>

In order to address the challenges mentioned above, we designed and synthesized six-coordinated EPR active enzyme analogs for Nap and Fdh intermediates (Figure 2.10). We have collected EPR data for these analogs and compared the spectra to analogous enzyme spectra in order to obtain additional details regarding the geometric and electronic structure of the enzyme sites, and to understand the roles of the ancillary ligands in the enzyme mechanism. Here, we have opted for a multi-component approach that includes spectroscopic studies (EPR, electronic absorption and X-ray absorption spectroscopy) and electronic structure and spectroscopic computations.

2.3 Synthesis of EPR active analogs for Fdh and Nap enzymes

**Solvent preparation:** Acetonitrile was first dried with powdered KOH and then filtered. The filtrate was distilled overnight with P<sub>2</sub>O<sub>5</sub> under a N<sub>2</sub> atmosphere. THF and diethyl ether were distilled in Na/benzophenone. All the distilled solvents (CH<sub>3</sub>CN, THF, and diethyl ether) were then stored over molecular sieves for three days.
Synthesis of Mo(cydt)$_2$SSe (1) (Scheme 2.2): 40 mg KBut'O was dissolved in 6 ml THF in a 10 ml round bottom flask. 30 μl 2-hydroselenolbenzenethiol was dissolved in 5 ml THF in another 10 ml round bottom flask. Both solutions were flushed with N$_2$ gas for 30 minutes. Then, the 2-hydroselenolbenzenethiol solution was transferred by canula into the KBut'O solution that was pre-cooled to -10 °C. The resultant mixture was stirred for 15 minutes. Then, the solution was subsequently transferred into a 50 ml round bottom flask containing a partially dissolved solution of Mo(cydt)$_2$(CO)$_2$ (50 mg in 6 ml CH$_3$CN) flushed with N$_2$ at -10°C. The mixture was stirred for ~15 minutes and then a benzyltriethylammonium chloride solution (51 mg in 5 ml CH$_3$CN) was added. The blue colored solution persisted for a few hours and then changed to a turquoise color. The solution was left to continue stirring overnight, then the solution was dried by purging with N$_2$ gas. The resulting dried powder was re-dissolved in dry CH$_3$CN, then filtered through Celite 545 under N$_2$ protection. The filtrate was transferred into a 100 ml
flask containing about 30 ml diethyl ether, then additional ether was added to get the solution to precipitate. The supernatant liquid was removed by a cannula with positive N₂ pressure. This recrystallization procedure was repeated twice, and the product was dried under N₂ gas. The product was stored in a glovebox antechamber for 3-4 hours. The yield was 55 mg (63.95%). The complex was characterized by mass spectrometry (observed mass 573.8391; theoretical 573.8388), EPR (experimental g_{iso} = 2.0129; computed g_{iso} = 2.0043), UV-Vis-NIR (λ_{max} 653nm, 730nm, 970nm). The nature of the coordinating ligands to Mo were determined by EXAFS. Since the complex is highly unstable, we could not obtain a crystal structure. Elemental analysis for this compound was unsuccessful due to the highly unstable nature of the compound.

Synthetic procedure and characterization methods for complexes 2 and 3 are the same as that for complex 1. However, the amounts of the individual reactants and the color of complexes are different, and this is discussed below.

**Synthesis of Mo(cydt)₂butdt (2):** For the synthesis of complex 2, 60 mg of KBut'O in 10 ml THF was treated with 30 μl of butane 2,3 dithiol dissolved in 10 ml THF). This mixture was added to 60 mg of Mo(cydt)₂(CO)₂ dissolved in acetonitrile (10 ml). 60 mg of benzyltriethylammonium chloride in 10 ml acetonitrile was added to the mixture. The blue color persisted for few hours and upon continued stirring overnight the color changed to dark blue. After recrystallization, the yield was 40 mg (~42%). The complex was characterized by mass spectrometry (observed mass 505.9211; theoretical mass 505.9251), EPR (experimental g_{iso} = 2.0035; computed g_{iso} = 2.0034), UV-Vis-NIR spectroscopy (λ_{max} 1030 nm, 592 nm) and
the nature of the coordinating ligands to Mo were determined using EXAFS. Since this complex is even more unstable than complex 1, we were not able to obtain a crystal structure and elemental analysis for this compound was unsuccessful.

**Synthesis of Mo(cydt)$_2$OS (3):** KBut$i'O$ 60 mg, 2-mercaptophenol 30 μl, Mo(cydt)$_2$(CO)$_2$ 60 mg, and benzyltriethylammonium chloride 60 mg were used in this reaction. The yield of the blue compound was 45 mg (~47%). The complex was characterized by mass spectrometry (observed mass 509.9169; theoretical mass 509.9168), EPR (experimental $g_{iso} = 1.9924$; computed $g_{iso} = 1.9929$), UV-Vis-NIR spectroscopy ($\lambda_{max}$ 1030 nm, 602 nm). Elemental analysis for this compound was unsuccessful due to the highly unstable nature of the compound.

### 2.4 Characterization of the Fdh and Nap enzyme models

The Nap and Fdh synthetic model complexes were characterized using a combination of mass spectrometry, EXAFS, and EPR spectroscopy.

#### 2.4.1 Mass spectrometry

Mass spectrometry data for complexes 1, 2 and 3 were determined by the electron spray ionization (ESI) technique in the Mass Spectrometry Facility in the Department of Chemistry and Chemical Biology at The University of New Mexico. The mass of complex 1 was determined to be 573.8391 (theoretical 573.8387), and the observed mass for complexes 2 and 3 were determined to be 505.9211 (theoretical 505.9251) and 509.9168 (theoretical 509.9169), respectively. Mass
spectral data for all three complexes are shown in Figure 2.11. Complexes 1 and 2 have clean mass spectrometry data whereas the complex 3 sample has other fragments in the full mass spectrum. Complex 3 was found to be the most unstable of these compounds when exposure to air. In the ESI mass spectrometry technique, the solution sample has to be exposed to air before injecting to the

Figure 2. 11: Mass spectra (with Mo isotope patterns) of Fdh and Nap model complexes. Complex- 1 (top), -2 (middle) and -3 (lower)
instrument. Thus, the most air sensitive samples decompose to some degree during the analysis. Our bis-dithiolene Mo complexes commonly decompose into more thermodynamically stable complexes such as \([\text{MoO(dithiolene)}_2]^n\), thiol-bridged Mo dimeric structures, and most commonly the \([\text{Mo(dithiolene)}_3]^n\) tris-dithiolene.\(^{40}\) As we see in the full mass spectrum of complex 3, the cyclohexenedithiolene molybdenum oxo complex fragment peak (mass 401.91) (Figure 2.11) appears.

2.4.2 Elemental Analysis

Multiple attempts to obtain quality analytical data for complexes 1, 2 and 3 all proved to be unsuccessful. We believe these results from the model complexes being highly unstable, even in the solid state, and particularly when they are exposed to air. As a result, we have relied heavily on mass spectrometric data and EXAFS to characterize complexes 1, 2 and 3.

2.4.3 EXAFS

The experimental details of the XAS technique were explained in Chapter 1. We collected EXAFS data (Figure 2.12) for complexes 1, 2 and 3 in order to provide additional structural support to supplement the characterization of these highly unstable molecules. Although it is difficult to determine the coordination number of these complexes solely from EXAFS, the technique can still provide considerable information and serve as a supplementary characterization tool if we carefully observe trends in the data for complexes 1, 2 and 3, and properly analyze
the results of the EXAFS data using a best fit analysis. In the EXAFS equation (Equation 2.1), the coordination number ($N$) and amplitude reduction factor ($S_0^2$) are inversely correlated. Thus, if one increases other decreases, and vice-versa.

$$\chi(k) = \sum \frac{N A(k) S_0^2}{k R^2} \exp\left(-\frac{2R}{\lambda(k)}\right) \exp(-2k^2\sigma^2).\sin(2kR + \phi(k))$$  \hspace{1cm} (2.1)

Figure 2. 12: EXAFS for 1 (top), 2 (middle) and 3 (lower). Left K-space representation; right Fourier transformed R-space representation.
In order to address this issue, we kept the amplitude reduction factor within a reasonable range from ~0.70 to ~1, and then fit the data to determine both the number and type of atoms coordinated to the Mo ion in the first coordination.

Table 2. 2: EXAFS fitting results 1 (a & b), 2 (c & d) and for 3 (e & f)

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<th>K-space fittings</th>
<th>Mo-S N</th>
<th>R fit (R guess)</th>
<th>σ²</th>
<th>Mo-Se N</th>
<th>R fit (R guess)</th>
<th>σ²</th>
<th>S ²</th>
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<td>Fit1</td>
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<td>1</td>
<td>2.546(2.552)</td>
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<tr>
<td></td>
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<td>2.415(2.408)</td>
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<td>R fit (R guess)</td>
<td>σ²</td>
<td>Mo-Se N</td>
<td>R fit (R guess)</td>
<td>σ²</td>
<td>S ²</td>
<td>∆E 0</td>
<td>Rf (%)</td>
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<td>σ²</td>
<td>Mo-Se N</td>
<td>R fit (R guess)</td>
<td>σ²</td>
<td>So²</td>
<td>∆E 0</td>
<td>Rf (%)</td>
</tr>
<tr>
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<td>R fit (R guess)</td>
<td>σ²</td>
<td>Mo-O N</td>
<td>R fit (R guess)</td>
<td>σ²</td>
<td>So²</td>
<td>∆E 0</td>
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</tr>
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<tr>
<td>Complex-3</td>
<td>Mo-S N</td>
<td>R fit (R guess)</td>
<td>σ²</td>
<td>Mo-O N</td>
<td>R fit (R guess)</td>
<td>σ²</td>
<td>So²</td>
<td>∆E 0</td>
<td>Rf (%)</td>
</tr>
<tr>
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<td>0.921</td>
<td>1.418</td>
<td>0.48</td>
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sphere. As shown in Table 2.2 the best fits to the data for complex 1 result in a Mo center bound to five sulfur scatterers and a single selenium scatterer. The best fits to the data for complex 2 have six sulfur scatterers bound to the Mo ion, while for complex 3 we find five sulfur scatterers and a single oxygen donor in the first coordination sphere. In this analysis, the best fit is that with the lowest \( R_f \) (R-factor), which is defined as percentage of misfit (see Eqn. 2.2) between the data and theory. However, the best fit for complex 2 as defined by \( R_f \) is not realistic, given the synthesis of this complex. For example, the lowest \( R_f \) for 2 is obtained with four sulfur scatterers instead of six, but with a correspondingly higher value for \( S_o^2 \). In complex 2, there is only one type of donor ligand coordinated to metal center, and this shows how difficult it is to determine the coordination number based on EXAFS data alone. This highlights the fact that \( N \), the number of scatterers of a given type, is highly correlated with the value of \( S_o^2 \) (see Eqn. 2.1) in the EXAFS equation. This problem can be overcome by performing a bond valence sum (BVS) analysis for complexes 2 and 3. Using this approach, the total BVS of all bonds in the first coordination sphere should be equal to the oxidation state of the metal center. Details about the BVS approach are discussed in Chapter 3.

The total BVS (calculated by using EXAFS determined bond lengths from six-coordinate fits) of two complexes (2 and 3) is 4.5 which is close to their oxidation state (+5). Whereas the total BVS for five-coordinate structures (bond lengths used from their five coordinate fits) is 3.8. We were unable to determine the BVS for the complex 1 because there is no reported crystallographic data for

\[
f(k_i) = \kappa(k_i|data)x(k_i|theory) \tag{2.2}
\]
Mo(V)-Se bond distances. We confirmed there is Mo-Se coordination in complex 1 by comparing the half height at full width (HHFW) of the normalized Fourier transformed peak with that of the corresponding peak of complex 2. For complex 1, the HHFW is 0.198 and for complex 2 the HHFW is 0.174, which is about 12% less than for complex 1. Regarding possible arguments about six-coordinate Mo(VI) structures in the EXAFS samples, we compared the electronic absorption data of complex 3, [Mo(V)(cydt)2OS]1−, [Mo(VI)(cydt)2OS]0 (Figure A11, Appendix E) and [Mo(VI)(bdt)3]4+. For the tris-chelating Mo(VI) dithiolene complexes, whether they are bis-dithiolenes, tris-chelating heteroleptic systems, or tris-dithiolene homoleptic complexes, there are two intense peaks at ~22,000 cm⁻¹ and at ~14,000 cm⁻¹. However, Mo(V) form of tris-chelating complexes has one very intense peak ~17,000 cm⁻¹. (Figure A10-11 in the Appendix E).

In summary, the EXAFS data and the analysis are consistent with the expected structures for all the complexes, which we synthesized as intermediate analogs for Fdh and Nap enzymes. The Fourier transform (FT) of the EXAFS modulations for complex 3 show an extra peak at ~1.3 Å, which was not able to be
removed. We hypothesize that this background peak is instrumental in nature.\textsuperscript{45, 46} This extra peak appears below the Mo=O bond peak at $R+\Delta \sim 1.7\text{Å}$. The EXAFS fitting results combined with the results of our BVS analysis strongly support the presence of five sulfur donors and a single non-oxo oxygen donor for complex 3. A summary of the best fit parameters for complexes 1, 2 and 3 are shown in Figure 2.12 and detailed further in Table 2.2. We also looked at the XANES spectra of these complexes (Figure 2.13) to determine whether there is an oxo-edge feature\textsuperscript{47, 48} but we did not observe one. Additionally, to check whether the samples have paramagnetic impurities we performed both room temperature and low temperature X-band EPR experiments. We were able to achieve clean EPR spectra for all three samples of compounds 1, 2 and 3. EPR experiments for these compounds are presented in section 2.4.4.

2.4.4 EPR spectroscopy of New Fdh and Nap Models

Since these complexes are paramagnetic Mo(V) species, EPR spectroscopy provides an exquisitely sensitive probe of this oxidation state and spectral simulations can be used to determine if multiple Mo(V) species are present in solution as a result of the formation of paramagnetic side products or degradation to other Mo(V) species. EPR data (Figure 2.14) for model complexes 1, 2 and 3 were collected at X-band using a Bruker EMX spectrometer with associated Bruker magnet control electronics and microwave bridge. The microwave frequency (mwFreq) was \textasciitilde 9.39 GHz. The exact mwFreqs used for the study of complexes 1, 2 and 3, at room temperature/77K resonance conditions
were, 9.4092/9.3891, 9.4050/9.3830, and 9.4083/9.3897, respectively. The modulation amplitude was adjusted between ~1-3 G and the frequency modulation was fixed 100 MHz. The field attenuation in all cases was fixed at 30 dB. The Mo(V) EPR resonance was observed at approximately 330 mT. For the low temperature EPR experiments a liquid helium cooled cryostat was used, and the sample space was purged with a positive pressure of N₂ gas. Since all three complexes are highly unstable, we prepared EPR samples using a Schlenk line for these experiments. The samples were dissolved in n-butyronitrile for low temperature EPR and in acetonitrile for room temperature EPR and were subsequently transferred via cannula to EPR tubes using positive nitrogen pressure. These EPR tubes were previously purged with N₂. For low temperature EPR spectroscopy, the EPR tube containing the sample was immersed (1 mm length of the tube in 2 sec) into liquid N₂ to ensure a proper glass was formed. In these studies, the use of toluene and DCM as solvents was avoided due to their incompatibility with the samples.

The X-band spin-Hamiltonian parameters of the model complexes are in reasonable agreement with those previously obtained for Nap and Fdh enzymes. The g-values and A²Mo values reflect the degree of metal-ligand covalency, while the dipolar contribution to A²Mo reflects the nature of the DFT-calculated spin density distribution and the nature of the singly occupied quasi-restricted molecular orbital (QRO, SOMO wave function). Slight variations in the bond lengths and dihedral angles (or twist angles) for these molecules result in the orientation of g- and A-tensors being different from one another. Spectral simulations of 1-3 yield EPR spin Hamiltonian parameters (Table 2.3) for direct comparison to the spin-
Hamiltonian parameters obtained for representative Mo(V) enzyme species. Mo hyperfine coupling has only been observed for very few Fdh and Nap enzymes. A more comprehensive list of enzyme g-values is provided in Table
2.1. The experimental and computed EPR spin Hamiltonian parameters have been reported in Table 2.3. The g-values for the synthetic complexes are consistent with as high degree of metal-ligand bond covalency that derives from the presence of multiple sulfur donors. This will be discussed in more detail in Chapter 4. Due to the presence of a selenium donor in complex 1, the $g_{ave}$ for this complex is the highest in this series, while the presence of a hard oxygen donor in complex 3 yields a value for $g_{ave}$ that is the lowest in the series. The dipolar hyperfine coupling, $A_{Mo}$, for all three complexes is observed to be rhombic, indicating that all the complexes have a QRO SOMO wavefunction that is comprised of a specific linear combination of canonical d-orbital wavefunctions. When the computed EPR spin Hamiltonian parameters are very similar to experimentally derived parameters, the computed relative orientation of the g- and A-tensors become very important to our understanding of the relative orientation of the SOMO with respect to the molecular geometry. Thus, direct comparison of the models with the enzymes can be used to assess the coordination environment and the geometry of a given paramagnetic enzyme species. The nature of the $\beta$-LUMO and $\alpha$-SOMO are similar, and we have

<table>
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<th>Enzymes/Models</th>
<th>g-tensor</th>
<th>$A_{Mo}$ tensor ($\times 10^4$ cm$^{-1}$)</th>
<th>Euler angles in degree</th>
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<tr>
<td></td>
<td>$g_1$</td>
<td>$g_2$</td>
<td>$g_3$</td>
</tr>
<tr>
<td>Fdh (Methanobacterium formicicum)*</td>
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<td>2.0060</td>
<td>1.9970</td>
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<tr>
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</tr>
<tr>
<td>Nap (Desulfovibrio desulfuricans)**</td>
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<td>2.0072</td>
<td>1.9900</td>
</tr>
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</tr>
<tr>
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<td>2.0131</td>
<td>1.9912</td>
<td>1.9819</td>
</tr>
</tbody>
</table>

References: * (Barber et al., 1986), **(Riva et al., 2007) & * (Gonzalez et al., 2006)
displayed the relative orientations of the A- and g-tensors superimposed on the β-LUMO orbitals of 1-3 in Figure 2.15. Thus, an analysis of EPR spin-Hamiltonian parameters and their relative orientation with respect to the molecular geometry and the α-SOMO/β-LUMO gives valuable information for comparing the geometric and electronic structures of the enzymes and the models. Understanding these basic geometric and electronic structure relationships will provide important input for understanding the electron transfer half reactions of Fdh and Nap. We will specifically discuss these spectroscopically and computationally derived EPR spin Hamiltonian parameters in a later section and correlate these parameters with the geometry of the models and enzymes.

Figure 2.15: Orientation of the g-tensor and the A-tensor for 1 (upper left), 2 (upper right) and 3 (lower) relative to the molecular geometry and the β-LUMO.
2.5 Magneto-Structural correlations

Six-coordinate DMSOr family enzymes have similar $g_{ave}$ and $g_{aniso}$ ($g_1$-$g_3$) values provided that they possess the same type of ligands in the first coordination sphere.\textsuperscript{24, 25} Slight variations in the observed $g$-values for these enzymes likely result from small differences in their metal-ligand bond lengths, dihedral angles, the experimental pH values, and differences in the second coordination sphere. Guigliarelli and coworkers have constructed plots of $g_{ave}$ vs. $g_{aniso}$ as a means of developing a EPR correlations that can be used to correlate EPR spin Hamiltonian parameters with enzyme family (XO, SO, DMSOr) and enzyme type (DMSOr Type I, II, and III).\textsuperscript{24, 25} The $g$-values for Mo enzymes that possess a single PDT ligand

![Figure 2. 16: g-anisotropy ($g_1$-$g_3$) correlations with g-average for enzymes XO, SO and DMSOr (Nap/Fdh, Nar and DMSOr subfamilies).]
are observed to fall below the virtual line that separates the $g$-values for Mo enzymes that are coordinated by two PDTs (Figure 2.16). Guigliarelli and coworkers have correlated individual $g_1$, $g_2$, and $g_3$ values with $g$-value anisotropy ($g_1 - g_2$) in order to develop insight into the structures of the high-$g$ and very high-$g$ Nap and Fdh species.\(^\text{25}\) In their plots of high-$g$ species, the values for $g_{\text{aniso}}$ range from $\sim 0.016$ to $\sim 0.03$ and the $g$-tensor is observed to be rhombic. Trigonal prismatic structures have been suggested for high-$g$ type enzymes.\(^\text{25}\) The potential for perchalcogenide bond formation between $S_{\text{Cys/SeSec}}$ and a terminal sulfido may contribute to the enzymes adopting a trigonal prismatic structure.\(^\text{25}\) For the very high-$g$ enzyme types, the $g_{\text{aniso}}$ values range from $\sim 0.020$ to $\sim 0.036$. If we look at the high anisotropy side of very high-$g$ enzyme plots (Figure 2.17), the enzymes have axial $g$-values with one high $g$ component and two lower $g$ components.\(^\text{25}\) On the low $g_{\text{aniso}}$ side, we also observe axial $g$ values but with two high $g$ components and a single lower $g$ component. It is apparent that the structure of these enzymes deviate from an idealized trigonal prismatic geometry, and it has been suggested that a terminal sulfido donor is coordinated to the Mo ion in oxidized forms of Fdh and Nap enzymes.\(^\text{25}\) To better understand these structure issues, we have replotted the $g_{\text{ave}}$ vs $g_{\text{aniso}}$ data in Figure 2.17 to include data from our model complexes. Only the enzyme data was used in the linear fits to the data. In Figure 2.17, the high-$g$ type enzyme data are shown in red and the very high-$g$ type data are given in blue. The solid green squares show the data for models 1-3. The $g$-values for our model complexes are most consistent with the Fdh and Nap enzyme high-$g$ pattern. In general, our model systems possess a higher degree of $g$-
anisotropy when compared with the enzymes. To obtain additional understanding of the high-g type enzyme structure, we performed DFT computations on computational models for the enzymes with a S\textsubscript{Cys} or Se\textsubscript{Sec} ligand bound to the Mo ion. A close inspection of the EPR spin Hamiltonian parameters (Table A1, Appendix C) for the computational and synthetic models (Figure 2.18) show that those which have one twist angle smaller than other two (Figure 2.18 (i) & (ii)) have \( g_{\text{aniso}} \) values similar to the high-g type enzyme forms. The higher twist angle anisotropy lowers the symmetry of these 6-coordinate complexes and results in rhombic g-tensors.

A rhombic dipolar hyperfine coupling is not commonly observed. However, it has been found in low symmetry molecules where the SOMO is comprised of a linear combination of canonical d-orbitals.\textsuperscript{49, 50} Both trigonal prismatic [Mo(cydt)\textsubscript{3}]\textsuperscript{1-}
and Donahue’s $D_{3h}$ type tris-dithiolenes possess nearly isotropic g-values. Thus, these complexes do not follow the correlations observed in Figure 2.17 (See Figure A8, Appendix C). Clearly, the high-g pattern observed for enzymes and model complexes is not characteristic of high symmetry trigonal prismatic coordination geometries. Also, Guigliarelli and coworkers suggest that the presence of a coordinated perchalcogenide using either a S$_{\text{Cys}}$ or Se$_{\text{Sec}}$ ligand results in the enzyme active site geometry being more trigonal prismatic in a constrained protein environment. This contrasts with an octahedral environment that minimizes the inter-ligand repulsion.$^{25}$ We have performed EPR calculations on computational models with a coordinated persulfide bound to Mo. In this case, one of the dithiolene twist angles is smaller than other two leading to a higher degree of correlation with high-g enzyme pattern. Therefore, our understanding is that in order to possess a high-g pattern (as in Figure 2.17), neither perchalcogenide

![Figure 2.18: Computational models of Fdh and Nap enzymes](image-url)
bonding nor trigonal prismatic structured are required. Instead, a degree of twist angle anisotropy should exit in the structures.

A more complete understanding of these geometric and electronic structure interpretations can be found in the literature,$^5, 16, 23, 32$ which also mentions key details about the methods of enzyme preparation for EPR spectroscopy. Except for the EPR results of Se-Fdh, which were collected$^{15}$ using the active form of the enzyme, EPR data for other enzyme species were collected using a different molecules (dithionite, NaN$_3$, CN$^-$ etc)$^{35, 51}$ other than the natural substrate, or they were collected on inactive forms of the enzymes. Our EPR calculations on sulfido containing enzyme models (ii) and (v) in Figure 2.18 yielded EPR spin Hamiltonian parameters that are close to the very high-g pattern. We conclude that very high-g enzyme forms are likely five-coordinate, with either a donor ligand from the protein or a non-dithiolene ligand detached. This conclusion is based on our EPR calculations on [Mo(V)O(bdt)$_2$]$^{1-}$ (Table A, Appendix C), which yielded g-values that are very close to those observed for very high-g enzyme forms. Also, the EPR data of Donahue and coworkers on distorted trigonal prismatic, [Mo(bdt)$_3$]$^{1-}$ type complexes$^{44}$ yield g-values that are similar to those of very high-g enzymes, although they do not contain a sulfido donor. This may mean that terminal sulfido coordination is not required in order to correlate with the low g-anisotropy side of the very high-g plot (Figure 2.17). The spin density (Figure A5, Appendix B) computed for [Mo(dithiolene)$_3$]$^{1-}$ and terminal sulfido containing models (Figure 2.17), possesses the shape of a canonical d-orbital, and this is responsible for the observed axial A-tensors in the case of either high g-anisotropy or low g-
anisotropy. Therefore, we conclude that the very high-g type enzymes are either sulfido containing, or they possess a terminal oxo ligand that arises from degradation.

2.6 Optical spectroscopy and electronic structure

After completing these magneto-structural correlations for the model complexes and enzymes, we sought to explore the electronic structure of these species further using electronic absorption spectroscopy. We have also used S K-edge XAS to probe the covalency and electronic structure of these Fdh and Nap model systems. These studies will be presented in Chapter 4. These synthetic model complexes represent essential benchmarks for determining the electronic structure of these enzymes since the structural and spectroscopic data for Mo(V) enzyme forms is sparse. This derives from the fact that these enzyme forms are poised or trapped intermediate species, and they possess other highly absorbing redox chromophores such as Fe-S clusters that hinder our ability to obtain meaningful optical data originating from the Mo site. Our electronic absorption spectroscopic results are discussed in the following sections.

2.6.1 Electronic absorption spectroscopy

Electronic absorption spectra for compounds 1, 2 and 3 are shown in Figure 2.19. These data were collected on a Hitachi double beam U-4100 UV-vis-NIR spectrometer. Solid samples were dissolved in pure dried acetonitrile. The dissolved samples were placed in 1 cm pathlength quartz cuvettes and sealed with
a rubber septum to ensure that the samples remain anaerobic and to prevent decomposition. Since the samples are highly sensitive to oxygen exposure, they were prepared under a nitrogen atmosphere.

![Graph of electronic absorption spectra](image)

Figure 2.19: Electronic absorption spectra of complexes 1 (red), 2 (blue) and 3 (purple).

2.6.2 Electronic absorption data analysis

A brief theoretical description of electronic transitions is presented in section 1.2.1 (Chapter1). Complexes 1, 2 and 3 are low symmetry molecules and have two major absorption bands. One low intensity band is observed at ~ 10000 cm\(^{-1}\) and a second, higher intensity band is observed at ~16000 cm\(^{-1}\). Time-dependent density functional theory (TDDFT) calculations have been used to make computationally assisted band assignments for compounds 1-3 and the results are displayed in Figure 2.20. Here, the vertical lines represent the individual computed transition energies, and the height of these lines is proportional to the oscillator strength. These TDDFT computations employed the B3LYP hybrid functional and
the TZVP basis set. We assign the lower energy band (~10000 cm\(^{-1}\)) as a transition that originates from out-of-plane sulfur-based dithiolene orbitals to the metal centered orbitals in all the complexes. Due to the presence of a single electron in the redox orbital, there exists large exchange interaction in this d\(^1\) complex that leads to an inverted bonding scheme,\(^{52}\) (Figure 2.21), where the \(\alpha\)-HOMO possesses dominant ligand character and a deeper lying \(\alpha\)-SOMO is the close spatial counterpart to the \(\beta\)-LUMO. Thus, the computed splitting between the \(\beta\)-LUMO and \(\alpha\)-SOMO is quite large in all cases (1.5 – 3.0 eV). The \(\alpha\)-HOMO is mainly composed of out-of-plane dithiolene S orbitals and is expected to be important for the electron transfer half reaction. The \(\beta\)-LUMO is also important in Nap enzymes, which catalyze the oxidation of nitrate to nitrite. The \(\alpha\)-HOMOs (Figure 2.22-2.24) for all the complexes display distinct contributions from ancillary
Se, S, or O donors. This indicates that there should be a significant contribution from the non-dithiolene ligands ‘X’ and ‘Y’ in the electron transfer half reactions of the enzymes. The α-HOMO wave functions are delocalized unsymmetrically. This is clearly evident in the α-HOMO of complex 2, which has an aliphatic 2,3-butanedithiolate bound to Mo, and one of the dithiolates illustrating one has major contributions in electron transfer half reactions.

Figure 2. 21: Inverted bonding schemes for complexes 1, 3 (left) and 2 (right).

Figure 2. 22: Spin-unrestricted representations of the orbitals involved in charge transfer band 1 (upper) and band 2 (lower) for complex 1.
Another major transition occurs at $\sim 1600\text{cm}^{-1}$. This transition occurs from the $\beta$-HOMO, which is mainly comprised of dithiolene out-of-plane orbitals, to the $\beta$-LUMO and $\beta$-LUMO+1, which are metal centered orbitals. The electronic

Figure 2. 23: Spin-unrestricted representations of the orbitals involved in charge transfer band 1 (upper) and band 2 (lower two) for complex 2.

Figure 2. 24: Spin-unrestricted representations of the orbitals involved in charge transfer band 1 (upper) and band 2 (lower) for complex 3.
transitions are observed to be similar in these DMSOr model complexes 1-3. This tells us that the DMSOr reductase enzyme family members have a general similarity in their geometric and electronic structure properties. The molecular orbital diagrams indicate an inverted bonding scheme and are shown in the Figures 2.25, 2.26 & 2.27. In the inverted bonding scheme\textsuperscript{52-54} the $\alpha$-HOMO orbitals in the model system are ligand-centered and the $\alpha$-SOMO orbital, due to high exchange interaction\textsuperscript{52, 55} with $\beta$-counterpart, lies below some of the ligand orbitals. The ligand-based delocalized $\alpha$-HOMO is expected to be important in electron transfer reactivity in order to shuttle electrons to the Fe-S cluster (Figure 2.2). Once an electron transfers to redox partner, another electron from non-redox orbital (in our model system it is $\alpha$-SOMO) shifts to the hole produced upon oxidation. The phenomenon is known as electronic relaxation.\textsuperscript{56} Another important thing we observed in the Fdh computational model is that the $\alpha$-HOMO wave

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2_23}
\caption{MO diagram of complex 1}
\end{figure}
function is delocalized unsymmetrically with respect to the two dithiolenes (Figure A19, Appendix E). This suggests that in the enzyme the P-PDT (proximal PDT, closer to the Fe-S cluster) plays a more important role in the electron transfer process compared to the D-PDT (distal PDT). The unsymmetrically delocalized \( \alpha \)-HOMO wavefunction has also been observed in the Fdh model 2, but not in model

Figure 2. 24: MO diagram of complex 2

Figure 2. 25: MO diagram of complex 3
1. For model 1, the $\alpha$-HOMO is delocalized towards the aromatic S,Se dichalcogenolene. The $\alpha$-HOMO wave functions for the synthetic and computational Fdh model compounds have been reported in the Appendix E, Figure A19.

2.7 Conclusions

To better understand the structural and functional properties of the EPR active catalytic intermediates of Fdh and Nap enzymes, we synthesized model complexes 1, 2 and 3. From our EPR spectroscopic study, we correlated spin-Hamiltonian parameters with specific enzyme structures, providing deep insight into the electronic structure of high-g and very high-g enzyme forms. This EPR correlation indicates that high-g type enzymes are most likely 6-coordinate with four sulfur donors originating from two dithiolenes (i.e. the two PDTs), a $S_{Cys}$ or $Se_{Sec}$ donor, and an additional ligand bound to the metal. The study also indicates that the structures of Mo(V) enzyme forms lie between octahedral and trigonal prismatic geometries. The nature of the $\alpha$-HOMO shows that it is delocalized more on one of the dithiolenes ligands, supporting a hypothesis that a single PDT ligand is involved as a conduit in the electron transfer regeneration of the active site for catalysis.\(^9\,^{57}\)
2.8 References


Chapter 3

New insights into the geometric and electronic structure of the
DMSO reductase high-g split catalytic intermediate

3.1 Introduction to the DMSO reductase (DMSOr) enzyme subfamily

The canonical DMSOr enzyme is a class III enzyme of the dimethyl sulfoxide reductase sub-family of molybdenum-tungsten enzymes.\(^1\) The enzyme reduces dimethyl sulfoxide (DMSO) obtained from the breakdown of methylsulfiniopropionate,\(^2\) to dimethyl sulfide (DMS).\(^3\) The oxidized form of the enzyme has a six coordinate distorted trigonal prismatic structure with four sulfur donors from two pyranopterin dithiolenes (PDTs) one oxygen donor from a coordinated serine residue and one oxo group bound to Mo.\(^4-6\) The reduced form of the enzyme has been observed as a five coordinate distorted square pyramidal structure with four sulfur donors from two PDTs and one oxygen donor from a serine residue bound to Mo.\(^6\) In the following sections, we will discuss the geometric and electronic structure of the enzyme, and their contributions to catalysis.

3.1.1. Roles of DMSOr in the global biogeochemical cycles

DMSOr is important in the biogeochemical cycle of sulfur, in addition to its role in anaerobic respiration of some marine bacteria and algae.\(^3,7\) The enzyme is found in marine bacteria such as *Rhodobacter sphaeroids* and *Rhodobacter capsulatus*, and it reduces dimethyl sulfoxide to dimethyl sulfide ((CH\(_3\))\(_2\)S), a
volatile sulfur gas. Once \((\text{CH}_3)_2\text{S}\) reaches the atmosphere, it is oxidized to sulfate \((\text{SO}_4^{2-})\) or methane sulfonate \((\text{CH}_3\text{SO}_3^-)\), which acts as cloud formation nuclei (CCN) and contributes to the earth’s albedo, enabling one to estimate climate change in the earth’s atmosphere.\(^3\)

### 3.1.2 Proposed catalytic mechanism of DMSOr

There are a number of studies on the DMSOr enzyme catalytic mechanism.\(^2\) Cobb and coworkers proposed, from their the UV-Vis and EPR spectroscopic studies, the reaction mechanism shown in Scheme 3.1.\(^2\) By monitoring changes in the electronic absorption spectra, the oxidative half reaction was observed to be pH dependent and involved 2 e⁻ transfer via a substrate-bound

\[
\text{Me}_2\text{SO} + \text{Mo}^{\text{IV(DMSOR)}} + 2\text{H}^+ \rightarrow \text{Me}_2\text{S} + \text{Mo}^{\text{VI(DMSOR)}} + \text{H}_2\text{O}
\]

![Scheme 3.1: Proposed catalytic cycle of DMSOr by Cobb et al.\(^2\)](image)
The individual steps in the reductive half reaction, observed from reductive titrations and EPR spectroscopy, are one electron transfer processes. The high-g split EPR active species has been observed as a Mo(V) intermediate in the reductive half reaction with DMSO, and this intermediate builds up quantitatively (~100%) using TMAO as substrate. A previous theoretical study by Webster and coworkers is also in agreement with an associative mechanism in the oxidative half reaction. Interestingly, regarding the structure of the high-g split species, there are opposing interpretations as to whether it is a six-coordinate species or a five-coordinate species with the OSer dissociated from the Mo ion. Thus, it has been suggested that the mechanism shown in the Scheme 3.1 may require revision.

### 3.1.3 DMSO enzyme structure

A combination of X-ray crystallography, EXAFS, EPR, resonance Raman, MCD, and computational studies have been conducted in order to probe the structure of the enzyme. Some of the spectroscopic interpretations regarding the structure of the enzyme will be discussed in the following sections.

#### 3.1.3.1 DMSO X-ray crystallography

Crystal structures of DMSO isolated from *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* have been determined. Schneider and coworkers used X-ray crystallography to determine the structure of the DMSO from *R. capsulatus* and found that the oxidized form of the enzyme is penta-coordinate.
with two terminal oxo ligands, two dithiolene S-donors from a single PDT and one O$_{\text{Ser}}$ bound to the Mo center.$^{12}$ However, Schindelin and coworkers determined the crystal structure of this enzyme from *R. sphaeroides* and found that the oxidized enzyme is six-coordinate with four S-donors from two PDTs, one O$_{\text{Ser}}$, and one oxo group bound to the Mo center.$^{11}$ In the dithionite reduced enzyme, they observed that one Mo-S bond was very long (3.1 Å). In this structure, only three S-donors and one O$_{\text{Ser}}$ are coordinated to Mo. Another X-ray structure from McAlpine and coworkers on the DMS reduced DMSOr enzyme from *R. capsulatus* showed that the reduced enzyme coordination geometry is very similar to that of the oxidized form. In this heptacoordinate structure, the extra ligand coordinated to Mo center can be interpreted as the oxygen from DMSO.$^{14}$ To understand the plasticity of the active site, Schindelin et al. studied a high resolution (1.3 Å) X-ray structure of the oxidized DMSOr from *R. sphaeroids*, and determined that the structure (Figure 3.1) is similar to that suggested from resonance Raman spectroscopy by Garton and coworkers.$^{15}$ In this oxidized active site structure, four dithiolene S-donors from
two PDTs are observed at a distance ~2.43 Å, an oxo ligand was found at a
distance 1.76 Å, and the $O_{\text{Ser}}$ was found at a distance of 1.84 Å to the Mo. In a
study using Hepes buffer, a five-coordinate structure was observed by Schneider
et al. in the enzyme isolated from *R. capsulatus*.\textsuperscript{15}

### 3.1.3.2 XANES and EXAFS Studies

George and coworkers conducted several studies using XAS to investigate
DMSOr structures in different oxidation states.\textsuperscript{5, 6, 9, 16} Specifically, they performed
XAS experiments on the oxidized Mo(VI), reduced Mo(IV), and glycerol inhibited
Mo(V) forms of DMSOr. In their Mo-XAS XANES spectra, the rising edge of the
reduced form of the enzyme shifted by 1.5 eV lower than that of the oxidized
enzyme, and the edge for the Mo(V) form shifted by 0.5 eV to lower energy. None
of the spectra of these three different enzyme forms showed dominant pre-edge
features. As a result, they argued that there might be a low number (one or zero)
of oxo ligands coordinated to Mo. In the EXAFS study of the oxidized Mo(VI)
enzyme form, the active site structure was determined to be 6-coordinate, with four
S-donors from two dithiolenes, an oxo ligand, and one light O/N atom coordinated
to Mo at distances of ~2.44 Å, 1.68 Å and 1.92 Å, respectively (Figure 3.2).\textsuperscript{5} On

![Figure 3.2: Proposed structures of DMSOr from EXAFS study by George et al.\textsuperscript{5} Mo(V) (right) is glycerol inhibited.](image)
the other hand, EXAFS of the reduced form of the enzyme showed that one of the four dithiolene S-donors should have bonded to Mo at a long distance which cannot be measured accurately from EXAFS technique, with a light O/N scatterer observed at two different distances (2.16 Å and 1.92 Å, Figure 3.2 middle).\(^5\)

George and coworkers also studied the glycerol-inhibited Mo(V) form of the enzyme and it was observed that the four sulfur donors from the two dithiolene ligands are bound to the Mo center at an distance of ~2.4 Å, and one O or N donor is bound at a distance of 1.96 Å.\(^5\)

In another EXAFS study\(^17\) of oxidized DMSOr obtained from \textit{R. capsulatus}, Baugh and coworkers found that the active site coordination was similar to that observed by McAlpine and coworkers\(^14\) from their X-ray structure. Baugh and coworkers’ EXAFS result shows two S-donor ligands coordinated to Mo at a distance ~2.32 Å, and another two S-donor ligands coordinated at a distance 2.47 Å. The Mo-oxo bond is 1.71 Å and two light O donors were observed at 1.92 and 2.27 Å distances from the Mo center as shown in Figure 3.3a.

The EXAFS study by George et al. on the DMS- and dithionite-reduced enzymes indicated a des-oxo active site structure.\(^6\) In the DMS-reduced enzyme,
four S-donors were observed at ~2.36 Å, one oxygen at 1.94 Å, and a second oxygen donor at 2.14 Å from the Mo ion. In the dithionite-reduced enzyme, it was observed that four S-donors were bound to Mo at 2.37 Å, and one oxygen at 2.12 Å. In yet another EXAFS study by George et al., the active site structure of the Mo(V) high-g split EPR active species was found to be five coordinate, in marked contrast to the six coordinate structure suggested by Kirk and coworkers from a combined UV-Vis, MCD, EPR and computational study. EXAFS showed that the 5-coordinate active site geometry of this Mo(V) enzyme form had four S-donors at a distance 2.354 Å and a single oxygen scatterer at a distance 1.99 Å from the Mo ion as shown in Figure 3.3b. The best fit EXAFS parameters for this five coordinate species was also supported from the minimum root mean square bond deviation (RMSD, 0.044) of the EXAFS determined structure and a constrained computational model of the Mo site.

3.1.3.3 Conclusions from previous structural data (X-ray Crystallography and EXAFS)

Despite extensive studies of X-ray crystal structures and Mo-Kedge EXAFS data for DMSOr enzymes from *R. sphaeroides* and *R. capsulatus*, there is a lack of consistency on the views about the coordination environment of the DMSOr active site. There is supporting evidence from X-ray crystallography for a distorted trigonal prismatic structure for the oxidized active site, and from EXAFS a square pyramidal structure for the reduced form of the enzyme. However, a debate remains regarding the EPR active species as to whether it is a five or six coordinate
structure. Understanding the structure of the EPR active intermediate is crucial for understanding the nature the electron transfer half reaction in the catalytic cycle of the DMSOr enzyme. Some earlier spectroscopic results on this high-g split species will be discussed in the following sections.

3.2 Spectroscopic studies on the high-g split DMSOr catalytic intermediate

A DMSOr bona fide catalytic intermediate (I_{Mo(v)}) was trapped under turnover conditions using TMAO as the substrate. This intermediate was studied via electronic absorption, MCD, EPR and computational techniques.¹⁰ The results of the study revealed that the intermediate (I_{Mo(v)}) has a distorted trigonal prismatic structure. Interestingly, the transition state geometry in the oxo transfer process is very similar to that of the fully optimized structure for the computational intermediate model. We will review some key spectroscopic studies on high-g split DMSOr in the following sections.

3.2.1. Electronic absorption spectroscopy

Due to the absence of other chromophores in the DMSOr subfamily, optical spectroscopy has been a very useful probe of the active site electronic structure. UV-Vis and MCD spectroscopic studies have been performed on DMSOR enzymes obtained from R. sphaeroides¹⁰,ª and R. captulatus.²¹ Bastian and coworkers collected electronic absorption data for oxidized DMSOr from R. sphaeroides and observed absorption bands at 13889 cm⁻¹, 18182 cm⁻¹, 21277 cm⁻¹, 28571 cm⁻¹ and 35714 cm⁻¹.²⁰ In 2011, Kirk and coworkers studied the
electronic absorption spectra of DMSO\textsubscript{r} high-g split from \textit{R. sphaeroides} and observed bands at 12150 cm\textsuperscript{-1}, 14960 cm\textsuperscript{-1} and 18525 cm\textsuperscript{-1} spanning the near-IR and UV-Visible regions. From MCD spectroscopy, Kirk and coworkers observed negative C-term bands at 12375 cm\textsuperscript{-1} and 15375 cm\textsuperscript{-1} and a positive C-term band at 15660 cm\textsuperscript{-1} (Figure 3.4). The low energy charge transfer band (~12000 cm\textsuperscript{-1}) was assigned as the transition from the HOMO ($d_{z^2}$ orbital; $S_0$ in Figure 3.4) to the LUMO and LUMO+1 ($d_{xy}$ and $d_{x^2-y^2}$; $U_0$ and $U_0+1$) respectively. The charge transfer bands at ~15000 cm\textsuperscript{-1} and ~18000 cm\textsuperscript{-1} were assigned as transitions from the ligand-centered $D_0$ (HOMO-1) orbital to the $U_0+1$ and $U_0$ orbitals, respectively.

3.2.2 EPR spectroscopic studies on the DMSO\textsubscript{r} intermediate ($I_{Mo(V)}$)

Multiple research groups have used electron paramagnetic resonance (EPR) spectroscopy to study the DMSOR from \textit{R. sphaeroides} and \textit{R. capsulatus}.\textsuperscript{10,22,23} In all these studies, a rhombic g-tensor has been observed. An EPR study of DMSOR from \textit{R. sphaeroides} by Bastian and coworkers yielded g-
values of $g_1 = 1.998$, $g_2 = 1.977$ and $g_3 = 1.961$. The EPR active form of the enzyme was obtained by benzyl viologen reduction of the fully oxidized enzyme.\textsuperscript{20} In another EPR study of DMSOr enzyme from the same species by Kirk and coworkers, rhombic $g$-tensors were observed yielding $g_1 = 1.999$, $g_2 = 1.989$ and $g_3 = 1.972$ and the data is shown in Figure 3.5.\textsuperscript{10} These researchers investigated the high-$g$ split EPR species obtained from the dithionite reduction of TMAO-oxidized form of the enzyme. The high-$g$ split species was trapped in near quantitative yield under turnover conditions. An EPR study of the enzyme from \textit{R. capsulatus} by Benson and coworkers showed a rhombic $g$-tensor with $g_1 = 1.99$, $g_2 = 1.98$ and $g_3 = 1.96$,\textsuperscript{23} while a similar study of the enzyme from \textit{R. capsulatus} by Bennett and coworkers, using different preparation procedures, displayed different $g$-values.\textsuperscript{23} The different $g$-values obtained for the enzyme likely arise from the plasticity of the DMSOr enzyme active site, as described by Schindelin and coworkers in their work on the crystal structure.\textsuperscript{24}
Guigliarelli and coworkers have developed magnetostructural correlations for a variety of pyranopterin Mo/W enzymes. Based on their observations, the nature of the g-tensor mainly depends on the type of ligands that are bound to Mo in the first coordination sphere. Slight variations in g-values depend on the bond lengths, dihedral angles, experimental pH changes, etc. in the protein. Plots of g-tensor anisotropies vs g_{ave} are shown in Figure 2.16 (Chapter 2). For DMSOr and Nar enzymes, the higher rhombicity (g_{1}-g_{3}) appears to correlate primarily with an increase in g_{1} (Figure 3.6). All of these enzymes (functional) are similar in that they possess four dithiolene S donors, one O_{Ser} donor, and one OH/OH_{2} bound to Mo. In 2018, Leimkuhler and coworkers’ study on the TMAOr enzyme showed the sixth ligand to be a S donor and not OH/OH_{2}. It is also possible that the O_{Ser} donor might have been detached during enzymatic catalysis. According to their argument, the previously observed oxygen donor ligand might replace a sulfur
donor during enzyme isolation, purification, or preparation. Their observations made us rethink our assumptions about the structure of DMSOr enzyme sub-families II and III. To understand thoroughly the correlation of EPR g-values and g-tensor anisotropies, we performed EPR computations on a model system (Figure 3.7), as proposed by Leimkuhler.\textsuperscript{26} Leimkuhler’s TMAO EPR data (1 in figure 3.6) are comparable to that of the computed data for the model (Figure 3.7; 2 in Figure 3.6). This model (Figure 3.7) is a monodithiolene structure and its computed spin-Hamiltonian parameters correlate well to that of low-pH sulfite oxidase.\textsuperscript{27} So, from the \textit{g} anisotropy analysis of computational models, synthetic models, and enzymes, it appears most likely that Leimkuhler’s TMAO structure might be from degraded enzyme and likely contains only single PDT bound to Mo.

X-ray crystallography, EXAFS, MCD, and EPR studies on DMSOr enzymes provide evidence for different active site structures. Thus, we have synthesized and studied a new high-\textit{g} split model system (Figure 3.8) and have interrogated their electronic and geometric structures using a variety of spectroscopic techniques. This study will help us to understand the nature of the high-\textit{g} split species and its role in the catalytic cycle of DMSOr.

Figure 3. 7: Proposed computational model from Leimkuhler’s TMAO enzyme active site structure interpretations\textsuperscript{24}.

Figure 3. 8: Model framework for the DMSOr catalytic intermediate, \textit{I}_{Mo(V)}.
3.3 Synthesis of new paramagnetic models for the “high-g split” intermediate

3.3.1 Synthesis of [Mo(V)(cydt)$_2$Cat]$^{1-}$ (1):

**Solvent preparation:** Acetonitrile was first dried with powdered KOH and then filtered. The filtrate was distilled overnight in P$_2$O$_5$ under a N$_2$ atmosphere. THF and diethyl ether were distilled in Na/Benzophenone. All these distilled solvents (CH$_3$CN, THF, and diethyl ether) were then stored over molecular sieves for three days.

For the synthesis of complex 1, 35 mg KBut'O was dissolved in 6 ml THF in a 10 ml round bottom flask. 30 mg 3,5-di-tert-butylcatechol was dissolved in 5 ml THF in another 10 ml round bottom flask. Both solutions were flushed with dry N$_2$ gas for 30 minutes, then the catechol solution was transferred into the KBut'O solution that was pre-cooled to -10 °C. The mixture was stirred for 15 minutes. The solution was then subsequently transferred into a 50 ml round bottom flask containing a partially dissolved solution of Mo(cydt)$_2$(CO)$_2$ (30 mg in 6 ml CH$_3$CN) flushed with N$_2$ at -10°C. The mixture was stirred for 15 minutes and then a
benzyltriethylammonium chloride solution (30 mg in 5 ml CH$_3$CN) was added. The blue colored solution persisted for a few hours and then changed to a purple color. The solution was left to continue stirring overnight, then the solution was dried by purging with N$_2$ gas. The resulting dried powder was re-dissolved in CH$_3$CN and filtered through Ceilite545 under N$_2$ protection. The filtrate was transferred into a 100 ml flask containing about 30 ml ether, then additional ether was added to produce a precipitate. The supernatant liquid was removed through a canula with positive N$_2$ pressure. This recrystallization procedure was repeated twice, and the product was dried under N$_2$ gas and was kept in glovebox antechamber for 3-4 hours. Yield was 25 mg (46%). The complex was then characterized by mass spectrometry (observed mass 606.0673 & theoretical mass 606.0652), EPR, and UV-Vis-NIR. The nature of the coordinating ligands to Mo were determined from EXAFS. Since the complex is highly unstable, a crystal structure determination was not successful. The elemental analysis for this intermediate was also unsuccessful due to the highly unstable nature of the complex.

3.3.2 Synthesis of [(Mo(V)(pdt)$_2$Cat)]$^{1-}$ (2):

Synthetic (Scheme 3.3) and characterization methods for complex 2 were similar to that for complex 1. To synthesize this complex, 210 mg of KBut$^t$O (in 10 ml THF) was treated with 100 mg of 3,5-di-tert-butylcatechol (in 10ml THF). The acid-base mixture was added to 100 mg of Mo(pdt)$_2$(CO)$_2$ (10 ml acetonitrile) dissolved in acetonitrile. 70 mg of benzyltriethylammonium chloride in 10 ml acetonitrile was then added to the mixture. The green color persisted for few hours
and upon stirring overnight the color changed to a reddish purple. After two recrystallizations, the yield was 70 mg (44.6%). The complex was characterized by mass spectrometry (observed mass 802.0970 and theoretical mass 802.0965), EPR, and UV-Vis-NIR. The nature of the coordinating ligands to Mo were determined using EXAFS. Since the complex was highly unstable (albeit, more stable compared to complex 1) we were not to obtain a crystal structure. The elemental analysis for this complex was unsuccessful.

### 3.4. Characterizations of DMSO reductase enzyme models 1 and 2

We were able to get both mass spectrometry data and EXAFS data, and we used these to analytically characterize our samples. Due to the instability issue with 1 and 2, X-ray crystallography and elemental analysis were not successful.
3.4.1 Mass spectrometry

Mass spectrometry data for complexes 1 and 2 (Figure 3.9) were determined by the electron spray ionization (ESI) technique at the Mass Spectrometry Facility in the Department of Chemistry and Chemical Biology at The University of New Mexico. The mass of complex 1 was determined to be 606.065 (theoretical 606.062) and the observed mass for complex 2 was determined to

Figure 3. 9: Mass spectra of 1 (upper) and 2 (lower); full spectrum (left) and isotope patter (left; computed above and experimental lower each of the complexes)
802.098 (theoretical 802.097). In our application of ESI mass spectrometry, the solution sample had to be exposed to air once before injecting the sample into the instrument. Thus, there is the possibility for some air sensitive samples to partially decompose. Our bis-dithiolene Mo complexes commonly decompose into more thermodynamically stable complexes such as \([\text{MoO(dithiolene)}_2]^n\), thiol-bridged Mo dimeric structures, and most commonly the \([\text{Mo(dithiolene)}_3]^n\) tris-dithiolene.\(^{28}\) As we see in the full mass spectra of complexes 1 and 2 (Figure 3.9), we observe as fragments the bis-cyclohexenedithiolene molybdenum-oxo complex peak (mass \(~401\)) and the bis-1,2-dipheylethenedithiolene molybdenum-oxo complex peak (mass \(~595\)) respectively.

### 3.4.2 EPR spectroscopy

EPR cannot be used as a single means to properly characterize synthetic complexes. Nevertheless it can be a powerful supplementary tool along with EXAFS and mass spectrometry to characterize highly air-sensitive molecules like models 1 and 2. To address whether our complexes are five or six coordinate, we optimized and performed EPR calculations on the computational models (i), (ii), (iii) and (iv) (Figure 3.10). All these models are potential derivative structures for compound 2. Computational model (i) is the same as the optimized structure of complex 2 except one of the oxygens in (i) has been replaced by hydrogen and this hydrogen was optimized. The DFT computed EPR g-values for (i) are markedly lower than that of complex 2 (Table 3.1). Poor agreement between the
spin-Hamiltonian values for 2, and (i) – (iv) strongly support a 6-coordinate structure for these new models.

A combination of EXAFS and an associated bond valence sum (BVS) analysis can be employed to further characterize the coordination number of complexes 1 and 2 given that the Mo(V) oxidation state has been determined by EPR. According to this approach, the total BVS contribution from the first coordination donors of a complex should be equal to the oxidation state of the metal. We performed a BVS calculation for (i) and determined a BVS of 3.7, whereas for 2 this value is 4.7 when using EXAFS determined bond lengths and 4.4 using bond lengths computed for a fully optimized structure (Table 3.2). To obtain additional information regarding the coordination number, we fully optimized the five-coordinate structure (i) to obtain structure (iv). As noted previously, the

![Figure 3.10: Optimized structures of synthetic (2) and computational (i-iv) models. In (i) one of the ‘O’ has been replaced (from model 2) by H and optimized (H only). (ii) is same as (i) but phenolate is replaced by ‘OCH3’. In (iii) dithiolene dihedral angle is constrained from 2. Structure (iv) is fully optimized.](image-url)
computed g-values for (iv) are not similar to those obtained for 2. Additionally, the Mo dipolar hyperfine coupling constants, $A_{\text{dip}}$, computed for (iv) are axial whereas the experimental and computed $A_{\text{dip}}$ couplings for 2 are rhombic. The BVS for model (iv) is 4.6, which is closer to the EPR-determined oxidation state of Mo. Thus, from our BVS study and the computed EPR spin Hamiltonian parameters for models (i) – (iv), we show that complexes 1 and 2 are six-coordinate with 4 S donors and 2 O donors bound to Mo.

### Table 3. 2: Bond lengths and BVS for 2 and computational models

<table>
<thead>
<tr>
<th>Bond length and BVS of complexes</th>
<th>$\text{Mo-S}$ (Å)</th>
<th>$\text{Mo-O}$ (Å)</th>
<th>BVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2) (Experimental)</td>
<td>2.380</td>
<td>2.015</td>
<td>4.7</td>
</tr>
<tr>
<td>(Computed)</td>
<td>2.402</td>
<td>2.026</td>
<td>4.4</td>
</tr>
<tr>
<td>(i) (Computed)</td>
<td>2.402</td>
<td>2.026</td>
<td>3.7</td>
</tr>
<tr>
<td>(ii) (Computed)</td>
<td>2.402</td>
<td>2.026</td>
<td>3.7</td>
</tr>
<tr>
<td>(iii) (Computed)</td>
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<td>4.5</td>
</tr>
<tr>
<td>(iv) (Computed)</td>
<td>2.346</td>
<td>1.856</td>
<td>4.6</td>
</tr>
</tbody>
</table>
3.5. XAS and EXAFS experiments on model compounds 1 and 2

3.5.1. Experimental

Mo-K edge XAS data (Figure 3.11) for complexes 1 and 2 were collected at SSRL on beamline 7-3. BL 7-3 has 20 magnetic poles at 2T magnetic field. The photon energy at BL 7-3 is 5-33 keV. A liquid N\textsubscript{2} cooled Si double beam monochromator was used for energy (~20000 eV) selection and it was calibrated using the energy established for a Mo foil standard. The temperature was maintained ~11 K with a He cryostat and data were collected in fluorescence mode using a Lytle detector.

![Figure 3.11: Mo-Kedge XAS spectra of complex 1 and complex 2 (inset XANES region zoomed in)](image-url)
3.5.2 Data observation and analysis

Background subtracted Mo K-edge XAS data were analyzed using the IFEFFIT program within the Artemis interface. Using this program, the EXAFS is calculated via equation 3.1.29,30 Despite some of its pitfalls31, EXAFS yields valuable data that allows for the determination of the coordination number (N), type of ligand (A), as well as the absorber-scatterer distance (R). EXAFS is particularly useful for accurate determinations of R. Due to the correlation of parameters in equation 3.1, extreme care must be taken when evaluating the coordination number. Complexes 1 and 2 were characterized by mass spectrometry, UV-Vis, and EPR spectroscopy. As a result,

$$\chi(k) = \sum \frac{NA(k)S^2_0}{kR^2} \exp\left(-\frac{2R}{\lambda(k)}\right) \exp(-2k^2\sigma^2) \cdot \sin(2kR + \phi(k))$$

(3.1)

we were able to fix the coordination number at six. Initial input parameters for R were obtained from density functional theory (DFT) geometry optimizations for models of complexes 1 and 2. The Debye-Waller factor ($\sigma^2$) and amplitude reduction factor ($S_0^2$) were then obtained within a reasonable range for a MoS$_4$O$_2$ coordination sphere. We obtained $S_0^2 \sim 1$ and $\sigma^2 \sim 0.0035$ (Mo-O shell) and $\sigma^2 \sim 0.002$ (Mo-S shell) for both complexes (Table 3.3 for 1 and 3.4 for 2). The observed EXAFS oscillations are a result of the interaction between outgoing photoelectrons and the backscattering of photoelectrons after the creation of the core hole.32 The EXAFS amplitude is reduced due to both intrinsic and extrinsic, which depends on the mean free path, $\lambda$, of the photoelectrons, properties. As an intrinsic property, some photoelectrons may interact with secondary (passive) electrons to cause (i) the incomplete overlap of initial and final orbitals after core hole creation, (ii) shake off, and (iii) shake up
phenomenon leading to the EXAFS amplitude being reduced.\textsuperscript{32} Rehr and coworkers introduced a reduction factor, $S_0^2$, into the EXAFS equation in order to correct the amplitude.\textsuperscript{30} The probability of the excitation of secondary electrons ($P(\omega)$) and amplitude reduction factors are related by $S_0^2 = (1-P(\omega))$.\textsuperscript{33} Therefore, $S_0^2$ should theoretically not be greater than unity for a homogeneous and thin sample.\textsuperscript{34} Inhomogeneity and sample thickness can affect the EXAFS amplitude.\textsuperscript{35} There is still debate\textsuperscript{32,36} on the transferability of $S_0^2$ from one sample to another, even when data are collected on the same beamline and during the same run. If the absorber is in a different chemical environment, such as Mo in Mo foil versus Mo in MoO$_4$, the value for $S_0^2$ cannot be transferable from one sample to another.\textsuperscript{32} In our experience, the value of $S_0^2$ is also dependent on the quality of the data, specifically with respect to

### Table 3.3: EXAFS fitting (R-space) parameters for 1

<table>
<thead>
<tr>
<th>N</th>
<th>Mo-S</th>
<th>$\sigma^2$</th>
<th>N</th>
<th>Mo-O</th>
<th>$\sigma^2$</th>
<th>$S_0^2$</th>
<th>$\Delta E_0$</th>
<th>$R_i$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fit1 4</td>
<td>2.393(2.407)</td>
<td>0.0020</td>
<td>2</td>
<td>1.983(2.030)</td>
<td>0.0021</td>
<td>0.916</td>
<td>0.258</td>
<td>1.03</td>
</tr>
<tr>
<td>Fit2 4</td>
<td>2.396(2.407)</td>
<td>0.0021</td>
<td>1</td>
<td>2.006(2.030)</td>
<td>-0.0008</td>
<td>0.916</td>
<td>1.327</td>
<td>1.30</td>
</tr>
<tr>
<td>Fit3 4</td>
<td>2.394(2.407)</td>
<td>0.0028</td>
<td>1</td>
<td>2.022(2.030)</td>
<td>-0.0010</td>
<td>1.013</td>
<td>1.060</td>
<td>1.14</td>
</tr>
<tr>
<td>Fit4 5</td>
<td>2.393(2.407)</td>
<td>0.0030</td>
<td>1</td>
<td>2.027(2.030)</td>
<td>-0.0017</td>
<td>0.916</td>
<td>-1.154</td>
<td>1.28</td>
</tr>
<tr>
<td>Fit5 6</td>
<td>2.389(2.407)</td>
<td>0.0044</td>
<td>0</td>
<td></td>
<td></td>
<td>0.916</td>
<td>0.08</td>
<td>3.58</td>
</tr>
</tbody>
</table>

### Table 3.4: EXAFS fitting (R-space) parameters for 2

<table>
<thead>
<tr>
<th>N</th>
<th>Mo-S</th>
<th>$\sigma^2$</th>
<th>N</th>
<th>Mo-O</th>
<th>$\sigma^2$</th>
<th>$S_0^2$</th>
<th>$\Delta E_0$</th>
<th>$R_i$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fit1 4</td>
<td>2.380(2.402)</td>
<td>0.0027</td>
<td>2</td>
<td>2.015(2.026)</td>
<td>0.0037</td>
<td>1.039</td>
<td>0.43</td>
<td>1.44</td>
</tr>
<tr>
<td>Fit2 4</td>
<td>2.381(2.402)</td>
<td>0.0026</td>
<td>1</td>
<td>2.041(2.026)</td>
<td>0.0015</td>
<td>1.039</td>
<td>0.87</td>
<td>1.60</td>
</tr>
<tr>
<td>Fit3 4</td>
<td>2.378(2.402)</td>
<td>0.0033</td>
<td>1</td>
<td>2.030(2.026)</td>
<td>-0.0003</td>
<td>1.172</td>
<td>0.33</td>
<td>1.38</td>
</tr>
<tr>
<td>Fit4 4</td>
<td>2.375(2.359)</td>
<td>0.0024</td>
<td>1</td>
<td>1.859(1.866)</td>
<td>-0.0008</td>
<td>1.039</td>
<td>-0.87</td>
<td>1.87</td>
</tr>
<tr>
<td>Fit5 5</td>
<td>2.375(2.402)</td>
<td>0.0041</td>
<td>1</td>
<td>2.030(2.026)</td>
<td>-0.0016</td>
<td>1.039</td>
<td>0.05</td>
<td>1.49</td>
</tr>
<tr>
<td>Fit6 6</td>
<td>2.367(2.402)</td>
<td>0.0058</td>
<td>0</td>
<td></td>
<td></td>
<td>1.039</td>
<td>-1.13</td>
<td>3.81</td>
</tr>
</tbody>
</table>
how large of a k-range or R-range is used in the spectral fitting procedure. For complexes 1 and 2, when the coordination number is fixed at five (MoS₄O₁ coordination sphere) the amplitude reduction factor increases and the Debye-Waller factor for the Mo-O shell becomes too low to achieve a similar fit to a 6-coordination model. However, when σ² and S₀² are constrained for N = 5 from the fit1 (MoS₄O₂ coordination sphere), the R-factor is increased by ~1%. From our study of these two DMSO₅ model complexes and the Fdh and Nap model complexes detailed in chapter 2, good fits to the data can be achieved for different N values by varying the amplitude reduction factor.

Figure 3.12: EXAFS (Fourier transformed) six- vs five-coordinate fits for complex 1 (upper two) and complex 2 (lower two).
3.5.3. Bond valence sum (BVS) analysis

EXAFS is a powerful tool for determining the local structure of metallocofactors in enzymes and their small molecule analogs. Bond distances can be determined by this technique more accurately than their coordination numbers. EXAFS determined bond distances can be used to evaluate the coordination number by using a bond valance sum (BVS) argument. According to this argument, the sum of metal-ligand bond valences should be equal to the oxidation state of the metal center. This is given by the equation, \( \text{BVS} = e^{(r_0 - r)/B} \).

Brown has used the empirical parameter, \( r_0 \), obtained from BVS tables or by fitting to BVS equations with the parameter B, which is usually taken as 0.37. BVS analyses are commonly used to determine the oxidation state of the metal center using EXAFS determined metal-ligand bond distances (\( r \)). Conversely, if the oxidation state is known, the BVS approach can be used to better define the coordination number. Using EXAFS determined bond distances from fitting to our R-space data (Table 3.3 and 3.4), the BVS for both complexes was determined to be 4.7. This is remarkably close to 5, the EPR-determined oxidation state for these complexes. From George’s EXAFS work on the structure of the high-g split DMSOR intermediate, the BVS determined for a 6-coordination (MoS\(_4\)O\(_2\)) site yields 5.0.

Thus, the coupling of a BVS analysis with EXAFS and EPR data can be used to provide very accurate assessments of metal-ligand bond distances, oxidation states, and coordination numbers for both metalloenzymes and their small molecule analogs.
3.6 EPR Spectroscopy

3.6.1 Experimental

EPR data were collected at X-band using a Bruker EMX spectrometer with associated Bruker magnet control electronics and microwave bridges. The microwave frequency was ~9.39 GHz (For complexes 1 and 2 at RT/77K microwave frequencies were 9.3970/9.3881GHz and 9.4102/9.3873 GHz respectively). The modulation amplitude was set at ~1mT and the frequency modulation was adjusted 20 MHz for complex 2 and 100 MHz for complex 1 at low temperature. At room temperature, the frequency modulation was fixed at 100 MHz for all the complexes. The field attenuation was set at 30 dB. The Mo(V) EPR resonance field was observed at approximately 330mT. For the low temperature EPR experiments, a liquid helium cooled cryostat was used, and the sample space was purged with a positive pressure of N₂ gas. Since both the complexes are highly unstable, the EPR samples were prepared using a Schlenk line. The compounds were dissolved in n-butyronitrile (BN) for low temperature studies and were dissolved in acetonitrile for the room temperature EPR experiments, and subsequently transferred by canula under positive nitrogen pressure to the N₂ purged dry EPR tubes. For low temperature EPR measurements, the sample in the EPR tube was immersed, slowly, into liquid N₂ to ensure a proper glass was formed. In these studies, the use of toluene and DCM as solvents was avoided due to their incompatibility with the samples. We also tried to prepare the samples in distilled 2-methyl-THF, but the samples were also incompatible in this solvent.
3.6.2 Data collection and analysis

The X-band EPR spin-Hamiltonian parameters of model complexes 1 and 2 are in close agreement with those previously obtained for the high-g split DMSO\textsubscript{r} catalytic intermediate. The magnitude of the g-values reflect a combination of spin-orbit coupling, covalency, and charge transfer excited states,\textsuperscript{40} while the Mo hyperfine coupling (A\textsubscript{Mo}) contains Fermi contact, dipolar, and indirect dipolar contributions.\textsuperscript{41, 42} The anisotropy in A\textsubscript{Mo} is reflected in the spin density distribution, and the spin density distribution in the molecule closely resembles the shape and of the singly occupied quasi restricted molecular orbital (QRO, SOMO) or, in a spin-unrestricted DFT calculation, the \(\beta\)-LUMO. We have observed that slight variations in bond lengths and dithiolene dihedral angles (twist angle, Figure 3.13) changes the relative orientation of the g- and A-tensors.\textsuperscript{25} Spectral simulations of complexes 1 and 2 reveal similar EPR spin-Hamiltonian parameters (Table 3.5) and similar Euler angles (Figure 3.14, Table 3.5), and these are comparable to those obtained previously for DMSO\textsubscript{r} high-g split.\textsuperscript{10} In marked contrast to the DMSO\textsubscript{r} high-g split intermediate, which displays a highly resolved sharp EPR spectrum, models 1 and 2 needed to be simulated using a higher degree of g-strain (Figure 3.15). The observed spectral broadening for 1 and 2 is due to sample heterogeneity that is
likely a function of a distribution of molecular geometries that arise from low-frequency distortions that are frozen out during cooling of the sample in the cryostat. Effectively, these small structural variations result in a distribution of the spin Hamiltonian parameter values. For the high-\( g \) split intermediate, the constraints of the protein matrix appear to yield a markedly more narrow distribution of structural conformers and a sharp EPR spectrum is observed.\(^{43} \)

**Figure 3.14:** Orientations of \( A-g \) tensors along with respective \( \beta \)-LUMO in complex 1 (left) and 2 (right)

**Table 3.5:** EPR spin-Hamiltonian parameters of complexes 1 and 2

<table>
<thead>
<tr>
<th>Enzyme/Model</th>
<th>( g_1 )</th>
<th>( g_2 )</th>
<th>( g_3 )</th>
<th>( g_{\text{ave}} )</th>
<th>( A_{\text{Mo}} )</th>
<th>( A_{\text{e}} )</th>
<th>( A_{\text{d}} )</th>
<th>( \alpha, \beta, \gamma )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSOR</td>
<td>1.9988</td>
<td>1.9885</td>
<td>1.9722</td>
<td>1.9865</td>
<td>17.78</td>
<td>-20.89</td>
<td>3.11</td>
<td>34.22 ( 15.5, 91.7 &amp; 11.5 )</td>
</tr>
<tr>
<td>Complex 1</td>
<td>2.0110</td>
<td>1.9856</td>
<td>1.9636</td>
<td>1.9867(1.9836)</td>
<td>13.54</td>
<td>-22.29</td>
<td>9.08</td>
<td>33.62(33.02) ( 3.66, 75.63 &amp; 1.03 )</td>
</tr>
<tr>
<td></td>
<td>2.0121</td>
<td>1.9869</td>
<td>1.9639</td>
<td>1.9877</td>
<td>11.25</td>
<td>-20.65</td>
<td>9.51</td>
<td>41.07 ( 5.8, 67.0 &amp; -2.3 )</td>
</tr>
<tr>
<td>Complex 2</td>
<td>2.0066</td>
<td>1.9881</td>
<td>1.9655</td>
<td>1.9867(1.9843)</td>
<td>16.38</td>
<td>-17.76</td>
<td>1.39</td>
<td>32.28(31.69) ( -0.88, 58.16 &amp; 3.32 )</td>
</tr>
<tr>
<td></td>
<td>2.0028</td>
<td>1.9889</td>
<td>1.9602</td>
<td>1.9840</td>
<td>15.98</td>
<td>-18.00</td>
<td>3.02</td>
<td>38.62 ( -5.8, 67.3 &amp; 2.2 )</td>
</tr>
</tbody>
</table>
Our EPR spectral simulations indicate that complex 1 possesses a rhombic dipolar hyperfine coupling tensor, while complex 2 possesses an axial dipolar hyperfine tensor. The rhombic nature of the dipolar hyperfine couplings was previously observed in the DMSO\textsubscript{r} high-g split intermediate. This has been interpreted as having a SOMO that is formed from a specific admixture of canonical d-orbitals.\textsuperscript{10} For model complex 2, one of the twist angles is small and this effectively lowers the symmetry of the molecule leading to an admixture of canonical d-orbitals to give a hybrid-type singly occupied quasi-restricted molecular orbital (QRO, SOMO) that is responsible for the rhombic hyperfine couplings determined from EPR spectroscopy. The \(\lambda\)-twist structure for the complex 1 and \(\Delta\)-twist structure for the
complex 2 have lower optimized energies (by ~1500 cm$^{-1}$) than their corresponding isomers. So, throughout Chapter 3, the $\lambda$ isomer for complex 1 and the $\Delta$- isomer for complex 2 will be discussed. The presence of a rhombic dipolar hyperfine tensor in model 2 more closely mimics the nature of the Mo hyperfine tensor in high-g split than the axial nature of the dipolar hyperfine tensor observed for complex 1.

The high-g split DMSOr intermediate and specific small molecule analogs of this intermediate display rhombic $^{95,97}$Mo dipolar hyperfine tensors. If the electron spin is located in an orbital with a high degree of s-orbital character, or if the spin density (SD) distribution is such that it is highly delocalized on to the ligands, the $^{95,97}$Mo dipolar hyperfine coupling is reduced, and the hyperfine interaction is dominated by the isotropic Fermi contact term. If two components of the dipolar A-tensor are negative and one is positive, the SOMO has a $d_{x^2-y^2}$ appearance. If two components of the A-tensor are positive and one is negative, the SOMO orbital is a $d_{z^2}$ type orbital.\textsuperscript{10} If the unpaired spin on Mo is predominantly localized in an orbital that resembles one of the five canonical d-orbitals, the spin dipolar component of the $^{95,97}$Mo hyperfine tensor is axial. However, a rhombic dipolar hyperfine tensor can occur if specific canonical d orbitals mix in a low-symmetry environment. We investigated the origin of rhombic dipolar hyperfine tensors in our EPR active DMSOr model complexes. Interestingly, one of our DMSOr high-g split model complexes possesses axial dipolar hyperfine tensor (complex 1) and the other possesses a rhombic dipolar hyperfine tensor (complex 2). Inspection of the computed dithiolene dihedral angles in the optimized
structures of both complexes reveal that the dihedral angle for both is ~131°. Then, we computed the respective twist angles for these complexes using the twist angle definition used by Donahue and coworkers.\(^{44}\) All three of the computed twist angles (Figure 3.13, \(\lambda\)) in model complex 1 are comparable. However, for model complex 2 (Figure 3.13, \(\Delta\)), which has a rhombic dipolar hyperfine tensor, two twist angles are comparable to one another and the third is smaller. We came to the conclusion that this twist angle anisotropy lowers the symmetry in complex 2 and causes the mixing of canonical d-orbitals. To illustrate that twist angle anisotropy is responsible for whether the dipolar hyperfine coupling, \(A^\text{Mo}\), is rhombic or axial we performed computational calculations on \([\text{Mo}(V)(\text{mdt})_2(\text{OMe})(\text{OH})]^{1-}\) (Table A3, Appendix C). From the fully optimized structure of this model, we obtained a rhombic Mo dipolar hyperfine coupling, \(A^\text{Mo}\). The Mo dipolar hyperfine couplings and the twist angles for the complex \([\text{Mo}(V)(\text{mdt})_2(\text{OMe})(\text{OH})]^{1-}\) are similar to complex 2. We then performed a constrained optimization, making the three twist angles comparable (~30°) to that of complex 1 and we computed an axial dipolar hyperfine coupling, \(A^\text{Mo}\). We also tested this trend in the complex with monodentate ligands, \([\text{Mo}(V)(\text{SH})_4(\text{OH})_2]^{1-}\) (Table A3, Appendix C), and we saw similar computed EPR results as we did for complexes 1 and 2. However, when we constrained all the twist angles (in complex with 6 monodentate ligands) to zero and optimized the geometry, the dihedral angle (defines as \(S_1S_2S_3S_4\) in Figure 3.16) was ~180°. The EPR result for this structure also indicate a rhombic dipolar hyperfine coupling. This tells us that when the dithiolene dihedral angle is large in a bis-dithiolene Mo molecule, it allows the mixing of canonical d-orbitals and gives a rhombic \(A^\text{Mo}\).
Thus, there are different ways to mix the canonical d-orbitals that yield a rhombic $A^{\text{Mo}}$. The rhombicity of the dipolar hyperfine couplings might be due to either high twist angles anisotropy or due to having a large dithiolene dihedral angle.

3.7 Mechanistic significance of the “high-g split” intermediate.

As discussed in previous sections, there is some controversy regarding the coordination number and geometry of the DMSOR high-g split species that primarily focuses on whether this is a six-coordinate or five-coordinate intermediate. Understanding the nature of the coordination geometry in this intermediate is important, since the nature of high-g split defines the catalytic sequence downstream of this intermediate. As such, we have investigated potential structures for high-g split computationally using DFT methods. We performed a series of Mo-O$_{\text{Ser}}$ bond scan calculations on the 6-coordinate enzyme model, [Mo(V)(pyranDT)$_2$(O$_{\text{Ser}}$)OH]$^+$ (Figure A20, Appendix F), while keeping the dithiolene dihedral angle at the value observed in the crystal structure of DMSOR (PDB→1eu1$^4$). Our calculations show that as the Mo-O$_{\text{Ser}}$ bond in lengthened, the O$_{\text{Ser}}$ abstracts the proton from the hydroxide coordinated to Mo in a manner consistent with their relative pK$_a$’s. When the Mo-O$_{\text{Ser}}$ bond is increased to a length of ~2.5 Å (mechanisms 2 and 3 Figure 2.16) the serine becomes fully protonated. Thus, we hypothesize that if O$_{\text{Ser}}$ dissociates to form a 5-coordinate high-g split structure, this would lead to a protonated serine. No strongly basic amino acid is observed in the vicinity of the serine residue in the crystal structure$^4$ of DMSO that could promote deprotonation of this detached serine.
Strong proton hyperfine coupling (~30MHz) is observed in the EPR spectrum of high-g split\textsuperscript{10}, and this supports the presence of a coordinated hydroxide ligand bound to Mo. It is known that high-g split accumulates under turnover conditions using DMSO as the oxidizing substrate, and this intermediate builds up to ~100\% when the substrate is TMAO. The rate-limiting step with DMSO as substrate is product (DMS) release, while Mo(V) reduction is rate-limiting with TMAO. This implies a significant activation barrier for the Mo(V) \( \rightarrow \) Mo (IV) step in the catalytic cycle. Since this is an electron transfer step, we hypothesized that the nature of the high-g split \( \beta \)-LUMO could provide information regarding this rate limiting electron transfer step. Our DFT computations and S-kedge data, which will be discussed in greater detail in Chapter 4, indicate that the \( \beta \)-LUMO wave function in models \textit{1} and \textit{2} (Figure 2.17) possesses a very high degree of Mo d-orbital character with very little ligand contribution. There is a high degree of electron-
electron repulsion associated with placing two electrons in a highly localized orbital on Mo, and this is reflected in the large exchange splitting computed for the high-g split species. This contributes to the stability of the Mo(V) state and lowers the reduction potential of the Mo(V/IV) couple. Likely, the rate for Mo(V) \( \rightarrow \) Mo (IV) becomes limiting only when the Mo(V) geometry is 6-coordinate. In the 5-coordinate structure, the \( \beta \)-LUMO wavefunction (Figure 3.17) is delocalized onto the dithiolene structures.

Serine detachment from 6-coordinate [Mo(PDT)\(_2\)O\(_{\text{Ser}}\)(OH)]\(^{1-}\) results in serine protonation and the formation of a 5-coordinate Mo(V)-oxo species, [MoO(PDT)\(_2\)]\(^{1-}\), which would have to acquire at proton (e.g. [Mo(PDT)\(_2\)(OH)]\(^{1-}\)) to account for the observed proton hyperfine in high-g split. Since such a 5-coordinate structure (Figure 3.18) is not compatible with observed EPR spin-Hamiltonian parameters (Table 3.6) or our combined EXAFS/BVS analysis, a pentacoordinate structure with a dissociated serine ligand seems highly unlikely. In order to further

![Figure 3.17: \( \beta \)-LUMO of 2 (left) and model [Mo(V)(pyran-dt)\(_2\) (O\(_{\text{Ser}}\))(OH)]\(^{1-}\) (middle) and [Mo(V)(pyran-dt)\(_2\) (OH)]\(^{1-}\) (right) (The dihedral angle in the latter two have same (145.5°) as in DMSOr crystal structure - 1eu1.](image-url)
address the issue of coordination number and electronic structure of DMSOr high-
g split, we will compare the electronic absorption spectra of high-g split and models 1 and 2 in the following section.

![Image of molecular structures]

a. $[\text{Mo}(\text{V})(\text{pyran-dt})_2(\text{O}_\text{Ser})(\text{OH})]^-$  
b. $[\text{Mo}(\text{V})(\text{pyran-dt})_2(\text{OH})]^-$

Figure 3. 18: 6-coordinate (a) and 5-coordinate DMSOr high-g split model (b). Both have same dihedral angle (145.5°) as in DMSOr crystal structure -1eu1.

<table>
<thead>
<tr>
<th>Enzyme/Models</th>
<th>g1</th>
<th>g2</th>
<th>g3</th>
<th>$g_{av}(g_{iso})$</th>
<th>$A_1^{dipolar}$</th>
<th>$A_2^{dipolar}$</th>
<th>$A_3^{dipolar}$</th>
<th>$A_{av}(A_{iso})$</th>
</tr>
</thead>
</table>
| DMSOr  
1. DMSOr  
2. 6-coordinate  
(Experimental)  
(Computed)  
(a) 6-coordinate  
(Computed)  
(b) 5-coordinate  
(Computed) | 1.9988 | 1.9885 | 1.9722 | 1.9865 | 17.78 | -20.89 | 3.11 | 34.22 |
| | 2.0066 | 1.9881 | 1.9655 | 1.9867(1.9843) | 16.38 | -17.76 | 1.39 | 32.28(31.69) |
| | 2.0028 | 1.9869 | 1.9602 | 1.9640 | 15.98 | -18.00 | 3.02 | 38.62 |
| | 1.9979 | 1.9836 | 1.9614 | 1.9810 | 18.87 | -19.26 | 1.4 | 40.01 |
| | 2.0273 | 1.9830 | 1.9554 | 1.9886 | 18.26 | -10.68 | -7.68 | 29.12 |
3.8 Electronic absorption spectroscopy

3.8.1 Experimental

Electronic absorption spectra for compounds 1 and 2 were collected on a Hitachi double beam U-4100 UV-vis-NIR spectrometer. Solids samples were dissolved in pure dried acetonitrile. The dissolved samples were placed in 1 cm pathlength quartz cuvettes and sealed with a rubber septum to ensure that the samples remained anaerobic to prevent decomposition. Due to the high atmospheric sensitivity of the samples to oxygen exposure, all samples were prepared under a nitrogen atmosphere.

3.8.2 Electronic absorption spectra and band assignments

![Electronic absorption spectra for the DMSOr high-g split intermediate and model complexes 1 and 2](image)

The DMSOr high-g split catalytic intermediate displays two major charge transfer bands at \(\sim 14960 \text{ cm}^{-1}\) (band 2) and at \(\sim 18525 \text{ cm}^{-1}\) (band 3). Band 2 was assigned as a charge transfer from the ligand-centered doubly-occupied molecular orbital (\(D_0\),HOMO-1) to the metal-centered orbital in that is represented...
by U+1 (the LUMO+1) in a spin-restricted formalism (Figure 3.4). Band 3 has also been assigned and is attributed as a charge transfer excitation from \( \text{D}_0 \) to the metal-centered orbitals, \( U_0 \) (LUMO) (Figure 3.4). The molar extinction coefficient for band 3 is higher by 790 \( \text{M}^{-1} \text{cm}^{-1} \) than that of band 2. In addition to these two bands, there is a lower energy band, band 1, at 12150 \( \text{cm}^{-1} \) that possesses very low intensity. This band was observed in the MCD spectrum (Figure 3.4) and assigned as the \( S_0 \) (HOMO) → \( U_0 \) \( \rightarrow \) \( S_0 \) (HOMO) → \( U+1 \) ligand field excitation.

Similar to the electronic absorption spectra of the high-g split DMSO intermediate, the electronic absorption spectra of model complexes 1 and 2 display two major bands at \( \sim 13000 \text{ cm}^{-1} \) (band 2) and at \( \sim 18000 \text{ cm}^{-1} \) (band 3). There is also a less intense band (band 1) at \( \sim 10000 \text{ cm}^{-1} \). Bands 2 and 3 are characteristic of LMCT bands. The orbitals involved in the one-electron promotions describing the electronic transitions for bands 2 & 3 are given in Figure 3.20 for compound 1.

![Spin-unrestricted representations of the orbitals involved in charge transfer bands 2 (upper) and 3 (lower) for complex 1.](image)

Figure 3. 20: Spin-unrestricted representations of the orbitals involved in charge transfer bands 2 (upper) and 3 (lower) for complex 1.
and in Figure 3.21 for complex 2. The higher energy band (band 3) is more intense in both the model complexes than it is in the high-g split DMSOr intermediate.

3.8.3 Electron density difference maps (EDDMs)

Electron density difference maps (EDDMs) have been computed using the Orca program. EDDMs are a convenient way to understand how the electron density changes between donor and acceptor orbitals involved in an electronic transition. The results of our EDDM calculations are reported in Appendix E. If we look closely at the EDDM for band 2 (Figure 3.22) of complex 2, the charge transfer transition is mostly from one of the two dithiolenes to the metal. However, for complex 1 both dithiolenes are involved for in this charge transfer transition. The common occurrence in the two complexes is the involvement of the catecholate in band 2. The intensity of an electronic transition depends on the square of the
transition dipole moment integral. A possible reason for the higher intensity of band 3 relative to band 2 likely derives from a greater degree of dithiolene overlap in the donor and acceptor orbitals that define the charge transfer transition that in the enzyme intermediate. Small electronic structure differences between the high-g split intermediate and the model complexes are reflected in their computed dithiolene dihedral angles, twist angles, and metal-ligand bond distances. This strongly suggests that the metal-ligand overlap responsible for the intensity of band 3 is slightly different in the models than in high-g split.

Figure 3.22: EDDMs (on truncated molecular structure) for electronic transitions 3 for complex 1 (left) and for complex 2 (right).

3.8.4 Molecular orbital diagrams

The frontier orbital energy diagrams for 1 and 2 are presented in Figure 3.23. These MO diagrams are computed by DFT using the Orca program with a B3LYP hybrid functional and a TZVP basis set. The α-HOMO (157a for complex 1 and 257a for complex 2) in Figure 3.20 is comprised of out-of-plane dithiolene orbitals. The metal-centered SOMO (156a for complex 1 and 206a for complex 2) in Figure 3.24 is found at energies below the α-HOMO. This is due to the large exchange interaction in this d¹ Mo(V) system that results in a strong spin polarization. Thus, the lowest energy, half-occupied, α-spin orbital (the α-SOMO)
is stabilized with respect to the \( \beta \)-LUMO, which is occupied upon one-electron reduction to the Mo(IV) low-spin \( d^2 \) state. One-electron occupation of the \( \beta \)-LUMO to yield a low-spin \( d^2 \) state results in a more destabilized Mo (IV) enzyme form due to the increased electron-electron repulsion in the doubly occupied, metal-
centered HOMO. This contributes to an effective lowering of the activation energy barrier for oxidative half reaction, where substrate reduction occurs. Since the \( \beta \)-LUMO is a dominantly metal-centered orbital, and its occupation leads to a high degree of electron-electron repulsion, this might be the reason for the Mo (V)\( \rightarrow \)Mo (IV) electron transfer step being rate limiting in the course of enzyme catalysis. As mentioned in the previous section, the SOMO of the DMSOr model complexes, which possesses less metal d-orbital character than the \( \beta \)-LUMO, is stabilized such that it lies lower in energy than the ligand-centered \( \alpha \)-HOMO. A similar situation is also observed in a [Mo(V)(mdt)\( _2 \)(OMe)(OH)]\(^{1-} \) computational model of high-g split, which has been probed in detail by EPR, electronic absorption, and MCD spectroscopies.

3.9 Conclusions

We have successfully synthesized complexes 1 and 2 as electronic and geometric structural analogs for the DMSOr high-g split catalytic intermediate. We highlighted the inherent problems associated with the use of EXAFS to determine the coordination number of this intermediate. This problem derives from the fact that the coordination number and the amplitude reduction factor are highly correlated in the EXAFS equation. Fortunately, we were able to apply a BVS approach in combination with the EXAFS determined bond lengths to correlate hexa-coordinate 1 and 2 with high-g split. This work showed that our synthetic model complexes and high-g split are both 6-coordinate species. For the enzyme, this means that serine is coordinated to Mo in the high-g split intermediate. The EPR determined spin Hamiltonian parameters for our synthetic 6-coordinate
complexes are observed to be remarkably similar to those we determined previously for high-g split. The axial dipolar hyperfine tensor determined for 1 is differed than the rhombic tensor obtained for 2 and high-g split. We hypothesize that a rhombic dipolar hyperfine tensor derives from twist angle anisotropy.

We have also assigned the two major electronic absorption bands at ~13000 cm⁻¹ and at ~18000 cm⁻¹ for complexes 1 and 2. These charge transfer bands are similar in energy to those previously observed for high-g split. The electronic absorption spectra of high-g split and 2 (we are not able to obtain the extinction coefficient for 1) differ only in the relative intensity of bands 2 and 3. Taken together with our EPR and XAS data, the current model study supports a six-coordinate MoS₄O₂ first coordination sphere structure for DMSOr high-g split, which was previously studied by electronic absorption, EPR and MCD spectroscopies by the Kirk research group¹⁰. We further observed a potential reason for high-g split being six-coordinate, namely, the absence of a strong base near the active site would not enable the facile deprotonation and re-coordination of the dissociated and protonated serine due to the high pKₐ of the serine hydroxyl proton.

In summary, our DMSOr model study strengthens our understanding of key geometric and electronic structure relationships for the high-g split catalytic intermediate and the mechanism of enzyme activity. Our work also validates an earlier study on high-g split by Kirk and coworkers ¹⁰ regarding the nature of the DMSOr reaction coordinate, where the geometry of high-g split is distorted trigonal prismatic with a low percentage of S p-orbital character in β-LUMO redox orbital.
3.10 References


46. VANDERAVOIRD, A.; ROS, P., TRANSITION PROBABILITIES IN CUCL2/4-COMPLEX. Theoretica Chimica Acta 1966, 4 (1), 13+-.
Chapter 4

S K-edge probes of Mo-S covalency in models for paramagnetic DMSO reductase family intermediates

4.1 Introduction to sulfur K-edge spectroscopy

Sulfur K-edge spectroscopy is an important technique for determining metal-sulfur covalency and the electronic structure of biomimetic model compounds. However, the common occurrence of sulfur-containing amino acids (cysteine and methionine) render this technique difficult in metalloenzymes. Understanding the electronic structure of DMSO family enzyme models is of great utility in furthering our understanding of their electron transfer, oxo transfer, and hydride transfer reactivity.\(^1\) Importantly, the intensity of the pre-edge features in S K-edge spectra reflects the degree of metal-ligand covalency, excited state multiplet effects, and ligand field excited state mixing.\(^2\)

In Mo-containing DMSO model complexes that possess two coordinated ditiholenes, the intensity of the S K-edge pre-edge feature arises due to transitions from sulfur 1s orbitals to low-lying acceptor orbitals primarily comprised of Mo d-orbital character admixed with S p-orbital character from the ligands. It is important to note that there may be multiple excitations to different Mo d-orbitals that possess some degree of S 3p character. This fact, in conjunction with the large number of S donors, leads to relatively intense pre-edge features that are comprised of overlapping peaks that derive from numerous S(1s) →Mo(4d) + S(p) one-electron promotions. For the DMSO family Fdh, and Nap model complexes presented
here, the intensity of the pre-edge peak does not directly reflect the number of the S donors that are bonded to the metal. This results from the fact that all the model complexes have different structures with varying twist angles and dithiolene dihedral angles. The variation of these angles in the different complexes allows for differing degrees of S 3p character to mix into the d-orbitals manifolds of the different Mo model complexes.

Since the sulfur 1s orbital is highly localized, S(1s) → Mo(4d) + S(p) electric dipole transitions follow strict selection rules. Since s → p transitions are electric dipole allowed, the percentage of sulfur 3p orbital character covalently mixed with the Mo d-orbitals reflects the degree of metal-ligand covalency. When there is only one hole in a Mo d orbital (e.g. the d1 SOMO) the S K-edge pre-edge intensity is a direct probe of the metal-ligand covalency in that orbital. However, when there is more than one electron or hole, one can observe multiplet effects that affect the intensity of the transition. With an increase in the nuclear charge of the metal, the d-orbitals are at deeper binding energy, and this corresponds to lower energy pre-edge features. When the pre-edge feature overlaps with the rising edge, the determination of the intensity depends on the accuracy of the background subtraction. The energy shift of the pre-edge feature also depends on the effective nuclear charge of ligands.

The pre-edge transition intensity is the intensity of pure 1s→3p electric dipole allowed transition weighted by the parameter $\beta$, which is related to the dipole integral ($I_s$) according to; $\text{Pre-edge intensity} = \beta^2 l_s$. The $\beta^2$ value is related to the area under the S K-edge pre-edge peak, and to the dipole integral ($I_s$) for the
1s→3p electric dipole allowed transition. The $I_s$ value for a 1s→3p transition has been determined by Solomon and coworkers by using the formula $D_0 = (h/3n) \beta^2 I_s\), where $D_0$ is the total area under the pre-edge peak, $h$ is the number of holes in metal d orbitals with ligand p orbital character, and $n$ is the number of absorbing atoms. The degree of covalency, $\beta^2$, has been determined from the $^{33}$S EPR superhyperfine interaction in [Cu(mnt)$_2$]$^-$, allowing for the determination of $I_s$. The value of $I_s$ also depends on the S-donor charge. Solomon and coworkers have established a linear correlation between $I_s$ and the 1s→3p energy via a combination of photoelectron and S-Kedge spectroscopies on KFeS$_2$, and EPR, and S-Kedge studies on [Cu(mnt)$_2$]$^2$.

4.2 Structural overview of the active sites

As mentioned in chapter 3, there have been numerous interpretations of various DMSOR crystal structures in their oxidized enzyme forms. The most accepted Mo coordination geometry for the oxidized form of DMSOR is distorted octahedral, with 4 S donors from two PDTs, an O$_{ser}$, and an oxo ligand as observed by Li and coworkers. The twist angles observed for the oxidized DMSOR Mo site

Figure 4. 1: Oxidized active site structure of Fdh (left), Nap (middle) and DMSOR (right)
(PDB-1eu1)⁹, as defined¹⁰ for the models in chapter 2 and 3, are 14.71°, 23.91° and 16.57°. The dithiolene dihedral angle is 145.51°. The twist angles and dithiolene dihedral angle (PDB-1fdo)¹¹ in the oxidized form of Fdh are; 14.55°, 23.37°, 4.46° and 148.23° respectively,¹¹ and the twist and dihedral angles (PDB-3ml1)¹² in oxidized Nap are 4.51°, 11.56°, 5.095° and 163.76°, respectively. In addition to X-ray crystallography, XAS, resonance Raman and EPR studies have been performed to further interrogate the geometric and electronic structure of these enzymes.¹³⁻²⁵

4.3 The degree of Mo-S covalency is not known for the enzymes

DMSOₐ family enzymes all contain the molybdopterin cofactor, with 4 S donors from two PDTs coordinated to the metal. In addition to the PDT ligands, the enzymes possess other S-containing entities such as methionine and cysteine. The presence of these other S containing amino acids in DMSOₐ family enzymes dramatically hinders the use S K-edge XAS as a probe of Mo-S covalency. However, EPR spectroscopy can be quantitatively utilized to probe metal-ligand covalent interactions in the enzymes. Metal-sulfur covalency can therefore only be realistically studied by S K-edge XAS using small molecule enzyme analogs. Solomon and coworkers have employed S-Kedge XAS on a variety of enzyme analogs including [Mo(IV)O(bdt)₂]²⁻, [Mo(IV)O₂(bdt)₂]²⁻, [Mo(IV)OSi(bdt)₂]⁻ and [Mo(IV)O(OSi)(bdt)₂]⁻ in order to understand their electronic structures and to determine the roles of the PDTs in active site oxidative and reductive redox processes.¹,²⁶
4.4 No ligand S K-edge studies have been performed on EPR active models

Holm\textsuperscript{27-30}, Okamura\textsuperscript{31} and others\textsuperscript{32-35} have synthesized DMSOr model compounds in their fully oxidized and reduced forms, and some studies of metal-ligand covalency have been performed. Due to the dearth of des-oxo Mo(V) model compounds, we have synthesized new EPR-active models for DMSOr family enzymes. These model systems are observed to have closely related EPR spin-Hamiltonian parameters (compare with Fdh and Nap in chapter 2 and DMSOr in chapter 3) and electronic absorption spectra (see DMSOr in chapter 3) when compared with the respective paramagnetic forms of the enzymes. However, prior to our work, no one has synthesized accurate EPR-active model complexes and studied their S K-edge spectra. In addition to electronic absorption, EPR, and Mo K-edge XAS spectroscopies, we have used S-edge spectroscopy to probe the electronic structure of these molecule as analogs for bona fide catalytic intermediates.

4.5 Electronic structure determinations for DMSO reductase family enzymes are limited

As mentioned earlier, there have been many structural studies performed on oxidized and reduced forms of DMSOr enzymes. Kirk and coworkers studied the high-g split DMSOr catalytic intermediate which was trapped using TMAO as the reducing substrate instead of the native DMSO substrate.\textsuperscript{19} The high-g split species has been studied by electronic absorption, EPR, and MCD spectroscopies, in addition to a variety of computational methods including DFT
and CASSCF (SORCI). Many synthetic models for the oxidized and reduced form of the enzymes have been studied with the goal of understanding their respective electronic structures in order to better understand electronic structure contributions to reactivity in the enzymes. \textsuperscript{1, 20, 26, 36-40} We have now synthesized new models for DMSO \textsubscript{r} enzyme intermediates and have rigorously studied them using a combination of EPR, S K-edge, and electronic absorption spectroscopies with the goal of understanding the paramagnetic Mo(V) form of the enzyme in the electron transfer half-reaction. More importantly, S K-edge spectroscopic studies on the model compounds reveal important metal-ligand covalency information.

\textbf{4.6 Experimental S K-edge XAS data collection and processing for model systems}

Ligand (S) K-edge XAS of molybdoenzyme model compounds allows us to obtain critical information regarding the covalent nature the Mo-S bonds and the magnitude of the ligand field splitting.\textsuperscript{41} The pre-edge intensity reflects the degree of metal-ligand covalency, the pre-edge intensity is also affected by excited state mixing and statistical probability concerns (i.e. the total number of acceptor holes).\textsuperscript{2} Symmetry equivalent ligands contribute equally to the pre-edge intensity, but in cases where the donor atoms are not symmetry equivalent, as in our DMSO \textsubscript{r} model compounds, only those S donors that have S 3p orbital character mixed into the Mo d-orbitals will contribute to the pre-edge intensity.\textsuperscript{41}

\textit{Sample preparation:} Each model compound solid sample was thinly dispersed on Mylar tape (a S-free acrylic adhesive) and mounted on the window
of an Al plate. Thin polypropylene tape was placed on the sample to avoid exposing the sample to the air and to prevent oxidative degradation. Since our model complexes are highly air-sensitive, all sample preparation was performed inside an inert atmosphere glovebox. The samples were subsequently placed into the He-purged sample holder (Figure 4.2), and the data were collected from the Web XAS data collection program installed on the computer outside of the beamline hutch.

*X-ray absorption experiment:* S-Kedge data for our DMSO\(\text{r}\) model complexes were collected on beamline 4-3 (BL 4-3) at the Stanford Synchrotron Radiation Light Source (SSRL). BL 4-3 utilizes 20-pole, 2.00 T wigglers as the radiation source, and the beamline energy ranges from 2.4-5 KeV. The beam goes through an entrance slit to the LN\(_2\) cooled double crystal Si-monochromator. The photon energy was calibrated to the S-Kedge of a Na\(_2\)S\(_2\)O\(_3\).5H\(_2\)O standard, and energy was assigned as 2472.02 eV. The scan range was fixed from 2400eV to 2510 eV and the step size was set at 1eV (at 2400-2460eV), 0.1eV (at 2460-2490eV) and 0.5eV (at 2490-2510eV). For all samples, data were collected using a Lytle fluorescence detector.

![Figure 4. 3: Schematic diagram of BL-4-3 at SSRL](image)
**Data processing:** All data were processed using the program Athena. XAS peaks were assigned from the negative peak of the second derivative for all samples. Peak fitting functions used were pseudo-Voigt (combination of Gaussian and Lorentzian) functions, and these were utilized for both the pre-edge and rising edge line shape fittings. An arctangent function was used for the edge jump. The peak height for the edge jump was fixed at 1 since the pre-edge and post-edge data were already normalized. \( \eta \) (eta), a mixing coefficient for pseudo-Voigt function, was adjusted between 0.35 to 0.65 to fit in line shape (default is 0.5).

**Metal-ligand covalency calculations:** Theoretical calculations for metal-ligand covalency were performed in ORCA v.4.0.0. Model structures were optimized using the B3LYP hybrid functional and a Triple-Zeta-Valence-Polarization (TZVP) basis set was utilized for all computations involving these structures. The 1s orbital from each sulfur atom was set as the donor orbital. Since the S atoms in the complexes are not equivalent, the 1s orbitals for all S atoms were not localized as single 1s orbital.

In order to evaluate the experimental metal-ligand bond covalency, we used the formula \( D_0 = (h/3n) \beta^2 I_s \), as has been employed previously.\(^5\) In this formula, \( D_0 \) is the total area under the pre-edge peak, \( h \) is the number of holes in metal d- or ligand p-orbitals and \( n \) is the number of absorbing atoms. The number of holes for the transition under the peak 1 is equal to one (half-filled \( d^1 \) redox orbital) and the number of holes corresponding to the second peak are equal four (unoccupied \( d(xz,yz) \) orbital set) for complexes 1-5. The number of absorbing S atoms are equal to 5, 6, 5, 4 and 4 for complexes 1-5, respectively. The dipole integral \( (I_s) \) for the
electric dipole allowed S 1s→ Mo(4d)+S(p) transition was determined from a linear correlation of \( I_s \) to the S1s→Mo(4d)+S(p) energy (i.e. the edge energy).\(^5\)

### 4.7 Results

For all five model complexes, we observe an intense peak in the pre-edge region, and the peak intensity does not appear to be proportional to the number of S-donor atoms in these complexes. However, if we compare the integrated areas under the pre-edge peak, we find that these areas are proportional to the number of S-donors except for [Mo(V)(cydt)\(_2\)OS]\(^1\) (complex 3). Unfortunately, the data for

![Figure 4. 5: XANES spectra for model complexes](image-url)

Complexes (Chem draw structures, Figure A1, Appendix A)

1 [Mo(V)(cydt)\(_2\)SSe]\(^1\)  
2 [Mo(V)(cydt)\(_2\)butdt]\(^1\)  
3 [Mo(V)(cydt)\(_2\)OS]\(^1\)  
4 [Mo(V)(cydt)\(_2\)Cat]\(^1\)  
5 [Mo(V)(pdt)\(_2\)Cat]\(^1\)
this complex appears to have suffered from extensive radiation damage and degradation (Figure A26, Appendix G). For complexes 1-3, inspection of the second derivative spectra (Figure A21-25, Appendix G) indicates that there are

![Graphs](image1.png)

![Graphs](image2.png)

![Graphs](image3.png)

Figure 4. 7: Experimental and TDDFT XANES spectra of complexes 1 (top left), 2 (top right), 3 (middle), 4 (lower left) and 5 (lower right), fit(--), peak1, (--), peak2(--), peak3(--), peak4, peak5(--), peak6(--), and step(--). Vertical lines TDDFT oscillatory strength
two distinct peaks under the pre-edge feature, with one very weak peak at lower energy and a more intense peak at higher energy (Figure 4.4).

The low energy peak is assigned as the S(1s) → β-LUMO transition from our TDDFT calculations. In complexes 4 and 5, since there are four S 1s orbitals from the two dithiolenes, a total of four S(1s) → β-LUMO transitions contribute to the intensity. In complexes 1 and 3, there are five S 1s orbitals (four from two dithiolenes and one from OS/SeS, aromatic structure) contributing S(1s) → β-LUMO transitions. The more intense pre-edge contribution derives from these same four S 1s orbitals to two low-lying α-LUMO and two β-LUMO orbitals. The

Table 4. 2: Mo–S covalency (%S in 3p) and energy of peaks under pre-edges for DMSOr model complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Peak1</th>
<th>Peak2</th>
<th>Peak1 and Peak2 Energy difference (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex 1</td>
<td>7.80(7.2)</td>
<td>29.55(27.7)</td>
<td>1.10(0.79)</td>
</tr>
<tr>
<td>%S 3p</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (eV)</td>
<td>2470.10</td>
<td>2471.20</td>
<td></td>
</tr>
<tr>
<td>Complex 2</td>
<td>8.64(10.8)</td>
<td>36.72(32.73)</td>
<td>0.83(0.77)</td>
</tr>
<tr>
<td>%S 3p</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (eV)</td>
<td>2469.90</td>
<td>2470.73</td>
<td></td>
</tr>
<tr>
<td>Complex 3</td>
<td>5.49(10.7)</td>
<td>19.27(28.18)</td>
<td>1.05(0.46)</td>
</tr>
<tr>
<td>%S 3p</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (eV)</td>
<td>2470.20</td>
<td>2471.25</td>
<td></td>
</tr>
<tr>
<td>Complex 4</td>
<td>7.42(7.3)</td>
<td>22.36(21.48)</td>
<td>0.61(0.47)</td>
</tr>
<tr>
<td>%S 3p</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (eV)</td>
<td>2470.66</td>
<td>2471.27</td>
<td></td>
</tr>
<tr>
<td>Complex 5</td>
<td>9.45(6.3)</td>
<td>23.86(19.53)</td>
<td>0.73(0.48)</td>
</tr>
<tr>
<td>%S 3p</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (eV)</td>
<td>2470.70</td>
<td>2471.43</td>
<td></td>
</tr>
</tbody>
</table>

Note: Values in parentheses are DFT computed
energy of these transitions has been determined from time dependent DFT calculations.

The pre-edge peak for complex 5 appears to be quite intense compared to that of complex 4, which has same number of sulfur ligands bound to the Mo center. From our TDDFT calculation results for complex 5, we observe that peak 3, representing the transition to the LUMO+3 and LUMO+4 orbitals, overlaps with

Figure 4. 9: Demonstrating the DFT computed energy gap between upper lying LUMOs and low lying LUMOs for complex 1 (upper) and 2 (lower).
peak 2. We noticed the energy gap between the average energy LUMO+3 and LUMO+4 orbitals, and that between the average energy of the LUMO+1 and LUMO+2 orbitals is relatively small (1.2eV) for complex 5. The corresponding energy gaps (shown in Figure 4.5) for the other complexes is approximately double (~2eV) that observed in complex 5. This energy gap for the complex 4 is shown in Figure 4.5.

The pre-edge energy (Figure 4.6) depends on the ligand charge (S in our model complexes) the effective nuclear charge on the metal, the ligand field splitting, and the geometry of the complexes. Complexes 1-5 all have distorted trigonal prismatic structures, but the degree of distortion is not constant. The twist angles for each of the complexes are shown in Figure 4.7 (Table 4.2). In some of the complexes the twist angle anisotropy is high. This, in part, reflects why

Figure 4. 11: Pre-edge energy in the first derivative of S Kedge spectra for complexes 1-5
the pre-edge intensity in the S K-edge spectrum does not always correlate with the number of S ligands bound to the Mo. The complex 3 which has one oxygen in the first coordination sphere, has higher pre-edge energy compared to the complex 4. Because of the radiation damaged spectrum of the complex 3, we are not in the position to analyze data further for this complex. The lowest pre-edge energy is for the complex 2 which has highest number (6) of the sulfur atoms, bound to the Mo center.

The rising edge energy (Figure 4.8) in S K-edge spectrum for these complexes correlates with the binding energy of the S atom core 1s electron. This
energy position can be determined from the point of inflection after the pre-edge in
the XANES part of the spectrum. This energy basically depends on the effective
nuclear charge on the absorber atom, which is a S atom in model complexes 1-5.

From our observations of the S K-edge of spectra for 1-5, the order of rising edge
energy for these model complexes is: 2 < 3 < 1 < 4 < 5. We had expected the
rising edge energy of model 3 to be higher than that of model complex 1 because
of the hard oxygen donor present in complex 3. However, this is not what was
observed. As we explained earlier, the data for the complex 3 appear to suffer from
radiation damage. Complexes 4 and 5 have two oxygen donors bound to the Mo
ion and show higher edge energies than the rest of the complexes. However, the
edge energy for the complex 5 is shifted by ~0.4eV higher than the edge energy
of complex 4. The higher edge energy in complex 5 may be due to the effective

Figure 4. 15: The first derivative of the rising edge
energy showing displaying an inflection point after pre-edge peak in the S K-edge spectra of complexes 1-5
nuclear charge on S ligands in 4 being different due to shorter Mo-S bond lengths than observed in complex 4. We observed by Mo-Kedge EXAFS (chapter 3) that...
the average Mo-S bond length for complex 5 is 2.38 Å and for that complex 4 is 2.39 Å (also in Table 4.3)

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Mo-S average bond length in Å</th>
<th>Mo-O (average) bond length in Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.40 (2.41)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.40 (2.41)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.39 (2.42)</td>
<td>2.04 (2.02)</td>
</tr>
<tr>
<td>4</td>
<td>2.39 (2.41)</td>
<td>2.05 (2.03)</td>
</tr>
<tr>
<td>5</td>
<td>2.38 (2.41)</td>
<td>2.06 (2.03)</td>
</tr>
</tbody>
</table>

*Note: Values in parentheses are DFT computed*

**4.8 Conclusions**

From our S-Kedge experiments and associated TDDFT calculations, Mo-S covalencies have been determined for these new DMSOr enzymes model complexes. All of the enzyme intermediate model complexes have similar degrees of Mo-S covalency (~9%) in the β-LUMO. However, in the β-LUMO+1 and β-LUMO+2 orbitals, the degree of Mo-S covalency correlate strongly with the number of S donors. As discussed in the previous chapters, our new model complexes are very similar in structure to those computed for the respective enzyme
intermediates. The degree of Mo-S covalency in DMSOr family enzyme intermediates should therefore be comparable to what we have observed in our small molecule synthetic analogs. The S 3p character in the LUMO, LUMO+1 and LUMO+2 orbitals of the complexes clearly indicates the PDT ligands play important roles in the electron transfer half reactions of all DMSOr family enzymes by modulating the effective nuclear charge of the Mo in and providing a covalent PDT superexchange pathway for electron transfer regeneration of the catalytically competent active site to begin substrate turnover.
4.9 References


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Appendices

Appendix A

Synthetic and computational models

Synthetic models

Figure A1: Fdh and Nap models (1-4), DMSOr models (5-8) and MsrP model (9)
In my synthetic research program, I first synthesized oxidized Mo(VI) complexes 10 and 11. These are not true models of the enzyme EPR active intermediates and the synthetic procedures for these complexes are not reported here. However, following parallel procedures for the synthesis of these compounds models 1-8 and 12 were synthesized. The synthetic procedures for the Fdh and Nap EPR active model complexes (1, 3, and 4) have been discussed in chapter 2 and for DMSOr models (7& 8) in chapter 3. The procedures for complexes 6 and 7 are same as for the complexes 7 and 8. The procedure for the MsrP (9) model complex has been reported, *vide infra*.

Figure A2: Nap (10) and Fdh models (11 & 12).
Synthesis of the precursor of 9, [BnNEt$_3$][Mo(IV)(cydt)Cl$_2$]: 3 equivalents (292.93mg) of PhSeCl$_2$ solution in THF was added to 1 equivalent (400mg) of [BnNEt$_3$]$_2$ [Mo(cydt)$_2$] solution in acetonitrile following the analogous procedure of [NEt$_4$] [MoCl$_2$(S$_2$C$_2$Me$_2$)]$^1$. Two equivalents of PhSeCl are for the substitution of two ‘S’ of one of the cydt ligands and one equivalent is for the oxidation from [BnNEt$_3$]$_2$[Mo(IV)(cydt)Cl$_2$] to [BnNEt$_3$] [Mo(V)(cydt)Cl$_2$]. The yellowish green solid obtained (yield 200mg, 75%) was characterized using mass spectrometry (exp. mass 327.85, theo mass 327.84), EPR ($g_{iso}$ exp. 1.9740 computed 1.9827) and electronic absorption spectroscopy ($\lambda_{max}$ 313.33nm, 313.33nm, 431.70nm, 829.90nm).

Synthesis of 9: HSC$_6$H$_2$-2,4,6-Pr$_3$ (92mg in 5ml THF) was deprotonated by KBut'O (44mg in 5ml THF). Then the deprotonated solution of [K][SC$_6$H$_2$-2,4,6-Pr$_3$] added to the solution of [BnNEt$_3$][Mo(V)(cydt)Cl$_2$] (100 mg in 10 ml acetonitrile). Procedural steps were followed as for compound 2 (see Holm et al. 2001). The blue-violet solid that was obtained was purified by recrystallization (dissolving in minimum quantity of acetonitrile and precipitating by adding dry ether). The yield was 75mg (42%). The recrystallized sample was characterized by mass spectrometry (exp. mass 728.20 theo mass 728.21), EPR ($g_{iso}$ exp. 1.9918 computed $g_{iso}$ 1.9917) and electronic absorption spectroscopy ($\lambda_{max}$ 319.90 nm, 495.90nm, 569.30nm).
Figure A3: Optimized computational derivatives of [Mo(V)(pdt)$_2$Cat]$^{1-}$. In (i) one of the ‘O’ donors has been replaced in the model [Mo(V)(pdt)$_2$Cat]$^{1-}$ by H and optimized (H only). (ii) is same as (i) but phenolate is replaced by ‘OMe”. In (iii) the dithiolene dihedral angle is constrained using values from [Mo(V)(pdt)$_2$Cat]$^{1-}$. Structure (iv) is fully optimized.
**Computational**

DFT calculations have been performed by using the hybrid functional, B3LYP, and the TZVP basis set for all atoms of most complexes. However, for frequency calculations in some other cases an SVP basis set has been used for ‘C’ and ‘H’ atoms to reduce the computational cost. In some calculations the RI (resolution of identity) approximation with Coulomb integral (J) and COSX (numerical integration) has been used to speed up the process. For the final calculations, a high grid level (up to 15) was used to obtain accurate energy. In magnetic property calculations like MCD and EPR, the Zeroth Order Relativistic Approximations (ZORA) has been used.

**XYZ-coordinates**

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C  7.364689952 -0.952853028 -1.341250975
C  8.330044312 -0.854116521 -1.847494455
C  6.818457969 -1.780030800 -1.798746389
C  7.553715883 -1.215447101  0.299957330
C  7.354947478  1.499447603 -0.804866652
C  8.320806441  1.627594432 -1.303500498
C  -5.396107000  -1.163679000  1.013575000
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**Mo(V)(cydt)O(2,4,6-Pr₃-benzenetriolate)₂**

* xyz -1 2 (charge multiplicity)

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H  3.926184000  -2.096919000  -2.457238000
C  2.447300000   2.911413000   0.304087000
C  2.589186000   2.721753000   1.816684000
H  1.788012000   3.248377000   2.338400000
H  3.546100000   3.106439000   2.180841000
H  2.517700000   1.668820000   2.084698000
C  2.486020000   4.397550000  -0.077017000
H  1.465197000   2.545044000   0.022057000
H  1.688797000   4.940077000   0.437609000
H  2.345564000   4.523921000  -1.152287000
H  3.439607000   4.858249000   0.195155000
C  7.299078000   2.193716000  -0.675050000
C  8.016160000   2.842830000  -1.864698000
H  9.044196600   3.114880000  -1.600564000
H  7.492988000   3.743617000  -2.190496000
H  8.060544000   2.158613000  -2.715019000
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H  8.091986000   0.189259000  -0.975369000
H  7.561737000   0.506537000   0.674339000
H -5.059258000  -1.777150000  -2.777467000
H -4.590307000   1.152959000  -2.066888000
H -4.602595000  -1.875981000   0.965417000
H -5.941389000  -0.287282000  -0.265893000
*
Appendix B

Spin densities of model compounds

Figure A4: Spin density distribution of DMSO(1), DMSO(3,4,6,8,9) model complexes 1, 3, 4, 6, 8 & 9
Spin Densities of Computational Models

Figure A5: Spin density distribution of \([\text{Mo}(V)(\text{bd})_3^{1-}]\) and \([\text{Mo}(V)(\text{cydt})_2(\text{Secys})S]^{2-}\)
Appendix C

EPR spin-Hamiltonian parameters

Table A1: EPR spin Hamiltonian parameters for synthetic and computational models of Fdh and Nap enzymes

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<tr>
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<th>$g_1$</th>
<th>$g_2$</th>
<th>$g_3$</th>
<th>$g_{Aniso}(g_1-g_3)$</th>
<th>$g_{ave(iso)}$</th>
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</thead>
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<tr>
<td>Fdh (<em>Methanobacterium formicium</em>)&lt;sup&gt;a&lt;/sup&gt; (Very high-g type)</td>
<td>2.0200</td>
<td>2.0060</td>
<td>1.9970</td>
<td>0.023</td>
<td>2.0077</td>
</tr>
<tr>
<td>Fdh (<em>Desulfovibrio desulfuricans</em>)&lt;sup&gt;b&lt;/sup&gt; (High-g type)</td>
<td>2.0120</td>
<td>1.9960</td>
<td>1.9850</td>
<td>0.027</td>
<td>1.9977</td>
</tr>
<tr>
<td>Nap (<em>Desulfovibrio desulfuricans</em>)&lt;sup&gt;c&lt;/sup&gt; (High-g type)</td>
<td>2.0000</td>
<td>1.9900</td>
<td>1.9820</td>
<td>0.018</td>
<td>1.9907</td>
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<tr>
<td>Complex 1 (Experimental)</td>
<td>2.0340</td>
<td>2.0093</td>
<td>1.9978</td>
<td>0.036</td>
<td>2.0138(2.0129)</td>
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<tr>
<td>(Computed)</td>
<td>2.0156</td>
<td>2.0072</td>
<td>1.9900</td>
<td>0.026</td>
<td>2.0043</td>
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<tr>
<td>Complex 3 (Experimental)</td>
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<td>2.0089</td>
<td>1.9896</td>
<td>0.030</td>
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<tr>
<td>(Computed)</td>
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<td>2.0080</td>
<td>1.9913</td>
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<td>Complex 4 (Experimental)</td>
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<td>1.9908</td>
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<td>(Computed)</td>
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<td>1.9819</td>
<td>0.031</td>
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<tr>
<td>(i) [Mo(V)(cyd)$_2$SCysSH]$^\text{1-}$</td>
<td>2.0061</td>
<td>1.9922</td>
<td>1.9867</td>
<td>0.019</td>
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<td>(ii) [Mo(V)(cyd)$_2$CyscS]$^\text{2-}$</td>
<td>2.0038</td>
<td>1.9836</td>
<td>1.9537</td>
<td>0.057</td>
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<td>(iii) [Mo(V)(cyd)$_2$]$^\text{3-}$</td>
<td>2.0121</td>
<td>2.0102</td>
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<td>(iv) [Mo(V)(cyd)$_2$SecSH]$^\text{1-}$</td>
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<td>(v) [Mo(V)(cyd)$_2$SecS]$^\text{2-}$</td>
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<td>(vi) [Mo(V)(bdt)$_2$O]$^\text{2-}$</td>
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<td>1.9777</td>
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Table A2: EPR spin Hamiltonian parameters for a synthetic model of the MsrP enzyme

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<th>$g_{Aniso}(g_1-g_3)$</th>
<th>$A_{1x} \times 10^4$ cm$^{-1}$ Dipolar</th>
<th>$A_{2x} \times 10^4$ cm$^{-1}$ Dipolar</th>
<th>$A_{3x} \times 10^4$ cm$^{-1}$ Dipolar</th>
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<td>1.9917(1.9917)</td>
<td>25.47</td>
<td>24.25</td>
<td>54.77</td>
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<td>-9.25</td>
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<td>X-Band</td>
<td>2.0318</td>
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<td>1.9954(1.9918)</td>
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Figure A6: Θ₁, Θ₂, and Θ₃ are the twist angles 1, 2 and 3 in the model complexes [Mo(V)(pdt)₂Cat]¹⁺, [Mo(V)(cydt)₂Cat]¹⁺, [Mo(V)(mdt)₂(OMe)(OH)]¹⁻ and [Mo(V)(SH)₂(OH)]¹⁻.

Table A3: Computed EPR spin Hamiltonian parameters for model compounds with twist angle variation

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<td>[Mo(V)(pdt)₂Cat]¹⁺</td>
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<tr>
<td>F.Opt Twist-1,2&amp;3= 18.0, 37.1 &amp; 34.2°</td>
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<td>[Mo(V)(cydt)₂Cat]¹⁺</td>
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<td>F.Opt Twist-1,2&amp;3= -25.2, -35.1 &amp; 29.6°</td>
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<td>[Mo(V)(mdt)₂(OMe)(OH)]¹⁻</td>
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<td>F.Opt Twist-1,2&amp;3= 17.1, 35.6 &amp; 33.2°</td>
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<td>[Mo(V)(mdt)₂(OMe)(OH)]¹⁻</td>
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<td>Cons. Twist-1,2&amp;3= 0.0°</td>
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<td>Cons. Twist-1,2&amp;3= 30.0°</td>
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*Note: F.Opt → Fully optimized and Cons → Constrained*
Figure A7: 77K X-band EPR spectra of 9; experimental (red) and simulated (black).

Figure A8: Very high-g and high-g $g_{\text{aniso}}$ ($g_1$-$g_3$) plots for enzymes and complexes 1, 2, 3, and Donahue’s tris bdt models (I and II). Enzyme data are from table 2.1 (Chapter 2).
Note: we synthesized the \([\text{Mo}(\text{cydt})_3]^-\) complex as a decomposition product while synthesizing DMSO\(_r\) model compounds. \([\text{Mo}(\text{bdt})_3]^-\) has been synthesized by Sproules et al. \(^2\)

Figure A9: Very high-\(g\) (red) and high-\(g\) (blue) \(g_{\text{aniso}}\) (\(g_1\)-\(g_3\)) plots for enzymes and complexes \([\text{Mo}(\text{cydt})_3]^-\) and \([\text{Mo}(\text{edt})_3]^-\) (see Sproules et al.)\(^2\). Enzyme data are from table 2.1 (Chapter 2).
Appendix D

EXAFS fitting parameters

Table A4: k-space EXAFS fittings for complex 6

<table>
<thead>
<tr>
<th>Fit</th>
<th>N</th>
<th>Mo-S R_{fit} (R_{guess})</th>
<th>σ²</th>
<th>Mo-O R_{fit} (R_{guess})</th>
<th>σ²</th>
<th>S_o²</th>
<th>ΔE₀</th>
<th>R_f (%)</th>
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<tbody>
<tr>
<td>Fit1</td>
<td>4</td>
<td>2.385(2.407)</td>
<td>0.0018</td>
<td>2</td>
<td>2.052(2.030)</td>
<td>0.0045</td>
<td>0.903</td>
<td>-1.912</td>
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<tr>
<td>Fit2</td>
<td>4</td>
<td>2.386(2.407)</td>
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<td>2.042(2.030)</td>
<td>0.0015</td>
<td>0.903</td>
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<td>Fit3</td>
<td>4</td>
<td>2.386(2.407)</td>
<td>0.0024</td>
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<td>2.037(2.030)</td>
<td>0.0013</td>
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<tr>
<td>Fit4</td>
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<td>2.386(2.407)</td>
<td>0.0029</td>
<td>1</td>
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<td>0.0004</td>
<td>0.903</td>
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Table A5: R-space EXAFS fittings for complex 6

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<th>Mo-O R_{fit} (R_{guess})</th>
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<th>S_o²</th>
<th>ΔE₀</th>
<th>R_f (%)</th>
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Table A6: k-space EXAFS fittings for complex 8

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<th>Mo-O R_{fit} (R_{guess})</th>
<th>σ²</th>
<th>S_o²</th>
<th>ΔE₀</th>
<th>R_f (%)</th>
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<td>0.08</td>
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Table A7: R-space EXAFS fittings for complex 8

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<th>σ²</th>
<th>Mo-O R_{fit} (R_{guess})</th>
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<th>S_o²</th>
<th>ΔE₀</th>
<th>R_f (%)</th>
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Appendix E

Electronic absorption spectra and EDDMs

Figure A10: Electronic absorption and TDDFT results of 1, 3, 4, 6, 8 & 9
Figure A11: Electronic absorption spectral comparison of [Mo(V)(cydt)₂OS]¹⁻ and [Mo(VI)(cydt)₂OS]⁰.

Figure A12: Electronic absorption spectra of [Mo(VI)(cydt)₂OS] with TDDFT results (red bars).
Figure A13: EDDMs for electronic transitions 2, 3, 8 and 9 of [Mo(V)(cydt)$_2$SSe]$^-$.

Figure A14: EDDMs for electronic transitions 2, 3, 4, 11 and 13 of [Mo(V)(cydt)$_2$butdt]$^-$.
Figure A15: EDDMs for electronic transitions 2, 3, 7, 8 and 9 of [Mo(V)(cydt)₂OS]⁺.

Figure A16: EDDMs for electronic transitions 1, 2, and 5 of Mo(V)(cydt)O(2,4,6-Pr₃-benzenetriolate)₂.
Figure A17: EDDMs for electronic transitions 2, 3, 6, 7 and 9 of [Mo(V)(cydt)\textsubscript{2}^\textsuperscript{-}Cat]\textsuperscript{1\textsuperscript{-}}.

Figure A18: EDDMs for electronic transitions 1, 2, 3, 8 and 9 of [Mo(pdt)\textsubscript{2}^\textsuperscript{-}Cat]\textsuperscript{1\textsuperscript{-}}.
Figure A19: α-HOMO wave functions for the Fdh computational model (upper right) and synthetic model 3 (lower right).
Appendix F

Relaxed and non-relaxed Mo-O\textsubscript{ser} bond scans in DMSOr(V) computational models

Figure A20: 6-coordinate DMSOr computational model (fully optimized, upper left and dihedral angle constrained from the DMSOr X-ray structure, lower left). Potential energies of Mo-O\textsubscript{ser} bond scans (Upper right for fully optimized structure and lower for lower left structure).
Appendix G

S K-edge spectra and associated tables

Figure A21: S-Kedge absorption (red), first derivative (blue), and second derivative (light blue) spectra (left) and TDDFT results (right) for complex 2.

Figure A22: S-Kedge absorption (red), first derivative (blue) and second derivative (light blue) spectra (left) and TDDFT results (right) for complex 3.
Figure A23: S-Kedge absorption (red), first derivative (blue) and second derivative (light blue) spectra (left) and TDDFT results (right) for complex 4.

Figure A24: S-Kedge absorption (red), first derivative (blue) and second derivative (light blue) spectra (left) and TDDFT results (right) for complex 6.

Figure A25: S-Kedge absorption (red), first derivative (blue) and second derivative (light blue) spectra (left) and TDDFT results (right) for complex 8.
S-Kedge Fitting Parameters for Complexes 1, 3, 4, 6 & 8

Table A8: S-Kedge Fitting Parameters for complex 1

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Table A9 S-Kedge Fitting Parameters for complex 3

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Table A10: S-Kedge Fitting Parameters for complex 4

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Note: hhlw = half height full linewidth. m= mixing coefficient for pseudo-Voigt function to fit the line shape.
Table A11: S-Kedge Fitting Parameters for complex 6

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Table A12: S-Kedge Fitting Parameters for complex 8

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*Note: hhlw = half height full linewidth. m = mixing coefficient*
Figure A26: Top: Radiation damage data shown for [Mo(V)(cydt)_2OS]^- S-Kedge spectra. Bottom: Expanded S-Kedge data region for scans 1-8.
References
