2022

Protonation and Non-Innocent Ligand Behavior in Pyranopterin Dithiolene Molybdenum Complexes

Cassandra Gates
Haley Varnum
Catherine Getty
Natalie Loui
Ju Chen

See next page for additional authors

Follow this and additional works at: https://repository.brynmawr.edu/chem_pubs

Part of the Chemistry Commons

Let us know how access to this document benefits you.

This paper is posted at Scholarship, Research, and Creative Work at Bryn Mawr College. https://repository.brynmawr.edu/chem_pubs/30

For more information, please contact repository@brynmawr.edu.
Authors
Cassandra Gates, Haley Varnum, Catherine Getty, Natalie Loui, Ju Chen, Martin L. Kirk, Jing Yang, and Sharon J. Nieter Burgmayer
Protonation and Non-Innocent Ligand Behavior in Pyranopterin Dithiolene Molybdenum Complexes

Cassandra Gatesa, Haley Varnuma, Catherine Gettya, Natalie Louia, Ju Chenb, Martin L. Kirk*b, Jing Yang*b, Sharon J. Nieter Burgmayer*a

aDepartment of Chemistry, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010, United States

bDepartment of Chemistry and Chemical Biology, The University of New Mexico, MSC03 2060, 1 University of New Mexico, Albuquerque, New Mexico 87131-0001, United States

KEYWORDS. Molybdenum enzymes, Moco, pyranopterin dithiolene, molybdopterin, intraligand charge transfer.

Abstract

The complex [TEA][Tp*MoIV(O)(S2BMOPP)] (1) (TEA = tetraethylammonium, Tp* = tris(3,5-dimethylpyrazolyl)hydroborate, BMOPP = 6-(3-butynyl-2-methyl-2-ol)-2-pivaloyl pterin) is a structural analog of the molybdenum cofactor common to all pyranopterin molybdenum enzymes since it possesses a pyranopterin-ene-1,2-dithiolate ligand (S2BMOPP) that exists primarily in the ring-closed pyrano structure as a resonance hybrid of ene-dithiolate and thione-thiolate forms. The protonated form [Tp*MoIV(O)(S2BMOPP-H)] (1-H), and the one-electron oxidized [Tp*MoV(O)(S2BMOPP)] (1-Mo(5+)) species have been studied using a combination of electrochemistry, electronic absorption and EPR spectroscopies. Additional insight into the nature of these molecules has been derived from electronic structure computations. Differences in dithiolene C-S bond lengths correlate with relative contributions from both ene-dithiolate and thione-thiolate resonance structures. Upon protonation of 1 to form 1-H, large spectroscopic changes are observed with transitions assigned as Mo(xy) → pyranopterin metal-to-ligand charge transfer (MLCT) and dithiolene → pyranopterin ILCT, respectively, and this underscores a dramatic change in electronic structure between 1 and 1-H. The electronic structure changes that occur upon protonation of 1 are also reflected in a large > 300 mV increase in the Mo(V/IV) redox potential for 1-H, resulting from the greater thione-thiolate resonance contribution and decreased charge donation that stabilize the Mo(IV) state in 1-H with respect to one-electron oxidation. EPR spin-Hamiltonian parameters for one-electron oxidized 1-Mo(5+) and uncyclized [Tp*MoV(O)(S2BDMPP)] (3-Mo(5+)) (BDMPP = 6-(3-butynyl-2,2-dimethyl)-2-pivaloyl pterin) are very similar to each other and to [Tp*MoV(O(bdt))] (bdt = 1,2-ene-dithiolate). This indicates that the dithiolate form of the ligand dominates at the Mo(V) level, consistent with the demand for a greater S → Mo charge donation and a corresponding increase in Mo-S covalency as the oxidation state of the metal is increased. Protonation of 1 represents a simple reaction that models how proton transfer from neighboring acidic amino acid residues to the Mo cofactor at a nitrogen atom within the pyranopterin dithiolene (PDT)
ligand in pyranopterin molybdenum enzymes can impact the electronic structure of the Mo-PDT unit. This work also illustrates how pyran ring-chain tautomerization drives changes in resonance contributions to the dithiolene chelate and may adjust the reduction potential of the Mo ion.

Introduction

Molybdenum and tungsten are essential metals used for a broad range of geochemical and biochemical processes including global C, N, and S cycles, respiration in bacteria, nitrogen assimilation in plants, and both sulfite detoxification and pro-drug conversion in humans.\textsuperscript{1-7} Although pyranopterin-containing molybdenum enzymes are ubiquitous across nearly all organisms, the similar tungsten enzymes are only found in bacteria.\textsuperscript{1-2, 5, 8-10} A pyranopterin dithiolene ligand (PDT), also known as molybdopterin (MPT), is found bound to Mo in all mononuclear Mo enzymes as the Mo cofactor, or Moco\textsuperscript{3, 11-17} (Fig. 1 top), and for the related W enzymes as the W cofactor. The PDT is an absolute requirement for a catalytically functional active site in the enzymes. The electronic structure of the PDT is complex, and the multi-ring structure of this ligand introduces the possibility of a novel redox interplay between the pterin, the dithiolene, and the metal ion.\textsuperscript{3, 18-25} Despite the ability for the PDT to be redox active, it remains to be determined whether ligand non-innocence is exploited during catalysis based on our current knowledge of the enzymes.\textsuperscript{3, 17, 26} Studies of Moco model compounds have provided strong evidence that the redox-flexibility of the dithiolene significantly impacts the behavior of the molybdenum ion.\textsuperscript{3, 4, 19-24, 27-40} The fact that a pyranopterin has been partnered with a dithiolene chelate in Mo- and W-enzymes since the earliest known organism, and has been retained throughout evolution beginning with the last universal common ancestor (LUCA) of all life forms,\textsuperscript{41-42} provides strong evidence that this unusual ligand functions to control enzymatic catalysis. While many studies have concluded that the PDT is involved in electron transfer reactivity,\textsuperscript{3, 31, 39, 43-51} and the dithiolene component functions to robustly bind the Mo or W ion and modulate redox potentials,\textsuperscript{5, 17, 28, 38, 44} additional roles in catalysis are still largely speculative.\textsuperscript{3} The plethora of hydrogen-bonding interactions between the pterin and the protein may affect the electronic structure of the pterin and are key to properly positioning the entire Moco unit for catalysis.\textsuperscript{17} In several enzymes, the pterin component of the PDT is oriented such that it can hydrogen bond to residues that are attached to iron-sulfur (Fe-S) electron transfer clusters.\textsuperscript{52-55} The proximity of these PDT-Fe/S cluster configurations provide compelling evidence that the pyranopterin dithiolene plays a key role as an electron transfer conduit and enables electronic coupling between the metal atom and the PDT,\textsuperscript{39} contributing to efficient electron transfer reactivity with other redox active sites in the protein, or in other proteins. There may be additional mechanisms that allow the protein environment to modulate the nature of the PDT and affect enzymatic catalysis.\textsuperscript{3} For example, an analysis of the residues around the pterin
component of Moco led to the discovery of trends in H-bonding among the different molybdenum enzyme families that appear to correlate with enzyme function.\textsuperscript{17, 56}

In an effort to understand the enigmatic role of the PDT in pterin containing Mo enzymes, we have been interrogating an expanding series of complexes (\textit{e.g.} 1-3, Fig. 1 middle) that possess a dithiolene chelate and a pterin substituent.\textsuperscript{3, 19-21, 57-62} Model compounds for Mo enzyme active sites often circumvent the incorporation of a pterin or pyranopterin for simplicity. However, we have shown that the inclusion of a full pyranopterin dithiolene component in model systems reveals that the pyran ring in 1 and 2 undergoes a low-energy (< 10 kJ mol\textsuperscript{-1}) reversible process of C-O bond scission and re-cyclization (Fig. 1, bottom) leading to the open-form (1\textsubscript{o}) or the ring-closed pyrano-form (1\textsubscript{p}).\textsuperscript{57} Collectively, our prior studies have also revealed that the electronic environment at the Mo ion can be dramatically affected when coordinated by

![Pyranopterin dithiolene ligand in Moco](image)

Figure 1. (top) The pyranopterin dithiolene (PDT) bound to Mo ion in molybdenum enzymes. B and C (blue) label the pyrazine and pyrimidine rings that form the pterin while A (red) labels the pyran ring. P* denotes a variety of substituents such as phosphate, guanosine, inosine and cytosine. (middle) Models for the molybdenum cofactor previously reported. 1, 2 and 3 are further investigated in this manuscript. (bottom) Reversible pyran ring cyclization and scission.
a partially oxidized pyranopterin dithiolene.\textsuperscript{19-21, 40} For 1\textsubscript{p} in the cyclized PDT conformation, an unusual thione-thiolate resonance structure can be drawn that depicts a redistribution of electron density from one of the dithiolene sulfur atoms to the electron-deficient partially oxidized pterin (Fig. 2).\textsuperscript{19} Although the PDT pterin in the enzymes is currently assumed to be in the reduced oxidation state (Fig. 1 top), there is structural evidence from protein X-ray crystallography for both open and closed (pyran) forms of the PDT, leading to increased speculation as to whether these various PDT forms permit further tuning of enzyme activity.\textsuperscript{3, 17, 54, 63}

![Figure 2](image_url)

Figure 2. Admixture of a thione-thiolate chelate resonance structure (middle) contributes to the delocalized electronic structure of pyranopterin dithiolene ligands (right). Formal charges of 4+ on the Mo ion and 1- on each thiolate sulfur in the ene-dithiolate structure at left produce an overall charge of +2 on fragment shown. R = -C(=O)(t-Bu).

It is becoming clear that the reversible ring cleavage and cyclization of the pyran ring, through a process similar to ring-chain tautomerism of carbohydrates,\textsuperscript{64} allows access to pterin structures with increased unsaturation that are similar to oxidized pterin states albeit without a net loss of electrons and protons.\textsuperscript{19, 65} In Fig. 3 we show at top the three oxidation states of the pterin system, where reduction in the right hand pyrazine ring of each pterin increases the number of saturated C-N bonds. In Fig. 3, middle and bottom, we depict two examples of pyran ring cleavage that change the electronic structure of the pterin by increasing the unsaturation level of the pyrazine ring, a change that is similar to oxidation. For the tetrahydropyranopterin form of Moco (Fig. 3, B), pyran ring opening reveals a 5,6-dihydropterin structure that has the same level of unsaturation as the pyranopterin form of 1\textsubscript{p}. Pyran ring opening of 1\textsubscript{p} in C leads to the 1\textsubscript{o} pterin structure shown in Fig. 3, C (oxidized pterin).

Based on our prior model studies, we can hypothesize that there will be thione-thiolate resonance contributions to the ground state wavefunction in cases where the PDT is ring closed and partially oxidized.\textsuperscript{3, 19-21} The thione-dithiolate contribution leads to a dithiolene ligand with one thiolate being partially oxidized to a thione\textsuperscript{3, 19-21} resulting from electron density being removed from the dithiolene chelate to a pyrazine pterin nitrogen atom N5, which is effectively partially reduced. This results in a novel non-innocence with respect to the electron donor nature of this ligand and would be expected to result in a corresponding increase in dithiolene chelate ring
asymmetry, a modified Mo-PDT electron delocalization, and the propensity for protonation at a specific pterin ring N (Fig. 3).\textsuperscript{3, 19}

Although there has been a number of works that have focused on how a dithiolene ligand bound to Mo can affect the Mo electronic structure and reduction potential,\textsuperscript{3, 19-21, 57-59, 65-76} there is a dearth of such studies on pyranopterin and related dithiolene contributions to electronic structure and reduction potential modification and control.\textsuperscript{3, 19, 57-58} In this manuscript, we investigate the critical importance of the entire PDT moiety in model systems for Moco and report the consequences of pterin protonation in complexes 1, 2, and 3 (Fig. 1, middle), which represents a simple reaction that models how proton transfer from neighboring acidic amino acid residues in the protein PDT binding pocket might impact the electronic structure of the Mo-PDT unit. Pterin protonation at the N5 position exclusively stabilizes the pyranopterin structure and completely disfavors pyran ring opening. In addition, it is observed that pterin protonation facilitates redox reactions with oxidants in reactions that do not occur in the absence of protonation.

**Results and Discussion**

**Pterin Protonation.** Pterin protonation was investigated for the Mo pterin-dithiolene complexes 1, 2, and 3 by addition of trifluoroacetic acid (TFAA) to each complex dissolved in

![Figure 3. Pterin oxidation states and pyran ring opening outcomes for Moco and 1. (A) Three oxidation levels of pterins. (B) Pyran ring opening in Moco. (C) Pyran ring opening in 1.](image)
acetonitrile (ACN). Complexes 1 and 2 exist predominantly (> 80 %) in the ring-closed pyranof orm when solvated in neat ACN, and complexes 1-3 exhibit different responses to TFAA addition. Yellow solutions of 1 and 2 immediately acquire an intense magenta color after TFAA addition and display an intense absorption band at 526 nm (Fig. 4, left and Fig. S1a). In contrast, monitoring the protonation reaction of 3 by electronic absorption spectroscopy shows only minimal spectral changes (Fig. S5, right).

Figure 4. Room temperature electronic absorption spectra of 1 and 3 before and after addition of TFAA. Left: Compound 1 in ACN (black) and after adding 1 eq TFAA (red). Right: Compound 3 in ACN (black) and after adding 1 eq TFAA (red).

A synthetic procedure was devised to isolate the protonated 1 species, 1-H, for full characterization (Scheme 1). Addition of excess TFAA to 1 in acetonitrile forms 1-H, which is immediately precipitated by transferring the reaction solution to a chilled (-41 °C) mixture of hexanes and diethyl ether. Rapid precipitation is necessary because 1-H is unstable in solution. Unfortunately, it was found that the isolated red-purple solid 1-H has a limited stability of approximately two weeks when stored under a dinitrogen atmosphere in a glove box. Solutions prepared by dissolving the isolated solid 1-H under anaerobic conditions decay within hours as the initial magenta color fades and the solution becomes orange. Electronic absorption and EPR spectroscopies indicate that the orange species is the one-electron oxidized \([\text{Tp}^*\text{Mo}^\text{V}O(\text{S}_2\text{BMOPP})]} 1-\text{Mo}^{(5+)} \) (vide infra). For this reason, all solution characterization of 1-H was performed with samples generated in-situ and analyzed immediately, rather than from dissolved isolated samples. ESI-MS analysis (positive mode) of a solution sample of 1-H made from addition of 2 eq TFAA to 1 exhibits a major signal at m/z 935 corresponding to the cationic adduct \((1-H + \text{TEA}^+)\) (Fig. S13).
NMR Spectroscopy. NMR experiments on samples of 1-H prepared in situ indicate that only the cyclized pyranopterin structure exists in solution. This contrasts with the solution behavior of 1 which exists as two distinguishable isomers in equilibrium, the open pterin form (1_o) and the cyclized pyranopterin form (1_p) (Fig. 1, bottom). The 1H NMR of 1-H generated in ACN-d3 (Fig. S2a) is consistent with the pterin existing exclusively in the pyrano form since no signal for an open pterin species, characterized by the H7 resonance downfield near 9 ppm, was detected. A broad singlet resonance at 6.44 ppm is assigned to H7 in the pyranopterin form of 1-H. The H7 assignment is confirmed by an HSQC experiment showing the 6.44 ppm resonance is coupled to a 13C resonance at 75 ppm that is characteristic of the quaternary C7 in the pyranopterin structure (Fig. S3). The 1H NMR of 1 exhibits two signals for H7 due to the presence of both R-H7 and S-H7 diastereomers in equal proportion and a small 1.7 Hz coupling between H7 and H8 is observed in dry d3-ACN (Fig. S2b). In contrast, 1-H exhibits H7 as a broad singlet, from overlapping resonances of the two R-H7 and S-H7 diastereomers. The interpretation that both R-H7 and S-H7 diastereomers are present in approximately equal proportion is based on the number and integration of methyl resonances in the upfield region of the 1H NMR spectrum (Fig. S2a). It is both surprising and significant that a consequence of pterin protonation forming 1-H is that the pyranopterin conformation is strongly favored to the complete exclusion of the open form. While resonances for N-H protons on N at C2, N3 and N8 are assigned to signals at 6.93, 9.11 and 11.99 ppm, no new signal is detected for the added proton (Fig. S2a). This is most likely due to rapid exchange in the acidic environment of the TFAA. Scheme 1 depicts N5 as the site of protonation and justification for assigning N5 as the site is provided by data that follows below.

Vibrational Spectroscopy. FT-IR data were collected on the isolated solid 1-H. The v(Mo≡O) stretching vibration for 1-H is observed at 934 cm\(^{-1}\), which is 14 cm\(^{-1}\) higher than that for 1, and 4 cm\(^{-1}\) higher than the 934 cm\(^{-1}\) v(Mo≡O) vibration observed in one-electron oxidized [Tp*Mo\(^{\text{V}}\)O(S\(_2\)BMOPP)], 1-Mo(5+). Thus, pterin protonation results in a notable change in the electronic environment of the Mo≡O unit that is comparable to a one unit increase in Mo.
oxidation state. In 1-H, the strengthening of the Mo≡O bond compensates for a decrease in electron density at the Mo ion, and this can be attributed to decreased thione S → Mo(xz,yz) charge donation that derives from a greater contribution of the asymmetric thione/thiolate resonance form (Scheme 1, right).

**Resonance Contributions to the Nature of the Dithiolene Chelate.** The cyclized PDT conformation observed for 1p, has been shown to possess a dithiolene chelate ring asymmetry ascribed to contributions from a thione-thiolate resonance structure that effectively redistributes electron density from one dithiolene sulfur atom to the partially oxidized pterin. Interestingly, four Mo(IV) complexes (Cp)_2Mo(pyrrolo-quinoxalylidithiolene)^77 (Fig. 5, A), Tp*Mo(O)(pyrrolo-S_2BMOQO)^20-21 (Fig. 5, B), (Cp)_2Mo(quinoxalylidithiolene) (Fig. 5, C),^78 and [(Cp)_2Mo(H-quinoxalylidithiolene)]^+ (Fig. 5, D),^78 also display dithiolene chelate ring asymmetry. In A - D, thione-thiolate resonance contributions to the electronic structure of the chelate can be inferred from the C-S bond difference (Δ_C-S), which is defined as the difference between the longer C-S bond (which is primarily of thiolate character) and the shorter C-S bond (which is primarily of thione character) in the asymmetric dithiolene chelate ring. The Δ_C-S value for (Cp)_2Mo(pyrrolo-quinoxalylidithiolene) A is 0.056 Å (thione C=S = 1.706 Å and thiolate C-S = 1.762 Å)^77 and the corresponding Δ_C-S value for Tp*Mo(O)(pyrrolo-S_2BMOQO) B is 0.053 Å (thione C=S = 1.695 Å and thiolate C-S = 1.748 Å).^20 For C, the Δ_C-S value is 0.044 Å (thione C=S = 1.725 Å and thiolate C-S = 1.769 Å), whereas the protonated quinoxaline complex D has a Δ_C-S value of 0.075 Å (C=S = 1.695 Å and thiolate C-S = 1.770 Å).^78 To quantify the effect of thione-thiol character, we plot the percent of thione character versus Δ_C-S (Fig. 5), where standard C-S and C=S bond lengths are given as 1.75 Å and 1.64 Å, respectively. A further comparison can be made to the experimentally observed Δ_C-S = 0.060 Å value in MoO(SPh)_2[Pr_2Dt^0], a complex possessing a dithiolene ligand with dithiolate/dithione character (C=S) in addition to two coordinated thiolate ligands (C-S). A natural bond orbital (NBO) study of the Pr_2Dt^0 ligand showed two contributing resonance forms of the ligand that were interpreted in the context of a 63:37 dithione:dithiolate valence bond description in the electronic ground state. Thus, two of the blue dots in Fig. 6 represent systems where the percent thiol character is known from the structure (e.g. organic thiol or thione). The third is derived from NBO computations on the Pr_2Dt^0 ligand evaluated in the context of crystallographically defined S-Ph (C-S) and Pr_2Dt^0 (C=S) bond lengths found in MoO(SPh)_2(Pr_2Dt^0).^29,40 A straight line has been fit to these three data points to allow the percent thione character to be estimated for other systems where only the Δ_C-S values are known from X-ray crystallography or geometry optimization computations. Thus, using the Δ_C-S values obtained from the crystal structures of the quinoxaline-dithiolene complexes A, B, C, D, we can estimate the degree of C=S thione character admixed into the thiol donor to be ~53%, ~50%, ~41%, and ~71%, respectively. The computed Δ_C-S value for protonated 1-H yields a markedly larger 66% degree of thione character (Fig. 5) relative to 1 (30%), which possesses dominant one-dithiolate chelate character. Thus, protonation in 1-H effectively drives a large electronic structure change.
Figure 5. Plot of percent thione character as a function of the C-S bond length difference $\Delta$ C-S. The black line is a fit to the data corresponding to the three blue dots. The blue dots represent pure C-S (% thione = 0) and C=S (% thione = 100) bond lengths, and one point that was experimentally determined from the S-Ph C-S and $^{1}\text{Pr}_2\text{D}^0$ C=S bond lengths of MoO(SPh)$_2$($^{1}\text{Pr}_2\text{D}^0$) (% thione = 63). However, the % thione character in the ($^{1}\text{Pr}_2\text{D}^0$) ligand of MoO(SPh)$_2$($^{1}\text{Pr}_2\text{D}^0$) derives from a natural bond orbital electronic structure computation on the $^{1}\text{Pr}_2\text{D}^0$ free ligand. The observation that in the MoO(SPh)$_2$($^{1}\text{Pr}_2\text{D}^0$) data point using the ($^{1}\text{Pr}_2\text{D}^0$) NBO data indicate that the C-S (thiol) and C=S (thione) character in the free $^{1}\text{Pr}_2\text{D}^0$ ligand is quite similar the that of MoO(SPh)$_2$($^{1}\text{Pr}_2\text{D}^0$). The percent of thione character for related quinoxaline-dithiolene complexes A, B, C, D (green dots, structures shown on right) in addition to 1, and 1-H are interpolated using the $\Delta$C-S values either from the crystal structure (A-D and 1) or from the computationally optimized geometry (1-H only). Notice C and D behave similarly to 1 and 1-H with respect to the percent thione character increasing substantially after protonation in the ligand, which can be described as a partial oxidation of the dithiolene with a partial reduction at the protonated N atom in the pyrazine ring. This is a remarkable outcome for a protonation event that is four bonds removed from the Mo-dithiolene sulfur atom and which results in a large change ($\sim 300$ mV) in the Mo reduction potential, vide infra.

**Geometric and Electronic Structure Computations.** The heteroatom-rich pterin system offers several possible protonation sites. For 6- and 7- alkylated oxidized pterins, N1 is established as the more basic site, where in 7,8-dihydropterins, the more basic site shifts to N5. As the pyranopterin structure is formed from addition of -OH across the C7-N8 bond, the resultant structure more closely resembles a semi-reduced 7,8-dihydropterin although no net redox reaction has occurred (Fig. 4).

Geometries of 1-H protonated either at N5 on the pyrazine ring or at N1 on the pyrimidine ring were optimized at the DFT level with effectively treated solvation effects included using acetonitrile as the solvent. The computed energies suggest the N5-H isomers are significantly more stable than the N1-H isomers by 82 kJ mol$^{-1}$ and 81 kJ mol$^{-1}$ for their $S$- and $R$- H7 diastereomers, respectively. DFT calculations used to evaluate the relative energies of the two possible $R$- and $S$- H7 diastereomers of 1-H, where the N5 position of the pterin ring is protonated (Fig. 6), show that within the accuracy of the DFT computation these diastereomers are
effectively isoenergetic. The optimized $R$- and $S$-structures reveal somewhat different conformations, including the twist and the degree of co-planarity of the pterin and the dithiolene portion of the ligand. Torsion angles between the planes calculated for the four dithiolene atoms $S$-$C=C=S$ and the plane defined by pterin atoms N5 and the pyrimidine ring are 4.85 degree for $S$-$H7$ and 2.80 degree for $R$-$H7$ in the DFT optimized structures.

**Electronic Absorption Spectroscopy and Band Assignments.** Insight into the origins of the intense absorption bands of 1-H was obtained through a detailed spectroscopic study of 1-H and 1. Compound 1 displays a low-intensity ($\varepsilon \approx 2,000$ M$^{-1}$cm$^{-1}$) band at $\sim 20,000$ cm$^{-1}$ that derives from a combination of Mo(xy) (HOMO) $\rightarrow$ pterin (LUMO) MLCT and (HOMO) Mo(xy) $\rightarrow$ Mo(xz) ligand field one-electron promotions. Two more intense transitions are observed at $\sim 22,500$ cm$^{-1}$ and $\sim 26,000$ cm$^{-1}$. The lower energy band was assigned as a dithiolene $\rightarrow$ pterin intraligand charge transfer (ILCT) transition that derives from a symmetric dithiolene $\pi$ (HOMO-1) to pterin (LUMO) one-electron promotion, with the higher energy band being assigned as a dithiolene $\rightarrow$ pterin ILCT transition from the asymmetric dithiolene HOMO-2 orbital to the pterin LUMO. In an energy region between these two bands, a weaker dithiolene-based HOMO-1 to Mo(xz) LMCT transition was assigned at $\sim 24,000$ cm$^{-1}$ ($\varepsilon \approx 2,000$ M$^{-1}$cm$^{-1}$). Large spectroscopic changes are observed upon protonation, which indicate a dramatic change in the electronic structure of 1-H compared to 1. Adding TFAA into the yellow acetonitrile solution of 1 to form 1-H leads to an instant solution color change to magenta that is reflected by an intense peak at 19,000 cm$^{-1}$ ($\varepsilon \approx 27,500$ M$^{-1}$cm$^{-1}$) in the electronic absorption spectrum (Fig. 7A). The in situ generated spectrum of 1-H is shown in Figure 7B, which is overlaid with the results of a CASSCF (8e7o) computation that reveals the number of individual electronic transitions that contribute to the overall spectral

![Figure 6. S- and R- diastereomers of 1-H differentiated by the configuration at C7 and the position of H7 drawn in red. The S-diastereomer (left) is slightly more stable than the R-diastereomer (right) by 3.68 kJ mol$^{-1}$. The DFT optimized structures are shown below each diastereomer.](image-url)
band shape for this complex. Considering the intense band at 19,000 cm\(^{-1}\), there are two dominant transitions that contribute to this band which derive from linear combinations of HOMO \(\rightarrow\) LUMO and HOMO-1 \(\rightarrow\) LUMO one electron promotions (Fig. S7A). These one-electron promotions are best described as Mo(xy) \(\rightarrow\) pyranopterin metal-to-ligand charge transfer (MLCT) and dithiolene \(\rightarrow\) pyranopterin intraligand charge transfer (ILCT) transitions, respectively. Both of these transitions highlight the acceptor nature of the partially oxidized pyranopterin and are analogous to higher energy transitions in 1 that possess lower oscillator strengths.\(^{19}\) The band at \(~25,000\) cm\(^{-1}\) can be assigned as predominantly arising from a HOMO-1 \(\rightarrow\) LUMO+2, dithiolene \(\rightarrow\) Mo(xz) LMCT transition, and the higher energy band at \(~28,000\) cm\(^{-1}\) as the HOMO-2 \(\rightarrow\) LUMO transition that possesses dithiolene S\(_{\text{thiol}}\) \(\rightarrow\) pyranopterin ILCT character.

Note that the protonation at the N5 position results in an electron redistribution to form the unusual asymmetric dithiolene (thione-thiolate) chelate ligand (Fig. 2, middle). This dithiolene S\(_{\text{thiolate}}\) \(\rightarrow\) pyranopterin transition only involves the electron-rich thiolate sulfur atom of the dithiolene, and the thione S atom does not contribute to the charge transfer transition. Protonation of 1 to form 1-H also leads to decreased stability of the Mo pterin-dithiolene complex. This was initially observed for \textit{in-situ} generated solutions of 1-H, where the corresponding magenta solutions degraded and slowly changed over hours to an orange color (Fig. 7A). When this color change was monitored by electronic absorption spectroscopy, a

![Figure 7](https://via.placeholder.com/150)

Figure 7. (A) Decay of protonated 1-H species (solid magenta line) in ACN over 7 h. Absorption at 19,000 cm\(^{-1}\) (526 nm, magenta lines) decreases (dashed lines) in tandem with growth of absorption at 25,000 cm\(^{-1}\) (395 nm, solid orange line) of 1-Mo(5\(^{+}\)). (B and C) Overlay of computed electronic absorption spectra, computed oscillator strength (f), and the experimental data of 1-H and 1-Mo(5\(^{+}\)).
decrease in the characteristic intense 1-H absorption at 19,000 cm\(^{-1}\) proceeded in parallel to growth of an absorption feature at 25,000 cm\(^{-1}\) that possessed an isosbestic point at 22,000 cm\(^{-1}\). The species absorbing at 25,000 cm\(^{-1}\) matched the spectroscopic signature of 1-Mo(5+),\(^{58}\) and EPR experiments confirm formation of the one-electron oxidized 1-Mo(5+) species from 1-H (Fig. S4). In the absence of any added reagents, the oxidant was eventually determined to be dioxygen from trace amounts of air. The orange-colored solution is observed with a weak transition at 19,100 cm\(^{-1}\) and an intense transition at 25,000 cm\(^{-1}\). A TDDFT computation of 1-Mo(5+) in acetonitrile suggests both bands derive from ligand-to-metal charge transfer (LMCT) transitions (dithiolene-pyranopterin \(\rightarrow\) Mo(xz,yz) (Fig. S7B).

**Redox reactivity of protonated pterin dithiolene complexes.** To confirm the stoichiometry of the increased redox reactivity of 1-H, the redox dye 2,6-dichloroindophenolindophenol (DCIP) was titrated into a solution of 1-H generated in-situ in acetonitrile then monitored by electronic absorption spectroscopy (Fig. 8). The oxidized form of DCIP in acetonitrile forms a blue solution with a strong band (640 nm) and becomes colorless after reduction to \(\text{H}_2\text{DCIP}\) by 2 electrons and 2 protons per molecule. Addition of aliquots of blue DCIP solution to the magenta solution of 1-H causes immediate bleaching of the blue color and a decrease in the absorption at 526 nm in parallel with increased absorption at 394 nm due to 1-Mo(5+) (Fig. 8). Plotting the changes in absorbance at 526 nm and 394 nm shows that the reaction stoichiometry is 1 eq 1-H : 0.5 eq DCIP, establishing 1-H as a 1 e/1 H\(^+\) reductant. In contrast, the parent complex 1 exhibits no reaction with DCIP (Fig. S5) and no decomposition by trace O\(_2\), underscoring that the redox reactivity of 1-H is directly related to pterin protonation. However, adding TFAA to a mixture of 1 and DCIP causes the redox reaction to proceed to 1-Mo(5+) and reduced \(\text{H}_2\text{DCIP}\) (Fig. S5).

**Determination of pK\(_a\).** The strong absorption at 526 nm is a useful probe of the protonation state in 1-H and allowed the determination of the pK\(_a\) by titration of 1 with pyridinium tetraphenylborate in acetonitrile.\(^{78}\) The pK\(_a\) value of 13.43 measured for 1-H and the assignment
of N5 as the site of protonation is consistent with previous work on related Mo(4+) dithiolene complexes of the general formula [Cp$_2$Mo(S$_2$C$_2$(H-quinoxaline))(R)]$^+$ (Fig. 5, D).$^{78}$ The pK$_a$ value of 13.43 shows that 1-H is more basic by an order of magnitude than the quinoxaline-dithiolene complexes [Cp$_2$Mo(S$_2$C$_2$(H-quinoxaline)))$^+$ where pK$_a$ values are 11.1 and 12.1 for R = H and Me, respectively. This increased basicity is likely due to a stronger electron withdrawing effect of pterin as compared to quinoxaline. The pK$_a$ analysis in the [Cp$_2$Mo(S$_2$C$_2$(H-quinoxaline))](R))$^+$ study revealed that the pK$_a$ values of protonated Mo-dithiolene complexes were 1-3 units larger than the pK$_a$ values of the free heterocycles. This observation was rationalized as the result of resonance stabilization of quinoxalinium by conjugation with the metallo-1,2-enedithiolate, an explanation consistent with the observation that quinoxaline protonation causes co-planarity between the dithiolene and quinoxaline structures. The pK$_a$ in 1-H can also be compared with the pK$_a$ values obtained in acetonitrile for a family of pteridines substituted to resemble aspects of PDT.$^{62}$ The objective in that study was to synthesize soluble PDT analogs to obtain the pK$_a$ values under non-aqueous conditions. These pteridine analogs in acetonitrile exhibit pK$_a$ values in the range 11.09 - 11.82, and theoretical computations of various protonated structures indicate that protonation is slightly more favored at N1 than at the N5 or N8 positions. Since the pK$_a$ of 13.43 in 1-H is 1-2 units larger than is observed for this pteridine series, it likewise indicates that appending a dithiolene at pyranopterin position C6 can significantly alter pterin properties. In striking contrast to the characteristics of 1 and 1-H, protonation of 3 causes little change in the absorption spectrum (Fig. 4, right), indicating minimal pterin protonation at a weakly basic pterin group. The negligible change in spectral features for 3 under acidic conditions prevented a parallel determination of pK$_a$ through pyridinium titration.

An extensive listing of pK$_a$ values for a plethora of pterin and pteridine derivatives in different oxidation states is available$^{83}$ but since these values were measured under aqueous conditions, it is not possible to directly compare those values with the pK$_a$ of 1-H. However, it is useful to consider the pKa trends established for pterins in different oxidation states to the protonation outcomes observed for 1 vs 3. Oxidized pterin and 6,7-alkylated derivatives, including biopterin, are protonated at position N1 with a pK$_a$ in the range pK$_a$ 2.0 – 2.5. Semi-reduced 7,8-dihydropterins shift the protonation site to N5 which has increased basicity and a pK$_a$ of 4 – 4.5. Fully reduced pterins are yet more basic with pK$_a$ values of 5.6 for the first protonation at N5 followed by a second protonation of pK$_a$ 1.3 that occurs at N1.$^{81}$ In comparison to these simple parent structures, the pyranopterin dithiolene chelate in 1 behaves like the 7,8-H$_2$pterin it structurally resembles where protonation occurs at N5 and its increased basicity can be attributed to the more electron-rich semi-reduced pterin ring as well as the thiolate-thione resonance stabilization afforded by conjugation between the pterin and dithiolene chelate. In contrast, the pterin group in 3 has low basicity because the out-of-plane pterin lacks access to the stabilizing electronic delocalization with the dithiolene chelate, hence, it exhibits low basicity similar to the 6-substituted oxidized pterins with pK$_a$ values in the 2- 2.5 range for protonation at
N1. We note that the cyclic voltammetry data for TFAA added to 3 is also consistent with little protonation of a very weak base. We attribute the striking difference in protonation behavior of 1 and 3 to the difference in the pterin structure and orientation, specifically how the presence of a pyran ring and dithiolene at C6 can dramatically alter pterin properties. It is notable that although 3 appears to be a very weak base, 3 in the presence of TFAA also reduces DCIP in a similar manner observed for 1-H. Titration of 3 with DCIP shows the same 1 eq Mo: 0.5 eq DCIP stoichiometry as observed for 1-H. Monitoring this redox reaction of 3 and DCIP by EPR confirms a one electron oxidation of the Mo(4+) atom in 3 to Mo(5+). These results also establish 3 as a 1e-, 1H+ reductant (Fig. S6).

**EPR Spectroscopy.** Although complexes 1 and 3 behave differently when TFAA is added to solutions of these compounds (Fig. 5), both protonated complexes can be oxidized to the Mo(+5) state. This is evidenced by strong Mo(V) EPR resonances being observed with acid and oxidant additions, regardless of the addition order (Fig. 9). The spin Hamiltonian parameters for 1 Mo(5+) and 3-Mo(5+) are provided in Table 1, and the results suggest that the Mo(+5) species generated from 1 and 3 have similar, but not identical, first coordination spheres around the Mo ion. Most importantly, the EPR data indicate that protonation is a necessary condition for the metal oxidation irrespective of whether a pyrano ring is associated with the pterin. This sheds new light on PDT functionality for DMSO family enzymes, which possess two distinct pyranopterin dithiolene ligands bound to Mo in the active site (one ring-opened PDT and a ring-closed PDT, as in the *E. coli* periplasmic nitrate reductase NarGHI (PDB 1Q16)). The EPR spin Hamiltonian parameters have also been computed for geometry optimized 3-Mo(5+) and both the R- and S-structures of 1-Mo(5+). In the pyranopterin dithiolene systems, the Mo-S covalency positively correlates with the Mo-S2-C2 dithiolene ring fold angle, where larger fold angles result in better Mo(xy)-S(pz) overlap. The fold angles that are defined with the S-Mo-S plane and S=C=C-S plane are 29°, 32° and 24° for the computed R-, S- structures of 1-Mo(5+) and 3-Mo(5+), respectively. The high degree of folding in these optimized structures results in greater g values and a smaller Mo hyperfine interaction, predicting a more delocalized electronic environment around Mo ion. Importantly, the EPR spin-Hamiltonian parameters for one-electron oxidized 1-Mo(5+) and the pyran ring opened (3-Mo(5+)) are quite similar. In fact, the spin-Hamiltonian parameters that we determine are remarkably similar to those of Tp*MoVO(bdt), which possesses a “dithiolate-type” dithiolene ligand. Our EPR computations for protonated forms of 1-Mo(5+) and 3-Mo(5+) do not result in markedly different spin-Hamiltonian parameters compared to the experimental unprotonated forms. Our EPR study allows us to conclude that the dithiolate form of the ligand in both 1-Mo(5+) and 3-Mo(5+) is the dominant resonance contributor at the Mo(V) level and pyranopterin protonation does not likely modify the Mo-S bonding scheme, consistent with the metal ion demand for increased Mo-S covalency and S → Mo charge donation as the oxidation state of the Mo ion is increased.
Figure 9. Room temperature and 77K EPR spectra and corresponding simulations for 1-Mo(5+) (A and C) and 3-Mo(5+) (B and D) that are generated in-situ by adding stoichiometric TFAA and DCIP in 1 and 3. X-band microwave frequencies: A, 9.3936 GHz; B, 9.3975 GHz; C, 9.3979 GHz; D, 9.3983 GHz.

Table 1. Experimental and computed EPR spin-Hamiltonian parameters for Mo(+5) species that are generated by adding TFAA and DCIP in 1 and 3.

<table>
<thead>
<tr>
<th>Species</th>
<th>g-tensor</th>
<th>g&lt;sub&gt;iso&lt;/sub&gt;</th>
<th>A&lt;sub&gt;iso&lt;/sub&gt; (×10&lt;sup&gt;-4&lt;/sup&gt; cm&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Euler angles (deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+TFAA+DCIP (rt, in ACN)</td>
<td>1.9694</td>
<td></td>
<td></td>
<td>34.3</td>
</tr>
<tr>
<td>1+TFAA+DCIP (77K in n-butyronitrile)</td>
<td>2.0026 1.9716 1.9335 1.9692 58.0 20.7 23.3</td>
<td>34.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Mo(5+) (77K, in n-butyronitrile)</td>
<td>2.0022 1.9720 1.9351 1.9698 58.0 20.7 23.3</td>
<td>34.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col, R-1-Mo(5+)</td>
<td>2.0142 1.9717 1.9335 1.9732 50.7 20.1 17.2</td>
<td>29.3</td>
<td></td>
<td>176.8, 35.9, -175.6</td>
</tr>
<tr>
<td>Col, S-1-Mo(5+)</td>
<td>2.0110 1.9749 1.9387 1.9749 51.1 19.0 17.7</td>
<td>29.3</td>
<td></td>
<td>4.3, 39.3, -1.9</td>
</tr>
<tr>
<td>Col, S-1-Mo(5+)-H</td>
<td>2.0029 1.9719 1.9436 1.9728 52.2 19.2 18.4</td>
<td>30.0</td>
<td></td>
<td>6.3, 41.7, -3.9</td>
</tr>
<tr>
<td>3+TFAA+DCIP (rt, in ACN)</td>
<td>1.9709</td>
<td></td>
<td></td>
<td>32.6</td>
</tr>
<tr>
<td>3+TFAA+DCIP (77K in n-butyronitrile)</td>
<td>2.0032 1.9775 1.9385 1.9730 52.7 26.0 20.7</td>
<td>33.1</td>
<td></td>
<td>-2.0, 31.0, 13.0</td>
</tr>
<tr>
<td>Col, 3-Mo(5+)</td>
<td>2.0105 1.9775 1.9383 1.9754 50.5 17.4 16.9</td>
<td>28.3</td>
<td></td>
<td>-7.7, 41.3, 7.6</td>
</tr>
<tr>
<td>Col, 3-Mo(5+)+H&lt;sup&gt;+&lt;/sup&gt;</td>
<td>2.0057 1.9776 1.9442 1.9758 50.7 16.7 17.1</td>
<td>28.1</td>
<td></td>
<td>-9.0, 44.9, 8.0</td>
</tr>
<tr>
<td>Tp*MoO(bdt) (77K)</td>
<td>2.004 1.972 1.934 1.970 60 26 25</td>
<td>37</td>
<td></td>
<td>0.45, 0</td>
</tr>
</tbody>
</table>

Euler angles are defined as R(α, β, γ) = R(α)R(β)R(γ) (see ref. 77). Col represents the computed spin-Hamiltonian parameters using the optimized geometries.
**Electrochemical Characterization.**

Monitoring the electrochemical behavior of 1 and 3 by cyclic voltammetry following TFAA addition reveals different protonation outcomes for each complex. Voltammograms of 1 and 3 in the absence of acid are nearly identical. These voltammograms exhibit the same features of a reversible Mo(V/IV) redox couple near -550 mV vs ferrocenium/ferrocene (Fc+/0) and an irreversible oxidation (+390 mV vs Fc+/0) presumed to be the Mo(V/VI) oxidation. We have previously interpreted the small difference (54 mV) in the Mo(V/IV) potential between 1 and 3 as a consequence of the electronic influence of the pyranopterin structure in 1 stabilizing a partially oxidized thione-thiolate resonance form of the dithiolene (Fig. 2, middle).

Fig. 10 shows changes in the cyclic voltammograms following addition of 2 eq TFAA to 1 (top) and 3 (bottom), while Fig. S8 illustrates the changes monitored by cyclic voltammetry when 1 and 3 are titrated with TFAA. The reversible Mo(V/IV) couple of 1 (-520 mV vs Fc+/0) (Fig. 10, red trace) undergoes a dramatic shift of +315 mV (Fig. 10, blue trace) following addition of 2 eq of TFAA to form 1-H, where the new process displays a pseudo-reversible couple at E_{1/2} -205 mV vs Fc+/0 with an E_p-E_c separation of 130 mV. In contrast, addition of 2 eq of TFAA to 3 results in a much smaller positive shift in the Mo (V/IV) potential (~ +50 mV) (Fig. 10, bottom). When TFAA is titrated into 1 (Fig. S8), a new process located +260 mV more positive than the Mo(V/IV) couple of 1 appears with substoichiometric aliquots of TFAA (0.33 eq and 0.66 eq) until addition of 1 eq TFAA, which results in the complete loss of initial Mo(V/IV) couple of 1. If the 1-H produced from 2 eq TFAA is repeatedly cycled to negative potentials, the new Mo(V/IV) couple at -205 mV disappears and regenerates the Mo(V/IV) couple of 1 (Fig. S9). Reduction of protons occurs at negative potential, therefore cycling through the potential range +500 to -1600 mV would consume protons and deplete 1-H to regenerate 1. Titration of 3 with TFAA produces small positive shifts in the Mo (V/IV) potential without any new redox processes appearing (Fig. S8, bottom). Further addition of TFAA to 3 up to 4 eq continues...
to shift the Mo (V/IV) couple positive to -460 mV (shift of +104 mV), but the quality of voltammograms deteriorates under these high acid conditions.

The differences in electrochemical behavior between 1 and 3 in regard to changes of the Mo (V/IV) couple are interpreted as the consequence of protonation at N5 in 1. Protonation at N5 stabilizes the pyranopterin structure in 1-H to the exclusion of the open pterin structure and favors the dominance of the thione-thiolate resonance structure within the dithiolene ligand. The thione-thiolate resonance form leads to the dithiolene chelate being a poorer donor to the Mo ion. Protonation at the N5 position of the ligand also results in a change in the charge of the molecule. Remote charge effects on the ligand can be substantial,28,84 and have been previously shown to result in large redox potential shifts (350 mV) in oxomolybdenum dithiolene complexes.28 Thus, the combined effects of an increase in thione-thiolate character and an increase in the molecular charge effectively stabilize the redox orbital, and this results in a >300 mV positive shift in Mo(V/IV) Mo couple of 1 relative to 1-H. The electrochemical behavior of 3 is consistent with the pterin group having negligible basic character. Since 3 lacks a side chain hydroxyl group, there is no possibility of pyran formation and 3 cannot access the pseudo-semi-reduced pterin structure present in 1. We hypothesize that the lack of a pseudo-semi-reduced structure in 3 is the reason why BDMPP, which lacks a pyran ring, is much less basic. Furthermore, steric pressure from the t-Bu substituent on the dithiolene in 3 prevents pterin rotation and the ability to achieve a coplanar pterin-dithiolene conformation that would favor N5 protonation.19 Consistent with the interpretation from CV experiments that 3 has very low basicity compared to 1, it is observed that addition of 1-2 eq of TFAA to 3 causes no change in the 1H NMR spectrum. In summary, the presence of a ring-closed PDT structure in 1 results in a thione-thiolate resonance contribution to the electronic structure that increases the pKₐ of the N5 nitrogen, which upon protonation drives a dramatic +300 mV shift in the Mo(V/IV) redox potential that stabilizes the Mo(IV) state.

**Relationship to Pyranopterin Mo Enzymes.**

This study provides an unprecedented view into the special nature of the PDT ligand present in all molybdenum and tungsten enzymes.1 The full suite of data from NMR, electronic absorption, and FTIR spectroscopies, coupled with cyclic voltammetric and computational studies, have enabled a detailed description of 1-H. Protonation of 1 shifts the pyran ring opening equilibrium exclusively toward the cyclized pyranopterin structure in 1-H. Complex 1 has ~30% thione-thiolate character in the dithiolene ligand, which redistributes electron density from one dithiolene sulfur atom to the partially oxidized pterin and increases the asymmetry within the chelate (Scheme 1). This resonance contribution results in a partial negative charge buildup on N5 (Fig. 3, middle). The change in electronic structure upon protonation of 1 leads to the appearance of a new intense absorption band at 19,000 cm⁻¹ (526 nm), which is comprised of transitions with MLCT and ILCT character. Notably, this interpretation of the electronic structure
in 1-H reveals that the pterin unit acts as a π-acid for Mo(IV) that is made possible by the increased thione-thiolate character of the coordinated dithiolene. Additionally, the increase in thione-thiolate character of the dithiolene ligand upon pterin protonation results in the chelate being a poorer π-donor. Electron density loss at Mo(4+) is compensated by increased π-donation from the terminal oxo ligand, which is experimentally observed as an increase in the ν(Mo≡O) stretching frequency. This interpretation is consistent with our previous work with a molybdenum quinoxaline dithiolene analog of 1, where we reported that TFAA addition induces pyran ring cyclization and produces a similar intense red-shifted absorption. However, the outcome in that study differed since quinoxaline protonation resulted in a loss of the hydroxyl group that triggered an intramolecular cyclization to form a pyrrolo-pterin dithiolene ligand. Hence, the present results also serve to underscore the differences between pterin and quinoxaline, and how use of quinoxaline as a simpler heterocycle mimic of pterin may not show the full scope of reactivity.

The results of this study also show how pterin protonation impacts redox reactivity. Moco model complexes 1 and 3 show that the pterin can serve as a proton relay for proton dependent redox processes. In addition to facilitating proton coupled redox behavior, the presence of the PDT structure creates a proton sensitive electronic switch where protonation at the pterin triggers an electronic reorganization that increases the thione-thiolate resonance contribution. This results in a dramatic Mo(V/IV) redox potential increased by more than 300 mV. This observation reveals a critical feature of the pyranopterin dithiolene structure that may be exploited in Moco. The presence of the pyran ring will favor protonation by acidic amino acids specifically at the most basic N5 atom of the pterin, whose electronic communication with the dithiolene chelate is facilitated by the co-planarity of the pterin and dithiolene structures held in place by the pyran ring. Protonation also accesses the redox capability of the dithiolene by increasing the amount of oxidized thione-thiolate character in the chelate. Since the thione-thiolate chelate is a poorer electron donor to Mo, the Mo(5+/4+) reduction potential becomes more favorable. The impact of thione-thiolate character on the metal in the catalytic site of Mo and W enzymes has been proposed by us, and the work reported here provides data for the magnitude of the effect tied directly to the extraordinary non-innocence of the pyranopterin-dithiolene in PDT. It is striking that pterin protonation occurring several bonds remote from the Mo ion has such a substantial effect on the metal environment to dramatically modify the reduction potential of the Mo ion.

Conclusions

This study of an analog system for Moco provides an example of how variation in the environment around the PDT pterin may impact the electronic structure of the Mo ion in the enzymes. Our observations correlate with a recently proposed notion that variability in the protein environment near the pterin of PDT correlates with the diversity of substrates accepted
by different members of the large group of molybdenum and tungsten enzymes. This proposal arose from the motivation to expand the typical metallocentric focus on the catalytic center to a wider view that considers roles that the PDT may bring to enzyme function and catalysis. In particular, the authors highlight H-bonding between N5 and nearby amino acid residues that link PDT to the Mo≡O active site. Our results are also relevant to recent Moco model studies where N-heterocycle protonation impacts reactivity. It is observed that quinoxaline protonation occurs prior to proton reduction to H₂, and that the quinoxaline reduction state determines the selectivity of proton vs CO₂ reduction. Finally, this work demonstrates that the Mo-pyranopterin-dithiolene is a highly interacting system, within which the effects of remote protonation can be transmitted throughout the entire structure to affect reduction potentials at a distant Mo ion.

Experimental

**Materials and Methods.** The synthesis and full characterization of compounds 1, 2, and 3 have been previously reported, and these procedures were used to prepare 1, 2, and 3 for the experiments below. The purity of 1 and 3 was determined by ESI-MS and ^1^H NMR and representative data from these methods are provided in Fig. S11-S12, S14-S15. ESI-MS analyses were performed using a Waters Micromass-ZQ mass spectrometer. All NMR experiments were performed on a Bruker 400 MHz FT-NMR. Infrared spectra were obtained using a PerkinElmer Frontier FT-IR with direct samples on an ATR. Anhydrous solvents were purchased from Sigma-Aldrich stored under nitrogen. Some solvents used in synthesis were from Pharmco-AAPER/Greenfield Global and were de-aerated with nitrogen gas and dried over activated alumina.

**Synthesis of [Tp*Mo^IV^O(S₂H-BMOPP)] (1-H).** All glassware was dried in oven at 125 °C at least 24 hours, then cooled under high vacuum to prevent advantageous moisture from re-adhering to the glass. In a glovebox, [TEA][Tp*Mo^IV^O(S₂BMOPP)] (1) (0.1002 g, 0.107 mmol) was solvated with 20-30 mL of acetonitrile in a 150 ml Schlenk flask. The golden solution was stirred while 1.10 mL of 1% trifluoroacetic acid in ACN (0.141 mmol, 1.3 eq) was added drop-wise, resulting in an intense magenta colored solution. The magenta solution was stirred for 5 minutes to equilibrate before being moved to a Schlenk line. The magenta solution was transferred via cannula to a 200 mL de-aerated solution of v/v 30:70 hexane:ether, chilled over a acetonitrile-dry ice bath (-41°C). The mixture was stirred vigorously causing a magenta solid to precipitate. The filtrate was immersion-filtered leaving a magenta solid which was washed twice with 25 mL of the hexane:ether mixture. The solid was dried under high vacuum to yield [Tp*Mo^IV^O(S₂H-BMOPP)] (0.61g, 61%) as a deep magenta solid. ^1^H NMR, δ (ppm): 11.99 (1H, s, br, N-H3), 9.11 (1H, s, N-H2), 6.94, 6.92 (1H, 2s, br, N-H8 R/S ), 6.45 (1H, s, C-H7), 6.07 (1H, s, Tp* C-H equatorial), 6.05, 6.04 (1H, 2s, br, Tp* C-H equatorial R/S), 5.44 (1H, s, Tp* C-H axial), 2.66, 2.65, 2.62 (6H, 3s, br, 2xTp*-CH₃ equatorial), 2.49, 2.48, 2.47 (6H, 3s, br, 2xTp*-CH₃ equatorial), 2.20 (3H, s, Tp*-CH₃...
axial-outer), 1.88 (1.5H, s, pyran-CH$_3$ R/S), 1.83 (1.5H, s, pyran-CH$_3$ R/S), 1.79 (1.5H, s, pyran-CH$_3$ R/S), 1.78 (1.5H, s, pyran-CH$_3$ R/S), 1.76 (1.5H, s, Tp*-CH$_3$ axial inner R/S), 1.72 (1.5H, s, Tp*-CH$_3$ axial inner R/S). FT-IR (ATR): $\nu$(Mo≡O) 934 cm$^{-1}$. ESI+MS: m/z 935 (+TEA). UV/vis (CH$_3$CN): 526nm ($\varepsilon$, 27, 800 M$^{-1}$ cm$^{-1}$)

**In-situ generation of [Tp*Mo$^{IV}$(O)(S$_2$H-BMOPP)] (1-H).** [TEA][Tp*Mo$^{IV}$(O)(S$_2$BMOPP)] (1) (0.0020g, 2.14 x 10$^{-3}$ mmol) was solvated in acetonitrile (ACN) (1.00 mL) in a glovebox to generate a stock solution (a) (2.14 mM). 100.0 µl of stock solution (a) was added to 3.00 mL acetonitrile in a quartz cuvette (0.071 mM). 20.0 µl of 0.1% trifluoroacetic acid (2.57 x 10$^{-3}$ mmol, 1.2 eq) was added to the cuvette, generating a magenta solution of [Tp*Mo$^{IV}$(O)(S$_2$HBMOPP)] (0.0686 mM). The [Tp*Mo$^{IV}$(O)(S$_2$HBMOPP)] solution was used to carry out titrations with solutions of DCIP (anhydrous 290.08 g/mol) in 10% MeOH/ACN.

**Absorption Monitored Titration Methodology.** Two main methods of titrations were utilized, one at the UV-Vis instrument and the other inside a nitrogen-environment glovebox.

For the first method, at the UV-Vis instrument aliquots of DCIP solution were added to a 1-H solution (0.0686 mM) in a septum capped cuvette. The 1-H and DCIP solutions were prepared and capped inside the glovebox to minimize air exposure. A micro-syringe was used to transfer DCIP to the cuvette, through the septum. The cuvette mixture was mixed for 30 seconds, then left to equilibrate for 1 minute. After blanking the instrument with ACN, the cuvette was placed in the instrument holder and allowed 30 seconds of quiet before an absorbance measurement was taken. This process was repeated for each volume of DCIP added to the same cuvette. This method has the advantage of many aliquot points and quick UV-Vis measurements. However, advantageous air was able to access the cuvette reaction.

The second method is the least affected by air. In a glovebox, the 1-H solution was added to the multiple cuvettes at the same concentration (0.0686 mM). Serial amounts of DCIP solution were added to each cuvette using a micro-syringe. The cuvettes were capped, mixed, and then removed from the glovebox to take measurements at the UV-Vis.

**EPR Spectroscopy.** Room temperature and 77K CW X-band (9.4 GHz) EPR spectra were collected using a Bruker ESP 300 spectrometer with associated Bruker magnet control electronics and microwave bridge. Low temperature spectra were collected using an Oxford Instruments ESR 910 liquid helium flow cryostat and an Oxford Instruments temperature controller (ITC 503). Compounds 1 and 3 were dissolved in anhydrous acetonitrile for the room temperature experiments, and in n-butyronitrile to form a glassy matrix for the low temperature experiments. 1-H was freshly generated by adding 99% trifluoroacetic acid (TFAA) into 1 with a 1:1 stoichiometric ratio, and then the EPR-active species, 1-Mo(S$^+$), was generated by adding 2,6-dichlorophenolindophenol (DCIP) in 1-H with a 1:2 stoichiometric ratio. All samples were prepared under a dry N$_2$ atmosphere to avoid any oxygen contamination and the addition of adventitious moisture. EPR spectral simulations were performed using EasySpin (version: 5.2.30) embedded in the Matlab (version: R2020a) platform.
**pK\textsubscript{a} Determination.** The pK\textsubscript{a} was calculated using the methodology from published work, where pK\textsubscript{a}′ for [Py-H][BPh\textsubscript{4}] is 12.3 in acetonitrile.\textsuperscript{78} A cuvette solution of 1-H (3.10 mL, 2.14 mM) was prepared in the glovebox. A solution of [Py-H][BPh\textsubscript{4}] (10.7 mM) was added to a septa-screw top vial. Both solutions were prepared in ACN. The absorbance was monitored of the cuvette solution with 2 µL serial increments of [Py-H][BPh\textsubscript{4}]. After each addition of [Py-H][BPh\textsubscript{4}], the cuvette solution was stirred for one minute with one minute of quiet before absorbance measurements. The adapted equations are shown below.

\[
\text{Reaction: } \text{1 } + \text{PyH} \rightarrow \text{1H } + \text{Py}
\]

\[
\text{Eq. 1 } K_{eq} = \frac{[\text{1H}][\text{Py}]}{[\text{PyH}][\text{1}]}
\]

\[
\text{Eq. 2 } K_{eq} = \frac{[\text{1H}]^2}{([\text{PyH}]\text{added} - ([\text{1H}]) + (1)}
\]

\[
\text{Eq. 3 } pK_a = pK_a' + \log K_{eq}
\]

**Cyclic Voltammetry.** Electrochemical analyses were performed using a BASi Epsilon-EC potentiostat with 0.1 M tetrabutylammonium perchlorate (TBAP) as the electrolyte in anhydrous acetonitrile, platinum working and auxiliary electrodes, and a Ag/AgCl sealed reference electrode (BASi). Addition of ferrocene to CV experiments provided the internal reference ferrocenium/ferrocene couple (+435 mV in ACN vs. the Ag/AgCl electrode; \(\Delta E_p\) 71 mV at 100 mV sec\(^{-1}\)) which is used as a standard potential against which all potentials reported are referenced.\textsuperscript{86} Samples of 1 and 3 (1.6 mM) were titrated with aliquots of a 1% TFAA in acetonitrile stock solution.

**DFT Computations.** Geometry optimizations and TDDFT computations on 1, 3, 1-H, 1-Mo(5+), and 3-Mo(5+) were performed at the density functional theory (DFT) level using the hybrid exchange-correlation functional (B3LYP) that were performed using the ORCA suite (v 4.0.0).\textsuperscript{87} Valence triple-zeta polarization basis sets were employed, with def2-TZVP for the light atoms and def2-TZVPP for Mo and S atoms. An 8-electron 7-orbital CASSCF calculation was conducted for 1-H with the basis sets def2-SVP for light atoms and def2-TZVPP used for Mo and S atoms. The n-electron valence perturbation theory (NEVPT2) is turned on to produce the correct electronic absorption spectroscopy. EPR spin-Hamiltonian calculations on optimized structures of 3-Mo(5+) and R- and S- 1-Mo(5+) employed the zeroth order relativistic approximation (ZORA) Hamiltonian for the relativistic correction and the old-ZORA-TZVP basis set for Mo, ZORA-def2-TZVPP basis set for S atom, and ZORA-def2-TZVP for all other elements. The solvation effect, conductor-like polarizable continuum model (CPCM), has been included for all geometry optimizations and spectroscopic property calculations.

**Supporting Information.**
$^1$H, and HSQC NMR data for 1-H; electronic absorption spectra of 1-H, and 2-H in acetonitrile; electronic absorption and EPR spectra of 1-H, and 1-Mo(S+) in acetonitrile; band assignments from electronic absorption spectroscopy and associated MOs for in 1-H and 1-Mo(S+); CV of 1-H in acetonitrile. This material is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgments
The authors acknowledge the National Institutes of Health (GM-057378 to M. L. K. and GM-081848 to S. J. N. B.) for continued financial support of our work on molybdoenzymes. S. J. N. B. also acknowledges the National Science Foundation (CHE-0958996). We thank the University of New Mexico Center for Advanced Research Computing, supported in part by the National Science Foundation, for providing high-performance computing resources used in this work.

References


An investigation of pyranopterin dithiolene-Mo(IV) complexes that model the molybdenum cofactor reveals how pterin protonation impacts redox reactivity and illustrates that the pterin can serve as a proton relay for proton dependent redox processes. The Mo-pyranopterin dithiolene structure creates a proton sensitive electronic switch where protonation accesses the redox capability of the dithiolene by increasing the thione-thiolate resonance contribution and leads to a 300 mV increase in the Mo(V/IV) redox potential.