Heterobimetallic models of the [NiFe] hydrogenases: A structural and spectroscopic comparison

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Abstract

Hydrogen has emerged as a leading alternative fuel; however, efficient chemical methods for generating hydrogen from water are lacking. Thus, chemists have turned to nature for inspiration. The [NiFe] hydrogenases possess rich structural, spectroscopic, and functional properties, motivating extensive synthetic efforts to model the complex active sites. In this Comment, the geometric and electronic structures of the [NiFe] hydrogenases are compared to model compounds that most closely reproduce features of the native enzyme. The implications of this comparison and opportunities for advancing the catalytic activity of model compounds are discussed.

Keywords: biomimetic, metal hydrides, FTIR spectroscopy, EPR spectroscopy, redox chemistry

Introduction

Global demand for energy has increased at an alarming rate over the past 50 years, with great economic, political, and ecological consequences.[1],[2] To address some of these rising challenges, it is becoming imperative to replace fuels such as coal, oil, and natural gas with alternative modes of energy storage that can be generated and consumed in a sustainable fashion.[3] One such molecular fuel is hydrogen. While deceptively simple, the efficient combination of two protons and two electrons, or the reverse reaction, to generate and/or utilize hydrogen typically requires either complex chemical active sites or expensive and rare heavy metals. In contrast, specialized enzymes called hydrogenases (H_2ases) carry out bidirectional hydrogen production and oxidation at rates rivaling those of platinum but using only iron and nickel at their active sites (Figure 1).[4],[5] H_2ases operate at the highest possible thermodynamic efficiency but are large and fragile, sensitive to oxygen inactivation, and costly from a biosynthetic standpoint, rendering large-scale application of these systems impractical. However, understanding the mechanisms of these natural systems can inform development of increasingly efficient molecular catalysts. Thus, a great deal of effort has been expended towards the study of H_2ase enzymes, with biochemistry research complementing synthetic and theoretical efforts to model and characterize active site structure, spectroscopy and reactivity.

In this Comment, the structure and mechanism of the [NiFe] hydrogenases will be considered in comparison to model compounds; the two other classes of hydrogenases, the [FeFe] and the [Fe]-only enzymes, have been recently discussed in other reports.[4],[6] The [NiFe] hydrogenases represent the largest and most structurally and phylogenetically diverse class of hydrogenase enzyme.[7] They are found throughout the archaeal and prokaryotic kingdoms and play important roles as both hydrogen oxidizing and hydrogen producing enzymes, depending on the local

cellular environment. The accessory cofactors found in hydrogenase-associated subunits are known to vary widely across different classes, which impacts the catalytic bias and/or likely physiological role of the enzyme;[8]–[10] however, for all [NiFe] hydrogenases characterized to date, a common active site has been presumed based on spectroscopic and functional properties.[11] We will focus on this hydrogen binding and evolving active site. The heterobimetallic cluster contains an iron center coordinated by one carbonyl and two cyanide ligands, bridged via two cysteine thiolates to a nickel center that also contains two terminally coordinated cysteine thiolates (Figure 1). The only variation that has been definitively observed in the primary coordination sphere of a [NiFe] hydrogenase in an active state is the substitution of one terminal cysteine ligand by a selenocysteine, though some X-ray crystal structures of oxidized proteins show further changes, including sulfinate- and sulfenate-modified cysteines.[12]

A large part of bioinorganic chemistry focuses on creating synthetic mimics of active sites that reproduce spectral or functional metrics to gain insight into the enzymatic mechanism; this approach has been highly successful in the case of the [NiFe] H_2ase. Diverse sets of ligands have been developed by various groups in order to mimic the electronic structure of both metal centers.[6],[13]–[16] Initial models were primarily structural mimics, but now highly functional [NiFe] compounds also exist. We are interested in the comparison of structural and spectroscopic markers of the [NiFe] hydrogenase to heterobimetallic model compounds, for the "structure = function" paradigm can exist both for enzymes and for small molecule catalysts. The compounds on which the focus of this Comment lies are shown in Scheme 1. As the biomimetic compounds directly reproduce parameters of the native site, their functionality may correspondingly increase. Ultimately, we as a community seek to reproduce the high activities of the enzymes in a

simple molecule, an objective that is now beginning to be realized, albeit with bioinspired rather than biomimetic ligand and metal platforms.[17]–[31]

Through global efforts to understand these complex enzymes, a self-consistent picture is beginning to emerge about the mechanism of hydrogen oxidation at the active site of the "standard" hydrogenases (Figure 2).[4] However, questions still remain about the site of hydrogen binding and the role of iron, which remains redox-inactive, and there are no bimetallic compounds that directly reproduce the electronic structure of the nickel(III) center found in the enzyme. These ongoing issues set the stage for continued synthetic and spectroscopic investigations of both model complexes and the native [NiFe] H_2ases.

[NiFe] model compounds reproduce key structural features of the hydrogenase enzymes

Initial synthetic models of the [NiFe] hydrogenase were created almost immediately following the first structural determination of the enzyme in 1995;[32] one of the first mimics was synthesized by the Darensbourg group (**1**),[25] with a similar model following shortly after by Pohl and coworkers (**2**).[33] These mimics successfully reproduced the heterometallic nature of the active site, including both iron and nickel within two distinct ligand environments (Figure 1).[25],[33] The metal-metal bond distance, inherently one of the most well defined features in the crystal structure, formed an initial point of comparison between the models and the native enzyme. While the first protein crystal structure revealed a Ni-Fe bond distance of 2.7 Å,[32] **1** and **2** displayed Ni-Fe distances of 3.76 Å and 2.80 Å, respectively.[25] As ligand platforms developed, these values began to converge. A model compound that approached the very short distance seen in the protein, with a metal-metal separation of 2.62 Å, was synthesized by the Sellmann group a few years later (**3**).[34] This complex reproduced two other key features seen in

the enzyme: tetrathiolate coordination around the nickel center and bridging thiolates between the two metal centers; however, the square planar geometry at the nickel center and saturated coordination sphere around iron precluded any reactivity.

One of the first examples of distorted coordination around the nickel site, which may be required for activity, analogous to the so-called protein entatic state, was seen in **4**. This molecule was synthesized by Schröder and coworkers in 2005.[35] Upon coordination of the nickel precursor to the iron fragment, significant geometric rearrangement around the nickel center was observed, from square planar to near tetrahedral. The Ni-Fe distance in **4** is very short, only 2.47 Å, directly comparable to the 2.5 Å metal-metal separation seen in the crystal structure of the reduced [NiFe] H_2ase.[36] Theoretical analysis of the electronic structure of **4** suggested the presence of a bent metal-metal bond, demonstrating an electron rich, coordinatively unsaturated core with potential for reactivity at the metal centers. This work laid the foundation for establishment of the highly successful, catalytically active [Ni(bis-phosphino)(μ-dithiolate)Fe$(L)_3$] platform, developed and advanced by Rauchfuss and coworkers (Figure 3).[37]–[41]

The first heterobimetallic model compound containing a bridging hydride was reported in 2009 by Rauchfuss and coworkers, generated by protonation of **4** with an organic acid across the metal centers (**5**).[37] Structural determination by X-ray crystallography revealed Fe-H and Ni-H distances of 1.46 Å and 1.64 Å, respectively.[37] It should be noted, however, that typically X-ray diffraction underestimates metal-hydride bond distances by 0.1 – 0.2 Å due to distortions of the electron density from the hydrogen nucleus; thus, distances determined by X-ray crystallography are likely to be systematically shorter than those determined by other techniques, such as neutron diffraction.[42] Subsequent studies generated a range of [NiFe] molecules with different

phosphine substituents, terminal iron ligands, and dithiolate bridges, of which many could be isolated with a bridging hydride. Even more promising was the fact that most were seen to be electrocatalytically active for proton reduction, albeit with moderate overpotentials and modest rates.[38],[39] By replacing two of the carbonyl ligands with borane-protected cyanide groups, a model that more closely resembled the primary coordination sphere around iron was synthesized (**6**).[40] Removal of one of the carbonyl ligands gives an open coordination site and, in the presence of a base, **6** is capable of activating dihydrogen and also features a bridging hydride. Across this series of bimetallic bridging hydrides, the metal-hydride distances are always asymmetric; however, iron remains the metal to which the hydride is closer.[38]–[40] In more recent work, a set of molecules using nickel, palladium, and platinum confirms a strong iron-hydride interaction and, correspondingly, weak interaction between the hydride and nickel site.[43] This work also demonstrated that hydride formation occurs via a transient, mixed-valence isomer in which Fe(0) can be protonated, rather than by direct protonation of the metal-metal sigma bond as was previously suggested.[37]

The significant discrepancy between metal-hydride bond lengths in the heterobimetallic model compounds is markedly distinct from the asymmetry observed in the [NiFe] hydrogenase. In the enzyme, the 1.60 Å Ni-H bond lengths are 0.1 Å and 0.2 Å shorter than the Fe-H bond lengths in the Ni-C and Ni-R states, respectively, as determined by EPR measurements and DFT calculations along with recent X-ray crystallography studies.[44],[45] Though formally, the series shown in Figure 3 is isoelectronic with the Ni-R state, the different metal-hydride bond lengths and reversal in asymmetry suggest that there are distinctions between the electronic structures of the enzyme and these models that may be relevant to the enzymatic mechanism. For example, in the model systems, it is clear that the iron center is responsible for hydride generation and

contributes to catalytic activity. In the enzyme, the iron site remains formally redox-inactive, though it likely plays an important role in substrate binding, similar to what is seen in the model compounds.[25],[46]–[49] It will be interesting to see if, as these models develop further, the original paradigm of iron-centered reactivity will re-emerge in models for the catalytic mechanism of the [NiFe] H_2ases.[25],[50]

Another structural feature recently shown in the Ni-R state is a terminal, protonated thiolate ligand.[5] This motif had been proposed, as heterolytic hydrogen cleavage requires a base to accept the proton, and the only base in the immediate vicinity of the active site is a cysteine thiolate. However, evidence for this proton has now been demonstrated in a high-resolution crystal structure of the reduced enzyme.[5] This moiety has also recently been reproduced in a heterobimetallic model compound, **7**, which shows reversible protonation on the terminal nickel thiolate and is active for proton reduction.[51] However, a bridging hydride cannot be formed, and well-defined, isolable redox couples cannot be accessed in **7**. Thus, as highlighted in a recent comment by Schilter,[52] reproducing both of these key motifs, a bridging hydride and a protonated thiolate, within a model system remains a challenge for synthetic chemistry.

Comparison of spectroscopic signatures across the native hydrogenases and [NiFe] model compounds

Spectroscopy has played a key role in bioinorganic chemistry throughout the years, providing complementary means to investigate metalloenzyme active sites in the absence of a crystal structure. The native [NiFe] H_2ases are large and highly complex, with cofactors ranging from FeS clusters to flavins to hemes,[7] which initially limited application of many types of traditional spectroscopy and analysis. As a result, selective techniques such as EPR and FTIR found the

most utility in the study of hydrogenases. Through those first studies, which largely predated the enzyme structural determination via X-ray crystallography, it was found that the active site of the [NiFe] hydrogenases indeed contained a nickel center along with CO and CN^- ligands.[53]–[57] The unique stretching frequencies of the diatomic ligands, outside the congested water and protein regions of the IR spectrum, renders these features unique markers for hydrogenase, and vibrational frequency shifts are still used as indicators of the protein redox state.[58]–[60]

Along these lines, many of the bimetallic model compounds for the [NiFe] hydrogenase have at least one carbonyl ligand to iron, with more advanced models containing cyanide or even nitrosyl ligands. It was through the use of model compounds that the identities of the diatomic ligands were first proposed, as similar vibrational frequencies and weak intraligand vibrational couplings seen in the protein spectra were observed in a synthetic $[Fe(CN)_2(CO)]$ fragment (**8**).[50] Further confirmation of the presence of two cyanides and one carbonyl ligand within the protein were provided by the synthesis of a binuclear [NiFe] mimic, **9**, that exactly reproduced the frequencies of an inactive state in the *A. vinosum* enzyme.[62] However, as with other structural models, **9** is coordinatively saturated at iron and exhibits no catalytic activity.[62]

Vibrational frequencies are often thought to provide a straightforward way to probe redox state changes, particularly for small molecules. Carbonyl ligands are common in organometallic chemistry, with frequency shifts on the order of +30 cm^{-1} typically seen for each 1 e^- oxidation. One example of this is seen upon oxidation and protonation of **4** to form [**4**]BF4 and **5**, in which a mean shift of +33 cm^{-1} is observed for each electron removed from the bimetallic core[43]; similar shifts are observed for analogous reactions across that series of compounds (Figure 3). However, significant changes in carbonyl frequencies can be observed even when formal oxidation states do not change. This phenomenon is seen in **7**,[51] where thiolate protonation

induces frequency shifts of +25 cm^{-1} for the terminal carbonyl ligands despite remaining a Ni(I)Fe(I) compound in both states. These shifts are reproduced by calculations and can be explained by considering the decreased electron donation of a thiol relative to a thiolate, lowering the electron density on the metal centers. This, in turn, will reduce the amount of pi-donation and backbonding from iron to the carbonyl ligand, thereby increasing the vibrational frequency.

Considering CO and CN^- frequencies as a tool to probe local electron density rather than redox state is a more germane way to consider these changes in the context of the native enzyme. Across the range of redox states for a given hydrogenase enzyme, the CO frequency shifts by ~50 cm^{-1}, though, as mentioned above, the iron center remains low-spin Fe(II) throughout catalysis and even the inactivation processes. The most significant degree of perturbation is observed in the Ni-L redox state, which features a dative bond from nickel to iron.[58],[68] This state has recently been shown to be present during turnover and is suggested to be a key intermediate between Ni-C and Ni-SI, as depicted in Figure 2.[69] The increased electron density on iron in the Ni-L state suggests a role for this metal during catalysis beyond structure or simple redox tuning. As more model compounds are developed, reproducing some of these subtle electronic features may confer increased activity.

Electron paramagnetic resonance (EPR) spectroscopy has also played an important role in characterizing the [NiFe] H_2ase due to the prevalence of Ni(III) states in the enzyme.[4] However, within synthetic hydrogenase mimics, only the Ni(II/I) or Ni(I/0) couples can be accessed, with most model compounds limited to the diamagnetic Ni(II)Fe(II) and Ni(I)Fe(I) states, as shown in Scheme 1. To the best of the authors' knowledge, there is to date only one report of an aliphatic Ni(III) thiolate hydrogenase mimic,[70] though a recently established, catalytically active model

protein system based on nickel-substituted rubredoxin is capable of accessing a stable Ni(III) state.[71] However, a heterobimetallic Ni(III)Fe(II) compound has yet to be synthesized and isolated; this remains an area for further development. Due to these limitations, direct comparison of the electronic structure of the enzyme to the models can only be made for the Ni-L state, which features a formal Ni(I)Fe(II) active site with *g*-values of 2.28, 2.11, and 2.05.[54],[68] The first models of the Ni-L state were generated upon one-electron oxidation of **4** and the related Rauchfuss-type compounds (Figure 3); however, though a number of oxidized variants have been generated, all contain a Ni(II)Fe(I) state that poorly mimics the enzyme.[72] Upon replacement of the [$Fe(CO)_3$] fragment with a Ru(arene) moiety, a paramagnetic nickel species with a Ni(I)Ru(II) state was recently generated (**10**).[65] The g-tensor of **10**, with principal values of 2.24, 2.05, and 2.03, closely resembles that seen in Ni-L, highlighting the similarity in electronic as well as formal redox state.[65]

In contrast to the Ni(III) and Ni(I) states of the enzyme, which have been thoroughly investigated using pulsed EPR techniques,[64] the EPR-silent, Ni(II)Fe(II) states of the enzyme, Ni-SI and Ni-R, present greater experimental challenges. The Ni-SI state is generally thought to feature an open coordination site between the metals,[73] while the Ni-R state has recently been demonstrated to carry a bridging hydride and protonated thiolate ligand (Figure 2).[5] In some theoretical reports, high-spin nickel has been invoked as necessary for binding or release of hydrogen,[46] though experimental reports regarding the spin state of divalent nickel in the [NiFe] H_2ase remain ambiguous.[74]–[76] However, to the best of the authors' knowledge, there are no heterobimetallic model compounds featuring a high-spin Ni(II) center, likely due to the challenge in building a distorted Ni(II) site within a small synthetic model. This represents another direction for future synthetic modeling efforts, as determining spin state within the

enzyme will be difficult in the absence of appropriate model compounds to provide spectroscopic metrics.

As mentioned above, the majority of model [NiFe] compounds are diamagnetic and have been straightforward to characterize using NMR spectroscopy. Because metal-hydride shifts are typically found far upfield of other protons, these are thought to be unambiguous indicators of the presence of hydride species. The extent to which these are shifted upfield may also correlate with other electronic properties. For example, in the series of [NiFe] bridging hydrides shown in Figure 3, only a weak correlation is observed between metal hydride chemical shifts and electron donating ability of the phosphine substituents; however, the electrocatalytic activity of the compounds seems to be weakly inversely correlated with chemical shift.[77]

Despite the utility of NMR spectroscopy to characterize the [NiFe] mimics, few examples are available showing NMR spectra of the native enzyme. There is certainly experimental justification for this. At least one of the auxiliary clusters in the standard [NiFe] hydrogenase is paramagnetic, whether the enzyme is in an active or inactive redox state, and the size of the enzyme precludes structural determination using traditional methods. Minor paramagnetic impurities, either weakly associated metal centers or small amounts of inactive or degraded protein, could obscure relevant signals, and selective labeling of the enzyme is complicated by the multistep bioassembly processes. Therefore, while NMR spectroscopy has potential to provide a great deal of information on the structure of the active site in the EPR-silent states of the [NiFe] H_2ases, application of this technique to the protein is likely to be a challenging endeavor.

However, there are other spectroscopic techniques that are increasingly being applied to the study of [NiFe] H_2ases and model compounds. One such method is Mössbauer spectroscopy.

Due to the prevalence of iron in bioinorganic chemistry, Mössbauer spectroscopy has found great utility in characterizing metalloprotein active sites; however, up to this point, application of this technique to the [NiFe] H_2ases or model compounds has been limited. There are two primary reasons for this, reflecting biochemical, spectroscopic, and synthetic challenges. In the native enzymes, the three, multimetallic iron-sulfur clusters in the small subunit have typically obscured signals from the single active-site iron center, though early Mössbauer spectra that were obtained prior to the first crystallographic structure of a [NiFe] H_2ase hinted at the presence of a single iron center in close proximity to the nickel.[49] Significant steps towards lowering this barrier have been made within the last year. In a recent report by Lubitz and coworkers, Mössbauer analysis of the regulatory [NiFe] hydrogenase (RH) reveal clear features from a low-spin Fe(II) that can be attributed to the active site metal.[63] The changes in Mössbauer parameters upon reduction from the Ni-SI to the Ni-C state, from δ = 0.10 mm/s, $|\Delta E_Q|$ = 1.60 mm/s to δ = 0.07 mm/s, $|\Delta E_Q|$ = 0.69 mm/s, are consistent with an increase in coordination number from 5 to 6 without a formal oxidation state change.[63] These can be compared to the few Mössbauer isomer shifts and quadrupole splittings that have been reported for model compounds, including **3** (δ =0.04, ΔE_Q = 0.68),[65] **7** (δ = 0.06, ΔE_Q = 1.39),[51], **11** (δ =0.18, ΔE_Q = -1.52),[78],[79] and **12** (δ =0.07, ΔE_Q = 0.56).[80] In the case of **11**, the hydride is coordinated terminally to iron, showing only minimal interaction with the nickel center. The Mössbauer spectrum reflects this strong covalent bond. However, analogous features are not seen in the hydride-bound Ni-C state of the RH, which shows a lower isomer shift and quadrupole splitting than **11** and instead more closely resembles **12**, which lacks a hydride and instead has a terminal thiolate coordinated to the Fe(II) center. Despite formal oxidation states at iron of +1, both **5** and **7** exhibit greater similarity to the enzyme Mössbauer spectra than **11**, suggesting that the electronic structure of the iron center in

the [NiFe] H_2ases might be closer to that of an Fe(I) species than Fe(II).[19]

From a synthetic standpoint, the challenge of constructing pure ^{57}Fe-carbonyl precursors hindered preparation of selectively labeled model compounds; to combat this, a new synthetic method was developed for isotopic labeling of the heterometallic compounds **5** using elemental ^{57}Fe.[81] Using this procedure, selective insertion of isotopes into heterobimetallic compounds and characterization using Mössbauer techniques should be feasible. It is anticipated that these methods will be of increasing utility in coming years to investigate hydrogenases and their model compounds.

Along with traditional Mössbauer spectroscopy, nuclear resonance vibrational spectroscopy (NRVS), an inelastic scattering technique that selectively reports on normal modes in which the iron is displaced, has begun to be applied to the study of [NiFe] hydrogenases and model compounds.[52],[81],[82] This is a highly sophisticated, synchrotron-based method currently only carried out at third-generation beamlines, requiring special equipment, high concentrations of protein, and large volumes; however, the inherent selectivity coupled with the structural information accessible in vibrational spectroscopy renders NRVS particularly valuable for the characterization of the electronic and geometric structures of these systems. For example, recent work comparing the Ni-R state of the [NiFe] hydrogenase to **5** provided the first direct, spectroscopic evidence for a hydride bridge and protonated thiolate as key structural motifs within the most reduced state of the enzyme, thus supporting recent crystallographic studies indicating these moieties.[5],[52] There is great promise in the future to use this technique as a general method for studying iron-hydride species, with opportunity to better understand the role that iron plays within the [NiFe] H_2ase systems.

Another vibrational technique that had been underutilized in the study of the [NiFe] hydrogenases and model compounds is resonance Raman spectroscopy. This is likely also due to the iron sulfur clusters in the protein, which have strong optical absorption features throughout the near-UV and visible regions of the spectrum. However, work by Hildebrandt and coworkers on multiple types of [NiFe] hydrogenases shows resonance enhancement of the Fe-CO and Fe-CN bending modes of the active site using visible excitation, with analogous frequencies to those seen in NRVS studies.[66],[83]–[85] At this time, bands sensitive to H/D exchange have not yet been identified in the RR spectra of any H_2ase; however, work on **5** demonstrated that metal-hydride stretching modes can be clearly identified using RR spectroscopy.[67] Future application of this technique to the study of the EPR-silent Ni-SI and Ni-R states would provide complementary insight into the local structure of these states, especially around the nickel center, as NRVS is strictly sensitive to iron displacements.[83] Through these types of studies, the structural asymmetry between the iron-hydride and nickel-hydride bond lengths can be directly investigated via the large difference in stretching frequencies. One factor that must be considered is the importance of identifying relevant electronic transitions; in RR studies of **7**, S-H/S-D vibrations could not be directly observed, likely due to a lack of low-lying electronic transitions that were delocalized onto the protonated thiolate. However, use of more advanced instrumentation with access to variable wavelength excitation will enable comprehensive investigations of metal-hydride and protonated thiolate modes, providing an experimental handle on moieties that are otherwise quite challenging to probe.

Historically, EPR and FTIR spectroscopy represent the primary spectroscopic methods used to investigate the different redox states of the [NiFe] hydrogenases, while the main techniques applied to the study of [NiFe] H_2ase model compounds were FTIR and NMR spectroscopy. In

recent years, advances in instrumentation, synthetic techniques, and sample preparation have enabled researchers to use other physical bioinorganic methods, including Mossbauer, NRVS, and resonance Raman spectroscopy, to provide a comprehensive structural and electronic picture of these heterometallic active sites both in the native enzyme and in synthetic models.

Conclusions

Comparison of the [NiFe] H_2ases to bimetallic model systems can occur across three fronts: structure, spectroscopy, and activity. In this Comment, we address structural and spectroscopic signatures of the enzyme in direct comparison to the models that most closely resemble the native systems. Key differences still remain between many structural and spectroscopic parameters. In all models structurally characterized to date, the asymmetry of the bridging metal-hydride is such that the iron-hydride distance is shorter than the nickel-hydride distance, directly opposite of what is seen in the enzyme. Only one model featuring a protonated thiolate has been developed, and a model that can sustain a protonated thiolate with a hydride ligand, reproducing the Ni-R state, has not yet been synthesized. Redox chemistry at iron rather than nickel is seen in many of the models, and typically the non-physiological +2/+1 couple is accessed. While the spin state remains ambiguous within the enzyme active site, all of the model complexes feature low-spin nickel, and the highest oxidation state accessed is divalent rather than the trivalent nickel commonly seen in the hydrogenases. However, with each year, increasingly active model compounds with structural elements resembling those of the native enzyme, including bridging hydrides and protonated thiolates, are developed. Characterization of these systems is being pursued using diverse techniques, beyond the traditional FTIR and EPR spectroscopies, which permits a more complete comparison between the models and the native systems. Through this

combination of structural and spectroscopic analyses, synthetic chemists can ensure that both geometric and electronic structures of the enzyme are effectively captured in model compounds. As these features are reproduced, molecules that mimic the high catalytic activity and low overpotential seen in the enzyme may also become accessible.

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