

Achieving Reversible H₂/H⁺ Interconversion at Room Temperature with Enzyme-Inspired Molecular Complexes: A Mechanistic Study

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Supporting Information

ABSTRACT: Inspired by the contribution of the protein scaffold to the efficiency with which enzymes function, we used outer coordination sphere features to develop a molecular electrocatalyst for the reversible production/oxidation of H₂ at 25 °C: $[Ni(P^{Cy}_2N^{Phe}_2)_2]^{2+}$ (CyPhe; $P^{R}_2N^{R'}_2 = 1,5$ -diaza-3,7diphosphacyclooctane, Cy = cyclohexyl, Phe = phenylalanine). Electrocatalytic reversibility is observed in aqueous, acidic methanol. The aromatic rings in the peripheral phenylalanine groups appear to be essential to achieving reversibility based on the observation that reversibility for arginine (CyArg) or glycine (CyGly) complexes is only achieved with elevated temperature (>50 °C) in 100% water. A complex with a hydroxyl group in the *para*-position of the aromatic ring, R' =



tyrosine (CyTyr), shows similar reversible behavior. NMR spectroscopy and molecular dynamics studies suggest that interactions between the aromatic groups as well as between the carboxylic acid groups limit conformational flexibility, contributing to reversibility. NMR spectroscopy studies also show extremely fast proton exchange along a pathway from the Ni-H through the pendant amine to the carboxyl group. Further, a complex containing a side chain similar to tyrosine but without the carboxyl group (CyTym; Tym = tyramine) does not display reversible catalysis and has limited proton exchange from the pendant amine, demonstrating an essential role for the carboxylic acid and the proton pathway in achieving catalytic reversibility. This minimal pathway mimics proton pathways found in hydrogenases. The influence of multiple factors on lowering barriers and optimizing relative energies to achieve reversibility for this synthetic catalyst is a clear indication of the intricate interplay between the first, second, and outer coordination spheres that begins to mimic the complexity observed in metalloenzymes.

KEYWORDS: reversible electrocatalysis, hydrogen production/oxidation, outer coordination sphere, renewable energy, enzyme mimic

INTRODUCTION

Nature uses hydrogenase enzymes to efficiently interconvert hydrogen (H_2) with protons and electrons,¹ reactions of considerable interest in the development of renewable energies. Efficiency is demonstrated in the ability of enzymes to operate with catalytic reversibility,^{2,3} meaning that enzymes can catalyze the reaction in either direction at or just beyond the equilibrium potential. This desirable feature is a demonstration of the thermodynamic and kinetic matching of each step in the catalytic cycle, something that has been evolutionarily optimized in enzymes.³ Several of the constituents of the protein structure are needed to achieve this efficiency, including the active site (first coordination sphere), the second coordination sphere, and the outer coordination sphere.^{2,4-7} While many molecular models of hydrogenase with fast rates or low overpotentials have been reported,⁸⁻¹³ there have been no reports of molecular catalysts that operate reversibly for H₂/H⁺ interconversion at room temperature.

Hydrogenase active sites are either monometallic or bimetallic, consisting of either a single iron atom, two iron

atoms, or a nickel atom and an iron atom,^{1,5,6} and mimics of hydrogenases have largely focused on the active site. $^{8,14-16}\ensuremath{\mathsf{A}}$ feature identified to be important in the [FeFe]-hydrogenase is an amine group positioned relative to one of the Fe atoms to aid in formation or cleavage of H2. Studies of second coordination sphere contributions have focused on the socalled pendant amine, $^{8,9,11,14-17}$ with the Ni($P_{2}^{R}N_{2}^{R'}$) molecular complexes a demonstration of one of the highly successful mimics.^{9,17,18} While the rest of the protein scaffold contributes significantly to catalysis in hydrogenases,^{4,6,19-21} there have been limited studies in this area in molecular mimics. The focus of our research,^{12,13,22-31} along with several other research groups,^{10,32-38} has been to attempt to utilize the influence of contributions even more remote from the active site to achieve efficient catalysis by investigating the effect of enzyme-inspired outer coordination spheres on molecular

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Figure 1. (A) CyPhe ($[Ni(P^{Cy}_2N^{Phe}_2)_2]^{2+}$), (B) CyTyr ($[Ni(P^{Cy}_2N^{Tyr}_2)_2]^{2+}$), and (C) CyTym ($[Ni(P^{Cy}_2N^{Tym}_2)_2]^{2+}$) complexes. The protic functional groups in the outer coordination sphere are highlighted in red.



Figure 2. Cyclic voltammetry for (A) 0.55 mM CyPhe, (B) 0.2 mM CyTyr, and (C) 1.1 mM CyTym in 0.1 M $^{n}Bu_{4}N^{+}BF_{4}^{-}$ in methanol at a scan rate of 0.2 V/s using a 1 mm glassy carbon electrode. The arrow indicates initial scanning direction.

catalysts. One of the recent outcomes of our approach is the achievement of reversible catalysis at elevated temperatures (>50 °C) in water by including arginines in the outer coordination sphere of the well-understood H₂ oxidation complex, $[Ni(P^{Cy}_2N^{R}_2)_2]^{2+}$, to give $[Ni(P^{Cy}_2N^{Arg}_2)_2]^{2+}$ (CyArg).¹² In spite of this advancement, room temperature catalytic reversibility (25 °C) has not yet been achieved, demonstrating that there are many molecular interactions in enzymes that still need to be understood and duplicated in molecular catalysts.

Electrocatalytic reversibility requires that all steps in the catalytic cycle (Figure S1), including H₂ addition, deprotonation, and electron transfer, are fast and reversible. This is achieved in enzymes by having low barriers and thermodynamically matched intermediates. Attempts to synthesize reversible catalysts using the $[Ni(P^{Cy}_{2}N^{R}_{2})_{2}]^{2+}$ platform have resulted in bidirectional catalysis rather than reversible catalysis when R is not an amino acid,^{17,23,39,40} where bidirectional catalysts operate in both directions but with the catalytic onset potential removed from the equilibrium potential.^{2,3} The result is catalysts that operate with a significant overpotential (70-400 mV in either direction),^{22,23} and/or exceedingly slow turnover frequencies (TOFs < 1 s^{-1}).³⁹ In our previous studies with CyArg in water, only elevated temperature (>50 °C) enabled reversible H₂ addition and fast electron transfer, suggesting that there are higher barriers to both processes at room temperature.²⁸ For the **CyArg** complex, the amines, α -carboxyl groups, and amino acid side chains were all proposed to contribute to catalytic reversibility.¹² The amine groups function as proton relays, aid in binding H₂, and assist in the heterolytic cleavage of H_{2} , as is true for all derivatives of this class of complex,¹⁷ while the carboxyl groups contribute to proton transfer.²² On the

basis of electrochemical evidence, we postulated that the side chains in **CyArg** form an intramolecular guanidinium pair that controls the positioning of the pendant amine relative to the Ni, facilitating H₂ addition to aid in reversible catalysis.^{12,24} Direct evidence of the importance of the side chain interactions in achieving fast, reversible catalysis was demonstrated with the complex **CyGly**, a similar complex with a glycine replacing the arginine. This complex was reversible under similar conditions, but nearly an order of magnitude slower in both directions.¹²

On the basis of these results, we hypothesized that if the side chains contained aromatic rings, stronger interactions between the aromatic rings may decrease conformational flexibility even more than those between guanidinium groups^{41,42} (~4 kcal/ mol^{41} compared to $\sim 1-2$ kcal/mol for Arg-Arg interactions^{43,44}). We further hypothesized that these interactions would enhance catalytic performance and allow us to advance our mechanistic understanding of the role of the outer coordination sphere on catalytic reversibility. Therefore, to achieve room temperature catalytic reversibility, in this work we probed the role of amino acids with aromatic side chains (Figure 1): CyPhe ($[Ni(P^{Cy}_2N^{Phe}_2)_2]^{2+}$; Phe = phenylalanine) and **CyTyr** ($[Ni(P^{Cy}_2N^{Tyr}_2)_2]^{2+}$; Tyr = tyrosine). We observed reversible H₂ oxidation/production catalysis in aqueous, acidic methanol at room temperature (25 °C) under 1 atm 25% H₂/ Ar for CyPhe and CyTyr while CyArg and CyGly were not reversible under these conditions. The same fundamental interactions found to be essential for CyArg, i.e., the pendant amine, the carboxyl group, and the side chain interactions, are still important here, but are functioning differently enough to result in room temperature catalytic reversibility. Using NMR spectroscopy, we directly demonstrate for the first time the movement of protons through the carboxyl group as a critical member of the proton pathway and link this to catalytic reversibility via a complex similar to tyrosine but lacking a carboxyl group ($\mathbf{R} = tyramine$; CyTym). We are able to provide novel structural evidence of the conformational control achieved with the aromatic groups using computational and experimental studies. To develop design principles for molecular catalysts, predicting the contributions of the outer coordination sphere and how it functions in concert with the first and second coordination spheres is essential. This work describes a significant advance toward this goal by demonstrating correlated mechanistic function for specific features in the outer coordination sphere with those in the first and second coordination spheres introduced in a model catalyst.

RESULTS AND DISCUSSION

Synthesis and Characterization of CyPhe, CyTyr, CyTym. The three complexes reported in this work were synthesized according to reported procedures in fair (CyTym) to excellent (CyPhe, CyTyr) yield. Characterization by a variety of methods (¹H, ³¹P{¹H}, ¹⁵N{¹H}, and ¹³C{¹H} NMR; ¹H TOCSY NMR; mass spectrometry; electrochemistry; elemental analysis) yields results that are consistent with the proposed structures. Unlike the previously reported CyGly and CyArg derivatives, CyPhe, CyTyr, and CyTym are insoluble in water. Under N₂, cyclic voltammetry of CyPhe and CyTyr showed a single wave in neutral methanol (Figure 2), similar to observations for previously reported $[Ni(P^{Cy}_2N^{AminoAcid}_2)_2]^{2+}$ complexes. Controlled potential coulometric experiments with CyPhe revealed that this wave corresponds to a two-electron process, with a peak-to-peak separation in the CV ($\Delta E_{\rm p}$) of 70 mV. This is consistent with two overlapping one-electron waves.⁴⁵ CyTym showed two distinct waves for the Ni^{II/I} and $Ni^{1/0}$ couples with a ~520 mV separation (Figure 2), consistent with non-amino-acid-containing $[Ni(P^{Cy}_2N^R_2)_2]^{2+}$ complexes.^{17,18} The irreversible Ni^{I/0} wave observed for CyTym in methanol is likely due to insolubility of the Ni(0) derivative, observed previously for these complexes and supported by the reversibility in THF (Figure S2);²⁹ reversal of the potential prior to the Ni^{1/0} in methanol for CyTym results in reversibility of the Ni^{II/I} wave.

Electrocatalyic Behavior. Upon addition of acid (protonated bis-triflimide; HTFSI) and 1 atm 25% H₂/Ar to a solution of **CyPhe** in 10% water/methanol at 25 °C, a fully reversible catalytic wave was observed, operating at the H^+/H_2 equilibrium potential in both directions (Figure 3). Room temperature catalytic reversibility with this complex when it has not been observed for similar complexes¹² implies a unique contribution of the aromatic groups.

In dry methanol the wave has a minor discontinuity (Figure 3), suggesting that water is facilitating a slightly more energetically favorable mechanism on the H₂ oxidation side, as has been observed for unidirectional H₂ oxidation catalysts.^{29,40,46} The shift observed here may stem from several sources, including a more easily oxidized isomer (endo/exo instead of endo/endo, for instance; Figure S3),^{29,40,46} better access of water to the carboxyl groups than methanol, an altered pK_a as a function of added water,⁴⁷ or enhanced Grotthuss proton transport⁴⁸ that is possible with water molecules. While the effect of water causing electrochemical shifts of tens of millivolts in this case is much smaller than that observed in unidirectional catalysts (100 to 300 mV),^{17,28,29,46} it serves to demonstrate the sensitivity of the reversibility of the catalytic process to all of the outer coordination sphere features,



Figure 3. Cyclic voltamogram of 0.36 mM **CyPhe** shows a fully reversible electrocatalytic H_2 oxidation/production wave at 25 °C in 10% water/methanol with 15 equiv of acid (HTFSI), 0.1 M ⁿBu₄N⁺BF₄⁻, and 1 atm 25% H₂/Ar (red). A nearly reversible wave is observed in the absence of water (blue). Note that while the addition of water results in a shift in the equilibrium potential of about -60 mV, it has been overlaid for direct comparison of the wave shape in 100% methanol. The vertical gray line indicates the H⁺/H₂ equilibrium potential for the data in the absence of water; the horizontal line indicates zero current, and the horizontal arrow indicates initial scanning direction. Data were collected with a 1 mm glassy carbon electrode at a scan rate of 0.2 V/s.

namely, the aromatic groups, the carboxyl groups, and the solvent.

Under the same conditions, reversible catalytic behavior with similar current enhancements was observed for CyTyr (Table 1 and Figure S4), indicating that the para-hydroxy group on the side chain of tyrosine does not influence reversibility. However, the absence of the carboxyl group of tyrosine (CyTym) had a significant impact on the catalytic properties of the complex. Under 1 atm 25% H₂/Ar and acidic methanol, conditions where CyPhe and CyTyr are catalytically reversible, H₂ production, and, consequently, reversibility, is not observed with CyTym (Table 1 and Figure 4). However, the amino acid carboxyl group alone is not responsible for catalytic reversibility in CyPhe and CyTyr because the CyGly and CyArg complexes were also tested under 1 atm 25% H₂/Ar in acidic methanol, and reversibility was not observed (Figure 4), implying a unique role of the aromatic groups in the side chains of phenylalanine and tyrosine.

The observation that **CyArg** is not catalytically reversible in methanol is notable. If **CyArg** had been reversible in methanol at room temperature, while it required elevated temperature in water, a dominant role of solvent would be implied. Likewise, **CyPhe** is catalytically reversible in up to 40% water (Figure S5), at which point it becomes insoluble, but this data implies that water is not hindering reversibility for **CyPhe**, suggesting that the reversible catalytic behavior is inherent in the catalyst, not the solvent. Collectively, these data suggest that **CyPhe** has unique properties which result in catalytic reversibility.

Scan rate independence was observed for CyPhe for both H_2 production and oxidation (Figure S6). The TOFs for CyPhe and CyTyr are faster for H_2 oxidation than H_2 production under reversible conditions, suggesting a slight catalytic bias for H_2 oxidation under these conditions (Figure 3 and Table 1). At 25 °C, the TOFs in either direction under reversible conditions for CyPhe, ~4 s⁻¹ for H_2 oxidation and ~1 s⁻¹ for H_2 production, are slower than TOFs observed for CyArg in

Fable 1. TOFs as a Function o	f Conditions	for CyTyr, (CyPhe, and O	CyTym in th	e Presence of	Acid and Water
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			conditions for reversible catalysis, 25% $\rm H_2/Ar~(1~atm)$	
complex	conditions biased for H_2 production, N_2 (1 atm) H_2 production (s ⁻¹)	conditions biased for H_2 oxidation, H_2 (1 atm) H_2 oxidation (s ⁻¹)	H ₂ production (s ⁻¹)	H_2 oxidation (s^{-1})
CyTyr	4 ± 0.1	90 ± 15	0.7 ± 0.1	4 ± 0.3
CyPhe	2 ± 0.1	135 ± 8	0.7 ± 0.4	4 ± 2
CyTym	N.D.	11 ± 0.6^{a}	N.D.	4 ± 1

^{*a*}TOF increased to 40 s⁻¹ with base (triethylamine). N.D.: not detected at the equilibrium potential. In all cases, data were collected at 25 °C in methanol with 15 equiv of acid (HTFSI) and 0.1 M $^{n}Bu_{4}N^{+}BF_{4}^{-}$ at a scan rate of 0.2 V/s with a 1 mm glassy carbon electrode.



Figure 4. Cyclic voltammograms for (A) 0.1 mM CyGly, (B) 0.1 mM CyArg, and (C) 0.2 mM CyTym in 0.1 M $^{n}Bu_{4}N^{+}BF_{4}^{-}$ in methanol under 1 atm 25% H₂/Ar with 15 equiv of acid (HTFSI). Reversible catalysis is not observed under any of the tested conditions for any of the three complexes. The data were recorded at a scan rate of 0.2 V/s using a 1 mm glassy carbon electrode.

water at ~75 °C, ~20 s⁻¹ for H₂ oxidation and ~300 s⁻¹ for H₂ production at pH \leq 1. With the assumption of similar barriers, these relative TOFs can be fully explained by the difference in temperature.^{12,47} The bias for H₂ oxidation is maintained for **CyPhe** under reversible conditions, while **CyArg** is biased to H₂ production under reversible conditions found for this catalyst, providing further support that **CyPhe** is influencing H₂ addition.

The preference for either H_2 production or oxidation can be altered by changing the catalytic conditions (Table 1, Figure 5, and Figure S7). Under 1 atm H_2 and acid, only H_2 oxidation is observed, with TOFs (90–135 s⁻¹) rivaling or surpassing other complexes of this type at room temperature. Two of the fastest $[Ni(P^{Cy}_2N^R_2)_2]^{2+}$ catalysts previously reported were **Cy^tBu** in



Figure 5. Cyclic voltammograms of 0.36 mM CyPhe in methanol under conditions optimized for H_2 production, 15 equiv of acid (HTFSI) under N_2 (red), and optimized for H_2 oxidation, 20 equiv of acid (HTFSI) and 2% water under 1 atm H_2 (blue). The black arrows indicate initial scanning direction. Data were collected with a 1 mm glassy carbon electrode at a scan rate of 0.2 V/s.

basic acetonitrile (58 s⁻¹) and **CyArg** in acidic water (210 s⁻¹).^{24,40,49,50} Under 1 atm N₂ and acid, primarily H₂ production is observed, with a small H₂ oxidation wave resulting from the H₂ produced at negative potentials. The TOF for H₂ production observed for **CyPhe** and **CyTyr** under N₂ in acidic solutions $(2-4 \text{ s}^{-1})$ is significantly slower than H₂ oxidation under 1 atm H₂, also consistent with these complexes having a thermodynamic bias for H₂ oxidation. Above 15 equiv of acid, no further increase is observed in the rate of H₂ production for **CyPhe** and **CyTyr**, indicating acid concentration independence (Figure S8). **CyTym** was also fastest under 1 atm of H₂ (Table 1 and Figure S7), although the TOF is about an order of magnitude slower than for **CyPhe** or **CyTyr** (Table 1). Adding acid to **CyTym** had little effect on the catalytic TOF.

Role of the Aromatic Groups. The aromatic side chains of CyPhe and CyTyr contribute to the enhanced performance over previous $[Ni(P^{Cy}N^{AminoAcid})_2]^{2+}$ catalysts, based on the lack of reversible catalysis for CyArg or CyGly in acidic methanol under 1 atm 25% H₂/Ar at room temperature and that, in water, elevated temperature was required for CyArg and CyGly to be reversible. The hypothesized role of the aromatic groups is to provide conformational stability via intramolecular interactions which could modify the position of the phosphorus groups or the pendant amine relative to the Ni atom. One of the features of the $[Ni(P^{R}_{2}N^{R'}_{2})_{2}]^{2+}$ family of complexes is rapid chair-to-boat interconversions of the six-membered rings (Figure 6).⁵¹⁻⁵⁵ If the proposed interactions between the aromatic groups are present, a higher barrier for the chair-toboat interconversion process (Figure 6, right) resulting in a decrease in the rate of interconversion would be expected, providing indirect evidence of this interaction. To assess the contribution of the aromatic side chains in hindering the chair-



Figure 6. Variable temperature ³¹P NMR spectra for unlabeled Ni^{II} **CyPhe** (*) in methanol under N₂ at 500 MHz ¹H resonance frequency. The coalescence temperature for **CyPhe** is -10 °C. The proposed side chain interaction of phenylalanine hindering the chair to-boat dynamics is shown on the right, with the color coded P's representing the two observed resonances below -30 °C. The cyclohexyl substituents on phosphorus are not shown for clarity.

to-boat conformational dynamics, NMR and computational studies were undertaken.

Using ³¹P NMR spectroscopy, we evaluated the chair-to-boat isomerization for **CyPhe** as a function of temperature. The isomerization process is observed by monitoring the phosphorus atoms in the Ni^{II} species (~5 ppm; indicated with an asterisk in Figure 6), as previously demonstrated.⁵¹ The second resonance in the ³¹P NMR spectrum for **CyPhe** at 25 °C (Figure 6) is attributed to a protonated species, with the proton residing on a pendant amine, supported by a strong ¹H–¹⁵N HSQC cross peak (Figure S9).

The ³¹P NMR spectra were recorded every 10–15 °C between 25 °C and –90 °C in methanol (Figure 6). At room temperature, the single resonance for the Ni^{II} **CyPhe** species results from an average of the isomerization process depicted in Figure 6. As the temperature is lowered, the isomerization process slows, resulting first in the resonance broadening to the point that it is not observed (about –10 °C, the coalescence temperature), and then separating into two unique resonances due to the inequivalence of the equatorial and apical phosphorus atoms in the five coordinate complex that is the stable product. The coalescence temperature is proportional to the barrier, ⁵⁶ and relative coalescence temperatures for different complexes can be used to compare relative barriers investigated under the same conditions.

For comparison, under the same conditions we evaluated the conformational dynamics of the Ni^{II} oxidation state of **CyGly** and **CyTym**, complexes which lack the possibility of intramolecular side chain or carboxyl group interactions, respectively. **CyGly** had a coalescence temperature of about -50 °C (Figure S10). The lower coalescence temperature implies a lower barrier to interconversion for **CyGly** than for **CyPhe**, consistent with our interpretation that interactions between the aromatic rings hinder the chair-to-boat interconversion process. **CyTym** had an even lower coalescence temperature of approximately -70 to -80 °C (Figure S10). These results indicate that both the carboxyl groups and the aromatic rings are needed to stabilize the conformational dynamics, and, when functioning together, provide significantly more stability than either functional group alone. Adding acid to **CyPhe** results in nearly identical variable temperature data as for **CyPhe** in neutral methanol (Figure S11). The $[Ni(P^{Cy}_2N^R_2)_2]^{2+}$ class of H₂ oxidation catalyst is limited by H₂ addition;²⁴ therefore, we believe that the aromatic groups are imparting a conformational stabilization that facilitates H₂ addition.

Computational studies also provide evidence of interactions between the aromatic groups. In an evaluation of **CyTyr** in an implicit representation of methanol using classical molecular dynamics simulations and umbrella sampling,⁵⁷ the free energy of the complexes was calculated with respect to the distance between the *para*-carbons on the aromatic groups (Figure 7).



Figure 7. Molecular dynamics simulations show that interactions between the tyrosine groups in CyTyr (red) are energetically favored with a minimum at \sim 5 Å, and are more favorable than the interaction between arginines in CyArg (blue). This interaction may be a key contribution in achieving room temperature catalytic reversibility.

CyArg was evaluated as a control measuring the distance between the terminal ε carbons of the arginine side chains, since similar side chain interactions have been proposed to enhance reactivity for this complex.²⁴ The **CyArg** complex was investigated with both implicit and explicit solvent, as explicit solvent–solute interaction has been proposed to be essential to capture guanidinium pairing.^{58,59}

The results of these calculations for the *inter*ligand intramolecular interactions for **CyTyr** and **CyArg** are shown in Figure 7, with a comparison of the *intra*ligand intramolecular interactions for the side chains of tyrosine (**CyTyr**) and arginine (**CyArg**) shown in Figure S12. For **CyTyr**, interactions between the interligand or intraligand aromatic groups result in



Figure 8. Variable temperature 1D ¹H NMR spectra for unlabeled (H)₂-**CyPhe** in THF- d_8 collected at 500 MHz ¹H resonance frequency. The splitting of the carboxyl protons into two resonances below -30 °C in both ¹³C and ¹H NMR spectra indicates two distinct environments for the carboxylic acid proton, consistent with the endo/endo (ee) conformation illustrated in the inset, with two carboxylic acids hydrogen bonding with the protonated pendant amines and positioned toward the metal, and two pointing away from the metal. The amine protons (red) are in rapid exchange at room temperature and in slow exchange at low temperature.



Figure 9. Variable temperature 1D NMR spectra of CyPhe in methanol after the addition of H_2 ((H)₂-CyPhe) as a function of temperature, collected at 500 MHz ¹H resonance frequency: (A) ¹³C{¹H} spectra of ¹⁵N/¹³C-labeled (H)₂-CyPhe, (B) ³¹P{¹H} spectra of unlabeled (H)₂-CyPhe, and (C) ¹⁵N spectra (-90 °C) and ¹⁵N{¹H} spectra (25 °C) of ¹⁵N/¹³C-labeled (H)₂-CyPhe. The carbon numbering scheme for the phenylalanine is shown on top of the ¹³C NMR spectra; the 54 Hz splitting of the carboxyl group in the ¹³C spectra is due to coupling with the C_{ar}.

a free energy minimum at approximately 5 Å. Representations of the lowest energy structures in Figure 7 show that the interligand aromatic groups stack in an offset face-on arrangement with a slight offset angle. For **CyArg**, no distinct minimum is observed under any conditions, providing no indication of a short-range attractive interaction. Arginine side chain interactions have been proposed to enhance reactivity for **CyArg**²⁴ based on guanidinium pairing observed in proteins.^{43,58,59} From the molecular dynamics data of Jungwirth, the Arg–Arg stabilization is very small (~1 kcal/mol);²⁴ the energy penalty to enforce the geometry required to position the guanidinium groups in this complex may be larger than 1 kcal/ mol. While these observations may suggest that our previous hypothesis regarding arginine pairing is incorrect for **CyArg**, they do agree with our hypothesis for **CyTyr** and show that interactions between aromatic side chains is possible and can stabilize the complex.

Positioning of the COOH Groups for the H₂ Addition Product of CyPhe ((H)₂-CyPhe). Structural studies were also performed for CyPhe under N₂, and for CyPhe after the addition of H₂, (H)₂-CyPhe, in methanol using ¹H, ¹⁵N, ³¹P, and ¹³C NMR spectroscopy. The ¹⁵N-labeled complex, the ¹³COOH-labeled complex, and the complex with all phenylalanine ¹⁵N and ¹³C species labeled were prepared and used to facilitate data collection and interpretation. The most notable changes were observed for (H)₂-CyPhe, and these data are summarized in Figures 8 and 9. Spectra of CyPhe under N₂ can be found in the Supporting Information (Figures S9, S11, S13, and S14). Upon the addition of H_2 to **CyPhe** at room temperature in THF- d_8 , we observe the carboxyl protons, endo and exo positioned amine protons, and hyride protons in the ¹H NMR spectra for the resulting (**H**)₂-**CyPhe** complex (Figure 8). These protons were not observed in the ¹H NMR spectrum in methanol- d_3 likely due to rapid exchange between these protons and the solvent resulting in an averaged resonance obscured by other resonances (Figure S15). Two resonances for the carboxyl protons are observed below -30 °C, consistent with two different carboxyl proton environments. These data are consistent with two carboxyl protons positioned next to the amines and two positioned away, further supported by the ¹³C, ³¹P, and ¹⁵N data discussed below.

The endo positioned amine protons are not visible at room temperature, likely due to exchange with the upfield hydride resonance; however, at lower temperatures the endo positioned amine proton resonance shifts downfield and is visible at and below 0 °C in the 1D spectra. Such downfield movement with decreasing temperature is consistent with a more stable hydrogen bond.^{60,61} A hydride resonance is visible in the 1D spectrum only at temperatures below 10 °C (Figure S16). We also observe two exo positioned amine proton resonances at room temperature which become obscured at approximately -20 °C by the emergence of the downfield resonance of the carboxyl protons.

As shown in Figure 9A, at -30 °C and below, the ${}^{13}C{}^{1}H{}$ NMR spectra of $(H)_2$ -CyPhe in methanol have two distinct resonances for the carboxyl (C1), methylene (C3), and ipso ring (C4) carbons. The observation of dual resonances for these three carbons is consistent with two environments at low temperature, as observed in the ¹H NMR spectra. Of particular interest are the two unique environments for the carboxyl groups. The most likely endo/exo and chair/boat combination of ligands about the Ni to expose the carboxyl carbons to two unique environments is the ee isomer shown in Figure 8, with the two amine protons positioned endo to the Ni (red) on the ligands in boat conformations, and the other pair oriented away from the Ni, on the ligands in chair conformations. This location of the carbons would position the protons as indicated in Figure 8, fully consistent with the ¹H NMR data. The observation that the non-ipso ring carbon resonances do not separate into two resonances as the temperature is lowered to -90 °C (C5, C6, C7; Figure S17) is further support for the interpretation that the carboxyl group is hydrogen bonding to the pendant amine.

The ³¹P NMR spectrum for (H)₂-CyPhe in methanol (Figure 9B, ~20 ppm) is best described as an endo/endo (ee) species where both protons on the amines are positioned next to the metal (Figure 8) in rapid exchange with an endo/hydride (eH, Figure S3). This is consistent with the ¹H and ¹³C NMR data since endo positioning of the amine is required to place the carboxyl protons next to the amine. A minor species (6%) is also observed in the room temperature ³¹P NMR spectrum at -10 ppm (Figure 9B), that has been attributed either to the presence of the endo/exo isomer (ex; Figure S3) which has one amine proton next to the metal and one positioned away, or the exo/exo isomer (xx; Figure S3), where the protons on both amines are positioned away from the metal.

Upon cooling to -50 °C and below, the predominant species in the ³¹P spectra is the endo/endo complex (23 and 27 ppm), with only a slight amount of residual endo/hydride (~20 ppm; Figure 9B). The two resonances observed at low temperature for the endo/endo complex are consistent with previous observations, interpreted as two phosphorus environments resulting from two phosphorus atoms positioned next to protonated pendant amines and two phosphorus atoms positioned away (Figure 8 and Figure S3).

¹⁵N NMR spectra of **CyPhe** in methanol are also suggestive of two conformers of the six-membered rings at low temperature (Figure 9C and Figure S18). The ¹H-coupled ¹⁵N NMR spectra at -90 °C shows two N environments in equal amounts, one protonated (-302 ppm) and the other with no proton (-343 ppm). This is the expected spectrum for the endo/endo complex, where two amines have residing protons and two do not, and is therefore consistent with the ¹H, ¹³C, and ³¹P data. In summary, the spectra of all of the NMR active nuclei are consistent with the structure shown in Figure 8, with the carboxyl protons positioned next to the pendant amines ready to facilitate proton transport.

Proton Transfer. In addition to the aromatic rings and carboxyl groups influencing structural flexibility, the positioning of the carboxyl groups will influence proton transfer. Electrochemically, we observe that reversibility is lost when the carboxyl group is removed, i.e., for the CyTym complex, indicating the importance of this additional proton relay in facilitating reversibility by enhancing proton movement during catalysis. Unfortunately, evaluating proton exchange under similar conditions as those under which the electrochemistry was performed was hindered by rapid exchange of the protons with methanol that effectively rendered them invisible (Figure S15). However, by using THF- d_8 to limit the exchange of these protons with the solvent, not only was it possible to observe these protons as illustrated in Figure 8, it was also possible to collect 2D-EXSY ¹H NMR spectra for these samples to evaluate proton movement at low temperature (-55 °C) as shown in Figure 10. This data was collected at -55 °C due to the rapid exchange that still existed at 25 °C in THF- d_8 .



Figure 10. Two-dimensional ${}^{1}\text{H} - {}^{1}\text{H}$ EXSY spectrum for (H)₂-**CyPhe** in THF- d_8 collected at -55 °C and 500 MHz ${}^{1}\text{H}$ resonance frequency. Stepwise proton exchange from the hydride to the endo positioned amine proton and finally the carboxyl group is observed, traced with the red dashed line in the EXSY spectrum, and with blue arrows in the structural diagram. Direct proton exchange from the hydride to the carboxyl group is not observed. The arrow depicting proton movement to methanol is based on separate NMR experiments. The asterisks (*) identify NOEs between the carboxyl groups and the protons on the β -carbon.

As illustrated in Figure 10, there are cross peaks between the two carboxyl proton resonances in the 2D-EXSY ¹H NMR at -55 °C, indicating that the carboxyl groups next to and away from the metal are interconverting with one another. The upfield carboxyl proton resonance (11.8 ppm) is exchanging with the endo proton, and the endo proton is exchanging with the hydride, as outlined with red dashed lines in Figure 10. No cross peak is observed between the hydride and the upfield carboxyl proton, providing confirmation that the proton transfers from the hydride, through the pendant amine, and then to the carboxyl group, resulting in a proton transfer pathway involving three sites. This stepwise proton pathway is reminiscent of the pathway identified in [FeFe]-hydrogenase, which transfers the proton from the metal to the pendant amine, a cysteine side chain, a conserved water, and then to the side chains of a glutamic acid, serine, and glutamic acid residue.⁴ While the molecular complex has only three relays, rather than the six found in the enzyme, this may be due to the smaller size of the molecular complex relative to the enzyme. Conversely, an even longer pathway may enhance catalysis even further in the molecular complex, particularly as the outer coordination sphere becomes longer, and as H₂ addition is enhanced.

The downfield carboxyl proton does not exchange with the endo positioned amine proton or hydride. These observations allow us to assign the upfield carboxyl proton as the one positioned next to the Ni center and the downfield carboxyl proton as the one positioned away from the metal center. The cross peaks between the carboxyl group protons and the resonances at 3.2 ppm are NOEs with phenylalanine β -carbon protons, with the latter assigned on the basis of ¹H–¹H TOCSY experiments.

Attempts to quantify the rate of proton exchange from the EXSY data are limited by the extensive overlap in the spectra with nonexchangeable protons. Furthermore, the rapid exchange of the carboxyl proton resonances results in cross peaks even at 0 ms mixing time, suggesting that the exchange process is too rapid for quantification using EXSY. Reducing the temperature and slowing exchange enough to allow quantitation for the carboxyl proton coincides with limited exchange between the hydride and amine resonances, further hindering quantitation.

While the above data provide strong evidence that in THF- d_8 there is a stepwise proton transfer from the Ni-hydride through the endopositioned pendant amine then to the carboxyl group before being handed off to the solvent, it is possible that this process is altered in methanol. To provide evidence that the carboxyl group is essential to the transfer of protons in these complexes in methanol, we added 2 equiv of methanol to $(H)_{2}$ -CyPhe in THF. Addition of methanol resulted in much faster exchange between all three exchangeable protons at -55 °C, with cross peaks between the hydride, endo, carboxyl group, and methanol, likely as a result of spin diffusion (Figure S19).⁶² At -78 °C, the only exchange observed was between the carboxyl groups and methanol, pointing to its importance as the solvent exposed proton relay (Figure S19). To further evaluate the importance of the carboxyl group in proton transfer with the solvent, we collected NMR data on CyTym, a complex lacking the carboxyl group, under several conditions in the presence of methanol. As summarized in Figure 11 and Figure S20, the endo protons were observable in the presence of H_2 with no evidence of exchange observed in a ¹H-¹H EXSY experiment (Figure 11). Confirmation of the endo proton



Figure 11. ¹H–¹H EXSY spectrum of 20 mM **CyTym** in 10% methanol in THF- d_8 at 25 °C and 500 MHz ¹H resonance frequency showing exchange between methanol (~3 ppm) and the hydroxyl group in the tyramine (red dashed lines), but not the endo proton, demonstrating the importance of the carboxyl group in **CyPhe** for transferring protons from the active site to the solvent.

assignment was provided by its disappearance in the presence of the strong base, triethyl amine (Figure S20).

The ³¹P NMR spectra for $(H)_2$ -CyPhe in methanol- d_3 shown in Figure 9B are also indirectly consistent with rapid proton movement. In methanol at room temperature, the endo/hydride complex (eH; Figure S3) and the endo/endo species are rapidly exchanged (~20 ppm; Figure 9B), supported by the downfield shift in the resonance and the residual hydride at low temperature, an exchange which requires a proton transfer. The variable temperature ³¹P NMR spectra in THF- d_8 are largely similar to the data in methanol, with the exception that all three H₂ addition isomers, endo/endo, endo/exo, and exo/exo, are observed at room temperature (Figure S21). Finally, ¹⁵N NMR spectra are also consistent with rapid proton exchange (Figure 9C). While the two ¹⁵N resonances at -90 °C are consistent with pendant amines with and without protons, at room temperature only one resonance is observed, suggesting rapid proton transport between the pendant amines resulting in an averaged resonance.53

CONCLUSIONS AND SUMMARY

Our initial hypothesis that side chain interactions are a critical element in catalytic reversibility is supported by the resulting observation that room temperature reversible catalysis for H₂ oxidation/production is achieved from complexes which contain an aromatic group in the side chain (CyPhe and CyTyr). We suggest that room temperature catalytic reversibility is a result of the stronger interaction between the aromatic rings than that between guanidinium groups in CyArg. Structural studies demonstrate that both the aromatic groups and the carboxyl groups work together to hinder chairto-boat isomerization, and further that the carboxyl groups also hinder isomerization. The role of the carboxyl groups in structural stability was unexpected, but suggests that these groups play two critical roles: providing structural stabilization and acting as proton relays. The results from NMR spectroscopy studies demonstrate that the proton moves stepwise through a proton pathway involving four sites, from the metal center to the solvent (Ni \rightarrow pendant amine \rightarrow carboxyl \rightarrow

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solvent), the first demonstration of an extended proton pathway for this family of complexes, and very reminiscent of the proton pathways found in enzymes,⁶³ such as the one identified in [FeFe]-hydrogenase (Figure 12).⁴



Figure 12. Proton pathway in the molecular complex is shown on the left in a methanol solvent, and has some similarities to the proton pathway in [FeFe]-hydrogenase (right).

The precise mechanism resulting in room temperature catalytic reversibility for CyPhe is not clear; however, elevated temperatures to achieve reversibility for CyArg enabled both reversible H_2 addition and fast electron transfer.¹² Both of these steps must be faster and more reversible at room temperature for CyPhe than for CyArg. Is it possible that, in addition to the demonstrated facile H₂ addition, CyPhe has more facile electron transfer? That CyPhe is catalytically reversible at room temperature is evidence that electron transfer is easier and may be the result of a structural twist in the NiP₄ environment closer to a tetrahedral geometry that facilitates electron transfer in the transition from Ni^{II} (square planar) to Ni^I (tetrahedral).^{9,17} A tetrahedral twist is manipulated with different substituents on the phosphorus atom to bias the complex from H₂ oxidation (cyclohexyl) or H₂ production (phenyl),^{9,17,18} and the aromatic groups in the periphery of CyPhe may be providing enough steric constraint to influence this twist. Additional evidence that CyPhe may have enhanced electron transfer can be observed in comparing the catalytic current response for H₂ oxidation for CyArg (Figure 4) and CyPhe (Figure 5) in acidic methanol. The current response is more rapid for CyPhe than for CyArg, consistent with more facile electron transfer.³¹ Clearly, this hypothesized phenomenon will need further evaluation to more fully understand the contribution of the aromatic groups to catalytic reversibility; however, enzymes are known to stabilize unique active site structures,⁶⁴ and it is possible that the simple scaffold on this molecular complex is serving a similar role. What is clear is that the aromatic groups impart unique functionality, based on achieving room temperature catalytic reversibility only in their presence.

EXPERIMENTAL METHODS

General Procedures. Samples were prepared under an N₂ atmosphere using either an anaerobic glovebox or a Schlenk line. Anhydrous methanol (Sigma-Aldrich, Sure-Seal) was used as received. Ultrapure water, 18.2 M Ω cm, was obtained from a Millipore unit. Solution state ¹H, ¹³C, ¹⁵N, and ³¹P NMR spectra were recorded on Agilent VNMR spectrometers (300 or 500 MHz ¹H resonance frequency). Direct detect dual-band or OneNMR probes were used. Typical ³¹P 90° pulses were ~8 μ s, and ³¹P NMR spectra were collected with ¹H decoupling.

All ¹H chemical shifts were internally referenced to the monoprotic solvent impurity; ³¹P chemical shifts were externally referenced to concentrated H_3PO_4 (0 ppm). ¹³C spectra were referenced to the deuterated solvent in which the experiment was run; ¹⁵N spectra were externally referenced to CH₃NO₂ (0 ppm).

Synthesis. Synthesis of $P^{Cy}_2 N^{Phe}_2$. The ligand $P^{Cy}_2 N^{Phe}_2$ was prepared similar to methods previously described.^{22,24} Bis-(hydroxymethyl)cyclohexylphosphine (1.04 g, 5.88 mmol) and phenylalanine (Phe) (0.97 g, 5.88 mmol) were dissolved in 20 mL of absolute ethanol in a Schlenk flask and heated at 70 °C for 15 h. The resulting white precipitate was collected on a fritted funnel by vacuum filtration and was washed thoroughly with ethanol and acetonitrile to obtain a white solid powder. Yield: 1.6 g (2.64 mmol) (90%). ¹H NMR (CD₃OD): δ 0.72– 2.12 (Cy-H, 22H, m); 2.78-3.50 (PCH₂N, NCH(CH₂C₆H₅)-COOH, 12H, m); 4.07 (NCH(CH₂C₆H₅)COOH, 2H, br); 6.99–7.59 (C₆H₅, 10 H, m). ³¹P NMR (CH₃OH): δ –27.0 ppm. ESI MS (positive mode): $m/z [P^{Cy}_2N^{Phe}_2 + H^+]$: 611.32 (calcd 611.31). Due to the limited solubility of the ligand in all solvents tested, ¹³C NMR spectra were not recorded. Anal. Calcd for $[P^{Cy}_2N^{Phe}_2 + 1.5EtOH]$: C, 64.61 H, 8.35; N, 4.07. Found: C, 64.80; H, 8.38; N, 4.17. Three isotopically labeled ligands were also prepared using this procedure with labeled phenylalanine purchased from Cambridge Isotopes: (1) ¹⁵Nlabeled, (2) ¹³COOH-labeled, and (3) all ¹³C- and ¹⁵N-labeled. Similar results were obtained for each complex.

Synthesis of $P^{Cy}_2 N^{Tyr}_2$. The $P^{Cy}_2 N^{Tyr}_2$ ligand was synthesized following a procedure similar to the $P^{Cy}_2 N^{Phe}_2$ ligand synthesis, using bis(hydroxymethyl)cyclohexylphosphine (0.88 g, 5 mmol) and tyrosine (Tyr) (0.90 g, 5 mmol), and collected as a white powder. Yield: 1.5 g (2.33 mmol) (93%). ESI MS (negative mode): m/z [$P^{Cy}_2 N^{Tyr}_2 - H^+$]⁻: 641.29 (calcd 641.30). Due to the limited solubility of the ligand in all solvents tested, NMR spectra were not recorded. Anal. Calcd for [$P^{Cy}_2 N^{Tyr}_2$ + EtOH]: C, 62.78; H, 7.90; N, 4.07. Found: C, 63.01; H, 7.53; N, 4.39.

Synthesis of $P^{Cy}_2 N^{Tym}_2$. The $P^{Cy}_2 N^{Tym}_2$ ligand was synthesized following a procedure similar to the $P^{Cy}_2 N^{Phe}_2$ ligand synthesis, using bis(hydroxymethyl)cyclohexylphosphine (0.348 g, 1.97 mmol) and tyramine (Tym) (0.274 g; 1.98 mmol). Yield: 0.4 g (0.73 mmol) (74%). ¹H NMR (CD₃OH): δ 0.94–1.99 (Cy-H, 22H, m); 2.55–3.06 (PCH₂N, NCH₂CH₂C₆H₅, 10H, m); 3.13 (NCH₂CH₂, 2H, br); 6.73– 7.07 (NCH₂CH₂C₆H₅, 8H, m). ³¹P{¹H} NMR (CD₃OH): -42.0 (br), -29.0. ¹³C NMR (CD₃OD): 23.73–28.14 (m, C– Cy), 31.20 (CH₂–CH₂–Ph), 33.84 (P–CH₂–N), 51.08–55.26 (m, N–CH₂–CH₂–Ph), 112.89, 127.21, 127.65, 128.69, 128.99, 153.42 (CH₂–C–Ph). ESI MS (positive mode): m/z[$P^{Cy}_2 N^{Tym}_2$ + H⁺]: 555.16 (calcd 555.32). Anal. Calcd for [$P^{Cy}_2 N^{Tym}_2$ + 2EtOH + H₂O]; C, 65.04; H, 9.40; N, 4.21. Found: C, 65.08; H, 9.09; N, 3.72.

Synthesis of $[Ni(P^{Cy}_2N^{Phe}_2)_2](BF_4)_2$ (**CyPhe**). $[Ni(CH_3CN)_6]$ -(BF₄)₂ (120.0 mg, 0.251 mmol) was dissolved in 3 mL of methanol, added dropwise to a suspension of $P^{Cy}_2N^{Phe}_2$ (305.0 mg, 0.500 mmol) in 10 mL of methanol, and stirred for 6 h. The solution turned reddish brown after the addition of the Ni²⁺ solution. The solvent was removed under reduced pressure. The resulting reddish brown powder was collected on a fritted filter under vacuum after thorough washing with diethyl ether. Yield: 350.0 mg (0.24 mmol) (96%). ¹H NMR (CD₃CN): δ 1.12–2.31 (Cy–H, 44H, m); 2.72–3.55 (PCH₂N, NCH(CH₂C₆H₅)COOH, 24H, m); 3.78 (NCH(CH₂C₆H₅)-

COOH, 4H, t); 6.99–8.01 (C_6H_5 , 20H, m); 9.74 (COOH, br s, 4H). ³¹P{¹H} NMR (CD₃OH): 7.5 (br), 9.9; ¹H–¹H TOCSY cross peaks at 3.78 (NCH(CH₂C₆H₅)COOH) and 2.99 (NCH(CH₂C₆H₅)COOH) confirmed the assignments. ¹³C NMR (for ¹³C enriched Phe samples, d_8 -THF): 34.49 (m, Ph–CH₂–CH), 70.32 (s, α -CH–N), 126.31, 129.29, 137.94 (m, Ph–C), 171.45, and 171.47 (s, COOH). ESI MS (negative mode): m/z [Ni(P^{Cy}₂N^{Phe}₂)₂(BF₄)]⁺: 1369.52 (calcd 1369.59). Anal. Calcd for {[Ni(P^{Cy}₂N^{Phe}₂)₂](BF₄)₂ + 4H₂O + 1MeCN}: C, 53.66; H, 6.88; N, 4.47. Found: C, 53.19; H, 6.57; N, 4.15.

Synthesis of $[Ni(P^{Cy}_2N^{Tyr}_2)_2](BF_4)_2$ (**CyTyr**). $P^{Cy}_2N^{Tyr}_2$ ligand (131.0 mg, 0.20 mmol) was mixed with 1 equiv of LiOH (5.0 mg, 0.200 mmol) in 10 mL of methanol to obtain a cloudy white solution. Then, ~0.5 equiv of $[Ni(CH_3CN)_6](BF_4)_2$ (49.0 mg, 0.10 mmol) dissolved in 3 mL of methanol was added dropwise to the ligand solution and stirred for 1 h. Upon mixing, the solution cleared and turned reddish brown. After 1 h, the solution was filtered to remove any unreacted ligand, and the solvent was removed under reduced pressure to obtain a reddish brown powder. It was collected on a fritted filter under vacuum after thorough washing with diethyl ether. Yield: 70 mg (0.036 mmol) (23%). ¹H NMR (CD₃OH): δ 0.64–2.22 (Cy– H, 44H, m); 2.49-3.78 ppm (PCH₂N, NCH(CH₂C₆H₅OH)-COOH, 28H, m); 7.19–7.35 (C_6H_5 , 16H, m). ³¹P{¹H} NMR (CD₃OH): 9.0, 10.1 (br). ¹³C NMR (CD₃OD): 25.05-28.45 (m, Cy-C), 33.18 $(s, Ph-CH_2-CH)$, 36.77 $(s, P-CH_2-N)$, 69.51 (s, Ph-CH₂-CH₂-N), 115.00, 115.63, 115.92, 116.88, 118.64, 120.95, 123.64, 127.60, 130.12, 155.59, 172.53, 173.59. ESI MS (positive mode): $m/z [Ni(P^{Cy}_2N^{Tyr}_2)_2 + 4H - BF_4]^+$: 1429.49 (calcd 1429.54). Anal. Calcd for $\{[Ni(P^{Cy}_2N^{Tyr}_2)_2]$ - $(BF_4)_2$ + 15LiOH + 2MeCN}: C, 44.14; H, 6.02; N, 4.29. Found: C, 43.88; H, 5.89; N, 4.62.

Synthesis of $[Ni(P^{Cy}_2N^{Tym}_2)_2](BF_4)_2$ (**CyTym**). [Ni-(P^{Cy}_2N^{Tym}_2)_2](BF_4)_2 was synthesized following the procedure for the $[Ni(P^{Cy}_2N^{Phe}_2)_2](BF_4)_2$ complex, from 228 mg of $P^{Cy}_2N^{Tym}_2$ ligand (0.411 mmol) and 98.0 mg of [Ni-(CH₃CN)₆](BF₄)₂ (0.20 mmol) in 10 mL of methanol. Yield: 340.0 mg (0.252 mmol) (62%). ¹H NMR (THF-*d*₈): δ 0.65–2.22 (Cy–H, 44H, m); 2.29–3.46 ppm (PCH₂N, CH₂CH₂C₆H₅); 32H, 6.28–7.43 (C₆H₅, 16H, m), 7.85 (C₆H₅OH). ³¹P{¹H} NMR (CD₃OH): δ 7.9. ¹³C NMR (CD₃CN): 22.51–30.65 (m, Cy–C); 35.16 (s, Ph–CH₂– CH₂), 48.44 (s, P–CH₂–N), 59.38 (s, Ph–CH₂–CH₂–N), 113.02, 115.15, 127.70, 153.52 (m, Ph–C). ESI MS (positive mode): m/z [Ni(P^{Cy}₂N^{Tym}₂)₂(BF₄)₂]⁺: 1170.56 (calcd 1170.60). Anal. Calcd for {[Ni(P^{Cy}₂N^{Tym}₂)₂](BF₄)₂ + 4H₂O}: C, 54.37; H, 7.42; N, 3.96. Found: C, 54.26; H, 7.09; N, 3.98.

Electrochemistry. Cyclic voltammetry was performed on solutions with the complex in 0.1 M ${}^{n}Bu_{4}N^{+}BF_{4}^{-}$ electrolyte in methanol using a glassy-carbon electrode (1 mm diameter), polished with 0.25 μ m MetaDi diamond polishing paste (Buehler). Cyclic voltammetry experiments were performed at 25 °C on a CH Instruments 1100A or 600D electrochemical analyzer using a standard three-electrode configuration. A glassy carbon rod was used as the counter electrode, and a AgClcoated Ag wire (in 0.1 M "Bu₄N⁺BF₄") separated from the analyte solution by a Vycor frit was used as the reference electrode. All couples were referenced to the internal reference ferrocenium/ferrocene couple (0.0 V vs $FeCp_2^{+/0}$). All electrocatalysis experiments were performed as previously reported,²² in an inert atmosphere, in 0.1 M ⁿBu₄N⁺BF₄⁻ in the desired solvent (methanol or THF) with catalyst concentrations 0.2-0.3 mM. A scan rate of 200 mV/s was

typically used. Hydrogen oxidation experiments were carried out by purging 100% H_2 gas into the reaction vial. Reversible electrocatalysis were performed by purging 25% H_2 /Ar into the reaction. Acid additions were made with HTFSI using a microliter syringe for both acid and water additions until no additional enhancement was observed. All experiments were repeated at least three times for statistical accuracy.

TOFs. Due to the complexity of the waves under noncatalytic conditions, TOFs were determined with eq 1, where *D* is the diffusion coefficient determined by DOSY NMR (**CyPhe** (2.99 × 10⁻⁶ cm²/s), **CyTyr** (2.69 × 10⁻⁶ cm²/s), and **CyTym** (3.60 × 10⁻⁶ cm²/s)), *A* is the electrode surface area (9.23 × 10⁻³ cm²), ²² *n* is the number of electrons (two), and *F* is Faraday's constant.

$$\text{TOF} = \left(\frac{i_{\text{cat}}}{nFA[\text{cat}]\sqrt{D_{\text{cat}}}}\right)^2 \tag{1}$$

Controlled-Potential Coulometry. A 20 mL electrochemical cell covered with a septum cap was filled with 10 mL of methanol and 5.0 mg $(3.3 \times 10^{-3} \text{ mmol})$ of **CyPhe** along with 1.0 mmol of ${}^{n}\text{Bu}_4\text{N}^+\text{BF}_4^-$ and a stir bar. The working electrode was prepared from reticulated vitreous carbon (1 cm diameter by 2.5 cm length) and connected with a copper wire. A coiled nickel–chromium wire, immersed in a 0.10 M ${}^{n}\text{Bu}_4\text{N}^+\text{BF}_4^-$ acetonitrile solution containing a fine frit at the end, was used as the counter electrode. The reference electrode consisted of a silver wire immersed in a 0.10 M ${}^{n}\text{Bu}_4\text{N}^+\text{BF}_4^-$ acetonitrile solution along with a Vycor frit. All of the electrodes were placed into the electrochemical cell through the septum cap.

The amount of charge passed for CyPhe was recorded for 10 min at -1.00 V versus the ferrocenium/ferrocene couple, during which time, the current dropped to 9% of its original value. During the experiment, the reddish brown Ni(II) CyPhe complex turned pale yellow. A control experiment was performed at -1.00 V versus the ferrocenium/ferrocene couple with the same setup using a blank containing only methanol/ 1.0 mmol $^{n}Bu_{4}N^{+}BF_{4}^{-}$. CyPhe exhibited 0.61 C of charge passed in 10 min. This is an average value collected over four independent runs and was corrected from background as 0.02 C charge was passed for the blank solution in the same amount of time. The expected current for a one-electron process with that amount of complex was 0.318 C (9.64853 \times 10⁴ C mol⁻¹ \times 3.3 \times 10⁻³ mmol), which corresponds to a 96% current efficiency. This is consistent with the wave observed in the cyclic voltammetry for CyPhe in methanol consisting of two electrons.

Mass Spectrometry (MS). MS analysis was performed using a LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, San Jose, CA) outfitted with a custom electrospray ionization (ESI) interface. Electrospray emitters were custommade using 360 μ m o.d. × 20 μ m i.d. chemically etched fused silica. The ion transfer tube temperature and spray voltage were 300 °C and 2.2 kV, respectively. Orbitrap spectra (AGC 1 × 10⁶) were collected from 600 to 2000 *m*/*z* or from 300 to 600 *m*/*z* at a resolution of 100k. Samples were directly infused using a 250 μ L Hamilton syringe at a flow rate of 1 μ L/min. The concentrations for both the (P^{Cy}₂N^R₂) ligands and Ni-(P^{Cy}₂N^R₂)(BF₄)₂ complexes (R = Phe, Tyr, Tym) were adjusted to ~50 μ M in methanol for the mass spectrometry experiments.

NMR Spectroscopy. Variable Temperature NMR Spectroscopy. Variable temperature NMR data were collected from

25 to -90 °C, using either liquid nitrogen (temperatures lower than -60 °C) or an XRII 852 sample cooler (FTS Systems, Stone Ridge, NY) (temperatures between -60 to 20 °C) to cool the samples. For each data point, the actual temperature was internally or externally calibrated using methanol as a standard.^{65,66} The NMR spectra for ¹⁵N and ¹⁵N{¹H} were collected using 30 mM uniformly ¹⁵N- and ¹³C-labeled **CyPhe**. The ¹³C, ¹³C{¹H}, and ³¹P{¹H} NMR spectra were collected on 20–30 mM **CyPhe** in THF-*d*₈, methanol-*d*₃, methanol-*d*₄, or methanol using a combination of unlabeled, ¹³COOH-labeled, and uniformly ¹⁵N/¹³C-labeled complexes. Protonated bistriflimide (HTFSI) was used as the acid for all acid additions.

Two-Dimensional NMR Spectroscopy. Two-dimensional NMR EXSY experiments were recorded for 30 mM **CyPhe** solutions at temperatures of -90, -78, -55, and 25 °C in THF- d_8 or THF- d_8 plus 2 equiv of methanol (relative to the complex). The standard phase-sensitive VNMRJ 2D NOESY pulse program was used with 256–512 increments, 16–32 scans per increment, and 50–200 ms mixing times. A 1 H– 15 N HSQC spectrum was collected for 30 mM **CyPhe** under N₂ methanol.

Determination of the Diffusion Coefficient. Diffusion measurements were performed at 25 °C on a 300 MHz Varian spectrometer. The system is equipped with a single axis gradient probe that has a maximum gradient strength of 20 G/cm. Gradient calibration utilized a standard sample (1% H₂O in 99% D₂O) that yielded a diffusion coefficient of 1.9×10^{-9} m²/s for ¹H₂O using the bipolar pulsed-field-gradient sequence. The NMR signal attenuates as described by the Stejskal–Tanner equation (eq 2):

$$I = I_0 e^{-D\gamma^2 g^2 \delta^2 (\Delta - \frac{\delta}{3})}$$
(2)

Here, the following abbreviations apply: I_0 denotes the signal intensity in the absence of gradient, γ is the gyromagnetic ratio of the studied nuclei, g is the gradient strength, δ is the gradient pulse duration (3 ms), and Δ is the time interval (50–100 ms) between two gradient pairs. In our measurements, we varied the gradient strength from 0 to 20 G/cm in 10 steps with 16 scans at each step. Normal signal attenuation yielded a single diffusion coefficient for the catalyst, with an experimental error bar of <10%. Diffusion coefficients for the [Ni(P^{Cy}₂N^R₂)₂]-(BF₄)₂ complexes (R = Phe, Tyr, Tym) were determined using 15 mM complex in 0.1 M ⁿBu₄N⁺BF₄⁻ in methanol- d_4 .

Computational Studies. Classical molecular dynamics (MD) simulations were carried out using the Amber11 program.⁶⁷⁻⁶⁹ The parameters for the ligands were taken from the GAFF force field.⁷⁰ For the $[Ni(P^{Cy}_2N^{AminoAcid}_2)_2]^{2+}$ core we used the parameters developed for the [Ni- $(P^{Cy}_2N^{Me}_2)_2]^{2+}$ complex³¹ and supplemented the missing bonding interaction with parameters from the GAFF force field. Charges for the $[Ni(P^{Cy}_2N^{AminoAcid}_2)_2]^{2+}$ core were calculated using the standard RESP procedure.⁴⁵ The solvent (water for CyArg, methanol for CyPhe) was treated implicitly using the generalized Born (GB) model.⁷¹ All simulations were done at a temperature of 298.15 K and 1 atm pressure. The free energy calculations were done using potential of mean force (PMF) simulations with umbrella sampling⁵⁷ and the weighted histogram analysis method.⁷² The reaction coordinate for the umbrella sampling was the C–C distance between the ε carbons of the two arginine groups for CyArg or the two paracarbons of the aromatic rings for CyPhe. A range of distances from 3.0 to 9 Å was covered in increments of 0.5 Å for a total of 14 umbrella sampling windows. For each window a 2 ns simulation was carried out. A control PMF calculation was done for **CyArg** with an explicit solvent (water) in a periodic cubic simulation box with 1935 water molecules. The water model used in the simulation is TIP3P.⁷³

ASSOCIATED CONTENT

S Supporting Information

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Catalytic cycle, electrochemistry, variable temperature NMR, and computational data (PDF)

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Notes

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