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Moving Protons and Electrons in Biomimetic Systems

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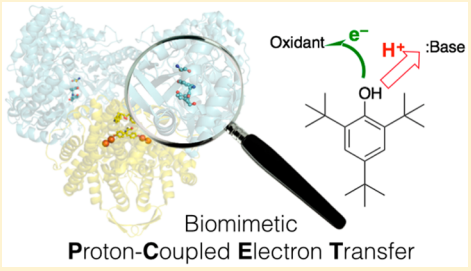
ABSTRACT: An enormous variety of biological redox reactions are accompanied by changes in proton content at enzyme active sites, in their associated cofactors, in substrates and/or products, and between protein interfaces. Understanding this breadth of reactivity is an ongoing chemical challenge. A great many workers have developed and investigated biomimetic model complexes to build new ways of thinking about the mechanistic underpinnings of such complex biological proton-coupled electron transfer (PCET) reactions. Of particular importance are those model reactions that involve transfer of one proton (H^+) and one electron (e^-), which is equivalent to transfer of a hydrogen atom ($H^\bullet$). In this Current Topic, we review key concepts in PCET reactivity and describe important advances in biomimetic PCET chemistry, with a special emphasis on research that has enhanced efforts to understand biological PCET reactions.



INTRODUCTION AND HISTORICAL PERSPECTIVE

The importance of atom transfers in redox reactions (electron transfers) was established in the late 1800s, culminating most famously with the formulation of the Nernst equation.[1] In a compendium published in 1963, Pourbaix applied the Nernst equation to an enormous variety of redox reactions that depend on proton concentration in widely used pH versus *E* diagrams that bear his name.[2] Transfer of one electron (e^-) and one proton (H^+) together, often more easily described as hydrogen atom ($H^\bullet$) transfer (HAT), has long been a focus of physical organic chemistry. HAT reactions of organic molecules were intensely investigated in the 1950s and 1960s,[3] and in 1960, Wiberg showed that chromium(VI) compounds could oxidize organic molecules by HAT.[4]

To the best of our knowledge, the first paper explicitly invoking the coupling of H^+ and e^- transfers in a metalloenzyme was by Edward Stiefel in 1973, entitled "Proposed Molecular Mechanism for the Action of Molybdenum in Enzymes: Coupled Proton and Electron Transfer". It is a particularly prescient essay, including the phrase "If both protons and electrons are delivered to the substrate in a concerted process, ..." In 1980, Eberson (likely independently) used the phrase "concerted electron/proton transfer" to describe the oxidation of toluene by tungsten polyoxometallate complexes,[5] though the mechanism was later revised.[6] Less than a year later, in 1981, Meyer coined the term "proton-coupled electron transfer" (PCET) to describe reactions between Ru^{IV}–oxo and Ru^{II}–aquo complexes (Figure 1).[7]

Researchers working on chemical and biological problems during the intervening 40 years have found that many, even most, electron transfer (ET) reactions are coupled in some way to proton transfer (PT) events.[8] In many cases, biological "electron transport chains" could be better described as "electron–proton transport chains". Sometimes, the transfer

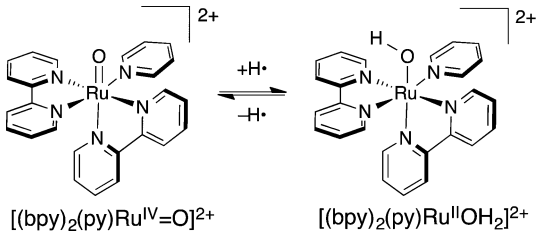


Figure 1. Prototype for the first named PCET reaction between $[(bpy)_2(py)Ru^{II}OH_2]^{2+}$ and $[(bpy)_2(py)Ru^{IV}{=}O]^{2+}$ (bpy = 2,2′-bipyridyl; py = pyridine).

of the e^- and H^+ is tightly coupled [e.g., transfer of a hydride from NAD(P)H or abstraction of hydrogen from hydrocarbons by compound I of cytochrome P450s]. At other times, the degree of coupling is less clear (e.g., pH-dependent reduction potentials of redox proteins such as cytochromes *c*). The complexity of many of the biochemical processes, and the challenges of monitoring H^+ in water, lead to the use of biomimetic small molecule complexes to provide an understanding of such complex biological phenomena at a detailed (atomic) level. In many ways, this parallels the use of simpler chemical systems in the early work on pure ET reactions.[9]

The goal of this Current Topic is to summarize key developments in biomimetic PCET chemistry and their relation to biological systems, rationalize different PCET reactivity patterns, and outline important frontiers to improve our understanding of biological PCET reactions. We also touch upon important challenges associated with investigating and

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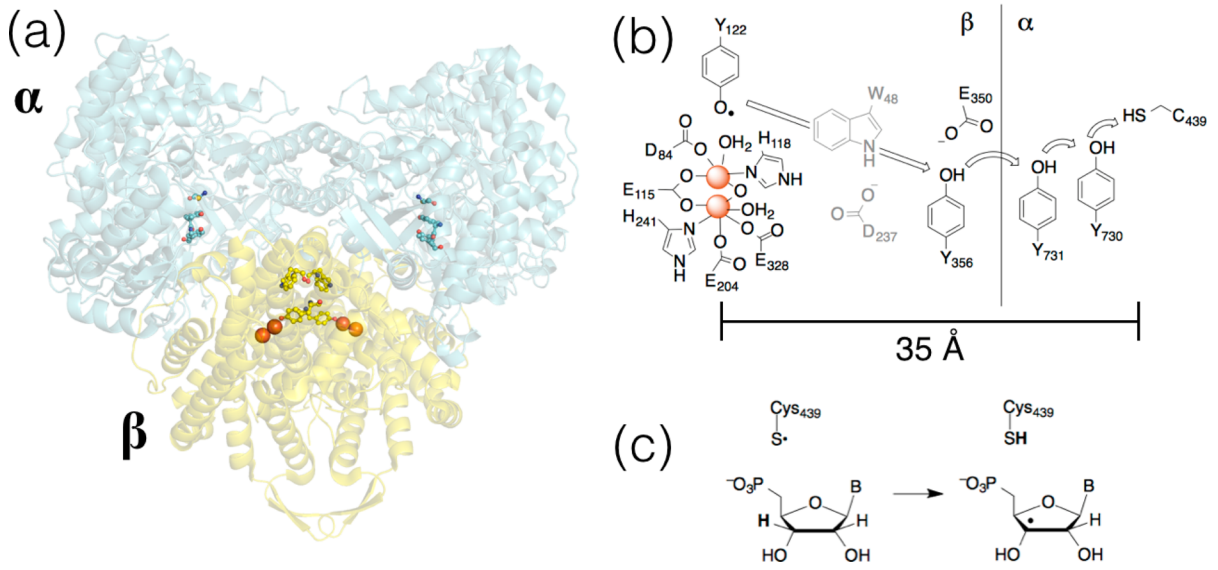

Figure 2. (a) Docking model of *E. coli* RNR.[11] (b) Schematic of the amino acid residues involved in PCET activation of Cys429. (c) Abstraction of hydrogen from ribonucleotide substrate by Cys349.

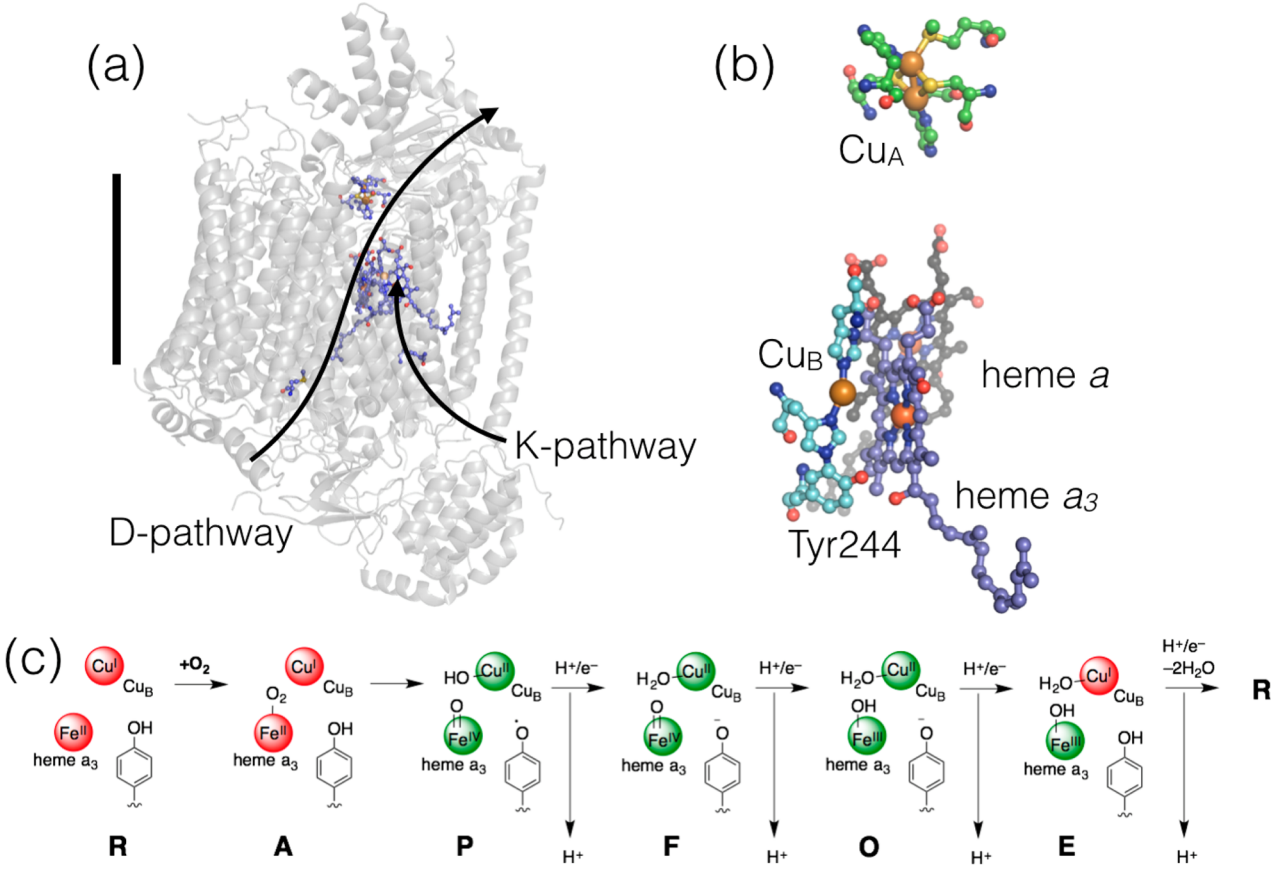

Figure 3. Structure and mechanism of cytochrome *c* oxidase proteins. (a) Structure of C*c*O from bovine (Protein Data Bank entry 2EIJ[20]) where the H^+ loading pathways are labeled and the black bar indicates the approximate width of a lipid bilayer. (b) Close-up view of the redox cofactors in the active site. (c) Mechanism of reduction of O_2, including commonly used one-letter abbreviations for the intermediate species. The red-colored spheres indicated reduced metal sites, and the green spheres indicate oxidized sites.

rationalizing PCET reactivity, both in small molecule models and more broadly in redox proteins.

View from the Top: Examples of Diverse PCET in Enzymes. PCET is recognized in many biological systems, but we think that it is underappreciated (or undiscovered) in an even greater number of biological processes and biomolecules. In our view, any system in which the reduction potential varies with pH is exhibiting PCET in some form. In this section, we develop a picture of PCET using two examples: class Ia ribonucleotide reductase (RNR) and cytochrome *c* oxidase

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(C*c*O). While no single system embodies all aspects of PCET, these two systems capture many salient points. In this section, we use these two examples to lay the groundwork for our subsequent discussion of the kinetics and thermodynamics of PCET reactions in small molecule models, underscoring the importance of those models in appreciating biological PCET.

RNR enzymes are essential for DNA biosynthesis, converting ribonucleotides to deoxyribonucleotides. Perhaps the best studied RNR is the class Ia enzyme from *Escherichia coli*, but we note that detailed data for other enzymes recently became available.[10] *E. coli* RNR (termed RNR hereafter, for convenience) is a heterotetrameric protein, consisting of homodimeric α and β subunits (Figure 2a).[11] Each turnover of the catalytic cycle involves net transfer of e^- ~35 Å from the substrate-binding site (on the α subunit) to a di-iron-tyrosyl site in the β subunit (Figure 2b); substrate- and effector-triggered conformational changes play key roles in the redox events.[12] For these, and any PCET reaction, the pK_a (describing the PT component) and $E°$ (describing the ET component) values provide valuable insights. To that end, workers have incorporated modified tyrosine into the PCET chain of RNR (Figure 2b) to test for pK_a perturbations[13] and relative redox levels of the redox active amino acids.[14] Likewise, these studies also provide kinetic data that can be used to rationalize fundamental reactivity in a complex system involving conformational and redox changes.[15]

The redox chemistry of tyrosine is central to RNR function. As outlined below, redox reactions of tyrosine are inherently proton-coupled because of the strong acidity of the oxidized (radical cation) form. By moving the electron and proton together, one can avoid this high-energy form. Therefore, PCET likely is involved in the activation of the diiron-tyrosyl active oxidant to form the initial tyrosyl radical.[16] Most notable is the long PCET cascade from the α to the β subunits in RNR, which also features two distinct types of PCET. The first involves $1H^+/1e^-$ oxidation of tyrosine where H^+ and e^- have distinct acceptors (Figure 2b); the second involves direct abstraction of $H^•$ from a ribonucleotide C−H by a cysteinyl radical (Figure 2c). The former type of PCET highlights the disparity in distance scales for PT and ET, raising the interesting questions of mechanism (stepwise, with separate H^+ and e^- transfer steps, or concerted, $H^•$ transfer) and PCET distance dependence.

Cytochrome *c* oxidases (C*c*Os, Complex IV in the mitochondrial respiratory chain or, more broadly, heme-copper oxidases) are the terminal electron acceptors in the respiratory chain. In these transmembrane enzymes (e.g., Figure 3a), reduction of O_2 to $2H_2O$ is coupled to transmembrane H^+ pumping.[17] The mammalian enzyme contains 13 subunits, but studies of very similar and more easily obtained bacterial proteins have facilitated understanding of the remarkable mechanism of C*c*O enzymes.[18] PCET is involved in every aspect of C*c*O function: reduction of O_2 to H_2O is a $4H^+/4e^-$ PCET reaction, and for each input of an e^- to the active site, H^+ is translocated across the membrane (where the H^+ stoichiometry is enzyme-dependent[18a]). In fact, PCET is critical throughout the mitochondrial and photosynthetic "electron−proton transport chains", as highlighted above.

The C*c*O active site contains one heme (heme a_3), a Cu (Cu_B), and a tyrosine residue that is cross-linked with a nearby His (which ligates Cu_B) (Figure 3b). The protein also contains two redox cofactors: heme *a* and a purple copper (Cu_A) site. These sites serve as e^- reservoirs for O_2 reduction, and the latter is the primary redox partner with cytochromes *c*.[19] Reduction of O_2 starts from the fully reduced active site (R, Figure 3c). Binding of O_2 gives state A. Four-electron reduction of the O_2 substrate in an apparent single step yields the Fe^{IV} form of heme a_3, Cu^{II}_B, and a tyrosyl radical. Subsequent additions of $1H^+$ and $1e^-$ reduce the active site back to the resting state (R) and release 2 equiv of water, and each redox step is coupled to a H^+ pumping step. This is only a bare overview of a complex process,[17,18] but as for RNR, it demonstrates several aspects of PCET chemistry. First, the step going from state A to state P is an example of a proton-coupled bond breaking (O═O and TyrO−H). Subsequent H^+/e^- additions are examples in which e^- are delivered to a metal site and H^+ are delivered to a metal-ligated group. As for RNR, these latter steps (P to R) feature PCET reactions in which e^- and H^+ have distinct donors and acceptors. Finally, each ET reaction at the active site is coupled to H^+ translocation over a great distance, which is required for net transmembrane H^+ pumping.

The two examples cited above set the groundwork for our ensuing discussion. A great many small molecule model systems have been examined to provide atomic-level details to answer important questions about biological PCET. What are the factors that dictate H^+ and e^- and transfer between the same donors and acceptors or different donors and acceptors? Are such reactions described by component theories of PT and ET, or is a new framework required? How can a cofactor (such as tyrosine) mediate different types of PCET? Importantly, how should these seemingly different reactions be rationalized, and what is the "best" experimental approach for answering such questions?

PCET REACTION CLASSES

The term PCET was initially used[7] to distinguish concerted H^+/e^- transfer reactions of inorganic complexes from hydrogen atom transfer (HAT) reactions long studied by organic chemists. However, because of extensive and varied use, experimentalists describing H^+/e^- transfer reactions typically use the term "PCET", regardless of stoichiometry or mechanism.[21] In the literature of chemical theory, PCET is still used mostly to refer just to the concerted transfer of $1e^-$ and $1H^+$.[8a] Because of this ambiguity, we encourage workers in the field to use very clear descriptions of H^+/e^- transfer reactions.

Studies of small molecule models have made possible important mechanistic distinctions for PCET reactions. There are three limiting mechanisms for those PCET reactions in which only one H^+ and one e^- are transferred: stepwise ET, and then PT; stepwise PT, and then ET; and a concerted mechanism in which H^+ and e^- are transferred in a single kinetic step. We recommend following the Savéant group and calling the third mechanism concerted proton−electron transfer (CPET).[22] Other workers term such reactions electron−proton transfer (EPT)[21b] or concerted electron−proton transfer (CEP).[23]

It has been suggested that HAT reactions might be considered as a special class of CPET in which the transferring H^+/e^- come from the same chemical bond.[21b] The distinction is subtle, but the implications for reactivity can be profound. One example is the transfer of H^+/e^- from $[(bpy)_2(py)Ru^{II}OH_2]^{2+}$ to $[(bpy)_2(py)Ru^{IV}═O]^{2+}$. The ground state products, from a CPET reaction, are 2 equiv of $[(bpy)_2(py)Ru^{III}OH]^{2+}$. Using the definition of HAT given above, the $H^•$

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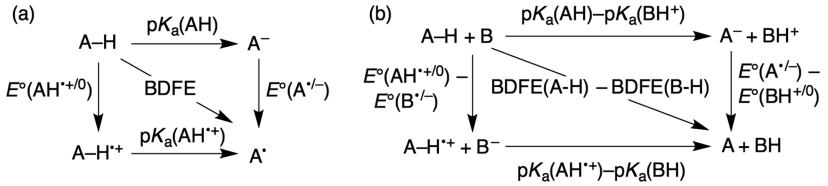

Figure 4. Thermodynamic square schemes (a) for a single PCET reagent (AH) and (b) for a PCET reaction (AH + B).

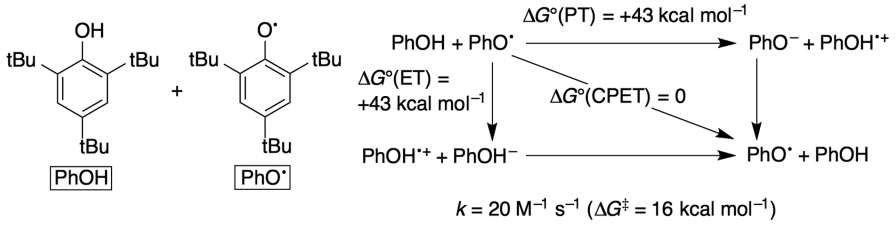

Figure 5. Thermodynamic and kinetic parameters for PCET reactions of $^{t}Bu_3PhOH$.[21a]

being transferred from $[(bpy)_2(py)Ru^{II}OH_2]^{2+}$ would come from an O–H bond to give the high-energy species $[(bpy)_2(py)Ru^{II}(^{\bullet}OH)]^{2+}$ as the initial product, before relaxing to the ground state. Here, and in a few other cases,[24] there are clear energetic consequences for a CPET/HAT orbital distinction, but this is a rare example in which a thorough mechanistic investigation can be used to distinguish two related, concerted mechanisms for net $H^{\bullet}$ transfer. The definition of HAT given above is also problematic because it often gives cases in which a single reaction is called HAT in the forward direction, but not in the reverse. For instance, the net transfer of $H^{\bullet}$ ($e^- + H^+$) from a tyrosine to a peroxyl radical would not be HAT, because the tyrosyl e^- comes from the π orbitals and not from the O–H bond, but the reverse reaction would be classified as HAT. To further complicate matters, there are quite different theoretical arguments that concerted $1e^-/1H^+$ transfers should be termed HAT when they are adiabatic, while those that are nonadiabatic (involve excited state energy surfaces) should be called CPET.[25]

Given the wide breadth of PCET reactivity, we think that restrictive and subtle distinctions such as HAT versus CPET can be easily misinterpreted and sometimes be (unintentionally) misleading. Furthermore, for the vast majority of PCET reactions, these distinctions are difficult, if not impossible, to draw from experimental results. This is an ongoing challenge in bridging concepts from new PCET theories with experimental results. For now, we prefer to use the term PCET for any reaction involving overall H^+/e^- transfers, CPET for all concerted $1e^-/1H^+$ transfers, and ET-PT or PT-ET for stepwise mechanisms.

In addition to the examples in which $H^{\bullet}$ is formally transferred from one donor molecule to one acceptor molecule given above, one of the most biologically diverse subsets of PCET reactions is that in which the H^+ and e^- being transferred start from (or go to) distinct donors (or acceptors). This is shown schematically in eq 1, in which AH forms $A^{\bullet}$ by delivering H^+ to a Brønsted base B and e^- to an oxidant X^+. These types of reactions are termed separated or multiple-site PCET (MS-CPET or sometimes MS-EPT or bidirectional PCET[26]).

$$AH + B{:}{+}X^+ \rightarrow A^{\bullet} + BH^+ + X \quad (1)$$

The canonical biological example of MS-CPET is the reversible oxidation of $tyrosine_Z$ (YOH_Z) in photosystem II. YOH_Z is oxidized by the chlorophyll P680 radical cation, likely concerted with transfer of H^+ to histidine 191.[27] The action of C*c*O, described above, is an even more complex example of MS-PCET.[18] In that enzyme, all of the redox and H^+ transfer processes must be coupled in some way, but they probably are not all CPET. Inspired by some of the issues in MS-PCET, we recently compiled and analyzed a more general series of combinations of oxidants and bases.[28]

Energetics and Mechanisms. The energetics and mechanism of a PCET reaction are typically rationalized using thermodynamic square schemes. One version of such schemes describes the thermochemistry of a single reactant (Figure 4a). For a molecule AH, this includes two reduction potentials, $E(AH^{0/+})$ and $E(A^{-/0})$ (E° in water or typically $E_{1/2}$ in organic media), two pK_as (for AH and AH^+), and a homolytic bond strength for the A–H bond. We strongly recommend the use of bond dissociation free energies (BDFEs) because they are directly related to the free energy changes associated with pK_a and E°, as well as the equilibrium constants (K_{eq}) for PCET reactions.[29] The bond strength literature, however, has for many decades emphasized bond dissociation enthalpies (BDEs). The use of BDEs in PCET square schemes requires the assumption that any entropy change be negligible. While this is usually a reasonable assumption for reactions of organic molecules, it is not appropriate for redox reactions of transition metals.[30] BDFEs are related to E° and pK_a values by eq 2. The constant C_G accounts for the free energy or formation and solvation of $H^{\bullet}$ in a given solvent. A complete derivation is given elsewhere.[31]

$$BDFE = 1.37pK_a + 23.06E^{\circ} + C_G \quad (2)$$

Information about the free energy landscape of a bimolecular PCET reaction AH + B is obtained by combining square schemes for two reactants (Figure 4b). For a separated MS-PCET (eq 1), the thermodynamics can be understood by combining the square scheme for AH with the reduction potential of the oxidant X^+ and the basicity of B: (the pK_a HB^+).[32] The overall driving force is given by the difference in BDFEs,[33] and the free energy change for individual PT or ET steps is given by the appropriate differences in pK_a or E°, respectively. Such information can provide mechanistic insight when kinetic data are available. In cases in which kinetic barriers calculated from the Eyring equation ($\Delta G^{\ddagger}$) are lower than the free energy change (ΔG°) for a stepwise ET or PT

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step, then a concerted mechanism is implicated. An example for the H• self-exchange reaction of 2,4,6-tri-*tert*-butylphenol in MeCN solvent is shown in Figure 5.[21a] The free energy required to produce intermediates required for stepwise ET-PT or PT-ET is much higher than the observed Eyring barrier; the self-exchange reaction must then proceed via a different pathway (CPET). In this case, the large differences between the $\Delta G^{\ddagger}$ and the $\Delta G°$(ET) and $\Delta G°$(PT) make the mechanistic conclusion very convincing. Given the typical uncertainties in the pK_a or $E°$ values, mechanistic conclusions require these differences to be at least a few kilocalories per mole. Still, when the energy differences are large, even crude kinetics can be sufficient for strong mechanistic conclusions because $\Delta G^{\ddagger}$ varies with the logarithm of the rate constant.

The unambiguous difference in energetics in Figure 5 does not hold for every PCET system. Some systems [e.g., ascorbate (see below)] have a "flat" energetic landscape and are able to undergo facile CPET, ET, or PT reactions. A sense of the energetic preference of a reagent to undergo concerted versus stepwise PCET can be obtained from its square scheme, from the difference in the pK_a of the oxidized and reduced forms. This is equivalent (in $\Delta G°$) to the difference between the $E°$ of the protonated and deprotonated forms. When the difference is large, as for toluene or for phenol,[21a] the reagent has a preference for concerted reactions, while it is small for ascorbate. Medium effects (e.g., protein active sites, solvent) can perturb such preferences for different PCET mechanisms; in this regard, what is given above is a rough guideline. Finally, note that "concerted" does not imply synchronous transfer of the e^- and H^+, only that the reaction does not proceed through any discrete intermediate.[34]

There are few systems in which the kinetics and thermodynamics of all (or even most) of the relevant ET, PT, and CPET reactions are well-defined. One example is the self-exchange reaction $Fe^{II}(H_2bim)_3^{2+}$ + $Fe^{III}(H_2bim)(Hbim)^{2+}$ (H_2bim = 2,2′-biimidazoline) complexes that were developed as a model for C−H bond activations in non-heme iron enzymes such as lipoxygenase.[35] For other selected organometallic examples, see ref 36. In general, concerted transfer of H• avoids the buildup of charge and other higher-energy intermediates that can be associated with an increased level of reorganization of the solvent. ET and PT reactions also can be facile, resulting in cases in which both concerted and stepwise reactions are possible depending on reaction conditions; we return to this concept below.

Medium Effects. The effects of the surrounding medium on the rates and efficiencies of ET reactions are well-established.[37] PCET reactions introduce another level of complexity because H^+ (and H•) transfers must occur over short, well-defined reaction coordinates, in contrast to the more diffuse tunneling of e^- through space or via multiple through-bond paths.[38] Studies of medium effects on PCET reactions are limited, except for the extensive studies of organic HAT reactions.[39] When H^+ is transferred between two electronegative atoms, PCET is typically predicated by the presence of a hydrogen bond (H-bond). The H-bonding properties of a solvent can markedly affect a PCET reaction X−H + Y by forming X−H···solvent complexes that "block" formation of the reactive X−H···Y H-bonded precursor complex. Likewise, specific interactions supported by protein active sites can play key roles in modulating the H^+/e^- transfer reactivity of a given cofactor. We demonstrated that it is essential to account for solvent H-bonding properties for a series of solution CPET reactions of oxyl radicals using expressions that describe the H-bonding donating and accepting equilibria involving the solvent and the H• donors and acceptors.[40] In a related study, we showed that the thermodynamics and kinetics of simple reactions of ascorbate are affected by its local environment; this likely influences how this ubiquitous PCET cofactor behaves within an enzyme active site or at a lipid interface, for instance.[22b,41]

To understand a biological PCET process, or to build and study a model system, it is important to consider the role of the surrounding chemical environment. As described in the following sections, the reactivity patterns (thermodynamic and kinetic) of different cofactors are strongly influenced by solvents or other changes in the chemical environment. The mechanistic propensity of a give enzyme active site likely is steered by specific hydrophobic interactions (e.g., exclusion of water), salt bridges, H-bonding, and dielectric "tuning" by nearby amino acids. Such specific interactions can be cumbersome to reproduce in solution biomimetic studies, but progress has been made, for example, the effect of H-bonding additives on CPET reactions of ascorbate,[42] the effect of different proton-accepting groups in oxidation of phenols with appended bases,[43] and even a subset of PCET reactions in which other bonds (e.g., O−O) are broken.[44] In addition, biomimetic interactions between ligands and metal centers can help stabilize reactive intermediates [e.g., M^{n+}=O[45] or heme(M^{n+}−OO)[46]] that model biologically important PCET intermediates. Such rationally designed ligands are inspired by the discrete native interactions in protein active sites that stabilize the analogous intermediates.

■ PCET THEORIES

A number of different theoretical approaches have been developed to understand PCET reactivity. Many studies use traditional transition state theory, typically calculating barrier heights and energy surfaces with density functional theory (DFT).[47] However, PCET is not well treated in this fashion, so a number of groups have developed more sophisticated approaches,[48] drawing heavily on the framework of semiclassical ET theory.[49] A full treatment is not possible in this article, and interested readers are pointed to the reviews given above. One of the most important features of PCET theories includes explicit terms that account for vibronic coupling between reactant and product states, emphasizing that the behavior of H^+ is as important as that of e^-. The notation used in PCET and ET theories is very similar, but the parameters are not interchangeable. For example, the driving force ($-\Delta G°$) and reorganization energy (λ) for a given ET or PCET process correspond to different chemical reactions.

■ PCET IN ORGANIC MOLECULES

Organic cofactors are the most widely studied class of PCET molecules. This is largely because of the enormous breadth of biological systems that use organic PCET cofactors [e.g., NAD(P)H and flavins], as well as the ready availability of many reasonable model compounds (e.g., phenol as a model for tyrosine). The biological ubiquity of organic redox cofactors, and their frequent reactions with metal-containing cofactors, makes them a good starting point. Furthermore, the reactions conducted at biological metal sites can be predicated by the reactivity of the molecule being acted upon (e.g., C−H bond activations). In this section, we describe important general

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features of the PCET reactivity of different classes of organic compounds.

Carbon−Hydrogen Bonds. Carbon−hydrogen bonds are among the most intrinsically unreactive PCET functional groups. This is likely in part because they do not form strong hydrogen bonds. One way of demonstrating this is the hydrogen atom self-exchange rate constant reactions of C−H bonds, which are sluggish compared to those of sterically congested phenols. For instance, the $k_{self\text{-}exchange}$ for toluene + benzyl radical[50] has a $k_{s.e.}$ of ~8×10^{-5} M^{-1} s^{-1} compared to a value of 20 M^{-1} s^{-1} for the sterically congested 2,4,6-tri-*tert*-butyl-phenol (Figure 5)[21a] and ~10^6 M^{-1} s^{-1} for phenol.[51] In addition, the thermodynamics of stepwise ET-PT activation of C−H bonds are typically quite unfavorable. For example, in MeCN solvent, toluene has a $pK_a(C_6H_5CH_2-H)$ of ~54 and an $E_{1/2}(C_6H_5-CH_3^{\bullet+/0})$ of ~1.87 V versus $Cp_2Fe^{+/0}$ (Cp = cyclopentadienyl).[21a]

Linear free energy relationships often are used to correlate the observed reactivity of a series of C−H bonds because of the propensity of C−H bonds to react predominantly[3,52] by concerted $H^{\bullet}$ transfer. The energetics of net C−H bond activations also are less sensitive to solvent composition because, as noted above, C−H groups do not form strong H-bonds. C−H BDFEs are slightly solvent-dependent because of differences in the free energy of solvation of $H^{\bullet}$.[31] As noted above, medium effects can modulate the specific reactivity (and energetics) of different substrates.

One exception to the reactivity pattern for C−H bonds described above is the reactivity of nicotinamide-containing molecules, which are net hydride (H^-) donors. Reactions of model complexes can proceed via initial $H^{\bullet}$ followed by e^- transfer,[53] but biological reactions, such as in the mitochondrial respiratory Complex I,[54] are thought to proceed via direct H^- transfer mechanisms. Hydride transfer is another kind of PCET, although not always grouped that way. Like other PCET reactions, H^- transfer reactions can be rationalized using thermodynamic cycles[55] (e.g., Figure 6[56]). The thermodynamics of the discrete H^+, $H^{\bullet}$, or e^- steps are often better known in aprotic media, though some data are available in aqueous solutions for NADH models.[57] To the best of our knowledge, $H^{\bullet}$ transfer reactions of nicotinamides in biological systems are not well-established. However, the biomimetic solution reactivity of nicotinamide models suggests that, under appropriate conditions, single e^-, single H^+, $H^{\bullet}$, and H^- reactivity are all possible modes of reactivity.[58]

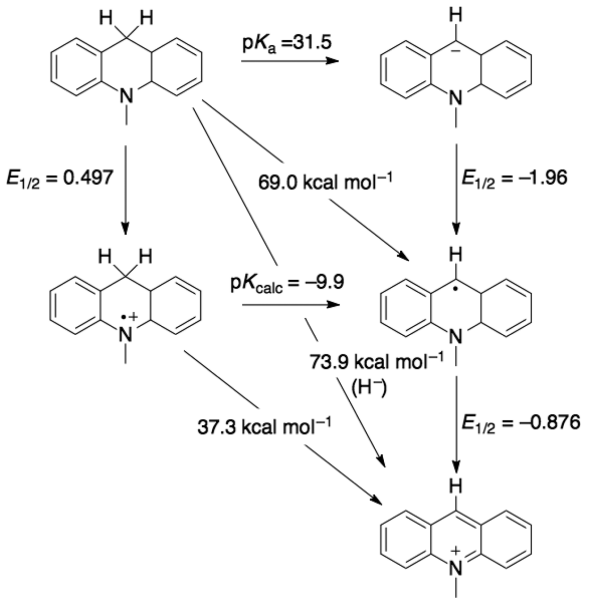

Figure 6. Thermodynamic square scheme for 10-methyl-9,10-dihydroacridine in DMSO.[56]

Phenols and Quinones. PCET reactions of phenols have been the subject of intense research, and some controversy, over the past 30 years. As such, it is impossible to highlight this work in great detail. Research on phenol PCET chemistry is a result of the recognition that redox reactions of the amino acid tyrosine (YOH) are integral to the function of many proteins, including ribonucleotide reductase,[59] photosystem II,[60] prostaglandin-H-synthase/cyclooxygenase (PGSH/COX),[61] and cytochrome *c* oxidase. Furthermore, products of radical reactions of YOH are markers for oxidative stress, which is implicated in the pathology of many disease states, such as atherosclerosis, asthma, some cancers, and neurodegenerative diseases.[62] Note that we prefer the unusual notation "YOH" for tyrosine because it keeps track of H^+ and e^- in deprotonated/oxidized species, using YO^- for tyrosinate and $YO^{\bullet}$ for tyrosyl radical.

The solution properties of many phenol (PhOH) derivatives are known in many different solvents,[21a] with the most extensive data available in DMSO[63] and water.[64] In all cases, PhO^- is more easily oxidized than PhOH by hundreds of millivolts, and the PhO^- is a good Brønsted base. In aprotic media, concerted reactions (Figure 7) are preferred to prevent

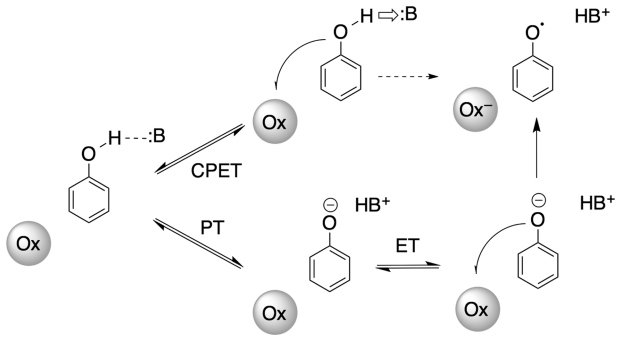

Figure 7. Stepwise and concerted PCET mechanisms that can be accessed by phenols using separate oxidants (Ox) and Brønsted bases (:B). Initial ET is rare because of the high $PhOH^{+\bullet/0}$ reduction potential, but such a pathway has been suggested in a few systems.[66]

the formation of the high-energy species PhO^- (from initial PT) or $PhOH^{\bullet+}$ (from initial ET). While the same arguments can hold in water, PT is much more facile. The ratio of rate constants for forward and reverse PT, K_{PT}, is dictated by the pK_as of PhOH and the H^+ acceptor. Exoergic PT between electronegative atoms, as in this case, occurs without barrier at the diffusion limit, so the rate constant for the reverse reaction is simply the diffusion limit times K_{PT}. The $PhO^-/PhO^{\bullet}$ electron self-exchange reaction is also very rapid[65] (1.9×10^8 M^{-1} s^{-1} in water, pH 12), so ET can be very rapid, even the in the low-ET driving force regime typical for biological systems [where $|\Delta G°| \ll \lambda$ (Marcus reorganization energy)]. Therefore, tyrosine and phenols are known to react by PT-ET, CPET, and sometimes ET-PT mechanisms[66] (Figure 7), depending on the other reactant and the medium. Among the most detailed analyses of phenol oxidations are reactions with outer-sphere ET partners,[67] electrochemical oxidations,[68] and laser flash-

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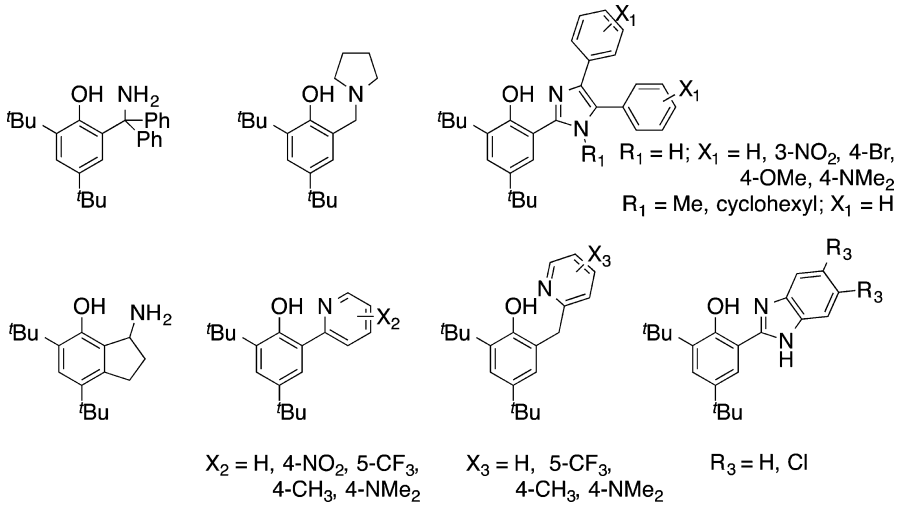
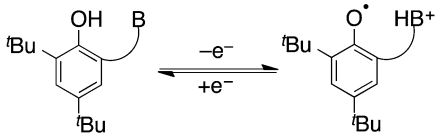


Figure 8. Phenol PCET model complexes that incorporate a pendant Brønsted base.

quench oxidations,[69] including cases in which the modified phenol is covalently linked to the photosensitizer.[70] Finally, we highlight recent work on PCET to electronically excited oxidants,[71] a mode of reactivity that is receiving new interest in H^+/e^- transfer model chemistry.

In groundbreaking work, Linschitz et al. surveyed MS-PCET reactions of phenols using combinations of a photooxidant and a large excess of Brønsted bases.[72] To address the issue of proton release/return on oxidation/reduction, workers developed a variety of small molecule model compounds that incorporate a Brønsted base proximal to a phenol OH group. Some of the molecules we have used in our laboratories are shown in Figure 8, based in part on related systems developed by other groups (see below).[73] In these molecules, $1e^-$ oxidation results in production of the corresponding distonic radical cation, where H^+ has migrated to the pendant base (eq 3). In many cases, electrochemical oxidation−reduction is



(3)

reversible, in contrast to the parent phenol radicals, which undergo H^+ loss and dehydrodimerization at nearly the diffusion limit. The electrochemical reversibility indicates that the H^+ transfer steps associated with oxidation and reduction are reversible, making these excellent small molecule models with which to explore the details of PCET reactivity, most notably the tyrosine-histidine pair in Photosystem II mentioned above. A remarkable example of reversible YOH/YO$^\bullet$ radical redox cycling occurs in RNR (see above), in which a hole is transferred along a series of YOH residues to ultimately initiate catalysis at a cysteine in another subunit.[74] This long-range PCET appears to occur by the redox equivalent hopping across multiple residues, including at least two intermediate tyrosines.[59]

The phenol−base systems are examples of MS-PCET because the oxidant is distinct from the Brønsted base. This is the situation for a great many biological PCET reactions, especially in oxidations of amino acids (tyrosine, tryptophan, and cysteine). The small molecule models have allowed for many tests of PCET parameters that are very difficult to probe in complex biomolecules. These parameters include driving force dependence,[68b,69,73d,75] reactivity modulation due to the pK_a of the base,[68b,70c,73,76,77] the role of conjugation between the phenol and the base,[78] and the effect of PT distance.[79] Furthermore, because of their small size and a variety of well-defined experimental observables (e.g., IR and NMR characterization, X-ray structures), these systems have proven to be valuable tests of modern PCET theories.[48b,80] The important lesson from these molecules is that the nature of the phenol···base hydrogen bond plays a central role in reactivity. Many systems that feature such strong H-bonds react primarily through concerted processes.

The demonstration of different regimes of phenol PCET (stepwise PT-ET vs concerted) can be used to rationalize many protein redox reactions. Solution models can sometimes be misleading because a "bulk" model poorly describes protein active sites, which use carefully arranged amino acids to promote a given function. For example, the ready return of H^+ lost upon oxidation of YOH_Z in photosystem II (PSII) is imperative for maintaining the high energy required to oxidize water. By taking advantage of a reversible bond homolysis step, PSII is able to maintain ≥1 eV in energy,[81] which is necessary to oxidize water. Loss of H^+ from the vicinity of $YO^\bullet{}_Z$ would shift the redox couple to that of $YO^{\bullet/-}$ (E° = 0.71 V in H_2O), which is not sufficient to oxidize water. Conversely, for abstraction of H from arachidonic acid by YO$^\bullet$ in PGHS, the fate of H^+ lost upon oxidative activation of YOH should not affect function in the following C−H bond abstraction step.

Many of the lessons from phenol chemistry apply to quinone/semiquinone/hydroquinone PCET. Quinones are ubiquitous redox cofactors. The quinone/quinol cycle is a $2H^+/2e^-$ cycle with mechanistic preferences that are sensitive to solvent and hydrogen bonding.[82] The fully reduced (quinol) form displays reactivity patterns like those of phenols; $1e^-$ oxidation or deprotonation is an unfavorable pathway in most cases (e.g., Figure 9a), especially in polar, aprotic solvents. On the other hand, stepwise PT-ET can be facile in water. The fully oxidized quinones have mild reduction potentials for single ET (e.g., 0.1 V[83] for benzoquinone$^{0/-}$) that allow facile $1e^-$ reduction to the semiquinone radical anion [whose protonated form HQ$^\bullet$ also is weakly acidic (pK_a = 4.1)]. Meyer and co-workers recently evaluated the many PCET pathways that are possible for quinones.[84] We note that the relative stability of

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Biochemistry 2015, 54, 1863−1878

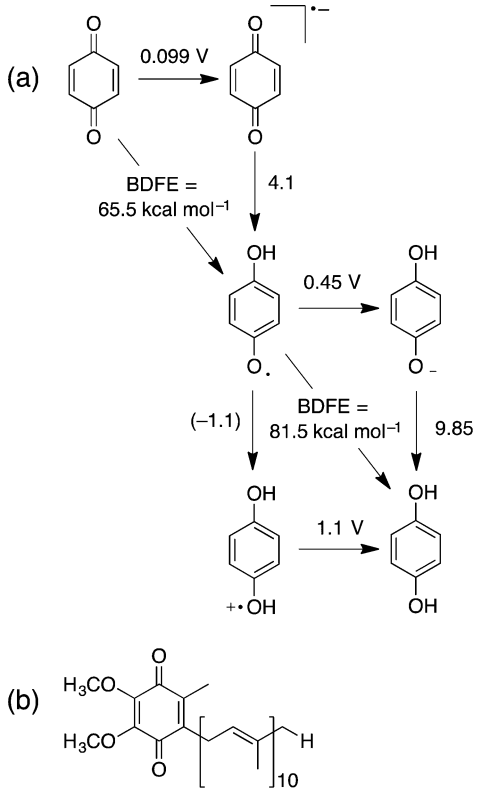

Figure 9. (a) Partial square scheme for the benzoquinone/hydroquinone thermochemistry.[21a] (b) Coenzyme Q_{10}.

these odd electron intermediates and the "tunability" of reactivity are important features that underlie function in biological quinone/quinol cycles, as for coenzyme Q (Figure 9b). The average BDFEs (corresponding to net $2H^+/2e^-$ conversions) cover a narrow range from approximately 74 (hydroquinone) to 69 (tetramethylhydroquinone) kcal mol^{-1}, and the individual $E°$ and pK_a values follow Hammett-type trends.[21a] Finally, some work suggests that transfer of H^- to quinones is possible, but stepwise mechanisms (e.g., $H^\bullet + e^-$ or $e^- + H^+ + e^-$) are likely preferred.[85]

Antioxidants. Historically, antioxidant chemistry places emphasis on "free radicals", but it was a comparatively recent notion that biological antioxidants can formally donate $H^\bullet$. In 1981, Creutz outlined the H^+/e^- reactivity of ascorbate ($HAsc^-$) with model complexes, even before the term PCET was coined,[86] and in 1991, Njus and co-workers highlighted the CPET reactivity of $HAsc^-$ *in vivo*.[22] As another example, oxidation of 2·glutatinone (GSH) gives glutathione disulfide (GSSG), a process that involves loss of $H^+ + e^-$ from each GSH. Vitamin E (α-tocopherol) also is a good example of antioxidant $H^\bullet$ donors. Indeed, a great many of the net reactions conducted by antioxidants are inherently PCET processes, including reactions with reactive oxygen species and reactions with alkyl radicals.

Ascorbate ($HAsc^-$) redox chemistry has often been considered in the context of outer-sphere ET, but H^+ have profound effects on reactivity, even with $1e^-$ oxidants.[87] Oxidation under biological conditions is always accompanied by loss of H^+ because $pK_a(HAsc^\bullet) = -0.45$. The O−H bond in ascorbate is weak, so $HAsc^-$ readily transfers $H^\bullet$ to other radical species. We demonstrated that derivatives of $HAsc^-$ can participate in CPET reactions with organic radicals[88] and with iron−porphyrin model complexes,[89] and that the CPET reactivity of ascorbate is sensitive to its chemical (H-bonding) environment.[42] One biological example in which this sort of thermodynamic tuning would be important is redox reactions at membrane interfaces.[41] In summary, the weak O−H bond, the mild reduction potentials for different $HAsc^-$ species, and the facile PT in water mean that ascorbate likely can react by CPET, PT-ET, or ET-PT, depending on the reacting partners and biological environment.

The PCET reactivity of tocopherols and related phenolic antioxidants resembles that of phenols and YOH, with a few caveats. Tocopherols have a lipid tail that confers membrane solubility,[90] and it is established that tocopherols readily react with lipid alkyl ($R^\bullet$) or peroxyl ($ROO^\bullet$) radicals produced from reactive oxygen species.[91] It is thought that such reactions proceed via the transfer of $H^\bullet$ from the tocopherol-OH to the radical (HAT or CPET in the terminology used here), and that this is the primary *in vivo* function.[90,91] This is a reasonable proposal (in our view) as the hydrophobic membrane environment will disfavor formation of the charged intermediates required for a stepwise mechanism. In some cases, tocopherol radicals can be reduced at membrane interfaces by PCET from $HAsc^-$, which likely also follows an HAT/CPET mechanism.[22,92]

Flavins. Flavins are $2H^+/2e^-$ PCET reagents that serve as important "switches" between $2e^-$ and $1e^-$ cofactors (e.g., NADH and hemes in cytochromes P450). One important biological example is the $2e^-$ reduction of the oxidized (quinonoid) form of flavins by NADH, which is followed by two sequential $1e^-$ oxidations, in which the electrons are usually transferred to a heme or [Fe-S] cluster. Flavins also are involved with O_2 activation (e.g., phenylalanine hydroxylase),[93] which is an inherently PCET process as O_2 is converted to H_2O or H_2O_2. The fundamental data required to construct thermochemical square schemes for flavins are available, but these data are probably only a starting point for thinking about PCET in flavins[94] because of the breadth of reactions that they conduct.[95] The variety of $2e^-$ transformations of flavins is aided by the relative stability and mild acid/base properties ($pK_a \sim 8.5$) of the flavosemiquinones.

While the PCET chemistry of flavins is widely appreciated, relatively few model studies have been reported. However, PCET reactivity in a variety of natural flavoenzymes has been described in a variety of contexts because of their remarkable range of PCET and non-PCET reactivity.[96] Flavins also are involved with the PCET reduction of O_2 to H_2O_2 by glucose oxidase, the process of which has been investigated in some detail.[97] In these examples, the extended structure of the protein active sites is very important, so they remain a significant challenge to fully model using small molecules. In these cases, careful kinetic and thermodynamic analyses of the natural enzymes have proven to be more fruitful.

Hydroxylamines. Hydroxylamines are not typical biological PCET substrates (with the exception of the nitrogen cycle), but they are invaluable reagents for studying biomimetic PCET reactions. Like most hydrocarbons and their C−H bonds, alkyl hydroxylamines (R_2NO-H) are very poor Brønsted acids and very poor reductants, so they strongly prefer to react via CPET. In many cases, the resulting aminoxyl radical is stable, the most prevalent example being 2,2′:6,6′-tetramethylpiperidinoxyl (TEMPO) and its relatives. The radicals also are poor bases and have very low reduction potentials, leading them to also prefer a CPET mechanism. Finally, the O−H

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Biochemistry 2015, 54, 1863−1878

bond in hydroxylamines is fairly weak [BDFE(TEMPO-H) = 71 kcal mol^{-1} in water], facilitating investigations of metal complexes and active sites with BDFEs from 60 to >80 kcal mol^{-1}.

INORGANIC PCET COFACTORS AND REACTIVITY

The idea that transition metal sites can participate in reactions involving e^- and H^+ gained momentum in the 1980s, well after the focus on this for organic cofactors. Investigations of a remarkable breadth of inorganic PCET model complexes provide a basis for understanding function in many metalloproteins. It is not possible here to provide a comprehensive picture of this work; we restrict ourselves to the prevalent and important patterns of reactivity. In the concluding section, we place special emphasis on new challenges for biomimetic inorganic chemistries.

Small Gaseous Molecules. The PCET chemistries of H_2, O_2, N_2, and CO_2 are at the heart of life, and the PCET thermodynamics of these building blocks have long been understood in isolation.[21a,98] The PCET reactivity of the small molecules mentioned above at transition metal sites has recently received a great deal of attention, especially for the respective reduction reactions: $2H^+ + 2e^- \rightarrow H_2$; $CO_2 + 2H^+ + 2e^- \rightarrow CO + H_2O$;[99] $N_2 + 6H^+ + 6e^- \rightarrow 2NH_3$;[100] and $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$.[101] Understanding the details of how enzymes use metal centers to conduct respiration, nitrogen fixation, and photosynthesis could provide pivotal insights across diverse areas, such as understanding biological functions and addressing emerging energy challenges across the planet.

Porphyrin-Based Models. One of the earliest and most important heme model compounds was Groves' $TMP^{\bullet+}Fe^{IV}$=O complex (TMP = 5,10,15,20-tetramesitylporphyrin).[102] This complex exhibited diverse PCET and oxygen atom transfer reactivity. It led to the proposal of the rebound mechanism that is now widely accepted for the widespread net oxygen insertion reaction accomplished by cysteine-ligated heme oxygenases.[103] Conceptually related porphyrin models with very bulky substituents in the periphery are models for oxygen-binding and -activating enzymes. Hydrogen-bonding moieties can stabilize bound superoxide [Fe^{III}-O_2], facilitating the intramolecular ET, as in oxygen-binding heme enzymes.[101a]

One of the final pieces of the P450 cycle was demonstrated recently, with Green's direct observation of the O=Fe^{IV}($P^{\bullet+}$) intermediate (Compound I, P = protoporphyrin IX), the observation of its abstraction of $H^\bullet$ from R-H to give Fe^{III}(P) and R-OH, and the determination of the pK_a value of HO=Fe^{IV}(P) (Compound II).[104] Though the substrate preference and reactivity of subclasses of P450 enzymes are distinct,[105] the C−H activating moieties share common features: all are oxidizing, and their reduced form is basic. In accord with the outline of C−H thermochemistry given above, activation of those bonds almost never proceeds by initial ET because that would require a very strong oxidant, which would be nonselective. Similarly, an unreasonably strong base would be required for initial deprotonation of most C−H bonds. Therefore, the enzymes use a less demanding combination of a milder oxidant and a moderate base for PCET activation. The activation of C−H bonds by Compound I is always described as a hydrogen atom transfer, but it is probably better described as MS-CPET: the H^+ removed from the C−H bonds adds to the oxo group, but the e^- is transferred to a half-occupied orbital located on the porphyrin and thiolate.

PCET reactions of hemes are not necessarily mediated by high-valent intermediates. Other early uses of model porphyrin complexes used to study PCET are the photochemical studies of Nocera et al., in which photoinduced ET is modulated by H^+ in a hydrogen-bonded bridge.[106] These models are useful for investigations of light-induced PCET, and they serve as scaffolds for investigation of the importance of H-bonded bridges in mediating PCET in ways related to the pathway model for biological ET.[38]

Later, we demonstrated that imidazolate-ligated complexes of Fe^{III}-5,10,15,20-tetraphenylporphyrin (Figure 10a) can act as

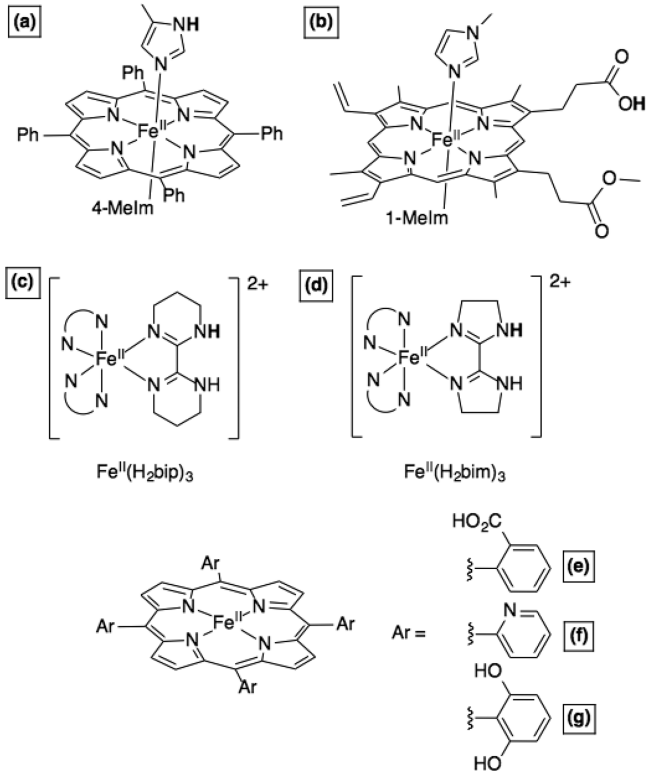

Figure 10. Iron heme and non-heme model complexes for PCET.[89b,101d,e,107,110,113,117] The transferring H^+ are shown in bold.

$H^\bullet$ acceptors, with reduction of the iron and protonation of the imidazolate. TEMPOH, hydroquinone, or 5,6-isopropylidene ascorbate are all effective donors.[107] Analogous complexes of Fe^{III}-protoporphyin IX dimethyl ester display very similar reactivity patterns.[108] The monomethyl ester complexes (Figure 10b) can undergo PCET coupling redox activity at the iron with protonation/deprotonation of the distant carboxylate, for instance with ascorbate.[89b] These compounds are models for 6-coordinate heme proteins that have pH-dependent reduction potentials.[109] Coordinatively saturated hemes are typically considered electron carriers (e.g., cytochrome *c*), but our model systems, and the pH-dependent potentials, suggest that bis(His)-ligated hemes can participate in PCET reactions. This is consistent with some biochemical investigations in which alkylation of a ligating His destroys enzyme function.[41]

Catalysis at heme sites often relies upon ready delivery or extraction of H^+. To that end, we[101d,e] and others[99b,110] illustrated the importance of Brønsted acid/base positions around the metal (Figure 10e−g); the delivery of H^+ was suggested to play a key role in the PCET reduction of O_2 and

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Biochemistry 2015, 54, 1863−1878

CO_2. As noted above, the distinct mechanism of these transformations could involve stepwise or concreted steps, but it is the controlled delivery of both e^- and H^+ that is central to reactivity and function.

Non-Heme Iron. Non-heme iron enzymes undergo a wide variety of PCET processes. For instance, the oxidation of a YOH to YO$^\bullet$ by a diiron site RNR was mentioned above. This field has grown enormously in recent years with the isolation of Fe^{IV}–oxo (ferryl) compounds and their identification in enzyme catalytic cycles. The interested reader is referred to relevant reviews[111] and those highlighted below. In addition to the ferryl intermediates, we and others set out to provide an understanding of lipoxygenase enzymes, which abstract H$^\bullet$ from the weak C–H bonds in polyunsaturated fatty acids using an Fe^{III}-OH active site, i.e., R-H + Fe^{III}-OH → R$^\bullet$ + Fe^{II}-OH_2. The first model for this reaction is likely the report by Stack et al., using an Fe^{III}–methoxide complex.[112] We were concurrently working on less biomimetic iron complexes with three biimidazoline or three bipyrimidine ligands (Figure 10c,d).[113] The oxidized/deprotonated forms of these complexes can activate weak C–H and O–H bonds, and the reduced/protonated complexes will react with phenoxyl radicals (e.g., 2,4,6-tri-*tert*-butylphenoxyl[114]) to give the corresponding phenols and the oxidized/deprotonated congener. These Fe^{III} systems are very mild oxidants and are able to oxidize weak C–H bonds only because they are fairly strong bases. The discovery that C–H bonds are oxidized by Fe^{III} active sites, as well as ferryl cofactors, reinforces the PCET perspective that both redox potential and basicity are required.

The iron systems with N-heterocyclic ligands proved to be robust and powerful systems for testing fundamental properties of CPET reactions. First, our investigation of CPET reactions of Fe(bip) and Fe(bim) complexes (Figure 10c,d), as well as other systems, showed that the Marcus Cross Relation could be applied to reactions in which H$^\bullet$ is transferred (as opposed its original derivation for outer-sphere ET).[115] We later expanded upon this idea to include a more comprehensive range of CPET reagents, including many of biochemical interest.[40,116] This demonstration that a version of Marcus theory holds for many reactions has some overlap with current, more sophisticated theories of CPET. In addition, some of these non-heme metal couples were found to have very large ground state entropic changes, in contrast to those of related organic reagents.[117] These studies demonstrated that the historical use of bond dissociation enthalpies (BDEs) in rationalizing hydrogen atom transfer reactivity was not appropriate for closely related reactions of transition metal complexes. We strongly encourage workers to employ bond dissociation free energies (BDFEs) when discussing PCET reaction driving forces. This follows from the use of free energies in Marcus theory and current theories of PCET and is consistent with the dominant use of linear free energy relationships in physical organic chemistry. BDFEs are more appropriate and also are directly related to the commonly reported PT and ET thermodynamic values, the pK_a and $E°$.

Proton-coupled redox transformations at biological iron sites are very widespread and varied, including enzymatic[118] and nonenzymatic examples.[119] One major area is the activation of O_2 for substrate functionalization by a great many non-heme iron enzymes,[120] and such processes have been extensively modeled using a diverse array of ligands.[121] Modeling such reactions is challenging because of the fleeting nature of the intermediates, and the lack of a strong chromophore. In terms of PCET reactivity, among the most comprehensively investigated systems are those of Borovik,[45] Collins,[122] Nam,[123] and Que[124] (examples given in Figure 11). In addition, the design criteria and reaction chemistry from Collins and co-workers provide some insight into important enzymatic features that protect proteins from oxidation by their own cofactors.[111b] The number of PCET reactions conducted by these model systems (Figure 11) is too large to elaborate here. Each system also can conduct a range of oxo transfer reactions and hydroxylations, depending on the substrate. Importantly, these model systems provide a broad foundation for the spectroscopy and reactivity of difficult to study non-heme iron in proteins.

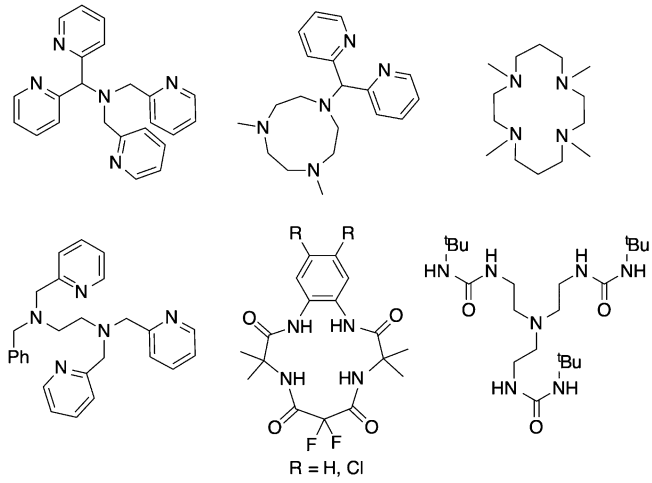

Figure 11. Examples of ligands that support formation of high-valent (Fe^{IV}/Fe^{V}) iron–oxo moieties.

Iron–sulfur clusters and related compounds have long been of interest for their range of ET properties and rich spectroscopy.[125] Of special interest to modern energy challenges are the hydrogenase enzymes, which catalyze perhaps the simplest PCET process ($2H^+ + 2e^- = H_2$). Early hydrogenase models roughly reproduced features of the enzyme active sites and reactivity, but a new generation of models provides a great deal of insight into reactivity and potential catalytic intermediates. Several groups demonstrated PCET reactivity in such models, including production of hydrides using combinations of reductants and acids.[126] An extremely important feature of some of the new models is the incorporation of a "proton relay" in the second (or outer) coordination sphere.[127] This idea was most clearly demonstrated in Ni catalysts[128] but was recently extended to those that contain Fe.[129] The detailed mechanisms of H^+ reduction and H_2 oxidation are beginning to become clear.[130] However, as emphasized above, the key feature required for function is facile ET chemistry and the ready availability of a H^+ donor/acceptor. The situation is recapitulated on a much larger scale in enzymes, notably the water channels (PT pathways) in laccase enzymes[131] or cytochrome *c* oxidase.

More recently, we[132] and others[133] reported the synthesis and PCET reactivity of small molecule models for Rieske iron–sulfur clusters (Figure 12). These models are inspired by the first structural and spectroscopic model[133a] (top left, Figure 12). Rieske proteins are involved with respiration and contain an unusual [2Fe-2S] cluster in which one iron is ligated by two histidine ligands and the other by two cysteine ligands. The

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Biochemistry 2015, 54, 1863–1878

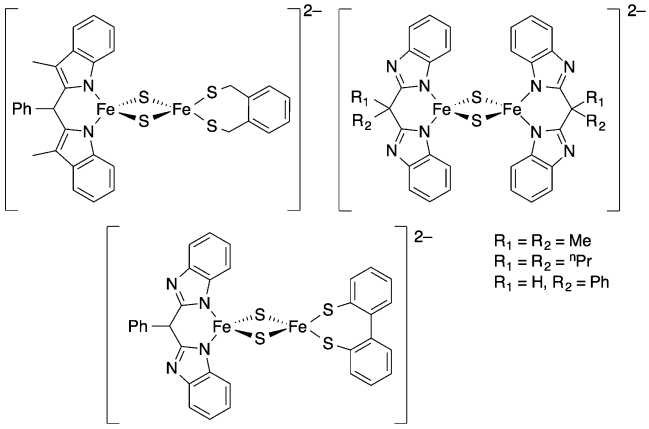

Figure 12. Structural and PCET-reactive Rieske model compounds.[132,133]

markedly pH-dependent reduction potentials of the protein suggest that it can be involved in PCET reactions, where PT is mediated by the ligated histidine(s).[134]

These Rieske models undergo CPET reactions with hydroxylamines and quinones (as models for natural redox partners). Both the homoleptic asymmetric models have approximately the same BDFE (60.2 kcal mol^{-1}), which is similar to those derived from thermodynamics data for the native protein.[134] However, the homoleptic complex undergoes CPET with TEMPO almost 50 times slower than the asymmetric complex, highlighting the importance of the contribution of ligands to differences in intrinsic reactivity. The facile ET and CPET reactivity of these clusters suggests that they have little bias for stepwise or concerted reactions.[132b] Such an observation is perfectly reasonable from a biological perspective; Rieske clusters react with quinones and quinols, the former usually preferring ET-PT and the latter favoring CPET, as discussed above.

Manganese-Containing Models. The PCET chemistries of PSII and Mn-superoxide dismutase (MnSOD) were historical drivers for the awareness of PCET in enzyme active sites, and it has stimulated the development of many model systems. The model chemistry of Mn−oxo cores was especially prevalent during the time at which PSII X-ray structures were disclosed with increasingly high resolution and different interpretations of the Mn_4Ca oxygen-evolving complex (OEC). Even with an atomic resolution structure available,[135] researchers are still trying to understand the discrete mechanism of water oxidation at the OEC, with improving X-ray methods,[136] and correlating structural models with spectroscopy and reactivity.[137]

The simplest Mn−O-containing PCET reagent is the permanganate anion (MnO_4^-), which has long been employed in organic oxidations.[138] The ability of MnO_4^- to abstract H• from X−H bonds is a result of the combination of the $MnO_4^{-/2-}$ reduction potential (0.564 V vs NHE) and the basicity of MnO_4^{2-} (pK_a = 7.4), yielding an aqueous BDFE of 80.7 kcal mol^{-1}. The thermodynamic parameters mentioned above are both reasonably mild, but together, they yield a PCET reagent with a strong affinity for H•.[139] The late Jerry Babcock, who was a very early proponent of the importance of PCET in biological water oxidation and O_2 reduction, was excited that this value was slightly below that for tyrosine (87.8 in water), supporting his hypothesis that Y_ZO• was abstracting H• from a MnOH group.[140] While this direct CPET mechanism is not supported by the high-resolution structure and other data, the focus on PCET thermochemistry that he and others emphasized is now a cornerstone of the field.

A great many SOD mimics are available, with many different metal sites (and even purely organic systems).[141] Many of these compounds are of interest as drug candidates that can be used to address health problems related to oxidative stress and the associate production of reaction oxygen species.[142] The classical SOD mechanism involves initial ET from Mn^{II} to O_2^- to give peroxide (O_2^{2-}) bound to Mn^{III}. Subsequently, Mn^{III} reacts with a second equivalent of O_2^- to give Mn^{II} and O_2. Studies of model complexes suggest that the first reaction could occur via PCET from Mn^{II}-OH_2 or via an inner-sphere mechanism involving displacement of water by O_2^-.[143] Detailed work on the native SOD enzymes suggests that PT and ET are closely related and are tightly controlled during turnover, and that diseases such as amyotrophic lateral sclerosis (ALS) can result if they are not tightly controlled.[144] These observations, in combination with PCET models involving phenol and hydrogenase mimics (see above), suggest that more efficacious artificial SOD systems (e.g., drug candidates) would incorporate a Brønsted acid proximal to the active site.

Other Transition Metal Systems. The PCET chemistry of a wide variety of other metal complexes has been studied in detail. Ruthenium model complexes play a preeminent role, starting from the groundbreaking studies of Meyer and coworkers starting in the early 1980s and continuing to this day.[21b] Many systems with first-row transition metals have been developed, many of them quite biomimetic and directly relevant to specific metalloenzyme systems. The elegant work on copper systems is particularly notable in this context.[145]

Workers have marched across the periodic table in their investigations of transition metal PCET. From vanadium−oxo complexes[146] and Cr−peroxide complexes[147] to second/third row metal hydrides[31] (to name only a few examples!), the basis of PCET reactivity has strong parallels to biochemical and biomimetic systems: good H atom abstractors display both moderate reduction potentials and moderate acidity/basicity; observed PCET reactivity often depends strongly on the substrate (e.g., CPET preference for C−H bonds); and intrinsic barriers and reorganization associated with redox change are central to rationalizing reactivity.

■ LONG-RANGE PCET AND SEPARATED PCET

Electrons can tunnel many angstroms between cofactors. In contrast, H^+ movement is limited to very short distances (less than ∼0.5 Å), often between donor and acceptor atoms separated by <3 Å in a well-defined hydrogen bond. To the best of our knowledge, there are two reports of the PT distance dependence in PCET reactions that offer contrasting views;[79] more work is needed to provide a comprehensive picture. The mismatch between ET and PT distance scales raises interesting questions about the nature of long-range PCET processes. For example, catalyses in RNR, PSII, and COX all involve transformation of YOH to YO• radical; the requisite ET reactions have at least 6 Å (edge-to-edge distances), while the distances between the H^+ donor and acceptor atoms are ≤3 Å. Long-range ET in biological systems is understood using semiclassical theory,[148] but it is still not clear what modifications are needed to rationalize PCET reactions in which long-range ET is coupled to PT. We focus on the ET component of PCET distance dependence in this section as it is

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Biochemistry 2015, 54, 1863−1878

an area of active research in many laboratories, on both biochemical and model systems.

Examples of a model system in which long-range ET is coupled to short-range PT are the indole- and phenol-appended Ru or Re complexes investigated by Hammarström and Nocera.[70] Wenger and co-workers recently reported on the distance dependence of PCET reactions in Ru−phenol complexes;[149] they used rigid xylene linkers to select defined separations between the Ru and the phenol (Figure 13a). They found that the distance decay constant (β) for CPET reactions involving phenol is similar to that for analogous ET reactions ($\beta \sim 0.5$–0.8 Å).[150]

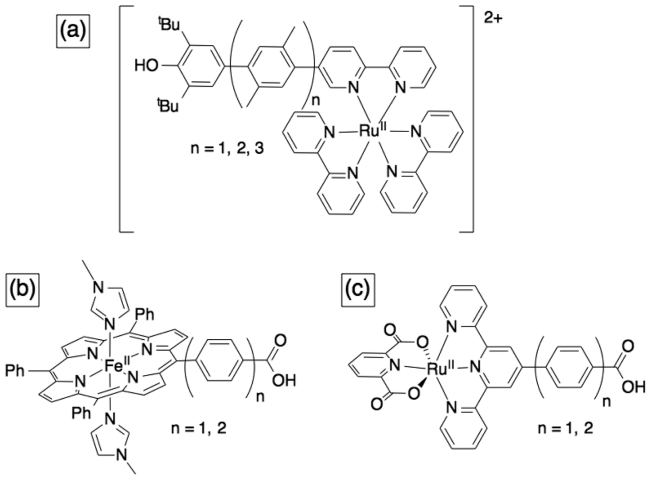

Figure 13. Examples of models used to investigate long-range PCET.[150,151]

We also investigated the ET distance dependence of CPET reactions, but using bimolecular reactions and organic H-donors with Fe−porphyrin[151] or Ru−terpyridine[152] complexes (panels b and c of Figure 13, respectively). We were not able to evaluate the distance decay constant (β) in the Ru complexes, but the observed CPET rates constants were slower with longer linkers, in accord with observations for ET reactions. With the help of computations, we evaluated β for CPET reactions of Fe-porphyrin-benzoates; $\beta = 0.23$ Å^{-1}, which is in accord with β for ET though phenyl bridges (0.2–0.5 Å^{-1}).

The models described above start to bridge the conceptual gap between "inner-sphere" PCET (e.g., X−H bond activation by metal oxos) and purely separated or multiple-site PCET, where the redox and acid base moieties are distinct.[32] In the stretched model systems described above, the protonation state of the acid/base group still affects the reduction potential of the metal site, although only slightly. In the porphyrin models, for example, the protonation state of the benzoate shifts the $Fe^{III/II}$ potential by 30 mV ($n = 1$) or 15 mV ($n = 2$).[151] This is an example of a "flat" square scheme, described above. In contrast, the protonation state of imidazole in related porphyrin models (Figure 11a) changes $E_{1/2}(Fe^{III/II})$ by 365 mV.[89a]

The coupling of thermodynamic parameters (E and pK_a) is of central importance to PCET reactivity, but the biological mechanistic implications are not yet clear. As noted above, activation of C−H bonds requires a combination of oxidant and base (a strongly coupled system). Conversely, long-range PCET in enzymes, (e.g., RNR) is not as strongly coupled, yet holes are efficiently steered through several distinct steps involving the PCET cofactor YOH. An important challenge is understanding how these distant steps are coupled.

FRONTIERS FOR BIOMIMETIC AND BIOCHEMICAL PCET

Having started this essay with Stiefel's 1973 paper on molybdenum enzymes, we believe it is fitting to close with a portion of his concluding section:

> *"The coupled proton-electron transfer may be involved in several chemical processes, e.g., ... However, the chemical mechanism may be inferior to the enzymatic process due to the role of the protein. Thus, the protein will have the capacity to precisely orient the substrate and metal so that the coupled transfer can facilely occur."*

More than 40 years later, this still is an excellent blueprint and challenge for the field. Much is understood about proton-coupled electron transfer processes. It is exciting to see the increasingly close interplay of biochemical, biomimetic, and theoretical studies.

Synthetic models for metalloenzymes are increasingly able to control not only the coordination environment around the metal but also the second coordination sphere, including groups to position protons and facilitate their transfer.[153] With substantial advances in the production of peptide-based models and modified metalloproteins, future biomimetic systems likely will more closely resemble actual biological systems. Recent examples include tyrosine-containing synthetic peptides,[154] proteins,[155] and peptide scaffolds on synthetic hydrogenase catalysts.[156] Likewise, investigations of intermediates in proteins can provide a great deal of insight from small molecule models. For instance, the comprehensive characterization of cytochrome P450 Compound I and measurement of the pK_a of Compound II were in part inspired by model systems showing the importance of the basicity of the reduced species for C−H bond activation.[157] Remarkably, the distinction between biomimetic and biochemical has recently been blurred by the report that a biomimetic iron complex is taken up by apo-hydrogenase protein scaffold and the resulting protein is an active catalyst.[158] Finally, PCET theory is showing how control of substrate orientation within an active site facilitates catalysis, and that both structure and dynamics of the active site are important.[159] We are optimistic that the confluence of these thrusts will enrich our understanding of not only how biology efficiently accomplishes complex PCET processes but also how valuable synthetic catalysts might be designed.

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Biochemistry 2015, 54, 1863−1878

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