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Light-driven water oxidation in algae, cyanobacteria, and higher plants generates dioxygen that supports life on Earth. The water-oxidation reaction is catalyzed by the oxygen-evolving complex (OEC) in photosystem II (PSII) that is comprised of the tetranuclear manganese calcium-oxo (Mn₄CaO₅) cluster, with participation of the redox-active tyrosine residue (YZ) and a hydrogen-bonded network of amino acids and water molecules. YZ mediates successive proton-coupled electron transfer (PCET) reactions that are essential for the oxidation of water to dioxygen at the Mn₄CaO₅ cluster. It has been proposed that the strong hydrogen bond between YZ and its conjugate base, D1-His190, likely renders YZ kinetically and thermodynamically competent leading to highly efficient water oxidation.¹ However, a detailed understanding of PCET at YZ remains elusive due to the transient nature of its intermediate states. In this study, we utilize a combination of high-resolution two-dimensional (2D) ¹⁴N hyperfine sublevel correlation (HYSCORE) spectroscopy and density functional theory (DFT) methods to investigate the electronic structure of a bioinspired artificial photosynthetic reaction center, benzimidazole-phenol porphyrin (BiP–PF₁₀), that mimics the PCET process at the YZ residue of PSII. The results of these studies underscore the importance of proximal water molecules and charge delocalization on the electronic structure of the artificial reaction center.

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HYSCORE and DFT Studies of Proton-Coupled Electron Transfer in a Bioinspired Artificial Photosynthetic Reaction Center

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Abstract

Light-driven water oxidation in algae, cyanobacteria, and higher plants generates dioxygen that supports life on Earth. The water-oxidation reaction is catalyzed by the oxygen-evolving complex (OEC) in photosystem II (PSII) that is comprised of the tetranuclear manganese calcium-oxo (Mn₄CaO₅) cluster, with participation of the redox-active tyrosine residue (Yₑ) and a hydrogen-bonded network of amino acids and water molecules. Yₑ mediates successive proton-coupled electron transfer (PCET) reactions that are essential for the oxidation of water to dioxygen at the Mn₄CaO₅ cluster. It has been proposed that the strong hydrogen bond between Yₑ and its conjugate base, D1-His190, likely renders Yₑ kinetically and thermodynamically competent leading to highly efficient water oxidation.¹ However, a detailed understanding of PCET at Yₑ remains elusive due to the transient nature of its intermediate states. In this study, we utilize a combination of high-resolution two-dimensional (2D) ¹⁴N hyperfine sublevel correlation (HYSCORE) spectroscopy and density functional theory (DFT) methods to investigate the electronic structure of a bioinspired artificial photosynthetic reaction center, benzimidazole-phenol porphyrin (BiP–PF₁₀), that mimics the PCET process at the Yₑ residue of PSII. The results of these studies underscore the importance of proximal water molecules and charge delocalization on the electronic structure of the artificial reaction center.

Keywords
Bio-inspired systems, Photosynthetic reaction center, Proton-coupled electron transfer, hydrogen bonds, electron spin delocalization, HYSCORE spectroscopy.
Introduction

The photosynthetic protein complex, photosystem II (PSII), catalyzes one of the most energetically demanding reactions in nature by using light energy to drive a catalyst capable of oxidizing water.\(^2\-^5\) Proton-coupled electron transfer (PCET) reactions, which are exquisitely tuned by smart protein matrix effects, are central to the water oxidation chemistry of PSII. Moreover, PCET is encountered in a wide variety of biological and synthetic catalysts where it diminishes the overall energetic penalty for multi-electron transfer and modulates the electrochemical driving force of reactions.\(^6\-^8\) In PSII, light-driven water oxidation is catalyzed in the oxygen-evolving complex (OEC) that is comprised of a tetrnuclear manganese-calcium-oxo (Mn\(_4\)Ca-oxo) cluster with an extensive network of hydrogen (H)-bonded amino acid residues and structured water molecules.\(^2\) In particular, there are two symmetrically placed tyrosine residues, Y\(_D\) and Y\(_Z\), one on each subunit of the heterodimeric core polypeptide, D2 and D1, respectively (Figure 1A).\(^3\-^5\) The function of these tyrosines is distinct as the ‘smart’ matrix effects from the surrounding protein environment greatly influence their behavior.\(^9\-^{12}\) The Y\(_D\) redox is suggested to poise the catalytic Mn\(_4\)Ca-oxo cluster\(^13\) and may be involved in the electrostatic tuning of the adjacent monomeric redox-active chlorophyll and \(\beta\)-carotene in a secondary photoprotection pathway.\(^14\) In contrast, Y\(_Z\) is kinetically competent and directly participates in water oxidation.\(^15\-^{17}\) Previous biochemical, spectroscopic and computational studies have demonstrated that both Y\(_D\) and Y\(_Z\) undergo photo-induced PCET with the formation of a neutral tyrosyl radical, Y\(^\bullet\), but the formation and decay of the Y\(_Z\)^\(\bullet\) is a thousand times more rapid than Y\(_D\)^\(\bullet\).\(^12\,^{18}\-^{26}\)

The difference in the kinetics of light-driven PCET at Y\(_Z\) and Y\(_D\) has been suggested to arise from differences in their respective microenvironment. The protein matrix surrounding Y\(_Z\) is highly hydrophilic with several H-bonded water molecules, while the Y\(_D\) site contains a single water molecule in the X-ray crystal structure of PSII (Figure 1B-C).\(^5\) Both Y\(_D\) and Y\(_Z\) are proximal to a histidine residue, D2-His189 and D1-His190, respectively, which suggests that the imidazole side chain could act as a conjugate base that accepts the proton during PCET. This is supported by recent computational studies that predict a remarkably short H-bond between Y\(_Z\) and D1-His190, which is in agreement with the X-ray crystal structure of PSII.\(^27\,^5\) Additionally, quantum mechanical
(all-QM) and hybrid quantum mechanical/molecular modeling (QM/MM) studies have suggested that the protonation state of the conjugate base, microsolvation and hydrogen bonding could influence the redox activity of Y\textsubscript{D} and Y\textsubscript{Z}.\textsuperscript{9,27–29} Since the Y\textsubscript{Z}• radical intermediate is short lived, previous mechanistic proposals for PCET were based on the dark-stable Y\textsubscript{D}• radical that displays slower decay kinetics. But the striking difference in the respective environments suggests that there may be important mechanistic differences in PCET at Y\textsubscript{Z} and Y\textsubscript{D}. It is important to understand the mechanism of PCET at Y\textsubscript{Z} and its role in water oxidation as the OEC of PSII is a blueprint for the design of bio-inspired molecular assemblies for new and efficient catalysts for solar fuels production that incorporate multi-electron and PCET reactions.\textsuperscript{7,30–38}

Since the transient Y\textsubscript{Z}• radical in intact PSII is not amenable to structural studies, artificial photosynthetic models of Y\textsubscript{Z} that allow for the generation of long-lived PCET intermediates could facilitate in depth analyses of its electronic structure. In particular, there is a need for models that mimic the key structural features of Y\textsubscript{Z}, namely, (i) the unusually short hydrogen bond with its conjugate base partner, D1-His190,\textsuperscript{27,5} and (ii) the presence of water molecules that interact closely with both the H-bond donor and acceptor. In the present study, we demonstrate the use of an artificial photosynthetic reaction center, benzimidazole-phenol porphyrin, BiP–PF\textsubscript{10}, as a model for PCET between the oxidized primary donor (P\textsubscript{680}•+) and the Y\textsubscript{Z}-D1-His190 pair in PSII (Figure 2). The BiP–PF\textsubscript{10} dyad is comprised of a benzimidazole-phenol (BiP) moiety that is covalently attached to a high oxidation potential porphyrin (PF\textsubscript{10}). Prior to PCET, the phenolic proton participates in a strong H-bond with the imino nitrogen of benzimidazole that makes the phenol essentially coplanar with the benzimidazole and mimics the Y\textsubscript{Z}-D1-His190 interaction in PSII.\textsuperscript{7} Upon photooxidation of PF\textsubscript{10}, BiP undergoes PCET that results in the formation of a long-lived radical at 77 K.

We have previously shown that BiP acts as an effective mediator in the electrochemical oxidation of water.\textsuperscript{39} More recently, we found that a hybrid organic/inorganic heterogeneous triad consisting of a dyad of BiP–PF\textsubscript{10} linked to TiO\textsubscript{2} nanoparticles could undergo concerted PCET in the BiP moiety.\textsuperscript{33} In this system, the excitation of PF\textsubscript{10} led to formation of the charge-separated state, TiO\textsubscript{2}–PF\textsubscript{10}–BiP•+. This study had suggested that computational models of the (PF\textsubscript{10}–BiP)*+ radical in the charge-
separated state, TiO$_2$–PF$_{10}$–BiP$^{•−}$, required the inclusion of explicit water molecules in the vicinity of the phenolic proton to better describe the $g$-tensor that was obtained from high-frequency D-band (130 GHz) EPR spectroscopy.

This observation presented the possibility that solvent molecules could play an important role in the spectroscopic signatures of molecular systems and more importantly, they could influence the mechanism of PCET in BiP–PF$_{10}$. Similar observations have previously been reported on the role of water molecules in the prediction of the physical behavior of PSII. Recent QM/MM studies by Saito and coworkers have suggested that the structured water molecules that were observed in the 1.9 Å resolution X-ray crystal structure of PSII could play a key role in shortening the distance between Y$_Z$ and its conjugate base partner, D1-His190. This could result in an ionic hydrogen bond, where the proton is delocalized in a nearly barrier-less potential well and the energy levels of the states with the proton associated with Y$_Z$ or D1-His190 are essentially the same. This is important as the presence of a low-barrier hydrogen bond in the Y$_Z$-D1-His190 couple could explain the rapid kinetics of PCET as well as the lack of a pronounced deuterium isotope effect at Y$_Z$ in comparison with that of the Y$_D$ residue. Additionally, the $g_x$ component of the $g$-tensors that were determined in our previous study for the BiP–PF$_{10}^{••}$ were lower than the corresponding values in literature that have been attributed solely to H-bonding effects in phenoxy groups. This observation suggested the presence of spin delocalization on both, the phenoxy and porphyrin moieties, in the charge-separated state and has raised the possibility that charge delocalization could also influence the PCET reactions and thereby render the BiP an effective mediator for water oxidation.

In the present study, in order to directly probe the role of H-bonded water molecules and charge delocalization in the tuning of PCET in the artificial reaction center, BiP–PF$_{10}$ (Figure 2), we use a combination of high-resolution two-dimensional (2D) $^{14}$N hyperfine sublevel correlation (2D HYSCORE) spectroscopy and density functional theory (DFT) to determine the electronic structure of BiP–PF$_{10}^{••}$ radical that is generated following PCET by the BiP moiety. Our results indicate that it is important to include hydrogen bonding effects from explicit water molecules in order to reproduce the hyperfine coupling constants and delocalization of electron spin density that is observed in the
experimental measurements. This study provides insight on the significance of solvation and hydrogen bonding in tuning the PCET reactions that mediate water oxidation in artificial and natural photosynthetic reaction centers.

**Results and Discussion**

We generated the radical intermediate state of the BiP–PF$_{10}$ dyad by illumination with white light at 77 K using DMSO as a solvent. In this case, initial light absorption leads to photoexcitation of the porphyrin, PF$_{10}$, which results in the formation of the photooxidized species, PF$_{10}^{1+}$, through electron transfer to the solvent matrix.$^{46,47–50}$ This is followed by rapid PCET at the BiP moiety, which reduces the PF$_{10}^{1+}$ cation to regenerate the neutral porphyrin with the formation of a phenoxyl radical where the phenolic proton is transferred to the proximal nitrogen atom on the benzimidazole.$^{18,33,39,51}$ The presence of continuous-wave (cw) and magnetic field sweep electron-spin-echo electron paramagnetic resonance (EPR) signals at a g value of 2.004 corresponding to a magnetic field position of 346.1 mT at X-band microwave frequency (9.64 GHz) confirm the formation of the BiP–PF$_{10}^{1+}$ radical (electron spin, S = $\frac{1}{2}$) upon cryogenic illumination (Figure 3A–B). Since the structure of BiP–PF$_{10}$ (Figure 2) exhibits partial conjugation, the g = 2.004 signal that is observed in Figure 3A–B could arise from overlapping spectral contributions from the distribution of the unpaired electron spin, S, of $\frac{1}{2}$ on both BiP and PF$_{10}$ in the BiP–PF$_{10}^{1+}$ radical as the respective signals are not resolved at X-band EPR frequency.

The electronic and local structure of the BiP–PF$_{10}^{1+}$ radical can be determined by measuring the electron-nuclear hyperfine interactions of the unpaired electron spin (S = $\frac{1}{2}$) on the radical cation with the magnetically interacting nitrogen-14 ($^{14}$N) atoms (nuclear spin, I = 1). The isotropic (A$_{iso}$) and anisotropic components of the $^{14}$N hyperfine interaction provide a measure of the distribution of the unpaired electron spin density at the nitrogen nuclei. Additionally, the quadrupolar coupling (K) and asymmetry parameter (η) of the $^{14}$N atoms reflect the interaction of the electric quadrupole moment with the electric field gradient due to the uneven distribution of electric charges around the nuclear spin. In principle, the values of K and η characterize the chemical nature of the nitrogen atom and its electronic state. Hence, these are valuable probes
of the chemical nature and electric field gradient of the magnetically coupled \(^{14}\)N atoms of BiP–PF\(_{10}\)\(^{••}\). However, the weak hyperfine interactions with the \(^{14}\)N atoms are not discernable from the line shape of the BiP–PF\(_{10}\)\(^{••}\) spectra (Figure 3A–B) as these are completely masked by inhomogeneous broadening of the EPR signals.

In the present study, we use two-dimensional (2D) hyperfine sublevel correlation (HYSCORE) spectroscopy to obtain a quantitative measure of the hyperfine and quadrupolar parameters of the BiP–PF\(_{10}\)\(^{••}\) radical.\(^{51–59}\) 2D HYSCORE spectroscopy is similar to the pulsed EPR spectroscopy methods, electron nuclear double resonance (ENDOR) and electron spin echo envelope modulation (ESEEM),\(^{60–64}\) that are used for the study of electron-nuclear hyperfine interactions in paramagnetic systems. However, in comparison with the other methods the observation of HYSCORE signals in two-dimensional (2D) frequency space results in enhanced resolution, which alleviates the problem of spectral overcrowding from the simultaneous detection of multiple nuclear spins,\(^62\) such as, the nitrogen atoms of the BiP–PF\(_{10}\)\(^{••}\) radical. Additionally, the nuclear transitions are correlated in the two dimensions which greatly facilitates the analysis and interpretation of the spectra.

The experimental 2D \(^{14}\)N HYSCORE spectrum of the BiP–PF\(_{10}\)\(^{••}\) displays cross-peaks that arise from the electron-nuclear hyperfine interactions of the unpaired electron spin with the nitrogen-14 atoms (nuclear spin, I = 1) of the BiP and PF\(_{10}\) moieties (Figure 4A). The position of the cross-peaks in the spectrum is determined by the Zeeman frequency of the \(^{14}\)N atoms arising from the nuclear magnetic interaction (\(\nu_{\text{I}}\) of 1.06 MHz) and the electron-nuclear hyperfine interaction, A. In the strong coupling limit (when A > 2\(\nu_{\text{I}}\)), the cross-peaks appear in the (–,+) quadrant, while in the weak coupling limit (when A < 2\(\nu_{\text{I}}\)) the cross-peaks are in the (+,+) quadrant of the spectrum.\(^{52,65}\)

Accordingly, the (–,+) quadrant of the 2D \(^{14}\)N spectrum of BiP–PF\(_{10}\)\(^{••}\) in Figure 4A displays three sets of cross-peaks that are symmetric about the anti-diagonal. The origin of these peaks are the nitrogen atoms, N\(^{\text{I}}\)–N\(^{\text{III}}\), that are strongly hyperfine coupled to the unpaired electron spin. In each case, the shift along the diagonal from multiples of the \(^{14}\)N Zeeman frequency, \(\nu_{\text{I}}\), (shown as dashed red lines in Figure 4A) is due to the quadrupolar interaction of the nitrogen atoms, while the separation of the cross peaks along the anti-diagonal is due to the hyperfine interaction with the unpaired electron spin.
In contrast, the (+,+) quadrant of the 2D $^{14}$N HYSCORE spectrum in Figure 4A displays cross-peaks that arise from the weakly hyperfine coupled nitrogen atoms, N$^{IV}$−N$^{VI}$. The two closely located cross-peaks in the (+,+ ) quadrant of the spectrum that are symmetric with respect to the diagonal are assigned to the double quantum transitions of the weakly-coupled nitrogen atoms, N$^{IV}$−N$^{V}$. In contrast, the absence of resolved structure of the cross-peak along the diagonal indicates that the nitrogen atom, N$^{VI}$, is very weakly hyperfine coupled to the unpaired electron spin. Additionally, while the cross-peaks from the N$^{I}$ nitrogen atom appear in only in the (−,+) quadrant the cross-peaks arising from N$^{II}$ are also present in the (+,+ ) quadrant (as labeled in Figure 4A–B).

The principal values of the spin Hamiltonian parameters of all of the contributing hyperfine coupled $^{14}$N atoms can be determined by numerical simulations to obtain a best fit of the cross-peaks in the experimental 2D $^{14}$N HYSCORE spectrum (Figure 4B). The simulations provide an accurate determination of isotropic hyperfine coupling, $A_{iso}$, as well as the value of $K^2(3+\eta^2)$, where the quadrupolar coupling constant, $K = e^2 qQ/4\hbar$, $Q$ is the quadrupole moment interacting with the electric field gradient $q$, and $\eta$ is the asymmetry parameter, for each nitrogen atom that is magnetically coupled to the unpaired electron spin (Table 1).

In the (−,+ ) quadrant of the 2D HYSCORE spectrum in Figure 4A, the most intense cross-peaks from the nitrogen atoms, N$^{I}$ and N$^{II}$, are overlapping at (± 2.6, ±1.3) MHz. This is because N$^{I}$ and N$^{II}$ possess similar magnetic parameters, namely, $K = -0.60$ MHz, $\eta = 0.50$, and $A_{iso} = 1.12$ MHz for N$^{I}$ and $K = -0.58$ MHz, $\eta = 0.52$, and $A_{iso} = 1.05$ MHz for N$^{II}$. Based on the values of $K$ of $-0.60$ – $-0.58$ MHz and $\eta$ of 0.50 – 0.52, we assigned N$^{I}$ and N$^{II}$ to the nitrogen atoms of the benzimidazole moiety, BiP. The assignment is based on previous nuclear quadrupole resonance (NQR) studies that have demonstrated that the quadrupolar coupling constant $(e^2 qQ/\hbar)$ for the amino nitrogen atom of an imidazole side chain is $\sim 1.4 – 2.4$ MHz. The position of the cross-peaks due to N$^{I}$ and N$^{II}$ is determined by the value of $A_{iso}$ while the anisotropy manifests itself as second order effects. As mentioned earlier, the cross-peaks display observable shifts from the nuclear Zeeman frequency (shown as dashed red lines in Figure 4B) due to the influence of the K
values. There is also a third set of cross-peaks located at (+3.9, ±1.3) MHz in the (-,+) quadrant that arise from a hyperfine interaction of the unpaired electron spin with the nitrogen atom, N^III, with a K of -0.66 MHz that is strongly coupled to the electron spin (A > 2\nu_i) (Table 1). Based on the value of K of -0.66 MHz, we assigned N^III to a pyrrole nitrogen in the PF_{10} ring.\textsuperscript{46,67}

In the (+,+) quadrant of the 2D HYSCORE spectrum in Figure 4A-B, there are two pairs of resolved cross-peaks separated from the diagonal points centered at (3.3, 3.3) MHz, which arise from weak hyperfine interactions of the unpaired electron spin with the nitrogen atoms, N^IV and N^V. The separation of the cross-peaks along the anti-diagonal corresponds to the weak coupling regime A < 2\nu_i and hence the peaks are located in a frequency region closer to the diagonal. Finally, the cross-peak arising from the weakest hyperfine interaction with the nitrogen atom, N^VI, with the smallest A_{iso} value is located along the diagonal. The K values of all three nitrogen atoms, N^IV – N^VI, are in the range of -0.69 – -0.72 MHz which is comparable to the K values that have previously been observed for pyrrole nitrogen atoms of a porphyrin ring.\textsuperscript{46,67} Thus, we assign N^IV – N^VI to the remaining pyrrole nitrogen atoms in the PF_{10} moiety.

It is notable that the porphyrin nitrogen atom, N^III, displays a strong isotropic hyperfine coupling, A_{iso}, of 2.15 MHz which indicates the presence of significant electron spin density at this location. In comparison, the remaining nitrogen atoms of the porphyrin ring, N^IV – N^VI, display relatively small values of isotropic hyperfine coupling constant of -0.2 – 0.5 MHz. The observation of finite isotropic hyperfine coupling constants, A_{iso}, for all of the nitrogen atoms, N^I – N^VI, clearly indicates that the electron spin density is delocalized on both the benzimidazole phenol, BiP, and high-potential porphyrin, PF_{10}, in BiP–PF_{10}•+.

The A_{iso} values of 1.05 MHz and 1.12 MHz for the nitrogen atoms, N^I and N^II, of BiP indicates that the magnitude of electron spin density that is distributed on the two nitrogen atoms is comparable. Typically, the A_{iso} values are determined by the unpaired spin density in the 2s orbital that is proportional to atomic
value of 1811 MHz for $^{14}\text{N}$, computed for unit spin density in this orbital.\(^6\) Therefore, the $A_{iso}$ value of 1.05 – 1.12 MHz that is determined for the $N^\text{I}$ and $N^\text{II}$ nitrogen atoms of BiP corresponds to a fractional spin density distribution, $\rho_s$, of $\approx 6.0 \times 10^{-4}$ in the 2s orbital of the each nitrogen atom. This suggests that the strength of the H-bonds between each nitrogen atom of BiP and its proximal water molecule are nearly equivalent. Moreover, the values of the isotropic hyperfine coupling constant, $A_{iso}$, indicate the presence of significant spin density on the strongly coupled nitrogen atom, $N^\text{III}$, of the porphyrin ring, PF$_{10}$, along with a smaller fraction of spin density on the three weakly coupled nitrogen atoms, $N^\text{IV} – N^\text{VI}$. The PF$_{10}$ molecule is an aromatic ring system, therefore, the $\pi$-electron density is delocalized over the nitrogen atoms of the PF$_{10}$ ring. The relative differences in $A_{iso}$ values reflect differences in the distribution of spin density on the nitrogen atoms, $N^\text{IV} – N^\text{VI}$, of the porphyrin ring (Table 1).

We performed DFT calculations on three computational models (A, B and C) shown in Figure 5A–C, respectively, to elucidate the high-resolution electronic structure of BiP–PF$_{10}^\ast$ and understand the role of water molecules play in the PCET process. All three structures correspond to the chemical species that would result from the transfer of the proton from the phenolic oxygen atom to the proximal imidazole nitrogen atom of BiP during PCET, thus resulting in a BiP–PF$_{10}^\ast$•. While model A does not include any water molecules (Figure 5A), the models B and C include explicit water molecules that are H-bonded to the BiP moiety of the BiP–PF$_{10}^\ast$• radical (Figure 5B–C). The structure of B (Figure 5B) includes two explicit water molecules; one water molecule that forms a H-bond with the distal N–H of BiP and another that forms a H-bond to the phenoxy oxygen atom. The alternate structure, C, also contains two water molecules, where one water acts as a H-bond acceptor to the distal amino N–H of BiP and a second water molecule that serves as both a H-bond acceptor and donor to the newly formed N–H and phenoxy oxygen atom, respectively (Figure 5C).

The DFT calculations allow for a comparison of the electron spin density distribution in each of the computational models, A, B, and C (Figure 6A–C, respectively). While the
electron spin density is distributed on the imidazole and phenol of the BiP moiety in all three models, we also observe delocalization of the unpaired electron spin onto the conjugated porphyrin, PF_{10}. The delocalization of the electron spin on both BiP and PF_{10} in these structures is in agreement with the experimental 2D ^{14}N HYSCORE measurements where we observe hyperfine interactions of the unpaired electron with the nitrogen atoms of both BiP (N^I and N^{II}) and PF_{10} (N^{III} – N^{VI}) (Figure 4A–B and Table 1). The delocalization of the electron spin density is also consistent with previous theoretical analysis on the methyl ester derivative of BiP–PF_{10}.^{33,45}

In addition to obtaining the electron spin density distribution of the structures, we calculated the quadrupolar and hyperfine parameters (K and A_{iso}, respectively) for comparison with the experimental values that were obtained from the 2D ^{14}N HYSCORE measurements. The calculated K values that are obtained for all of the nitrogen atoms, N^I – N^{VI}, for the A, B and C structures are comparable to the K values that are determined from the 2D HYSCORE measurements. In particular, the value of K for the benzimidazole (N^I – N^{II}) and porphyrin (N^{III} – N^{VI}) nitrogen atoms of the C structure was 0.62 ± 0.04 MHz and 0.77 ± 0.03 MHz, respectively (Table 1). This is in agreement with the experimental values of K for the BiP and PF_{10} nitrogen atoms, within an average error of 0.07 MHz.

The calculated value of the isotropic hyperfine coupling constant, A_{iso}, that is obtained for the benzimidazole nitrogens, N^I – N^{II}, in the C structure was 1.17 MHz and 0.88 MHz, respectively, while the corresponding value for the porphyrin nitrogens, N^{III} – N^{VI}, was 2.2 MHz, 0.41 MHz, 0.77 MHz and -0.17 MHz, respectively. The calculated values for A_{iso} are in agreement with the experimental couplings of N^I – N^{VI}; the average error was 0.15 MHz. In contrast, the average error for the calculated values A_{iso} for B and A structures was 0.52 MHz and 0.33 MHz, respectively (as shown in Table 1S and 2S).

The corresponding g-tensors that are obtained from the DFT calculations of the A, B and C structures are presented in Table 3S. Previous high-frequency EPR and ENDOR measurements on the redox-active tyrosine-D radical of photosystem II^{18,51,69,70} and TiO_{2}–PF_{10}BiP triad^{33} have demonstrated that the g_x component of the g-tensor for a tyrosyl radical that is oriented along the C-O molecular axis is sensitive to the local environment
of the phenolic oxygen atom. In this case, the spin–orbit coupling interaction between the unpaired electron in the singly occupied molecular orbital (SOMO) and the lone pairs of electrons on the phenolic oxygen atom induce a magnetic moment that results in a rather large deviation of the $g_x$ component of the $g$-tensor (e.g. $g_x$ of $\sim 2.008$) from the free electron $g$-value ($g_e = 2.0023$). However, the presence of H-bonding interaction(s) with the lone pair on the phenolic oxygen atom and/or delocalization of the unpaired spin density tend to decrease this deviation, yielding radicals with lower $g_x$ values. This is in agreement with the $g_x$ component of the $g$-tensor of 2.0056, 2.0052 and 2.0061 that is calculated for the A, B and C structures (Table 3S). The $g_x$ component that was observed for the A, B and C structures is lower than the calculated $g_x$ value of $\sim 2.0065$ that was obtained for a series of phenoxy radicals that were H-bonded to an imidazolium moiety in the absence of a covalently linked conjugated system. Similarly, the $g_x$ component of the A, B and C structures is also less than the $g_x$ value of 2.0065 that was previously reported for a BiP–PF$_{10}$ derivative in which the porphyrin molecule was covalently bonded to BiP through the imidazole moiety instead of the phenol which precluded delocalization of the electron spin density between the BiP and PF$_{10}$ moieties. Hence, in the present study the smaller value of 2.0056, 2.0052 and 2.0061 that is obtained for the $g_x$ component of the A, B and C structure is attributed to the effects from the presence of strong H-bonding interactions at the phenoxy oxygen atom and to a lesser degree the delocalization of the electron spin density on both BiP and PF$_{10}$.

In our previous work, we had demonstrated that calculated $g$-values for structure B were in better agreement with the corresponding experimental values of the radical species obtained by illumination at 13 K (prior to annealing the sample at 77 K). In contrast, the calculated $g$-values for structure C were in better agreement with those of the radical after annealing the sample at 77 K. We rationalized this with the fact that before PCET the nitrogen of the imidazole that is H-bonded to the phenol is a hydrogen bond acceptor, but after PCET the nitrogen accepts the phenolic proton forming an N–H group and is therefore a hydrogen bond donor. The PCET reactions occur at 13 K but lack the thermal energy for solvent reorganization and form a H-bond with the newly formed N–H group (structure B). Once the sample is annealed, the solvent is able to reorganize and form a bifurcated H-bond between the newly formed N–H and the phenolic oxygen.
atom (structure C). This indicates that in the present study the illumination of BiP–PF\textsubscript{10} with white light at 77 K should lead to the formation of a cation radical whereby upon PCET, the nitrogen atom of BiP accepts the phenolic proton resulting in an N–H that is in a bifurcated H-bond.

Based on the magnetic parameters of the computational models of BiP–PF\textsubscript{10}, A, B and C, we examine the geometric parameters and relative energies of the DFT structures. We find that the extent of electron spin delocalization between BiP and PF\textsubscript{10} in the three DFT structures (Figure 6A–C) can be explained in terms of the dihedral angle between the conjugated rings of BiP and PF\textsubscript{10}. The delocalization of the electron on BiP and PF\textsubscript{10} is greater when the dihedral angle between the planes of the BiP and PF\textsubscript{10} ring tends to 0º and is diminished when the dihedral angle tends to 90º (i.e. when the rings are orthogonal). As observed in Table 2, the dihedral angle between the BiP and PF\textsubscript{10} is ~47º for all of the structures.

The main structural difference between the computational models is the dihedral angle between the benzimidazole and phenolic group of BiP. As shown in Table 2, the BiP system is almost co-planar for the A and B structures, but it is twisted slightly out of plane for C structure (with a dihedral angle of ~7º). We hypothesize that this difference is caused by the relaxation of the water molecules around the newly formed N–H bond which forms a bifurcated H-bond with both the phenolic and water oxygen atoms thus lowering the strength of the intra-molecular hydrogen bond. The effects of the spin density delocalization can be also observed in the A\textsubscript{iso} values for specific nitrogen within the different structures. The deviation of the experimental and calculated A\textsubscript{iso} value for N\textsuperscript{II} is 0.24, 0.67 and 0.55 MHz for models C, B and A, respectively. The deviation of the experimental and calculated A\textsubscript{iso} value for N\textsuperscript{III} is 0.06, 0.85 and 0.30 MHz for models C, B and A, respectively. These results highlight the importance of incorporating explicit water molecules in the models in the right conformation. Finally, as shown in Table 2, the energy of structure C is ~3 kcal/mol lower than the energy of structure B. Therefore, the relaxation from structure B to structure C is thermodynamically favorable, which is consistent with the discussion above.

Conclusions:
In the present study, we determine the experimental and calculated electron spin density distribution on the radical species that is formed upon light-induced PCET in the artificial reaction center, BiP–PF\textsubscript{10}. We demonstrate excellent agreement between the hyperfine coupling constants of the nitrogen atoms that are determined by experimental 2D \textsuperscript{14}N HYSCORE spectroscopy and those calculated by DFT methods. Moreover, we observe that there is a direct correlation between the hyperfine coupling constants of the nitrogen atoms and delocalization of electron spin density in the presence and absence of structures water molecules in the vicinity of the BiP and porphyrin moieties of BiP–PF\textsubscript{10}. The presence of two explicit water molecules in the C structure that are hydrogen bonded to the N–H sites of the BiP moiety leads to a near perfect match of the experimental and calculated hyperfine couplings. This highlights the importance of hydrogen bonding interactions with water molecules in the favorable tuning of the electronic structure and respective energy levels that facilitates PCET reactions in the BiP–PF\textsubscript{10} reaction center. In contrast the experimental hyperfine coupling constants are not as well reproduced in the related structures, A and B, that exhibit differences in the hydrogen bonding pattern or a complete absence of such interactions with structured water molecules. These results present direct experimental and theoretical evidence of spin delocalization and highlights the effects of structured water molecules on the tuning of the electronic structure of artificial reaction centers. The results of this study emphasize the importance of considering these factors in the experimental and computational design of novel artificial photosynthetic systems that incorporate multi-electron and PCET processes.

**Methodology:**

**Synthesis and Characterization:** BiP–PF\textsubscript{10} was synthesized and characterized following the procedures reported by Megiatto \textit{et al.}\textsuperscript{33,74} The procedure consists of an acid-catalyzed porphyrin condensation of 5-formyl-3-tert-butyl-2-hydroxybenzaldehyde, 5-(pentafluorophenyl)dipyrromethane, and methyl-4-formylenzoate at room temperature to form 5, 15-bis(pentafluorophenyl)-10-(4-methoxycarbonylphenyl)-20-(3-formyl-4-hydroxy-5-tert-butylphenyl)porphyrin. After purification this porphyrin precursor was allowed to react with 1,2-phenylenediamine to form the ester form of the BiP–PF\textsubscript{10}, 5, 15-bis(pentafluorophenyl)-10-(4-methoxycarbonylphenyl)-20-[2’-(3”-tert-butyl-2”-
hydroxyphenyl)benzimidazole]porphyrin. Finally, the ester was hydrolyzed with trifluoroacetic acid and HCl to form the desired compound 5, 15-bis(pentafluorophenyl)-10-(4-carboxyphenyl)-20-[2’-(3”-tert-butyl-2”-hydroxyphenyl)benzimidazole]porphyrin.

**Continuous-wave and Pulsed EPR Spectroscopy:** The EPR spectra were obtained on a custom-built continuous-wave (cw)/pulsed X-band Bruker Elexsys 580 EPR spectrometer using a dielectric flex-line ER 4118-MD5 probe (Bruker BioSpin, Billerica, MA) and a dynamic continuous-flow cryostat CF935 (Oxford Instruments, Oxfordshire, U.K.) at 30 K temperature. The cw EPR spectrum of the BiP–PF$_{10}$$^{•+}$ was acquired at the operating microwave frequency of 9.71 GHz with modulation frequency of 100 kHz, modulation amplitude of 1 G and microwave power of 0.7 µW. For the magnetic-field-sweep electron-spin-echo EPR spectrum, the primary electron spin echo was generated using the pulse sequence ($\pi$/$\tau$–$\pi$–$\tau$–echo). The echo was integrated over a 32 ns time window that was centered at the maximum of the echo signal. The length of the $\pi$/$2$- and $\pi$-pulse was 8 ns and 16 ns, respectively. The inter-pulse separation, $\tau$, was 120 ns and the delay in the pulse sequence is defined as the difference in the starting point of the pulses.

The 2D $^{14}$N HYSCORE spectra were recorded at magnetic field position of 346.1 mT. For the 2D $^{14}$N HYSCORE spectra of BiP, the echo amplitude was measured using the pulse sequence ($\pi$/$2$–$\tau$–$\pi$–$\tau$–$t_1$–$\pi$–$t_2$–$\pi$/$2$–$\tau$–echo) with a $\tau$ value of 136 ns, an 8 ns and 16 ns length for the $\pi$/$2$- and $\pi$-pulse, respectively. The delays in the pulse sequence are defined as the difference in the starting point of the pulses. The echo intensity was measured as a function of $t_1$ and $t_2$, where $t_1$ and $t_2$ were incremented in steps of 16 ns from an initial value of 40 ns and 32 ns, respectively. 256 steps were used for each dimension. The 8 ns time difference between the initial value of $t_1$ and $t_2$ was set to account for the difference in length between the $\pi$/$2$- and $\pi$-pulse. The unwanted echoes were eliminated by applying a 16-step phase cycling procedure. The hyperfine parameters of the $^{14}$N nuclei were obtained by performing numerical simulations using the saffron function of the EasySpin software package. The simulated spectra were carefully compared with the experimental ones to match the position of the cross-peaks and signal intensity distribution over the different points of the ridges.
The frequency of the double-quantum transition is described by the following equation:

\[ v_{\text{dqt}} = 2\left[ v_{\text{eff}, \pm}^2 + K^2(3 + \eta^2) \right]^{1/2} \]

The effective frequency is \( v_{\text{eff}, \pm} = |v_I \pm a/2| \) where \( v_I \) is the nuclear Zeeman frequency for the given magnetic field and \( a \) is the hyperfine (isotropic) coupling value. The value of nuclear quadrupole coupling is described by \( K = e^2 q Q / 4\hbar \) where \( Q \) is the quadrupole moment interacting with the electric field gradient \( q \) and \( \eta \) is the asymmetry parameter. In general, the isotropic hyperfine coupling is calculated from the cross-peak positions of the double-quantum transitions. The detection of cross-peaks in the (-,+) and (+,+) quadrant depends mainly on the strength of the electron-nuclear hyperfine coupling and orientation dependence of the nuclear transition of inequivalent \( ^{14}\text{N} \) nuclei.

**Density Functional Theory Calculations**: The theoretical structure of the A, B and C models of the PF\(_{10}\)-BiP\(^+\) radical (Figure 5A-C) was optimized with Gaussian 09\(^{76,77} \) at the B3LYP/6-31G(d,p) level of theory. The electron spin density distributions, quadrupolar and hyperfine coupling parameters and g-tensors for all of three structures were obtained with ORCA\(^{78,79} \) at the B3LYP/EPR-II level of theory using the Conductor-like Polarizable Continuum Model (CPCM), with the value of epsilon set to 47.2 and refrac at 1.479 to simulate a dimethyl sulfoxide (DMSO) environment.

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Author Contributions
D.D.M.H., A.B., V.K., P.C., B. Mark, W.M., and B. Molnar conducted experiments, analyzed the data and performed calculations; D.D.M.H., K.V.L., A.L.M and T.A.M. designed the experiments and wrote the paper.

Declaration of Interest
The authors declare no competing interests.

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Figure 1. X-ray crystal structure of PSII. (A) The location of the charge-transfer cofactors that participate in the water-oxidation reaction of PSII. In the primary electron transfer (ET) pathway of PSII, the photooxidation of the primary donor, P$_{680}$ (green), leads to the formation of the initial charge-separated state, P$_{680}^+$-Pheo$_A^-$ (Pheo$_A$ is shown in blue), that is rapidly stabilized by forward ET to the primary and secondary quinone acceptors, Q$_A$ and Q$_B$ (red), respectively. P$_{680}^+$ is a strong oxidant that leads to rapid PCET at the redox-active tyrosine residue, tyrosine-Z (Y$_Z$) (black) which results in the oxidation of water at the Mn$_4$Ca-oxo cluster in the oxygen-evolving complex (OEC). The smart protein matrix in the vicinity of the redox-active (B) Y$_Z$ and (C) tyrosine-D (Y$_D$) residue (black) as observed in the 1.9 Å resolution X-ray crystal structure of PSII.$^5$

Figure 2. Schematic of the artificial photosynthetic reaction center, benzimidazole phenol-porphyrin, BiP–PF$_{10}$. The electron withdrawing ability of the two pentafluorophenyl substituents (purple) make the porphyrin ring (green) a high oxidation potential molecule. Photooxidation of PF$_{10}$ leads to PCET at the BiP moiety, where the electron is transferred to PF$_{10}^+$ and the phenolic (red) proton is transferred to the benzimidazole (blue) imino nitrogen atom. Also shown is the labeling scheme for the respective nitrogen atoms of BiP–PF$_{10}$.

Figure 3. The (A) continuous-wave (cw) and (B) electron-spin-echo magnetic-field-sweep EPR spectrum of BiP–PF$_{10}$. The arrow in part B indicates the magnetic field position of 346.1 mT that was used for the acquisition of the 2D $^{14}$N HYSCORE spectrum that is shown in Figure 4A.

Figure 4. The (A) experimental and (B) simulated 2D $^{14}$N HYSCORE spectrum of BiP–PF$_{10}^{++}$ radical. The (-,+)$^-$ quadrant displays cross-peaks from the strong hyperfine couplings of the electron spin with three nitrogen atoms, N$^I$ – N$^III$, that are symmetric about the anti-diagonal. In contrast, the (+,+)$^+$ quadrant contains cross-peaks from weaker hyperfine couplings with the N$^IV$ – N$^VI$ nitrogen atoms that are symmetric with respect to
the diagonal. The dashed red lines in parts A and B indicate multiples of the $^{14}$N Zeeman frequency, $\nu_i^n$, of 1.06 MHz.

**Figure 5.** The DFT optimized structure of the BiP moiety in the A, B, and C structure of the BiP–PF$_{10}^\bullet\bullet$, where PF$_{10}$ is omitted for clarity. Both structures B and C contain two water molecules, but differ in the H-bonding pattern between the phenolic O atom, imidazole nitrogen atom and proximal water molecule. In contrast, structure A does not contain any water molecules.

**Figure 6.** The electron spin density (purple and gold) distribution of the A, B, and C structures of BiP–PF$_{10}^\bullet\bullet$ as determined by DFT. The atoms are color coded as carbon (grey), hydrogen (white), nitrogen (blue), fluorine (cyan) and oxygen (red).
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**Table 1.** The $^{14}$N hyperfine and quadrupolar parameters that are obtained from spectral simulations of the experimental 2D $^{14}$N HYSCORE spectrum of the BiP–PF$_{10}^{**}$ and DFT calculations of the C structure of the BiP–PF$_{10}^{**}$.

**Table 2.** The relative energies (ΔE) and relevant dihedral angles of the A, B and C structure of BiP–PF$_{10}^{**}$ that were optimized by DFT calculations.
Table 1.

| Nitrogen | K (MHz) (experimental) | $A_{\text{iso}}$ (MHz) (experimental) | K (MHz) (calculated C structure) | $A_{\text{iso}}$ (MHz) (calculated C structure) |
|----------|------------------------|----------------------------------------|---------------------------------|-----------------------------------------------|
| N$^I$    | -0.59 ± 0.01           | 1.05 ± 0.1                             | -0.66                           | 1.17                                          |
| N$^{II}$ | -0.60 ± 0.02           | 1.12 ± 0.1                             | -0.58                           | 0.88                                          |
| N$^{III}$| -0.66 ± 0.01           | 2.15 ± 0.1                             | -0.80                           | 2.21                                          |
| N$^{IV}$ | -0.72 ± 0.01           | 0.50 ± 0.1                             | -0.75                           | 0.41                                          |
| N$^V$    | -0.70 ± 0.01           | 0.40 ± 0.2                             | -0.75                           | 0.77                                          |
| N$^{VI}$ | -0.69 ± 0.01           | -0.20 ± 0.1                            | -0.80                           | -0.18                                         |
Table 2.

| Structure | Benzimidazole-Phenol dihedral angle | BiP–Porphyrin dihedral angle | ΔE relative to structure A (kcal/mol) |
|-----------|------------------------------------|-----------------------------|--------------------------------------|
| A         | 1.7                                | 48.5                        | Reference                            |
| B         | 1.8                                | 44.5                        | −16.2                                |
| C         | 7.1                                | 47.6                        | −19.1                                |
HYSCORE and DFT Studies of Proton-Coupled Electron Transfer in a Bioinspired Artificial Photosynthetic Reaction Center

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Abstract

Light-driven water oxidation in algae, cyanobacteria, and higher plants generates dioxygen that supports life on Earth. The water-oxidation reaction is catalyzed by the oxygen-evolving complex (OEC) in photosystem II (PSII) that is comprised of the tetranuclear manganese calcium-oxo (Mn₄CaO₅) cluster, with participation of the redox-active tyrosine residue (Y_Z) and a hydrogen-bonded network of amino acids and water molecules. Y_Z mediates successive proton-coupled electron transfer (PCET) reactions that are essential for the oxidation of water to dioxygen at the Mn₄CaO₅ cluster. It has been proposed that the strong hydrogen bond between Y_Z and and its conjugate base, D1-His190, likely renders Y_Z kinetically and thermodynamically competent leading to highly efficient water oxidation.¹ However, a detailed understanding of PCET at Y_Z remains elusive due to the transient nature of its intermediate states. In this study, we utilize a combination of high-resolution two-dimensional (2D) $^{14}$N hyperfine sublevel correlation (HYSCORE) spectroscopy and density functional theory (DFT) methods to investigate the electronic structure of a bioinspired artificial photosynthetic reaction center, benzimidazole-phenol porphyrin (BiP–PF₁₀), that mimics the PCET process at the Y_Z residue of PSII. The results of these studies underscore the importance of proximal water molecules and charge delocalization on the electronic structure of the artificial reaction center.

Keywords
Bio-inspired systems, Photosynthetic reaction center, Proton-coupled electron transfer, hydrogen bonds, electron spin delocalization, HYSCORE spectroscopy.
Introduction

The photosynthetic protein complex, photosystem II (PSII), catalyzes one of the most energetically demanding reactions in nature by using light energy to drive a catalyst capable of oxidizing water.\(^2\)\(^{-5}\) Proton-coupled electron transfer (PCET) reactions, which are exquisitely tuned by smart protein matrix effects, are central to the water oxidation chemistry of PSII. Moreover, PCET is encountered in a wide variety of biological and synthetic catalysts where it diminishes the overall energetic penalty for multi-electron transfer and modulates the electrochemical driving force of reactions.\(^6\)\(^{-8}\) In PSII, light-driven water oxidation is catalyzed in the oxygen-evolving complex (OEC) that is comprised of a tetranuclear manganese-calcium-oxo (Mn\(_4\)Ca-oxo) cluster with an extensive network of hydrogen (H)-bonded amino acid residues and structured water molecules.\(^2\) In particular, there are two symmetrically placed tyrosine residues, Y\(_D\) and Y\(_Z\), one on each subunit of the heterodimeric core polypeptide, D2 and D1, respectively (Figure 1A).\(^3\)\(^{-5}\) The function of these tyrosines is distinct as the 'smart' matrix effects from the surrounding protein environment greatly influence their behavior.\(^9\)\(^{-12}\) The Y\(_D\) redox is suggested to poise the catalytic Mn\(_4\)Ca-oxo cluster\(^13\) and may be involved in the electrostatic tuning of the adjacent monomeric redox-active chlorophyll and β-carotene in a secondary photoprotection pathway.\(^14\) In contrast, Y\(_Z\) is kinetically competent and directly participates in water oxidation.\(^15\)\(^{-17}\) Previous biochemical, spectroscopic and computational studies have demonstrated that both Y\(_D\) and Y\(_Z\) undergo photo-induced PCET with the formation of a neutral tyrosyl radical, Y\(^●\), but the formation and decay of the Y\(_Z\)^\(^●\) is a thousand times more rapid than Y\(_D\)^\(^●\).\(^12\)\(^{-18}\)\(^{-26}\)

The difference in the kinetics of light-driven PCET at Y\(_Z\) and Y\(_D\) has been suggested to arise from differences in their respective microenvironment. The protein matrix surrounding Y\(_Z\) is highly hydrophilic with several H-bonded water molecules, while the Y\(_D\) site contains a single water molecule in the X-ray crystal structure of PSII (Figure 1B-C).\(^5\) Both Y\(_D\) and Y\(_Z\) are proximal to a histidine residue, D2-His189 and D1-His190, respectively, which suggests that the imidazole side chain could act as a conjugate base that accepts the proton during PCET. This is supported by recent computational studies that predict a remarkably short H-bond between Y\(_Z\) and D1-His190, which is in agreement with the X-ray crystal structure of PSII.\(^27\)\(^,\)\(^5\) Additionally, quantum mechanical
(all-QM) and hybrid quantum mechanical/molecular modeling (QM/MM) studies have suggested that the protonation state of the conjugate base, microsolvation and hydrogen bonding could influence the redox activity of \( Y_D \) and \( Y_Z \).\(^{9,27–29} \) Since the \( Y_Z^\bullet \) radical intermediate is short lived, previous mechanistic proposals for PCET were based on the dark-stable \( Y_D^\bullet \) radical that displays slower decay kinetics. But the striking difference in the respective environments suggests that there may be important mechanistic differences in PCET at \( Y_Z \) and \( Y_D \). It is important to understand the mechanism of PCET at \( Y_Z \) and its role in water oxidation as the OEC of PSII is a blueprint for the design of bio-inspired molecular assemblies for new and efficient catalysts for solar fuels production that incorporate multi-electron and PCET reactions.\(^{7,30–38} \)

Since the transient \( Y_Z^\bullet \) radical in intact PSII is not amenable to structural studies, artificial photosynthetic models of \( Y_Z \) that allow for the generation of long-lived PCET intermediates could facilitate in depth analyses of its electronic structure. In particular, there is a need for models that mimic the key structural features of \( Y_Z \), namely, (i) the unusually short hydrogen bond with its conjugate base partner, D1-His190,\(^{27,5} \) and (ii) the presence of water molecules that interact closely with both the H-bond donor and acceptor. In the present study, we demonstrate the use of an artificial photosynthetic reaction center, benzimidazole-phenol porphyrin, BiP–PF\(_{10}\), as a model for PCET between the oxidized primary donor (P\(_{680}^\bullet\)) and the \( Y_Z\)-D1-His190 pair in PSII (Figure 2). The BiP–PF\(_{10}\) dyad is comprised of a benzimidazole-phenol (BiP) moiety that is covalently attached to a high oxidation potential porphyrin (PF\(_{10}\)). Prior to PCET, the phenolic proton participates in a strong H-bond with the imino nitrogen of benzimidazole that makes the phenol essentially coplanar with the benzimidazole and mimics the \( Y_Z\)-D1-His190 interaction in PSII.\(^7 \) Upon photooxidation of PF\(_{10}\), BiP undergoes PCET that results in the formation of a long-lived radical at 77 K.

We have previously shown that BiP acts as an effective mediator in the electrochemical oxidation of water.\(^{39} \) More recently, we found that a hybrid organic/inorganic heterogeneous triad consisting of a dyad of BiP–PF\(_{10}\) linked to TiO\(_2\) nanoparticles could undergo concerted PCET in the BiP moiety.\(^{33} \) In this system, the excitation of PF\(_{10}\) led to formation of the charge-separated state, TiO\(_2^\bullet\)--PF\(_{10}\)--BiP\(^\bullet\). This study had suggested that computational models of the (PF\(_{10}\)--BiP\(^\bullet\))\(^\bullet\) radical in the charge-
separated state, TiO$_2^+$–PF$_{10}$–BiP$^+$, required the inclusion of explicit water molecules in the vicinity of the phenolic proton to better describe the $g$-tensor that was obtained from high-frequency D-band (130 GHz) EPR spectroscopy.

This observation presented the possibility that solvent molecules could play an important role in the spectroscopic signatures of molecular systems and more importantly, they could influence the mechanism of PCET in BiP–PF$_{10}$. Similar observations have previously been reported on the role of water molecules in the prediction of the physical behavior of PSII.$^{27,40,41}$ Recent QM/MM studies by Saito and coworkers have suggested that the structured water molecules that were observed in the 1.9 Å resolution X-ray crystal structure of PSII could play a key role in shortening the distance between Y$_Z$ and its conjugate base partner, D1-His190. This could result in an ionic hydrogen bond,$^{42}$ where the proton is delocalized in a nearly barrier-less potential well and the energy levels of the states with the proton associated with Y$_Z$ or D1-His190 are essentially the same.$^{26}$ This is important as the presence of a low-barrier hydrogen bond in the Y$_Z$-D1-His190 couple could explain the rapid kinetics of PCET as well as the lack of a pronounced deuterium isotope effect at Y$_Z$ in comparison with that of the Y$_D$ residue.$^{7,22,43-45}$ Additionally, the $g_s$ component of the $g$-tensors that were determined in our previous study$^{33}$ for the BiP–PF$_{10}$** were lower than the corresponding values in literature that have been attributed solely to H-bonding effects in phenoxy groups.$^1$ This observation suggested the presence of spin delocalization on both, the phenoxy and porphyrin moieties, in the charge-separated state and has raised the possibility that charge delocalization could also influence the PCET reactions and thereby render the BiP an effective mediator for water oxidation.

In the present study, in order to directly probe the role of H-bonded water molecules and charge delocalization in the tuning of PCET in the artificial reaction center, BiP–PF$_{10}$ (Figure 2), we use a combination of high-resolution two-dimensional (2D) $^{14}$N hyperfine sublevel correlation (2D HYSCORE) spectroscopy and density functional theory (DFT) to determine the electronic structure of BiP–PF$_{10}$** radical that is generated following PCET by the BiP moiety. Our results indicate that it is important to include hydrogen bonding effects from explicit water molecules in order to reproduce the hyperfine coupling constants and delocalization of electron spin density that is observed in the
experimental measurements. This study provides insight on the significance of solvation and hydrogen bonding in tuning the PCET reactions that mediate water oxidation in artificial and natural photosynthetic reaction centers.

**Results and Discussion**

We generated the radical intermediate state of the BiP–PF₁₀ dyad by illumination with white light at 77 K using DMSO as a solvent. In this case, initial light absorption leads to photoexcitation of the porphyrin, PF₁₀, which results in the formation of the photooxidized species, PF₁₀⁺⁺, through electron transfer to the solvent matrix.⁴⁶,⁴⁷–⁵⁰ This is followed by rapid PCET at the BiP moiety, which reduces the PF₁₀⁺⁺ cation to regenerate the neutral porphyrin with the formation of a phenoxy radical where the phenolic proton is transferred to the proximal nitrogen atom on the benzimidazole.¹⁸,³³,³⁹,⁵¹ The presence of continuous-wave (cw) and magnetic field sweep electron-spin-echo electron paramagnetic resonance (EPR) signals at a g value of 2.004 corresponding to a magnetic field position of 346.1 mT at X-band microwave frequency (9.64 GHz) confirm the formation of the BiP–PF₁₀⁺⁺ radical (electron spin, S = ½) upon cryogenic illumination (Figure 3A–B). Since the structure of BiP–PF₁₀ (Figure 2) exhibits partial conjugation, the g = 2.004 signal that is observed in Figure 3A–B could arise from overlapping spectral contributions from the distribution of the unpaired electron spin, S, of ½ on both BiP and PF₁₀ in the BiP–PF₁₀⁺⁺ radical as the respective signals are not resolved at X-band EPR frequency.

The electronic and local structure of the BiP–PF₁₀⁺⁺ radical can be determined by measuring the electron-nuclear hyperfine interactions of the unpaired electron spin (S = ½) on the radical cation with the magnetically interacting nitrogen-14 (¹⁴N) atoms (nuclear spin, I = 1). The isotropic (Aₗₒ) and anisotropic components of the ¹⁴N hyperfine interaction provide a measure of the distribution of the unpaired electron spin density at the nitrogen nuclei. Additionally, the quadrupolar coupling (K) and asymmetry parameter (η) of the ¹⁴N atoms reflect the interaction of the electric quadrupole moment with the electric field gradient due to the uneven distribution of electric charges around the nuclear spin. In principle, the values of K and η characterize the chemical nature of the nitrogen atom and its electronic state. Hence, these are valuable probes of the chemical
nature and electric field gradient of the magnetically coupled $^{14}$N atoms of BiP–PF$_{10}^{\bullet^+}$. However, the weak hyperfine interactions with the $^{14}$N atoms are not discernable from the line shape of the BiP–PF$_{10}^{\bullet^+}$ spectra (Figure 3A–B) as these are completely masked by inhomogeneous broadening of the EPR signals.

In the present study, we use two-dimensional (2D) hyperfine sublevel correlation (HYSCORE) spectroscopy to obtain a quantitative measure of the hyperfine and quadrupolar parameters of the BiP–PF$_{10}^{\bullet^+}$ radical.$^{51-59}$ 2D HYSCORE spectroscopy is similar to the pulsed EPR spectroscopy methods, electron nuclear double resonance (ENDOR) and electron spin echo envelope modulation (ESEEM),$^{60-64}$ that are used for the study of electron-nuclear hyperfine interactions in paramagnetic systems. However, in comparison with the other methods the observation of HYSCORE signals in two-dimensional (2D) frequency space results in enhanced resolution, which alleviates the problem of spectral overcrowding from the simultaneous detection of multiple nuclear spins,$^{62}$ such as, the nitrogen atoms of the BiP–PF$_{10}^{\bullet^+}$ radical. Additionally, the nuclear transitions are correlated in the two dimensions which greatly facilitates the analysis and interpretation of the spectra.

The experimental 2D $^{14}$N HYSCORE spectrum of the BiP–PF$_{10}^{\bullet^+}$ displays cross-peaks that arise from the electron-nuclear hyperfine interactions of the unpaired electron spin with the nitrogen-14 atoms (nuclear spin, I = 1) of the BiP and PF$_{10}$ moieties (Figure 4A). The position of the cross-peaks in the spectrum is determined by the Zeeman frequency of the $^{14}$N atoms arising from the nuclear magnetic interaction ($\nu_i^N$ of 1.06 MHz) and the electron-nuclear hyperfine interaction, A. In the strong coupling limit (when A > 2$\nu_i^N$), the cross-peaks appear in the (−,+) quadrant, while in the weak coupling limit (when A < 2$\nu_i^N$) the cross-peaks are in the (+,+ ) quadrant of the spectrum.$^{52,65}$

Accordingly, the (−,+) quadrant of the 2D $^{14}$N spectrum of BiP–PF$_{10}^{\bullet^+}$ in Figure 4A displays three sets of cross-peaks that are symmetric about the anti-diagonal. The origin of these peaks are the nitrogen atoms, N$^I$–N$^{III}$, that are strongly hyperfine coupled to the unpaired electron spin. In each case, the shift along the diagonal from multiples of the $^{14}$N Zeeman frequency, $\nu_i^N$, (shown as dashed red lines in Figure 4A) is due to the quadrupolar interaction of the nitrogen atoms, while the separation of the cross peaks along the anti-diagonal is due to the hyperfine interaction with the unpaired electron spin.
In contrast, the (+,+) quadrant of the 2D $^{14}$N HYSCORE spectrum in Figure 4A displays cross-peaks that arise from the weakly hyperfine coupled nitrogen atoms, N$^{IV}$–N$^{VI}$. The two closely located cross-peaks in the (+,+) quadrant of the spectrum that are symmetric with respect to the diagonal are assigned to the double quantum transitions of the weakly-coupled nitrogen atoms, N$^{IV}$–N$^{V}$. In contrast, the absence of resolved structure of the cross-peak along the diagonal indicates that the nitrogen atom, N$^{VI}$, is very weakly hyperfine coupled to the unpaired electron spin. Additionally, while the cross-peaks from the N$^{I}$ nitrogen atom appear in only in the (−,+) quadrant the cross-peaks arising from N$^{II}$ are also present in the (+,+) quadrant (as labeled in Figure 4A–B).

The principal values of the spin Hamiltonian parameters of all of the contributing hyperfine coupled $^{14}$N atoms can be determined by numerical simulations to obtain a best fit of the cross-peaks in the experimental 2D $^{14}$N HYSCORE spectrum (Figure 4B). The simulations provide an accurate determination of isotropic hyperfine coupling, $A_{iso}$, as well as the value of $K^2(3 + \eta^2)$, where the quadrupolar coupling constant, $K = e^2qQ/4\hbar$, $Q$ is the quadrupole moment interacting with the electric field gradient $q$, and $\eta$ is the asymmetry parameter, for each nitrogen atom that is magnetically coupled to the unpaired electron spin (Table 1). In the (−,+) quadrant of the 2D HYSCORE spectrum in Figure 4A, the most intense cross-peaks from the nitrogen atoms, N$^{I}$ and N$^{II}$, are overlapping at ($\mp$ 2.6, ±1.3) MHz. This is because N$^{I}$ and N$^{II}$ possess similar magnetic parameters, namely, $K = -0.60$ MHz, $\eta = 0.50$, and $A_{iso} = 1.12$ MHz for N$^{I}$ and $K = -0.58$ MHz, $\eta = 0.52$, and $A_{iso} = 1.05$ MHz for N$^{II}$. Based on the values of K of -0.60 – -0.58 MHz and $\eta$ of 0.50 – 0.52, we assigned N$^{I}$ and N$^{II}$ to the nitrogen atoms of the benzimidazole moiety, BiP. The assignment is based on previous nuclear quadrupole resonance (NQR) studies that have demonstrated that the quadrupolar coupling constant ($e^2qQ/\hbar$) for the amino nitrogen atom of an imidazole side chain is ~1.4 – 2.4 MHz.66

The position of the cross-peaks due to N$^{I}$ and N$^{II}$ is determined by the value of $A_{iso}$ while the anisotropy manifests itself as second order effects. As mentioned earlier, the cross-peaks display observable shifts from the nuclear Zeeman frequency (shown as dashed red lines in Figure 4B) due to the influence of the K values. There is also a third set of cross-peaks located at ($\mp$ 3.9, ±1.3) MHz in the (−,+) quadrant that arise from a hyperfine interaction of the unpaired electron spin with the nitrogen atom, N$^{III}$, with a K of -0.66
MHz that is strongly coupled to the electron spin ($A > 2\nu_I$) (Table 1). Based on the value of $K$ of -0.66 MHz, we assigned $N^{III}$ to a pyrrole nitrogen in the PF$_{10}$ ring.$^{46,67}$

In the (+,+) quadrant of the 2D HYSCORE spectrum in Figure 4A–B, there are two pairs of resolved cross-peaks separated from the diagonal points centered at (3.3, 3.3) MHz, which arise from weak hyperfine interactions of the unpaired electron spin with the nitrogen atoms, $N^{IV}$ and $N^V$. The separation of the cross-peaks along the anti-diagonal corresponds to the weak coupling regime $A < 2\nu_I$ and hence the peaks are located in a frequency region closer to the diagonal. Finally, the cross-peak arising from the weakest hyperfine interaction with the nitrogen atom, $N^{VI}$, with the smallest $A_{iso}$ value is located along the diagonal. The $K$ values of all three nitrogen atoms, $N^{IV} - N^{VI}$, are in the range of -0.69 – -0.72 MHz which is comparable to the $K$ values that have previously been observed for pyrrole nitrogen atoms of a porphyrin ring.$^{46,67}$ Thus, we assign $N^{IV} - N^{VI}$ to the remaining pyrrole nitrogen atoms in the PF$_{10}$ moiety.

It is notable that the porphyrin nitrogen atom, $N^{III}$, displays a strong isotropic hyperfine coupling, $A_{iso}$, of 2.15 MHz which indicates the presence of significant electron spin density at this location. In comparison, the remaining nitrogen atoms of the porphyrin ring, $N^{IV} - N^{VI}$, display relatively small values of isotropic hyperfine coupling constant of -0.2 – 0.5 MHz. The observation of finite isotropic hyperfine coupling constants, $A_{iso}$, for all of the nitrogen atoms, $N^{I} - N^{VI}$, clearly indicates that the electron spin density is delocalized on both the benzimidazole phenol, BiP, and high-potential porphyrin, PF$_{10}$, in BiP–PF$_{10}^+$.

The $A_{iso}$ values of 1.05 MHz and 1.12 MHz for the nitrogen atoms, $N^{I}$ and $N^{II}$, of BiP indicates that the magnitude of electron spin density that is distributed on the two nitrogen atoms is comparable. Typically, the $A_{iso}$ values are determined by the unpaired spin density in the 2s orbital that is proportional to atomic value of 1811 MHz for $^{14}$N, computed for unit spin density in this orbital.$^{68}$ Therefore, the $A_{iso}$ value of 1.05 – 1.12 MHz that is determined for the $N^{I}$ and $N^{II}$ nitrogen atoms of BiP corresponds to a fractional spin density distribution, $\rho_s$, of $\approx 6.0 \times 10^{-4}$ in the 2s orbital of the each nitrogen atom. This suggests that the strength of the H-bonds between each nitrogen atom of BiP and its proximal water molecule are nearly equivalent. Moreover, the values of the isotropic hyperfine coupling constant, $A_{iso}$, indicate the presence of significant spin
density on the strongly coupled nitrogen atom, N^{III}, of the porphyrin ring, PF_{10}, along with a smaller fraction of spin density on the three weakly coupled nitrogen atoms, N^{IV} – N^{VI}. The PF_{10} molecule is an aromatic ring system, therefore, the \( \pi \)-electron density is delocalized over the nitrogen atoms of the PF_{10} ring. The relative differences in A_{iso} values reflect differences in the distribution of spin density on the nitrogen atoms, N^{IV} – N^{VI}, of the porphyrin ring (Table 1).

We performed DFT calculations on three computational models (A, B and C) shown in Figure 5A–C, respectively, to elucidate the high-resolution electronic structure of BiP–PF_{10}°° and understand the role of water molecules play in the PCET process. All three structures correspond to the chemical species that would result from the transfer of the proton from the phenolic oxygen atom to the proximal imidazole nitrogen atom of BiP during PCET, thus resulting in a BiP–PF_{10}°°. While model A does not include any water molecules (Figure 5A), the models B and C include explicit water molecules that are H-bonded to the BiP moiety of the BiP–PF_{10}°° radical (Figure 5B-C). The structure of B (Figure 5B) includes two explicit water molecules; one water molecule that forms a H-bond with the distal N–H of BiP and another that forms a H-bond to the phenoxyl oxygen atom. The alternate structure, C, also contains two water molecules, where one water acts as a H-bond acceptor to the distal amino N–H of BiP and a second water molecule that serves as both a H-bond acceptor and donor to the newly formed N–H and phenoxyl oxygen atom, respectively (Figure 5C).

The DFT calculations allow for a comparison of the electron spin density distribution in each of the computational models, A, B, and C (Figure 6A–C, respectively). While the electron spin density is distributed on the imidazole and phenol of the BiP moiety in all three models, we also observe delocalization of the unpaired electron spin onto the conjugated porphyrin, PF_{10}. The delocalization of the electron spin on both BiP and PF_{10} in these structures is in agreement with the experimental 2D \(^{14}\)N HYSCORE measurements where we observe hyperfine interactions of the unpaired electron with the nitrogen atoms of both BiP (N\(^{I}\) and N\(^{II}\)) and PF_{10} (N\(^{III}\) – N\(^{VI}\)) (Figure 4A–B and Table 1). The delocalization of the electron spin density is also consistent with previous theoretical analysis on the methyl ester derivative of BiP–PF_{10}.\(^{33,45}\)
In addition to obtaining the electron spin density distribution of the structures, we calculated the quadrupolar and hyperfine parameters ($K$ and $A_{\text{iso}}$, respectively) for comparison with the experimental values that were obtained from the 2D $^{14}$N HYSCORE measurements. The calculated $K$ values that are obtained for all of the nitrogen atoms, N$^I$ – N$^{VI}$, for the A, B and C structures are comparable to the $K$ values that are determined from the 2D HYSCORE measurements. In particular, the value of $K$ for the benzimidazole (N$^I$ – N$^{II}$) and porphyrin (N$^{III}$ – N$^{VI}$) nitrogen atoms of the C structure was $0.62 \pm 0.04$ MHz and $0.77 \pm 0.03$ MHz, respectively (Table 1). This is in agreement with the experimental values of $K$ for the BiP and PF$_{10}$ nitrogen atoms, within an average error of 0.07 MHz.

The calculated value of the isotropic hyperfine coupling constant, $A_{\text{iso}}$, that is obtained for the benzimidazole nitrogens, N$^I$ – N$^{II}$, in the C structure was 1.17 MHz and 0.88 MHz, respectively, while the corresponding value for the porphyrin nitrogens, N$^{III}$ – N$^{VI}$, was 2.2 MHz, 0.41 MHz, 0.77 MHz and -0.17 MHz, respectively. The calculated values for $A_{\text{iso}}$ are in agreement with the experimental couplings of N$^I$ – N$^{VI}$; the average error was 0.15 MHz. In contrast, the average error for the calculated values $A_{\text{iso}}$ for B and A structures was 0.52 MHz and 0.33 MHz, respectively (as shown in Table 1S and 2S).

The corresponding $g$-tensors that are obtained from the DFT calculations of the A, B and C structures are presented in Table 3S. Previous high-frequency EPR and ENDOR measurements on the redox-active tyrosine-D radical of photosystem II$^{18,51,69,70}$ and TiO$_2$-PF$_{10}$BiP triad$^{33}$ have demonstrated that the $g_x$ component of the $g$-tensor for a tyrosyl radical that is oriented along the C-O molecular axis is sensitive to the local environment of the phenolic oxygen atom. In this case, the spin–orbit coupling interaction between the unpaired electron in the singly occupied molecular orbital (SOMO) and the lone pairs of electrons on the phenolic oxygen atom induce a magnetic moment that results in a rather large deviation of the $g_x$ component of the $g$-tensor (e.g. $g_x$ of ~2.008) from the free electron $g$-value ($g_e = 2.0023$).$^{33,71}$ However, the presence of H-bonding interaction(s) with the lone pair on the phenolic oxygen atom and/or delocalization of the unpaired spin density tend to decrease this deviation, yielding radicals with lower $g_x$ values.$^{18,51,69,70}$ This is in agreement with the $g_x$ component of the $g$-tensor of 2.0056, 2.0052 and 2.0061 that is calculated for the A, B and C structures
(Table 3S). The $g_x$ component that was observed for the A, B and C structures is lower than the calculated $g_x$ value of $\sim 2.0065$ that was obtained for a series of phenoxy radicals that were H-bonded to a imidazolium moiety in the absence of a covalently linked conjugated system.\cite{72} Similarly, the $g_x$ component of the A, B and C structures is also less than the $g_x$ value of 2.0065 that was previously reported for a BiP–PF$_{10}$ derivative in which the porphyrin molecule was covalently bonded to BiP through the imidazole moiety instead of the phenol which precluded delocalization of the electron spin density between the BiP and PF$_{10}$ moieties.\cite{73} Hence, in the present study the smaller value of 2.0056, 2.0052 and 2.0061 that is obtained for the $g_x$ component of the A, B and C structure is attributed to the effects from the presence of strong H-bonding interactions at the phenoxy oxygen atom and to a lesser degree the delocalization of the electron spin density on both BiP and PF$_{10}$.

In our previous work, we had demonstrated that calculated g-values for structure B were in better agreement with the corresponding experimental values of the radical species obtained by illumination at 13 K (prior to annealing the sample at 77 K). In contrast, the calculated g-values for structure C were in better agreement with those of the radical after annealing the sample at 77 K.\cite{33} We rationalized this with the fact that before PCET the nitrogen of the imidazole that is H-bonded to the phenol is a hydrogen bond acceptor, but after PCET the nitrogen accepts the phenolic proton forming an N–H group and is therefore a hydrogen bond donor. The PCET reactions occur at 13 K but lack the thermal energy for solvent reorganization and form a H-bond with the newly formed N–H group (structure B). Once the sample is annealed, the solvent is able to reorganize and form a bifurcated H-bond between the newly formed N–H and the phenolic oxygen atom (structure C). This indicates that in the present study the illumination of BiP–PF$_{10}$ with white light at 77 K should lead to the formation of a cation radical whereby upon PCET, the nitrogen atom of BiP accepts the phenolic proton resulting in an N–H that is in a bifurcated H-bond.

Based on the magnetic parameters of the computational models of BiP–PF$_{10}$, A, B and C, we examine the geometric parameters and relative energies of the DFT structures. We find that the extent of electron spin delocalization between BiP and PF$_{10}$ in the three DFT structures (Figure 6A–C) can be explained in terms of the dihedral angle between
the conjugated rings of BiP and PF$_{10}$. The delocalization of the electron on BiP and PF$_{10}$ is greater when the dihedral angle between the planes of the BiP and PF$_{10}$ ring tends to 0° and is diminished when the dihedral angle tends to 90° (i.e. when the rings are orthogonal). As observed in Table 2, the dihedral angle between the BiP and PF$_{10}$ is ~47° for all of the structures.

The main structural difference between the computational models is the dihedral angle between the benzimidazole and phenolic group of BiP. As shown in Table 2, the BiP system is almost co-planar for the A and B structures, but it is twisted slightly out of plane for C structure (with a dihedral angle of ~7°). We hypothesize that this difference is caused by the relaxation of the water molecules around the newly formed N–H bond which forms a bifurcated H-bond with both the phenolic and water oxygen atoms thus lowering the strength of the intra-molecular hydrogen bond. The effects of the spin density delocalization can be also observed in the $A_{\text{iso}}$ values for specific nitrogen within the different structures. The deviation of the experimental and calculated $A_{\text{iso}}$ value for N$^{\text{II}}$ is 0.24, 0.67 and 0.55 MHz for models C, B and A, respectively. The deviation of the experimental and calculated $A_{\text{iso}}$ value for N$^{\text{III}}$ is 0.06, 0.85 and 0.30 MHz for models C, B and A, respectively. These results highlight the importance of incorporating explicit water molecules in the models in the right conformation. Finally, as shown in Table 2, the energy of structure C is ~3 kcal/mol lower than the energy of structure B. Therefore, the relaxation from structure B to structure C is thermodynamically favorable, which is consistent with the discussion above.

Conclusions:

In the present study, we determine the experimental and calculated electron spin density distribution on the radical species that is formed upon light-induced PCET in the artificial reaction center, BiP–PF$_{10}$. We demonstrate excellent agreement between the hyperfine coupling constants of the nitrogen atoms that are determined by experimental 2D $^{14}$N HYSCORE spectroscopy and those calculated by DFT methods. Moreover, we observe that there is a direct correlation between the hyperfine coupling constants of the nitrogen atoms and delocalization of electron spin density in the presence and absence of structures water molecules in the vicinity of the BiP and porphyrin moieties of BiP–PF$_{10}$. 

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The presence of two explicit water molecules in the C structure that are hydrogen bonded to the N–H sites of the BiP moiety leads to a near perfect match of the experimental and calculated hyperfine couplings. This highlights the importance of hydrogen bonding interactions with water molecules in the favorable tuning of the electronic structure and respective energy levels that facilitates PCET reactions in the BiP–PF$_{10}$ reaction center. In contrast the experimental hyperfine coupling constants are not as well reproduced in the related structures, A and B, that exhibit differences in the hydrogen bonding pattern or a complete absence of such interactions with structured water molecules. These results present direct experimental and theoretical evidence of spin delocalization and highlights the effects of structured water molecules on the tuning of the electronic structure of artificial reaction centers. The results of this study emphasize the importance of considering these factors in the experimental and computational design of novel artificial photosynthetic systems that incorporate multi-electron and PCET processes.

**Methodology:**

**Synthesis and Characterization:** BiP–PF$_{10}$ was synthesized and characterized following the procedures reported by Megiatto et al.$^{33,74}$ The procedure consists of an acid-catalyzed porphyrin condensation of 5-formyl-3-tert-butyl-2-hydroxybenzaldehyde, 5-(pentafluorophenyl)dipyrrromethane, and methyl-4-formylbenzoate at room temperature to form 5, 15-bis(pentafluorophenyl)-10-(4-methoxycarbonylphenyl)-20-(3-formyl-4-hydroxy-5-tert-butylpheylnyl)porphyrin. After purification this porphyrin precursor was allowed to react with 1,2-phenylenediamine to form the ester form of the BiP–PF$_{10}$, 5, 15-bis(pentafluorophenyl)-10-(4-methoxycarbonylphenyl)-20-[2’-(3”-tert-butyln-2”-hydroxyphenyl)benzimidazole]porphyrin. Finally, the ester was hydrolyzed with trifluoroacetic acid and HCl to form the desired compound 5, 15-bis(pentafluorophenyl)-10-(4-carboxyphenyl)-20-[2’-(3”-tert-butyln-2”- hydroxyphenyl)benzimidazole]porphyrin.

**Continuous-wave and Pulsed EPR Spectroscopy:** The EPR spectra were obtained on a custom-built continuous-wave (cw)/pulsed X-band Bruker Elexsys 580 EPR spectrometer using a dielectric flex-line ER 4118-MD5 probe (Bruker BioSpin, Billerica, MA) and a dynamic continuous-flow cryostat CF935 (Oxford Instruments, Oxfordshire, U.K.) at 30
K temperature. The *cw* EPR spectrum of the BiP–PF$_{10}^{+}$ was acquired at the operating microwave frequency of 9.71 GHz with modulation frequency of 100 kHz, modulation amplitude of 1 G and microwave power of 0.7 μW. For the magnetic-field-sweep electron-spin-echo EPR spectrum, the primary electron spin echo was generated using the pulse sequence (π/2–τ–π–τ–echo). The echo was integrated over a 32 ns time window that was centered at the maximum of the echo signal. The length of the π/2- and π-pulse was 8 ns and 16 ns, respectively. The inter-pulse separation, τ, was 120 ns and the delay in the pulse sequence is defined as the difference in the starting point of the pulses.

The 2D $^{14}$N HYSCORE spectra were recorded at magnetic field position of 346.1 mT. For the 2D $^{14}$N HYSCORE spectra of BiP, the echo amplitude was measured using the pulse sequence (π/2–τ–π/2–t1–π–t2–π/2–τ–echo) with a τ value of 136 ns, an 8 ns and 16 ns length for the π/2- and π-pulse, respectively. The delays in the pulse sequence are defined as the difference in the starting point of the pulses. The echo intensity was measured as a function of $t_1$ and $t_2$, where $t_1$ and $t_2$ were incremented in steps of 16 ns from an initial value of 40 ns and 32 ns, respectively. 256 steps were used for each dimension. The 8 ns time difference between the initial value of $t_1$ and $t_2$ was set to account for the difference in length between the π/2- and π-pulse. The unwanted echoes were eliminated by applying a 16-step phase cycling procedure. The hyperfine parameters of the $^{14}$N nuclei were obtained by performing numerical simulations using the *saffron* function of the EasySpin software package.$^{75}$ The simulated spectra were carefully compared with the experimental ones to match the position of the cross-peaks and signal intensity distribution over the different points of the ridges.

The frequency of the double-quantum transition is described by the following equation:

$$
u_{dqk} = 2(\nu_{eff}^2 + K^2(3 + \eta^2))^{1/2}$$

The effective frequency is $\nu_{eff} = |\nu_1 \pm a/2|$ where $\nu_1$ is the nuclear Zeeman frequency for the given magnetic field and $a$ is the hyperfine (isotropic) coupling value. The value of nuclear quadrupole coupling is described by $K = e^2qQ/4\hbar$ where $Q$ is the quadrupole moment interacting with the electric field gradient $q$ and $\eta$ is the asymmetry parameter. In general, the isotropic hyperfine coupling is calculated from the cross-peak positions of the double-quantum transitions. The detection of cross-peaks in the (-,+)$^+$ and (+,+)
quadrant depends mainly on the strength of the electron-nuclear hyperfine coupling and orientation dependence of the nuclear transition of inequivalent $^{14}$N nuclei.

**Density Functional Theory Calculations:** The theoretical structure of the A, B and C models of the PF$_{10}$–BiP$^{\ast\ast}$ radical (Figure 5A-C) was optimized with Gaussian 09$^{76,77}$ at the B3LYP/6-31G(d,p) level of theory. The electron spin density distributions, quadrupolar and hyperfine coupling parameters and g-tensors for all of three structures were obtained with ORCA$^{78,79}$ at the B3LYP/EPR-II level of theory using the Conductor-like Polarizable Continuum Model (CPCM), with the value of epsilon set to 47.2 and refrac at 1.479 to simulate a dimethyl sulfoxide (DMSO) environment.

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Author Contributions
D.D.M.H., A.B., V.K., P.C., B. Mark, W.M., and B. Molnar conducted experiments, analyzed the data and performed calculations; D.D.M.H., K.V.L., A.L.M and T.A.M. designed the experiments and wrote the paper.

Declaration of Interest
The authors declare no competing interests.

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**Figure 1.** X-ray crystal structure of PSII. (A) The location of the charge-transfer cofactors that participate in the water-oxidation reaction of PSII. In the primary electron transfer (ET) pathway of PSII, the photooxidation of the primary donor, P$_{680}$ (green), leads to the formation of the initial charge-separated state, P$_{680}^+$-PheoA$^-$ (PheoA is shown in blue), that is rapidly stabilized by forward ET to the primary and secondary quinone acceptors, QA and QB (red), respectively. P$_{680}^+$ is a strong oxidant that leads to rapid PCET at the redox-active tyrosine residue, tyrosine-Z (YZ) (black) which results in the oxidation of water at the Mn$_4$Ca-oxo cluster in the oxygen-evolving complex (OEC). The smart protein matrix in the vicinity of the redox-active (B) YZ and (C) tyrosine-D (YD) residue (black) as observed in the 1.9 Å resolution X-ray crystal structure of PSII.\textsuperscript{5}

**Figure 2.** Schematic of the artificial photosynthetic reaction center, benzoimidazole phenol-porphyrin, BiP–PF$_{10}$. The electron withdrawing ability of the two pentafluorophenyl substituents (purple) make the porphyrin ring (green) a high oxidation potential molecule. Photooxidation of PF$_{10}$ leads to PCET at the BiP moiety, where the electron is transferred to PF$_{10}^+$ and the phenolic (red) proton is transferred to the benzoimidazole (blue) imino nitrogen atom. Also shown is the labeling scheme for the respective nitrogen atoms of BiP–PF$_{10}$.

**Figure 3.** The (A) continuous-wave (cw) and (B) electron-spin-echo magnetic-field-sweep EPR spectrum of BiP–PF$_{10}$. The arrow in part B indicates the magnetic field position of 346.1 mT that was used for the acquisition of the 2D $^{14}$N HYSCORE spectrum that is shown in Figure 4A.

**Figure 4.** The (A) experimental and (B) simulated 2D $^{14}$N HYSCORE spectrum of BiP–PF$_{10}^+$ radical. The (-,+), (+,-) quadrant displays cross-peaks from the strong hyperfine couplings of the electron spin with three nitrogen atoms, N$^I$–N$^III$, that are symmetric about the anti-diagonal. In contrast, the (+,+), (-,-) quadrant contains cross-peaks from weaker hyperfine couplings with the N$^{IV}$–N$^{VI}$ nitrogen atoms that are symmetric with respect to
the diagonal. The dashed red lines in parts A and B indicate multiples of the $^{14}$N Zeeman frequency, $\nu_N$, of 1.06 MHz.

**Figure 5.** The DFT optimized structure of the BiP moiety in the A, B, and C structure of the BiP–PF$_{10}^{\bullet^+}$, where PF$_{10}$ is omitted for clarity. Both structures B and C contain two water molecules, but differ in the H-bonding pattern between the phenolic O atom, imidazole nitrogen atom and proximal water molecule. In contrast, structure A does not contain any water molecules.

**Figure 6.** The electron spin density (purple and gold) distribution of the A, B, and C structures of BiP–PF$_{10}^{\bullet^+}$ as determined by DFT. The atoms are color coded as carbon (grey), hydrogen (white), nitrogen (blue), fluorine (cyan) and oxygen (red).
Figure 1
Figure 2
Figure 3
Figure 4

Quadrant

\( (-+) \) Quadrant
Strong hyperfine couplings

\( (++) \) Quadrant
Weak hyperfine couplings

\( \nu_1 [\text{MHz}] \)

\( \nu_2 [\text{MHz}] \)

\( \nu_1 [\text{MHz}] \)

\( \nu_2 [\text{MHz}] \)
Figure 5
Figure 6
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**Table 1.** The $^{14}$N hyperfine and quadrupolar parameters that are obtained from spectral simulations of the experimental 2D $^{14}$N HYSCORE spectrum of the BiP–PF$_{10}^{•+}$ and DFT calculations of the C structure of the BiP–PF$_{10}^{•+}$.

**Table 2.** The relative energies ($\Delta E$) and relevant dihedral angles of the A, B and C structure of BiP–PF$_{10}^{•+}$ that were optimized by DFT calculations.
Table 1.

| Nitrogen | K (MHz) (experimental) | $A_{iso}$ (MHz) (experimental) | K (MHz) (calculated C structure) | $A_{iso}$ (MHz) (calculated C structure) |
|----------|------------------------|--------------------------------|---------------------------------|----------------------------------------|
| N$^I$    | -0.59 ± 0.01           | 1.05 ± 0.1                     | -0.66                           | 1.17                                   |
| N$^II$   | -0.60 ± 0.02           | 1.12 ± 0.1                     | -0.58                           | 0.88                                   |
| N$^{III}$| -0.66 ± 0.01           | 2.15 ± 0.1                     | -0.80                           | 2.21                                   |
| N$^{IV}$ | -0.72 ± 0.01           | 0.50 ± 0.1                     | -0.75                           | 0.41                                   |
| N$^V$    | -0.70 ± 0.01           | 0.40 ± 0.2                     | -0.75                           | 0.77                                   |
| N$^{VI}$ | -0.69 ± 0.01           | -0.20 ± 0.1                    | -0.80                           | -0.18                                  |
Table 2.

| Structure | Benzimidazole-Phenol dihedral angle | BiP–Porphyrin dihedral angle | ΔE relative to structure A (kcal/mol) |
|-----------|------------------------------------|-----------------------------|-------------------------------------|
| A         | 1.7                                | 48.5                        | Reference                           |
| B         | 1.8                                | 44.5                        | −16.2                               |
| C         | 7.1                                | 47.6                        | −19.1                               |
HYSCORE and DFT Studies of Proton-Coupled Electron Transfer in a Bioinspired Artificial Photosynthetic Reaction Center

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Supporting Information
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Figure 1S: Schematic representing the numbering scheme of atoms in the A structure of the BiP–PF$_{10}^{**}$ PCET intermediate that was used in tabulating the results of the HYSCORE experiments and DFT calculations.

Figure 2S: Schematic representing the numbering scheme of atoms in the B structure of the BiP–PF$_{10}^{**}$ PCET intermediate that was used in tabulating the results of the HYSCORE experiments and DFT calculations.

Figure 3S: Schematic representing the numbering scheme of atoms in the C structure of the BiP–PF$_{10}^{**}$ PCET intermediate that was used in tabulating the results of the HYSCORE experiments and DFT calculations.

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Table 1S: The $^{14}$N hyperfine and quadrupolar parameters that are obtained from DFT calculations of the B structure of the BiP–PF$_{10}^{**}$.

Table 2S: The $^{14}$N hyperfine and quadrupolar parameters that are obtained from DFT calculations of the A structure of the BiP–PF$_{10}^{**}$.

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Figure 1S
Figure 2S
Figure 3S
Table 1S. The $^{14}$N hyperfine and quadrupolar parameters that are obtained from DFT calculations of the B structure of the BiP–PF$_{10}^{	ext{●}}$.

| Nitrogen | K (MHz) (calculated B structure) | A$_{iso}$ (MHz) (calculated B structure) |
|----------|---------------------------------|----------------------------------------|
| N$^I$    | -0.67                           | 0.86                                   |
| N$^{II}$ | -0.64                           | 0.45                                   |
| N$^{III}$| -0.81                           | 3.00                                   |
| N$^{IV}$ | -0.73                           | 0.65                                   |
| N$^{V}$  | -0.74                           | 1.11                                   |
| N$^{VI}$ | -0.82                           | 0.35                                   |
Table 2S. The $^{14}$N hyperfine and quadrupolar parameters that are obtained from DFT calculations of the A structure of the BiP–PF$_{10}$$^{2-}$.

| Nitrogen | $K$ (MHz) (calculated A structure) | $A_{iso}$ (MHz) (calculated A structure) |
|----------|-----------------------------------|------------------------------------------|
| $N^I$    | -0.61                             | 1.01                                     |
| $N^II$   | -0.61                             | 0.57                                     |
| $N^III$  | -0.80                             | 2.45                                     |
| $N^IV$   | -0.75                             | 0.59                                     |
| $N^V$    | -0.74                             | 0.94                                     |
| $N^VI$   | -0.80                             | 0.24                                     |
Table 3S. The g-tensors that are obtained from the DFT calculations of the A, B and C structure of the BiP–PF$_{10}^\ast$ PCET intermediate.

| Structure | $g_x$  | $g_y$  | $g_z$  |
|-----------|--------|--------|--------|
| A         | 2.0056 | 2.0042 | 2.0023 |
| B         | 2.0052 | 2.0041 | 2.0023 |
| C         | 2.0060 | 2.0044 | 2.0023 |
