**ABSTRACT:** Photosystem II (PSII) of oxygenic photosynthesis captures sunlight to drive the catalytic oxidation of water and the reduction of plastoquinone. Among the several redox-active cofactors that participate in intricate electron transfer pathways there are two tyrosine residues, YZ and YD. They are situated in symmetry-related electron transfer branches but have different environments and play distinct roles. YZ is the immediate oxidant of the oxygen-evolving Mn4CaO5 cluster, whereas YD serves regulatory and protective functions. The protonation states and hydrogen-bond network in the environment of YZ remain debated, while the role of microsolvation in stabilizing different redox states of YD and facilitating oxidation or mediating deprotonation, as well the fate of the phenolic proton, is unclear. Here we present detailed structural models of YD and its environment using large-scale quantum mechanical models and all-atom molecular dynamics of a complete PSII monomer. The energetics of water distribution within a hydrophobic cavity adjacent to YD are shown to correlate directly with electron paramagnetic resonance (EPR) parameters such as the tyrosyl g-tensor, allowing us to map the correspondence between specific structural models and available experimental observations. EPR spectra obtained under different conditions are explained with respect to the mode of interaction of the proximal water with the tyrosyl radical and the position of the phenolic proton within the cavity. Our results revise previous models of the energetics and build a detailed view of the role of confined water in the oxidation and deprotonation of YD. Finally, the model of microsolvation developed in the present work rationalizes in a straightforward way the biphasic oxidation kinetics of YD, offering new structural insights regarding the function of the radical in biological photosynthesis.

**INTRODUCTION**

Photosystem II (PSII) is the primary enzymatic complex in oxygenic photosynthesis. It uses the energy of sunlight to drive the oxidation of water to dioxygen and the reduction of a mobile plastoquinone, which carries reducing equivalents further along the photosynthetic chain to be eventually used for modulating the various S oxidation states (i = 0–4) of the Mn4CaO5 cluster of the OEC. Despite the large distance between YD and the OEC (ca. 30 Å), it is crucial for modulating the various S oxidation states (i = 0–4) of the Mn4CaO5 cluster of the OEC. YD is oxidized on a time scale of seconds by the OEC in its S2 or S3 state, while YD-O* can be reduced by the S0 state during dark adaptation, both processes aiding the OEC to reach the dark-stable S1 state. In addition, YD is proposed to enhance the rate of electron transfer at the YZ site by specific electrostatic interaction with P680+.

Although YD does not participate in the mainstream electron transfer processes, it plays important regulatory roles for the smooth and efficient functioning of PSII. Despite the large distance between YD and the OEC (ca. 30 Å), it is crucial for modulating the various S oxidation states (i = 0–4) of the Mn4CaO5 cluster of the OEC. YD is oxidized on a time scale of seconds by the OEC in its S2 or S3 state, while YD-O* can be reduced by the S0 state during dark adaptation, both processes aiding the OEC to reach the dark-stable S1 state. In addition, YD is proposed to enhance the rate of electron transfer at the YZ site by specific electrostatic interaction with P680+.

The two tyrosine residues have different properties, as YZ exhibits fast oxidation and reduction kinetics, whereas YD displays slower oxidation and reduction rates at physiological pH. The YD radical is short-lived and decays with a t1/2 of 0.03–1 ms whereas YD* decays with a t1/2 in the range of 0.03–1 ms.
minutes to hours. Additionally, they have different estimated redox potentials, i.e., $+900−1000$ mV for Y$_Z$ 19 and $+700−800$ mV for Y$_D$. 33 Looking at the immediate environment for potential structural explanations of these divergent properties, it becomes obvious that the two residues have clear similarities but also important differences. Both tyrosines have a hydrogen-bonded histidine partner, D1-His190 for Y$_Z$ and D2-His189 for Y$_D$. D1-His190 is further hydrogen-bonded to D1-Asn298, 38 while D2-His189 to D2-Arg294. A fundamental difference is that whereas Y$_Z$ is embedded in a water-rich hydrogen-bonding network, which includes waters directly coordinated to the calcium ion of the OEC cluster, Y$_D$ is situated at a hydrophobic phenylalanine-rich cavity that in recent crystallographic models appears to accommodate a single water molecule confined between Y$_D$ and an arginine–aspartate pair, D2-Arg180–Asp333. Two water positions have been identified in some, but not all, crystallographic models. 6,9,39–43 These are termed proximal (at a hydrogen-bonding distance of ca. 2.7 Å from the phenolic oxygen of Y$_D$) and distal (at a distance of $>4.0$ Å from the phenolic oxygen). Understanding the effect of microsolvation and the role of the histidine partner and the confined water is fundamental for understanding the mechanism of formation of the Y$_D$ radical, its spectroscopic properties and differences from Y$_Z$, the possible proton translocation pathways, and ultimately the functional role of Y$_D$ in the context of biological photosynthesis.

A fundamental question is whether deprotonation proceeds with the same mechanism in both tyrosine sites upon oxidation or not. Two contrasting ideas exist in the literature. The first is that Y$_D$ follows the same mechanism as Y$_Z$, 44–49 for which it is rather well established that the phenolic proton simply shifts toward the His partner upon oxidation and is available to return for fast reduction of the Y$_Z$ radical. This has been traditionally assumed to be the case with the Y$_D$–His189 pair and guided the interpretation of experimental data and past modeling of the system. The second idea is that proton transfer in Y$_D$ follows an entirely different mechanism; however on this point diverging views exist 45,57 and the available data are structurally ambiguous. Even before the crystallographic confirmation of the presence of a water molecule near Y$_D$, there were reports supporting the presence of one or more coupled water molecules or exchangeable protons near Y$_D$. 48,58–63 For example, the electron paramagnetic resonance (EPR) spectrum 64 of oxidized Y$_D$ in His189Gln mutants is characteristic of a neutral tyrosyl radical with no hydrogen bond, suggesting the existence of a proton acceptor other than His189. Similar conclusions were reached from FTIR studies. 55

Even more intriguingly, based on a proton inventory study Barry and co-workers 66,67 reported on a multiproton donation pathway to the Y$_D$ radical, suggesting that water plays a crucial role in proton transfer to and from the Y$_D$ site. Additionally, FTIR studies by Hienerwadel et al. 65 suggested the presence of two proton exit channels 68 and claimed the observation of proton release to the membrane surface upon Y$_D$ radical formation. 69,70 Currently it is not obvious how to cast all of these observations and interpretations into a unique and precise structural model.

The identification of the cavity water in recent crystallographic models of PSII led to renewed discussions and speculations on its role and importance. This has been recently highlighted experimentally by Sjöholm et al. 55 who employed continuous-wave EPR and two-pulse electron spin echo envelope modulation (ESEEM) spectroscopy to correlate the time of H/D exchange with the redox state of Y$_D$ and suggested that the position of the water molecule might be relevant for interpreting their observations. Further studies conjectured that the biphasic kinetics of Y$_D$ oxidation may be related to the position of the water molecule in the hydrophobic cavity. Using computational simulations Saito et al. 55 suggested that the two water positions reflect a mechanism of water-mediated proton removal upon oxidation of the Y$_D$ residue: it was suggested that the water molecule preferably occupies the proximal position, hydrogen bonding to the reduced Y$_D$ residue, but abstracts the phenolic proton upon formation of Y$_D^*$ and shifts to the distal position, releasing the proton outside the cavity with the involvement of Arg180. The above studies attribute functional significance to the presence of two crystallographic water positions, but the structural interpretations they propose are neither clear nor consistent with each other, while their agreement with all available data from crystallography and spectroscopy has not been explicitly examined.

A powerful way to clarify these open questions is to use quantum chemical methods in order to couple the detailed modeling of the Y$_D$ environment with calculation of spectroscopic parameters for possible oxidized forms of Y$_D$ in relation to protonation states and the position of the cavity water. EPR spectroscopy offers an invaluable source of electronic structure information and has been used extensively in the study of Y$_D$ oxidation. 46,47,64,71–79 but the various observations have not received atomistic explanations in the context of current structural information. In the present work...
we offer new insights into the mechanism of Y\textsubscript{D} oxidation using extended quantum chemical models as well as all-atom force-field molecular dynamics modeling of PSII to understand the energetics of water distribution and to relate available EPR observations with specific structural models. Our results lead to a revised model regarding the role of microsolvation for the oxidation of the Y\textsubscript{D} residue and suggest a coherent structure-based explanation of both the spectroscopic and the kinetic data reported for the Y\textsubscript{D} radical of PSII.

**METHODOLOGY**

**Molecular Dynamics Simulations.** Classical molecular dynamics simulations were performed on the PSII monomer obtained from the 1.9 Å crystal structure (PDB ID: 3WU2)\textsuperscript{32} to understand the stability and dynamics of the water molecule in the Y\textsubscript{D} cavity under the influence of the protein environment. The protonation states of Y\textsubscript{D} and the nearby residues were assigned manually. The AMBER03 force field\textsuperscript{80} parameters were used for standard protein residues and ions, and the TIP3P water model\textsuperscript{81} was employed for water molecules. Force-field parameters for the various cofactors were taken from the literature.\textsuperscript{82}–\textsuperscript{83} The PSII monomer was solvated with TIP3P water in a simulation box of dimensions 115.1 × 130.98 × 128.76 Å. Crystallographic waters were retained during system preparation, and counterions were added to maintain charge neutrality. The final system consists of 428 890 atoms. Energy minimization to remove the structural bad contacts included 2000 steps of steepest descent followed by conjugate-gradient minimization. The solvent around the protein is well equilibrated using a force constant of 10 kJ mol\textsuperscript{−1} Å\textsuperscript{−2} to avoid unnatural large-scale backbone movements due to absence of the membrane. The temperature and pressure were maintained using the Berendsen thermostat\textsuperscript{84} and Parinello-Rahman barostat\textsuperscript{85} with a coupling constant of 0.1 and 2.0 ps, respectively. The nonbonded interactions were treated explicitly up to 12 Å in the production run, and interactions above this cutoff were treated using the particle-mesh-Ewald (PME) summation algorithm.\textsuperscript{86,87} The LINCS constraint algorithm\textsuperscript{88} was employed for all bonds. The simulations were performed using the GROMACS software package (version 4.6.7).\textsuperscript{89}

**Quantum Cluster Models.** The starting point for the quantum chemical simulations was the same crystal structure of PSII (PDB ID: 3WU2) from which we built quantum cluster models that encompass a large region of the protein around the Y\textsubscript{D} residue (Figure 2). The model includes the D2 residues Ile159, Tyr160 (Y\textsubscript{D}), Pro161, Leu162, Glu163, Gln164, Phe169, Ala170, Arg180, Phe181, Leu182, Leu183, Phe184, Phe185, Glu186, Gly187, Phe188, His189 (the H-bonding partner of Y\textsubscript{D}), Asn292, Arg294, Asp333, and the backbone peptide bonds (CO-NH) of Phe169–Ala170 and Phe362–Phe363, the manually added hydrogens on C\textsubscript{α} atoms of these residues, and the cavity water.

**Computational Details.** Geometry optimizations were performed with the PBE functional\textsuperscript{90} using D3(RJ) dispersion corrections.\textsuperscript{91,92} The Def2-TZVP basis sets\textsuperscript{93} were used for all atoms in the fully optimized part, and the Def2-SV\textsubscript{D} basis sets\textsuperscript{95} were used for atoms in the constrained part of the model. This combination led to a total number of 3477 basis functions. The resolution of identity (RI) approximation\textsuperscript{94,95} was employed to speed up the calculations of Coulomb integrals, in combination with Weigend’s universal Def2/S auxiliary basis sets.\textsuperscript{96,97} The conductor-like polarizable continuum model (CPCM)\textsuperscript{98} was employed with a dielectric constant of ε = 6 throughout the investigation. Single-point energy calculations on selected models were performed using the hybrid B3LYP\textsuperscript{99} and the meta-hybrid TPSSh\textsuperscript{100} functionals in conjunction with the larger Def2-TZVPP basis set (total of 6795 basis functions). EPR parameters of the tyrosyl radical in our models were calculated using the TPSSh functional with Barone’s EPR-II basis set.\textsuperscript{101} This approach has been shown to be reliable for the calculation of EPR parameters in various related systems (see also the Supporting Information for a brief comparison of functionals).\textsuperscript{102}–\textsuperscript{108} The chain-of-spheres approximation\textsuperscript{109} was used in the evaluation of exchange integrals for the calculations employing hybrid functionals. Tight convergence settings were used throughout, along with higher than default integration grids (Grid6 and GridX6 in ORCA nomenclature).

![Figure 2. Residues considered for the construction of the QM cluster model used in the present work. The coordinates were obtained from PDB structure 3WU2. Both sites for the cavity water (red spheres) are shown, according to their crystallographic occupancies. Selected amino acid residues are labeled, all from the D2 protein of PSII unless otherwise indicated. The water cavity is defined by the side chain phenyl groups of Phe169, Phe181, Phe184, and CP47-Phe362. The side outward-pointing chains of certain peripheral helix residues were simplified in the final QM calculations as described in the main text. The residues shown in yellow correspond to the fully relaxed part of the QM model.](Image)
The $g$-factors of tyrosyl radical models were computed within the framework of a DFT-based coupled-perturbed self-consistent field approach, in conjunction with an efficient implementation of the spin–orbit mean-field approximation to the Breit–Pauli operator for the spin–orbit coupling. The gauge origin for the computation of $g$-factors was chosen to be the center of the tyrosyl radical ring. The hyperfine coupling constants calculations were performed for the protons present on the $Y_D^\ast$ radical ring, on the $C_P$ carbon, and also for protons that are directly hydrogen-bonded to the $Y_D^\ast$ radical, HisN$_H$ and H$_2$O. We have also computed the hyperfine coupling constants for the $^{13}$C nuclei and the $^{17}$O of $Y_D$, as well as of the $^{15}$N of His189. All quantum chemical calculations were performed with ORCA.112,113

### RESULTS

#### Analysis of Crystallographic Models

Table 1 collects representative data from crystallographic models of PSII (see Table S1 for a complete collection of data). The absence of a proximal water in many cases, the distribution of distances between the phenolic oxygen of $Y_D$ and the cavity water, and the relative occupancies of the two water positions when both sites are modeled as occupied in the re

| PDB     | organism | resolution (Å) | $O_{Y_D-O_{prox}}$ (Å) | $O_{Y_D-O_{dist}}$ (Å) | ref |
|---------|----------|----------------|-------------------------|------------------------|-----|
| 5MX2    | T. elongatus | 2.55          | 4.0                     | 40                     |
| 5MX2    | T. elongatus | 2.55          | 3.9                     | 40                     |
| 5H2F    | T. elongatus | 2.2           | 4.4                     | 41                     |
| 5H2F    | T. elongatus | 2.2           | 4.3                     | 41                     |
| 4L6     | T. vulcana  | 2.1           | 4.3                     | 42                     |
| 4L6     | T. vulcana  | 2.1           | 4.1                     | 42                     |
| 4UB6    | T. vulcana  | 1.95          | 2.7 (0.40)              | 4.5 (0.60)             | 9   |
| 4UB6    | T. vulcana  | 1.95          | 3.1 (0.65)              | 4.5 (0.35)             | 9   |
| 4UB8    | T. vulcana  | 1.95          | 2.6 (0.35)              | 4.3 (0.65)             | 9   |
| 4UB8    | T. vulcana  | 1.95          | 2.7 (0.40)              | 4.5 (0.60)             | 9   |
| 3WU2    | T. vulcana  | 1.9           | 2.9 (0.5)               | 4.4 (0.5)              | 8   |
| 3WU2    | T. vulcana  | 1.9           | 4.4                     | 8                      |
| 6DHP    | T. elongatus | 2.04          | 4.5                     | 114                    |
| 6DHP    | T. elongatus | 2.04          | 4.4                     | 114                    |

“Two entries per structure are provided, corresponding to each one of the PSII monomers. When both proximal and distal water sites are occupied, the numbers in parentheses correspond to crystallographic occupancies for the O atom. From the $S_0$ state enriched sample; see Table S1 for complete data.

Figure 3. Depiction of the central region extracted from three optimized QM models with reduced $Y_D$, featuring different proton arrangements in the Arg294–His189–$Y_D^\ast$ triad and different positions of the cavity water. Selected distances are indicated (in Å) between the O atom of the cavity water, the O atom of $Y_D$, the O atom of the Phe169 peptide carbonyl, and the N$_H$ atom of the NH$_2$ group of Arg180 that interacts with water at the distal position.

Therefore, the problem of $Y_D$ microsolvation needs to be revisited with refined energetics obtained using expanded models. Additionally, it is necessary to seek connections with spectroscopic data, particularly the EPR spectra of the radical, which we accomplish as described in the following by explicit computation of the tyrosyl $g$-tensor for a series of possible structural models.

#### Energetics of Cavity Water Distribution in the Reduced State

Various protonation states and patterns along with different positions and orientations of the cavity water were explored with QM cluster calculations. We single out three models for further discussion and analysis as representative of the major species obtained as stable minima under the assumption of a neutral ($Y_D$-OH) tyrosine residue (Figure 3). In models $1_R$ and $2_R$ (the subscript “R” is used to denote the reduced state of $Y_D$) the protonation of the N$_H$ (or
Nε) site of His189 is blocked by hydrogen bonding with the Nε-H group of Arg294, which acts as a proton donor. As a result, the Nε (or Nτ) site of the imidazole is protonated and acts as hydrogen bond donor to the phenolic oxygen of the YD, while the phenolic proton of YD points toward the cavity. Of the two models, one corresponds to the proximal (1R) and the other to the distal water position (2R). In model 1R the proximal water is stabilized by hydrogen bonds with YD-OH and the backbone carbonyl of Phe169. In model 2R the water in the distal position also interacts with the backbone carbonyl but forms a hydrogen bond with Arg180. The distances between YD(O) and Owater are 2.78 and 4.35 Å for the two optimized models. Importantly, the distal water position is computed to be more stable than the proximal by 4.4 kcal mol⁻¹ with the PBE functional. This value is similar to different hybrid functionals and with the larger basis set, i.e., 5.0 kcal mol⁻¹ (B3LYP) and 4.6 kcal mol⁻¹ (TPSSh). Apparently, the water is better stabilized at the distal position because the interaction with Arg180 is stronger than the interaction with YD-OH.

Additional protonation states and hydrogen-bonding patterns have been considered. Model 3R mimics the hydrogen-bonding pattern of the YZ site, with YD-OH acting as hydrogen bond donor to the His189. In this case the only stable distribution of protons among Arg294–His189–Tyr160 is that depicted in Figure 3 for model 3R; that is, the imidazole Nε is protonated and an unusual protonation state of Arg294 is obtained, with its deprotonated Nτ acting as acceptor for the Nε-H of His189. Note that the total number of protons in model 3R is reduced by one compared to 1R and 2R; therefore 3R is not an isomer of the other two models and their relative energies cannot be compared. A most important result regarding this protonation arrangement is that no optimized structure associated with the proximal water position could be located. Only the distal position of the water molecule gives rise to a stable minimum, with an optimized YD(O)–Owater distance of 4.25 Å. This is a crucial observation because it implies that under a protonation and hydrogen-bonding scenario directly analogous to that of YZ the cavity water cannot function as a hydrogen bond partner to YD, and as will be discussed below, this excludes a role of water in YD oxidation. By contrast, the protonation state of models 1R and 2R naturally gives rise to two minima and hence to the dual occupancy of the water molecule.

In terms of energetics, the results based on the protonation scheme of models 1R and 2R are consistent with the observations from crystallography discussed above, which indicate that the distal position should be more stable. However, our results are in contrast to the computed values reported by Saito et al.,50 who suggested that the proximal position is instead more stable than the distal position by a similar energy margin of ca. 4 kcal mol⁻¹. After close inspection of the computational models and methods used by Saito et al., we conclude that the reason for this large discrepancy on the order of 10 kcal mol⁻¹ is the very limited QM region employed in that QM/MM study, which likely leads to artifacts in the evaluation of hydrogen bonds. It appears that some parts of the model, despite being in hydrogen-bonding interaction with the cavity water, were not included in the QM region but treated with force-field parameters. Since the water molecule and the different hydrogen-bonding partners available in the cavity were not uniformly part of the same theoretical representation, their interactions were not treated with a common level and type of theory. The definition of the computational model and the unequal representation likely explains why the reported relative energetics in the study of Saito et al. deviate from those reported here. In the present work all hydrogen-bonding interactions are treated equally at a fully quantum mechanical level with large converged basis sets, and hence we suggest that the present values can be considered to be a qualitatively correct representation of the relative stabilities of the two water sites, even if the absolute numerical values may still be open to refinement.

**Molecular Dynamics Simulations of Cavity Water Distribution.** To further evaluate this assignment using a methodologically orthogonal approach, we performed molecular dynamics simulations on a complete PSII monomer. Along the trajectory of the MD simulation we observed that the water molecule explores the whole cavity. However, from the analysis of the results it is clear that two regions are most frequently visited on average, and these correlate directly with the proximal and distal sites discussed above. The proximal site in the MD simulations is more well-defined, having a rather sharp peak at ca. 2.75 Å in the graph depicting the time evolution of the distance between the O atom of the cavity...
water and the phenolic oxygen of YD. In contrast, there is no well-defined unique minimum that can be ascribed to the distal site, but rather a broad distance range at ca. 4.0−5.0 Å. This encompasses the range of “distal” water positions reported in various crystal structures (see Table 1), a fact that supports the validity of the simulations and at the same time justifies the spread of crystallographic values.

The difference between the two sites relates to the fact that the proximal position is spatially more restricted, and when the water occupies this position, there is one optimal hydrogen-bonding arrangement, which coincides with that of the QM model shown in Figure 2. On the other hand, there is greater conformational flexibility in the distal region as a combined result of the larger space and the flexibility of the Arg180 side chain. In fact, we observed that water may exit and re-enter the cavity (points with YD(O)−Owater distances longer than those that fall within the distal range in Figure 4), a motion facilitated by tilting of the Arg180 guanidinium group. What is most relevant for the preceding discussion is the distribution of the cavity water among the proximal and distal positions. In this respect the MD simulations show that the distal region is more frequently occupied by the cavity water molecule. This is also the region to which water tends to drift toward in MD runs initiated with water at the proximal position (see Supporting Information Figure S1 for an additional MD simulation that demonstrates this point). Using the distance of 3.5 Å as a cutoff point between proximal and distal regions, the frames with YD(O)−Owater distances shorter than 3.5 Å account for ca. 26.6% of the population, while those above 3.5 Å account for 73.4%. Discounting the frames where water is outside the cavity and using the distance of 5.5 Å as a second cutoff for the distal region, the relative populations become 27.3% and 72.7%, respectively.

In conclusion, both classical MM/MD simulations on a PSII monomer and quantum chemical optimizations with large QM cluster models support that in the reduced state of YD, the cavity water occupies preferentially the distal position, although access to the less favorable proximal position is not energetically inhibited.

Formation of the Tyrosyl Radical. Based on the protonation state of the residues discussed above, His189 cannot function as a proton acceptor unless the entire protonation state of the His189−Arg294 pair becomes altered so that His189 becomes a hydrogen bond acceptor in its interaction with YD. Interestingly, Arg294 was identified by targeted random mutagenesis studies as functionally important for PSII. With the models favoring the orientation of the phenolic proton of the tyrosine toward the cavity it can be concluded that the cavity water is the most likely recipient of the proton upon oxidation of YD. By attempting to oxidize the models shown in Figure 3, it becomes apparent that model 2n, which contains the water at the distal position, cannot be oxidized: upon electron removal from the model, we observed no coupled deprotonation of YD and no spin localization. Rather, the unpaired spin density was sparsely distributed over the model (see Figure S2). This result appears to be independent of the density functional used (e.g., PBE0, B3LYP, TPSS0, and TPSSH). This particular observation emphasizes that YD cannot be oxidized with distal occupancy of the water molecule when His189 is a hydrogen bond donor to YD. By contrast, oxidation of model 1n with the water at the proximal position proceeds easily and yields a tyrosyl radical with concomitant shift of the phenolic proton to the proximal water (model 1ox of Figure 5). The optimized geometry contains the tyrosyl radical bound with two hydrogen bonds, from His189 and the proximal water. As a result of the strong hydrogen-bonding interaction between the proximal water−YD pair, the hydrogen-bonding distance involving the YD−His189 pair becomes larger (O−N = 3.03 Å). Following the deprotonation of the YD−OH, we observed no explicit formation of a hydronium ion (H3O+), but rather a proton shift toward the peptide carbonyl of Phe169 (see Figure 5).
Table 2. Computed g Values and O Löwdin Spin Population for the YD• Models, Compared to the Experimental Ranges of Values Reported under Various Conditions

| model   | \(g_x\) | \(g_y\) | \(g_z\) | \(\rho_O\) |
|---------|---------|---------|---------|-----------|
| \(1_{Ox}\) | 2.0063 | 2.0044 | 2.0022 | 0.325     |
| \(2_{Ox}\) | 2.0073 | 2.0044 | 2.0021 | 0.347     |
| \(3_{Ox}\) | 2.0073 | 2.0043 | 2.0021 | 0.345     |
| experiment* | 2.0074–2.0078 or 2.0064* | 2.0042–2.0045 | 2.0020–2.0023 | 0.2819,120 |

*Detailed experimental g values from available EPR studies are listed in Table S2. \(g_x\) value from tyrosyl radical generated at 1.8 K at pH ca. 8.5.47

In model \(2_{Ox}\) the water is optimized in a position that is intermediate between proximal and distal (\(YD(O)−O_{\text{Water}} = 3.58\) Å) with the proton essentially attached to the backbone carbonyl of Phe169. Models \(1_{Ox}\) and \(2_{Ox}\) are distinct geometric minima, but they are not energetically distinguishable by DFT because the relative energy difference between the models is 0.0 or 0.3 kcal mol\(^{-1}\) with the B3LYP and TPSSh functionals, respectively, although \(1_{Ox}\) is 3.2 kcal mol\(^{-1}\) lower than \(2_{Ox}\) with the PBE functional. We note that structures \(1_{Ox}\) and \(2_{Ox}\) have not been previously identified in the literature. The third model shown in Figure 5 is \(3_{Ox}\) with \(YD(O)−O_{\text{Water}} = 4.18\) Å, i.e., with water at the distal position. This can be viewed in two ways: (1) as directly related to the other two models in a hypothetical sequence \(1_{Ox} → 2_{Ox} → [3_{Ox} + H^+]\), where the cavity water takes the proton at the proximal position (\(1_{Ox}\)), moves to the intermediate position (\(2_{Ox}\)), and then is stabilized at the distal position with the proton having left the cavity; or (2) as the oxidized form of model \(3_{R}\) in which a proton translocation has taken place from \(YD\) to His189-N\(_{E}\) and from His189-N\(_{E}\) to Arg294.

Our calculations suggest that a hydronium cation cannot be stabilized at the distal position. Additionally, no \(YD\)-oxidized model with an overall proton configuration similar to \(3_{Ox}\) could be obtained with the water at the proximal position. These results admit two interpretations. First, if \(3_{R}\) better reflects the reduced state of \(YD\), then oxidation follows a "\(Y_D\)"-like proton shift; that is, His189 is the immediate proton acceptor. In this scenario the water is exclusively stabilized at a unique minimum in the distal position and hence is only a spectator, playing no role in the oxidation and deprotonation of \(YD\). The obvious problem with this interpretation is that it does not allow for occupation of the proximal position under any redox state of \(YD\). Thus, on the other hand, the pair \(1_{B}/2_{B}\) better reflects the reduced state of \(YD\), then upon oxidation, the proton either remains within the cavity as a hydronium at proximal or intermediate positions (\(1_{Ox}\) and \(2_{Ox}\)) or leaves the cavity and the water is stabilized at the distal position (\(3_{Ox}\)). This accommodates the existence of distinct minima for the water position and implicates the cavity water directly in \(YD\) oxidation and the release of the phenolic proton. The precise mechanism of proton removal in the latter scenario cannot be directly deduced from the QM models described here, but it would likely involve participation of Arg180 as proposed by Saito et al.50

EPR Spectroscopy: g-Tensors. In an attempt to identify connections between the three models presented above and the available EPR observations, we examine the g-tensors of the three oxidized models. The computed g values of all models are tabulated in Table 2 and compared with the experimental values. The orientation of principal g-matrix components is shown in Figure 6.

The g values of phenoxy radicals depend on two factors, which are in turn influenced by the protein environment (local electrostatics) and hydrogen bonding: (1) the unpaired spin density on the oxygen atom, which has the largest spin–orbit coupling constant, and (2) the relative energy difference between the oxygen-based \(p_x\) and \(p_y\) orbitals. The \(p_x\) orbital of the oxygen atom is orthogonal to the ring plane and contributes to the SOMO of the radical. The \(g_x\) value is affected by the energy difference between the SOMO and the in-plane \(p_x\) lone pair of the phenolic oxygen. The relative energy of the in-plane \(p_x\) orbital with respect to the SOMO is influenced by the number, strength, and orientation of hydrogen bonds to the oxygen atom. In-plane hydrogen bonds (from D2-His189 in the case of \(YD\)) stabilize the \(p_x\) orbitals, increasing the energy difference between the \(p_x\) and the SOMO, which results in less effective spin–orbit coupling and decreased \(g_x\) values.

Shifts in g values as a response to the number and orientation of hydrogen bonds to tyrosine radicals have been studied for simple models121–124 as well as explicitly for the \(Y_D^*\) of PSII102 and the \(Y_D^*\) of ribonucleotide reductase (RNR).125,126 In the case of \(Y_D^*\) the \(g_x\) value was shown to decrease with the number of hydrogen bonds, and this change tracked also the decrease in oxygen spin population.102 For example, the associated \(g_x\) values of \(Y_D^*\) with the one, two, and three hydrogen bonds were reported to be 2.0072, 2.0063, and 2.0054, respectively. It was also demonstrated that the \(g_x\) value for any specific hydrogen-bonding scenario depends on the \(Y_D(O)··H\) distance that affects the unpaired spin density on the oxygen, the atom with the largest spin–orbit coupling. Elaborate studies conducted on the redox-active NH\(_2\)Y\(_{730}\) radical126 of RNR yielded a \(g_x\) value of 2.0052 as characteristic of three hydrogen bonds associated with the radical. The above observations clearly delineate the direct correlation between the number of hydrogen bonds and the \(g_x\) values of the phenoxyl radical.

This analysis is fully consistent with the present results for the \(Y_D\) models. Specifically, models \(2_{Ox}\) and \(3_{Ox}\) have similar \(g_x\) values (2.0073), as both contain only one hydrogen bond to the tyrosyl radical, from the N\(_{E}\) (N\(_J\)) of His189. By contrast, in

![Figure 6: Orientation of the principal g-matrix components for the YD• radical (model 3Ox is used for this plot).](image-url)
model $1_{Ox}$ the $g_c$ value decreases to 2.0063 as a result of the two hydrogen bonds, i.e., from His189 and from the proximal water/hydronium. Only the $g_a$ value differs between the three models, while $g_g$ and $g_e$ remain the same. Therefore, the difference in $g_c$ is directly correlated to the number of hydrogen bonds and therefore to the position of the cavity water. Even though the intermediate and distal water positions cannot be distinguished because the $Y_D^*$ radical in both cases appears with the same $g_c$, of 2.0073, the present results enable us to conclusively and uniquely correlate the occupation of the proximal water position with the $g_c$ value of 2.0063.

It has been observed experimentally that a $g_c \approx 2.0064$ signal is obtained when $Y_D$ is oxidized under high pH conditions at cryogenic temperatures.\textsuperscript{46,47,72} Importantly, upon increasing the temperature, the $g_c$ shifts to 2.0075–2.0078. As will be discussed in the following, this change in the $g_c$ value does not reflect the protonation state of His189, as assumed in past literature,\textsuperscript{47,127} but is correlated with the movement of the proton-accepting water inside the cavity.

**EPR Spectroscopy: Hyperfine Coupling Constants.** In addition to the $g$-tensor, an important parameter that can offer insight into the electronic structure of the tyrosyl radical and help in evaluating computational models is the hyperfine coupling constants (HFC) for protons and heavier nuclei bound to or strongly interacting with it.\textsuperscript{71,105,119,120,122,127–137} In addition to proton HFCs, Brynda and Britt have analyzed the $^{13}$C and $^{15}$N HFCs using a computational Tyr model,\textsuperscript{122} and we refer the interested reader to that work for a discussion of the data. Here we will briefly focus on selected data relating only to the protons/deuterons because they are most relevant to the subject of microsolvation of $Y_D^*$. Two types of $^2$H ENDOR HFCs have been reported, i.e., where the tyrosyl radical is generated under physiological pH (6.5)\textsuperscript{79} and high pH (8.7)\textsuperscript{46} conditions. Radicals generated under both conditions give the characteristic $g_c \approx 2.0074$ signal,\textsuperscript{71} which implies that $Y_D^*$ interacts only with His189; that is, $N_e$ ($N_d$) acts as the sole hydrogen bond donor to $Y_D^*$.\textsuperscript{131} The experimentally fitted HFC parameters for both cases appear the same (Table 3), suggesting that the pH difference does not change the immediate protonation environment of $Y_D^*$ under physiological temperatures. The computed HFCs for the His189 N-deuterium for models that correspond to the same class of $g_c$ signal, i.e., for models $2_{Ox}$ and $3_{Ox}$, agree well with the experimental values (Table 3), which again confirms that the radical is bound with only one hydrogen bond, to His189.

Similar agreement is obtained with the computed HFCs for the protons of the tyrosyl ring, $^{13}$C ($Y_D$) and $^{17}$O ($Y_D$) (Figure S3 and Tables S3–S7), which also agree well with experimental data.\textsuperscript{11,119,120,132} Support the orientation and environment of the $Y_D$ radical in the present models. The computed quadrupole tensors provided in Table 3 agree somewhat better with the results obtained under high pH conditions, but at this point we are running the risk of overinterpreting both our results and the information content of the experiment. The experimentally fitted distance between the tyrosyl oxygen and the His189-bound proton was reported as 1.84 and 1.75 Å at physiological and high pH, respectively. These are consistent with the computed distances (1.79 and 1.78 Å for models $2_{Ox}$ and $3_{Ox}$), but the calculations clearly suggest that the fitted parameters derived from experimental data independently of explicit atomistic models would be worth revisiting using QM-optimized distances and accounting for the cavity water. Overall, the agreement of the computed HFCs for the His189 proton with the experimental values is consistent with the structural interpretation derived from the $g$ values.

As discussed above, the $Y_D^*$ radical generated at 1.8 K and pH 8.5 shows a $g_c = 2.0064$, which we assigned to a structural configuration with a proximal water-like model $1_{Ox}$ Table 3 also reports computed HFCs for model $1_{Ox}$ in which $Y_D^*$ interacts directly with two hydrogens, the $N_e$-H of His189 and the proximal water molecule. The HFC parameters for $1_{Ox}$ suggest comparable HFCs for the two partners. Interestingly, the computed parameters for the proximal water resemble those of the His189 hydrogen in the other two models ($2_{Ox}$ and $3_{Ox}$), whereas the His189 hydrogen in $1_{Ox}$ experiences relatively weaker coupling. Both values can be considered consistent with experimental HFCs of higher-$g_c$ species, but comparisons with the cryogenic HFCs are not entirely reliable for two reasons. First, no spectral simulations have been reported with the assumption of the $Y_D^*$ radical interacting with more than one deutereron.\textsuperscript{46} Second, based on the $g$ value calculations, model $1_{Ox}$ is only an approximate structural model for $Y_D$ oxidation at cryogenic temperature and high pH, but cannot be an exact representation of the cryogenic state because the positions of heavy atoms are optimized. In our view it is not possible to either deduce from experiment the extent of proton shift toward the proximal water or to model reliably with standard QM models the evolution of proton movement along the $Y_D^*$ radical. The results of protonation are found to be 2.0076, which is consistent with the $g_c$ values computed for the same models, i.e., with the $Y_D^*$ radical bound with only one hydrogen bond to His189. Another result that corroborates this observation is the isotropic hyperfine coupling for the $^{15}$N ($N_d$ of His189). While for model $1_{Ox}$ a rather low value of 0.26 MHz was computed, for models $2_{Ox}$ and $3_{Ox}$ the computed values are 0.49 and 0.58 MHz, respectively, which agree well with the $^{15}$N ENDOR.

### Table 3. Computed Hyperfine and Quadrupole Tensor Components (MHz) for Exchangeable Deuterons

| model | H-bond partner | $A_x$ | $A_y$ | $A_z$ | $Q_x$ | $Q_y$ | $Q_z$ |
|-------|----------------|-------|-------|-------|-------|-------|-------|
| $1_{Ox}$ | His189,N,D | 1.01 | -0.60 | -0.47 | 0.110 | -0.062 | -0.048 |
| $2_{Ox}$ | His189,N,D | 1.39 | -0.86 | -0.77 | 0.078 | -0.049 | -0.029 |
| $3_{Ox}$ | His189,N,D | 1.34 | -0.82 | -0.73 | 0.096 | -0.055 | -0.041 |
| exp (pH 6.5)\textsuperscript{79} | 1.10 | -0.59 | -0.51 | 0.300 | -0.190 | -0.110 |
| exp (pH 8.7, relaxed)\textsuperscript{46} | 1.06 | -0.58 | -0.48 | 0.11 | -0.07 | -0.04 |
| exp (pH 8.7, unrelaxed)\textsuperscript{46} | 1.59 | -0.91 | -0.68 | 0.14 | -0.074 | -0.066 |

“Unrelaxed $Y_D^*$ intermediate trapped at 7 K. The uncertainty in the calculated parameters is estimated to be 20%.\textsuperscript{125,126} The components of the $A$ and $Q$ tensors are described such as $|x| > |y| > |z|$.
In the reduced state of YD, the cavity water molecule can occupy two positions in the cavity defined by the cluster of phenylalanine residues shown in Figure 2, proximal and distal with respect to the YD residue (Figure 3). In both positions the backbone carbonyl of Phe169 plays the role of H-bond acceptor; in the proximal position the water is additionally stabilized by a H-bond to YD which therefore acts as proton donor (model 1\(\text{b}\)), while in the distal (models 2\(\text{a}\) and 3\(\text{a}\)) it is stabilized by an additional H-bond to Arg180. The results presented above show that the distal position is energetically preferred compared to the proximal position when YD is reduced. This conclusion is in contrast with a previous suggestion, but it is in line with available crystallographic data and supported both by large-scale QM calculations and by MD simulations. Our models additionally suggest a correlation between the protonation pattern in the Arg294–His189–YD triad and the water position: if YD acts as a proton donor to His189, then the cavity water cannot be stabilized in the proximal position.

Two ideas can be formulated regarding the deprotonation of YD upon oxidation: deprotonation to His189 or to the cavity water molecule. The idea of YD deprotonation to the His189 parallels the mechanism proposed for the other redox-active YD residue, which deprotonates to the coupled His190. The computational models presented here can in principle accommodate the scenario of a YD-like proton shift, which is equivalent to model 3\(\text{a}\) being oxidized to model 3\(\text{b}\). In the following we will discuss why the latter scenario is disfavored and how the computed energetics of water distribution and the related EPR parameters explain the whole range of EPR observations, including the temperature dependence of EPR spectra, on the basis of the oxidized models presented here. Simultaneously, compelling evidence in favor of the present models and of water-assisted oxidation and deprotonation comes from the structural explanation of the bimodal kinetics of YD oxidation.

**Water Distribution and Kinetics of YD Oxidation.** The two results—(a) that the distal water position is more stable than the proximal when YD is reduced and (b) that YD can be oxidized only when water is found in the proximal position—imply that oxidation of YD for the majority of PSII centers would be inhibited because of the requirement for distal water to move to the less favorable proximal local minimum. This provides a natural explanation for a wide range of experimental observations. It is known that no YD centers undergo oxidation at cryogenic temperature (5 K, at pH 6.5). According to the present models, under such conditions almost all YD centers are expected to be associated with the distal water and the movement of water to the proximal position is expected to be blocked. This dependence of YD oxidation on the spatial availability of the cavity water as proton acceptor fundamentally differentiates the oxidation characteristics of YD from the Y\(\text{a}\) radical of PSI. It is also in line with the distinct rates of oxidation for YD and Y\(\text{a}\); under physiological conditions (pH \(\approx\) 6.5), YD is oxidized on the microsecond time scale \(t_{1/2} > 150\) µs, much slower compared to Y\(\text{a}\) \(t_{1/2} \approx 2–10\) µs.\(^{22}\)

Mamedov and co-workers studied the oxidation kinetics of YD at different pH values and concluded that at pH 4.7 and 6.3 the oxidation kinetics of YD are biphasic; that is, they exhibit a fast and a slow phase.\(^{35,54}\) Crucially, it was observed that the amplitude of the slow phase is always higher than that of the fast phase. The hypothesis that the two phases may correlate with the position of the cavity water is fully borne out by the detailed computational models presented here. The observation of the dominance of the slow phase is consistent with the result that the majority of YD centers have the cavity water present at the distal position, whereas the proximal position is occupied only in a minority of centers and gives rise to the small-amplitude fast phase in oxidation kinetics. Therefore, our computational models fully agree with the suggested correlation of oxidation kinetics and water distribution proposed for the low-pH situation in the studies by Ahmadova et al.\(^{53}\) and Sjöholm et al.\(^{54}\) (but not for the high-pH situation as will be discussed in the following).

Experiments performed with the presence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), an inhibitor of the QA site that blocks forward electron transfer in PSII, forcing QA\(\rightarrow\)S\(\text{b}\) recombination, show only the fast phase irrespective of the pH of the sample.\(^{53}\) This study concluded that the fast-phase YD oxidation outcompetes the QA\(\rightarrow\)S\(\text{b}\) recombination, whereas the rate of the slow-phase oxidation (according to the present interpretation, YD centers with distal water) lags compared to the QA\(\rightarrow\)S\(\text{b}\) recombination. This is why the slow phase is not observed. This analogy is evident from the fact that the amplitude of the fast phase is the same with or without DCMU. In addition, only 24% YD centers get oxidized at pH 6.3 with DCMU compared to 63% at the same pH without DCMU. The above results become transparent in terms of their structural interpretation in view of the present models that support simultaneously the enhanced stability of the distal water position and the necessity of a proximal water for YD oxidation.

**Structural Explanation of EPR Spectroscopy.** Compelling evidence for correlating experimental observations with the water position comes from comparing EPR data with the computed g-matrix values we report for our models. Two types of EPR signal can be distinguished based on the \(g\) value of the YD radical, close to either 2.0064 or 2.0075. YD exhibits interesting EPR properties under high pH conditions.\(^{22,25,48,47}\) Faller et al. showed that at high pH the reduced YD can be oxidized even at 1.8 K, giving rise to an EPR signal with a \(g\) value of 2.0064.\(^{47}\) Upon increasing the temperature (77 K), the increased \(g\) value of 2.0075 was observed.\(^{47}\) HF-EPR experiments by Chatterjee et al.\(^{50}\) are consistent with these observations: an unrelaxed state could be trapped at 7 K upon YD oxidation with a \(g\) value of 2.0067, while upon thermal relaxation, the \(g\) value increases to 2.0078. The \(g\) value of 2.0074–2.0078 is observed experimentally for the YD radical generated under physiological temperatures at any pH value.

The original structural explanation of the two signals implicated proton transfer from YD to the N\(_{\text{p}}\) of a singly protonated (at N\(_{\text{p}}\)) His189 and formation of a cationic His189 species (low \(g\)\(_{\text{a}}\)), which deprotonates (from N\(_{\text{a}}\)) at higher temperature, relaxing to the lower-field signal.\(^{47}\) The present calculations do not support this scenario. Instead, the “cryogenic” signal corresponds directly to the value computed for model 1\(\text{a}\) (\(g\) \(_{\text{a}}\) = 2.0063), and hence we attribute this to the presence of two hydrogen bonds to YD or, equivalently, to the presence of water (as proton acceptor) at the proximal position. According to this interpretation, the experimentally
observed change in $g_e$ is not related to the behavior of the histidine, but to the movement of the water/hydronium from the proximal position (model 1) to an intermediate position with the proton retained in the cavity (model 2) or a distal position with the proton removed from the cavity (model 3). Both 2 and 3 have $g_e$ values of 2.0073 and are consistent with the EPR of a tyrosyl radical having only one hydrogen bond (to His189). Therefore, this signal implies that the water has moved away from the proximal position after accepting the proton. However, the existing data do not allow us to determine whether the proton has left the cavity (model 3) or not (model 2).

**pH Dependence of YD Oxidation.** As already stated above, the pH affects the oxidation of YD. We suggest that the molecular basis of this effect relates to the change in the relative stability of the proximal and distal water positions. YD is known to outcompete $Y_z$ in high-pH conditions; for example, at pH 8.5 oxidation of YD becomes extremely fast ($t_{1/2} \approx 190$ ns), and recent results from Schlodder et al. report this rate to be even faster ($t_{1/2} \approx 30$ ns) at pH 9. These faster oxidation rates also depend on the location of the cation on the reaction center, and it is suggested that in high-pH (8.5) conditions the major proportion of cation resides on the P$_{D2}$ side of the reaction center, unlike in low-pH conditions, where the cation is mainly localized on the P$_{D1}$ side. This charge shift presumably makes electron transfer faster from the reduced YD to P$_{680}$ ($t_{1/2} \approx 30$–190 ns). Ahmadova et al. observed nearly 78% of the fully oxidized YD centers at pH 8.5. At pH 8.5 it is observed that the YD oxidation follows a single-exponential phase with fast oxidation rates. This was attributed to the deprotonation of YD in the reduced form, (i.e., a tyrosine anion), which would render the subsequent oxidation a pure electron transfer event. Our computational models however provide no support for this for two reasons: first, the identification of a minimum with a $Y_D^\cdot$O$^-$ form was not possible, and second, the radical formed upon oxidation of this hypothetical deprotonated form would be inconsistent with the low $g_e$ value observed in EPR and assigned to model 1. Therefore, we suggest that the observed effects are not attributable to changes in protonation state of the reduced YD residue, but are again associated with the distribution of water within the cavity. Specifically, the observations at high pH would be consistent with association of most YD centers with the proximal water. According to the model presented in the present work, the observations may reflect a shift in the relative energetics of proximal vs distal water positions, that is, a progressive stabilization of the proximal position at increasing pH values.

It is acknowledged that the present models and computational approaches cannot provide a detailed view of how protonation states of residues respond to bulk pH changes or how hydrogen-bonding networks are rearranged at large scales within PSII. Hence, it is also unclear how increasing bulk pH might stabilize the proximal position. However, based on the structure of the cavity we suggest that a possible local explanation of the observed effects is that at high pH the distal water position might be destabilized by perturbation of the Arg180–Asp333 salt bridge that is in contact with the protein surface through a rather short water channel. The result would be the preferential occupation of the proximal water position at high pH, rendering the YD readily oxidizable even at cryogenic temperatures since water movement is no longer required to switch on the electron transfer. The special importance of Arg180 was highlighted in site-directed mutagenesis studies by Manna et al. who reported that mutations at the Arg180 residue resulted in EPR signals attributed to the YD radical being of reduced intensity and altered line shape. More importantly, Arg180 mutants had limited oxygen evolution capacity of PSII, and the amount of enzyme present in thylakoids was reduced, demonstrating the functional importance of this residue for smooth redox behavior at the YD site.

**Fate of the Proton.** The fate of the phenolic proton after oxidation of the YD relates to all three factors: the relative stabilities of the cavity water positions, the effect of pH, and the role of the Arg180–Asp333 salt bridge. A detailed scenario on a possible deprotonation pathway has been presented by Ishikita and co-workers, who suggested that after YD oxidation the proton is transferred toward the bulk via proton exchange through Arg180–Asp333 and a series of water molecules beyond this salt bridge. A recent FTIR study from Nakamura and Naguchi reported the detection of the proton released upon YD oxidation to the bulk, assuming a correspondence of their observations with the model of Saito et al. On the other hand, that model required a very large energy for the return of the proton (ca. 80–120 kcal/mol) upon YD reduction.

The experimental observations on the deprotonation step remain debatable. A proton inventory study by Barry and co-workers supported the existence of multiple proton donation pathways to the YD radical at high pH, one of them involving multiple protons and the other a single proton. The proton-coupled electron transfer (PCET) mechanism under high pH conditions is supported by the difference FTIR study of Heinerwadel et al., where it is proposed that YD remains protonated under a pH range 6.0–10.0 and is involved in a strong hydrogen bond. A pure ET process upon oxidation is instead supported by a recent EPR study of Schlodder et al., where no change in oxidation rates was observed upon introducing exchangeable protons. In addition, their flash-induced absorbance study reports that YD oxidation is independent of temperature between 5 and 250 K at pH 9.

If we focus on the EPR results, a clear conclusion based on the present oxidized models 2 and 3 is that they correlate equally well with the $g_e \geq 2.0073$ tyrosyl EPR signals and hence accommodate two distinct possibilities equally well: that the proton remains in the cavity or that the proton has left the cavity. The latter model reflects the scenario described by Saito et al. The former, however, represents a possibility that has not been previously represented by computational models and can be of relevance in interpreting experimental results obtained at different pH values. Beyond the agreement of this “proton-in-the-cavity” model with the EPR, it is interesting to note that it would be consistent with one of the proposed roles of the YD residue. Specifically, it has been suggested that the oxidized YD might be exerting an electrostatic effect on the primary charge separation site, pushing the electron hole toward the P$_{D1}$ side of P$_{680}$ and hence enhancing the Y$_D$P$_{680}$ donation rates. For this function it would be required that the proton is retained near the Y$_D$ residue, i.e., in model 2. The oxidized models described here therefore provide a structural basis for discussing several observations and mechanistic possibilities, but further investigations will be required to clarify which one corresponds to the real system and under which conditions.
CONCLUSIONS

In this work we investigated the role of a confined water in regulating the properties of the redox-active tyrosine YD of PSII. Static and dynamic calculations showed that in the reduced form of the tyrosine both proximal and distal positions are stable, but the distal position of the cavity water is energetically favored. When we take into account simultaneously the energetics of cavity water distribution in relation to the ability of YD to be oxidized and in relation to the EPR data reported under various conditions, our results are consistent with the idea that the histidine partner plays a different role in YD and YP. Whereas D1-His190 accepts the proton of oxidized YD and keeps it in immediate availability to be returned to YD when it is reduced by the manganese cluster of the OEC, our models are consistent with assigning the role of YD proton acceptor to the cavity water. The proton can remain within the cavity or not; closer integration of computational modeling and experiment will be required to clarify which scenario is most likely under which conditions. Crucially, if His189 acts as a proton donor to YD, which can be considered as a “normal” situation due to its expected interaction with Arg294, then YD can be oxidized only when the cavity water is at the proximal position to accept the phenolic proton. In combination with the proposed energetics of water distribution in the cavity, this has profound implications for understanding and explaining the experimental observations of biphasic YD oxidation kinetics: the predominant slow phase is ascribed to the majority population where water is found at the distal position. The EPR calculations reported here lead to a natural interpretation of the spectroscopic observations, correlating the observed distribution of $g$ values with the position of the cavity water, as opposed to the protonation state or structural relaxation of the His189 residue as previously speculated. In addition, our results suggest a new structural rationalization of the observed pH effect. In contrast to a previous hypothesis that attributed the effect of pH to a direct change of the YD protonation state, we propose that at high pH the relative stabilities of the two water sites are simply inverted, enabling the oxidation of YD at cryogenic temperatures. The detailed structure–spectroscopy correlations described in the present work can serve as the basis for revisiting past experiments in light of the role of the cavity water and also for designing future experiments that will further probe the role of microsolvation in regulating the behavior and function of the redox-active tyrosine.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b13123.
Figures S1–S3, Tables S1–S7 (PDF)

AUTHOR INFORMATION

Corresponding Author
*dimitrios.pantazis@kofo.mpg.de

ORCID
Abhishek Sirohiwal: 0000-0002-4073-7627
Frank Neese: 0000-0003-4691-0547
Dimitrios A. Pantazis: 0000-0002-2146-9065

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Support by the Max Planck Society is gratefully acknowledged. This work was supported by the Cluster of Excellence RESOLV (EXC 1069) funded by the Deutsche Forschungsgemeinschaft.

REFERENCES

(1) Blankenship, R. E. Molecular Mechanisms of Photosynthesis, 2nd ed.; Wiley: Chichester, 2014; p 312.
(2) Messinger, J.; Noguchi, T.; Yano, J. Photosynthetic O2 Evolution. In Molecular Solar Fuels; Wydryniski, T. J.; Hillier, W., Eds.; The Royal Society of Chemistry: Cambridge, 2012; pp 163–207.
(3) Rutherford, A. W.; Osyczka, A.; Rappaport, F. Back-Reactions, Short-Circuits, Leaks and Other Energy Wasteful Reactions in Biological Electron Transfer: Redox Tuning to Survive Life in O2. FEBS Lett. 2012, 586, 603–616.
(4) Styring, S.; Sjøholm, J.; Mamedov, F. Two Tyrosines that Changed the World: Interfacing the Oxidizing Power of Photochemistry to Water Splitting in Photosystem II. Biochim. Biophys. Acta, Acta Bioenerg. 2012, 1817, 76–87.
(5) Diner, B. A.; Britt, R. D. The Redox-Active Tyrosines YZ and YD. In Photosystem II; Springer, 2005; pp 207–233.
(6) Nugent, J. H.; Ball, R. J.; Evans, M. C. Photosynthetic Water Oxidation: The Role of Tyrosine Radicals. Biochim. Biophys. Acta, Bioenerg. 2004, 1655, 217–221.
(7) Pujols-Ayala, I.; Barry, B. A. Tyrosyl Radicals in Photosystem II. Biochim. Biophys. Acta, Bioenerg. 2004, 1655, 205–216.
(8) Umena, Y.; Kawakami, K.; Shen, J.-R.; Kamiya, N. Crystal Structure of the Oxygen-Evolving Photosystem II at a Resolution of 1.9 Å. Nature 2011, 473, 55–60.
(9) Suga, M.; Akita, R.; Hirata, K.; Ueno, G.; Murakami, H.; Nakajima, Y.; Shimizu, T.; Yamashita, K.; Yamamoto, M.; Ago, H.; Shen, J.-R. Native Structure of Photosystem II at 1.95 Å Resolution Viewed by Femtosecond X-ray Pulses. Nature 2015, 517, 99–103.
(10) Cardona, T.; Sedoud, A.; Cox, N.; Rutherford, A. W. Charge Separation in Photosystem II: A Comparative and Evolutionary Overview. Biochim. Biophys. Acta, Bioenerg. 2012, 1817, 26–43.
(11) Grabolle, M.; Dau, H. Energetics of Primary and Secondary Electron Transfer in Photosystem II Membrane Particles of Spinach Revisited on Basis of Recombination-Fluorescence Measurements. Biochim. Biophys. Acta, Bioenerg. 2005, 1708, 209–218.
(12) McEvoy, J. P.; Brudvig, G. W. Water-Splitting Chemistry of Photosystem II. Chem. Rev. 2006, 106, 4455–4483.
(13) Dau, H.; Zaharieva, I. Principles, Efficiency, and Blueprint Character of Solar-Energy Conversion in Photosynthetic Water Oxidation. Acc. Chem. Res. 2009, 42, 1861–1870.
(14) Krewald, V.; Retegan, M.; Pantazis, D. A. Principles of Natural Photosynthesis. Top. Curr. Chem. 2015, 371, 23–48.
(15) Pantazis, D. A. Missing Pieces in the Puzzle of Biological Water Oxidation. ACS Catal. 2018, 8, 9477–9507.
(16) Vermaas, W. F. J.; Rutherford, A. W.; Hansson, Ö. Site-Directed Mutagenesis in Photosystem II of the Cyanobacterium Synechocystis sp. PCC 6803: Donor D is a Tyrosine Residue in the D2 Protein. Proc. Natl. Acad. Sci. U. S. A. 1988, 85, 8477–8481.
(17) Debus, R. J.; Barry, B. A.; Babcock, G. T.; McIntosh, L. Site-Directed Mutagenesis Identifies a Tyrosine Radical Involved in the Photosynthetic Oxygen-Evolving System. Proc. Natl. Acad. Sci. U. S. A. 1988, 85, 427–430.
(18) Styring, S.; Rutherford, A. W. In the Oxygen-Evolving Complex of Photosystem II the S2 State is Oxidized to the S1 State by D* (Signal I↓↓down). Biochemistry 1987, 26, 2401–2405.
(19) Vass, I.; Styring, S. pH-Dependent Charge Equilibria Between Tyrosine-D and the S States in Photosystem II. Estimation of Relative Midpoint Redox Potentials. Biochemistry 1991, 30, 830–839.
(20) Vermaas, W. F. J.; Renger, G.; Dohnt, G. The Reduction of the Oxygen-Evolving System in Chloroplasts by Thylakoid Components. Biochim. Biophys. Acta, Bioenerg. 1984, 764, 194–202.
of Tyrosine YZ and the Manganese Cluster in the Water Oxidizing 
II Slow in Tris-Washed Chloroplasts. Biochemistry 1993, 32, 
9379–9386.

(21) Messinger, J.; Renger, G. Generation, Oxidation by the 
Oxidized Form of the Tyrosine of Polypeptide D2, and Possible 
Electronic Configuration of the Redox States S0, S1, and S2, of the 
Water Oxidase in Isolated Spinach Thylakoids. Biochemistry 1993, 32, 
9379–9386.

(22) Faller, P.; Debus, R. J.; Brettle, K.; Sugira, M.; Rutherford, A. 
W.; Boussac, A. Rapid Formation of the Stable Tyrosyl Radical in 
Photosystem II. Proc. Natl. Acad. Sci. U. S. A. 2001, 98, 14368–14373.

(23) Ananyev, G. M.; Sakiyana, I.; Diner, B. A.; Dismukes, G. C. A. 
Functional Role for Tyrosine-D in Assembly of the Inorganic Core 
of the Water Oxidase Complex of Photosystem II and the Kinetics of 
Water Oxidation. Biochemistry 2002, 41, 974–980.

(24) Kok, B.; Forbush, B.; McGloin, M. Cooperation of Charges in 
Photosynthetic O2 Evolution − I. A Linear Four Step Mechanism. 
Photochem. Photobiol. 1970, 11, 457–475.

(25) Havelius, K. G. V.; Styring, S. pH Dependent Competition 
Between Y2 and Y1 in Photosystem II Probed by Illumination at S K. 
Biochemistry 2007, 46, 7865–7874.

(26) Deak, Z.; Vass, I.; Styring, S. Redox Interaction of Tyrosine-D 
with the S-States of the Water-Oxidizing Complex in Intact and 
Chloride-Depleted Photosystem II. Biochim. Biophys. Acta, Bienerg. 
1994, 1185, 65–74.

(27) Fezyiye, Y.; Rotterdam, B. J. v; Bernat, G.; Styring, S. Electron 
Transfer from Cytochrome b6f and Tyrosine-D to the S2 and 
S1 States of the Water Oxidizing Complex in Photosystem II. Chem. 
Phys. 2003, 294, 415–431.

(28) Mamedov, F.; Smith, P. J.; Styring, S.; Pace, R. J. Relaxation 
Behaviour of the Tyrosine Y2 Radical in Photosystem II: Evidence for 
Strong Dipolar Interaction with Paramagnetic Centers in the S1 and 
S2 states. Phys. Chem. Chem. Phys. 2004, 6, 4890–4896.

(29) Rutherford, A. W.; Boussac, A.; Faller, P. The Stable Tyrosyl 
Radical in Photosystem II: Why D? Biochim. Biophys. Acta, Bienerg. 
2004, 1655, 222–230.

(30) Saito, K.; Ishida, T.; Sugira, M.; Kawakami, K.; Umena, Y.; 
Kamiya, N.; Shen, J.-R.; Ishikawa, T.; Iwata, S.; Shen, 
−11 J.-R. Light-Induced Structural Changes and the Site of O2=O bond 
Formation in PSII Caught by XFEL. Nature 2017, 543, 131–135.

(31) Debus, R. J. Amino Acid Residues that Modulate the Properties 
of Tyrosine YZ and the Manganese Cluster in the Water Oxidizing 
II Slow in Tris-Washed Chloroplasts. Biochemistry 1993, 32, 
9379–9386.

(32) Boussac, A. Rapid Formation of the Stable Tyrosyl Radical in 
Photosystem II. Proc. Natl. Acad. Sci. U. S. A. 2001, 98, 14368–14373.

(33) Ananyev, G. M.; Sakiyana, I.; Diner, B. A.; Dismukes, G. C. A. 
Functional Role for Tyrosine-D in Assembly of the Inorganic Core 
of the Water Oxidase Complex of Photosystem II and the Kinetics of 
Water Oxidation. Biochemistry 2002, 41, 974–980.

(34) Kok, B.; Forbush, B.; McGloin, M. Cooperation of Charges in 
Photosynthetic O2 Evolution − I. A Linear Four Step Mechanism. 
Photochem. Photobiol. 1970, 11, 457–475.

(35) Havelius, K. G. V.; Styring, S. pH Dependent Competition 
Between Y2 and Y1 in Photosystem II Probed by Illumination at S K. 
Biochemistry 2007, 46, 7865–7874.
(51) Saito, K.; Sakashita, N.; Ishikita, H. Energetics of the Proton Transfer Pathway for Tyrosine D in Photosystem II. *Aust. J. Chem.* 2016, 69, 991–998.

(52) Nakamura, S.; Noguchi, T. Infrared Detection of a Proton Released from Tyrosine Y2 from the Bulk upon Its Photo-oxidation in Photosystem II. *Biochemistry 2015,* 54, 5045–5053.

(53) Ahmadova, N.; Ho, F. M.; Stirling, S.; Mamedov, F. Tyrosine D Oxidation and Redox Equilibrium in Photosystem II. *Biochim. Biophys. Acta, Bioenerg.* 2017, 1858, 407–417.

(54) Sjöholm, J.; Ho, F.; Ahmadova, N.; Brinkert, K.; Hammarström, L.; Mamedov, F.; Stirling, S. The Protonation State Around TyrD/Tyr* in Photosystem II is Reflected in its Biphasic Oxidation Kinetics. *Biochim. Biophys. Acta, Bioenerg.* 2017, 1858, 147–155.

(55) Sjöholm, J.; Mamedov, F.; Stirling, S. Spectroscopic Evidence for a Redox-Controlled Proton Gate at Tyrosine D in Photosystem II. *Biochemistry 2014,* 53, 5721–5723.

(56) Migliore, A.; Polizi, N. F.; Therien, M. J.; Beratan, D. N. Biochemistry and Theory of Proton-Coupled Electron Transfer. *Chem. Rev. 2014,* 114, 3381–3465.

(57) Linke, K.; Ho, F. M. Water in Photosystem II: Structural, Functional and Mechanistic Considerations. *Biochim. Biophys. Acta, Bioenerg.* 2014, 1837, 14–32.

(58) Takahashi, R.; Sugimura, M.; Noguchi, T. Water Molecules Coupled to the Redox-Active Tyrosine YD in Photosystem II as Detected by FTIR Spectroscopy. *Biochemistry 2007,* 46, 14245–14249.

(59) Mino, H.; Satoh, J.-i.; Kawamori, A.; Toriyama, K.; Zimmermann, J.-L. Matrix ENDOR of Tyrosine D* in Oriented Photosystem II Membranes. *Biochim. Biophys. Acta, Bioenerg.* 1993, 1144, 426–433.

(60) Force, D. A.; Randall, D. W.; Britt, R. D.; Diner, B. A. "H ESE-ENDOR Study of Hydrogen Bonding to the Tyrosine Radicals YD* and YD** of Photosystem II. *J. Am. Chem. Soc. 1995,* 117, 12643–12644.

(61) Tang, X.-S.; Zheng, M.; Chisholm, D. A.; Dismukes, G. C.; Diner, B. A. Investigation of the Differences in the Local Protein Environments Surrounding Tyrosine Radicals YD* and YD** in Photosystem II Using Wild-Type and the D2-Tyr160Phe Mutant of *A. thaliana.* *Biochemistry 2002,* 41, 12914–12920.

(62) Delmotte, P.; Rutherford, A. W.; Usoskin, I. G.; Hoff, A. J. High Frequency Electron Paramagnetic Resonance Spectroscopy: Comparison with Ribonucleotide Reductase and in Vitro Tyrosine. *Biochemistry 1992,* 31, 11874–11880.

(63) Faller, P.; Rutherford, A. W.; Usoskin, I. G. Orientation of the Tyrosyl Radical in *Rps.* *J. Chem. Phys. 2012,* 136, 144103.

(64) Force, D. A.; Randall, D. W.; Britt, R. D.; Tang, X.-S.; Diner, B. A. High-Frequency Electron Paramagnetic Resonance Spectroscopy and Density Functional Theoretical Study. *J. Phys. Chem. B 1997,* 101, 6634–6641.

(65) Gulin, V. I.; Dikanov, S. A.; Tsytova, Yu. D.; Eroko, R. G.; Hoff, A. J. Very High Frequency (153 GHz) EPR of the Oxidized Primary Donor of the Photosynthetic Bacteria *Rh. sphaeroides* R-26 and *Rps.* *J. Phys. Chem. B 2003,* 107, 1275–1282.

(66) Duan, Y.; Wu, C.; Chowdhury, S.; Lee, M. C.; Xiong, G.; Zhang, W.; Yang, R.; Cieplak, P.; Luo, R.; Lee, T.; Caldwell, J.; Wang, J.; Kollman, P. A Point-Charge Force Field for Molecular Mechanics Simulations of Proteins Based on Condensed-Phase Quantum Mechanical Calculations. *J. Comput. Chem.* 2003, 24, 1999–2012.

(67) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R.; Klein, M. L. Comparison of Simple Potential Functions for Simulating Liquid Water. *J. Chem. Phys. 1983,* 79, 926–935.

(68) Zhang, L.; Silva, D.-A.; Yan, Y.; Huang, X. Force Field Development for Cofactors in the Photosystem II. *J. Comput. Biol. 2012,* 20, 1969–1980.

(69) Retegan, M.; Pantazis, D. A. Differences in the Active Site of Water Oxidation among Photosynthetic Organisms. *J. Am. Chem. Soc.* 2017, 139, 14340–14343.

(70) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. Molecular Dynamics with Coupling to an External Bath. *J. Chem. Phys. 1984,* 81, 3684–3690.

(71) Parrinello, M.; Rahman, A. Polymorphic Transitions in Single Crystals: A New Molecular Dynamics Method. *J. Appl. Phys.* 1981, 52, 7182–7190.

(72) Darden, T.; York, D.; Pedersen, L. Particle mesh Ewald: An N·log(N) method for Ewald sums in large systems. *J. Chem. Phys.* 1993, 98, 10089–10092.
Mechanics Study of the Tyrosine Residue, TyrD, of Photosystem II.

17670. α Hydrogen-Bond Network Within ENDOR Spectroscopy and DFT Calculations: Evidence for the Ribonucleotide Reductase.

J. Am. Chem. Soc. 2015, 137, 289–298.

(126) Argirevic, T.; Riplinger, C.; Stubbe, J.; Neese, F.; Bennati, M. ENDOR Spectroscopy and DFT Calculations: Evidence for the Hydrogen-Bond Network Within α2 in the PCET of E. coli Ribonuclease Reductase. J. Am. Chem. Soc. 2012, 134, 17661–17670.

(127) Hart, R.; O’Malley, P. J. A Quantum Mechanics/Molecular Mechanics Study of the Tyrosine Residue, TyrD, of Photosystem II. Biochim. Biophys. Acta. 2010, 1797, 250–254.

(128) Mezzetti, A.; Maniero, A. L.; Brustolon, M.; Giacometti, G.; Brunel, L. C. A Tyrosyl Radical in an Irradiated Single Crystal of N-Acetyl-l-tyrosine Studied by X-band cw-EPR, High-Frequency EPR, and ENDOR Spectroscopies. J. Phys. Chem. A 1999, 103, 9636–9643.

(129) Hulsebosch, R. J.; van den Brink, J. S.; Nieuwenhuis, S. A. M.; Gast, P.; Raap, J.; Lugtenburg, J.; Hoff, A. J. Electronic Structure of the Neutral Tyrosine Radical in Frozen Solution. Selective 2H-, 13C-, and 17O-Isotope Labeling and EPR Spectroscopy at 9 and 35 GHz. J. Am. Chem. Soc. 1997, 119, 8685–8694.

(130) Hoganson, C. W.; Sahlin, M.; Sjöberg, B.-M.; Babcock, G. T. Electron Magnetic Resonance of the Tyrosyl Radical in Ribonuclease Reductase from Escherichia coli. J. Am. Chem. Soc. 1996, 118, 4672–4679.

(131) Campbell, K. A.; Peloquin, J. M.; Diner, B. A.; Tang, X.-S.; Chisholm, D. A.; Britt, R. D. The α-Nitrogen of D2 Histidine 189 is the Hydrogen Bond Donor to the Tyrosine Radical YD* of Photosystem II. J. Am. Chem. Soc. 1997, 119, 4787–4788.

(132) Teutloff, C.; Pudollet, S.; Keßen, S.; Broser, M.; Zouni, A.; Bittl, R. Electronic Structure of the Tyrosine D Radical and the Water-Splitting Complex from Pulsed ENDOR Spectroscopy on Photosystem II Single Crystals. Phys. Chem. Chem. Phys. 2009, 11, 6715–6726.

(133) O’Malley, P. J. Hybrid Density Functional Studies of the Oxidation of Phenol–Imidazole Hydrogen-Bonded Complexes: A Model for Tyrosine Oxidation in Oxygenic Photosynthesis. J. Am. Chem. Soc. 1998, 120, 11732–11737.

(134) O’Malley, P. J.; Ellson, D. H, 13C and 17O Isotropic and Anisotropic Hyperfine Coupling Prediction for the Tyrosyl Radical Using Hybrid Density Functional Methods. Biochim. Biophys. Acta, Bioenerg. 1997, 1320, 65–72.

(135) Nieuwenhuis, S. A. M.; Hulsebosch, R. J.; Raap, J.; Gast, P.; Lugtenburg, J.; Hoff, A. J. Structure of the YD Tyrosine Radical in Photosystem II. Determination of the Orientation of the Phenoxyl Ring by Enantioselective Deuteration of the Methylene Group. J. Am. Chem. Soc. 1998, 120, 829–830.

(136) Rigby, S. E. J.; Nugent, J. H. A.; O’Malley, P. J. The Dark Stable Tyrosine Radical of Photosystem 2 Studied in Three Species Using ENDOR and EPR Spectroscopies. Biochemistry 1994, 33, 1734–1742.

(137) Warncke, K.; McCracken, J.; Babcock, G. T. Structure of the YD Tyrosine Radical in Photosystem II as Revealed by 1H Electron Spin Echo Envelope Modulation (ESEEM) Spectroscopic Analysis of Hydrogen Hyperfine Interactions. J. Am. Chem. Soc. 1994, 116, 7332–7340.

(138) Schloeder, E.; Çetin, M.; Lendzian, F. Temperature Dependence of the Oxidation Kinetics of TyrZ and TyrD in Oxygen-Evolving Photosystem II Complexes Throughout the Range from 320 to 5 K. Biochim. Biophys. Acta, Bioenerg. 2015, 1847, 1283–1296.

(139) Ahmadova, N.; Mamedov, F. Formation of Tyrosine Radicals in Photosystem II Under Far-Red Illumination. Photosynth. Res. 2018, 136, 93–106.

DOI: 10.1021/jacs.8b13123
J. Am. Chem. Soc. 2019, 141, 3217–3231