Factors that differentiate the H-bond strengths of water near the Schiff bases in bacteriorhodopsin and *Anabaena* sensory rhodopsin

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Running Title: H-bond strength of water in BR and ASR

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**Background:** Bacteriorhodopsin (BR) functions as a proton pump whereas *Anabaena* sensory rhodopsin (ASR) function as a photosensor.

**Results:** pKa of the conserved Asp residues near the Schiff base significantly differ between BR and ASR.

**Conclusion:** The O-D stretching frequencies for D2O is correlated with pKa(Asp).

**Significance:** The presence of a strongly H-bonded water results from the proton-pumping activity in BR.

**SUMMARY**

Bacteriorhodopsin (BR) functions as a light-driven proton pump, whereas *Anabaena* sensory rhodopsin (ASR) is believed to function as a photosensor, despite the high similarity in their protein sequences. In Fourier transform infrared (FTIR) spectroscopic studies, the lowest O-D stretch for D2O was observed at ~2200 cm⁻¹ in BR but was significantly higher in ASR (>2500 cm⁻¹), which was previously attributed to a water molecule near the Schiff base (W402) that is H-bonded to Asp85 in BR and Asp75 in ASR. We investigated the factors that differentiate the lowest O-D stretches of W402 in BR and ASR. Quantum mechanical/molecular mechanical (QM/MM) calculations reproduced the H-bond geometries of the crystal structures and the calculated O-D stretching frequencies were corroborated by the FTIR band assignments. The potential-energy profiles indicate that the smaller O-D stretching frequency in BR originates from the significantly higher pKa(Asp85) in BR relative to the pKa(Asp75) in ASR, which were calculated to be 1.5 and ~5.1, respectively. The difference is mostly due to the influences of Ala53, Arg82, Glu194-Glu204, and Asp212 on pKa(Asp85) in BR, and the corresponding residues Ser47, Arg72, Ser188-Asp198, and Pro206 on pKa(Asp75) in ASR. Because these residues participate in proton transfer pathways in BR but not in ASR, the presence of a strongly H-bonded water molecule near the Schiff base ultimately results from the proton-pumping activity in BR.

Bacteriorhodopsin (BR) functions as a light-driven proton pump and the driving force for its action is provided by photoisomerization of the all-trans retinal chromophore, which is covalently attached to Lys216 via the Schiff base, to 13-cis. This leads to proton transfer pathways that proceed toward the extracellular side via Asp96, the Schiff base, Asp85, and Glu204 (1-3). Asp 85 is located near the Schiff
base and serves as the counter ion. The presence of water molecules in this region had been suggested by Fourier transform infrared (FTIR) studies (4,5), and these were later confirmed as W401, W402, and W406 in the high-resolution crystal structure of the BR ground state (PDB code 1C3W; Figure 1) (6). The three water molecules form an H-bond network with Asp85, Arg82, Asp212, and the Schiff base. Among the three water molecules, the chemical properties of W402 are of particular interest because the water molecule is located at H-bond distances of 2.63 Å and 2.87 Å from the carboxyl O atom of Asp85 and the Schiff base N atom, respectively (Table 1). From FTIR analysis of various mutants in D2O, the lowest O-D stretching frequency of 2171 cm⁻¹ in BR was assigned to the O_W402–H…O_Asps bond, implying that this is the strongest H-bond in the network near the Schiff base (7). The band assignment presented in FTIR studies was also supported by previous computational studies by Hayashi and coworkers (8,9).

Water molecule W402 was also found in the crystal structure of Anabaena sensory rhodopsin (ASR) (PDB code 1X1O; Figure 1) (10). In contrast to the proton pumping of BR, ASR is believed to function as a photosensor. Despite the high similarity of their protein sequences, some key residues that are functionally important in the proton-pumping event in BR are not conserved in ASR: Asp96 in BR, which serves as a proton donor to the Schiff base, is replaced with Ser86 in ASR; Glu194 and Glu204 in BR, which is located in the terminal region of the proton transfer pathway, are replaced with Ser188 and Asp198, respectively. Although W402 is present in both BR (6) and ASR (10), Asp212 in BR is replaced with Pro206 in ASR (Figure 1). The absence of W401 and W406 in the corresponding H-bond network of ASR may be associated with the absence of an acidic residue corresponding to Asp212 in BR. FTIR studies have suggested that the O-D stretch in water molecules was only observed at >2500 cm⁻¹ in ASR (11), which is ~300 cm⁻¹ higher than the lowest O-D stretching frequency of 2171 cm⁻¹ in BR (7).

The significant difference in the lowest O-D stretching frequency implies that the H-bond properties of W402 are significantly different in BR and ASR. It has been established by FTIR that the lowest O-D stretching frequency of water can be found at less than 2400 cm⁻¹ in a number of proton-pumping rhodopsins (12). The same tendency also holds true for BR and ASR.

In the BR and ASR crystal structures, the angle defined by the Schiff base N, W402 O, and Asp85 O atoms (N_Lys...O_W402...O_Asps) differs significantly: the angle is 106° in BR (6) and 83° in ASR (10). Thus, it was proposed that the angle difference may differentiate the H-bond strength of the water molecules in BR and ASR (11). On the other hand, it also appears that the O_W402–H…O_Asps angle is more crucial than the N_Lys...O_W402...O_Asps angle to the energetics of the H bond since it involves a H atom. In general, the O_donor–H…O_acceptor angle strongly depends on the O_donor–O_acceptor distance, i.e., the O_donor–H…O_acceptor angle is more linear when the O_donor–O_acceptor bond is shorter (13).

In addition, the N_Lys...O_W402...O_Asps angle in the crystal structure can be directly altered in response to changes in the O_W402–H…O_Asps distance. Because of the nature of H bonds (14-17), the O_W402–H…O_Asps distance could also be predominantly determined by the pKₐ difference between the H-bond donor (W402) and acceptor (Asp) moieties. In general, a smaller pKₐ difference yields a short, symmetrical H bond (14-17), and the H atom of O_W402–H migrates toward the acceptor moiety, Asp. Unfortunately, the energetics of the O_W402–H…O_Asps bond, particularly the pKₐ difference between W402 and the Asp residue, remain unclear. Thus, it is essential to clarify how the O_W402–H…O_Asps distance is energetically determined in the BR and ASR crystal structures.

Here, we present calculated O-D stretching frequencies of D₂O near W402 in BR and ASR in the ground state using a large-scale quantum mechanical/molecular mechanical (QM/MM) approach. We also present the potential-energy profiles of the H bond between W402 and Asp85 in BR, and W402 and Asp75 in ASR, and demonstrate that the frequencies and H-bond properties are ultimately determined predominantly by the pKₐ values of H-bond acceptors Asp85 and Asp75. By calculating pKₐ(Asp85) in BR and pKₐ(Asp75) in ASR through solving the linear Poisson-Boltzmann equation with explicit consideration of the protonation states for all titratable residues, we are finally able to pinpoint the factors that cause a difference of ~300 cm⁻¹ between the O-D stretching frequencies of W402 in BR and ASR.

**EXPERIMENTAL PROCEDURES**

As demonstrated in our previous work with photoactive yellow protein (18), we employed the following systematic modeling procedure.

First, we constructed initial molecular models of BR and ASR using their crystal structures and adding hydrogen atoms. Second, to gain better understanding of the electronic structure of W402 and the associated H-bond network, we performed large-scale QM/MM calculations for the entire BR and ASR proteins.
gain insight into the QM/MM potential-energy profiles of the H bonds, O\textsubscript{W402}–H...O\textsubscript{Asp85} in BR and O\textsubscript{W402}–H...O\textsubscript{Asp75} in ASR, we calculated p\textsubscript{K\textsubscript{a}}\textsubscript{(Asp85)} in BR and p\textsubscript{K\textsubscript{a}}\textsubscript{(Asp75)} in ASR by simultaneously titrating all titratable residues in BR and ASR. Technical details of each modeling procedure are summarized below.

Atomic coordinates and charges. As a basis for the computations, the crystal structures of BR (PDB codes 1C3W (6) and 2NTU (19)) and ASR (PDB code 1XIO (10)) were used. To generate the initial geometries, the positions of the H atoms were energetically optimized with CHARMM (20) using the CHARMM22 force field. During this procedure, the positions of all non-H atoms were fixed and the standard charge states of all the titratable groups were maintained (i.e., basic and acidic groups were considered to be protonated and deprotonated, respectively). The Schiff base was considered to be protonated. Atomic partial charges of the amino acids and the Schiff base were adopted from the all-atom CHARMM22 (20) parameter set.

Protonation pattern and p\textsubscript{K\textsubscript{a}}. The present computation is based on the electrostatic continuum model created by solving the linear Poisson-Boltzmann equation with the MEAD program (21). To facilitate a direct comparison with previous computational results (e.g., Refs. (22,23)), identical computational conditions and parameters, such as atomic partial charges and dielectric constants, were used. To obtain absolute p\textsubscript{K\textsubscript{a}} values of target sites (e.g., p\textsubscript{K\textsubscript{a}}\textsubscript{(Asp85)} of BR), we calculated the difference in electrostatic energy between the two protonation states, protonated and deprotonated, in a reference model system using a known experimentally measured p\textsubscript{K\textsubscript{a}} value (e.g., 4.0 for Asp (24)). The difference in the p\textsubscript{K\textsubscript{a}} value of the protein relative to the reference system was added to the known reference p\textsubscript{K\textsubscript{a}} value. The experimentally measured p\textsubscript{K\textsubscript{a}} values employed as references were 7.2 for the Schiff base (25,26), 12.0 for Arg, 4.0 for Asp, 9.5 for Cys, 4.4 for Glu, 10.4 for Lys, 9.6 for Tyr (24), and 7.0 and 6.6 for the N\textsubscript{e} and N\textsubscript{\delta} atoms of His, respectively (27-29). All other titratable sites were fully equilibrated to the protonation state of the target site during the titration. The ensemble of the protonation patterns was sampled by a Monte Carlo (MC) method with Karlsberg (30). The dielectric constants were set to \epsilon_{\text{prot}} = 4 inside the protein and \epsilon_{\text{water}} = 80 for water. All computations were performed at 300 K, pH 7.0, and an ionic strength of 100 mM. The linear Poisson-Boltzmann equation was solved using a three-step grid-focusing procedure at resolutions of 2.5 Å, 1.0 Å, and 0.3 Å. The MC sampling yielded the probabilities [protonated] and [deprotonated] of the two protonation states of the molecule. The p\textsubscript{K\textsubscript{a}} value was evaluated using the Henderson-Hasselbalch equation. A bias potential was applied to obtain an equal amount of both protonation states ([protonated] = [deprotonated]), yielding the p\textsubscript{K\textsubscript{a}} value as the resulting bias potential.

QM/MM calculations. We employed an electrostatic embedding QM/MM scheme and used the Qsite (31) program code, as performed in previous studies (32). We employed the restricted DFT method with B3LYP functional and LACVP** basis sets. The geometries were refined using a constrained QM/MM optimization, whereby the coordinates of the heavy atoms in the surrounding MM region were fixed to the original X-ray coordinates, whereas those of the H atoms in the MM region were optimized with the OPLS-2005 force field. To investigate the energetics of the entire H-bond network, the QM region was defined as follows (Figure 2): (a) W402 and the residues/groups in the same H-bond network, i.e., W401, W406, Tyr57, Arg82, Asp85, Ser85, Thr89, Tyr185, Asp212, Lys216 (the Schiff base), and retinal for BR; and (b) W402, and the residues/groups in the same H-bond network, i.e., Tyr11, Tyr51, Asp75, Trp76, Thr79, Ser47, the backbone of Phe202, Lys210 (the Schiff base), and retinal for ASR. Other residues that are not in the H-bond network of W402 (e.g., Trp86 in BR is not involved in the same H-bond network, even though the residue corresponds to Trp76 in ASR) were approximated by the MM force field. The resulting QM/MM optimized geometries are listed in Table S1 in the SI. The potential-energy profile of the H bond was obtained in the following manner: first, we optimized the geometry without constraints using QM/MM and used the resulting geometry as the initial geometry for the subsequent steps. Next, we moved the H atom from the H-bond donor atom (O\textsubscript{donor}) to the acceptor atom (O\textsubscript{acceptor}) by 0.05 Å, optimized the geometry by constraining the O\textsubscript{donor}–H and O\textsubscript{acceptor}...H distances, and then calculated the energy of the resulting geometry. This procedure was repeated until the H atom reached the O\textsubscript{acceptor} atom. After obtaining the stable geometry of the QM fragment, we calculated the O-D stretching frequencies of W401, W402, and W406 in BR, and of W402 in ASR. The frequency calculations were performed using a numerical differentiation method at the same level as the QM/MM geometry optimization. The calculated frequencies were scaled using a standard factor of 0.9614 for B3LYP (33).

Calculation of the O-D stretching frequencies of D\textsubscript{2}O from an empirical equation. The O-D stretching frequencies of D\textsubscript{2}O were also evaluated on the basis of the resultant QM/MM optimized geometries by using the correlation of stretching frequency with respect to bond distance of the O\textsubscript{donor}–...O\textsubscript{acceptor}...H...O\textsubscript{donor}...H distance.
D...O{acceptor} bond, as previously proposed by Mikenda (34). This correlation was empirically expressed with the O-D stretching frequency, \(\nu_{O-D}\), and the O{donor}...O{acceptor} bond distance, \(r_{O-O}\), by

\[
\nu_{O-D} [\text{cm}^{-1}] = 2727 - A \exp(-B r_{O-O} [\text{Å}]),
\]

(eq. 1)

where \(A = 2.11 \times 10^6\) and \(B = 3.23\) for the O{water}...D{water}...O{acceptor} bond (34). Note that these parameters were derived from spectroscopic and X-ray diffraction data for 61 different solid deuterates, e.g., CaSO\(_4\)•2D\(_2\)O and MnCl\(_2\)•6D\(_2\)O (34).

To predict the frequencies, eq. 1 requires only the O{donor}...O{acceptor} distance, which can be preferentially taken from the QM/MM optimized geometry. Remarkably, the O-D stretching frequencies calculated by a QM/MM numerical differentiation method were reasonably well-reproduced using eq. 1 solely from the optimized O{donor}...O{acceptor} distance, particularly for frequencies \(<2500\,\text{cm}^{-1}\) (Table 2b).

Notably, eq. 1 indicates that the O-D stretching frequency of 2500 cm\(^{-1}\) corresponds to the O{acceptor}...O{donor} distance of ~2.8 Å. The deviation from the values calculated with the QM/MM numerical differentiation method appears to be more pronounced for frequencies \(>2500\,\text{cm}^{-1}\) (Figure 3). Because the original parameters were derived from solid hydrates (34), the larger deviation for weak H bonds at these frequencies may be due to H-bond patterns or mode couplings specific to the protein environments.

RESULTS

H-bond geometries near W402 in BR. The O-D stretching frequencies of D\(_2\)O predominantly depend on the H-bond geometry, particularly the H bond donor-acceptor distance (O{donor}...O{acceptor}) (34). If the assumed H-bond pattern is not correct, the resulting QM/MM optimized geometries significantly deviate from the original crystal structure. Thus, although the QM/MM optimized geometries (especially the heavy atom positions) are not always consistent with the crystal structures, it is necessary to carefully evaluate the H-bond pattern with respect to the atomic coordinates of the crystal structures prior to frequency calculations.

In BR, two H-bond patterns are geometrically possible for the H-bond network of W402 (Figure 4). A model previously presented from FTIR studies (7) suggests that an H atom of W401 has no H-bond acceptor (model 1), while an alternative model suggests that an H atom of W406 has no H-bond acceptor (model 2). The QM/MM optimized geometries of the two models appear to represent the actual H bond patterns of the crystal structures because of the low RMSD (0.17–0.19 Å, Table 1), implying that the two models probably coexist as tautomers in the actual BR protein environment.

Model 1 resembles the QM/MM geometry reported by Hayashi and Ohmine (8). However, in their QM/MM geometry, the O{W402}...O{Asp212} distance was significantly elongated to 4.07 Å and the H bond is absent (8). This varies significantly from the crystal structures and the present QM/MM geometries (O{W402}...O{Asp212} = 2.85–3.01 Å, Table 2). In the reported QM/MM geometry, W402 forms a new H bond with another carboxyl O atom of Asp212 (Figure 4). Hence, it is to be considered that the band assignment reported by Hayashi and Ohmine (8) refers to another possible H-bond geometry that differs from the crystal structures.

The QM/MM geometries imply that model 1 can explain the O{donor}...O{acceptor} distances in the crystal structure provided by Luecke et al. (PDB code 1C3W) (6) more reasonably than model 2 (Table 1). On the other hand, model 2 appears to explain the O{donor}...O{acceptor} distances in the crystal structure determined by Lanyi et al. (PDB code 2NTU) (19) more reasonably, namely in terms of O{W402}...O{Asp85}, O{W402}...O{Asp212}, O{W401}...O{Asp85}, and O{Asp82}...O{W406} distances (Table 1). However, the small discrepancies could also be due to being artifacts of the crystallization process. Because the resolutions of the two crystal structures are 1.53 and 1.55 Å (which are essentially the same), both of the H-bond tautomers are likely to contribute to the O-D stretching frequencies measured by FTIR. If both tautomers are present, this might corroborate the rapid H/D exchange observed in this region.

Calculated O-D stretching frequencies in BR. In FTIR studies (7), the three H bonds O{W401}...H...O{Asp85}, O{W402}...H...O{Asp85}, and O{W406}...H...O{Asp212} were classified as being strong H bonds because of the assignment of low frequencies (Figure 4).

a) Model 1. In FTIR studies (7), the lowest frequency of 2171 cm\(^{-1}\) was assigned to O{W402}...H...O{Asp85}. However, in model 1, O{W402}...H...O{Asp85} was longer than O{W401}...H...O{Asp85} (Table 1), leading to a larger calculated O-D stretching frequency for O{W402}...H...O{Asp85} than O{W401}...H...O{Asp85} (Table 2a), as predicted from the empirical equation by Mikenda (eq. 1) (34).

In model 1, two mode couplings between O{W401}...H...O{Asp85} and O{W401}...H...O{Asp85}, and between O{W406}...H...O{Asp212} and O{W406}...H...O{W401} were observed. However, the latter coupling is not consistent with the FTIR assignments. In FTIR studies, H bonds O{W406}...
H...O_{Asp212} and O_{W406}...H...O_{W401} were assigned as strong (2292 cm\(^{-1}\)) and weak (2599 cm\(^{-1}\)), respectively (Table 2a) (7). Because mode couplings are unlikely to occur between a strong and weak H bond, model 1 fails to sufficiently explain the FTIR assignments.

\textbf{b) Model 2.} In contrast to model 1, model 2 can reasonably explain the strong H bond (2292 cm\(^{-1}\)) of O_{W406}...H...O_{Asp212} and the weak H bond (2636 cm\(^{-1}\)) of O_{W406}...H...O_{W401} that were assigned by FTIR studies (Table 2b). Furthermore, the lowest calculated frequency was observed for O_{W402}...H...O_{Asp85}, which is also consistent with FTIR studies (7). Significantly high correlations of the frequencies predicted from eq. 1 suggest that assignment of the O-D stretching frequencies on the basis of model 2 is also justified by the H-bond geometry. Note that no remarkable mode couplings were observed for model 2 in the QM/MM calculations.

In the following section, further analysis of BR will be discussed on the basis of model 2 using the crystal structure by Luecke et al. (PDB code 1C3W) (6) unless otherwise specified.

**H-bond geometries near W402 and O-D stretching frequencies in ASR.** In contrast to BR, the ASR crystal structure only possesses a single water molecule, W402, near the Schiff base (Figure 4). The H-bond geometry of the crystal structure was reasonably reproduced by the QM/MM geometry optimization (Table 3). In the QM/MM calculations, the lowest O-D stretching frequency of 2376 cm\(^{-1}\) in ASR, which was found for O_{W402}...H...O_{Asp75} (Table 4), was significantly larger (by ~300 cm\(^{-1}\)) than that for the corresponding O_{W402}...H...O_{Asp85} in BR (2078 cm\(^{-1}\), Table 2b). In FTIR studies, the O-D stretching of D_{2}O was only observed in the >2500 cm\(^{-1}\) region for ASR (11), which was also ~300 cm\(^{-1}\) higher than the lowest frequency of 2171 cm\(^{-1}\) in BR (7). Since the main purpose of the present study is to understand the reason for the significant discrepancy between the lowest frequencies of D_{2}O (assumed as W402 (11)) of BR and ASR, these computational results are sufficiently accurate for our purpose to describe the chemical properties of W402.

**DISCUSSION**

**Understanding frequencies from energetics.**

**Significant differences between the H-bond energy profiles of BR and ASR.** To identify the origin of the difference in O-D stretching frequencies of W402, we analyzed the potential-energy profiles of H-bonds O_{W402}...H...O_{Asp85} in BR and O_{W402}...H...O_{Asp75} in ASR by altering the H atom position along the O_{W402}...O_{Asp85/75} bond (Figure 5). In both H bonds, the energy minimum was found at the W402 moiety rather than at the Asp moiety, suggesting that in the ground state, W402 exists as H_{2}O in the presence of deprotonated Asp in BR and ASR.

On the other hand, the potential-energy profiles of the Asp moiety were significantly different for BR and ASR. For an H atom along the O_{W402}...O_{Asp85/75} bond, the energy of the Asp85 moiety of BR is significantly lower than that of the Asp75 moiety of ASR. Thus, it is more favorable for an H atom of O_{W402}...H to be at the Asp moiety in BR than in ASR, indicating that pK_{a}(Asp) is significantly higher for BR than ASR (arrow 1 in Figure 5). As clearly shown in Figure 5, the lower energy near the Asp85 moiety in BR leads to greater broadening of the potential-well width near W402 toward Asp85, which corresponds to an elongation of O_{W402}...H. Thus, the H atom of O_{W402}...H migrates toward the H-bond acceptor Asp more in BR than in ASR (arrow 2 in Figure 5). It should be noted that the longer O_{W402}...H corresponds to a smaller O-D stretching frequency. It is also obvious from the width of the entire well of the potential-energy profiles that the O_{W402}...O_{Asp85} length in BR (~2.6 Å, Table 1), which is shorter than the O_{W402}...O_{Asp75} length in ASR (~2.7 Å, Table 3), is a result of the smaller pK_{a} difference between W402 and the Asp residue in BR relative to the corresponding difference in ASR (arrow 3 in Figure 5).

Thus, from the potential-energy profiles (Figure 5), the difference of ~300 cm\(^{-1}\) in the O-D stretching frequency is mainly due to the difference between the pK_{a} values for the Asp residues in BR and ASR.

**pK_{a}(Asp85) in BR and pK_{a}(Asp75) in ASR**

**Factors that differentiate pK_{a}(Asp) of BR and ASR.** By solving the linear Poisson–Boltzmann equation, we calculated pK_{a}(Asp85) in BR and pK_{a}(Asp75) in ASR to be 1.5 and ~5.1, respectively, in the presence of the protonated Schiff base (Table 5). The calculated pK_{a}(Asp85) of 1.5 is consistent with an experimentally measured pK_{a}(Asp85) of 2.2–2.6 in the presence of the protonated Schiff base (2,35). Although pK_{a}(Asp75) is not known, the significantly low pK_{a}(Asp85) in BR relative to pK_{a}(Asp75) in ASR is consistent with the QM/MM-calculated potential-energy profiles (Figure 5). The significantly low pK_{a}(Asp75) in ASR appears to be consistent with the fact that Asp75 remains deprotonated throughout the photocycle (36).
**a) Asp212 in BR.** The most significant difference observed between $pK_a$(Asp85) and $pK_a$(Asp75) was induced by the substitution of Asp212 in BR for Pro206 in ASR (Figure 6), which is responsible for the $pK_a$ difference of ~6 (Table 5). Because Asp212 is an H-bond acceptor for $\text{O}_{\text{W}402}-\text{H}$ and is thus far the most proximal negative charge to Asp85 (5.1 Å), Asp212 upshifts $pK_a$(Asp85) by the largest amount among all residues in BR (Table 5).

It is widely known that both Asp85 and Asp212 are ionized in the ground state (2,37,38). However, the deprotonated state of Asp212 is more energetically stable than the deprotonated state of Asp85, as seen in the lower $pK_a$(Asp212) of ~2.0 (Table 6); this is consistent with previous $pK_a$ assignments of <1 for Asp212 (39,40) or $pK_a$(Asp85) < $pK_a$(Asp212) (41,42). The deprotonated state of Asp212 was facilitated by H-bond donations from Tyr57 (decreasing $pK_a$(Asp212) by 3.4) and Tyr185 (decreasing $pK_a$(Asp212) by 3.3). In addition, Arg82 is closer to Asp212 (3.8 Å) than Asp85 (6.6 Å) (Figure 6), stabilizing the ionized state of Asp212 more effectively than Asp85 (increasing $pK_a$ by ~6, Table 6).

Although the most significant difference between $pK_a$(Asp85) and $pK_a$(Asp75) induced by the substitution of Asp212 in BR for Pro206 in ASR is remarkable, this is not sufficient to entirely explain the difference between $pK_a$(Asp85) and $pK_a$(Asp75) (see below), which is in agreement with mutational studies of ASR mutant P206D (43).

**b) Ser47 in ASR.** Ser47 donates an H bond to Asp75 in ASR, decreasing $pK_a$(Asp75) by 3.2, whereas the corresponding residue in BR is the non-polar Ala53 (Table 5). This was responsible for a $pK_a$ difference of 2.7 between Asp85 and Asp75 (Table 5).

**c) Glu194-Glu204 pair in BR.** Another remarkable $pK_a$(Asp85) upshift in BR originates from the Glu194-Glu204 moiety near the terminal region of the proton transfer pathway (2,3) (Figure 6). It is likely that the two acidic residues share a proton. The corresponding acidic residue pair is absent in ASR, thereby contributing slightly to the larger $pK_a$(Asp) in BR relative to that in ASR (~0.5 $pK_a$ units, Table 5). In the present study, Glu204 is protonated and Glu194 is deprotonated when Ser193 donates an H bond to Glu194, which is consistent with the previously reported $pK_a$(Glu204) of ~9 (35,44,45).

Nevertheless, the geometry of the crystal structure also suggests that protonated Glu194 and deprotonated Glu204 are almost equally possible when Ser193 donates an H bond to Glu204, as suggested by Gerwert et al. (46). Thus, it would be more plausible to consider the two acidic residues as a pair that possesses ~1 H⁺ as concluded in previous QM/MM simulations (47). This resembles a pair of acidic residues, Glu-L212 and Asp-L213, in the proton transfer pathway of photosynthetic reaction centers, which has been interpreted as sharing ~1 H⁺ (48-50). Deprotonated Glu194 contributes to an increase in $pK_a$(Asp85) of ~1 (Table 5); this therefore corroborates the linkage between $pK_a$(Asp85) and $pK_a$(Glu204) previously reported in mutant BR studies (35) if we consider Glu194-Glu204 as a pair of acidic residues sharing ~1 H⁺.

**d) Orientation of Arg82 in BR and Arg72 ASR.** Arg82 was found to contribute to an increase in $pK_a$(Asp85) of 3.1 in BR (Table 5), which is in agreement with a similar increase (~4.5) reported for BR mutants R82Q and R82A (51,52). Irrespective of the conservation of Arg82/Arg72 in BR/ASR, their influence on $pK_a$(Asp85) and $pK_a$(Asp75) significantly differ by ~2 because the side chain of Arg72 is oriented away from the Schiff base and toward the extracellular side in ASR ($O_{\text{Asp75}}-N_{\text{Arg72}} = 9.6$ Å) (10), which is the opposite of the orientation of Arg82 in BR ($O_{\text{Asp85}}-N_{\text{Arg82}} = 6.6$ Å) (6) (Figure 6). Thus, the differing orientations of the Arg side chains decrease the $pK_a$(Asp) difference between BR and ASR (Table 6).

In summary, the significantly large $pK_a$(Asp85) in BR relative to $pK_a$(Asp75) in ASR is largely due to the presence of Asp212 near Asp85 in BR and the absence of the corresponding acidic residue in ASR. In addition, the donation of an H-bond from Ser47 to Asp75 lowers the $pK_a$(Asp75) in ASR by facilitating deprotonation. The corresponding polar residue is absent in BR; this upshifts $pK_a$(Asp85) in BR relative to $pK_a$(Asp75) in ASR. The Glu194-Glu204 pair is located near the terminal region of the proton transfer pathway in BR, whereas only a single acidic residue is located near the corresponding position in ASR. This also upshifts $pK_a$(Asp85) in BR relative to $pK_a$(Asp75) in ASR.

**CONCLUSIONS**

QM/MM calculations revealed that the heavy atom positions in the BR crystal structures could be explained by one of two models. Model 2 can reasonably explain the strong H bond (2292 cm⁻¹) of $\text{O}_{\text{W406}}-\cdots\text{O}_{\text{Asp212}}$ and the weak H bond (2636 cm⁻¹) of $\text{O}_{\text{W406}}-\cdots\text{O}_{\text{W401}}$, as previously assigned by FTIR (Table 2b). In model 2, the lowest calculated frequency was observed at $\text{O}_{\text{W402}}-\cdots\text{O}_{\text{Asp85}}$, which is also consistent with FTIR studies. Essentially the same frequencies were also predicted solely from the
The potential-energy profiles of two H bonds, $O_{W402}-H...O_{Asp85}$ in BR and $O_{W402}-H...O_{Asp75}$ in ASR, demonstrate that the significant $pK_a$ difference between Asp85 in BR and Asp75 in ASR is ultimately responsible for the difference of $\sim$300 cm$^{-1}$ in the O-D stretching frequency of W402 between BR and ASR (Figure 5). Electrostatic calculations resulted in a $pK_a(Asp85)$ of 1.5 in BR and a $pK_a(Asp75)$ of $\sim$5.1 in ASR (Table 5). The $pK_a$ difference is mainly due to differences in the influences of Ala53, Arg82, Glu194, and Asp212 on Asp85 in BR, and the corresponding residues Ser47, Arg72, Ser188, and Pro206 on Asp75 in ASR, indicating the presence of long-range electrostatic interactions along the proton transfer pathways (53). A strongly H-bonded water near the Schiff base, which results from electrostatic interactions between Asp85 and these acidic residues in BR, could play a key role in proton transfer.

REFERENCES

1. Lanyi, J. K. (1998) Understanding structure and function in the light-driven proton pump bacteriorhodopsin. *J Struct Biol* **124**, 164-178
2. Balashov, S. P. (2000) Protonation reactions and their coupling in bacteriorhodopsin. *Biochim Biophys Acta* **1460**, 75-94
3. Kandori, H. (2000) Role of internal water molecules in bacteriorhodopsin. *Biochim Biophys Acta* **1460**, 177-191
4. Maeda, A., Sasaki, J., Yamazaki, Y., Needleman, R., and Lanyi, J. K. (1994) Interaction of aspartate-85 with a water molecule and the protonated Schiff base in the L intermediate of bacteriorhodopsin: a Fourier-transform infrared spectroscopic study. *Biochemistry* **33**, 1713-1717
5. Kandori, H., Yamazaki, Y., Sasaki, J., Needleman, R., Lanyi, J. K., and Maeda, A. (1995) Water-mediated proton transfer in proteins: an FTIR study of bacteriorhodopsin. *J Am Chem Soc* **117**, 2118-2119
6. Luecke, H., Schobert, B., Richter, H. T., Cartailler, J. P., and Lanyi, J. K. (1999) Structure of bacteriorhodopsin at 1.55 A resolution. *J Mol Biol* **291**, 899-911
7. Shibata, M., and Kandori, H. (2005) FTIR studies of internal water molecules in the Schiff base region of bacteriorhodopsin. *Biochemistry* **44**, 7406-7413
8. Hayashi, S., and Ohmine, I. (2000) Proton transfer in bacteriorhodopsin: structure, excitation, IR spectra, and potential energy surface analyses by an ab initio QM/MM method. *J Phys Chem B* **104**, 10678-10691
9. Hayashi, S., Tajkhorshid, E., Kandori, H., and Schulten, K. (2004) Role of hydrogen-bond network in energy storage of bacteriorhodopsin's light-driven proton pump revealed by ab initio normal-mode analysis. *J Am Chem Soc* **126**, 10516-10517
10. Vogeley, L., Sineshchekov, O. A., Trivedi, V. D., Sasaki, J., Spudich, J. L., and Luecke, H. (2004) Anabaena sensory rhodopsin: a photochromic color sensor at 2.0 Å. *Science* **306**, 1390-1393
11. Furutani, Y., Kawanabe, A., Jung, K. H., and Kandori, H. (2005) FTIR spectroscopy of the all-trans form of Anabaena sensory rhodopsin at 77 K: hydrogen bond of a water between the Schiff base and Asp75. *Biochemistry* **44**, 12287-12296
12. Kandori, H. (2010) in Hydrogen bonds of protein-bound water molecules in rhodopsins, In Hydrogen bonding and transfer in the excited state (Han, K.-L. and Zhao, G., Eds.), pp 377-391, John-Wiley & Sons Ltd., West Sussex
13. Frey, P. A. (2006) in *Isotope Effects in Chemistry and Biology* (*Kohen, A. and Limbach, H.-H.*, Eds.), pp 975-993, CRC press, Boca Raton, FL
14. Cleland, W. W., and Kreevoy, M. M. (1994) Low-barrier hydrogen bonds and enzymatic catalysis. *Science* **264**, 1887-1890
15. Frey, P. A., Whitt, S. A., and Tobin, J. B. (1994) A low-barrier hydrogen bond in the catalytic triad of serine proteases. Science 264, 1927-1930
16. Perrin, C. L., and Nielson, J. B. (1997) "Strong" hydrogen bonds in chemistry and biology. Annu Rev Phys Chem 48, 511-544
17. Schutz, C. N., and Warshel, A. (2004) The low barrier hydrogen bond (LBHB) proposal revisited: the case of the Asp...His pair in serine proteases. Proteins 55, 711-723
18. Saito, K., and Ishikita, H. (2012) Energetics of short hydrogen bonds in photoactive yellow protein. Proc Natl Acad Sci U S A 109, 167-172
19. Lanyi, J. K., and Schober, B. (2007) Structural changes in the L photointermediate of bacteriorhodopsin. J Mol Biol 365, 1379-1392
20. Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S., and Karplus, M. (1983) CHARMM: a program for macromolecular energy minimization and dynamics calculations. J. Comput. Chem. 4, 187-217
21. Bashford, D., and Karplus, M. (1990) pK_a's of ionizable groups in proteins: atomic detail from a continuum electrostatic model. Biochemistry 29, 10219-10225
22. Ishikita, H., and Knapp, E.-W. (2007) Protonation states of ammonia/ammonium in the hydrophobic pore of ammonia transporter protein AmtB. J. Am. Chem. Soc. 129, 1210-1215
23. Ishikita, H. (2010) Origin of the pK_a shift of the catalytic lysine in acetoacetate decarboxylase. FEBS Lett 584, 3464-3468
24. Nozaki, Y., and Tanford, C. (1967) Acid-base titrations in concentrated guanidine hydrochloride. Dissociation constants of the guanidinium ion and of some amino acids. J Am Chem Soc 89, 736-742
25. Bassov, T., and Sheves, M. (1986) Alteration of pK_a of the bacteriorhodopsin protonated Schiff base. A study with model compounds. Biochemistry 25, 5249-5258
26. Rouss, I., Friedman, N., Sheves, M., and Ottolenghi, M. (1995) pK_a of the protonated Schiff base and aspartic 85 in the bacteriorhodopsin binding site is controlled by a specific geometry between the two residues. Biochemistry 34, 12059-12065
27. Tanokura, M. (1983) 1H-NMR study on the tautomerism of the imidazole ring of histidine residues. I. Microscopic pK values and molar ratios of tautomers in histidine-containing peptides. Biochim Biophys Acta 742, 576-585
28. Tanokura, M. (1983) 1H-NMR study on the tautomerism of the imidazole ring of histidine residues. II. Microenvironments of histidine-12 and histidine-119 of bovine pancreatic ribonuclease A. Biochim Biophys Acta 742, 586-596
29. Tanokura, M. (1983) 1H nuclear magnetic resonance titration curves and microenvironments of aromatic residues in bovine pancreatic ribonuclease A. J Biochem 94, 51-62
30. Rabenstein, B., and Knapp, E. W. (2001) Calculated pH-dependent population and protonation of carbon-monoxo-myoglobin conformers. Biophys J 80, 1141-1150
31. QSite, version 5.6, Schrödinger, LLC, New York, NY, 2010.
32. Saito, K., Ishida, T., Sugiura, M., Kawakami, K., Umena, Y., Kamiya, N., Shen, J.-R., and Ishikita, H. (2011) Distribution of the cationic state over the chlorophyll pair of photosystem II reaction center. J. Am. Chem. Soc. 133, 14379-14388
33. Scott, A. P., and Radom, L. (1996) Harmonic vibrational frequencies: an evaluation of Hartree–Fock, Møller–Plesset, quadratic configuration interaction, density functional theory, and semiempirical scale factors. J Phys Chem 100, 16502-16513
34. Mikenda, W. (1986) Stretching frequency versus bond distance correlation of O-D(H)...Y (Y = N, O, S, Se, Cl, Br, I) hydrogen bonds in solid hydrates. J Mol Struct 147, 1-15
35. Richter, H. T., Brown, L. S., Needleman, R., and Lanyi, J. K. (1996) A linkage of the pK_a's of asp-85 and glu-204 forms part of the reprotonation switch of bacteriorhodopsin. Biochemistry 35, 4054-4062
36. Bergo, V. B., Ntefidou, M., Trivedi, V. D., Amsden, J. J., Kralj, J. M., Rothschild, K. J., and Spudich, J. L. (2006) Conformational changes in the photocycle of Anabaena sensory rhodopsin: absence of the Schiff base counterion protonation signal. J Biol Chem 281, 15208-15214
37. Song, Y., Mao, J., and Gunner, M. R. (2003) Calculation of proton transfers in Bacteriorhodopsin bR and M intermediates. *Biochemistry* 42, 9875-9888
38. Bombarda, E., Becker, T., and Ullmann, G. M. (2006) Influence of the membrane potential on the protonation of bacteriorhodopsin: insights from electrostatic calculations into the regulation of proton pumping. *J Am Chem Soc* 128, 12129-12139
39. Metz, G., Siebert, F., and Engelhard, M. (1992) Asp85 is the only internal aspartic acid that gets protonated in the M intermediate and the purple-to-blue transition of bacteriorhodopsin. A solid-state 13C CP-MAS NMR investigation. *FEBS Lett* 303, 237-241
40. Balashov, S. P., Govindjee, R., Imasheva, E. S., Misra, S., Ebrey, T. G., Feng, Y., Crouch, R. K., and Menick, D. R. (1995) The two pKa's of aspartate-85 and control of thermal isomerization and proton release in the arginine-82 to lysine mutant of bacteriorhodopsin. *Biochemistry* 34, 8820-8834
41. Bashford, D., and Gerwert, K. (1992) Electrostatic calculations of the pKa values of ionizable groups in bacteriorhodopsin. *J Mol Biol* 224, 473-486
42. Sampogna, R. V., and Honig, B. (1994) Environmental effects on the protonation states of active site residues in bacteriorhodopsin. *Biophys J* 66, 1341-1352
43. Choi, A. R., Kim, S. Y., Yoon, S. R., Bae, K., and Jung, K. H. (2007) Substitution of Pro206 and Ser86 residues in the retinal binding pocket of Anabaena sensory rhodopsin is not sufficient for proton pumping function. *J Microbiol Biotechnol* 17, 138-145
44. Dioumaev, A. K., Richter, H. T., Brown, L. S., Tanio, M., Tuzi, S., Saito, H., Kimura, Y., Needleman, R., and Lanyi, J. K. (1998) Existence of a proton transfer chain in bacteriorhodopsin: participation of Glu-194 in the release of protons to the extracellular surface. *Biochemistry* 37, 2496-2506
45. Rammelsberg, R., Huhn, G., Lubben, M., and Gerwert, K. (1998) Bacteriorhodopsin's intramolecular proton-release pathway consists of a hydrogen-bonded network. *Biochemistry* 37, 5001-5009
46. Ishikita, H., Morra, G., and Knapp, E.-W. (2003) Redox potential of quinones in photosynthetic reaction centers from *Rhodobacter sphaeroides*: dependence on protonation of Glu-L212 and Asp-L213. *Biochemistry* 42, 3882-3892
47. Otto, H., Marti, T., Holz, M., Mogi, T., Stern, L. J., Engel, F., Khorana, H. G., and Heyn, M. P. (1990) Substitution of amino acids Asp-85, Asp-212, and Arg-82 in bacteriorhodopsin affects the proton release phase of the pump and the pK of the Schiff base. *Proc Natl Acad Sci U S A* 87, 1018-1022
48. Calimet, N., and Ullmann, G. M. (2004) The influence of a transmembrane pH gradient on protonation probabilities of bacteriorhodopsin: the structural basis of the back-pressure effect. *J Mol Biol* 339, 571-589
FOOTNOTES
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FIGURE LEGENDS

Figure 1. Overview of the BR (PDB code 1C3W) and ASR (PDB code 1XIO) structures. Red spheres indicate O atoms of water molecules. Yellow arrows indicate proton transfer pathways.

Figure 2. Residues and groups included in the QM regions for (a) BR and (b) ASR. Dotted lines indicate hydrogen bonds. (c) Geometry of moieties near Lys210 in ASR before (magenta) and after (yellow) the QM/MM optimization.

Figure 3. The geometric correlation of the O-D stretching frequencies with the $O_{\text{acceptor}} \cdots H - O_{\text{donor}}$ bond of H$_2$O (correlation with the $O_{\text{donor}} - O_{\text{acceptor}}$ distance). Each □ represents the $O_{\text{donor}} - O_{\text{acceptor}}$ distance of the QM/MM optimized geometries (Tables 1 and 3) and the O-D stretching frequency calculated by a QM/MM numerical differentiation method (Tables 2 and 4). The solid curve was determined using an empirical equation (eq. 1) proposed by Mikenda (34).

Figure 4. H-bond geometries of water molecules near the Schiff base in BR and ASR. Pink arrows in model 1 indicate mode couplings of the two O-D bonds. Red dotted lines indicate “strong H bonds” reported in FTIR studies (7).

Figure 5. Potential-energy profiles along the proton transfer coordinates of $O_{\text{W402}} - H \cdots O_{\text{Asp85}}$ in BR (black solid line) and $O_{\text{W402}} - H \cdots O_{\text{Asp75}}$ in ASR (blue solid line). $\Delta E$ describes the difference in energy relative to the energy minimum. Boxed arrows indicate the shifts in the potential energy curve accompanied by the $pK_a$(Asp) change from ASR to BR. The marginal energy drop near 1.74 Å in $O_{\text{W402}} - H$ of ASR was due to an alteration of the H-bond pattern; below 1.74 Å, Ser47 donates an H bond to the carboxyl O atom of Asp75, which is simultaneously H-bonded by W402. At 1.74 Å, the hydroxyl H atom is oriented toward another carboxyl O atom of Asp75 due to the unusual proximity of the H atom of W402 to Asp75.

Figure 6. Residues that contribute to differences between $pK_a$(Asp) of (a) BR and (b) ASR.
Table 1. Comparison of the H-bond geometries near W402 in the BR crystal structures (PDB ID codes 1C3W at 1.55 Å resolution and 2NTU at 1.53 Å resolution), in the QM/MM geometry by Hayashi and Ohmine (8), and in the present study (models 1 and 2, Figure 4). Distances in Å, angles in °. n.d. = not determined. An underlined number indicates a significant deviation from the distance in the crystal structure. See Table S1 in the SI for the atomic coordinates of the QM/MM geometries.

| Donor…acceptor | 1C3W | 2NTU |
|----------------|------|------|
|                | QM/MM Hayashi & Ohmine<sup>a</sup> (model 1) | QM/MM (model 1) | QM/MM (model 2) | QM/MM (model 1) | QM/MM (model 2) |
| W402…Asp85    | 2.63 | 2.58 | 2.61 | 2.57 | 2.52 | 2.63 | 2.59 |
| W402…Asp212   | 2.85 |        | 2.92 | 2.99 | 3.01 | 2.90 | 2.95 |
| W401…Asp85    | 2.59 | 2.63 | 2.51 | 2.64 | 2.69 | 2.59 | 2.68 |
| (OH without acceptor) <sup>b</sup> | (W401) | (W401) | (W406) | (W401) | (W406) |
| W406…W401     | 2.75 | 2.77 | 2.76 | 3.06 | 2.69 | 2.78 | 3.07 |
| W406…Asp212   | 2.75 | 2.78 | 2.64 | 2.64 | 2.81 | 2.73 | 2.65 |
| Lys216…W402   | 2.87 | 2.63 | 2.78 | 2.76 | 2.94 | 2.80 | 2.77 |
| Arg82…W406    | 2.49 | 2.72 | 2.74 | 2.87 | 2.87 | 2.73 | 2.86 |
| RMSD           | n. d. | n. d. | 0.19 | 0.18 | n. d. | 0.17 | 0.18 |
| angle(N<sub>Lys216</sub>…O<sub>W402</sub>…O<sub>Asp85</sub>) | 106 | n. d. | 108 | 108 | 111 | 111 | 111 |

<sup>a</sup> See Ref. (8).

<sup>b</sup> A water molecule in which one of the two O-H bonds does not possess the H-bond acceptor molecule; W401 in model 1 and W402 in model 2 (Figure 4).
Table 2. O–D stretching frequencies (in cm⁻¹) of the water molecules near W402 in BR. Connecting lines indicate the presence of coupling between the two modes. n.d. = not determined. $R^2$ = correlation coefficient.

(a) Model 1.

|                | FTIR-1$^a$ | Hayashi & Ohmine$^b$ (model 1) | QM/MM (model 1) | empirical eq.$^c$ (model 1) | QM/MM (model 1) | empirical eq.$^c$ (model 1) |
|----------------|------------|--------------------------------|----------------|--------------------------|----------------|--------------------------|
| W402…Asp85    | 2171       | 2221                           | 2258           | 2247                     | 2293           |
|                | (lowest)   |                                |                |                          |                |
| W402…Asp212   | 2636       | 2663                           | 2564           | 2646                     | 2548           |
| W401…Asp85    | 2323       | 2133                           | 2173           | 2169                     | 2229           |
|                | (lowest)   | (lowest)                       | (lowest)       | (lowest)                 | (lowest)       |
| W401…(n.d.)   | 2690       | 2703                           | 2727           | 2704                     | 2727           |
| W406…W401     | 2599       | 2515                           | 2454           | 2514                     | 2463           |
| W406…Asp212   | 2292       | 2434                           | 2375           | 2437                     | 2418           |

$R^2$ to FTIR: 0.89, 0.75, 0.75, 0.76, 0.71

$^a$ See Ref. (7).

$^b$ See Ref. (8).

$^c$ See Ref. (34).
(b) Model 2. The QM/MM results of model 2 on the basis of the 1C3W structure primarily discussed in the present study.

|                | 1C3W | 1C3W | 2NTU | 2NTU |
|----------------|------|------|------|------|
|                | FTIR-2<sup>a</sup> | QM/MM<sup>b</sup> | empirical eq.<sup>b</sup> | QM/MM<sup>b</sup> | empirical eq.<sup>b</sup> |
| W402…Asp85    | 2171 (lowest) | 2078 (lowest) | 2201 (lowest) | 2120 (lowest) | 2240 (lowest) |
| W402…Asp212   | 2599 | 2690 | 2592 | 2670 | 2572 |
| W401…Asp85    | 2323 | 2400 | 2311 | 2411 | 2359 |
| W406…(n.d.)   | 2690 | 2678 | 2727 | 2689 | 2727 |
| W406…W401     | 2636 | 2658 | 2620 | 2656 | 2622 |
| W406…Asp212   | 2292 | 2312 | 2303 | 2302 | 2328 |

<sup>a</sup> See Ref. (7).

<sup>b</sup> See Ref. (34).

<sup>c</sup> Relative to “FTIR-2.”

\[ R^2 \text{ to FTIR} \]

\[
\begin{array}{cccc}
& 0.94^c & 0.99^c & 0.96^c & 0.98^c \\
\end{array}
\]
Table 3. H-bond geometries near W402 in the ASR crystal structure (PDB ID code 1XIO at 2.00 Å resolution) and the QM/MM geometry. n.d. = not determined. See Table S1 in the SI for the atomic coordinates of the QM/MM geometries. Distances in Å, angles in °.

| Donor…acceptor       | 1XIO  | QM/MM |
|-----------------------|-------|-------|
| W402…Asp75           | 2.74  | 2.71  |
| W402…Tyr51           | 2.92  | 2.97  |
| Trp76…W402           | 2.94  | 2.84  |
| Lys210…W402          | 3.02  | 3.00  |
| RMSD<sup>a</sup>      | n. d. | 0.17  |

angle(N<sub>Lys210</sub>…O<sub>W402</sub>…O<sub>Asp75</sub>) 83 92

<sup>a</sup> Excluding Lys210 due to obvious displacement of the sidechain carbon atoms from the original crystal structure (Figure 2c).
Table 4. O–D stretching frequencies (in cm$^{-1}$) of W402 in ASR.

| deprotonated Asp75 | FTIR$^a$ | QM/MM | Empirical eq. |
|---------------------|----------|-------|---------------|
| W402…Asp75         | $>$2500  | 2376  | 2392          |
| W402…Tyr51         | $>$2500  | 2573  | 2583          |

$^a$ See Ref. (11).
Table 5. Contribution of residues to the p$K_a$ shifts of Asp85 in BR and Asp75 in ASR (relative to the bulk solvent) in the presence of the protonated Schiff base (in p$K_a$ units). For clarity, residue pairs that are responsible for differences of <0.7 between p$K_a$(Asp85) and p$K_a$(Asp75) are not listed, except for the Glu204/Asp198 pair.

side = sidechain, b.b. = backbone.

| BR           | p$K_a$(Asp85) = 1.5 | ASR        | p$K_a$(Asp75) = -5.1 | difference |
|--------------|---------------------|------------|----------------------|------------|
|              | side | b.b. | total | side | b.b. | total | total |
| (1C3W)       |      |      |       |      |      |       |       |
| Ile52        | -0.1 | 0.0  | -0.1  | Trp46 | -0.6 | -0.3  | -0.9  | 0.8     |
| Ala53        | 0.0  | -0.5 | -0.5  | Ser47 | -2.6 | -0.6  | -3.2  | 2.7     |
| Tyr57        | -0.5 | -0.4 | -0.9  | Tyr51 | 0.2  | -0.4  | -0.2  | -0.7    |
| Arg82        | -3.9 | 0.8  | -3.1  | Arg72 | -2.1 | 0.9   | -1.2  | -1.9    |
| Glu194       | 1.2  | -0.1 | 1.1   | Ser188| 0.0  | 0.0   | 0.0   | 1.1     |
| Glu204       | 0.0  | 0.1  | 0.1   | Asp198| 0.6  | 0.0   | 0.6   | -0.5    |
| Asp212       | 6.3  | -0.1 | 6.2   | Pro206| -0.1 |       |       | 6.3     |
| Schiff       | -9.3 | Schiff | -10.3 |       |       |       |       | 1.0     |
Table 6. Key residues that alter the pKₐ difference between Asp85 and Asp212 in the presence of the protonated Schiff base (in pKₐ units).

side = side chain, b.b. = backbone, n.d. = not determined.

| Residue | pKₐ(Asp85) = 1.5 | pKₐ(Asp212) = -2.0 |
|---------|------------------|-------------------|
|         | side  | b.b.  | total | side  | b.b.  | total |
| Met56   | -0.4  | -0.8  | -1.2  | -0.1  | -0.3  | -0.3  |
| Tyr57   | -0.5  | -0.3  | -0.9  | -3.2  | -0.2  | -3.4  |
| Ala81   | 0.0   | 0.7   | 0.8   | 0.0   | 0.1   | 0.1   |
| Arg82   | -3.9  | 0.8   | -3.1  | -6.4  | 0.1   | -6.3  |
| Asp85   | n.d.  | n.d.  | n.d.  | 6.4   | -0.4  | 6.0   |
| Trp86   | -0.2  | -0.6  | -0.8  | -0.8  | -0.3  | -1.0  |
| Thr89   | -2.0  | -0.9  | -2.9  | 0.1   | -0.2  | -0.1  |
| Tyr185  | -0.3  | -0.1  | -0.4  | -3.2  | -0.1  | -3.3  |
| Glu194  | 1.2   | -0.1  | 1.2   | 1.8   | -0.1  | 1.7   |
| Phe208  | 0.0   | 0.2   | 0.2   | 0.0   | 1.8   | 1.8   |
| Asp212  | 6.3   | -0.1  | 6.2   | n.d.  | n.d.  | n.d.  |
| Schiff  | -9.5  | 0.2   | -9.3  | -9.3  | 0.6   | -8.7  |
Figure 1.
Figure 2 (a, b).

Figure 2 (c).
Figure 3.

![Graph showing the relationship between donor-acceptor distance (Å) and frequency (cm⁻¹).]
Figure 4.
Figure 5.

BR

ASR

increase in $O_{W402}$-H (H migration toward Asp)

→ decrease in $O_{W402}$-D frequency

increase in $pK_a$(Asp)

3.

decrease in $O_{W402}$-$O_{Asp}$

1.

$pK_a$ difference between Asp85 and W402 (BR)

2.

(pKₐ)

$\Delta E$ [kcal/mol]

W402 moiety

Asp moiety

d($O_{W402}$-H) [Å]
Figure 6.
Factors that differentiate the H-bond strengths of water near the Schiff bases in bacteriorhodopsin and Anabaena sensory rhodopsin

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Table S1. Atomic coordinates of the QM/MM optimized geometry for BR [W401, W402, W406, Tyr57, Arg82, Asp85, Thr89, Tyr185, Asp212, Lys216 (the Shiff base) and the retinal].

(a) 1C3W (model 1)

| residue | atom | x    | y    | z    |
|---------|------|------|------|------|
| ARG A   | 82 CB| 14.733 | 36.929 | -2.302 |
| ARG A   | 82 HB1| 15.162 | 37.353 | -1.391 |
| ARG A   | 82 HB2| 14.176 | 37.735 | -2.799 |
| ARG A   | 82 CG | 15.861 | 36.483 | -3.21 |
| ARG A   | 82 HG1| 15.528 | 36.352 | -4.243 |
| ARG A   | 82 HG2| 16.291 | 35.541 | -2.87 |
| ARG A   | 82 CD | 16.925 | 37.572 | -3.181 |
| ARG A   | 82 HD1| 17.371 | 37.628 | -2.187 |
| ARG A   | 82 HD2| 16.494 | 38.545 | -3.42 |
| ARG A   | 82 NE | 17.958 | 37.321 | -4.161 |
| ARG A   | 82 HE | 17.955 | 37.866 | -5.04 |
| ARG A   | 82 CZ | 18.912 | 36.413 | -4.065 |
| ARG A   | 82 NH1| 19.066 | 35.659 | -2.979 |
| ARG A   | 82 HH11| 18.435 | 35.684 | -2.155 |
| ARG A   | 82 HH12| 19.894 | 35.071 | -2.935 |
| ARG A   | 82 NH2| 19.727 | 36.23 | -5.134 |
| ARG A   | 82 HH21| 19.679 | 36.905 | -5.893 |
| ARG A   | 82 HH22| 20.569 | 35.69 | -5.009 |
| ASP A   | 85 CB | 13.183 | 33.56 | 1.776 |
| ASP A   | 85 HB1| 13.478 | 34.483 | 1.278 |
| ASP A   | 85 HB2| 13.239 | 32.845 | 0.967 |
| ASP A   | 85 CG | 14.378 | 33.28 | 2.695 |
| ASP A   | 85 OD1| 14.364 | 33.431 | 3.929 |
| ASP A   | 85 OD2| 15.428 | 32.917 | 2.042 |
| ASP A   | 212 CB| 20.879 | 34.919 | 2.914 |
| ASP A   | 212 HB1| 20.234 | 34.146 | 3.344 |
| ASP A   | 212 HB2| 21.559 | 34.43 | 2.212 |
| ASP A   | 212 CG | 19.886 | 35.803 | 2.158 |
| ASP A   | 212 OD1| 19.395 | 36.831 | 2.662 |
| ASP A   | 212 OD2| 19.491 | 35.319 | 1.033 |
| TYR A   | 57 CB | 18.451 | 28.758 | -1.114 |
| TYR A   | 57 HB1| 19.199 | 28.139 | -1.589 |
| TYR A   | 57 HB2| 18.125 | 28.236 | -0.217 |
| TYR A   | 57 CG | 19.121 | 30.08 | -0.848 |
| TYR A   | 57 CD1| 18.793 | 30.923 | 0.216 |
| TYR A   | 57 HD1| 18.074 | 30.612 | 0.962 |
| TYR A   | 57 CE1| 19.357 | 32.189 | 0.332 |
| TYR A   | 57 HE1| 19.07 | 32.83 | 1.155 |
| TYR A   | 57 CZ | 20.257 | 32.65 | -0.64 |
| TYR A   | 57 OH | 20.72 | 33.915 | -0.634 |
| TYR A   | 57 HH | 20.274 | 34.489 | 0.098 |
| TYR A   | 57 CD2| 20.101 | 30.515 | -1.752 |
| TYR A   | 57 HD2| 20.394 | 29.853 | -2.564 |
| TYR A   | 57 CE2| 20.675 | 31.778 | -1.657 |
| TYR A   | 57 HE2| 21.412 | 32.105 | -2.382 |
| TYR A   | 185 CB| 19.172 | 43.743 | 2.258 |
| TYR A   | 185 HB1| 18.377 | 43.664 | 1.513 |
| Residue | Atom  | Type 1  | Type 2  | Type 3  |
|---------|-------|---------|---------|---------|
| TYR     | A     | 185 HB2 | 18.767  | 44.326  | 3.088  |
| TYR     | A     | 185 CG  | 19.52   | 42.353  | 2.737  |
| TYR     | A     | 185 CD1 | 20.268  | 42.19   | 3.9    |
| TYR     | A     | 185 HD1 | 20.633  | 43.08   | 4.378  |
| TYR     | A     | 185 CE1 | 20.548  | 40.951  | 4.451  |
| TYR     | A     | 185 HE1 | 21.122  | 40.855  | 5.366  |
| TYR     | A     | 185 CZ  | 20.084  | 39.807  | 3.801  |
| TYR     | A     | 185 OH  | 20.332  | 38.611  | 4.382  |
| TYR     | A     | 185 HH  | 20.005  | 37.882  | 3.796  |
| TYR     | A     | 185 CD2 | 19.06   | 41.197  | 2.104  |
| TYR     | A     | 185 HE2 | 19.001  | 39.039  | 2.114  |
| THR     | A     | 89 CB   | 13.525  | 34.153  | 7.186  |
| THR     | A     | 89 HB   | 14.346  | 34.097  | 7.913  |
| THR     | A     | 89 OG1  | 13.966  | 35.005  | 6.142  |
| THR     | A     | 89 HG1  | 13.87   | 34.535  | 5.292  |
| THR     | A     | 89 CG2  | 13.189  | 32.74   | 6.718  |
| THR     | A     | 89 HG21 | 12.422  | 32.755  | 5.943  |
| THR     | A     | 89 HG22 | 12.81   | 32.133  | 7.545  |
| THR     | A     | 89 HG23 | 14.065  | 32.249  | 6.293  |
| LYS     | A     | 216 CB  | 19.893  | 32.654  | 8.085  |
| LYS     | A     | 216 HB1 | 19.393  | 31.733  | 8.411  |
| LYS     | A     | 216 HB2 | 20.648  | 32.351  | 7.352  |
| LYS     | A     | 216 CG  | 18.877  | 33.605  | 7.446  |
| LYS     | A     | 216 HG1 | 19.386  | 34.456  | 6.987  |
| LYS     | A     | 216 HG2 | 18.239  | 34.032  | 8.227  |
| LYS     | A     | 216 CD  | 17.978  | 32.944  | 6.407  |
| LYS     | A     | 216 HD1 | 17.583  | 31.994  | 6.785  |
| LYS     | A     | 216 HD2 | 18.528  | 32.712  | 5.492  |
| LYS     | A     | 216 CE  | 16.805  | 33.859  | 6.066  |
| LYS     | A     | 216 HE1 | 16.292  | 34.19   | 6.969  |
| LYS     | A     | 216 HE2 | 16.06   | 33.387  | 5.427  |
| LYS     | A     | 216 NZ  | 17.246  | 35.053  | 5.368  |
| LYS     | A     | 216 HZ1 | 17.404  | 34.926  | 4.357  |
| HOH     |      | 402 O   | 17.687  | 33.915  | 2.868  |
| HOH     |      | 402 H1  | 17.98   | 34.384  | 2.073  |
| HOH     |      | 402 H2  | 16.81   | 33.489  | 2.627  |
| HOH     |      | 401 O   | 16.112  | 33.207  | -0.4   |
| HOH     |      | 401 H1  | 15.746  | 33.082  | 0.528  |
| HOH     |      | 401 H2  | 16.816  | 32.55   | -0.483 |
| HOH     |      | 406 O   | 17.486  | 35.589  | -0.744 |
| HOH     |      | 406 H1  | 16.939  | 34.786  | -0.588 |
| HOH     |      | 406 H2  | 18.131  | 35.647  | -0.008 |
| RTAL    | A     | 301 C1  | 14.874  | 45.074  | 0.303  |
| RTAL    | A     | 301 C2  | 14.774  | 45.988  | -0.938 |
| RTAL    | A     | 301 H21 | 14.308  | 45.416  | -1.741 |
| RTAL    | A     | 301 H22 | 15.791  | 46.237  | -1.265 |
| RTAL    | A     | 301 C3  | 14.02   | 47.282  | -0.724 |
| RTAL    | A     | 301 H31 | 12.981  | 47.066  | -0.471 |
| RTAL    | A     | 301 H32 | 13.995  | 47.853  | -1.658 |
| RTAL    | A     | 301 C4  | 14.724  | 48.066  | 0.379  |
| RTAL    | A     | 301 H41 | 14.15   | 48.946  | 0.693  |
| RTAL    | A     | 301 H42 | 15.652  | 48.492  | -0.025 |
| RTAL    | A     | 301 C5  | 15.062  | 47.233  | 1.599  |
| RTAL  | 301 C6 | 15.157 | 45.873 | 1.58 |
|-------|-------|--------|--------|------|
| RTAL  | 301 C7 | 15.498 | 45.176 | 2.812|
| RTAL  | 301 H71| 15.837 | 45.798 | 3.63 |
| RTAL  | 301 C8 | 15.497 | 43.842 | 3.061|
| RTAL  | 301 H81| 15.129 | 43.141 | 2.326|
| RTAL  | 301 C9 | 16.055 | 43.227 | 4.233|
| RTAL  | 301 C10| 16.193 | 41.863 | 4.193|
| RTAL  | 301 H101| 15.834 | 41.354 | 3.301|
| RTAL  | 301 C11| 16.824 | 41.027 | 5.149|
| RTAL  | 301 H111| 17.126 | 41.424 | 6.114|
| RTAL  | 301 C12| 17.031 | 39.71  | 4.84 |
| RTAL  | 301 H121| 16.723 | 39.382 | 3.853|
| RTAL  | 301 C13| 17.49  | 38.689 | 5.711|
| RTAL  | 301 C14| 17.499 | 37.406 | 5.192|
| RTAL  | 301 H141| 17.461 | 37.302 | 4.12 |
| RTAL  | 301 C15| 17.349 | 36.236 | 5.932|
| RTAL  | 301 H15| 17.194 | 36.268 | 7.005|
| RTAL  | 301 C16| 13.524 | 44.339 | 0.492|
| RTAL  | 301 H161| 13.238 | 43.888 | -0.457|
| RTAL  | 301 H162| 12.74  | 45.034 | 0.806|
| RTAL  | 301 H163| 13.563 | 43.545 | 1.238|
| RTAL  | 301 C17| 16.018 | 44.093 | -0.04|
| RTAL  | 301 H171| 16.959 | 44.637 | -0.16|
| RTAL  | 301 H172| 15.8   | 43.609 | -0.995|
| RTAL  | 301 H173| 16.179 | 43.311 | 0.697|
| RTAL  | 301 C18| 15.215 | 48.039 | 2.857|
| RTAL  | 301 H181| 14.243 | 48.181 | 3.345|
| RTAL  | 301 H182| 15.611 | 49.025 | 2.625|
| RTAL  | 301 H183| 15.866 | 47.594 | 3.603|
| RTAL  | 301 C19| 16.581 | 44.061 | 5.361|
| RTAL  | 301 H191| 15.867 | 44.825 | 5.666|
| RTAL  | 301 H192| 17.494 | 44.579 | 5.059|
| RTAL  | 301 H193| 16.808 | 43.445 | 6.225|
| RTAL  | 301 C20| 17.929 | 38.991 | 7.105|
| RTAL  | 301 H201| 17.237 | 39.646 | 7.628|
| RTAL  | 301 H202| 18.89  | 39.516 | 7.053|
| RTAL  | 301 H203| 18.086 | 38.088 | 7.695|
(b) 1C3W (model 2)

| residue | atom  | x    | y    | z    |
|---------|-------|------|------|------|
| ARG     | A 82 CB  | 14.716 | 36.94 | -2.32 |
| ARG     | A 82 HB1 | 15.108 | 37.429 | -1.422 |
| ARG     | A 82 HB2 | 14.149 | 37.713 | -2.855 |
| ARG     | A 82 CG  | 15.871 | 36.492 | -3.191 |
| ARG     | A 82 HG1 | 15.553 | 36.313 | -4.222 |
| ARG     | A 82 HG2 | 16.312 | 35.568 | -2.814 |
| ARG     | A 82 CD  | 16.91  | 37.607 | -3.198 |
| ARG     | A 82 HD1 | 17.336 | 37.739 | -2.201 |
| ARG     | A 82 HD2 | 16.466 | 38.557 | -3.494 |
| ARG     | A 82 NE  | 17.976 | 37.324 | -4.132 |
| ARG     | A 82 HE  | 18.016 | 37.848 | -5.026 |
| ARG     | A 82 CZ  | 18.914 | 36.414 | -3.944 |
| ARG     | A 82 NH1 | 19.004 | 35.725 | -2.804 |
| ARG     | A 82 HH11| 18.337 | 35.829 | -2.036 |
| ARG     | A 82 HH12| 19.779 | 36.767 | -5.764 |
| ARG     | A 82 HH21| 19.784 | 36.767 | -5.764 |
| ARG     | A 82 HH22| 20.589 | 35.589 | -4.743 |
| ASP     | A 85 CB  | 13.185 | 33.544 | 1.792 |
| ASP     | A 85 HB1 | 13.47  | 34.438 | 1.24  |
| ASP     | A 85 HB2 | 13.249 | 32.788 | 1.024 |
| ASP     | A 85 CG  | 14.391 | 33.314 | 2.718 |
| ASP     | A 85 OD1 | 14.376 | 33.501 | 3.951 |
| ASP     | A 85 OD2 | 15.441 | 32.952 | 2.073 |
| ASP     | A 212 CB | 20.87  | 34.929 | 2.914 |
| ASP     | A 212 HB1| 20.226 | 34.154 | 3.345 |
| ASP     | A 212 HB2| 21.545 | 34.442 | 2.208 |
| ASP     | A 212 CG | 19.871 | 35.814 | 2.165 |
| ASP     | A 212 OD1| 19.349 | 36.818 | 2.688 |
| ASP     | A 212 OD2| 19.496 | 35.354 | 1.023 |
| TYR     | A 57 CB  | 18.454 | 28.766 | -1.12 |
| TYR     | A 57 HB1 | 19.207 | 28.151 | -1.595 |
| TYR     | A 57 HB2 | 18.132 | 28.244 | -0.22 |
| TYR     | A 57 CG  | 19.11  | 30.096 | -0.859 |
| TYR     | A 57 CD1 | 18.677 | 30.993 | 0.119 |
| TYR     | A 57 HD1 | 17.859 | 30.744 | 0.78  |
| TYR     | A 57 CE1 | 19.239 | 32.257 | 0.226 |
| TYR     | A 57 HE1 | 18.867 | 32.943 | 0.973 |
| TYR     | A 57 CZ  | 20.235 | 32.663 | -0.668 |
| TYR     | A 57 OH  | 20.71  | 33.934 | -0.668 |
| TYR     | A 57 HH  | 20.283 | 34.5   | 0.065 |
| TYR     | A 57 CD2 | 20.161 | 30.49  | -1.699 |
| TYR     | A 57 HD2 | 20.519 | 29.792 | -2.453 |
| TYR     | A 57 CE2 | 20.732 | 31.756 | -1.612 |
| TYR     | A 57 HE2 | 21.529 | 32.055 | -2.286 |
| TYR     | A 185 CB | 19.175 | 43.742 | 2.26  |
| TYR     | A 185 HB1| 18.377 | 43.663 | 1.516 |
| TYR     | A 185 HB2| 18.772 | 44.322 | 3.091 |
| TYR     | A 185 CG | 19.524 | 42.352 | 2.737 |
| Residue |  |  |  |
|---------|---|---|---|
| TYR A 185 CD1 | 20.279 | 42.189 | 3.897 |
| TYR A 185 HD1 | 20.647 | 43.078 | 4.373 |
| TYR A 185 CE1 | 20.557 | 40.95 | 4.449 |
| TYR A 185 HE1 | 21.134 | 40.854 | 5.363 |
| TYR A 185 CZ | 20.086 | 39.806 | 3.804 |
| TYR A 185 OH | 20.332 | 38.61 | 4.388 |
| TYR A 185 HH | 19.984 | 37.882 | 3.815 |
| TYR A 185 CD2 | 19.058 | 41.196 | 2.109 |
| TYR A 185 HD2 | 18.436 | 41.276 | 1.223 |
| TYR A 185 CE2 | 19.355 | 39.932 | 2.615 |
| TYR A 185 HE2 | 18.995 | 39.038 | 2.124 |
| THR A 89 CB | 13.524 | 34.158 | 7.187 |
| THR A 89 HB | 14.34 | 34.092 | 7.921 |
| THR A 89 OG1 | 13.971 | 35.019 | 6.155 |
| THR A 89 HG1 | 13.886 | 34.553 | 5.299 |
| THR A 89 CG2 | 13.187 | 32.749 | 6.707 |
| THR A 89 HG21 | 12.427 | 32.769 | 5.926 |
| THR A 89 HG22 | 12.801 | 32.139 | 7.529 |
| THR A 89 HG23 | 14.065 | 32.257 | 6.286 |
| LYS A 216 CB | 19.897 | 32.655 | 8.084 |
| LYS A 216 HB1 | 19.398 | 31.733 | 8.408 |
| LYS A 216 HB2 | 20.653 | 32.354 | 7.352 |
| LYS A 216 CG | 18.881 | 33.604 | 7.445 |
| LYS A 216 HG1 | 19.39 | 34.545 | 6.983 |
| LYS A 216 HG2 | 18.245 | 34.034 | 8.227 |
| LYS A 216 CD | 17.977 | 32.942 | 6.411 |
| LYS A 216 HD1 | 17.587 | 31.99 | 6.789 |
| LYS A 216 HD2 | 18.52 | 32.713 | 5.49 |
| LYS A 216 CE | 16.798 | 33.854 | 6.081 |
| LYS A 216 HE1 | 16.29 | 34.183 | 6.986 |
| LYS A 216 HE2 | 16.051 | 33.384 | 5.442 |
| LYS A 216 NZ | 17.232 | 35.05 | 5.383 |
| LYS A 216 NZ | 17.388 | 34.918 | 4.369 |
| HOH 402 O | 17.669 | 33.922 | 2.906 |
| HOH 402 H1 | 17.968 | 34.368 | 2.101 |
| HOH 402 H2 | 16.789 | 33.486 | 2.653 |
| HOH 401 O | 15.754 | 33.013 | -0.549 |
| HOH 401 H1 | 15.588 | 32.944 | 0.422 |
| HOH 401 H2 | 16.345 | 33.777 | -0.634 |
| HOH 406 O | 17.34 | 35.631 | -0.467 |
| HOH 406 H1 | 16.641 | 36.19 | -0.093 |
| HOH 406 H2 | 18.102 | 35.669 | 0.17 |
| RTAL 301 C1 | 14.867 | 45.06 | 0.321 |
| RTAL 301 C2 | 14.764 | 45.965 | -0.927 |
| RTAL 301 H21 | 14.293 | 45.386 | -1.723 |
| RTAL 301 H22 | 15.779 | 46.209 | 1.26 |
| RTAL 301 C3 | 14.014 | 47.264 | -0.721 |
| RTAL 301 H31 | 12.974 | 47.055 | -0.467 |
| RTAL 301 H32 | 13.992 | 47.829 | -1.659 |
| RTAL 301 C4 | 14.719 | 48.053 | 0.377 |
| RTAL 301 H41 | 14.147 | 48.937 | 0.684 |
| RTAL 301 H42 | 15.648 | 48.473 | -0.03 |
| RTAL 301 C5 | 15.054 | 47.227 | 1.603 |
| RTAL 301 C6 | 15.151 | 45.867 | 1.592 |
| RTAL 301 C7 | 15.499 | 45.176 | 2.826 |
| RTAL  | 301 H71 | 15.836 | 45.803 | 3.642 |
|-------|---------|--------|--------|-------|
| RTAL  | 301 C8  | 15.51  | 43.843 | 3.078 |
| RTAL  | 301 H81 | 15.142 | 43.138 | 2.346 |
| RTAL  | 301 C9  | 16.079 | 43.231 | 4.248 |
| RTAL  | 301 C10 | 16.214 | 41.868 | 4.212 |
| RTAL  | 301 H101| 15.845 | 41.358 | 3.324 |
| RTAL  | 301 C11 | 16.844 | 40.28  | 5.167 |
| RTAL  | 301 H111| 17.152 | 41.421 | 6.131 |
| RTAL  | 301 C12 | 17.039 | 39.709 | 4.859 |
| RTAL  | 301 H121| 16.722 | 39.383 | 3.874 |
| RTAL  | 301 C13 | 17.491 | 38.683 | 5.729 |
| RTAL  | 301 C14 | 17.487 | 37.402 | 5.212 |
| RTAL  | 301 H141| 17.442 | 37.298 | 4.14  |
| RTAL  | 301 C15 | 17.337 | 36.229 | 5.949 |
| RTAL  | 301 H15 | 17.183 | 36.259 | 7.022 |
| RTAL  | 301 C16 | 13.52  | 44.326 | 0.519 |
| RTAL  | 301 H161| 13.243 | 43.838 | -0.415|
| RTAL  | 301 H162| 12.73  | 45.028 | 0.802 |
| RTAL  | 301 H163| 13.554 | 43.56  | 1.294 |
| RTAL  | 301 C17 | 16.014 | 44.08  | -0.015|
| RTAL  | 301 H171| 16.956 | 44.626 | -0.125|
| RTAL  | 301 H172| 15.806 | 43.599 | -0.974|
| RTAL  | 301 H173| 16.169 | 43.295 | 0.722 |
| RTAL  | 301 C18 | 15.209 | 48.042 | 2.857 |
| RTAL  | 301 H181| 14.24  | 48.173 | 3.353 |
| RTAL  | 301 H182| 15.589 | 49.031 | 2.617 |
| RTAL  | 301 H183| 15.872 | 47.608 | 3.599 |
| RTAL  | 301 C19 | 16.609 | 44.069 | 5.37  |
| RTAL  | 301 H191| 15.845 | 44.736 | 5.772 |
| RTAL  | 301 H192| 17.434 | 44.699 | 5.028 |
| RTAL  | 301 H193| 16.971 | 43.443 | 6.181 |
| RTAL  | 301 C20 | 17.934 | 38.98  | 7.123 |
| RTAL  | 301 H201| 17.233 | 39.618 | 7.657 |
| RTAL  | 301 H202| 18.885 | 39.523 | 7.072 |
| RTAL  | 301 H203| 18.109 | 38.074 | 7.702 |
| residue | atom  | x     | y     | z    |
|---------|-------|-------|-------|------|
| ARG A  | 82 CB | 14.748| 37.211| -2.273|
| ARG A  | 82 HB1| 15.184| 37.638| -1.365|
| ARG A  | 82 HB2| 14.193| 38.019| -2.77 |
| ARG A  | 82 CG | 15.87 | 36.75 | -3.19 |
| ARG A  | 82 HG1| 15.52 | 36.594| -4.217|
| ARG A  | 82 HG2| 16.306| 35.812| -2.842|
| ARG A  | 82 CD | 16.932| 37.845| -3.208|
| ARG A  | 82 HD1| 17.39 | 37.935| -2.221|
| ARG A  | 82 HD2| 16.491| 38.808| -3.469|
| ARG A  | 82 NE | 17.957| 37.582| -4.198|
| ARG A  | 82 HE | 17.942| 38.111| -5.083|
| ARG A  | 82 CZ | 18.929| 36.69 | -4.086|
| ARG A  | 82 NH1| 19.096| 35.953| -2.991|
| ARG A  | 82 HH11| 18.491| 35.985| -2.145|
| ARG A  | 82 HH12| 19.937| 35.38 | -2.952|
| ARG A  | 82 NH2 | 19.749| 36.504| -5.151|
| ARG A  | 82 HH21| 19.695| 37.164| -5.922|
| ARG A  | 82 HH22| 20.597| 35.974| -5.019|
| ASP A  | 85 CB | 13.226| 33.832| 1.815 |
| ASP A  | 85 HB1| 13.516| 34.753| 1.309 |
| ASP A  | 85 HB2 | 13.259| 33.102| 1.014 |
| ASP A  | 85 CG | 14.429| 33.547| 2.735 |
| ASP A  | 85 OD1| 14.412| 33.679| 3.974 |
| ASP A  | 85 OD2| 15.479| 33.198| 2.073 |
| ASP A  | 212 CB | 20.954| 35.152| 3.094 |
| ASP A  | 212 HB1| 20.298| 34.39 | 3.527 |
| ASP A  | 212 HB2| 21.642| 34.642| 2.413 |
| ASP A  | 212 CG | 19.991| 36.029| 2.285 |
| ASP A  | 212 OD1| 19.561| 37.115| 2.716 |
| ASP A  | 212 OD2| 19.572| 35.479| 1.198 |
| TYR A  | 57 CB | 18.602| 28.971| -1.201|
| TYR A  | 57 HB1 | 19.338| 28.349| -1.706|
| TYR A  | 57 HB2 | 18.284| 28.432| -0.306|
| TYR A  | 57 CG | 19.272| 30.289| -0.895|
| TYR A  | 57 CD1 | 18.921| 31.103| 0.187 |
| TYR A  | 57 HD1 | 18.197| 30.771| 0.917 |
| TYR A  | 57 CE1 | 19.47 | 32.371| 0.348 |
| TYR A  | 57 HE1 | 19.164| 32.989| 1.183 |
| TYR A  | 57 CZ | 20.388| 32.863| -0.59 |
| TYR A  | 57 OH | 20.847| 34.13 | -0.538|
| TYR A  | 57 HH | 20.397| 34.671| 0.202 |
| TYR A  | 57 CD2 | 20.265| 30.756| -1.766|
| TYR A  | 57 HD2 | 20.581| 30.12 | -2.59 |
| TYR A  | 57 CE2 | 20.831| 32.019| -1.621|
| TYR A  | 57 HE2 | 21.583| 32.368| -2.322|
| TYR A  | 185 CB | 19.289| 44.049| 2.317 |
| TYR A  | 185 HB1 | 18.483| 43.962| 1.584 |
| TYR A  | 185 HB2 | 18.897| 44.633| 3.152 |
| TYR A  | 185 CG | 19.643| 42.663| 2.815 |
| TYR A  | 185 CD1 | 20.383| 42.505| 3.987 |
| TYR A  | 185 HD1 | 20.742| 43.393| 4.48 |
| TYR A  | 185 CE1 | 20.658| 41.263| 4.539 |
| Residue | Atom  | X  | Y  | Z   |
|---------|-------|----|----|-----|
| TYR     | HE1   | 21.228 | 41.169 | 5.459 |
| TYR     | CZ    | 20.197 | 40.115 | 3.892 |
| TYR     | OH    | 20.451 | 38.914 | 4.468 |
| TYR     | HH    | 20.14  | 38.186 | 3.873 |
| TYR     | CD2   | 19.176 | 41.503 | 2.192 |
| TYR     | HD2   | 18.552 | 41.577 | 1.308 |
| THR     | CB    | 13.567 | 34.365 | 7.361 |
| THR     | HB    | 14.402 | 34.288 | 8.071 |
| THR     | OG1   | 13.997 | 35.215 | 6.309 |
| THR     | HG1   | 13.927 | 34.738 | 5.46  |
| THR     | CG2   | 13.208 | 32.958 | 6.882 |
| THR     | HG21  | 12.446 | 32.984 | 6.101 |
| THR     | HG22  | 12.815 | 32.348 | 7.702 |
| LYS     | CB    | 20.002 | 32.975 | 8.395 |
| LYS     | HB1   | 19.514 | 32.047 | 8.714 |
| LYS     | HB2   | 20.757 | 32.678 | 7.659 |
| LYS     | CG    | 18.983 | 33.908 | 7.732 |
| LYS     | HG1   | 19.487 | 34.774 | 7.294 |
| LYS     | HG2   | 18.312 | 34.312 | 8.496 |
| LYS     | CD    | 18.139 | 33.228 | 6.655 |
| LYS     | HD1   | 17.749 | 32.269 | 7.018 |
| LYS     | HD2   | 18.739 | 33.004 | 5.769 |
| LYS     | CE    | 16.955 | 34.104 | 6.244 |
| LYS     | HE1   | 16.39  | 34.434 | 7.117 |
| LYS     | HE2   | 16.252 | 33.59  | 5.589 |
| LYS     | NZ    | 17.378 | 35.296 | 5.529 |
| LYS     | HZ1   | 17.515 | 35.161 | 4.516 |
| HOH     | O     | 17.751 | 34.13  | 3.01  |
| HOH     | H1    | 18.114 | 34.562 | 2.222 |
| HOH     | H2    | 16.872 | 33.737 | 2.733 |
| HOH     | O     | 16.221 | 33.469 | -0.389 |
| HOH     | H1    | 15.83  | 33.367 | 0.53  |
| HOH     | H2    | 16.916 | 32.8   | -0.442 |
| HOH     | O     | 17.631 | 35.848 | -0.691 |
| HOH     | H1    | 18.266 | 35.884 | 0.056 |
| HOH     | H2    | 17.078 | 35.046 | -0.553 |
| RTAL    | C1    | 14.962 | 45.339 | 0.341 |
| RTAL    | C2    | 14.828 | 46.27  | -0.888 |
| RTAL    | H21   | 14.365 | 45.702 | -1.699 |
| RTAL    | H22   | 15.833 | 46.545 | -1.23 |
| RTAL    | C3    | 14.054 | 47.547 | -0.624 |
| RTAL    | H31   | 13.031 | 47.307 | -0.328 |
| RTAL    | H32   | 13.982 | 48.138 | -1.544 |
| RTAL    | C4    | 14.779 | 48.323 | 0.469 |
| RTAL    | H41   | 14.218 | 49.198 | 0.808 |
| RTAL    | H42   | 15.703 | 48.738 | 0.041 |
| RTAL    | C5    | 15.132 | 47.485 | 1.676 |
| RTAL    | C6    | 15.24  | 46.125 | 1.634 |
| RTAL    | C7    | 15.573 | 45.425 | 2.863 |
| RTAL    | H71   | 15.864 | 46.055 | 3.692 |
| RTAL    | C8    | 15.607 | 44.088 | 3.112 |
| RTAL    | H81   | 15.305 | 43.379 | 2.354 |
|   |   |   |   |
|---|---|---|---|
| RTAL | 301 C9 | 16.111 | 43.48 | 4.312 |
| RTAL | 301 C10 | 16.282 | 42.117 | 4.27 |
| RTAL | 301 H101 | 15.987 | 42.161 | 3.354 |
| RTAL | 301 C11 | 16.872 | 42.286 | 5.252 |
| RTAL | 301 H111 | 17.139 | 41.693 | 6.223 |
| RTAL | 301 C12 | 17.095 | 39.963 | 4.96 |
| RTAL | 301 H121 | 16.82 | 39.63 | 3.965 |
| RTAL | 301 C13 | 17.542 | 38.952 | 5.844 |
| RTAL | 301 H131 | 17.757 | 37.66 | 5.332 |
| RTAL | 301 H141 | 17.537 | 37.551 | 4.258 |
| RTAL | 301 C15 | 17.453 | 36.493 | 6.078 |
| RTAL | 301 H15 | 17.31 | 36.53 | 7.154 |
| RTAL | 301 C16 | 13.633 | 44.566 | 0.523 |
| RTAL | 301 H161 | 13.393 | 44.037 | -0.401 |
| RTAL | 301 H162 | 12.818 | 45.256 | 0.759 |
| RTAL | 301 H163 | 13.668 | 43.831 | 1.328 |
| RTAL | 301 C17 | 16.125 | 44.388 | -0.02 |
| RTAL | 301 H171 | 17.053 | 44.954 | -0.14 |
| RTAL | 301 H172 | 15.913 | 43.905 | -0.978 |
| RTAL | 301 H173 | 16.307 | 43.605 | 0.714 |
| RTAL | 301 C18 | 15.268 | 48.288 | 2.938 |
| RTAL | 301 H181 | 14.269 | 48.449 | 3.36 |
| RTAL | 301 H182 | 15.694 | 49.264 | 2.711 |
| RTAL | 301 H183 | 15.882 | 47.834 | 3.712 |
| RTAL | 301 C19 | 16.543 | 44.31 | 5.487 |
| RTAL | 301 H191 | 15.904 | 45.182 | 5.63 |
| RTAL | 301 H192 | 17.564 | 44.675 | 5.34 |
| RTAL | 301 H193 | 16.519 | 43.724 | 6.405 |
| RTAL | 301 C20 | 17.961 | 39.272 | 7.244 |
| RTAL | 301 H201 | 17.301 | 39.991 | 7.724 |
| RTAL | 301 H202 | 18.957 | 39.729 | 7.203 |
| RTAL | 301 H203 | 18.038 | 38.384 | 7.871 |
(d) 2NTU (model 2)

| residue | atom | x      | y      | z      |
|---------|------|--------|--------|--------|
| ARG     | A    | 82 CB  | 14.733 | 37.219 | -2.288 |
| ARG     | A    | 82 HB1 | 15.136 | 37.7   | -1.39  |
| ARG     | A    | 82 HB2 | 14.167 | 37.999 | -2.815 |
| ARG     | A    | 82 CG  | 15.88  | 36.76  | -3.176 |
| ARG     | A    | 82 HG1 | 15.542 | 36.555 | -4.197 |
| ARG     | A    | 82 HG2 | 16.334 | 35.844 | -2.792 |
| ARG     | A    | 82 CD  | 16.912 | 37.885 | -3.238 |
| ARG     | A    | 82 HD1 | 17.352 | 38.059 | -2.251 |
| ARG     | A    | 82 HD2 | 16.451 | 38.819 | -3.559 |
| ARG     | A    | 82 NE  | 17.97  | 37.594 | -4.185 |
| ARG     | A    | 82 HE  | 17.988 | 38.101 | -5.086 |
| ARG     | A    | 82 CZ  | 18.936 | 36.709 | -3.998 |
| ARG     | A    | 82 NH1 | 19.056 | 36.026 | -2.857 |
| ARG     | A    | 82 HH11| 18.425 | 36.134 | -2.058 |
| ARG     | A    | 82 HH12| 19.863 | 35.418 | -2.749 |
| ARG     | A    | 82 NH2 | 19.802 | 36.472 | -5.008 |
| ARG     | A    | 82 HH21| 19.782 | 37.064 | -5.831 |
| ARG     | A    | 82 HH22| 20.616 | 35.904 | -4.814 |
| ASP     | A    | 85 CB  | 13.228 | 33.821 | 1.827  |
| ASP     | A    | 85 HB1 | 13.515 | 34.727 | 1.296  |
| ASP     | A    | 85 HB2 | 13.265 | 33.073 | 1.043  |
| ASP     | A    | 85 CG  | 14.433 | 33.557 | 2.758  |
| ASP     | A    | 85 OD1 | 14.405 | 33.7   | 3.999  |
| ASP     | A    | 85 OD2 | 15.486 | 33.219 | 2.107  |
| ASP     | A    | 212 CB | 20.947 | 35.16  | 3.094  |
| ASP     | A    | 212 HB1| 20.291 | 34.398 | 3.528  |
| ASP     | A    | 212 HB2| 21.63  | 34.651 | 2.409  |
| ASP     | A    | 212 CG | 19.979 | 36.039 | 2.292  |
| ASP     | A    | 212 OD1| 19.525 | 37.111 | 2.737  |
| ASP     | A    | 212 OD2| 19.575 | 35.507 | 1.189  |
| TYR     | A    | 57 CB  | 18.607 | 28.985 | -1.208 |
| TYR     | A    | 57 HB1 | 19.348 | 28.367 | -1.711 |
| TYR     | A    | 57 HB2 | 18.295 | 28.45  | -0.308 |
| TYR     | A    | 57 CG  | 19.259 | 30.314 | -0.915 |
| TYR     | A    | 57 CD1 | 18.778 | 31.201 | 0.052  |
| TYR     | A    | 57 HD1 | 17.928 | 30.947 | 0.667  |
| TYR     | A    | 57 CE1 | 19.331 | 32.463 | 0.209  |
| TYR     | A    | 57 HE1 | 18.925 | 33.138 | 0.948  |
| TYR     | A    | 57 CZ  | 20.374 | 32.88  | -0.623 |
| TYR     | A    | 57 OH  | 20.853 | 34.15  | -0.573 |
| TYR     | A    | 57 HH  | 20.416 | 34.686 | 0.165  |
| TYR     | A    | 57 CD2 | 20.35  | 30.722 | -1.696 |
| TYR     | A    | 57 HD2 | 20.748 | 30.036 | -2.44  |
| TYR     | A    | 57 CE2 | 20.915 | 31.989 | -1.558 |
| TYR     | A    | 57 HE2 | 21.745 | 32.298 | -2.187 |
| TYR     | A    | 185 CB | 19.29  | 44.049 | 2.318  |
| TYR     | A    | 185 HB1| 18.482 | 43.962 | 1.586  |
| TYR     | A    | 185 HB2| 18.899 | 44.632 | 3.153  |
| TYR     | A    | 185 CG | 19.644 | 42.664 | 2.814  |
| TYR     | A    | 185 CD1| 20.391 | 42.508 | 3.981  |
| TYR     | A    | 185 HD1| 20.755 | 43.397 | 4.468  |
| TYR     | A    | 185 CE1| 20.665 | 41.267 | 4.537  |
| Residue | Atom  | X    | Y    | Z    |
|---------|-------|------|------|------|
| TYR A   | HE1   | 21.239 | 41.176 | 5.454 |
| TYR A   | CZ    | 20.196 | 40.118 | 3.896 |
| TYR A   | OH    | 20.451 | 38.918 | 4.475 |
| TYR A   | HH    | 20.125 | 38.19 | 3.89 |
| TYR A   | CD2   | 19.17 | 41.504 | 2.197 |
| TYR A   | HD2   | 18.54 | 41.578 | 1.317 |
| THR A   | CB    | 13.568 | 34.362 | 7.362 |
| THR A   | HB    | 14.401 | 34.278 | 8.075 |
| THR A   | OG1   | 14.002 | 35.214 | 6.313 |
| THR A   | CG    | 13.942 | 34.736 | 5.462 |
| THR A   | CD    | 18.986 | 33.908 | 7.73 |
| THR A   | HD1   | 17.75 | 32.65 | 7.028 |
| LYS A   | CB    | 20 | 32.974 | 8.398 |
| LYS A   | HB1   | 19.506 | 32.05 | 8.721 |
| LYS A   | HB2   | 20.754 | 32.669 | 7.663 |
| LYS A   | CG    | 18.986 | 33.908 | 7.73 |
| LYS A   | HG1   | 19.495 | 34.768 | 7.285 |
| LYS A   | HG2   | 18.32 | 34.321 | 8.493 |
| LYS A   | CD    | 18.134 | 33.224 | 6.661 |
| LYS A   | HD1   | 17.75 | 32.65 | 7.028 |
| LYS A   | HD2   | 18.726 | 33 | 5.77 |
| LYS A   | CE    | 16.943 | 34.096 | 6.26 |
| LYS A   | HE1   | 16.383 | 34.423 | 7.138 |
| LYS A   | HE2   | 16.238 | 33.582 | 5.607 |
| LYS A   | NZ    | 17.357 | 35.292 | 5.546 |
| LYS A   | HZ1   | 17.484 | 35.157 | 4.529 |
| HOH 402 | O     | 17.709 | 34.173 | 3.04 |
| HOH 402 | H1    | 18.096 | 34.556 | 2.24 |
| HOH 402 | H2    | 16.838 | 33.75 | 2.75 |
| HOH 401 | O     | 15.873 | 33.336 | -0.542 |
| HOH 401 | H1    | 15.675 | 33.256 | 0.422 |
| HOH 401 | H2    | 16.494 | 34.08 | -0.599 |
| HOH 406 | O     | 17.525 | 35.918 | -0.446 |
| HOH 406 | H1    | 18.262 | 35.901 | 0.223 |
| HOH 406 | H2    | 16.826 | 36.47 | -0.065 |
| RTAL 301| C1    | 14.962 | 45.529 | 0.352 |
| RTAL 301| C2    | 14.819 | 46.256 | -0.88 |
| RTAL 301| H21   | 14.352 | 45.683 | -1.686 |
| RTAL 301| H22   | 15.823 | 46.528 | -1.23 |
| RTAL 301| C3    | 14.048 | 47.534 | -0.619 |
| RTAL 301| H31   | 13.026 | 47.298 | -0.32 |
| RTAL 301| H32   | 13.976 | 48.124 | -1.54 |
| RTAL 301| C4    | 14.776 | 48.314 | 0.471 |
| RTAL 301| H41   | 14.216 | 49.189 | 0.808 |
| RTAL 301| H42   | 15.698 | 48.727 | 0.039 |
| RTAL 301| C5    | 15.132 | 47.48 | 1.681 |
| RTAL 301| C6    | 15.245 | 46.119 | 1.642 |
| RTAL 301| C7    | 15.591 | 45.421 | 2.87 |
| RTAL 301| H71   | 15.887 | 46.053 | 3.696 |
| RTAL 301| C8    | 15.632 | 44.085 | 3.122 |
| RTAL 301| H81   | 15.322 | 43.372 | 2.37 |
|   |   |   |   |
|---|---|---|---|
| RTAL | 301 C9 | 16.15 | 43.483 | 4.32 |
| RTAL | 301 C10 | 16.307 | 42.118 | 4.291 |
| RTAL | 301 H101 | 15.994 | 41.608 | 3.382 |
| RTAL | 301 C11 | 16.896 | 41.283 | 5.271 |
| RTAL | 301 H111 | 17.174 | 41.686 | 6.24 |
| RTAL | 301 C12 | 17.102 | 39.958 | 4.98 |
| RTAL | 301 H121 | 16.814 | 39.625 | 3.988 |
| RTAL | 301 C13 | 17.545 | 38.943 | 5.863 |
| RTAL | 301 C14 | 17.562 | 37.653 | 5.354 |
| RTAL | 301 H141 | 17.511 | 37.543 | 4.281 |
| RTAL | 301 C15 | 17.443 | 36.483 | 6.1 |
| RTAL | 301 H15 | 17.31 | 36.518 | 7.177 |
| RTAL | 301 C16 | 13.635 | 44.555 | 0.542 |
| RTAL | 301 H161 | 13.4 | 44.005 | -0.371 |
| RTAL | 301 H162 | 12.815 | 45.246 | 0.761 |
| RTAL | 301 H163 | 13.669 | 43.837 | 1.363 |
| RTAL | 301 C17 | 16.124 | 44.379 | -0.015 |
| RTAL | 301 H171 | 17.053 | 44.946 | -0.13 |
| RTAL | 301 H172 | 15.915 | 43.905 | -0.978 |
| RTAL | 301 H173 | 16.305 | 43.59 | 0.712 |
| RTAL | 301 C18 | 15.266 | 48.29 | 2.939 |
| RTAL | 301 H181 | 14.267 | 48.447 | 3.36 |
| RTAL | 301 H182 | 15.686 | 49.268 | 2.71 |
| RTAL | 301 H183 | 15.881 | 47.841 | 3.715 |
| RTAL | 301 C19 | 16.601 | 44.326 | 5.477 |
| RTAL | 301 H191 | 15.863 | 45.087 | 5.732 |
| RTAL | 301 H192 | 17.529 | 44.85 | 5.229 |
| RTAL | 301 H193 | 16.775 | 43.719 | 6.363 |
| RTAL | 301 C20 | 17.974 | 39.261 | 7.261 |
| RTAL | 301 H201 | 17.301 | 39.959 | 7.756 |
| RTAL | 301 H202 | 18.957 | 39.744 | 7.213 |
| RTAL | 301 H203 | 18.08 | 38.369 | 7.878 |
Table S2. Atomic coordinates of the QM/MM optimized geometry for ASR [W402, Tyr11, Tyr51, Asp75, Trp76, Thr79, Ser47, the backbone of Phe202, Lys210 (the Schiff base) and the retinal].

1X1O (deprotonated Asp75)

| residue | atom | x     | y     | z     |
|---------|------|-------|-------|-------|
| TYR A   | CB   | 28.683| 32.043| 23.475|
| TYR A   | HB1  | 29.208| 32.866| 23.952|
| TYR A   | HB2  | 28.175| 32.457| 22.597|
| TYR A   | CG   | 29.664| 30.969| 23.082|
| TYR A   | CD1  | 29.352| 30.066| 22.066|
| TYR A   | HD1  | 28.405| 30.148| 21.539|
| TYR A   | CE1  | 30.236| 29.054| 21.722|
| TYR A   | HE1  | 29.956| 28.336| 20.969|
| TYR A   | CZ   | 31.467| 28.925| 22.373|
| TYR A   | OH   | 32.302| 27.928| 21.976|
| TYR A   | HH   | 33.133| 27.936| 22.512|
| TYR A   | CD2  | 30.878| 30.802| 23.759|
| TYR A   | HD2  | 31.123| 31.475| 24.574|
| TYR A   | CE2  | 31.782| 30.802| 23.737|
| TYR A   | HE2  | 32.735| 31.711| 21.727|
| TYR A   | CB   | 33.276| 33.098| 25.01 |
| TYR A   | HB1  | 32.369| 33.707| 25.086|
| TYR A   | HB2  | 33.13 | 32.21 | 25.638|
| TYR A   | CG   | 33.493| 32.727| 23.547|
| TYR A   | CD1  | 34.614| 32.028| 23.071|
| TYR A   | HD1  | 35.404| 31.705| 23.737|
| TYR A   | CE1  | 34.764| 31.711| 21.727|
| TYR A   | HE1  | 35.648| 31.178| 21.404|
| TYR A   | CZ   | 33.767| 32.05 | 20.812|
| TYR A   | OH   | 33.953| 31.654| 19.506|
| TYR A   | HH   | 33.22 | 31.982| 18.944|
| TYR A   | CD2  | 32.518| 33.062| 22.602|
| TYR A   | HD2  | 31.614| 33.553| 22.948|
| TYR A   | CE2  | 32.632| 32.735| 21.25 |
| TYR A   | HE2  | 31.849| 32.996| 20.548|
| ASP A   | CB   | 38.833| 29.43 | 22.443|
| ASP A   | HB1  | 38.814| 30.216| 21.689|
| ASP A   | HB2  | 38.631| 29.939| 23.383|
| ASP A   | CG   | 37.618| 28.525| 22.171|
| ASP A   | OD1  | 37.662| 27.273| 22.244|
| ASP A   | OD2  | 36.54 | 29.182| 21.953|
| TRP A   | CB   | 40.705| 27.733| 17.857|
| TRP A   | HB1  | 40.923| 26.964| 17.11 |
| TRP A   | HB2  | 41.52 | 28.465| 17.807|
| TRP A   | CG   | 39.4  | 28.405| 17.529|
| TRP A   | CD1  | 38.175| 28.269| 18.132|
| TRP A   | HD1  | 37.904| 27.718| 19.022|
| TRP A   | NE1  | 37.205| 28.987| 17.456|
| TRP A   | HE1  | 36.34 | 29.236| 17.936|
| TRP A   | CE2  | 37.81 | 29.657| 16.41 |
| TRP A   | CD2  | 39.193| 29.315| 16.423|
| TRP A   | CE3  | 40.044| 29.927| 15.482|
|      |      |      |
|------|------|------|
| TRP  | A 76 | HE3 |
| TRP  | A 76 | CZ3 |
| TRP  | A 76 | HZ3 |
| TRP  | A 76 | CZ2 |
| TRP  | A 76 | HZ2 |
| TRP  | A 76 | CH2 |
| TRP  | A 76 | HH2 |
| THR  | A 79 | CB  |
| THR  | A 79 | HB  |
| THR  | A 79 | OG1 |
| THR  | A 79 | HG1 |
| THR  | A 79 | HG21|
| THR  | A 79 | HG22|
| THR  | A 79 | HG23|
| SER  | A 47 | CB  |
| SER  | A 47 | HB1 |
| SER  | A 47 | HG1 |
| LYS  | A 210| CB  |
| LYS  | A 210| HB1 |
| LYS  | A 210| HB2 |
| LYS  | A 210| CG  |
| LYS  | A 210| HG1 |
| LYS  | A 210| HG2 |
| LYS  | A 210| CD  |
| LYS  | A 210| HD1 |
| LYS  | A 210| HD2 |
| LYS  | A 210| CE  |
| LYS  | A 210| HE1 |
| LYS  | A 210| HE2 |
| LYS  | A 210| NZ  |
| LYS  | A 210| HZ1 |
| PHE  | A 202| CA  |
| PHE  | A 202| HA  |
| PHE  | A 202| C  |
| PHE  | A 202| O  |
| CYS  | A 203| N  |
| CYS  | A 203| HN |
| HOH  | 402  | O  |
| HOH  | 402  | H1 |
| HOH  | 402  | H2 |
| RTAL | 301  | C1 |
| RTAL | 301  | C2 |
| RTAL | 301  | H21|
| RTAL | 301  | H22|
| RTAL | 301  | C3 |
| RTAL | 301  | H31|
| RTAL | 301  | H32|
| RTAL | 301  | C4 |
| RTAL | 301  | H41|
| RTAL | 301  | H42|
| RTAL | 301  | C5 |
| RTAL | 301  | C6 |
| RTAL  | 301 C7 | 40.162 | 26.866 | 10.261 |
|-------|--------|--------|--------|--------|
| RTAL  | 301 H71| 40.012 | 25.985 | 9.646  |
| RTAL  | 301 C8 | 39.687 | 26.785 | 11.534 |
| RTAL  | 301 H81| 39.82  | 27.621 | 12.206 |
| RTAL  | 301 C9 | 38.957 | 25.685 | 12.123 |
| RTAL  | 301 C10| 38.455 | 25.913 | 13.382 |
| RTAL  | 301 H101| 38.671  | 25.892 | 14.2   |
| RTAL  | 301 C11| 37.625 | 25.092 | 13.904 |
| RTAL  | 301 C12| 37.11  | 25.618 | 15.358 |
| RTAL  | 301 H121| 37.359  | 26.653 | 15.577 |
| RTAL  | 301 C13| 36.321 | 24.963 | 16.353 |
| RTAL  | 301 C14| 35.941 | 25.734 | 17.44  |
| RTAL  | 301 H141| 36.093  | 26.804 | 17.366 |
| RTAL  | 301 C15| 35.553 | 25.244 | 18.7   |
| RTAL  | 301 H15| 35.623 | 24.183 | 18.924 |
| RTAL  | 301 C16| 41.961 | 29.212 | 11.546 |
| RTAL  | 301 H161| 42.022  | 30.174 | 12.068 |
| RTAL  | 301 H162| 42.967  | 28.917 | 11.236 |
| RTAL  | 301 H163| 41.622  | 28.473 | 12.272 |
| RTAL  | 301 C17| 39.642 | 29.893 | 10.784 |
| RTAL  | 301 H171| 38.928  | 29.904 | 9.956  |
| RTAL  | 301 H172| 39.771  | 30.924 | 11.123 |
| RTAL  | 301 H173| 39.191  | 29.346 | 11.611 |
| RTAL  | 301 C18| 41.306 | 26.466 | 7.592  |
| RTAL  | 301 H181| 42.24   | 25.899 | 7.673  |
| RTAL  | 301 H182| 41.185  | 26.701 | 6.53   |
| RTAL  | 301 H183| 40.485  | 25.807 | 7.875  |
| RTAL  | 301 C19| 38.729 | 24.409 | 11.358 |
| RTAL  | 301 H191| 38.228  | 24.596 | 10.403 |
| RTAL  | 301 H192| 38.113  | 23.708 | 11.921 |
| RTAL  | 301 H193| 39.684 | 23.917 | 11.135 |
| RTAL  | 301 C20 | 35.918 | 23.524 | 16.19  |
| RTAL  | 301 H201| 36.748  | 22.875 | 15.905 |
| RTAL  | 301 H202| 35.183  | 23.45  | 15.381 |
| RTAL  | 301 H203| 35.45  | 23.125 | 17.09  |