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Identification of Binding Sites of Non-I-Helix Water Molecules in Mammalian Cytochromes P450

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a) Running title: Water binding sites in mammalian P450s

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c)

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d) Non-standard abbreviations: P450, cytochrome P450; H-bond, hydrogen bond; pdb, Protein Data Bank

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Abstract

The cytochromes P450 enzymes are integral in determining the disposition of many therapeutic compounds. At the molecular level, the details of P450 catalysis are still under investigation, but the importance of water-mediated proton shuttles seems evident in the catalytic cycle as it progresses through various heme iron-oxygen enzyme intermediates. The study of P450-bound waters has been largely restricted to bacterial enzymes that may or may not reflect the location or function of waters in human drug-metabolizing P450s. However, in recent years, sixteen structures of mammalian P450s containing crystallographic waters have been deposited in the Protein Data Bank. Described herein is the identification of seven well-defined water clusters in mammalian P450s identified by calculating the density of globally aligned waters as reported by Tanner and co-workers [Bottoms CA, White TA, and Tanner JJ (2006) *Proteins* **64** (2): 404-421 (DOI: 10.1002/prot.21014)]. All water binding sites were in or within the immediate vicinity of the active sites of the P450s, but most were not near the conserved I-helix threonine often implicated in P450 catalysis. Therefore, it is possible that some of the water binding sites identified here ultimately determine P450 catalytic efficiency either by working as an extension of the I-helix water network, or by acting in novel proton shuttles that modulate the non-productive shunting of reactive oxygen species.

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The heme-thiolate enzymes known as cytochromes P450 (P450s) are widely recognized for their role in the metabolism of drugs and other xenobiotics. One property that makes the P450s such versatile catalysts is their ability to generate reactive iron-oxygen species (reviewed in (Denisov *et al.*, 2005)). Water networks have long been proposed to act as proton shuttles that help the P450s generate various iron-oxygen intermediates during the catalytic cycle (Denisov *et al.*, 2005). Much of the evidence for the existence and role of water networks in P450 catalysis has come from the study of the bacterial P450s, such as $P450_{cam}$ (CYP101A), for which the first P450 enzyme structure was determined (Poulos *et al.*, 1985). The position of the I-helix near the heme prosthetic group and the presence of a conserved threonine residue (Nelson and Strobel, 1989) near a solvated kink within this helix have prompted numerous studies aimed at describing the details of proton shuttling in P450. In particular, the biochemical (Gerber and Sligar, 1994;Imai *et al.*, 1989) and crystallographic (Clark *et al.*, 2006;Raag *et al.*, 1991) characterization of I-helix mutant enzymes and the ability to trap their iron-dioxygen intermediates (Nagano and Poulos, 2005;Schlichting *et al.*, 2000) with ordered water networks continues to reveal subtle enzyme conformational changes that appear critical in controlling water positioning.

In addition to being central in P450 catalysis, water is almost certain to affect enzyme stability, dynamics, and substrate binding. However, a substantial hurdle to studying water-protein interactions, even beyond understanding their function, is that of identifying where water actually binds. Until recently, the location of waters or water binding residues in mammalian, microsomal P450s could only be inferred based on information derived from bacterial enzymes. For instance, it has become apparent that the same highly conserved I-helix threonine residue studied in bacterial P450s is involved in several aspects of catalysis (Vaz, 2001) and the water-

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mediated reversibility of covalent heme adducts (Blobaum, 2006) in the mammalian P450s 2E1 and 2B4. However, over the past few years crystallization of mammalian P450s by Williams and co-workers, Johnson, Stout and co-workers, and Halpert and co-workers have greatly added to our knowledge of the drug metabolizing P450 isoforms by reporting some of their structural features (Johnson and Stout, 2005). There are currently sixteen crystal structures encompassing seven mammalian isoforms all with ordered waters, but no reports regarding the positions of these water molecules have been presented.

This work describes an attempt to evaluate whether there may be conserved water molecules and/or water networks in the mammalian xenobiotic-metabolizing P450s. After globally aligning the P450 structures, the three-dimensional coordinates of all the crystallographic waters were analyzed by calculating the density of water clusters. Searches for nearby H-bonding polar backbone and sidechain partners for water clusters were also conducted and appeared to correlate with observed isoform differences in water binding sites. Seven well-defined water clusters in close proximity to the active site were identified. The possible roles of these water clusters in P450 structure and function are discussed based on these findings.

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Materials and Methods

Hardware and software. All analyses were carried out on a Unix workstation using the *Maestro* (version 7.5) interface of Schrödinger (Schrödinger, LLC, New York, NY, 2006) and *CNS* version 1.1 (Brunger *et al.*, 1998). Water density maps and contouring were visualized with *PyMOL* version 0.99 (DeLano Scientific, San Carlos, CA, 2002).

Alignment of P450 crystal structures. The following pdb files were obtained from the Protein Data Bank (http://www.rcsb.org) (Berman *et al.*, 2000): $P450_{cam}$ (1DZ4) (Schlichting *et al.*, 2000), CYP2C9 (1OG2 and 1OG5) (Williams *et al.*, 2003), CYP2C5 (1N6B) (Wester *et al.*, 2003a), CYP2C5 (1NR6) (Wester *et al.*, 2003b), CYP2B4 (1PO5) (Scott *et al.*, 2003), $P450_{BM3}$ (1P0V) (Ost *et al.*, 2003), CYP2C8 (1PQ2) (Schoch *et al.*, 2004), CYP2C9 (1R9O) (Wester *et al.*, 2004), CYP2B4 (1SUO) (Scott *et al.*, 2004), CYP3A4 (1TQN) (Yano *et al.*, 2004), CYP3A4 (1W0E, 1W0F, and 1W0G) (Williams *et al.*, 2004), CYP2A6 (1Z10 and 1Z11) (Yano *et al.*, 2005), CYP2B4 (2BDM) (Zhao *et al.*, 2006), and CYP2D6 (2F9Q) (Rowland *et al.*, 2006), representing all of the currently deposited crystal structures of human and rabbit microsomal P450s. In addition, the model bacterial isoforms $P450_{cam}$ (CYP101A1) and P450 BM3 (CYP102A1) were added for comparison. After trimming the pdb files of any extra polypeptide chains in the unit cell, the files were imported into *Maestro* with all of their assigned water oxygens. A global structure alignment performed using the Protein Alignment Tool also successfully translated the positions of all water oxygens allowing all coordinates to be updated and saved. The rmsd of the I-helix C_α atoms between any two P450 structures did not exceed 2.01 Å, and averaged 0.99 Å betweeen all structures when the 2B4 structure, 2BDM, was excluded. Furthermore, the rmsd values between the heavy atoms of the heme groups averaged

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0.96 Å without the 2BDM structure. At this point, the coordinates of the waters from all structures were added to a single pdb file for further analysis.

Water density peak calculation. The density of overlapping water oxygens was calculated exactly as described by Tanner and co-workers using the program *CNS* (Bottoms *et al.*, 2006). By allowing *CNS* to search for water clusters, record their coordinates, and assign a quantitative number describing their density, the identification of potential water binding sites remains objective since no previous knowledge is needed. Four CNS scripts were used to generate the "pseudo-electron density" map and water cluster density by using the theoretical reflections of the pdb file containing the water oxygens. A P1 lattice with unit cell angles of 90 degrees and unit cell lengths of 30 Å was used in the following analysis. The first three scripts (generate_easy.inp, model_fcalc.inp, and make_cv.inp) generate the necessary coordinate, topology, and cross-validated (10 % in the test set) reflection files for input into the fourth script, model_map, as described previously (Bottoms *et al.*, 2006). Model_map.inp was then used with a uniform B-factor of 20 $Å^2$ for all water to output a list of coordinates for each water peak, which can be thought of as the average position of waters belonging to a single cluster. The *CNS* script also lists the density of the water peaks, and outputs a density map ($2|F_o|-|F_c|$).

Waters from an enzyme were classified as belonging to a specific water peak by using a cutoff distance of 2.0 Å between the water oxygen atom and calculated water peak. Potential H-bond donors and acceptors near the water peaks were manually identified using the polar group distance tool in *PyMOL*. The two angstrom cutoff was chosen because distances between water oxygens in the water network ranged from 2.03 to 2.10 Å based on the protein structure alignment. Therefore, the assignment of multiple water molecules to a single binding site was avoided. Furthermore, all but three of the waters from all seven water binding sites were less

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than 2.0 angstroms from their respective calculated water peaks. There were a total of 66 water molecules in the alignment (excluding I-helix waters bound to unique sites), which means any given water was correctly assigned to a particular water binding site 95 % of the time. In those rare cases where the water oxygen lies 2.0 angstroms beyond a water peak calculated from our alignment, an examination of the H-bonding partners confirms whether the water belongs to a conserved site, or whether it is unique.

Results and Discussion

The unbiased water cluster analysis method used here with the thirteen microsomal P450 and two bacterial P450 structures listed in Table 1 identified seven distinct water binding sites in or within a few angstroms of the enzyme active sites. Numbering of the sites follows the N- to C-terminus direction as close as possible. Interestingly, the locations of the highest density water binding sites are not associated with the conserved I-helix threonine. In fact, waters near the conserved threonine (site 2, Fig. 1a) were considerably scattered into multiple, smaller clusters of low density. The position of the equivalent T252-bound water and associated network described in $P450_{cam}$ (Nagano and Poulos, 2005;Schlichting *et al.*, 2000) was shown to be dependent on the presence of an oxygen ligand. Therefore, an oxygen ligand in mammalian enzymes may be necessary to draw any conclusions about proton relay in this region (Fig. 1a). Furthermore, the bending of the I-helix controls the position of water so this most likely adds to the more dispersive water density found at water binding site 2 (Schlichting *et al.*, 2000). On the other hand, well-defined water clusters were discovered in two other regions of the data set, which largely reflects the mammalian 2 family of P450s. Four clusters defining a water network (sites 3, 4, 5, and 6, Fig. 1a) were found around the plane of the heme group a and b rings that are

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bound to the K-helix and loop between the K-helix and β1-4 strand. Another cluster in this region (site 7) lying slightly higher above the distal face of the heme is not part of the water network, but receives H-bonds from backbone groups in multiple loops near the C-terminus (Fig. 1a and b). Finally, a water binding site was found situated in the twisting C-terminal end of the B-C loop (site 1, Fig. 1b) in four out of seven mammalian enzymes. Except for site 3, which H-bonds with a threonine one helical turn away from the conserved threonine position, the H-bonding partners of the waters were found to be primarily backbone amide and carbonyl groups.

Five of the enzymes included in the analysis, P450s 2C8, 2D6, 3A4, BM3, and cam, contained only a few of the waters clustered at sites 1-7 (Table 1). For P450s 3A4, BM3, and cam, the absence of polar groups can account for the lack of crystallographic waters in most cases. Threonine and glutamine sidechains involved in water binding to site 3 are replaced with valine, leucine, and phenylalanine in these enzymes, and hence, would be predicted to disrupt the water network at sites 3 and 4. Additionally, the water at site 5 of P450s 3A4, BM3, and cam forms an H-bond with the enzyme directly rather than networking with another water, thereby eliminating site 6. P450s 3A4 and cam were the only two enzymes which had significantly different loop structures in the region where site 7 is proposed to exist in the P450 2 family enzymes, and no corresponding water was found at this site in these well-hydrated structures (Fig. 1c). No water at site 1 in P450 3A4 is found, presumably because it contains an isoleucine instead of an asparate in the I-helix and because the position of the helix in the B-C loop allows it to form H-bonds with itself, decreasing the available space for a water to bind (Fig. 1d). Hence, isoforms lack particular water(s) when there is an unfavorable geometry of polar backbone groups or substitution of polar sidechains for hydrophobic sidechains. When water

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was absent from any of the water binding sites, there did not appear to be any compensation by polar protein sidechains.

Conversely, very few waters were assigned throughout the P450 2C8 and 2D6 structures and it is speculated that this may be due to their lower resolution (> 2.8 Å) rather than a lack of water binding sites, since the positions and residues at most of the water binding sites are the same as the 2C and 2B isoforms. Water was also sparse in the lower resolution P450 3A4 structures reported by Williams and co-workers so these enzymes were excluded from the analysis (Williams *et al.*, 2004).

The locations of the non-I-helix waters may prove to be significant for several reasons. First, site 5 appears to be conserved across species, though this might not be detected by sequence alignments since the H-bonding partners are either other waters or backbone groups of different residues (e.g. Pro or Thr). This region is also suspiciously close to two positions of naturally occurring P450 2C9 variants, 2C9.3 (I359L) and 2C9.5 (D360E), and might explain, in part, the altered kinetic parameters of these enzymes (Fig. 1a) (Dickmann *et al.*, 2001). For example, the D360 sidechain forms part of water binding site 6. Since the networked waters rely, to some degree, on each other, and because D360 also accepts an H-bond from the K'-helix, the seemingly conservative D360E substitution may produce changes in both the water network and tertiary structure of P450 2C9. Second, the location of a water binding site in the B-C loop (site 1) may enable a water to bind directly with substrate or help control the egress of metabolites out of the P450 because this glycine-rich region has been postulated to be flexible and adopt multiple conformations (Honma *et al.*, 2005;Li *et al.*, 2005;Wester *et al.*, 2003b). Third, like site 1, water binding site 7 is in a position where it could regulate the structure or stability of a loop (region

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between the two β3 strands) that a substrate would encounter if it enters the enzyme near the F-G loop region (Schleinkofer *et al.*, 2005).

It should be pointed out that this analysis was carried out with multiple structures of the same P450 isoform under varying conditions (e.g. alternate substrates) due to the limited number of mammalian P450 structures currently available. Because of this, the density of some water clusters is weighted toward the enzymes for which multiple structures were available. On the other hand, Table 1 demonstrates that some waters absent from one structure of an isoform are present in other structures of the same isoform. In these cases, such as with P450s 2A6 and 2C9, we can only speculate that the experimental conditions and or refinement altered the assignment of crystallographic waters. Given that the enzyme conformation and positions of nearby polar groups remain remarkably constant in these situations, such as in the two 2A6 structures (rmsd is 0.15 Å around the active site (Yano *et al.*, 2005)), the binding site most likely exists, but its occupancy was simply not detected. The presence of different substrates, remarkably, did not alter the site 1 or site 7 active site waters in P450s 2A6 or 2C9. Conversely, the differences in the P450 2B4 structures are all explained by differences in enzyme conformation and/or the presence of detergent molecules.

In conclusion, several hypotheses regarding the shuttling of protons within P450s have been reported (Nagano and Poulos, 2005;Oprea *et al.*, 1997;Schlichting *et al.*, 2000). Here, seven distinct water clusters were found in close proximity to the active site of multiple microsomal P450s. Bacterial structures were also included in the alignment and water density calculation because most of the literature describing water binding sites originated from studies with these enzymes. In addition, the apparent overlap of water binding site 5 between the mammalian and bacterial enzymes suggest this previously unrecognized water has some

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unknown significance. The well-defined nature of these clusters suggests each one truly represents a water binding site, though waters that move during catalysis may not have been identified. Moreover, visual inspection of a P450 structure aligned to the water peaks described here allows one to predict whether a water binds to a site in a given P450 isoform based on available H-bonding partners and a pocket of sufficient volume. Interestingly, six of these sites are not associated with the proton shuttle near the conserved I-helix threonine residue. Though this may not preclude them from having a role in catalysis, these water binding sites may also carry out important roles related to protein folding, stability, and/or dynamics.

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DMD Fast Forward. Published on September 6, 2006 as DOI: 10.1124/dmd.106.011890
This article has not been copyedited and formatted. The final version may differ from this version.

Reference List

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat T N, Weissig H, Shindyalov I N and Bourne P E (2000) The Protein Data Bank. *Nucleic Acids Res* **28**:235-242.

Blobaum AL (2006) Mechanism-Based Inactivation and Reversibility: Is There a New Trend in the Inactivation of Cytochrome P450 Enzymes? *Drug Metab Dispos* **34**:1-7.

Bottoms C, White T and Tanner J (2006) Exploring Structurally Conserved Solvent Sites in Protein Families. *Proteins* **64**:404-421.

Brunger AT, Adams P D, Clore G M, DeLano W L, Gros P, Grosse-Kunstleve R W, Jiang J S, Kuszewski J, Nilges M, Pannu N S, Read R J, Rice L M, Simonson T and Warren G L (1998) Crystallography & NMR System: A New Software Suite for Macromolecular Structure Determination. *Acta Crystallogr D Biol Crystallogr* **54**:905-921.

Clark JP, Miles C S, Mowat C G, Walkinshaw M D, Reid G A, Daff S N and Chapman S K (2006) The Role of Thr268 and Phe393 in Cytochrome P450 BM3. *J Inorg Biochem* **100**:1075-1090.

Denisov IG, Makris T M, Sligar S G and Schlichting I (2005) Structure and Chemistry of Cytochrome P450. *Chem Rev* **105**:2253-2277.

Dickmann LJ, Rettie A E, Kneller M B, Kim R B, Wood A J, Stein C M, Wilkinson G R and Schwarz U I (2001) Identification and Functional Characterization of a New CYP2C9 Variant (CYP2C9*5) Expressed Among African Americans. *Mol Pharmacol* **60**:382-387.

Gerber NC and Sligar S G (1994) A Role for Asp-251 in Cytochrome P-450cam Oxygen Activation. *J Biol Chem* **269**:4260-4266.

Honma W, Li W, Liu H, Scott E E and Halpert J R (2005) Functional Role of Residues in the Helix B' Region of Cytochrome P450 2B1. *Arch Biochem Biophys* **435**:157-165.

Imai M, Shimada H, Watanabe Y, Matsushima-Hibiya Y, Makino R, Koga H, Horiuchi T and Ishimura Y (1989) Uncoupling of the Cytochrome P-450cam Monooxygenase Reaction by a Single Mutation, Threonine-252 to Alanine or Valine: Possible Role of the Hydroxy Amino Acid in Oxygen Activation. *Proc Natl Acad Sci U S A* **86**:7823-7827.

Johnson EF and Stout C D (2005) Structural Diversity of Human Xenobiotic-Metabolizing Cytochrome P450 Monooxygenases. *Biochem Biophys Res Commun* **338**:331-336.

Li W, Liu H, Scott E E, Grater F, Halpert J R, Luo X, Shen J and Jiang H (2005) Possible Pathway(s) of Testosterone Egress From the Active Site of Cytochrome P450 2B1: a Steered Molecular Dynamics Simulation. *Drug Metab Dispos* **33**:910-919.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 17, 2024

DMD Fast Forward. Published on September 6, 2006 as DOI: 10.1124/dmd.106.011890
This article has not been copyedited and formatted. The final version may differ from this version.

Nagano S and Poulos T L (2005) Crystallographic Study on the Dioxygen Complex of Wild-Type and Mutant Cytochrome P450cam. Implications for the Dioxygen Activation Mechanism. *J Biol Chem* **280**:31659-31663.

Nelson DR and Strobel H W (1989) Secondary Structure Prediction of 52 Membrane-Bound Cytochromes P450 Shows a Strong Structural Similarity to P450cam. *Biochemistry* **28**:656-660.

Oprea TI, Hummer G and Garcia A E (1997) Identification of a Functional Water Channel in Cytochrome P450 Enzymes. *Proc Natl Acad Sci U S A* **94**:2133-2138.

Ost T, Clark J, Miles C, Walkinshaw M, Reid G, Chapman S, Daff S and Mowat C (2003) F393A Mutant Heme Domain of Flavocytochrome P450 BM3. *J Am Chem Soc* **125**:15010-15020.

Poulos TL, Finzel B C, Gunsalus I C, Wagner G C and Kraut J (1985) The 2.6-A Crystal Structure of Pseudomonas Putida Cytochrome P-450. *J Biol Chem* **260**:16122-16130.

Raag R, Martinis S A, Sligar S G and Poulos T L (1991) Crystal Structure of the Cytochrome P-450CAM Active Site Mutant Thr252Ala. *Biochemistry* **30**:11420-11429.

Rowland P, Blaney F E, Smyth M G, Jones J J, Leydon V R, Oxbrow A K, Lewis C J, Tennant M G, Modi S, Eggleston D S, Chenery R J and Bridges A M (2006) Crystal Structure of Human Cytochrome P450 2D6. *J Biol Chem* **281**:7614-7622.

Schleinkofer K, Sudarko, Winn P J, Ludemann S K and Wade R C (2005) Do Mammalian Cytochrome P450s Show Multiple Ligand Access Pathways and Ligand Channelling? *EMBO Rep* **6**:584-589.

Schlichting I, Berendzen J, Chu K, Stock A M, Maves S A, Benson D E, Sweet R M, Ringe D, Petsko G A and Sligar S G (2000) The Catalytic Pathway of Cytochrome P450cam at Atomic Resolution. *Science* **287**:1615-1622.

Schoch GA, Yano J K, Wester M R, Griffin K J, Stout C D and Johnson E F (2004) Structure of Human Microsomal Cytochrome P450 2C8. Evidence for a Peripheral Fatty Acid Binding Site. *J Biol Chem* **279**:9497-9503.

Scott EE, He Y A, Wester M R, White M A, Chin C C, Halpert J R, Johnson E F and Stout C D (2003) An Open Conformation of Mammalian Cytochrome P450 2B4 at 1.6-A Resolution. *Proc Natl Acad Sci U S A* **100**:13196-13201.

Scott EE, White M A, He Y A, Johnson E F, Stout C D and Halpert J R (2004) Structure of Mammalian Cytochrome P450 2B4 Complexed With 4-(4-Chlorophenyl)Imidazole at 1.9-A Resolution: Insight into the Range of P450 Conformations and the Coordination of Redox Partner Binding. *J Biol Chem* **279**:27294-27301.

Vaz AD (2001) Multiple Oxidants in Cytochrome P450 Catalyzed Reactions: Implications for Drug Metabolism. *Curr Drug Metab* **2**:1-16.

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Wester MR, Johnson E F, Marques-Soares C, Dansette P M, Mansuy D and Stout C D (2003a) Structure of a Substrate Complex of Mammalian Cytochrome P450 2C5 at 2.3 A Resolution: Evidence for Multiple Substrate Binding Modes. *Biochemistry* **42**:6370-6379.

Wester MR, Johnson E F, Marques-Soares C, Dijols S, Dansette P M, Mansuy D and Stout C D (2003b) Structure of Mammalian Cytochrome P450 2C5 Complexed With Diclofenac at 2.1 A Resolution: Evidence for an Induced Fit Model of Substrate Binding. *Biochemistry* **42**:9335-9345.

Wester MR, Yano J K, Schoch G A, Yang C, Griffin K J, Stout C D and Johnson E F (2004) The Structure of Human Cytochrome P450 2C9 Complexed With Flurbiprofen at 2.0-A Resolution. *J Biol Chem* **279**:35630-35637.

Williams PA, Cosme J, Vinkovic D M, Ward A, Angove H C, Day P J, Vonrhein C, Tickle I J and Jhoti H (2004) Crystal Structures of Human Cytochrome P450 3A4 Bound to Metyrapone and Progesterone. *Science* **305**:683-686.

Williams PA, Cosme J, Ward A, Angove H C, Matak V D and Jhoti H (2003) Crystal Structure of Human Cytochrome P450 2C9 With Bound Warfarin. *Nature* **424**:464-468.

Yano JK, Hsu M H, Griffin K J, Stout C D and Johnson E F (2005) Structures of Human Microsomal Cytochrome P450 2A6 Complexed With Coumarin and Methoxsalen. *Nat Struct Mol Biol* **12**:822-823.

Yano JK, Wester M R, Schoch G A, Griffin K J, Stout C D and Johnson E F (2004) The Structure of Human Microsomal Cytochrome P450 3A4 Determined by X-Ray Crystallography to 2.05-A Resolution. *J Biol Chem* **279**:38091-38094.

Zhao Y, White M A, Muralidhara B K, Sun L, Halpert J R and Stout C D (2006) Structure of Microsomal Cytochrome P450 2B4 Complexed With the Antifungal Drug Bifonazole: Insight into P450 Conformational Plasticity and Membrane Interaction. *J Biol Chem* **281**:5973-5981.

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Figure 1. Location and numbering of conserved water binding sites in mammalian P450 isoforms. (a) Human P450 2C9 (pdb 1R9O) shown with water sites 2 through 7. (b) Rabbit P450 2C5 (pdb 1N6B) shown with water sites 1 and 7. (c) Comparison of water binding site 7 near the loop N-terminal to helix K in P450 2C5 (gray) versus the lack of water in P450s 3A4 (blue) and cam (magenta). The position of this loop in P450s 3A4 and cam explain the absence of water binding site 7. (d) Comparison of water binding site 1 in P450 2C5 (gray) with P450 3A4 (blue) explains how a lack of polar sidechains and a different B'-helix structure does not allow P450 3A4 to bind water at this site. Waters aligned from all of the structures listed in Table 1 are shown as red spheres and the corresponding density map contoured at 5.0σ denotes distinct water binding sites. The heme group is shown in black. H-bonding partners of conserved waters are shown with the numbering of the corresponding enzyme; a complete list is available in Table 1.

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Table 1. Location and ID number of crystallographic waters in mammalian cytochrome P450 structures.

	B-C loop/ I-helix	*I-helix*	*I-helix/ K-helix*	*K-helix*	*K''-L loop*	*K-helix/ K-K' loop*	*K-K' loop*	
pdb ID	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Chain ID[a]
1Z11:2A6	7		2	46	97		37	A
1Z10:2A6	41		1	43	100	167	28	A
1PO5:2B4		9023	9001	9002	9003	9007	9055	A
1SUO:2B4	1087	1172	1049	1071	1047	1088	1106	A
2BDM:2B4			614	619	608	628		A
1N6B:2C5	722	616	612	625	626	611	620	A
1NR6:2C5	861	850	602	607	615	659	601	A
1PQ2:2C8			605					A
1OG2:2C9	30	33	35	50		51	52	A,Z
1OG5:2C9	33	109	135	136		137	138	A,Z
1R9O:2C9	648	819	609	642	637	640	610	A
2F9Q:2D6				1001			1006	C
1TQN:3A4		139			26			A
1DZ4:cam		687			252			A,Z
1P0V:BM3	9	325			32		445	A
representative H-bonds (1R9O:2C9)[b]	G111 amide, F114 amide, D293 sidechain	G296 carbonyl, A297 carbonyl, E300 amide, T301 amide	T305 sidechain, Q356 sidechain, site 4 H_2O	Q356 carbonyl, site 3 H_2O, site 5 H_2O	P427 carbonyl, site 4 H_2O, site 6 H_2O	D360 sidechain, L362 amide and carbonyl, site 5 H_2O, site 7 H_2O	T364 amide, S365 carbonyl, F476 carbonyl	
density (σ)[c]	36.8	20.4	54.2	54.8	39.5	45.6	35.2	

[a]Chain ID of the enzyme followed by the chain ID of the solvent when applicable.
[b]H-bonds are defined by distances of 3.2 Å or less between heteroatoms.
[c]Density peak value of coordinates representing the seven highest clustered water sites determined using CNS; see Methods.

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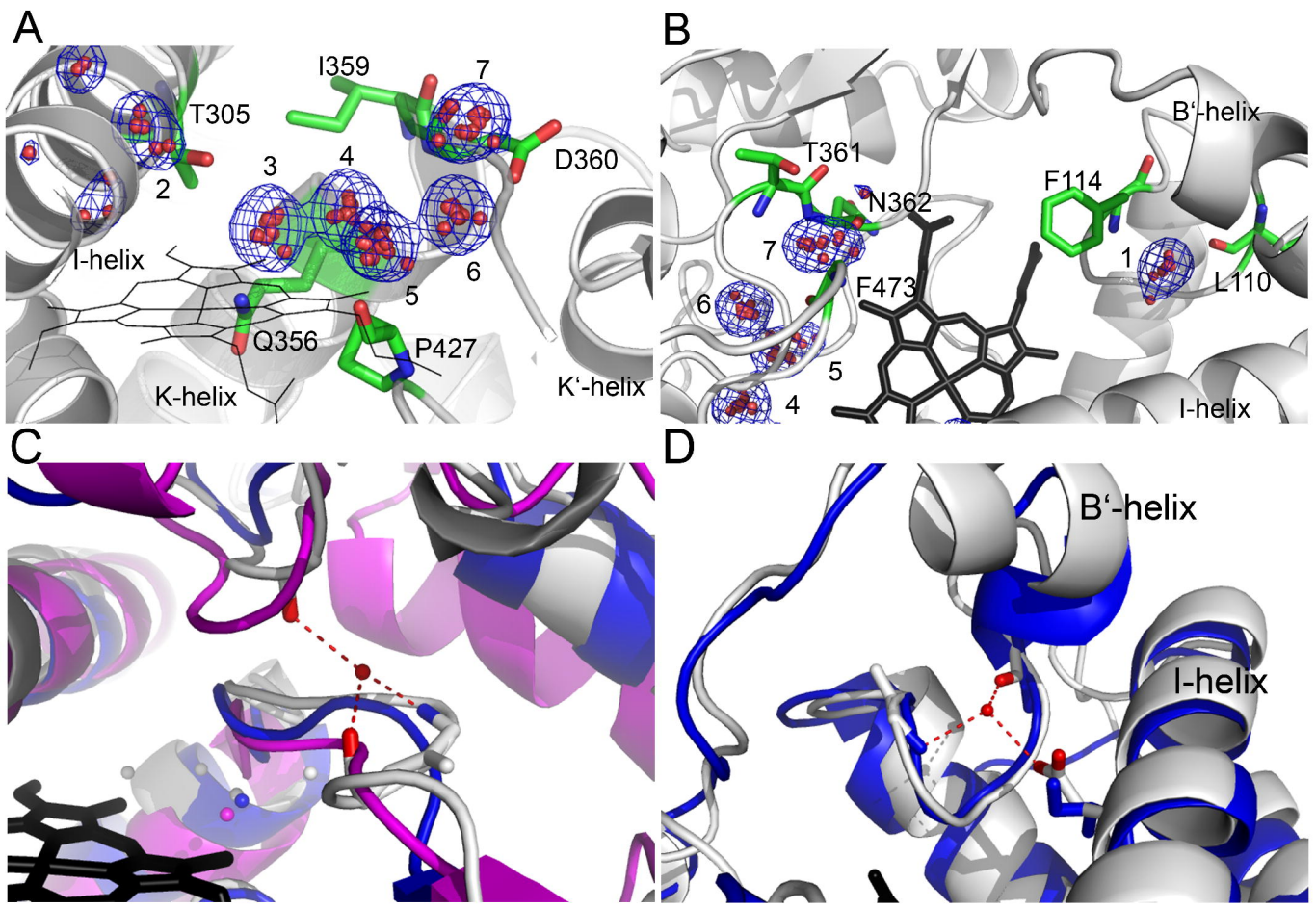
A
I359
7
T305
D360
3
4
2
I-helix
6
5
Q356
P427
K-helix
K'-helix
B
B'-helix
T361
F114
N362
7
1
L110
F473
6
5
4
I-helix
C
D
B'-helix
I-helix