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Incorporating Fast Protein Dynamics into Enzyme Design: A Proposed Mutant Aromatic Amine Dehydrogenase

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Abstract

In recent years, there has been encouraging progress in the engineering of enzymes that are designed to catalyze reactions not accelerated by natural enzymes. We tested the possibility of reengineering an existing enzyme by introducing a fast protein motion that couples to the reaction. Aromatic amine dehydrogenase is a system that has been shown to use a fast substrate motion as part of the reaction mechanism. We identified a mutation that preserves this fast motion but also introduces a favorable fast motion near the active site that did not exist in the native enzyme. Transition path sampling was used for the analysis of the atomic details of the mechanism.

Graphical abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.7b05319. Figures (S1) the reductive half reaction mechanism of AADH with tryptamine; (S2) starting structures of WT and F97K AADH; (S3) D–A distance projection on the plane of bond-breaking and bond-forming distances; (S4) reactive trajectories projection on the plane of bond-breaking and bond-forming distances; (S5) TS structures of WT and F97K AADH; (S6) linker angle comparison between WT and F97K AADH (PDF)

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Notes

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INTRODUCTION

Although the progress in designing artificial enzymes is encouraging^{1–6} their proficiency is still far below naturally occurring enzymes.⁷ One possibility that has not been explored in enzyme design is the incorporation of subpicosecond motions that couple to the reactive event. There are strong indications, both theoretical and experimental,^{8–11} that such fast motions play an important role in catalysis. However, other researchers feel that the evidence is not convincing.^{12,13} The identification and elucidation of these rate-promoting motions is a challenging task. Our group has used transition path sampling (TPS)¹⁴ to extract atomistic details of the catalytic mechanism and identify such motions.^{15–18} This study is part of a design program^{19,20} that examines the possibility of including rapid motions in computational enzyme design. The purpose of this paper is to show that we can perform similar design studies on enzymes that function in a very different chemical space. It should be viewed as further proof of principle of our eventual ability to bring these techniques to bear in artificial enzymes. Unlike the previous cited papers, which began with theory and proceeded to experimental confirmation, this work is purely theoretical showing that we can develop protein designs in a wide range of enzymatic activities.

We studied the enzyme aromatic amine dehydrogenase (AADH) whose mechanism of catalysis is well-understood.^{21–25} AADH is a tryptophan tryptophylquinone (TTQ)-dependent quinoprotein that catalyzes proton transfer. It oxidatively deaminates aromatic primary amines to form the corresponding aldehyde. AADH is a $\alpha_2\beta_2$ heterotetramer and its structure consists of two large and two small subunits with the cofactor TTQ attached covalently to the small subunit. The cofactor is derived from two tryptophan residues (Trp109 and Trp160) after post-translational modification. Trp109 is the residue that is converted to tryptophylquinone and Trp160 is cross-linked without further modification. We study the reductive half-reaction of aromatic amine dehydrogenase with tryptamine. Specifically, we are interested in the proton transfer step (Figure 1a), which is thought to proceed via quantum tunneling.²⁶ The starting point is the Schiff base (iminoquinone) intermediate of the catalytic cycle, and Asp128 is deprotonated and acts as the catalytic base

abstracting the proton. We will identify a mutation that introduces a favorable fast motion that favorably orients the oxygens of the catalytic base to perform this abstraction without disturbing the essential dynamics and electrostatics in the active site.

METHODS

All molecular dynamics simulations were performed using the CHARMM^{27,28} molecular dynamics package. The starting point for the simulations was the crystal structure 2AGY from the Protein Data Bank. This is the related Schiff base intermediate (structure V) which had to be modified to structure III (Figure S1). To build our model, we modified the TTQ cofactor and TSH substrate in Accelrys Discovery Studio and we obtained parameters for the CHARMM force field. All crystallographic waters were retained for both native and mutated structures. Simulations for the wild-type (WT) and its mutated proteins were performed separately following the same protocol. The system was partitioned into quantum and molecular mechanics regions. The QM region consisted of 56 atoms including the cofactor (TTQ), the substrate tryptamine (TSH), and the catalytic base Asp128 (Figure 1b). The MM region included the rest of the protein, ions, and solvent. The generalized hybrid orbital (GHO)²⁹ method was used to couple the two regions through the CB atoms of Trp160/Trp109 and Asp128. The QM region was modeled using the PM3 method and the rest of the system was modeled using the CHARMM27 force field.

The protein was then solvated in a sphere of water of 60 Å radius using explicit TIP3 water molecules, and then the system was neutralized by placing 19 sodium ions for the WT and 18 for the mutant. Then the protein was minimized using 150 steps of steepest descent followed by two stages of adopted basis Newton–Raphson (ABNR) minimization of 1000 steps each. In the first ABNR stage, we applied harmonic constraints to the protein backbone and the QM region that were released in the second stage of ABNR minimization.

The heating was conducted slowly from 0 to 300 K for 45 ps while any harmonic constraints applied to the system were gradually released. During the heating, we switched the QM/MM on. Following the heating process, the system was equilibrated at 300 K with the QM/MM on for 100 ps.

Transition path sampling was used to generate and analyze the ensemble of reactive trajectories. First, we defined the order parameters using the D–H bond-breaking and H–A bonding-forming distances: the reactant region contained all configurations with the D–H bond length <1.35 Å and the H–A bond length >1.35 Å, whereas the product region contained all configurations with D–H bond length >1.35 Å and H–A bond length <1.35 Å. The generation of reactive trajectories was done with the shooting algorithm: all momenta at a randomly chosen slice along a reactive trajectory are perturbed and the system is propagated using the new momenta, and this process is iterated until a new reactive trajectory is generated. The initial reactive trajectory was generated by applying a harmonic constraint with force constant 45 (kcal/mol)/Å² on both the bond-breaking and bond-forming distances followed by propagation for 250 fs. Using this biased first reactive trajectory as a seed, we iterated until a sufficient set of reactive trajectories is generated. We

generated 160 reactive trajectories of 500 fs length for the WT, and 160 reactive trajectories for the mutated AADH enzyme.

Next, we performed committor analysis to locate the transition state (TS), defined as the time slice with the property that new trajectories initiated from it with random momenta have probability 0.5 to reach reactants or products. The set of these isocommittor structures forms the transition state ensemble (TSE). From the harvested reactive trajectories, we identified a transition state ensemble of 15 uncorrelated TS structures for each enzyme. This TSE was used as starting points for the algorithm that leads to the identification of the reaction coordinate: (i) one makes a guess for the reaction coordinate; (ii) starting from a TS structure, one evolves the system while restraining the degrees of freedom of the guess for the reaction coordinate; (iii) at various time slices along the constrained trajectory, one initiates trajectories with random initial velocities; (iv) one tabulates the ratios of the trajectories in the previous step that end up in reactants or products; (v) if the guess for the reaction coordinate were correct, because it was constrained from moving and the constrained trajectories started from a TS structure, the commitment probabilities calculated in the previous step would be 0.5 for either reactants or products; (vi) if the probability is not 0.5, one makes a new guess for the reaction coordinate and repeats.

Mulliken charges were calculated with the PM3 method in CHARMM. We selected five timeslices of chemical interest of every 10th reactive trajectory, excluding the first 20 trajectories, and averaged the results over trajectories. The contour maps in Figure 3 were calculated with the statistics package R, using the two-dimensional kernel density estimation package kde2d (MASS).

RESULTS AND DISCUSSION

The donor (CAH) in Figure 1b is in hydrogen-bonding distance to OD2 and van der Waals distance to OD1 (acceptor). In principle, either oxygen could be the proton acceptor. Previous studies³⁰ demonstrated that the hydrogen attachment to OD1 is the most favorable pathway, even though it is positioned further away. We generated a TPS ensemble that agrees with the previous work and shows that the hydrogen transfers to OD1. We also identified the promoting motion³¹ that Scrutton and co-workers first reported. This motion consists of a rotation of the donor–transferring hydrogen, so that the proton is repositioned across the acceptor OD1 shortening the distance for transfer. The rotation of the donor group causes the secondary hydrogen H2 of CAH to become more in-plane with the iminoquinone, and the CAH–H3 bond more perpendicular to it, thus making the sp^3 -hybridized donor carbon more sp^2 -like (i.e., product-like).

Our enzyme design goal was to identify a mutation near the active site that (a) introduces important dynamics, (b) does not affect the promoting rotating motion that already exists in the native enzyme, and (c) shortens the hydrogen–acceptor distance (which would enhance tunneling).

Examining the geometry of the active site of this enzyme, we note that most residues are facing the reaction region with their backbone atoms. There are two residues “side chain

facing”, Phe169 and Thr172, but they are important for product stabilization:³² Phe169 pushes against OD1 and facilitates the breakage of the Thr172–Asp128 hydrogen bond, allowing Asp128 to move further away from the donor and therefore stabilize the product. Furthermore, there are no residues directly behind the donor or the acceptor that could be candidates for a mutation that affects the donor–acceptor distance as in alcohol dehydrogenase enzymes. We identified as a possible mutation candidate a residue from the neighboring unit, Phe97b (Figure S2), with an orientation that favors interactions with the substrate ring. Its side chain is pointing toward the active site, but is neither in hydrogen-bonding nor in van der Waals interaction with the reactants. The position of its aromatic ring is perpendicular to the substrate ring and is placed close to atoms NE1H and CBH and is therefore in a position to influence the motion of either the ring or the iminoquinone region. Mutation of Phe97b to lysine (hereafter F97K) alters its character by removing the aromatic ring and adding an elongated side chain instead. We note that this is a nonconservative mutation that changes hydrophobicity, polarizability, charge, and aromaticity. There is a possibility that such a mutation might change important electrostatics in the active site but as we will show later this is not the case.

TPS analysis of the reactive trajectories showed that in the native enzyme Phe97b acted as a “wall”, blocking possible movement of the substrate ring. Our expectation was that the mutated residue would increase the flexibility of the substrate and the probability of optimizing interactions favoring formation of the transition state.

Comparing representative trajectories from WT AADH (Figure 2a) and F97K AADH (Figure 2b), we see that when the bond starts to break (black line), the distance between hydrogen and acceptor (red line) is shorter by more than 0.3 Å in the mutant. Also, in the mutant the other possible acceptor, the nearby oxygen OD2, has moved away (blue line), long before the bond starts to break compared to the WT, leaving OD1 as the only option for accepting the hydrogen. At the time of bond breaking, the transferring hydrogen is at equal distances from either OD1 or OD2 oxygen, whereas in the mutant the difference between the distances hydrogen–OD2 and hydrogen–OD1 (acceptor) is 0.5 Å. We also note that the donor–acceptor distance in the mutant is shorter by approximately 0.25 Å and has its minimum right at the TS, as identified by committor analysis. We present the projections of histogrammed densities of structures along all reaction trajectories on the plane of the D–A distance versus bond breaking (D–H) minus bond forming (H–A) distance for WT AADH (Figure S3a) and F97K AADH (Figure S3b). Also, a similar figure for the projection on the plane of bond-breaking and bond-forming distances (Figure S4a,b)

Analyzing the TS ensemble obtained by TPS for both enzymes (Table 1), we find that the average TS structure distances for WT AADH are 1.56 Å for donor–hydrogen and 1.42 Å for hydrogen–acceptor; for the F97K enzyme, the donor–hydrogen distance is 1.34 Å and the hydrogen–acceptor distance is 1.30 Å. There is a significant decrease in the donor–hydrogen distance in the mutant, indicating the mutant exhibits an early TS. Transfer via tunneling will be significantly enhanced by even a 0.2 Å decrease in transfer distance. Although the equilibrated structures of the two enzymes are similar, in their TS structures along the true reaction coordinate there are differences (Table 1, Figure S5) In the mutant the substrate ring is pushed toward the residue Val158 (we will return to this point).

This discussion shows that the proposed mutation fulfilled one of the design goals, shortening the donor–acceptor and hydrogen–acceptor distances, which should enhance quantum tunneling. Next, we have to verify that the promoting motion (rotation of the carbon–hydrogen group) that was present in the wild-type (WT), is also present in the mutant.

The important rotational motion of the C–H group has complicated time dependence, in particular it is not a simple harmonic motion. To analyze the time series, we used the empirical mode decomposition (EMD)^{33,34} which is an extension of Fourier analysis, that allows the Fourier frequencies to be time-dependent. EMD allows us to isolate the instantaneous frequencies of the rotation motion just before the reactive event. We found a rotational frequency at 180 cm⁻¹ for the native enzyme and 195 cm⁻¹ for the F97K enzyme, both close to the previously reported value³¹ of 167 cm⁻¹ (this was calculated using spectral density analysis). In Figure 3, we plot the rotation of the C–H group as a function of reaction coordinate progression, measured as the difference of the breaking and forming bond distances, for all the reactive trajectories. The rotational motion of interest is the time progression of the angle D–H–OD2, from Figure 3 one can see that is occurring in both WT AADH (from 80°–160°, with the TS at 110°) and F97K AADH (from 100°–160°, with the TS again at 110°). We also plot the linker dihedral formed by atoms CH2Q–NH–CAH–CBH (Figure S6), which in the mutant undergoes a larger change going from reactants to products. In both enzymes, this dihedral has the same starting value, but its value at the TS is 10° higher in F97K and at the product region 15° higher in F97K.

Finally, we turn to the last design goal and examine whether the proposed mutation introduced favorable dynamics. A major benefit of a TPS analysis is that it allows identifying the reaction coordinate,^{35,36} as described in Methods.

The committor analysis for the case where only the QM region was constrained shows that for WT AADH (Figure 4a) that there is no protein motion involvement in the reaction coordinate, unlike the F97K enzyme (Figure 4b): the probability distribution for the wild-type peaks at 0.5 (indicating a correct choice for the reaction coordinate), unlike for the mutant where the commitment probability is flat. For the F97K mutant, testing different sets of residues finally identified the reaction coordinate as consisting of residues Phe169, Thr172, Val158, and Lys97b (Figure 4c).

Now we will explain the dynamical role of the identified residues in the F97K reaction and we will compare to the WT AADH. Figure 5a,b examines how two residues stabilize the product for WT and F97K enzyme, respectively: Phe169 is pushing OD1 to assist in the hydrogen bond breakage between OD1 and Thr172 so that the catalytic base can move away from the donor and thus stabilize the product. We monitor the distances between two pairs of atoms: CE1 (Phe169)–OD1(acceptor) and OH (Thr172)–OD1 (acceptor). For WT AADH, Phe169 comes closer to the acceptor, reaching a minimum of 2.7 Å about 25 fs after the TS, whereas the distance between Thr172 and acceptor decreases, reaching a minimum at around the same time as Phe169. The hydrogen bond between OD1 and Thr172 starts to break about 70 fs after the TS is reached. For F97K, the distance between residue Phe169 and the acceptor is minimized just before the TS to facilitate the Thr172–OD1 hydrogen

bond cleavage. This bond breaks right after the TS is reached, indicating an immediate product stabilization compared to WT. In both enzymes, the distances between all the aforementioned residues are about the same at the transition state. We need to clarify that the two residues Phe169 and Thr172 not affecting the committor distribution in WT does not mean that they are not necessarily part of the reaction coordinate. The committor distribution analysis captures events very close to the TS, so it does not capture motions that are happening well after the TS is reached.

We calculated the RMSF of all QM atoms (from every 10th trajectory, excluding the first 20 trajectories and averaged over all trajectories) to verify that the mutation introduced a new protein motion near the active site and made the substrate ring more flexible. Figure 6 shows that the QM region of F97K is overall more flexible compared to the WT, the acceptor's flexibility (atom 15) is the same in both enzymes, the donor is more flexible in the mutant (atom 32), whereas the transferring hydrogen (atom 45) is more flexible in the WT. Most importantly, the substrate ring (atoms 33–42) is clearly more flexible in the mutant.

Figure 7a,b examines the behavior of Phe97b (Lys97b for the mutant) and Val158 with respect to the substrate ring. In Figure 8, we see that the substrate ring is positioned between Phe97b/Lys97b and Val158. In WT (Figure 7a), the Val158 pushes the ring and Phe97b acts as a block that stops its motion. Lys is a more flexible residue than Phe and it is charged, therefore we surmised it would behave differently. In fact, Figure 7b shows that it no longer acts as a block to substrate movement (as it did in the wild-type). We note that when Val158 pushes the substrate ring, in the mutant Lys97b pushes the ring back toward Val158, as a result the substrate is more flexible. In particular, in the mutant the distance of Val158 reaches a minimum distance from the ring equal to 3 Å at the TS, whereas in WT the same distance was 4.2 Å.

Snapshots of representative trajectories in Figure 8 along the reaction for both enzymes, focusing on the motions of the substrate ring, Phe97b/Lys97b and Val158, showcase the differences between wild-type and mutant. The top figure in Figure 8 shows that the substrate ring in the wild-type is practically immobile, whereas in the mutant it moves (bottom figure).

As a final test of our design, we must verify that our designed mutation has no deleterious effects on active site electrostatics. As mentioned earlier, mutating from a neutral to a charged amino acid near the active site may change crucial electrostatics of the reaction. We will now show that in this system, this particular mutation did not affect the charge distribution of the active site atoms during the reactive event. We performed Mulliken charge analysis of the QM atoms for the WT and mutant at five different times along a reactive trajectory: 50, 30, and 10 fs before the TS, at bond-breaking, and at the TS, and compared the results atom by atom. Because the Mulliken charges were calculated inside CHARMM in the presence of all MM atoms, our analysis can capture possible electrostatic changes affected by the nearby MM residue we mutated. The Mulliken charges at these time slices for both enzymes are shown in Figure 9 and Table 2.

The charge distributions for the two enzymes are the same, and there is no evidence of charge delocalizations because Figure 9 shows that the charge distribution of the mutant, compared to WT, did not shift toward different atoms. The charges change similarly for both enzymes along the reaction event, and neither the donor (atom 32) nor the acceptor (atom 15) seem to be influenced (see the atom numbering reference panel in Figure 9). Specifically, as we approach the TS, in both enzymes the acceptor (atom 15) becomes less negative, the donor (atom 32) becomes more negative, and the transferring hydrogen (atom 45) becomes more positive. The atomic partial charges of OD1 (acceptor, atom 15) is more negative than the charge of OD2 (atom 16) when the bond starts to break (Figure 9D) for both the WT and F97K AADH. The two oxygens in the mutant are more negative compared to the WT. For both enzymes, the partial atomic charges of the TTQ (atoms 1–13, 17–30) are more affected from the hydrogen transfer than the tryptamine (atoms 33–53). Because the target of the mutation was the tryptamine ring, more specifically the nitrogen NE1H (atom 41) and carbon CD1H (atom 42), we expect that these atoms would show the largest change between the WT and F97K. Interestingly, the NE1H partial charge is the same for both enzymes 30 fs before the TS, and as we reach the TS. The CD1H, on the other hand, is slightly different in WT compared to the F97K 10 fs before the bond breaks, and at the TS it is more negative. All other atoms of the substrate ring have similar partial charges in both enzymes. According to our analysis, a notable change is the partial charge of NH (atom 31, the nitrogen bonded to the donor), which is different at 10 fs before the bond breaking, but this difference becomes small as we reach the TS. The change in this charge is bigger in the mutant (+0.17 e) than in the WT (+0.08 e). In the WT this charge, from right before the bond breaking to the TS, does not change as much compared to the mutant. However, in both enzymes the atomic charges of the NH and the donor (CAH) change in a similar way to form the double bond, as the former becomes more positive and the latter more negative. Furthermore, we note that in the WT the charge on the transferring proton H3 increases from 0.234 to 0.332 45 fs before the TS, a significant change that could at least partially account for the decrease of the D–A and H–A distances seen in the mutant.

CONCLUSION

Some time ago, using operator theory,^{37,38} we identified the importance of symmetrically coupled vibrations in chemical reactions. The discovery that such motions exist in proteins as promoting vibrations that are central to the reaction coordinate of enzymatic catalysis has informed our design approach. In conclusion, we studied the enzyme AADH in which a rate-promoting motion, as has been previously identified, is part of the reaction mechanism. We identified a mutation that does not affect that motion and in addition introduces favorable dynamical motions that augment the natural promoting vibration. This mutation achieves greater D–A compression, which might itself have an energy cost, but will lower the free energy barrier to reaction and we thus expect the overall reaction probability to be significantly accelerated; of course, the final test of this would be experimental. We also verified that the mutation did not affect the electrostatics of the reactive event. This work is further evidence, along with our previous publications, that our design and analysis methods are capable of producing desired results. We emphasize that it is likely impossible to “beat” evolution with any dynamic design. This work, and the previous papers in this series,

demonstrate a proof of principle that we may apply these methods to artificial enzymes in which chemistry is likely rate limiting and far less optimally designed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. (a) The rate-limiting proton transfer step of the reductive half-reaction of AADH with tryptamine. (b) QM region of the enzyme AADH.

Figure 2.

Representative trajectories showing the donor–hydrogen distance (black), the hydrogen–acceptor (OD1) distance (red) and the hydrogen–OD2 distance (dashed blue), the donor–acceptor distance (green) near the transition state crossing for (a) WT AADH, (b) F97K AADH. The vertical dashed line (magenta) refers to the TS slice.

Figure 3.

Projections of histogrammed densities of the structures along all reaction trajectories on the plane of the D–H–OD2 angle versus bond breaking (D–H) minus bond forming (H–A) distance for WT AADH (a) and F97K AADH (b). The colormap (white for “plains”, that is, zero density, up to red for “mountains”, that is, maximum density of structures) represents the allocation of a pair of the above geometric properties among structures along the reaction path. Contour maps that join points with equal density have also been drawn.

Figure 4. Committor distribution histograms for (a) WT AADH, constraining only the QM atoms, (b) F97K AADH, constraining only the QM region, (c) F97K AADH, constraining residues Phe169, Thr172, Val158, and Lys97b.

Figure 5. Representative trajectories showing the Phe169 (black) and Thr172 motion (red) for (a) WT AADH and (b) F97K AADH. The location of the transition state is shown with a vertical dashed line.

Figure 6. RMSF of QM atoms for WT AADH (black) and F97K AADH (red) with atom numbering in Figure 9.

Figure 7. Representative trajectories showing the Phe97b/Lys97b (black), and Val158 (red) motion for (a) WT AADH and (b) F97K AADH. The location of the transition state is shown with a vertical dashed line.

Figure 8.
Snapshots of representative trajectories along the reaction event for (top) WT AADH,
(bottom) F97K AADH, focusing on the substrate ring movement and residues 158,97.

Figure 9.

Partial atomic charge distribution of all QM atoms for WT AADH (black) and F97K AADH (red) (a) 50 fs before the TS, (b) 30 fs before the TS, (c) 10 fs before the TS, (d) when the bond breaks, and (e) at the TS. The correspondence between atom numbers and atoms is shown in the panel.

Table 1

Average Distances of Important Active Site Residues at the Equilibrated and Transition State Structures for WT AADH and F97K AADH

	distances	equil. (Å)	TS (Å)
WT	D-H	1.16	1.56 ± 0.08
	H-A	2.67	1.42 ± 0.07
	D-A	3.60	2.84 ± 0.07
	H-OD2	1.68	1.83 ± 0.09
	Phe169 OE1 - A	3.11	3.04 ± 0.12
	Thr172 OG - A	2.66	2.71 ± 0.22
	Val158 O - NE1H	3.86	4.42 ± 0.11
	Phe97b CE1 - NE1H	3.95	4.32 ± 0.21
F97K	D-H	1.11	1.35 ± 0.03
	H-A	2.24	1.30 ± 0.03
	D-A	3.30	2.60 ± 0.03
	H-OD2	1.79	1.98 ± 0.07
	Phe169 OE1 - A	3.10	3.34 ± 0.19
	Thr172 OG - A	2.94	3.06 ± 0.16
	Val158 O - NE1H	3.63	3.01 ± 0.14
	Lys97b CE1 - NE1H	3.72	4.01 ± 0.33

Table 2

Average Partial Atomic Charges Derived from Mulliken Analysis for Selected QM Atoms 45 fs before the TS and at the TS for WT AADH and F97K AADH

	atom	45 fs before TS (e)	at the TS (e)
WT	CAH (D)	-0.324	-0.566
	H3 (H)	0.234	0.364
	OD1 (A)	-0.645	-0.520
	NH	0.529	0.777
	NE1H	0.361	0.327
	CD1H	-0.178	-0.178
F97K	CAH (D)	-0.350	-0.532
	H3 (H)	0.332	0.356
	OD1 (A)	-0.648	-0.586
	NH	0.594	0.730
	NE1H	0.328	0.337
	CD1H	-0.152	-0.101