Supporting Figure Legends

Supporting Figure 1. Superimposition of monomers of HADH (subunit A, shown in light blue, PDB: 3K30.pdb) and TMADH (subunit B, shown in light green, PDB: 1DJN.pdb). In HADH, 6-S-Cys-FMN is shown in red, [4Fe-4S] is in orange, and ADP is in blue. In TMADH, 6-S-Cys-FMN is shown in pink, [4Fe-4S] is in yellow, and ADP is in green.

Supporting Figure 2. Stereoview showing a 2Fo-Fc electron density map of the [4Fe-4S] cluster contoured at 1σ.

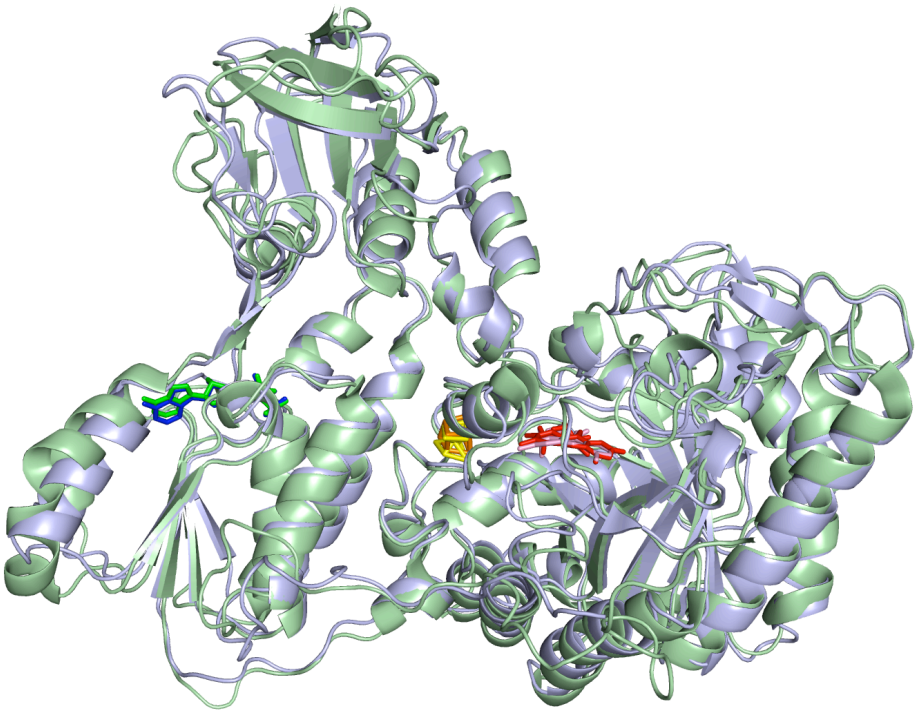
Supporting Figure 3. Amino acid sequence alignment of HADH and TMADH. ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html) (1) and ESPript (2) were used to generate the alignment. Identical residues and homologous residues are boxed. (●)Tyr-His-Asp triads. (□) Residues providing hydrogen bonding and/or salt bridge interactions in the proposed histamine binding site in HADH. (▲) Residues providing the hydrophobic pocket in the proposed histamine binding site in HADH. (○) Aromatic bowl in TMADH. (■) Residues involved in the electron transfer pathway in TMADH (3). (△) Residues that interact with the proposed recognition loop of ETF (4).

Supporting Figure 4. a) Electrostatic surface representation of a) the groove proposed to facilitate electron-transfer reaction to external electron acceptors in TMADH and b) the corresponding groove in HADH. Val344 and Tyr442 in TMADH are proposed to play important roles in electron-transfer reaction to ferricenium (artificial electron acceptor) and ETF, respectively (3).

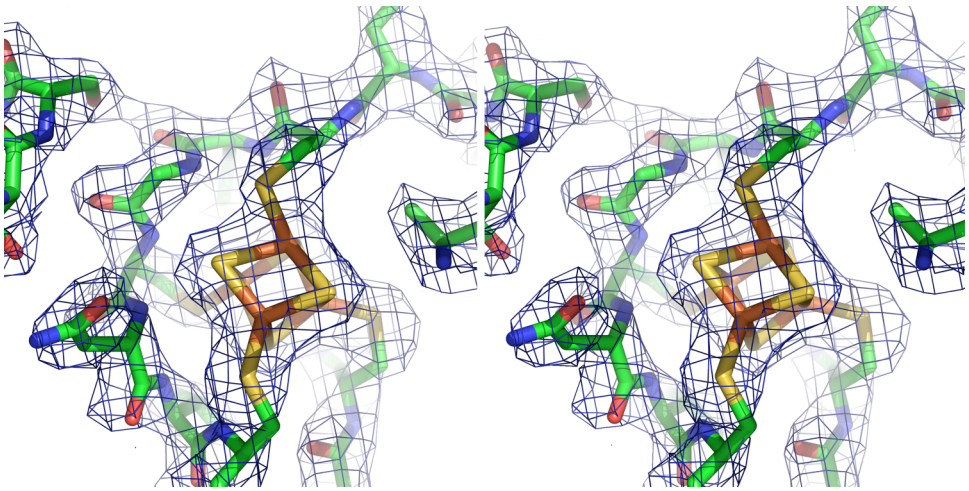
Supporting Figure 5. a) Two proposed electron-transfer pathways from the [4Fe-4S] cluster to the surface residues Val344 (in red arrows), and Tyr442 (in blue arrows). b). Two structually comparable electron-pathways in HADH from the [4Fe-4S] cluster to the surface residue Ala441 (in red arrows), and Arg444 (in blue arrows).

Supporting Figure 6. Docking trimethylamine and histamine into the active site of TMADH. 6-S-Cys-FMN is shown as a stick model. a) A graphic showing the docking pattern of trimethylamine in the aromatic bowl. b) A graphic showing the incompatible sterics between histamine and the receptor cavity. This figure was generated by manually superimposing histamine onto the position occupied by the docked trimethylamine.

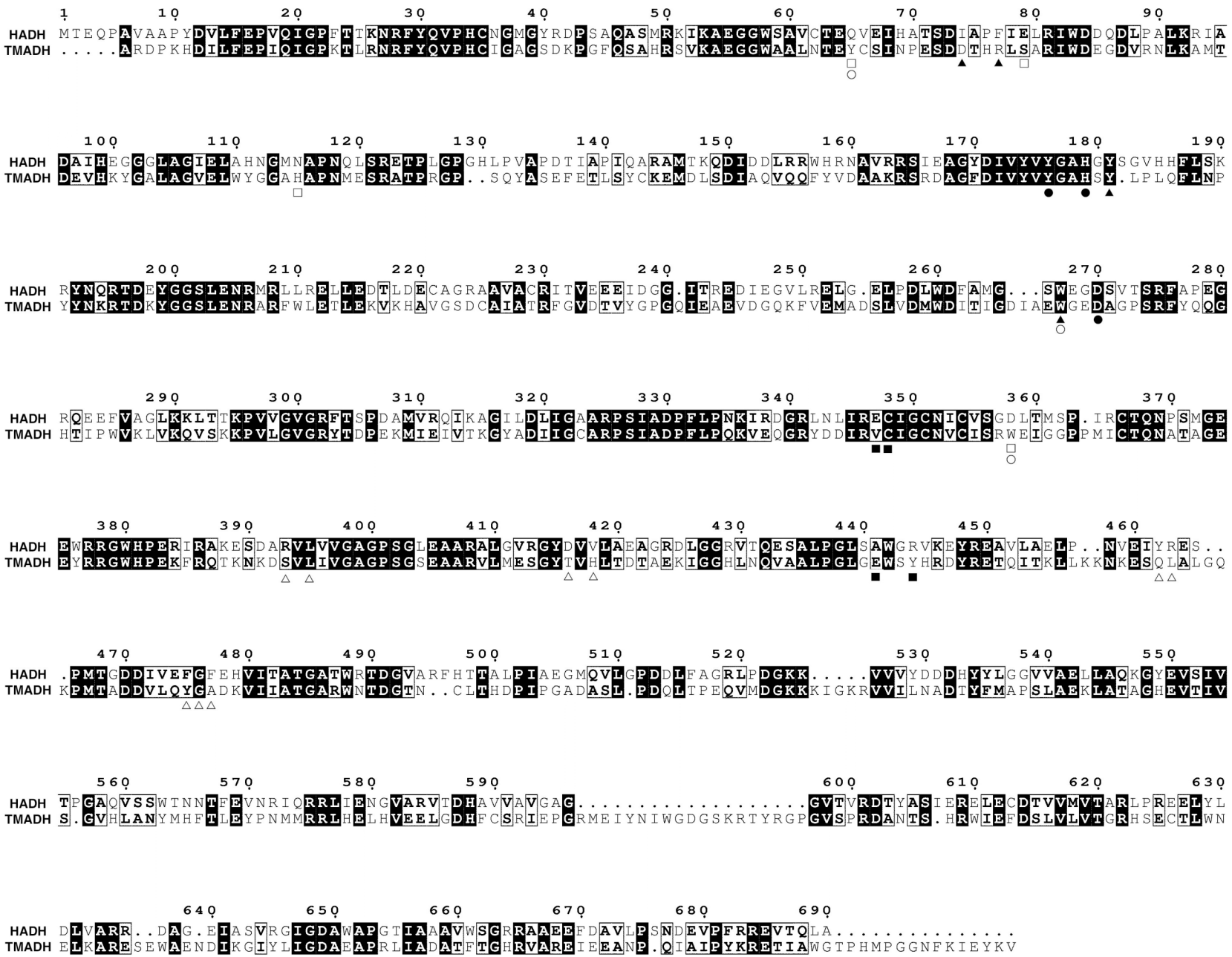
Supporting Figure 7. Histamine binding sites in HADH in comparison to other structurally characterized proteins. Hydrogen bond interactions are shown in orange dashed lines. Hydrophobic residues are shown in blue. Water molecule is shown in red sphere.



Supporting Figure 1.

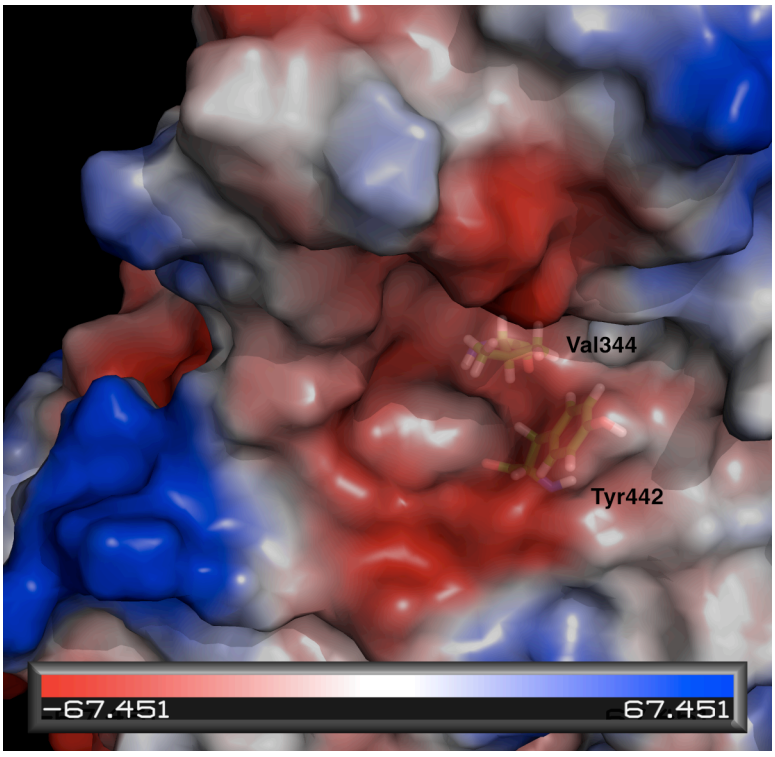


Supporting Figure 2.

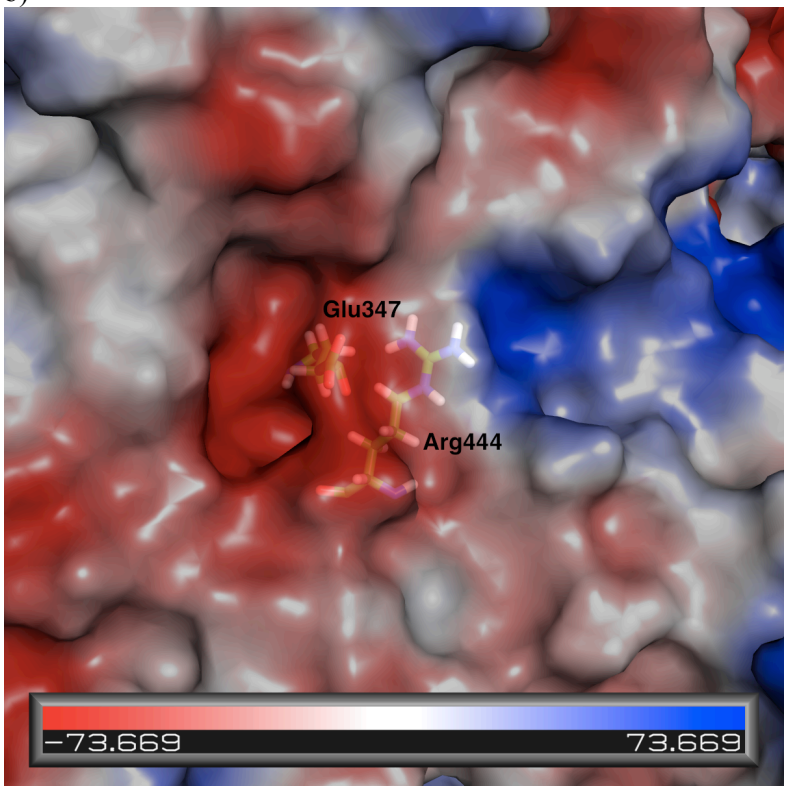



Supporting Figure 3.

a)

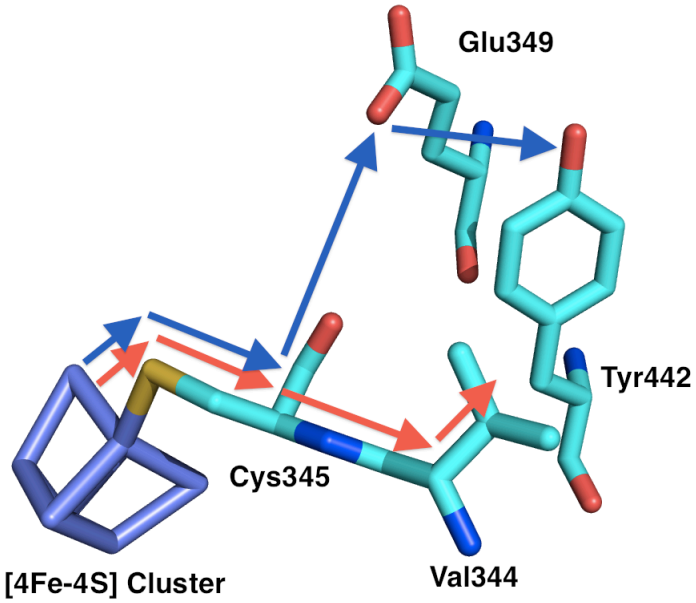



b)




Supporting Figure 4

a)

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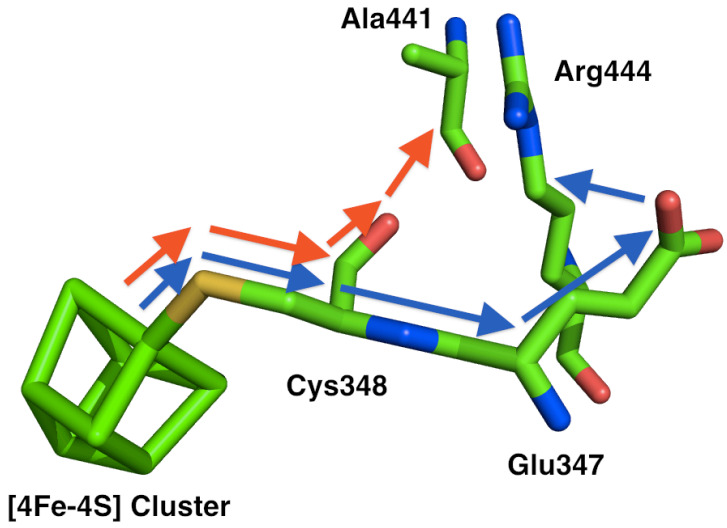
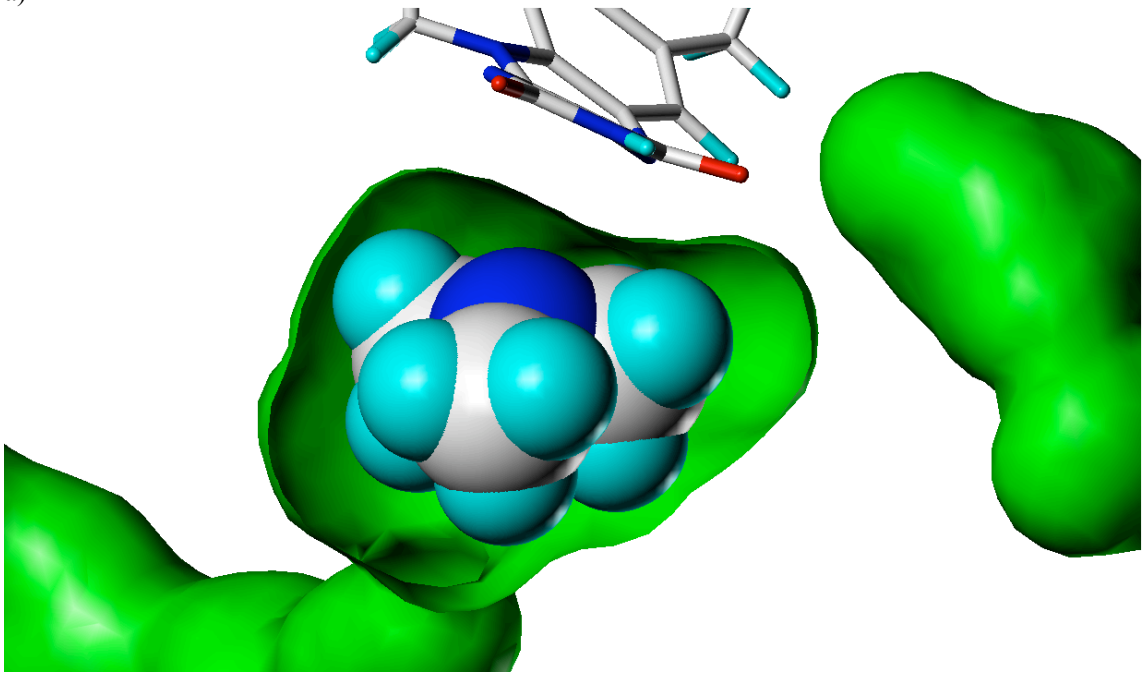



Supporting Figure 5

a)



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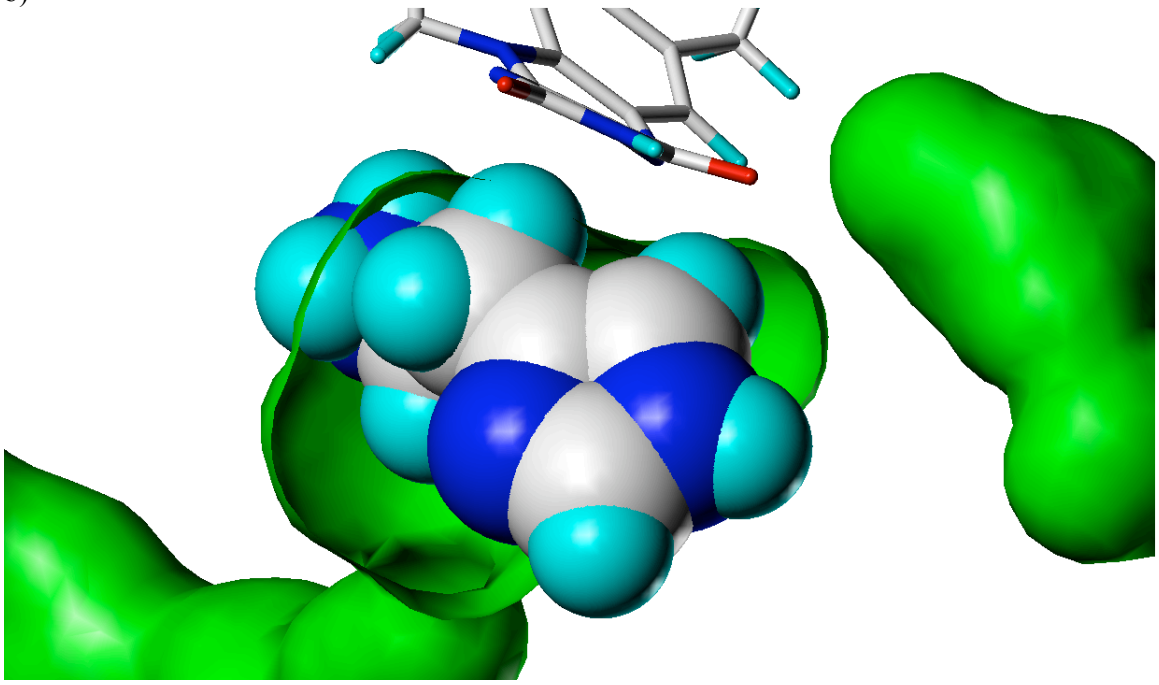
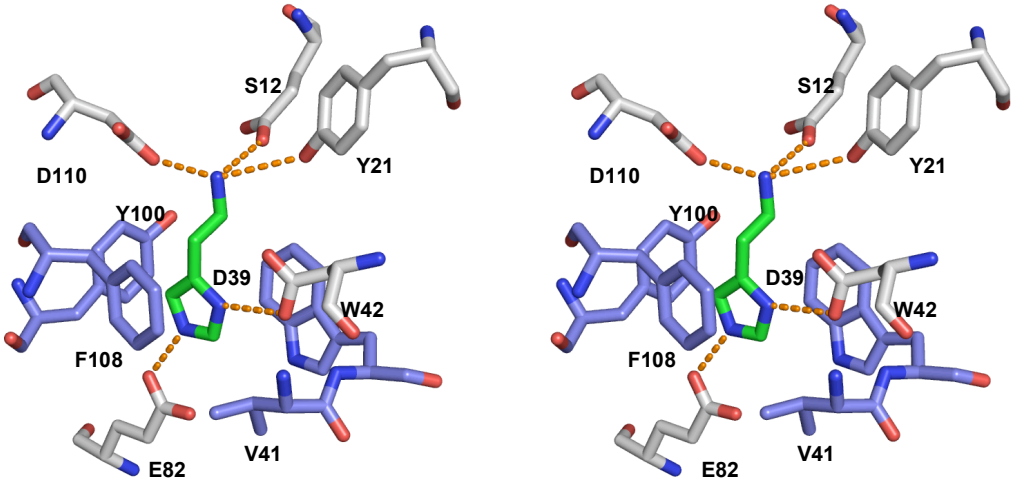
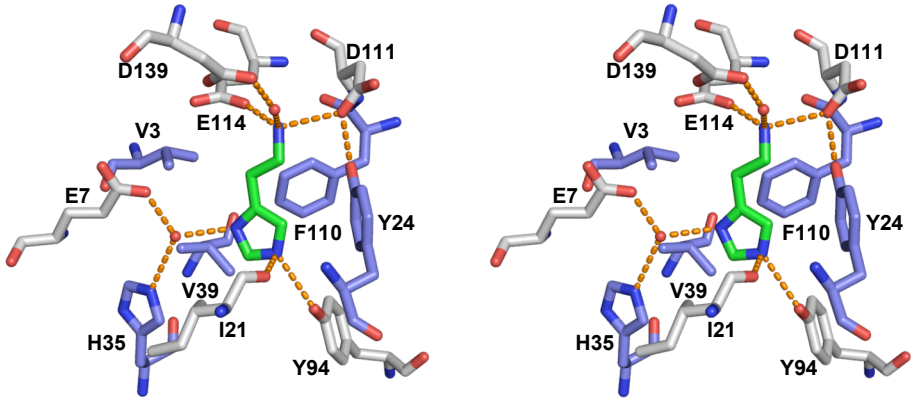


Supporting Figure 6.

a)




b)




Supporting Figure 7.

Reference:


1. Thompson, J. D., Gibson, T. J., and Higgins, D. G. (2002) Multiple sequence alignment using ClustalW and ClustalX, *Curr Protoc Bioinformatics Chapter 2*, Unit 2 3.
2. Gouet, P., Courcelle, E., Stuart, D. I., and Metoz, F. (1999) ESPript: analysis of multiple sequence alignments in PostScript, *Bioinformatics 15*, 305-308.
3. Basran, J., Chohan, K. K., Sutcliffe, M. J., and Scrutton, N. S. (2000) Differential coupling through Val-344 and Tyr-442 of trimethylamine dehydrogenase in electron transfer reactions with ferricenium ions and electron transferring flavoprotein, *Biochemistry 39*, 9188-9200.
4. Leys, D., Basran, J., Talfournier, F., Sutcliffe, M. J., and Scrutton, N. S. (2003) Extensive conformational sampling in a ternary electron transfer complex, *Nat Struct Biol 10*, 219-225.

Supporting Table 1. Kinetic parameters for histamine oxidation by recombinant HADH and native HADH.

| | Specific Activity (mmol $min^{-1}$ $mg^{-1}$) | $k_{cat}$ ($s^{-1}$) | $K_m$ (μM) | $K_i$[c] (μM) | $k_{cat}/K_m$ ($M^{-1}s^{-1}$) |
|---|---|---|---|---|---|
| Recombinant HADH[a] | 40 | 2.6 ± 0.1 | 34 ± 3 | 1270 ± 165 | 0.8 (± 0.1) x $10^5$ |
| Native HADH[b] | 45 | 6.6 ± 2.3 | 31 ± 11 | 1200 ± 300 | 2.1 (± 1.1) x $10^5$ |

a. This study, b. reference 6, c. for histamine

The kinetic parameters were obtained from least squares-fitting analysis using the substrate-inhibition Michaelis-Menten kinetics equation as described in reference 6.