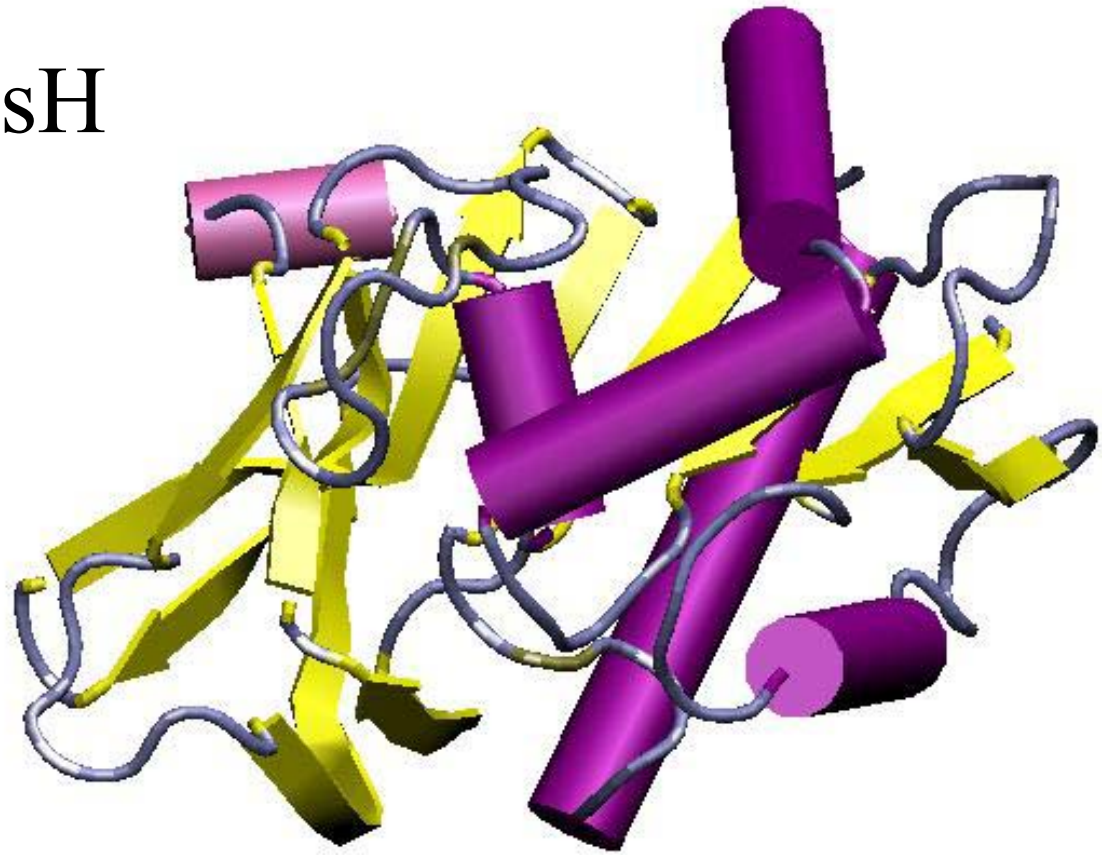
HisH - HisF

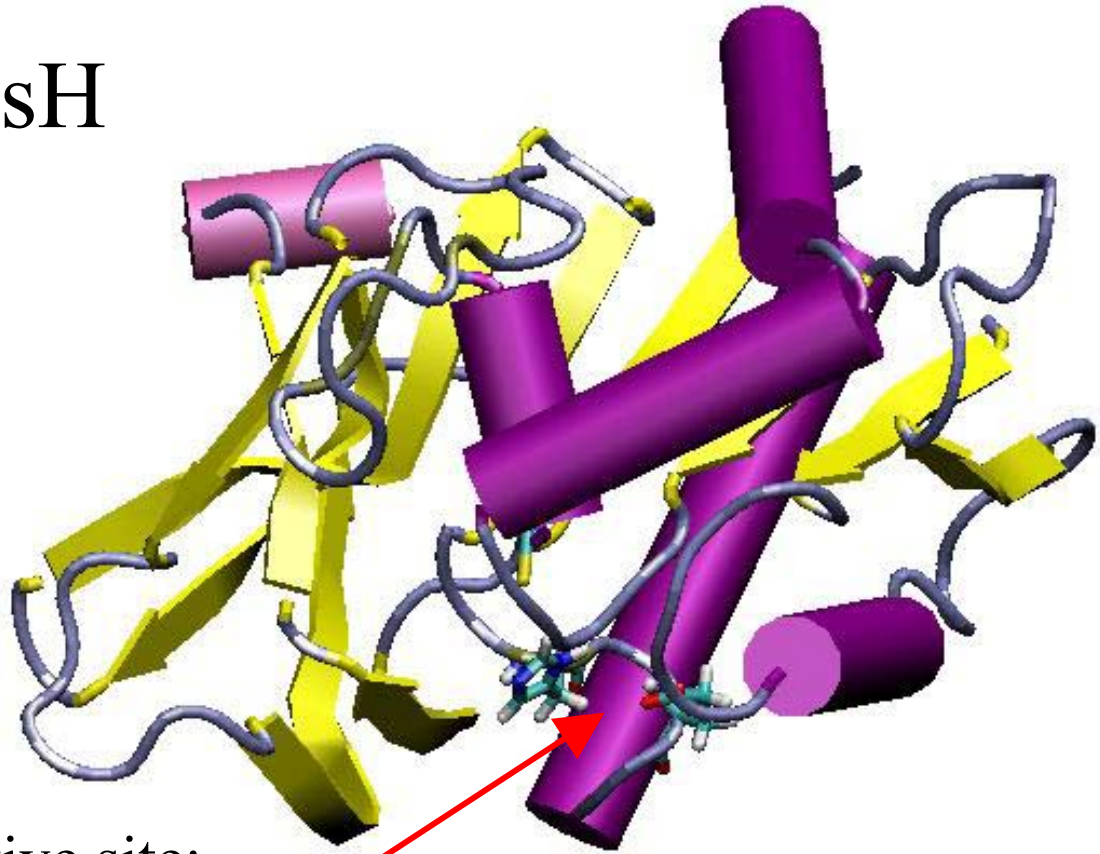
- Imidazole Glycerol Phosphate Synthase: regulates 5th step histidine biosynthesis
- HisH class I glutamine amidotransferase
- HisF alpha-beta barrel fold, cyclase rxn
- Recently suggested hisF uses barrel as efficient intermediate channel
- Ammonia conduction, gating mechanism
- Modeling complete, activated complex requires parameterization

P. O'Donoughue, R. Amaro, Z. Schulten, **J Struct Biol**, 2001.

HisH



HisH

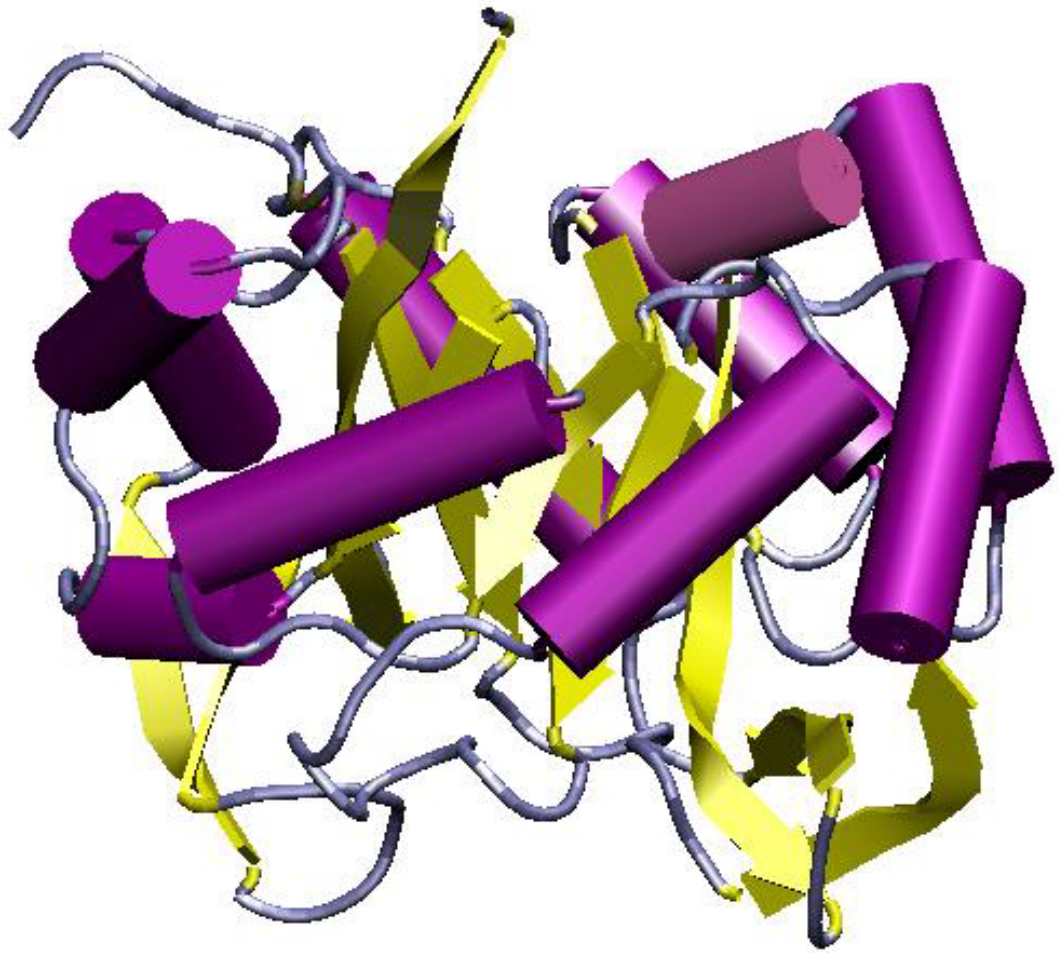


HisH active site:

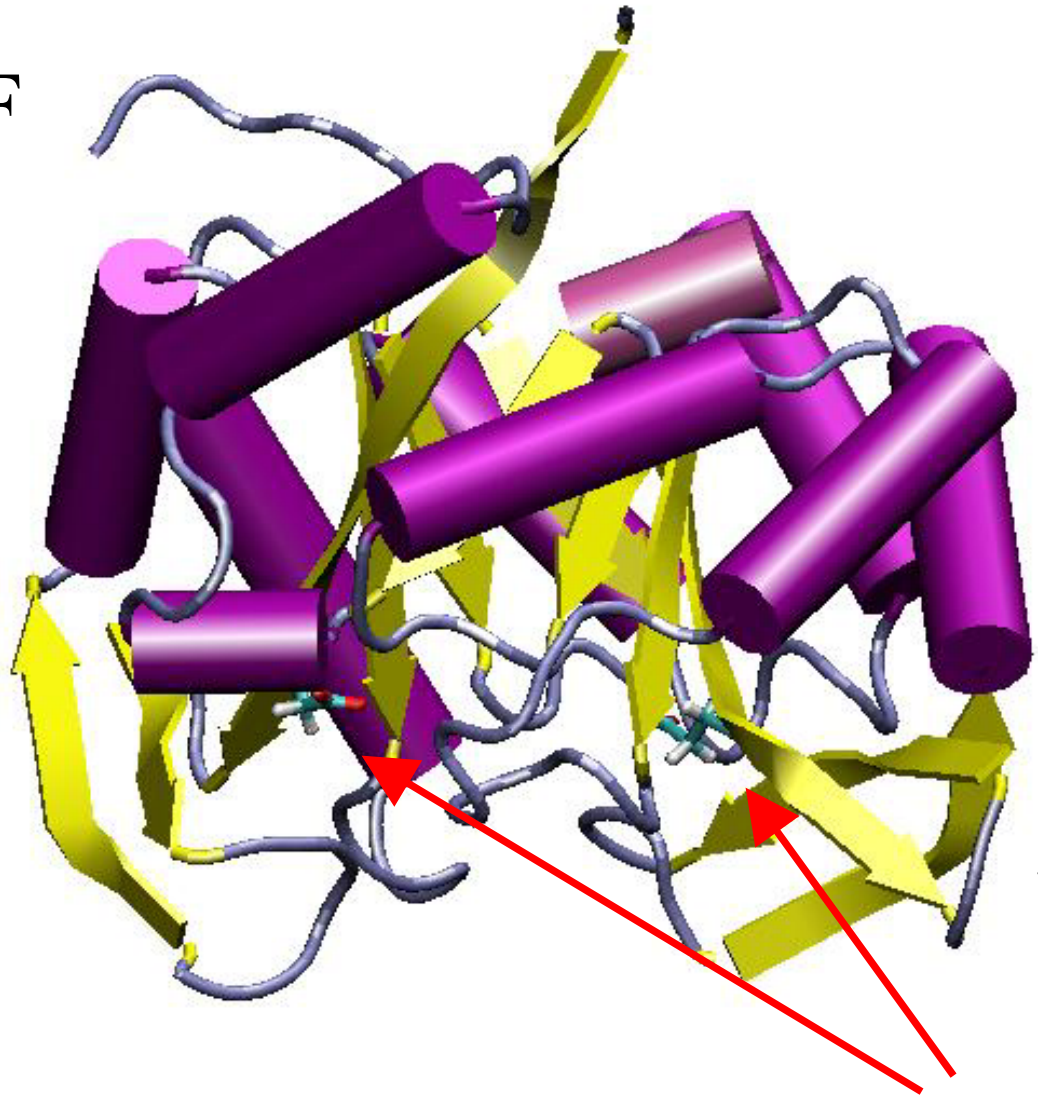
Catalytic triad

CYS84 – HIS178 – GLU180

HisF

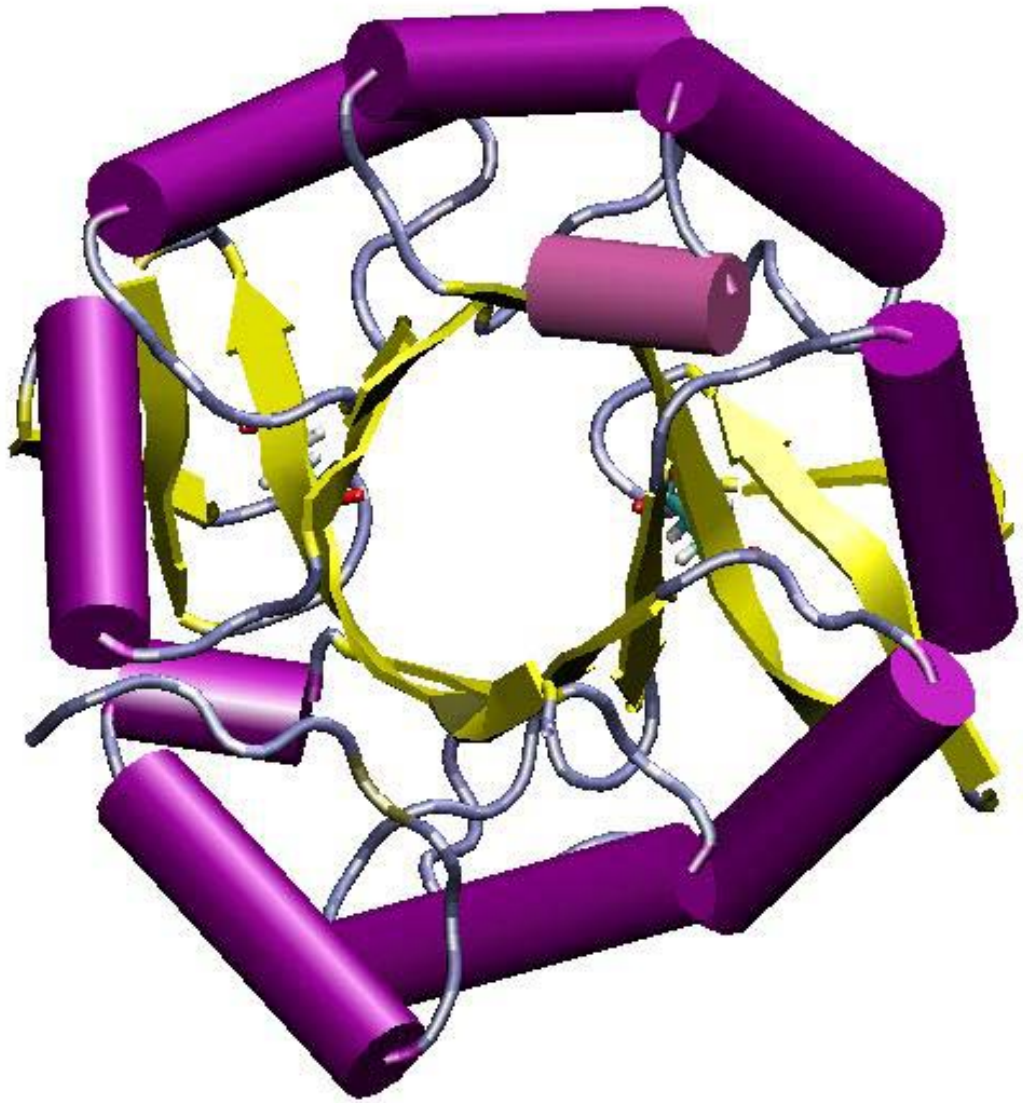


HisF



Active
Site
Residues

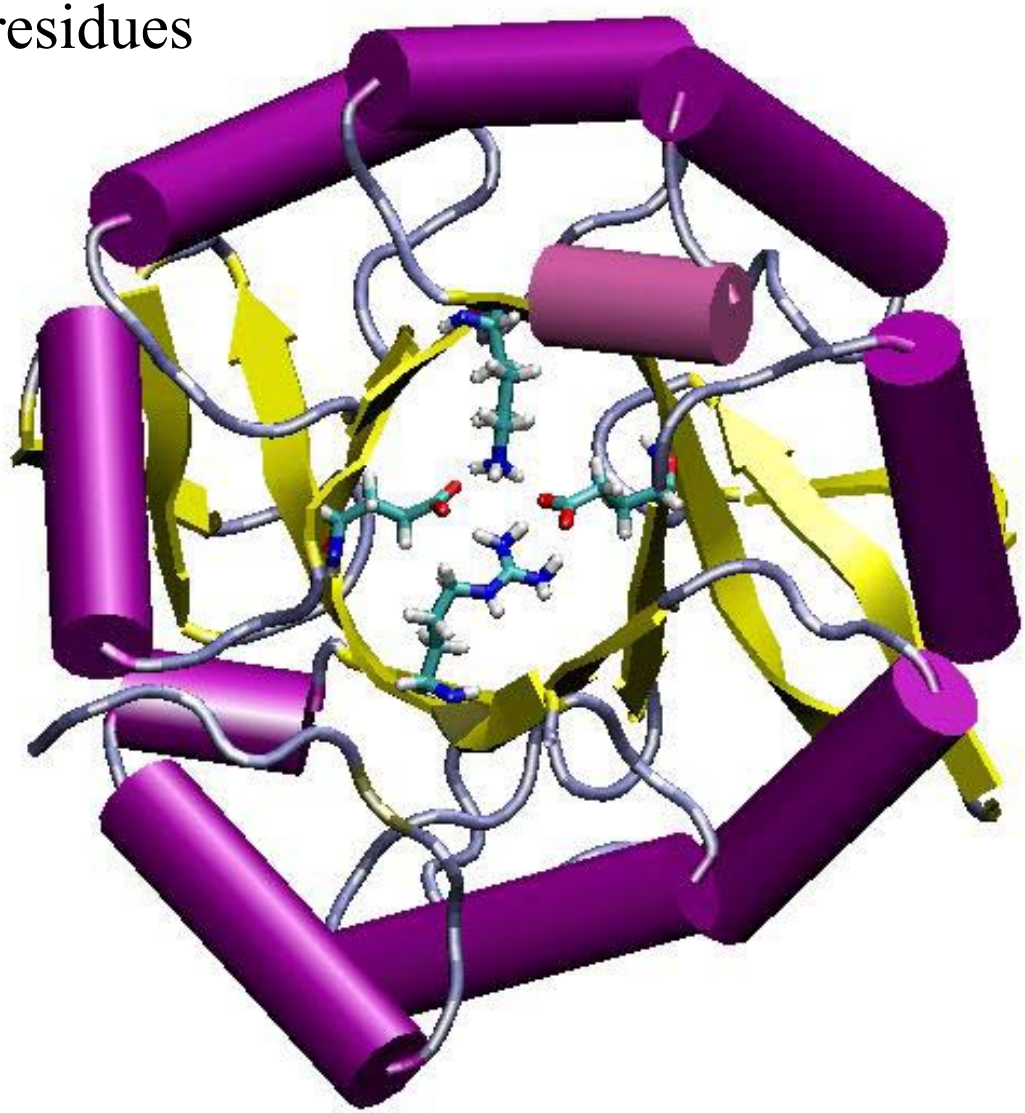
Top View of HisF



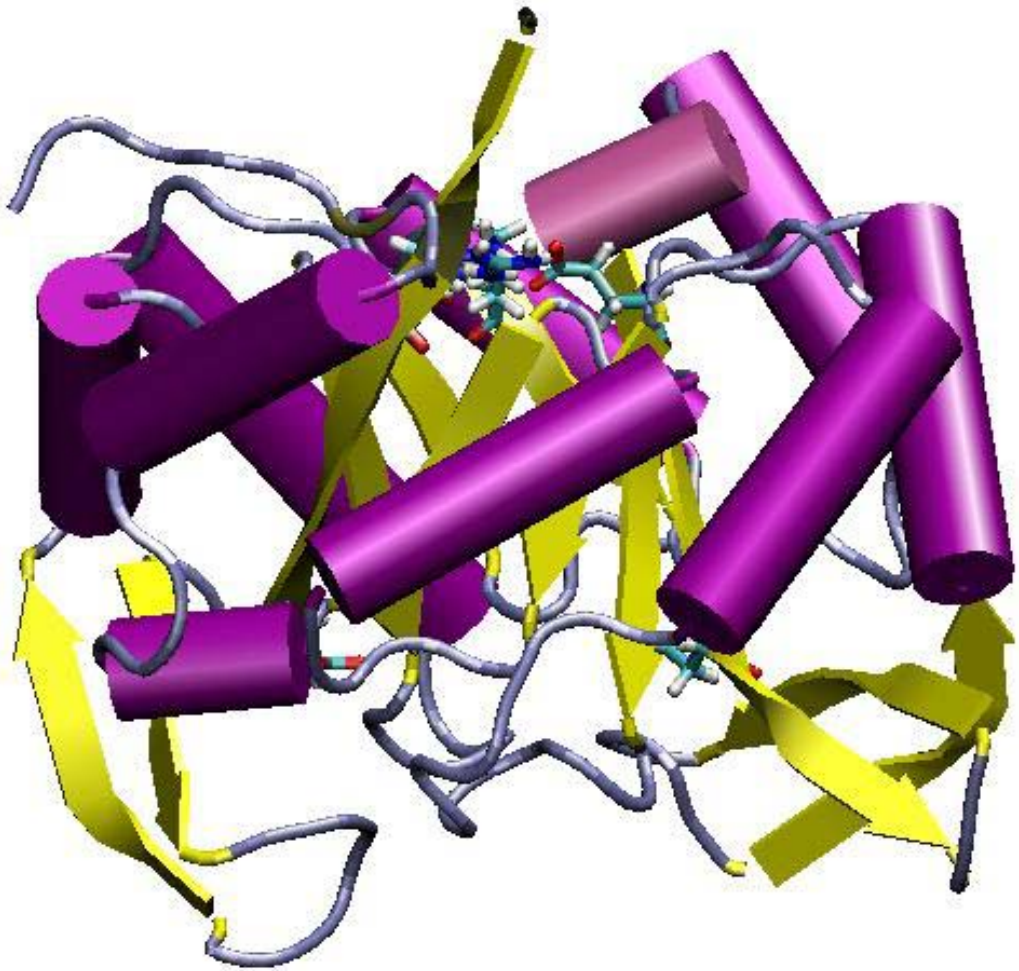
Conserved gate residues

Form stable salt bridges

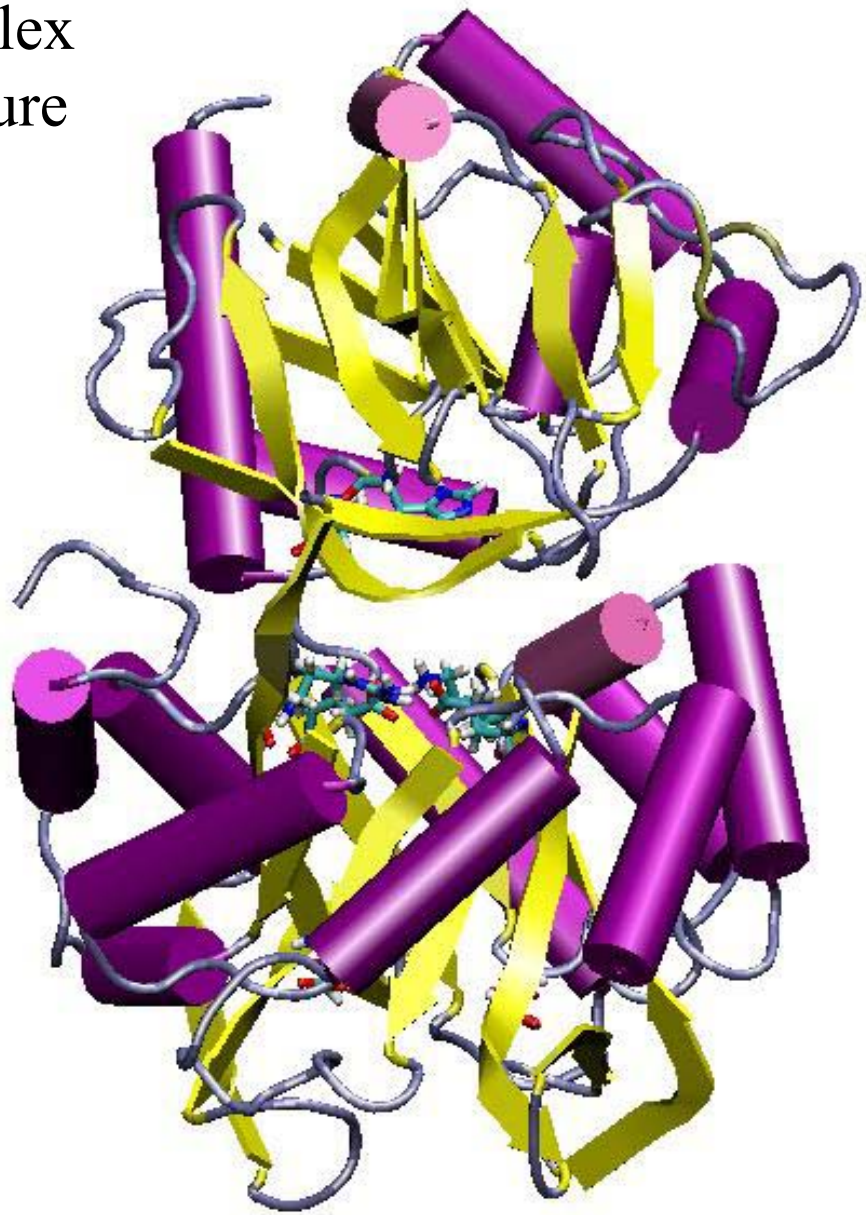
Gate diameter ~ 3 Å



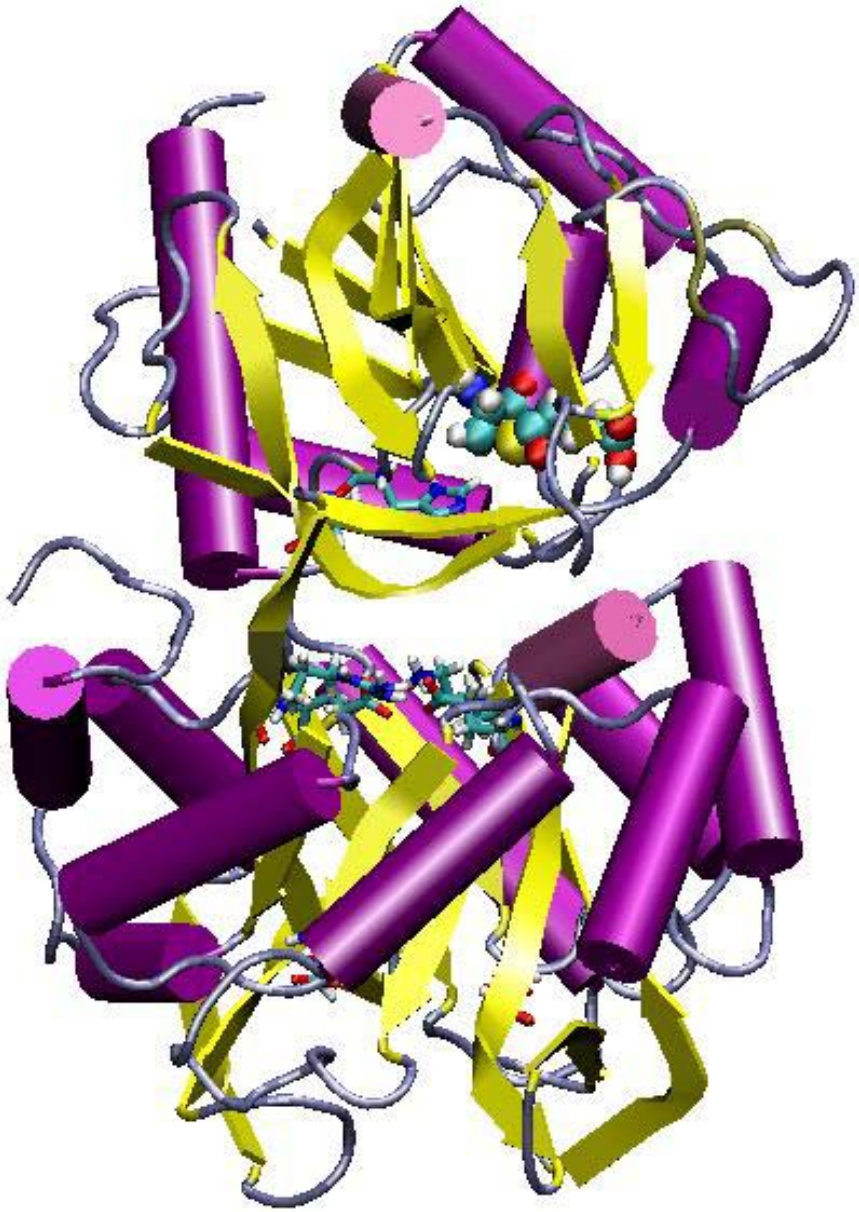
Predominantly hydrophobic channel



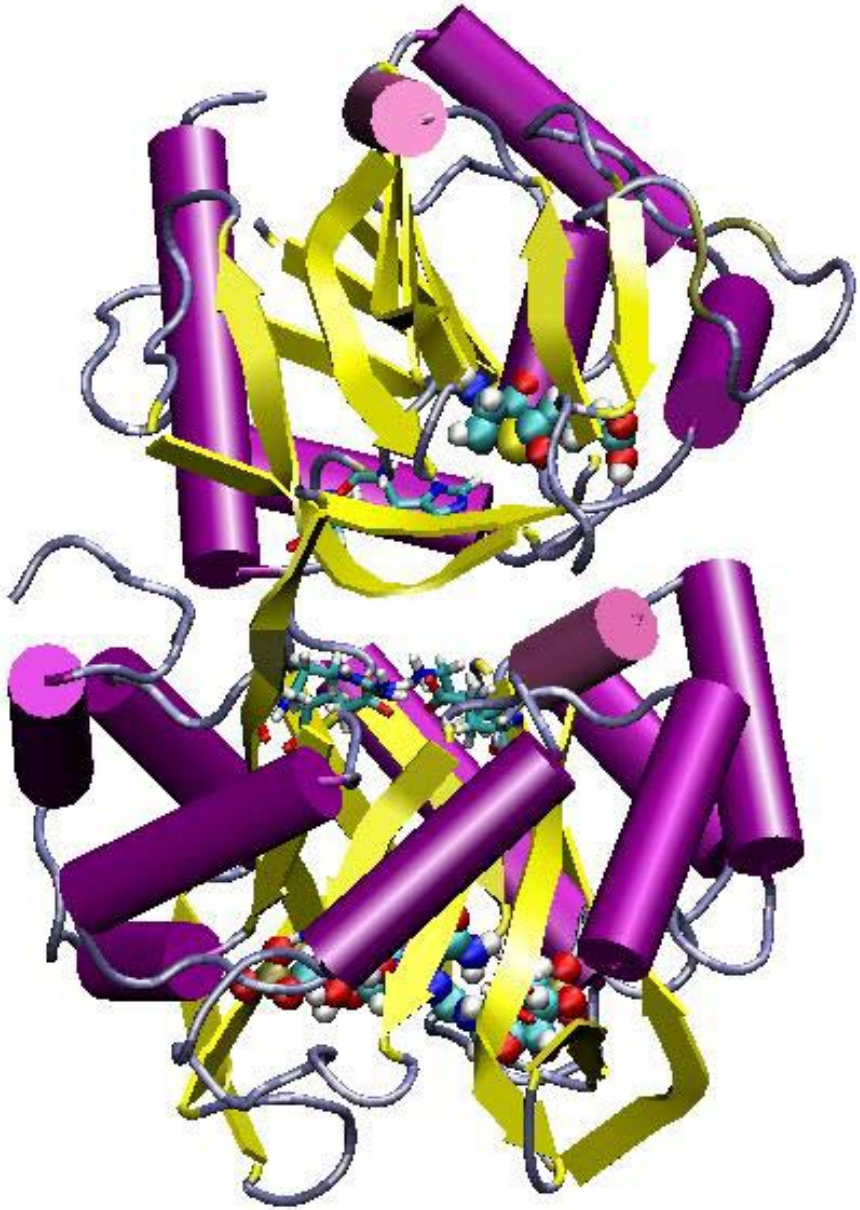
Docked Complex
Crystal Structure



Douangamath et al., **Structure**, Feb. 2002. PDB code: 1GPW

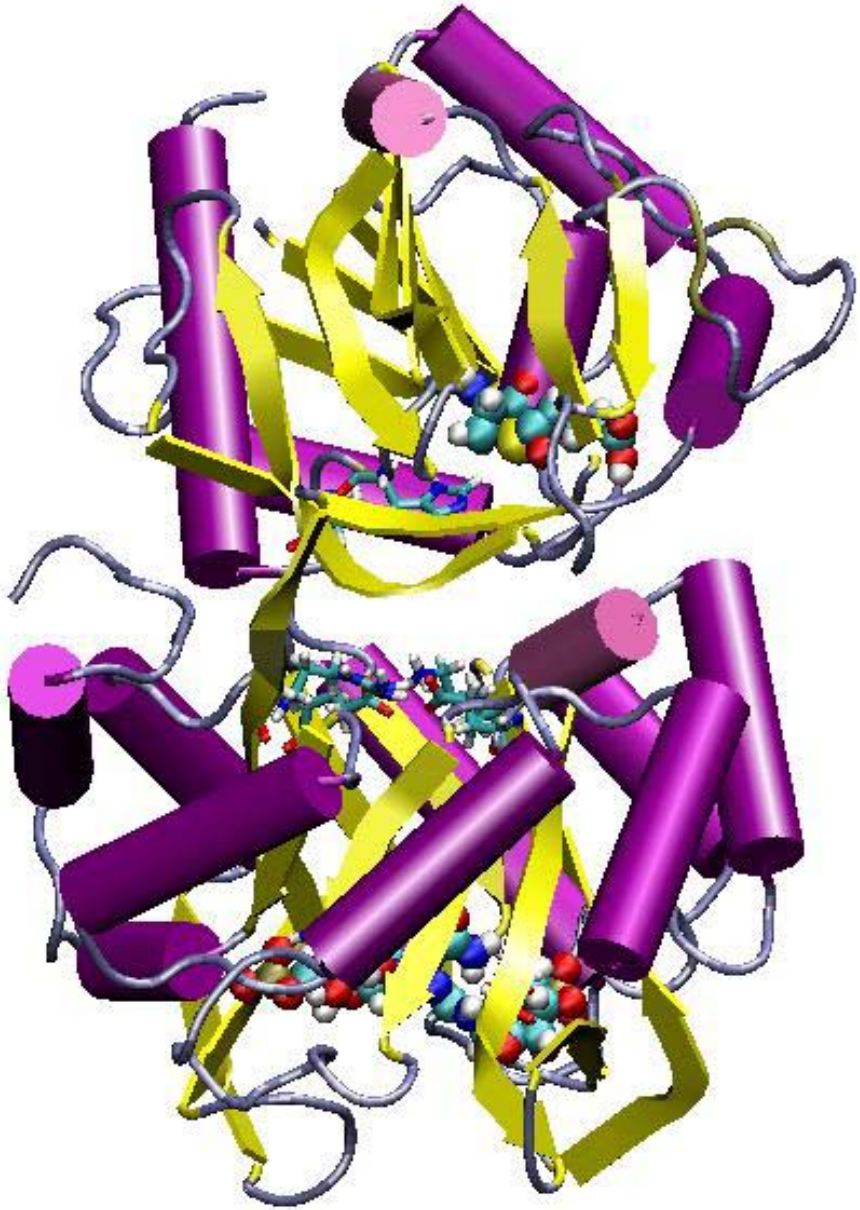


Glutamine binds in hisH active site



Glutamine binds in hisH active site

PRFAR binds to hisF active site



Glutamine binds in hisH active site

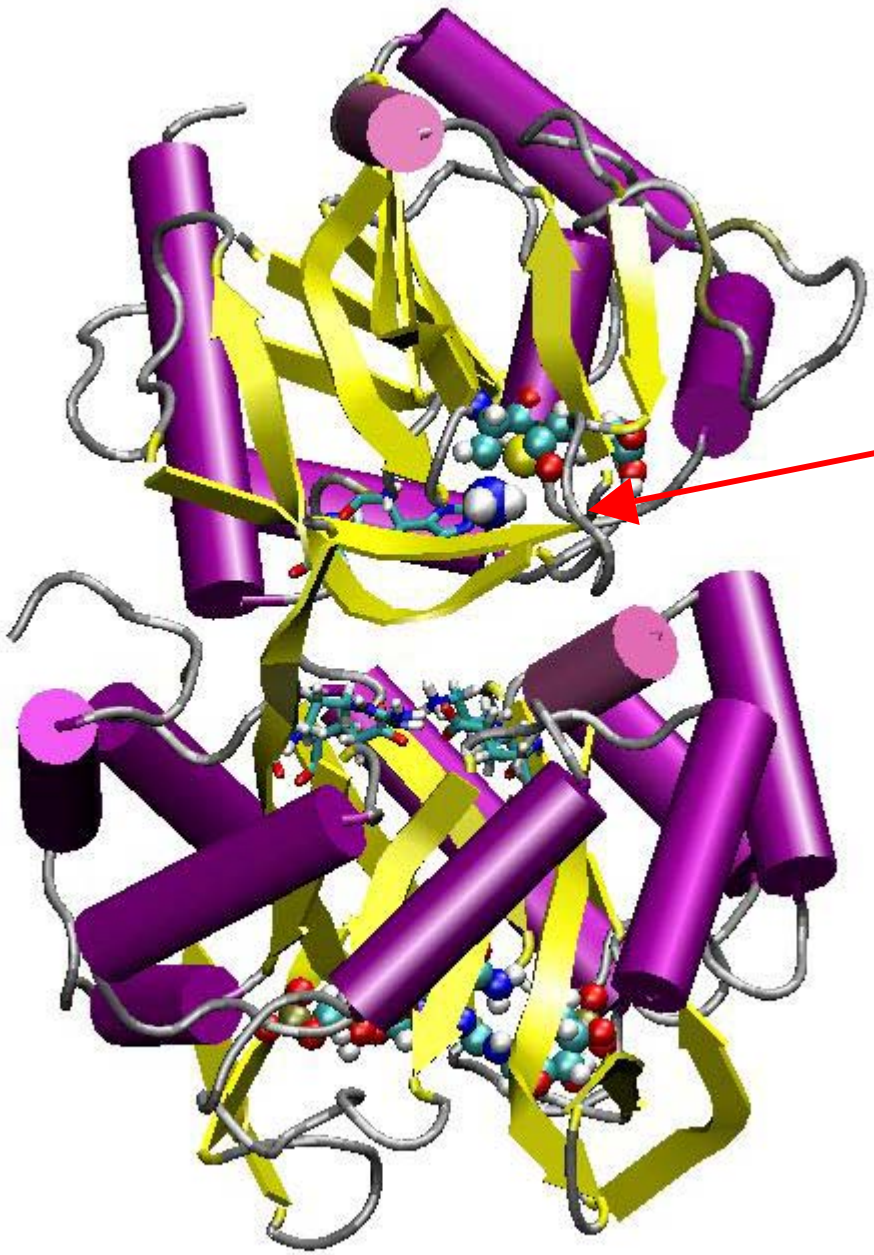
↓

PRFAR binds to hisF active site

↓

Activated event

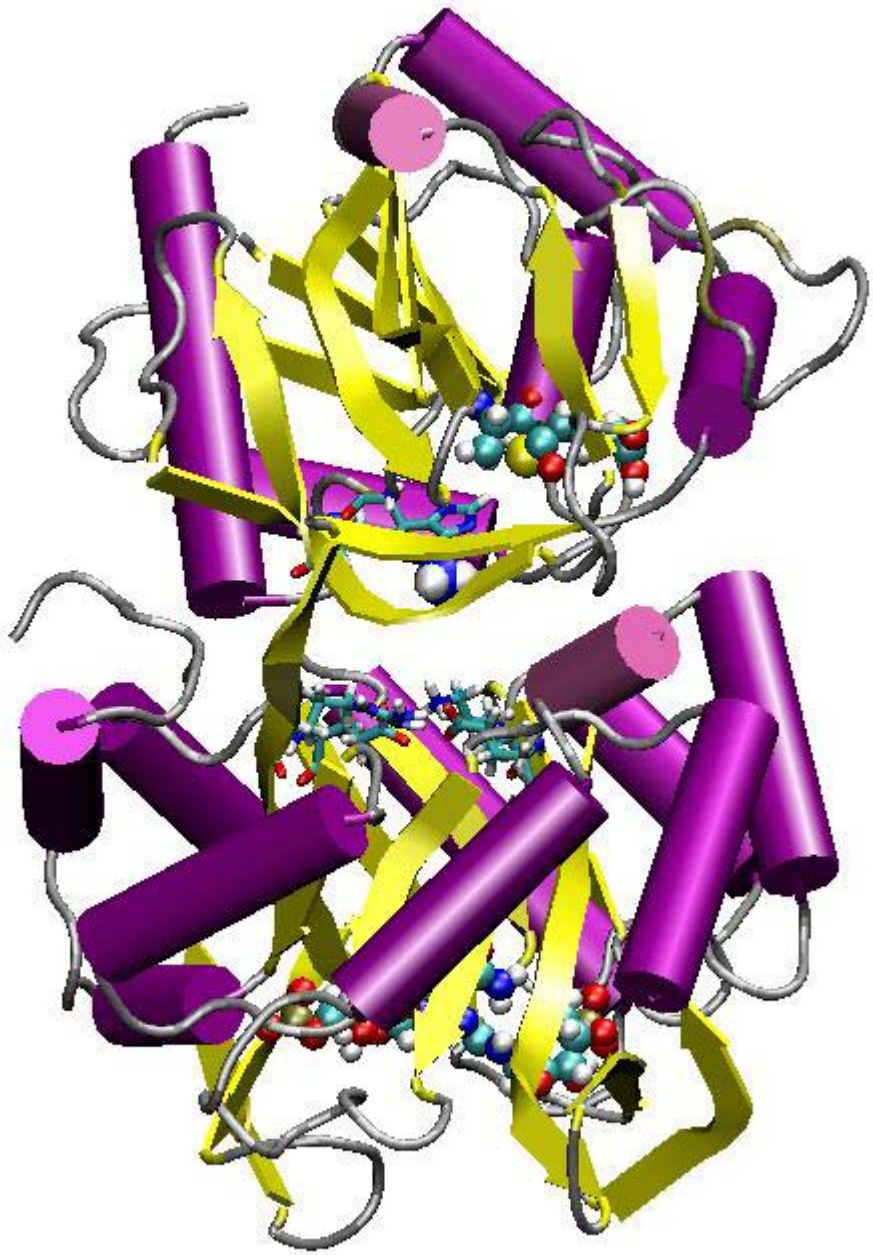
Hypothetical Coupling Mechanism



NH_3 released in 5th reaction

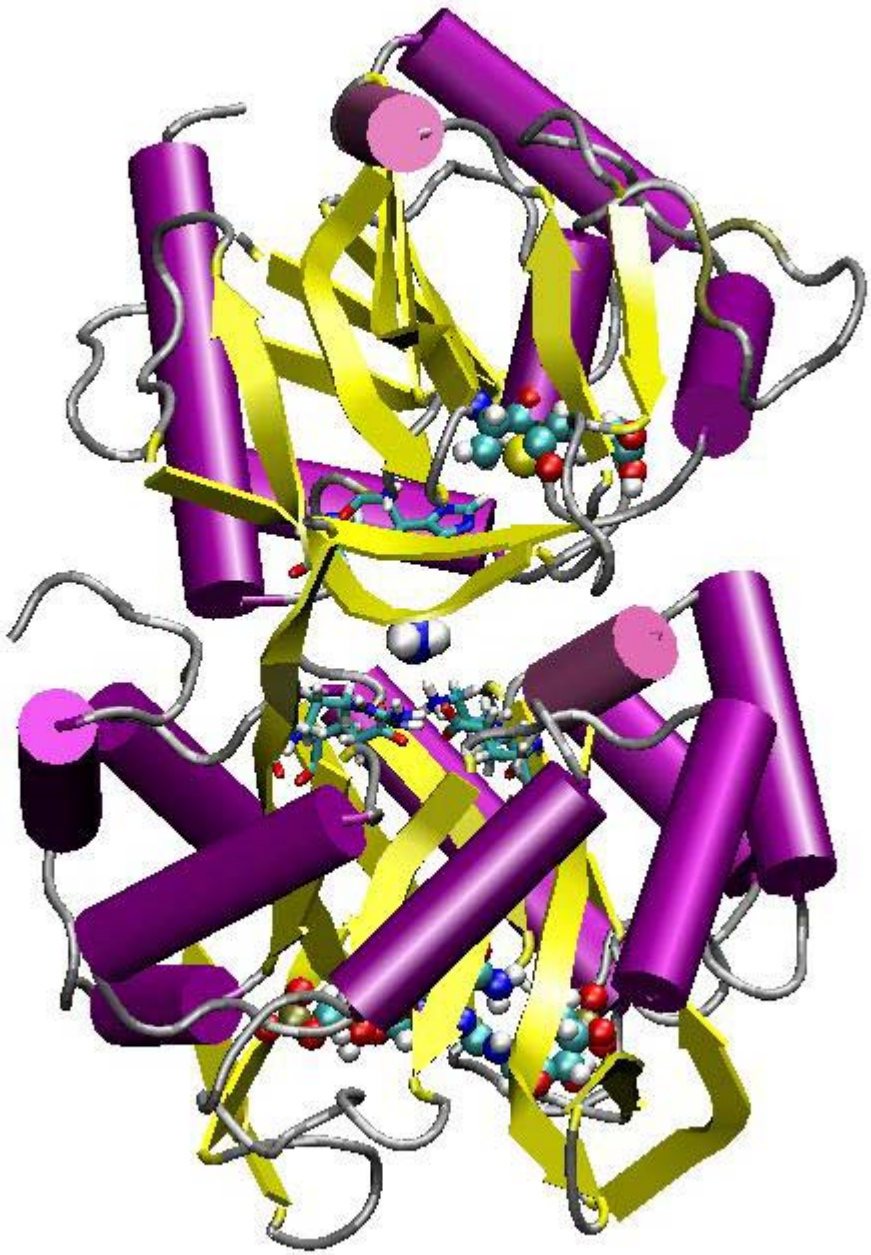
Hypothetical Coupling Mechanism

NH_3 diffuses across interface



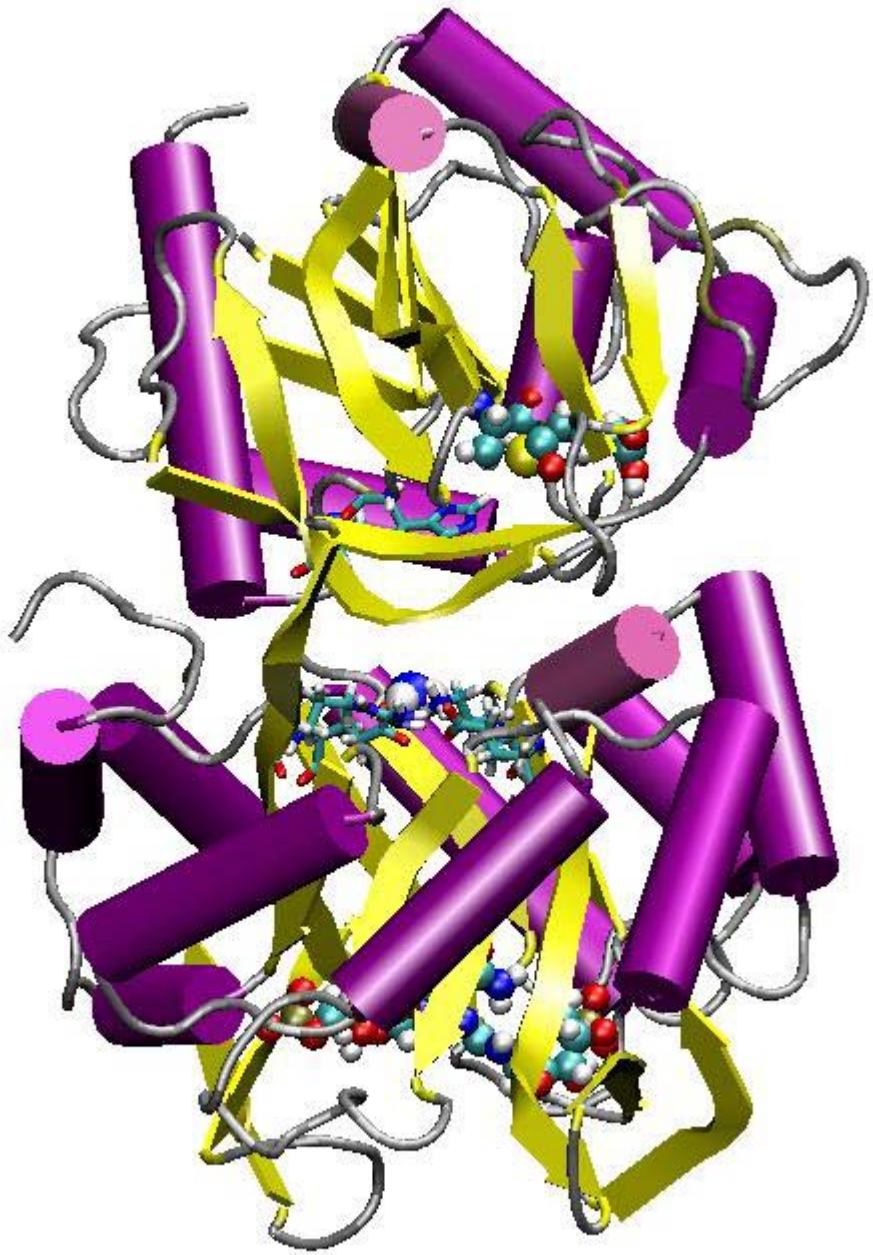
Hypothetical Coupling Mechanism

NH_3 diffuses across interface



Hypothetical Coupling Mechanism

NH_3 travels ~10Å to mouth of hisF



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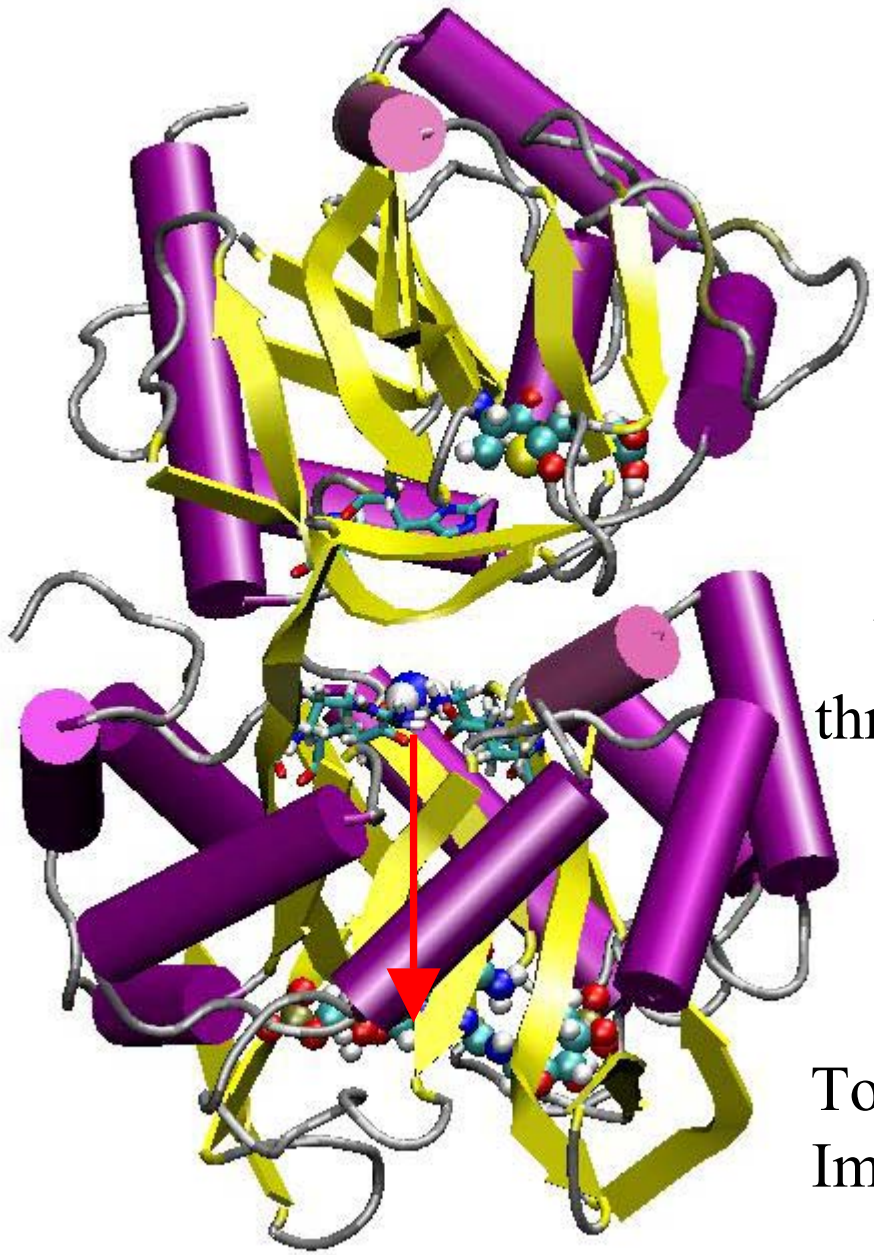


NH_3 passes through channel ~15Å

To participate in ImGP formation

What is known experimentally

- Crystal structures of both bacterial and eukaryotic[1] organisms (2001)
- Mutational studies involving residues of both subunits in gate and at interface[2]
  - ARG5 and GLU46 play essential roles in rxn
- The activity of hisH is dependent on the binding of the substrate at the hisF active site

[1]Chaudhuri et al., **Structure**, 2001.

[2]Klem et al., J Bactero., 2001; Beismann-Driemeyer, **J Biol Chem**, 2001

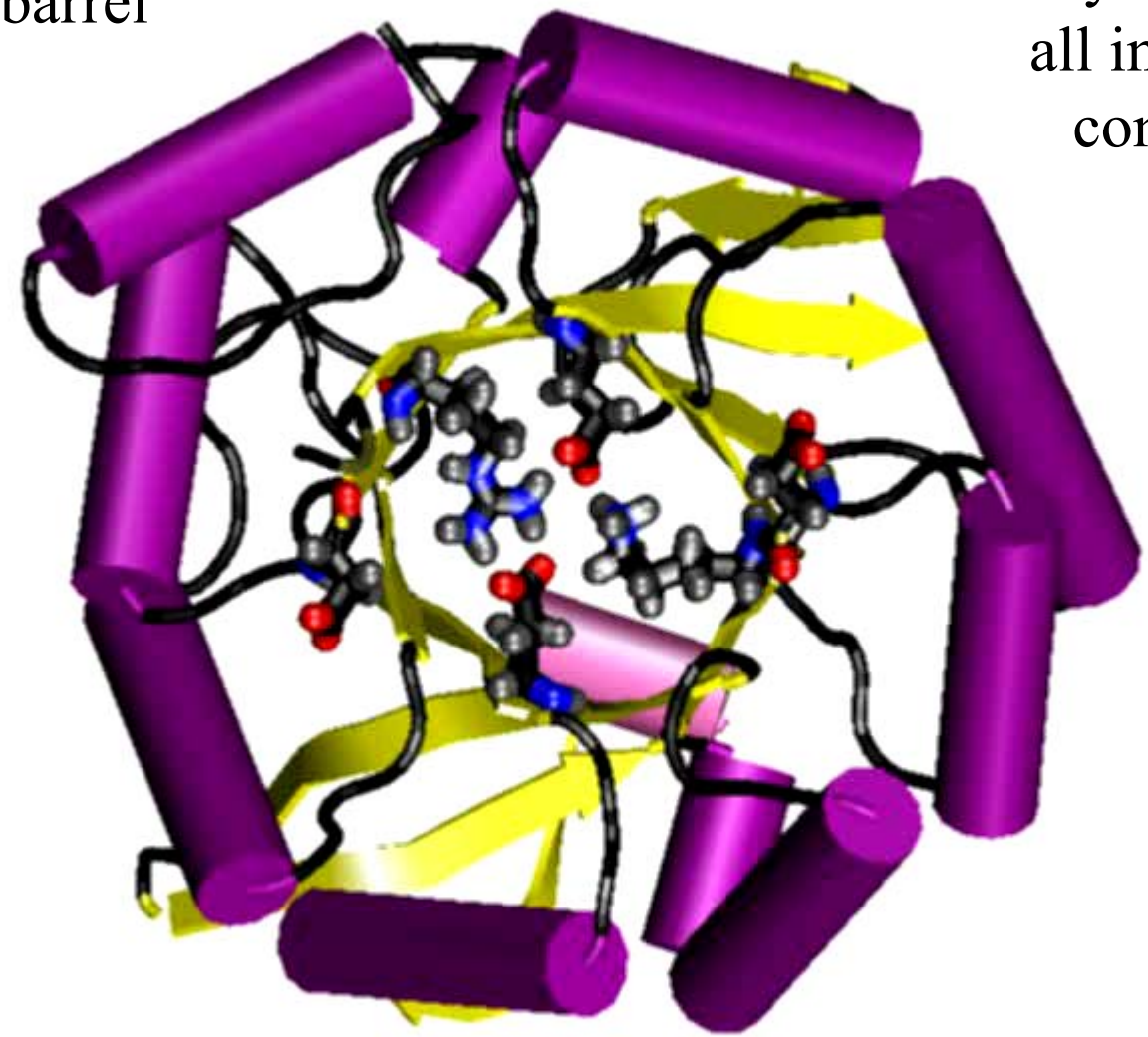
Why Substrate Channeling?

- Common in glutamine amidotransferases since coupled to second reaction requiring reactive ammonia
- Allows protected travel of intermediate
  - NH_3 at physiological conditions usually found as NH_4^+
- Allows directed travel of intermediate
- First time α/β barrel proposed to be used as an intermediate channel !

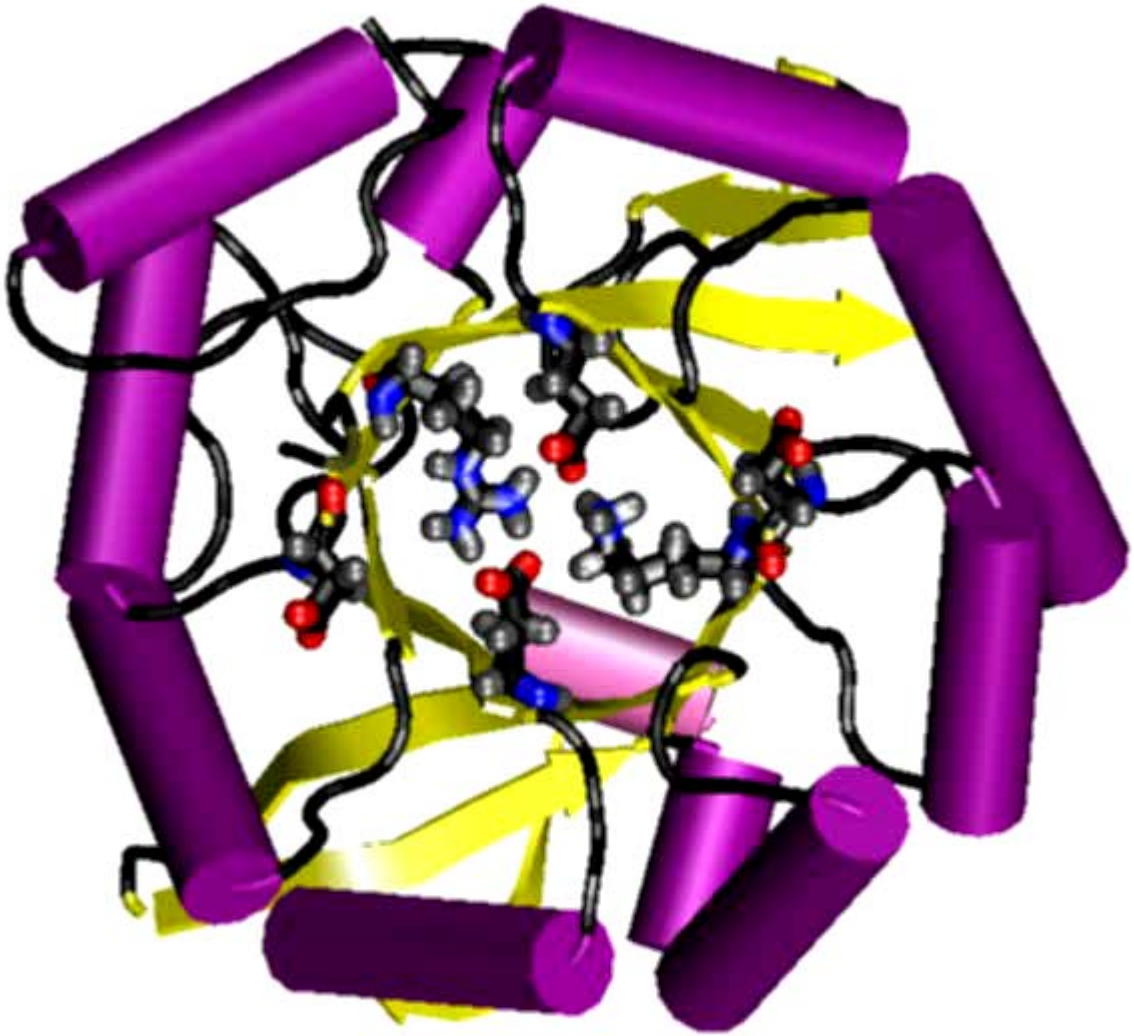
Investigating the Gate Mechanism

- Gate seems closed in crystal structures
- Diameter of gate 3.2Å, NH_3 is ~ 2Å
- Use bioinformatics to narrow the search
- 2 conserved ASP's near gate
- Salt bridges could be formed between ASP98 – LYS99 and/or ASP219—ARG5
- Increases diameter of gate to 6.9Å
- Stable! Stay in formation for ps

Gate at entrance of hisF barrel



Crystal structures all in closed gate conformation

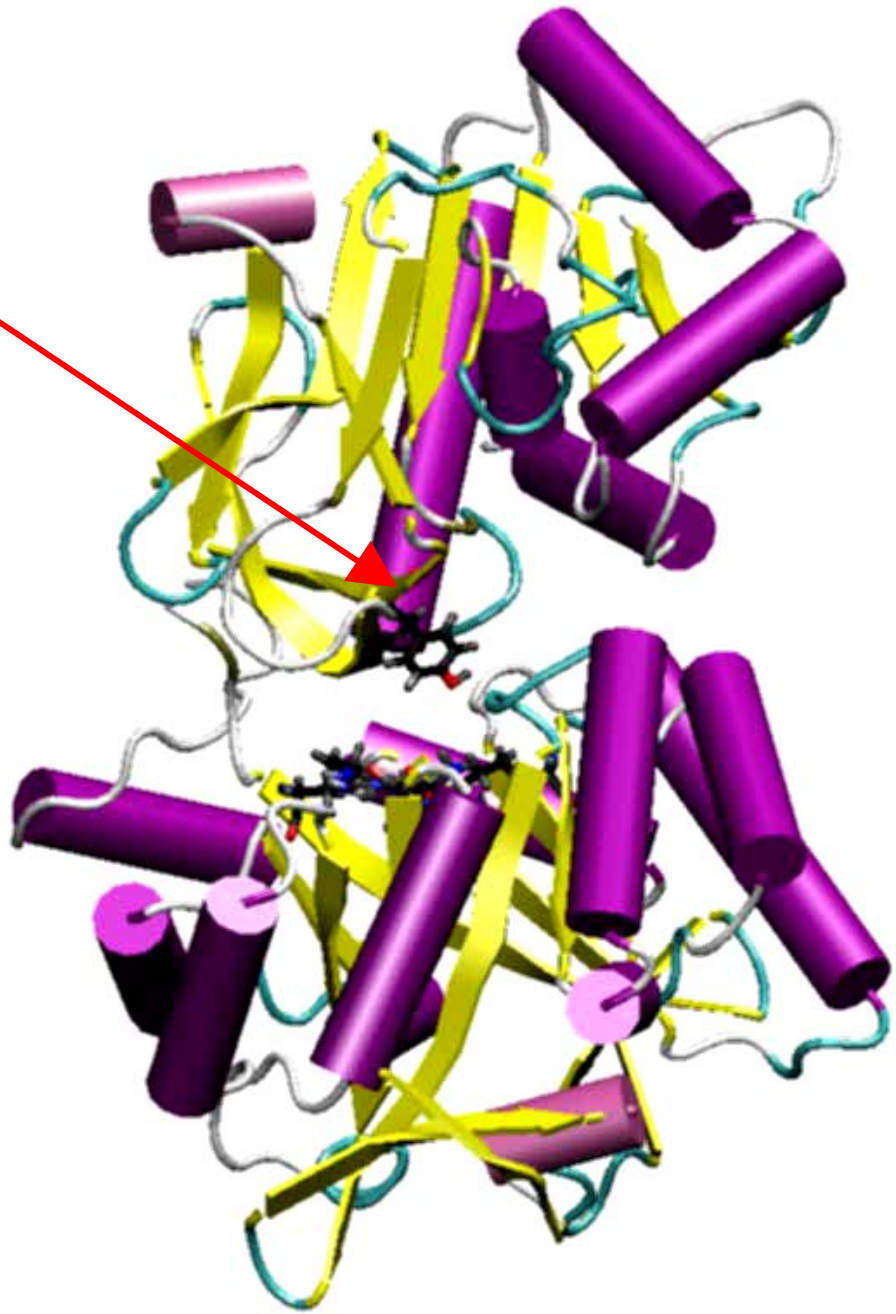


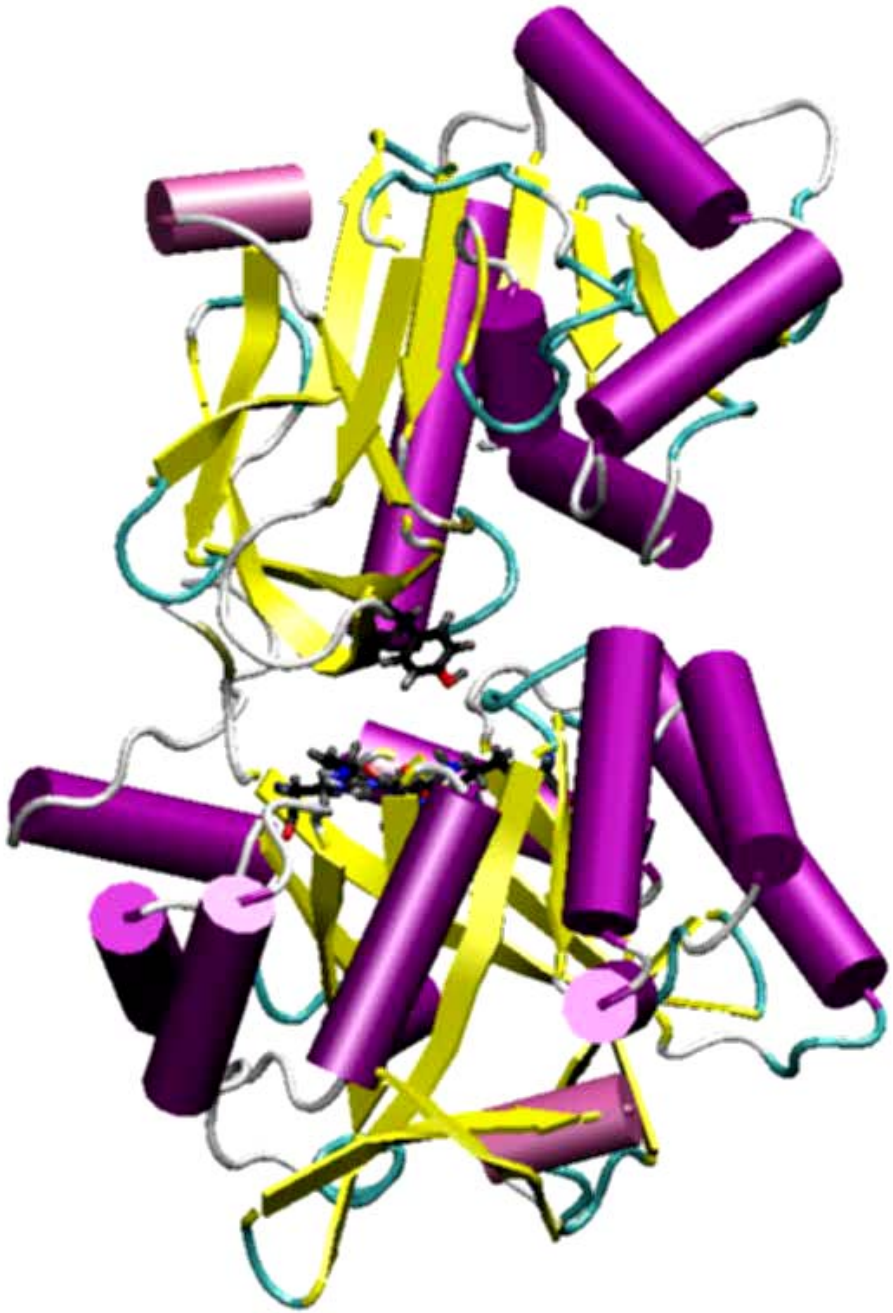
Simulated Gating Mechanisms

- Followed suggestion by Chaudhuri et al. to form a hydrogen bond between 2 strictly conserved residues at the interface: TYR138 of hisH and LYS99 of hisF's gate
- Increased the diameter of the channel from 3.2Å to 5.8Å
- Since no experimental evidence for any gating mechanism, also simulated the closed gate

Strictly conserved TYR 138 of hisH

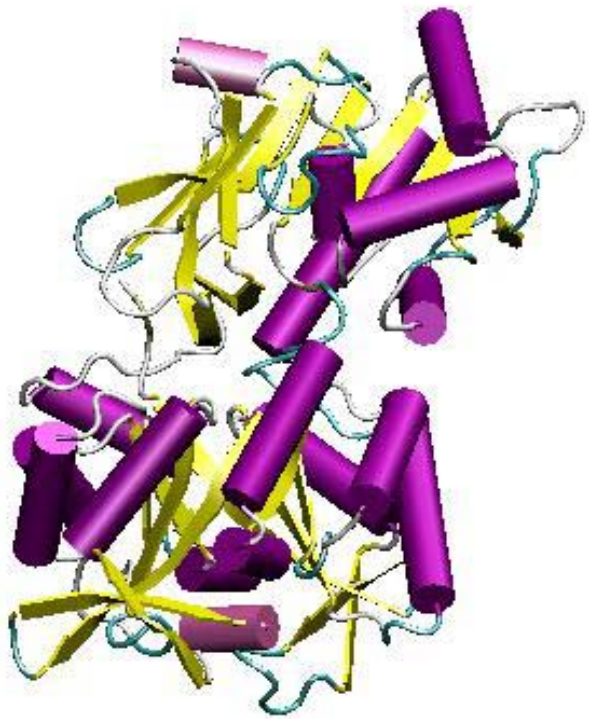
Possible gating mechanism?





System Setup

Started with 2.4Å resolution crystal structure



System Setup

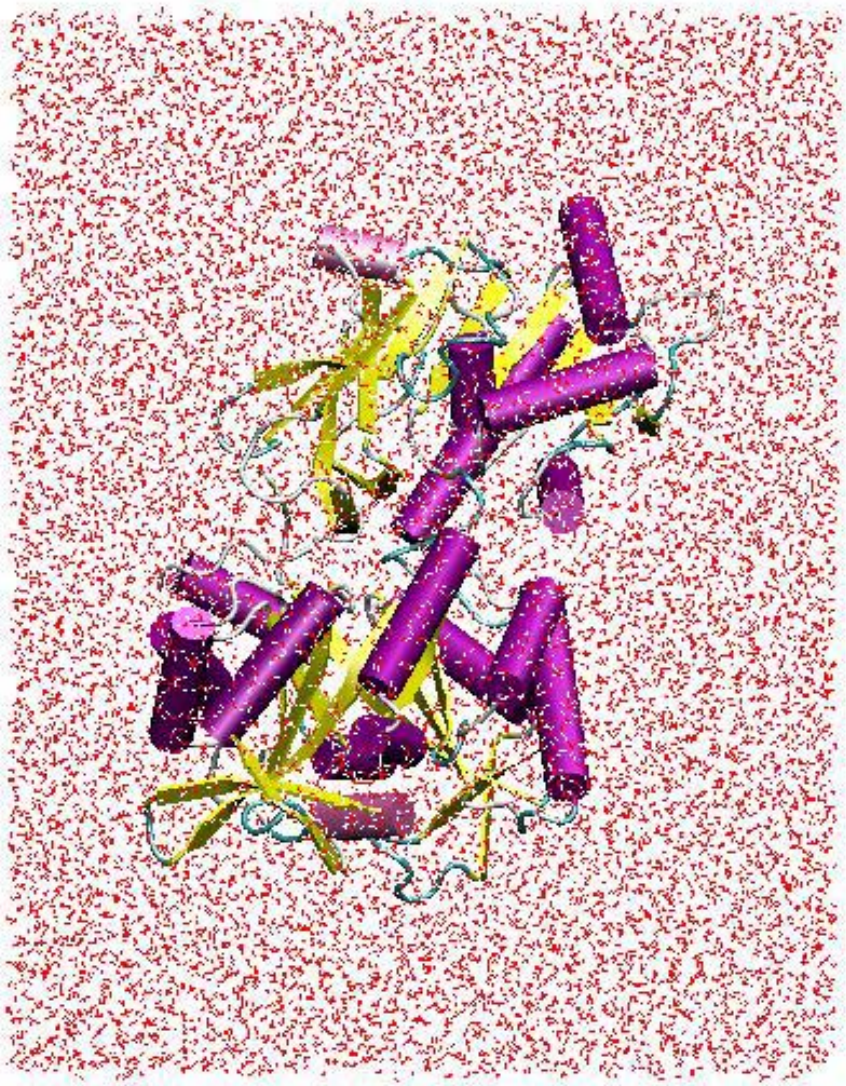
Started with 2.4Å resolution crystal structure

↓

Solvated complex with explicit waters

↓

Minimized, equilibrated using NAMD2 and Charmm27 forcefield in NPT ensemble



Theoretical Biophysics Group, K. Schulten et al.

Ammonia Conduction

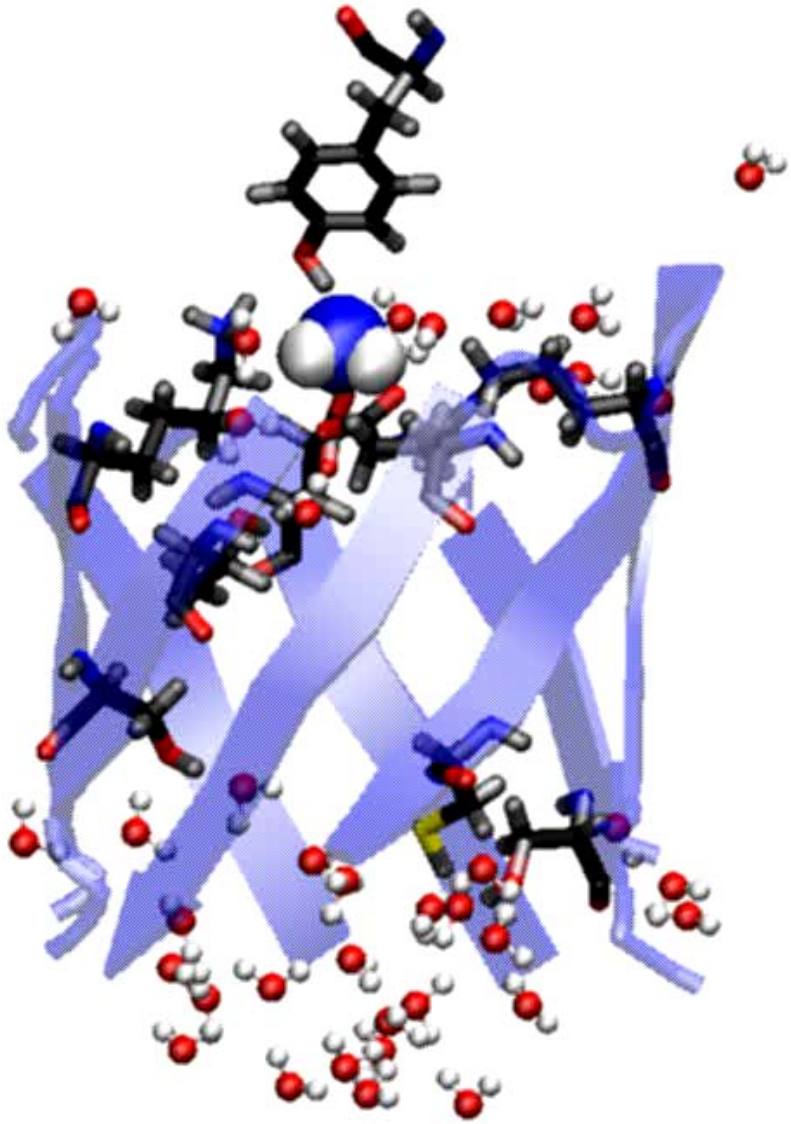
- SMD to induce the passage of ammonia through the channel on the ns timescale

- Hamiltonian of the system becomes:

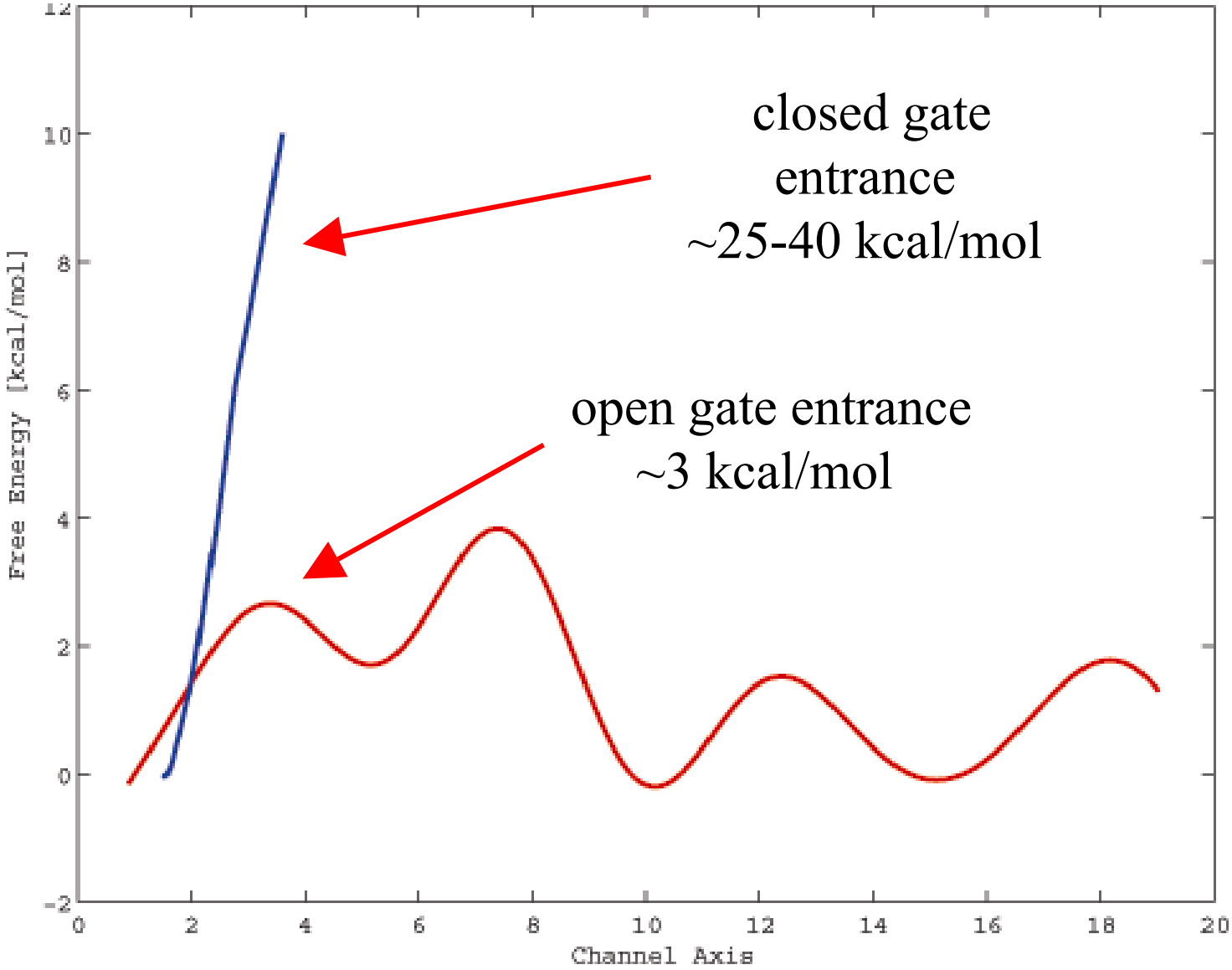
$$H(r,t) = \mathrm{H}_0(r) + \frac{k}{2}[z(r) - z_0 - vt]^2$$

- NH_3 through the channel at constant $v = 15$Å/ns

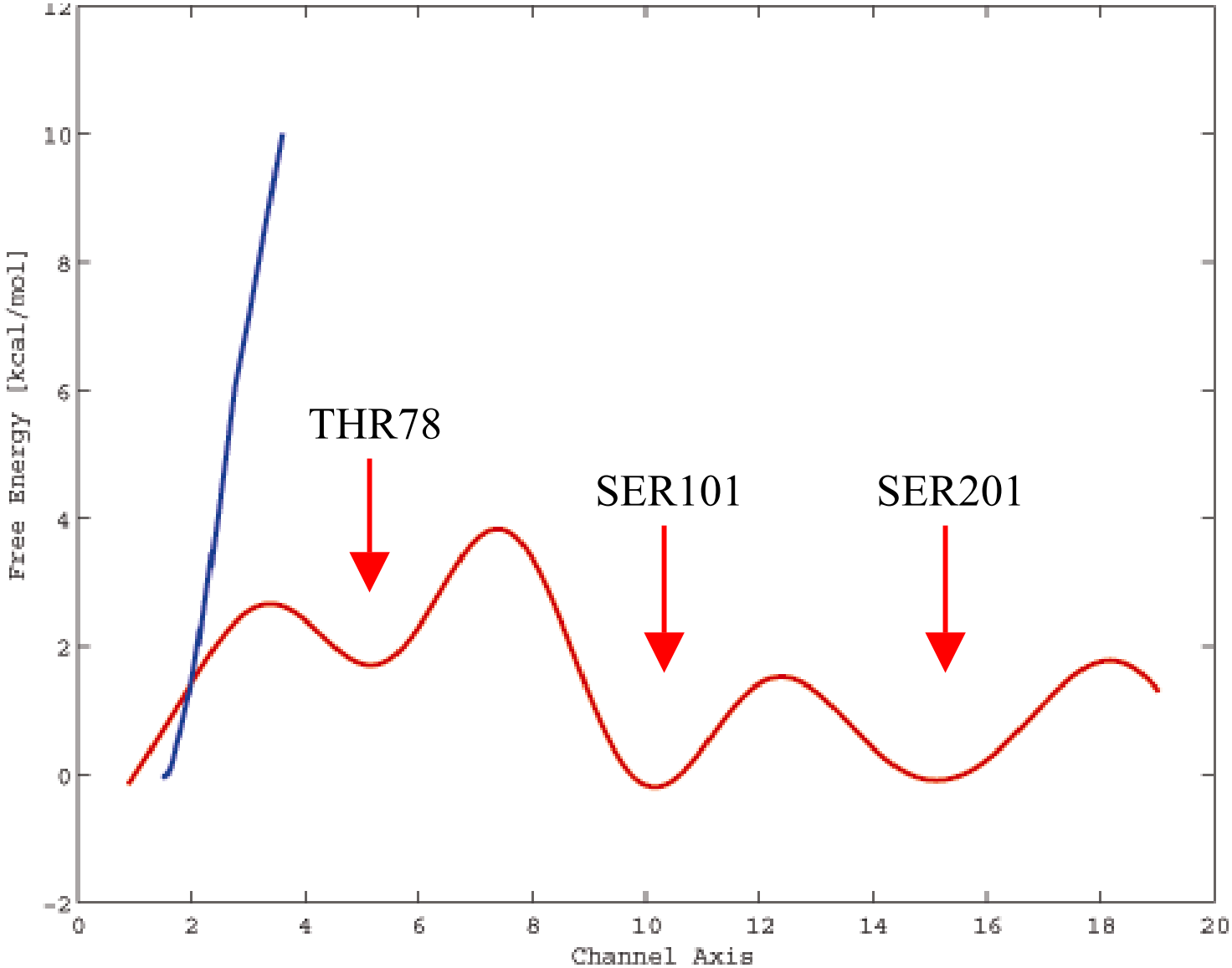
- Analyzed the resulting trajectories, forces



Energy Barrier of Gating Mechanism



Free Energy Profile in Channel

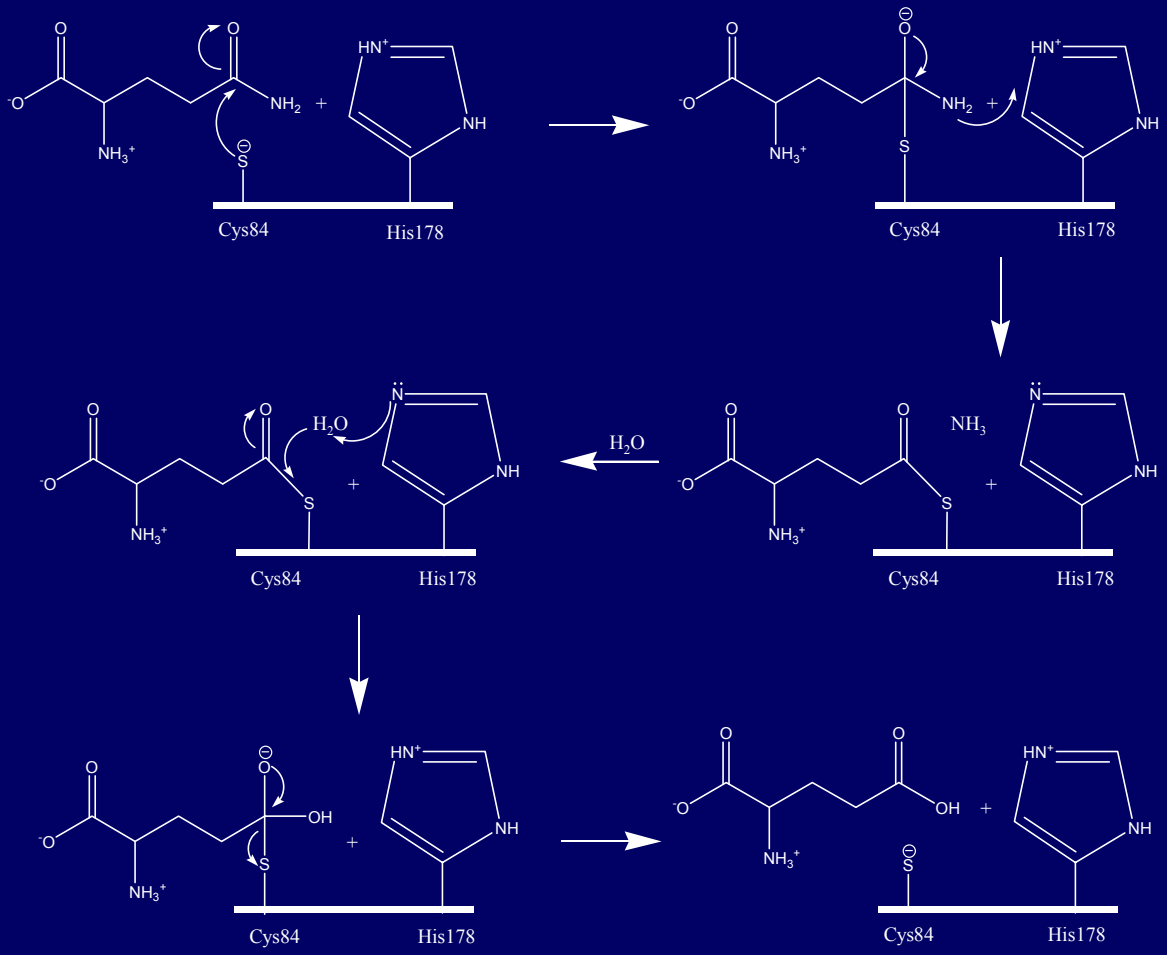


A more realistic model

- Would like to model “activated” complex
- Needed to build in both substrates
- hisH: covalently bound glutamine / glutamate
- hisF: substrate PRFAR
- But no publicly available parameters for parts of hisH substrate (thioester) and PRFAR
- Today’s lab exercise will walk you through the parameterization of the hisH substrate

HisH Mechanism

- HisH glutamine amidotransferase
- Conserved catalytic triad: C84, H178, E180



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