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

HisH
HisH active site:
Catalytic triad
CYS84 – HIS178 – GLU180
HisF
Top View of HisF
Conserved gate residues

Form stable salt bridges

Gate diameter
\( \sim 3 \text{ Å} \)
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 5$^{th}$ reaction
Hypothetical Coupling Mechanism

$\text{NH}_3$ diffuses across interface
Hypothetical Coupling Mechanism

$\text{NH}_3$ diffuses across interface
Hypothetical Coupling Mechanism

$\text{NH}_3$ travels $\sim10\text{Å}$ to mouth of hisF
Hypothetical Coupling Mechanism

NH₃ travels ~10Å to mouth of hisF

NH₃ passes through channel ~15Å

To participate in ImGP formation
What is known experimentally

• Crystal structures of both bacterial and eukaryotic\textsuperscript{1} organisms (2001)

• Mutational studies involving residues of both subunits in gate and at interface\textsuperscript{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

\textsuperscript{1}Chaudhuri et al., \textit{Structure}, 2001.
\textsuperscript{2}Klem et al., \textit{J Bactero.}, 2001; Beismann-Driemeyer, \textit{J Biol Chem}, 2001
Why Substrate Channeling?

- Common in glutamine amidotransferases since coupled to second reaction requiring reactive ammonia
- Allows protected travel of intermediate
  - $\text{NH}_3$ at physiological conditions usually found as $\text{NH}_4^+$
- Allows directed travel of intermediate
- First time $\alpha/\beta$ barrel proposed to be used as an intermediate channel!
Investigating the Gate Mechanism

- Gate seems closed in crystal structures
- Diameter of gate $3.2\text{Å}$, $\text{NH}_3$ is $\sim 2\text{Å}$
- 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 $6.9\text{Å}$
- 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?
Started with 2.4Å resolution crystal structure
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:

\[ H(r,t) = H_0(r) + \frac{k}{2} [z(r) - z_0 - vt]^2 \]

- \( \text{NH}_3 \) through the channel at constant \( \nu = 15\text{Å/ns} \)

- Analyzed the resulting trajectories, forces
Energy Barrier of Gating Mechanism

- Closed gate entrance: ~25-40 kcal/mol
- Open gate entrance: ~3 kcal/mol
Free Energy Profile in Channel

THR78

SER101

SER201
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
HisH Mechanism

- HisH glutamine amidotransferase
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