Why the histidine biosynthesis pathway?
Why hisH-hisF?

De novo purine biosynthesis

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HisH

triad glutamine amidotransferase

HisH

Catalytic triad active site

CYS84 – HIS178 – GLU180

All GATases coupled to a second reaction requiring reactive NH$_3$
HisF
HisF

Active site residues
Top View of HisF
Conserved gate residues

Form stable salt bridges

Gate diameter
\(~ 3 \text{ Å}~\)
Predominantly hydrophobic channel
Glutamine (mM)

PRFAR (sub-µM)
NH₃ released in 5th reaction
NH$_3$ diffuses across interface ~10Å to mouth of hisF
NH₃ diffuses across interface ~10Å to mouth of hisF
Mutating conserved gate residues drastically reduces cyclase rxn efficiency!

... where it meets the gate
NH₃ passes through channel ~15Å
to participate in ImGP formation

Novel function for ubiquitous fold!
Talk Outline

• Many interesting aspects: gating mechanism, NH₃ conduction, allosteric effects, chemistry of catalytic reactions

• Main tools are molecular dynamics simulations and bioinformatic analyses

• Highlights of the research on the *apo*-system

• Building active system requires parameterization of substrates

• New results regarding *active*-system
Ammonia Conduction

• Steered Molecular Dynamics (SMD) to induce NH$_3$ conduction on ns timescale

• Apply an external force to the system:

\[ H \[ x(t), t \] = H_0 \[ x(t) \] + 0.5k \[ z(x) - z_0 - vt \]^2 \]

• To quantify the energetics of conduction we use:

\[ e^{-\beta \Delta F} = \langle e^{-\beta \Delta W} \rangle_{\text{traj}} \]

• This new identity allows us to determine equilibrium information from repeated nonequilibrium measurements
Results through partially open gate in \textit{apo}-complex

Dipole Moment Analysis

\begin{figure}
\begin{center}
\includegraphics[width=0.8\textwidth]{dipole_moment_analysis.png}
\end{center}
\end{figure}

Free energy profile of ammonia in barrel

\begin{figure}
\begin{center}
\includegraphics[width=0.8\textwidth]{free_energy_profile.png}
\end{center}
\end{figure}

Mean First Passage Time Analysis:

\textit{Without substrates, passage of NH}_3 \sim 110 \text{ ns}

\textit{Overall this step is \textbf{not} rate limiting!}

We can model various functional states
Modeling the *active*-complex: including substrates

**HisH:**
Glutamyl thioester intermediate corresponding to post-NH$_3$ release state
Parameterization required for thioester linkage

**HisF:**
N1-(5’-phosphoribosyl)-formimino-5-aminoimidazole-4-carboxamide ribonucleotide (… or PRFAR) cryo-trapped in hisF active site*
Parameterization according to existing CHARM MM protocol

Including substrates produced a surprising result!

Same gate configuration, higher barriers?!?
How could PRFAR change the energetics of conduction?

PRFAR introduces large electrostatic effects!
Net effect: a torque on ammonia’s dipole

Electrostatic field from PRFAR makes it more difficult for NH$_3$ to flip orientations

Modeling the *active*-complex: today’s tutorial
VMD to Attach the substrate GLN to the active site of hisH
Class I Potential Energy function

\[ E_{\text{Total}} = \sum_{\text{bonds}} k_b (b - b_0)^2 + \sum_{\text{angles}} k_\theta (\theta - \theta_0)^2 \]

\[ + \sum_{\text{dihedrals}} \frac{V_n}{2} [1 + \cos(n\phi - \delta)] \]

\[ + \sum_{\text{improvers}} k_\omega (\omega - \omega_0)^2 + \sum_{\text{Urey-Bradley}} k_v (r_{1,3} - r_{1,3,0})^2 \]

Non-bonded Interaction Terms

\[ + \sum_{\text{electrostatics}} \left( \frac{q_i q_j}{\epsilon r_{ij}} \right) + \sum_{\text{VDW}} \epsilon_{ij} \left[ \left( \frac{R_{\text{min},ij}}{r_{ij}} \right)^{12} - 2 \left( \frac{R_{\text{min},ij}}{r_{ij}} \right)^{6} \right] \]

From MacKerell
Class I Potential Energy function

\[ E_{\text{Total}} = \sum_{\text{bonds}} k_b (b - b_0)^2 + \sum_{\text{angles}} k_\theta (\theta - \theta_0)^2 \]
\[ + \sum_{\text{dihedrals}} \frac{V}{2} [1 + \cos(n\phi - \delta)] \]
\[ + \sum_{\text{impropers}} k_\omega (\omega - \omega_0)^2 + \sum_{\text{Urey-Bradley}} k_v (r_{1,3} - r_{1,3,0})^2 \]

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Specify in topology file from MacKerell
Class I Potential Energy function

\[ E_{\text{Total}} = \sum_{\text{bonds}} k_b (b - b_0)^2 + \sum_{\text{angles}} k_\theta (\theta - \theta_0)^2 \]

\[ + \sum_{\text{dihedrals}} \frac{V_n}{2} [1 + \cos(n\phi - \delta)] \]

\[ + \sum_{\text{impropers}} k_\omega (\omega - \omega_0)^2 + \sum_{\text{Urey-Bradley}} k_x (r_{1,3} - r_{1,3,0})^2 \]

Non-bonded Interaction Terms

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From MacKerell

Specify in parameter file
Creating a new topology file entry

HG1 deleted from CYS and the charge was moved to SG (-0.23 +0.16=0.07) so that the SG charge becomes 0.07 in final compound and the group remains neutral

Changes annotated!
Creating new parameters

BONDS
!
!V(bond) = Kb(b - b0)**2
!
!Kb: kcal/mole/A**2
!b0: A
!
!atom type Kb b0
!
! Modified for CYG residue after 6-31G* geometry optimization
! S CC 240.000 1.7814 ! ALLOW ALI SUL ION

ANGLES
!
!V(angle) = Ktheta(Theta - Theta0)**2
!
!Ktheta: kcal/mole/deg**2
!
!V(Urey-Bradley) = Kub(S - S0)**2
!
!Kub: kcal/mole/deg**2
!Theta0: degrees
!S0: A
!
!atom types Ktheta Theta0 Kub S0
!
! Modified for CYG residue after 6-31G* geometry optimization
! CT2 S CC 34.000 100.2000 ! ALLOW ALI SUL ION
! CT2 CC S 50.000 114.5000 ! ALLOW ALI SUL ION
! O CC S 75.000 122.2000 ! ALLOW ALI SUL ION

DIHEDRALS
!
!V(dihedral) = Kchi(1 + cos(n(chi) - delta))
!
!Kchi: kcal/mole
!n: multiplicity
!delta: degrees
!
!atom types Kchi n delta
! CC S CT2 CT1 0.2400 1 180.00
! CC S CT2 CT1 0.3700 3 0.00
! HA CT2 S CC 0.2800 3 0.00
! CT2 S CC CT2 2.05 2 180.00
! CT2 S CC 0 2.05 2 180.00
Semi-empirical Parameter Estimation Using SPARTAN

Main Spartan Window

You build a part of CYG

Be careful with the dihedral drive section!
Acknowledgements

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The TCBG Resource