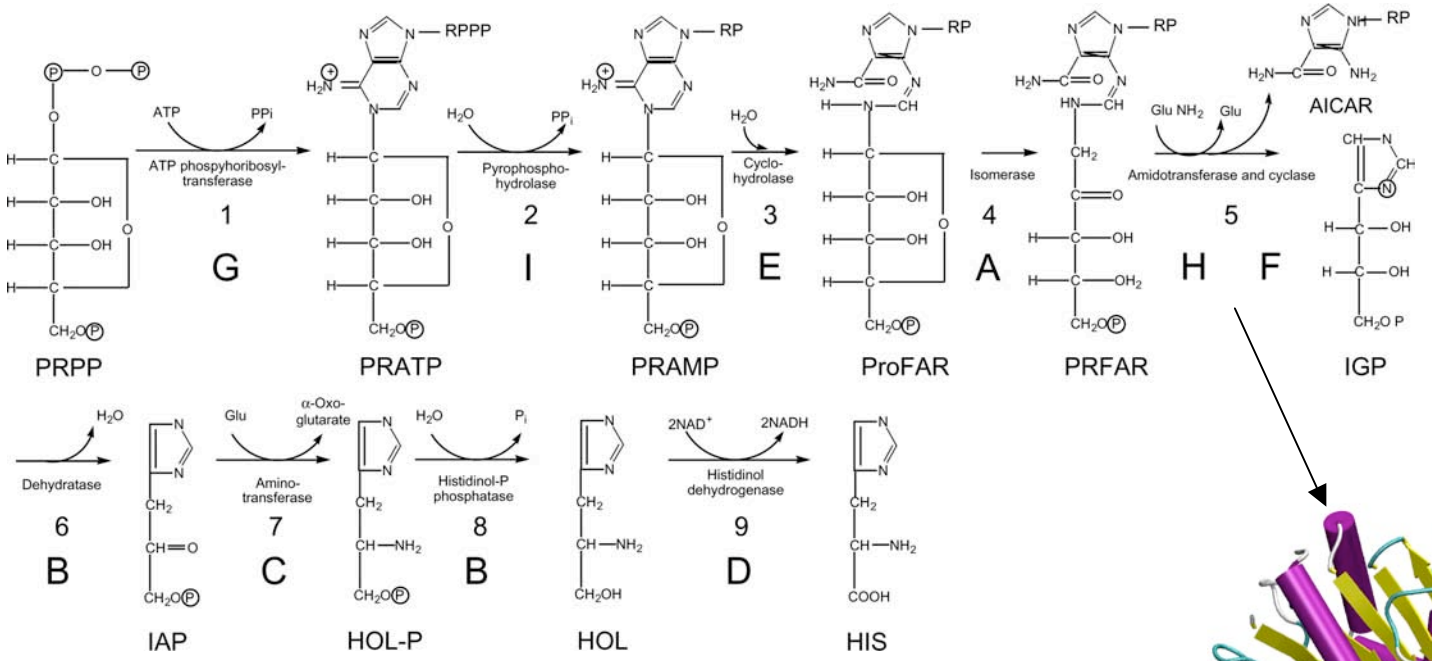
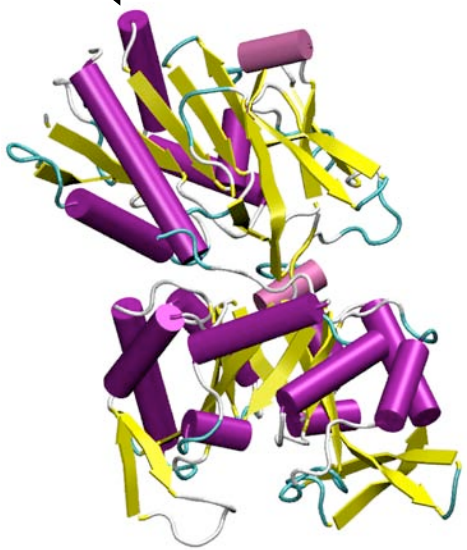
Why the histidine biosynthesis pathway?
Why hisH-hisF?

De novo purine biosynthesis



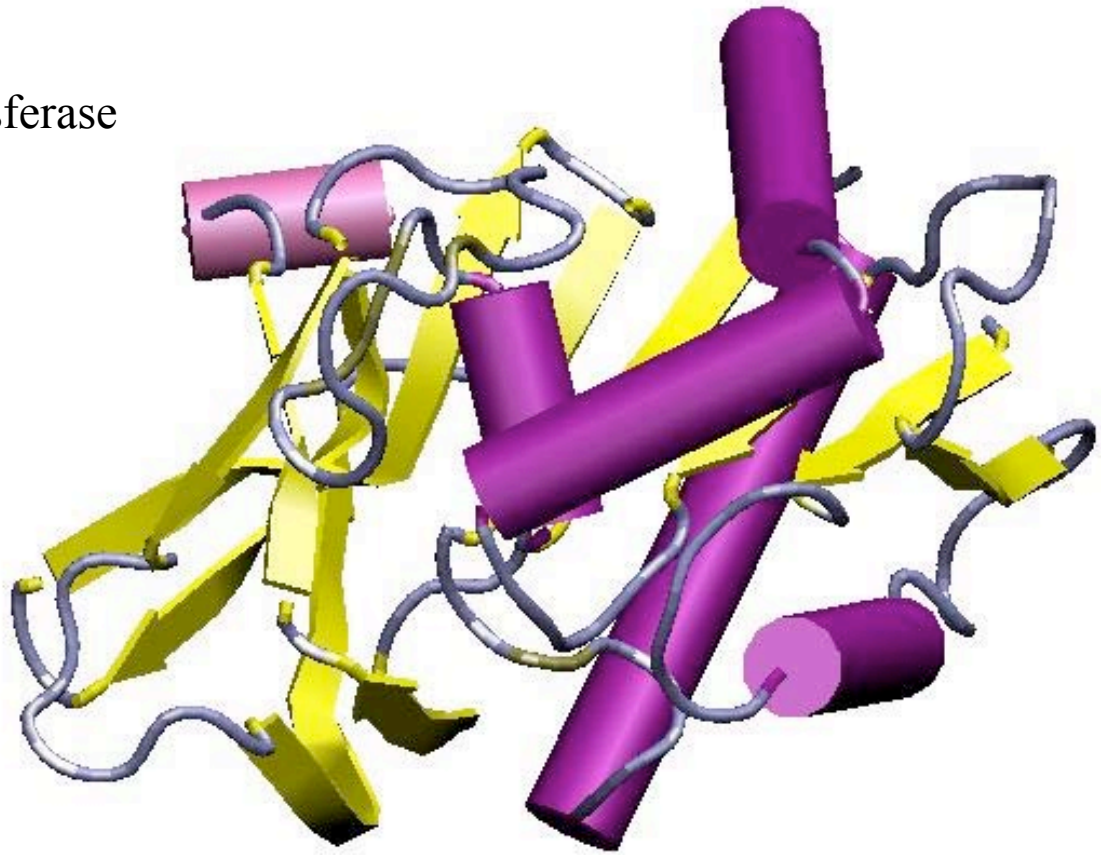


Rommie Amaro

June 2004 - UWA

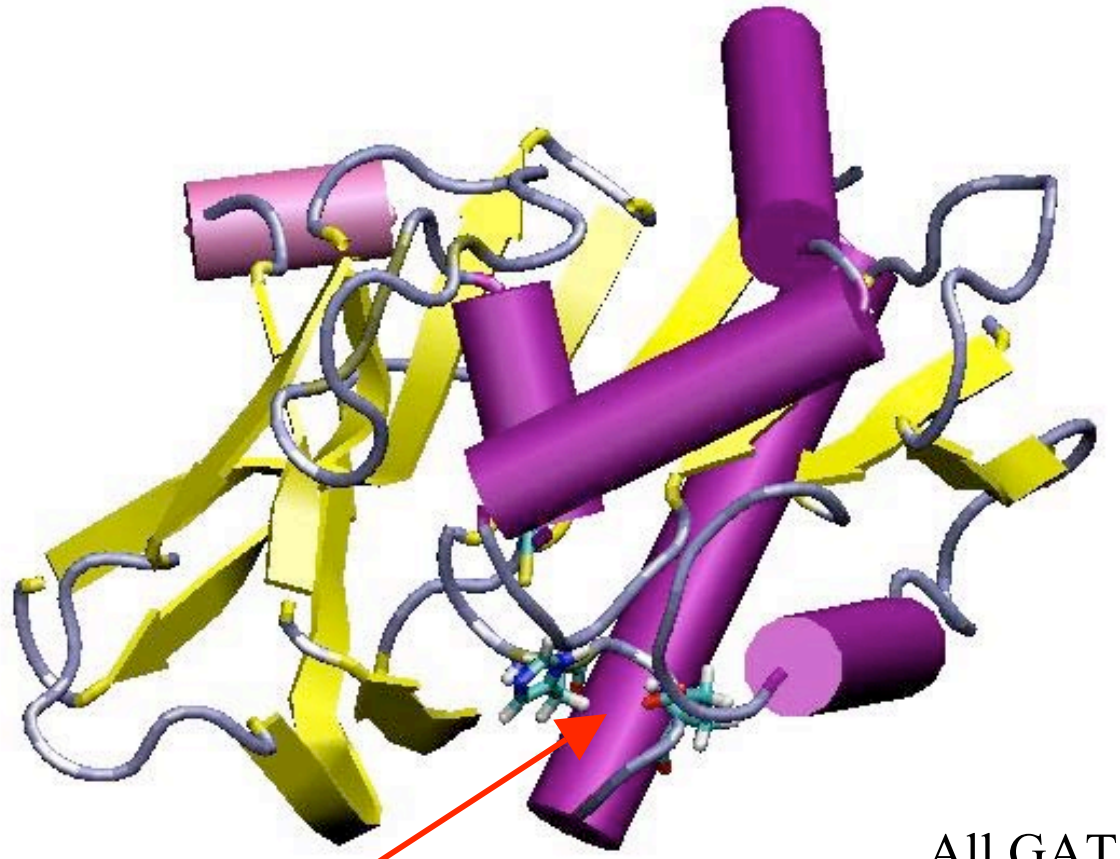
HisH

Glutamine
amidotransferase



P. O'Donoughue, R. Amaro, Z. Schulten, **J Struct Biol**, *134*, 257 (2001)

HisH

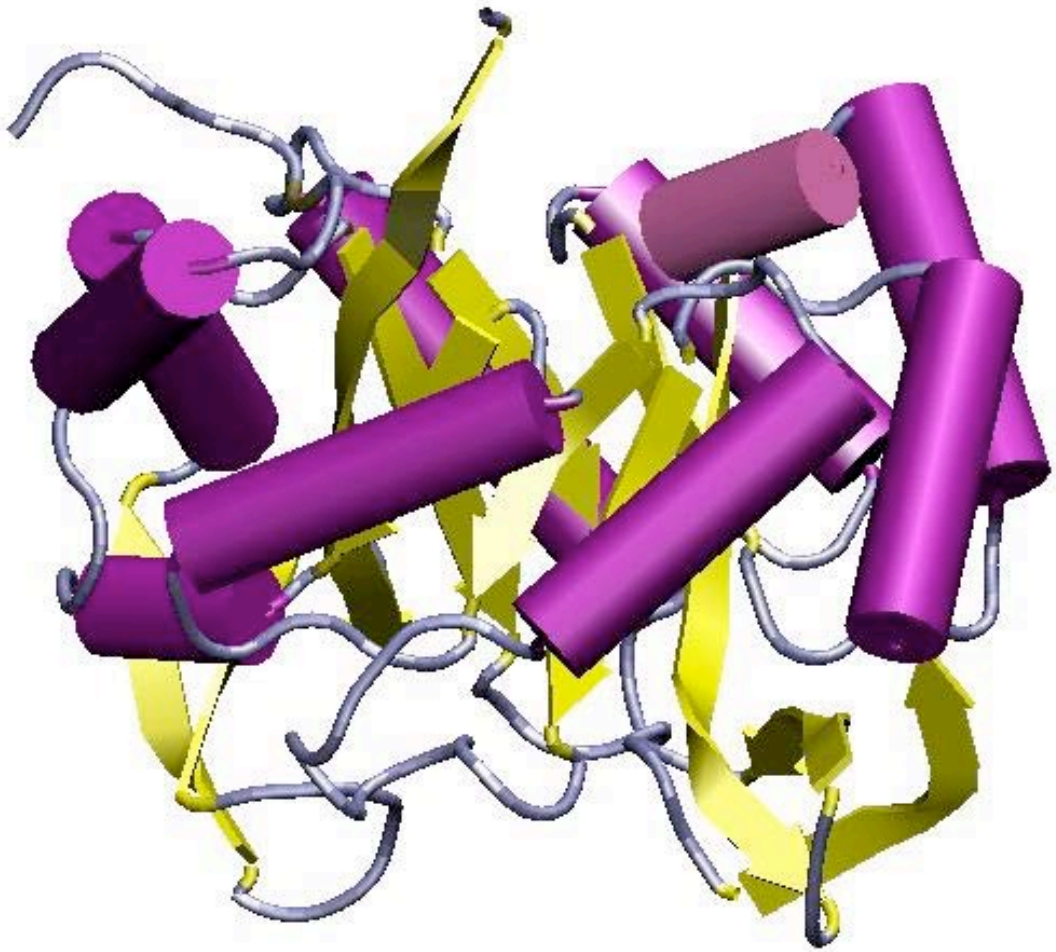


Catalytic triad active site

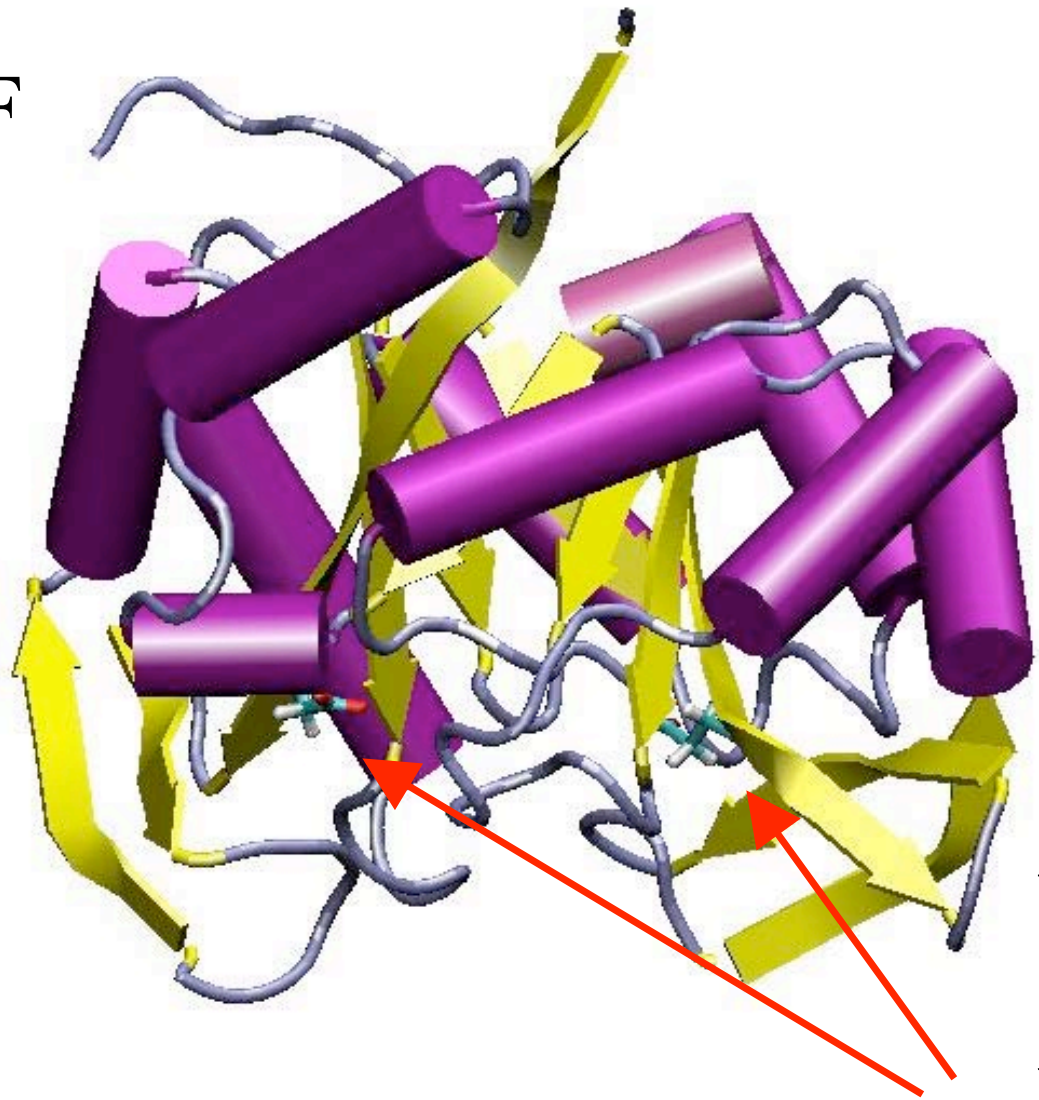
CYS84 – HIS178 – GLU180

All GATases coupled to a second reaction requiring reactive NH_3

HisF

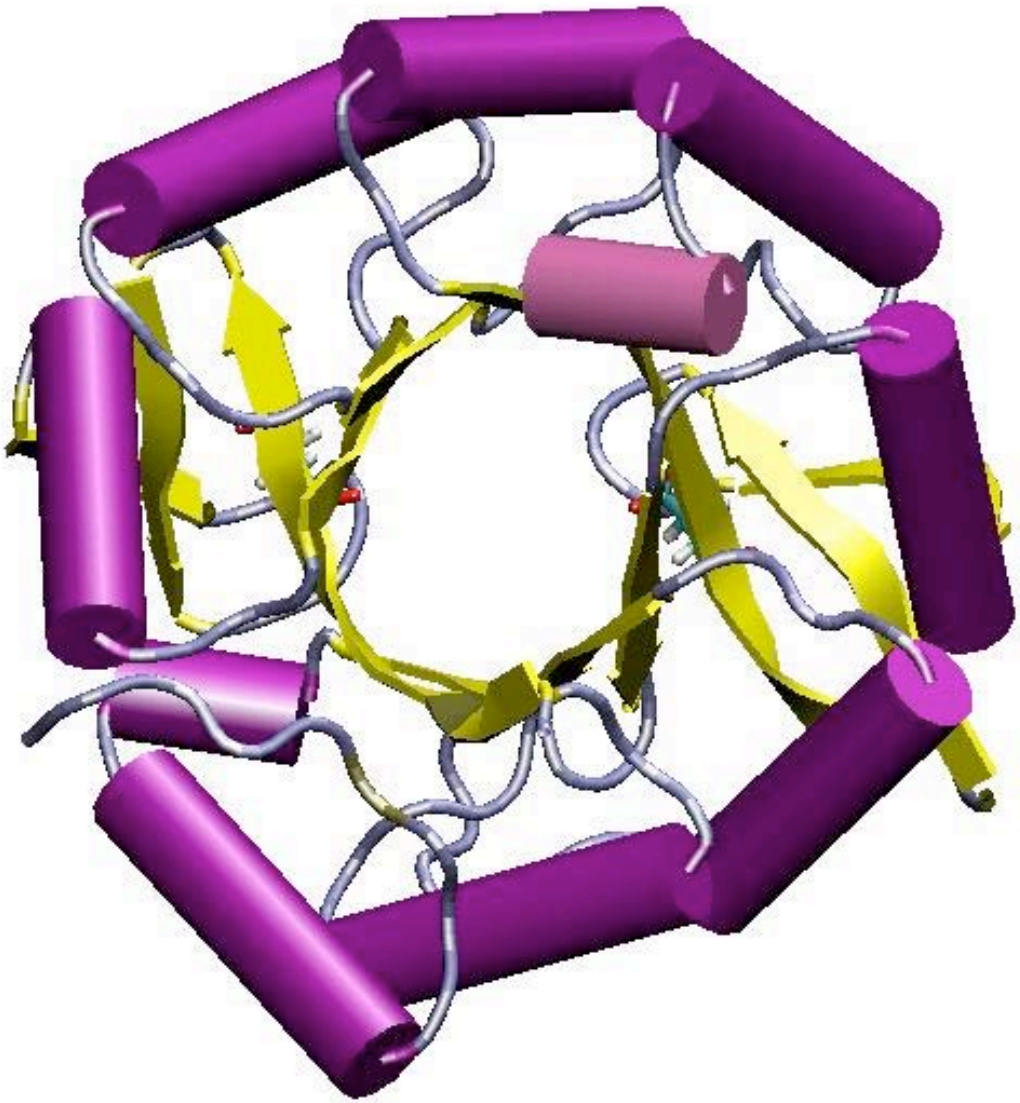


HisF



Active site residues

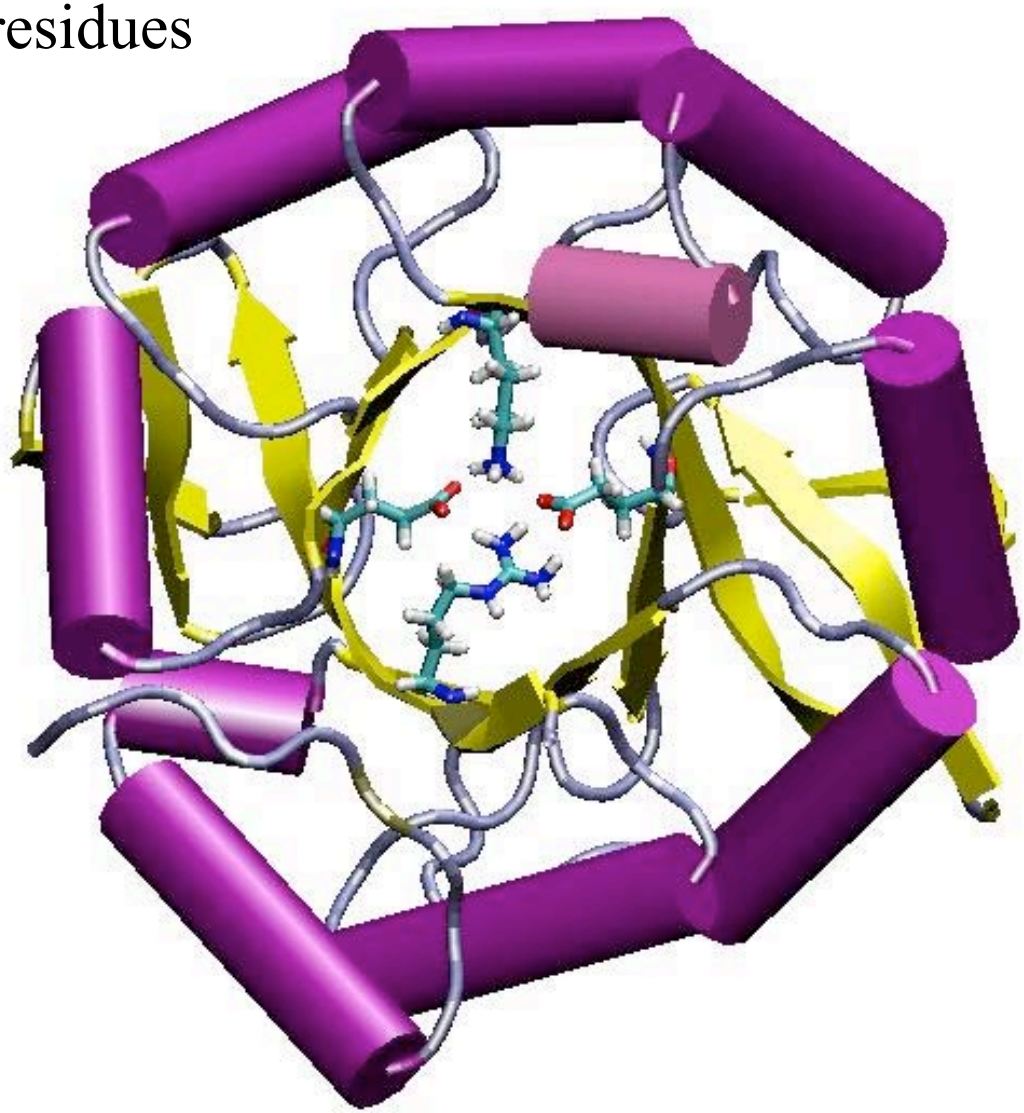
Top View
of HisF



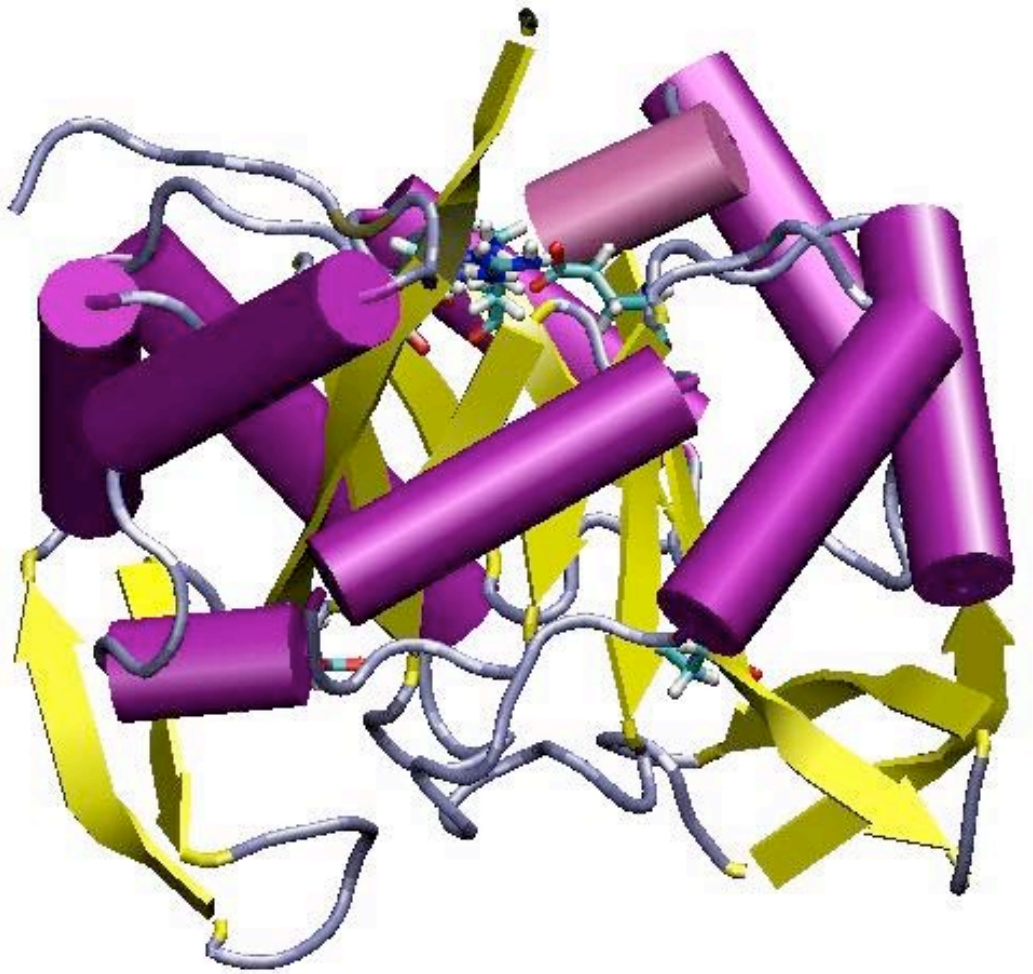
Conserved gate residues

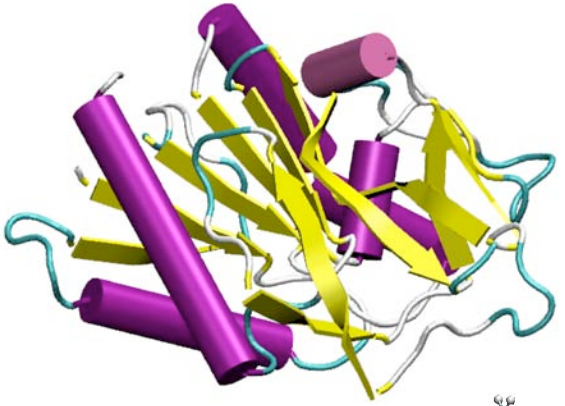
Form stable salt bridges

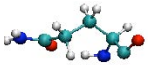
Gate diameter ~ 3 Å



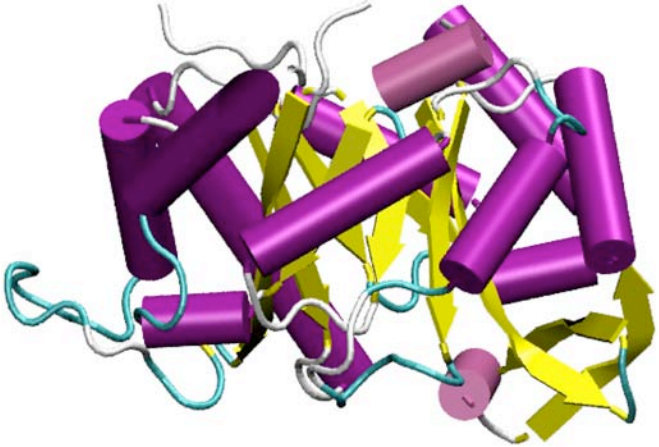
Predominantly hydrophobic channel

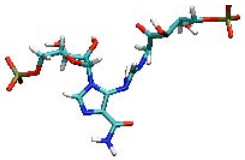




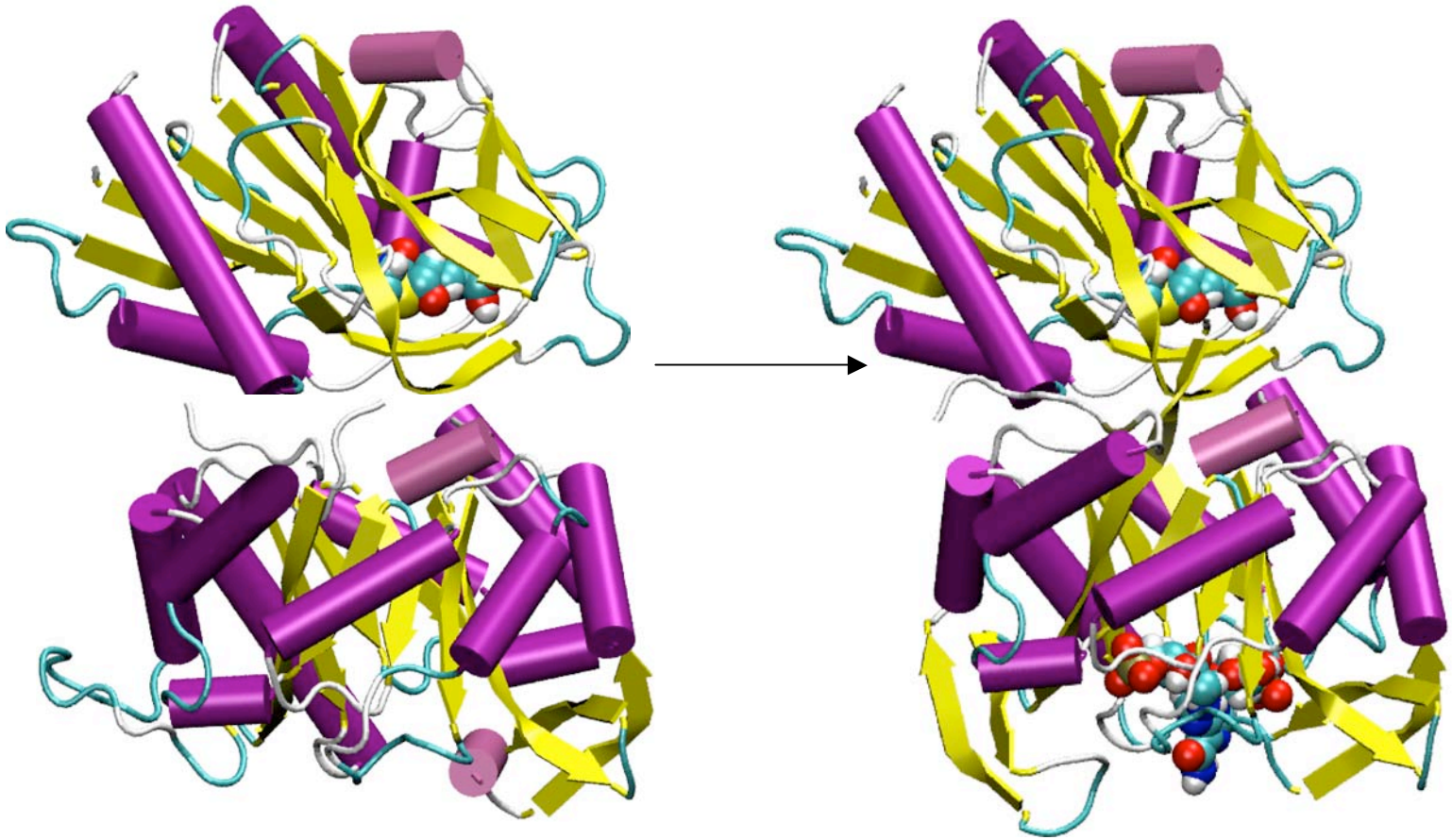


Glutamine
(mM)





PRFAR
(sub-μM)



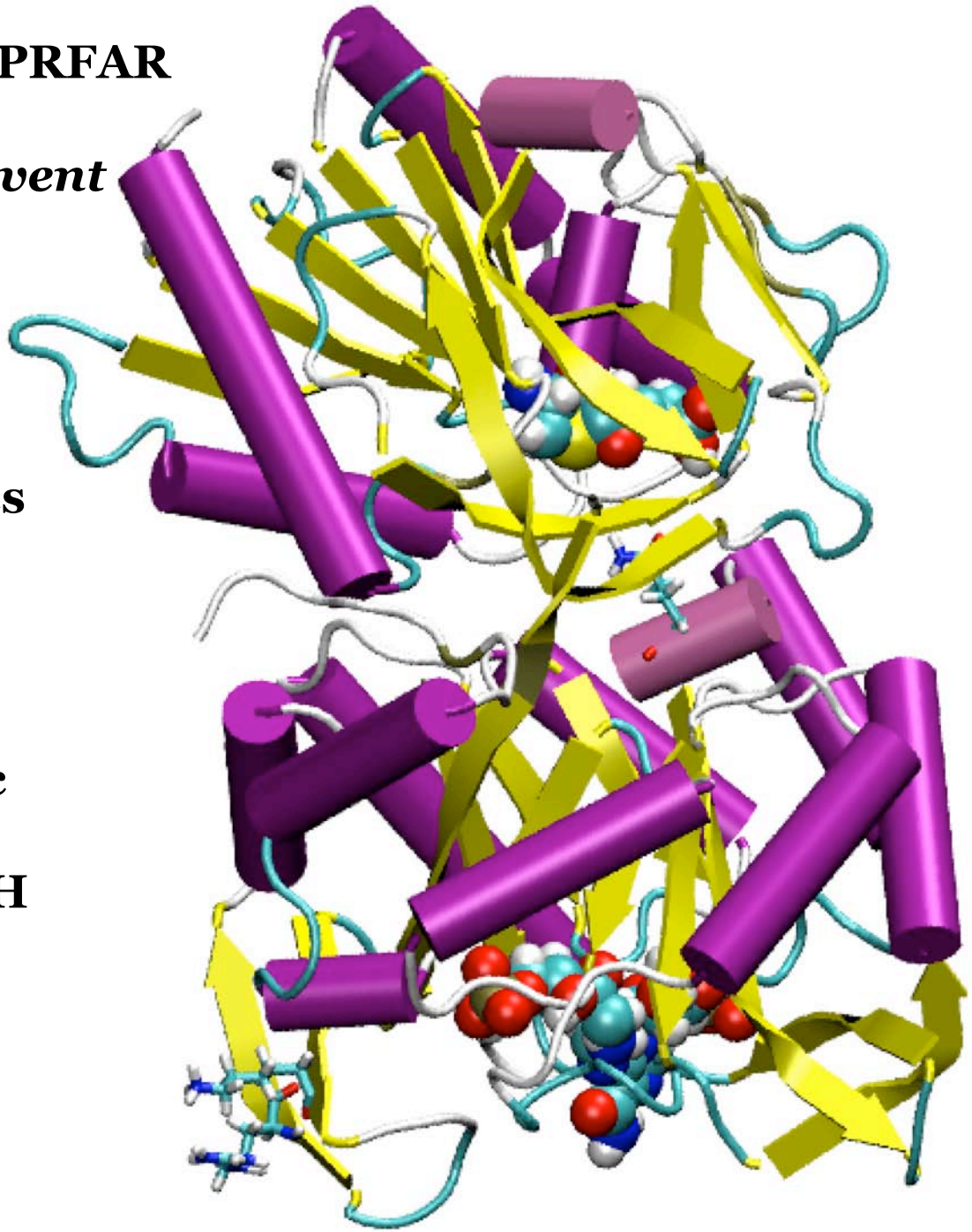
Binding of PRFAR causes an *activated event*

↓

Loop closes

↓

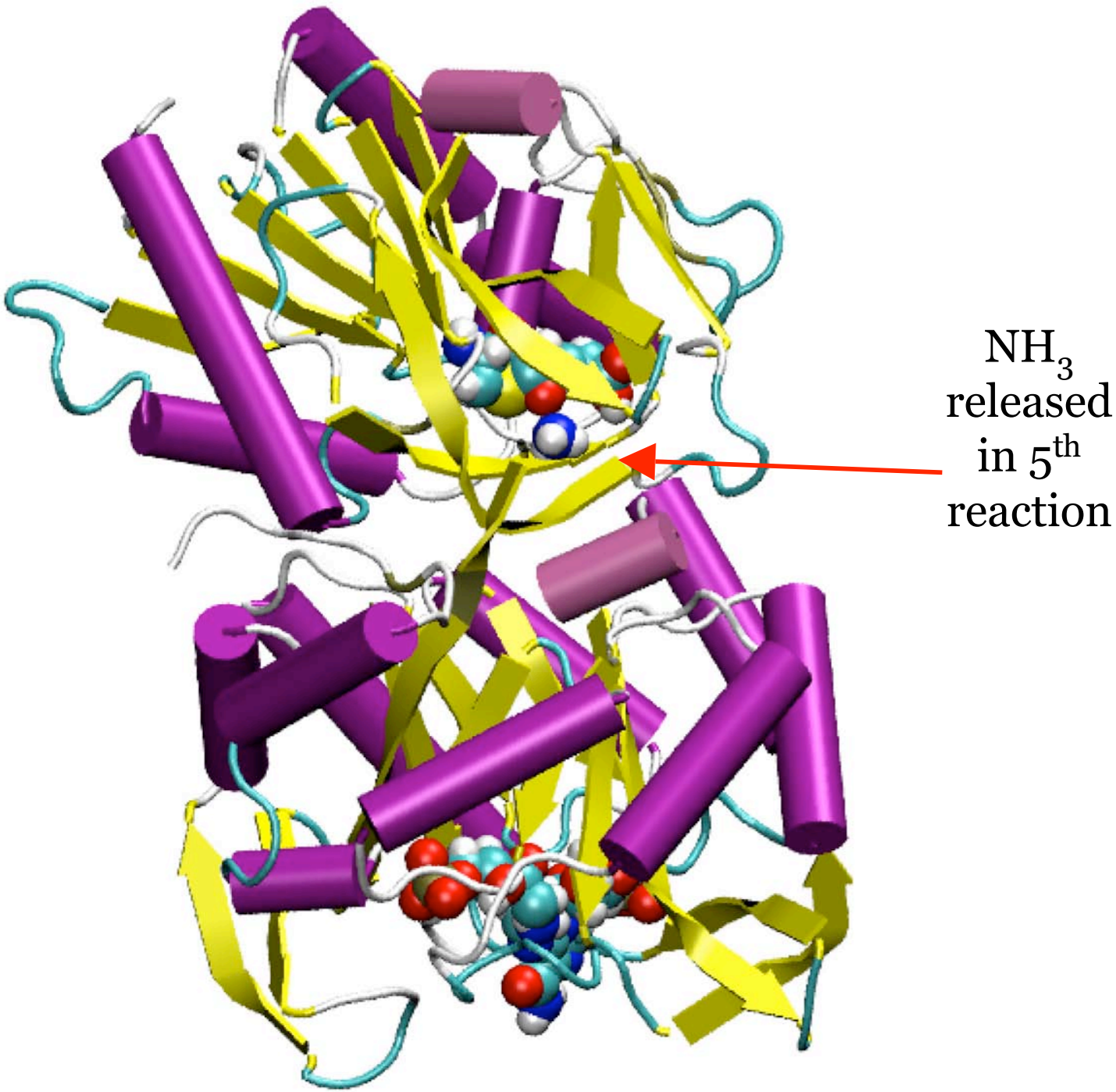
Allosteric signal drives hisH reaction

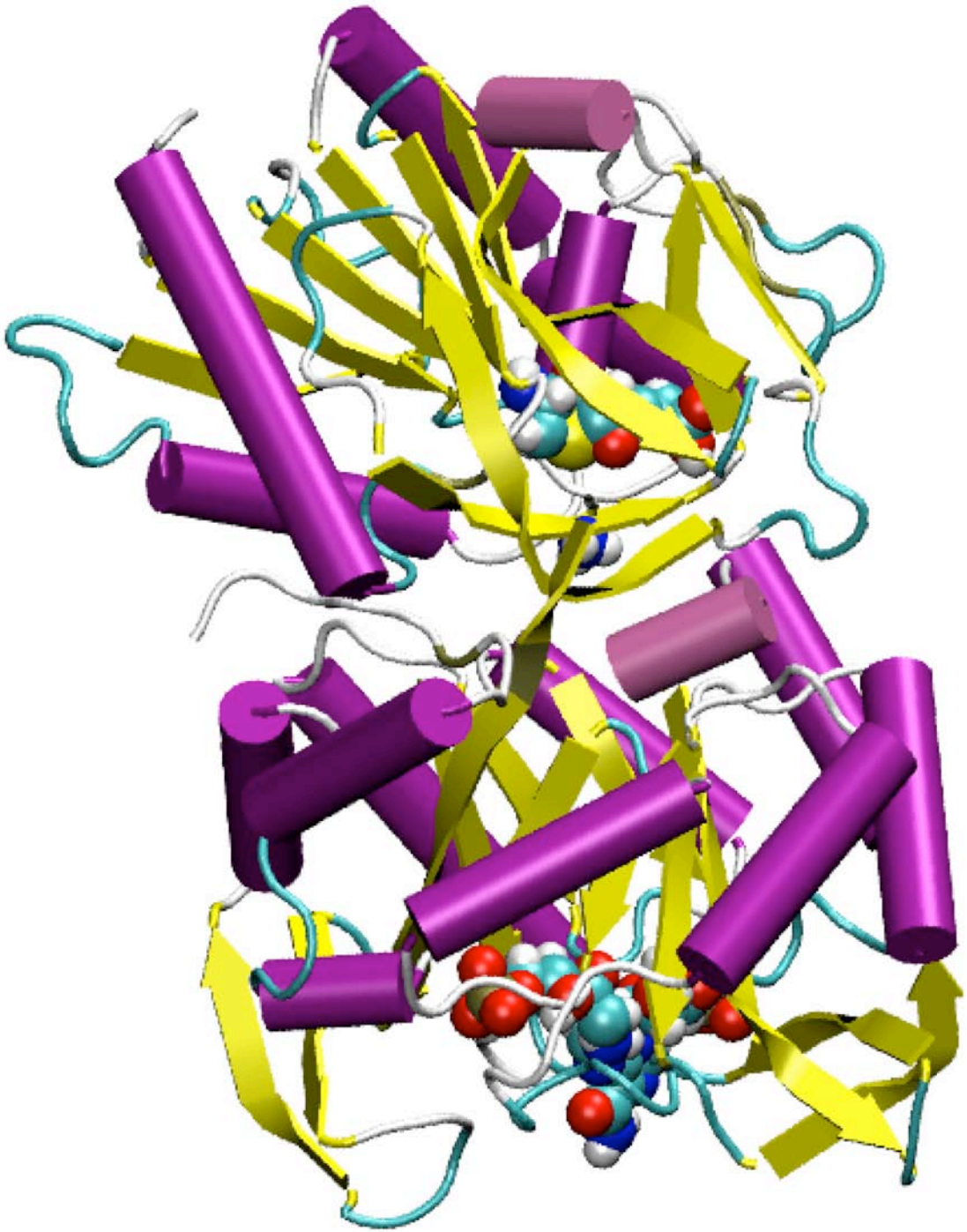


Lys-19

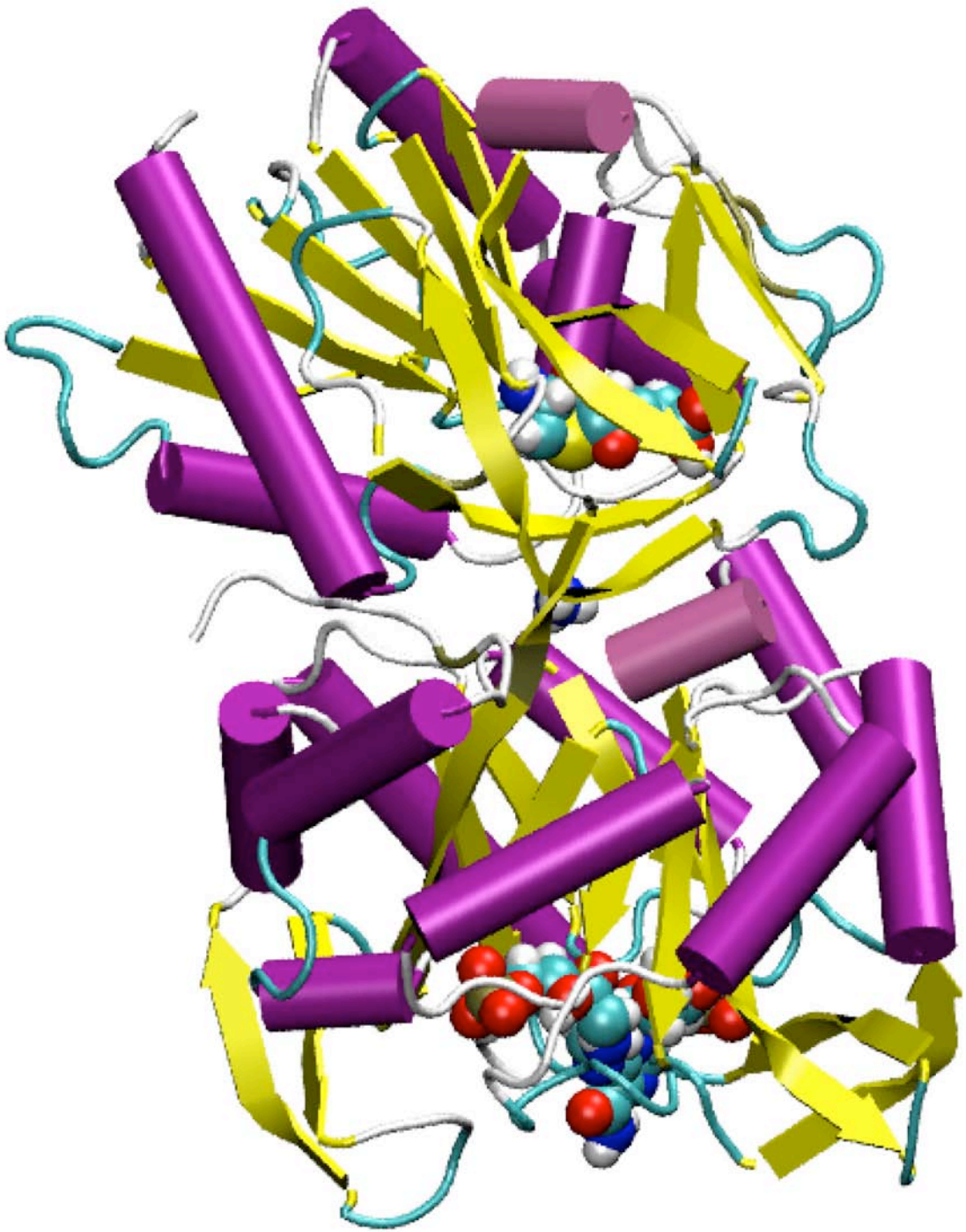
Arg-27

Gln-123

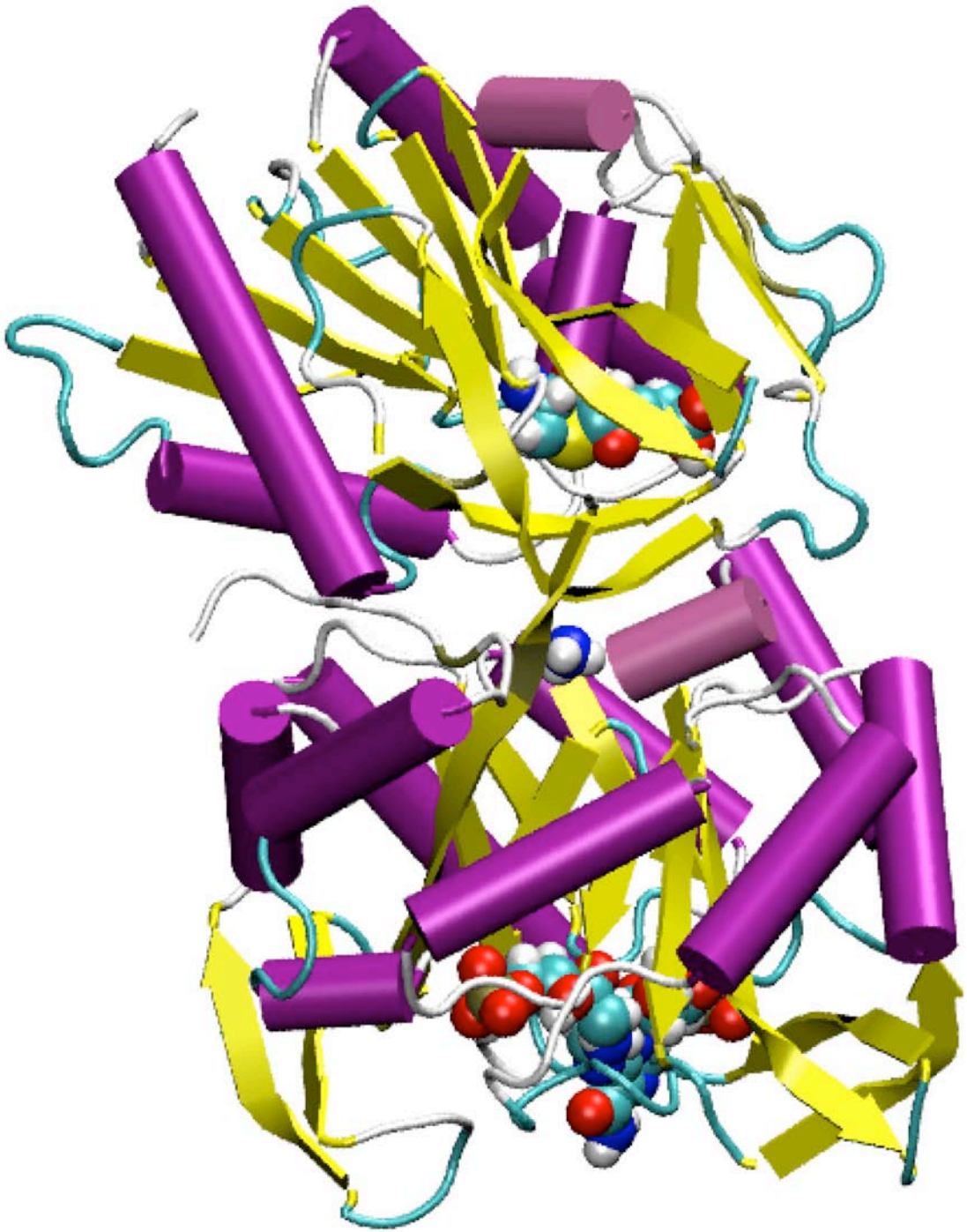

NH_3 released in 5th reaction



NH_3 diffuses across interface ~10Å to mouth of hisF

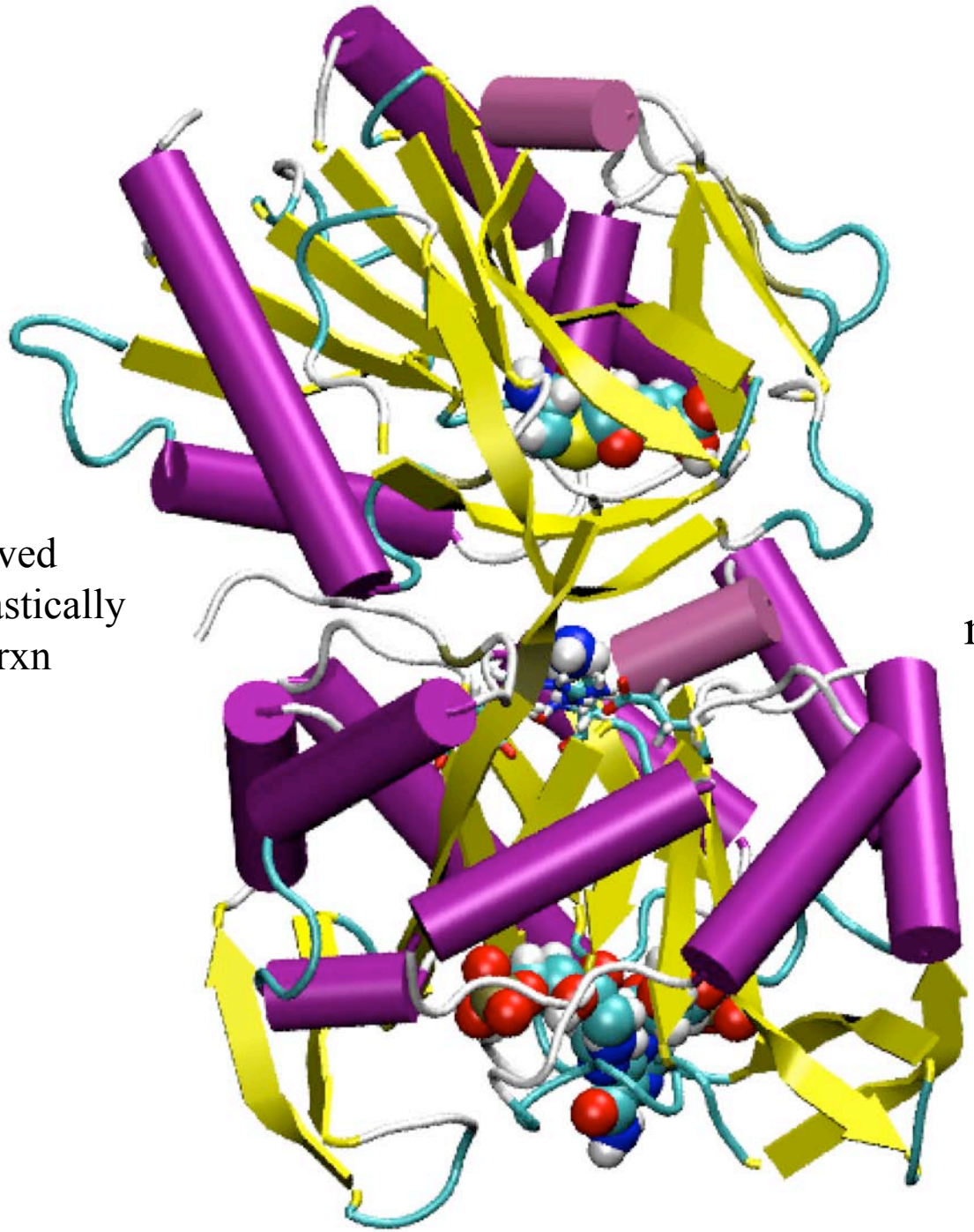


NH_3 diffuses across interface ~10Å to mouth of hisF



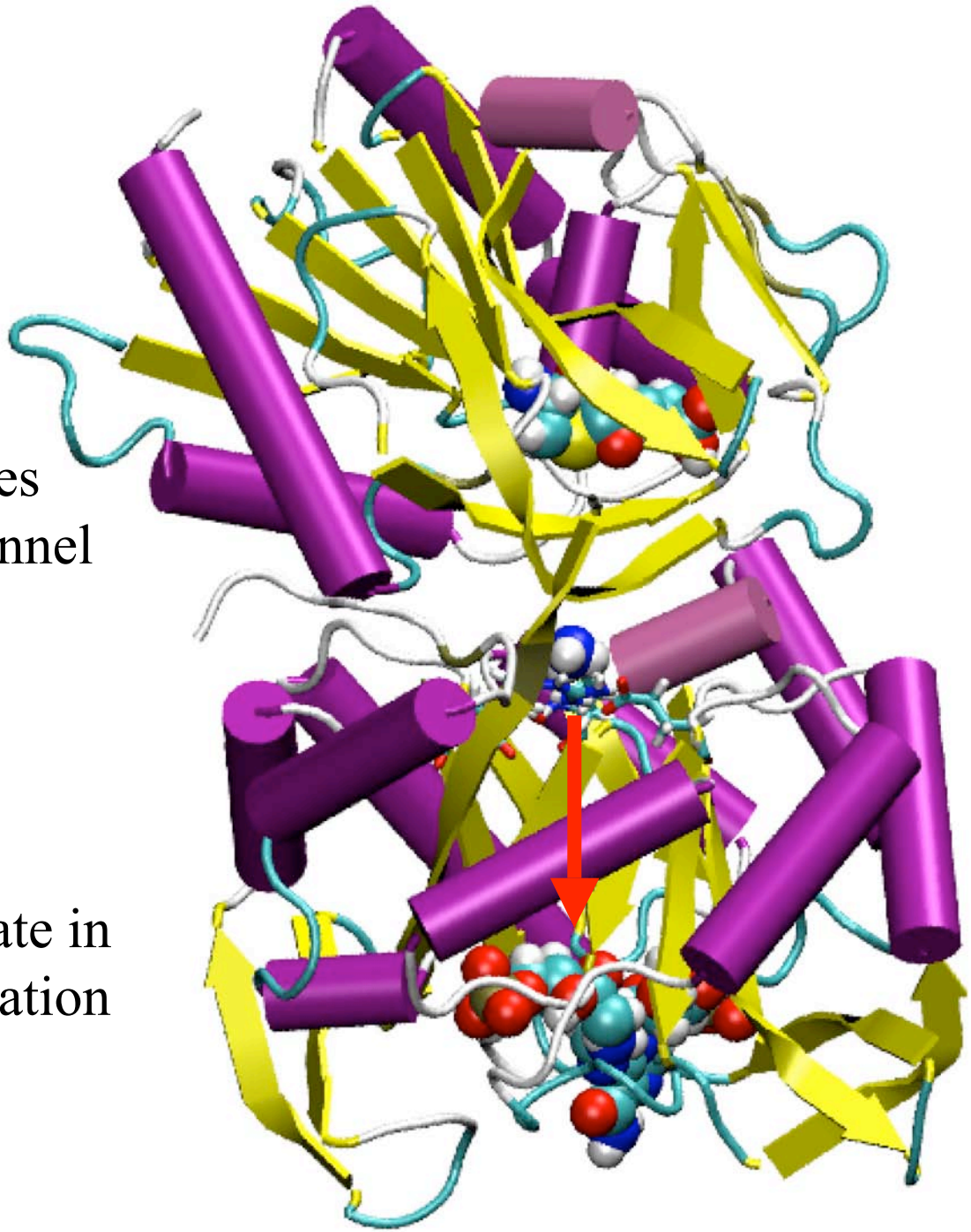
NH_3 diffuses across interface ~10Å to mouth of hisF

Mutating conserved gate residues drastically reduces cyclase rxn efficiency!



… where it meets the *gate*

NH_3 passes through channel ~15Å



To participate in ImGP formation

Novel function for ubiquitous fold!

Talk Outline

•Many interesting aspects: gating mechanism, NH_3 conduction, allosteric effects, chemistry of catalytic reactions

•Main tools are molecular dynamics simulations and bioinformatic analyses

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

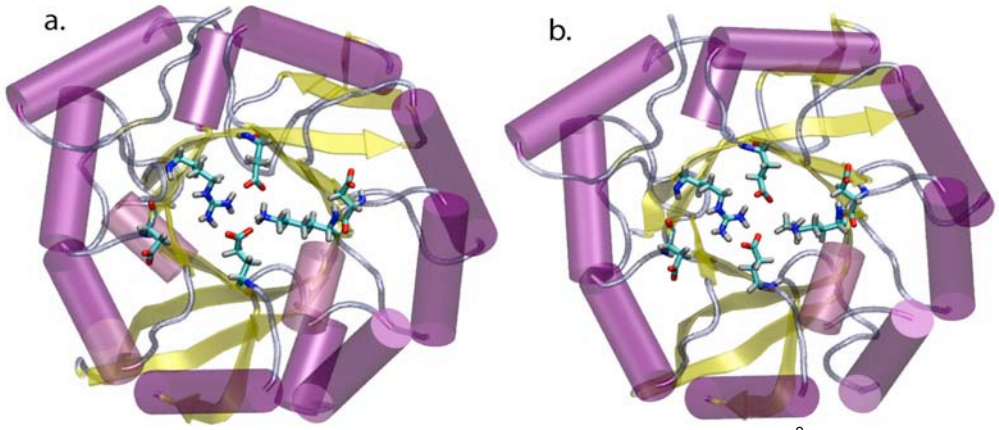
- Building active system requires parameterization of substrates

- New results regarding *active*-system

Investigating the Gate Mechanism

- Gate seems closed in crystal structures
- Diameter of NH_3 is ~ 2Å
- Used bioinformatics to narrow the search
- Lys-99 already in 2 different conformations
- Followed experimentalist's suggestion to form H-bond with TYR138*

Closed *Partially open*




```
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THEMA   -------MLAKRIIACLDVKD-----GRVVKGTN--------FENLRDSGDPVELGKFYSEIGIDELVFLDITAS---VEKRKTMLE  64
METTH   -------MLAKRIIPCLDVRD-----GQVVKGVQ--------FRNHEIIGDIVPLAQRYAEEGADELVFYDITAS---SDGRTIDKS
BUCAP   -------MLKTRIIPCLDVAD-----GRVVKGVN--------FVNLIDAGDPVEAAKAYDAAGADELCFLDIHAT---HENRGTMFD
                      *                                                       *
YEAST   VLKQAAKTVFVPLTVGGGIKDIVDVDGTKIPALEVASLYFRSGADKVSIGTDAVYAAEKYYELGNRGDGTSPIETISKAYGAQAVVI
THEMA   LVEKVAEQIDIPFTVGGGIHDFET-----------ASELILRGADKVSINTAAVENPSLITQIAQT-------------FGSQAVVV 127
METTH   WVERVAQVIDIPFCVAGGIKSVED-----------AEKLFAFGADKISINSPALADPDLINRLADR-------------FGVQAIVV
BUCAP   LVTRTAEQCFMPLTVGGGVRTQED-----------VRALLLAGADKVSFNSAAVANPD-VVRSRRS-------------LRSQCIVV
                                                  **
YEAST   SVDPKRVYVNSQADTKNKVFETEYPGPNGEKYCWYQCTIKGG----RESRDLGVWELTRACEALGAGEILLNCIDKDGSNSGYDLEL
THEMA   AIDAKRVD--------------------G----EFMVFTYSG----KKNTGILLRDWVVEVEKRGAGEILLTSIDRDGTKSGYDTEM 186
METTH   GIDSWFEKE-----------------TG----KYWVNQYTGDESRTRQTHWQLLDWVKEVQQRGAGEIVLNMMNQDGVRQGYDIAQ
BUCAP   AIDAKTVE-----------------PG----RWEIFTHGG----RKSTGIDAVEFARHVEAKGAGEILLTSMDRDGTKAGFNLPL
                                                                          *
YEAST   IEHVKDAVK-IPVIASSGAGVPEHFEEAFLKTRADACLGAGMFHRGEFTVNDVKEYLLEHGLKVRMDEE
THEMA   IRFVRPLTT-LPIIASGGAGKMEHFLEAFLAG-ADAALAASVFHFREIDVRELKEYLKKHGVNVRLEGL
METTH   LKLVRNLCH-IPLIASGGAGEMVHFRDAFIEANVDGALAASVFHKRIIDIGELKDYLRKEKIKIR----
BUCAP   TRAIADAVK-IPVIASGGVGTLDHLVEGVTEGHASAVLAASIFHFGTYTIGEAKAHMAPAGIPMRLA—
                                        *
```

*Chaudhuri, Lange, Myers, Chittur, Davisson, and Smith, *Structure*, 2001.

Ammonia Conduction

- Steered Molecular Dynamics (SMD) to induce NH_3 conduction on ns timescale

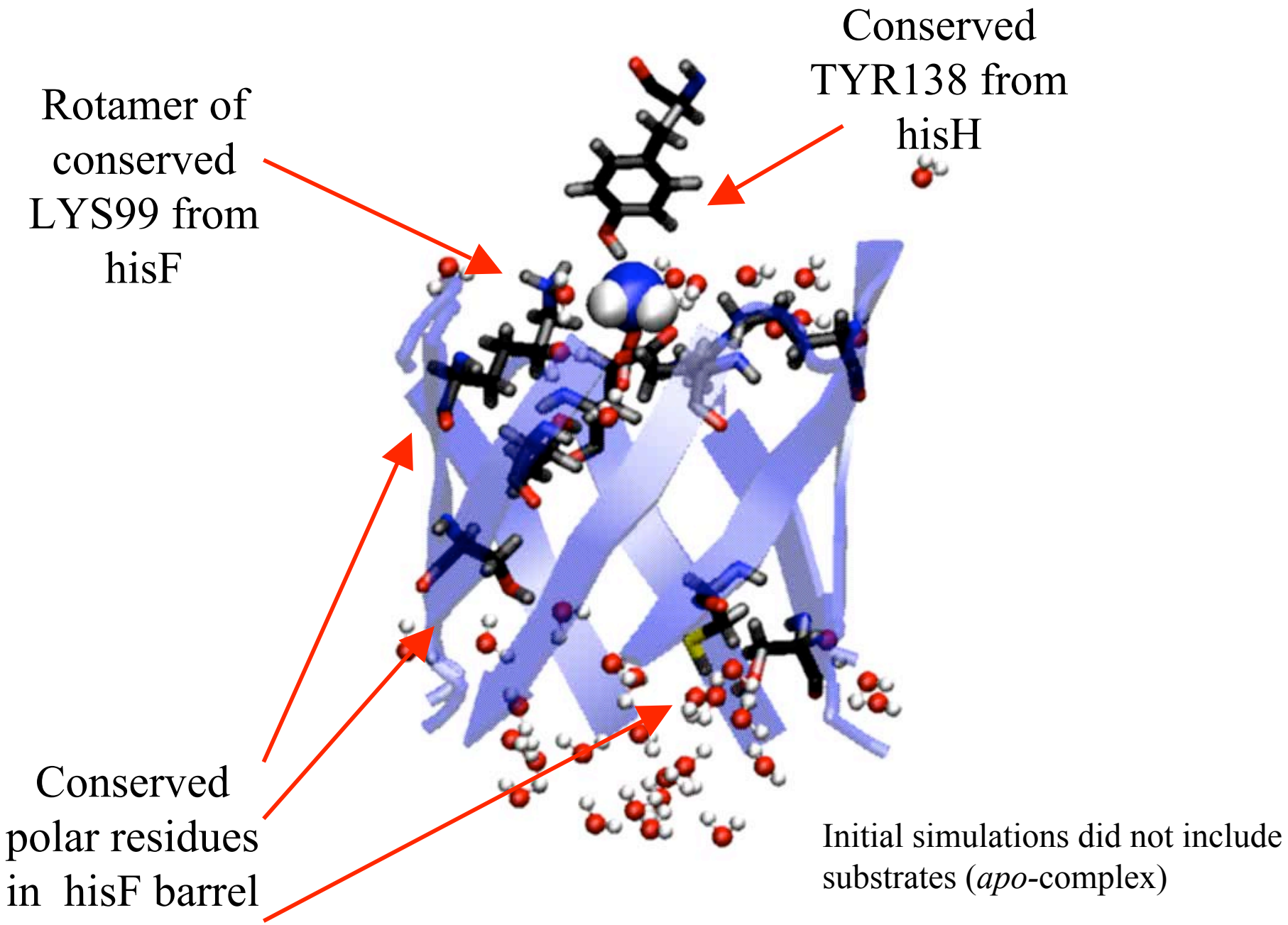
- Apply an external force to the system:

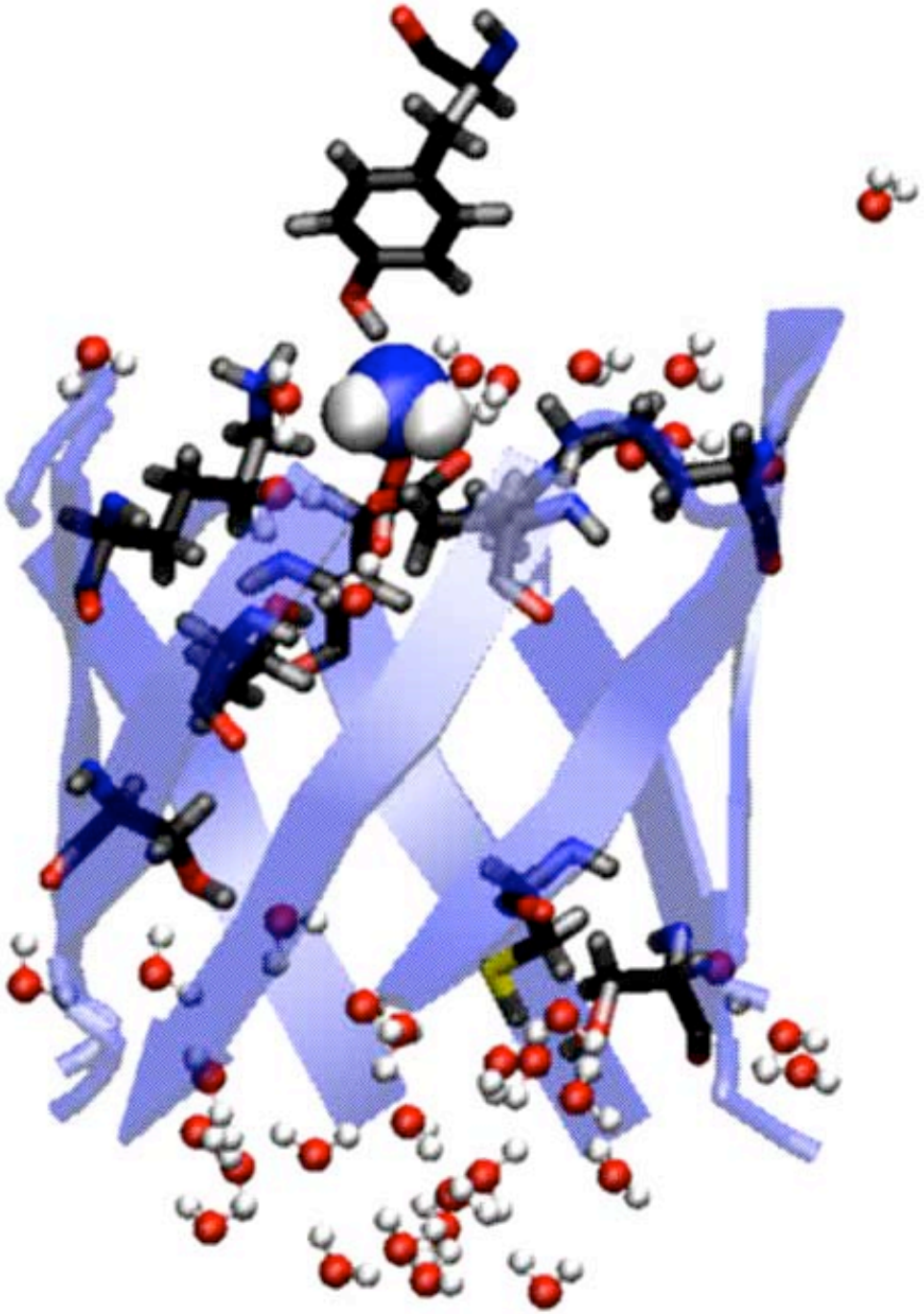
$$H\left[\underline{x}(t), t\right] = H_0\left[\underline{x}(t)\right] + 0.5k\left[z(\underline{x}) - z_0 - vt\right]^2$$

- To quantify the energetics of conduction we use:

Jarzynski's Identity: $$e^{-\beta\Delta F} = \left\langle e^{-\beta\Delta W} \right\rangle_{traj}$$

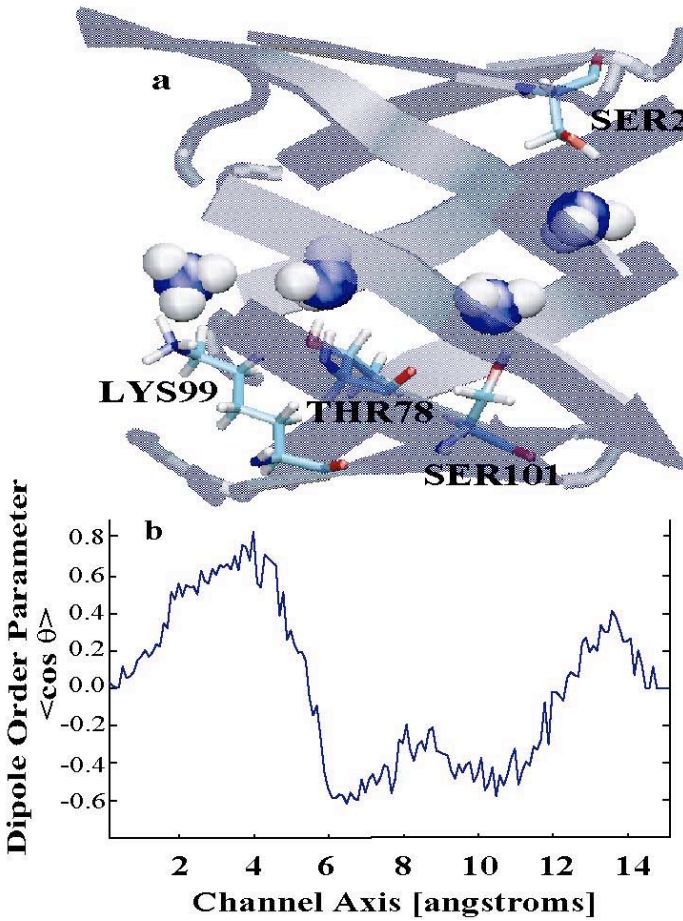
- This new identity allows us to determine *equilibrium* information from repeated *nonequilibrium* measurements


Conserved TYR138 from hisH
Rotamer of conserved LYS99 from hisF
Conserved polar residues in hisF barrel
Initial simulations did not include substrates (*apo*-complex)

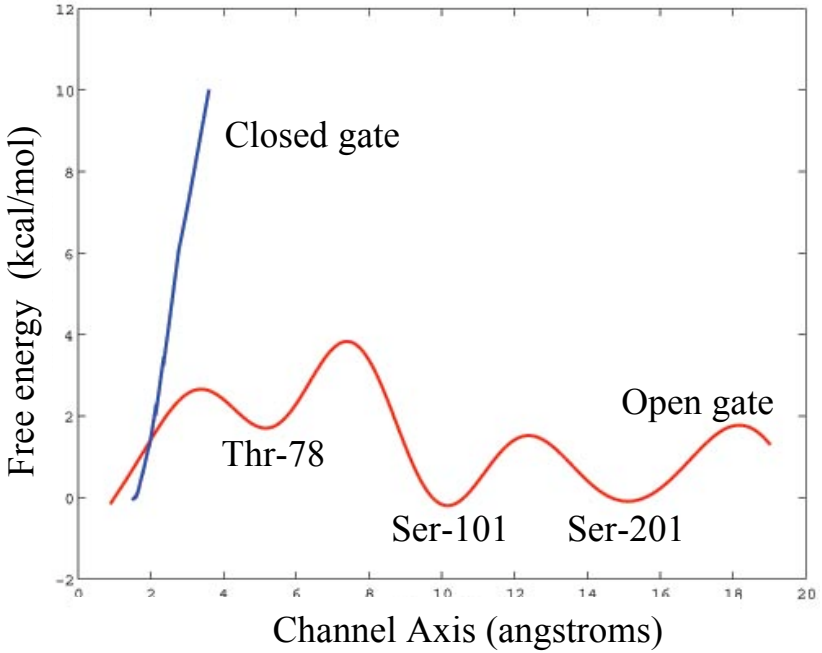


Results through partially open gate in *apo*-complex

Dipole Moment Analysis



Free energy profile of ammonia in barrel



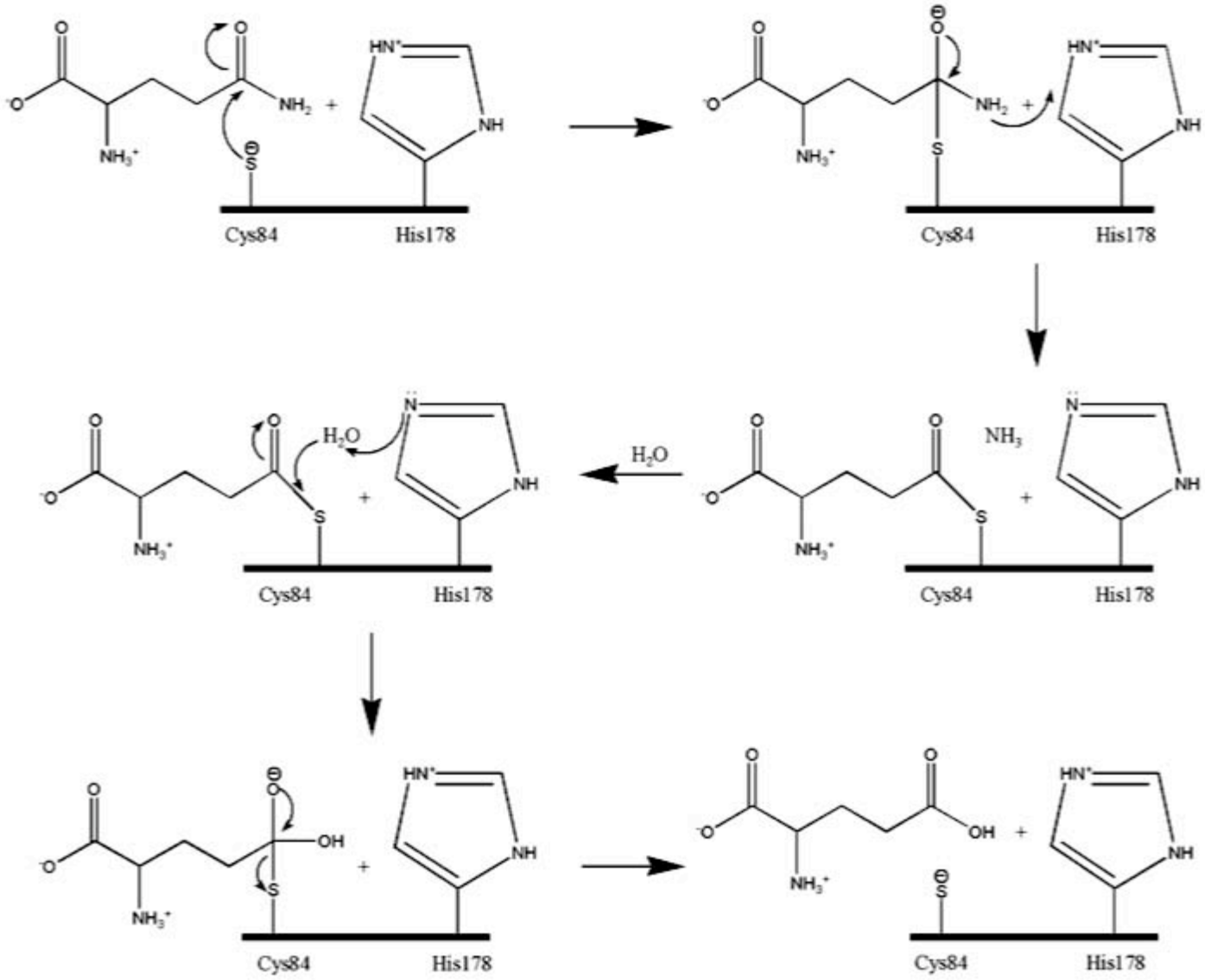
Mean First Passage Time Analysis:

Without substrates, passage of NH_3 ~ 110 ns

*Overall this step is **not** rate limiting!*

R. Amaro, E. Tajkhorshid, Z. Luthey-Schulten, **PNAS**, *100*, 7599 (2003)

Modeling the *active*-complex

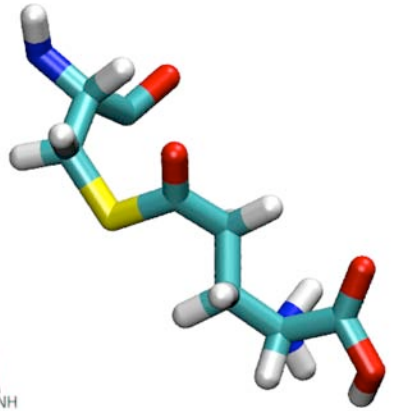


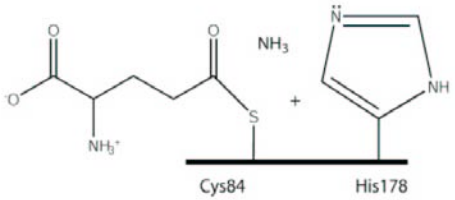
Modeling the *active*-complex: including substrates

HisH:

Glutamyl thioester intermediate corresponding to post-NH_3 release state

Parameterization required for thioester linkage

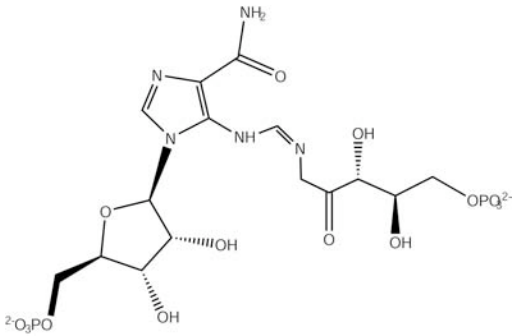


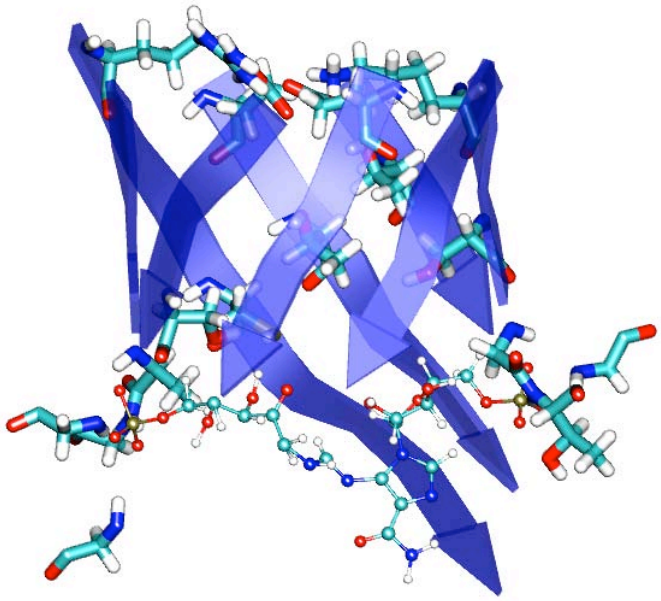


HisF:

N1-(5'-phosphoribulosyl)-formimino-5-aminoimidazole-4-carboxamide ribonucleotide (… or PRFAR) cryo-trapped in hisF active site*

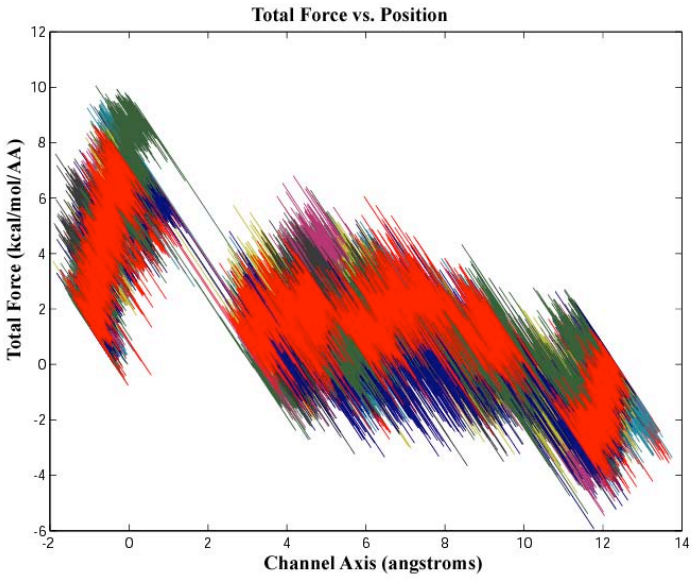
Parameterization according to existing CHARMM protocol



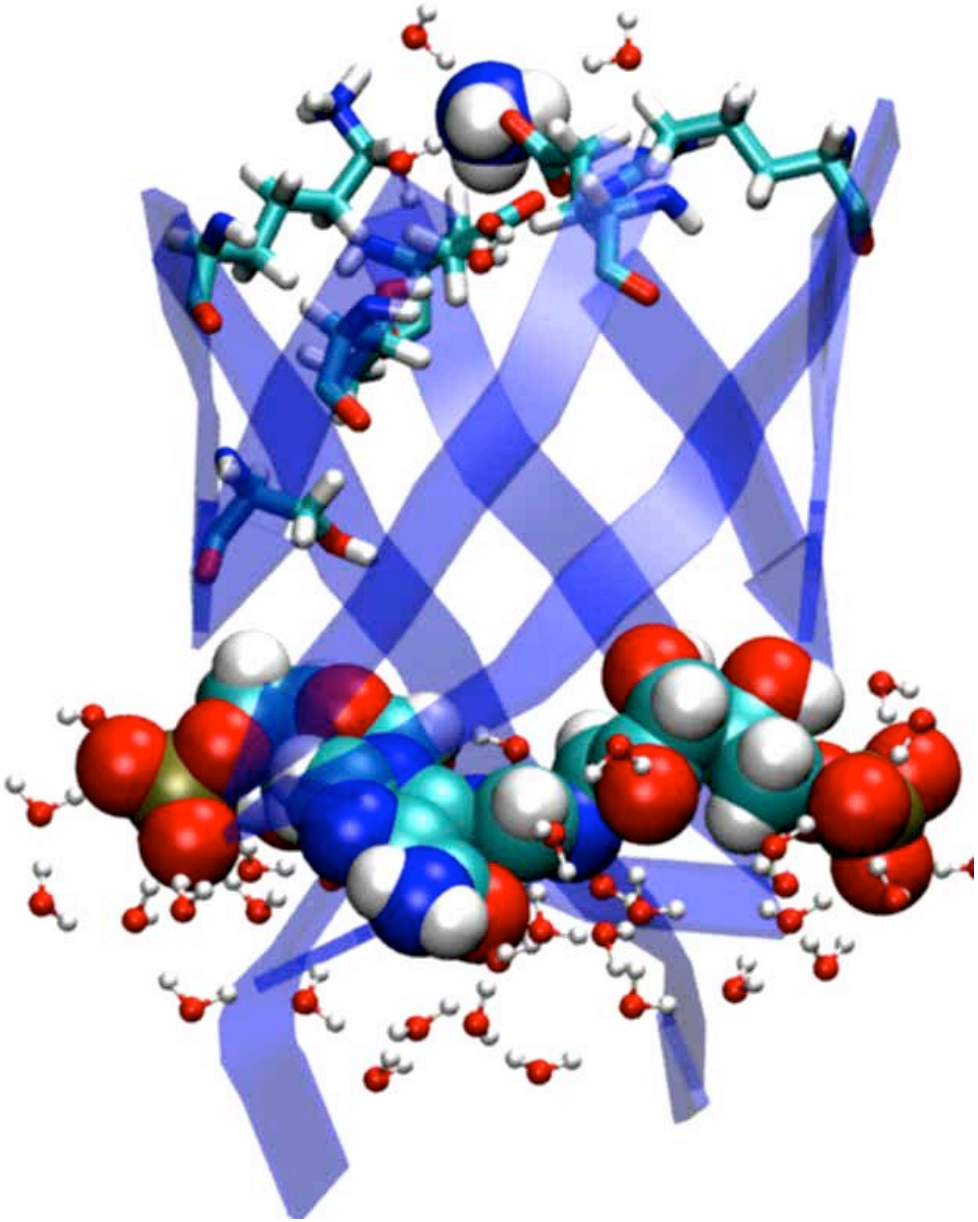


* Chaudhuri, Lange, Myers, Davisson, and Smith, *Biochemistry*, 2003; Myers, Jensen, Deras, Smith, and Davisson, *Biochemistry,* 2003.

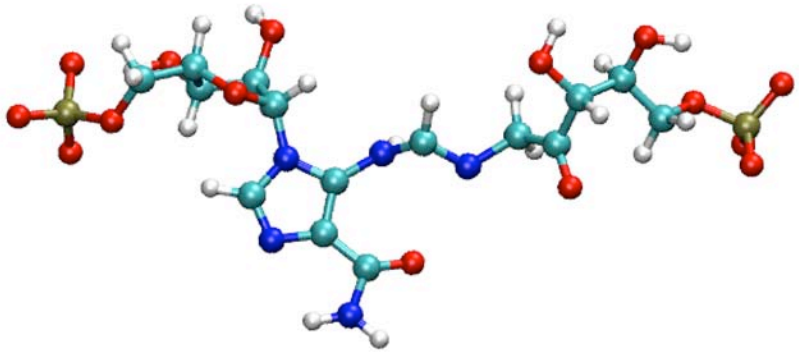
Including substrates produced a surprising result!

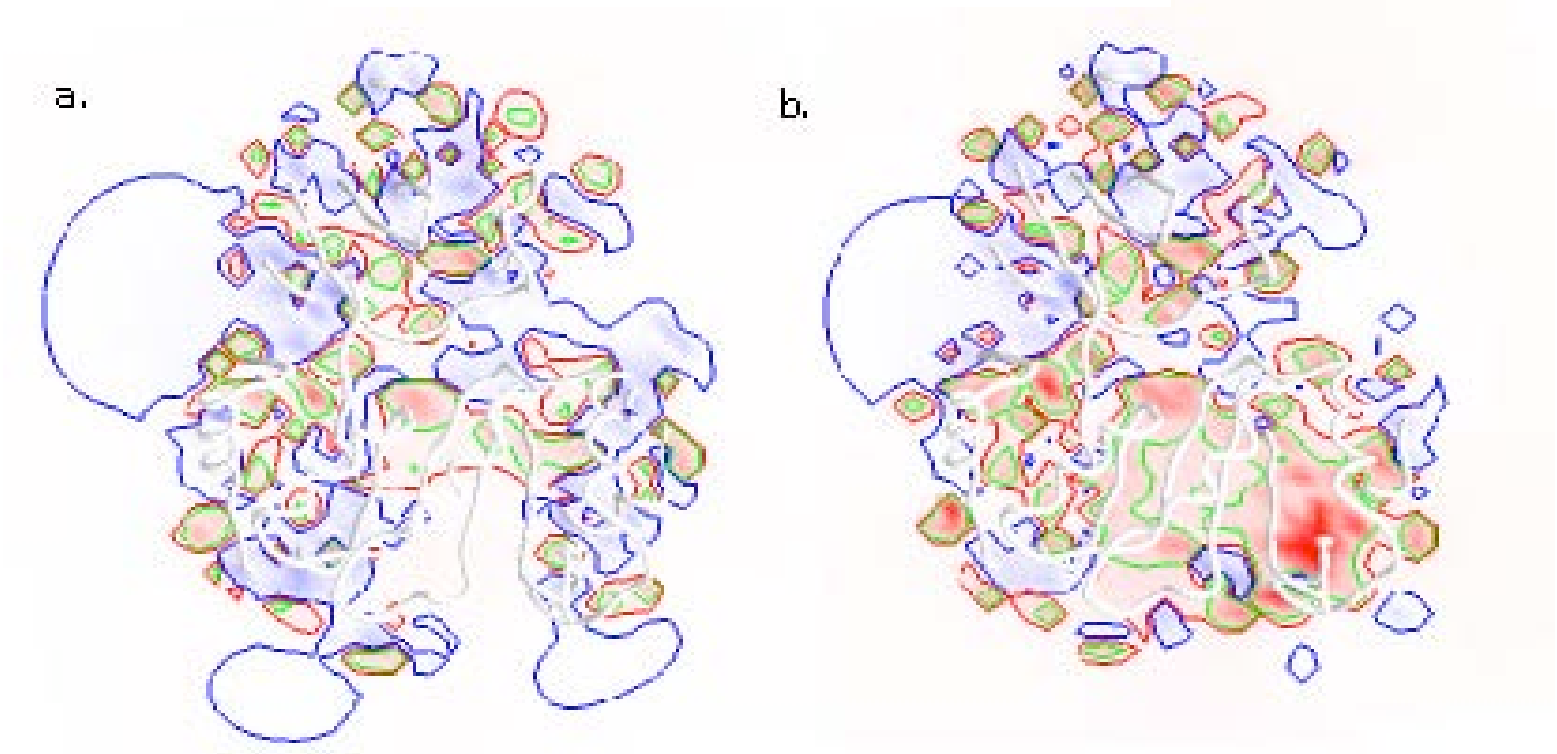


Same gate configuration, higher barriers?!?



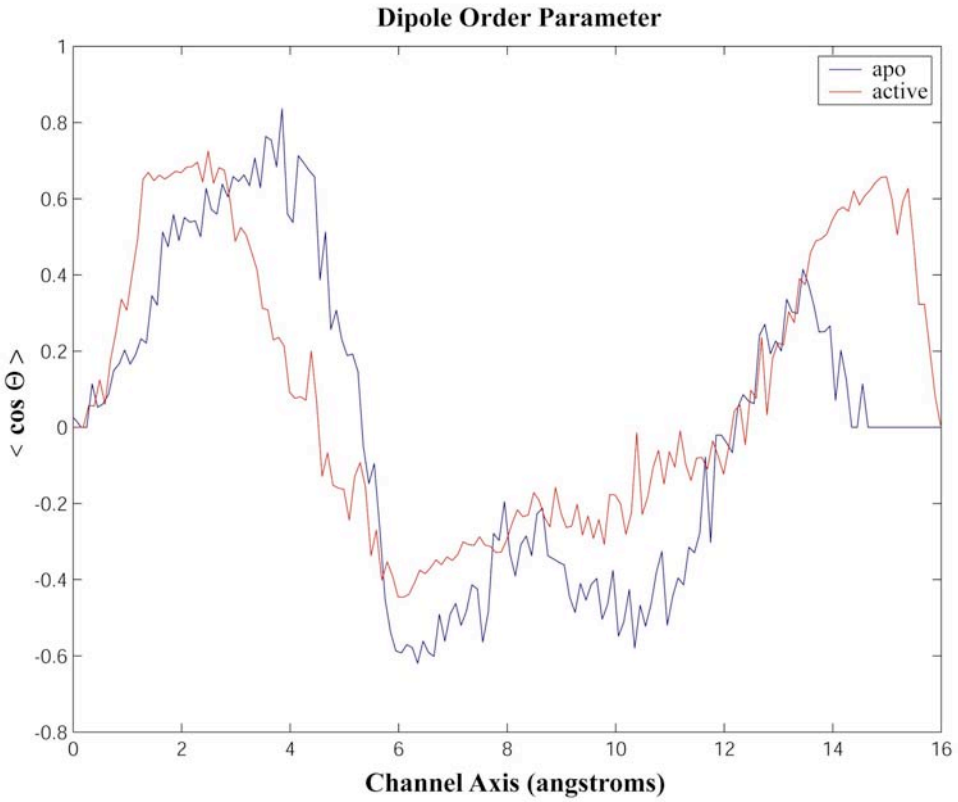
How could PRFAR change the energetics of conduction?

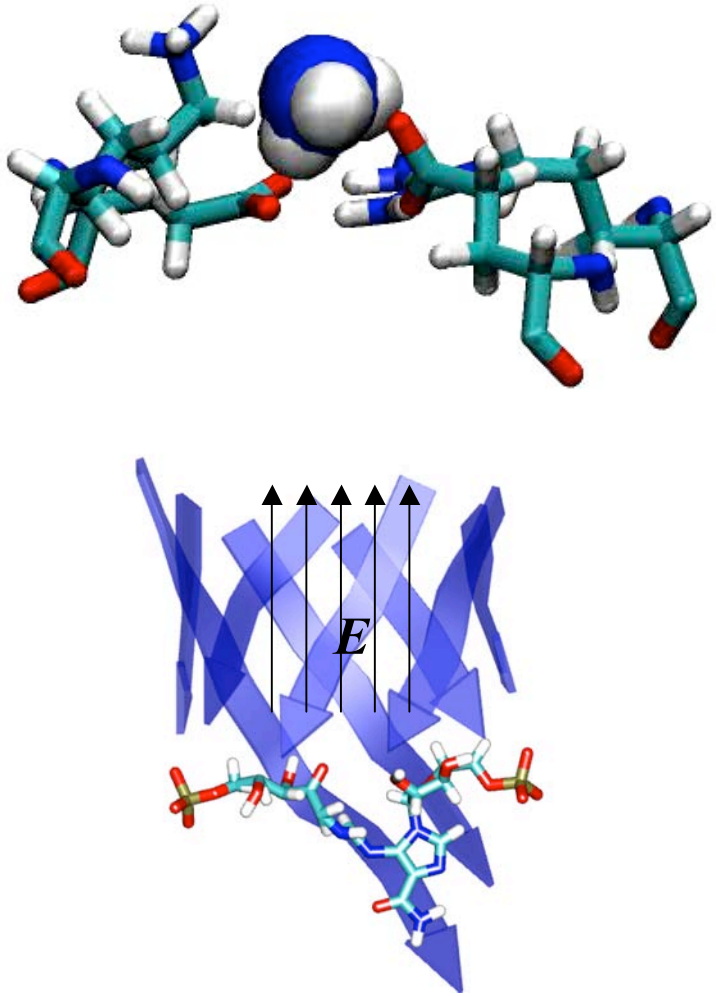




PRFAR introduces large electrostatic effects!

Net effect: a torque on ammonia's dipole

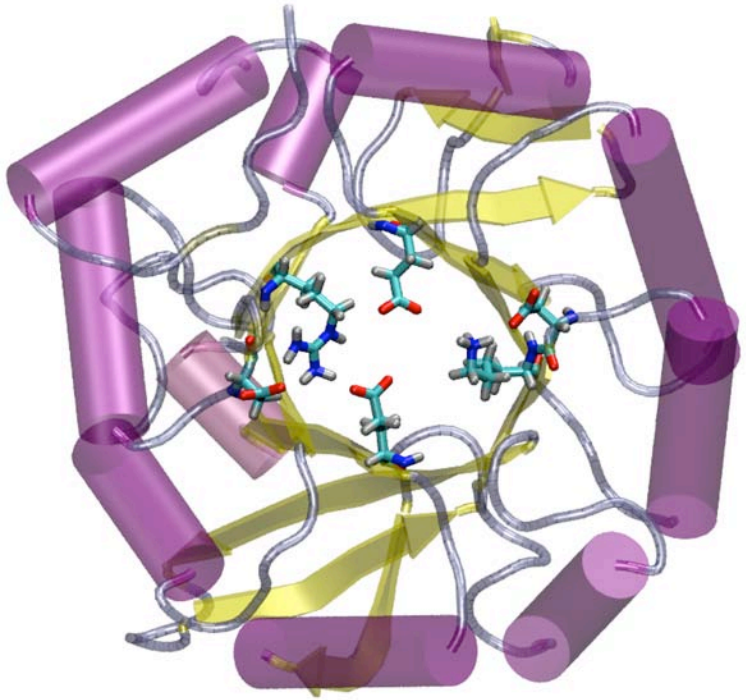


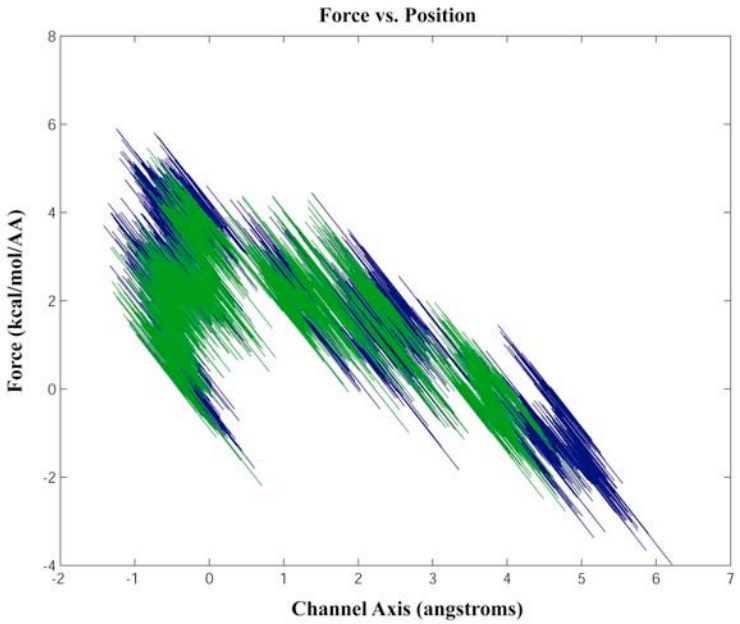


Electrostatic field from PRFAR makes it more difficult for NH_3 to flip orientations

An opportunity to model our open gate hypothesis

- Bioinformatic analysis revealed 2 conserved Asp's near positively charged gate residues
- Salt bridges are stable! Stay in contact for ps
- *Fully open* conformation ~ 7 Å





- Hypothetical fully open gate configuration did initially seem to have lower forces!

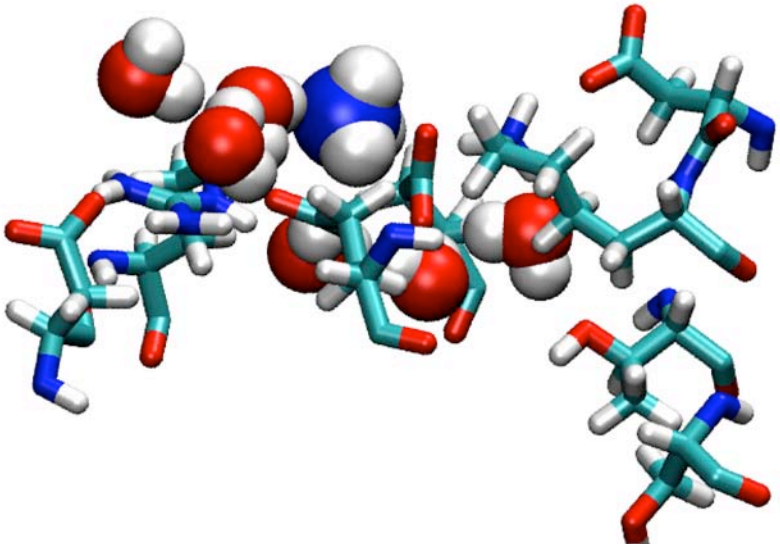
Number of water molecules affects barrier height

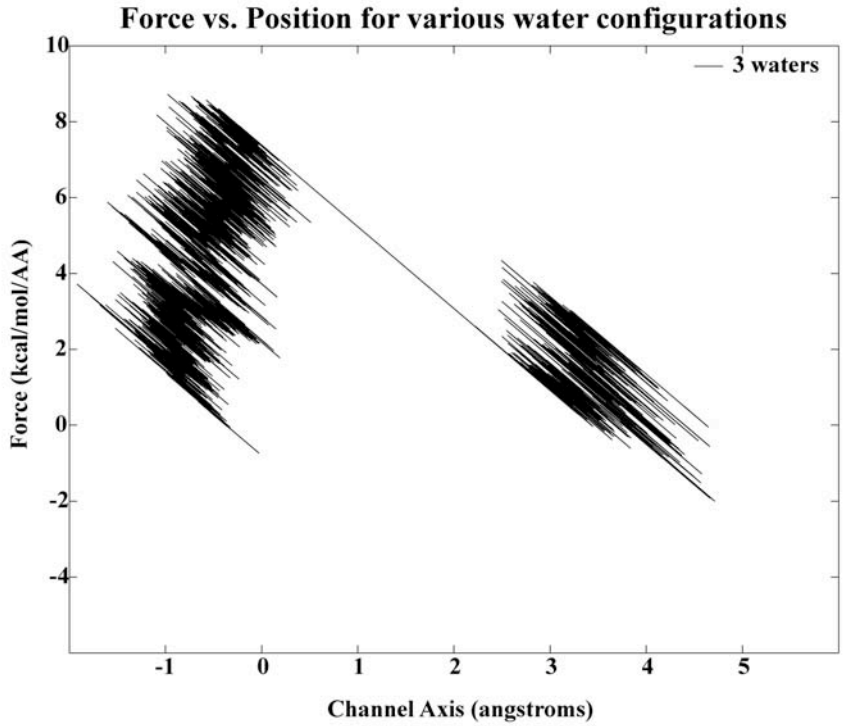
- Some fully open gate trajectories had a higher barrier

- During equilibration, 1-2 water molecules entered into first vestibule

- Presence of additional water molecules affects barrier to NH_3 entry (optimum is 1)

- In crystal structures, multiple water conformations present (between 0 – 2 water molecules in first vestibule)





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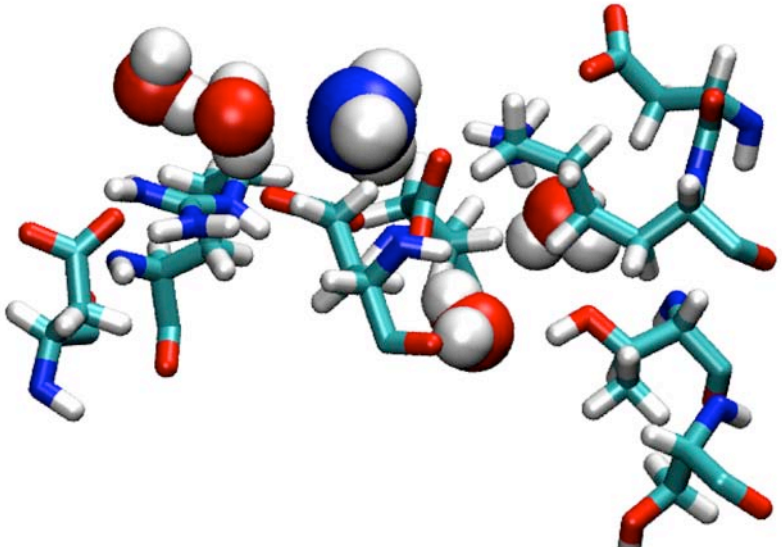

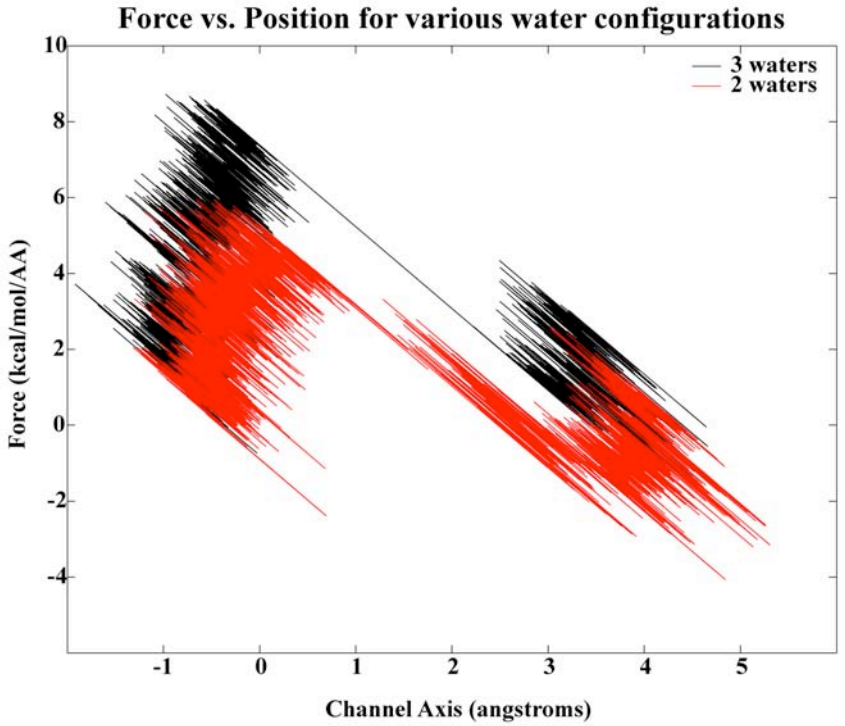

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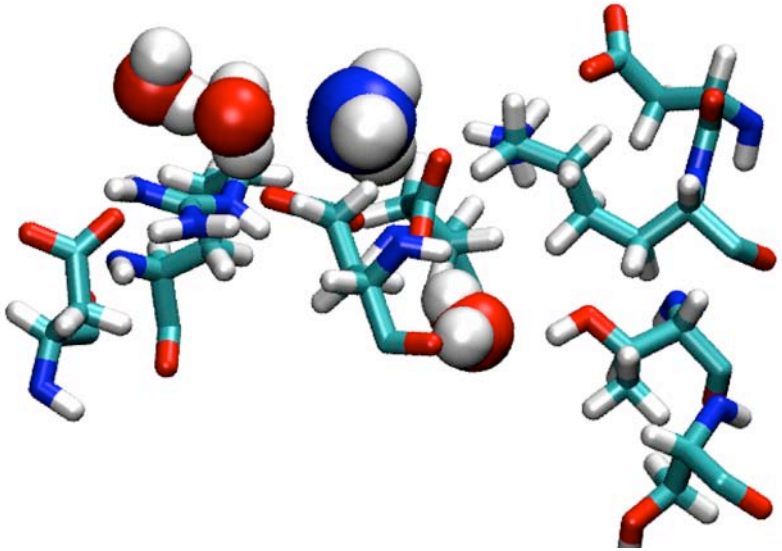

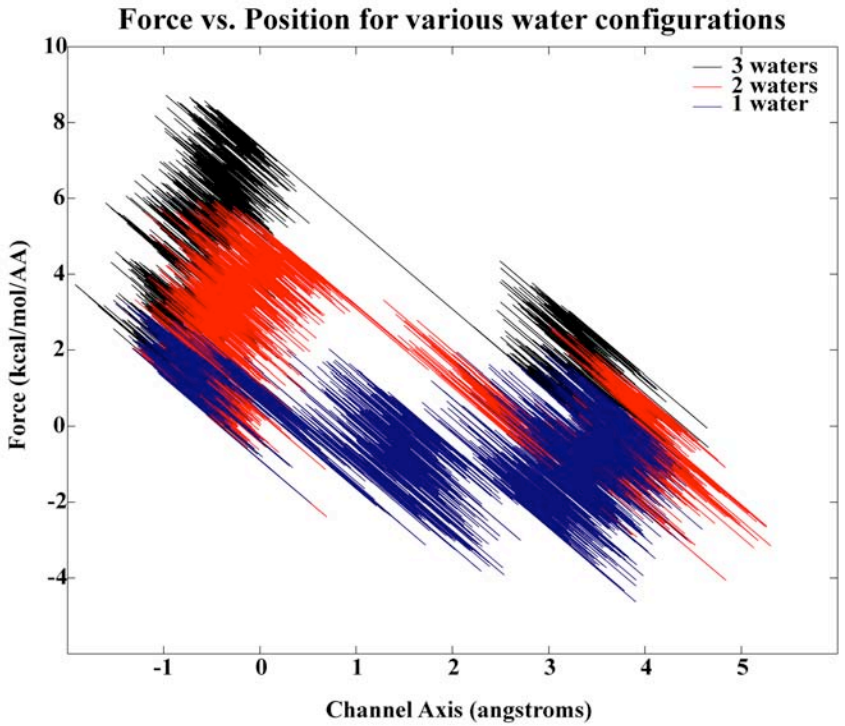

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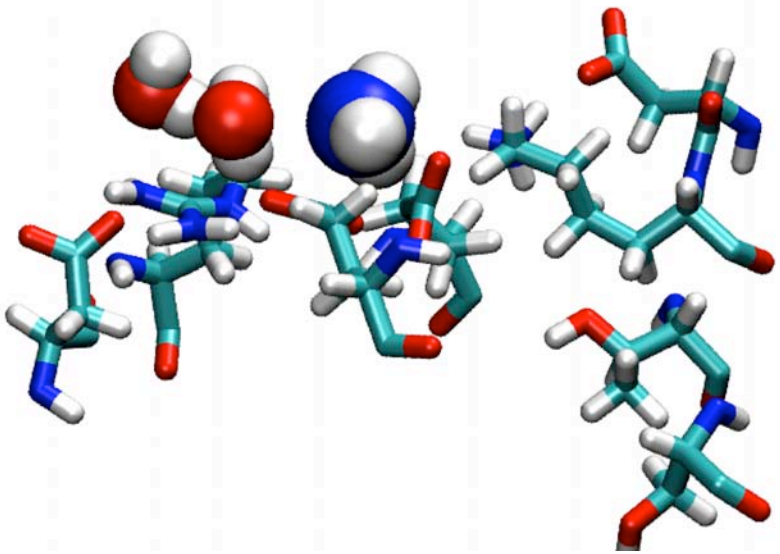
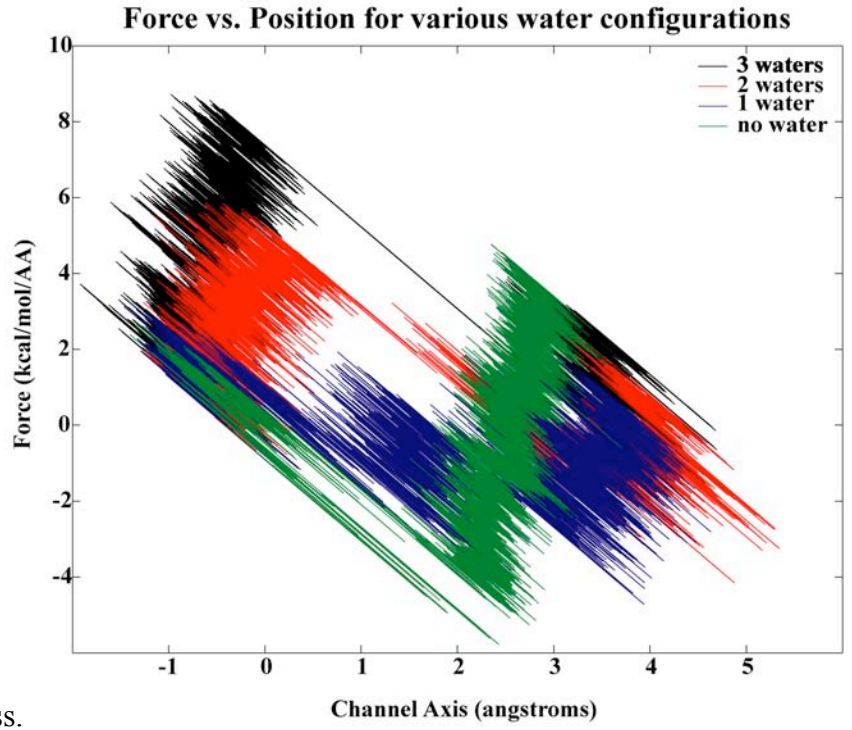
- Presence of additional water molecules affects barrier to NH_3 entry (optimum is 1)

- In crystal structures, multiple water conformations present (between 0 – 2 water molecules in first vestibule)





R. Amaro and Z. Luthey-Schulten, *Chem. Phys*., June 2004, in press.

Movement of Residues at the Interface

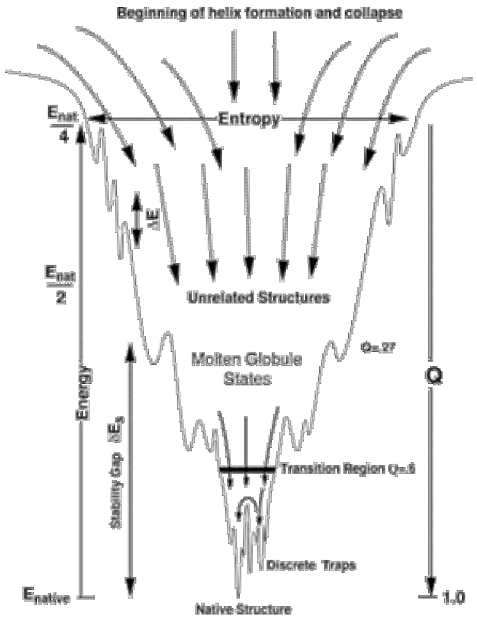
• Presently simulating the complex in different “snapshots” along the reaction pathway

• Comparison of fluctuations may indicate which residues are affected by the binding of PRFAR, pulling of the switching loop, presence of hisH substrate

•Preliminary results show increased fluctuations in the gate residues and switching loop residues when PRFAR is bound

• What else can we do to probe the possible motions?

Acknowledgements



The Luthey-Schulten Group

The TCBG Resource