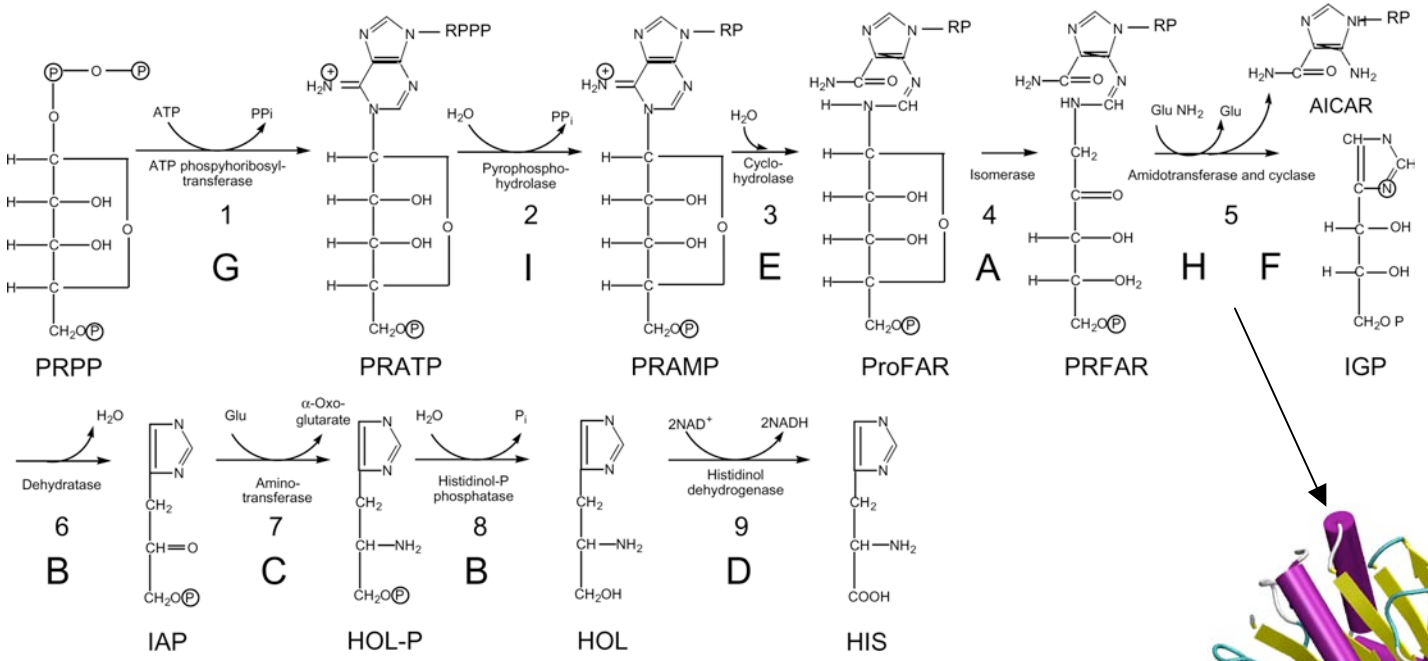
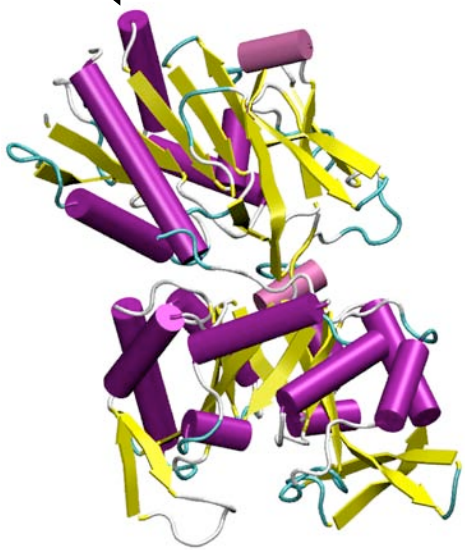
Why the histidine biosynthesis pathway?
Why hisH-hisF?

De novo purine biosynthesis



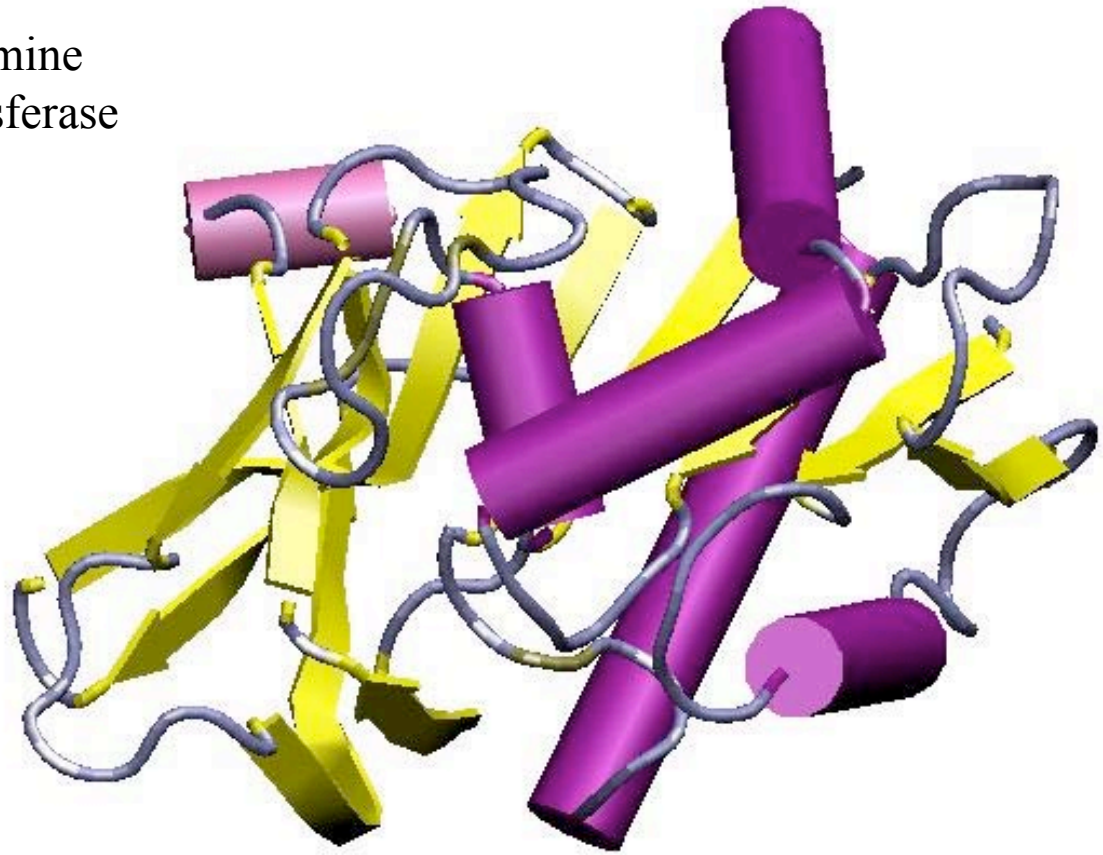


Rommie Amaro

Nov 2004 - TCBG Workshop

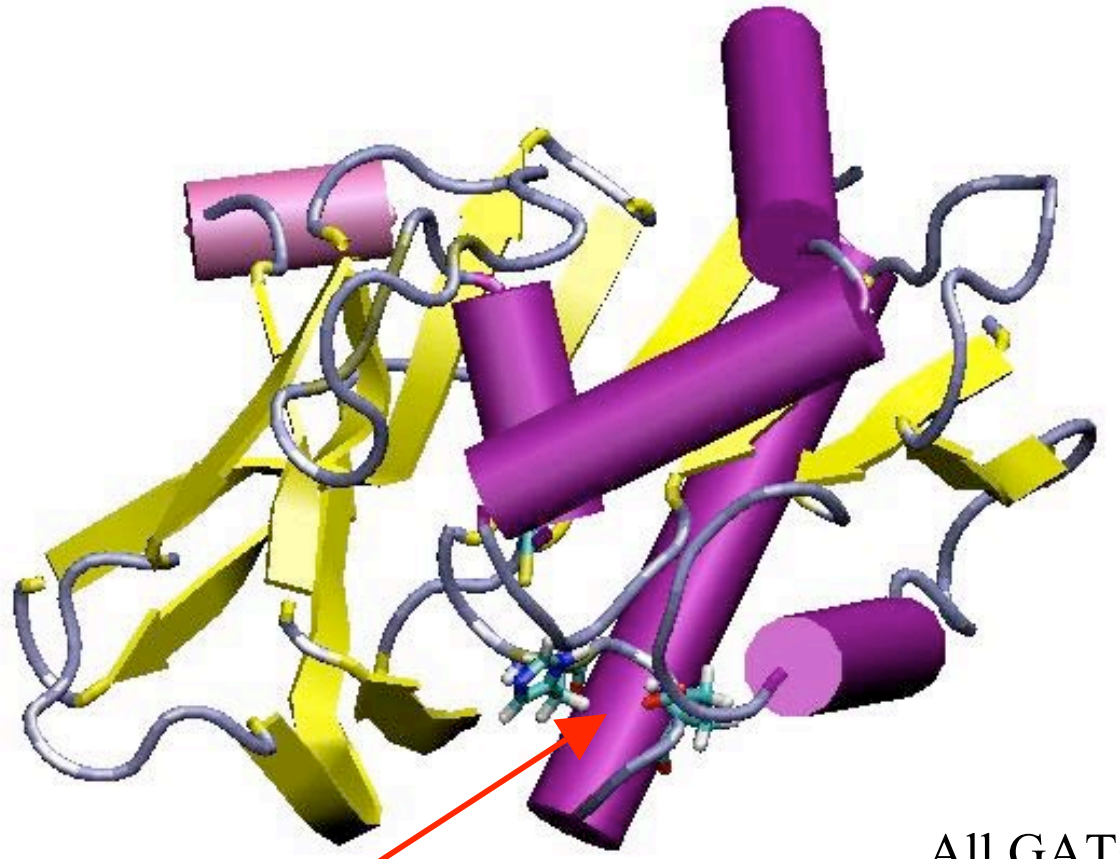
HisH

triad glutamine
amidotransferase



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

HisH

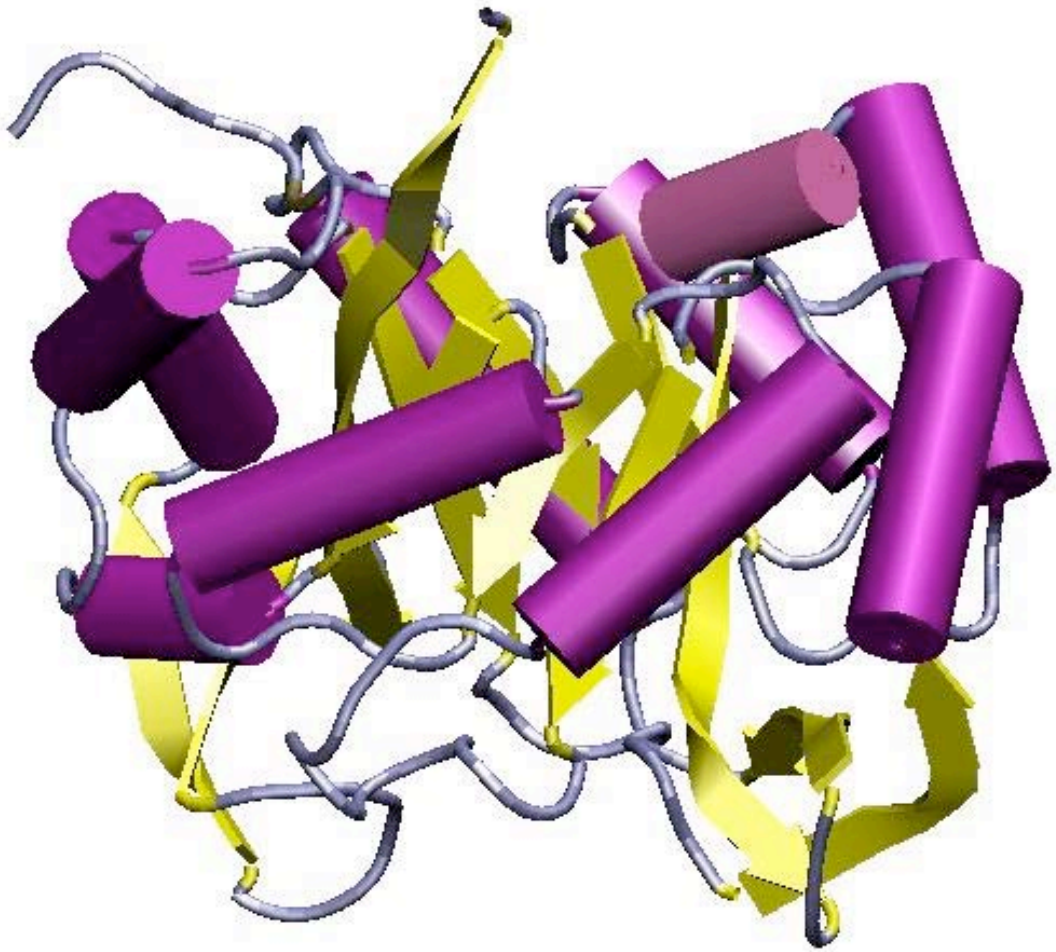


Catalytic triad active site

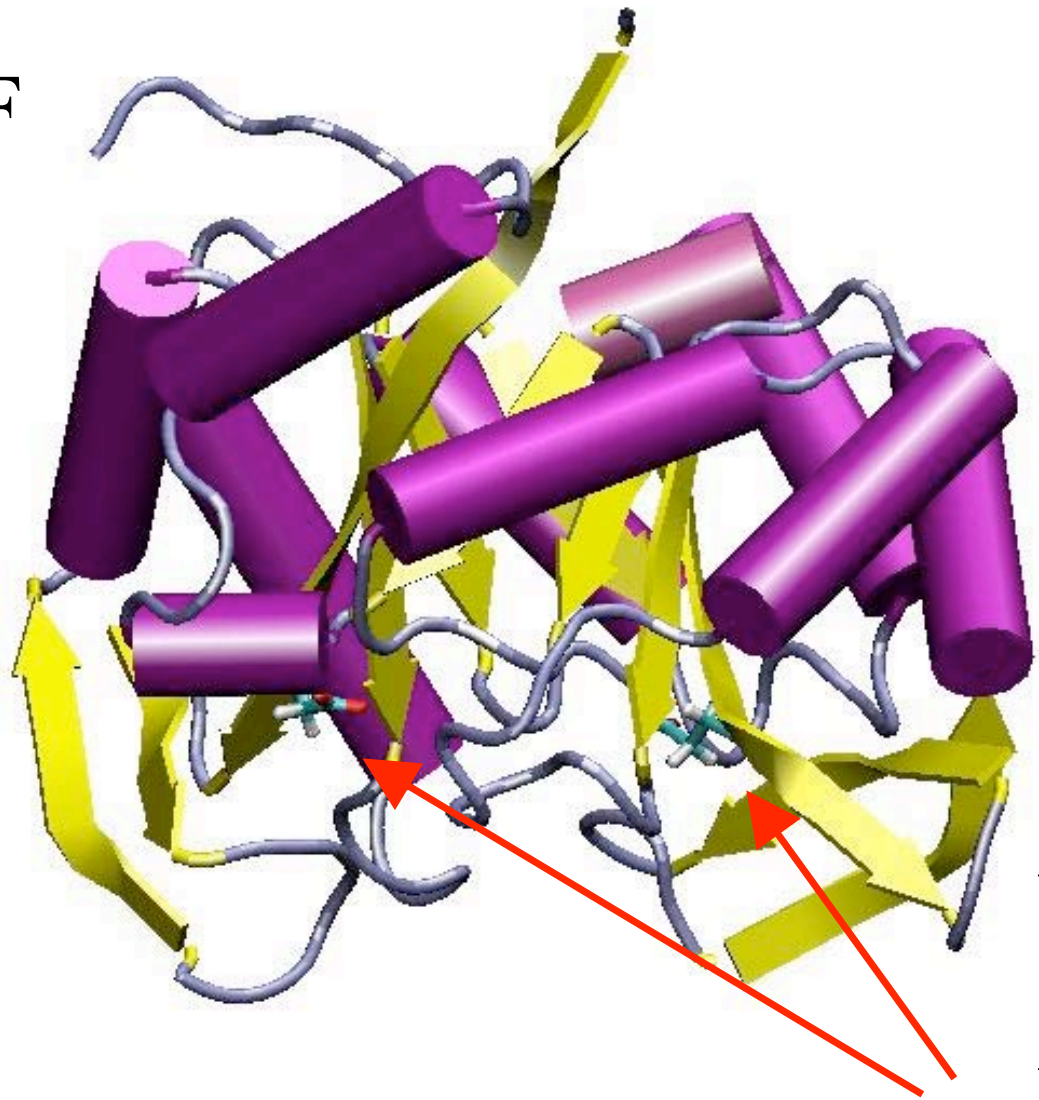
CYS84 – HIS178 – GLU180

All GATases coupled to a second reaction requiring reactive NH_3

HisF

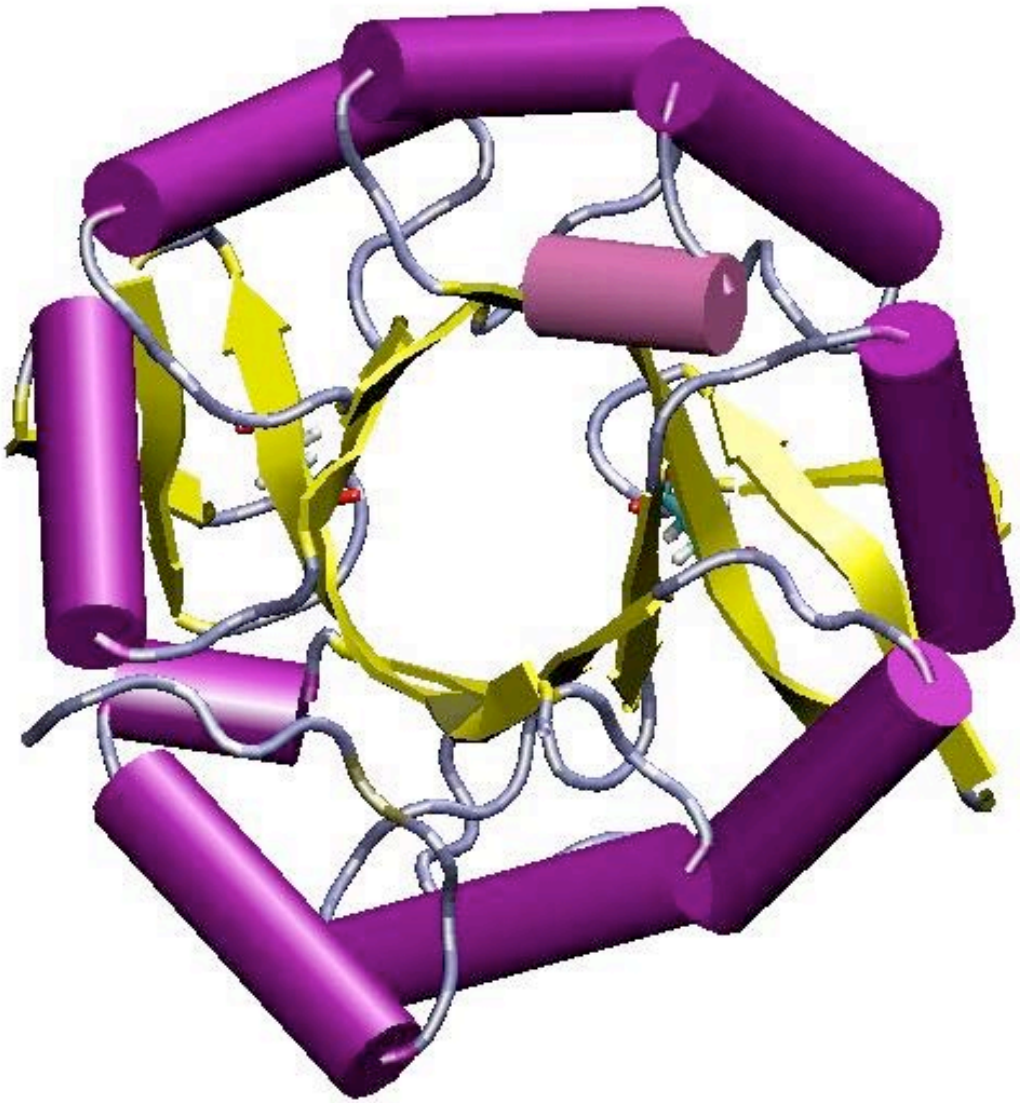


HisF



Active site residues

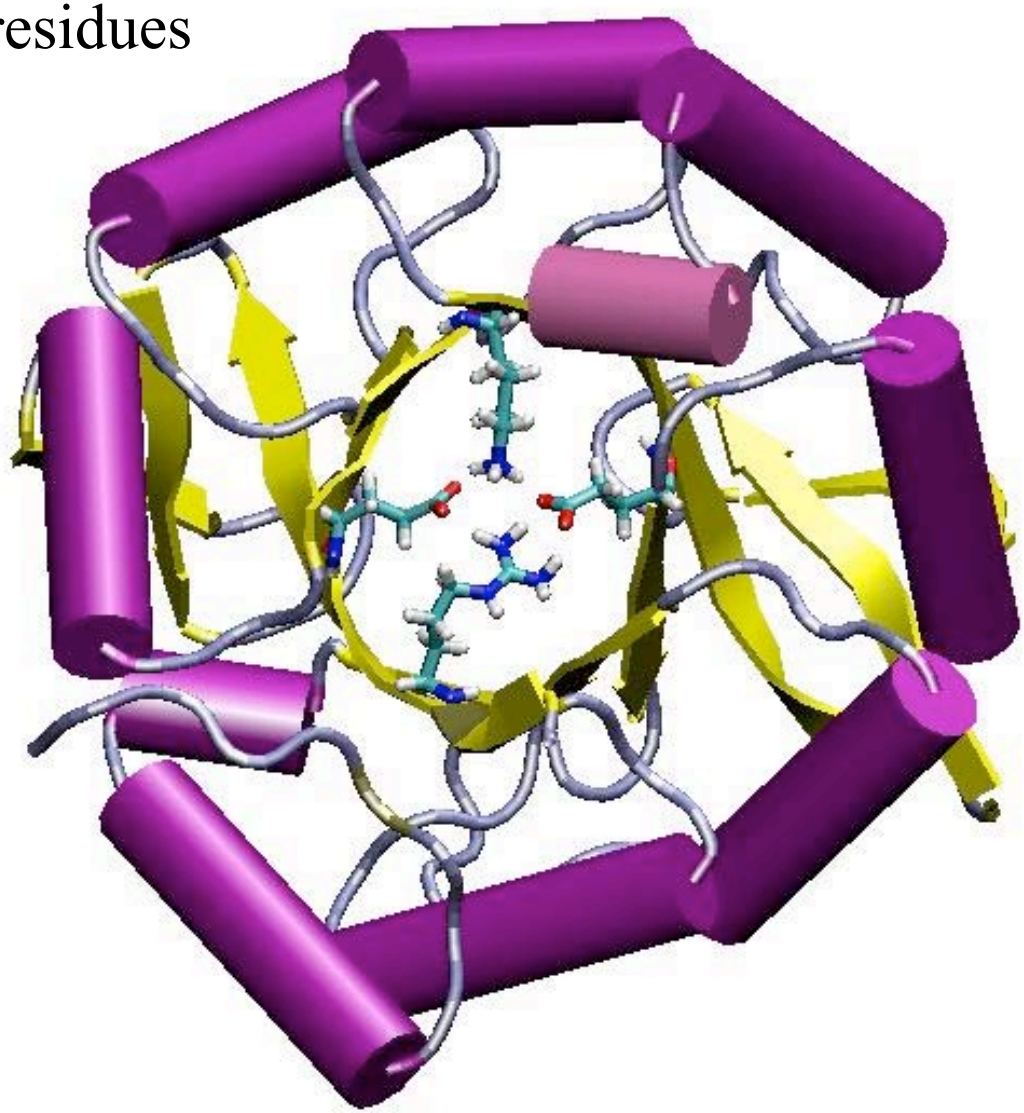
Top View
of HisF



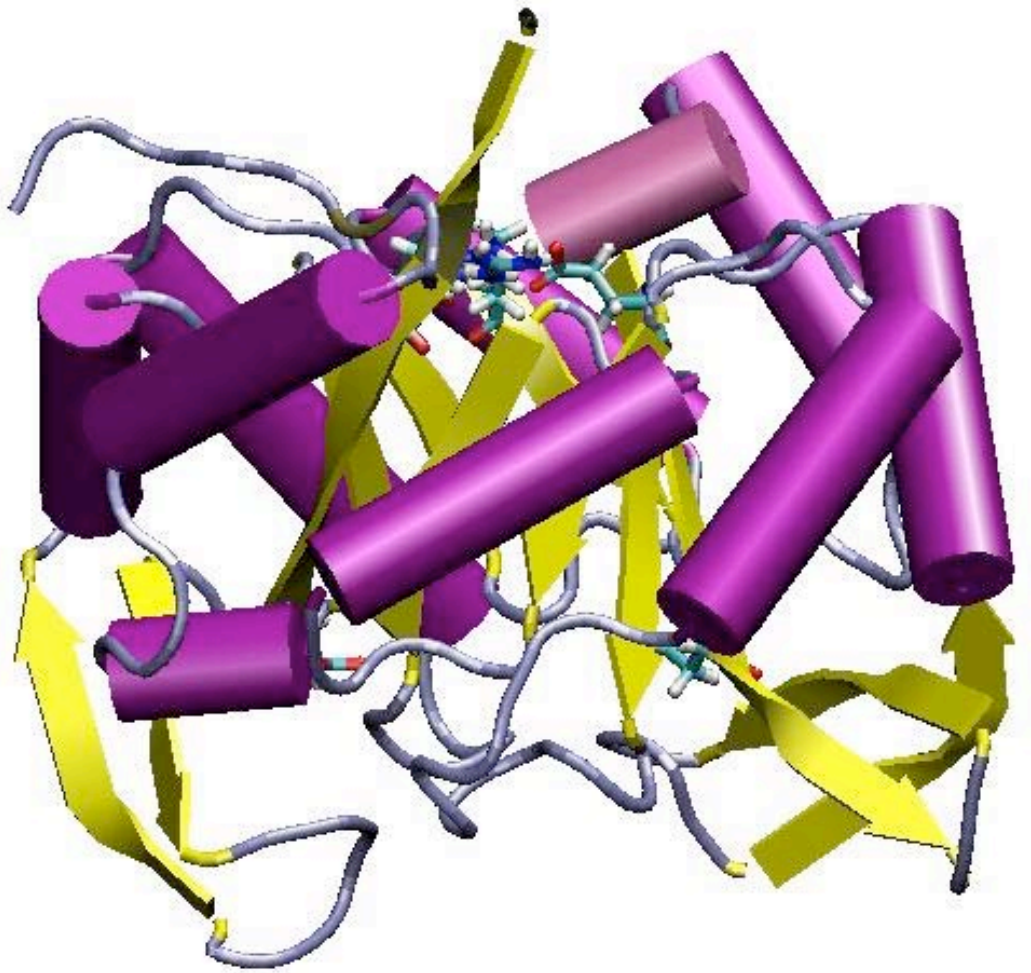
Conserved gate residues

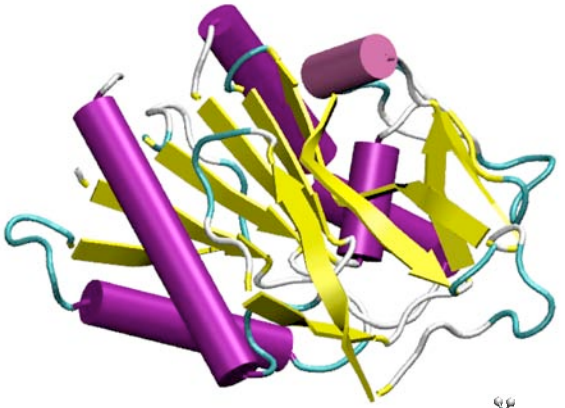
Form stable salt bridges

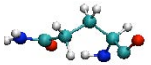
Gate diameter ~ 3 Å



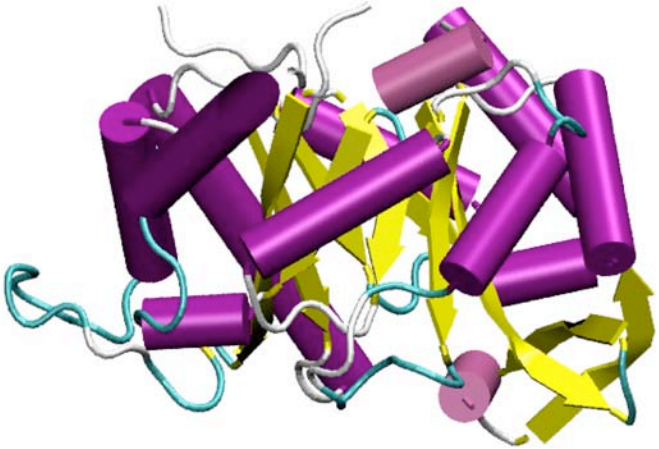
Predominantly hydrophobic channel

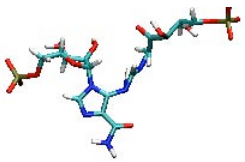




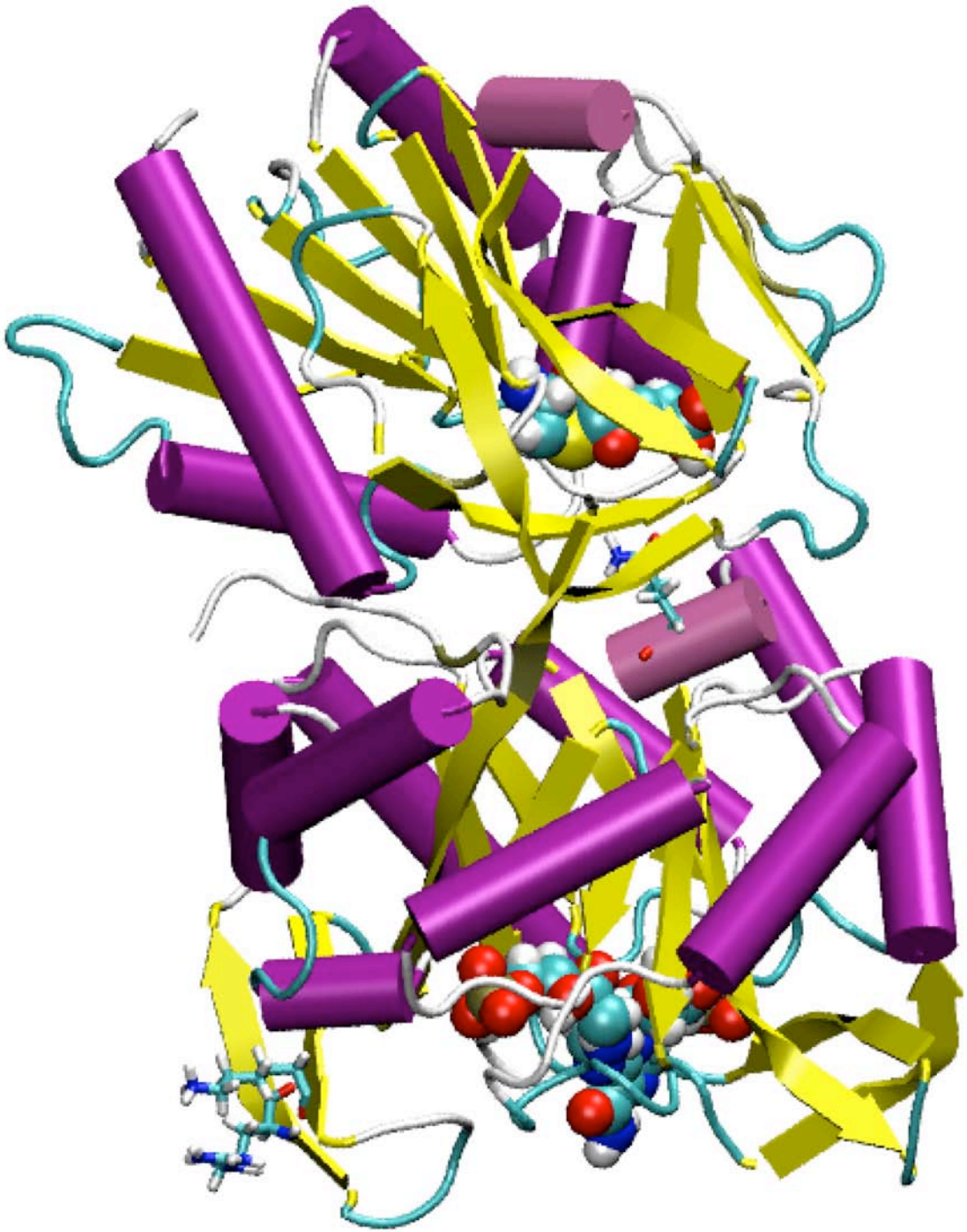


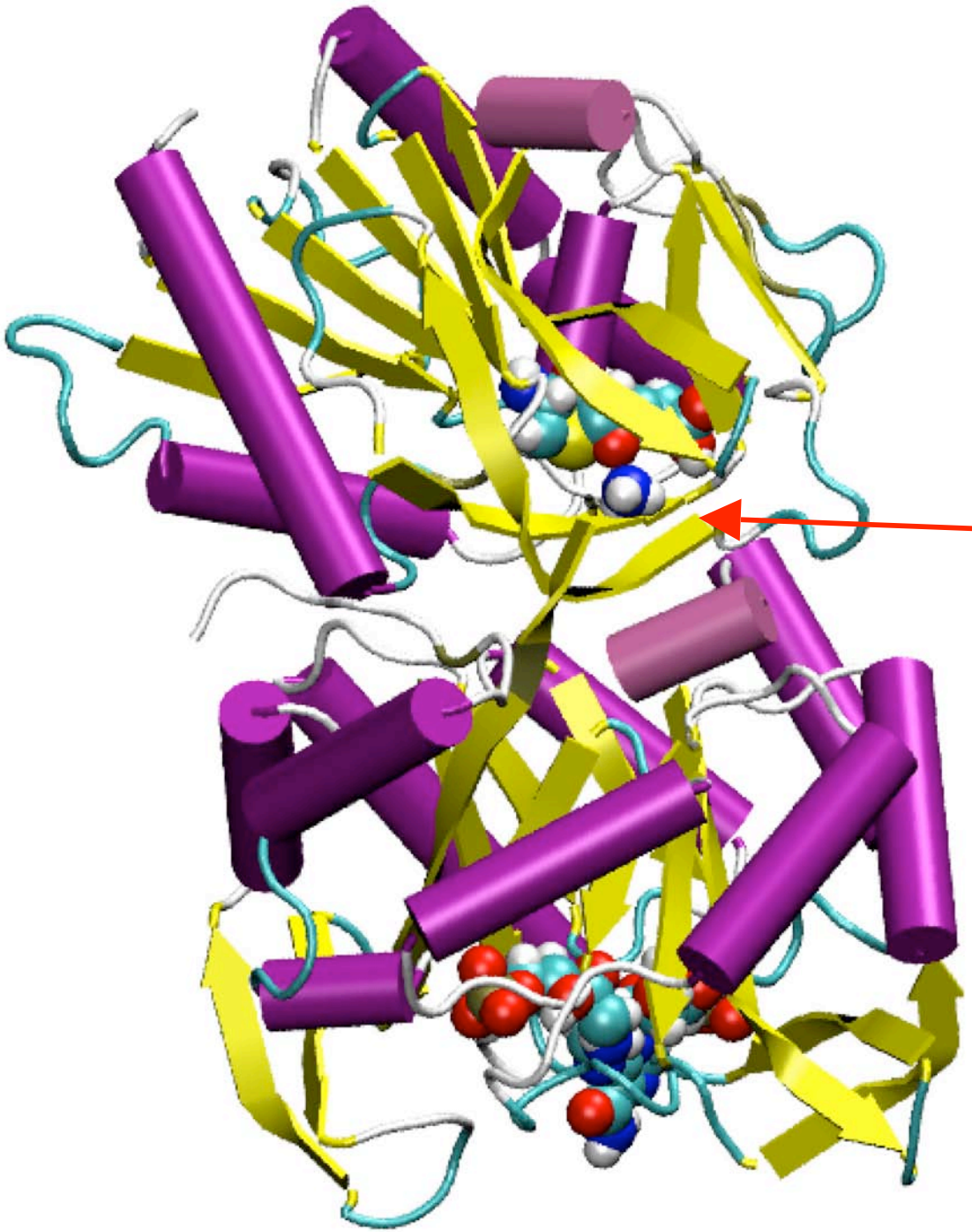
Glutamine
(mM)



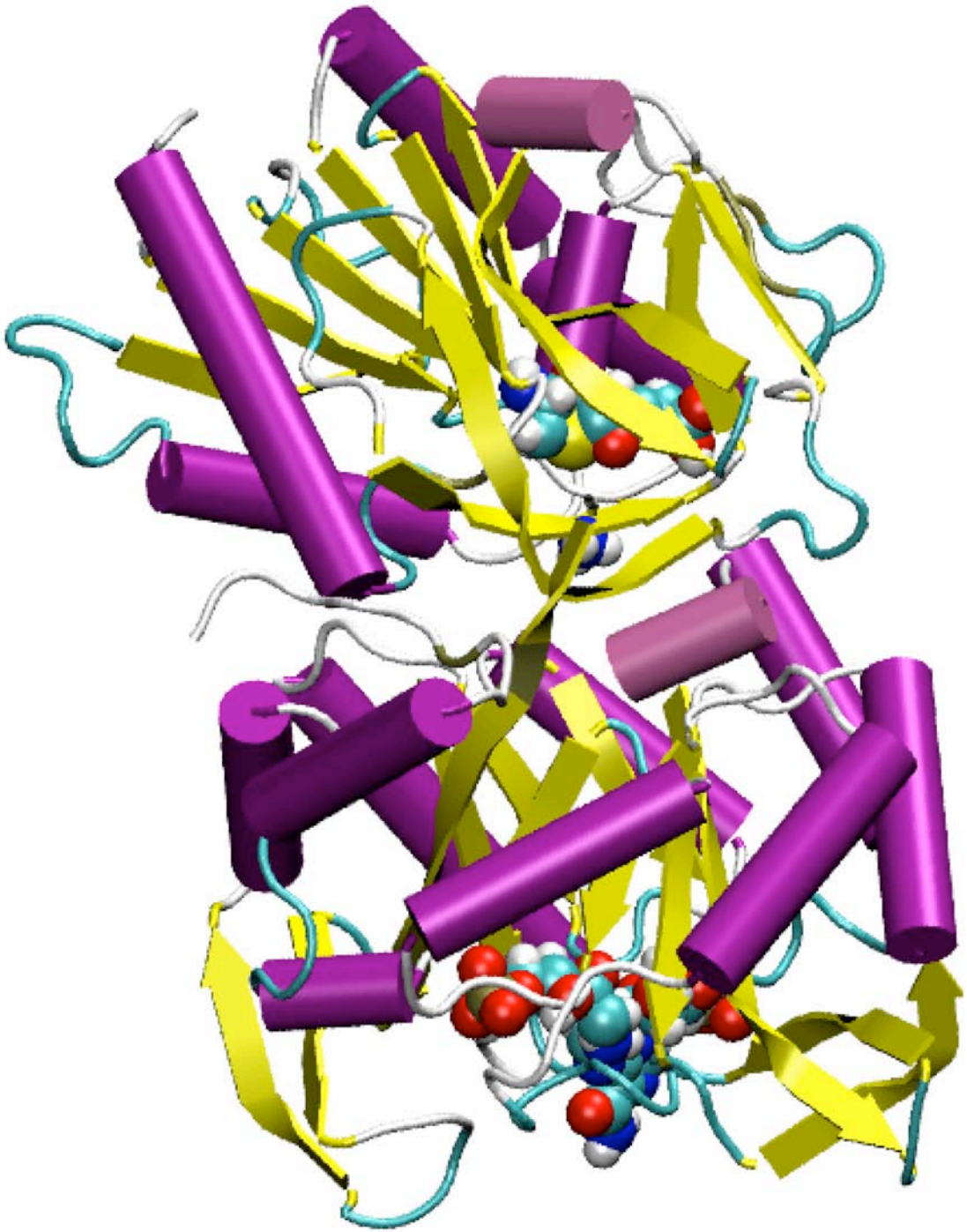


PRFAR
(sub-µM)

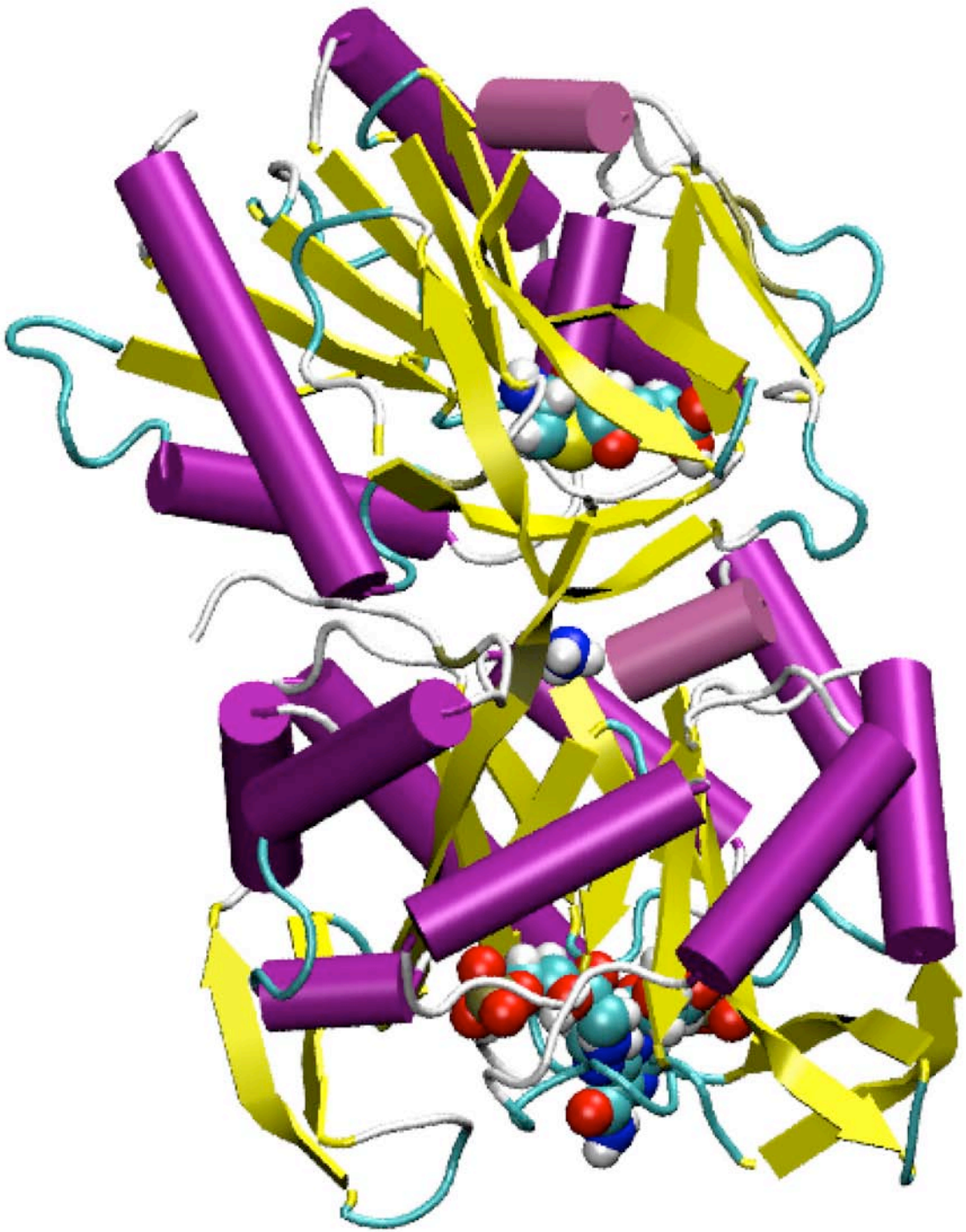




NH_3 released in 5th reaction

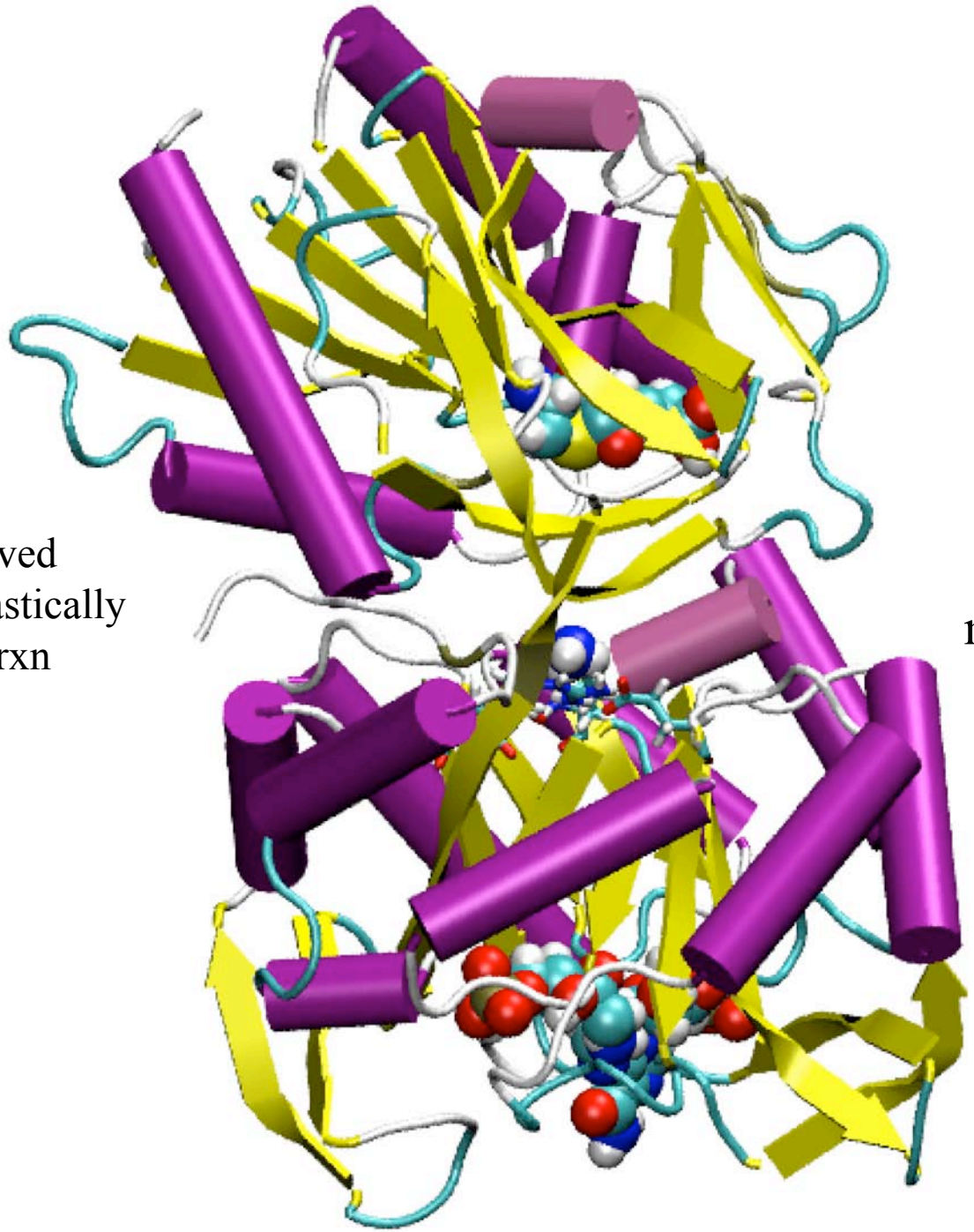


NH_3 diffuses across interface ~10Å to mouth of hisF



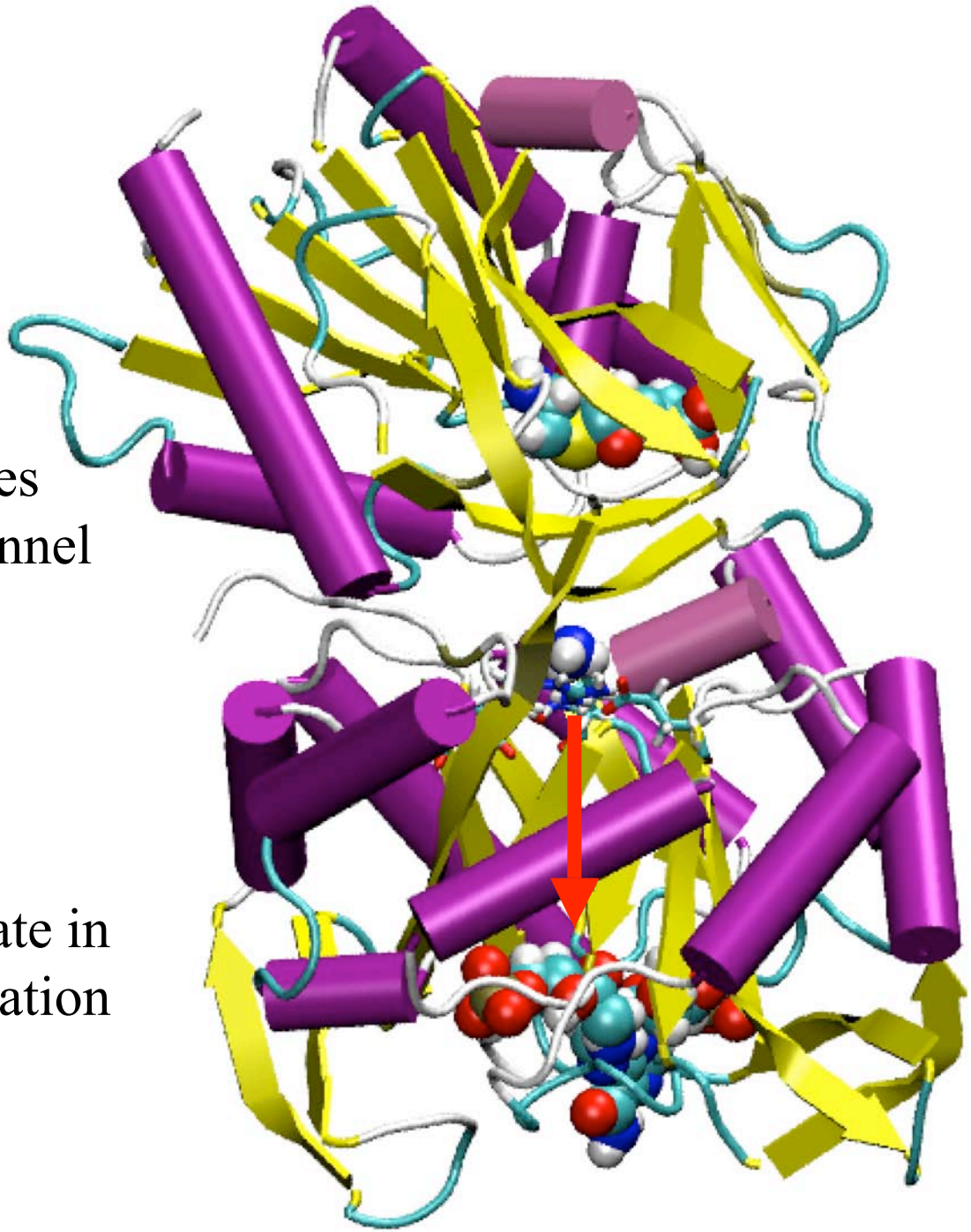
NH_3 diffuses across interface ~10Å to mouth of hisF

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

Ammonia Conduction

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

- Apply an external force to the system:

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

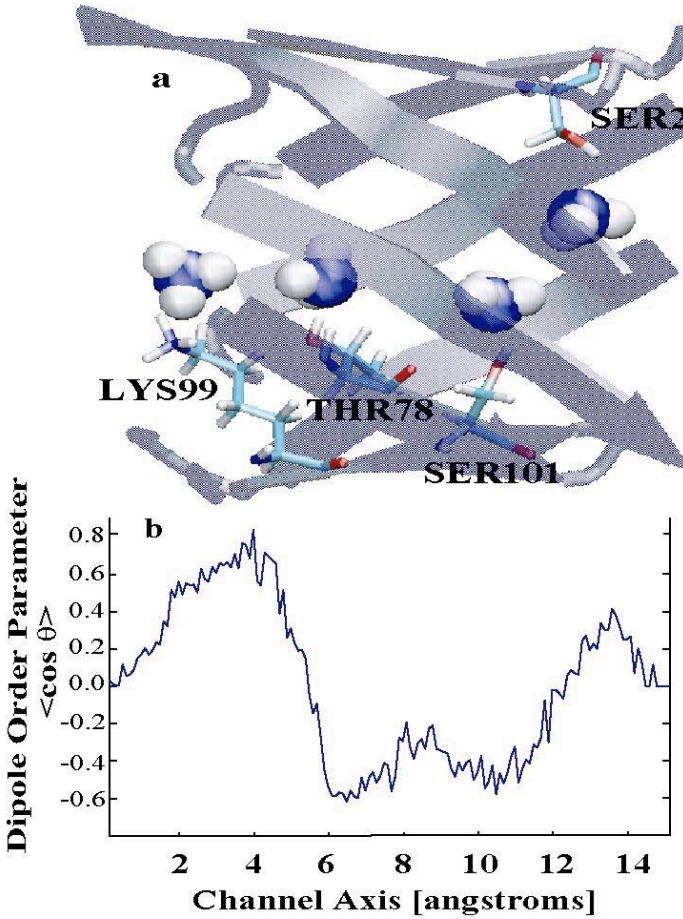
- To quantify the energetics of conduction we use:

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

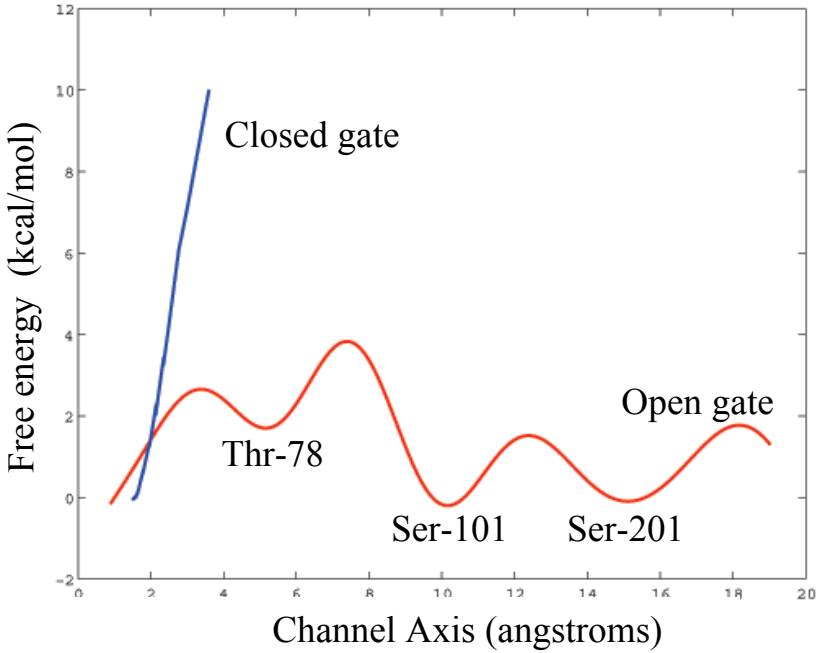
- This new identity allows us to determine *equilibrium* information from repeated *nonequilibrium* measurements

Results through partially open gate in *apo*-complex

Dipole Moment Analysis



Free energy profile of ammonia in barrel



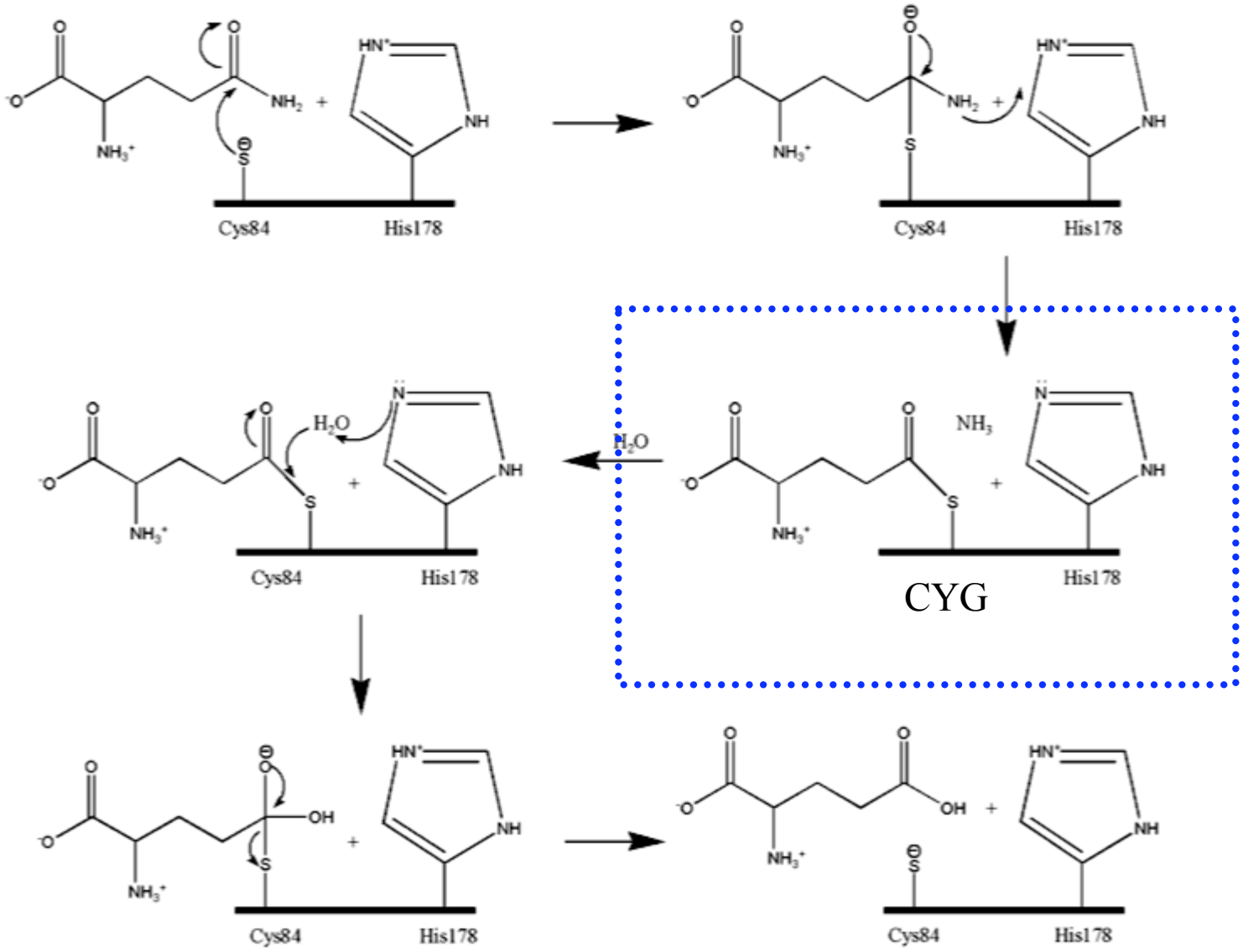
Mean First Passage Time Analysis:

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)

We can model various functional states

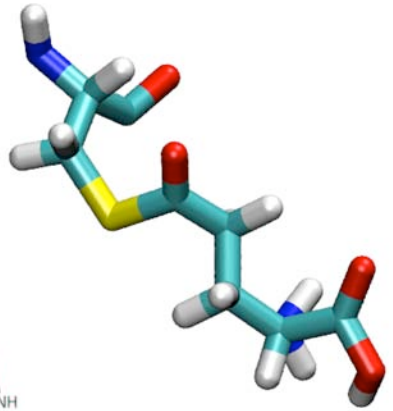


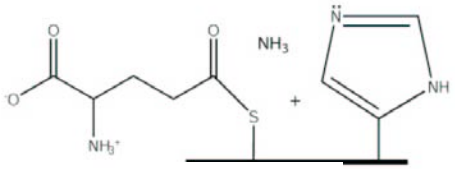
Modeling the *active*-complex: including substrates

HisH:

Glutamyl thioester intermediate corresponding to post-NH_3 release state

Parameterization required for thioester linkage



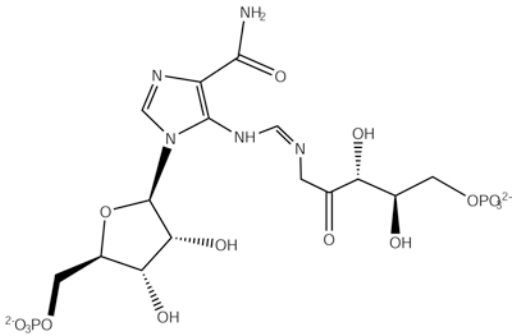


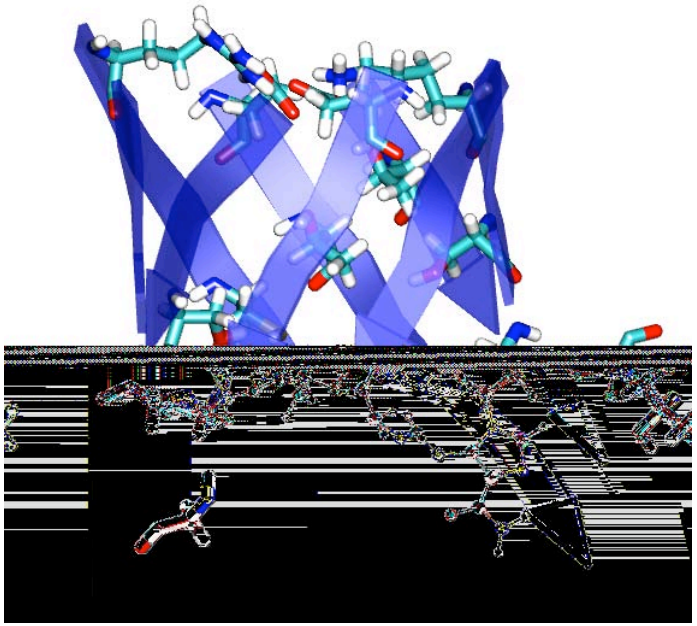
CYG

HisF:

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

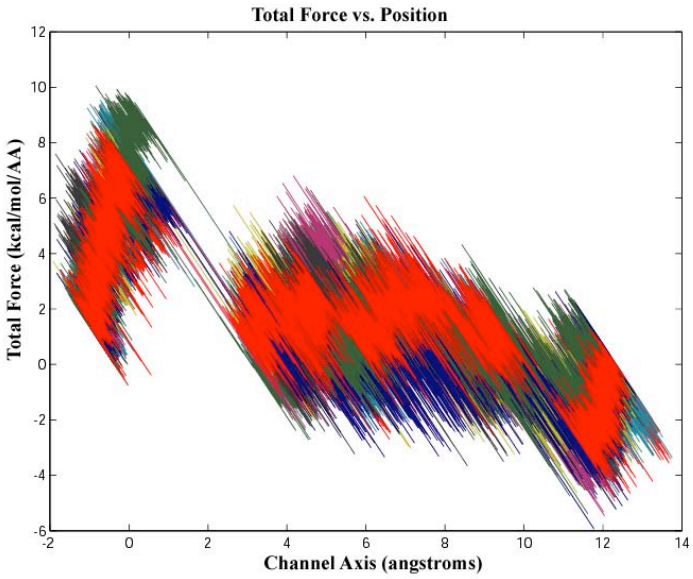
Parameterization according to existing CHARMM protocol



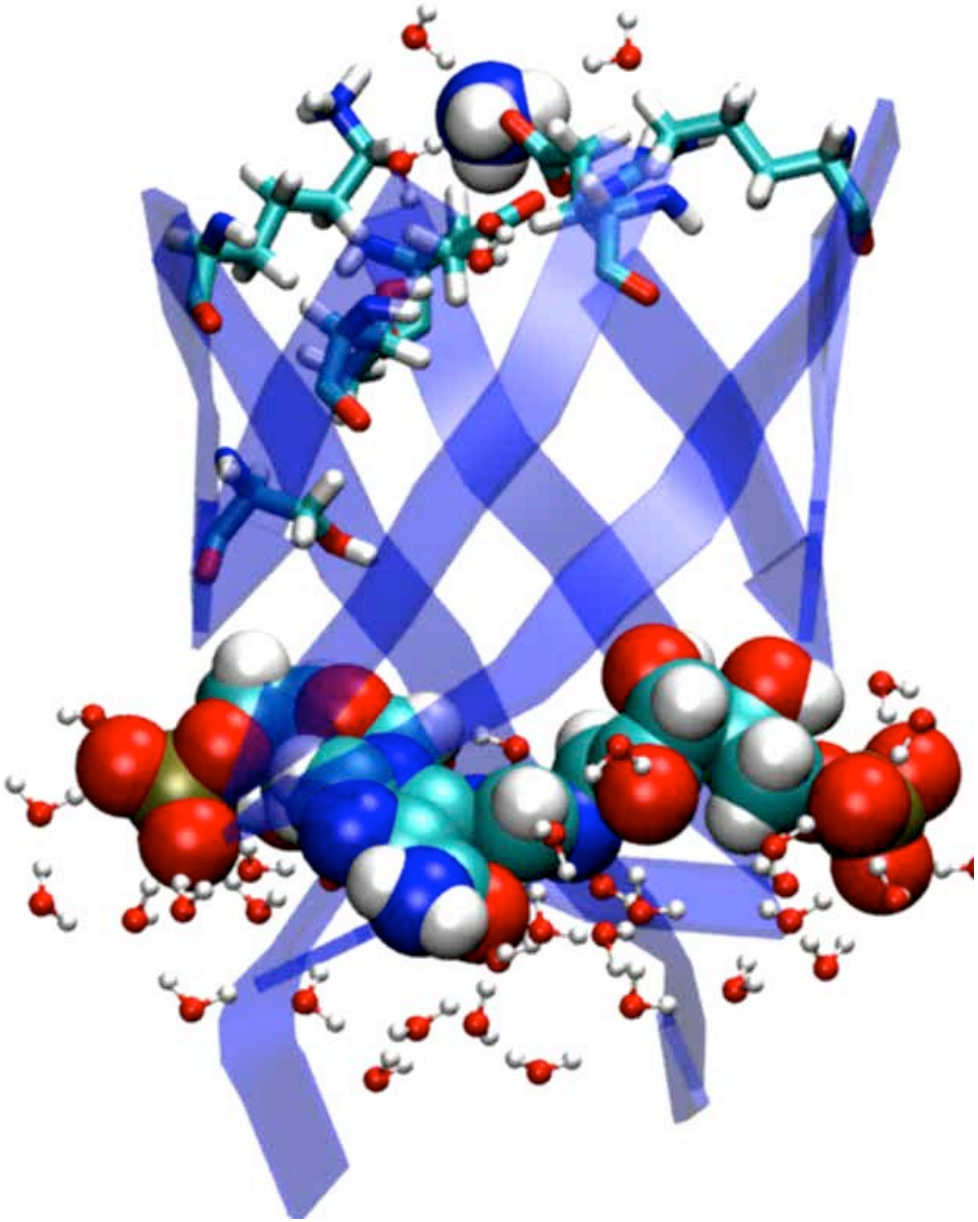


* Chaudhuri, Lange, Myers, Davisson, and Smith, *Biochemistry*, 2003; Myers, Jensen, Deras, Smith, and Davisson, *Biochemistry,* 2003.

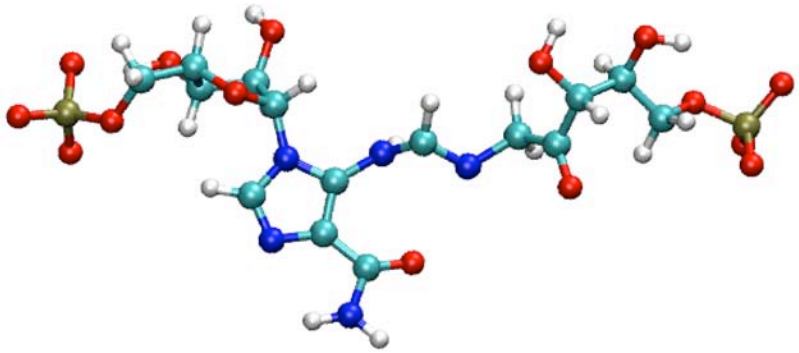
Including substrates produced a surprising result!

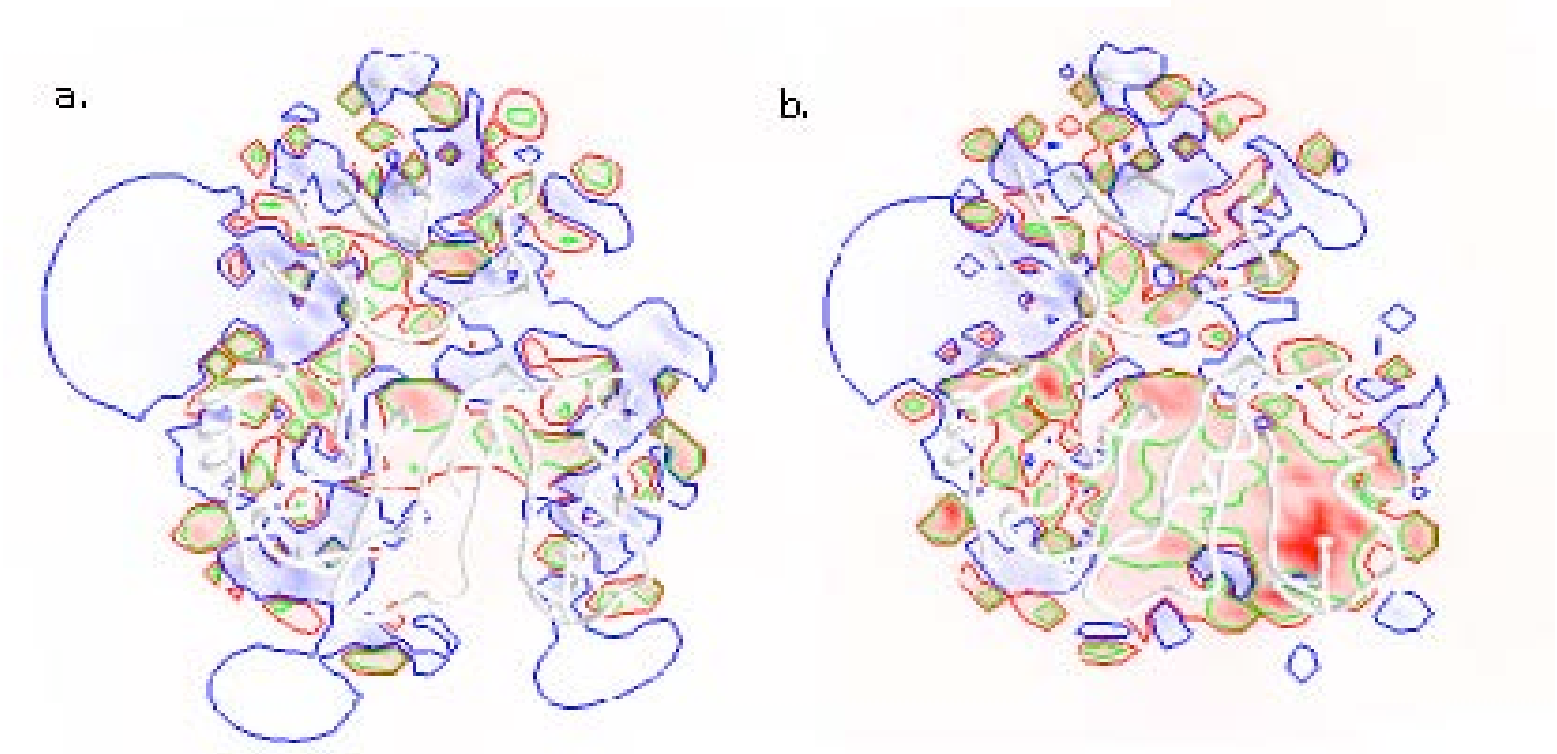


Same gate configuration, higher barriers?!?



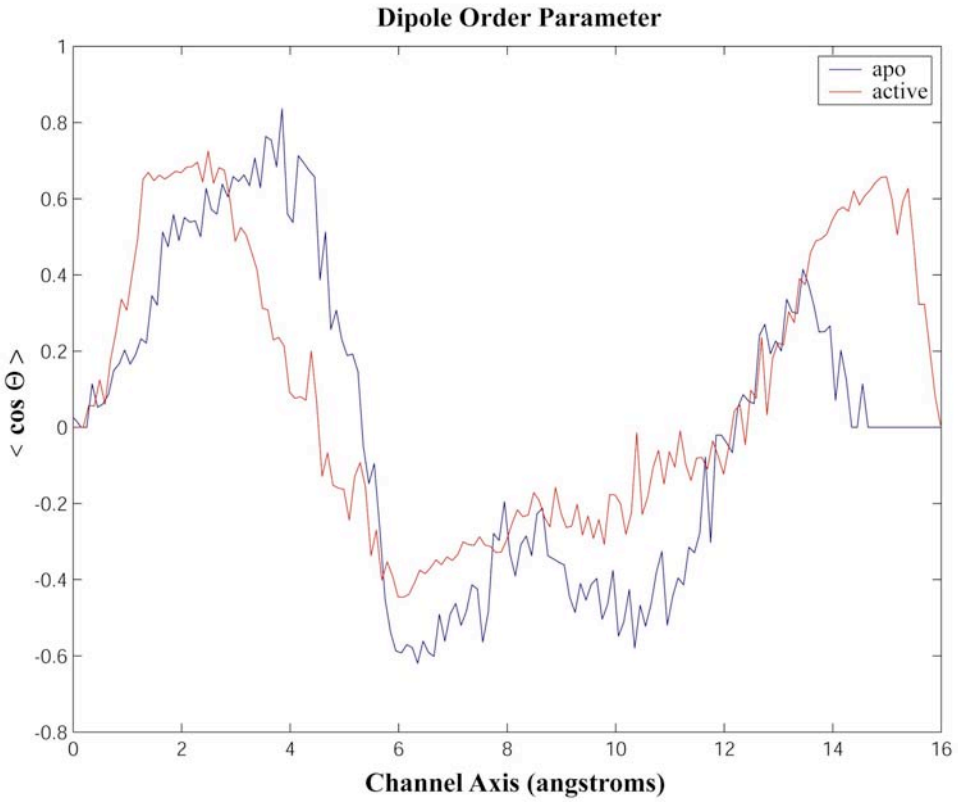
How could PRFAR change the energetics of conduction?

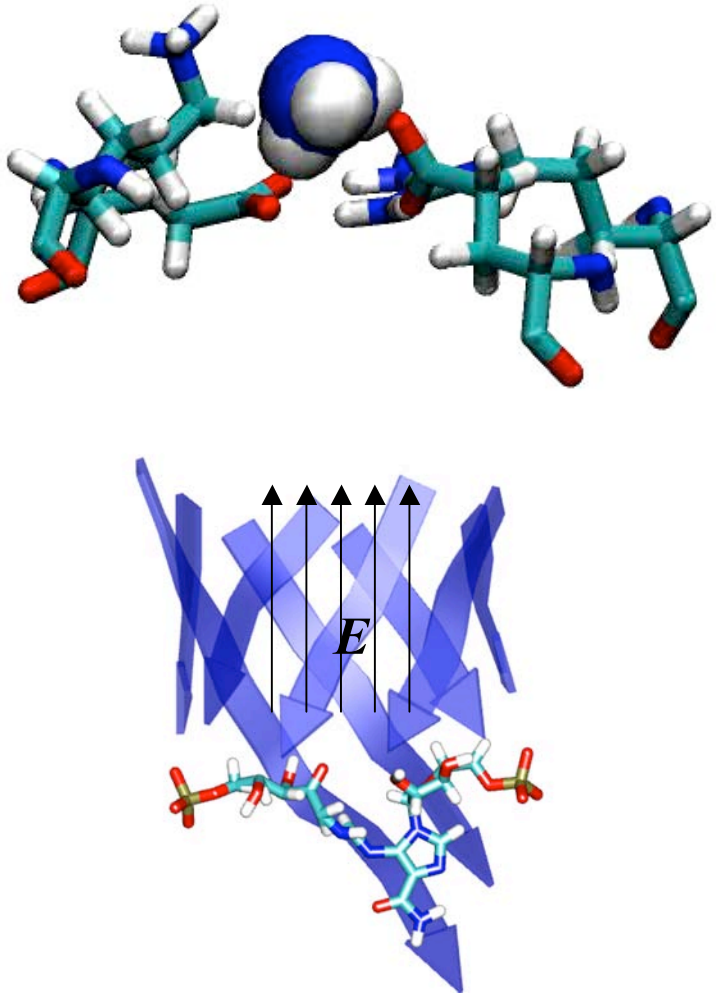




PRFAR introduces large electrostatic effects!

Net effect: a torque on ammonia's dipole

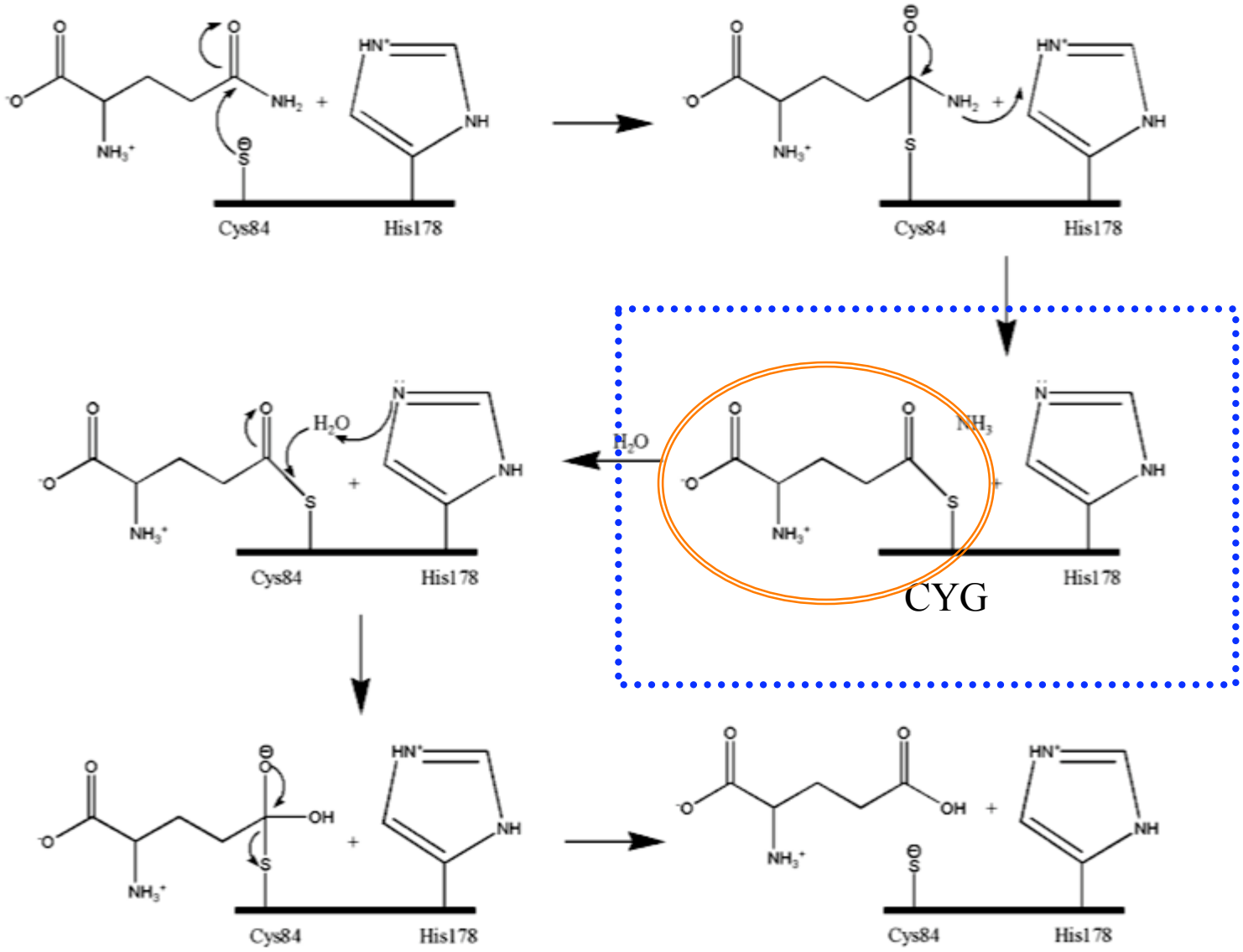




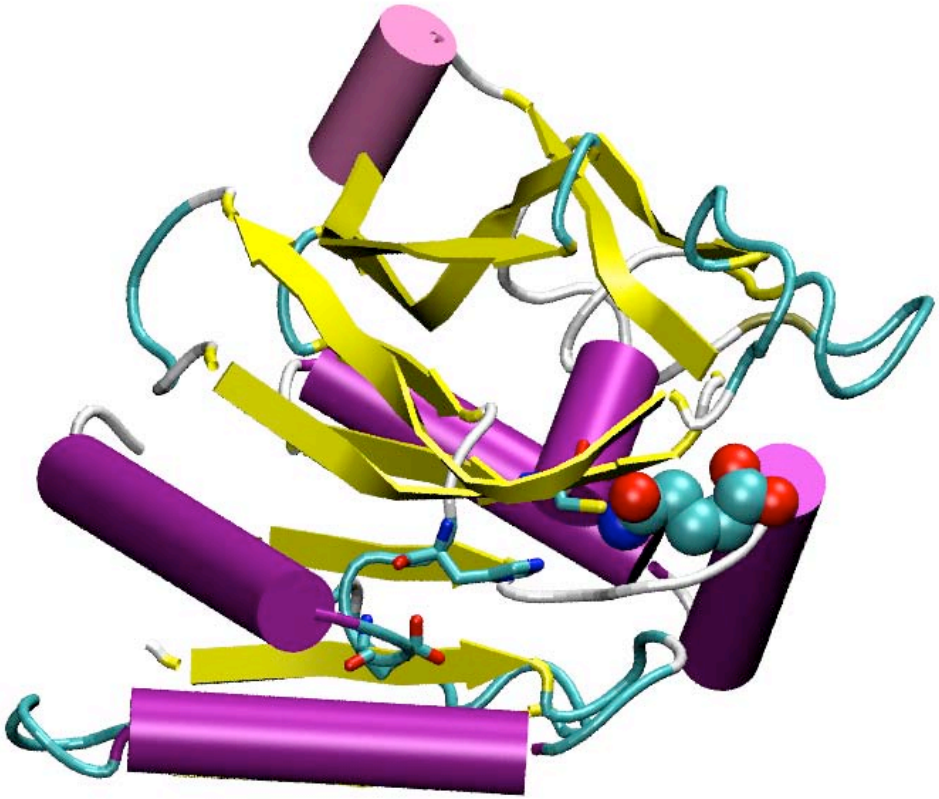
Electrostatic field from PRFAR makes it more difficult for NH_3 to flip orientations

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

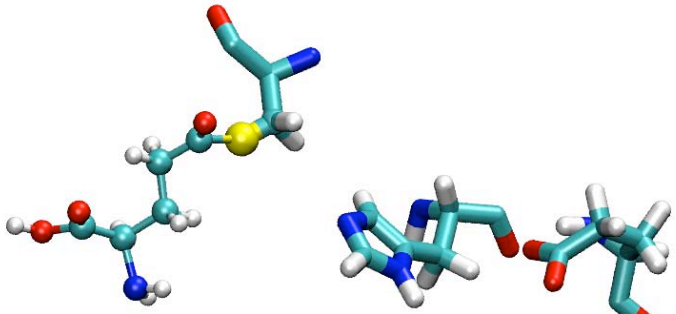
Modeling the *active*-complex: today's tutorial



VMD to Attach the substrate GLN to the active site of hisH



CYS & GLN



CYG

Class I Potential Energy function

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

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

$$+ \sum_{impropers} k_\omega (\omega - \omega_0)^2 + \sum_{Urey-Bradley} k_u (r_{1,3} - r_{1,3,0})^2$$

Non-bonded Interaction Terms

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

From MacKerell

Class I Potential Energy function

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From MacKerell

Specify in topology file

Class I Potential Energy function

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

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Non-bonded Interaction Terms

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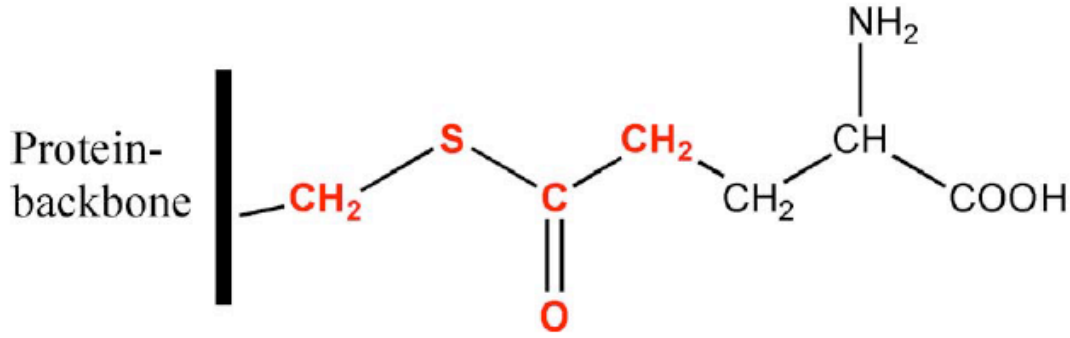
From MacKerell

Specify in parameter file

Creating a new topology file entry

```
RESI CYG 0.00
GROUP
ATOM N    NH1    -0.47  !      |
ATOM HN   H       0.31  !  HN-N
ATOM CA   CT1     0.07  !      |   HB1
ATOM HA   HB      0.09  !      |   |
GROUP                   !  HA-CA--CB--SG
ATOM CB   CT2    -0.11  !      |   |   |
ATOM HB1  HA      0.09  !      |   HB2 |
ATOM HB2  HA      0.09  !   O=C        |
ATOM SG   S      -0.07  !      |         \
!ATOM HG1  HS     0.16 !                  \
GROUP                  !                   \
ATOM CDG  CC      0.55 !                    \
ATOM OE1  O      -0.55 !                     \
GROUP                      !     HN2G          \
ATOM CGG  CT2    -0.18     !       |             \
ATOM HG1G HA      0.09     ! HN1G-NG   HB1G HG1G\
ATOM HG2G HA      0.09     !       |    |     |   \
GROUP                      !  HAG-CAG--CBG--CGG--CDG=OE1
ATOM CBG  CT2    -0.18     !       |    |     |
ATOM HB1G HA      0.09     !       |    HB2G HG2G
ATOM HB2G HA      0.09     !  O1G=CG
GROUP                      !       |
ATOM CG   CD      0.75     !      O2G-HO2G
ATOM O1G  OB     -0.55
ATOM O2G  OH1    -0.61
ATOM HO2G H       0.44
ATOM CAG  CT1    -0.12
ATOM HAG  HB      0.09
ATOM NG   NH3     -0.62
ATOM HN1G HC       0.31
ATOM HN2G HC     0.31
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
```



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

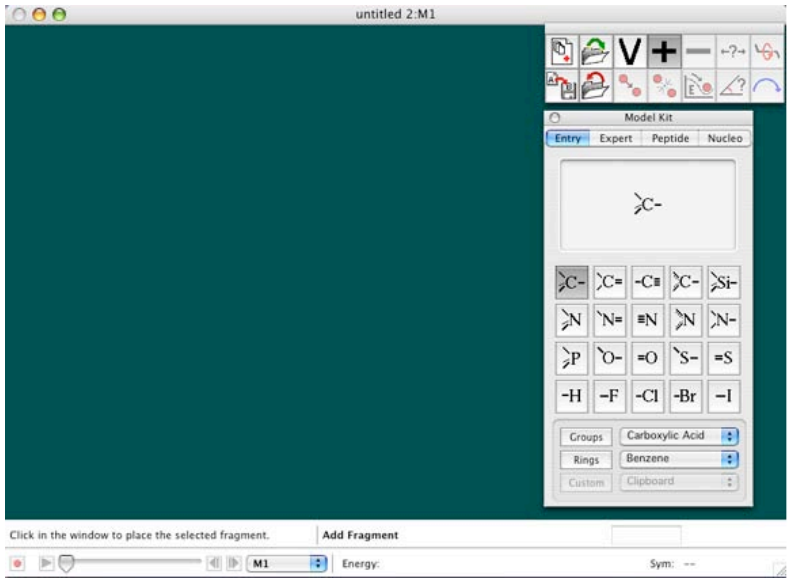
Changes annotated!

Creating new parameters

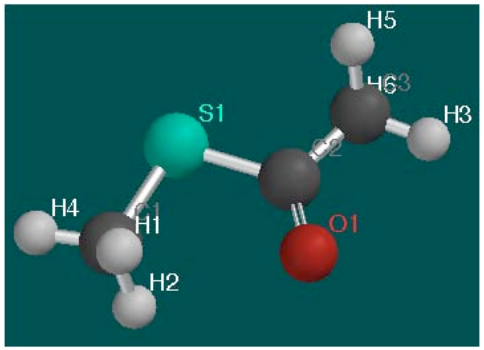
```
BONDS
!
!V(bond) = Kb(b - b0)**2
!
!Kb: kcal/mole/A**2
!b0: A
!
!atom type Kb          b0
! Modified for CYG residue after 6-31G* geometry optimization
S    CC   240.000      1.7814 ! ALLOW   ALI SUL ION

ANGLES
!
!V(angle) = Ktheta(Theta - Theta0)**2
!
!V(Urey-Bradley) = Kub(S - S0)**2
!
!Ktheta: kcal/mole/rad**2
!Theta0: degrees
!Kub: kcal/mole/A**2 (Urey-Bradley)
!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   O        2.05    2     180.00
```

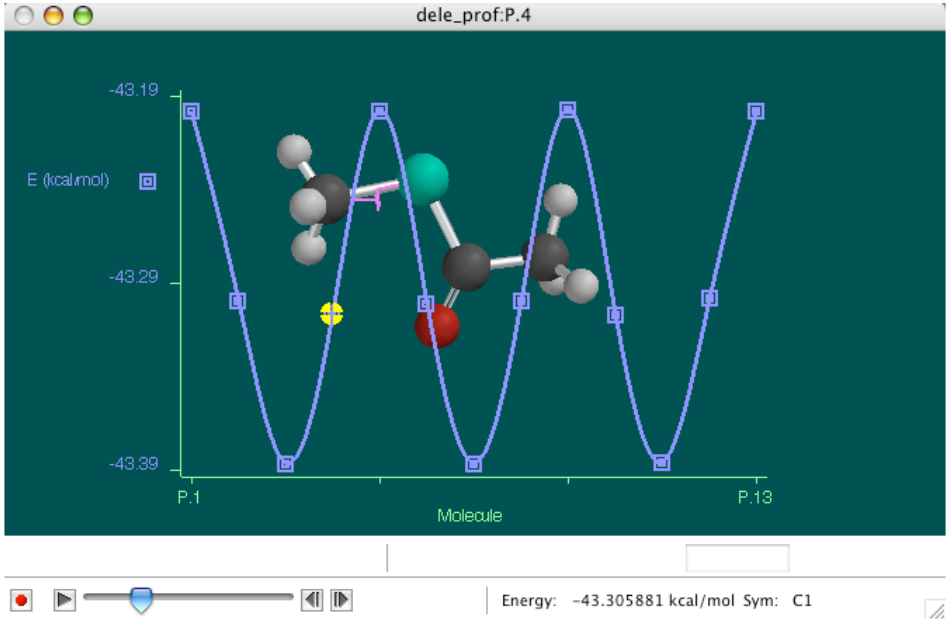
Semi-empirical Parameter Estimation Using SPARTAN



Main Spartan Window

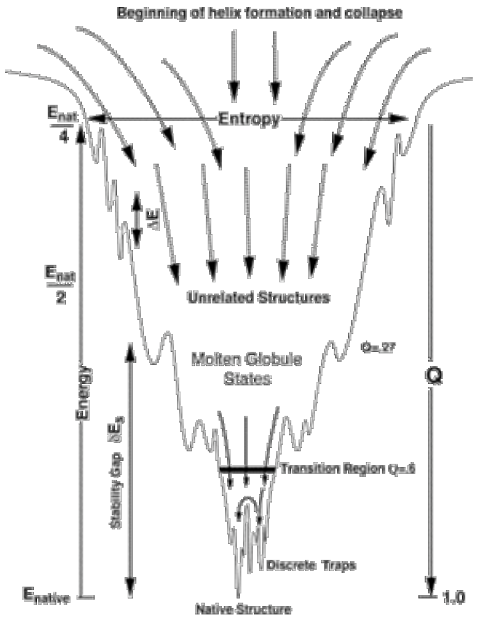


You build a part of CYG



Be careful with the dihedral drive section!

Acknowledgements



The Luthey-Schulten Group

The TCBG Resource

NSF

NCSA