Computational Studies on Reaction Mechanisms of Nylon-oligomer Hydrolase

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ka Univ.

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H. Ando (M1: Interaction analyses of Nylonoligomer hydrolase)

M. Nakano (Prof.: Nylonoligomer hydrolase)

Dr. T. Matsui (JSPS Fellow: pKa & redox potential estimation)

Dr. R. Harada (PD: Induced fit and substrate binding of Nylonoligomer hydrolase

gawa Institute of Technology

Dr. K. Kamiya (Prpf: Enzymatic reaction of Nylonoligomer hydrolase)

yo Univ. & Univ. Tokyo

Dr. Y. Mochizuki (Prof: Interfragment interaction energy analyses)

Dr. C. Watanabe (PD: Interfragment interaction energy analyses)

Dr. Y. Okiyama (PD: Interfragment interaction energy analyses)

ersity of Hyogo

Dr. S. Negoro (Prof: Biochemical analyses on Nylonoligomer hydrolase)

Dr. Y. Higuchi (Prof: Xray structural analyses on nylonoligomer hydrolase)

Dr. N. Shibata (Prof: Xray structural analyses on nylonoligomer hydrolase)

sbourg Univ./CNRS/IPCMS

Dr. M. Boero (Prof :Metadynamics Simulation using CPMD)

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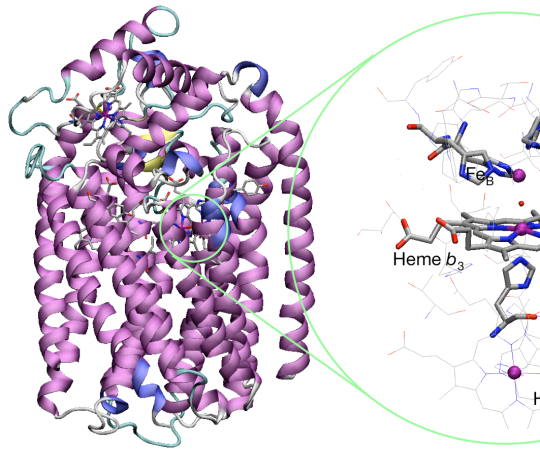
Analyses on
Interaction energy

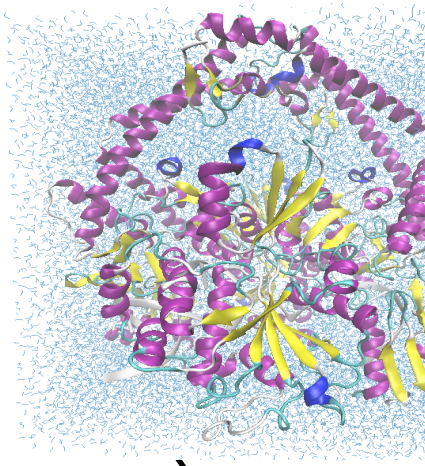
RSCPMD/PWCPMD

Dynamics on
Enzymatic reactions



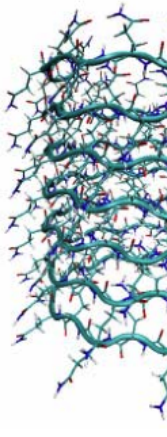
QM/MM

Static Enzymatic
Reaction Analyses



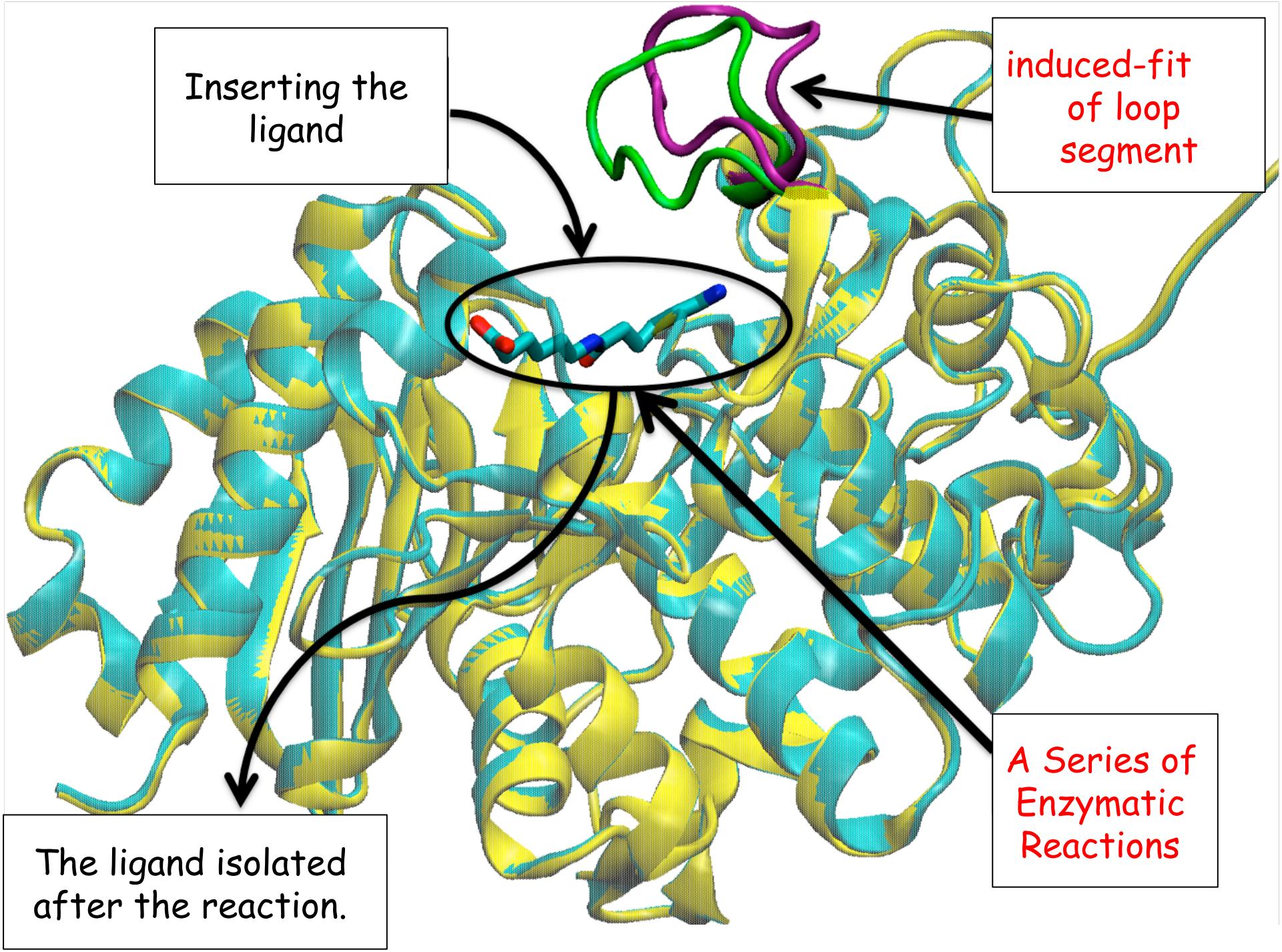
Classical MD

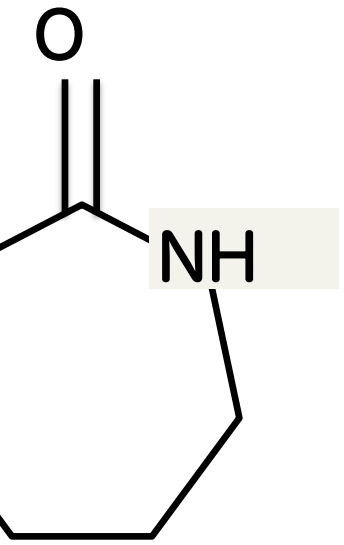
Analyses on structures, large amplitude motion, etc.



Coarse-grained/ Accelerated MD

Protein-Protein, Protein-Enzyme complex formation


Inserting the ligand
induced-fit of loop segment
A Series of Enzymatic Reactions
The ligand isolated after the reaction.



ring opening polymerization

$$H\text{-}(HN\text{-}(CH_2)_5\text{-}CO)_n\text{-}OH$$

- n > 200 Nylon-6
- n < 20 Ahx-linear oligomer
- n = 2 Ahx-linear dimer (A

Ahx-cyclic oligomer

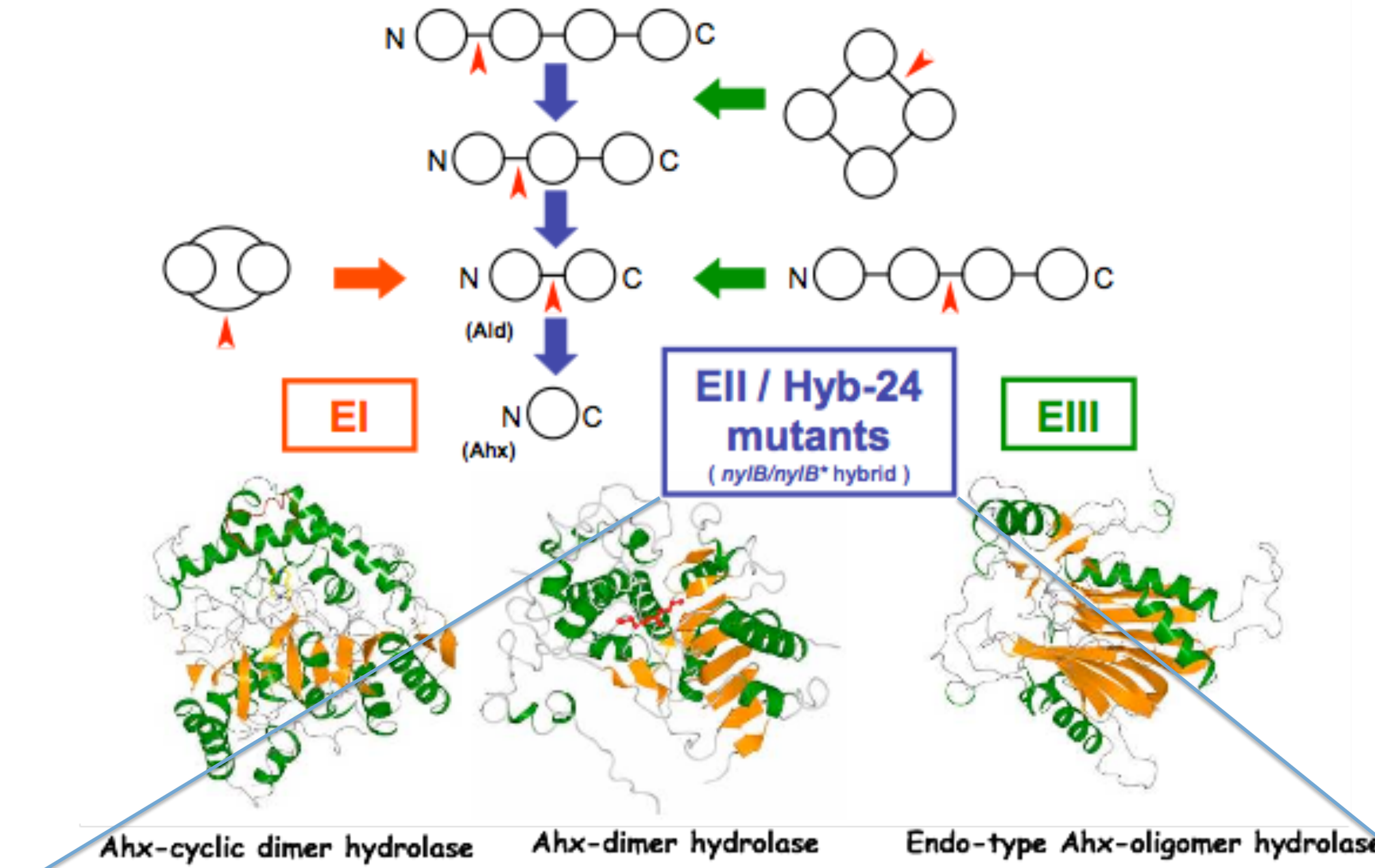
prolactum

. Biol. Chem., 39, 1219-1223, 1975

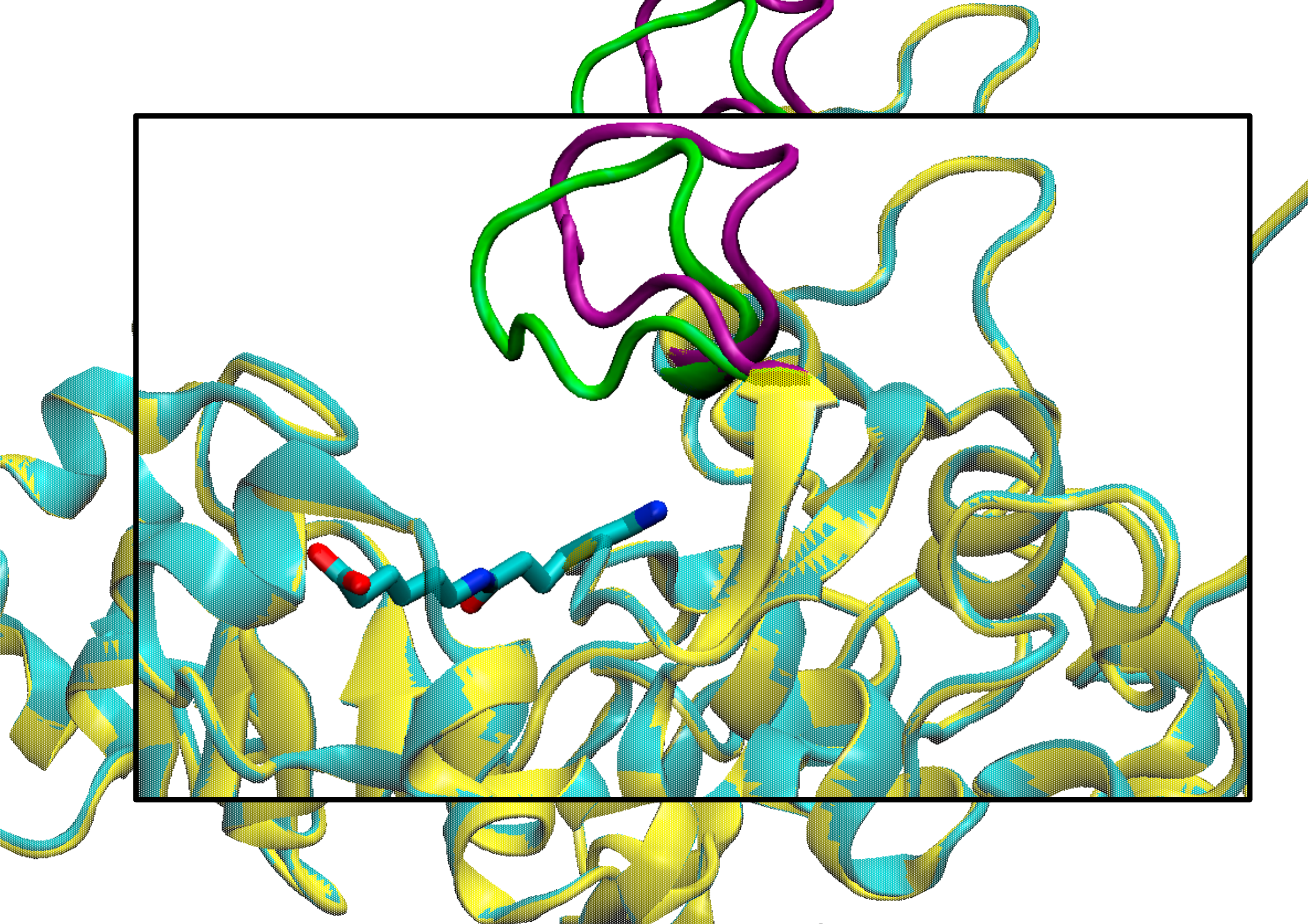
By products of Nylon

⇒ Main industrial waste

teria that degrades Nylon-oligomers to its monomer was f
he soil near Nylon factory in 1975 and X-ray structures of

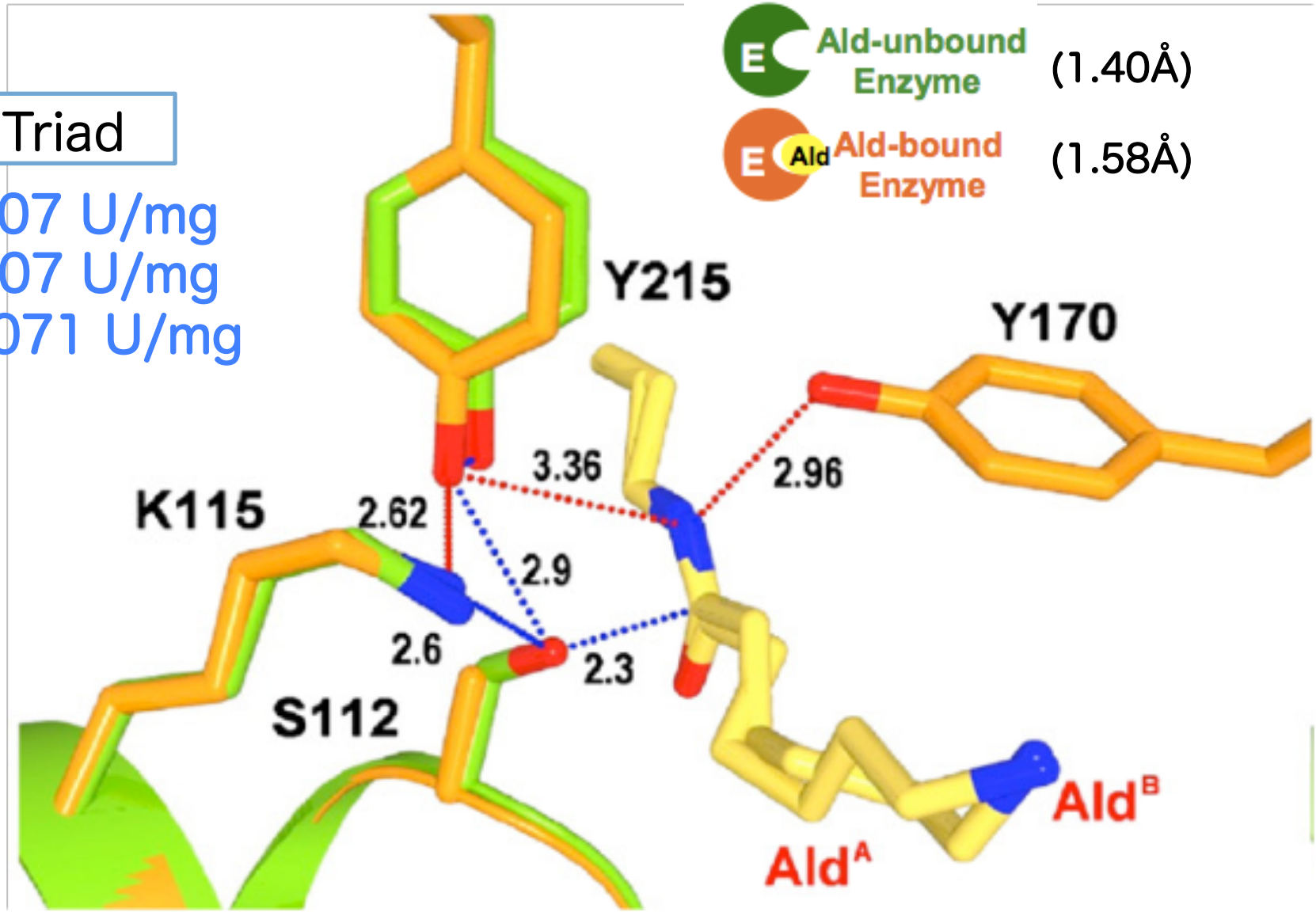

N
C
(Ald)
(Ahx)
EI
EII / Hyb-24 mutants
(nylB/nylB* hybrid)
EIII
Ahx-cyclic dimer hydrolase
Ahx-dimer hydrolase
Endo-type Ahx-oligomer hydrolase

EII



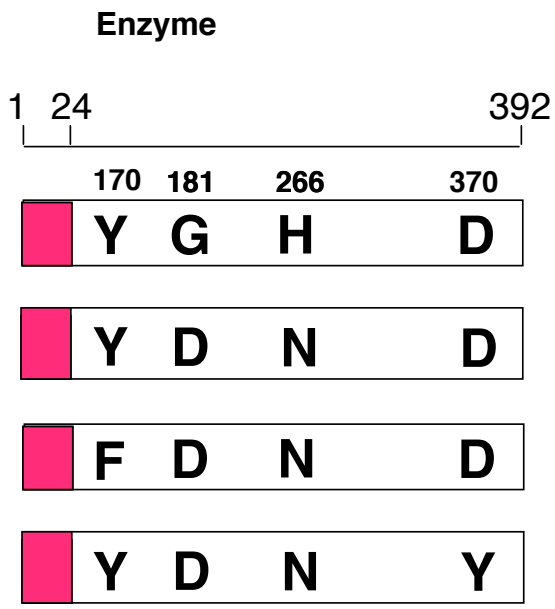
Catalytic Triad

2A <0.0007 U/mg
5A <0.0007 U/mg
5A 0.00071 U/mg

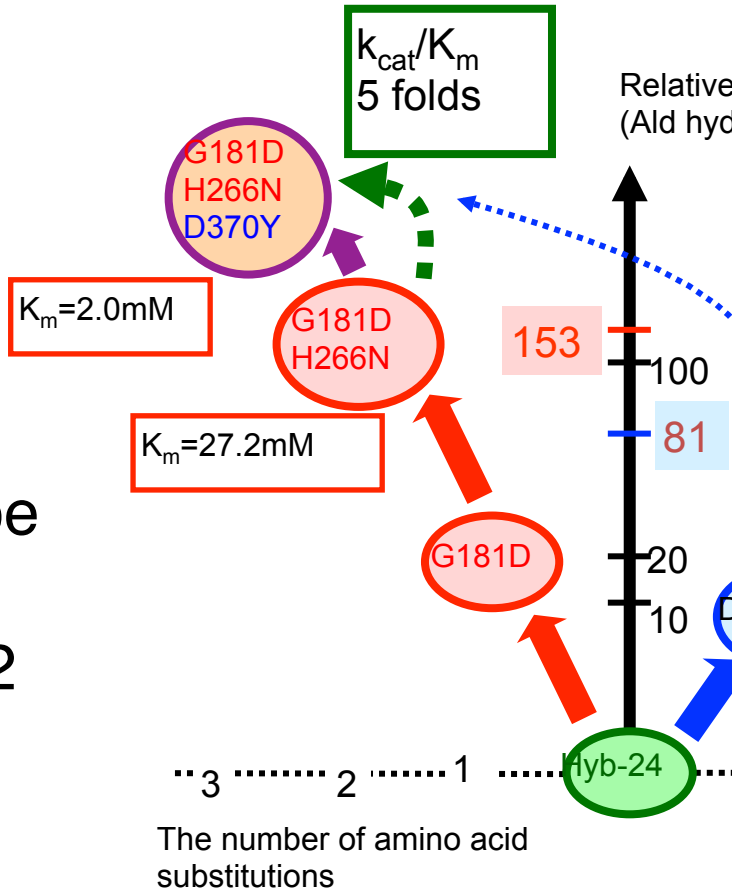


(Hyb24-DNY：3.53 U/mg)

Y170F mutant **0.05 U/mg**



	Ald-hydrolytic activity (U/mg)	
24	0.023	Original
24DN	3.53	
24DNF	0.05	
4DNY	3.60	Wild-type



ar Enzyme EII' is encoded in the same plasmid pOAD2
osed of 392 AA residues (the same as EII)

larity in AAs between EII and EII* is 88%
vity of EII* to Ald is 1/200 less than EII

-24 (24 AAs from N-terminal of EII* is taken into EII)

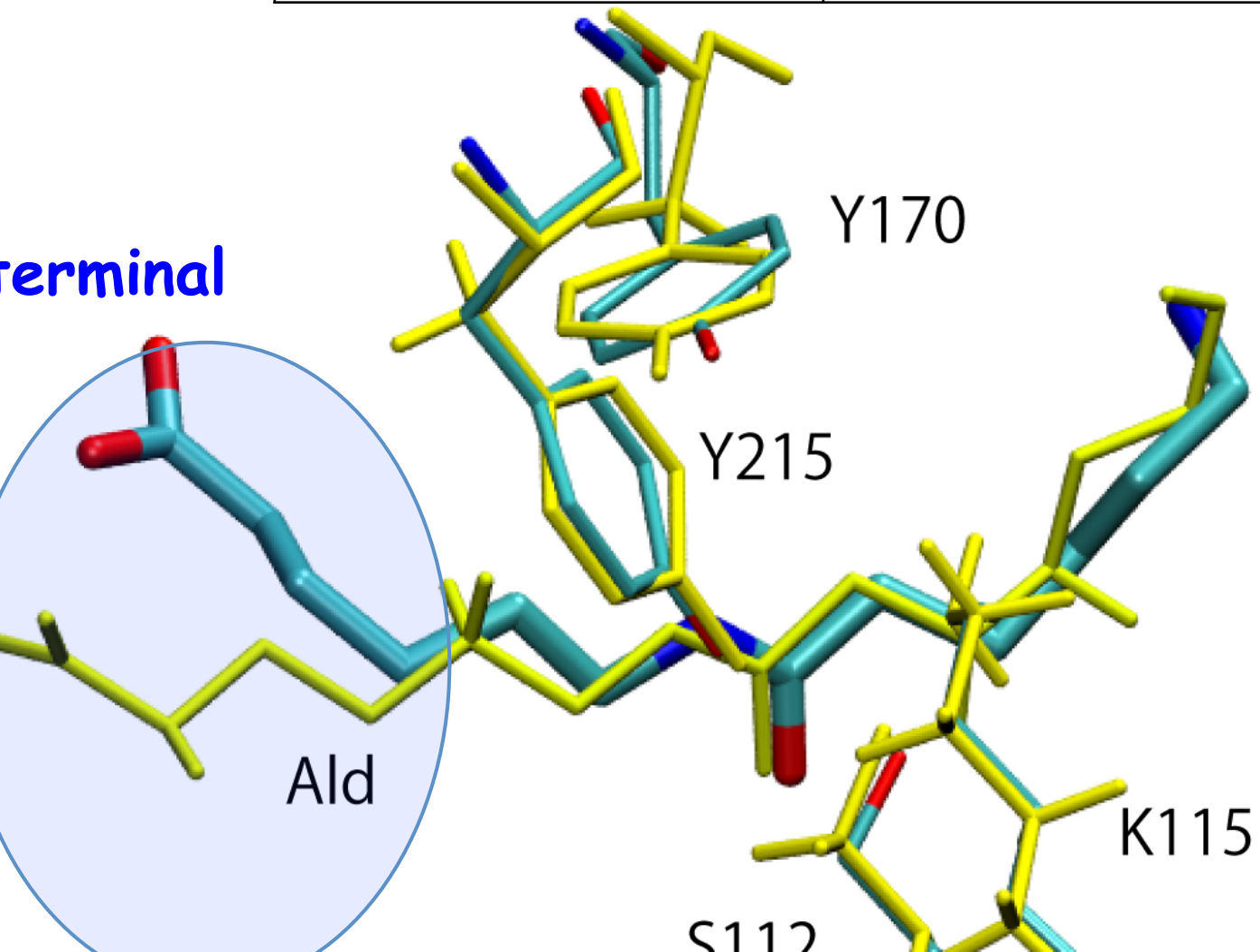
ce the mutagenesis studies were mostly based on the
perience, we would like to contribute to propose highly

Analyses on Structural Stability of Enzyme and its Mutant by Molecular Dynamics Simulations

Collaborators：T. Baba（OU）, K. Kamiya（KIT）
S. Negoro（HU）, Y. Higuchi（HU）, N. Shibata（HU）

~Hydrogen bonds around the catalytic residues~

Distance(Å)	MD	X-ray structure
N345-Ald393	2.9±0.10	2.86
SN112-Ald393	3.14±0.17	3.03
O170-Ald393	3.05±0.16	3.04



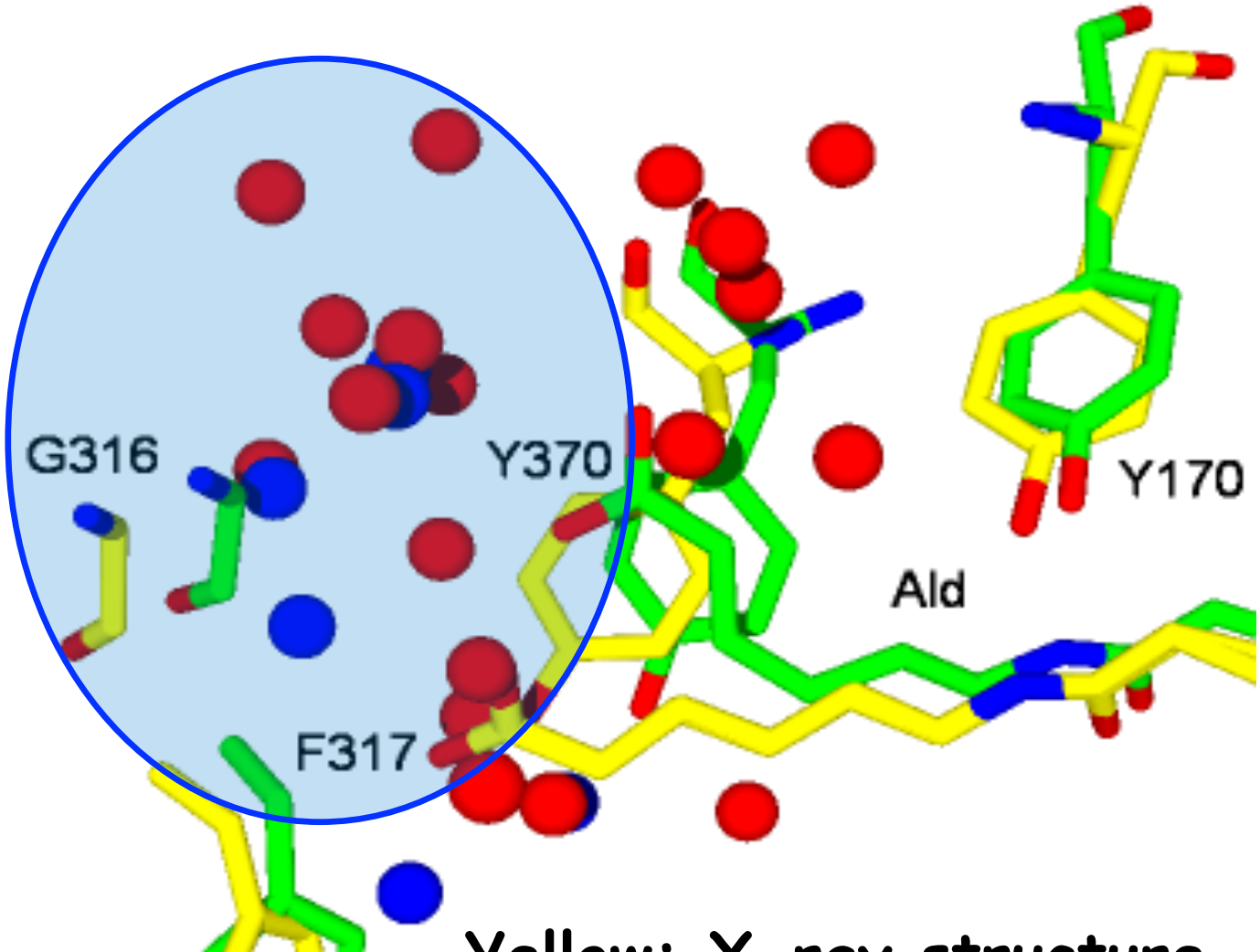
Yellow: X-ray

Blue : MD

Both structures are similar to each othe except for C-termi

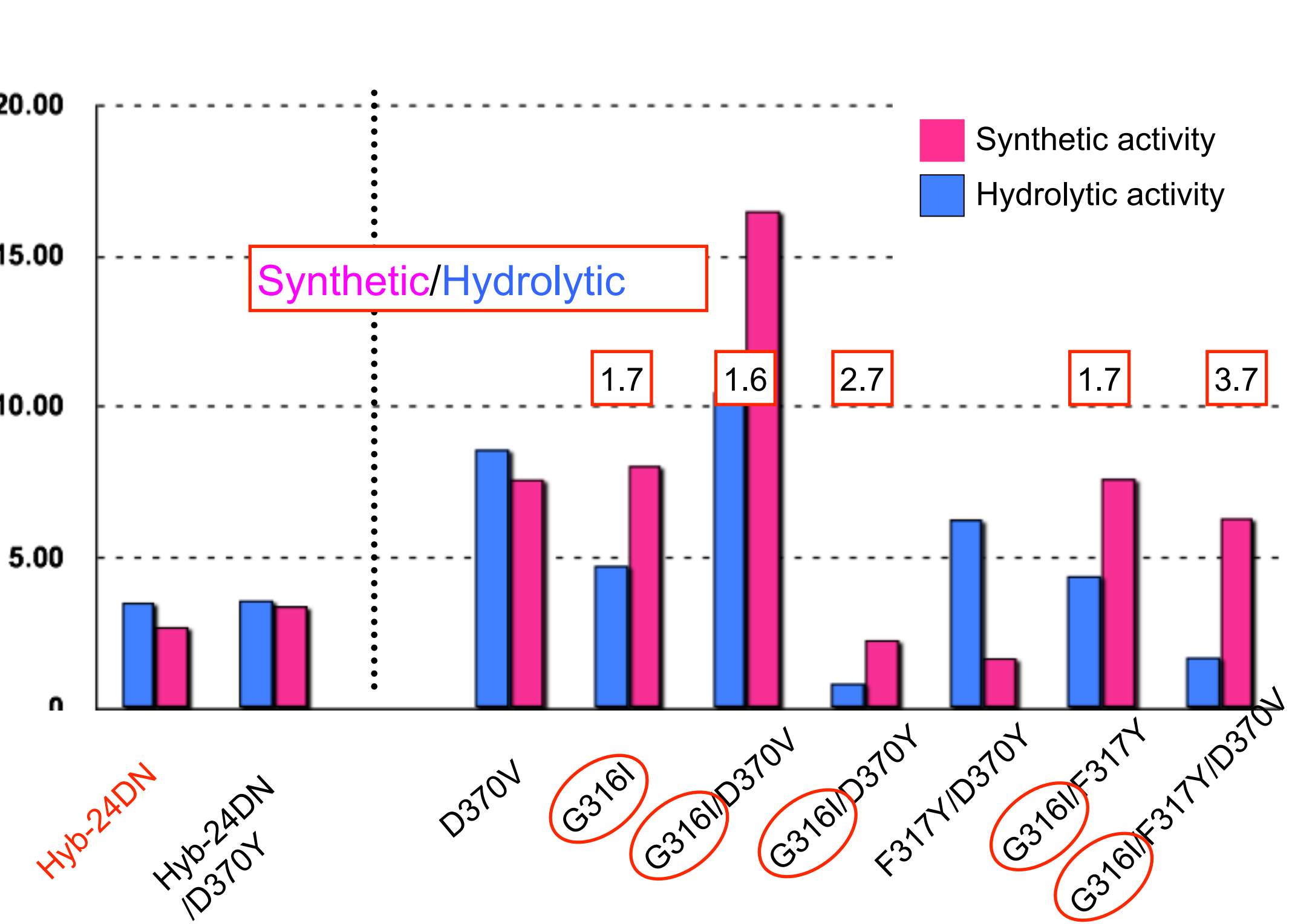
In X-ray structure Glycerol occupies th

	112 115 170 215 370	K_m	k_{cat}
rthrobacter sp.	S K Y Y D	21	9.0
	115 118 173 221 375		
seudomonas sp.	S K Y Y V	1.16	9.2



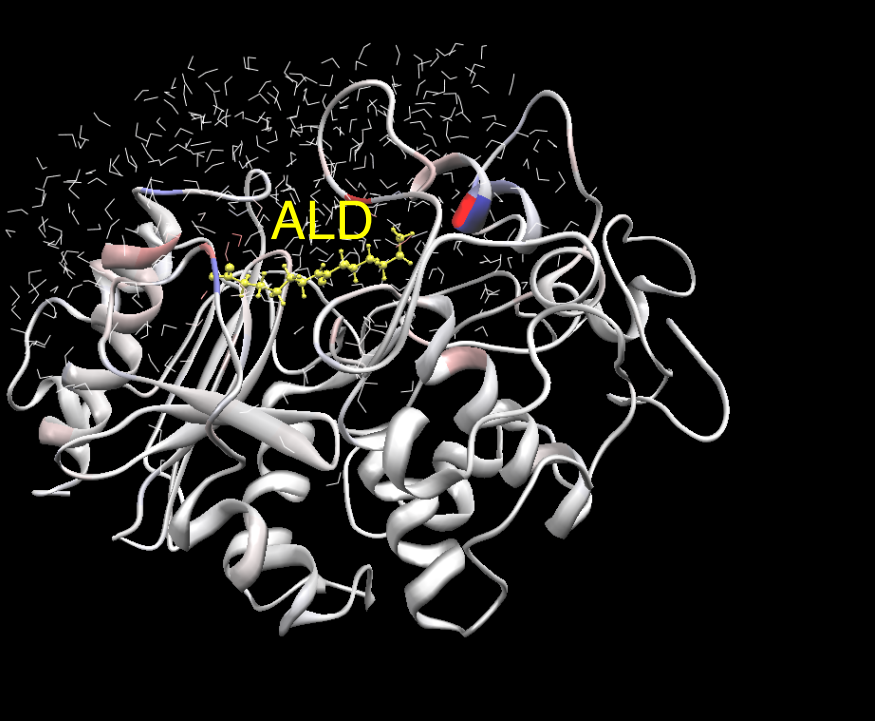
G316I
F317Y
D370V

Yellow: X-ray structure

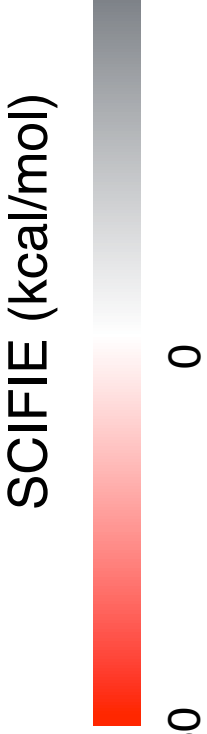

20.00
15.00
10.00
5.00
0
Synthetic activity
Hydrolytic activity
Synthetic/Hydrolytic
1.7
1.6
2.7
1.7
3.7
Hyb-24DN
Hyb-24DN /D370Y
D370V
G316I
G316I/D370V
G316I/D370Y
F317Y/D370Y
G316I/F317Y
G316I/F317Y/D370V

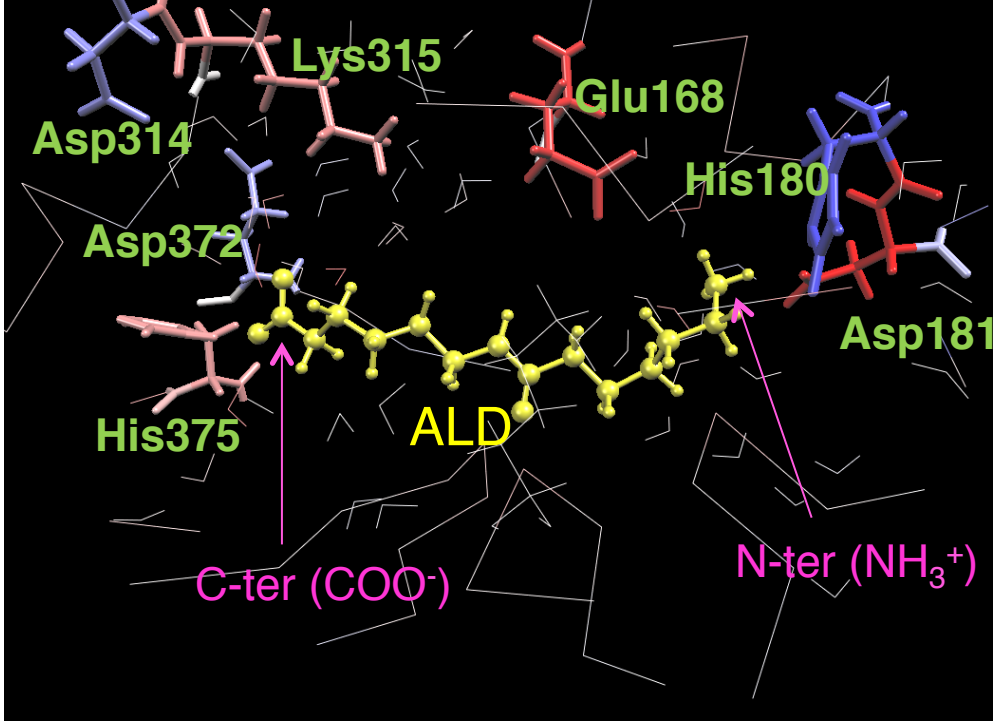
Analyses on NylB-substrate Complex by Fragment Molecular Orbital-based Inter-Fragment Interaction Energy Analyses

Collaborators：H. Ando（OU）, T. Baba（OU）, M. Nakano（OU）,
Y. Mochizuki（Rikkyo U）, C. Watanabe（U. Tokyo）, Y. Okiyama（U. Tokyo）



Total NylB

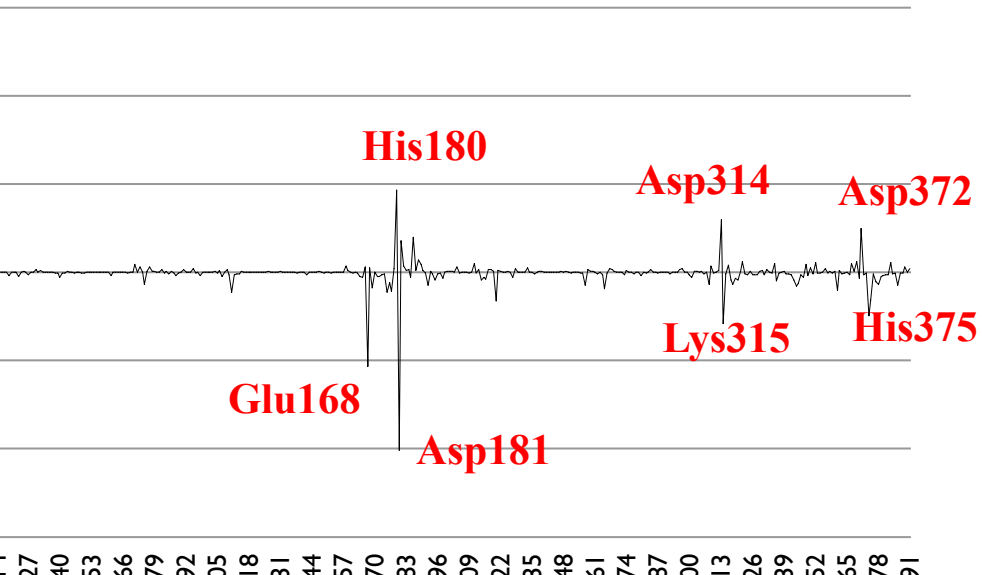




Around Active Site

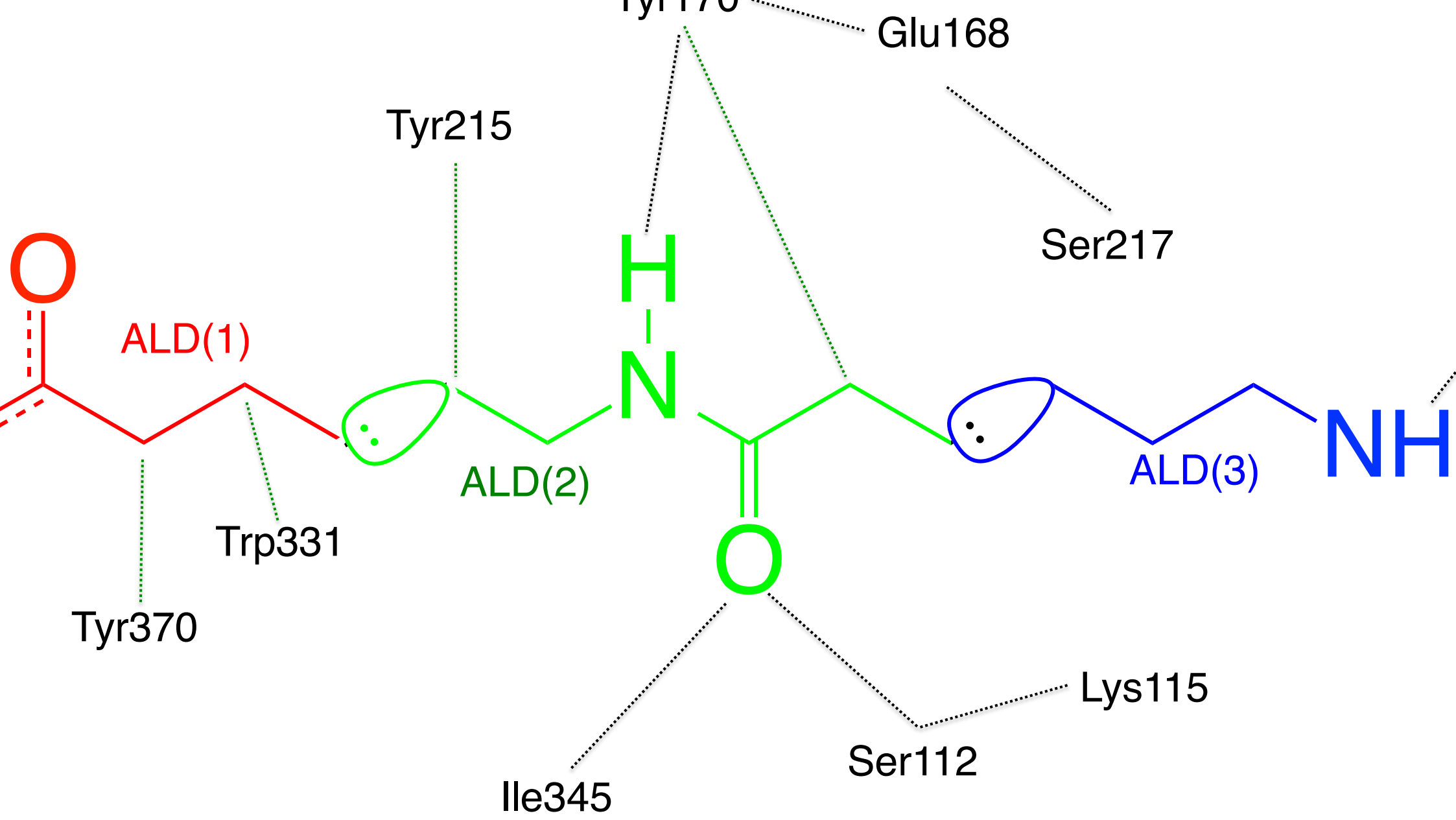
※At MP2/6-31G

SCIFIE between ALD and AAs



- Both terminals of ALD (COO^-、NH_3^+) strongly with charged AA residues
- We wants to precisely know the intera with amide moiety of ALD with AA resid

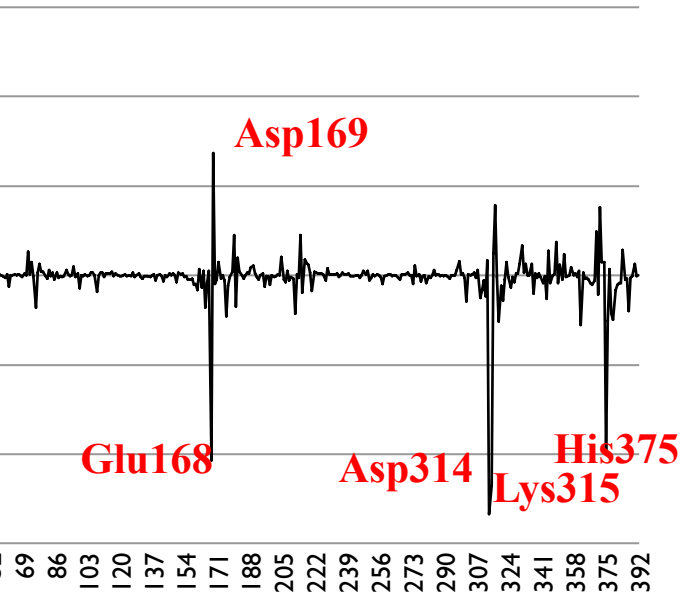
→ { Dividing ALD into N-ter (NH_3^+) , C-te (COO^-), and Amide (CO-NH) moieti


Tyr170
Glu168
Tyr215
Ser217
O
ALD(1)
H
N
ALD(2)
ALD(3)
NH
Trp331
Tyr370
O
Lys115
Ser112
Ile345
Hydrogen bonds
3 different moiety=fragme
ALD(1) → C-terminal
ALD(2) → Amide group
ALD(3) → N-terminal

$$\Delta SCIFIE = [SCIFIE_{Close}] - [SCICIE_{Open}]$$

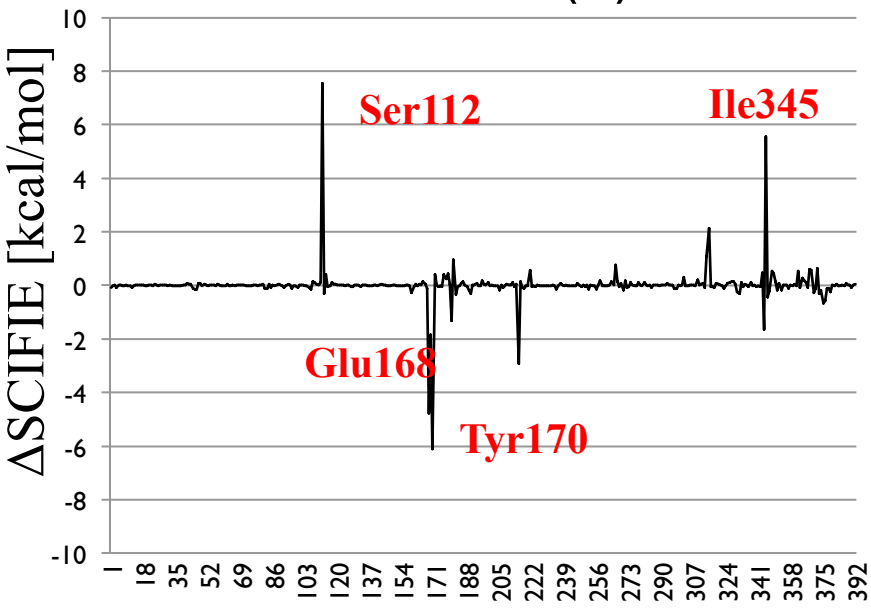
※MP2/6-31G* I

ALD(1)



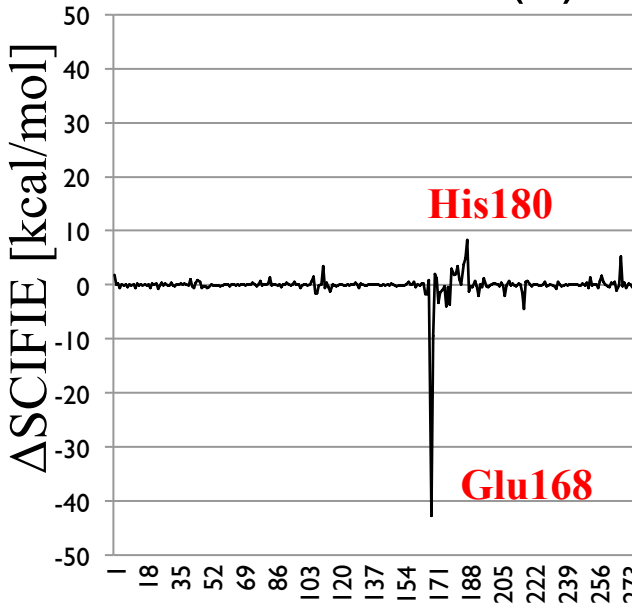
Amino acid residue

ALD(2)



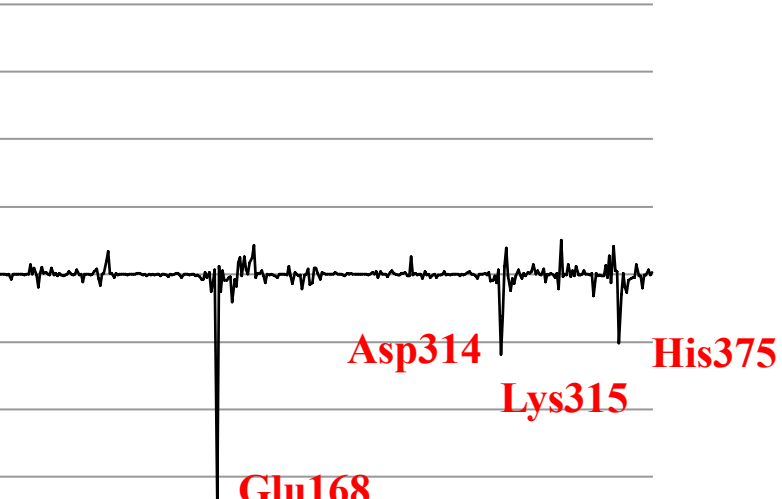
Amino acid residue

ALD(3)



Amino acid resid

otal Changes



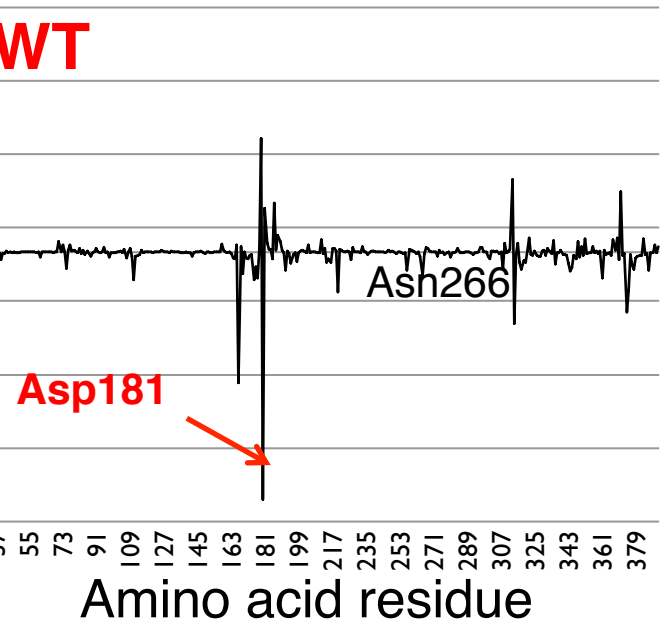
Forming closed structure results in

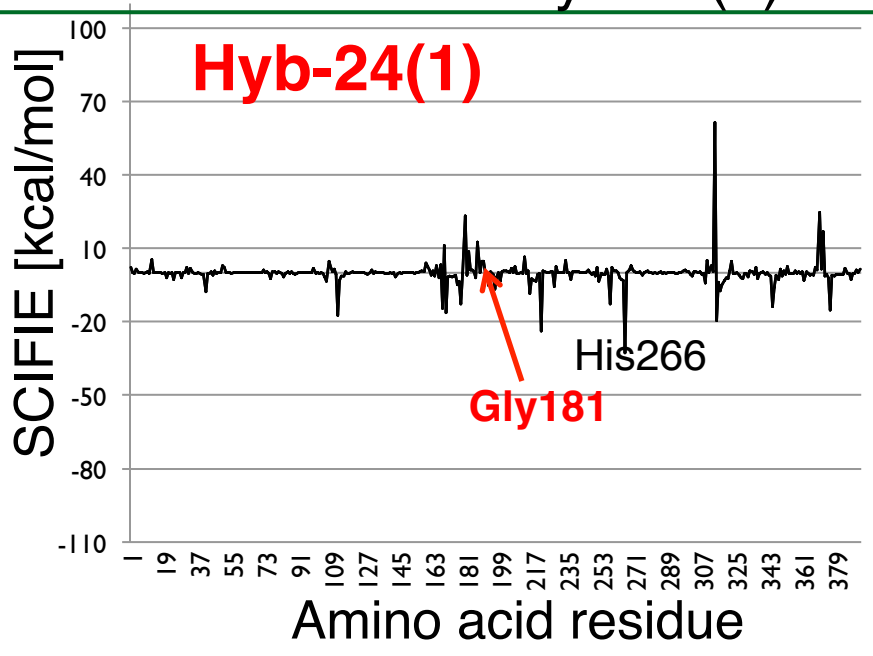
- ALD (1) → Gaining attractive interaction with neighboring AAs such as Asp314, Ly His375 and also with Glu168 in loop segment by totally 20 kcal/mol
- ALD (2) → Stabilizing amid moiety interacting w Glu168 and Tyr 170, but destabilizin Ser 112 and Ile 345

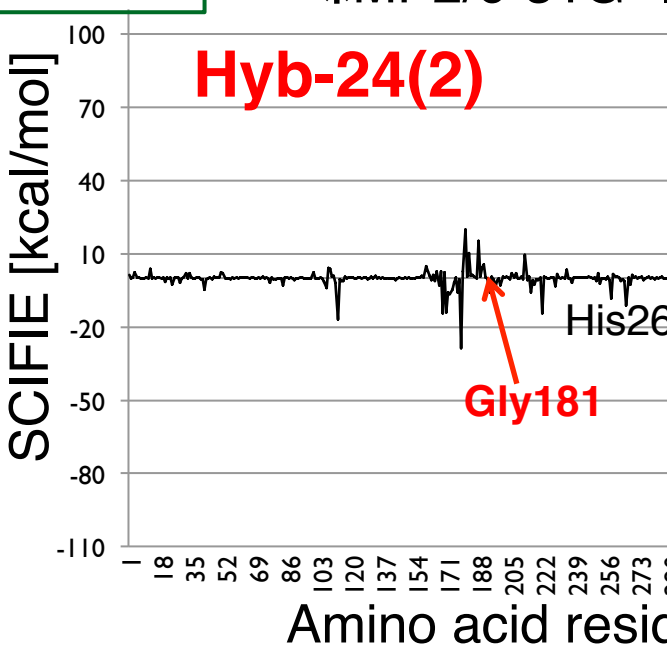
Hyb-24 is mutant with D181G and N266H

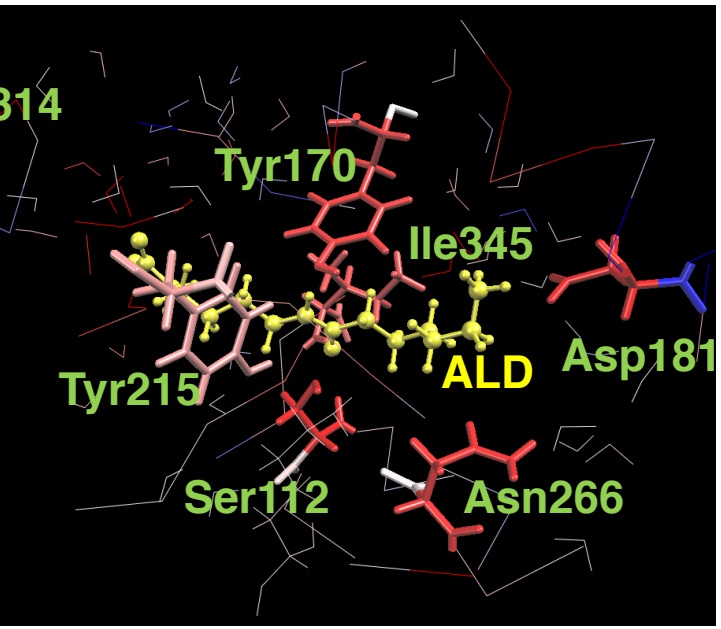
Hyb-24(1) is taken from MD at 9.475 ns and Hyb-24(2) at 10 ns

※MP2/6-31G*



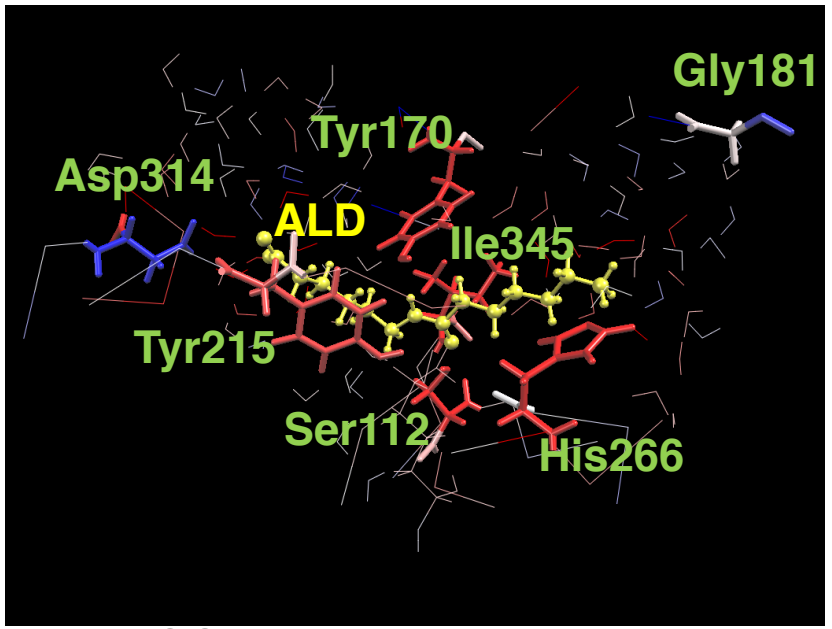






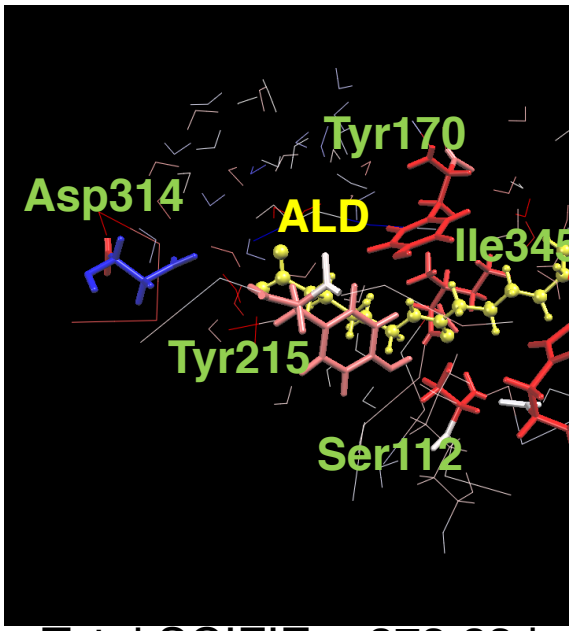
SCIFIE : -378.31 kcal/mol

81 SCIFIE : -100.94 kcal/mol



Total SCIFIE : -286.29 kcal/mol

Gly181 SCIFIE : -0.82 kcal/mol

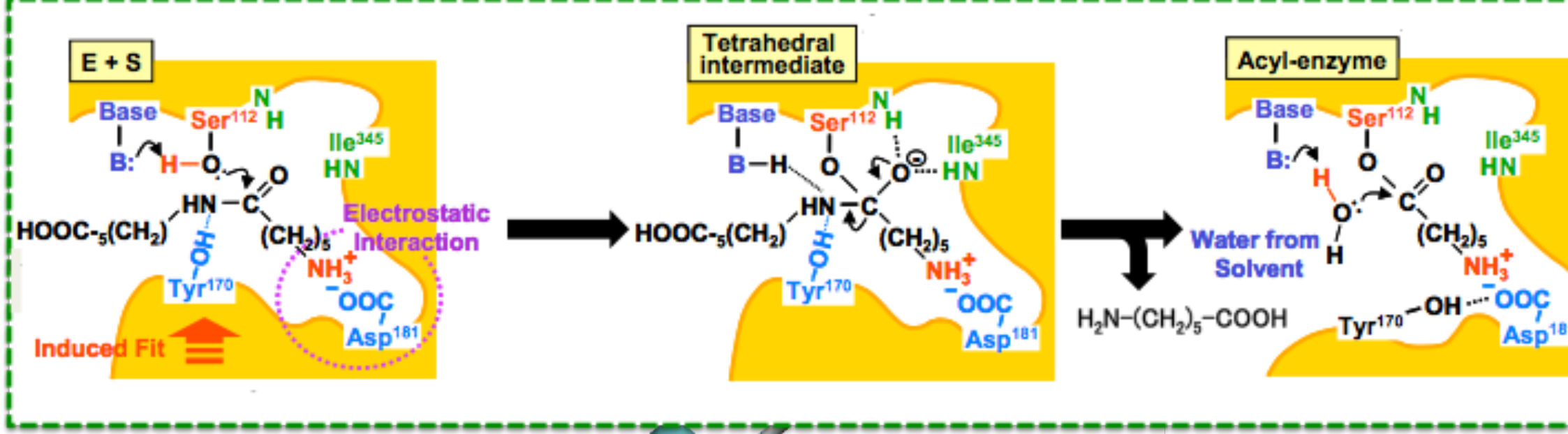


Total SCIFIE : -273.23 kc

Gly181 SCIFIE : 0.68 kc

Enzymatic Reactions of NylB By QM/MM Molecular Dynamics Simulations

Collaborators：K. Kamiya（KIT）, T. Baba （OU）, T. Matsui (AICS),
M. Boero (IPCMS, France), S. Negoro（HU)



eaction Coordinates

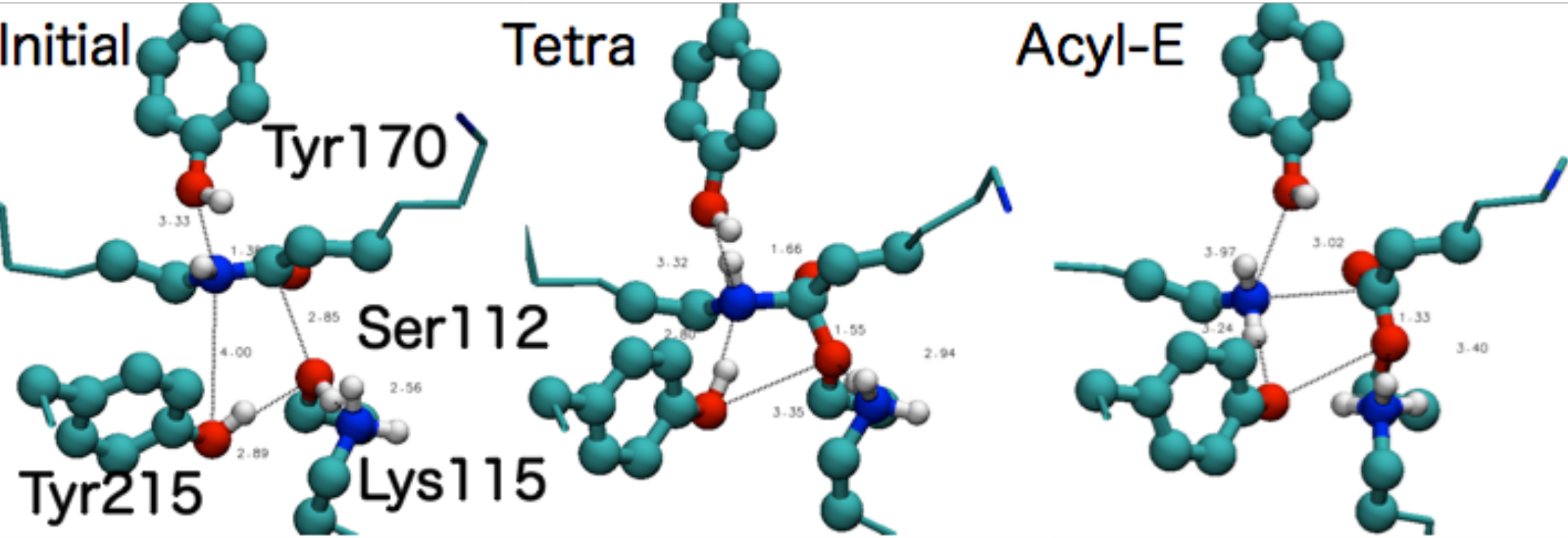
Tyr170

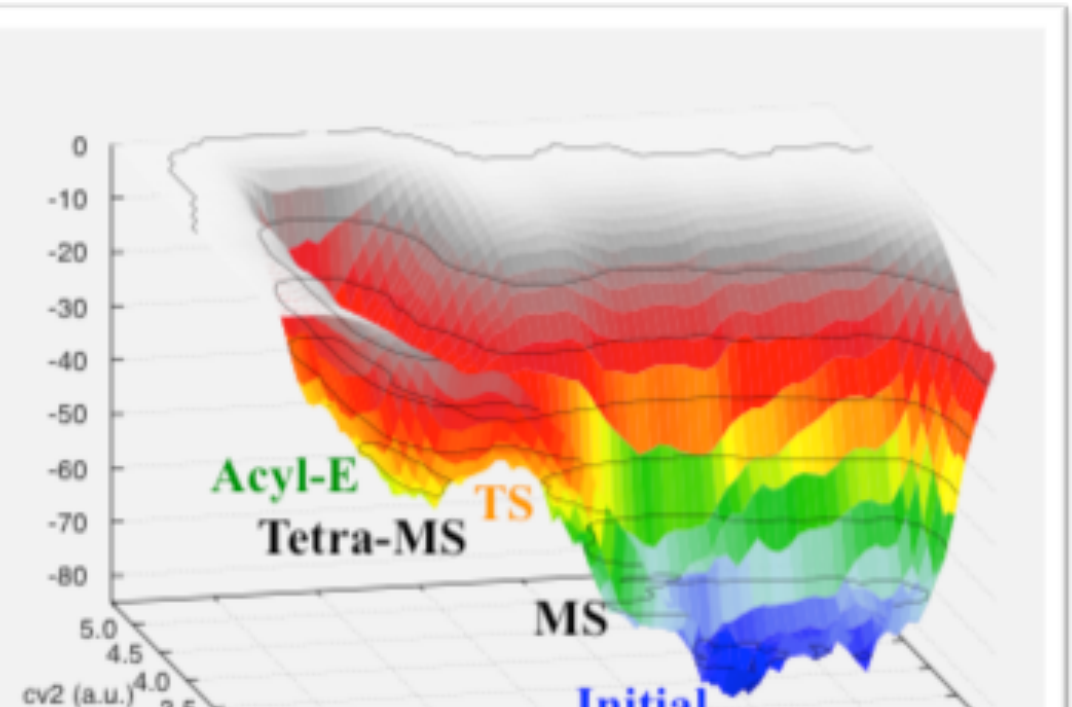
ALD

C-N Cleavage（s_1）

Ser nucleophylic attack（s_2）

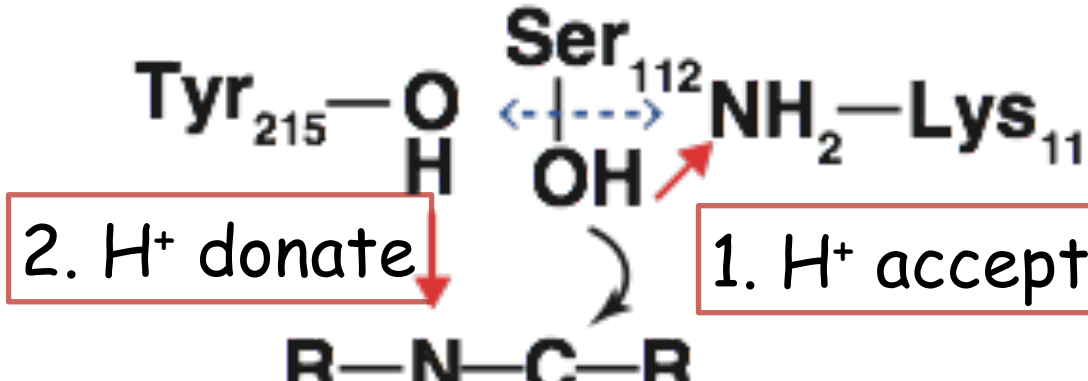
Try170 always binds to subst

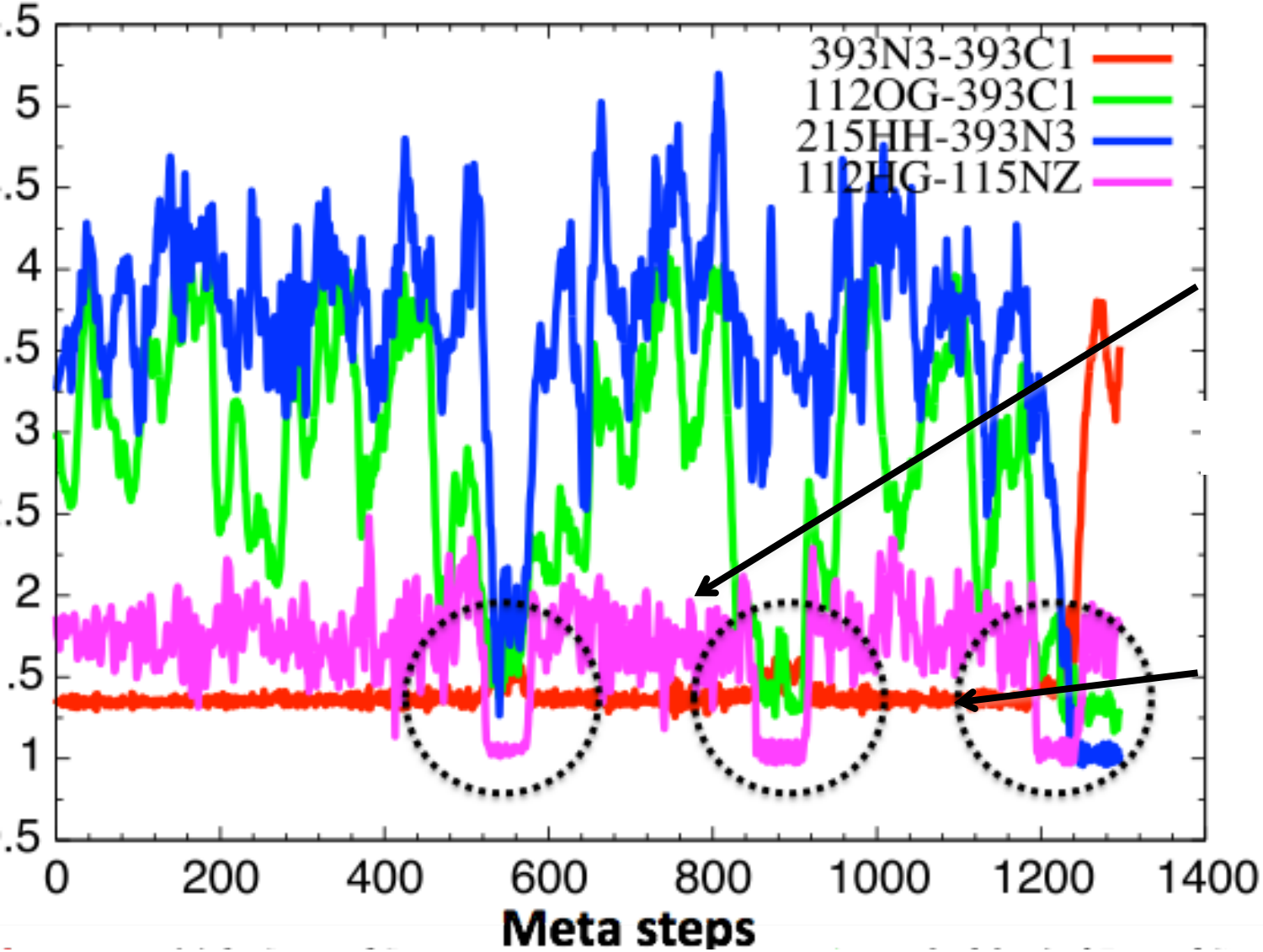


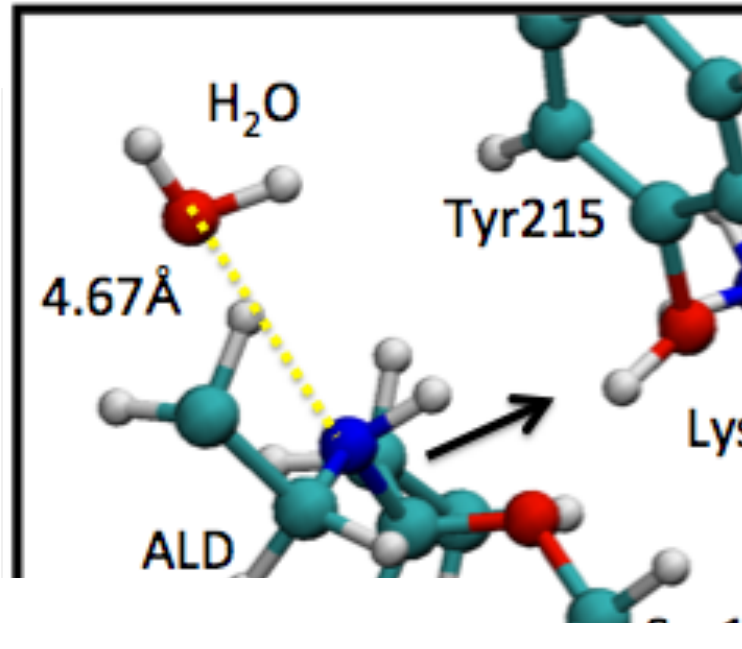


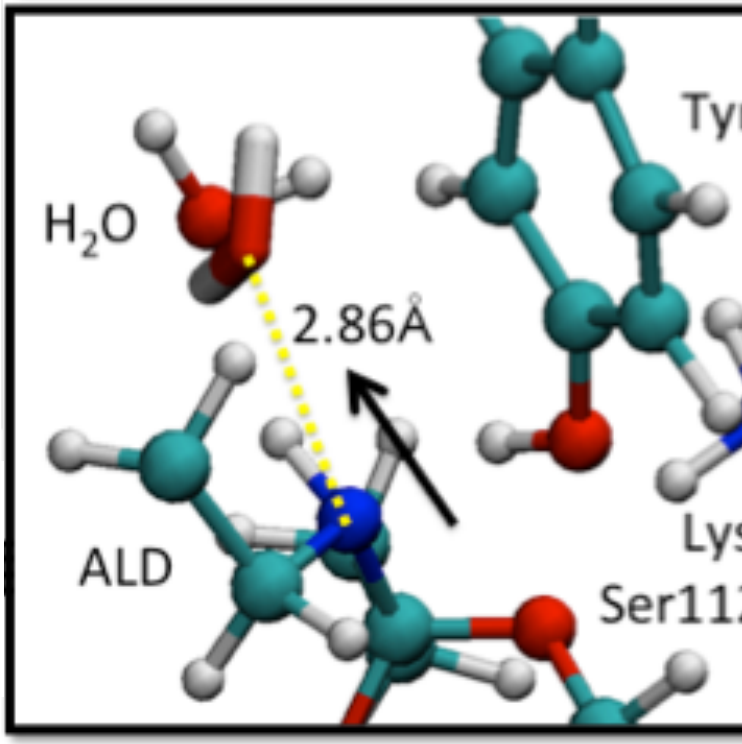
Ser nucleophilic reaction

3. HB bw NH_3^+ & OH







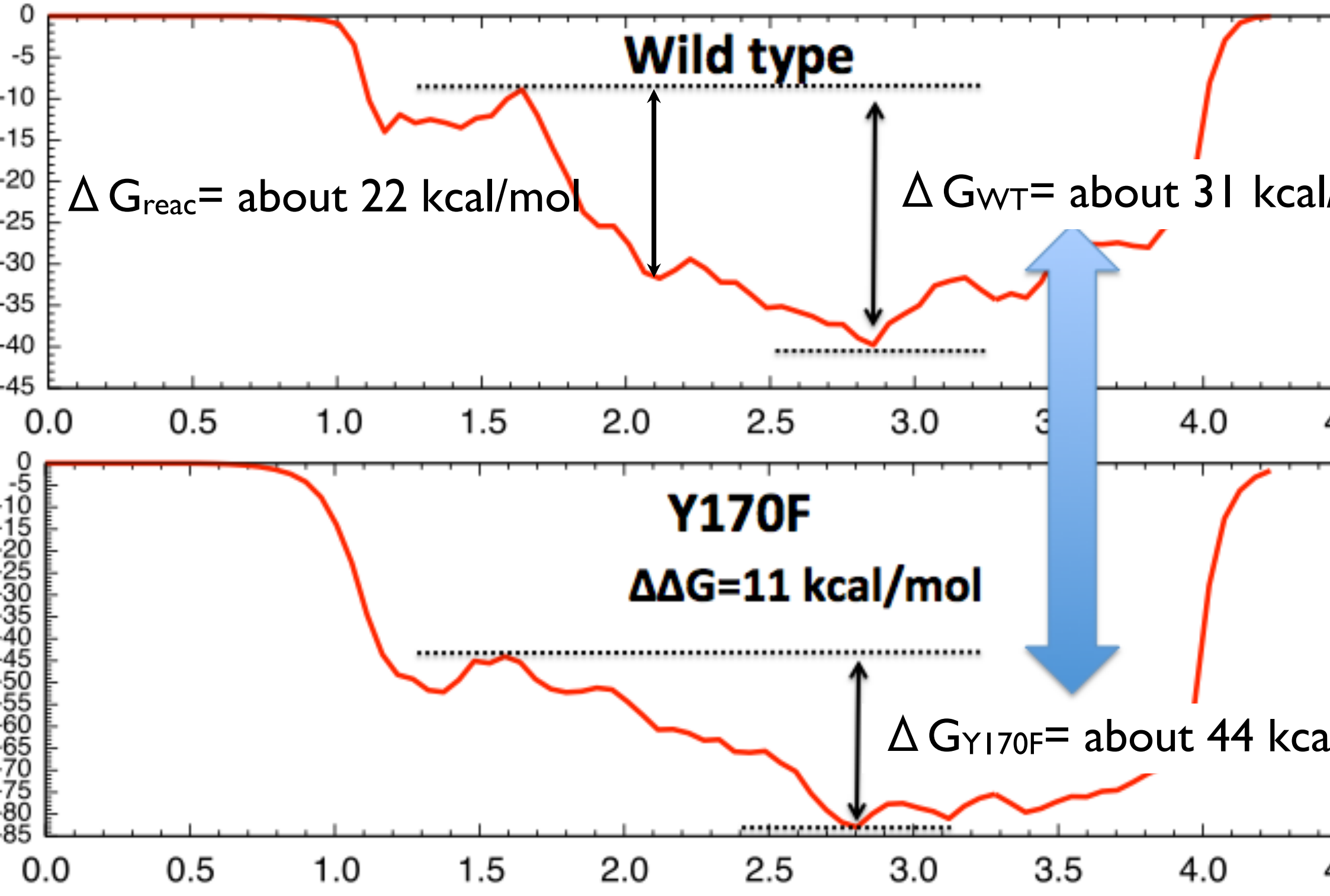


: $d_{Amide,ALD}$

: $d_{Hy215\text{-}NALD}$

Green : $d_{OK115\text{-}CALD}$

Purple : $d_{HS112\text{-}NK115}$


Wild type
Δ Greac= about 22 kcal/mol
Δ GWT= about 31 kcal/
Y170F
ΔΔG=11 kcal/mol
Δ GY170F= about 44 kca

Analyses on various processes that carried by NylB were perfromed with several methods.

Structural Analyses（MD）

・ Differences between X-ray and MD structures

Interaction Analyses（FMO）

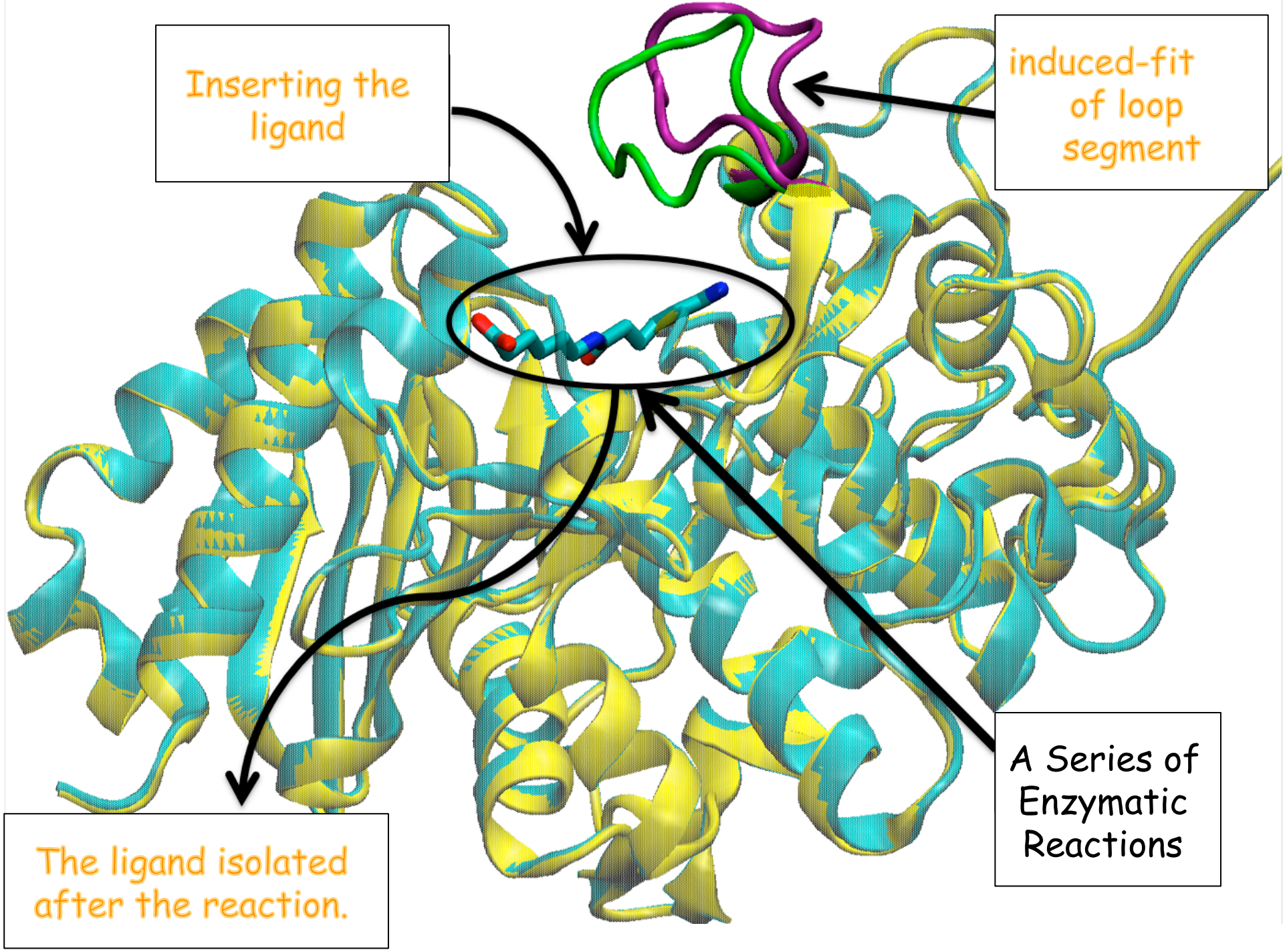
・ Role of Asp181 is quantitatively clarified

Reaction Analyses（QM･MM-CPMD）

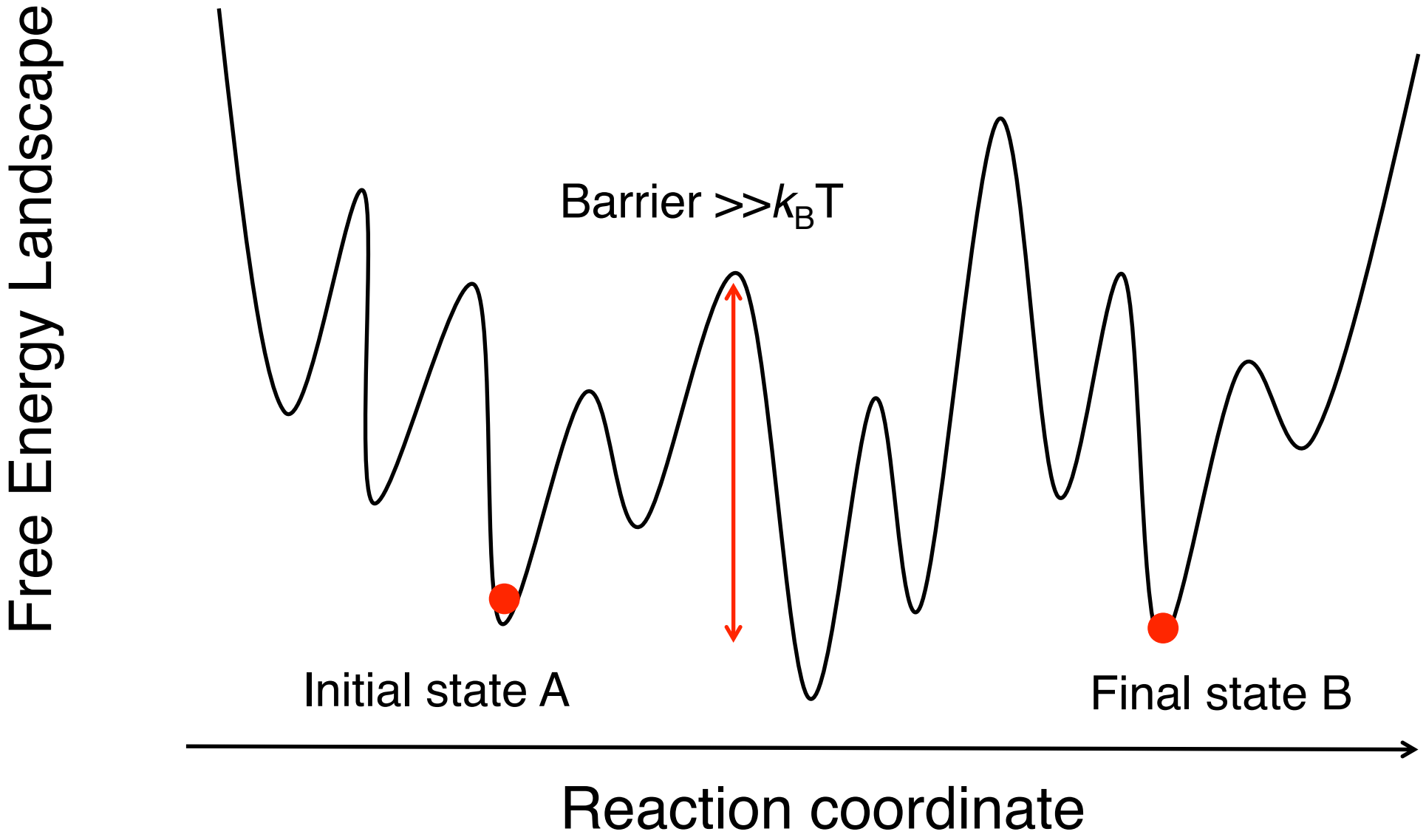
・ Interplay of catalytic triad Ser/Lys/Tyr and Tyr170 results in regulation mechanism of orientation of a hydrogen bond

Protein-Substrate Binding Processes by PaCS-MD Methods

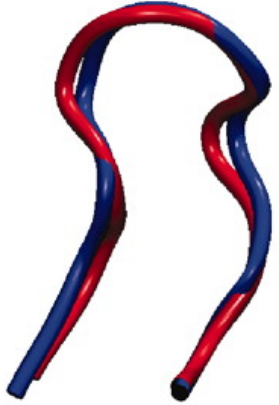
Collaborators：T. Baba（OU）, R. Harada (AICS), M. Nakano（OU）,



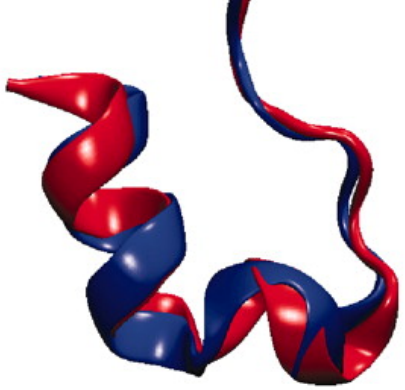
Difficult problems even no



Large amplitude motion takes long time and Folding occurs as a stochastic processes, so it is difficult to accomplish with available MD simulations. (Except now for long time dynamics done by David Shaw with Anton)

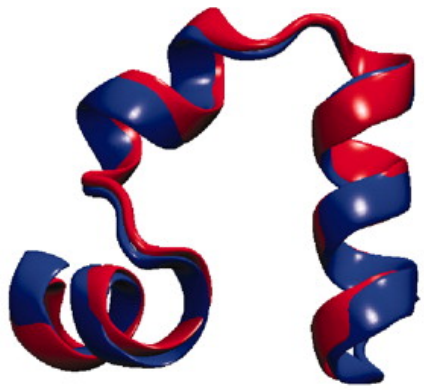


Chignolin 106 *μs*
cln025 1.0 Å 0.6 *μs*



Trp-cage 208 *μs*
2JOF 1.4 Å 14 *μs*

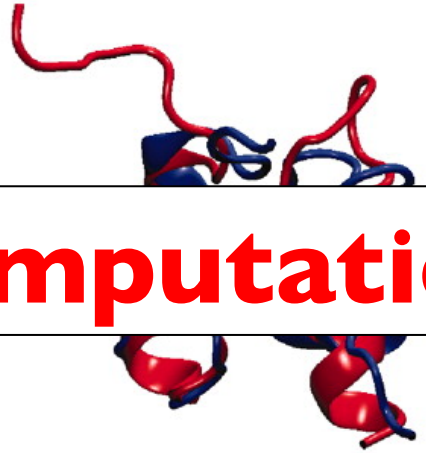
BBA 325 *μs*
1FME 1.6 Å 18 *μs*



Villin 125 *μs*
2F4K 1.3 Å 2.8 *μs*

WW domain 1137 *μs*
2F21 1.2 Å 21 *μs*

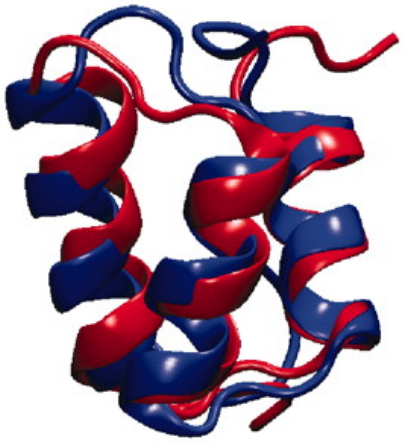
NTL9 2936 *μs*
2HBA 0.5 Å 29 *μs*

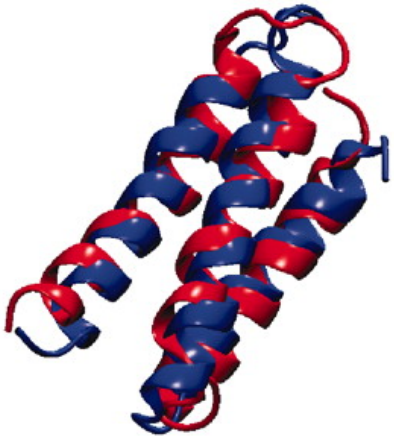


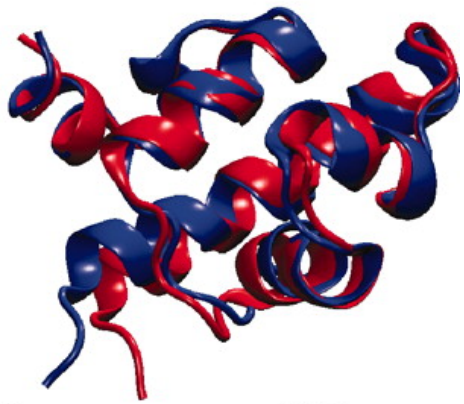
BBL 429 *μs*
2WXC 4.8 Å 29 *μs*

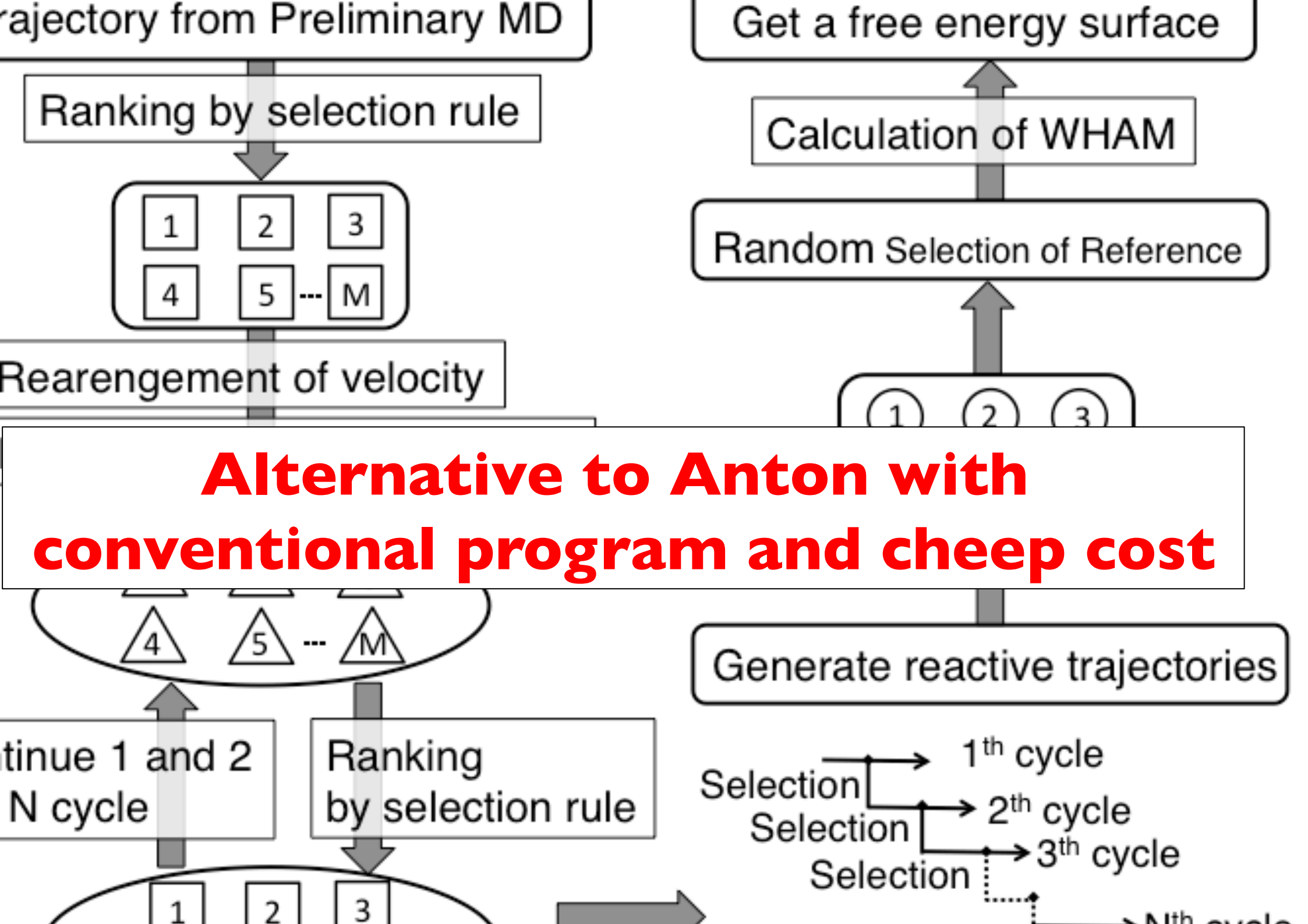
Protein B 104 *μs*
1PRB 3.3 Å 3.9 *μs*

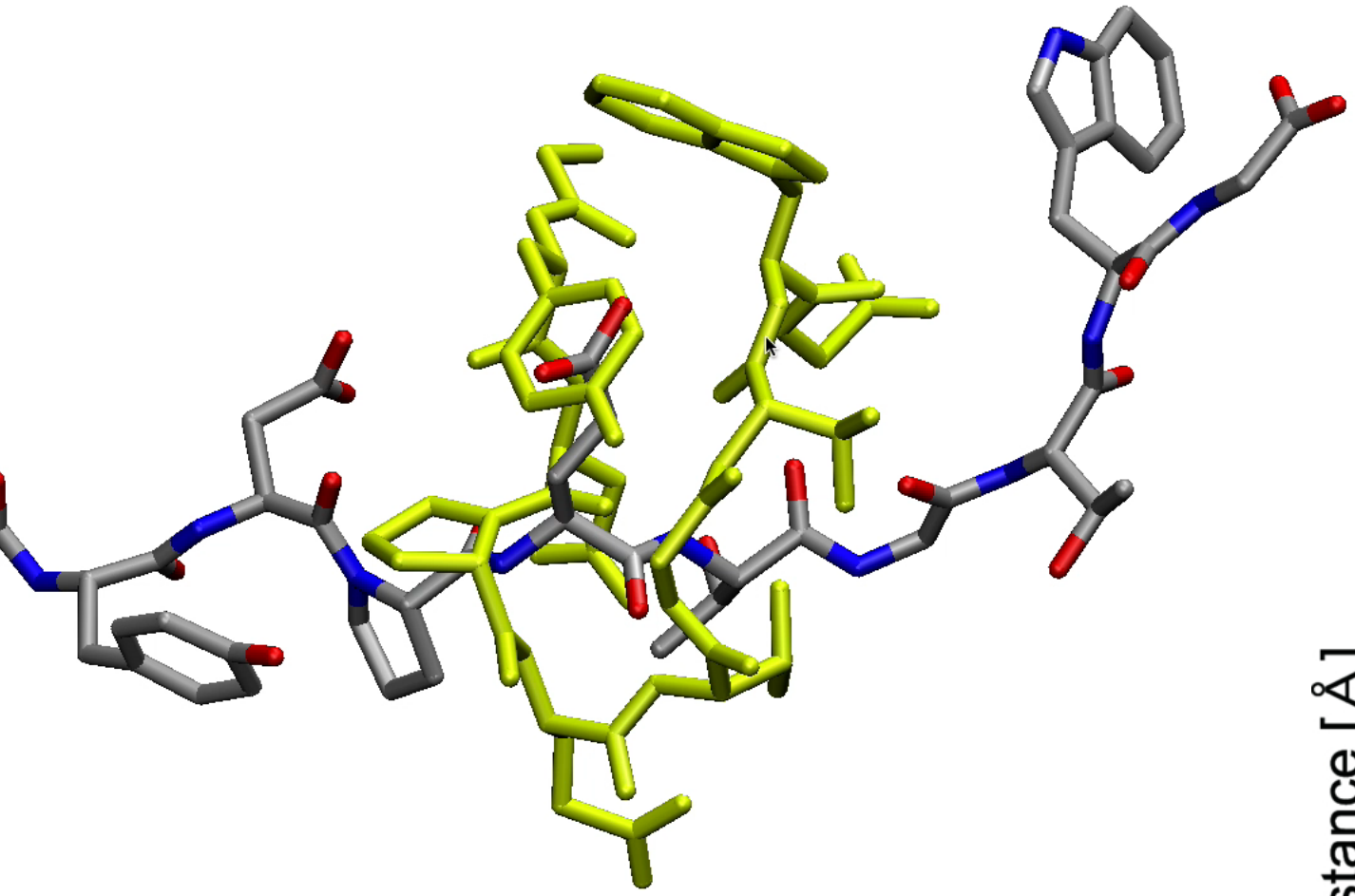
It needs “Vast” computational cost

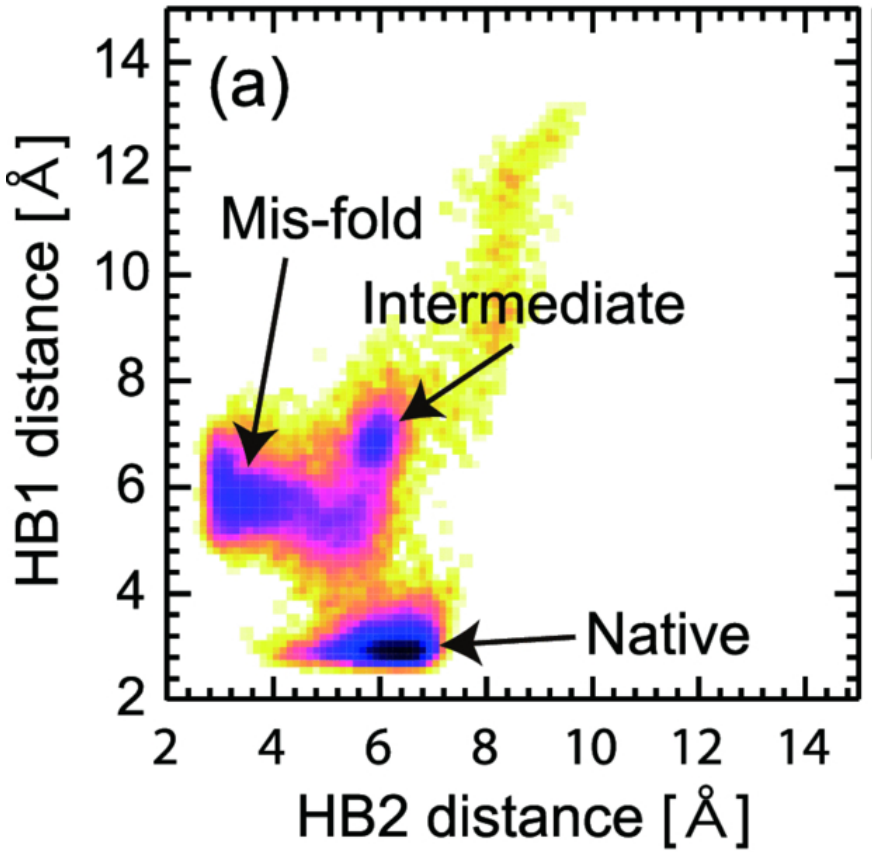


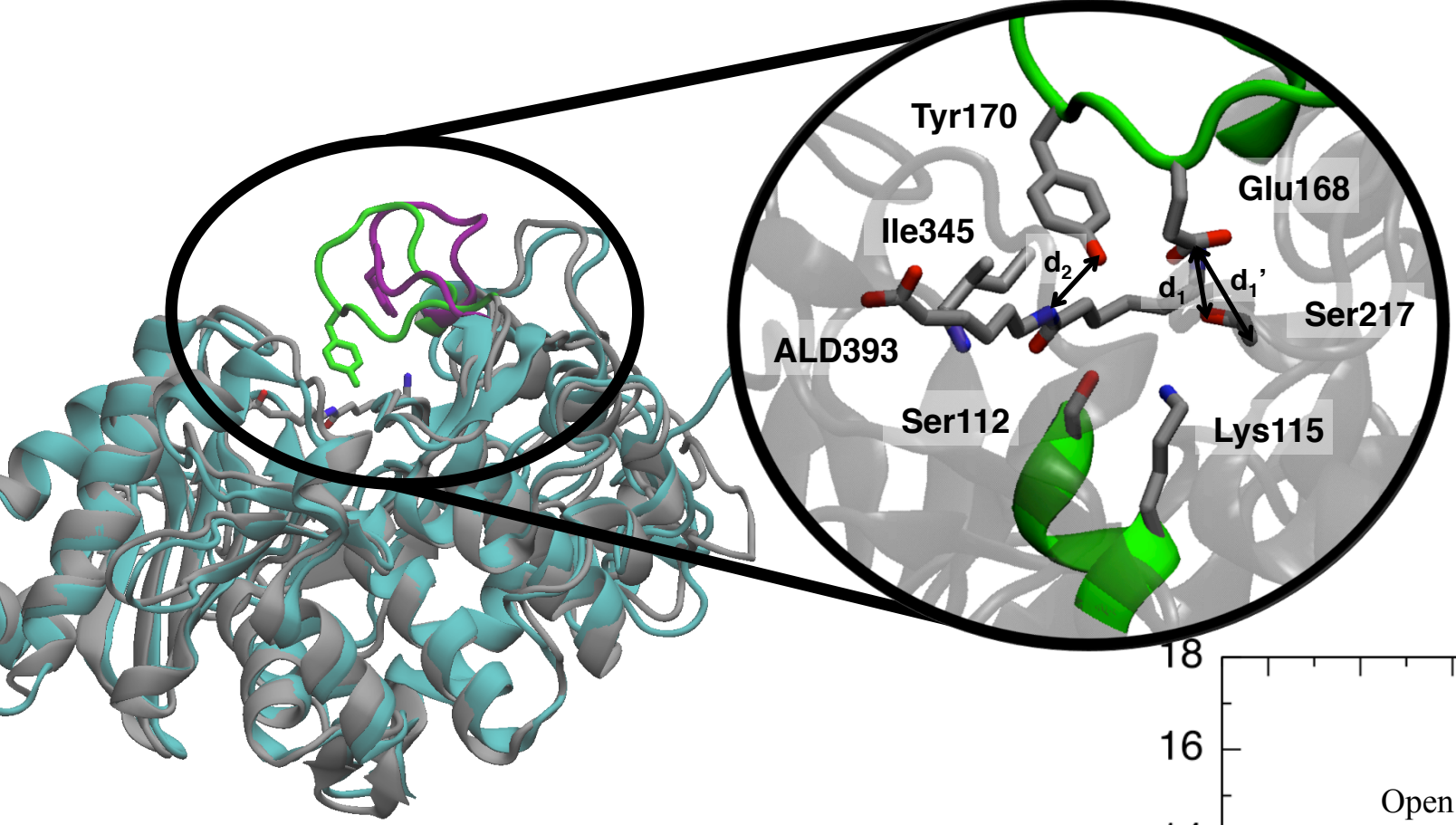




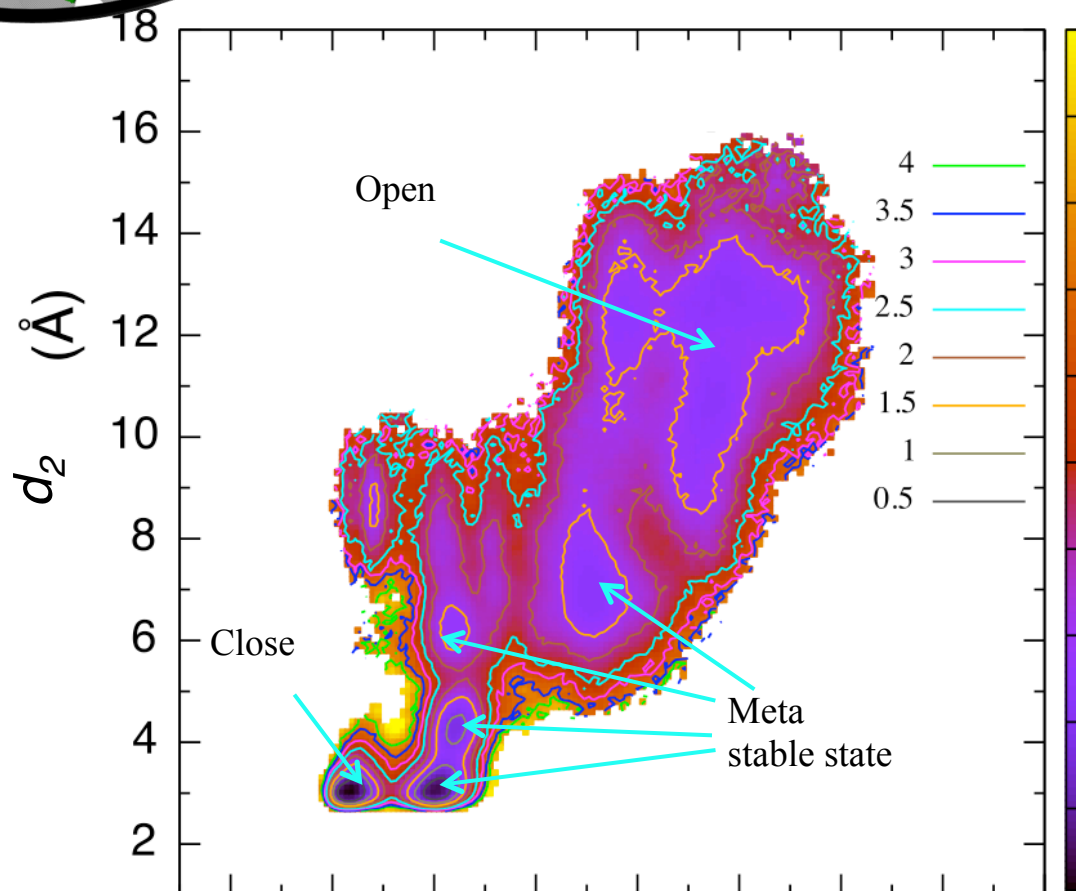

rajectory from Preliminary MD
Ranking by selection rule
1
2
3
4
5
M
Rearengement of velocity
Get a free energy surface
Calculation of WHAM
Random Selection of Reference
Generate reactive trajectories
Alternative to Anton with conventional program and cheep cost
tinue 1 and 2
N cycle
Ranking by selection rule
Selection
1th cycle
Selection
2th cycle
Selection
3th cycle



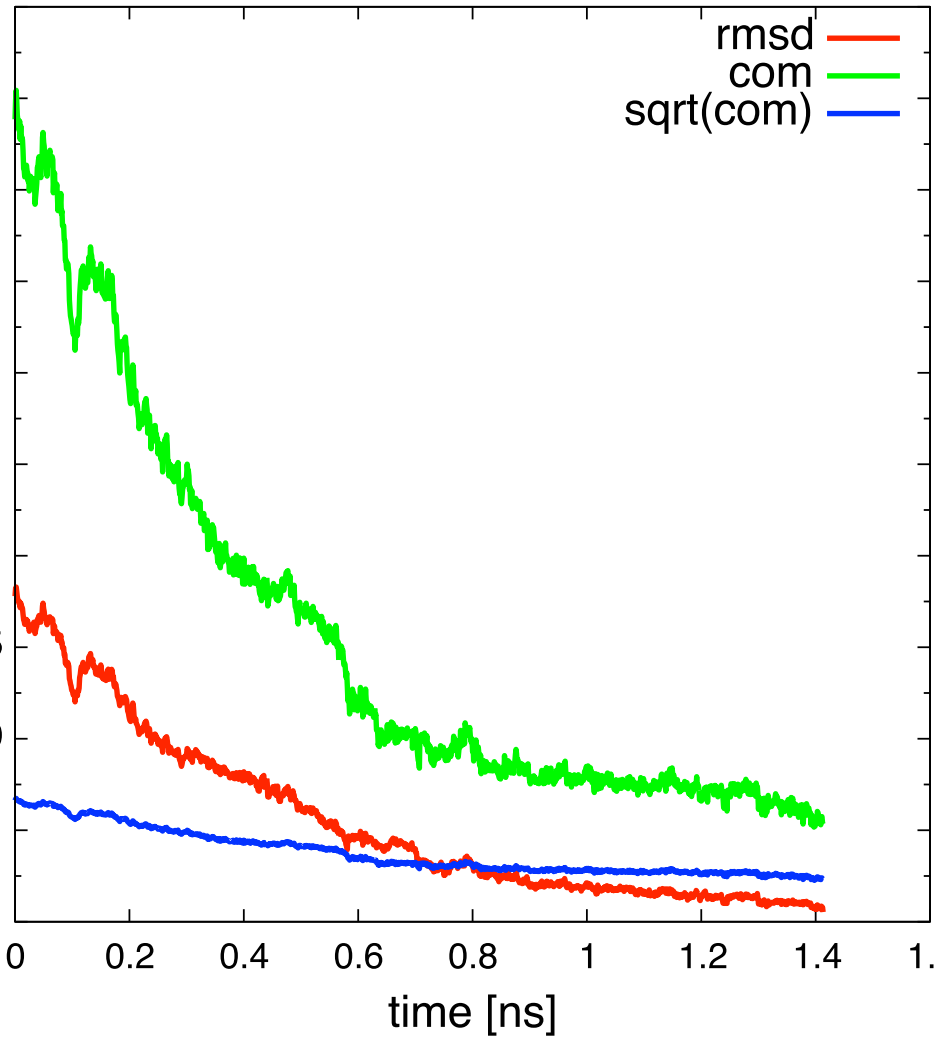

(a)
Mis-fold
Intermediate
Native
HB1 distance [Å]
HB2 distance [Å]
2
4
6
8
10
12
14

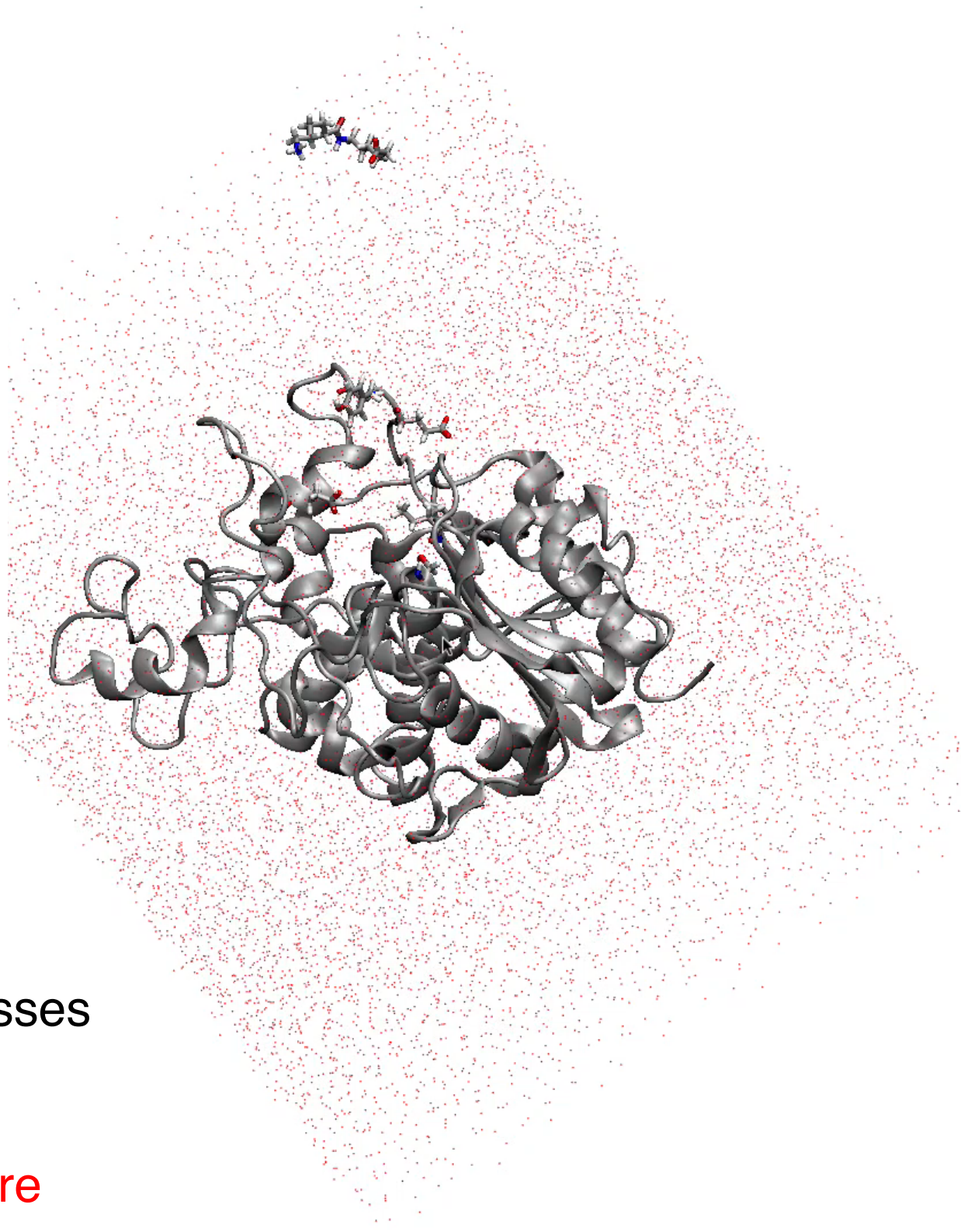


- Distance bw Glu168 – Ser217
- Distance bw Tyr170 – N_{ALD}



ere are many metastable structure from
en state with vast FEL space to Closed
te with narrow FEL basin.





: Distance bw two Center-of-Masses

SD : RMSD toward X-ray Structure

e can precisely predict X-ray Structure