ハイブリッド分子動力学計算による新規の生体反応機構の解析

筑波大学計算科学研究センタ
物質生命研究部門・生命科学分野

舘野　賢

Development of Gamess/Amber hybrid program

Gamess … program package for *ab initio* calculation

Amber … program package for molecular mechanics calculation

Force calculation

Gamess

Introduction of the one-electron integral term with respect to MM atoms

$$\hat{H}_{QM} = -\frac{1}{2}\nabla_i^2 + \sum_{ij}\frac{1}{r_{ij}} - \sum_{i\alpha}\frac{Z_\alpha}{r_{i\alpha}} + \sum_{\alpha\beta}\frac{Z_\alpha Z_\beta}{R_{\alpha\beta}} - \sum_{iM}\frac{q_M}{r_{iM}}$$

$$\left\{-\frac{\hbar}{2m}\nabla^2 + V[\rho]\right\}\psi_n(\mathbf{r}) = \varepsilon_{nQM}\psi_n(\mathbf{r}),\ \rho(\mathbf{r}) = \sum_n f_n|\psi_n(\mathbf{r})|^2$$

$$F_{QM}(\mathbf{r}) = -\nabla E_{QM}$$

Amber

Deletion of bonded and non-bonded interactions between QM atoms. Switching of charges of QM atoms.

$$E_{MM} = \sum_{bonds} K_r(r - r_{req})^2 + \sum_{angles} K_\theta(\theta - \theta_{req})^2 + \sum_{dihedrals}\frac{V_n}{2}(1 + \cos[n\phi - \gamma])$$
$$+ \sum_{i<j}^{atoms}\frac{q_i q_j}{\varepsilon R_{ij}} + \sum_{i<j}^{atoms}\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \sum_{\alpha M}\frac{Z_\alpha q_M}{R_{\alpha M}}$$

$$F_{MM}(\mathbf{r}) = -\nabla E_{MM}$$

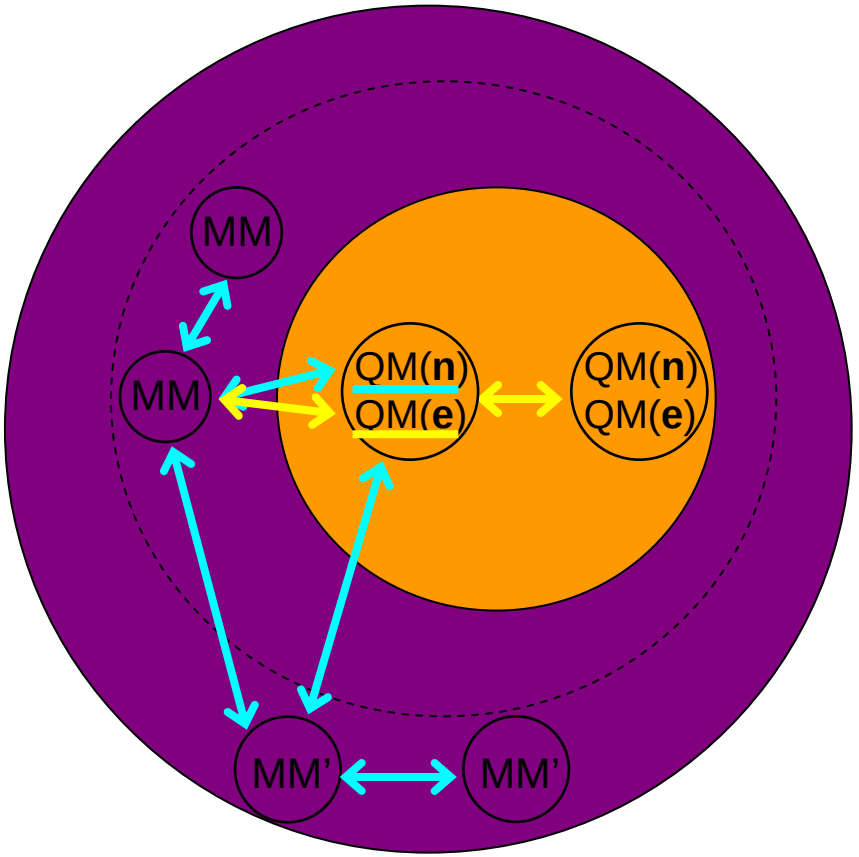
Merging of the forces **Making consistency between the two programs**

$$F(\mathbf{r}) = F_{QM} + F_{MM}$$

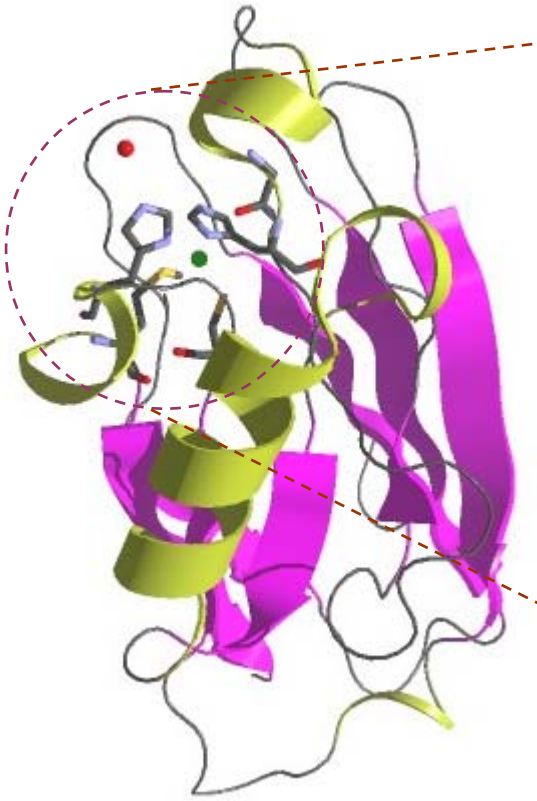
Amber

$$M\frac{d^2\mathbf{r}}{dt^2} = F(\mathbf{r})$$

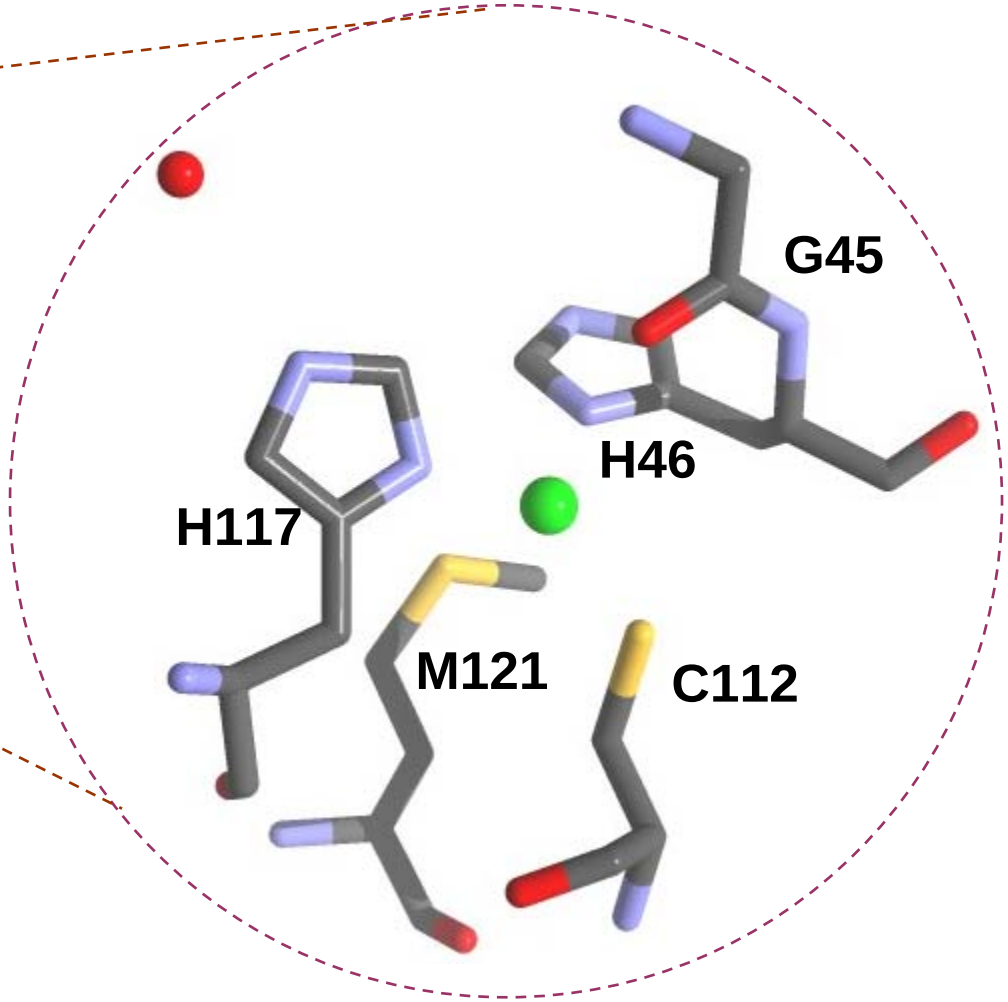
Updating coordinates



Crystal Structure of Azurin

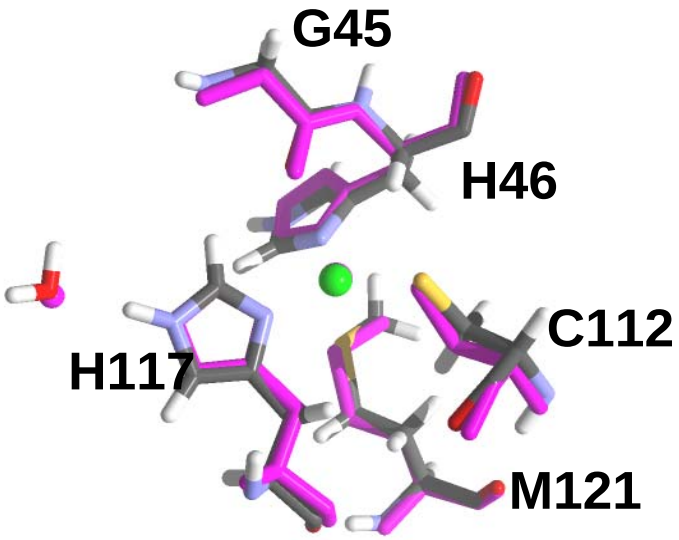


Azurin (pdb id : 4azu)
Pseudomonas aeruginosa



active
site

Molecular Dynamics Simulation



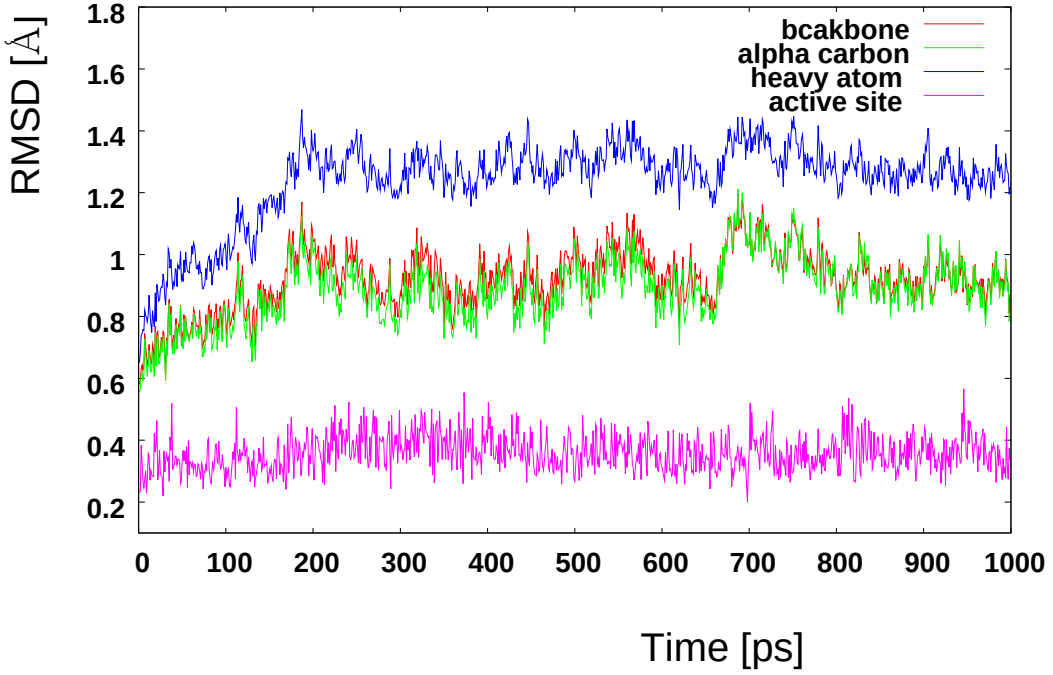
Crystal structure

vs

MD snapshot (1ns)

RMSD 0.3301 Å

RMSD plot for the 1-ns MD simulation



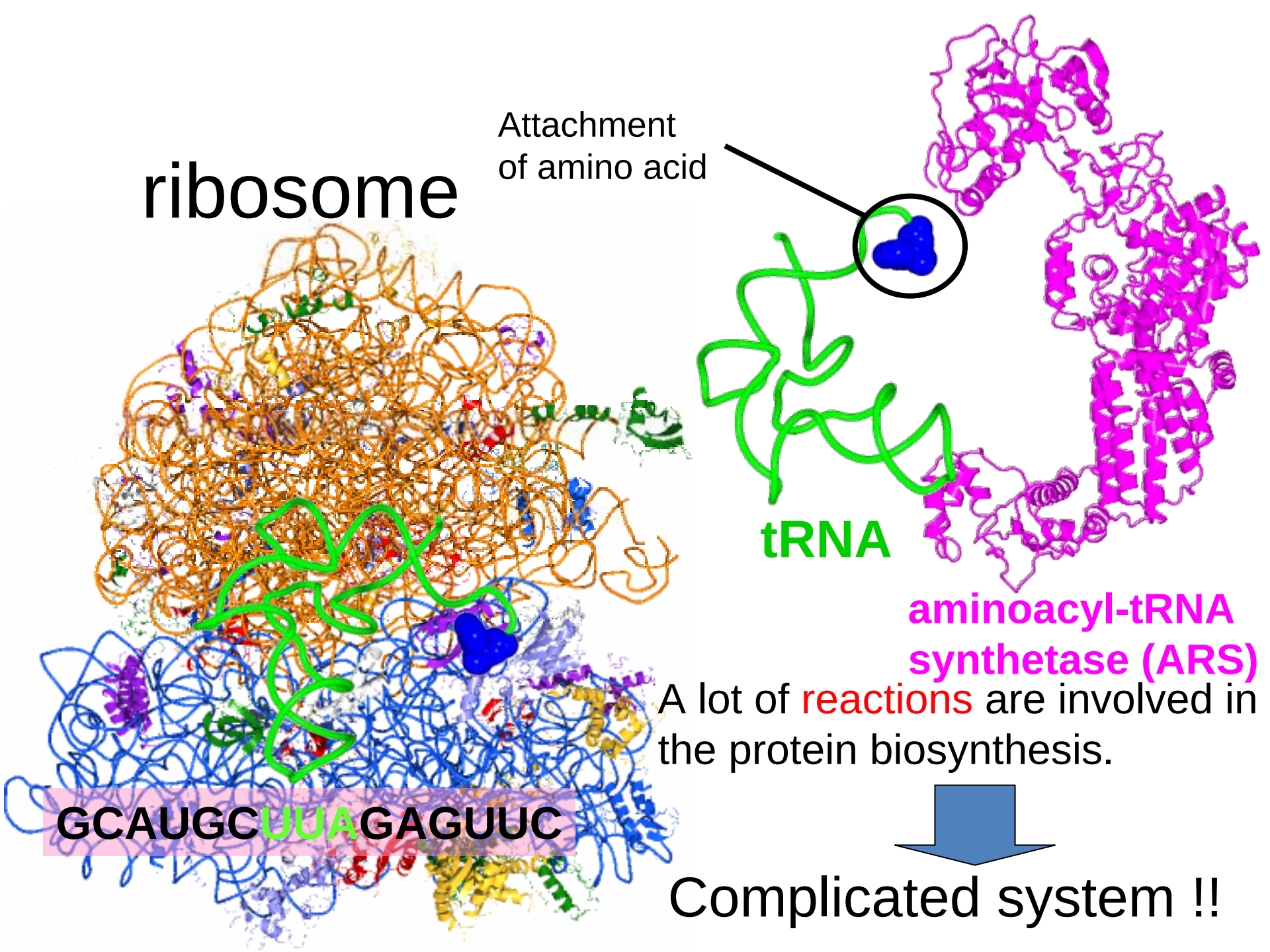
Geometry of Optimized structure (QM/MM) – Oxidized state

	PA Xtal[a]	MD–MM	Model I	Model II	ONIOM[b]	CPMD[c]	EXAFS[d]	A. denitrificans	A. xylosoxidans	P. aeruginosa azurin	Xtal[e]
PDB	**4azu (B-chain)**	**calculation**	**calculation**	**calculation**	**calculation**	**calculation**		**2AZA**	**1DYZ**	**EXAFS Data**	**4azu 4chains**
resolution	**1.90**							**1.80**	**1.75**		**1.90**
pH	**5.50**									**5.5**	**5.50**
$Cu–S_{Met121}$	3.16	3.35	3.49	3.5	3.53(3.41)	3.32+-0.28	3.39	3.11	3.26		2.87–3.26
$Cu–S_{Cys112}$	2.27	2.25	2.20	2.24	2.17(2.17)	2.13+-0.04	2.12	2.15	2.14	2.14	2.12–2.27
$Cu–O_{Gly45}$	2.95	2.96	2.98	2.81	2.55(2.49)	3.20+-0.22	2.82	3.13	2.72		2.75–3.16
$Cu–N_{His117}$	1.98	1.95	2.01	2.1	2.01(2.03)	1.99+-0.06	1.94	2.00	1.99	1.95	1.99–2.12
$Cu–N_{His46}$	2.06	2.06	2.04	1.93	1.99(2.01)	1.98+-0.05	1.86	2.08	2.04		1.99–2.12
$O_{H2O}–N_{His117}$	2.93	2.95	2.97	3.04	-	-	-	-			

Geometry of Optimized structure (QM/MM) – Reduced state

	PA Xtal[a]	MD–MM	Model I	Model II	ONIOM[b]	CPMD[c]	EXAFS[d]	A. denitrificans	A. xylosoxidans	P. aeruginosa azurin	Xtal[c]
PDB	**4azu (B-chain)**	**calculation**	**calculation**	**calculation**	**calculation**	**calculation**		**b/**	**1DZ0/**	**EXAFS Data**	**-**
resolution								**1.90**	**1.75**		**-**
pH	**5.50**									**5.50**	**-**
$Cu–S_{Met121}$	3.16	3.35	3.67	3.56	3.67(3.48)	3.25+-0.28	3.35	3.23	3.26		-
$Cu–S_{Cys112}$	2.27	2.25	2.27	2.24	2.21(2.21)	2.13+-0.06	2.19	2.26	2.16	2.21	-
$Cu–O_{Gly45}$	2.95	2.96	2.98	2.98	2.59(2.74)	3.15+-0.22	2.98	3.22	2.75		-
$Cu–N_{His117}$	1.98	1.95	2.06	2.03	2.25(2.25)	1.99+-0.06	2.01	2.05	2.02	2.00	-
$Cu–N_{His46}$	2.06	2.06	2.20	2.17	2.02(2.01)	1.98+-0.05	1.91	2.13	2.03	2.00	-
$O_{H2O}–N_{His117}$	2.93	2.95	3.01	2.83	-	-					-

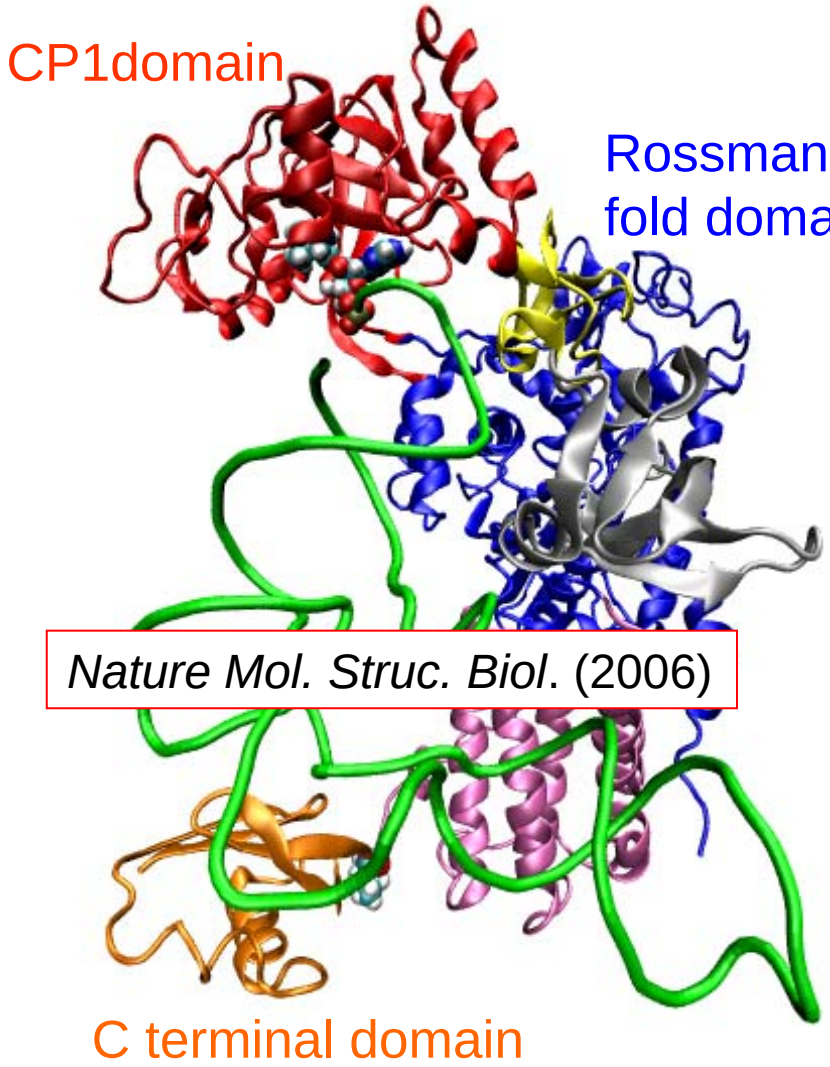
[a] Experimental values in the crystal structure used in the present study as the initial structure of the MD simulation.
[b] See ref. [14]; EE(ME) optimized geometries
[c] CPMD U. Rothlisberger PNAS, vol 103, 19641
[d] Experimental values summarized in ref. [11]
[e] Experimental values listed in ref. [14]



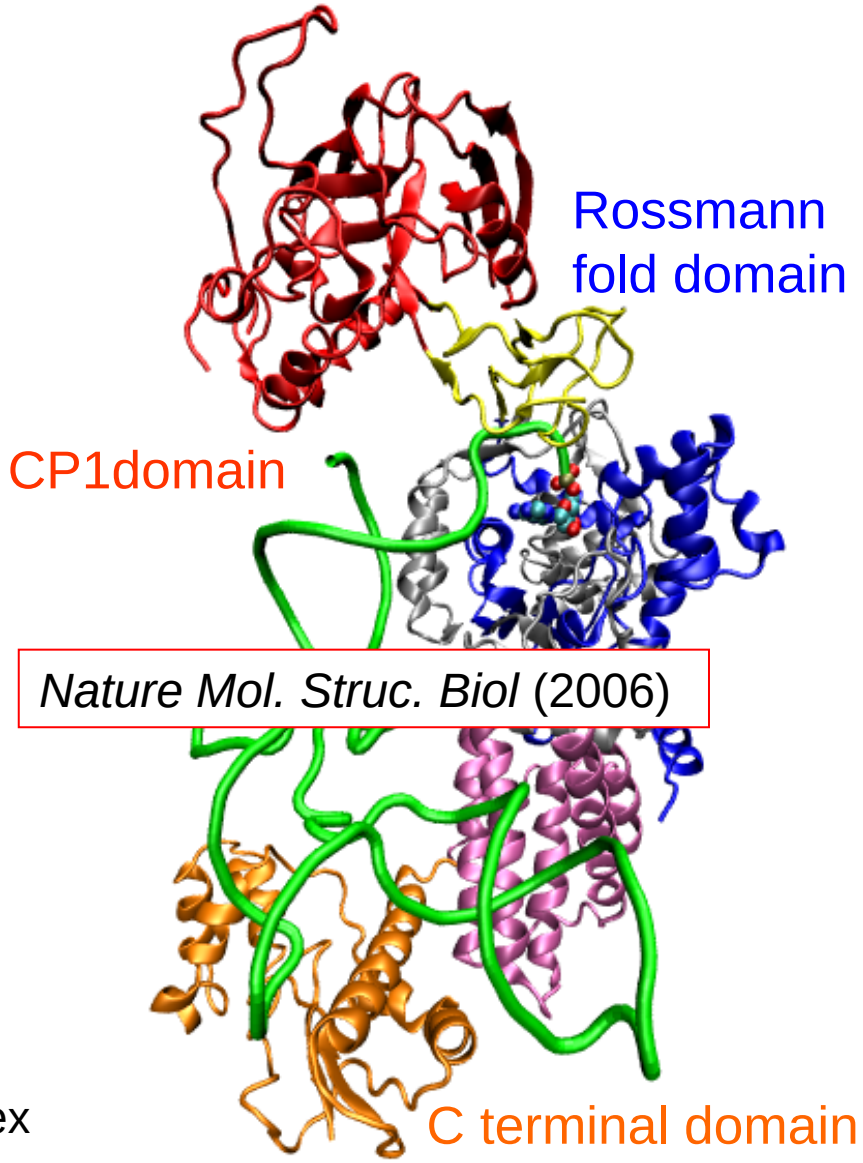
A lot of reactions are involved in the protein biosynthesis.

Complicated system !!

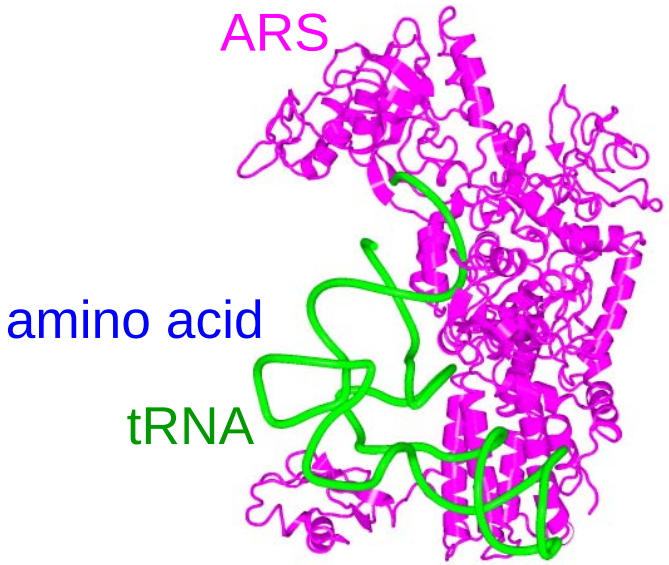
The editing site is located in the CP-1 domain (class I aaRSs)



Thermus thermophilus LeuRS•tRNA^{Leu} complex



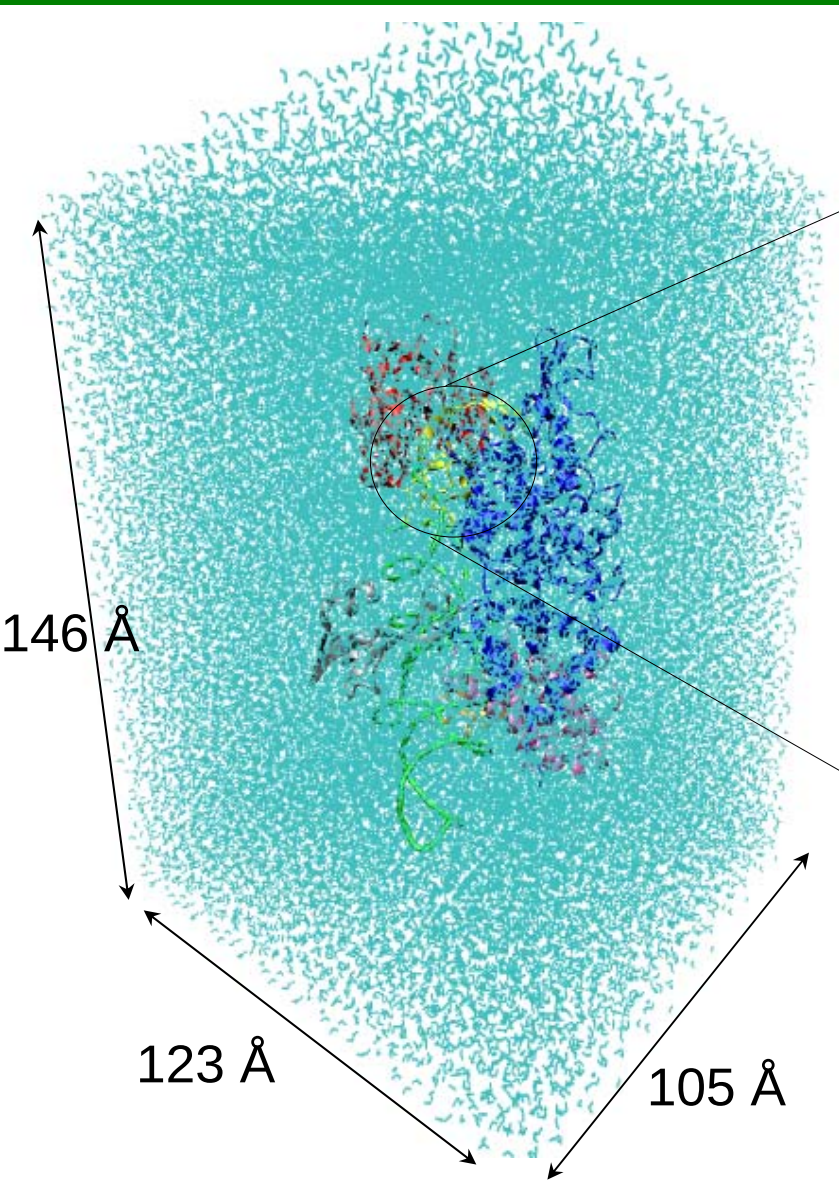
Crystal structures related to the editing

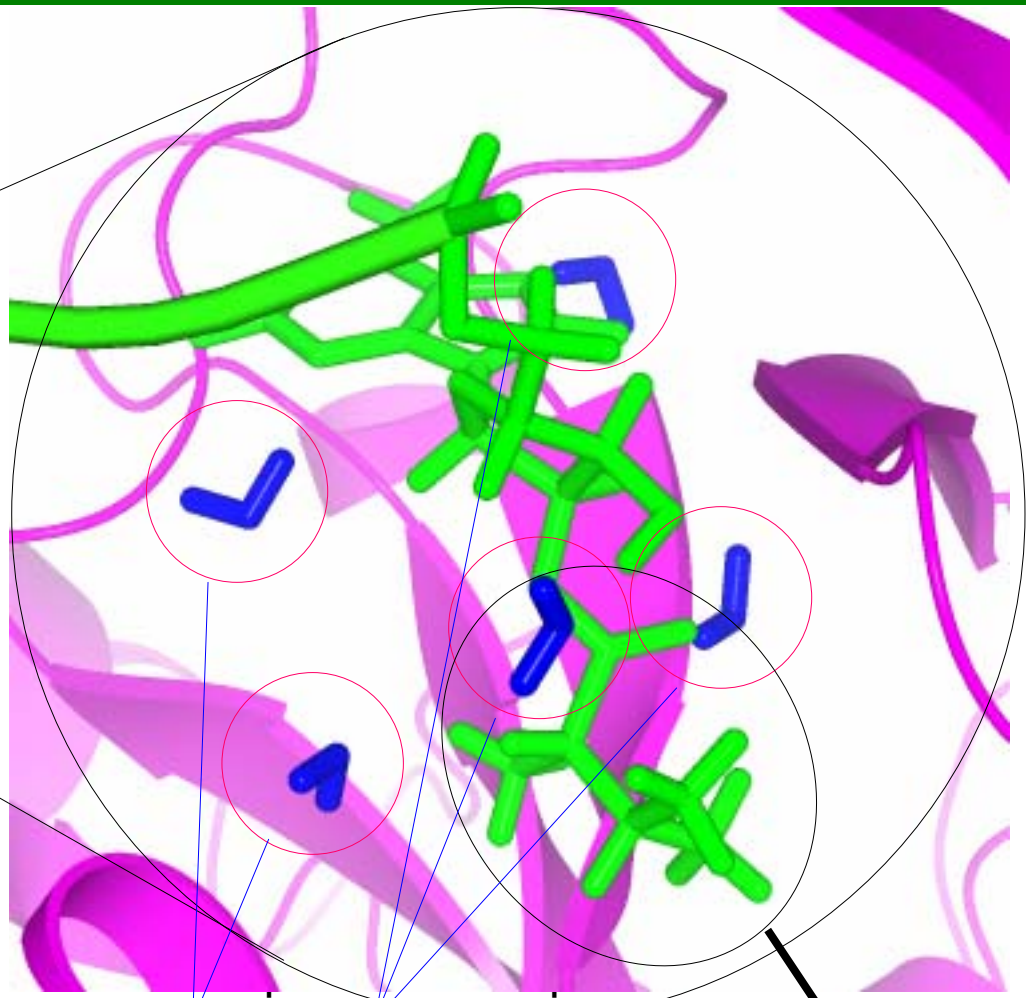


X-ray structure	protein	$tRNA^{Leu}$	amino acid	crystallographic water
2BYT	○	○	×	×
1OBC	○	×	○	○
1H3N	○	×	×	○

Identification of the conformation of the substrate (amino acid moiety) and the configuration of water molecules are required to analyze the mechanisms of the editing

Application of FSDD to construct the model structure for the reaction analyses





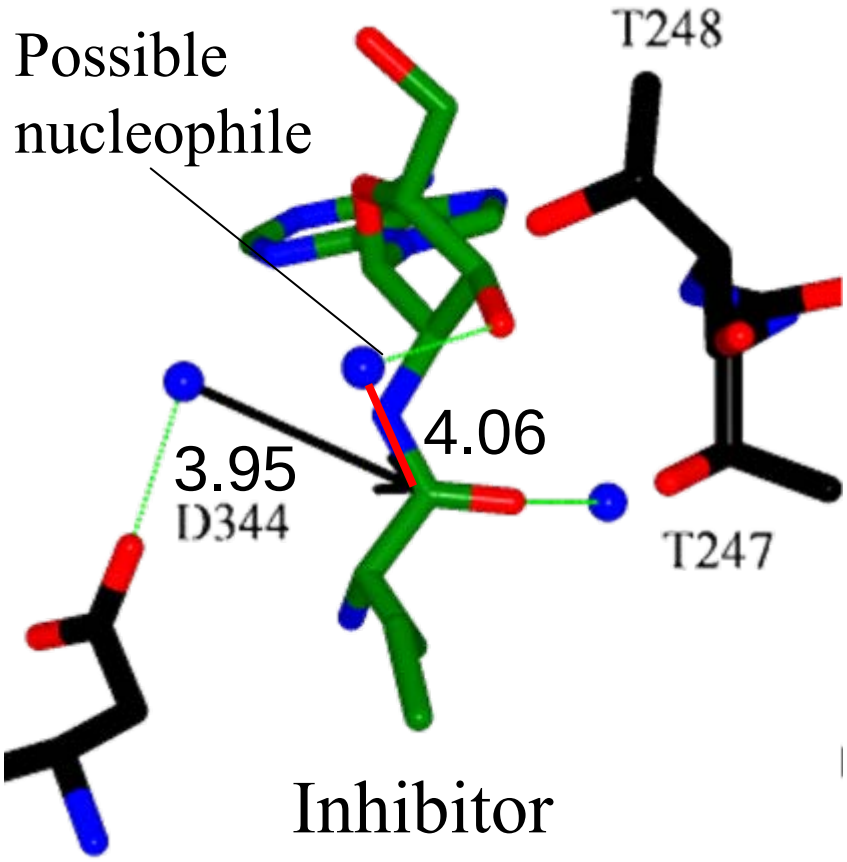
controlled by using Berendsen algorithm.

water molecules **amino acid**

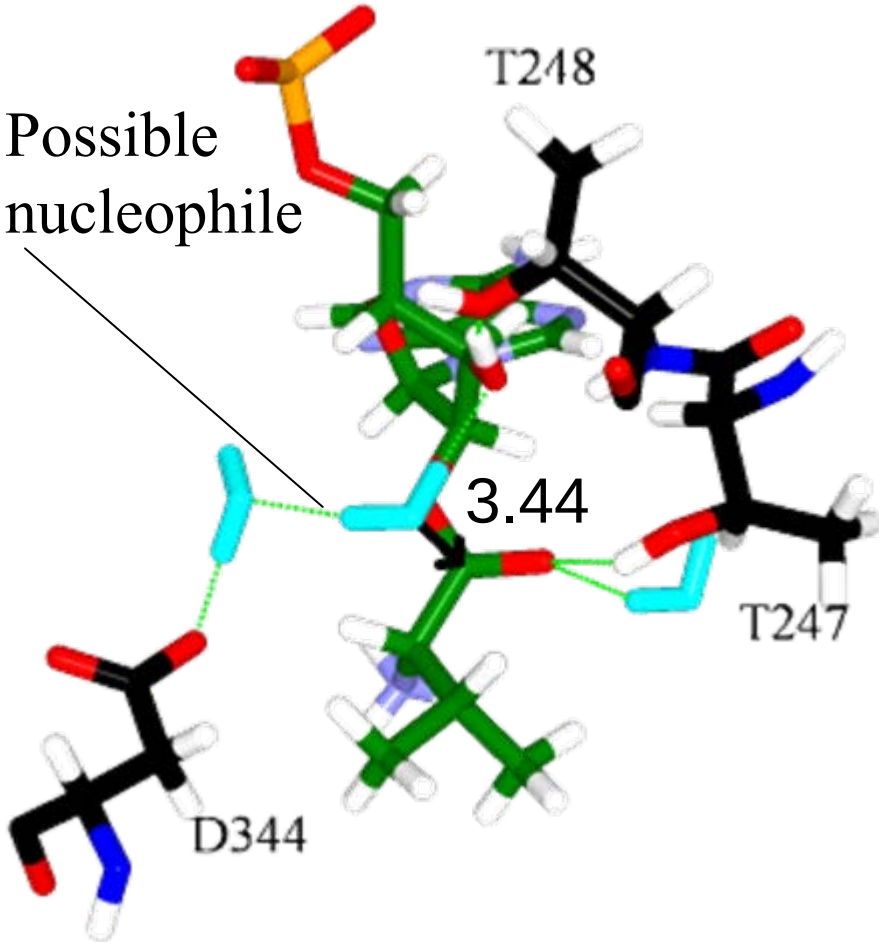
docking !! **moiety**

Protein (ARS) + Val-tRNA^{Leu}

Comparison of the configurations of the active center between the crystal structure (in a complex with an inhibitor) and the resultant obtained using the FSDD calculation



Crystal structure of the *Thermus Thermophilus* LeuRS (1OBC)



The structure obtained using FSDD calculations

QM/MM-MD simulations

QM atoms: 77-atom (in the catalytic site) in the case of
LeuRS・Val-tRNA^{Leu} system

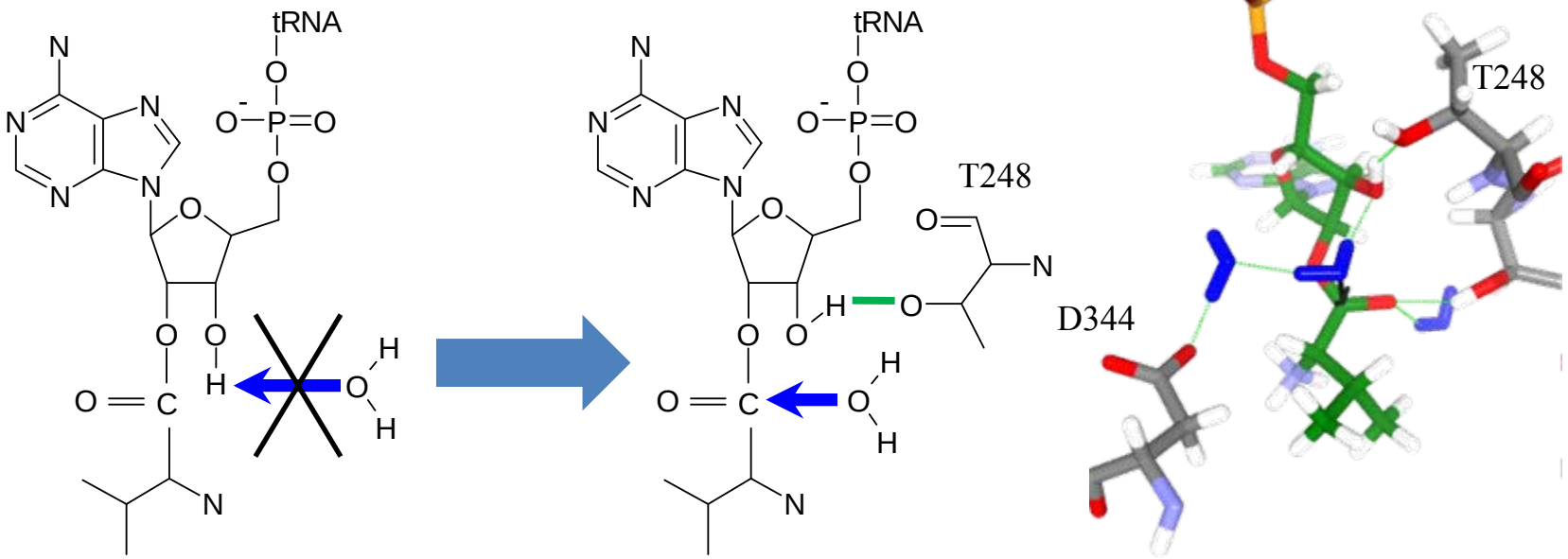
Total number of atoms … 165,721
QM calculation → **Density Functional Theory (DFT)**, B3LYP, all-electron calc.
MM calculation → AMBER (parm99)
MD calculation:
Temperature: 300 K Integration steps: ~10000-step
Time step for integration … 0.1fs
Free energy estimation: PMF (Potential of Mean Force)

Recently, we have developed a new interface program to connect QM (gamess) and MM (amber) engines.

Our related papers

1) Ohta, T., Hagiwara, Y., Kang J., Nishikawa, K., Yamamoto, T., Nagao, H., and Tateno, M., Evaluation of Electronic and Geometrical Properties of the Blue Copper Site in Fully Solvated Azurin by QM/MM Hybrid calculations Using a New Interface Program Connecting QM and MM Engine, *J. Comp. Theor. Nanosci*., in press.
2) Hagiwara, Y. and Tateno, M., Evaluation of stabilization energies in $\pi-\pi$ and cation–π interactions involved in biological macromolecules by ab initio calculations, *J. Phys. Cond. Mat.*, in press.
3) Boero, M., Kang. J., Tokumoto, S., and Tateno, M., A First-Principle Exploration of Heme *a* and Heme *a*3 of the Bovine Cytochrome c Oxidase in Reduced and Oxidized Charge States, *J. Comp. Theor. Nanosci*., in press.
4) Kang, J., Ohta, T., Hagiwara, Y., Nishikawa, K., Yamamoto, T., Nagao, H. and Tateno, M., Electronic and geometric
structures of the blue copper site of azurin investigated by QM/MM hybrid calculations, *J. Phys. Cond. Mat*., in press.

Mechanism of approaches of the nucleophilc water : the “H-gate” → Open/Close



distance O_w-C is 3.44 Å

The access of the nucleophile is inhibited by the excluded volume of the 3'-HO.

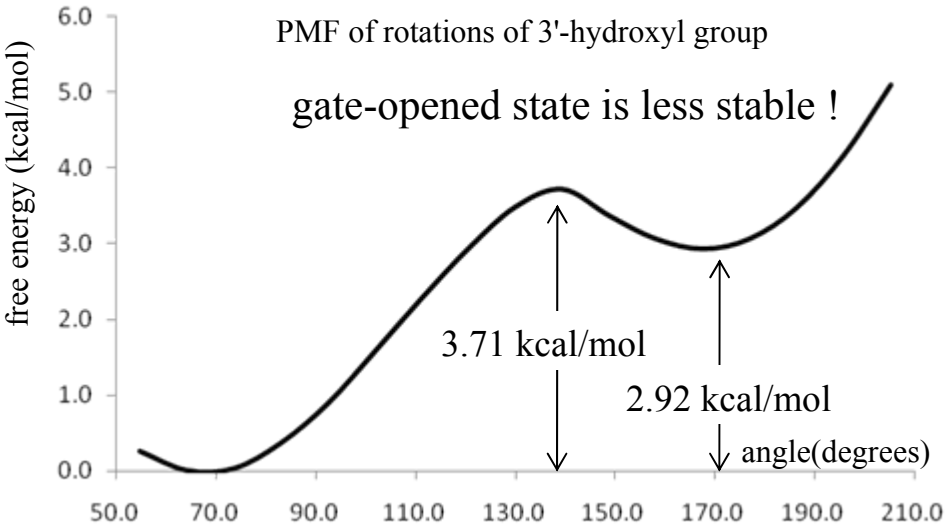
distance O_w-C is 2.38 Å

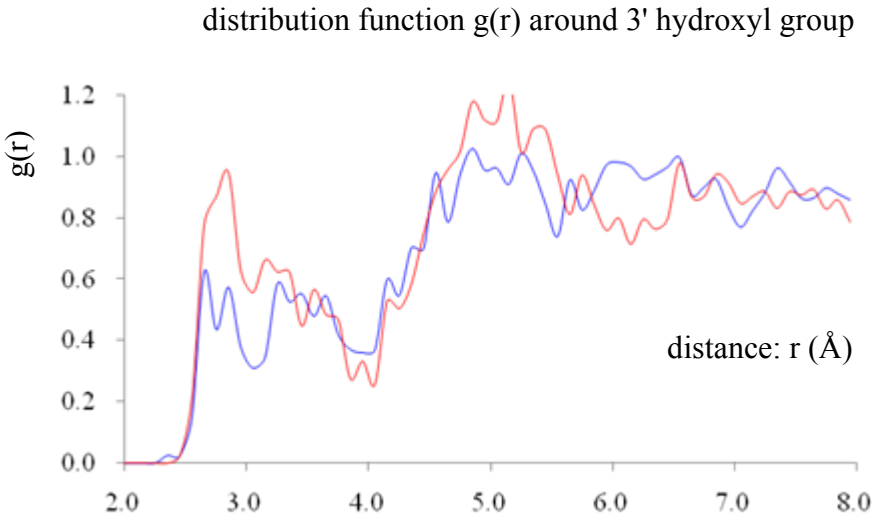
The rotation of 3'-HO enables the nucleophile to attack the carbonyl carbon.

Close

Open

The gate (3‘-hydroxyl group) can be opened ? --- Yes !



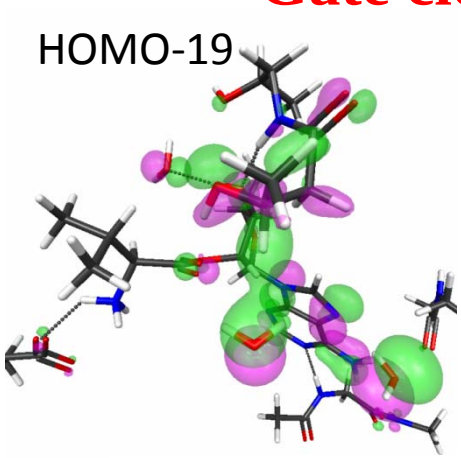


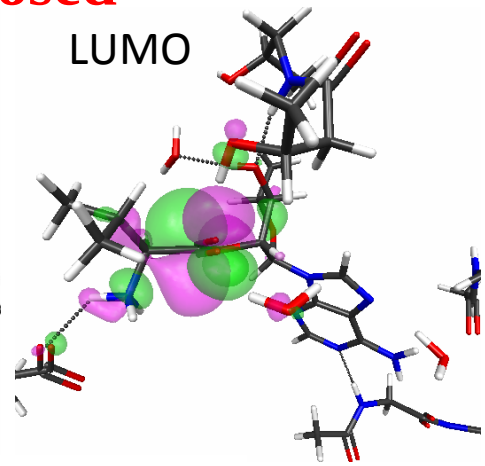
Free energy barrier for rotation do exists, and the opened state is less stable, i.e., the 3' hydroxyl group seems to work as a 'gate'.

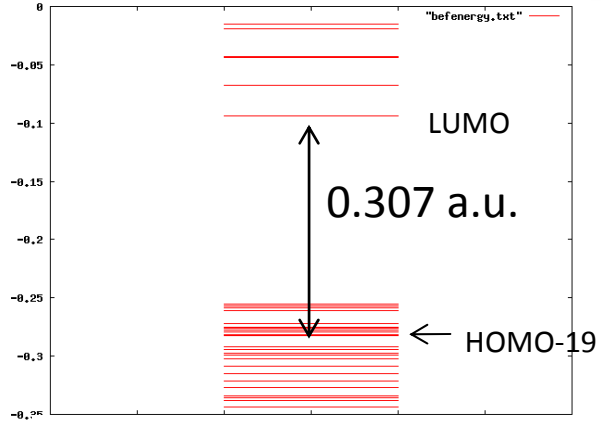
The functional role of the gate is considered to prevent the hydrolysis of Leu-tRNA^{Leu}. Actually, in the case of Leu-tRNA^{Leu}, although the bulkiness of the side chain of leucine limits the access of water molecules to the reaction point, the probability of the water access remains one half, compared with Val-tRNA^{Leu} case.
Thus, it is supposed that double mechanisms are present to prevent the hydrolysis of Leu-tRNA^{Leu} ; the presence of the gate and limitation of the access of a nucleophile water by bulkiness of leucine side chain.

Comparison of electronic structures between the close and open conformations of the H-gate

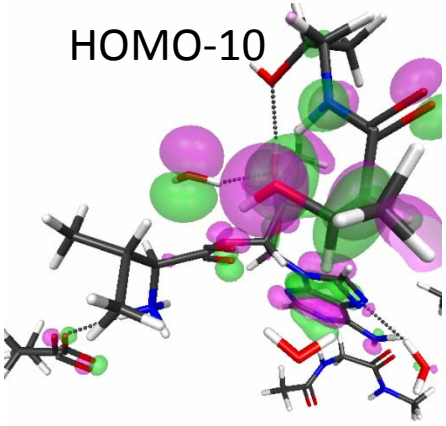
Gate closed

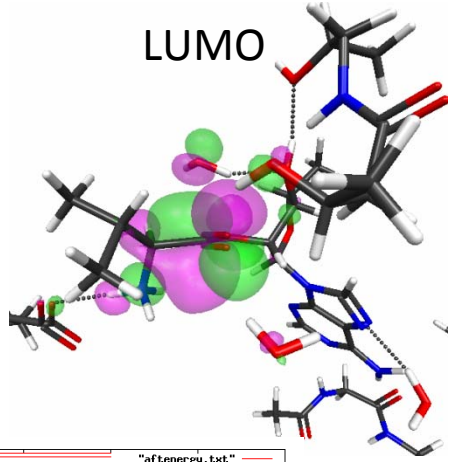


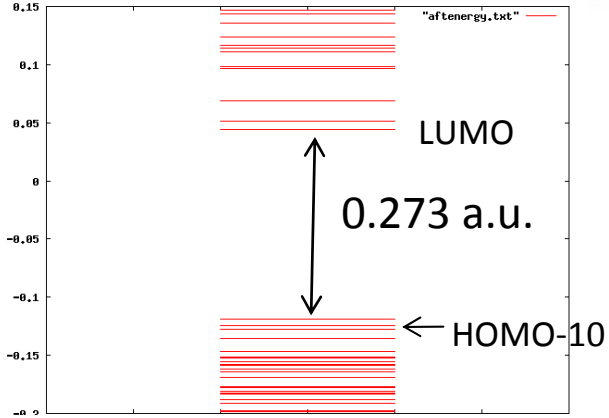




Gate opened





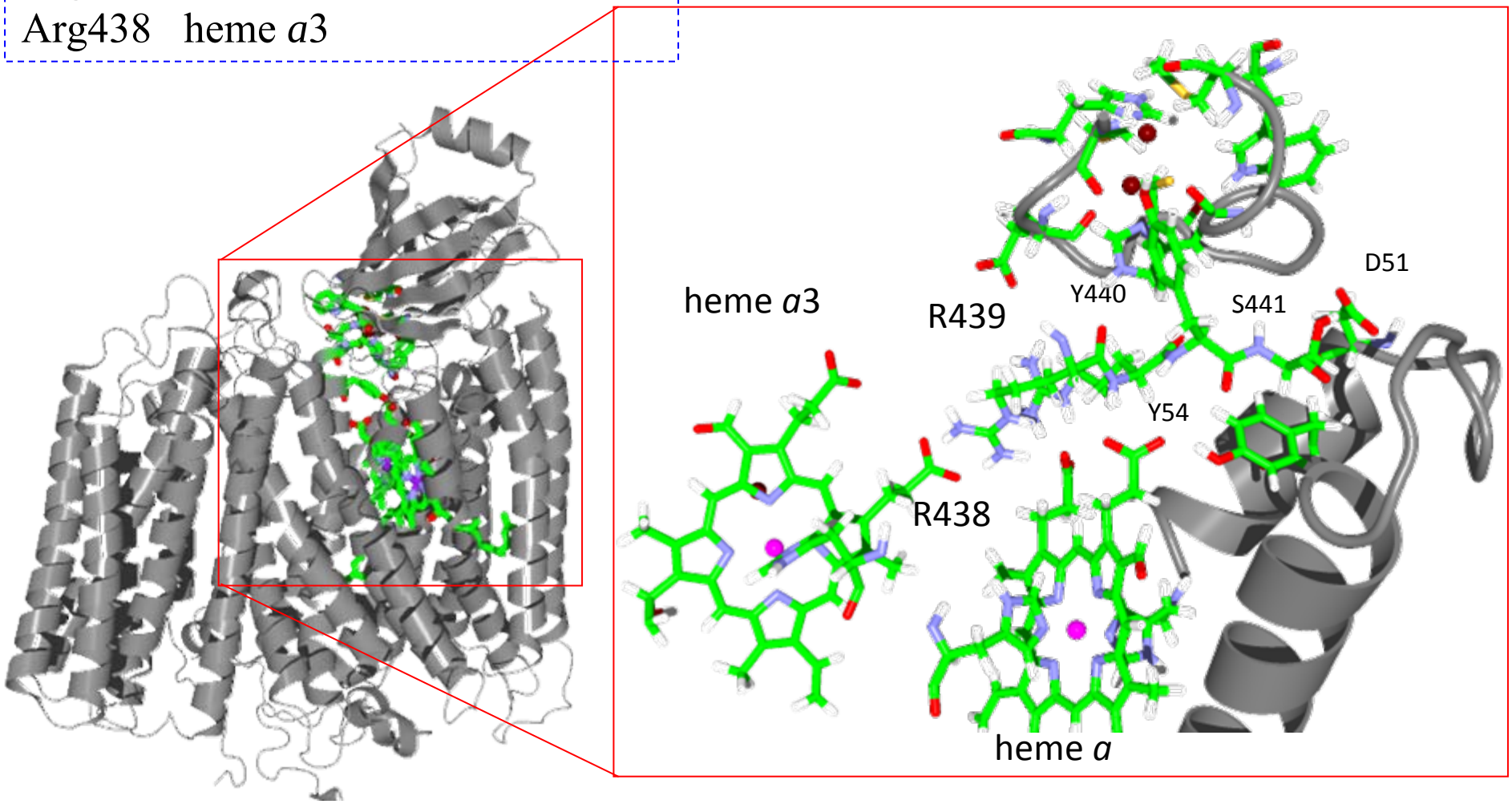


In both states, LUMO is localized on the carbonyl group of the substrate, and the character of the antibonding orbital of the C-O2' π bond are included, indicating the C-O2' bond is weakened.

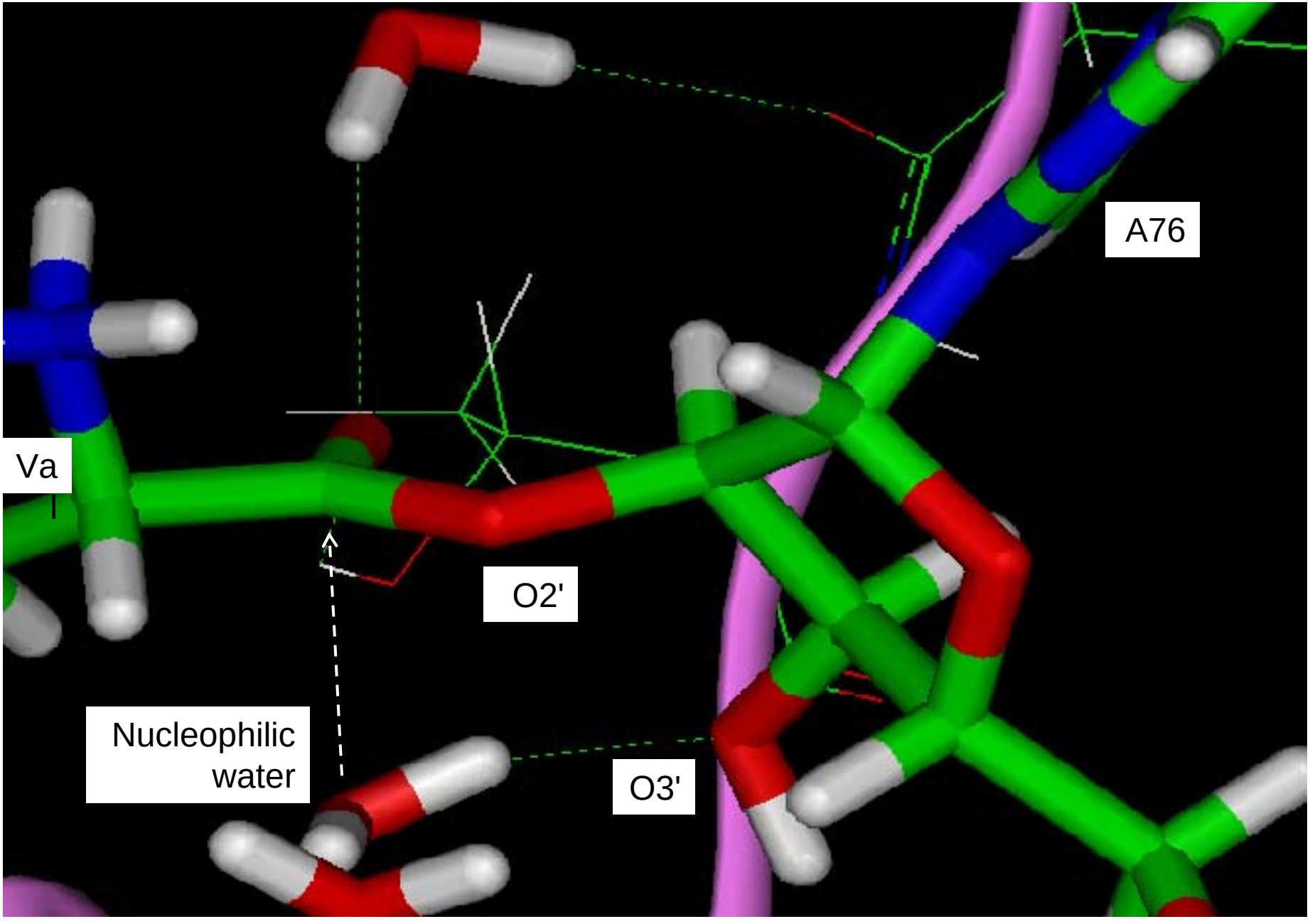
The gate is opened, and thereby, the nucleophilic water accesses to the carbonyl; then, the electronic structure is activated.

Intramolecular interaction network system related to the biological function of the bovine cytochrome *c* oxydase

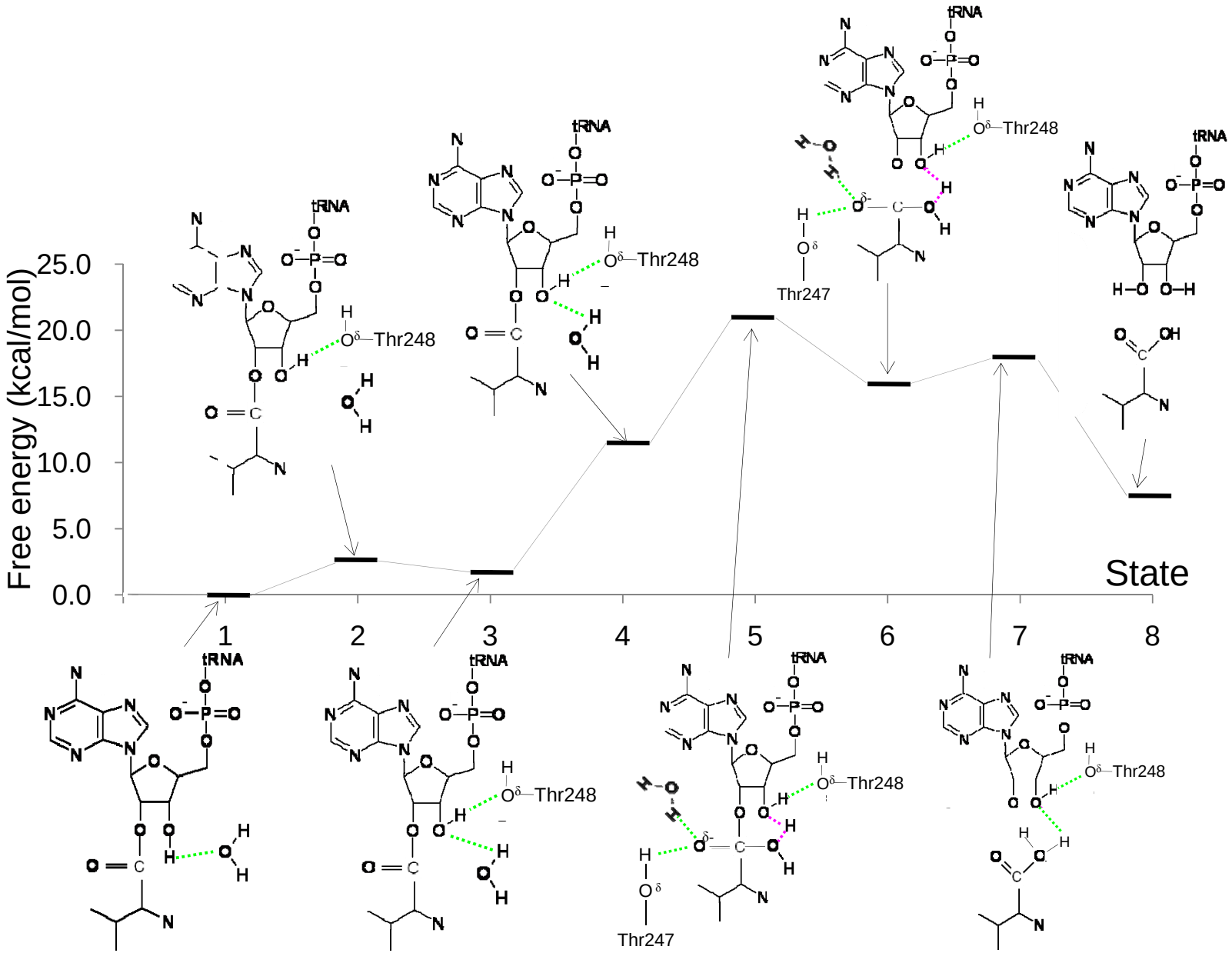
Tyr440-Ser441 Tyr54 Cys196 Cu_A
Trp104
Arg439 heme *a*
Arg438 heme *a*3



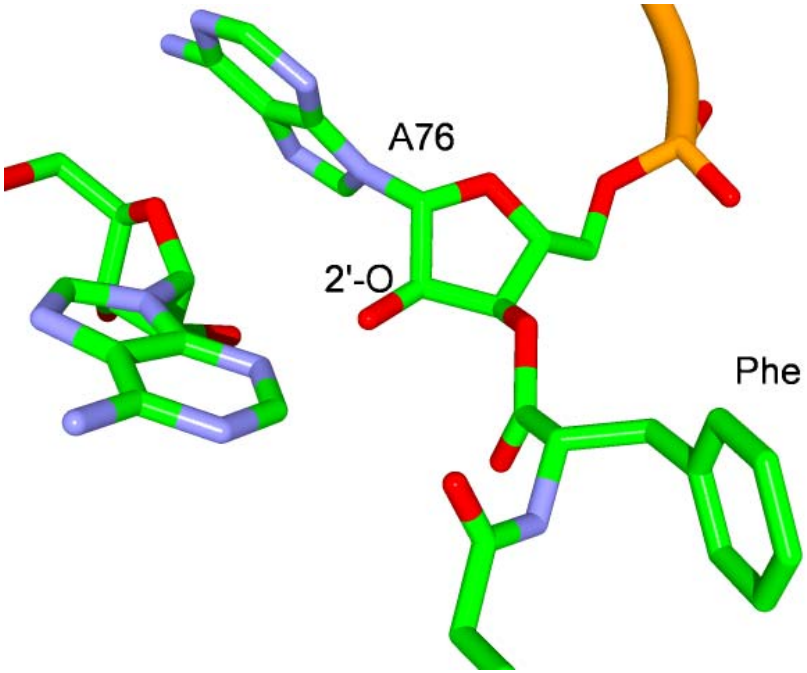
QM/MM Molecular Dynamics Simulation of Editing



Energy Diagram of the editing by LeuRS

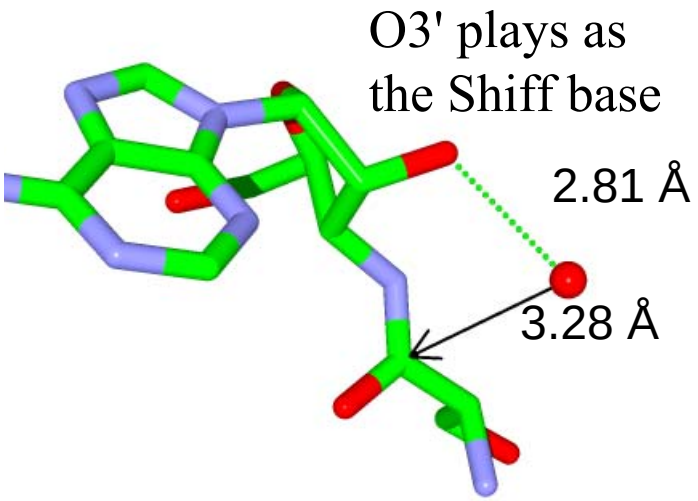


Also in other systems …..



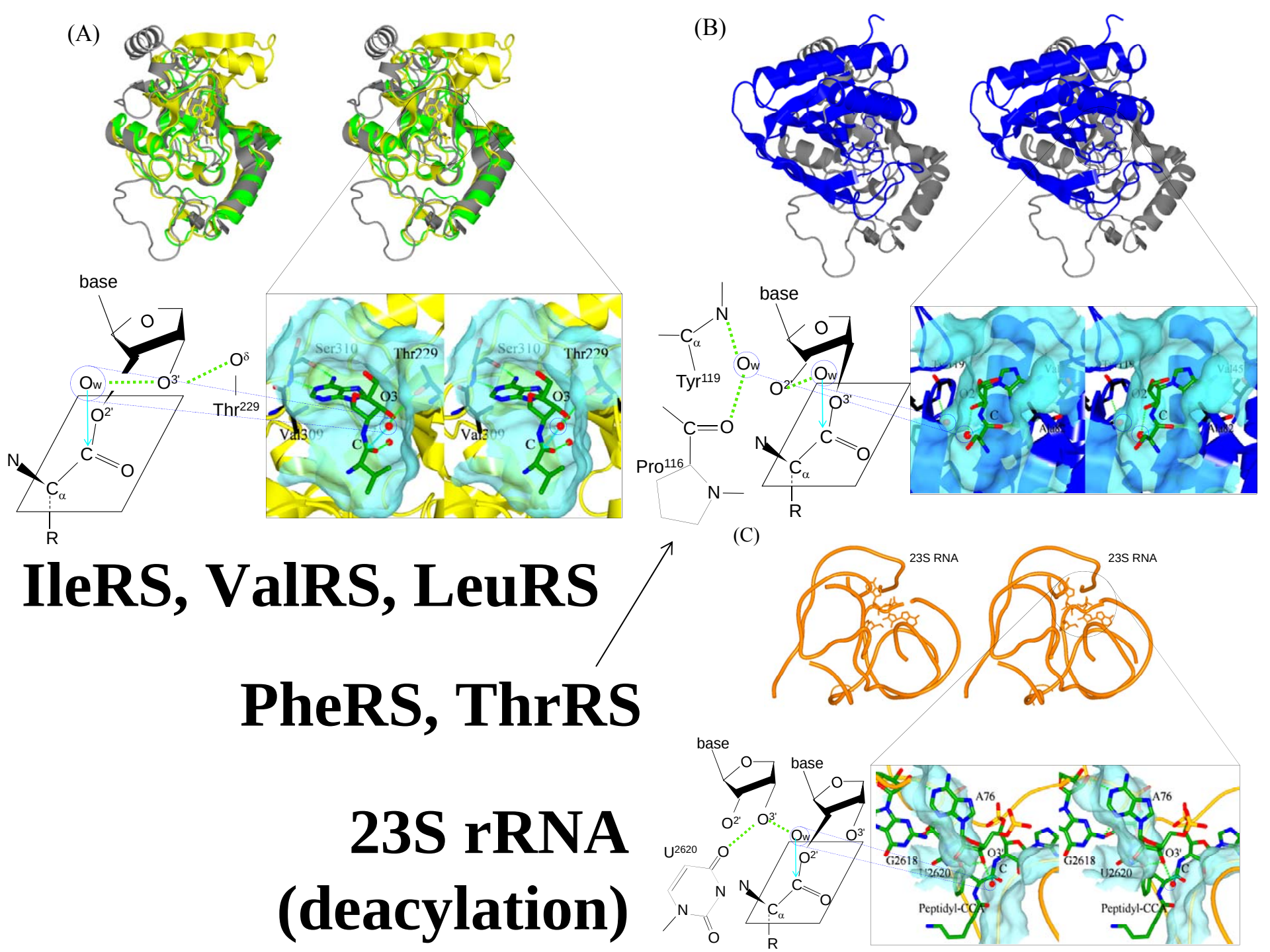
CCA-Phe bound *Haloarcula Marismortui* ribosome

Schmeing, T. M., Huang, K. S., Kitchen, D.E., Strobel, S. A., Steitz, T. A. 2005, *Mol.Cell.* 20, 437-448



Pyrococcus abyssi ThrRS

Hussain, et al. *EMBO. J.* 2006, 25, 4152-4162


(A)
(B)
(C)
base
O
Ow
O3'
Oδ
O2'
C
O
N
Cα
R
Thr229
Ser310
Thr229
O3
Val309
C
Tyr119
Pro116
23S RNA
23S RNA
U2620
A76
G2618
Peptidyl-CCA
IleRS, ValRS, LeuRS
PheRS, ThrRS
23S rRNA
(deacylation)

References

[1] Hagiwara, Y., et al., "QM/MM hybrid calculation of biological macromolecules using new interface program connecting QM and MM engines", *J. Phys. : Condens. Matter*, **21** (2009), 064224.

[2] Kang, J., et al., "Electronic and geometric structures of the blue copper site of azurin investigated by QM/MM hybrid calculations", *J. Phys. : Condens. Matter*, **21** (2009), 064235.

[3] Tateno, M., et al., "Evaluation of stabilization energies in p– p and cation– p interactions involved in biological macromolecules by *ab initio* calculations", *J. Phys. : Condens. Matter*, **21** (2009), 064243.

[4] Ohta, T., et al., "Evaluation of Electronic and Geometrical Properties of the Blue Copper Site in Fully Solvated Azurin by QM/MM Hybrid Calculations Using a New Interface Program Connecting QM and MM Engine", *J. Comp. Theor. Nanosci.*, in press.

[5] Boero, M., et al., "A First-Principle Exploration of Heme *a* and Heme *a*3 of the Bovine Cytochrome *c* Oxidase in Reduced and Oxidized Charge States", *J. Comp. Theor. Nanosci.*, in press.

[6] a) Hagiwara, Y. and Tateno, M., submitted. b) Hagiwara, Y. and Tateno, M., submitted. (density-based descriptions of van der Waals and electrostatic interactions)

[7] Hagiwara, Y., Nureki, O., and Tateno, M., *FEBS Lett.*, in press.

[8] Hagiwara, Y., Nureki, O., and Tateno, M., submitted.

[9] Hagiwara, Y., Kino, H., and Tateno, M., submitted.

[10] Hagiwara, Y., Matsumura, H., and Tateno, M., submitted.

Conclusions

１） 特異的に結合した溶媒水分子の孤立電子対が，基質のLUMOを攻撃することによって反応が開始される機構を解明した。

２） ハイブリッドMD計算により，反応過程における電子構造の動的な変化過程を解明した。

３） この酵素は，求核剤としての水分子の電子構造と配置とを巧みに制御することにより，高い反応効率と選択性を同時に生み出していることを明らかにした。

４） 鍵となる機構は，「水素原子（プロトン）の共有」であり，有機電子論の有効な有機化合物（プロトン移動）などとは異なる，生体酵素反応の原理のひとつと考えられる。