QM/MM分子動力学法による生体機能解析

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QM/MM分子動力学（MD）シミュレーション（QM/MM-MD；ハイブリッドMD）

DFTなどの第一原理計算を用いた生体高分子の反応機構の研究
→ 多くの研究が蓄積されている，が，しかし。。。

計算精度 → できるだけ高度に
　　数kcal/mol（～meV）のエネルギ差が決定的に重要。
計算速度 → できるだけ速く
　　～10,000-stepの時間積分を実行することが必要。

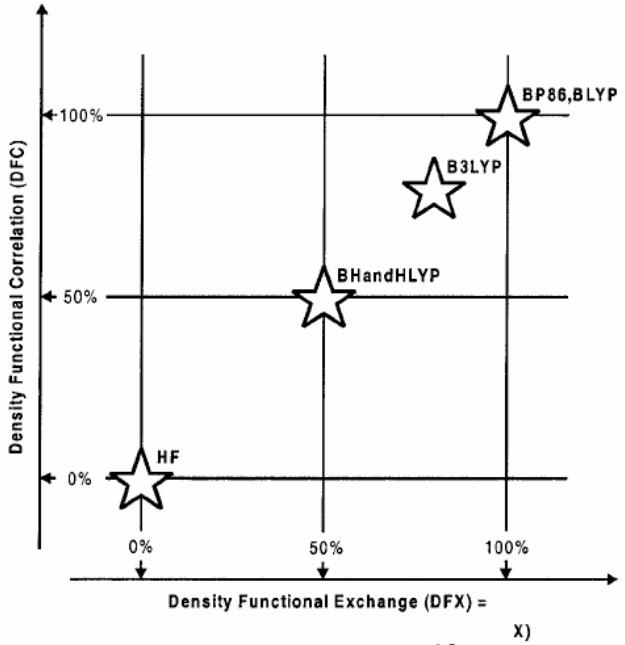
以上をバランスさせることが必要

　　なぜQM/MMか？
　　なぜMDか？

Spectroscopic calibration of HF/DFT hybrid (all electron) by using $[CuCl_4]^{2-}$

- B(38HF)P86 reproduces experimental Cu spin density (0.62 ± 0.02e)
- Spin density is sensitive to the ratio of HF and DF exchanges
- Cu-Cl bond length is sensitive to DF correlation functional

SCHEME 1: Conceptual Relationship among GGA, Hybrid Density Functional and Hartree–Fock Theory



Szilagyi, R.K.; Mets, M.; Solomon, E.I. *J. Phys. Chem. A* **2002**, *106*, 2994-3007

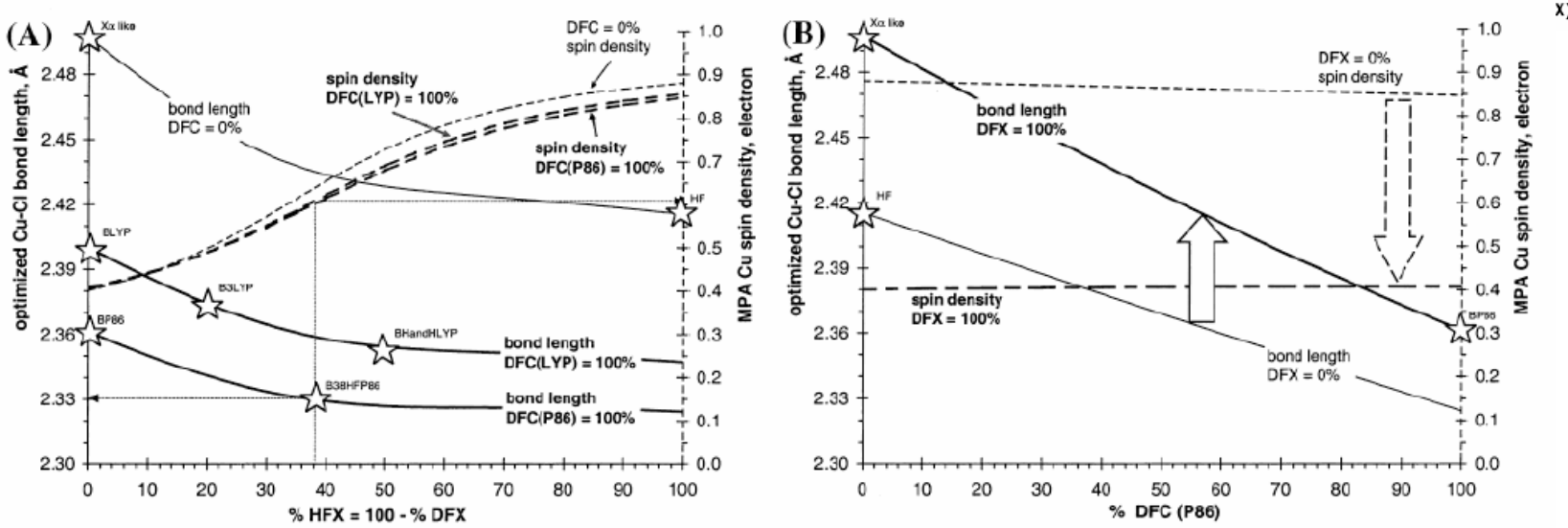


Figure 5. (A) Optimized Cu–Cl bond lengths (Å) and MPA Cu spin densities (electron) in D_{4h} $[CuCl_4]^{2-}$ as function of density functional exchange (DFX: B88) substitution by Hartree–Fock exchange (HFX) at maximal and minimal density functional correlation (DFC: solid black P86; solid gray LYP) limits using BS5. (B) Optimized Cu–Cl bond lengths (Å) and MPA Cu spin densities (electron) in D_{4h} $[CuCl_4]^{2-}$ as function of the amount of density functional correlation (DFC: P86) at maximal and minimal density functional exchange (DFX: B88) limits using BS5.

QM/MM分子動力学（MD）シミュレーション（QM/MM-MD；ハイブリッドMD）

計算精度 → できるだけ高度に

数kcal/mol（～meV）のエネルギ差が決定的に重要。

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～10,000-stepの時間積分を実行することが必要。

以上をバランスさせることが必要

→ B3LYPによるHF/DFTハイブリッド全電子計算

なぜQM/MMか？

なぜMDか？

生体高分子のQM/MM計算の現状

HF/DFTハイブリッド全電子計算（QM）と力場（MM）のジョイント

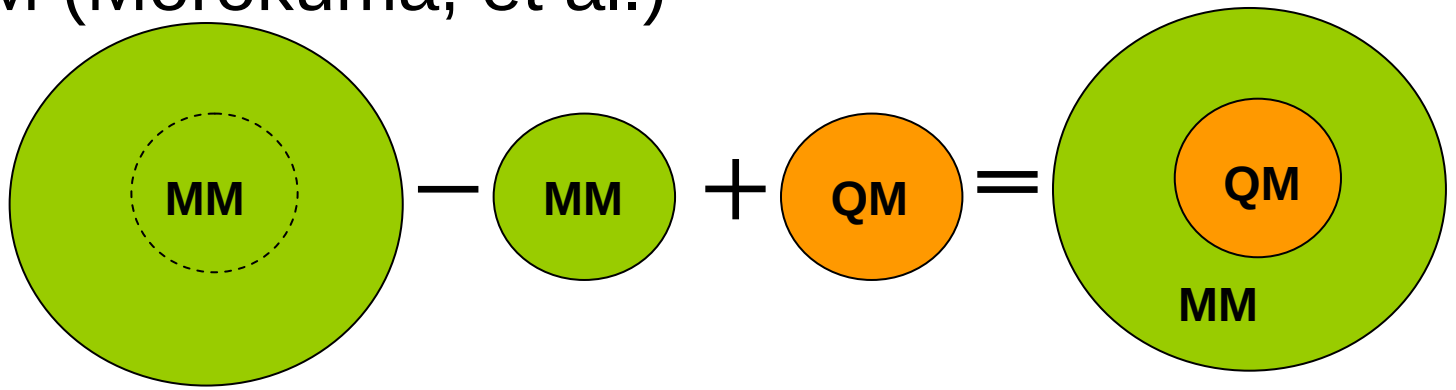
QM calculation	interface	MM calculation
GAMESS MNDO TURBOMOLE GAUSSIAN Molpro Orca NWChem Dmol	← ChemShell PUPYL →	CHARMM GLUP GROMACS AMBER

Applications

Sherwood, P. et al., *J.Mol.Struct*. **2003**, 632,1.
Senn, H, M. et al., *J.Chem.Theory.Comput.* **2005**, 1,494.
Kastner, J. et al., *J.Chem.Phys.* **2005**, 123,144104.
Claeyssens, F. et al., *Angew. Chem.* **2006**, 118,7010.
Grrerke, S. et al., *J.Chem.Theory.Comput.* **2007**, 3,1499.
Senn, H, M. et al., *Top.Curr.Chem.* **2007**, 268,173.

Schemes of QM/MM calculation

ONIOM (Morokuma, et al.)

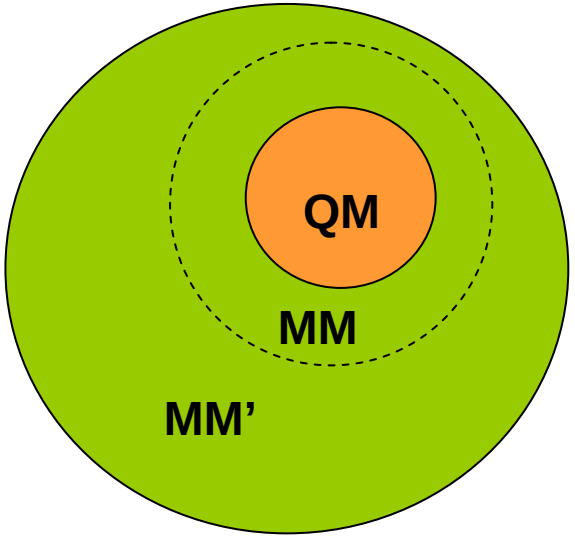


$$E^{MM,All} - E^{MM,Extract} + E^{QM,Extract} = E^{ONIOM}$$

Polarization effect induced by MM region (MM→QM interaction) is not considered.

Hybrid (implemented in our system)

Both of "QM→MM" and "MM→QM" interactions are included in the calculation



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

$$H_{MM} = \sum_{\alpha M}\frac{Z_\alpha q_M}{R_{\alpha M}} + E_{MM}$$

$$E = \langle \Psi | \hat{H}_{QM} + H_{MM} | \Psi \rangle = \langle \Psi | \hat{H}_{QM} | \Psi \rangle + H_{MM}$$

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 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.

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

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

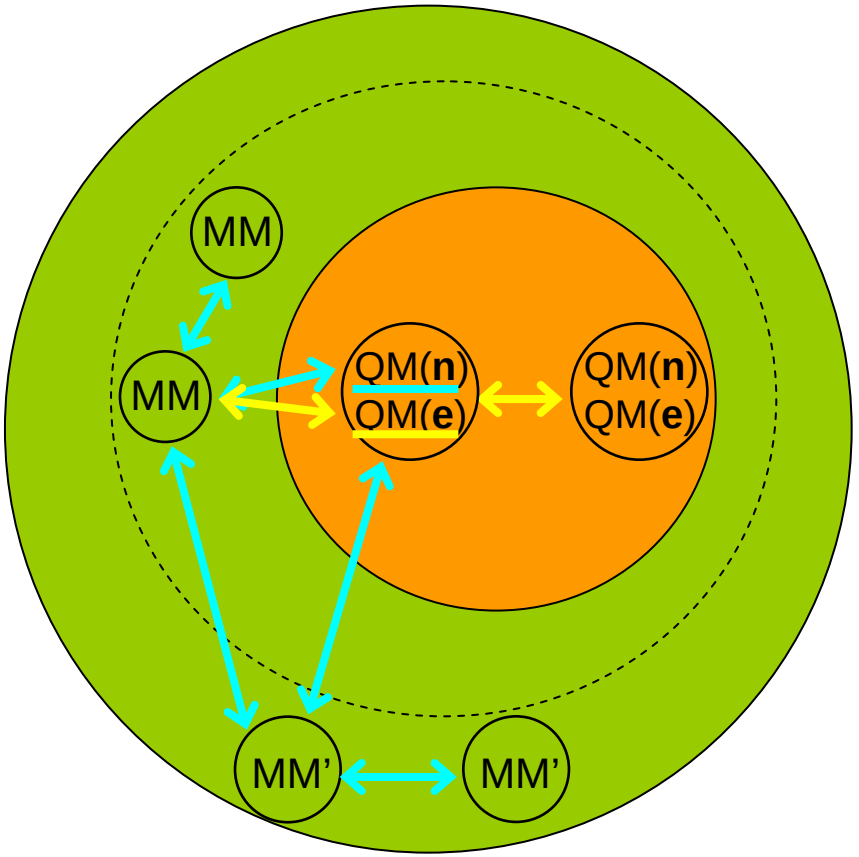
Merging of the forces **Making consistency between the two programs**

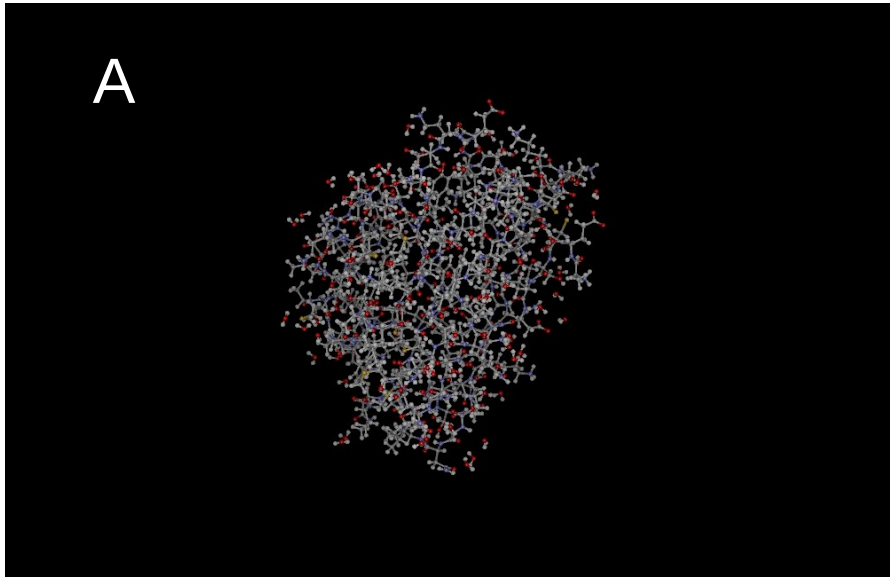
$$F(\mathbf{r}) = F_{QM} + F_{MM}$$

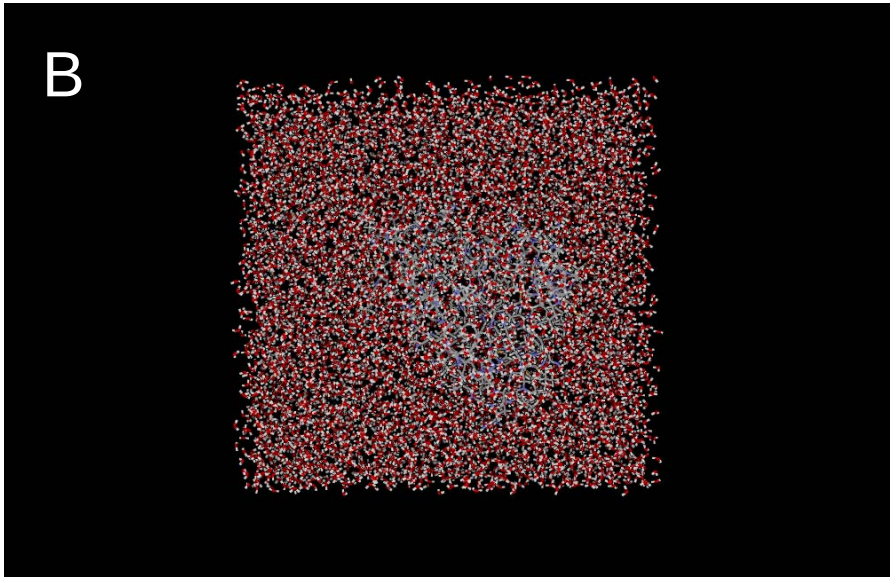
Amber

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

Updating coordinates



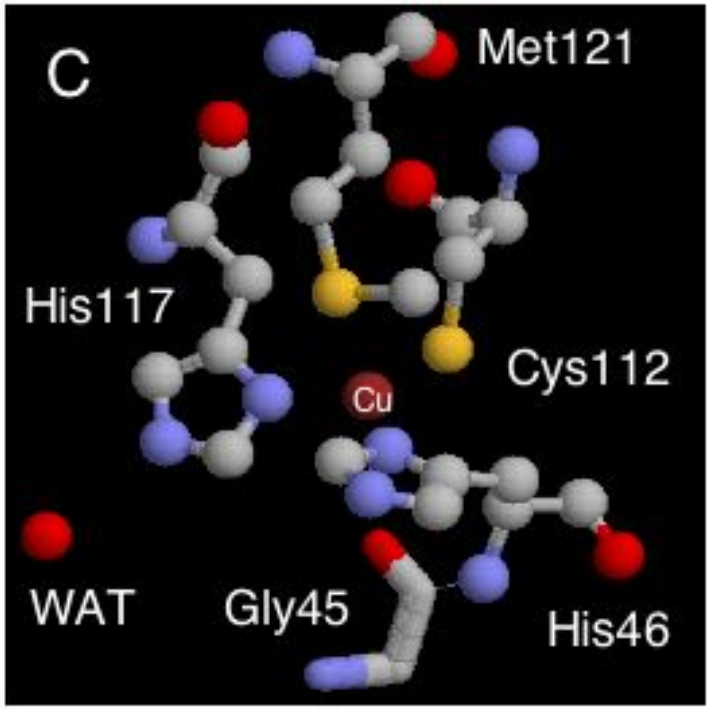




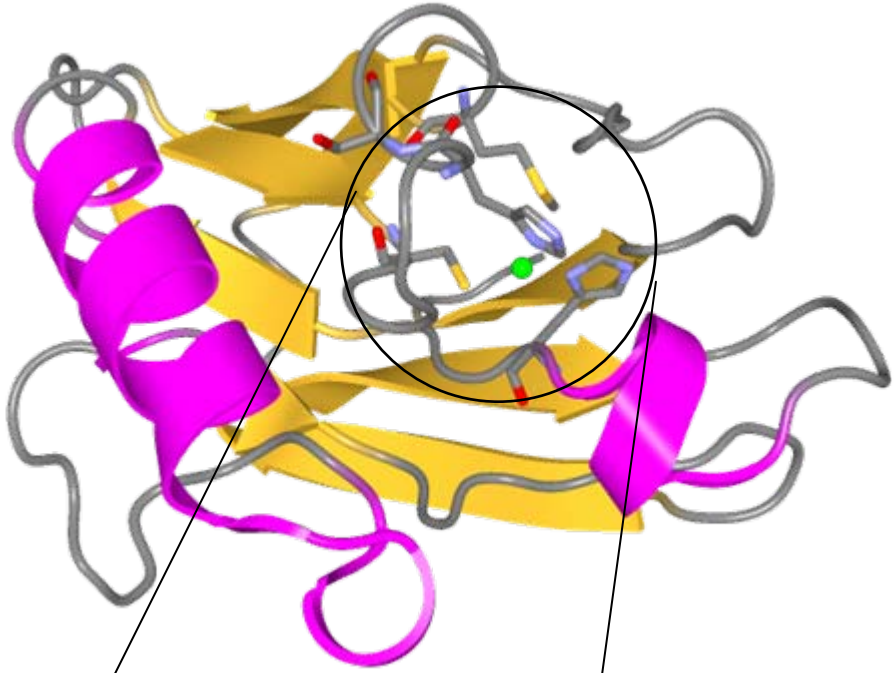
Coordination environment of Azurin

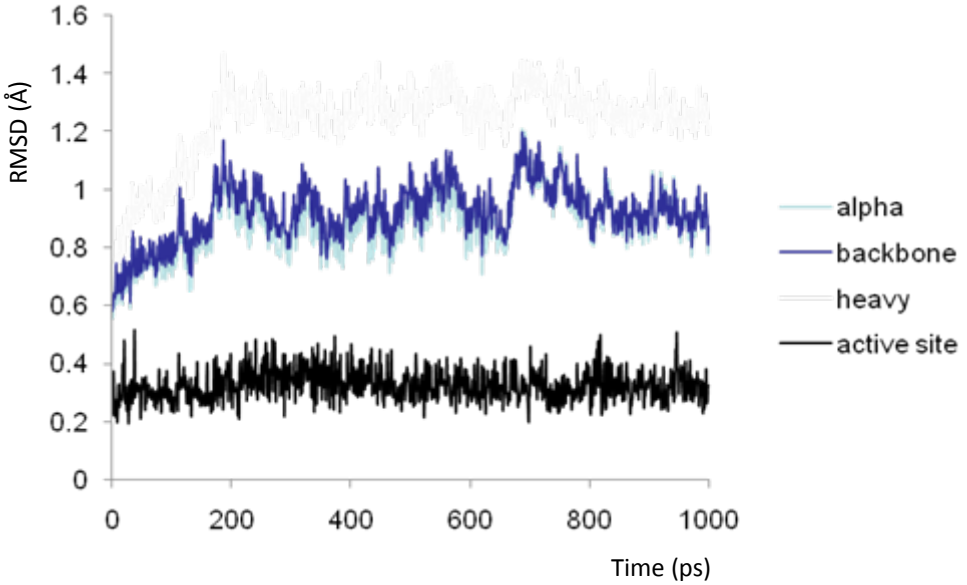
- Trigonal coordination by Cys112, His46, and His117
- Weakly coordinated Met121 and O=C (backbone of Gly45)

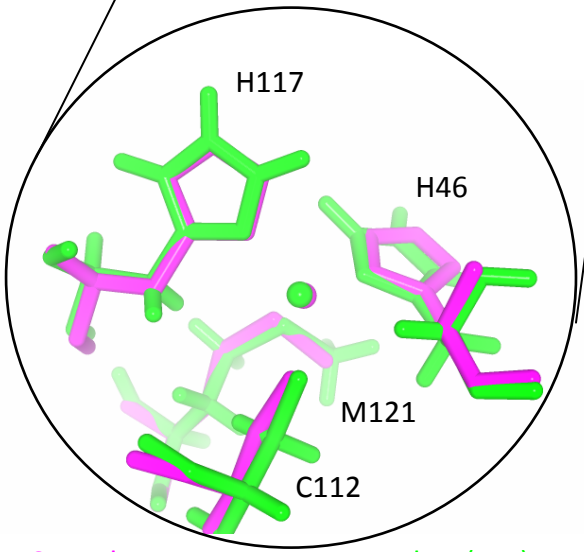
Plastocyanin and Stellacyanin involve four coordinate Cu with methionine and glutamine as an axial ligand, respectively



Electrostatic effect on the coordination environment of Azurin Cu

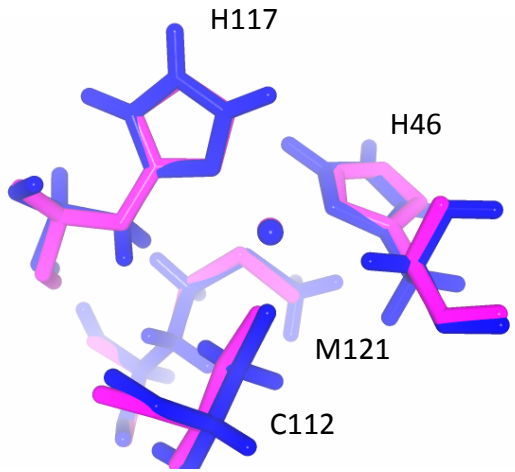






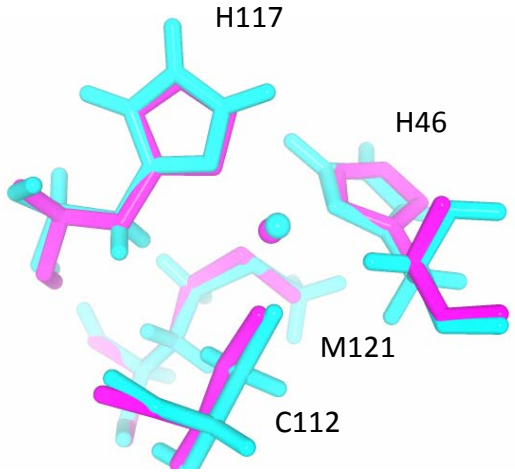
Crystal structure vs MD snapshot (1ns)

RMSD 0.316 Å



Crystal structure vs QM/MM opt (model I)

0.292 Å



Crystal structure vs QM/MM opt (model II)

0.320 Å

Schemes of QM/MM calculation

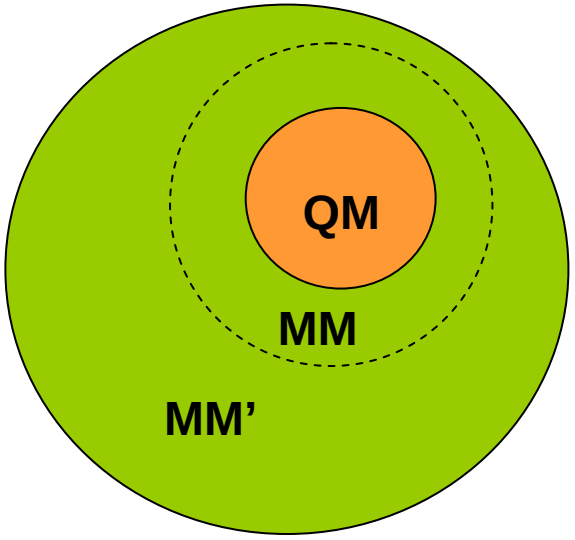
ふたつのモデルによる評価:

Model I : CutOff = 50 A

Model II: CutOff = 0 A

Hybrid (implemented in our system)

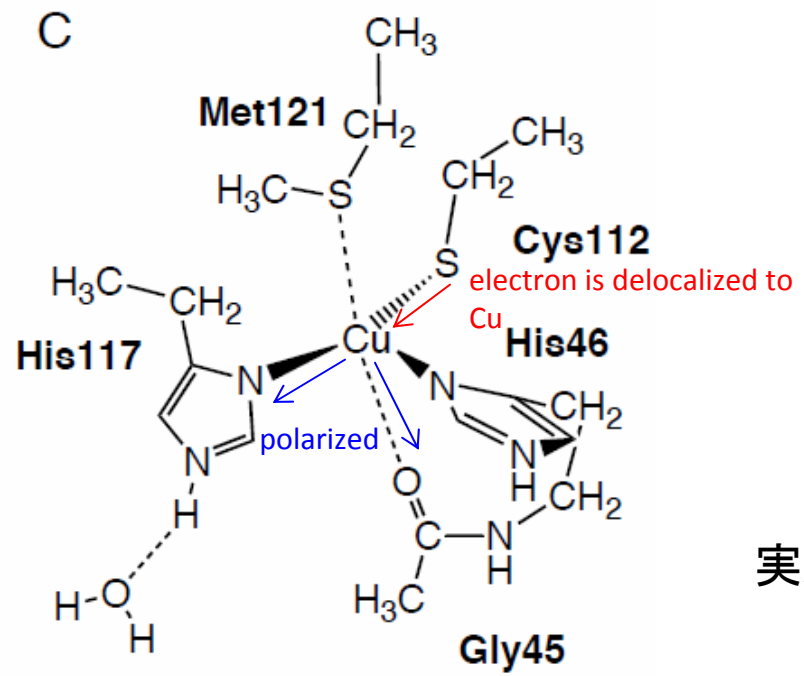
Both of “QM→MM” and “MM→QM” interactions are included in the calculation



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

$$H_{MM} = \sum_{\alpha M}\frac{Z_\alpha q_M}{R_{\alpha M}} + E_{MM}$$

$$E = \left\langle \Psi \middle| \hat{H}_{QM} + H_{MM} \middle| \Psi \right\rangle = \left\langle \Psi \middle| \hat{H}_{QM} \middle| \Psi \right\rangle + H_{MM}$$



Mulliken Charge (Spin Population)

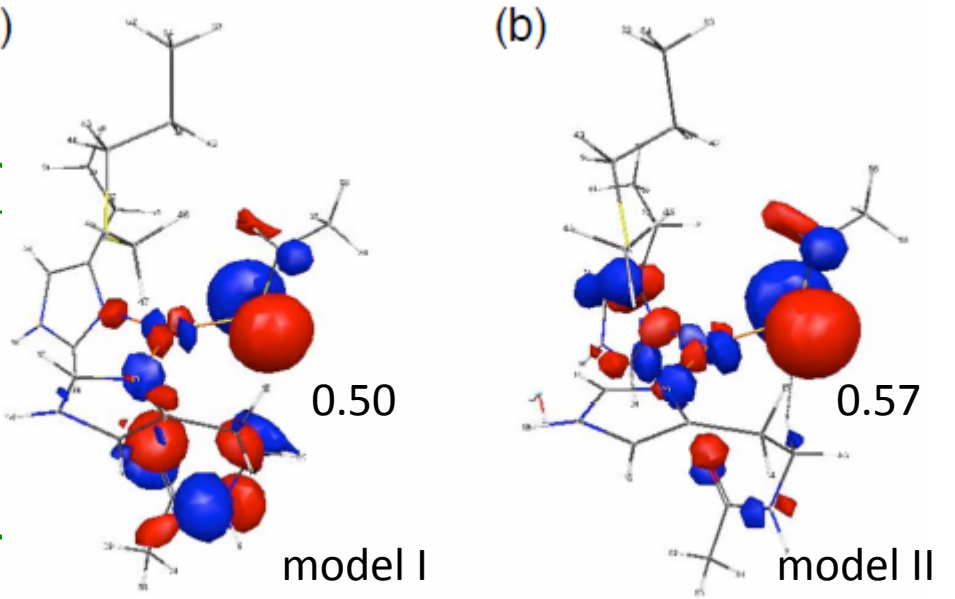
	Model 1	Model II
Cu	0.49(0.39)	0.48(0.34)
S_{Cys112}	-0.09(0.50)	-0.06(0.57)
S_{Met121}	0.06(0.00)	0.07(0.00)
O_{Gly}	-0.5(0.00)	-0.51(0.00)
His117	0.17	0.22

いずれのモデルもCu, S_{Cys112}がSOMOに寄与

実験事実：S_{Cys112} 3p orbitalがSOMOに占める割合は45%

Molecular geometry

	MD-MM	Model 1	Model II	ONIOM	Xtal[a]
Cu-S_{Met121}	3.35	3.49	3.50	3.53	2.87-3.26
Cu-S_{Cys112}	2.25	2.20	2.24	2.17	2.12-2.27
Cu-O_{Gly45}	2.96	3.01	2.81	2.55	2.75-3.16
Cu-N_{His117}	1.95	2.00	2.10	2.01	1.99-2.12
Cu-N_{His46}	2.06	2.03	1.93	1.99	1.99-2.12
O_{H2O}-H_{His117}	1.95	1.98	2.03	-	-
H-N_{His117}	1.02	1.03	1.02	-	-

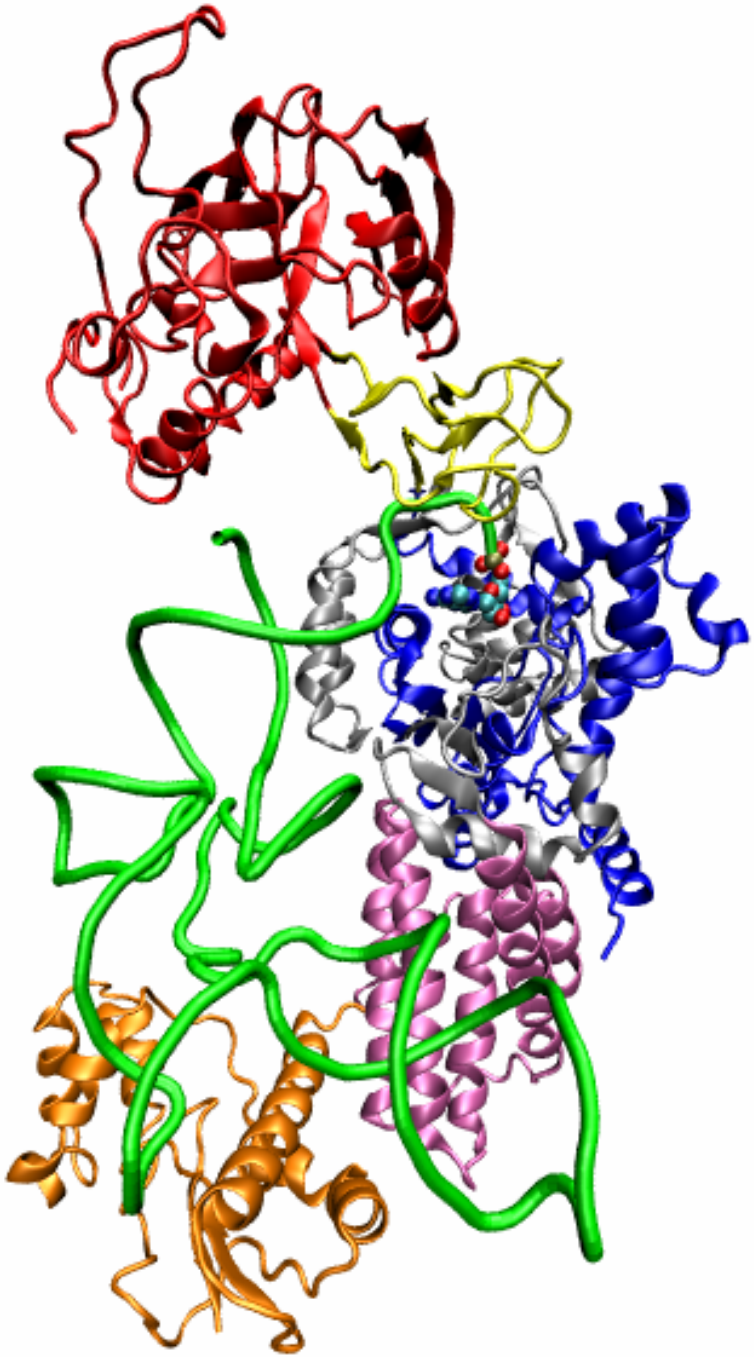


- Model IはSpin densityの測定値とより近い
- 分極した結合長に違いが生じる → Cu-O(G45), Cu-N(H117)
- Cu-N(H117) の分極は結晶水との水素結合の強さに依存する

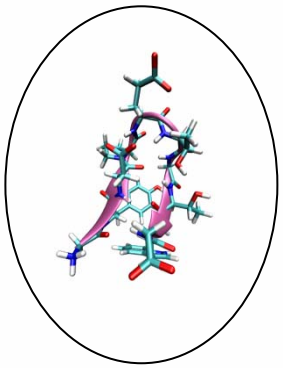
Model Iの方がより実験事実を再現

Protein-RNA complex

Aminoacyl-tRNA synthetase: 1200 amino acid residues

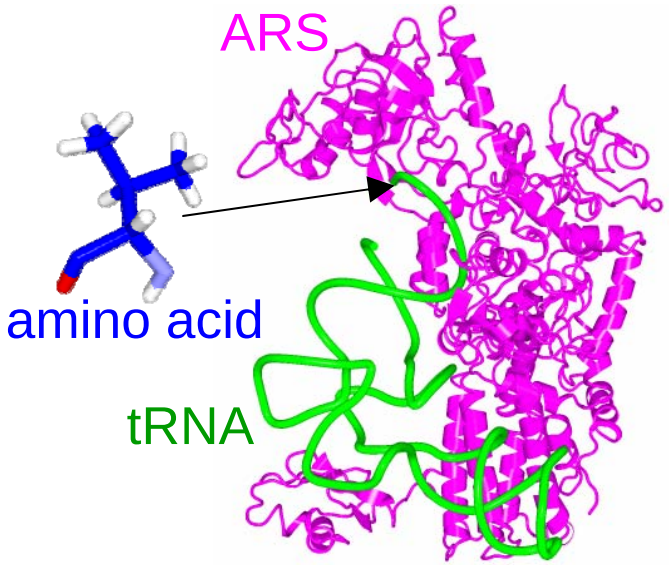


Chignolin: 10 amino acid residues



Honda, et al, *Nature Mol Struc Biol.*

Incompleteness of experimental structures

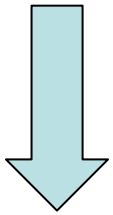


X-ray structure	protein	$tRNA^{Leu}$	amino acid	crystallographic water
2BYT	○	○	×	×
1OBC	○	×	○	○
1H3N	○	×	×	○

We dock the amino acid / solvent water molecules toward the experimental structure by using a novel molecular docking algorithm.

Problems in the molecular docking simulation

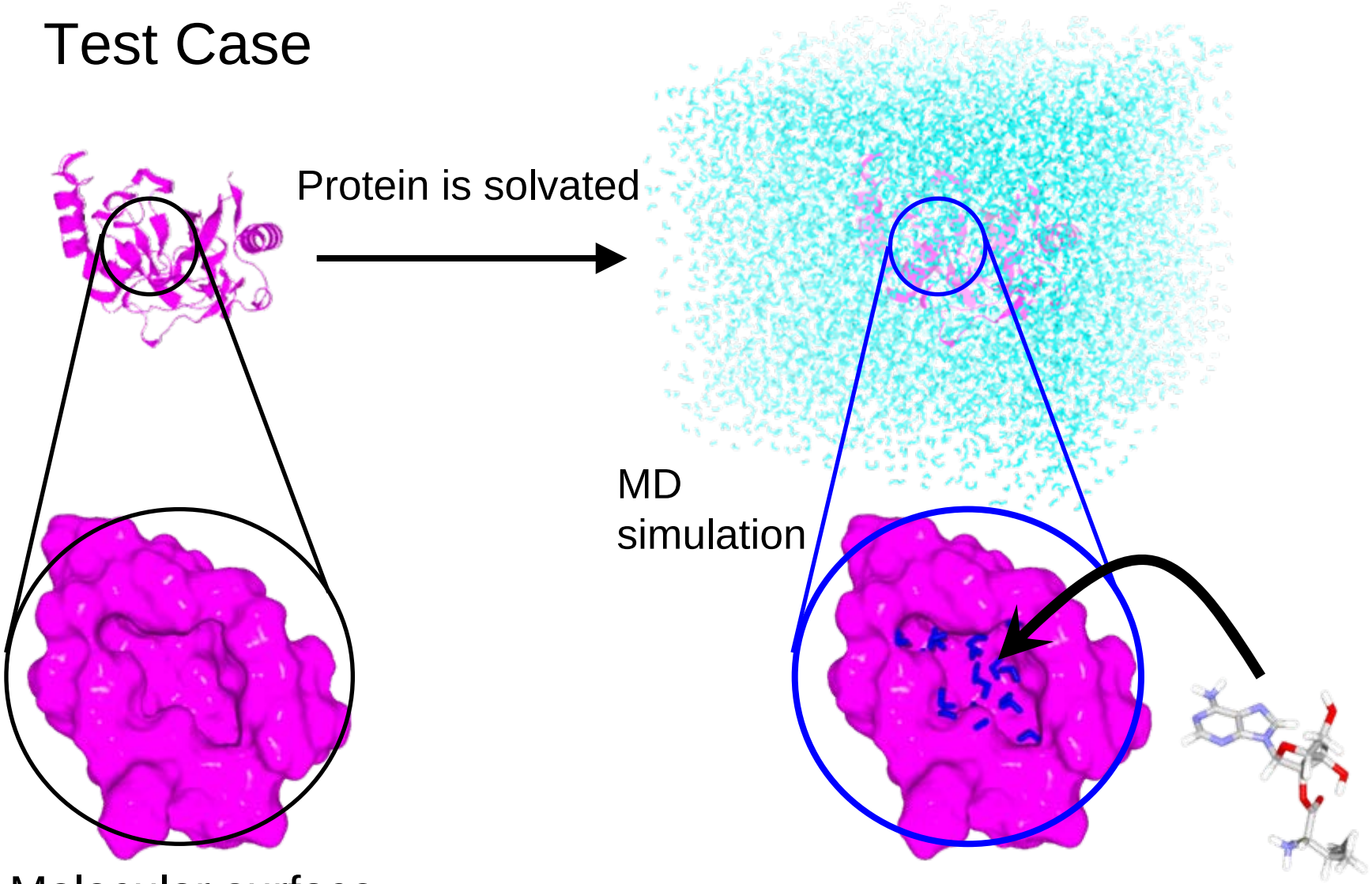
Problem:
Effects of **explicit solvent water molecules**



We have developed a novel algorithm for sophisticated molecular docking calculation, which introduces effects of **explicit solvent water molecules**.

Development of molecular docking simulation

Test Case



Development of molecular docking simulation

Step 1;
Relaxation of solvent water molecules

Step 2;

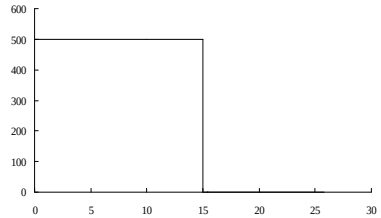
- Atomic volumes and charges of substrate are increased on the basis of the following equation.

$$E(\lambda) = E_0 + \lambda(E_1 - E_0)$$

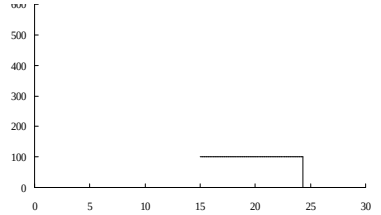
- Temperature, the relaxation time constant of pressure regulation and force constants of the positional constrains are regulated.

Force constants of positional constraints (kcal mol^{-1} Å^{-2})

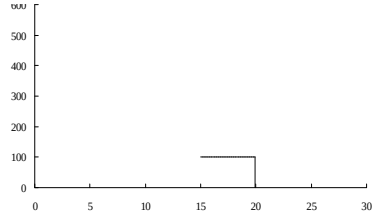
protein atoms of main chain



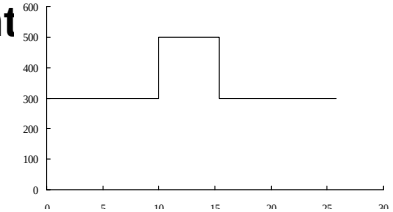
ligand atoms of fragment 1



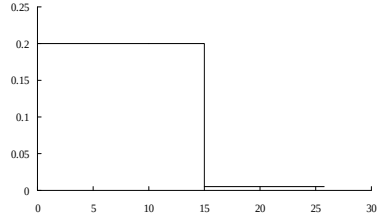
ligand atoms of fragment 2



Relaxation time constant of heat bath coupling for the system (ps)

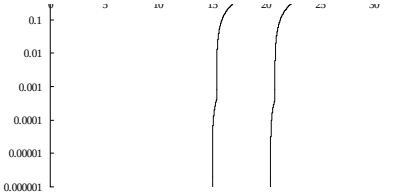


Temperature (T)



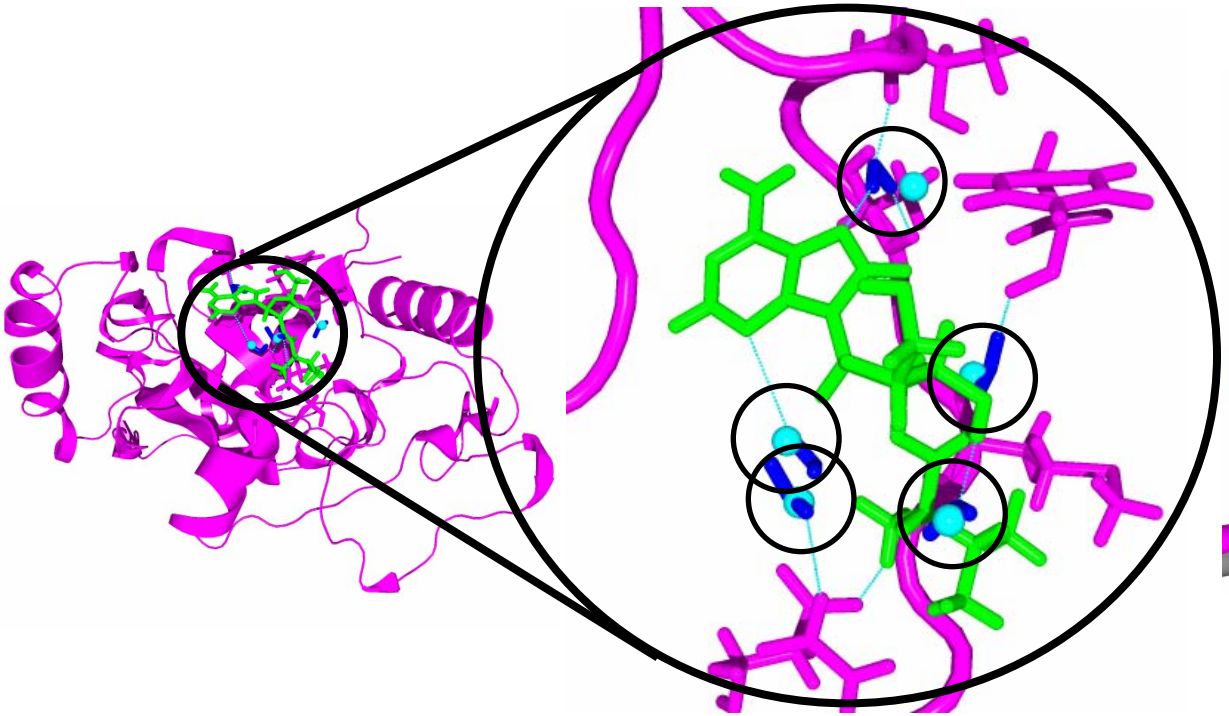
log λ

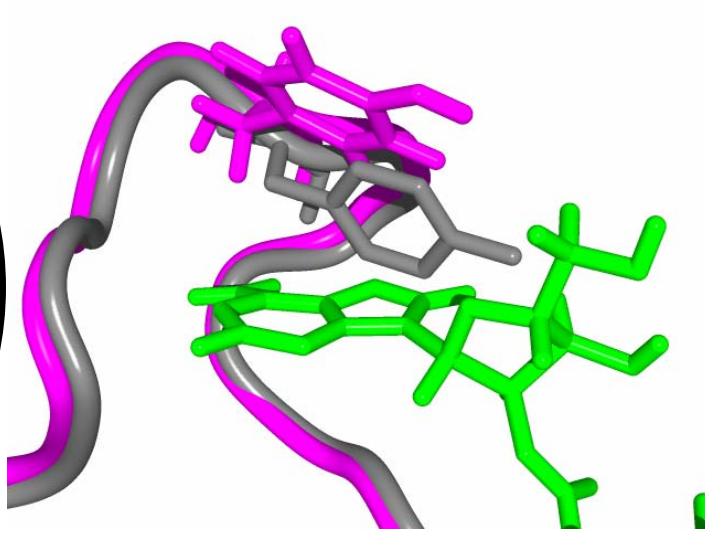
time (ps)



Fully Solvated Dynamical Docking (FSDD)

Results of the test case

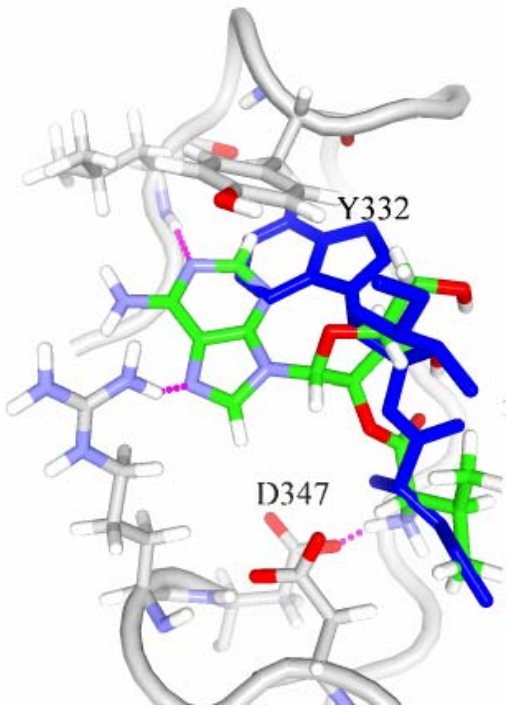




All of five crystallographic water molecules bound to the protein and the ligand could be predicted.

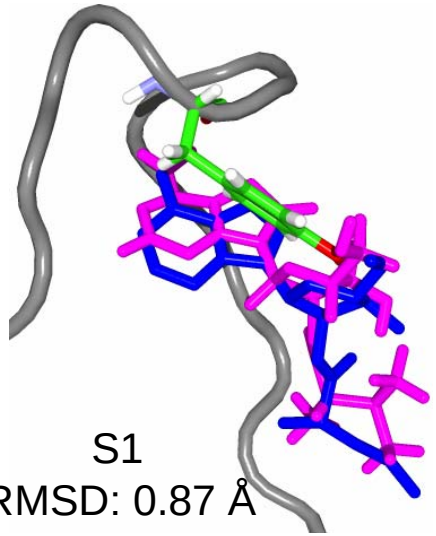
Both of the ligand and protein conformations could be predicted.

Initial structures for FSDD calculation

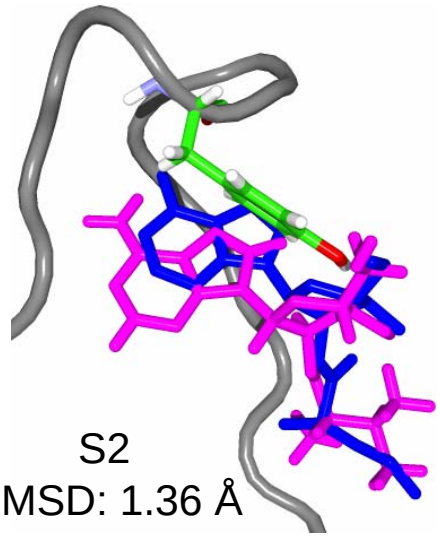


1. Initial structures for FSDD calculation are obtained by conventional docking calculation without considering explicit water molecules and conformational changes of the protein

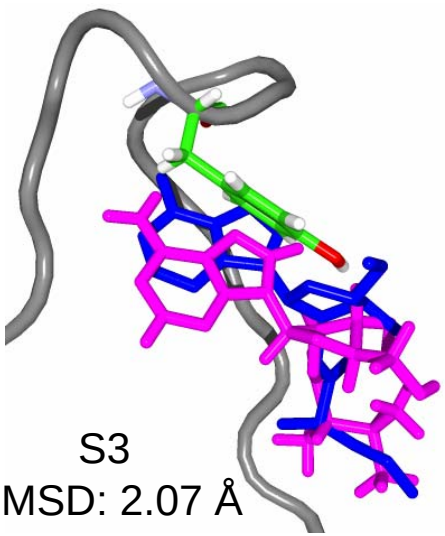
2. Structural modeling of the structures obtained above for finer docking; adenosine moiety in substrate form syn-conformation which corresponds to high energy state, and so, its conformation is changed to anti-conformation.



S1
RMSD: 0.87 Å



S2
RMSD: 1.36 Å



S3
RMSD: 2.07 Å

blue : crystal structure
magenta: initial structure

Advantages of FSDD

1. Inclusion of **effects of explicit solvent water**

2. Inclusion of **conformational changes of the protein**

3. Simulation time

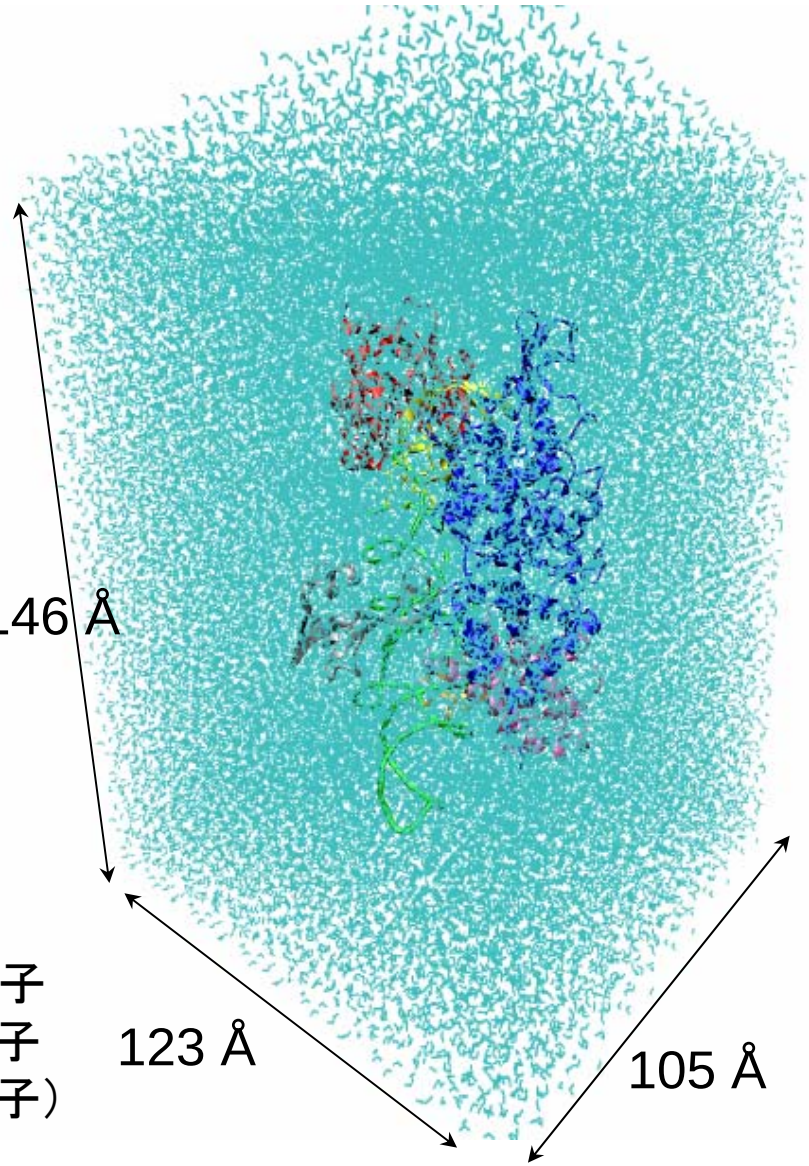
   It takes 70,000 ps to include above two things in the multi-canonical sampling scheme
   (Kamiya et al., *Proteins: Struct., Funct., Genet*. 2007)

protein	1H3N	1HI5	1HI2	4RSK	3RSK	1E3V
time (ps)	**30.8**	**26.0**	**25.6**	**25.6**	**25.6**	**25.6**

FSDD calculation for LeuRS-tRNA complex

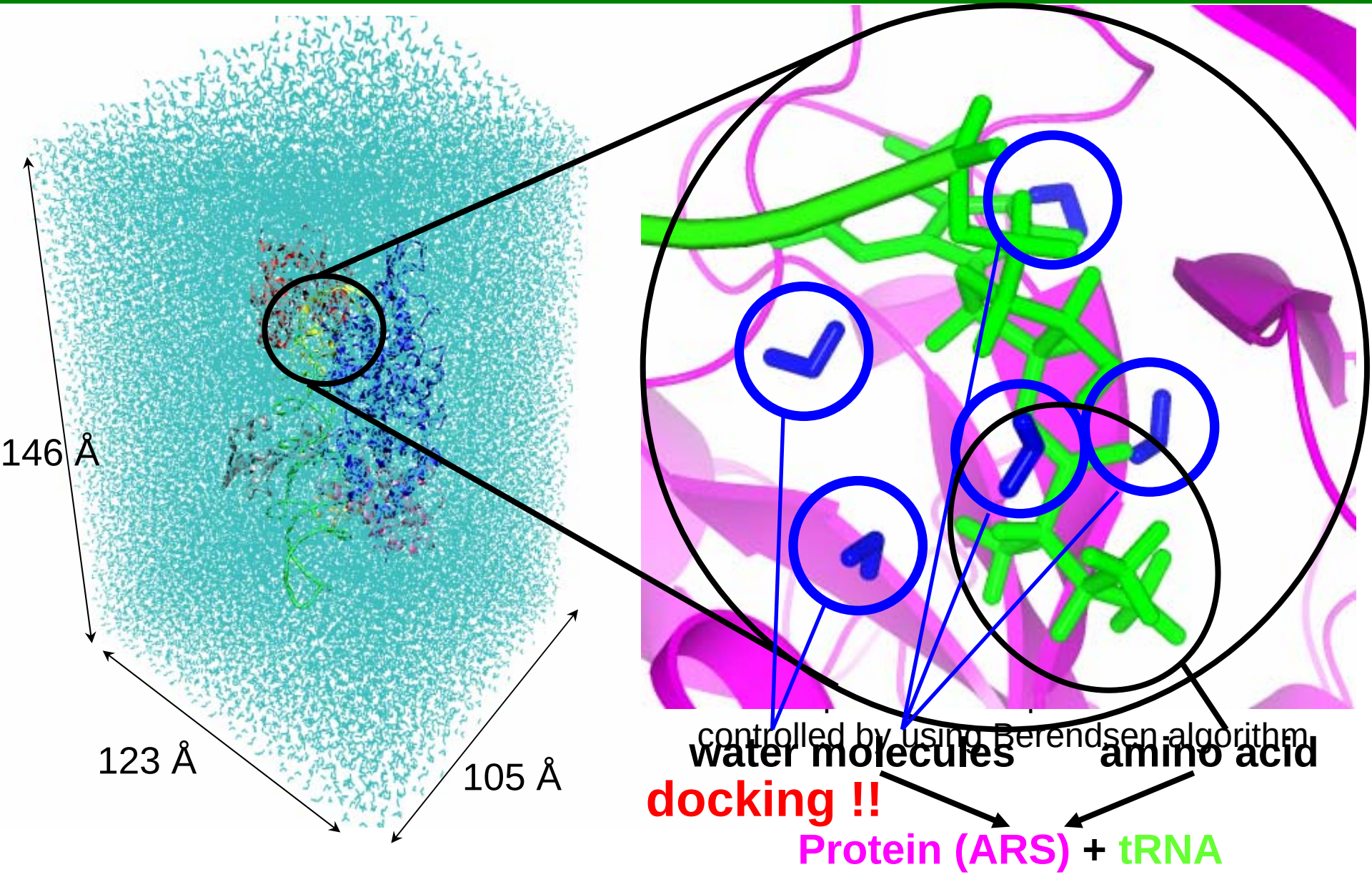
Setup of the system analyzed:

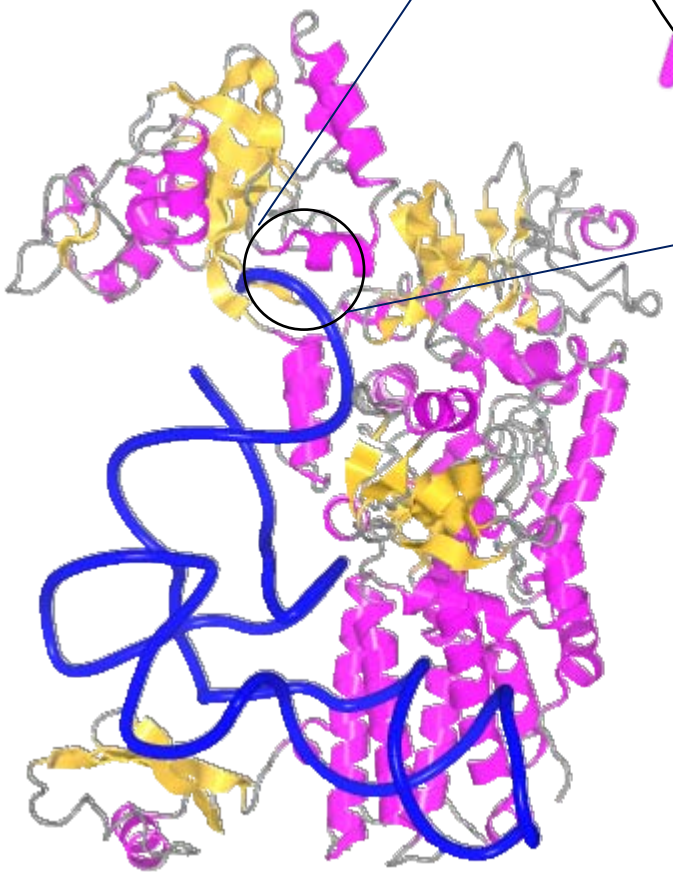
1) Box water → Solvation
2) Ions
3) Periodic Boundary Condition
4) Ewald summation → Electrostatic Interactions

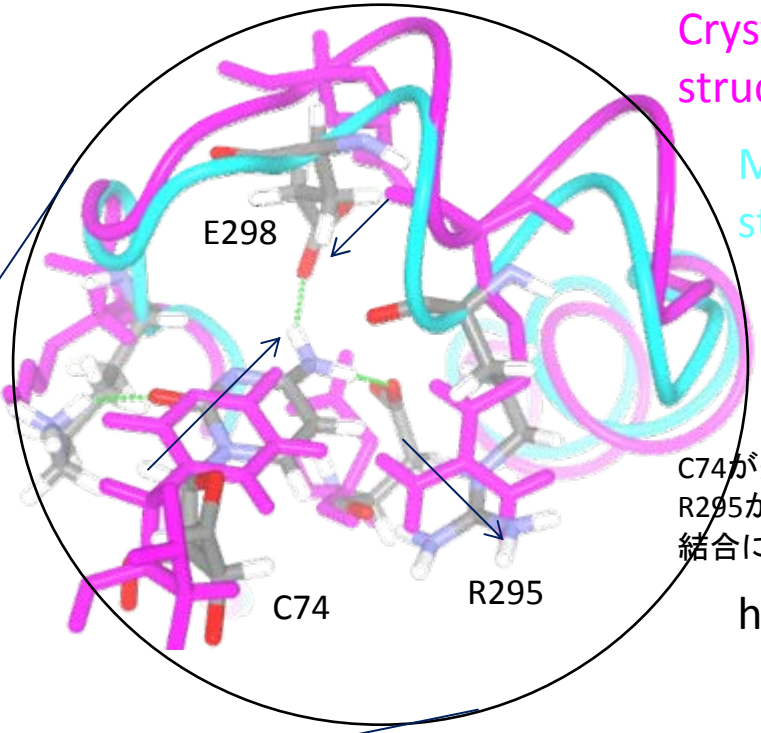


タンパク質・RNA複合体: ～ 10,000原子
溶媒を含めると: ～100,000原子
（本研究: ～170,000原子）

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







Crystal structure

MD structure

C74が矢印方向に入り込むことで、helix上のR295が押しのけられるのと同時にE298が水素結合によって引きつけられる

helixのずれを引き起こす

QM/MM計算　MDおよびOptimization

QM原子数

QM/MM-MD:　QM:　121-atom

QM/MM-Opt:　QM:1,000-atom

総原子数　… 165,721

QM領域 →DFT (B3LYP)

MM領域 →AMBER parm99

QM原子数	77	121	1,023
MM原子数（1電子積分に含む）	4,252	4,134	3,845
MM原子数（1電子積分に含まない）	161,423	161,490	160,519

Simulation 1 (反応座標 : $d_{Ow\text{-}C}$)

水分子（求核剤）の攻撃：結合生成

手法 : QM/MM MD

温度　300 K

Time step for integration … 0.1fs

Window計算の設定：3.4 – 1.4 Åまで0.1 Å刻み（各Windowあたり0.01 ps）。

Force Constantは200kcal/molÅ^2

Simulation 2 (反応座標 : $d_{Hw\text{-}O2'}$,$d_{C\text{-}O2'}$)

結合の切断

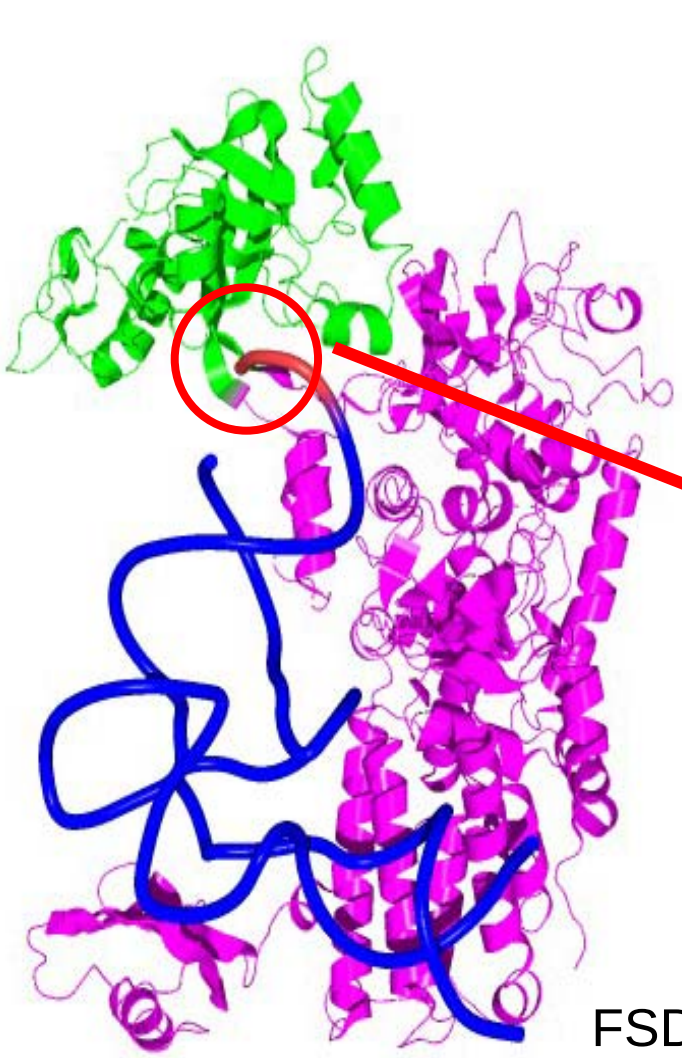
手法 : QM/MM minimization

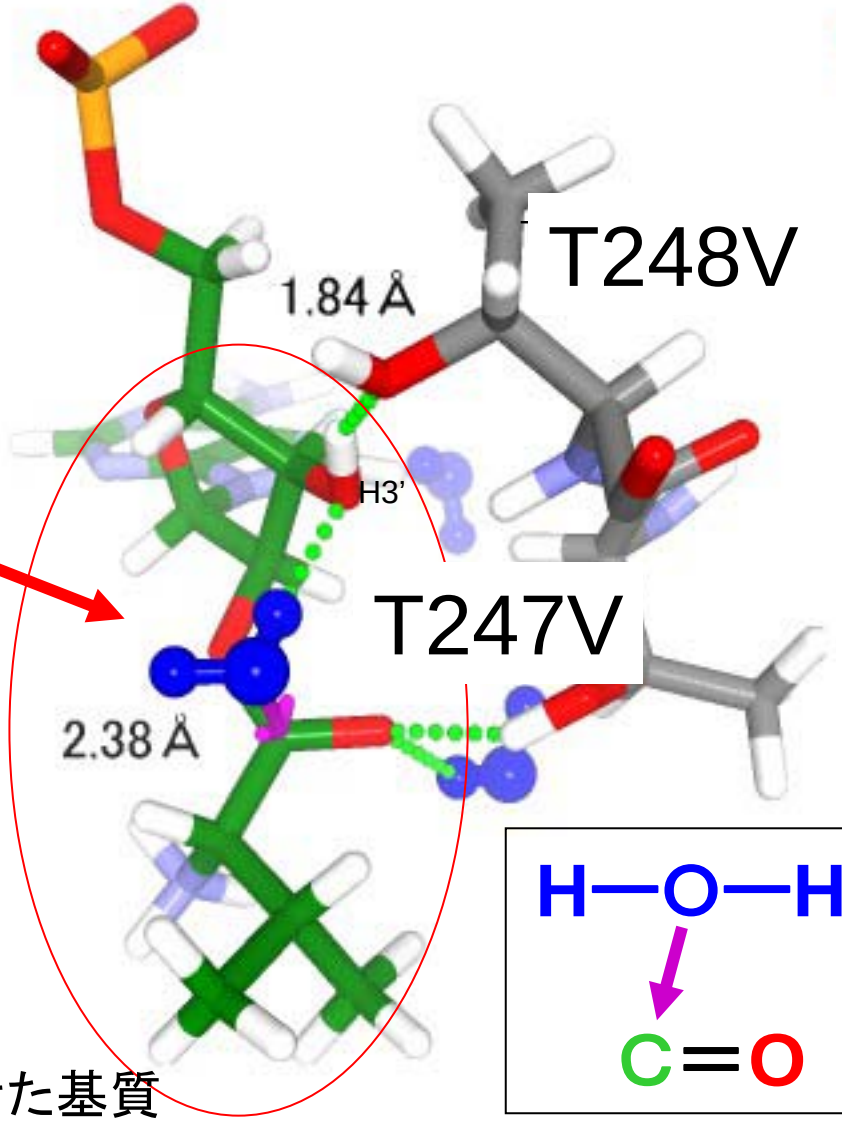
Distance Constrainの設定： $d_{Hw\text{-}O2'}$,$d_{C\text{-}O2'}$の平衡距離はそれぞれ1.0, 2.0 Åに設定。

Force ConstantはMDと同様

機能改変への挑戦 → アミノ酸残基の変異

QM/MM hybrid MD simulation





FSDDにより
ドッキングさせた基質

H—O—H
↓
C=O

まとめ

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） QM/MM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，**新しい反応機構**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。