# QM/MM study on inhibitor of HIV-1 protease

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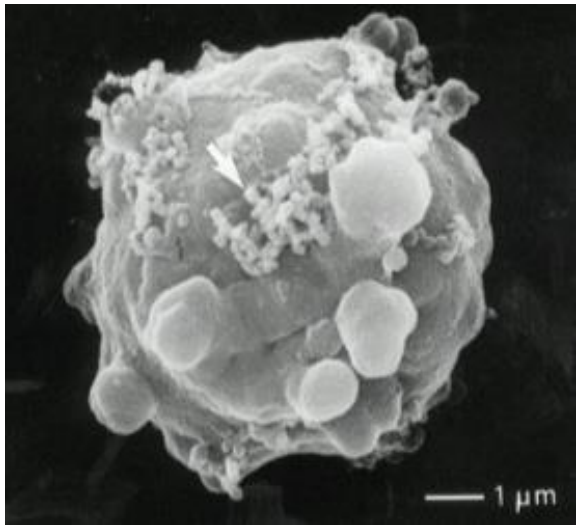
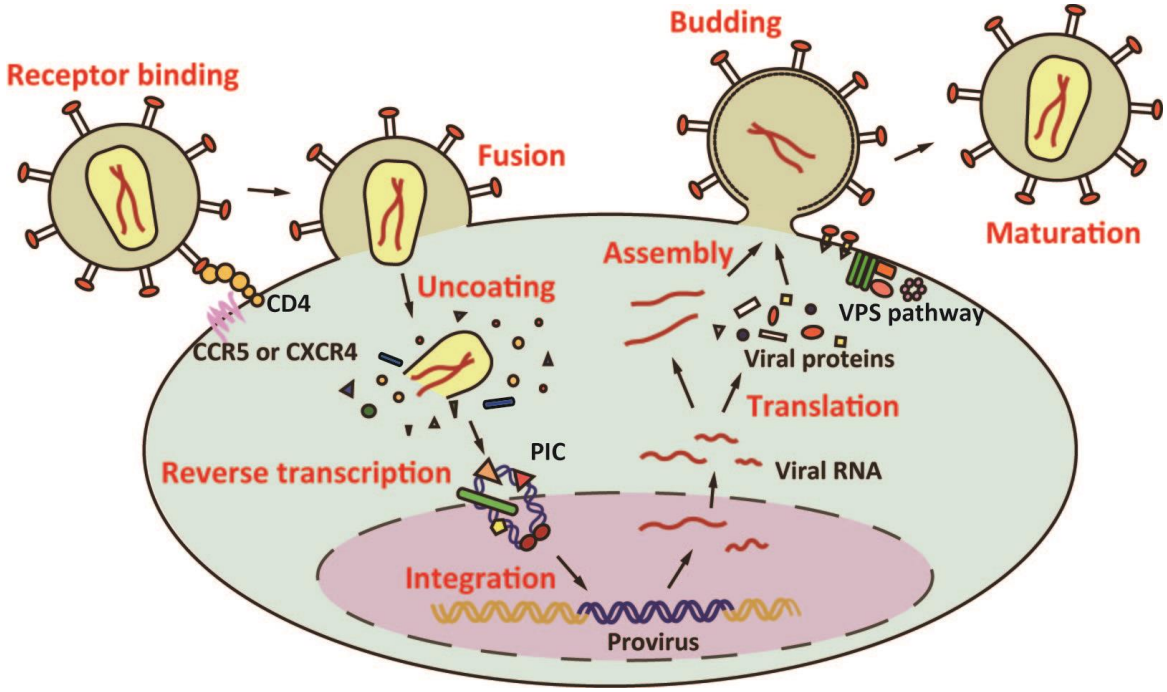
# HIV

- Human Immunodeficiency Virus
- Origin of AIDS



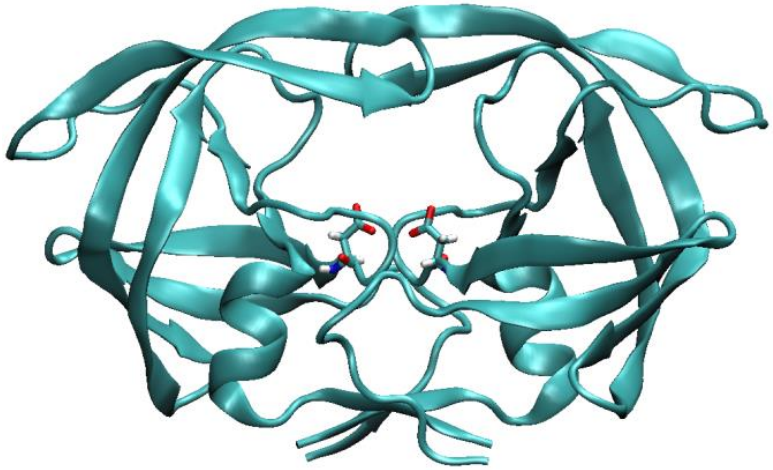

HIV appearing from lymphocytes
Ref: https://www.pref.hiroshima.lg.jp/soshiki/25/bu-biseibutu2-hivvirus.html



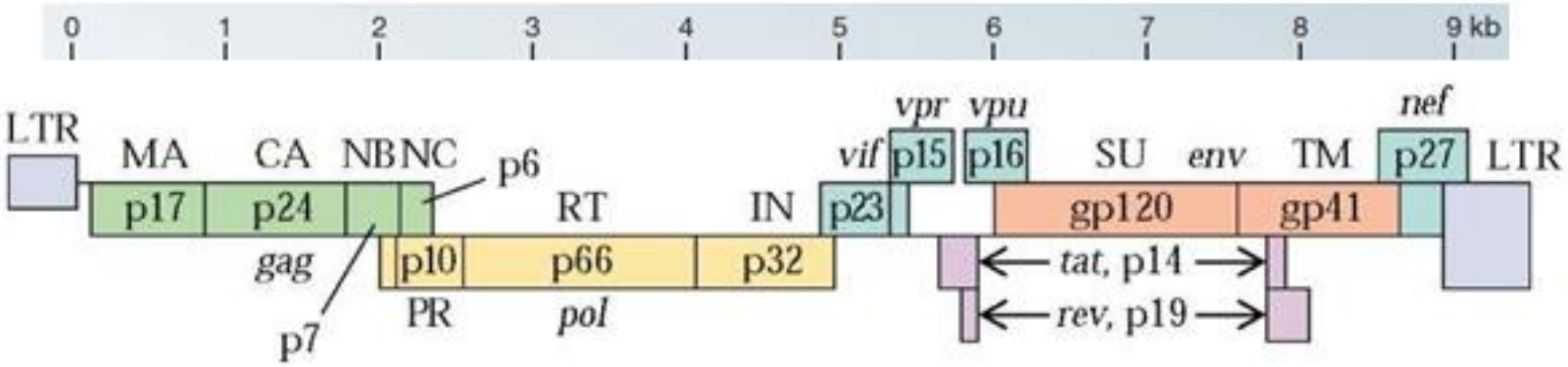

HIV replication in a cell
Ref: http://colgateimmunology.blogspot.de/2013/12/a-new-mechanism-of-cell-death-by-hiv.html

# HIV-1 protease

- One of protein in HIV virus
- Activated by cutting polyprotein (gag, pol) with hydrolysis



HIV-1 protease

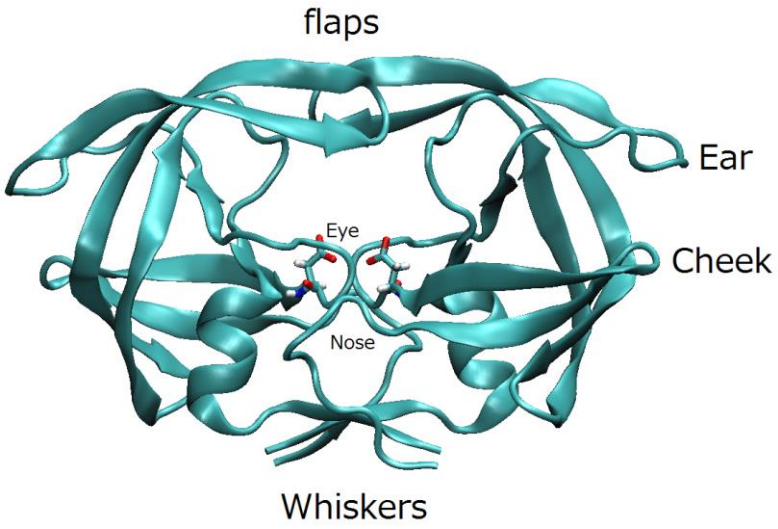


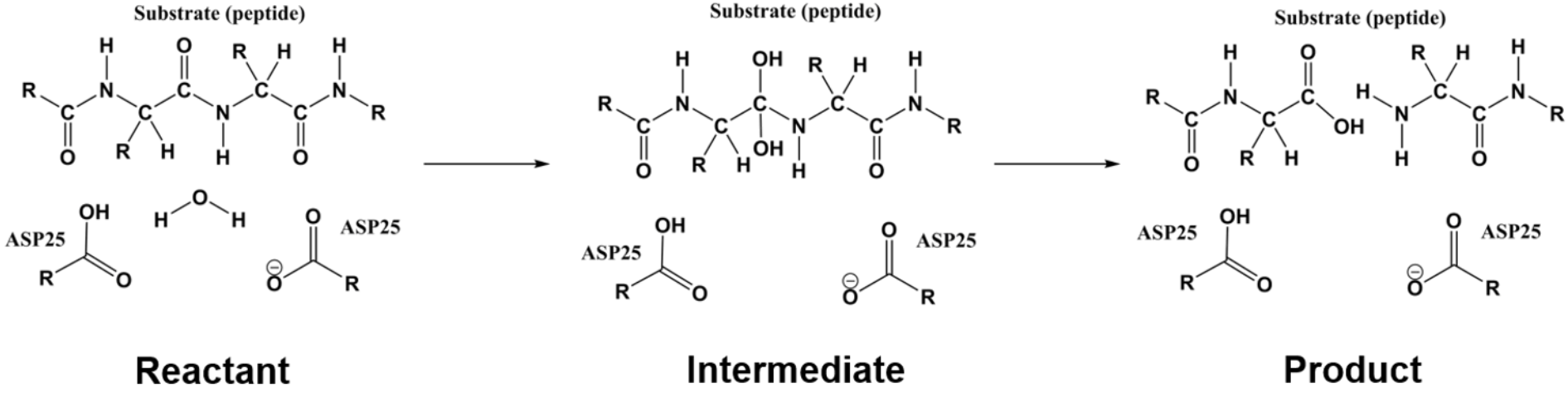

Part of gene of HIV-1 protease

Ref: http://edoc.rki.de/documents/dissertationen/wittwer-malte-2014-10-01/PDF/wittwer.pdf

# HIV-1 protease

- Homodimer consisted of 99 residues × 2
- Activation part: two Asp25
- Catalyzing hydrolysis of peptide bonds

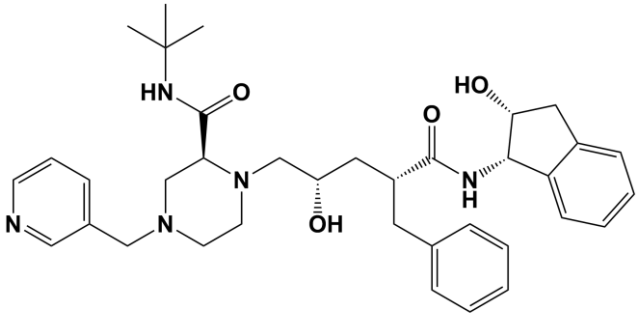


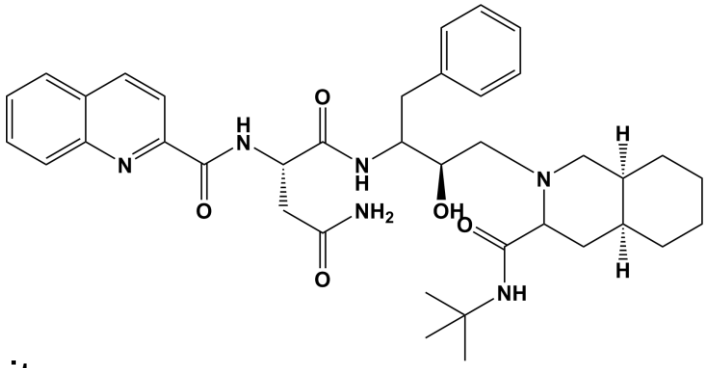

*water molecule near chemical reaction part is important!*

# Inhibitors of HIV-1 protease

- AIDS medicine
- Transition state(TS) analog molecule
- Serious drug-resistance(high mutation rate)

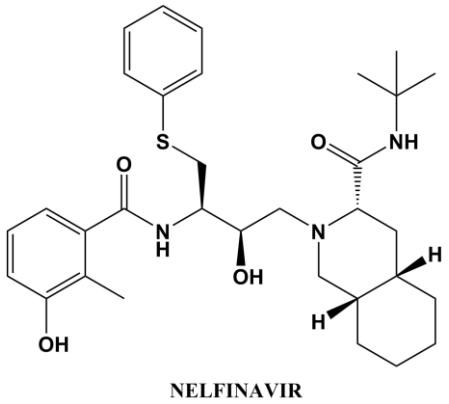


INDINAVIR

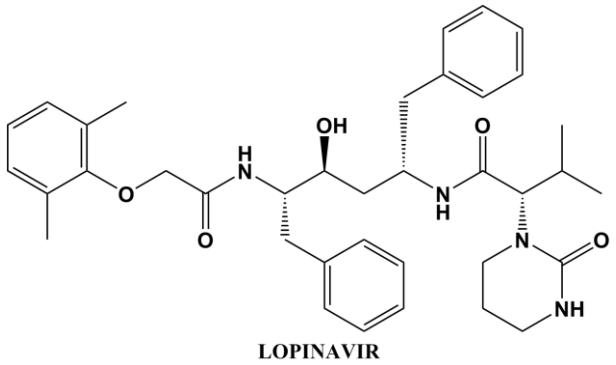


SAQUINAVIR

Example of inhibitor
of HIV-1 protease



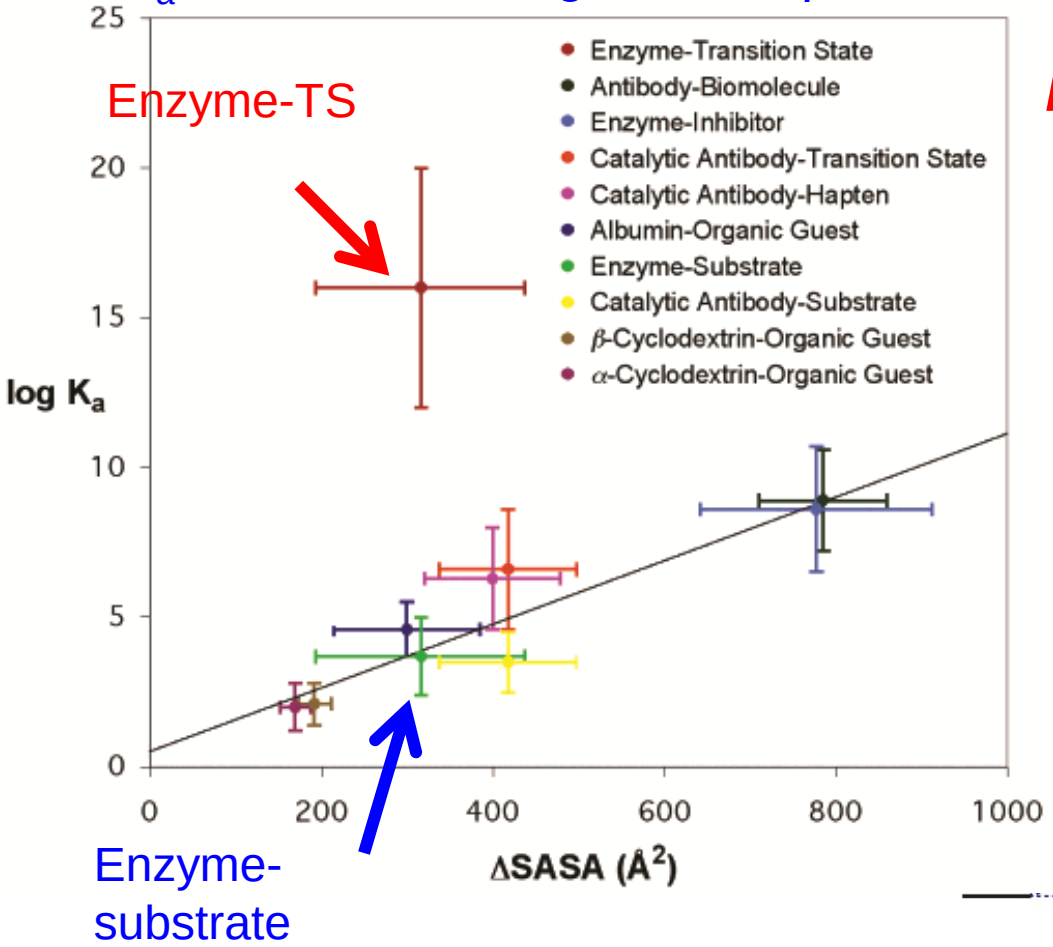
NELFINAVIR



LOPINAVIR

# Stabilization of TS in enzyme

$K_a$ of various host-guest complexes

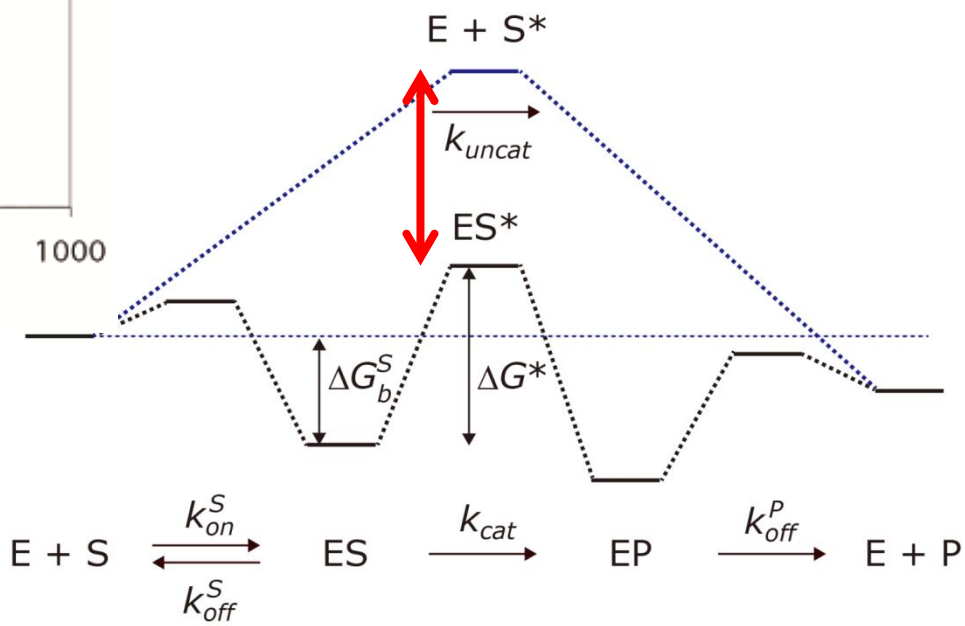



Zhang, Houk, ACR (2005)

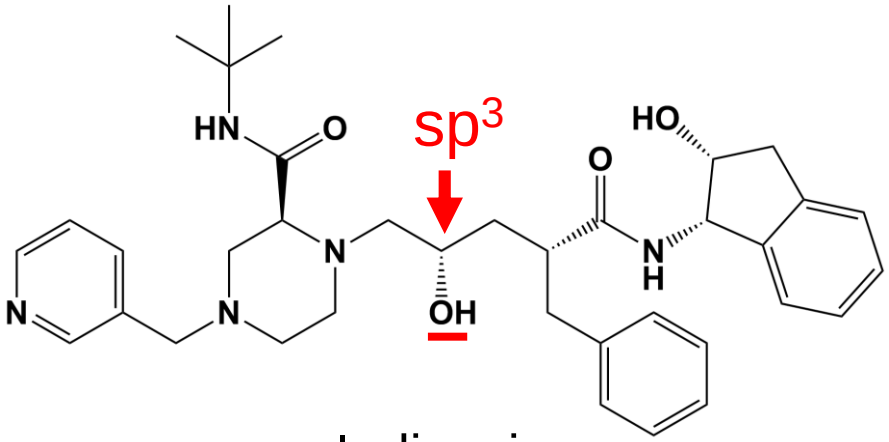
$K_a$ of TS substrate

$$\log K_a \propto -\log \frac{k_{cat}}{K_m k_{uncat}}$$

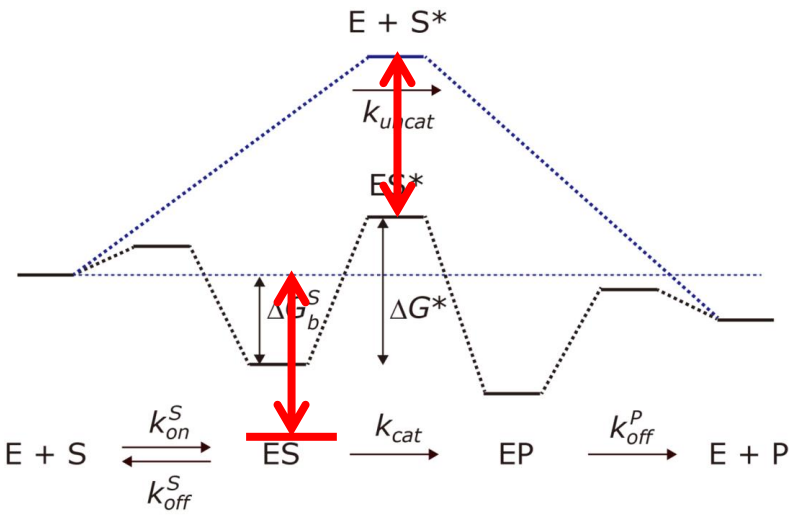
Exceptionally large

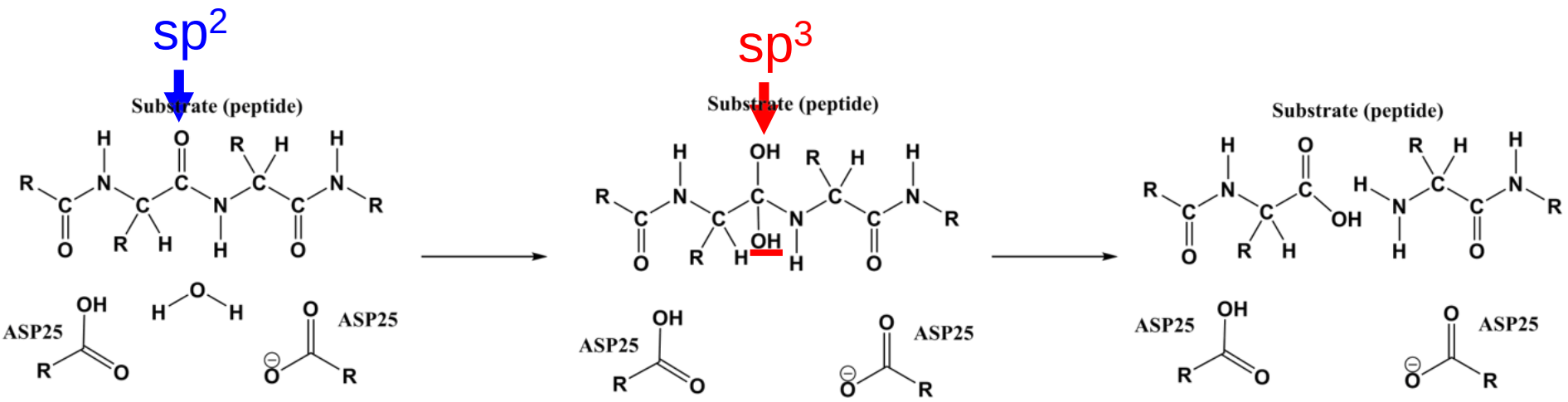


# TS analog inhibitor



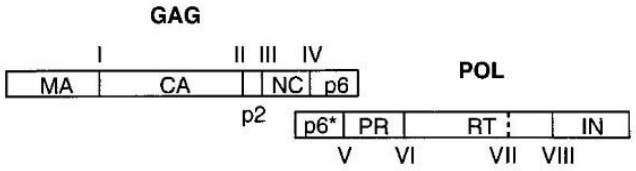

Indinavir




**Reactant** **Intermediate** **Product**

**(close to TS)**




# Drug Resistance

Shock *et al.*, J. Biol. Chem. **271**, 31957 (1996)

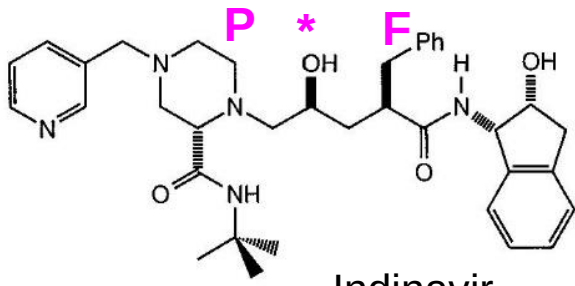
## Reaction rate of natural substrate molecule

| Cleavage site | Peptide sequence | Wild-type | | 4X | | M46I/L63P | V82T/I84V |
|---|---|---|---|---|---|---|---|
| | | $M^{-1}\,s^{-1}$ | | $M^{-1}\,s^{-1}$ | | $M^{-1}\,s^{-1}$ | $M^{-1}\,s^{-1}$ |
| *I.* MA(p17)/CA(p24) | NQVSQNY*PIVQNI | 17,980 | | 940 | | 65,100 | 830 |
| *II.* CA/p2 | GHKARVL*AEAMSQ | 720 | → | 310 | ~1/2 | 790 | 220 |
| *III.* p2/NC(p7) | TNSATIM*MQRGNF | 27,970 | | 3,440 | | 76,300 | 1,770 |
| *IV.* NC/p6 | KGRPGNF*LQSRPE | 370 | | 140 | | 490 | 43 |
| *V.* p6*/PR | GTVSFNF*PQVTLW | 113,400 | | 6,810 | | 221,400 | 3,760 |
| *VI.* PR/RT | IGCTLNF*PISPIE | 8,930 | → | 430 | ~1/20 | 14,600 | 300 |
| *VII.* RT (internal) | IVGAETF*YVDGAA | 11,400 | | 227 | | 14,160 | 130 |
| *VIII.* RT/IN | AGIRKVL*FLDGID | 845 | | 58 | | 1,850 | 38 |

## Dissociation constant of inhibitor molecule

| Inhibitor | Wild-type | | 4X | | M46I/L63P | V82T/I84V |
|---|---|---|---|---|---|---|
| | *nM* | | *nM* | | *nM* | *nM* |
| MK-639 | 0.40 | → | 21.6 | x 50 | 0.47 | 23.7 |
| ABT-538 | 0.19 | | 28.5 | | 0.15 | 30.1 |
| Ro 31–8959 | 0.33 | | 11.9 | | 0.37 | 13.3 |
| VX-478 | 0.57 | | 5.8 | | 0.53 | 10.1 |

1. **High activation part is largely effected by its mutation**
2. **Inhibitor is a mimic of high activation site (e.g. F * P)?**
3. **Low activation part (determines overall activation) is smally effected by its mutation (e.g. site II)**



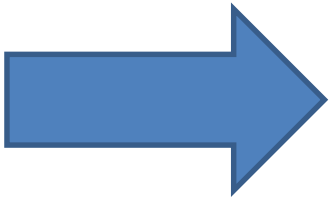

Indinavir

# Purpose

1. Precise identification on transition state of natural substrate molecule

2. Clarifying drug binding affinity and molecular mechanism of enzymatic reactive activity for drug-resistant mutant

3. Design of drug molecule for controlling both drug binding affinity and reactivity of natural substrate



***Correlation between chemical reaction part and surrounding protein environment is important***

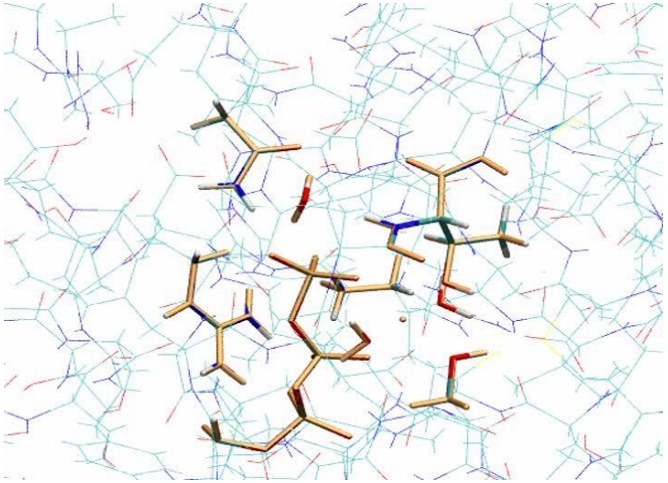
**QM/MM RWFE method**

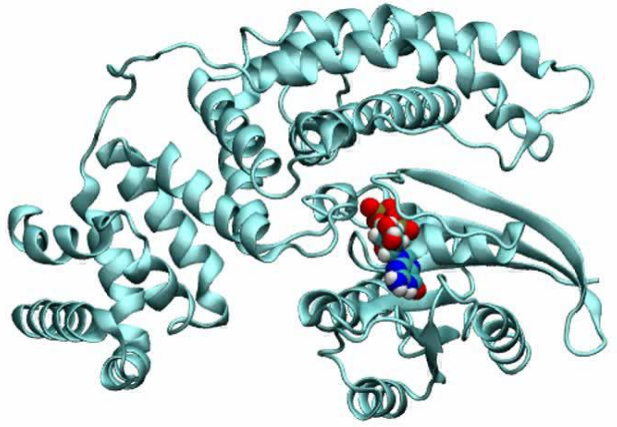
**Complete separation between**

- **QM/MM calculation of a chemical reaction and**
- **MD simulation for protein conformational sampling**

## QM/MM free energy (FE) geometry optimization

**Geometry optimization of the QM molecule on a *free energy surface* defined with the MM conformational thermal distribution obtained by MD simulations**





~ 1 μ second (> 1,000 times longer than direct QM/MM-MD)

# QM/MM-FE Geometry Optimization

Nagaoka and co-workers

## Free energy functional

$$F[\Psi] = -k_B T \ln \int\int d\mathbf{R} d\mathbf{X} \exp\left[-\beta E^{QM/MM}\left(\{\Psi(\mathbf{R},\mathbf{X})\},\mathbf{R},\mathbf{X}\right)\right]$$

$\Psi$ : electronic wf, R : QM coordinates, X : MM coordinates

Give up sampling of R ↓

$$F[\Psi,\mathbf{R}] = -k_B T \ln \int d\mathbf{X} \exp\left[-\beta E^{QM/MM}\left(\{\Psi(\mathbf{R},\mathbf{X})\},\mathbf{R},\mathbf{X}\right)\right]$$

Free energy surface of QM coordinates

Gradient on FE surface: mean gradient

$$\frac{\partial F}{\partial R_i} = \left\langle \frac{\partial E^{QM/MM}}{\partial R_i} \right\rangle_{\mathbf{X}}$$

**Problem:** Electronic WF depends on X.

Its computational cost is not very different from a direct QM/MM-MD.

# Mean Field (MF) QM/MM Method

Yamamoto

$$F[\Psi,\mathbf{R}] = -k_B T \ln \int d\mathbf{X} \exp\left[-\beta E^{QM/MM}\left(\{\Psi(\mathbf{R},\mathbf{X})\},\mathbf{R},\mathbf{X}\right)\right]$$

MF approximation

$$\Psi(\mathbf{R},\mathbf{X}) \rightarrow \Psi_{MF}(\mathbf{R})$$

↓ MF-QM/MM

$$F[\Psi_{MF},\mathbf{R}] = -k_B T \ln \int d\mathbf{X} \exp\left[-\beta E^{QM/MM}\left(\{\Psi_{MF}(\mathbf{R})\},\mathbf{R},\mathbf{X}\right)\right]$$

↓

Variational solution

$$f^{QM/MM}\left(\mathbf{d},\mathbf{R}\right) = f^{0}\left(\mathbf{d},\mathbf{R}\right) + \hat{\mathbf{q}}^{t}\left(\mathbf{R}\right)\bar{\mathbf{V}}^{Cl}\left(\mathbf{d},\mathbf{R}\right)$$

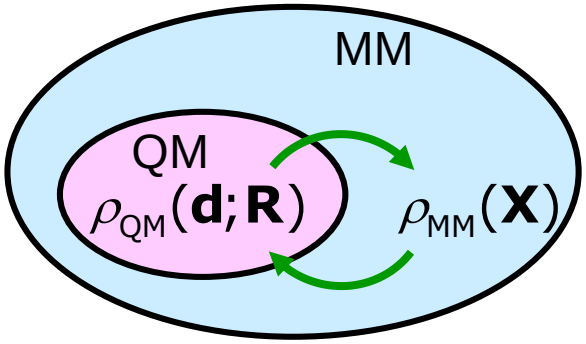
MF of MM region is computed by MD.

# QM/MM RWFE-SCF Method

Kosugi and SH, JCTC (2012)
Kosugi and SH, JACS (2012)

## Problem of QM/MM-FE

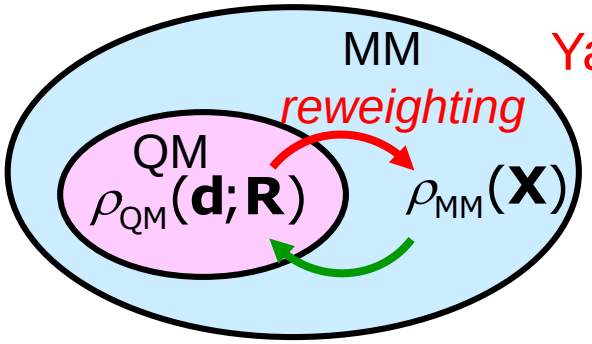
Conventional




Frequent iteration of QM/MM opt. and MD sampling is necessary.
Convergence problem!

Present method

Yang et al.



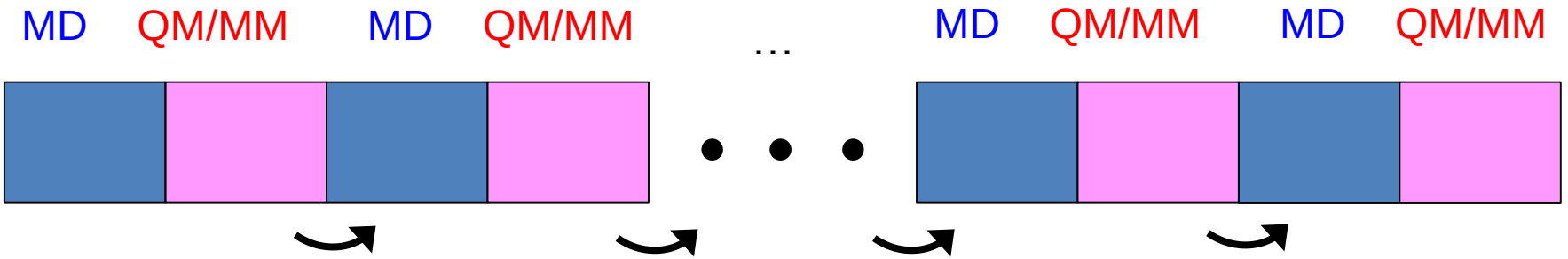

MM distribution is reweighted.

Much quicker convergence of MM thermal distribution

$$\rho_{MM}(\mathbf{d},\mathbf{R};\mathbf{X}) = \frac{\exp\left[-\beta\left\{E^{QM-MM}(\mathbf{d},\mathbf{R};\mathbf{X}) - E^{QM-MM}(\mathbf{d}_0,\mathbf{R}_0;\mathbf{X})\right\}\right]}{\left\langle \exp\left[-\beta\left\{E^{QM-MM}(\mathbf{d},\mathbf{R};\mathbf{X}) - E^{QM-MM}(\mathbf{d}_0,\mathbf{R}_0;\mathbf{X})\right\}\right]\right\rangle_0} \times \rho_{MM}(\mathbf{d}_0,\mathbf{R}_0;\mathbf{X})$$

Well-defined variational method

# Procedure



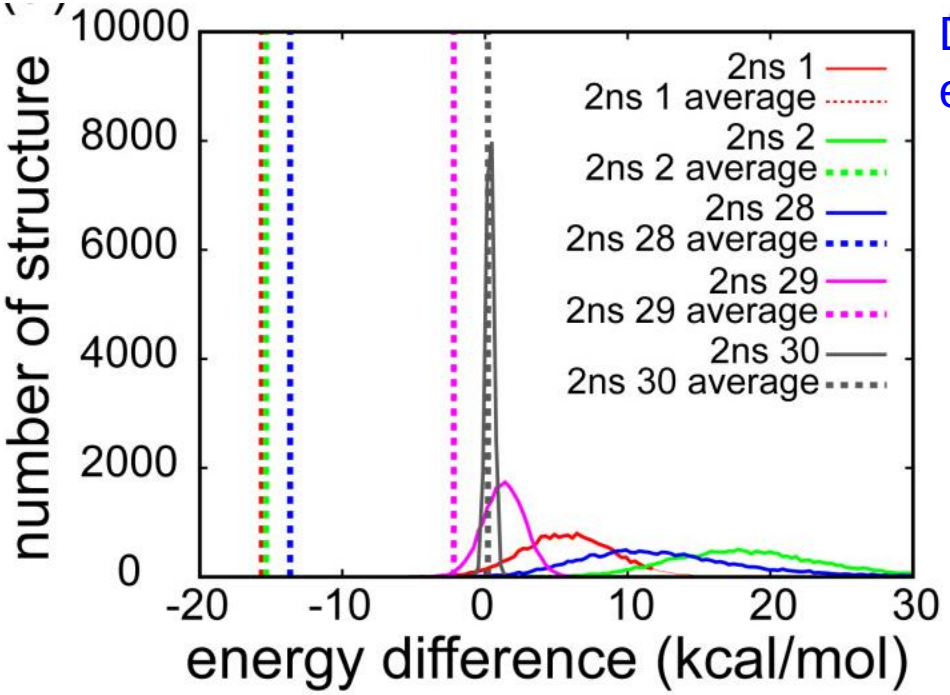

$$\mathbf{R}_{opt}^{i} \rightarrow \mathbf{R}_{0}^{i+1}$$

$$\mathbf{q}_{opt}^{i} \rightarrow \mathbf{q}_{0}^{i+1}$$

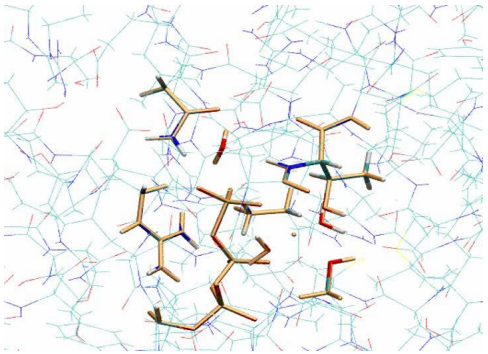
Update

## MD

3 – 15 ns
~20,000 confs.



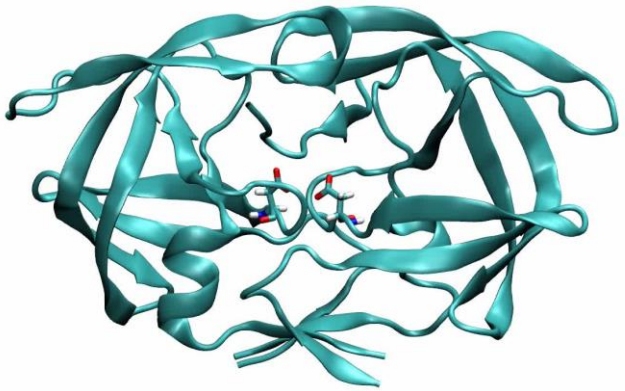

Distribution of energy difference



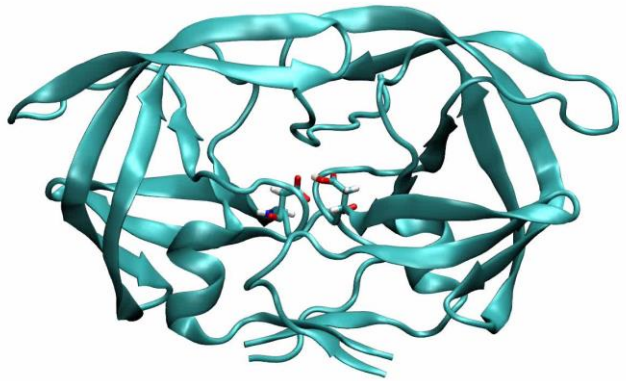
$$\rho_{MM}(\mathbf{d},\mathbf{R};\mathbf{X}) = \frac{\exp\left[-\beta\left\{E^{QM-MM}(\mathbf{d},\mathbf{R};\mathbf{X}) - E^{QM-MM}(\mathbf{d}_0,\mathbf{R}_0;\mathbf{X})\right\}\right]}{\left\langle \exp\left[-\beta\left\{E^{QM-MM}(\mathbf{d},\mathbf{R};\mathbf{X}) - E^{QM-MM}(\mathbf{d}_0,\mathbf{R}_0;\mathbf{X})\right\}\right]\right\rangle_0} \times \rho_{MM}(\mathbf{d}_0,\mathbf{R}_0;\mathbf{X})$$

# Determination of protonated state (classical MD)

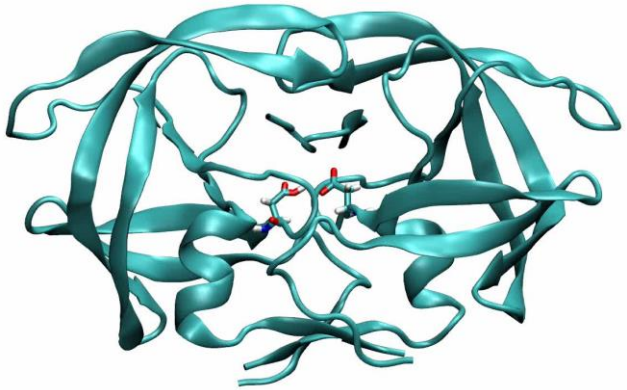
*We cannot determine protonated state by X-ray structural analysis!*



non-protonated: 300 ns



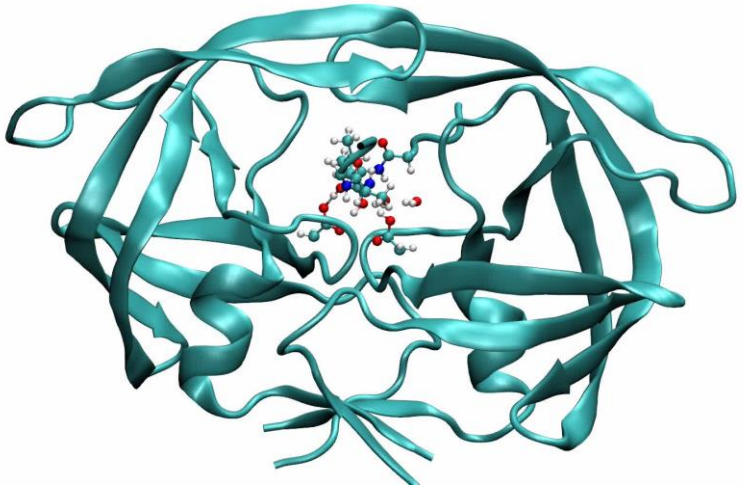
mono-protonated(A): 700 ns

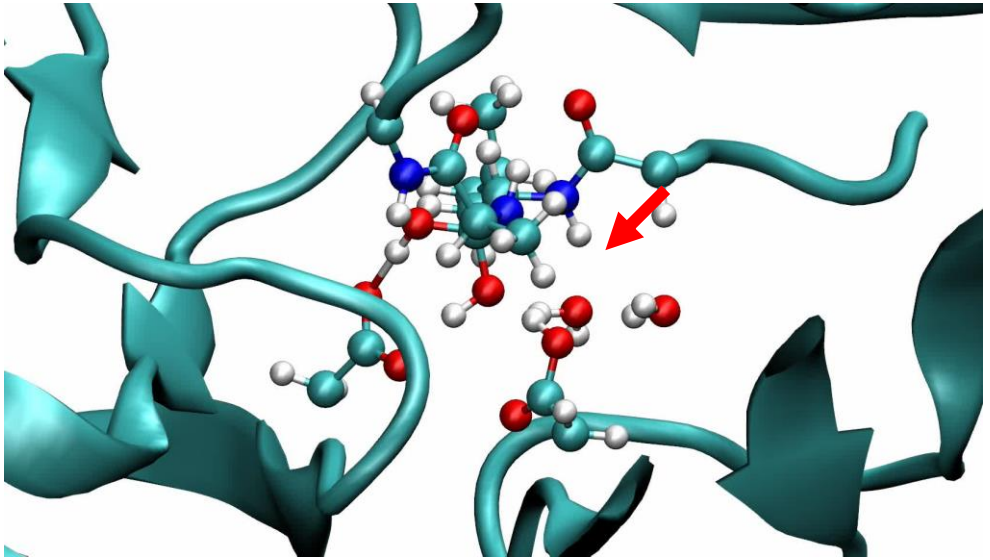


mono-protonated(B): 1000 ns

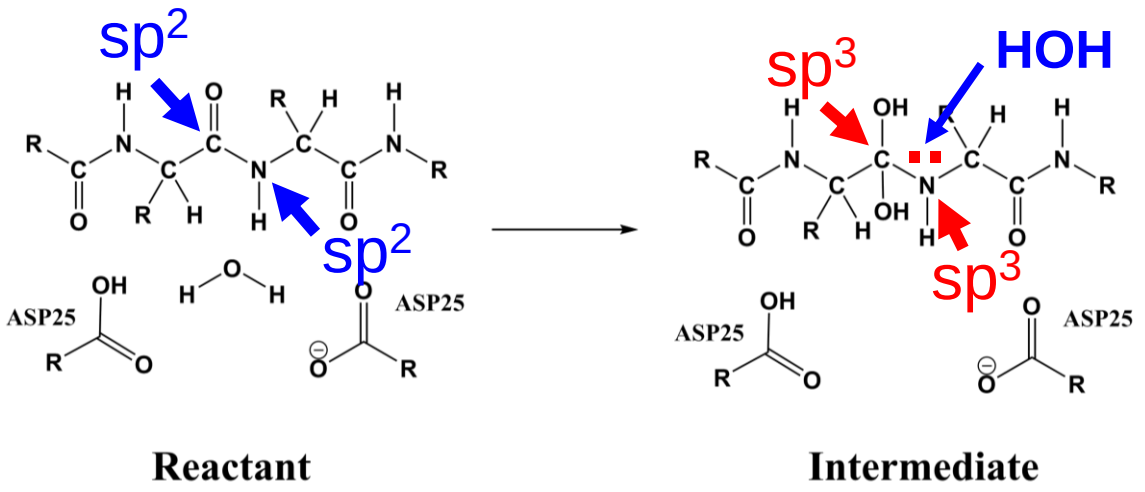
- non-protonated and mono-protonated (A): substrate dissociated
- mono-protonated(B): stable under 1μs MD and water molecules entered activation part

# Intermediate state (QM/MM)





Not yet converged（~6 μs）

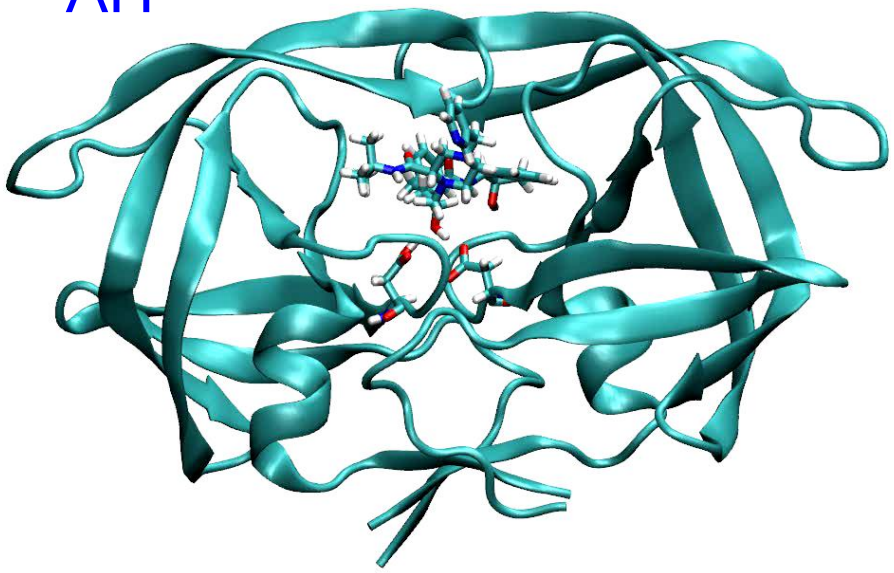



Water molecule attached a lone pair of amine

**New mechanism for stabilization of intermediate state**

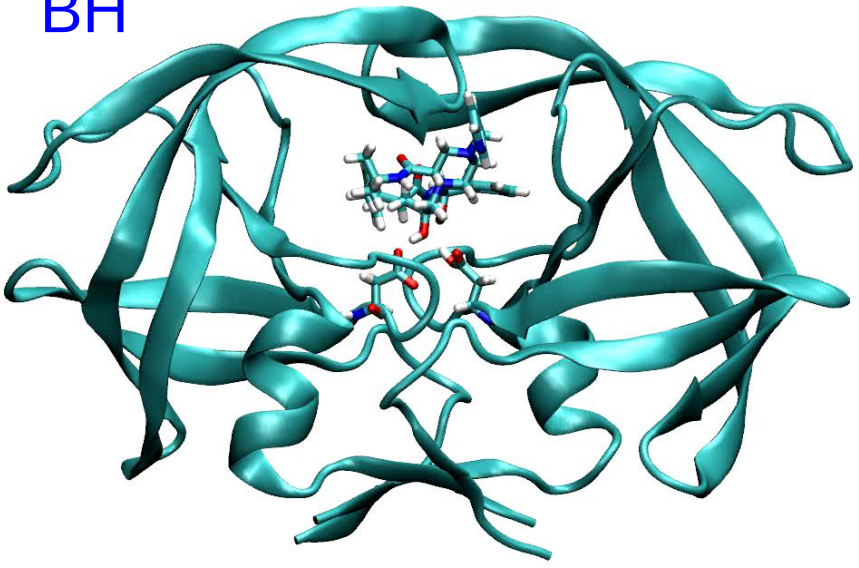
# Inhibitor molecule (Indinavir) binding state (QM/MM)

AH



1100 ns

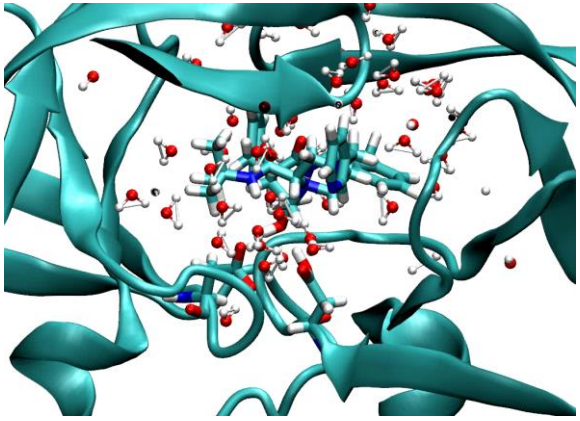
BH



700 ns

Two types of protonated states(AH, BH)

- BH: water molecules entered between protease and inhibitor
- AH: more stable than BH



# Summary

- Different protonated states caused different structural changes for both natural substrate and inhibitor cases

→ **Reasonable state is spontaneously determined**

- We clarified new stabilization mechanism of intermediate state

- We will calculate mutation case of protease

→ **Comparing it with wild type and try to make TS analog drug**