

Complex Structure Modeling

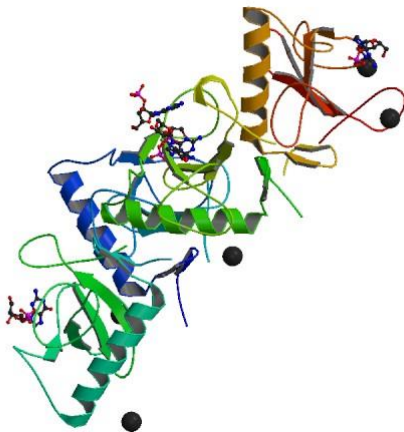
Agricultural Bioinformatics Research Unit,
Graduate School of Agricultural and Life Sciences,
The University of Tokyo
Tohru Terada

Today's topics

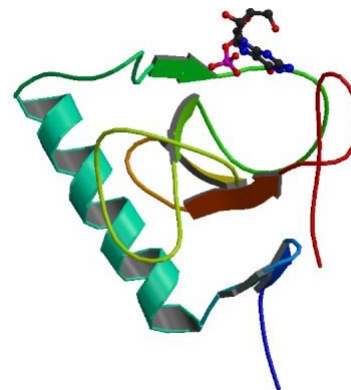
- Notes for conducting simulations
- Protein-protein docking
- Protein-small molecule docking
 - Exercise
- An application of molecular simulation

Notes for conducting simulations (1)

- Obtain the structure
 - Download the experimental structure from PDB (<http://www.rcsb.org/>)
 - Usually, simulations are performed for the biological units of the biomacromolecules.
 - Example: Ribonuclease T1 (PDB ID: 1I0X)



Asymmetric unit

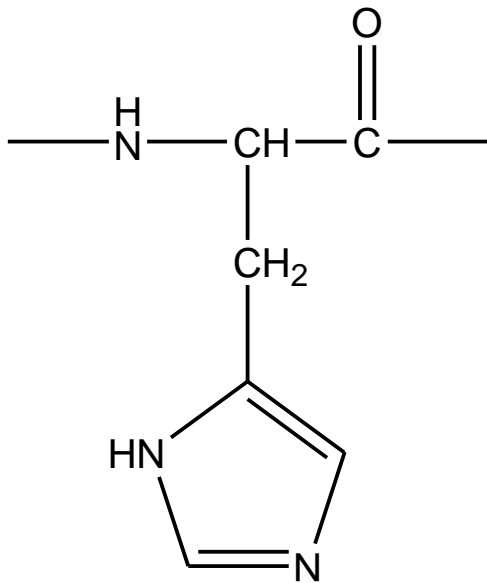


Biological Unit

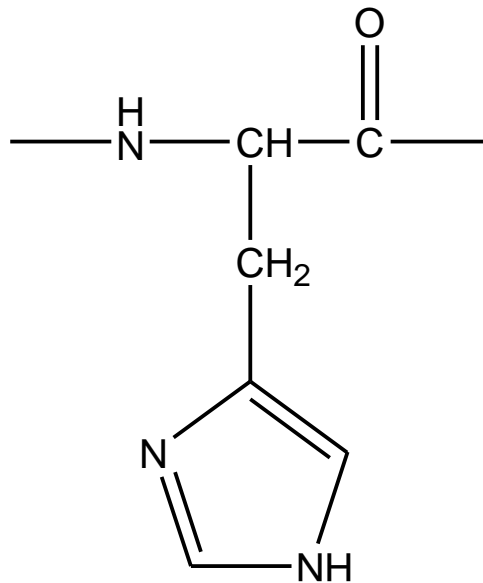
Notes for conducting simulations (2)

- Add missing atoms and residues using modeling software
 - When N- or C-terminal residues are missing, you can block the terminus with an acetyl or N-methyl group.
- Add hydrogen atoms
 - Most of them are added automatically.
 - Pay special attention to SS bonds and protonation states of His.

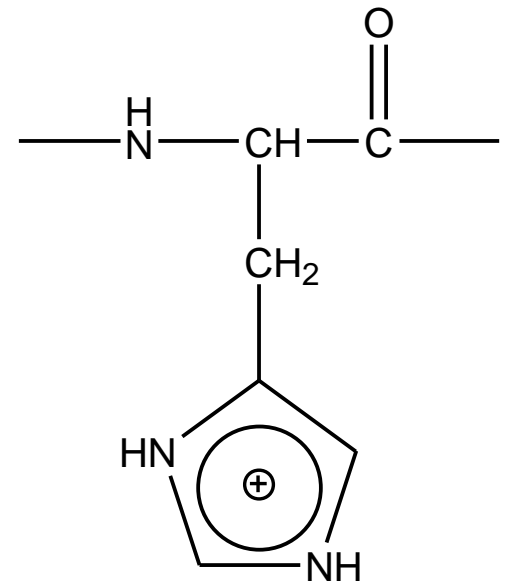
Protonation states of His



Protonation at δ



Protonation at ϵ



Protonation at δ and ϵ

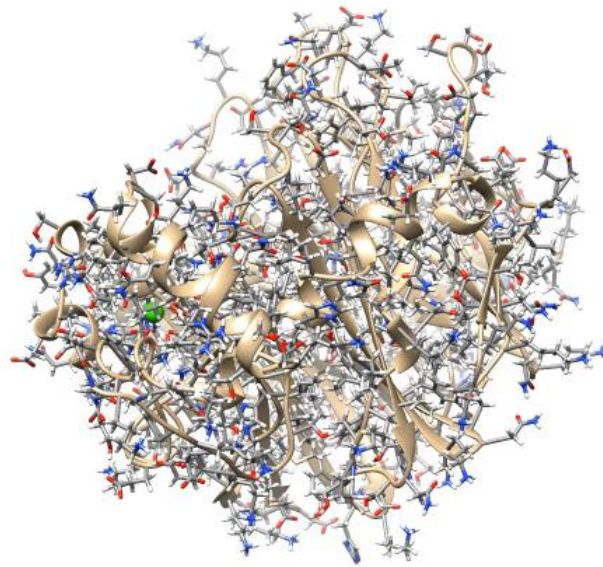
- $\text{p}K_a$ of His side chain is close to neutral.
- You can determine the protonation state based on the hydrogen bond network where His is involved.

Notes for conducting simulations (3)

- Obtain ligand force field parameters
 - Ligand force field parameters are not included in the molecular dynamics software. It is necessary to make them by yourself or to obtain them from e.g. Amber Parameter Database.*
- Add counterions to neutralize the system, when using PME.
- The equilibration run should be sufficiently long.
 - The length should be at least 1 ns.
 - During the equilibration run, use position restraints to avoid large deviation from the initial structure and gradually reduce the restraining force.

*<http://www.pharmacy.manchester.ac.uk/bryce/amber>⁶

An example of a soluble protein

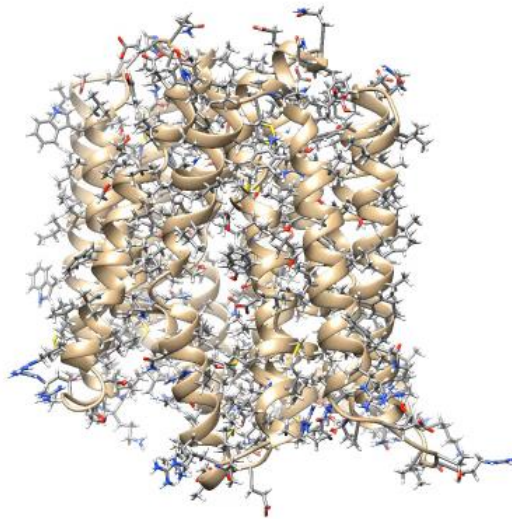


Levansucrase (PDB ID: 1PT2)

Simulations of membrane proteins

- Membrane proteins are embedded in solvated lipid bilayers.
- Lipid models
 - Prokaryote: phosphatidylethanolamine
 - Eukaryote: phosphatidylcholine etc.
- Database of protein orientations in membrane
 - Orientations of Proteins in Membranes (OPM) database
 - <http://opm.phar.umich.edu/>
- Initial structure generator
 - <http://www.charmm-gui.org/>

An example of a membrane protein



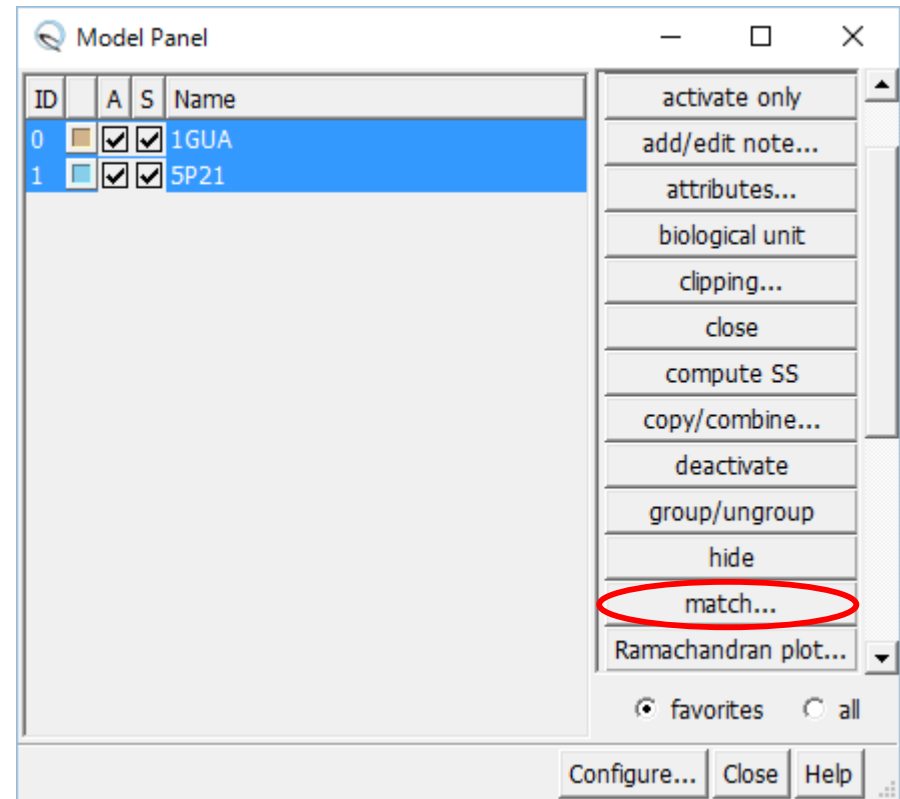
E. coli multidrug transporter MdfA (PDB ID: 4ZOW)

Complex structure modeling

- Predicts protein-protein or protein-small molecule complex structure.
- If an experimental structure of similar complex is available, you should try following methods:
 - Homology modeling
 - Structure superposition
- If not, try
 - Docking simulation

Structure superposition (1)

1. Start UCSF Chimera
2. Choose “File” → “Fetch by ID” to open the complex structure between Rap1A and the Ras-binding domain of Raf-1 with PDB ID of “1GUA.”
3. Open the uncomplexed structure of Ras with PDB ID of “5P21.”
4. Choose “Favorites” → “Model Panel,” select “1GUA” and “5P21,” and click “match” and “OK.”
 - RMSD value is displayed at the bottom of the main window.

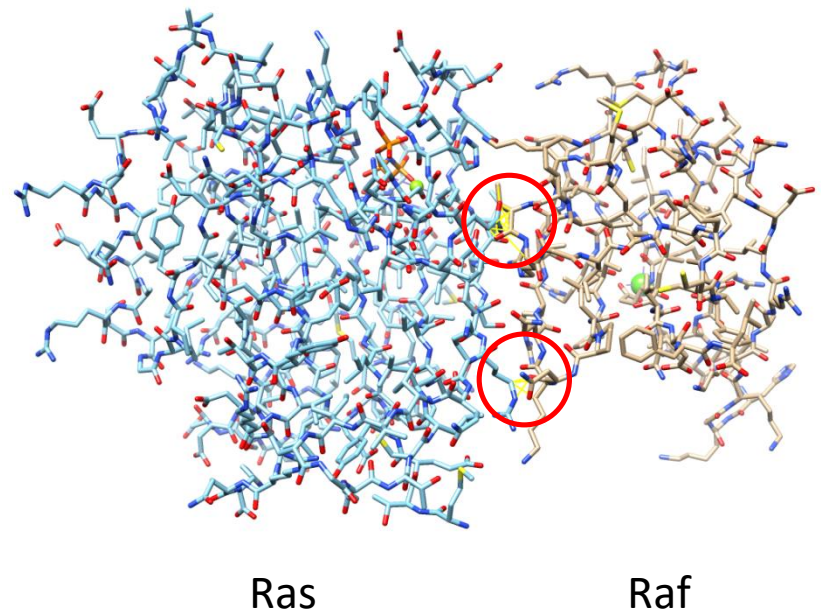


Structure superposition (2)

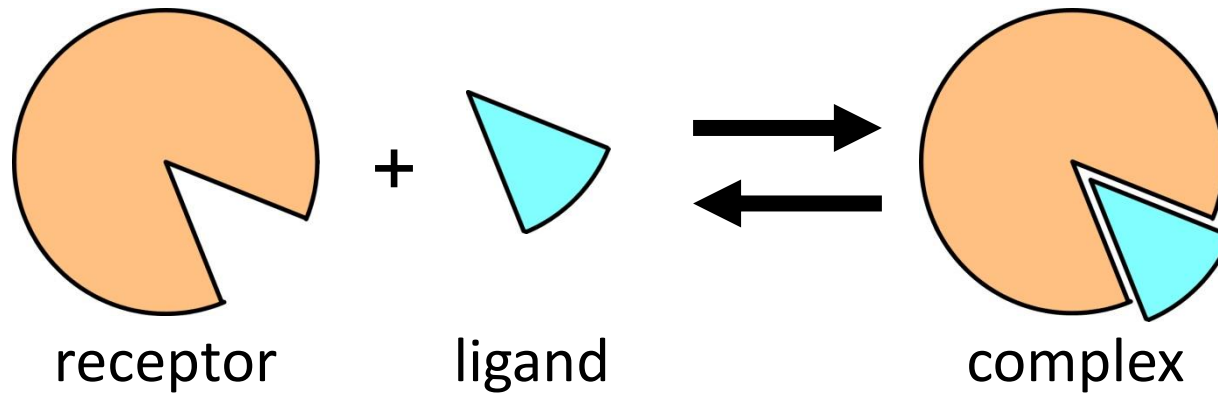
5. Choose “Select” → “Chain” → “A” → “1GUA” to select the Rap1A atoms and delete them by choosing “Actions” → “Atoms/Bonds” → “delete.”
6. Delete water molecules after selecting them by choosing “Select” → “Residue” → “HOH.”
7. Change the representation to sticks
8. Choose “Tools” → “Structure Analysis” → “Find Clashes/Contacts”
9. Choose “Select” → “Chain” → “A” to select the Ras atoms and click the “Designate” button in the Find Clashes/Contacts window.

Structure superposition (3)

10. Select the “second set of designated atoms” option for Check designated atoms against.
11. Choose “Select” → “Chain” → “B” to select the Raf atoms, click the “Designate selection as second set” button, and click “OK.”
 - Clashing atoms are indicated by yellow lines.



Docking simulation



- Dock a ligand into ligand-binding site on the surface of a receptor protein.
- Different methods are used depending on the type of ligand (protein or small molecule).

Binding free energy

- The complex structure has the minimum free energy.
- Free energy difference between the complex and the uncomplexed states is given by:

$$\Delta G_{\text{bind}}^{\circ} = G_{\text{complex}}^{\circ} - (G_{\text{receptor}}^{\circ} + G_{\text{ligand}}^{\circ})$$

$$K_{\text{D}} = \exp(\Delta G_{\text{bind}}^{\circ} / RT)$$

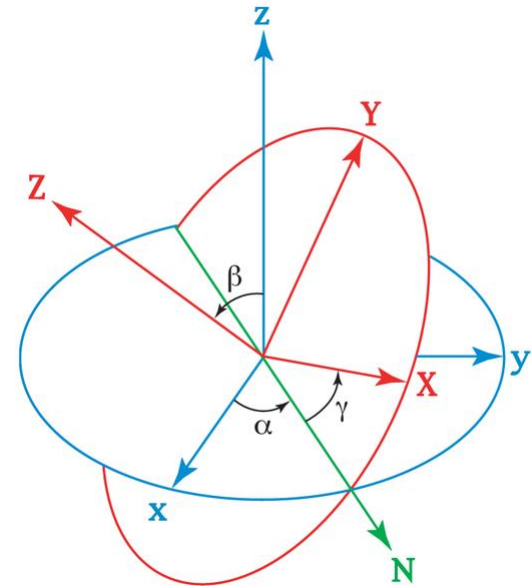
- The complex structure is predicted as the one having the lowest ΔG_{bind} in the candidate structures generated by docking the ligand into all the possible sites on the protein surface in all the possible orientations and conformations.

Calculation of binding free energy

- When calculation is done for a small number of the targets:
 - e.g. calculation for the crystal structure of a complex
 - MM-PB/SA method
 - Free-energy perturbation, thermodynamic integration
- When calculation is done for a large number of the targets:
 - e.g. docking simulation
 - Score function

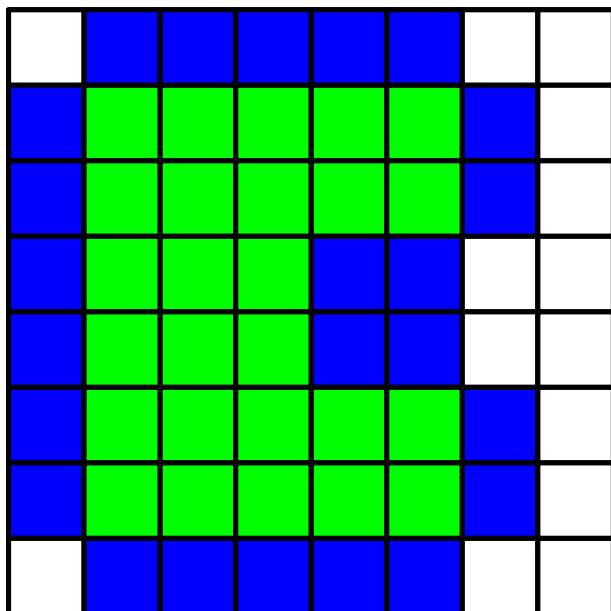
Protein-protein docking

- Both of receptor and ligand are treated as rigid bodies. Conformational changes upon complex formation are not considered.
- Three translational and three rotational degrees of freedom of ligand are considered.
 - Rotation is described with Euler angle.
- Shape complementarity is important.

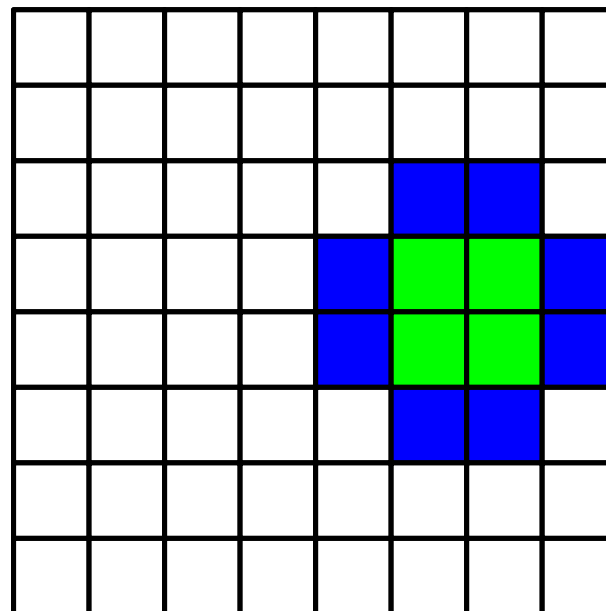


Shape complementarity (1)

Receptor



Ligand

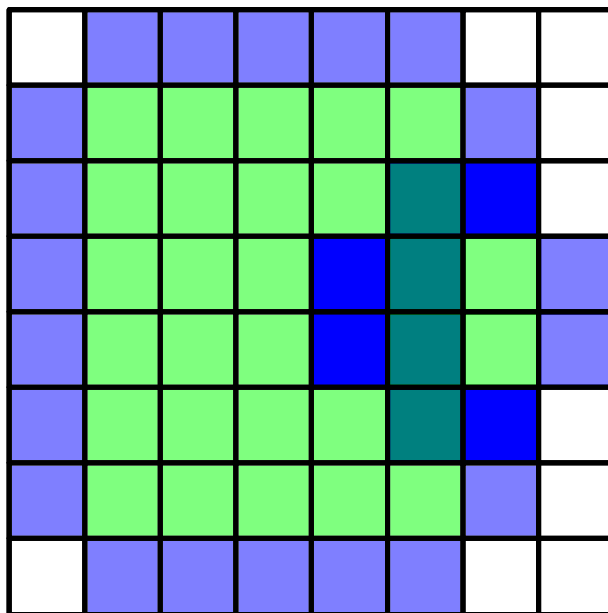


= 1 (solvent accessible surface layer)



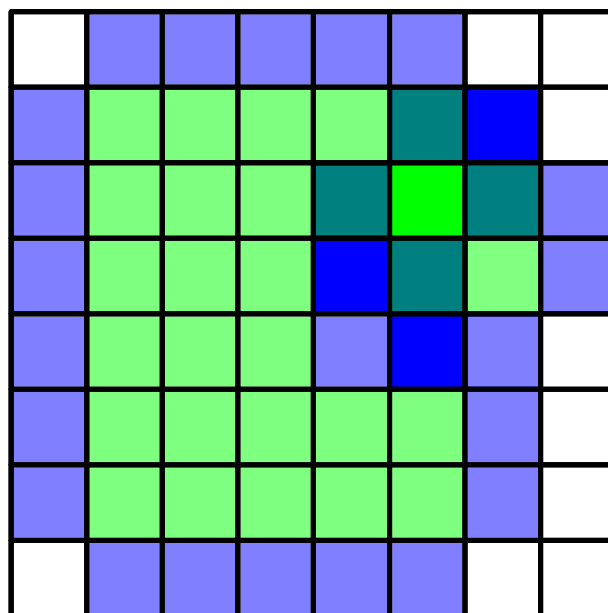
= 9*i* (solvent excluding surface layer)


Shape complementarity (2)



Calculate product of scores for each grid.
Real part of sum of products = Docking score = 4

Shape complementarity (3)



 = -81

Calculate product of scores for each grid.

Real part of sum of products = Docking score = $3 - 81 = -78$

Efficient calculation

- Generalization

$$S(a,b,c) = \sum_{x,y,z} f(x,y,z)g(x+a,y+b,z+c)$$

Find ligand position (a, b, c) that maximizes S .

- S can be efficiently calculated with fast Fourier transform (FFT).

$$\tilde{S}(h,k,l) = \tilde{f}(h,k,l)\tilde{g}(h,k,l)$$

- S is calculated for different ligand orientation.
- It is possible to calculate electrostatic and other interactions in a similar manner.

Docking software

- DOT

<http://www.sdsc.edu/CCMS/DOT/>

- FTDock

<http://www.sbg.bio.ic.ac.uk/docking/ftdock.html>

- GRAMM-X

<http://vakser.bioinformatics.ku.edu/resources/gramm/grammx>

- HEX

<http://hex.loria.fr/>

- ZDOCK

<http://zlab.umassmed.edu/zdock/index.shtml>

An application of ZDOCK (1)

1. Access <http://zdock.umassmed.edu/>
2. Put the PDB ID of β -lactamase, “1ZG4,” in the textbox of Input Protein 1
3. Put the PDB ID of β -lactamase inhibitory protein, “3GMU,” in the textbox of Input Protein 2
4. Enter your e-mail address and click “Submit.”

An application of ZDOCK (2)

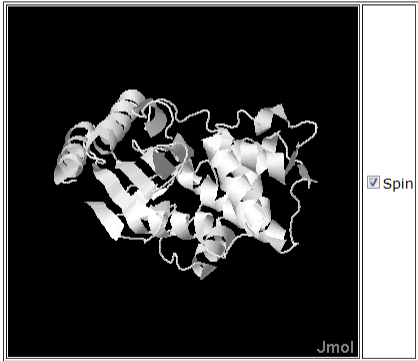
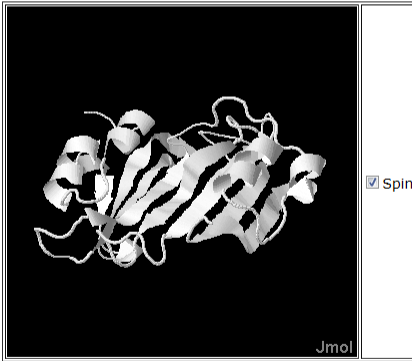
5. You can specify residues that are not involved in the binding or that are involved in the binding.
Do not click “submit” in this lecture.

ZDOCK SERVER

[ZDOCK](#) [M-ZDOCK](#) [Help](#) [Links](#) [References](#)

Step 2: Pick Contact and Blocking Residues

[Select Residues to Block from the Binding Site:](#)

<p>1ZG4</p>  <p><input checked="" type="checkbox"/> Spin</p> <p>Jmol</p>	<p>1ZG4</p> <div>26 Chain A HIS ▲ 27 Chain A PRO (E) 28 Chain A GLU 29 Chain A THR 30 Chain A LEU 31 Chain A VAL 32 Chain A LYS 33 Chain A VAL 34 Chain A LYS 35 Chain A ASP ▼</div>	<p>3GMU</p> <div>1 Chain B ALA ▲ 2 Chain B GLY (E) 3 Chain B VAL 4 Chain B MET 5 Chain B THR 6 Chain B GLY 7 Chain B ALA 8 Chain B LYS 9 Chain B PHE 10 Chain B THR ▼</div>	<p>3GMU</p>  <p><input checked="" type="checkbox"/> Spin</p> <p>Jmol</p>
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[Select Binding Site Residues for Filtering Output:](#)

<p>1ZG4</p> <div>26 Chain A HIS ▲ 27 Chain A PRO (E) 28 Chain A GLU 29 Chain A THR 30 Chain A LEU 31 Chain A VAL 32 Chain A LYS 33 Chain A VAL 34 Chain A LYS 35 Chain A ASP ▼</div>	<p>3GMU</p> <div>1 Chain B ALA ▲ 2 Chain B GLY (E) 3 Chain B VAL 4 Chain B MET 5 Chain B THR 6 Chain B GLY 7 Chain B ALA 8 Chain B LYS 9 Chain B PHE 10 Chain B THR ▼</div>
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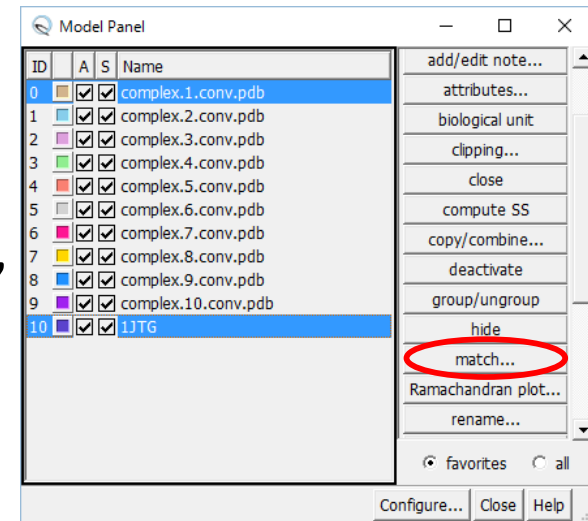
Submit

An application of ZDOCK (3)

6. When the calculation is finished, you will receive an e-mail containing the URL of the calculation result page.
7. Download top_preds.zip from the web page of this lecture and unzip it on the desktop.
8. The top_preds folder contains ten PDB files named from complex.1.pdb to complex.10.pdb in the order of the score. Because they cannot be displayed in Chimera, convert them by using conv.pl available on the web page of this lecture.
 - Ten more files named from complex.1.conv.pdb to complex.10.conv.pdb are generated

An application of ZDOCK (4)

9. Start Chimera and open complex.1.cov.pdb, ..., and complex.10.conv.pdb.
10. Open the experimental complex structure, 1JTG.
11. Choose “Favorites” → “Model Panel,” select complex.1.conv.pdb and 1JTG, and click “match” and “OK.”
12. Delete chains C and D of 1JTG



Which is the closest to the experimental structure among complex.1.cov.pdb, ..., and complex.10.conv.pdb?

Protein-small molecule docking

- Find the ligand-binding site on the surface of the receptor protein. Then, dock the ligand into the site.
- Search the conformational space of ligand for the free-energy minimum “pose” by translating and rotating the ligand and rotating all the rotatable bonds in the ligand.
- Usually, the receptor atoms are not moved. The receptor is treated as a rigid body.

Docking software

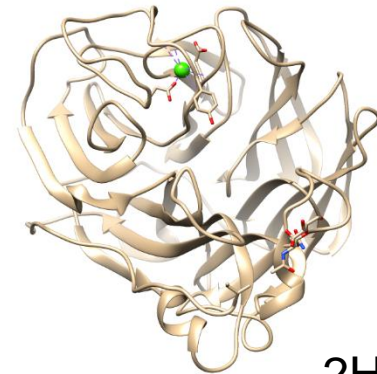
- AutoDock Vina
 - <http://vina.scripps.edu/>
- DOCK
 - <http://dock.compbio.ucsf.edu/>
- Glide
 - <http://www.schrodinger.com/Glide>
- GOLD
 - <http://www.ccdc.cam.ac.uk/solutions/csd-discovery/components/gold/>
- AutoDock Vina and DOCK are free, but Glide and GOLD incur a charge.
- Only the translational, rotational, and dihedral degrees of freedom of small molecules are considered. Proteins are treated as rigid bodies.

Hands-on training

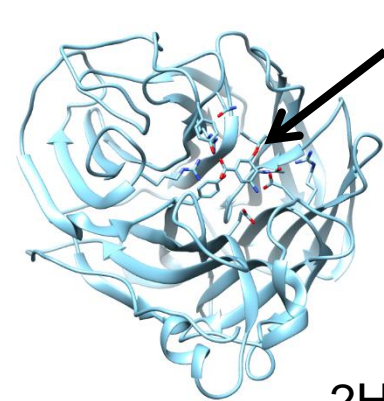
- Dock an inhibitor into N1 neuraminidase using AutoDock Vina.
 1. Obtain a crystal structure of N1 neuraminidase
 2. Generate the structure of the inhibitor
 3. Detect cavities on the protein surface
 4. Perform a docking simulation
 5. Analyze the result

1. Obtaining a crystal structure

1. Start Chimera
2. Choose “File” → “Fetch by ID” to open “2HTY.”
3. Choose “Select” → “Chain” → “A” to select chain A.
4. Choose “File” → “Save PDB,” check the checkbox of “Save selected atoms only,” and save the coordinates as “2HTY_A.pdb” on the Desktop.
5. After choosing “File” → “Close session”, fetch “2HU4” and save the coordinates of chain A as “2HU4_A.pdb” on the Desktop.



2HTY chain A



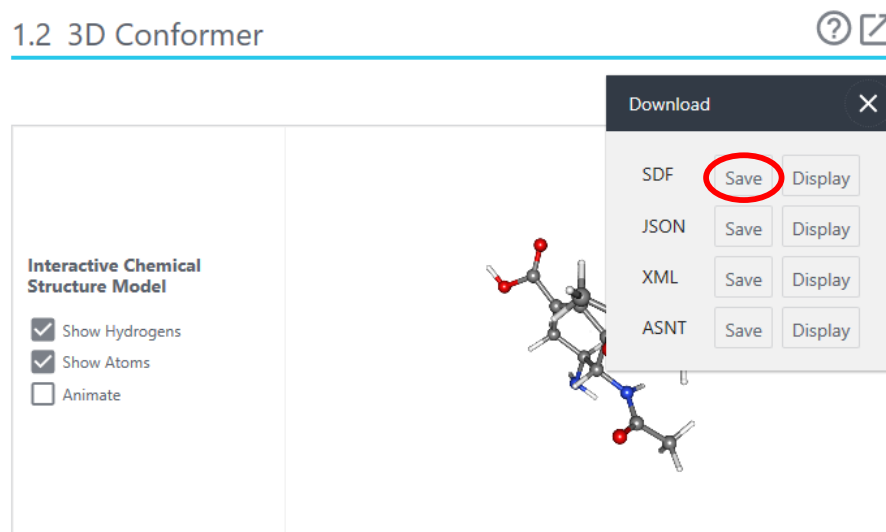
oseltamivir
carboxylate

2HU4 chain A

2. Generating inhibitor structure (1)

1. Access PubChem at <https://pubchem.ncbi.nlm.nih.gov/>
2. Enter “oseltamivir carboxylate” and search.
3. Ascertain that CID of the Compound Best Match is 449381 and click it.
4. Save 3D Conformer in SDF format.
5. Open the file in UCSF Chimera.

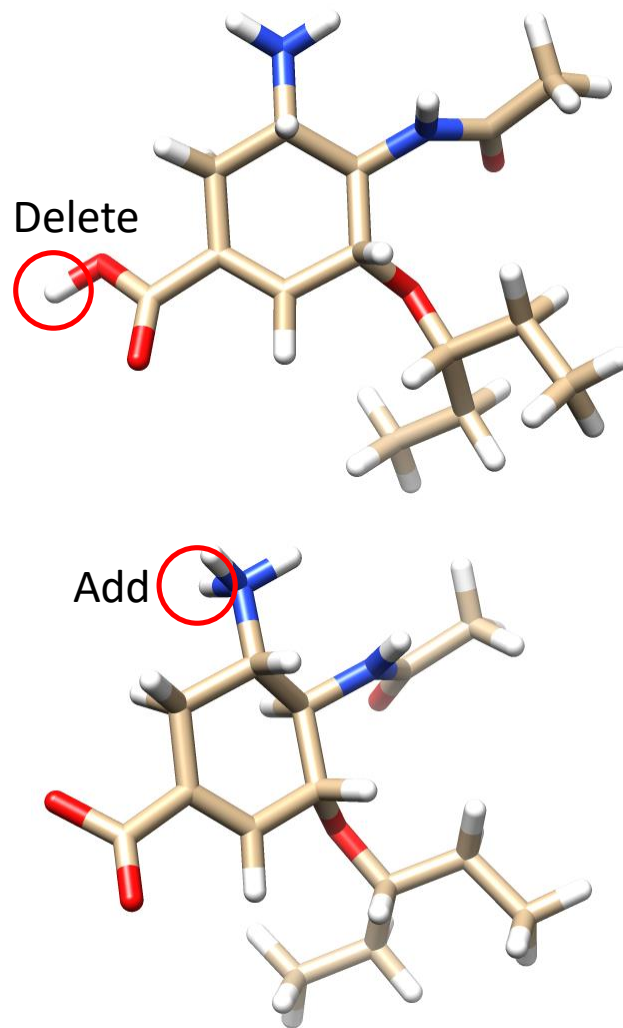
1.2 3D Conformer



File name is:
Conformer3D_CID_449381.sdf

2. Generating inhibitor structure (2)

6. Select the hydrogen atom of the carboxy group by clicking on the atom while pressing the Ctrl key.
7. Delete the selected atom by using “Actions” → “Atoms/Bonds” → “delete.”
8. Add hydrogen to the amino group by using “Tools” → “Structure Editing” → “AddH.”
9. Calculate charges by using “Tools” → “Structure Editing” → “Add Charge.”
 - The net charge is +0.
10. Optimize the structure by using “Tools” → “Structure Editing” → “Minimize Structure.”
11. Choose “File” → “Save Mol2” to save the coordinates as “ose.mol2” on the Desktop.

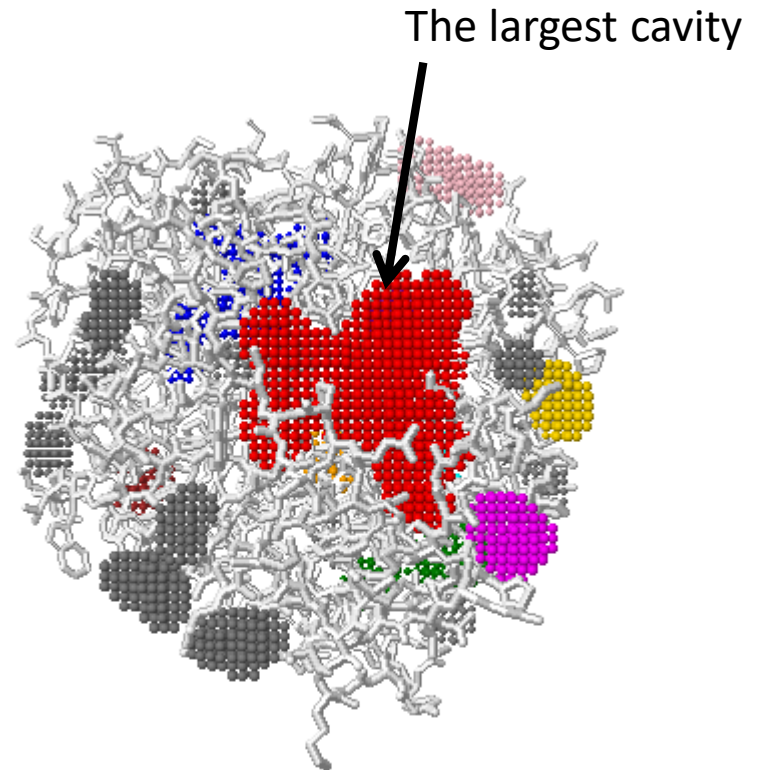


Compound library

- Available Chemicals Directory (ACD)
 - A commercial compound library
 - <http://accelrys.com/products/collaborative-science/databases/sourcing-databases/biovia-available-chemicals-directory.html>
- DrugBank
 - Database of drugs and their targets
 - <http://www.drugbank.ca/>
- PubChem
 - Database managed by NCBI
 - <https://pubchem.ncbi.nlm.nih.gov/>
- ZINC
 - Database managed by USCF
 - <http://zinc.docking.org/>

3. Cavity detection

- Detect cavities on the protein surface to which small molecules bind
 - Here, we use the GHECOM server at <http://strcomp.protein.osaka-u.ac.jp/ghecom/>
 - Put “2hty” in INPUT PDBID
 - Center of the largest cavity: $(-0.260, 78.567, 112.835)$



JSmol

Cavity detection software

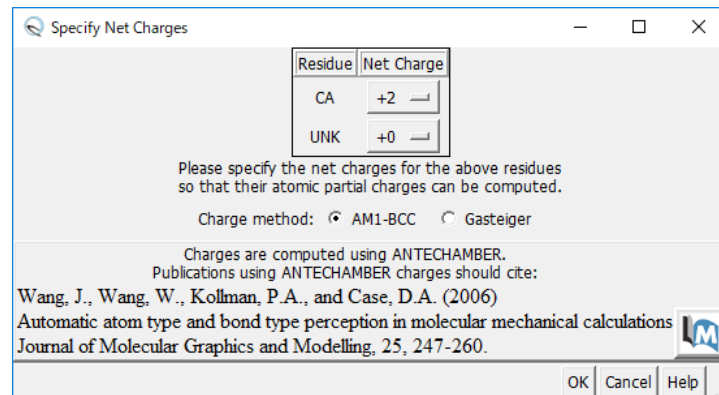
- SURFNET
 - <http://www.ebi.ac.uk/thornton-srv/software/SURFNET/>
 - Detects “gap regions” on the protein surface.
- PASS
 - <http://www.ccl.net/cca/software/UNIX/pass/overview.shtml>
 - Detects cavities on the protein surface and ranks them.
- Q-SiteFinder
 - Detects cavities on the protein surface and ranks them based on the interaction energy with CH₄ probe.

4. Docking simulation (1)

1. Make a folder named “docking” on the Desktop and move 2HTY_A.pdb, 2HU4_A.pdb, and ose.mol2 to the folder.
2. Download vina.exe from the web page of this lecture and save it on the Desktop.
3. Choose “File” → “Close Session” in Chimera.
4. Choose “File” → “Open” to open 2HTY_A.pdb
5. Choose “Select” → “Residues” → “NDG” to select the sugar and delete it by choosing “Actions” → “Atoms/Bonds” → “delete.”
6. Delete water molecules (residue name: HOH) in a similar manner.

4. Docking simulation (2)

7. Choose “File” → “Open” to open ose.mol2.
8. Choose “Tools” → “Surface/Binding Analysis” → “Dock Prep.”
9. Make sure that “2HTY_A” and “449381” are selected in Molecules to prep, uncheck the check box of Write Mol2 file, and click “OK.”
10. Click “OK” in the Add Hydrogens for Dock Prep window.
11. Select “Amber ff14SB” for Standard residues and “AM1-BCC” for Other residues in the Assign Charges for Dock Prep window and click “OK.”
12. Select “+0” for Specify Net Charges and click “OK.”
(You can ignore the warning.)



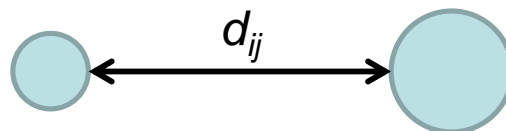
4. Docking simulation (3)

13. Choose “Tools” → “Surface/Binding Analysis” → “AutoDock Vina.”
14. Set “ose.pdbqt” in the docking folder to Output File.
15. Select “2HTY_A.pdb” and “449381” as Receptor and Ligand, respectively.
16. Unfold Receptor search volume options and set “-0.260 78.567 112.853” and “20 20 20” to Center and Size, respectively.
17. Unfold Executable location and select “Local” and set “vina.exe” on the Desktop to Path.
18. Click “OK” to start the simulation. (It will take about one minute.)

Score function

- Complex structure is optimized to minimize c :

$$c = \sum_X w_X \sum_{i < j} h(d_{ij})$$



- X is gauss1, gauss2, repulsion, hydrophobic, or hydrogen bonding.
- Binding free energy is calculated as:

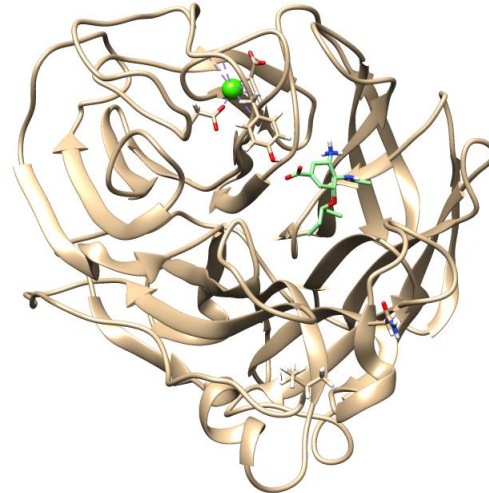
$$s = \frac{c - c_{\text{intra1}}}{1 + w_{\text{rot}} N_{\text{rot}}}$$

c_{intra1} : c for intramolecular interaction of the model with the smallest c

- w_X is optimized so that s correlates with the experimental value of ΔG_{bind} .

5. Analysis of the result (1)

- The result is displayed when the calculation is completed.
- The docking scores are listed in the ViewDock window in the order of the score.
- Clicking on the row of the list changes docking pose displayed.



ViewDock - C:\Users\Hterada\Desktop\170629\ose.pdbqt

File Compounds Column Selection Chimera HBonds Movie

S	Score	RMSD l.b.	RMSD u.b.
V	-6.7	0.0	0.0
V	-6.2	2.384	5.788
V	-6.2	2.37	4.171
V	-6.2	2.773	4.81
V	-6.1	2.057	4.404
V	-5.8	1.85	2.642
V	-5.8	2.339	6.32
V	-5.7	3.543	6.613
V	-5.7	2.175	4.829
...

Chimera Model #3.1

REMARK VINA RESULT: -6.7 0.000 0.000

REMARK 7 active torsions:

REMARK status: ('A' for Active; 'I' for Inactive)

REMARK 1 A between atoms: O1_1 and C3_9

REMARK 2 A between atoms: O1_1 and C7_13

REMARK 3 A between atoms: N1_5 and C1_7

REMARK 1 I between atoms: N1_5 and C11_17

REMARK 4 A between atoms: N2_6 and C2_8

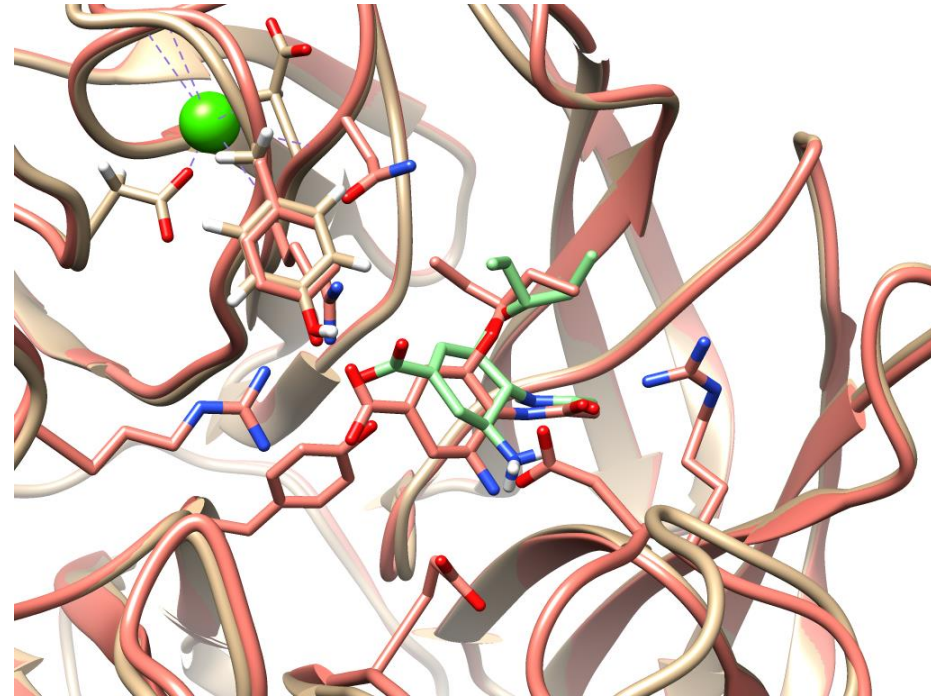
Change Compound State

☒ Viable ☐ Deleted ☐ Purged

Hide Quit Help

5. Analysis of the result (2)

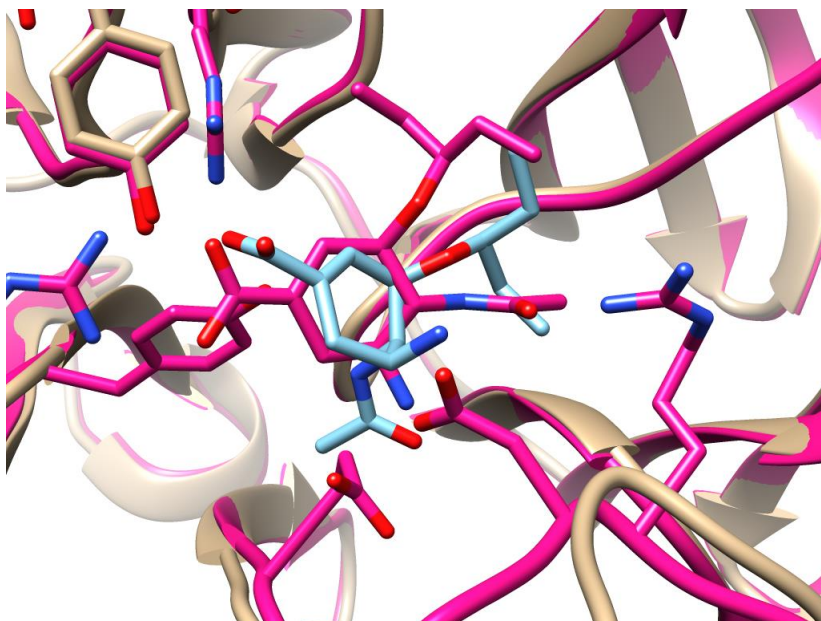
- Compare with the crystal structure.
 1. Open 2HU4_A.pdb.
 2. Choose “Favorites” → “Model Panel” to hide the model of 449381.
 3. Select “2HTY_A.pdb” and “2HU4_A.pdb” in Model Panel and click “match” and “OK.”
- Which pose is the closest to the crystal structure?



Comparison between the 5-th ranked pose and the crystal structure

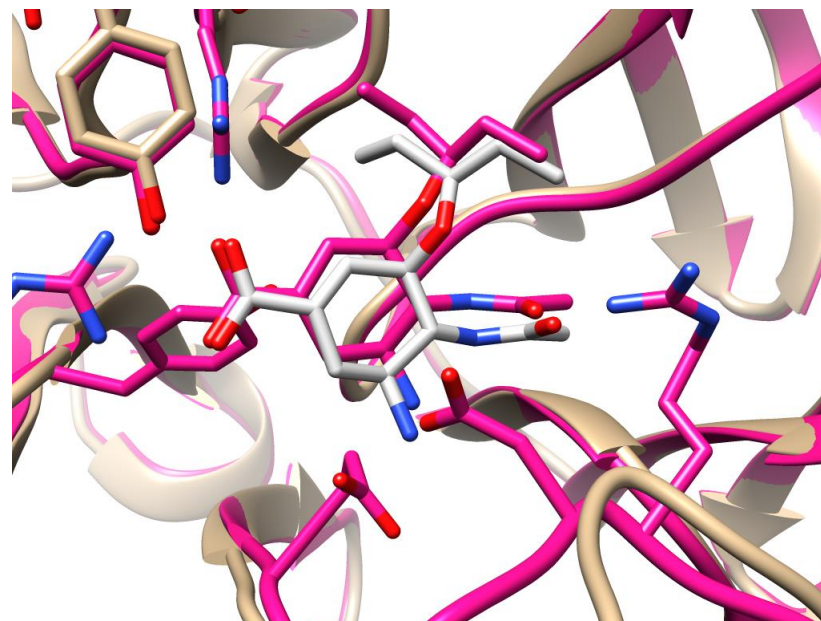
Results from Glide

Top ranked pose



Light blue: top ranked pose
Pink: 2HU4

5-th ranked pose

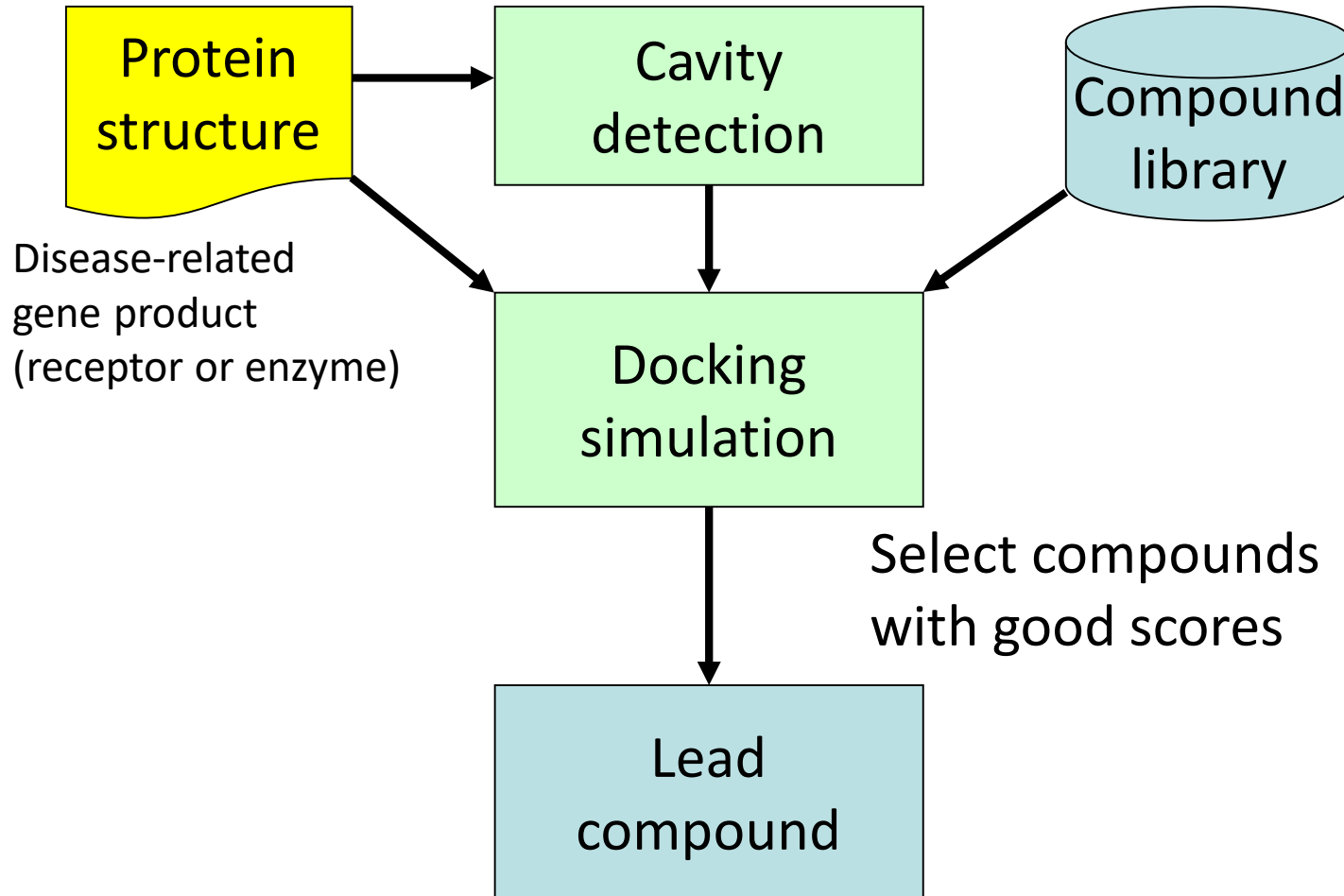


Gray: 5-th ranked pose
Pink: 2HU4

Application to drug discovery

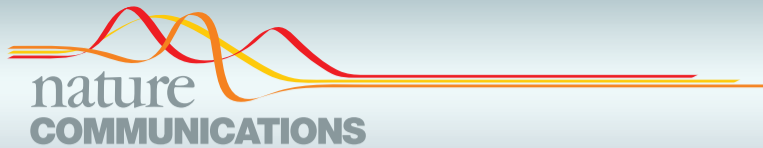
- A high-throughput screening (HTS), which searches compound libraries for compounds tightly binding to the target protein, is used to find drug candidates in the field of drug discovery.
- It costs huge sums of money to establish compound libraries and to measure the affinity of each compound to the protein.
- The affinity can be estimated by reproducing the protein-compound binding in a computer, *i.e.*, by docking simulation. → virtual screening

Virtual screening



An application of molecular simulation

- Conformational change mechanism of multidrug transporter MdfA
 - Nagarathinam *et al.* *Nat. Commun.* **9**, 4005 (2018).



ARTICLE

DOI: 10.1038/s41467-018-06306-x

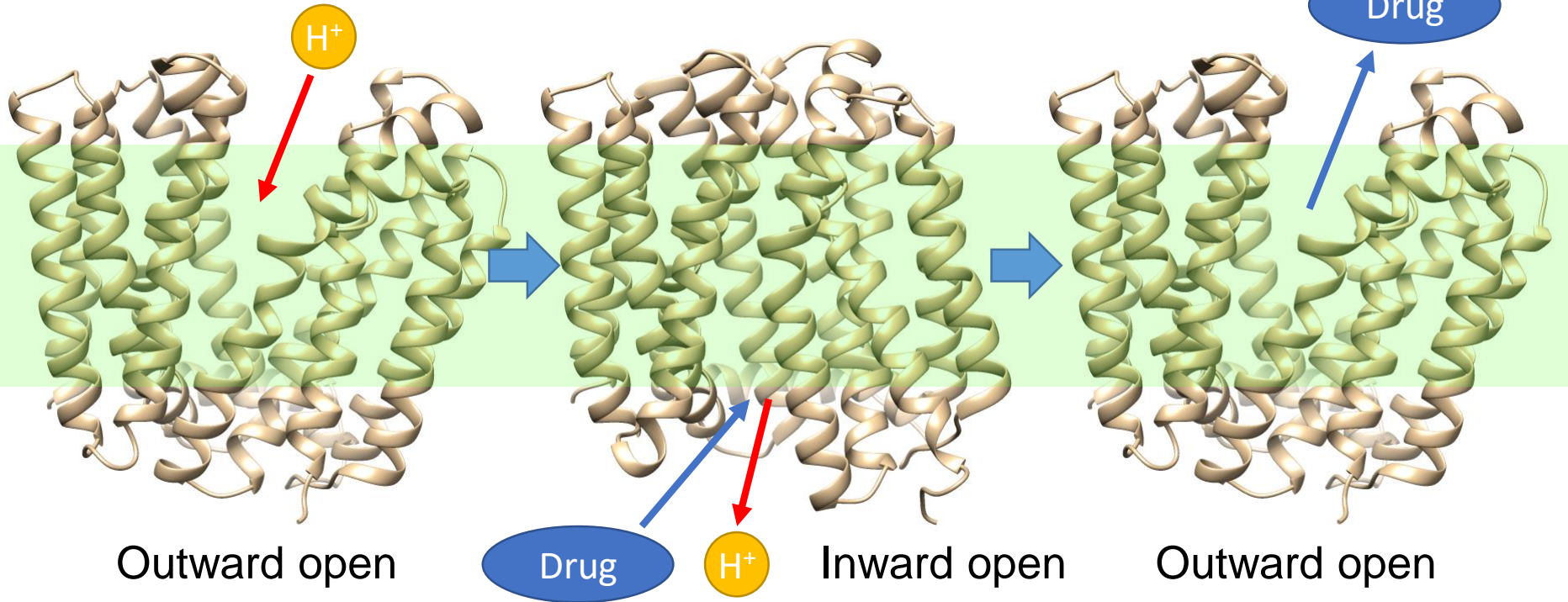
OPEN

Outward open conformation of a Major Facilitator Superfamily multidrug/H⁺ antiporter provides insights into switching mechanism

Kumar Nagarathinam^{1,2,9}, Yoshiko Nakada-Nakura³, Christoph Parthier², Tohru Terada⁴, Narinobu Juge⁵, Frank Jaenecke¹, Kehong Liu³, Yunhon Hotta³, Takaaki Miyaji⁵, Hiroshi Omote⁶, So Iwata^{3,7}, Norimichi Nomura³, Milton T. Stubbs^{1,2} & Mikio Tanabe^{1,8}

Multidrug transporter MdfA

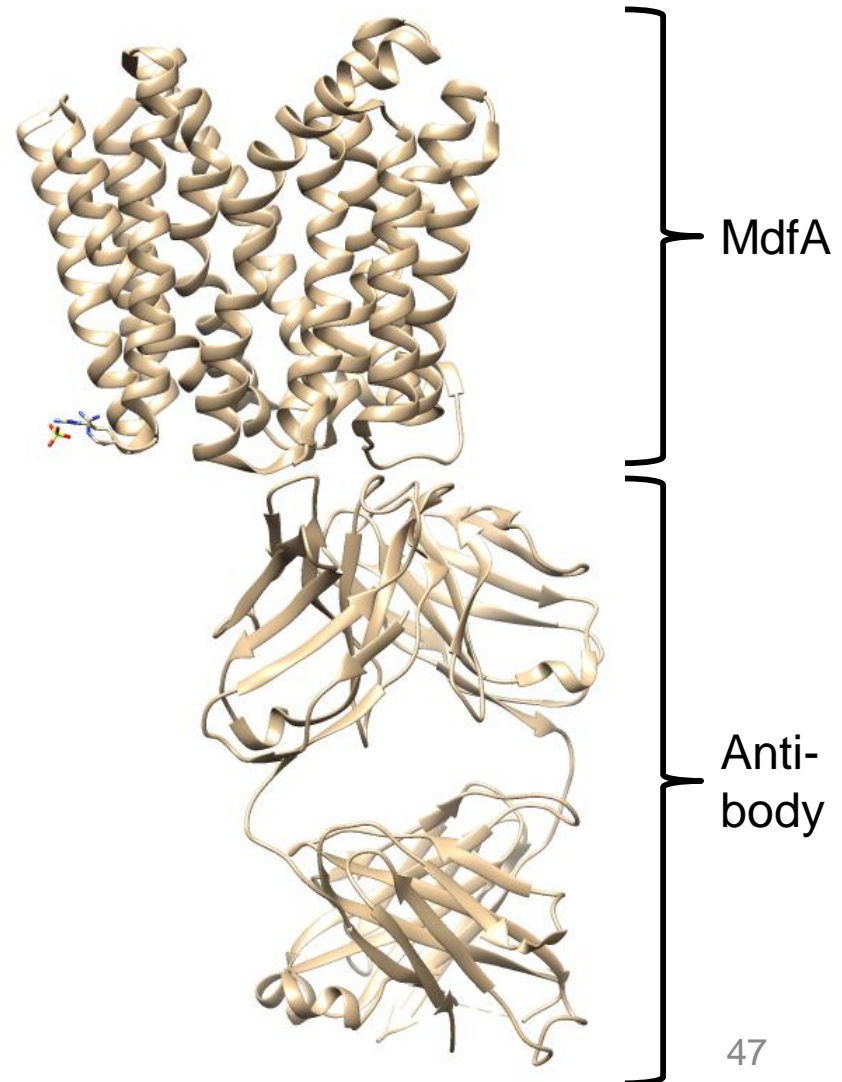
Periplasm (low pH)



Cytoplasm (high pH)

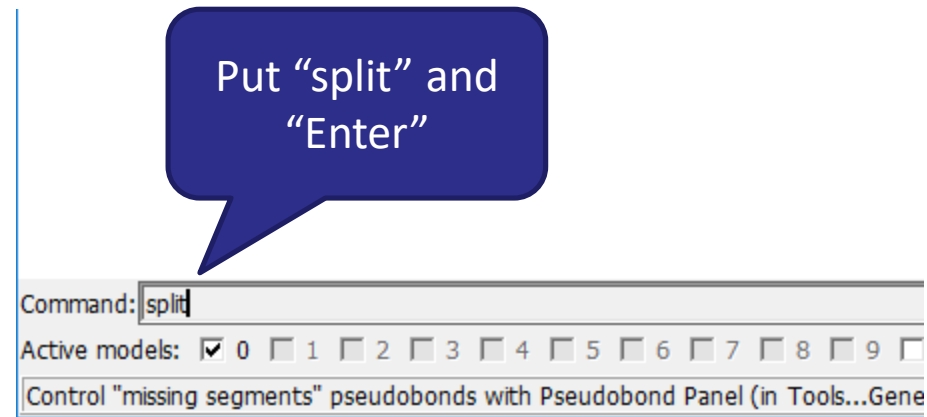
Tertiary structure of MdfA (1)

1. Start Chimera, choose “File” → “Fetch by ID,” put “6GV1” for PDB ID, and “Fetch.”
 - An antibody binds to MdfA to stabilize the outward open structure and to facilitate crystallization.

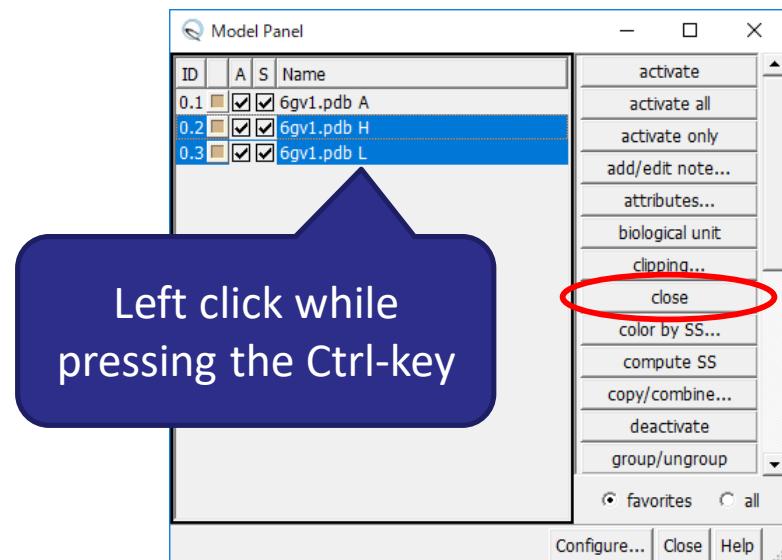


Tertiary structure of MdfA (2)

2. Choose “Favorites” → “Command Line” to display a command input field and put “split” in this field.

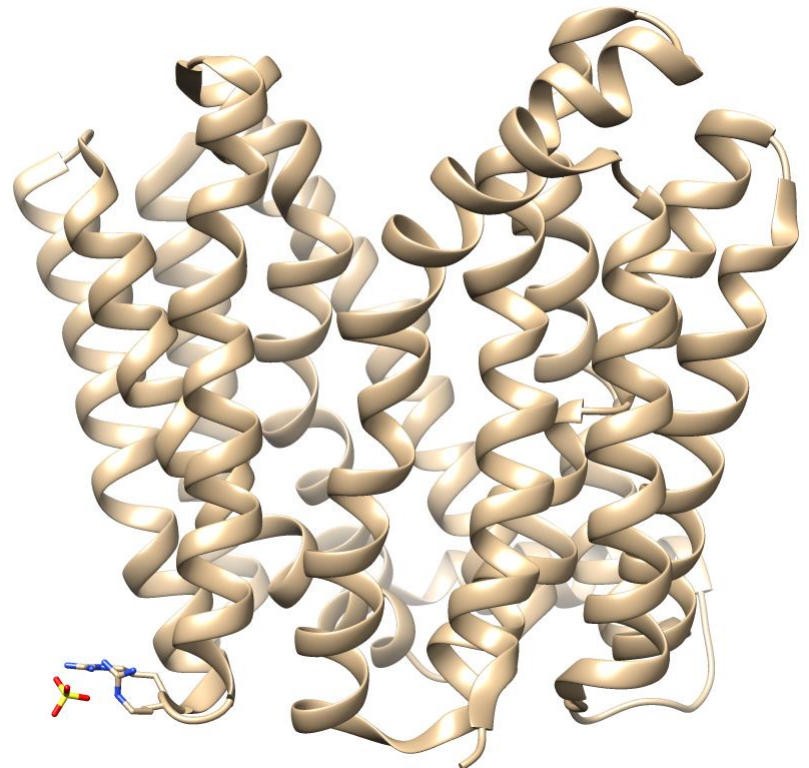


3. Choose “Favorites” → “Model Panel,” select “6gv1.pdb H” and “6gv1.pdb L” and “close.”



Tertiary structure of MdfA (3)

4. Choose “Actions” → “Focus” to fit the molecular image to the window.
 - Confirm that the protein adopts the outward open structure.

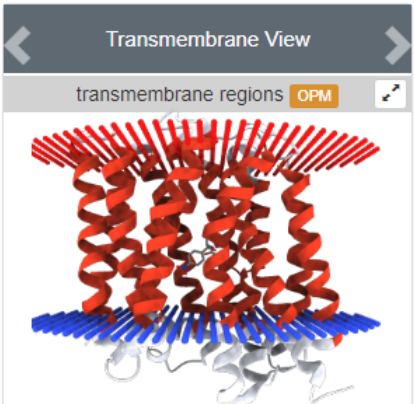


Comparison of the structures (1)

- The inward-open structure has been determined by Chinese researchers.

1. Fetch the “4ZOW” structure.

[Structure Summary](#) [3D View](#) [Annotations](#) [Sequence](#) [Sequence Similarity](#) [Structure Similarity](#) [Experiment](#)



Transmembrane View

transmembrane regions OPM

The image shows a 3D ribbon diagram of the MdfA multidrug transporter from E. coli, embedded in a lipid bilayer. The protein is shown in red and blue, with the lipid bilayer in grey. The structure is viewed from the transmembrane perspective, showing the arrangement of the transmembrane helices and the lipid environment.

4ZOW

Crystal structure of E. coli multidrug transporter MdfA in complex with chloramphenicol

DOI: [10.2210/pdb4ZOW/pdb](https://doi.org/10.2210/pdb4ZOW/pdb)

Classification: [TRANSPORT PROTEIN](#)

Organism(s): [Escherichia coli \(strain K12\)](#)

Expression System: [Escherichia coli 'BL21-Gold\(DE3\)pLysS AG'](#)

Mutation(s): 1

Deposited: 2015-05-07 Released: 2015-08-19

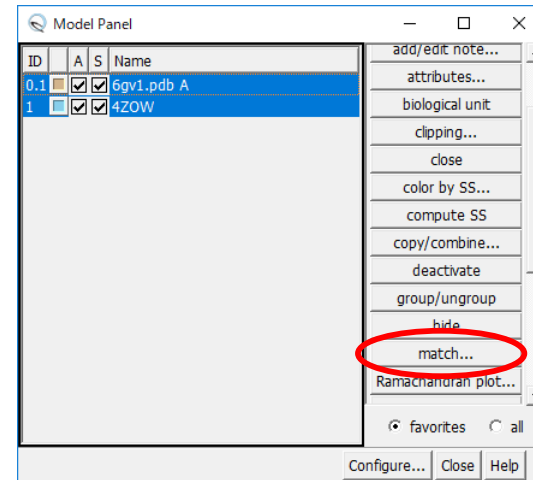
Deposition Author(s): [Zhang, X.C.](#), [Heng, J.](#), [Zhao, Y.](#), [Wang, X.](#)

[Display Files](#) [Download Files](#)

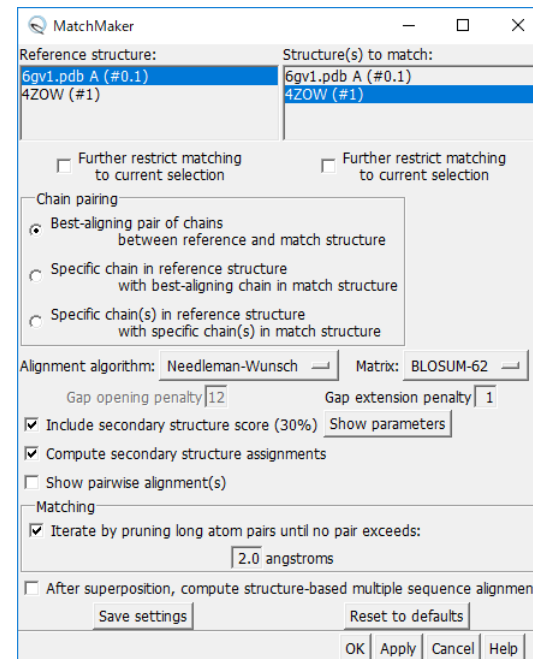
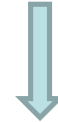
[Contact Us](#)

Comparison of the structures (2)

2. Select “6gv1.pdb A” and “4ZOW” on the Model Panel and “match.”
3. Click “OK” on the MatchMaker window.
4. Choose “File” → “Open” to open “color.com” in the MdfA_MD folder.

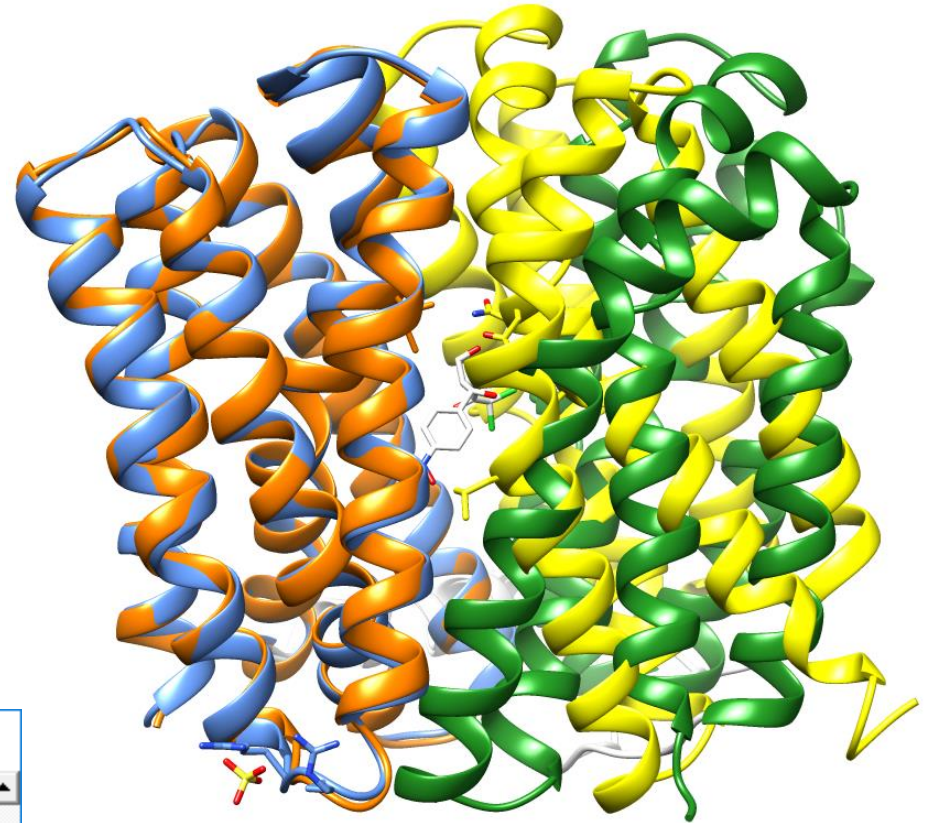
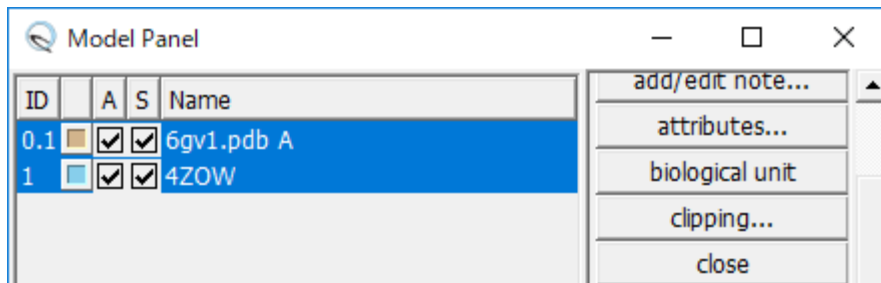


Scroll down



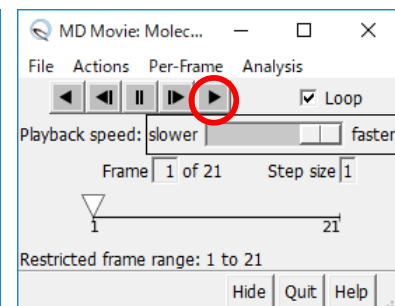
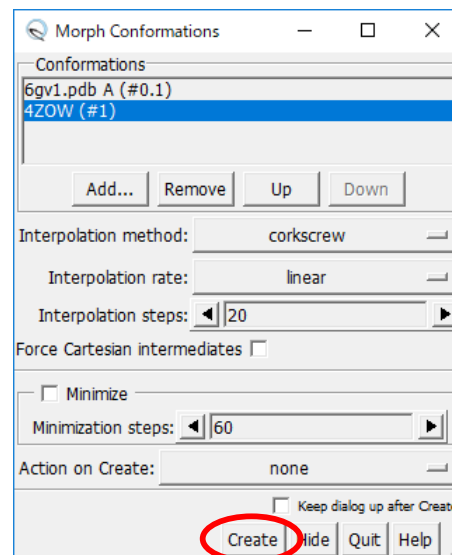
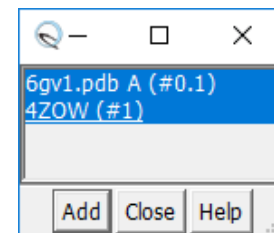
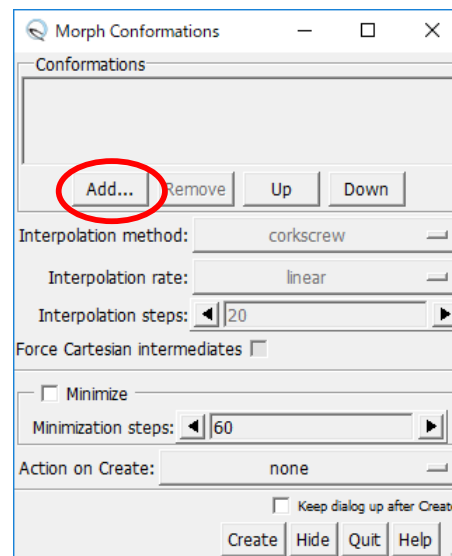
Comparison of the structures (3)

- The C-lobes of the two structures are aligned.
- Each structure can be shown or hidden by checking or unchecking the checkbox in the S column.



Comparison of the structures (4)

5. Choose “Tools” → “Structure Comparison” → “Morph Conformations.”
6. Click “Add” in the Morph Conformations window.
7. Select “6gv1.pdb A” and “4ZOW,” and click “Add” and “Close.”
8. Click “Create” in the Morph Conformations window.
9. Click the play button.



Objectives

- “Morph Conformations” does not shows the real process of the conformational change.
- The mechanism of the conformational change remains unclear.
- The mechanism of the H⁺/drug anti-transport also remains unclear.

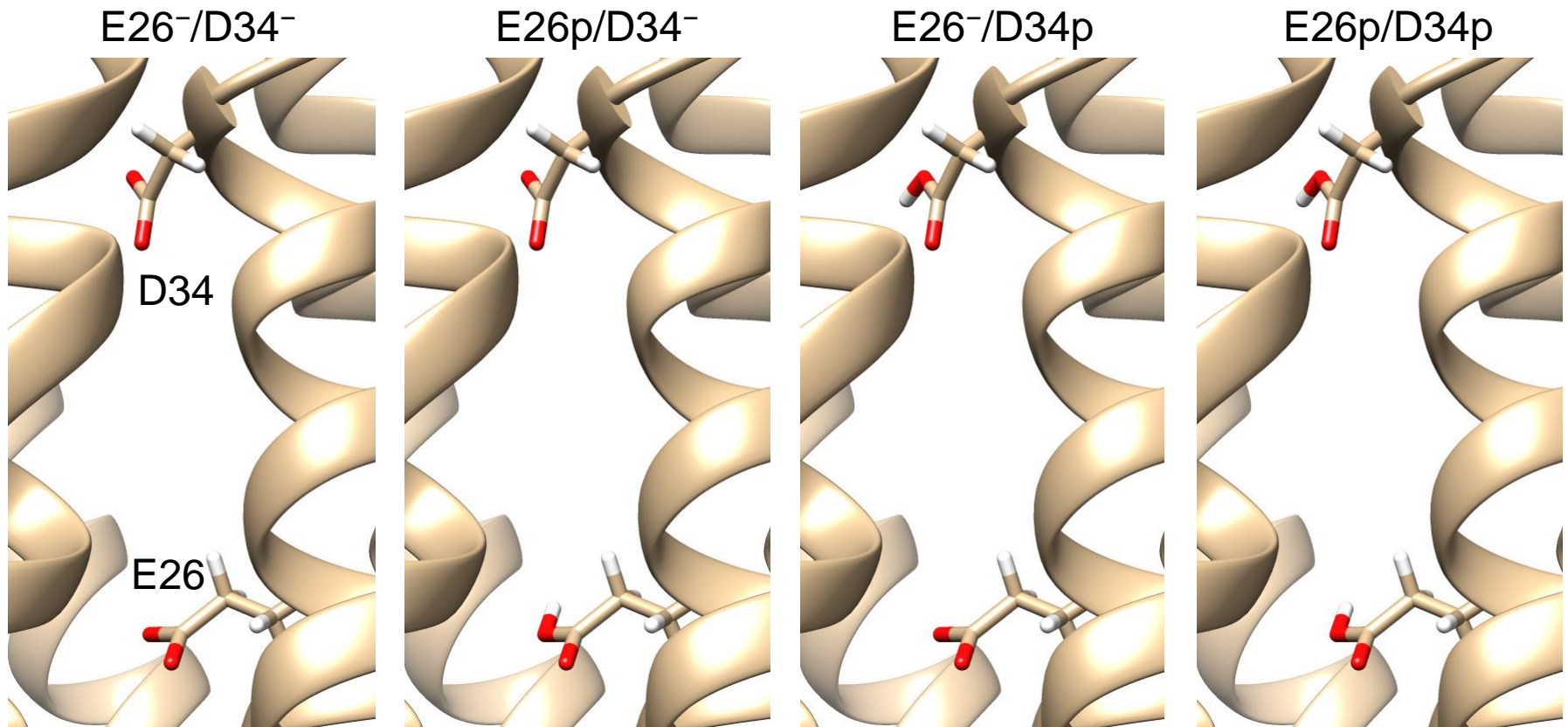


- We aimed to clarify these mechanisms by MD simulations.

Methods

- Each of the outward-open and the inward-open structures was embedded in a solvated lipid bilayer.
- The two acidic residues (E26 and D34) are known to be important for the transport activity. We performed an MD simulation for each protonation state of these residues.
- We monitored the conformational changes during the MD simulations.

Protonation state



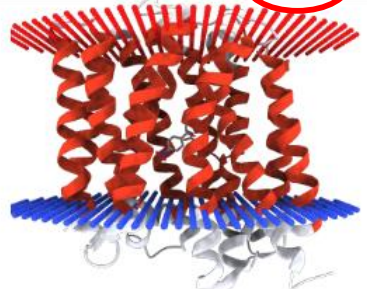
Modeling of membrane protein (1)

1. Open the 4ZOW page at the RCSB PDB site and click “OPM.”
2. Move to the OPM (Orientations of Proteins in Membranes) site.
3. Download the PDB file by clicking the “Download File” link and save the file on the Desktop.

Structure Summary 3D View Annotations Sequence

Transmembrane View

transmembrane regions OPM

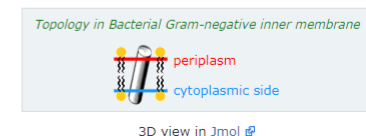
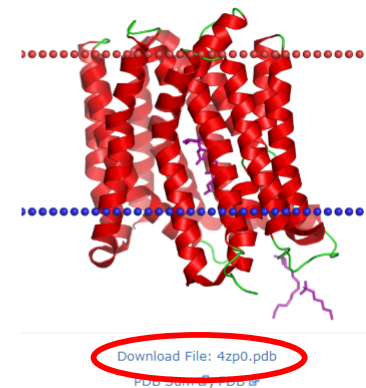


4ZOW
Crystal structure of chloramphenicol acetyltransferase (CAT) from *Escherichia coli* (PDB ID: 4ZOW).
DOI: 10.2210/pdb.4ZOW.rcsb
Classification: [TF](#)
Organism(s): [Escherichia coli](#)
Expression System: [E. coli](#)
Mutation(s): 1
Deposited: 2015-
Deposition Authority: [RCSB PDB](#)

4zp0 » Multidrug transporter MdfA, structure 1

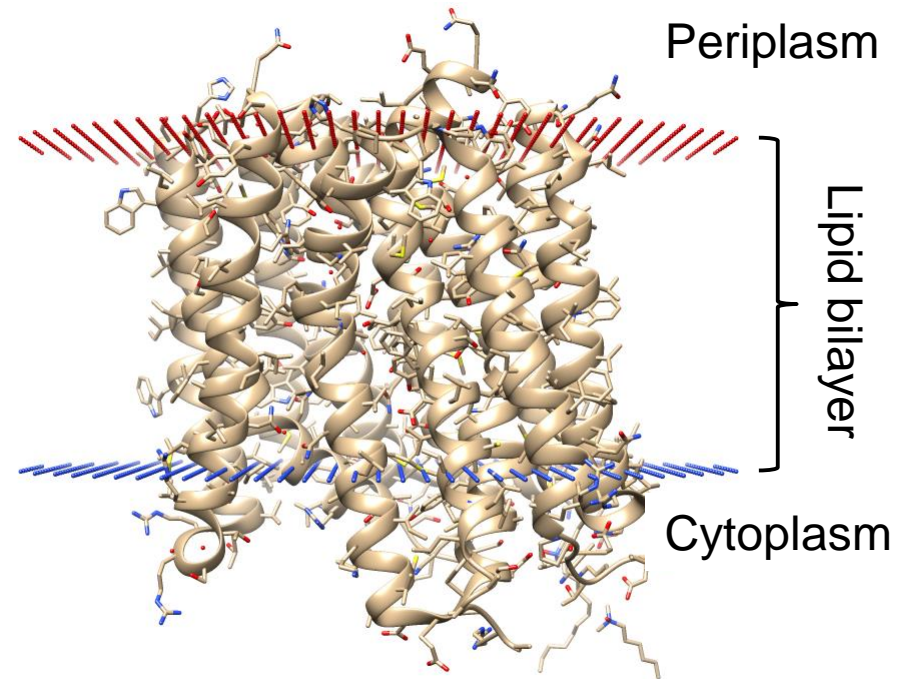
- Type: Transmembrane (3 classes)
- Class: Alpha-helical polytopic (116 superfamilies)
- Superfamily: Major Facilitator Superfamily (MFS) (9 families) 2.A.1 (TCDB) [CL0015](#)
- Family: Drug/proton antiporter (4 proteins) 2.A.1.2 (TCDB) [PF07690](#) [PDBsum](#)
- Species: *Escherichia coli* (279 proteins)
- Localization: Bacterial Gram-negative inner membrane (564 proteins)

4zp0 » Multidrug transporter MdfA, structure 1	
Hydrophobic Thickness or Depth	29.8 ± 1.3 Å
Tilt Angle	10 ± 0°
ΔG _{transfer}	-77.2 kcal/mol
Links to 4zp0	PDB Sum PDB , SCOP PDB , MSD PDB , MMDB PDB , Encompass PDB
Topology	subunit A (N terminus cytoplasmic side)
Resolution	2.00
Primary PDB representation	4zp0
Other PDB entries representing this structure	4zow, 4zp2, 6euq
Number of TM Secondary Structures	12



Modeling of membrane protein (2)

4. Choose “Files” → “Close Session” to initialize Chimera.
5. Choose “Files” → “Open” to open “4zp0.pdb.”
6. Choose “Actions” → “Atoms/Bonds” → “show.”

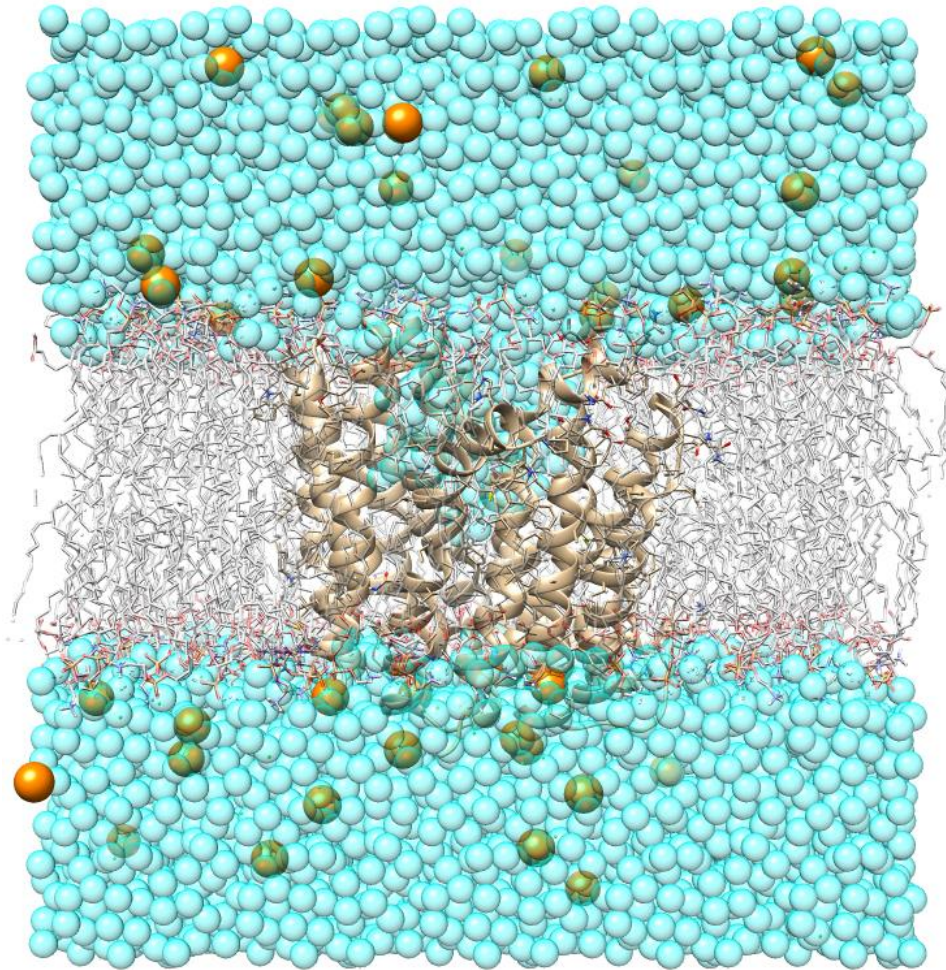


Modeling of membrane protein (3)

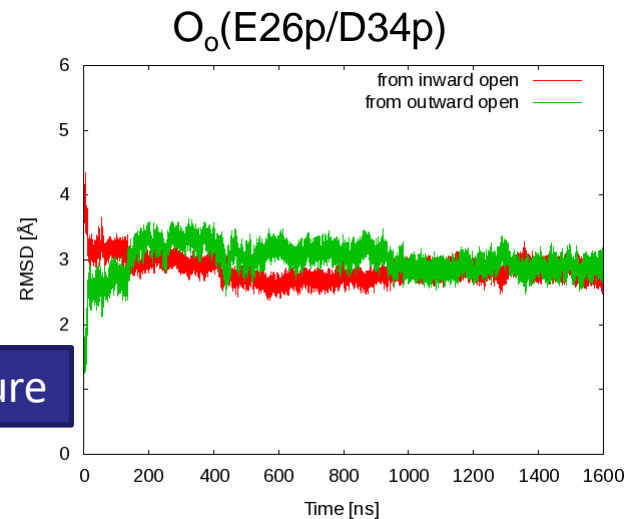
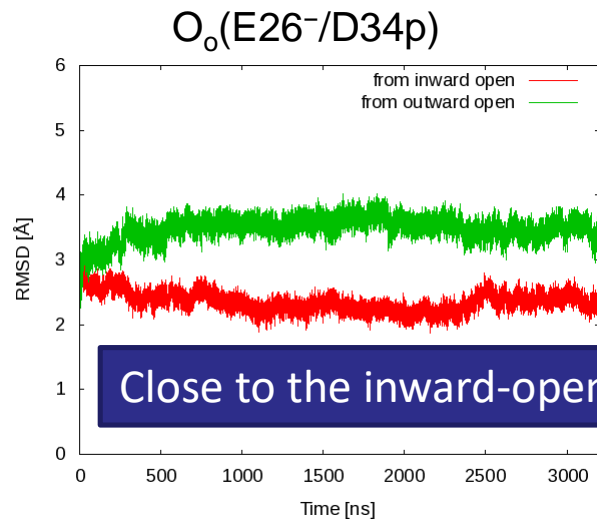
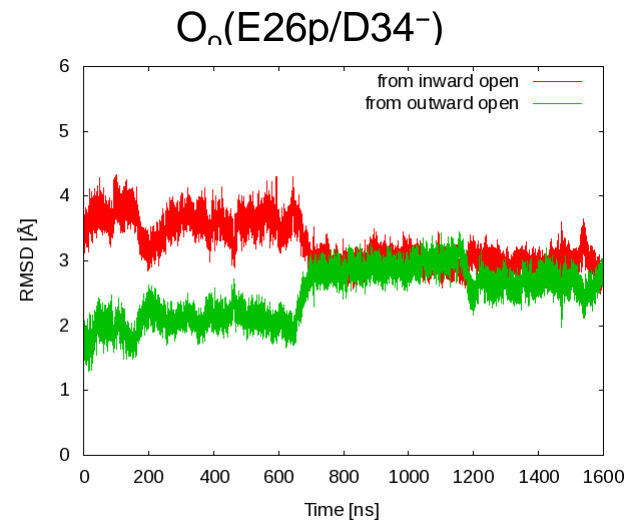
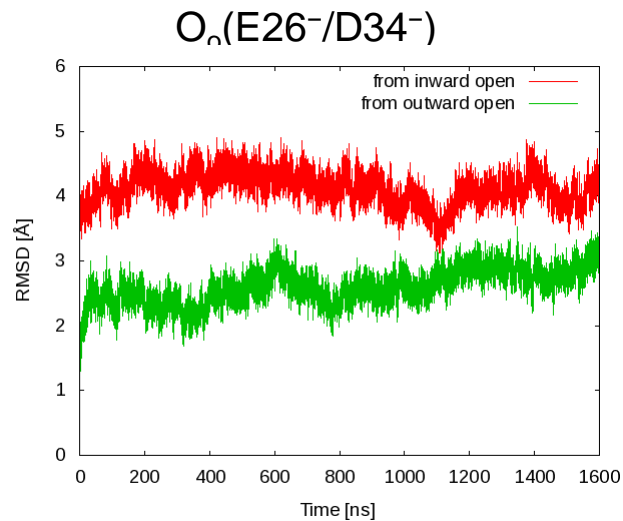
- The tertiary structure of a membrane protein itself does not have an explicit information about how the protein is embedded in the lipid bilayer.
- Because transmembrane regions are rich in hydrophobic residues, they can be predicted from the tertiary structure and the amino-acid composition.
- OPM is a database of the predictions for the membrane proteins in PDB of how each protein is embedded in a lipid bilayer.

Modeling of membrane protein (4)

- The CHARMM-GUI server generates a model of a protein embedded in a solvated lipid bilayer.
 - <http://www.charmm-gui.org/>
1. Choose “File” → “Open” to open “step5_assembly.pdb” in the MdfA_MD folder.
 2. Choose “File” → “Open” to open “color2.com” in the MdfA_MD folder.



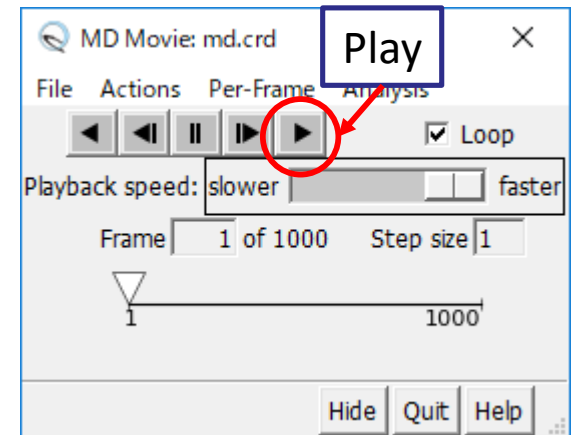
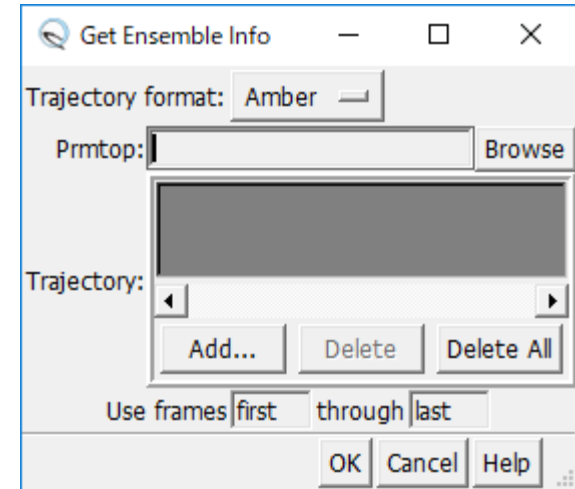
Time evolutions of RMSDs



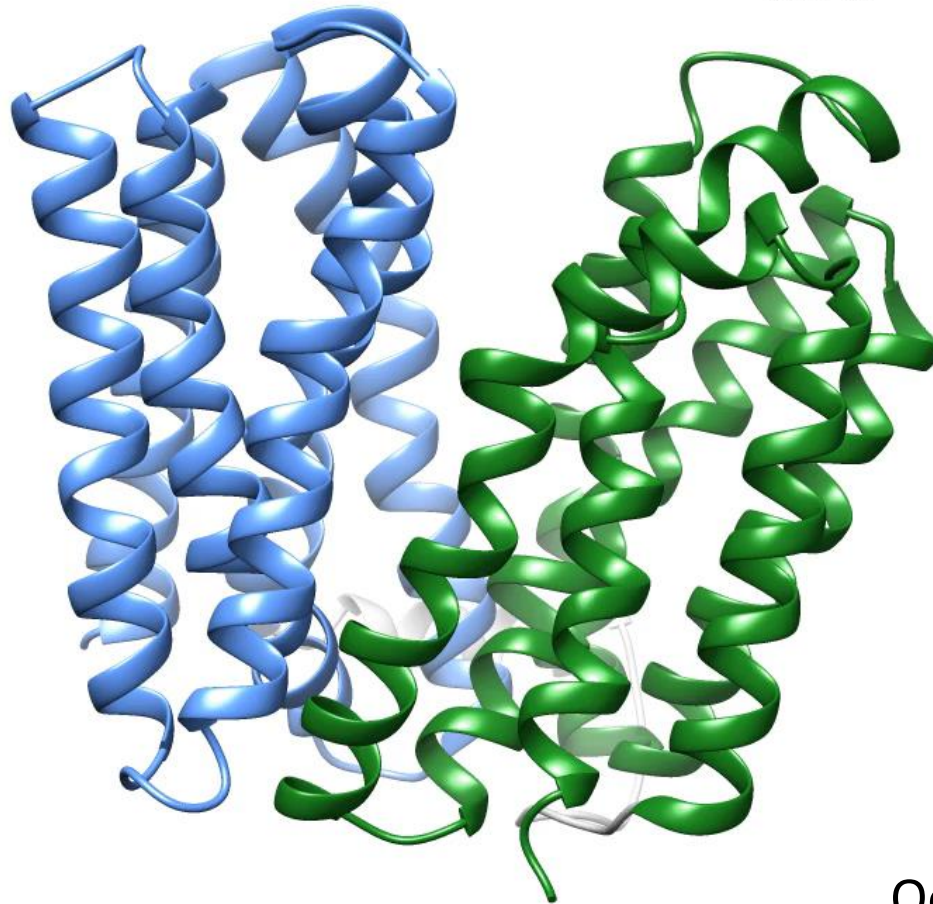
Close to the inward-open structure

Watching the trajectory

1. Choose “File” → “Close Session” to initialize Chimea.
2. Choose “Tools” → “MD/Ensemble Analysis” → “MD Movie.”
3. Select “Amber” as Trajectory format.
4. Set “leap_nowat.top” in the MdfA_MD folder to Prmtop.
5. Click “Add” to add “md.crd” in the MdfA_MD folder to the trajectory list and click “OK.”
6. Choose “File” → “Open” to open “color3.com” in the MdfA_MD folder.



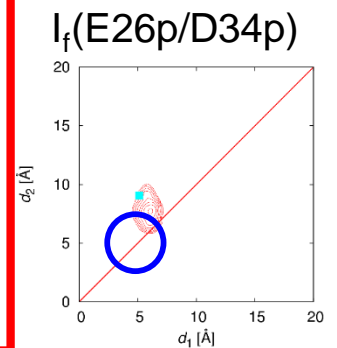
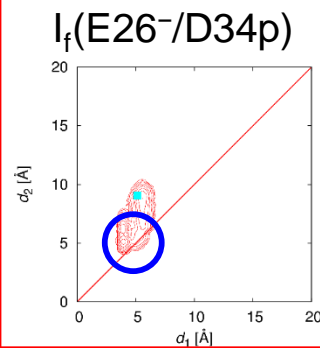
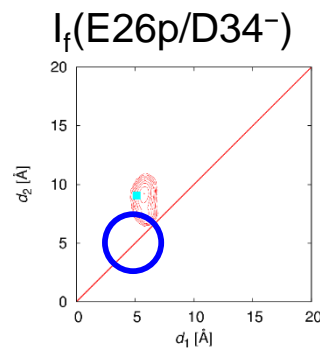
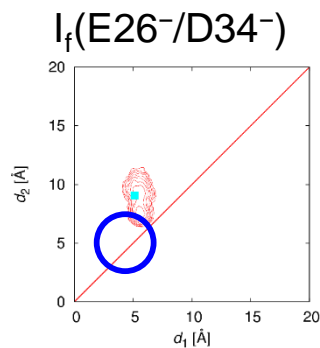
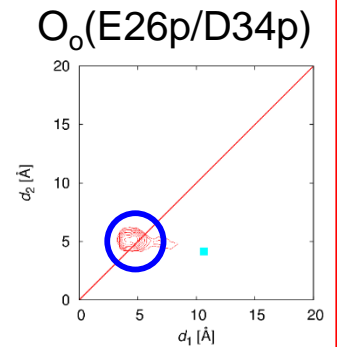
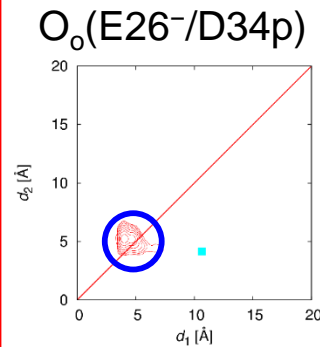
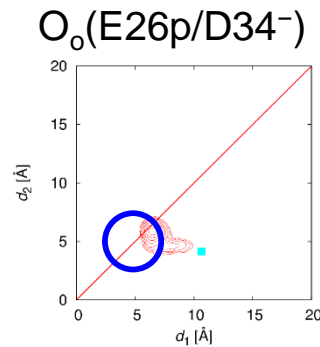
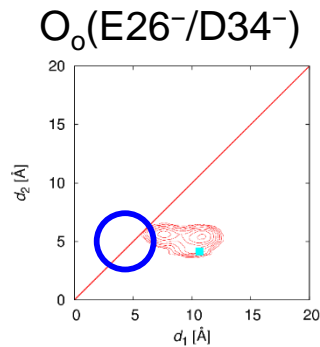
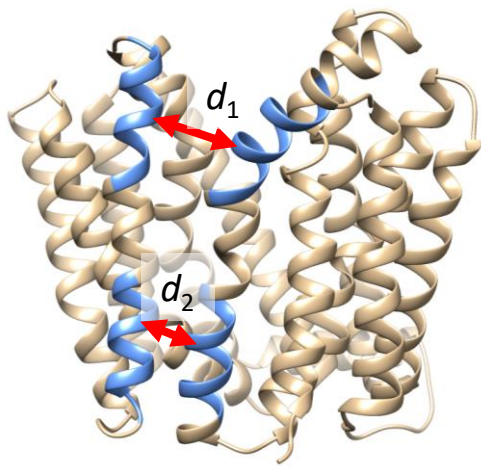
0.01 ns



Oo (E26⁻/D34p)

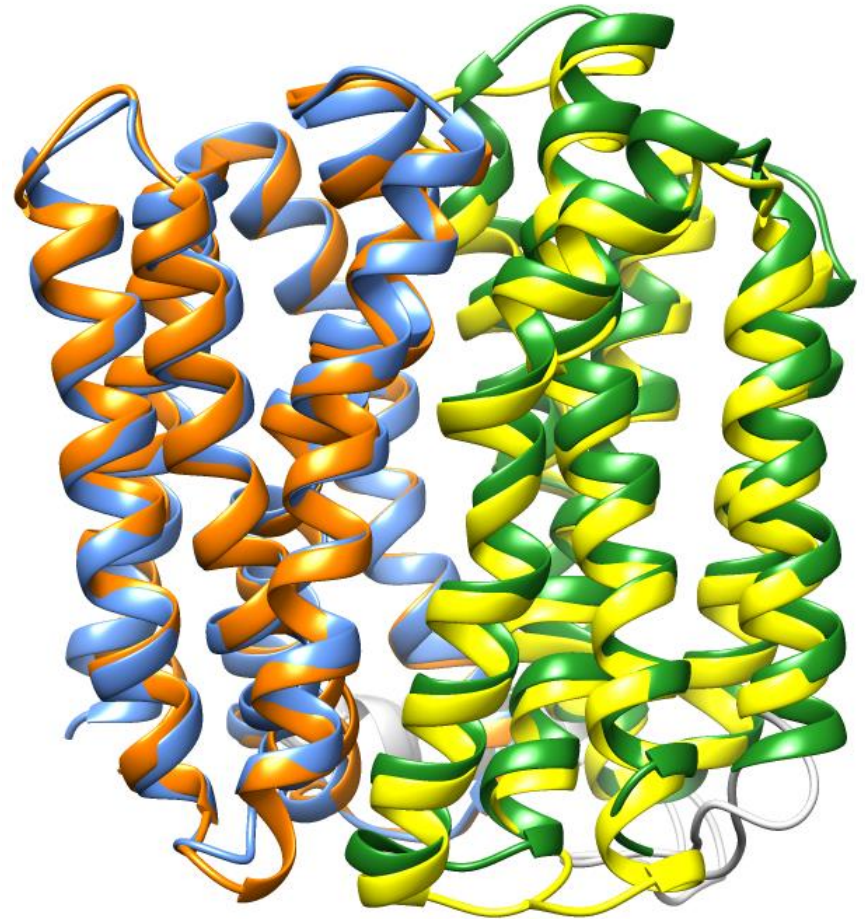
Conformational distribution

Transitions to the structure with both side closed

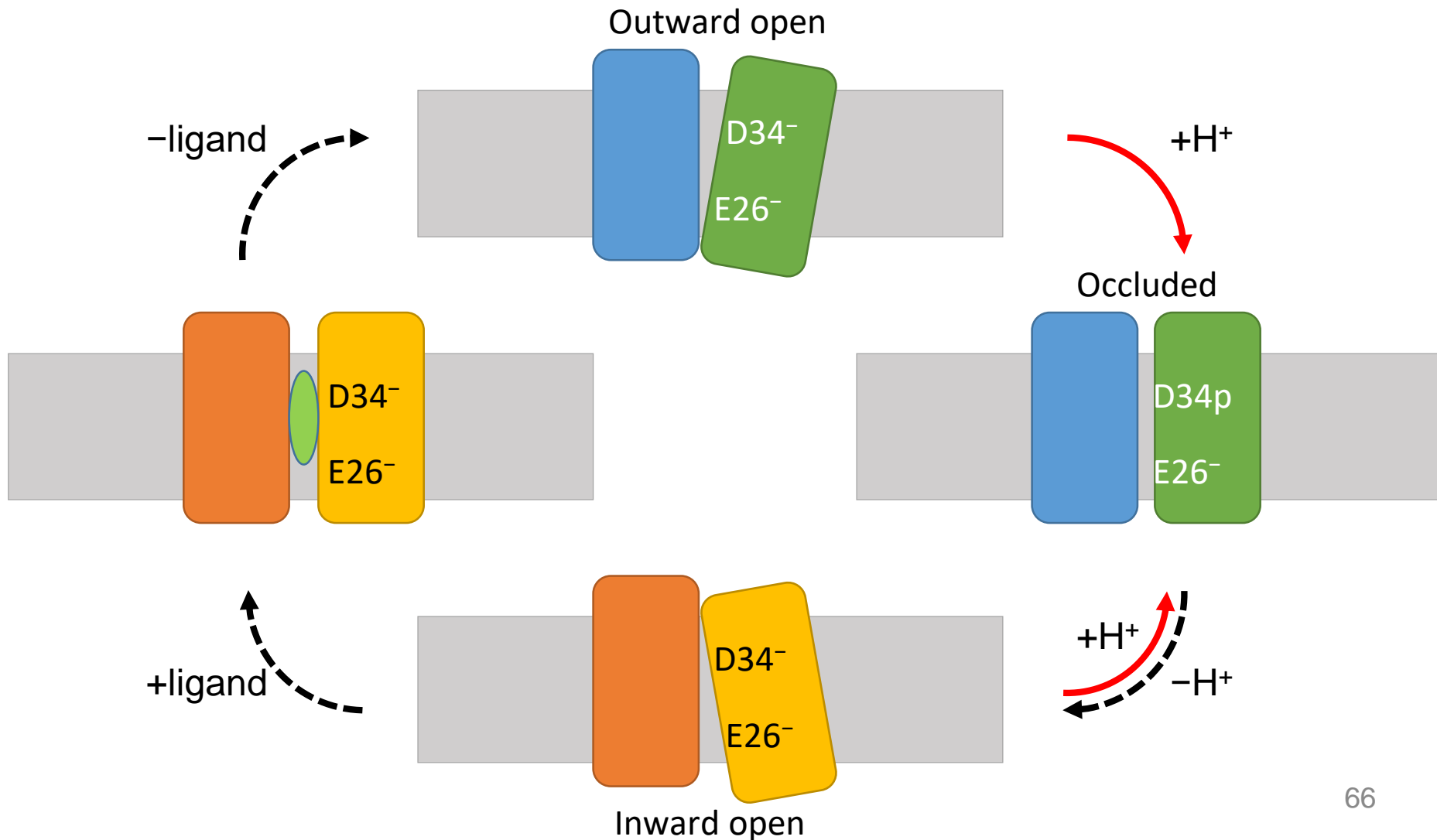


Occluded structure

- When D34 was protonated, both the outward-open and the inward-open structures changed to the structure with both ends closed (occluded structure).
- RMSD between the final structures was 1.5 Å.
→ Almost the same structure.

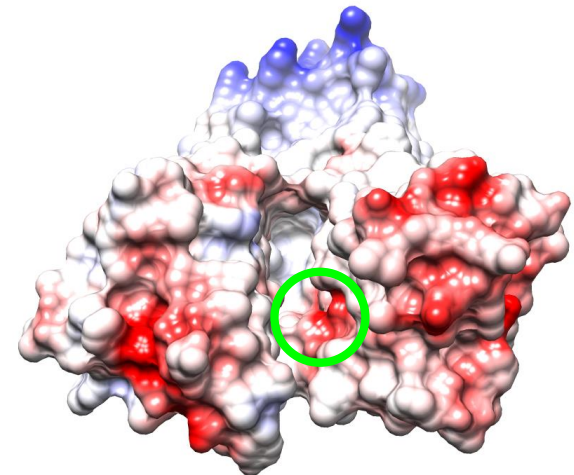
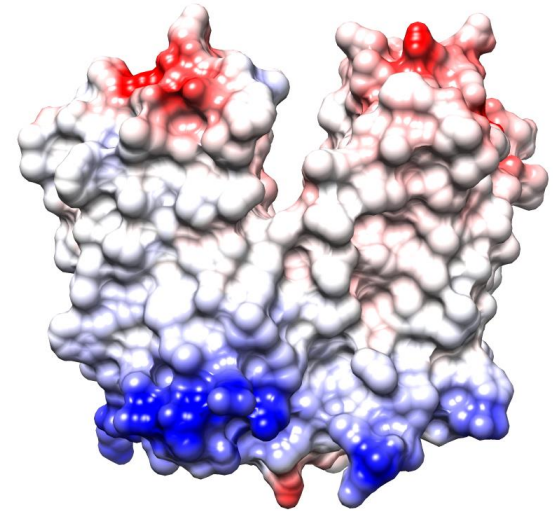


Conformational change mechanism (1)



Conformational change mechanism (2)

- There are many acidic residues on the periplasmic side, while there are many basic residues on the cytoplasmic side.
- Protonation on D34 may weaken the electrostatic repulsion between the N- and C-lobes.



Exercise

- What is the rank and the score of the pose closest to the crystal structure in the docking of oseltamivir carboxylate to chain A of 2HTY?
- Create a figure of the closest pose superimposed on the crystal structure.
- Create a similar figure for the top ranked pose. Discuss why this pose has such a good score.

Instructions for submission

- Put the results and discussion in the body of email.
- Send the two figures (image files) as attachments.
- Put “Molecular modeling” in the Subject field.
- Be sure to put your name, ID card number (if you are a student), and registration ID (e.g. 19001) in the body of the email.
- Send the email to Prof. Tohru Terada (tterada@iu.a.u-tokyo.ac.jp).