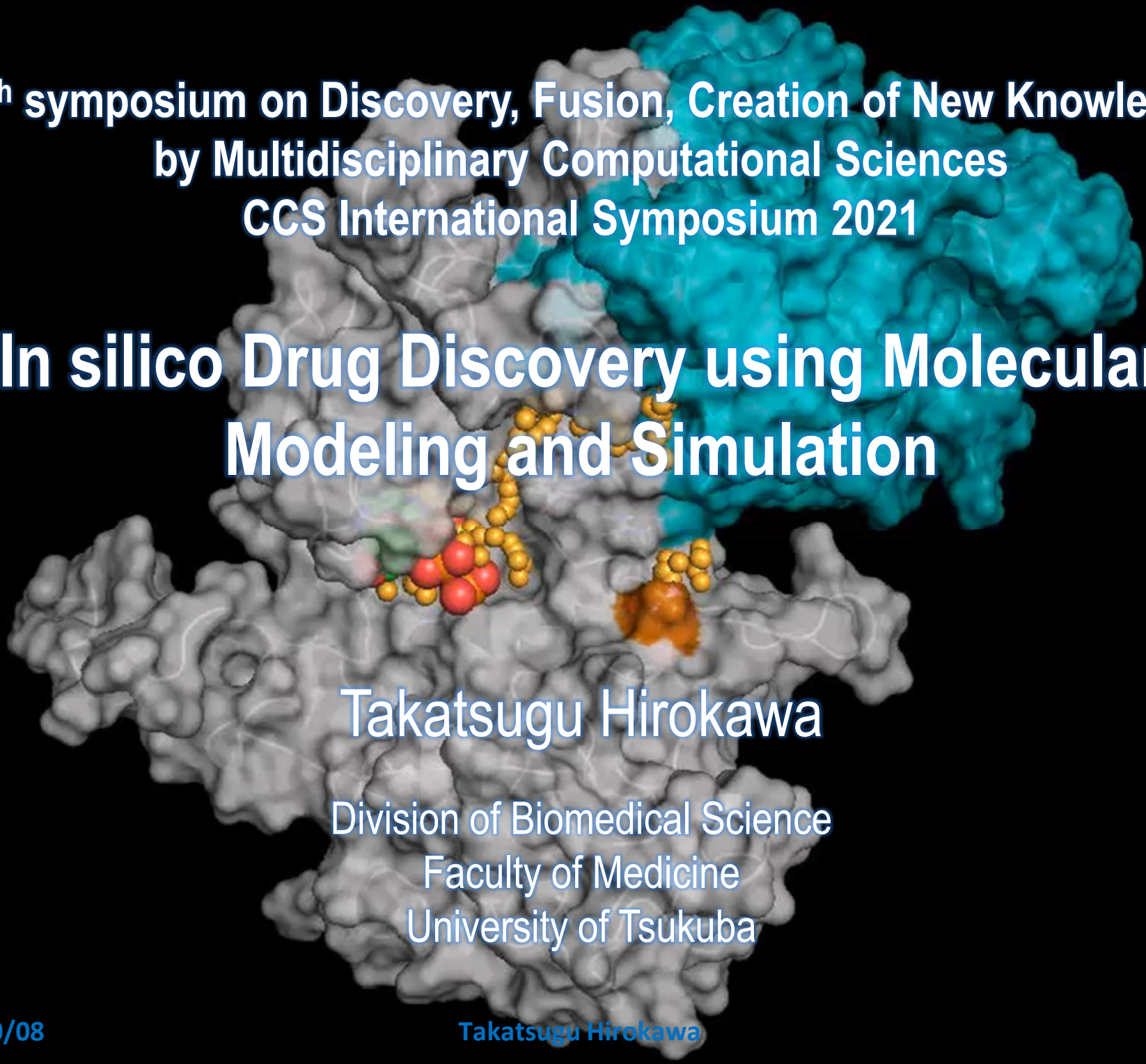
13th symposium on Discovery, Fusion, Creation of New Knowledge by Multidisciplinary Computational Sciences
CCS International Symposium 2021

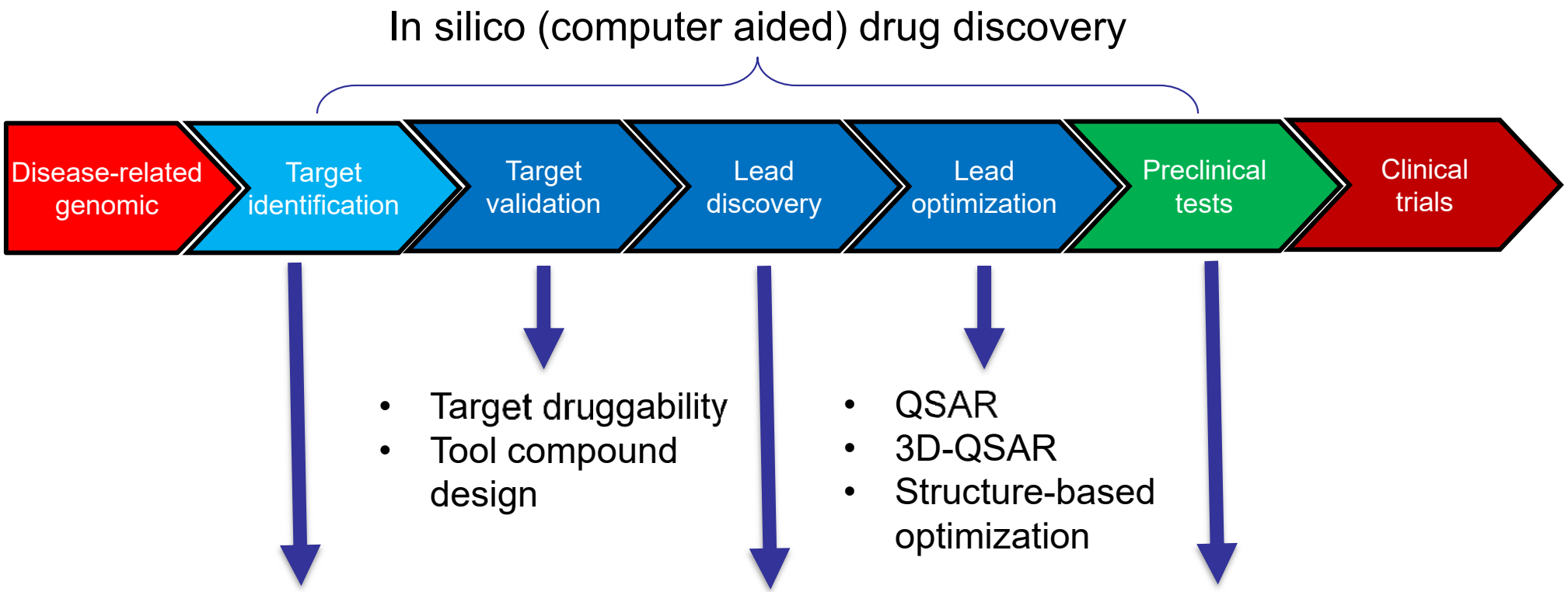
In silico Drug Discovery using Molecular Modeling and Simulation



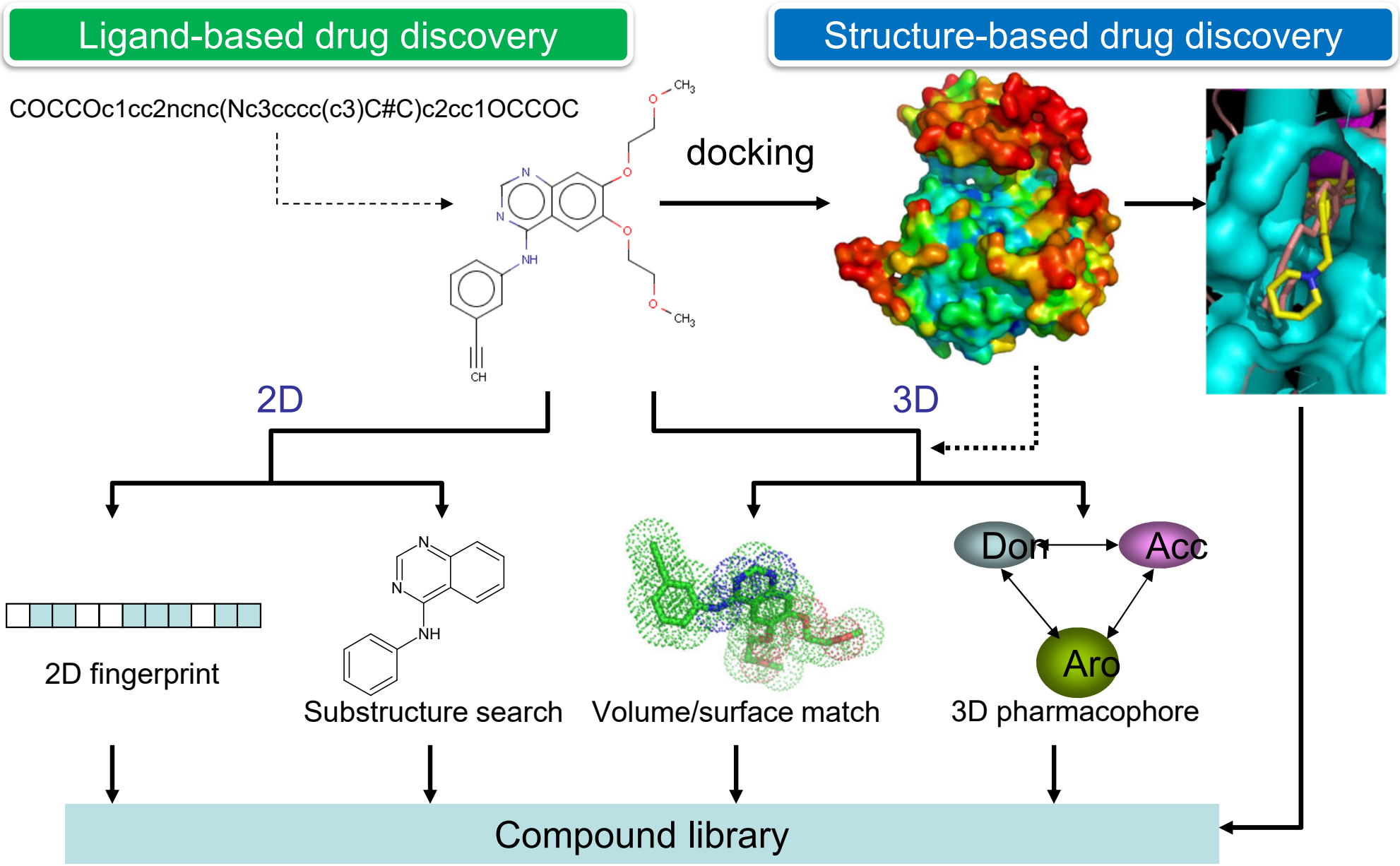
Takatsugu Hirokawa

Division of Biomedical Science
Faculty of Medicine
University of Tsukuba

In silico DD to the various stages of drug development



In silico drug discovery approaches



In silico drug discovery approaches

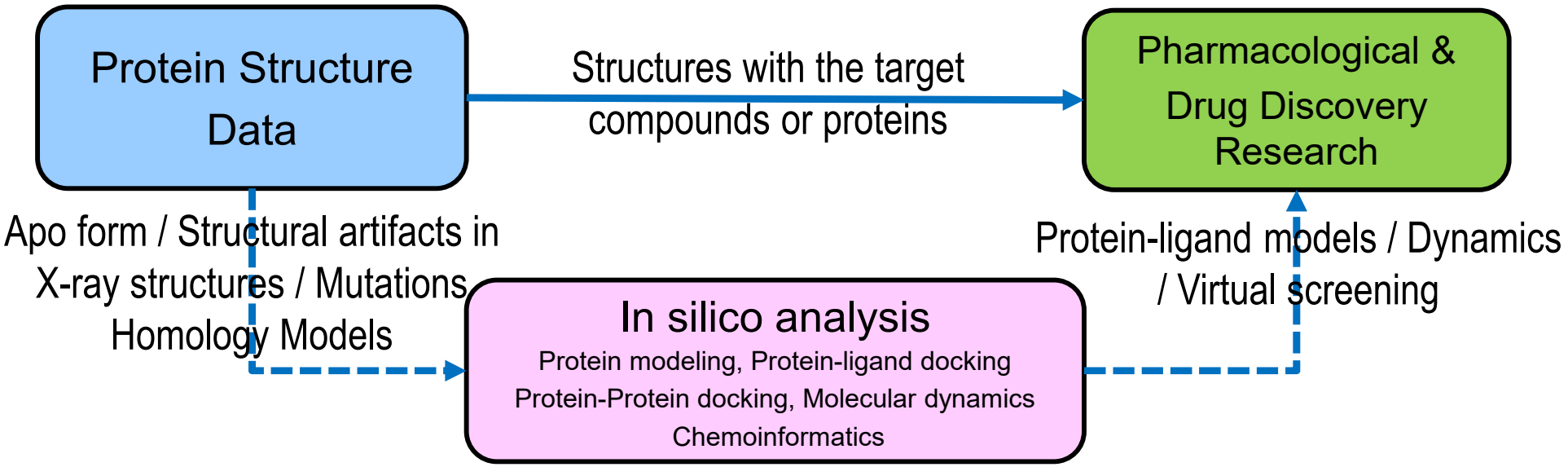
Structure-Based Drug Design (SBDD)

Pros	Cons	Recent topics
Free active ligand information	Hight computational cost for accurate simulation	• Long Time Simulation • GPCR structures • Cryo-EM technology
Structural novelty and chemical diversity		
Understanding the energy contribution of ligand binding		

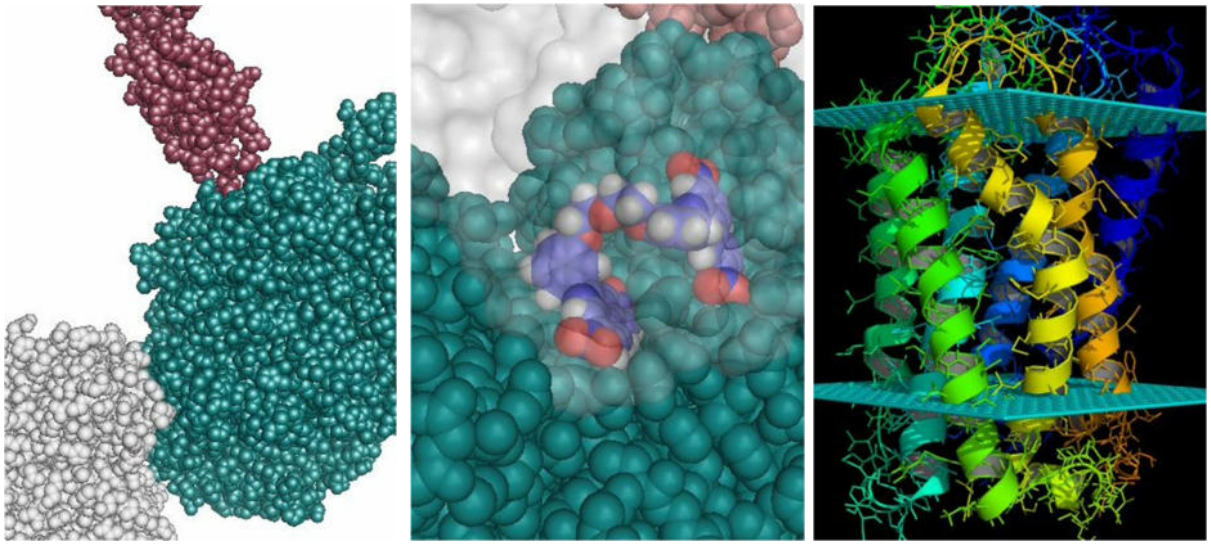
Ligand-Based Drug Design (LBDD)

Pros	Cons	Recent topics
Low computational cost	Rely on known active ligands, narrow chemical diversity	• Open database and big data analysis • Molecular Matched Pair • Artificial Intelligence
Do not rely on protein structures		
Reasonable Hit rate		

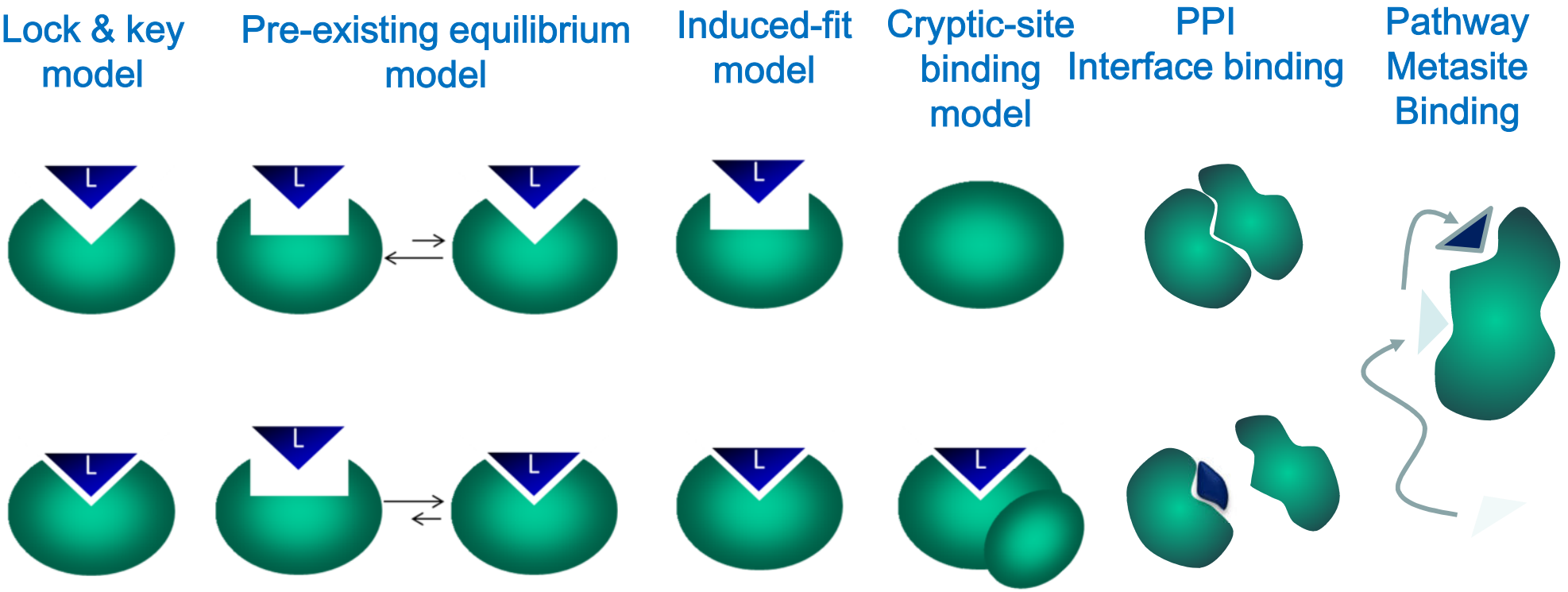
Role of in silico analysis for drug discovery



- **Protein modeling**
- **Protein-Ligand docking**
  - Binding site prediction
  - Induced Fit Docking
  - Protein-ligand interaction fingerprint
  - Virtual screening
- **Protein-Protein docking**
  - PPI interface analysis for drug discovery
- **Molecular dynamics simulation**
  - Conventional MD and biased MD
- **Cheminformatics**



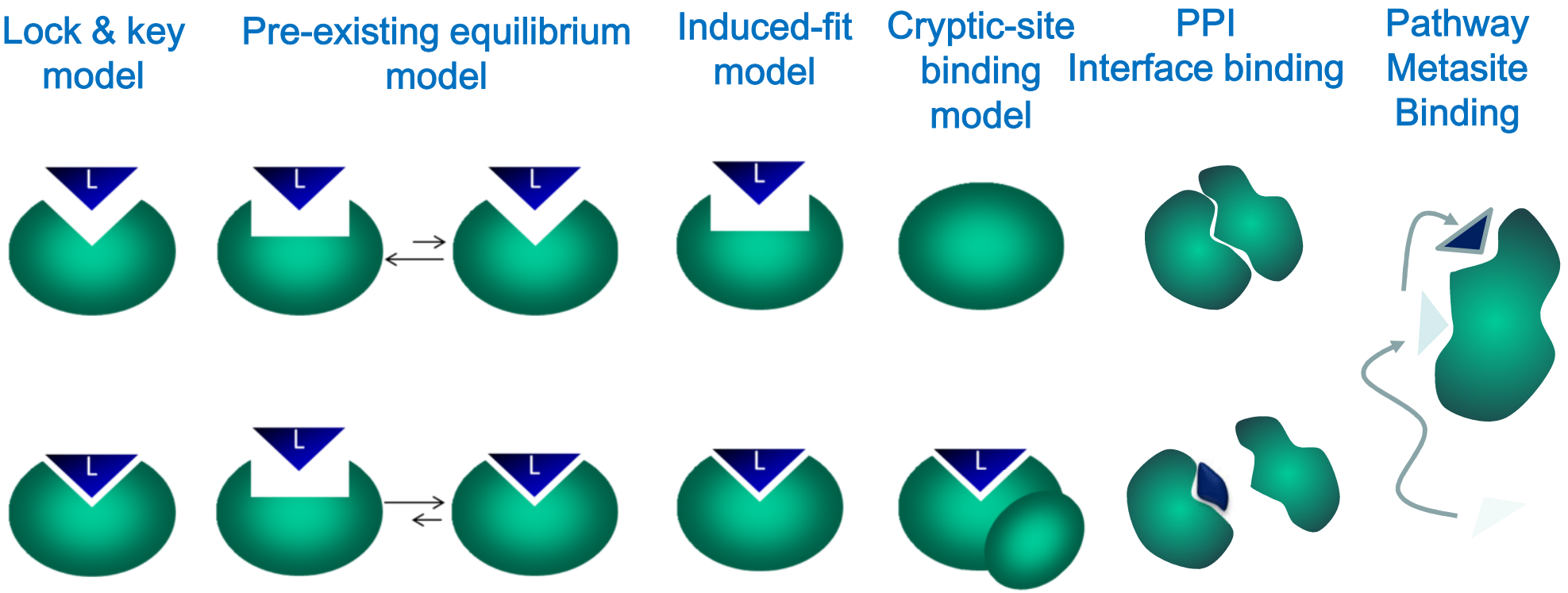
In silico strategies for protein-ligand interactions



Easy — *in silico* strategies — *Hard*

Conventional Docking	Conventional MD & Docking	Conventional / Biased MD & Advanced Docking	Biased MD & Advanced Docking	PPI Docking Conventional / Biased MD & Advanced Docking and Scoring	Biased MD & Advanced Docking & Chem-informatics

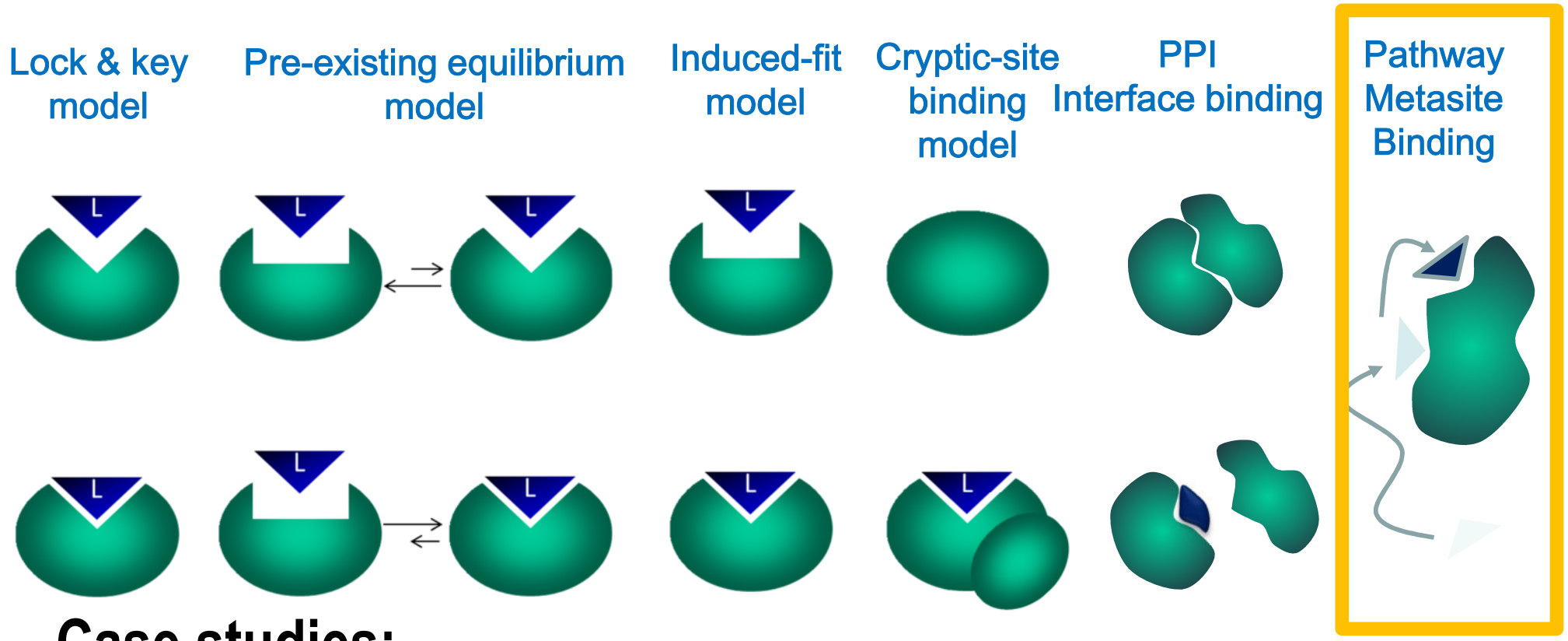
Outline



Case studies:

1. MetaD simulations of ligand entry into EP4 receptor.
2. Analysis by MetaD simulation of binding pathway of influenza virus M2 channel blockers.
3. Identification of the druggable Hidden Catalytic Cavity within the CDK9 Molecule Upon Tat Binding (short topic)

Outline



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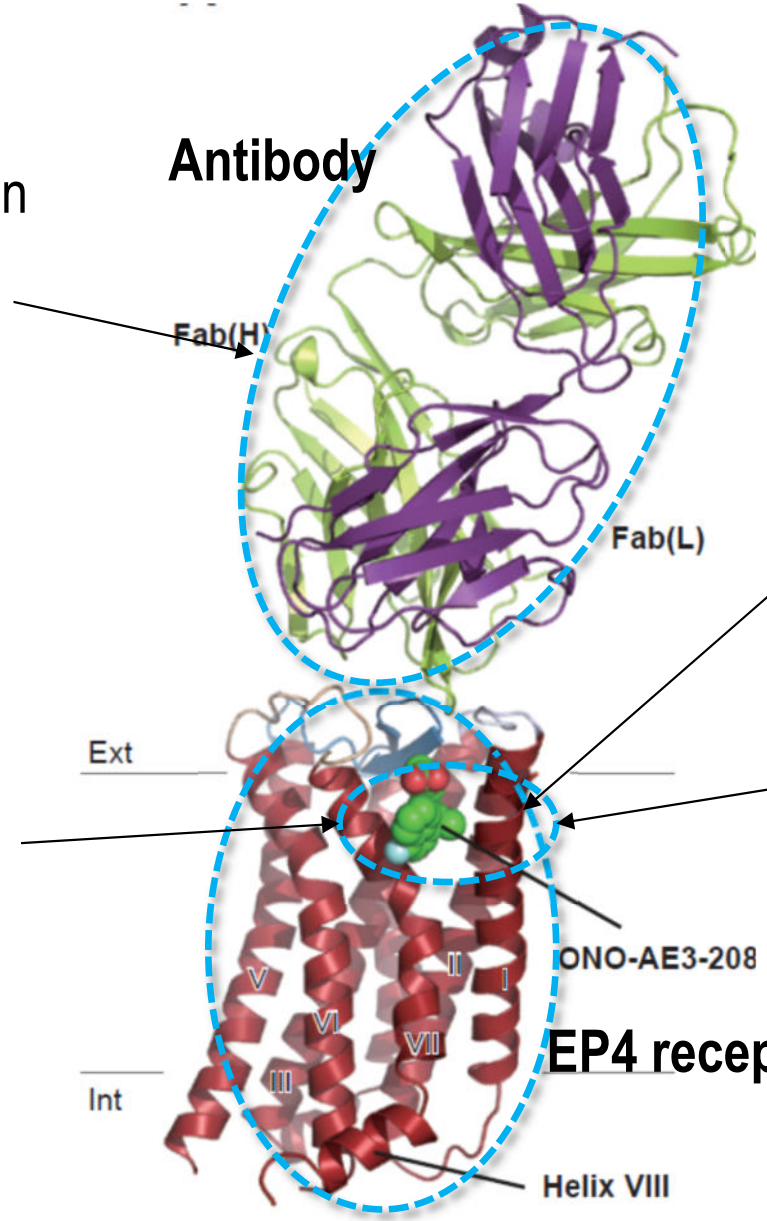
In silico analysis to post-structure determination for GPCRs

Antibody binding contribution on ligand binding

☟

Antibody free simulation



Diversity of structural changes (Active/Inactive)

☟

Expansion of structural template in homology modeling

Ligand-protein contacts
Contribution of key residues for ligand recognition

☟

In silico screening
Ligand design
Understanding of ligand function

Ligand binding pathway simulation
(cMD or Biased MD)

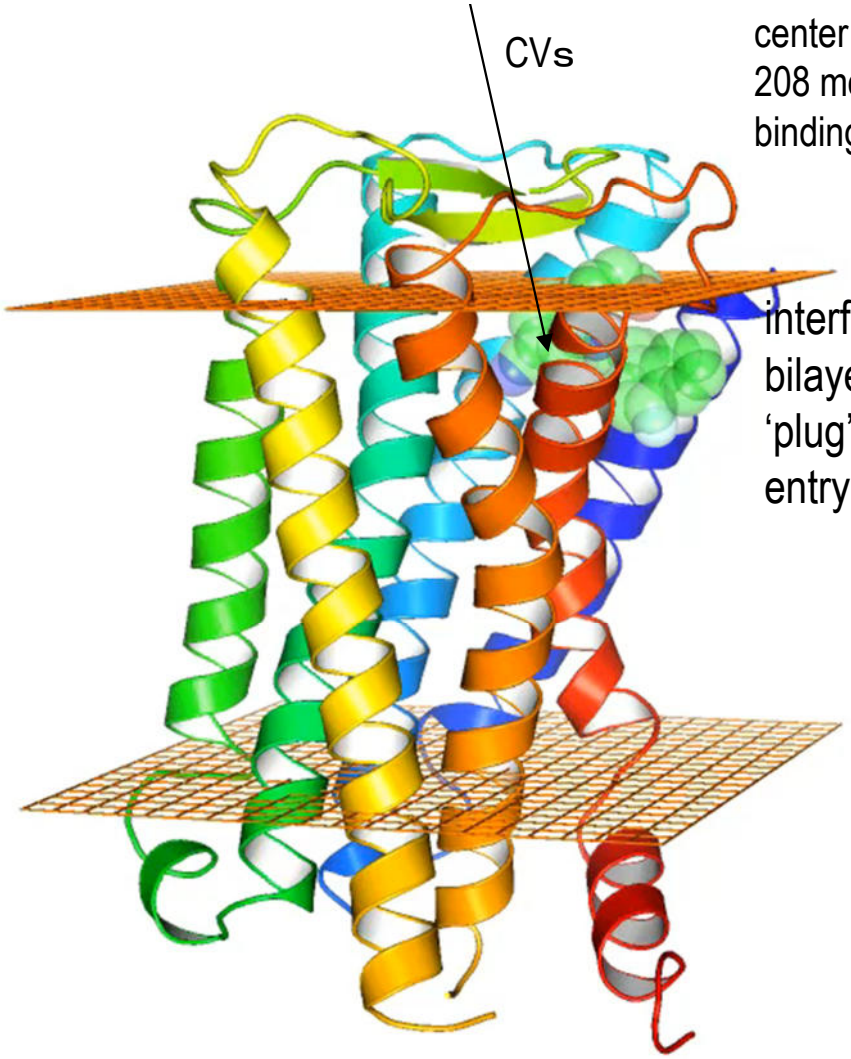
☟

Allosteric or meta site
Mode of action

Toyoda et al., Nat Chem Biol. 2019, 15, 18-26.

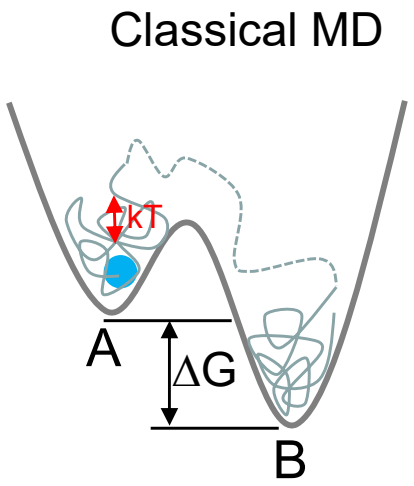
Metadynamics (MetaD) simulation of ligand entry

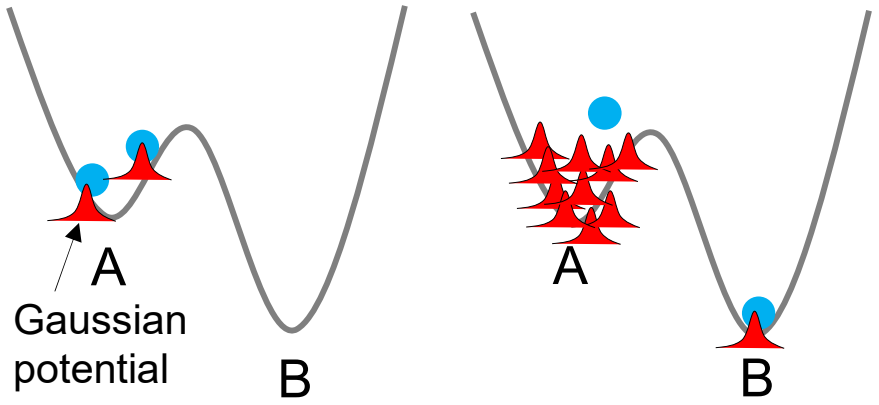
ONO-AE3-208



Biasing collective variables (CVs) as the distance between the center of mass of the ONO-AE3-208 molecule and the ligand-binding residues

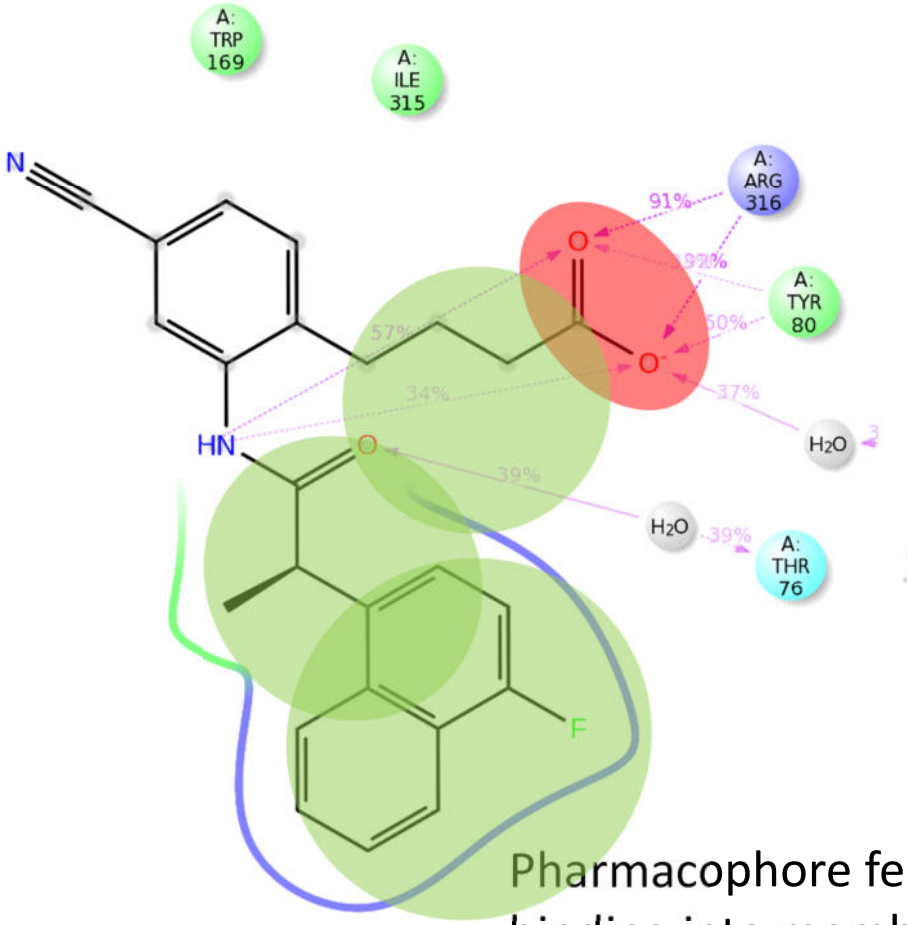
interface of the lipid bilayer, acting as a 'plug' to prevent the entry of agonist



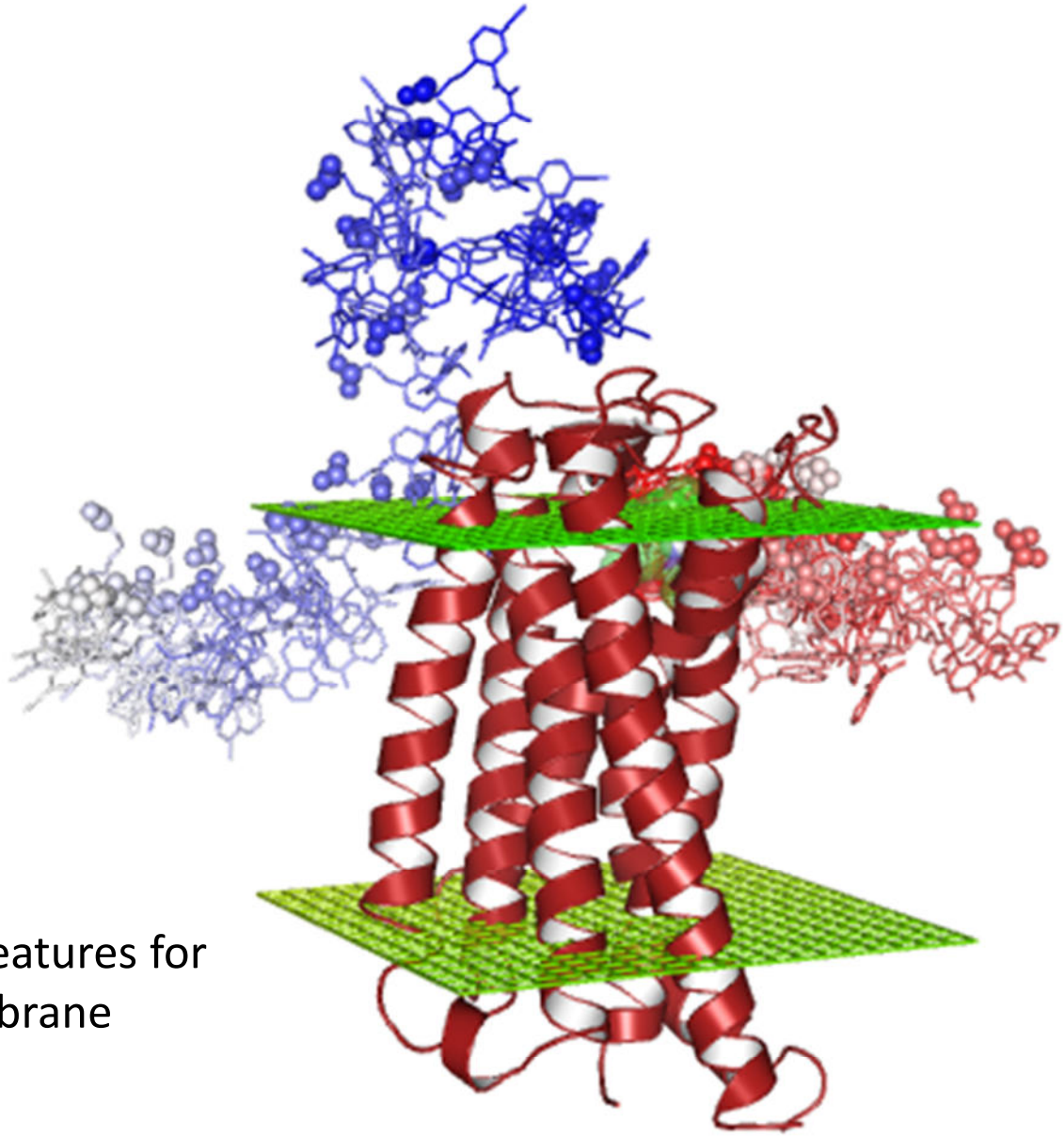


Toyoda et al., Nat Chem Biol. 2019, 15, 18-26.

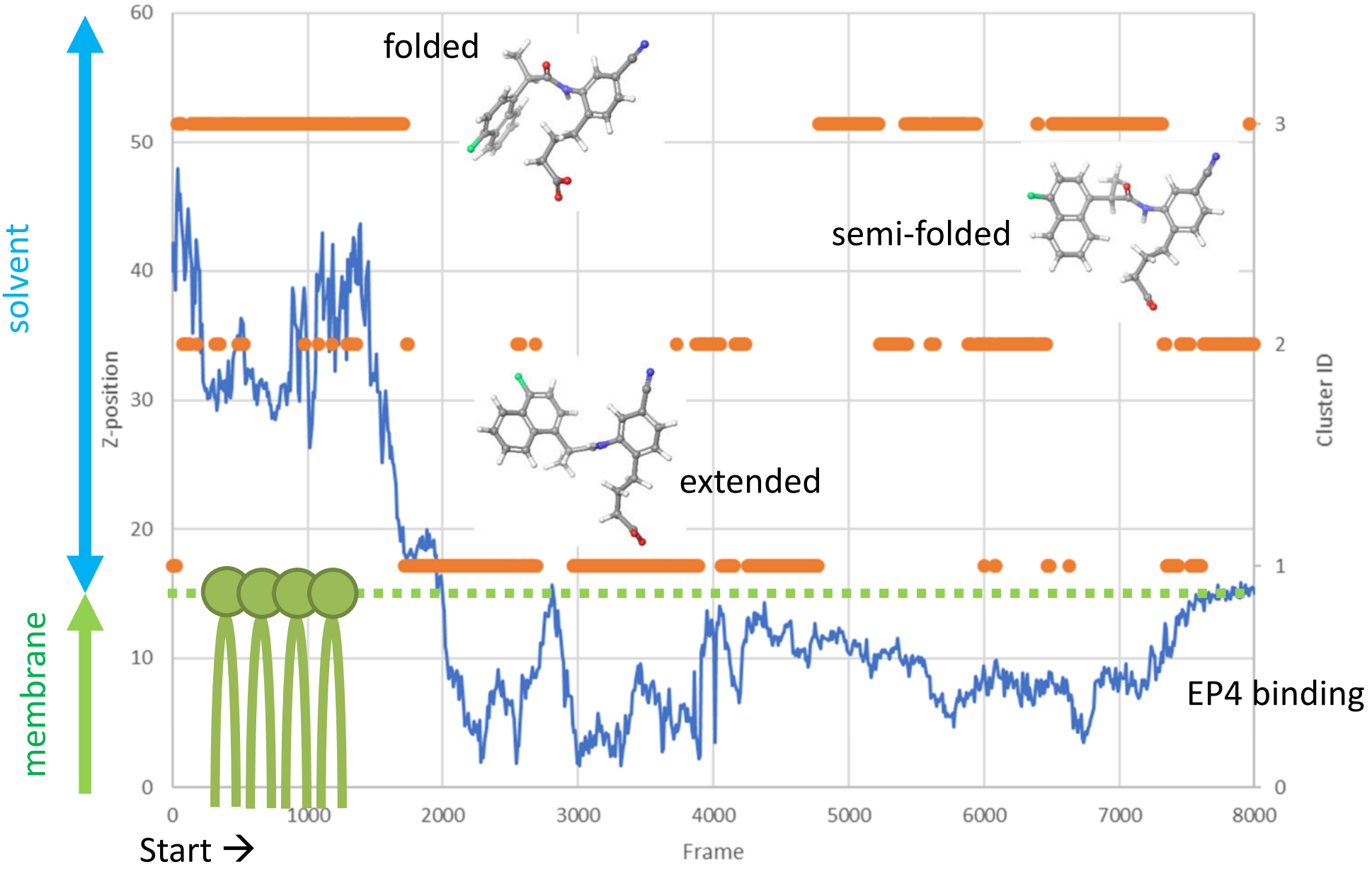
Metadynamics (MetaD) simulation of ligand entry



Pharmacophore features for binding into membrane

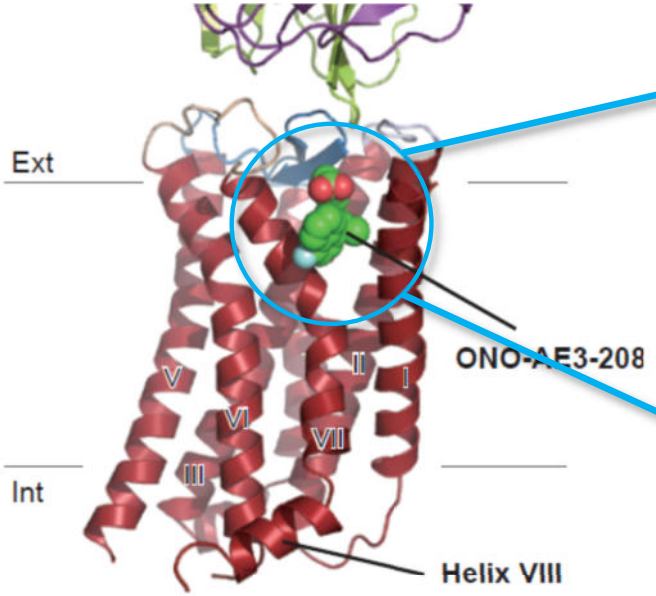


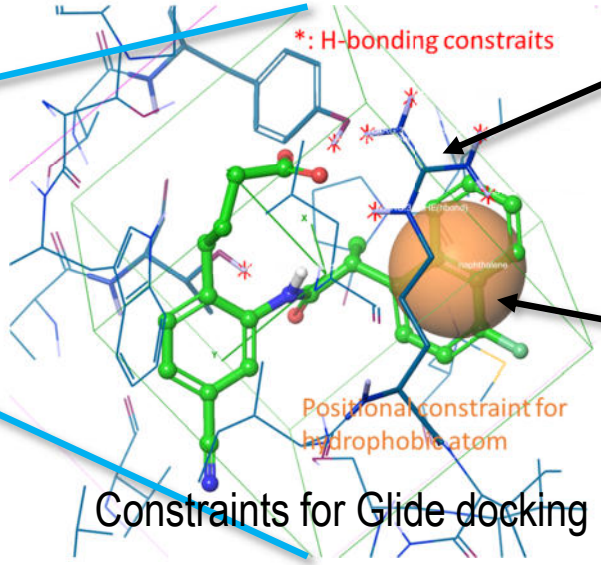
Toyoda et al., Nat Chem Biol. 2019, 15, 18-26.



Ligand conformational changes on ligand entry pathway from the membrane bilayer to the EP4 binding pocket

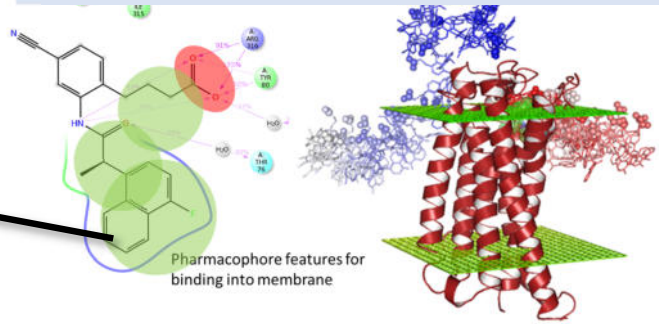
Evaluation for virtual screening test



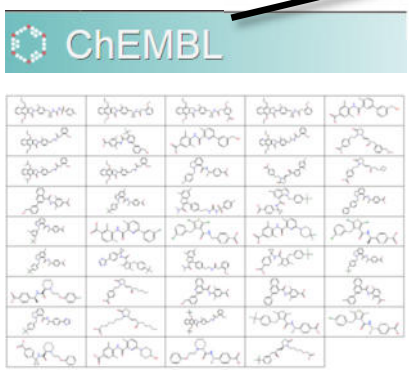


Constraints for Glide docking

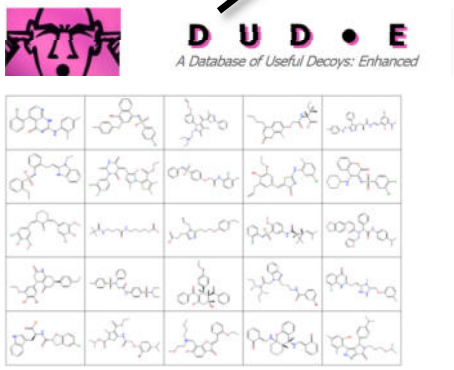
With constrained hydrogen bonding between the compounds and Arg316



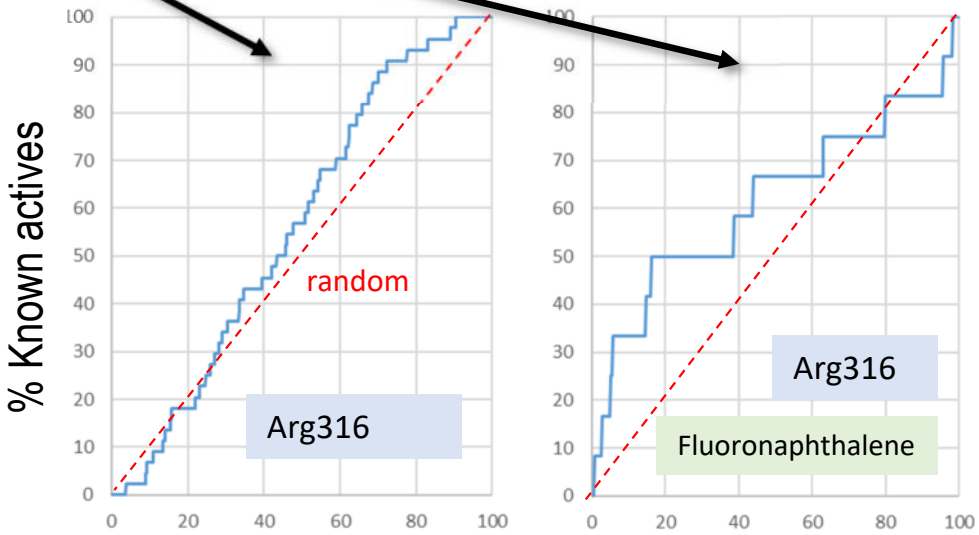
Positional hydrophobic constraint based on Fluoronaphthalene functional group of ligand



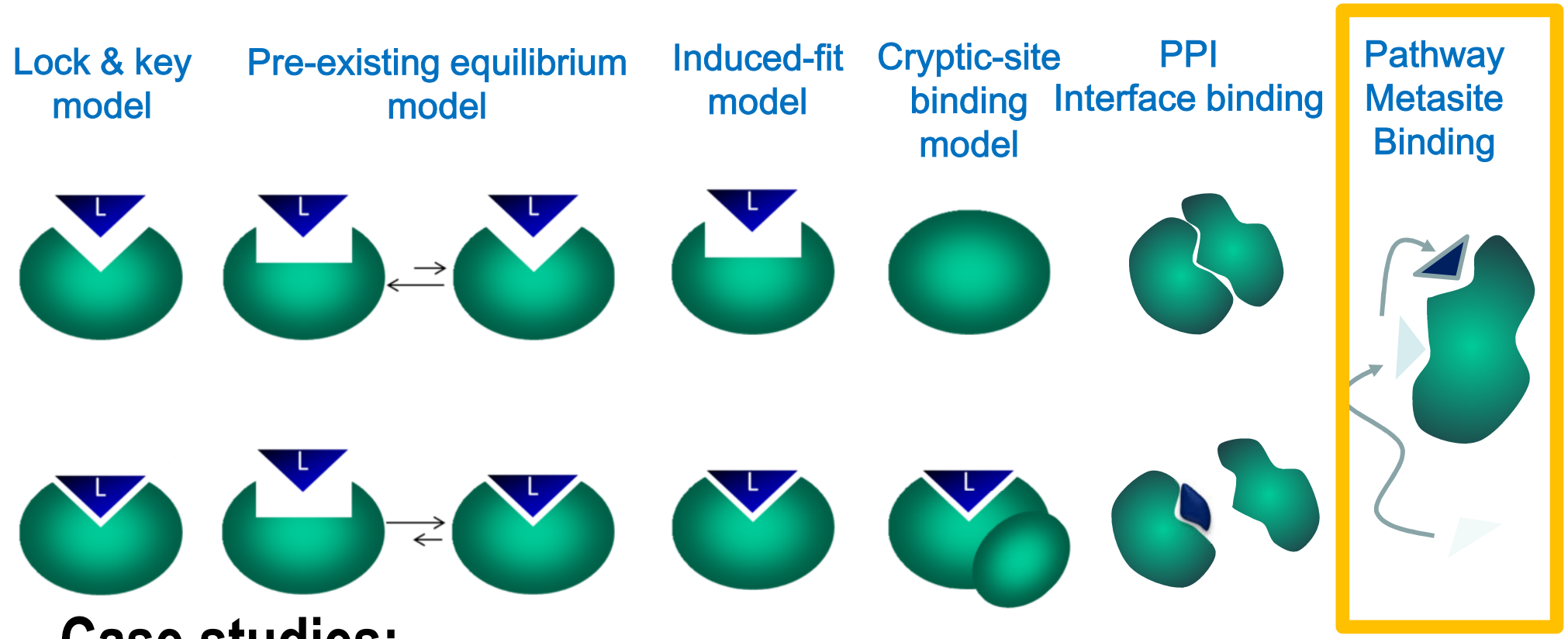
44 Known active ligands of EP4 from ChEMBL



Generation of 2,200 decoys against 44 actives using DUD-E



Outline



Case studies:

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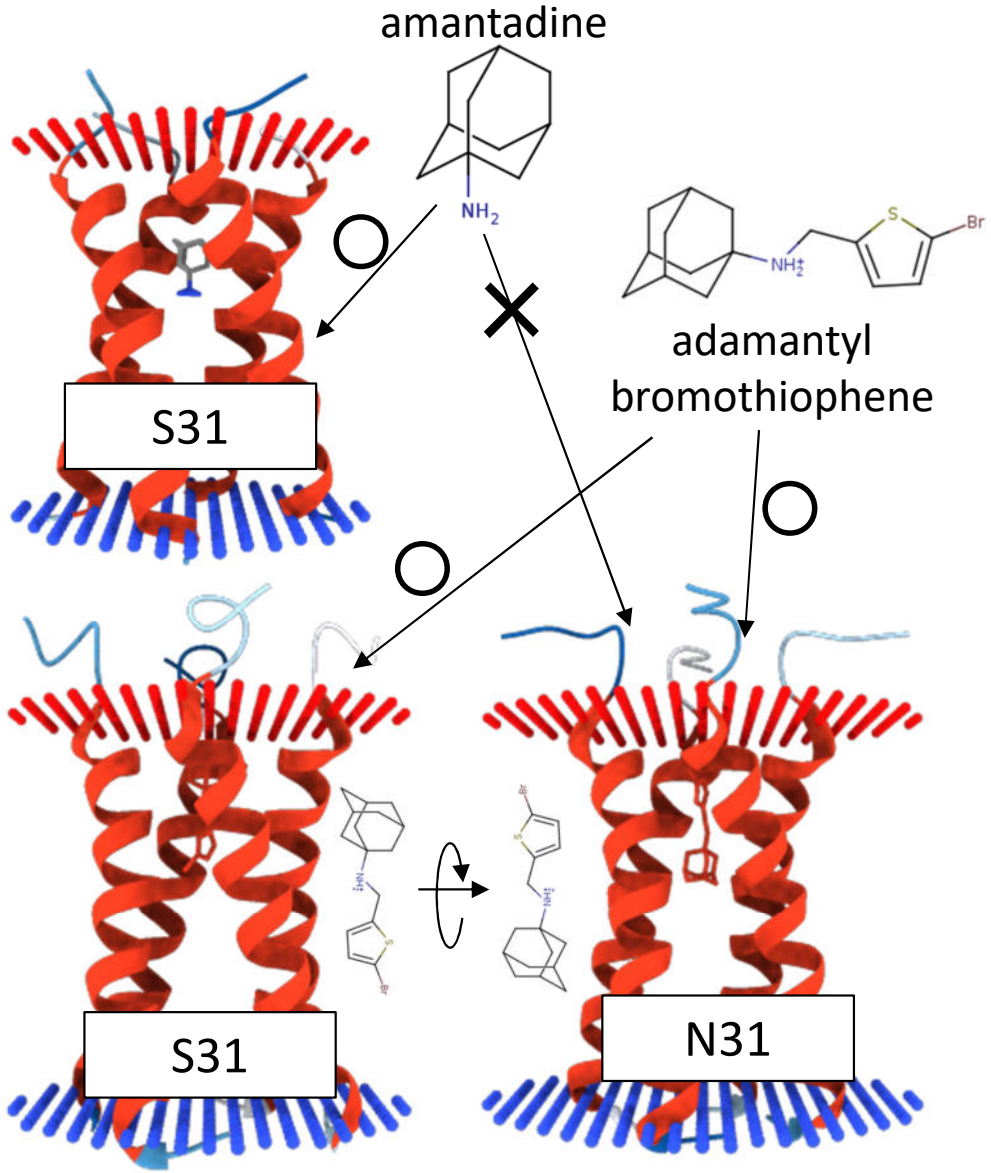
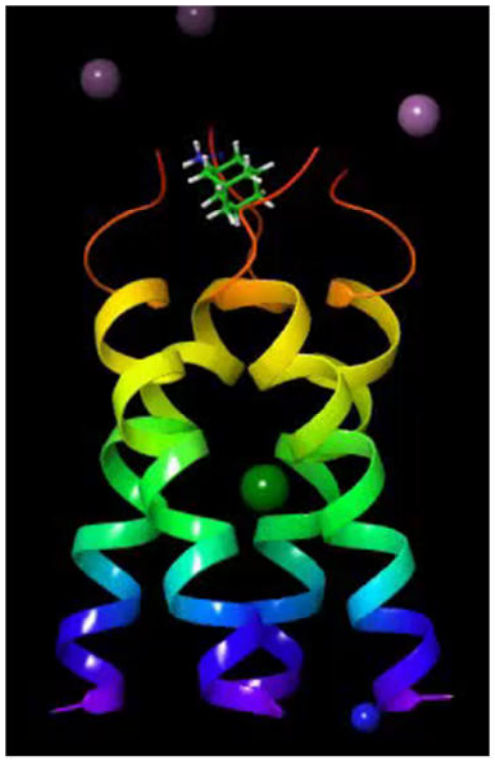
Analysis by metadynamics simulation of binding pathway of influenza virus M2 channel blockers.

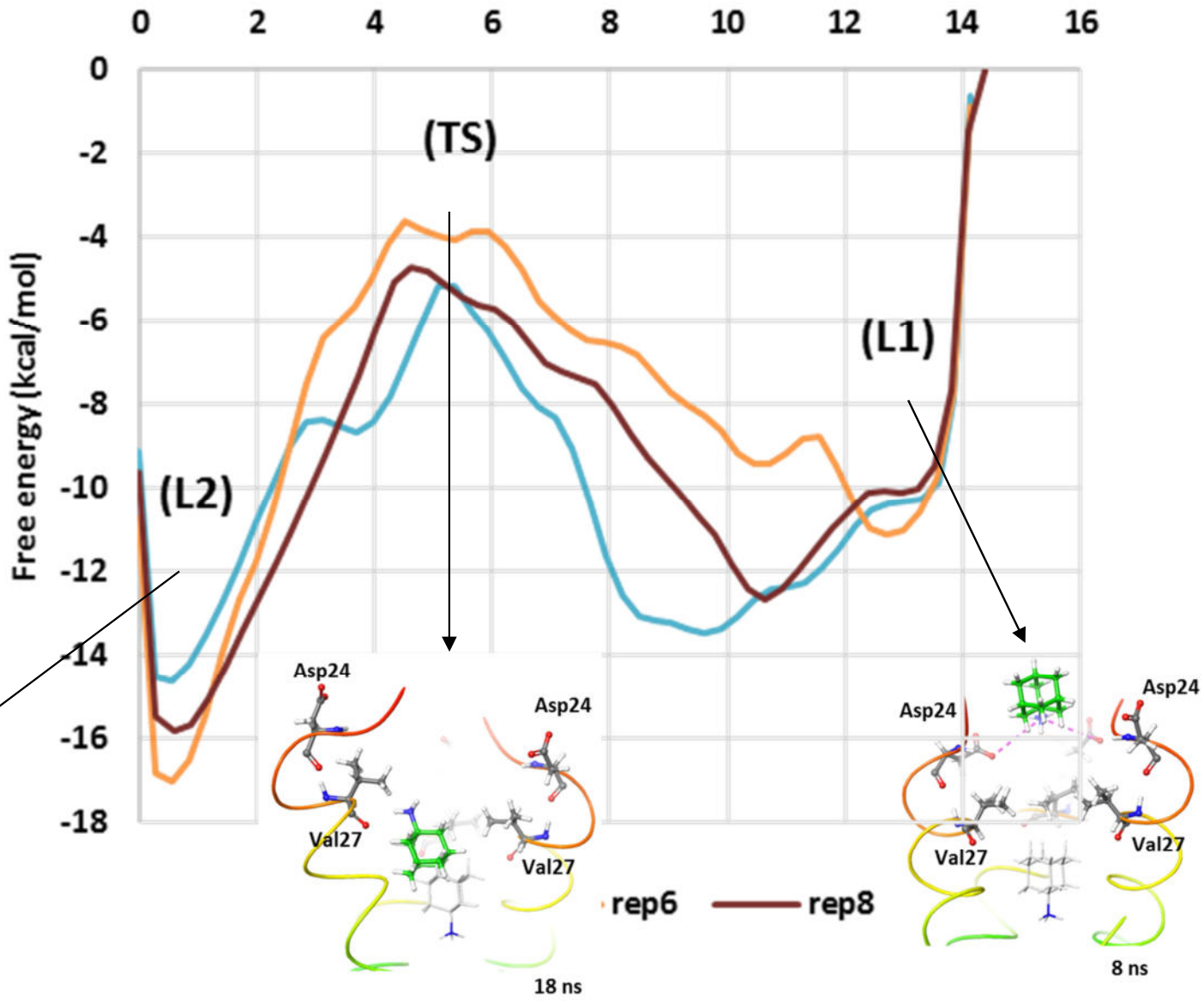
- Proton channel spanning the viral envelope
- Amantadine inhibits M2 proton channel activity by binding to the channel pore
- Most currently circulating influenza A viruses are amantadine-resistant.
- The most prevalent resistant mutation is a substitution from Ser to Asn at position 31 in M2.
- Adamantyl bromothiophene (ABT) was reported to be a dual inhibitor, targeting both S31 M2 and N31 M2.
- Solution NMR structures revealed that the adamantane ring in ABT is oriented up toward the N-terminus of S31 M2, but down toward the C-terminus of N31 M2

Free energy profiles of the binding kinetics of M2 channel blockers by MetaD (100ns x 10 replica for each target)

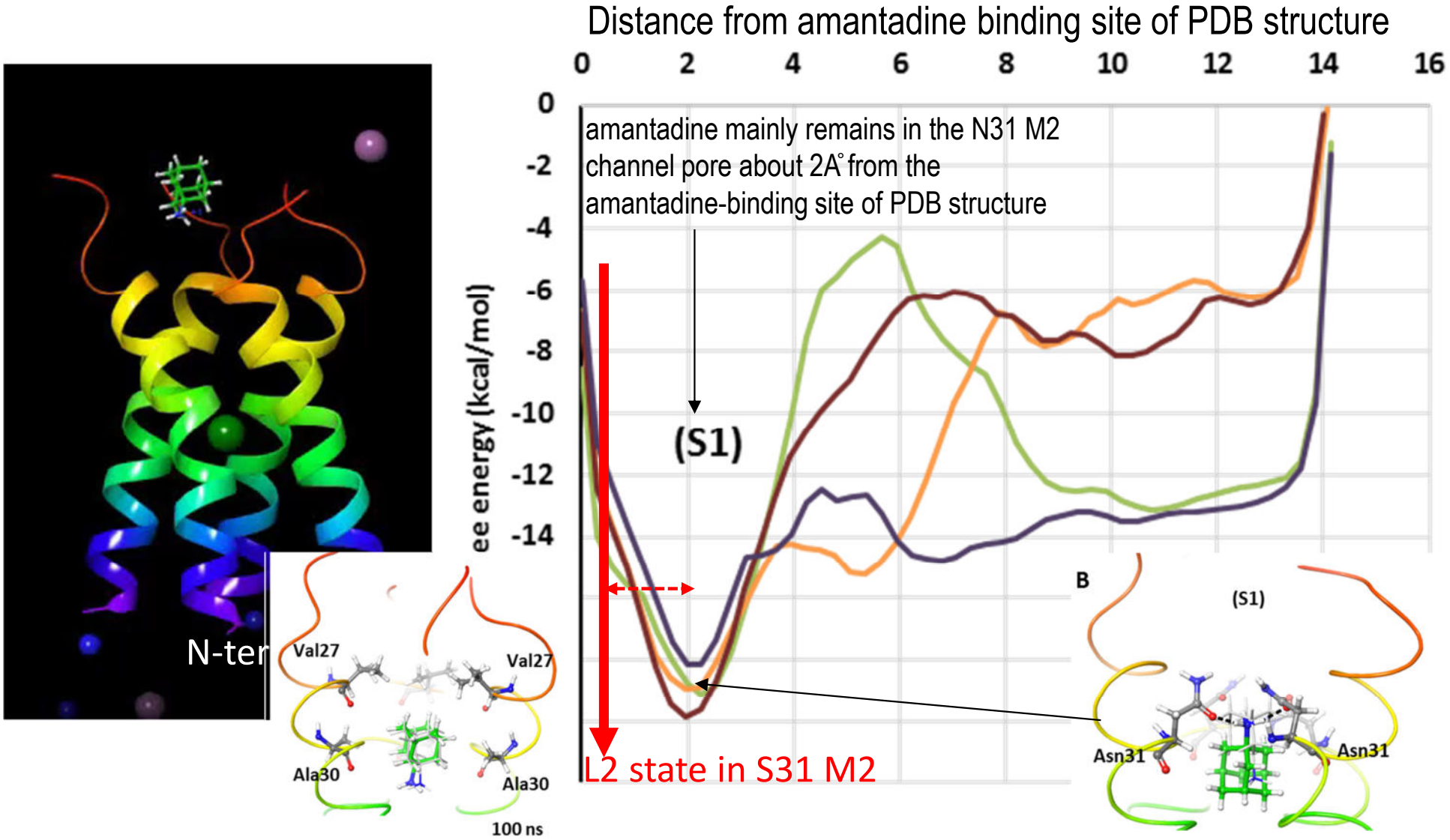


MetaD simulations of amantadine binding in S31 M2.



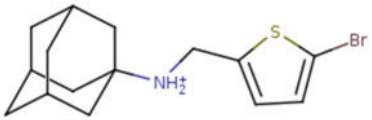


MetaD simulations of amantadine binding in N31 M2.

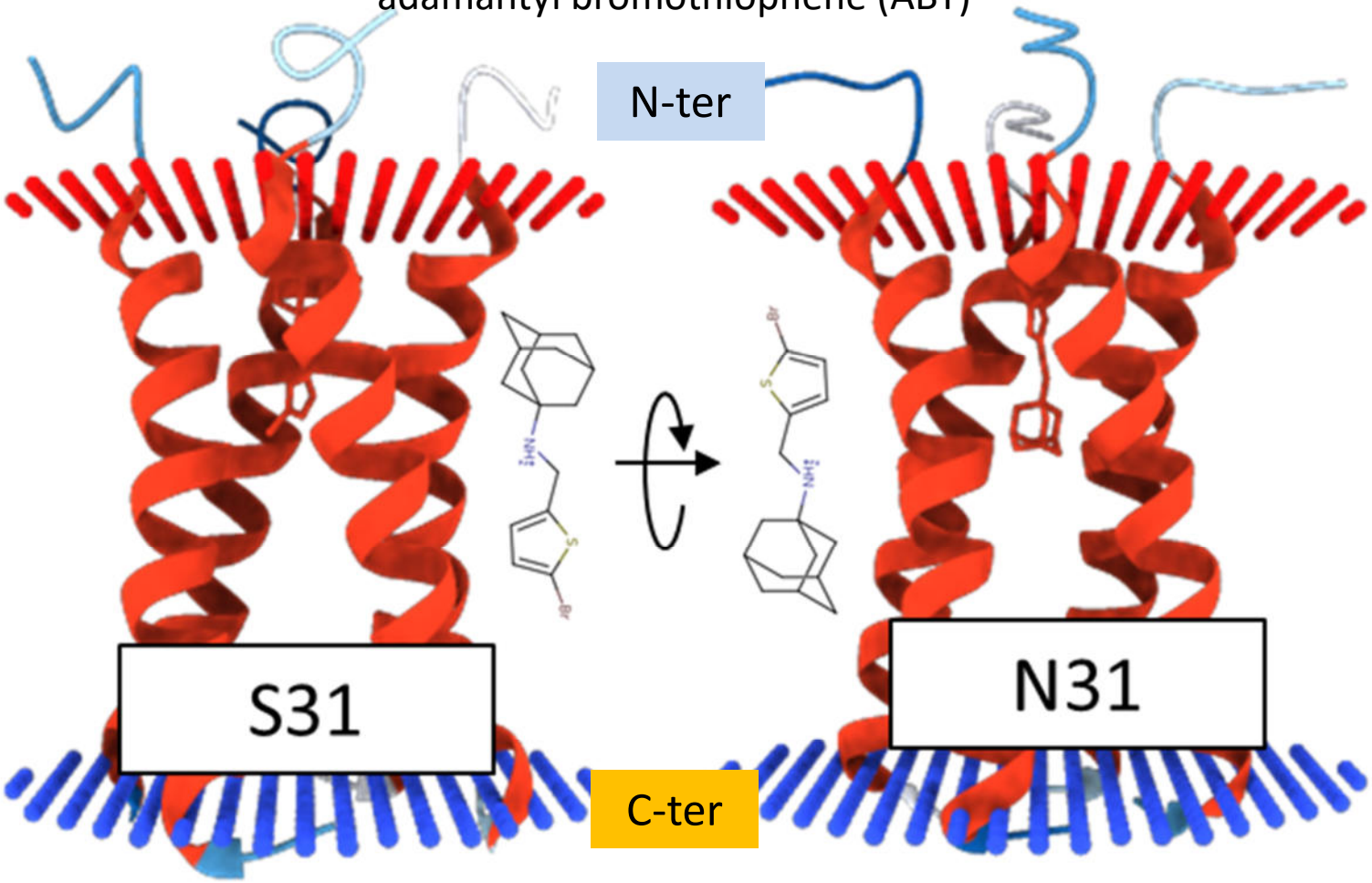


The amino group of amantadine was found to form direct hydrogen bonds with the side chains of Asn31 during the whole simulation time and to be oriented toward the N-terminus of M2

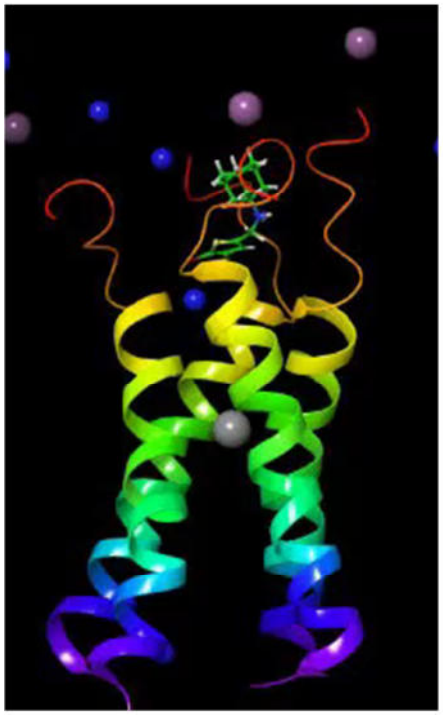
Binding kinetics of ABT, which is an amantadine derivative that inhibits both S31 M2 and N31 M2 proteins.

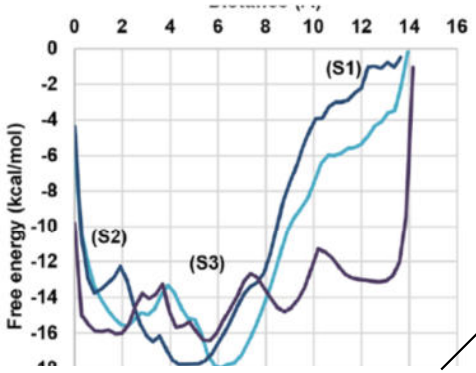


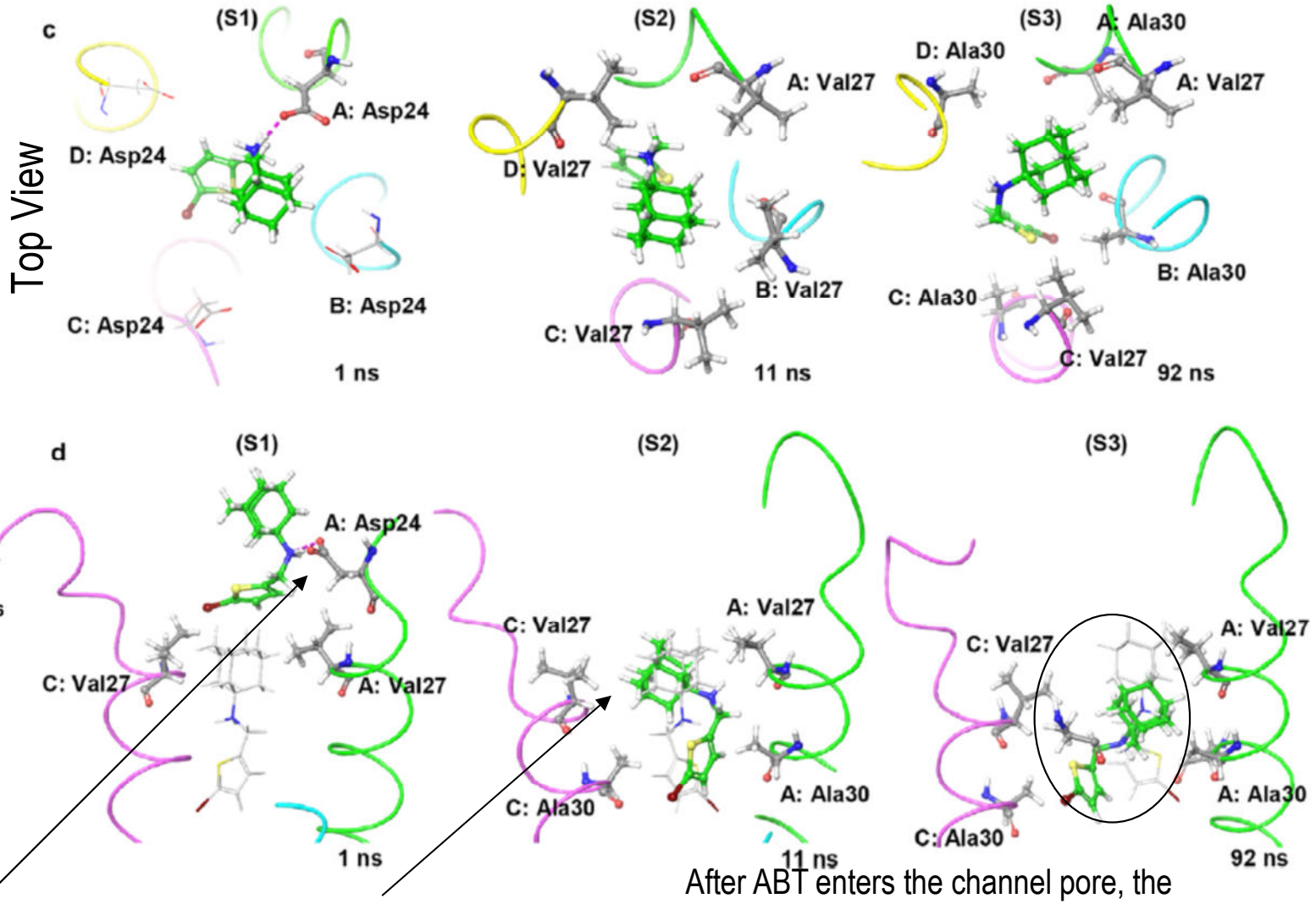
adamantyl bromothiophene (ABT)



MetaD simulations of ABT binding in S31 M2.





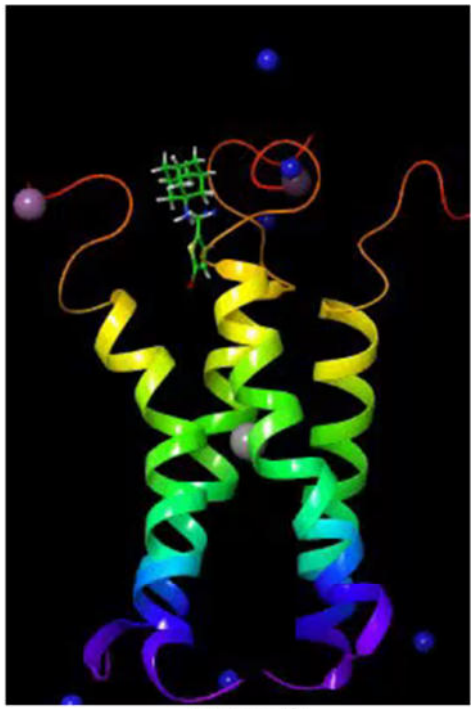


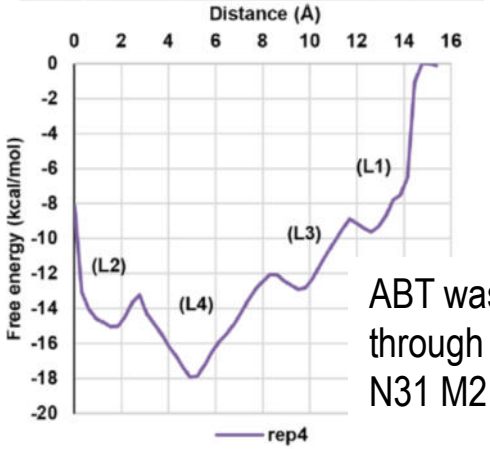
ABT binds to the entrance of the channel pore of S31 M2 through interaction of the amino group with one of the four Asp24 residues by a salt bridge.

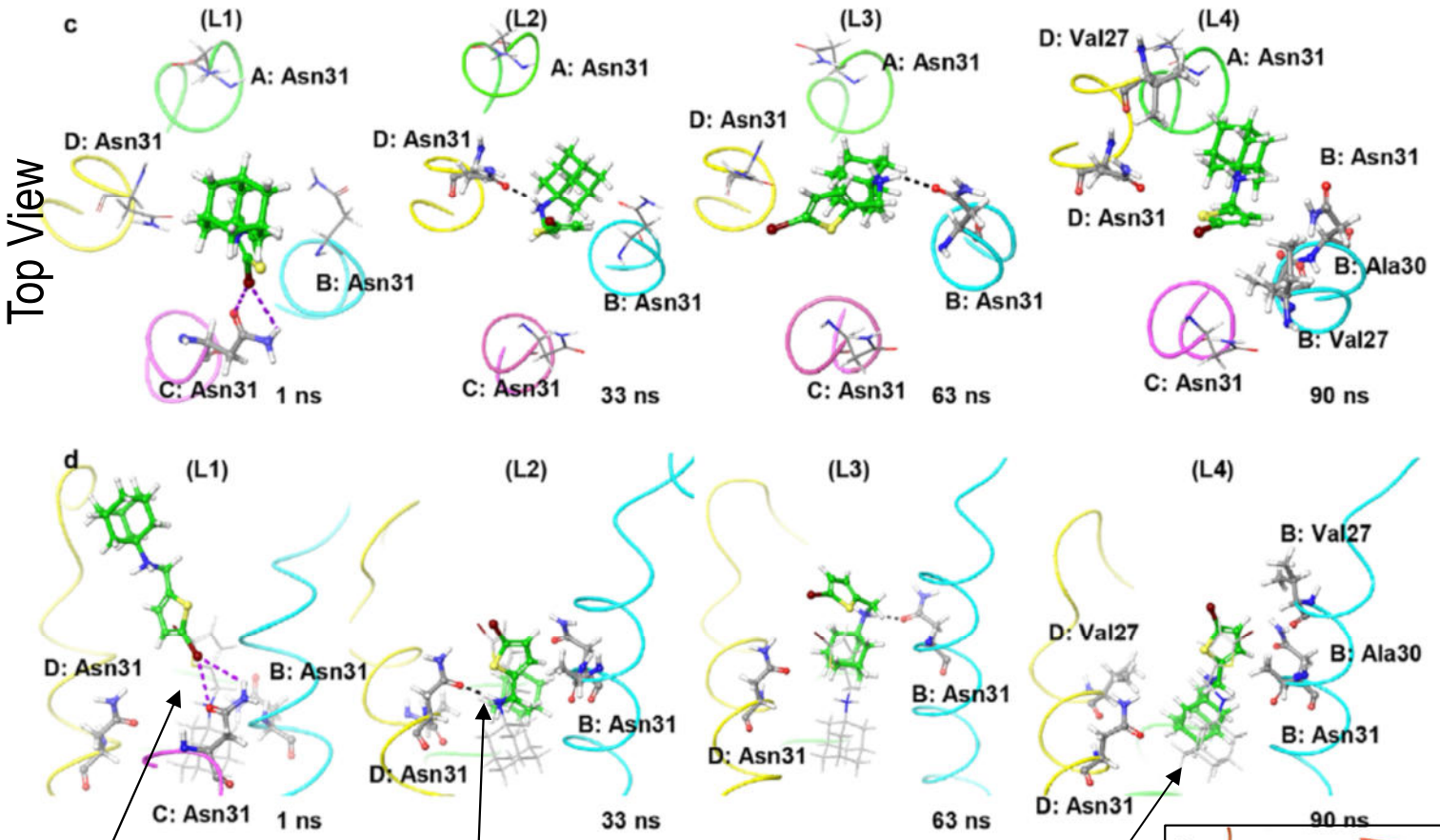
Enters the channel pore through hydrophobic interactions between the adamantane ring and Val27.

After ABT enters the channel pore, the bromothiophene group oriented toward the N-terminus of S31 M2 and ABT remains stable in the channel pore by means of water-bridged hydrogen bonds with the carbonyl oxygen atoms of Ala30.

MetaD simulations of ABT binding in N31 M2.

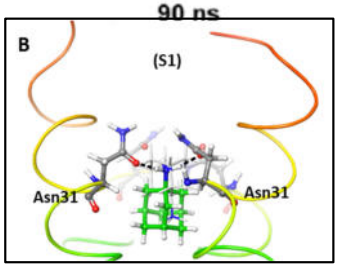






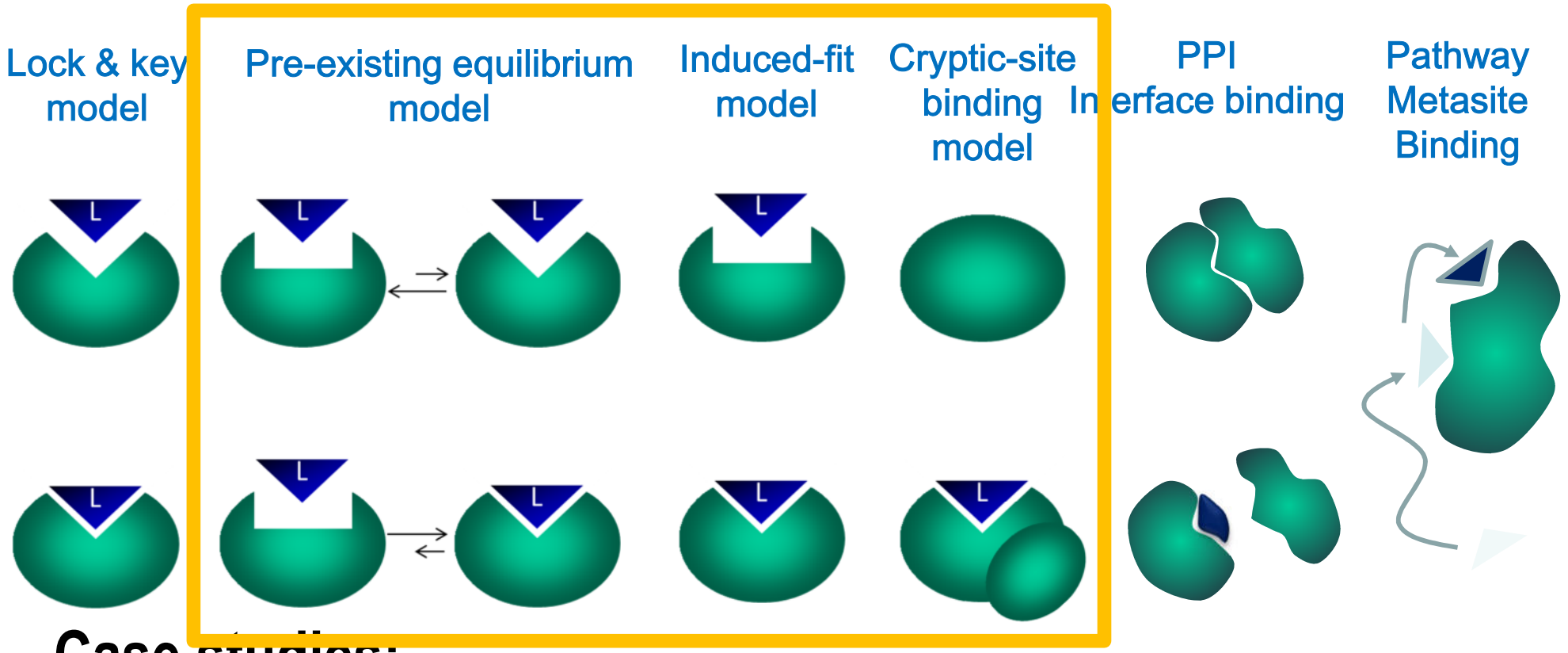
ABT was found to enter the channel pore through a halogen bond with Asn31 of N31 M2

ABT forms stable bonds in the channel pore through hydrophobic interactions between the bromothiophene group and Ala30 and the hydrogen bond between the amino group and Asn31



The amino group of ABT then interacts with the side chain of Asn31 through a transient hydrogen bond for 30–40 ns The hydrogen bond between the amino group and Asn31 is relatively unstable because of the lack of a high free energy barrier, there is repeated binding and unbinding of ABT with Asn31. In addition, the bromothiophene group is oriented toward the C-terminus of N31 M2

Outline



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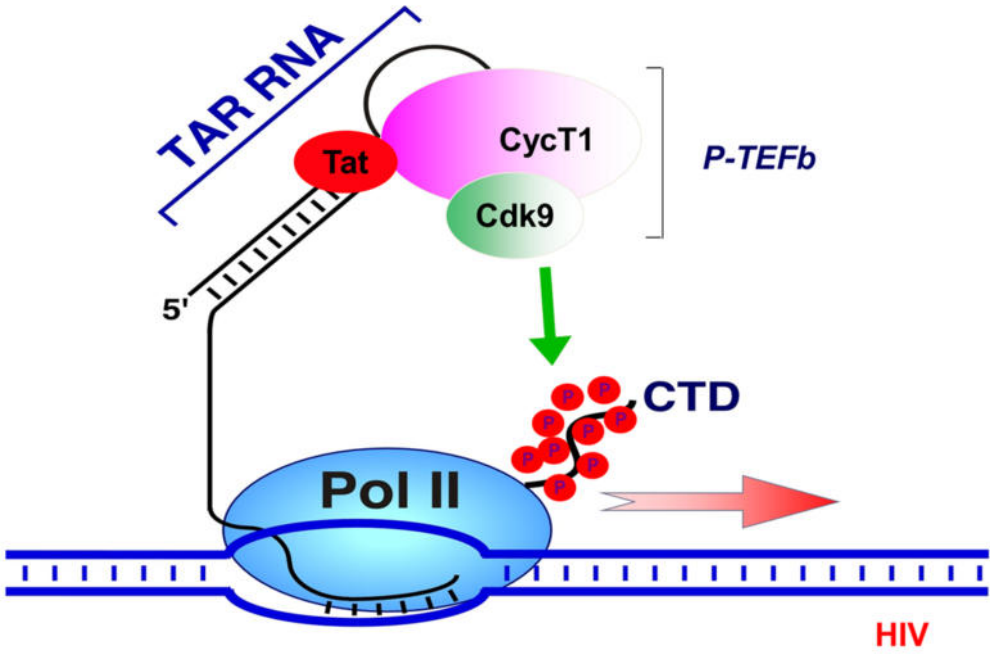
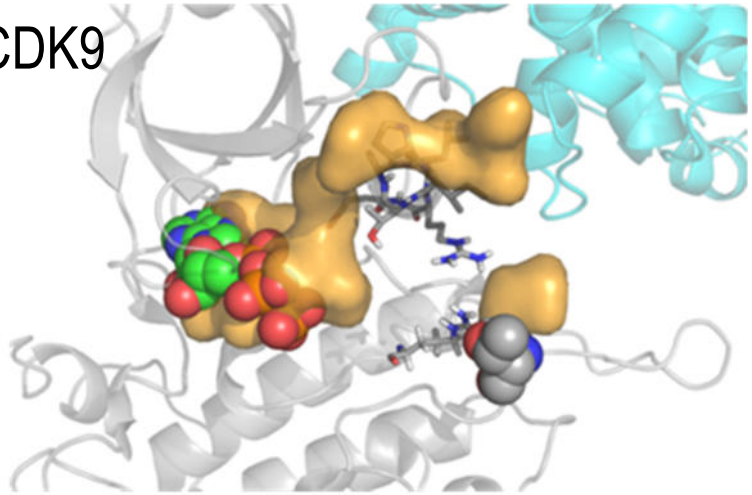
Identification of the druggable Hidden Catalytic Cavity within the CDK9 Molecule Upon Tat Binding

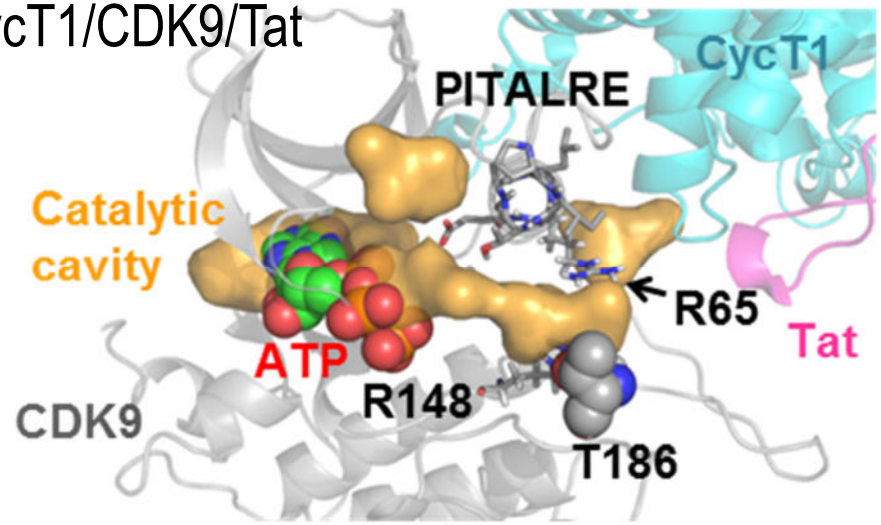


Transcription from the integrated proviral DNA of human immune-deficiency virus type 1 (HIV-1) is tightly regulated by a virus-encoded transcription factor Tat.

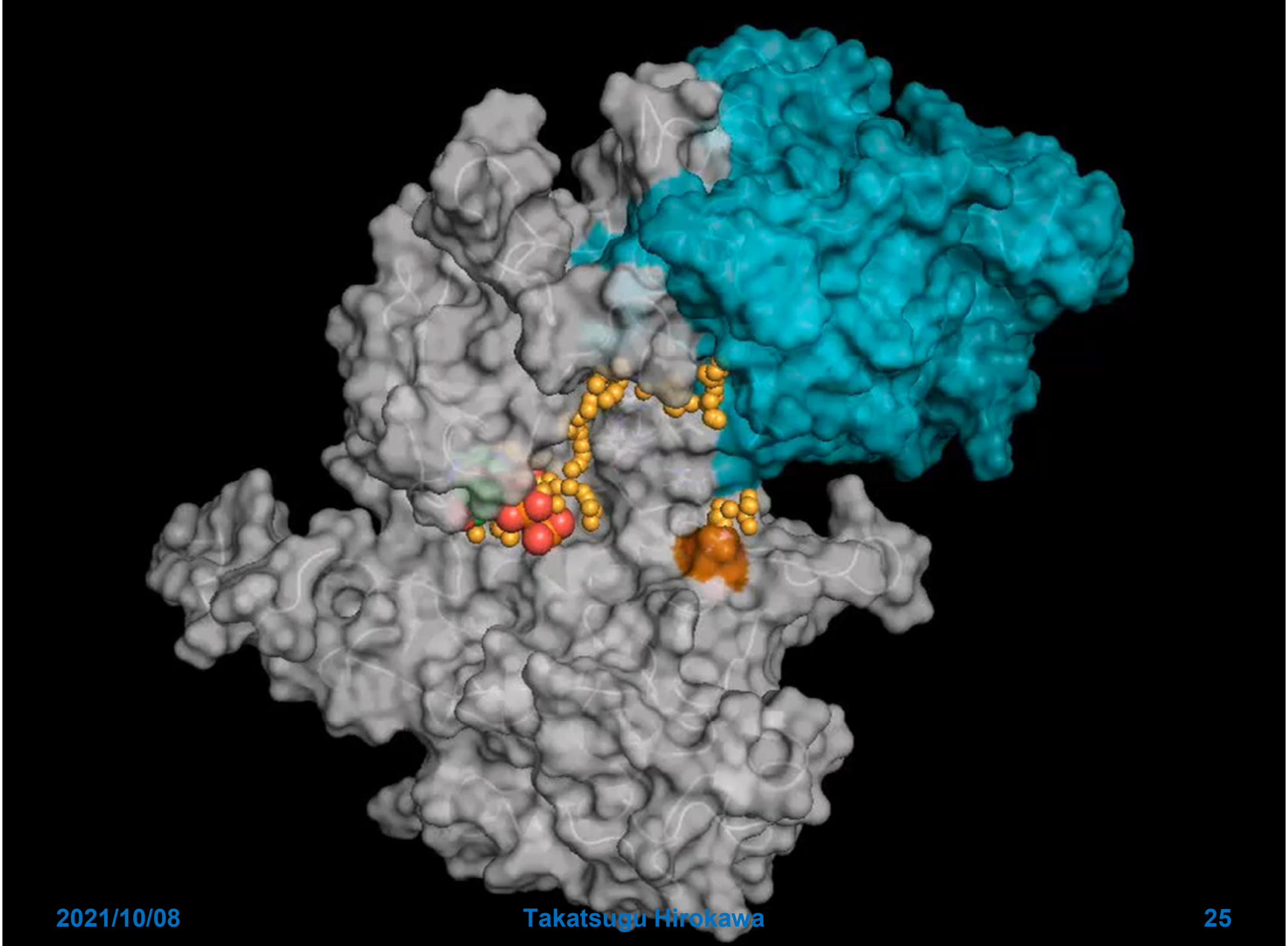
CycT1/CDK9

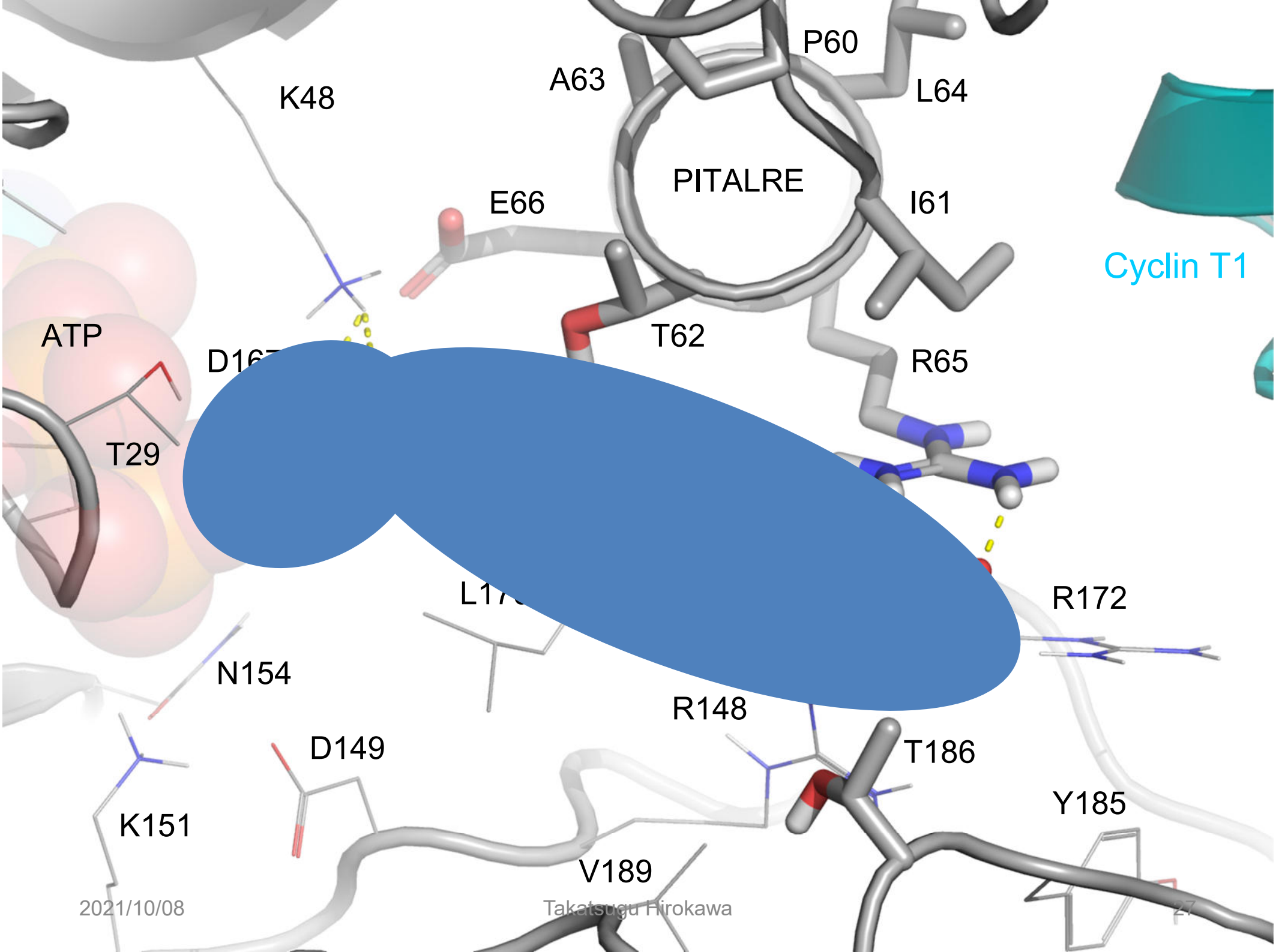


CycT1/CDK9/Tat



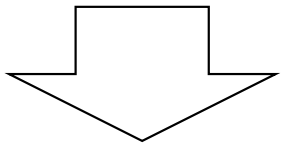
Asamitsu K, Hirokawa T, Okamoto T. PLoS One. 2017 Feb 8;12(2):e0171727.




P60
A63
L64
K48
PITALRE
E66
I61
Cyclin T1
ATP
T62
R65
T29
R172
N154
R148
D149
T186
K151
Y185
V189

Summary

- **Molecular simulation tell us**
  - Conformational payment or behavior to the environment in ligand pathway
  - Conformational changes of ligand in binding pathway simulation
  - Providing new approaches to designing further ligands for resistant mutations
  - Finding the novel hot spot for ligand binding
  - and more ..



high accurate ligand design and virtual screening

Collaborators and Publications

- Toyoda Y, Morimoto K, Suno R, Horita S, Yamashita K, Hirata K, Sekiguchi Y, Yasuda S, Shiroishi M, Shimizu T, Urushibata Y, Kajiwara Y, Inazumi T, Hotta Y, Asada H, Nakane T, Shiimura Y, Nakagita T, Tsuge K, Yoshida S, Kuribara T, Hosoya T, Sugimoto Y, Nomura N, Sato M, Hirokawa T, Kinoshita M, Murata T, Takayama K, Yamamoto M, Narumiya S, Iwata S, Kobayashi T. **"Ligand binding to human prostaglandin E receptor EP4 at the lipid-bilayer interface"** *Nat Chem Biol*. 2019 Jan;15(1):18-26.
- Sakai Y, Kawaguchi A, Nagata K, Hirokawa T. **"Analysis by metadynamics simulation of binding pathway of influenza virus M2 channel blockers"** *Microbiol Immunol*. 2018 Jan;62(1):34-43.
- Asamitsu K, Hirokawa T, Okamoto T. **"MD simulation of the Tat/Cyclin T1/CDK9 complex revealing the hidden catalytic cavity within the CDK9 molecule upon Tat binding"** *PLoS One*. 2017 Feb 8;12(2):e0171727.

Acknowledgment

- Platform for Drug Discovery, Informatics, and Structural Life Science from the Ministry of Education, Culture, Sports, Science and Technology, Japan.
- This research was supported by MEXT as "Priority Issue on Post-K computer" (Building Innovative Drug Discovery Infrastructure Through Functional Control of Biomolecular Systems).(HP160213)