香港中文大學（深圳）
The Chinese University of Hong Kong, Shenzhen

Molecular Dynamics Simulations of Penicillin-Binding Protein 2a

Ying-Chih Chiang, Mabel Y T Wong , and Jonathan W Essex

QSAR Fall 2021

UNIVERSITY OF Southampton

Antibiotics

Antibiotics are drugs active against bacteria. They target relevant biological processes inside bacteria, such as

- **Cell-wall synthesis.**
  β-lactams, Vancomycin, etc.

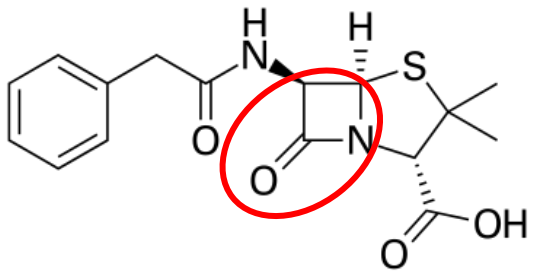
- **Cell-membrane.**
  Polymixins.

- **Protein synthesis.**
  Aminoglycosides, lincosamide, etc.

- **DNA duplication**.
  Quinolones.

- **Folic acid synthesis or other processes.**

β-lactam



Penicillin G

Mechanism of Antibiotic Resistance

- **Upregulate the efflux pumps**
- **Metabolize the antibiotics**
- **Protect target receptor**

J. M. A. Blair, M. A. Webber, A. J. Baylay, D. O. Ogbolu and L. J. V. Piddock, *Nat. Rev. Microbiol.*, **13**, 42-51 (2015).

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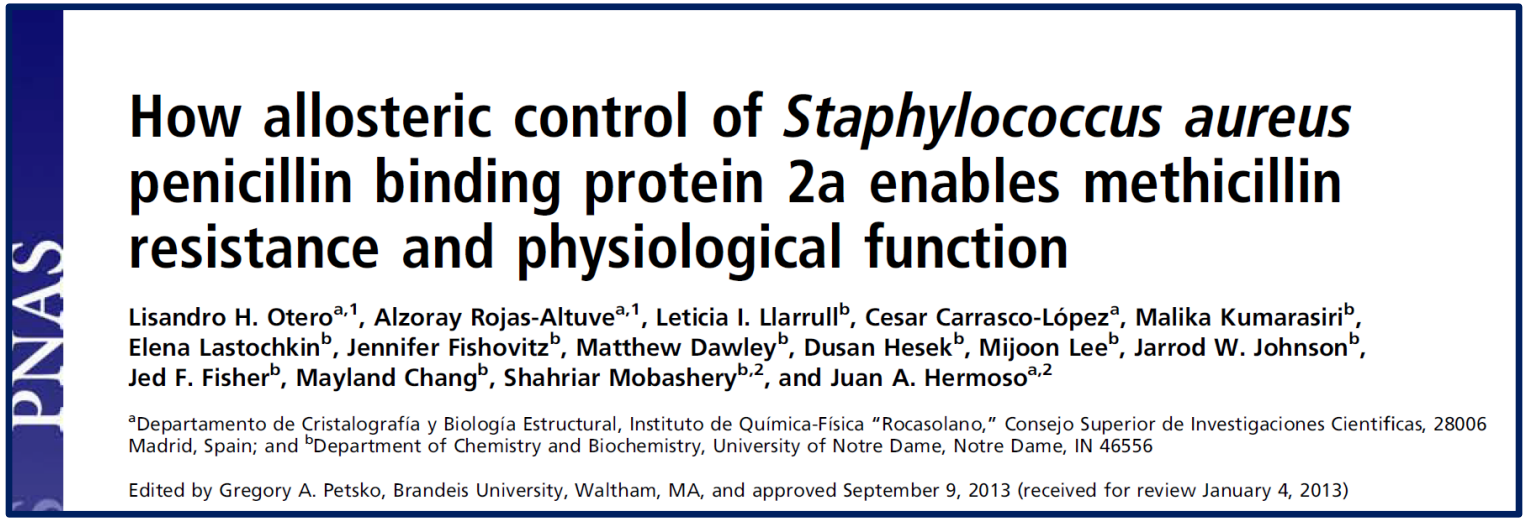
β-lactam Resistance Mechanism in MRSA

FIGURE REMOVED

The Methicillin resistant *Staphylococcus aureus* (MRSA) achieves its resistance against β-lactams in two ways:

- Producing **β-lactamases** to decompose β-lactams.
- Producing a Penicillin-binding protein **PBP2a** that does not bind with most of the β-lactams, to carry out cell wall synthesis under the presence of β-lactams.



PNAS

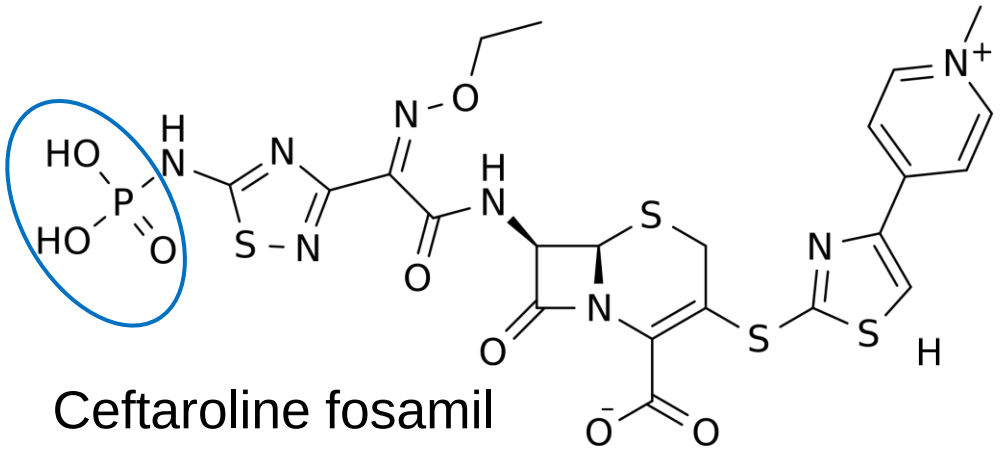
How allosteric control of *Staphylococcus aureus* penicillin binding protein 2a enables methicillin resistance and physiological function

Lisandro H. Otero[a,1], Alzoray Rojas-Altuve[a,1], Leticia I. Llarrull[b], Cesar Carrasco-López[a], Malika Kumarasiri[b], Elena Lastochkin[b], Jennifer Fishovitz[b], Matthew Dawley[b], Dusan Hesek[b], Mijoon Lee[b], Jarrod W. Johnson[b], Jed F. Fisher[b], Mayland Chang[b], Shahriar Mobashery[b,2], and Juan A. Hermoso[a,2]

[a]Departamento de Cristalografía y Biología Estructural, Instituto de Química-Física "Rocasolano," Consejo Superior de Investigaciones Cientificas, 28006 Madrid, Spain; and [b]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556

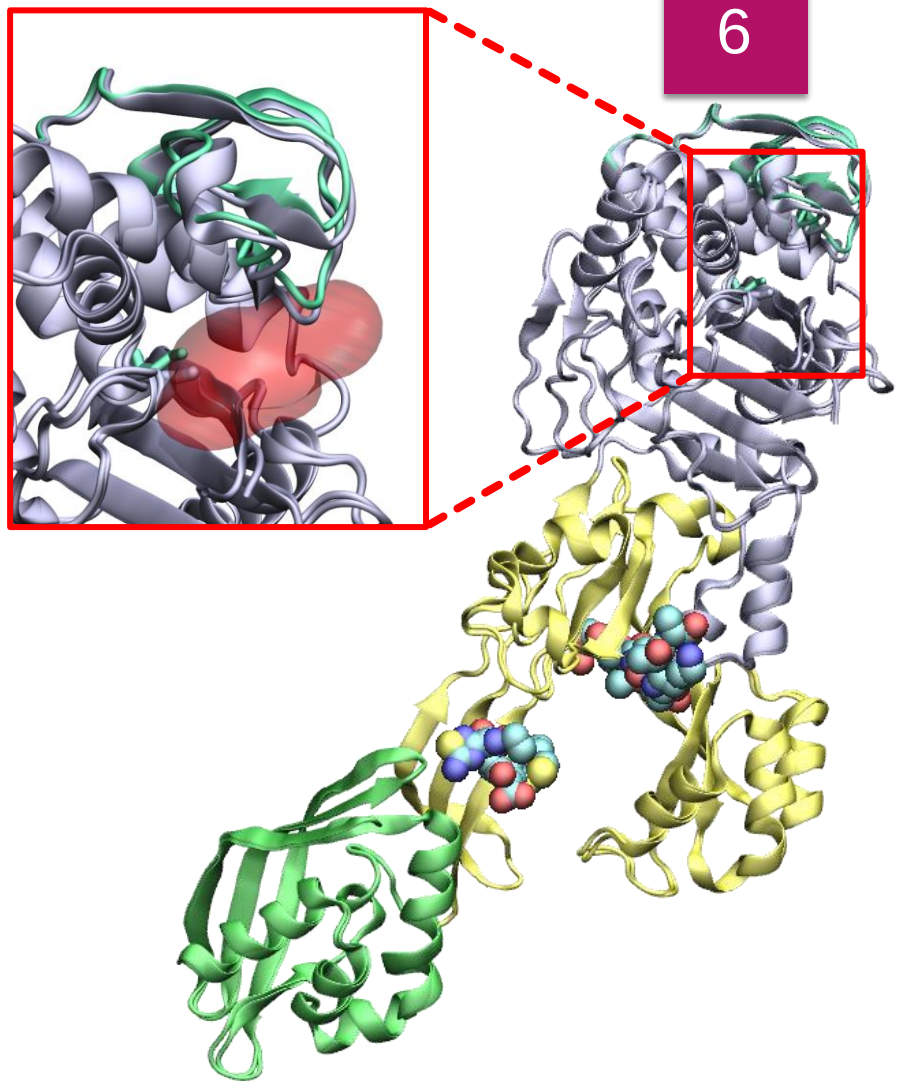
Edited by Gregory A. Petsko, Brandeis University, Waltham, MA, and approved September 9, 2013 (received for review January 4, 2013)

Ceftaroline & Allosteric Mechanism



Ceftaroline fosamil

Ceftaroline fosamil (brand name Teflaro or Zinforo) is a cephalosporin antibiotic **with anti-MRSA activity**.



https://en.wikipedia.org/wiki/Ceftaroline_fosamil

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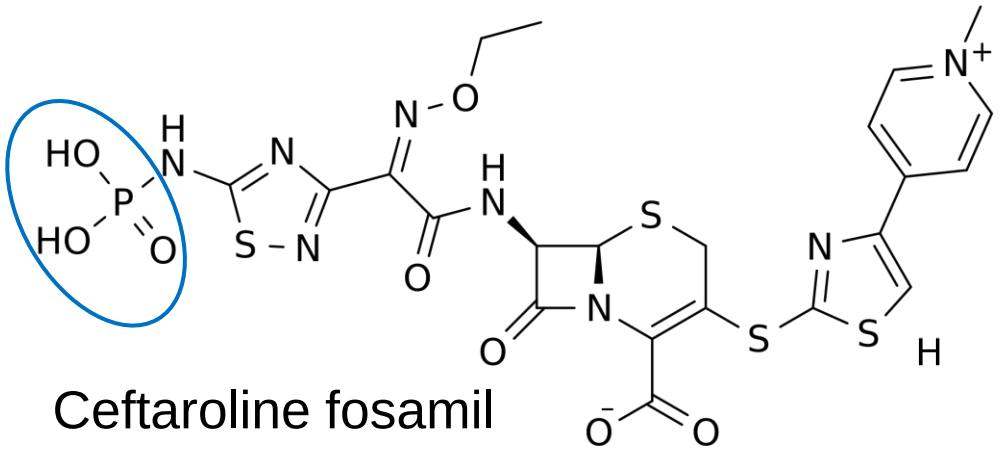

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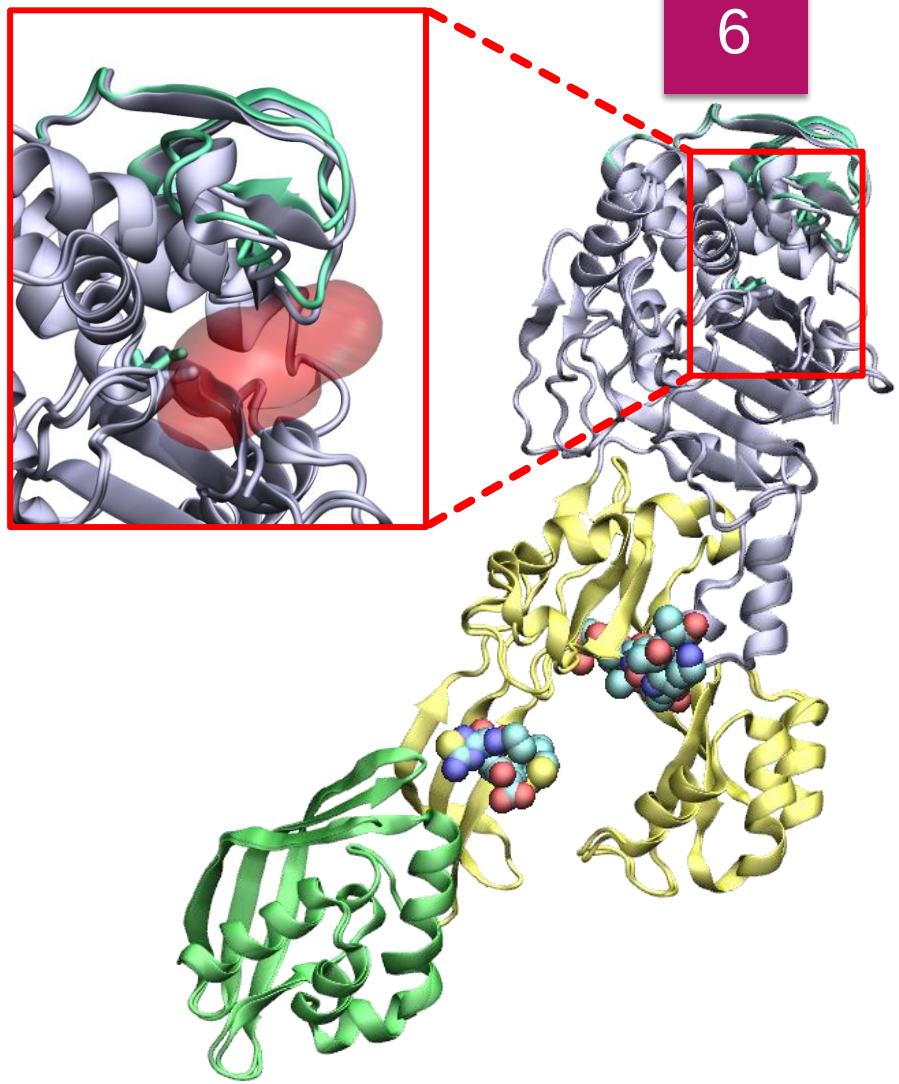

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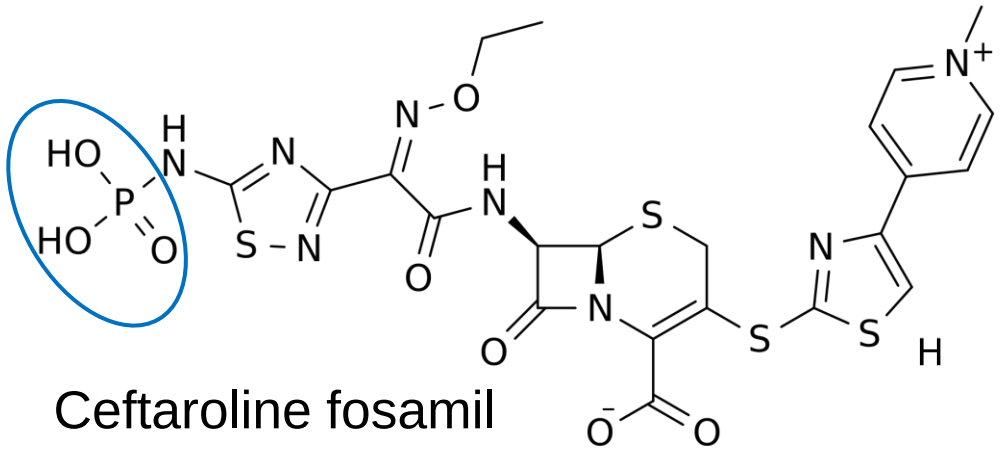

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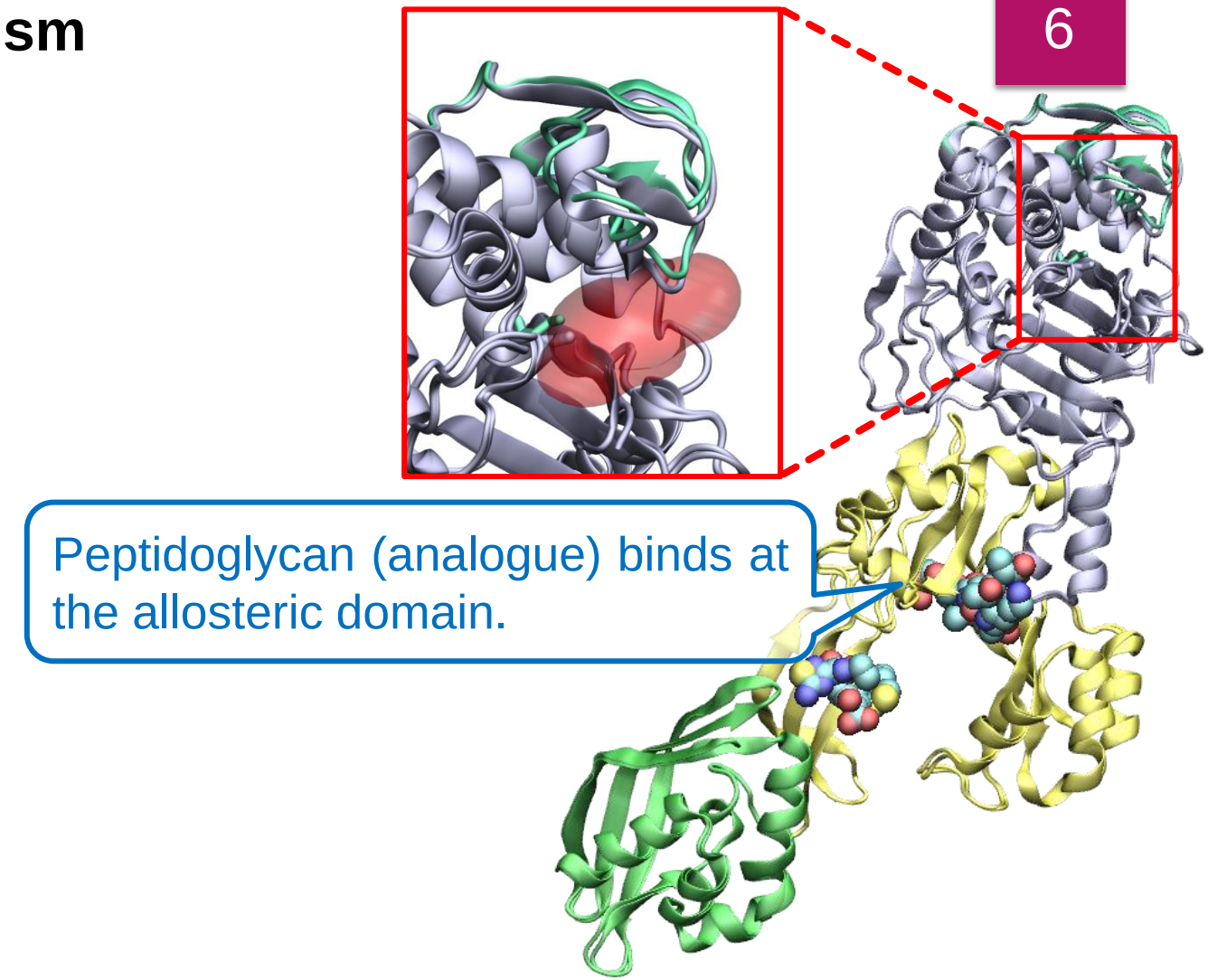

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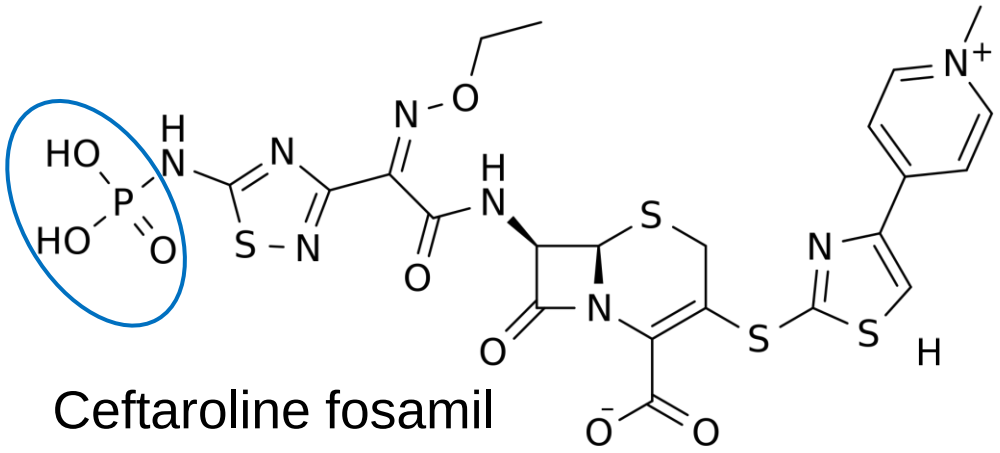

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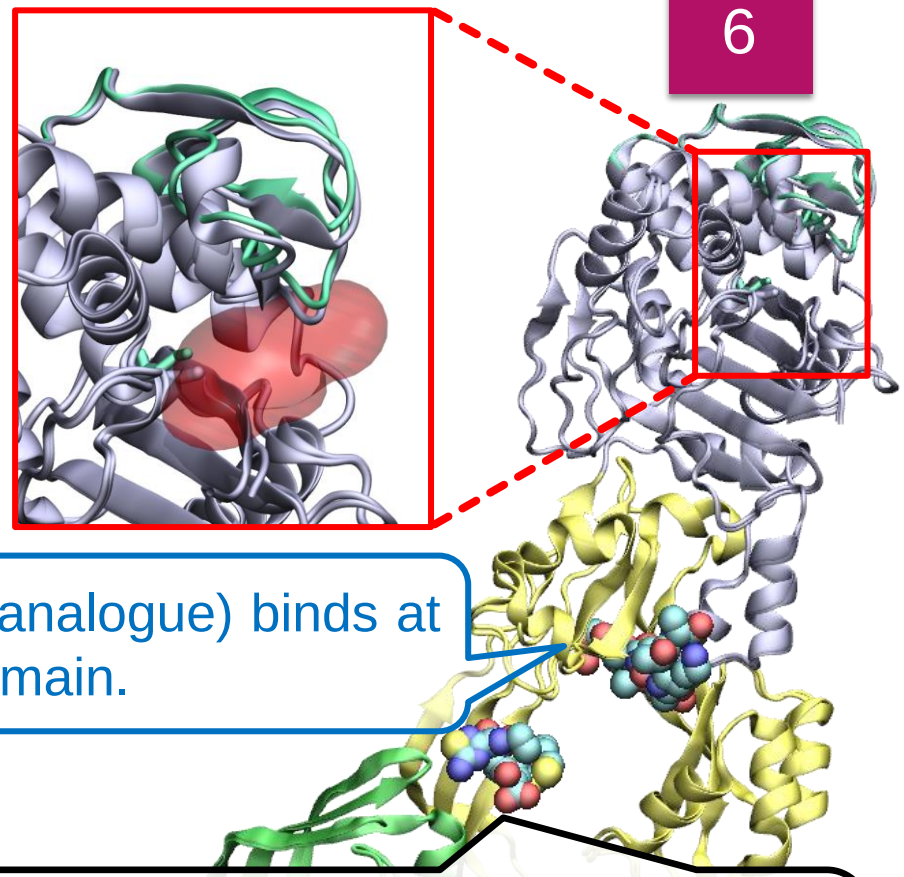
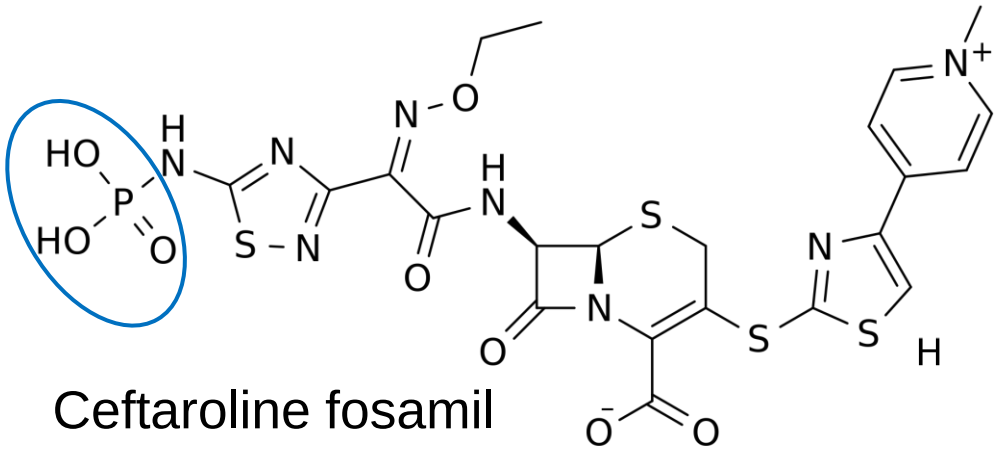


Peptidoglycan (analogue) binds at the allosteric domain.

Ceftaroline binds at the allosteric domain. The orthosteric site then opens up for ligand binding.

Ceftaroline & Allosteric Mechanism



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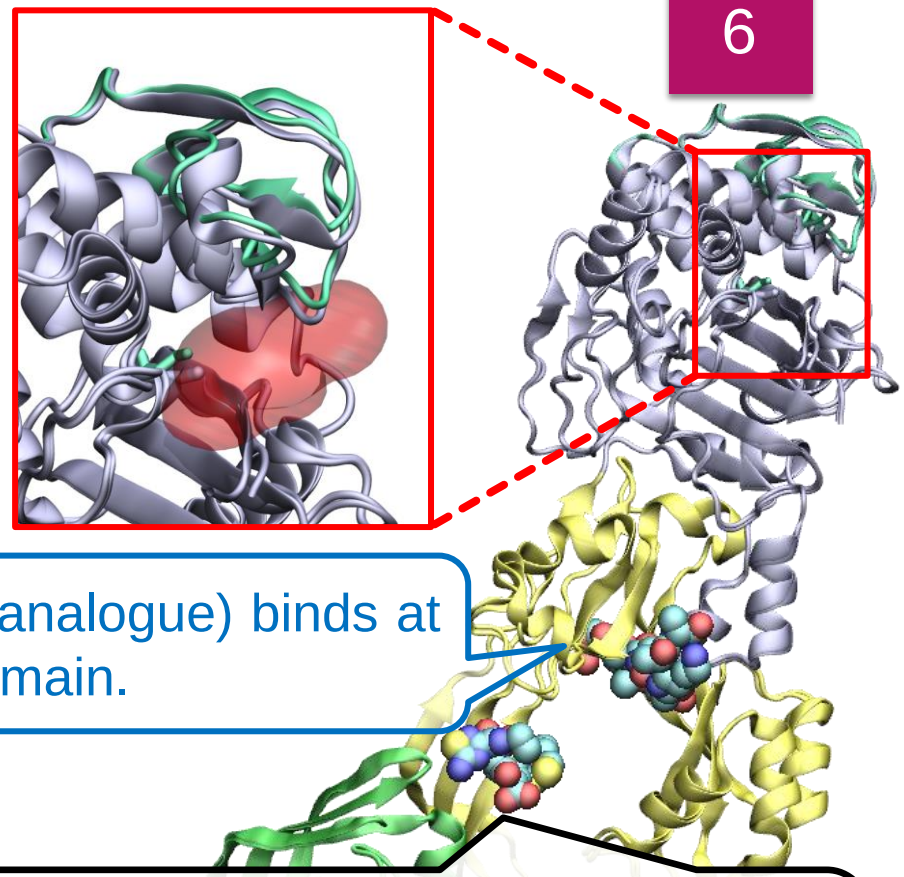

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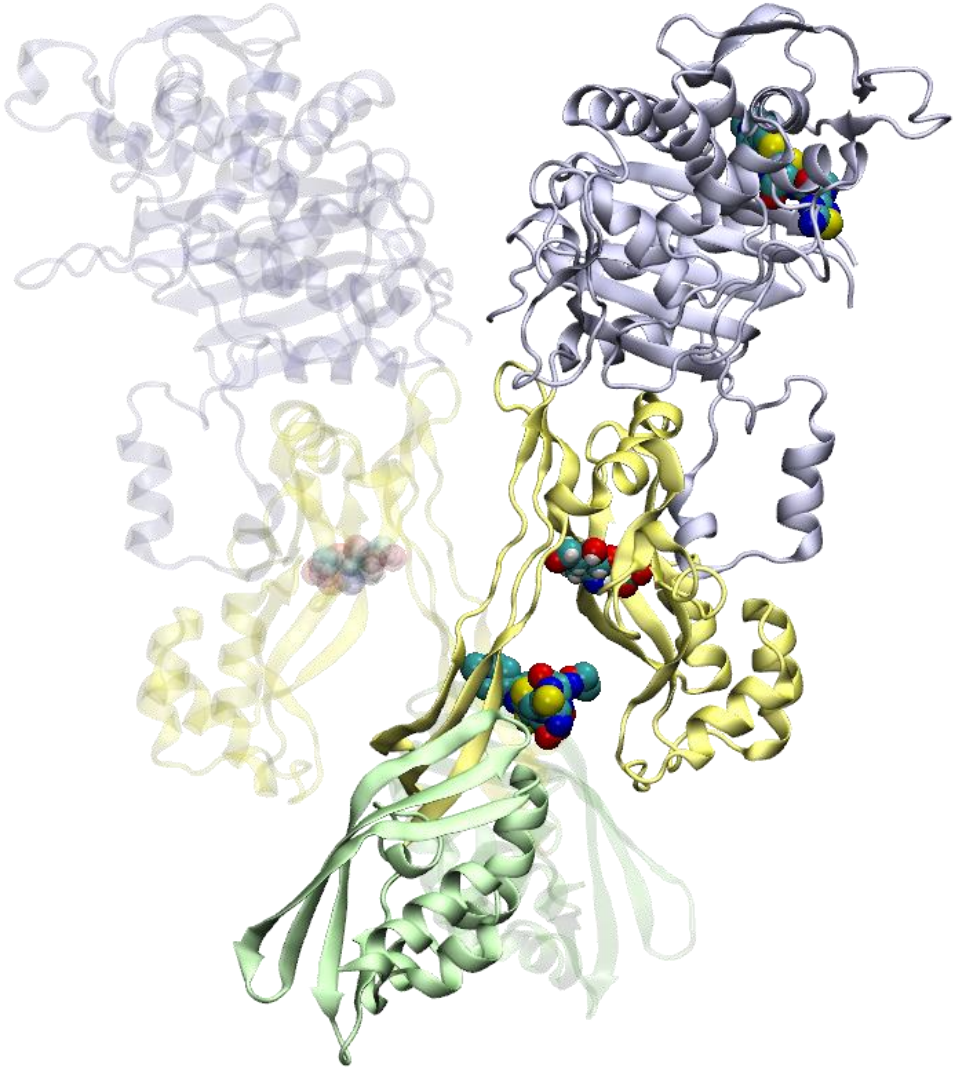
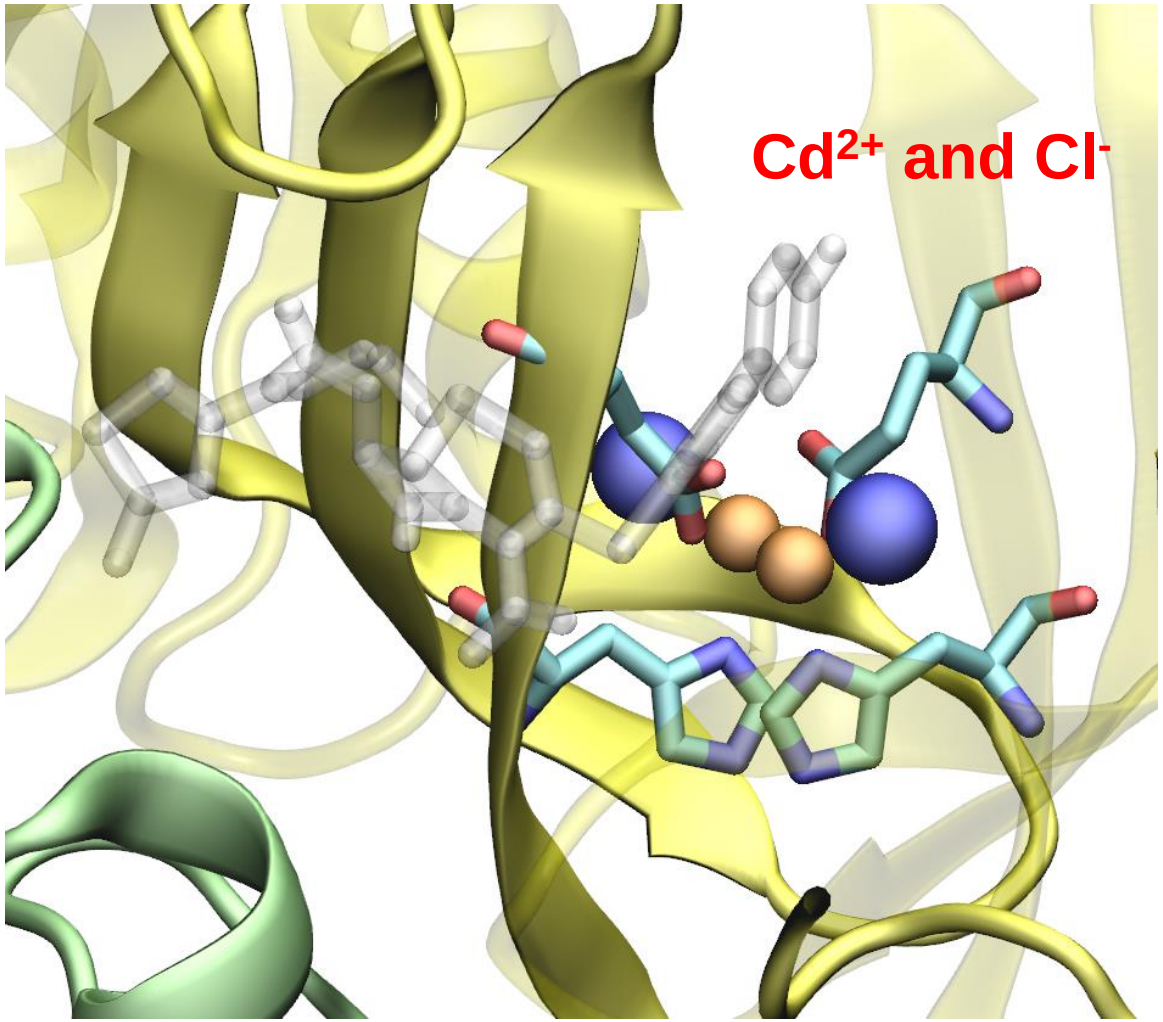
Q: Which residues are responsible for anchoring the ligand at the allosteric site?

Crystal Structure (3zfz)





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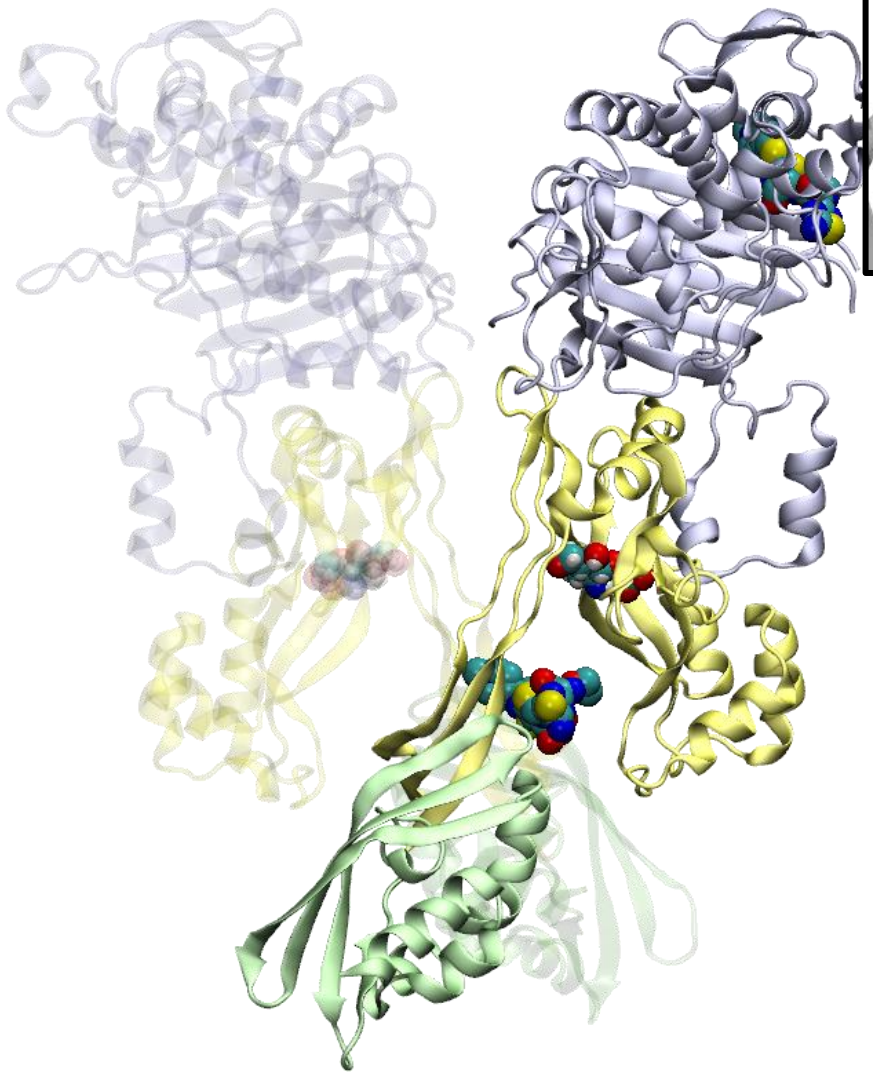
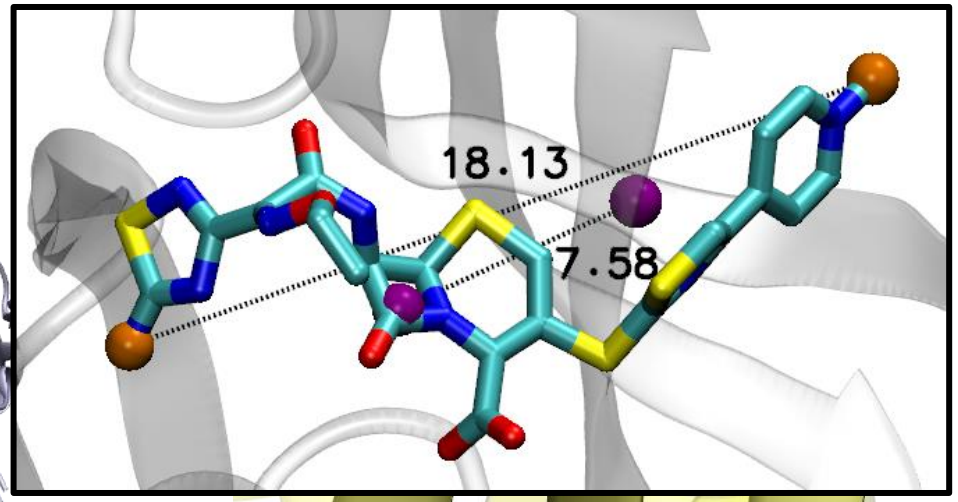
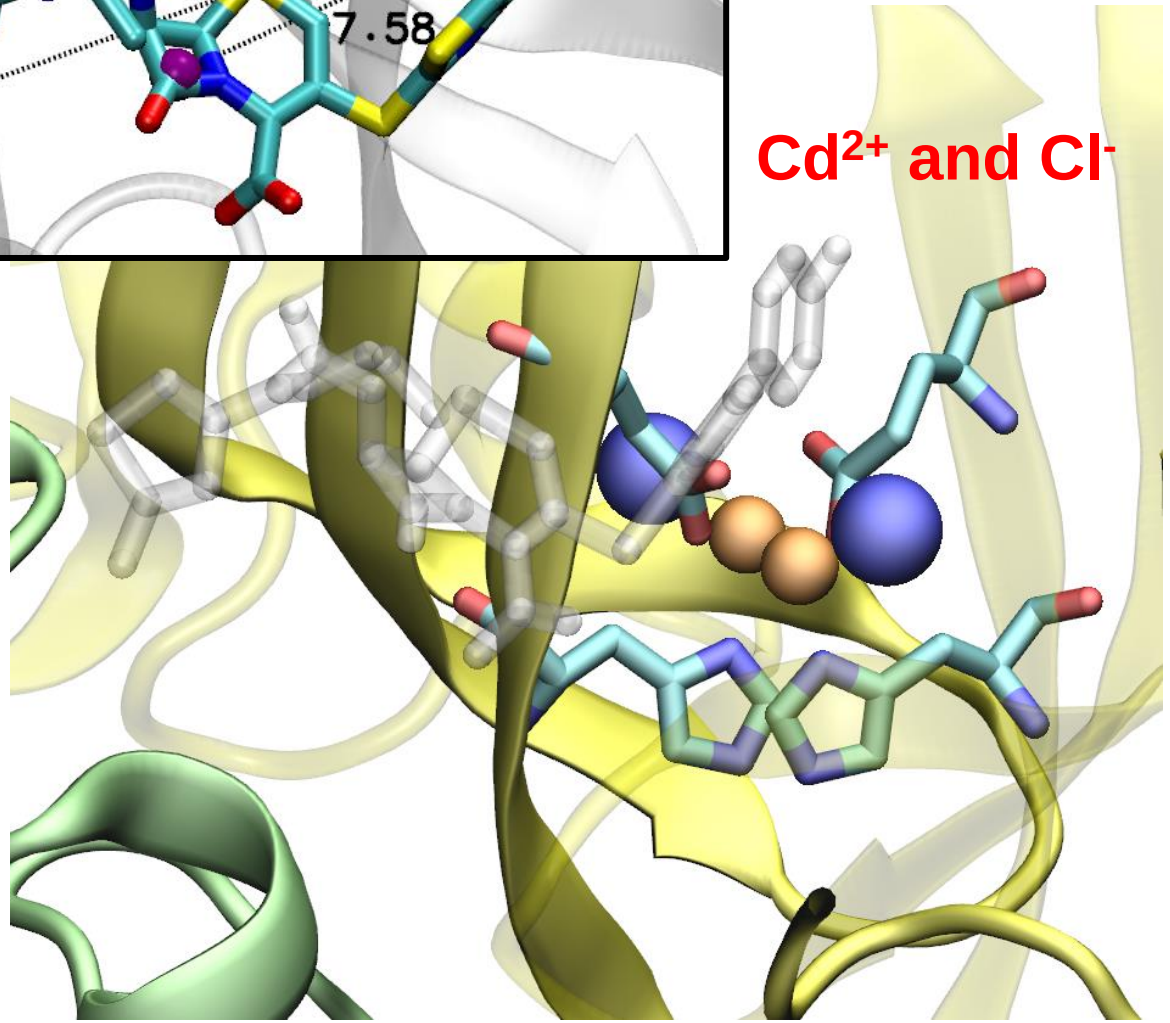
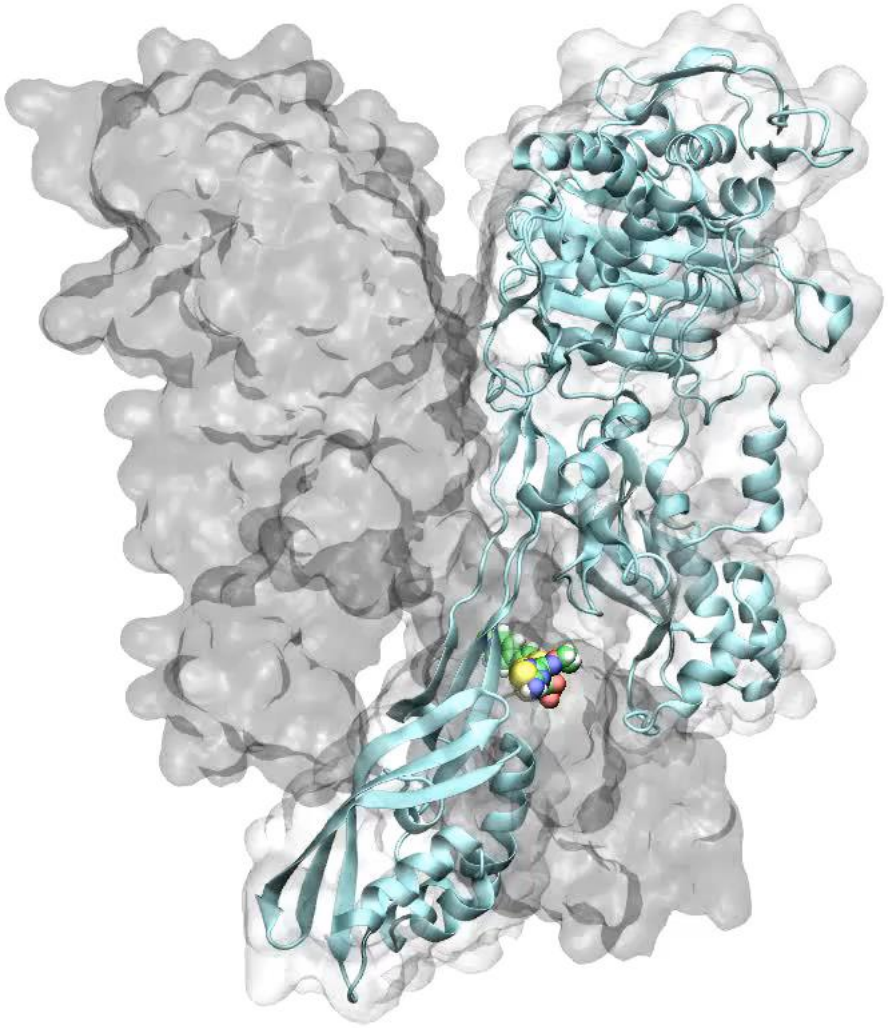




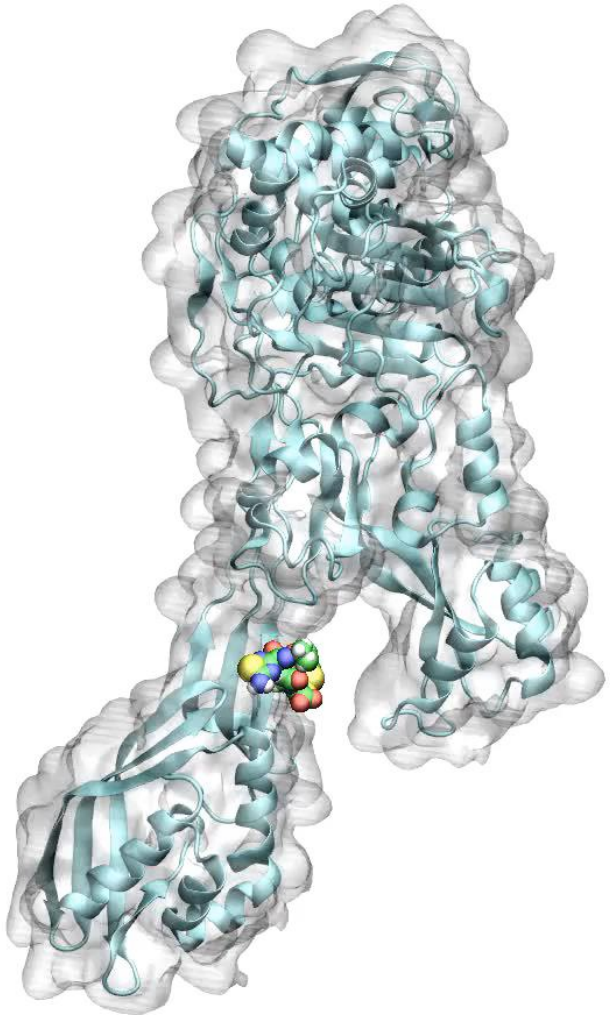


Cd^{2+} and Cl^{-}

Molecular Dynamics (MD) Simulation

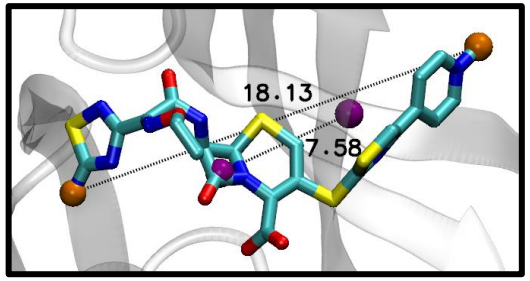


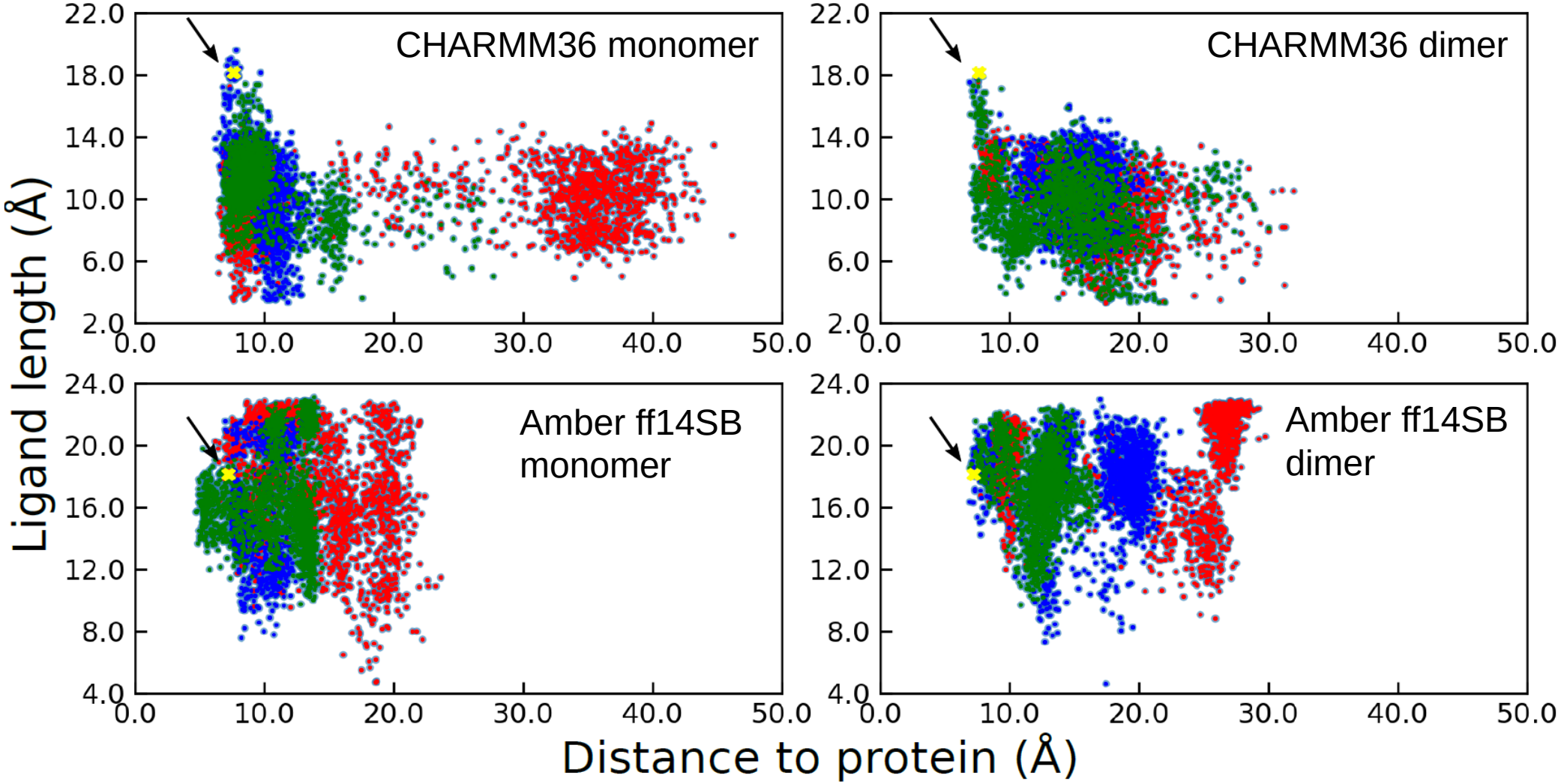
Amber ff14SB + AM1-BCC, 0.1 M NaCl, Cd^{2+}->Ca^{2+}



CHARMM36 + CGenFF with FFTK, 0.1 M $CaCl_2$, Cd^{2+}->Ca^{2+}

Ligand Distribution at the Allosteric Site

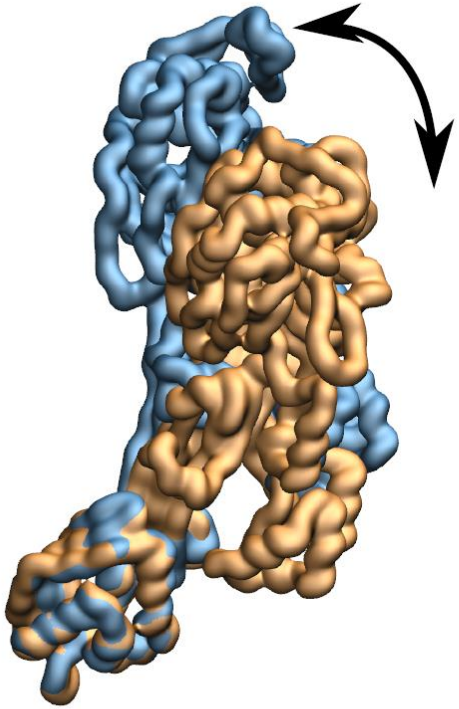




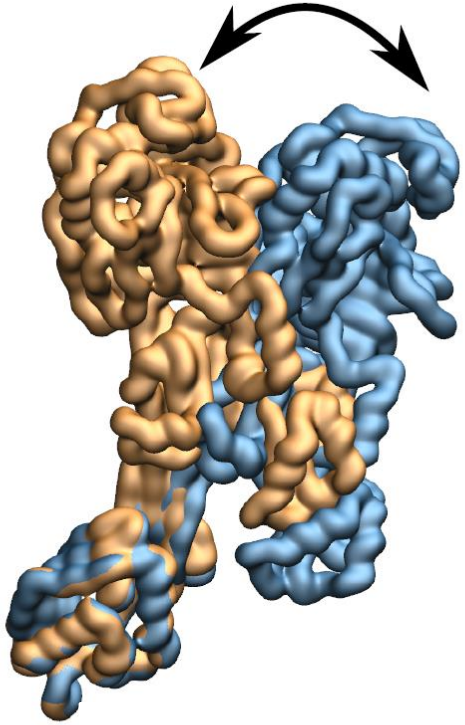
Y.-C. Chiang, M. T. Y. Wong, J. W. Essex, *Isr. J. Chem.* **2020**, *60*, 754.

Motion of PBP2a Revealed by Principle Component Analysis (PCA)

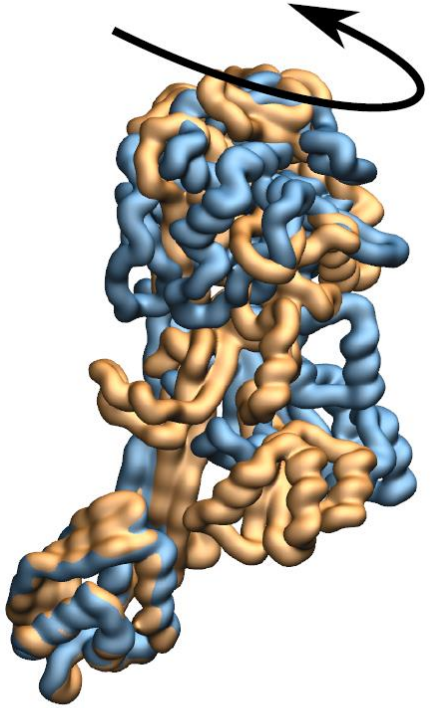
PCA: 53.3%, 26.8%, and 14.1% of the variance in Cartesian coordinates.



Bending Mode

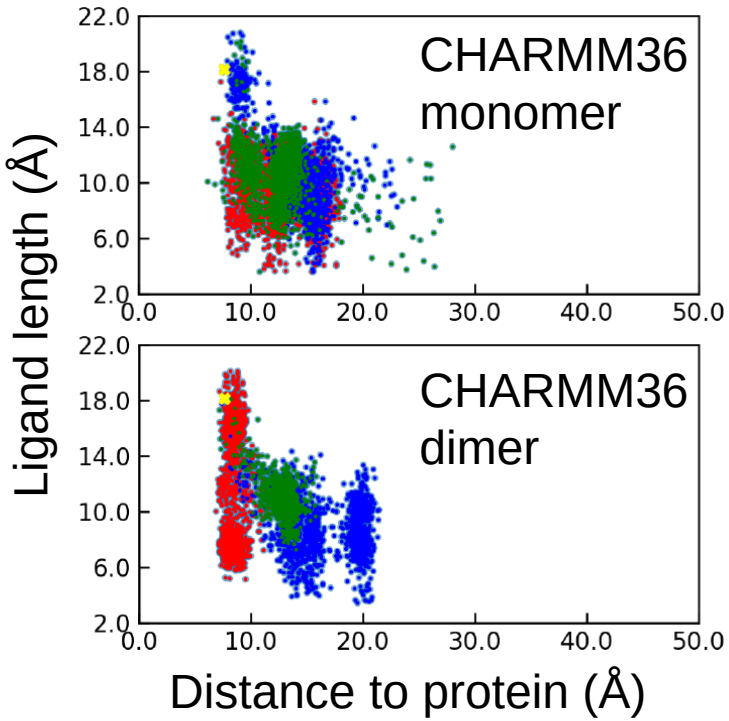


Wiggling Mode

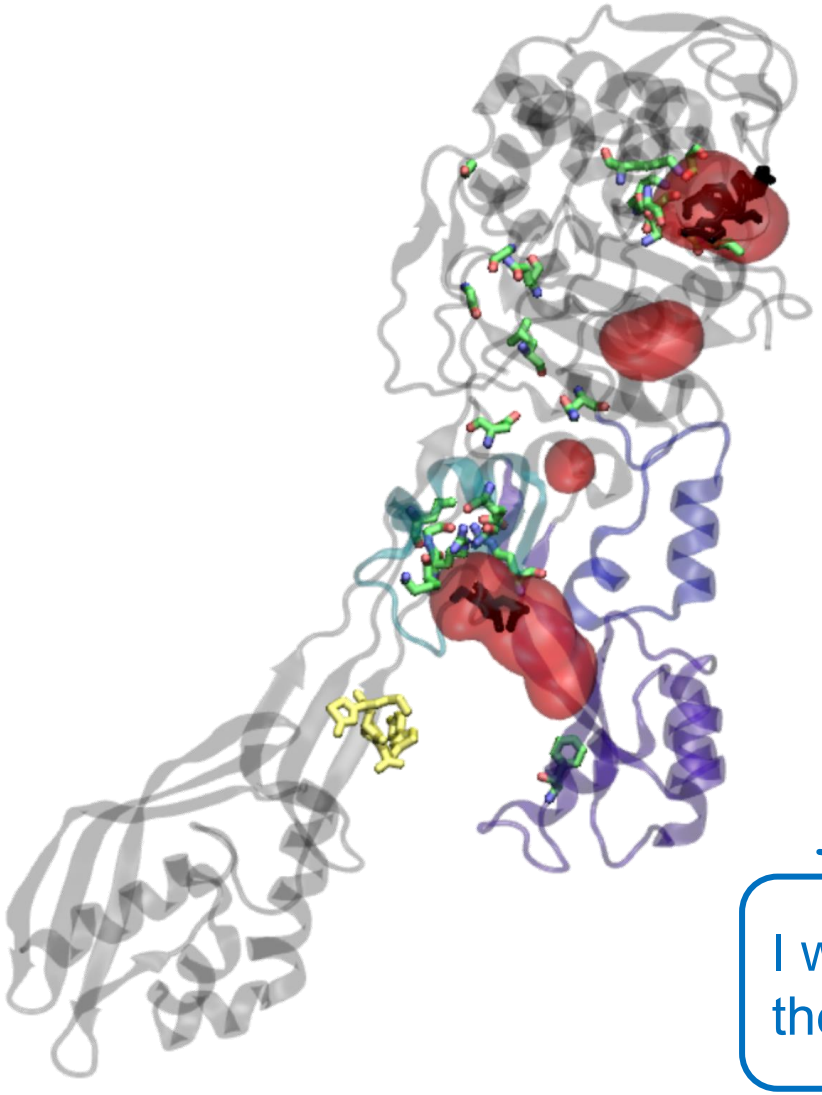


Twisting Mode

Perhaps the large domain motion causes the ligand to be shaken off.



Other Evidence?



Perhaps we should double check where the binding sites are?

- **Binding hot spot prediction via FTMAP**

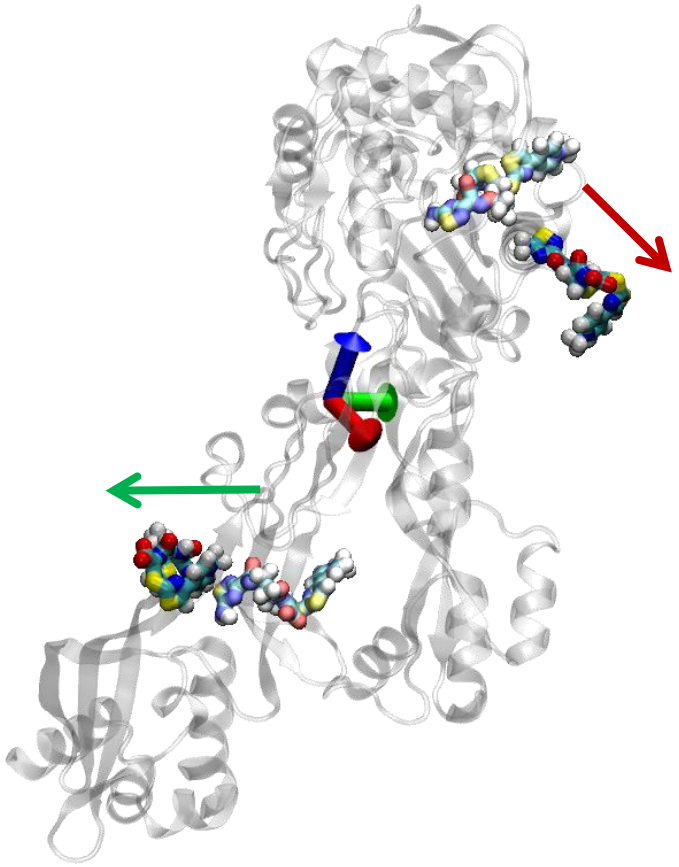
  FTMAP docks 16 different small molecules (fragment docking) to the protein. The consensus sites are then identified as the binding hot spots.

- **Sequence alignment via Clustal Omega**

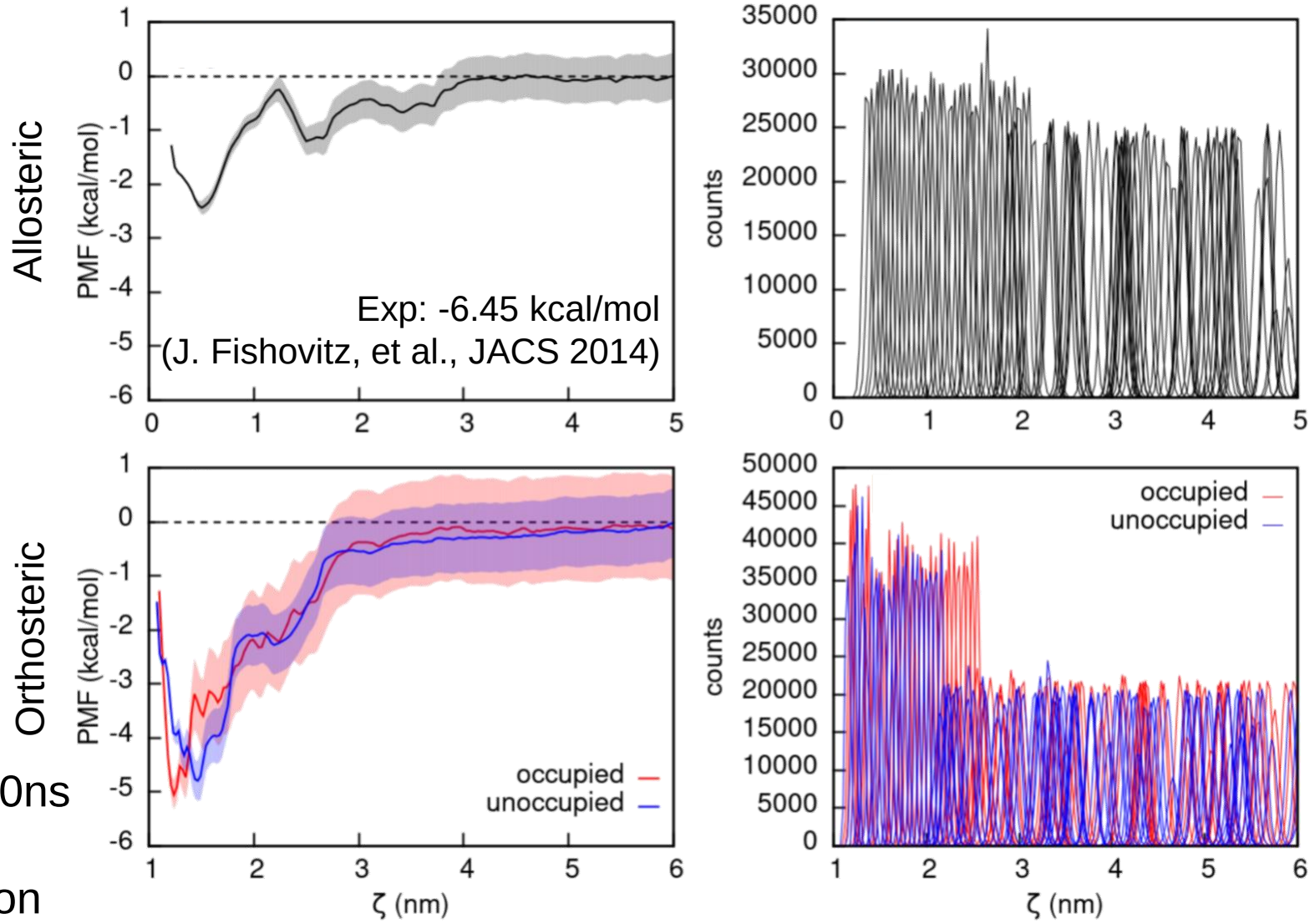
  First we found the 9 different PBP isomers identified by searching the PBP2a sequence on Pfam, and then we used Clustal Omega to find the conserved residues.

I wish that someone would have told me that one has to perform the binding site prediction before picking up a project…

Binding Free Energy Calculations via Umbrella Sampling

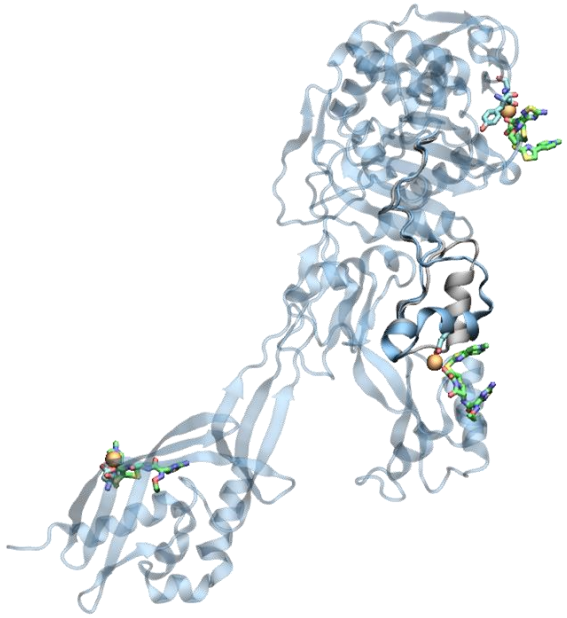


- 100+ windows, each samples 10ns
- Umbrella sampling + WHAM
- Bootstrap 200 for error estimation



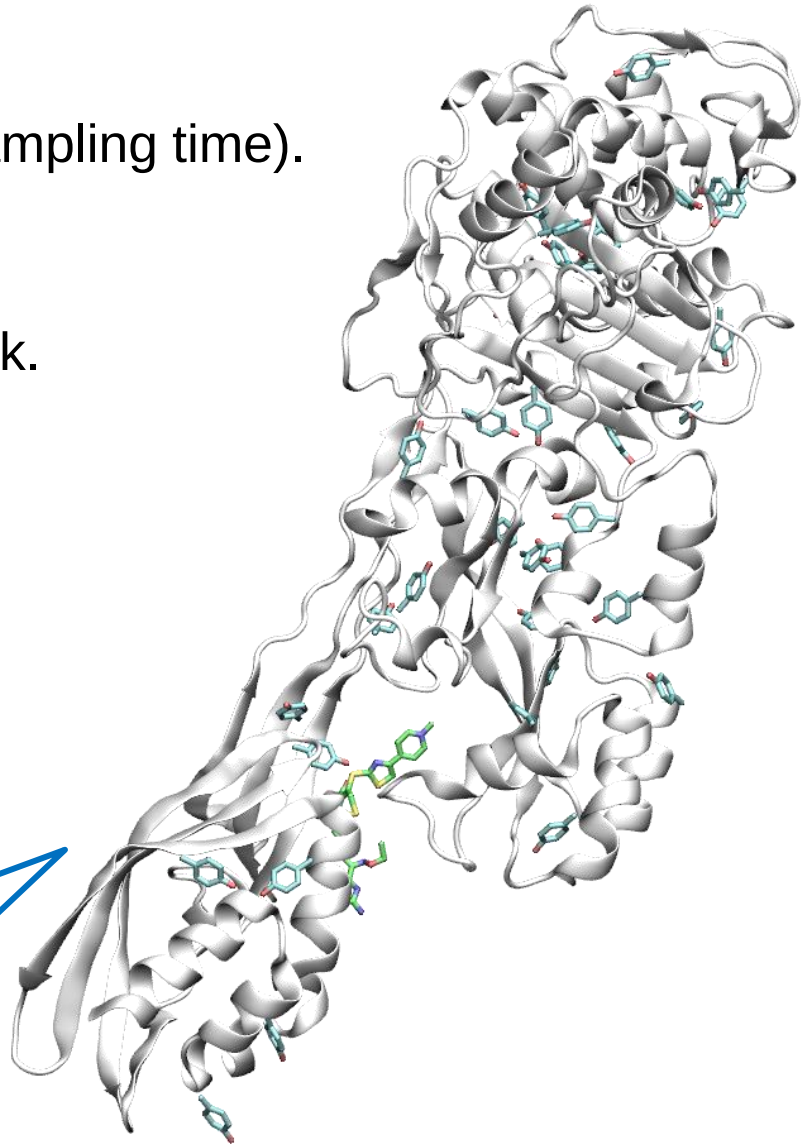
Reasons for The Binding Free Energy Mismatch

- Calculation not converged?
  → Checked with longer simulation time (double the sampling time).
- Bad force field?
  → We are currently optimizing the parameters to check.
- Other binding hot spots that can also attract ligands?

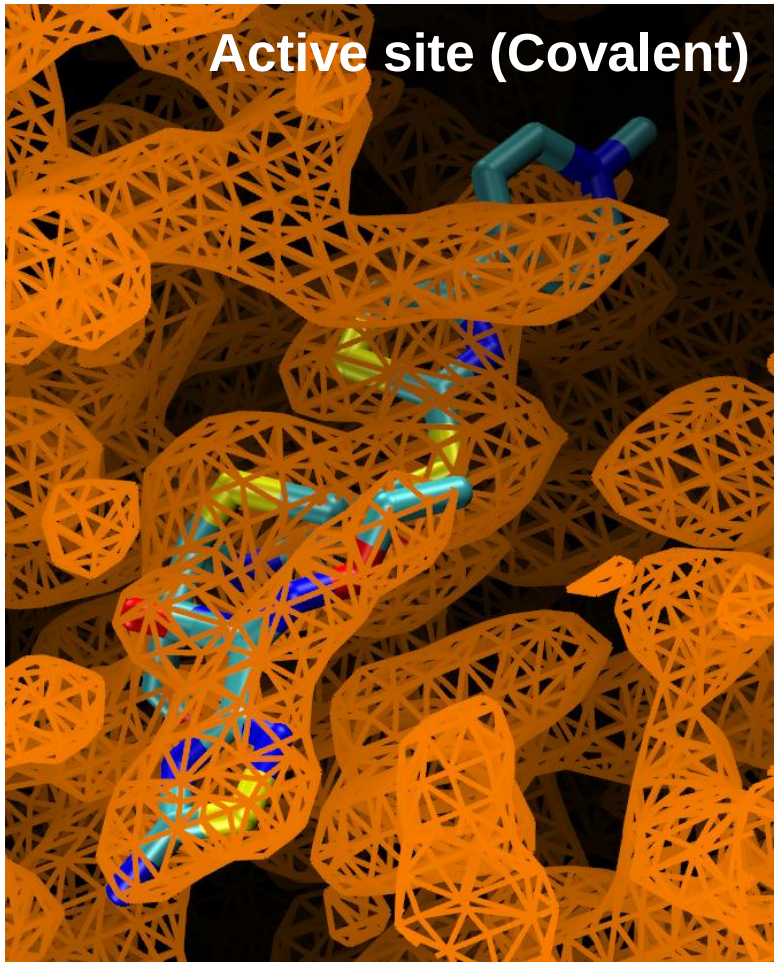


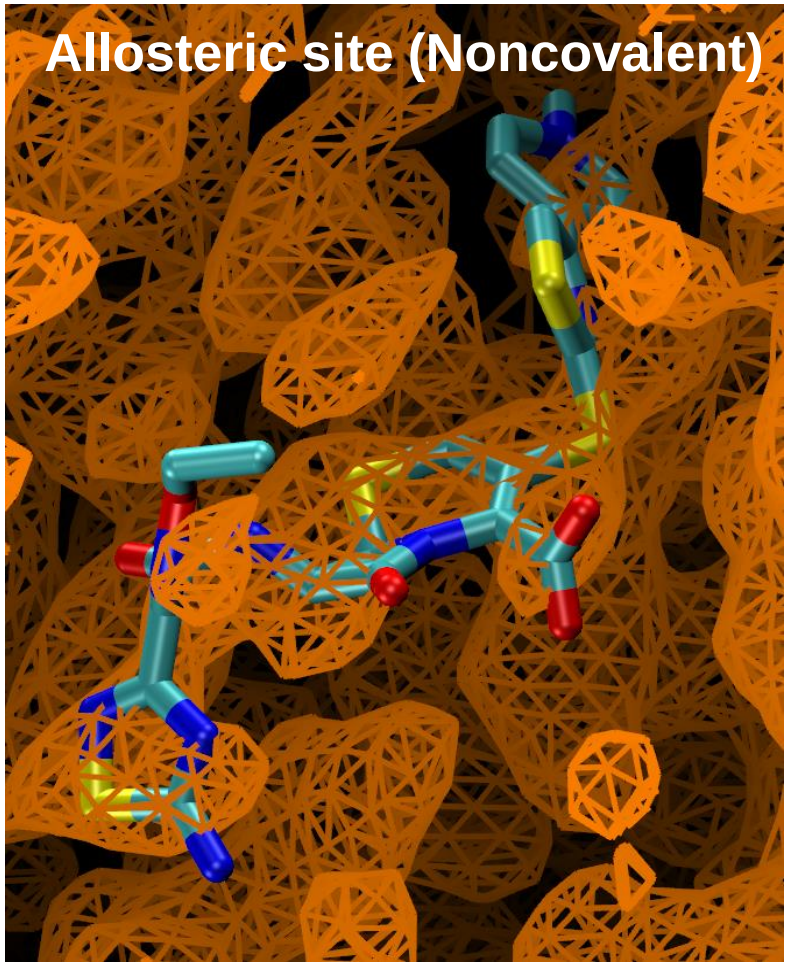
PBP2a + 6 free ceftaroline molecules explore some transient binding hot spots with Tyrosine.

TYR are all over the PBP2a, and apparently there are many places ceftaroline can bind.



Electron Density





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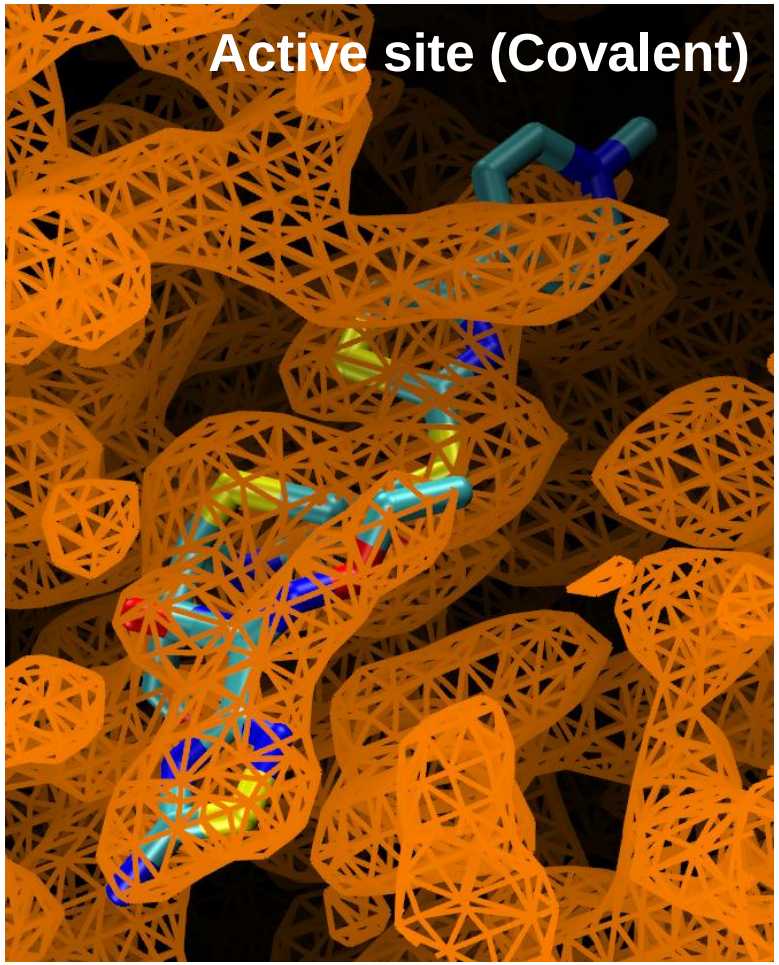
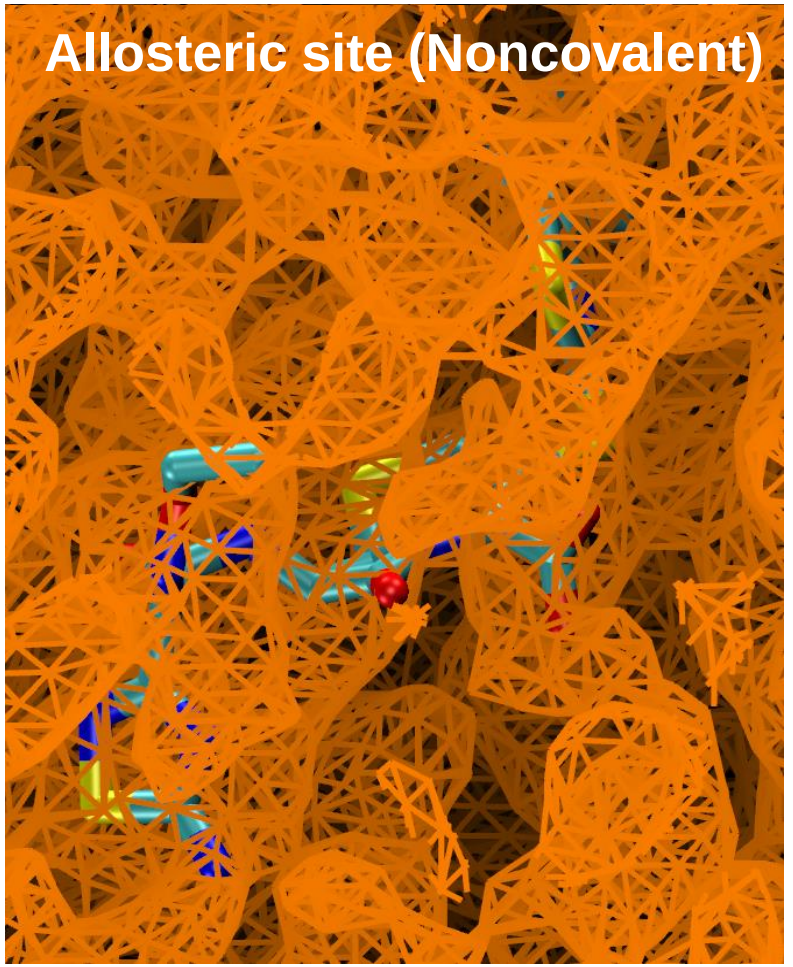
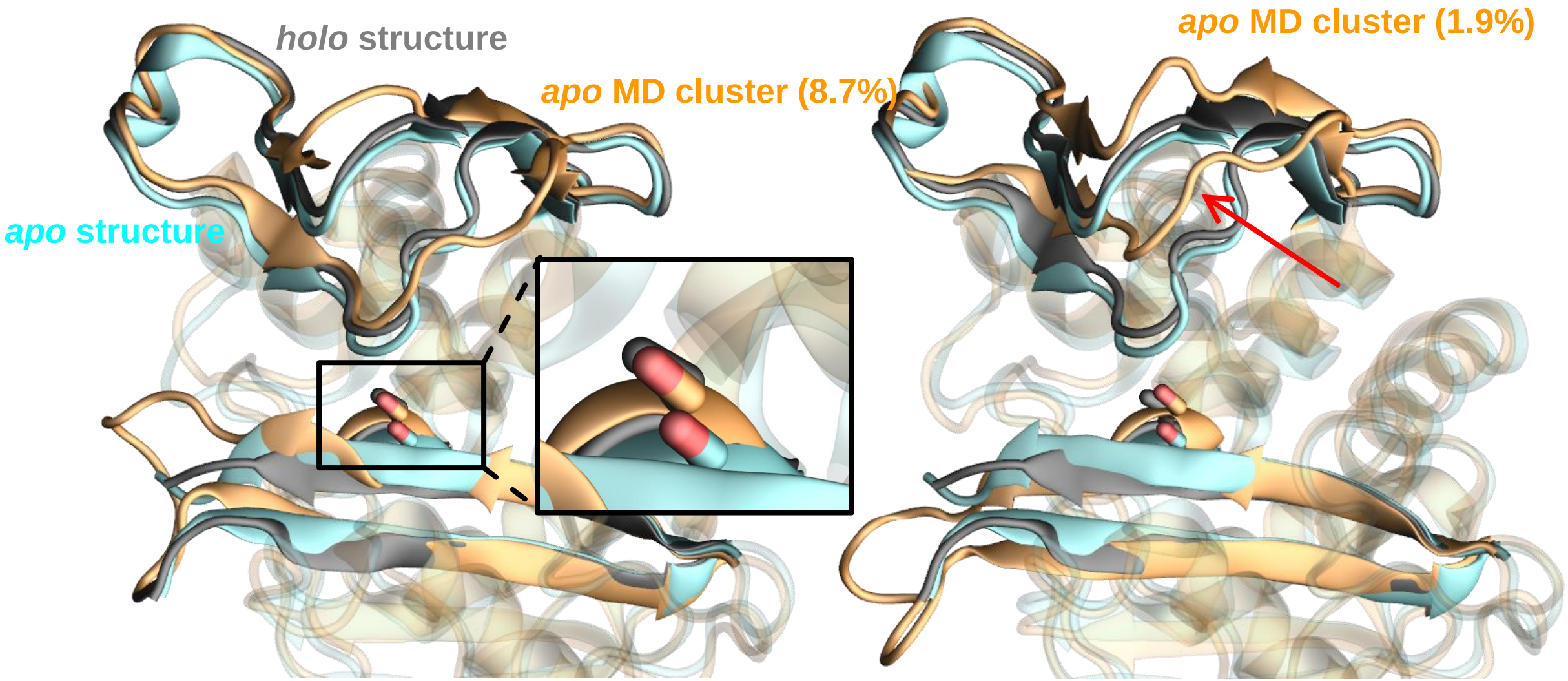




Can the Orthosteric Site Open for Ligand Binding without Allosteric Mechanism?



Conclusion & Other Possibility?

- MD simulations reveal an unstable binding of ceftaroline at the allosteric site “observed” in the crystal structures.
- PMFs obtained from umbrella sampling show insignificant allosteric effect.
- MD simulations show that thermal fluctuations could support the active site conformational change for reaction.
- Always check if the binding sites are real before starting a project: FTMAP + Multiple Sequence Alignment via Clustal Omega.

Acknowledgements

Supervisor: Prof. Jonathan W Essex

Electron Density: Prof. Peter L Roach and Prof. Gergely Katona

Biology: Ms. Mabel T. Y. Wong

FFTK parameterization: Mr. Yui Tik Pang (Georgia Institute of Technology)

THE ROYAL SOCIETY

Newton International Fellowship (NF171278)

Thank you for your attention!

What Michaelis–Menten Kinetics Taught Us

For an enzymatic reaction (E: enzyme, S: substrate, P: product),

$$E + S \underset{k_r}{\overset{k_f}{\rightleftarrows}} ES \xrightarrow{k_{cat}} E + P$$

The turnover rate is given by,

$$\frac{d[P]}{dt} = V_{\max} \frac{[S]}{K_M + [S]}$$

maximum reaction rate $V_{\max} = k_{cat}[E]_0$

Michaelis constant $K_M = \frac{k_r + k_{cat}}{k_f}$

To account for the difficulty of forming a stable enzyme-substrate complex, one often defines the catalytic efficiency as

$$\frac{k_{cat}}{K_M} = \frac{k_{cat}k_f}{k_r + k_{cat}} = \frac{k_{cat}}{K_d + \frac{k_{cat}}{k_f}}$$

If now we want to compare two β-lactams' efficiency,

- compare k_{cat}
- compare K_M (or the dissociation constant K_d)

Protocols

- Force Fields: CHARMM36 FF with CGenFF + FFTK ligand optimization or Amber ff14SB with AM1-BCC without ligand FF optimization.
- System setup: Dodecahedron water box solvation + 0.1 M $CaCl_2$ for CHARMM systems. Dodecahedron water box solvation + 0.1 M NaCl for Amber systems.
- MD parameters: PME (spline order 6, grid spacing 0.12 nm, cutoff 1.2 nm), van der Waals cutoff 1.2 nm, Verlet update 20fs, integration step 2fs, Langevin dynamics thermostat, Parrinello-Rahman barostat
- MD simulations: Energy minimization + NVT 500ps + NPT 500ps + Backbone restrained 5ns + Production Run 100ns (x 3)