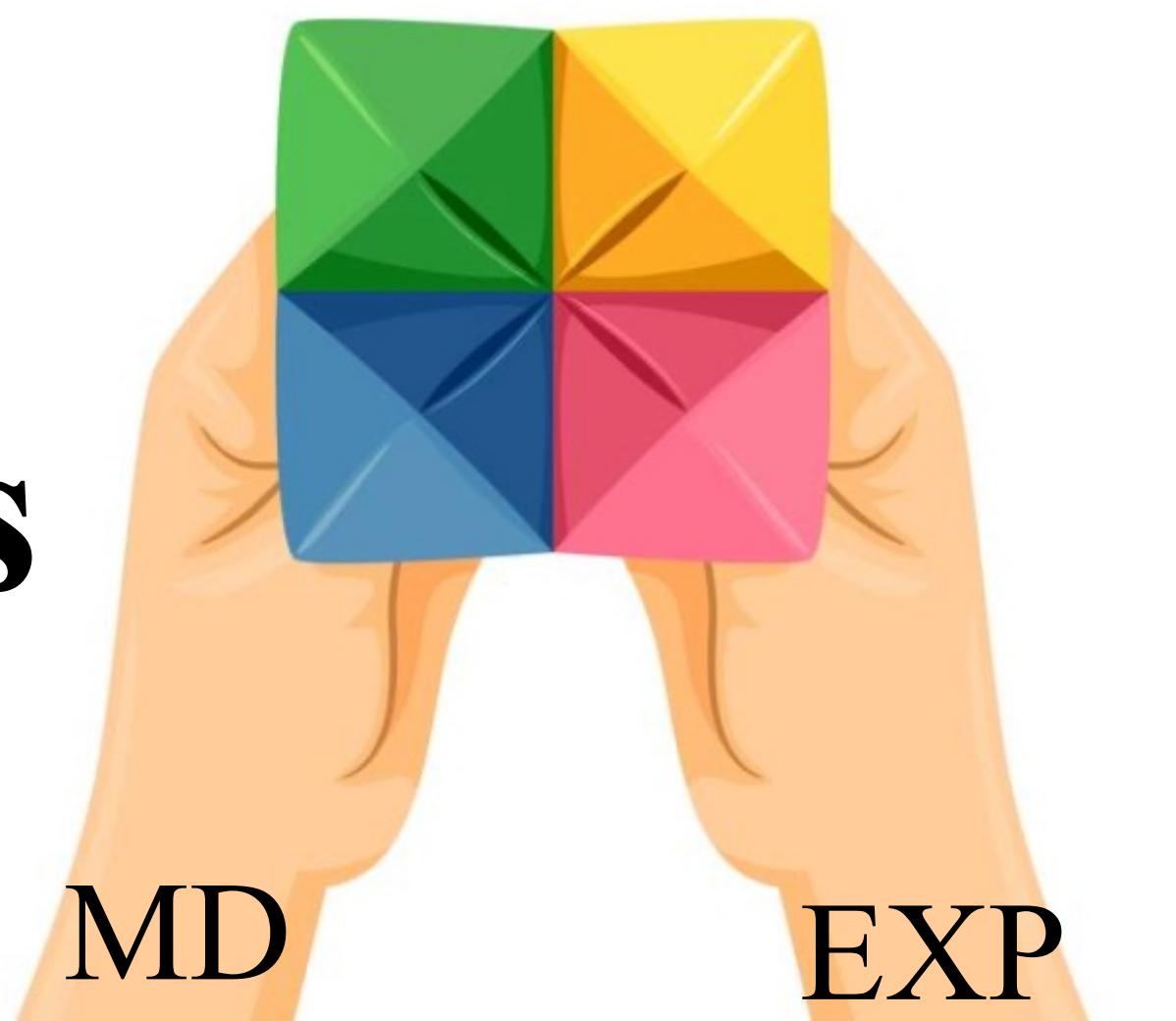


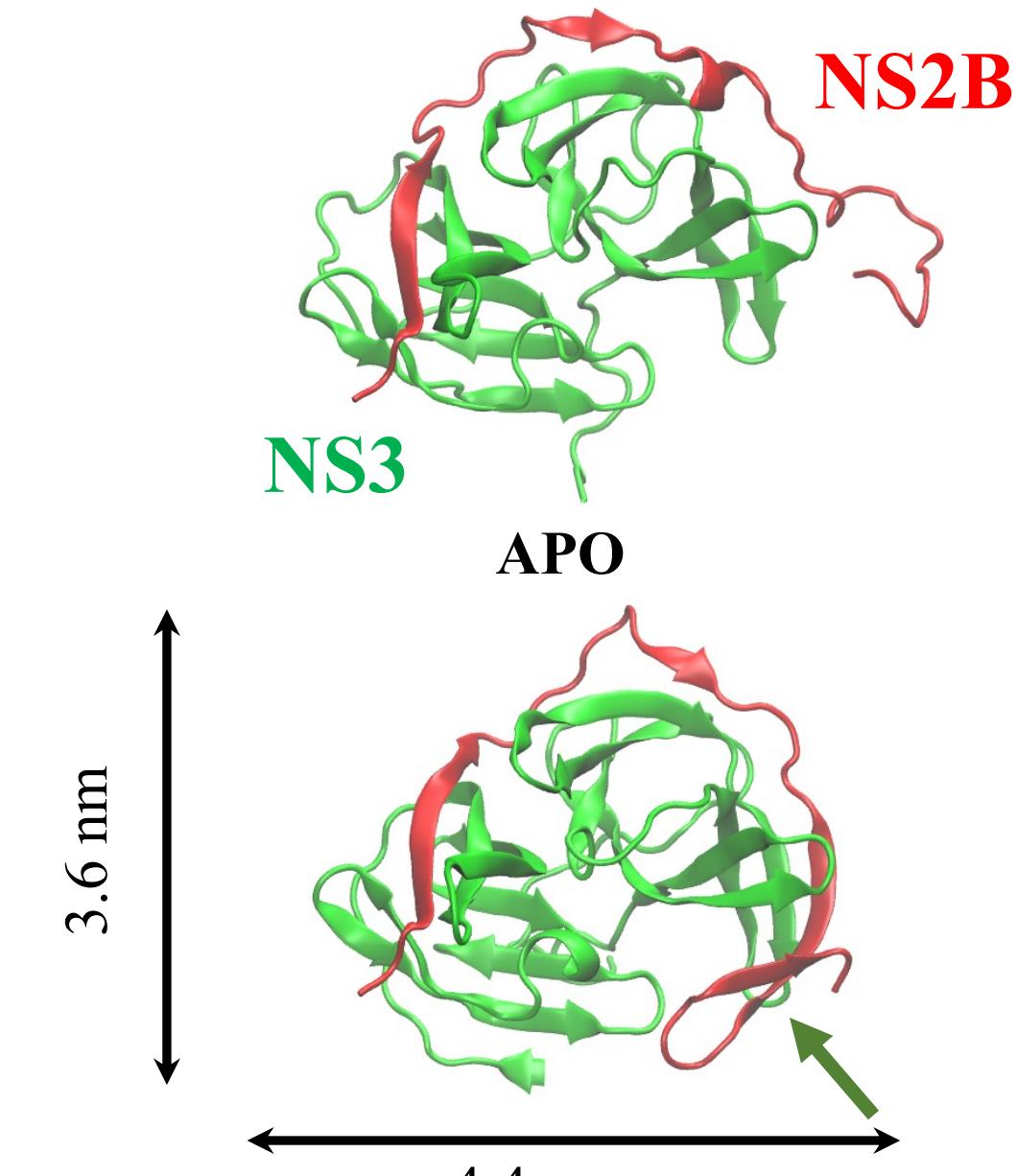
“ZIKA_Proteases Teller”

--- The Integration of MD and Experiments

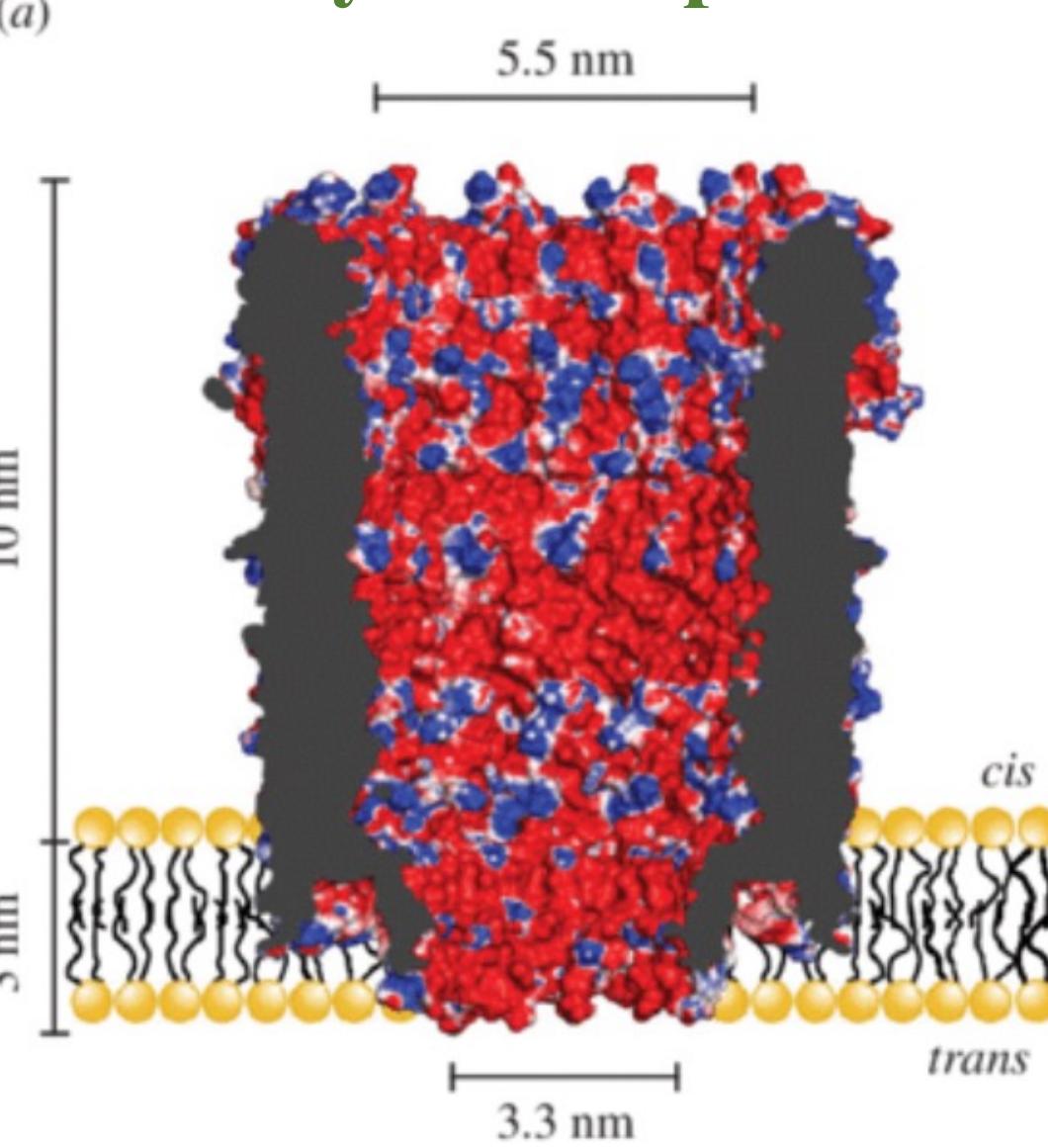
Yumeng Zhang, Spencer Shorkey, Min Chen, Jianhan Chen
Department of Chemistry, University of Massachusetts Amherst



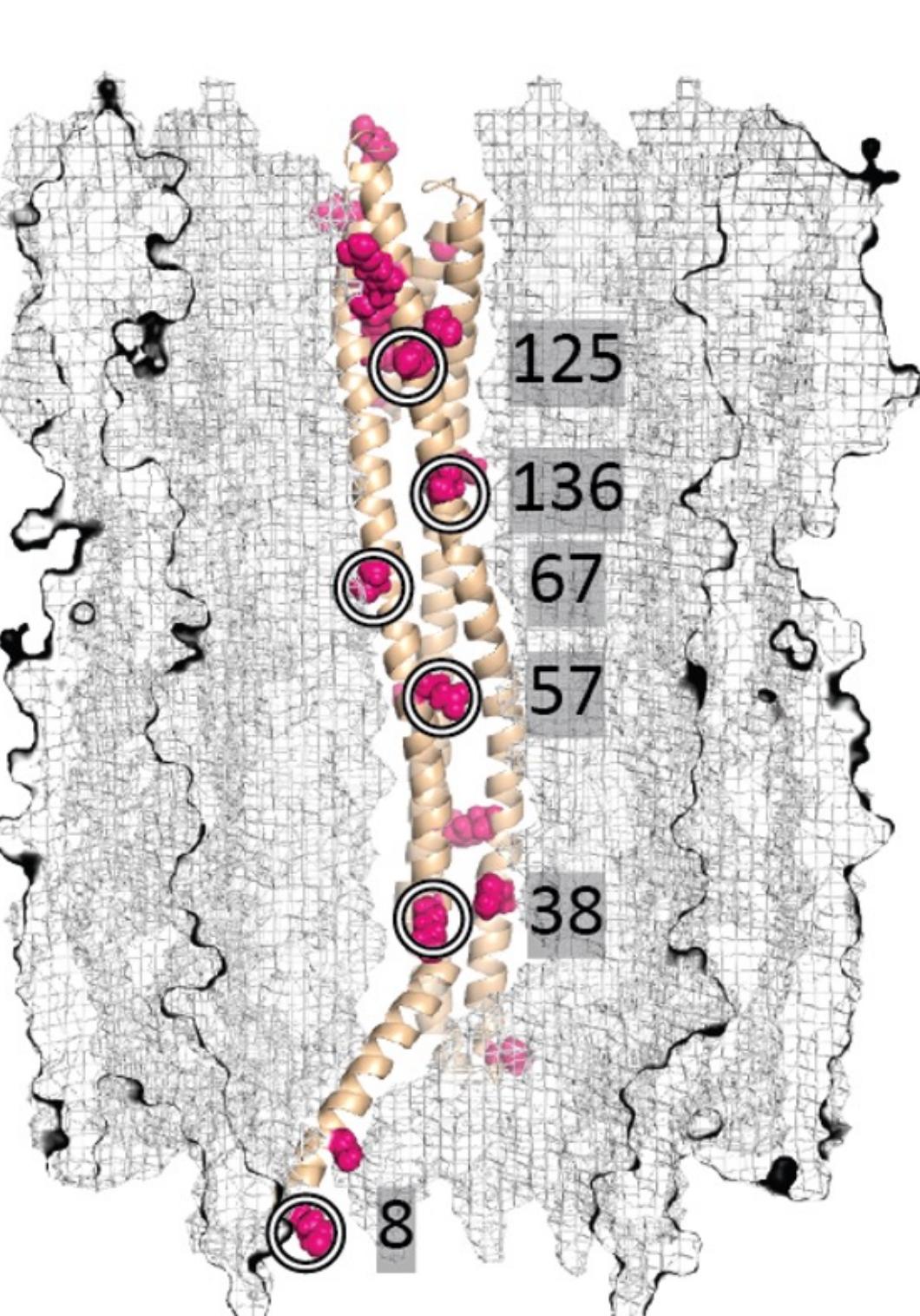
NS2B/NS3 Proteases



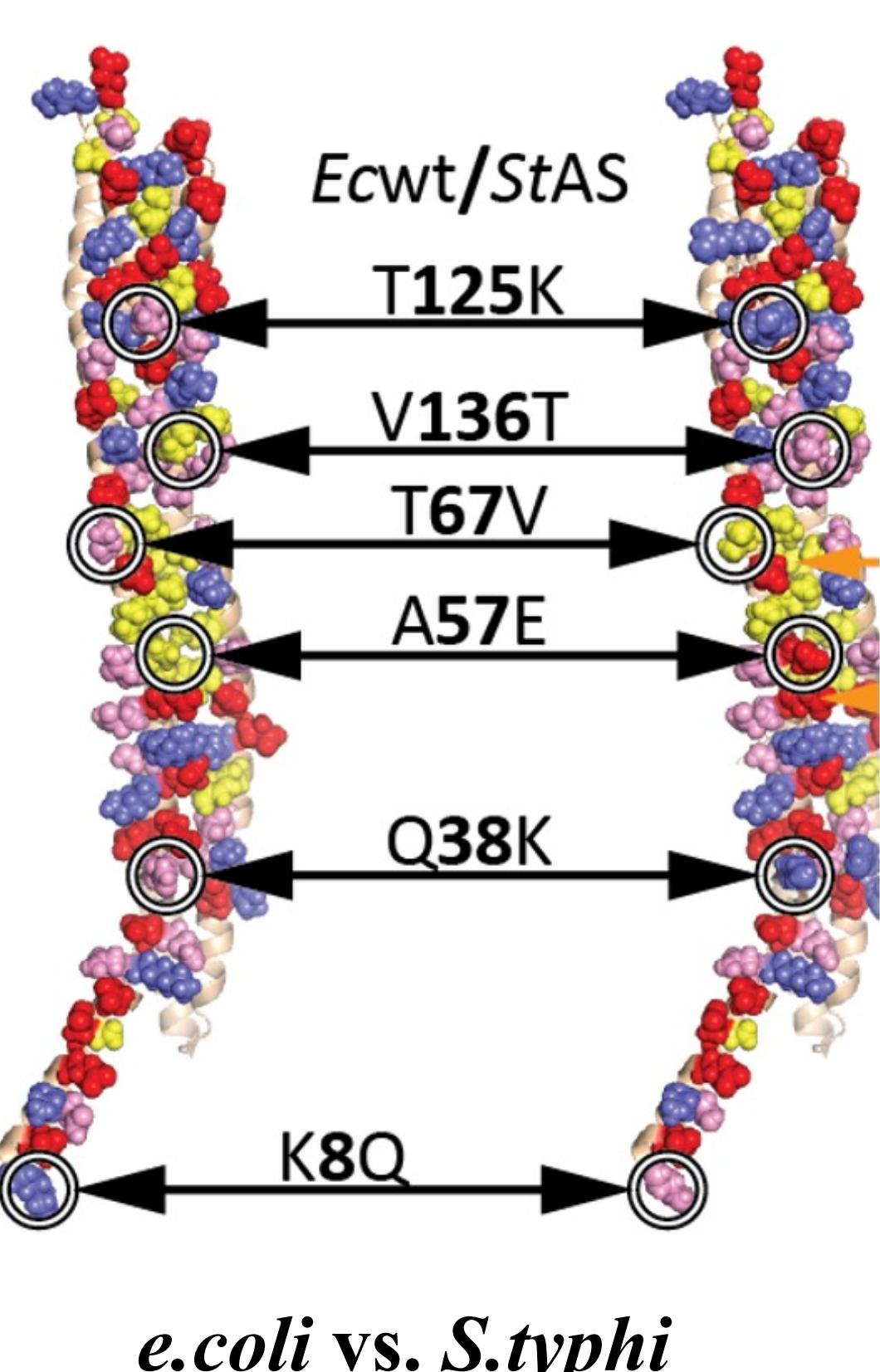
ClyA Nanopore



Key sites on ClyA



Engineerable sites.



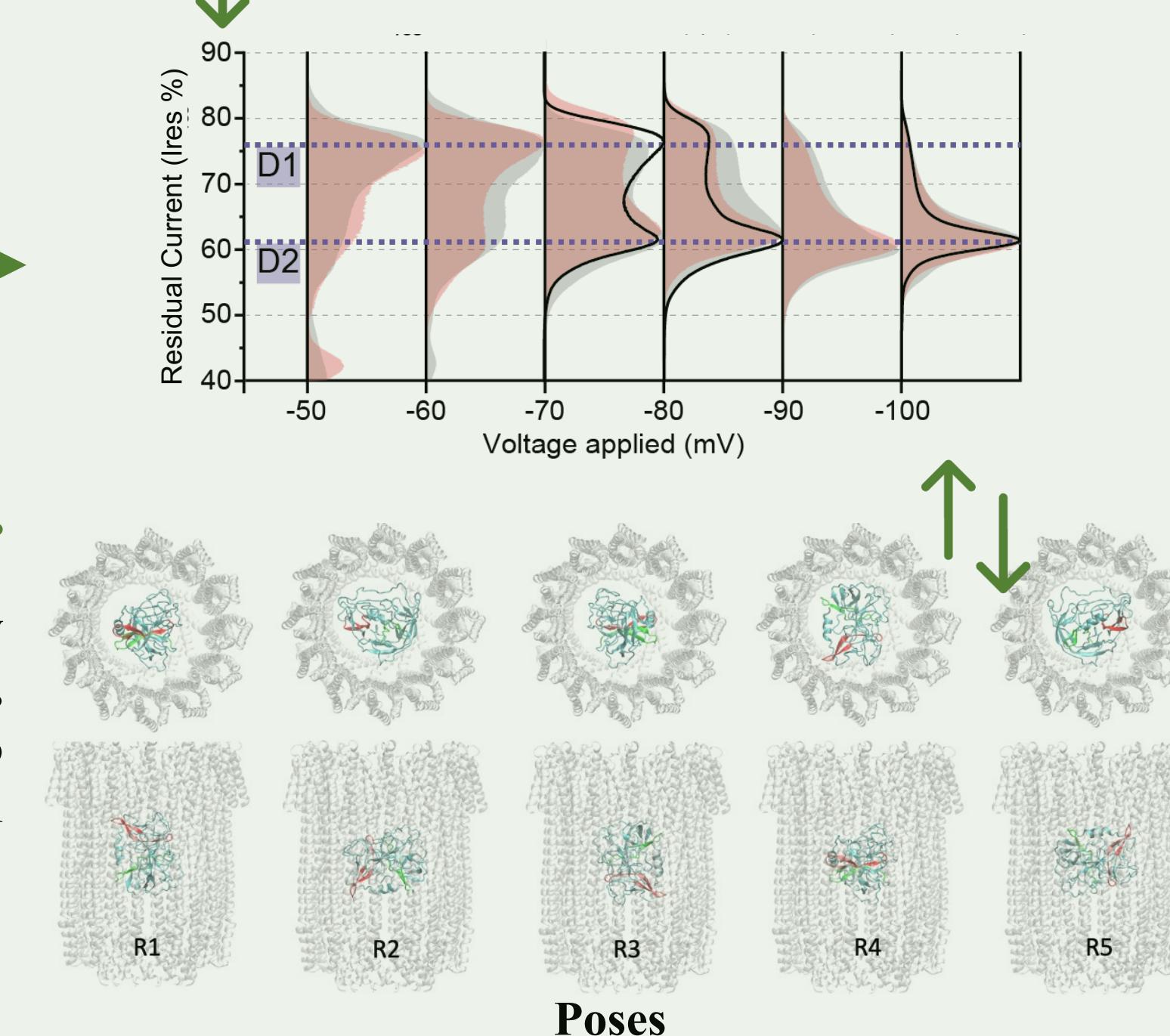
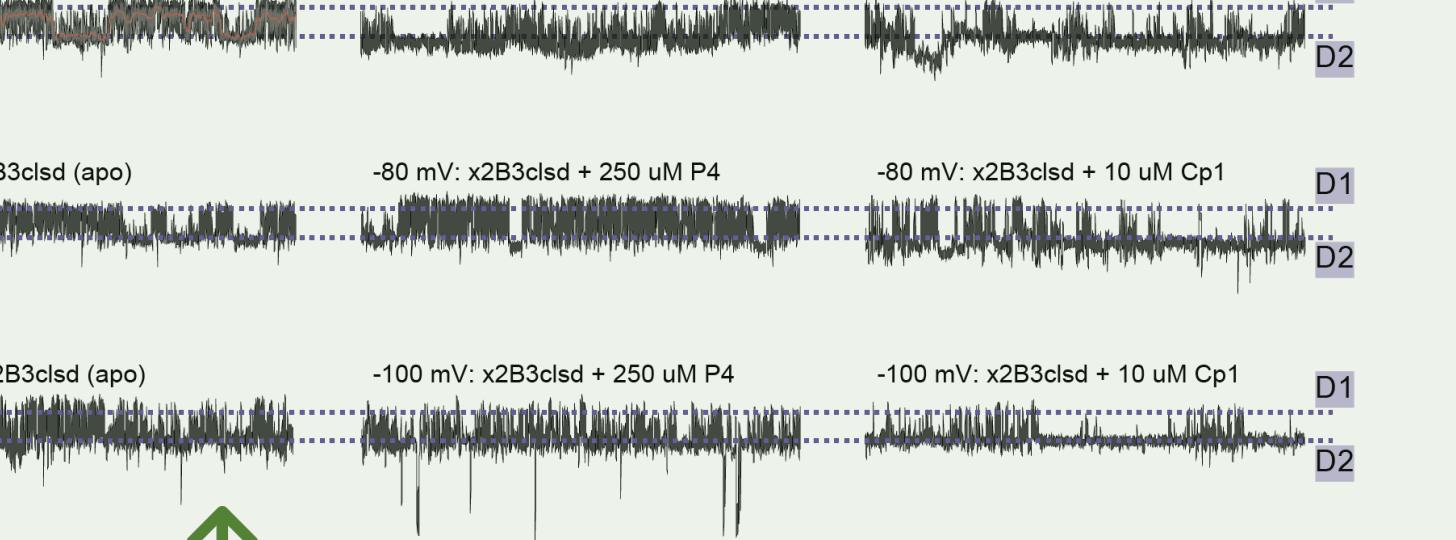
1st Teller --- The Integration of Nanopore Tweezer and MD simulations

Flavivirus Proteases: The flaviviruses Zika (ZIKV), Dengue (DENV) and West Nile (WNV) are major mosquito-borne pathogens that cause an estimate of ~100 million infections globally every year. Despite intensive efforts, no antiviral treatment is currently available. The flaviviral two-component NS2B/NS3 serine protease is required for viral replication and considered an attractive antiviral drug target.

Nanopore tweezers: can trap a single protein within its lumen and monitors conformation changes to as electrical current fluctuations in real time (100 μ s).



Molecular Dynamic Simulations: The validity of the CG-based MD (HyRes II) approach can assist to determine the molecular detail of proteases-ClyA interaction.



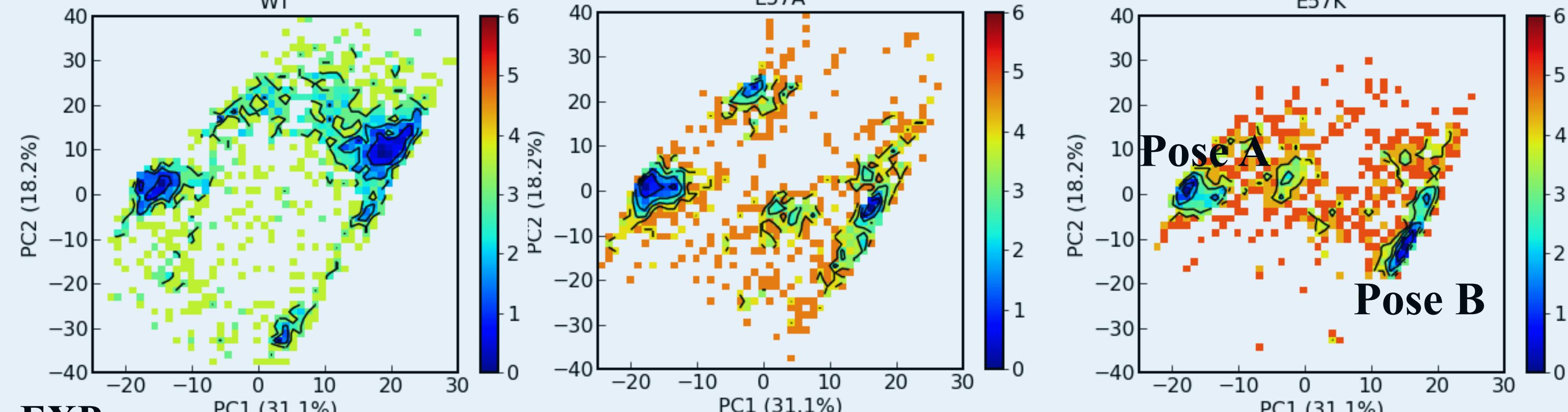
The integration of ClyA nanopore and MD approach, will help us to achieve our objective: capture the functional conformational states of Proteases.

3rd Teller --- Proteases/ClyA Binding Poses

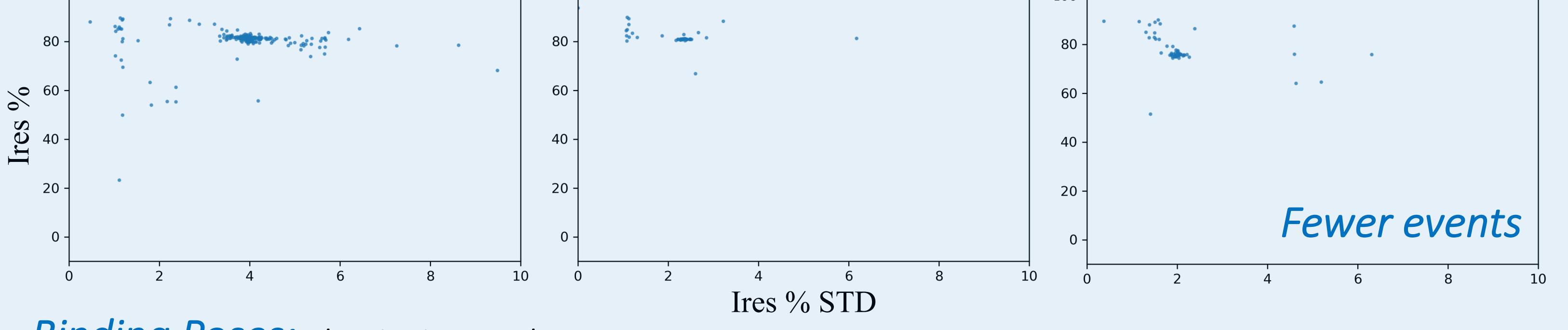
Objective 2: ‘Lock’ the mid-trapping states by pore engineering for further dynamics detection upon ligand binding or catalytic analysis.

E57: The mutation on S.typhi ClyA pore with E57 site significantly improved the mid-trapping probability and stabilized the binding states.

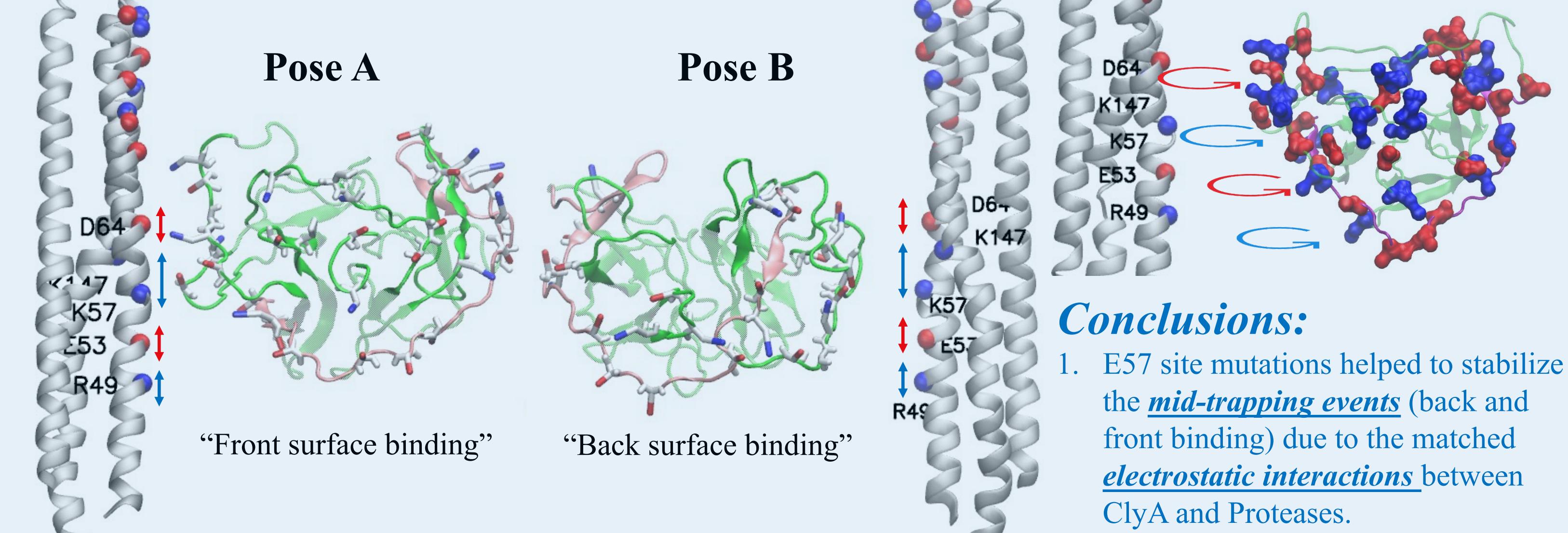
MD



EXP



Binding Poses: Electric Ring match.

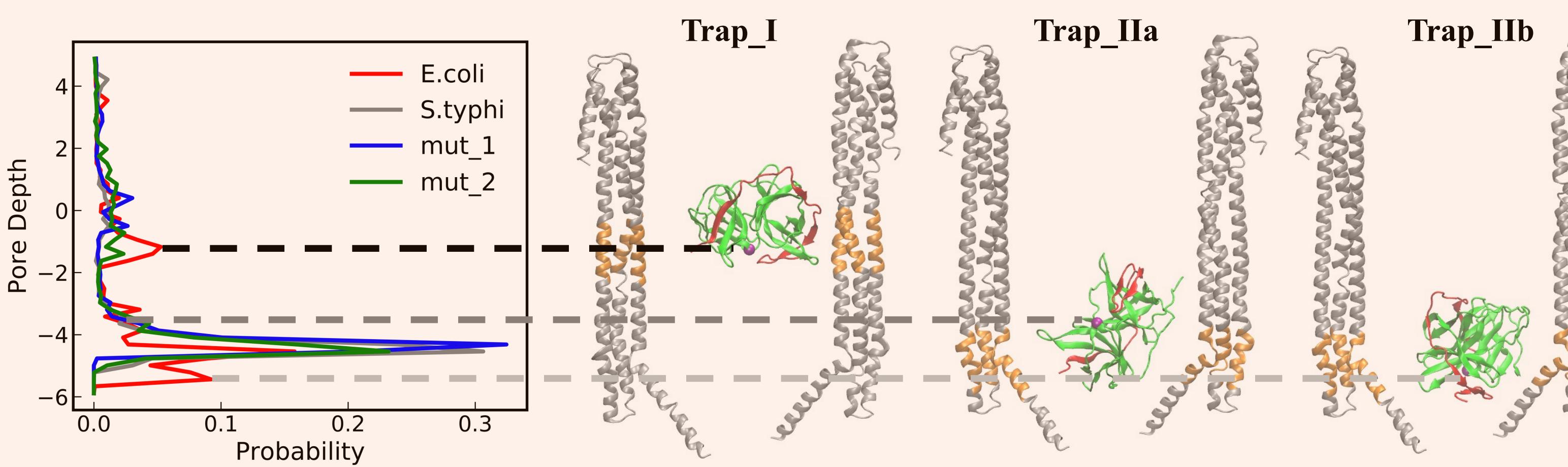


Conclusions:

1. E57 site mutations helped to stabilize the mid-trapping events (back and front binding) due to the matched electrostatic interactions between ClyA and Proteases.
2. E57K has better effects.

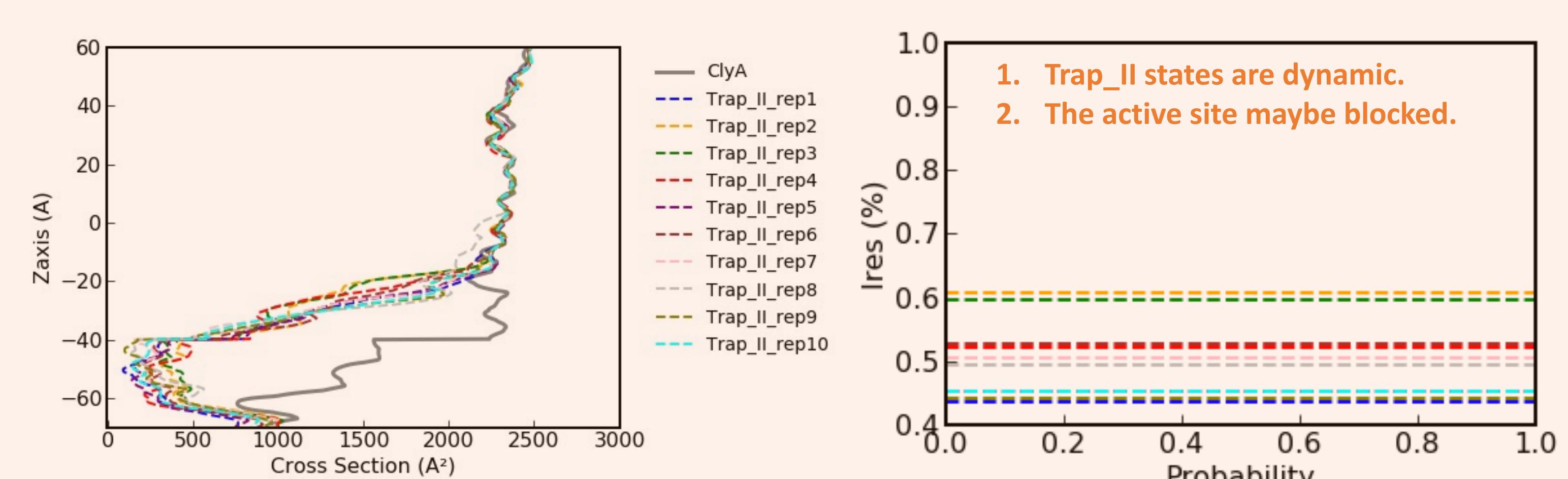
2nd Teller --- Hot Spots Locating

Objective 1: obtain a globular understanding of Proteases/ClyA dynamics from orientation selection and site selection studies.



Orientation: Little effects. It is plausible due to the globular charged property of Proteases.

Side Selection: Big influence. Can be engineered to improve or specify the certain binding position probabilities.

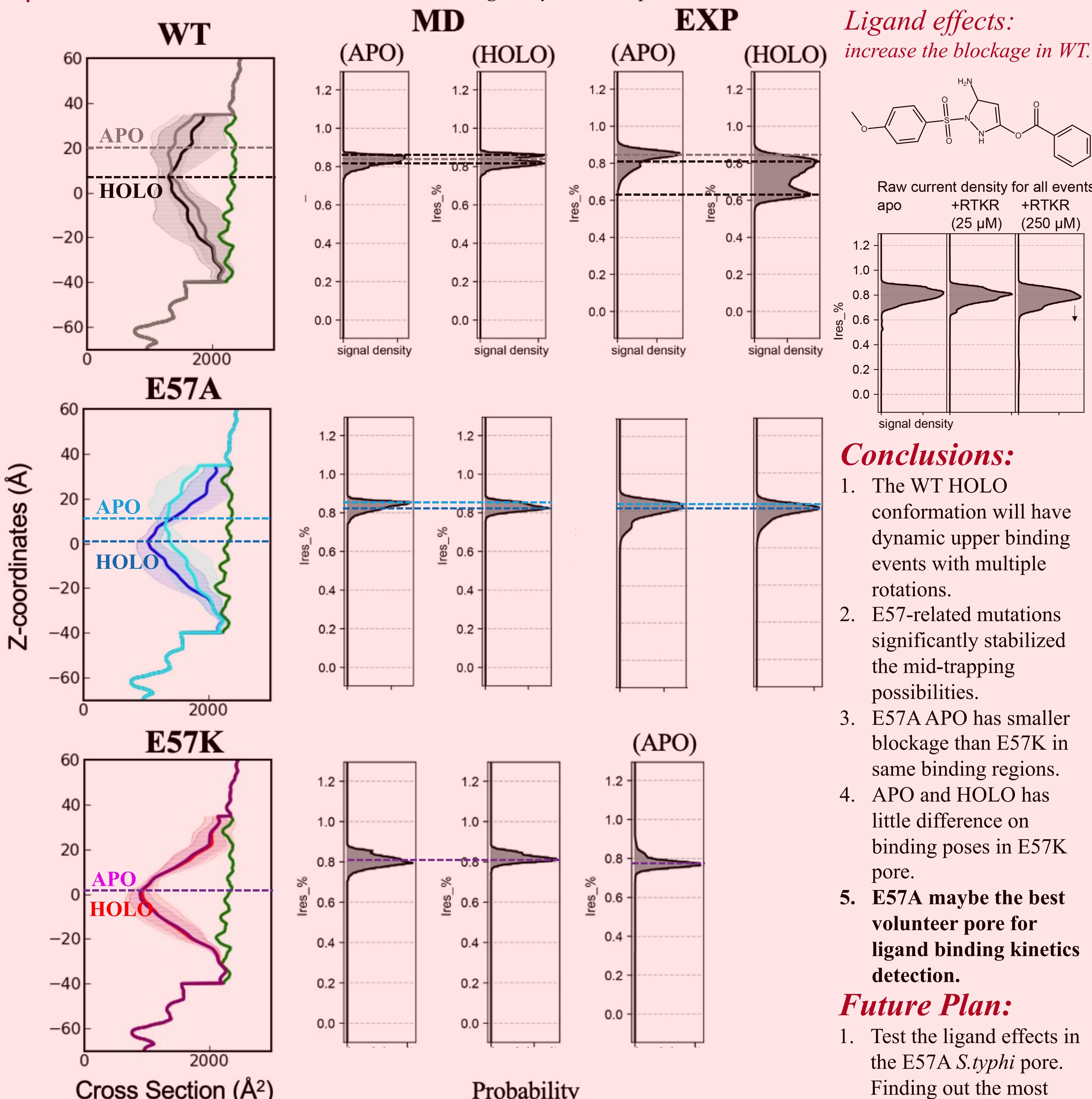


We want to realize stable mid-trapping events to avoid the active-site blocking of Proteases when trapped at the constriction region.

4th Teller --- Dynamics of ZIKA Proteases

Objective 3: Exploring the conformational dynamics and possible kinetics of Proteases.

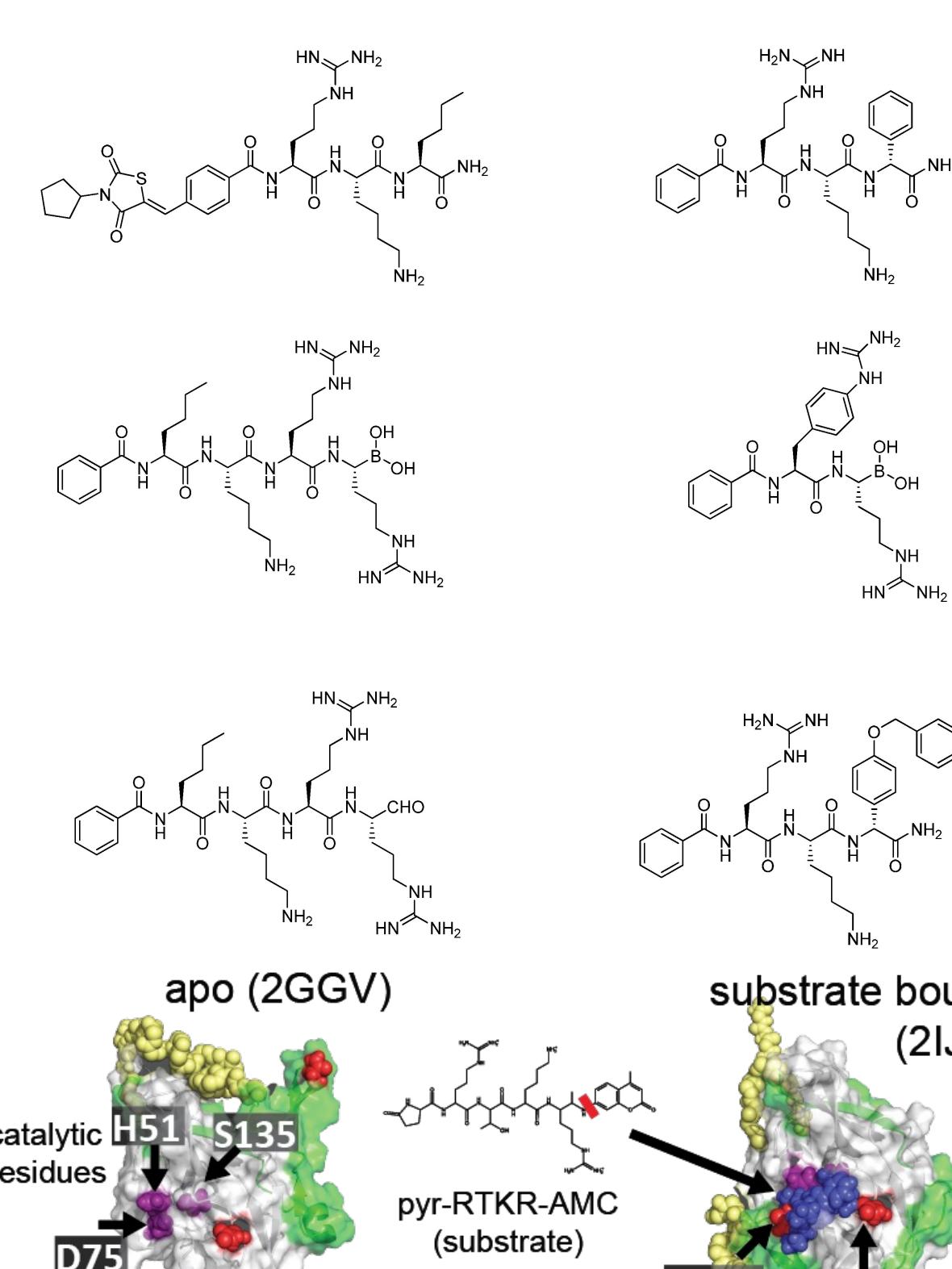
Open&Close states: The APO states showed higher dynamics in pore.



Ligand effects:

increase the blockage in WT.

Potential Inhibitors



• Reference

- [1] *J. Med. Chem.* 2020, 63, 1, 140–156
Publication Date: December 5, 2019

- [2] *Phys Chem Chem Phys.* 2017 Dec 13; 19(48): 32421–32432.

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Min Chen research group
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Future Plan:

1. Test the ligand effects in the E57A S.typhi pore. Finding out the most