"ZIKA_Proteases Teller"

--- The Integration of MD and Experiments

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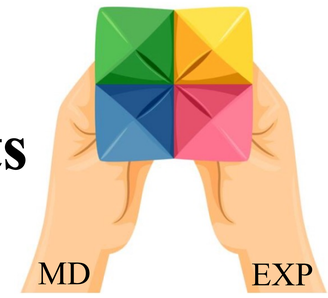
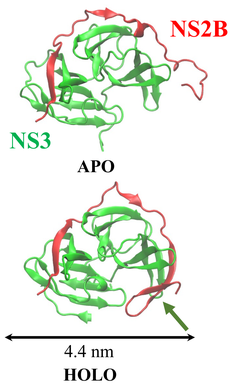
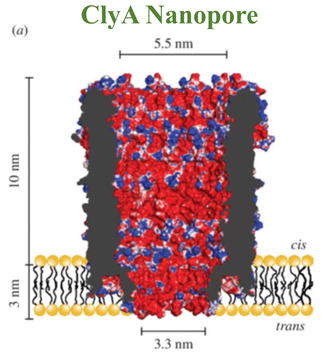


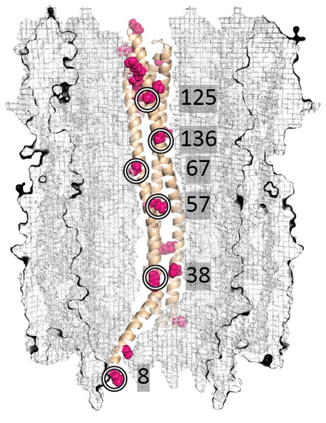
NS2B/NS3 Proteases



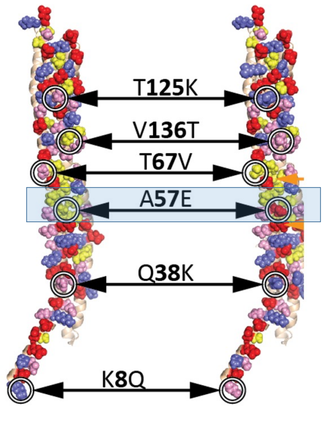
ClyA Nanopore



Key sites on ClyA



Engineerable sites

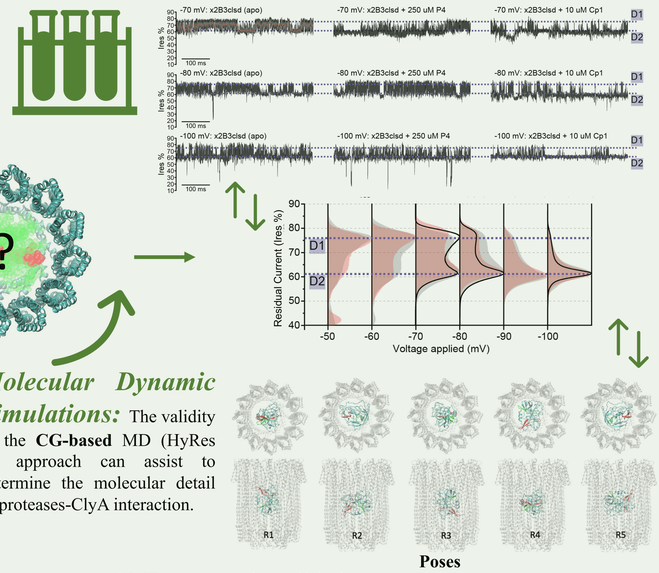


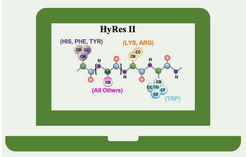
e.Coli **vs.** ***S.typhi***

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 μs).





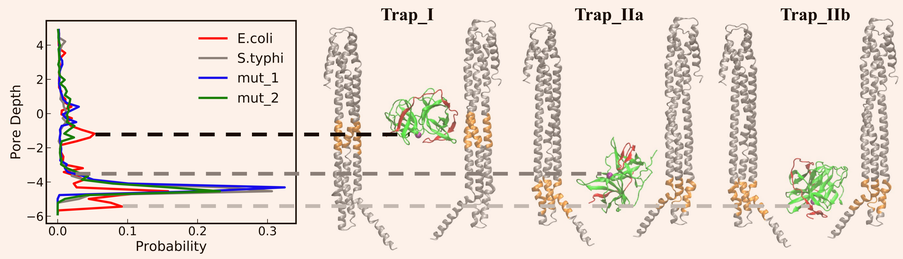
Molecular Dynamic Simulations: The validity of the **CG-based** MD (HyRes II) approach can assist to determine the molecular detail of proteases-ClyA interaction.

Poses

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

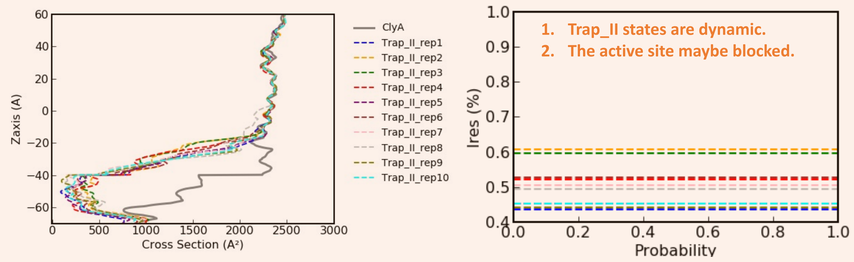
2nd Teller --- Hot Spots Locating

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.

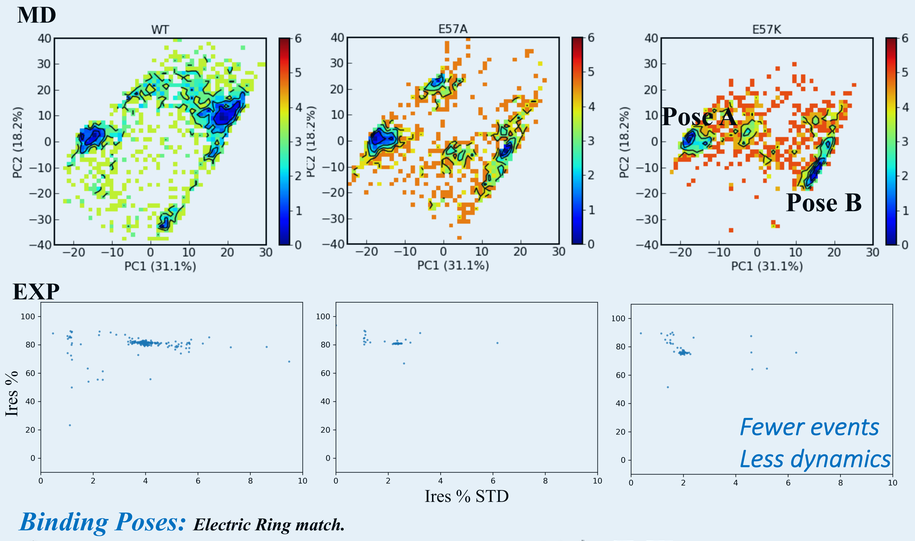


We want to realize stable mid-trapping events to ***avoid the active-site blocking of Proteases when trapped at the constriction region.***

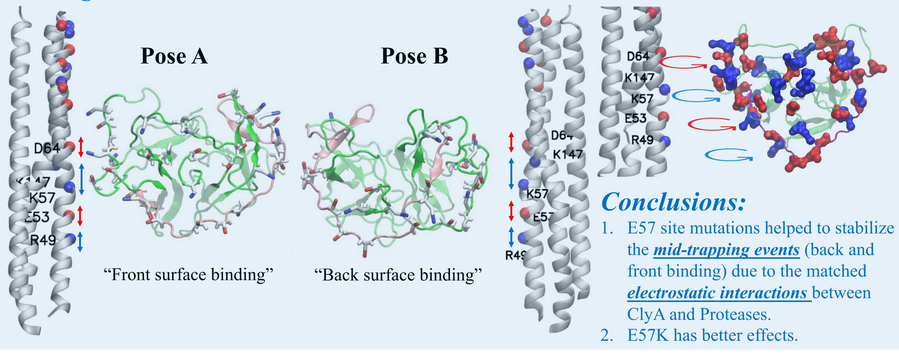
3rd Teller --- Proteases/ClyA Binding Poses

Objective 2: ***'Lock' the mid-trapping states*** *via pore engineering.*

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



Binding Poses: ***Electric Ring match.***



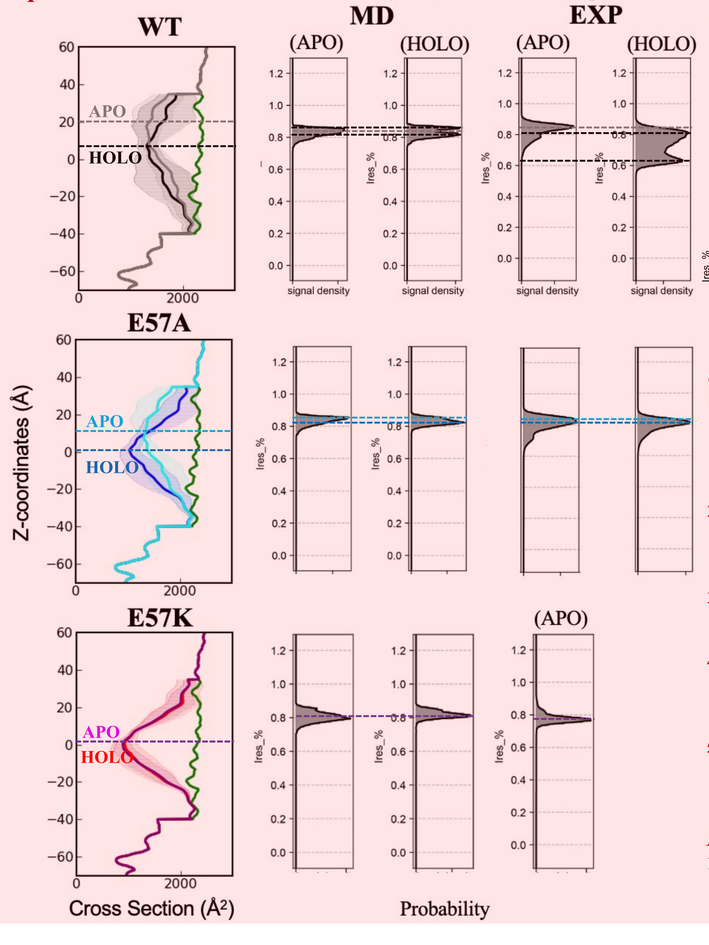
Conclusions:

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.

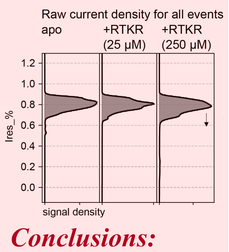
4th Teller --- Dynamics of ZIKA Proteases

Objective 3: *Exploring the* ***conformational dynamics*** *of Proteases.*

Open&Close states: *The APO states are differed from HOLO in E57A pore.*



Ligand effects: *increase the blockage in WT.*



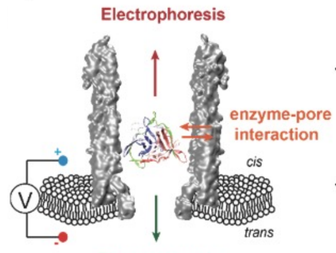
Conclusions:

1. The WT HOLO conformation will have dynamic upper binding events with multiple rotations.
2. E57-related mutations significantly stabilized the mid-trapping possibilities.
3. E57A APO has smaller blockage than E57K in same binding regions.
4. APO and HOLO has little difference on binding poses in E57K pore.
5. **E57A maybe the best volunteer pore for ligand binding kinetics detection.**

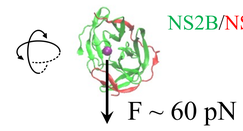
Future Plan:

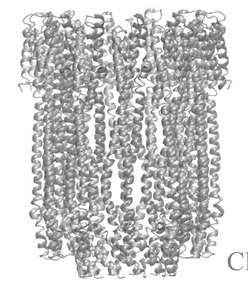
1. Characterize the inhibitor effects on Proteases in E57A *S.typhi* pore.

Nanopore Trapping

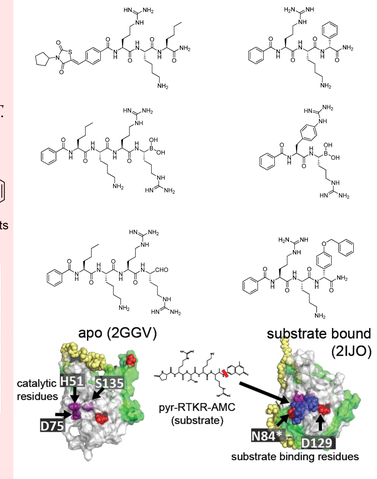


Steered MD





Potential Inhibitors



- **Reference**

[1] *J. Med. Chem.* 2020, 63, 1, 140–156
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[2] *Phys Chem Chem Phys*. 2017 Dec 13; 19(48): 32421-32432.

- **Acknowledgments**

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