# Millisecond protein dynamics does not control catalysis in Cyclophilin A – evidence from molecular dynamics simulations

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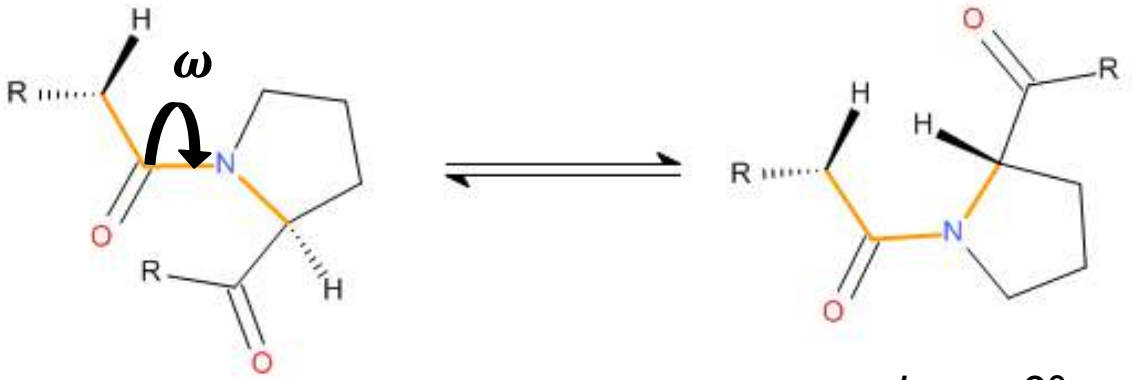
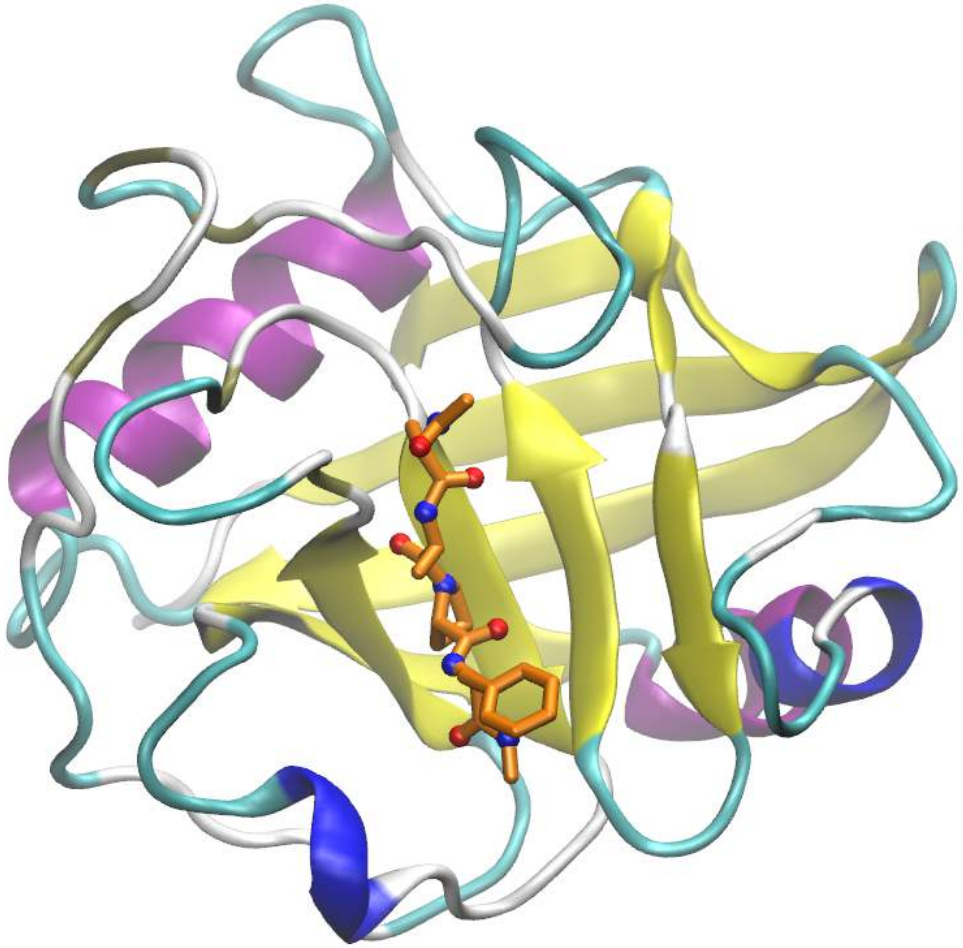
# Cyclophilin A (CypA)

CypA plays an essential role in protein folding and regulation, gene expression, cellular signaling and the immune system. It catalyzes the ***cis/trans* isomerization** of amide groups in **Proline** residues.



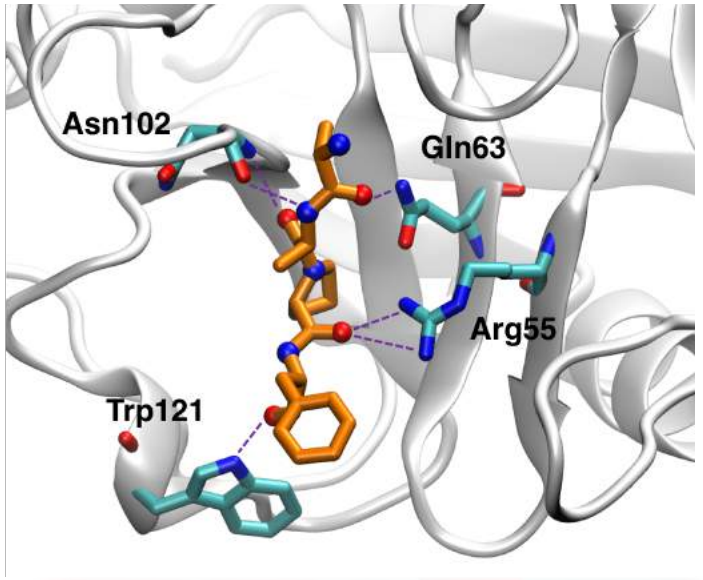

*trans* $\omega$= 180º

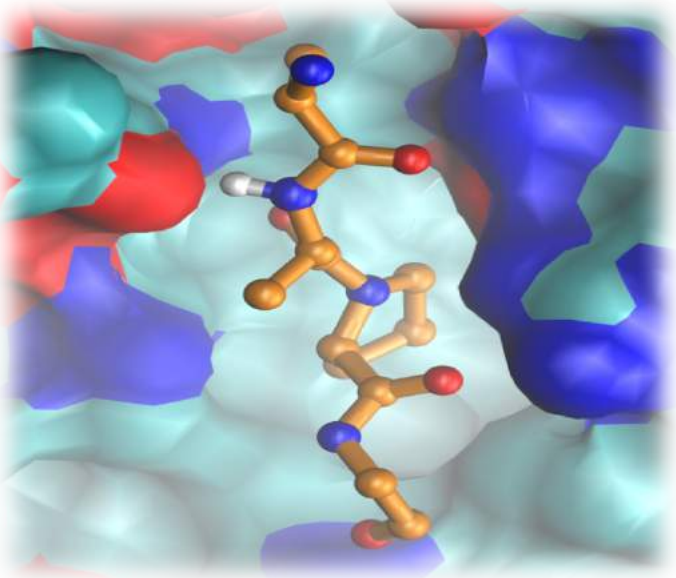
*cis* $\omega$= 0º



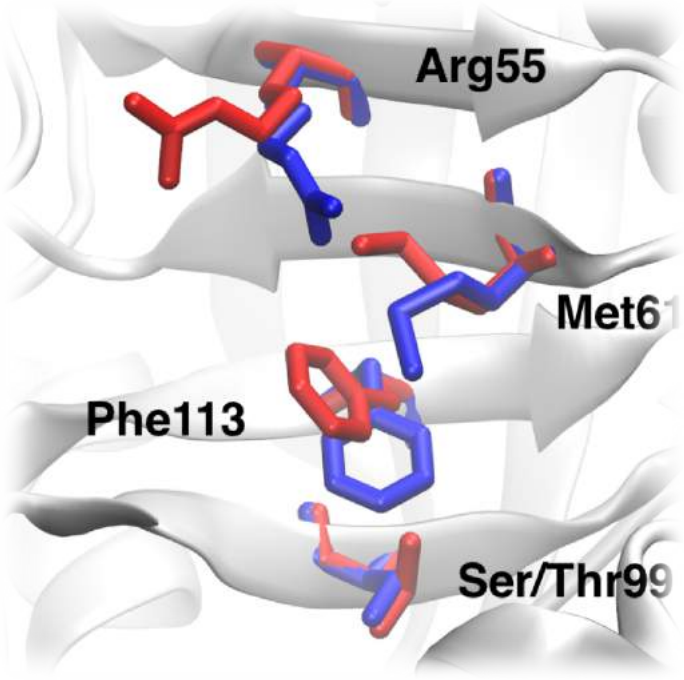
# The catalytic mechanism of CypA

- The Catalytic mechanism of Cyclophylin A is due to **the stabilization and preferential binding of the transition state**.
- **The hydrogen bonding interaction** at the active site help to stabilize the transition state of substrate during catalysis
- The binding site of CypA has a very **hydrophobic pocket** which fit into the side chain ring of proline residue.



# Catalysis & Enzyme Dynamics



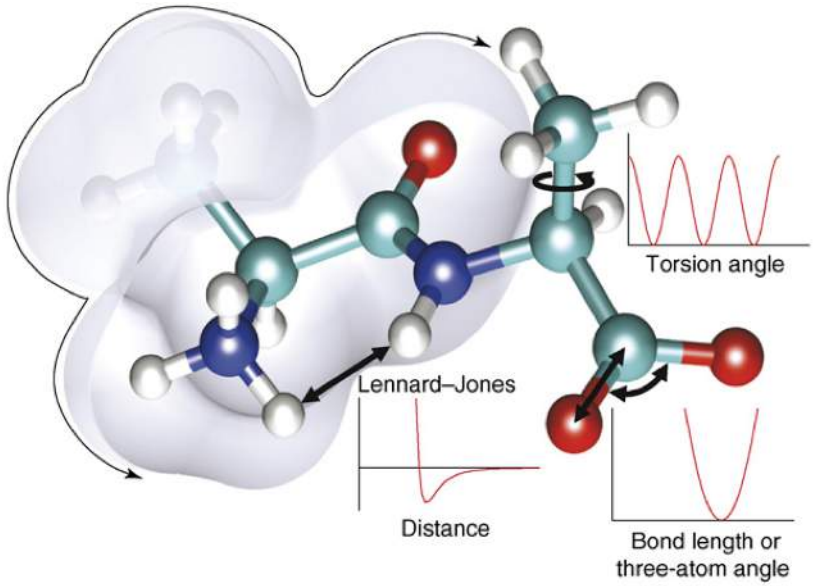

**Minor($\chi_1$ F113= -60°) = 'out' rotamer**
**Major ($\chi_1$ F113= +60°) = 'in' rotamer**

- The previous NMR studies observed the **millisecond internal motions** of CypA during catalysis. These intrinsic motions is also observed in free enzyme characterized as **'major'** and **'minor'** states.

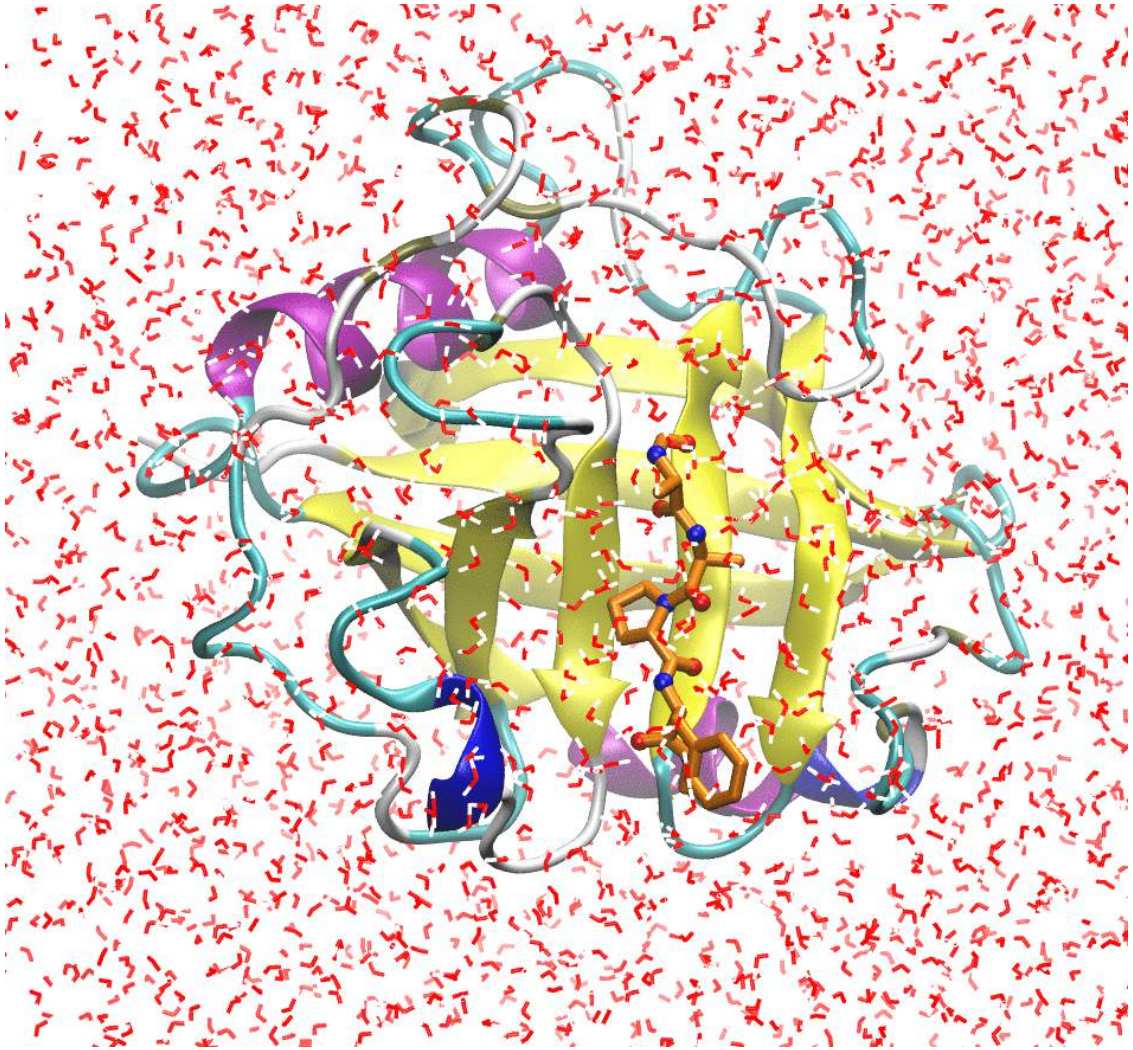
- The S99T mutant increased the population of this minor state
- The S99T mutant showed **a 70-fold reduction in the bidirectional *cis/trans* isomerization rate** of model substrate with respect to WT.

Eisenmesser et al. (2002), Eisenmesser et al. (2005), Fraser et al. (2009)

# Methods



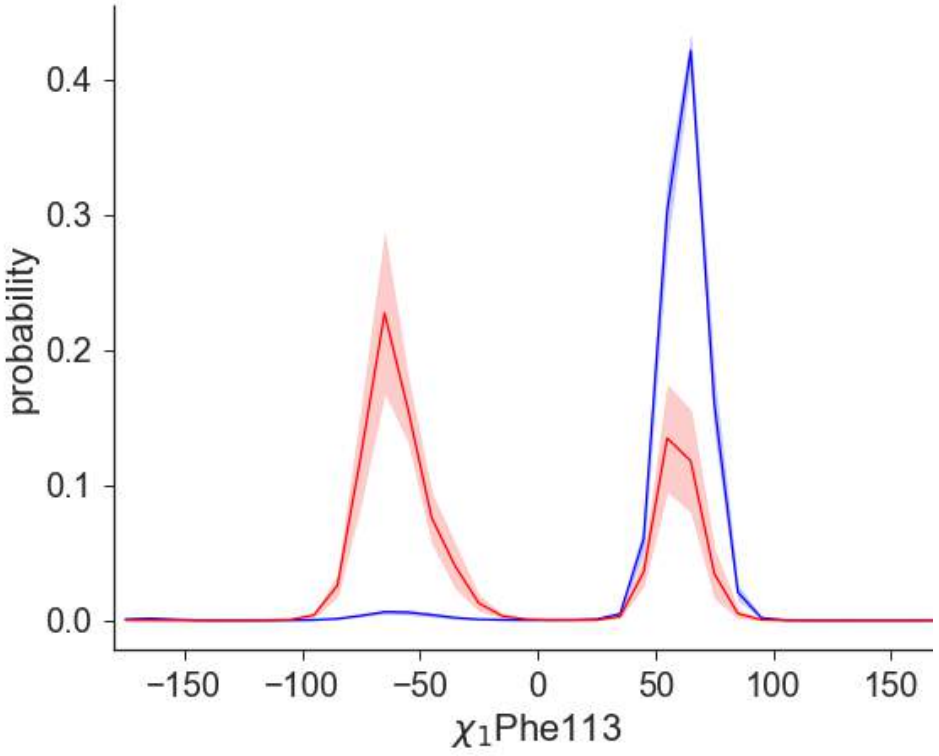

- Calculated the behavior of molecular system
- Based on the classical mechanics

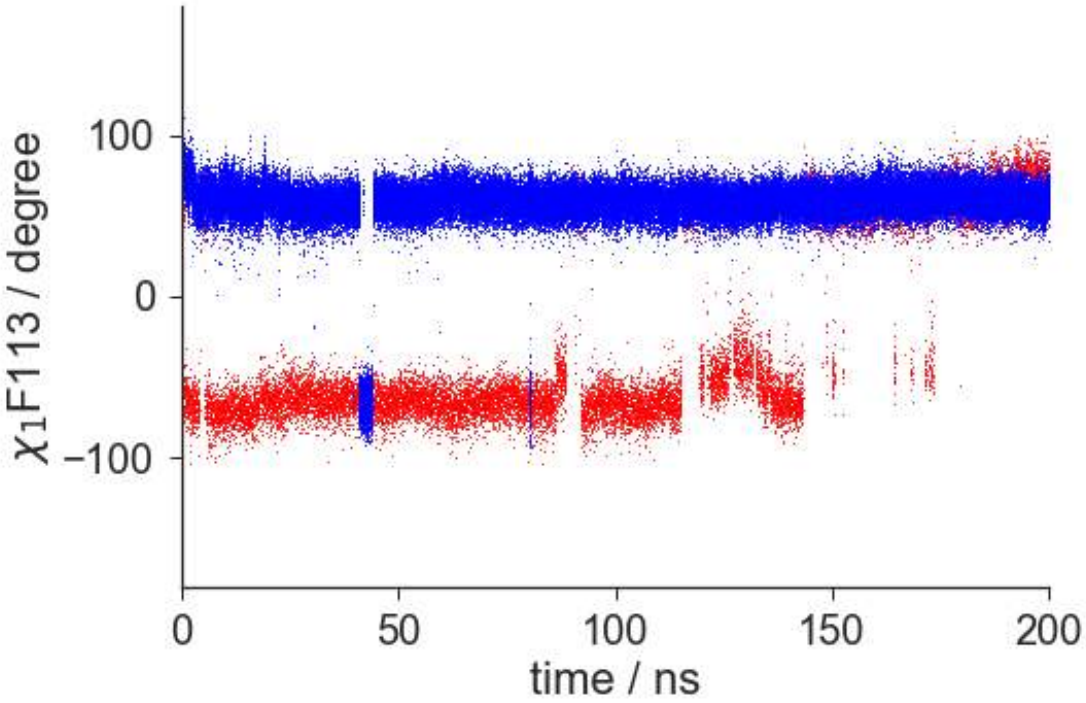


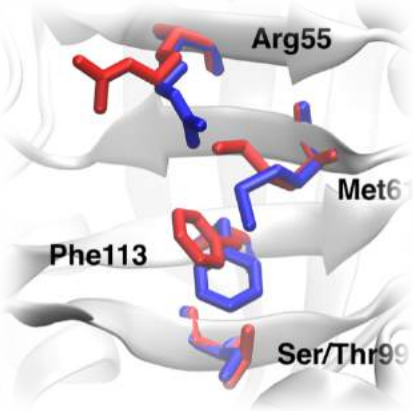
# ‘Major’ and ‘Minor’ CypA Conformations Exchange

the minor ($\chi_1$ = -60°) ‘out’ rotamer
the major ($\chi_1$= +60°) ‘in’ rotamer

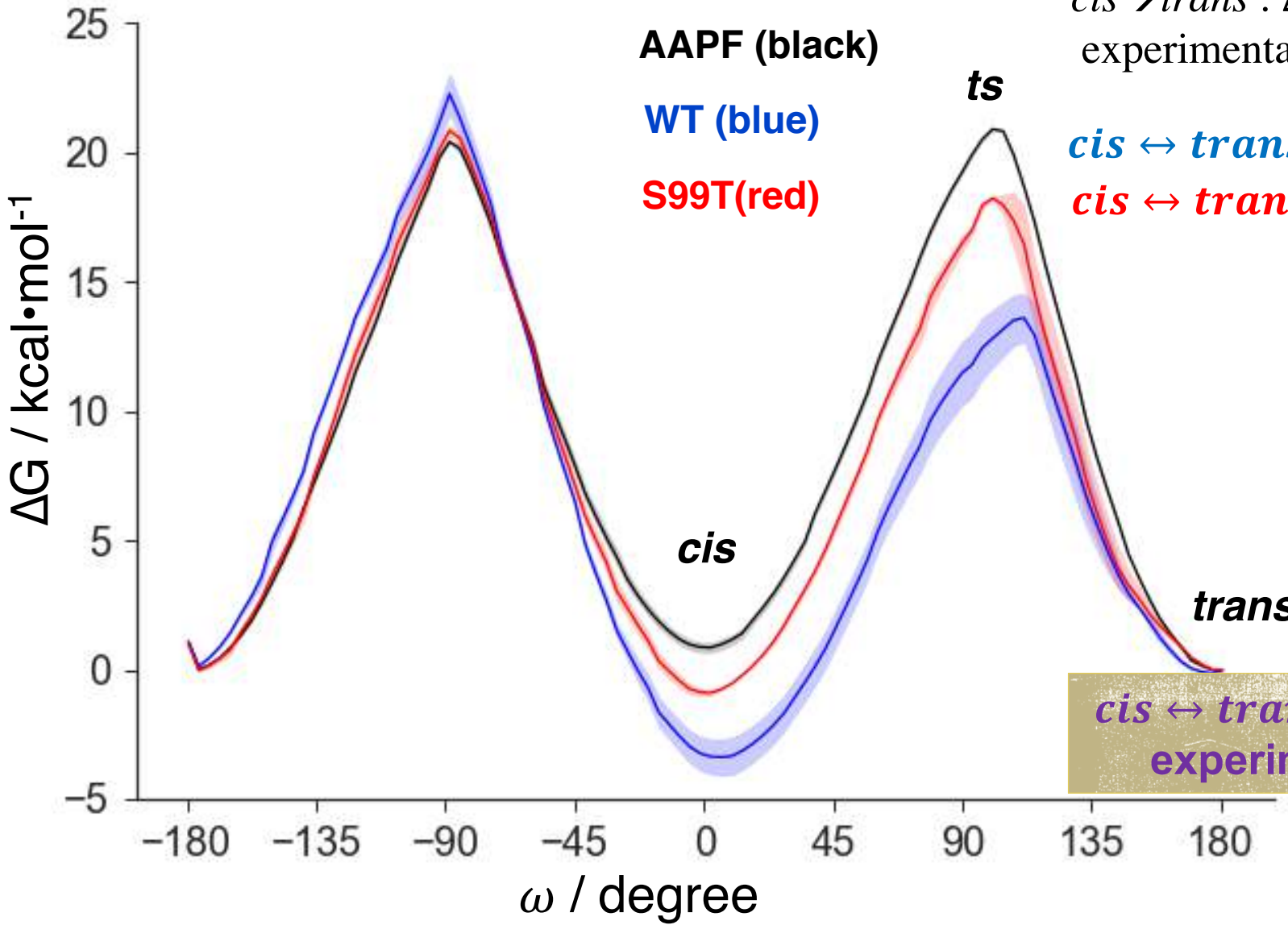
**WT (blue)**
**S99T(red)**



# Nanosecond protein dynamics is sufficient to explain differential catalytic activity




$cis \rightarrow trans$ : $\Delta G_{AAPF}$= 20.1 kcal/mol
experimental data is 19.3 kcal/mol

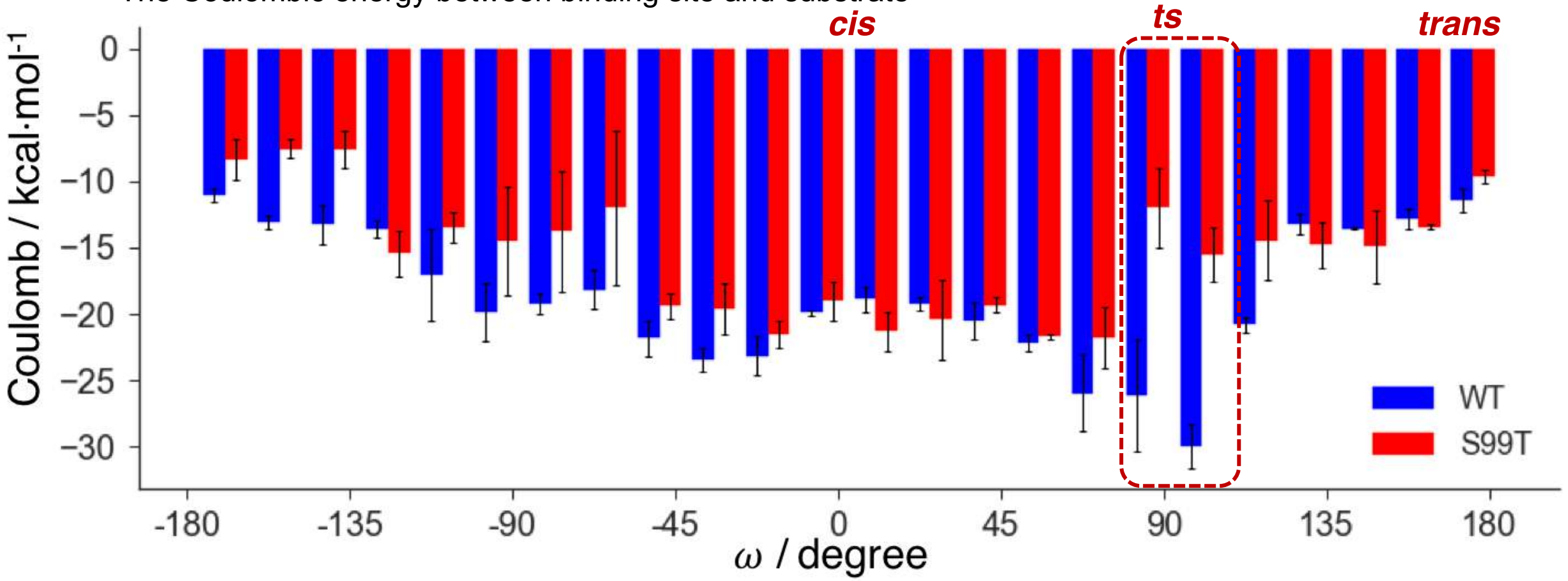
$cis \leftrightarrow trans$: $\Delta G_{AAPF-WT}$ = -5.3 kcal/mol

$cis \leftrightarrow trans$: $\Delta G_{AAPF-S99T}$ = -1.8 kcal/mol

$cis \leftrightarrow trans$: $\Delta G_{S99T-WT} = -3.5\ kcal/mol$
experimental data is -2.3 $kcal/mol$

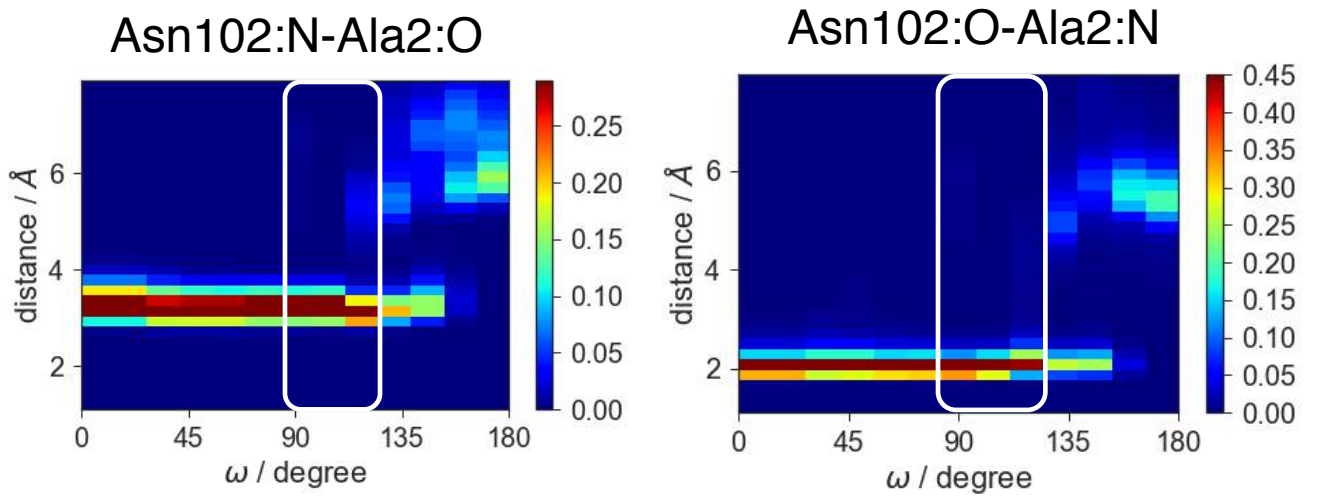
# Transition Destabilization in S99T Mutant

The Coulombic energy between binding site and substrate

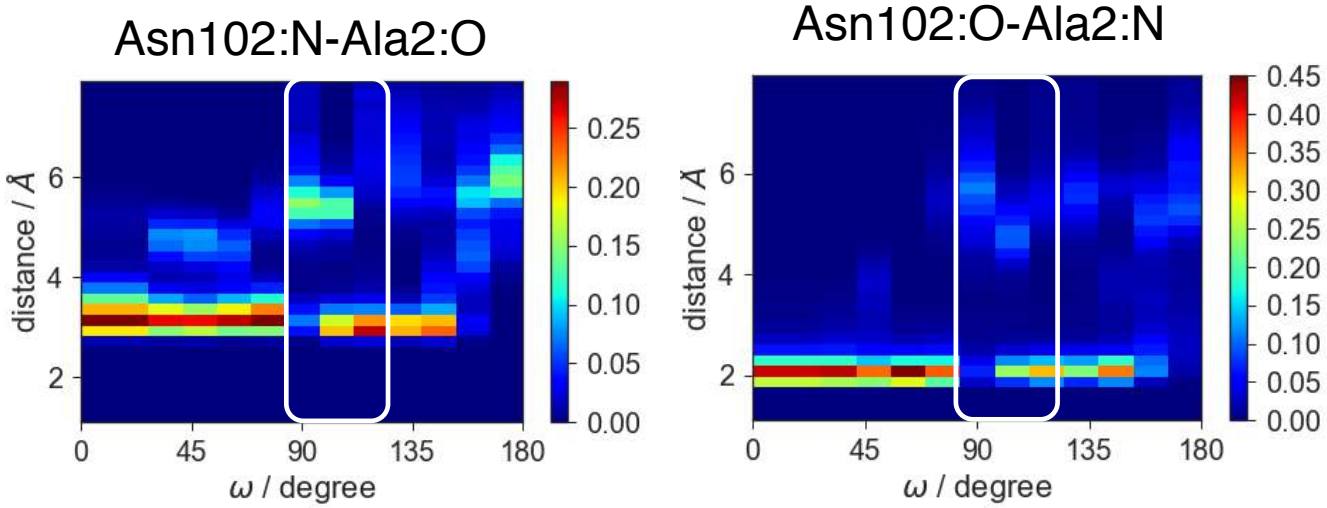



**S99T decrease the transition state stabilization from the weaker electrostatic interaction with binding site**

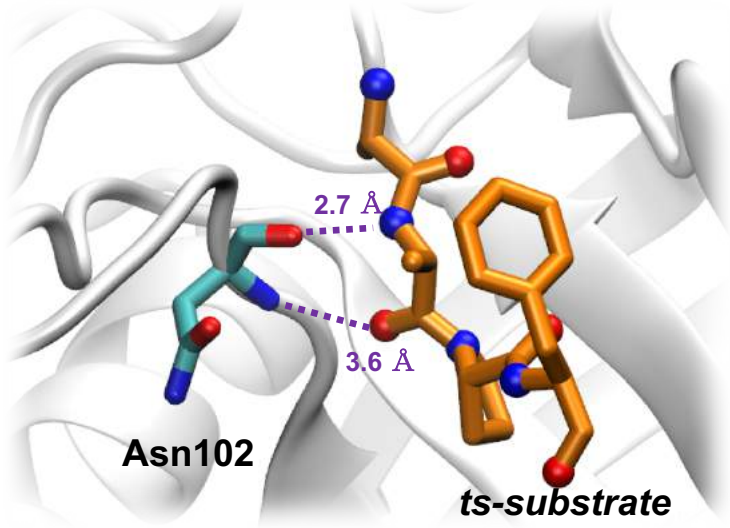
# Decreased hydrogen-bonding interactions of S99T

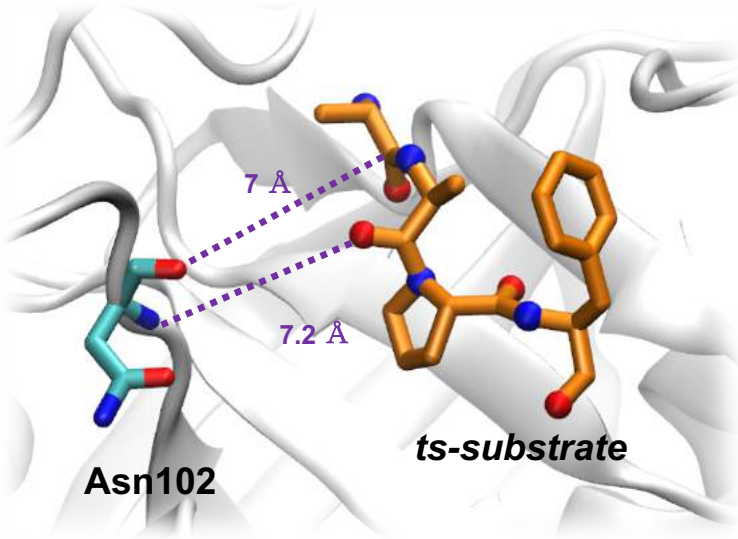



**WT+AAPF**




**S99T+AAPF**

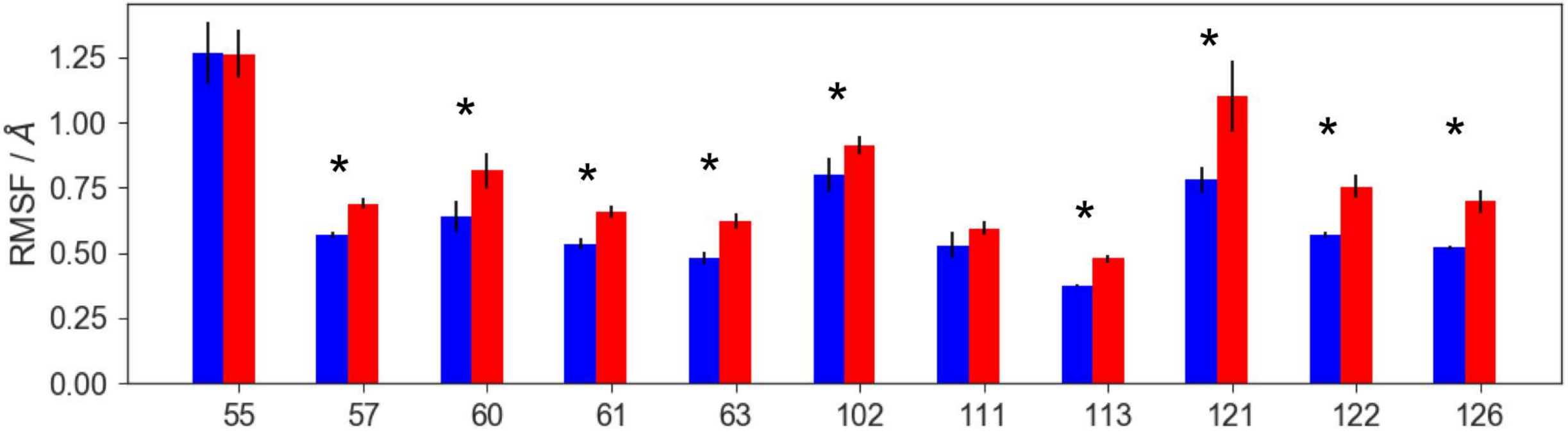


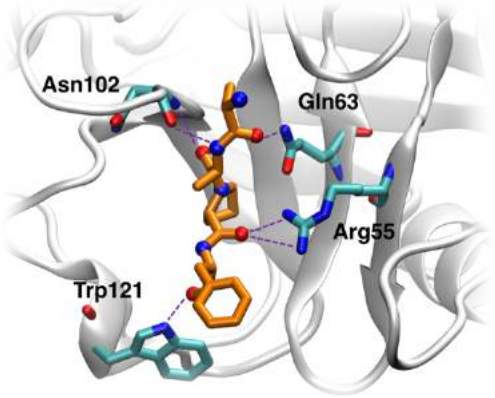



# S99T increases fast dynamics of active site residues

WT (blue)
S99T(red)

RMSFs are calculated on all heavy atoms




**A destabilization of the transition state configuration of the substrate in S99T is due to an increased side chain flexibility of active site residues.**

# Conclusion

- The fast conformational exchange of the S99T mutant from the minor to the major state was observed at a nanosecond time scale.
- Free energy profiles show an increase in activation energy for the S99T mutant to catalyze the isomerization reaction compared to that of the WT system.
- The decreased catalytic activity of the S99T mutant is a result of weakened hydrogen bonding interactions between Asn102 and the transition state conformations of the substrate.
- The weakened transition state stabilisation in S99T is due to an overall increase in fast (nanosecond) dynamics of active site residues.

# Acknowledgements

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aListery

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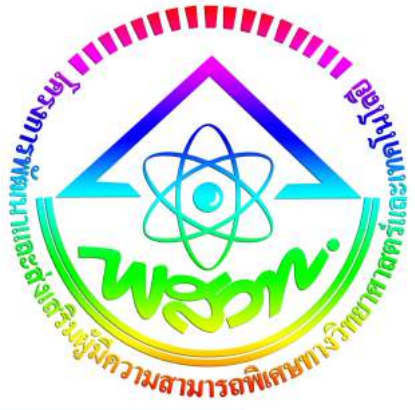
Hipster Alchemist

Greek energizer

Fragment-ed

Spin Doctor

CycloPatlins

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