



Faculty of Science,  
University of Zagreb

# **Allosteric Regulation and Structural Dynamics of Thermostable L-Lactate Dehydrogenase: Insights from Molecular Dynamics Simulations and Experimental Analysis**

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Computational Chemistry Day 2025

Zagreb

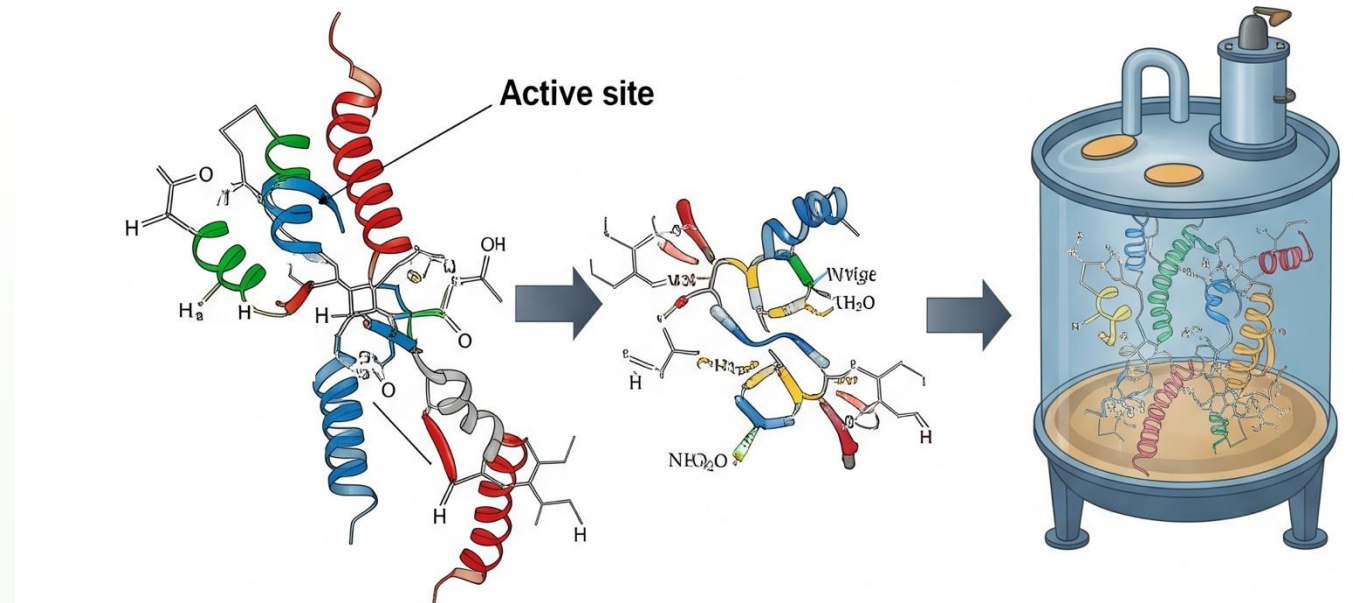
# Outline

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2. Allostery
3. Molecular dynamic simulation captures the dimerization process
4. Kinetic analysis
5. Activator-induced conformational change
  - Overall structure
  - Substrate binding (pyruvate)
  - Cofactor binding (NADH)
6. Single point mutant Q189L
7. Summary

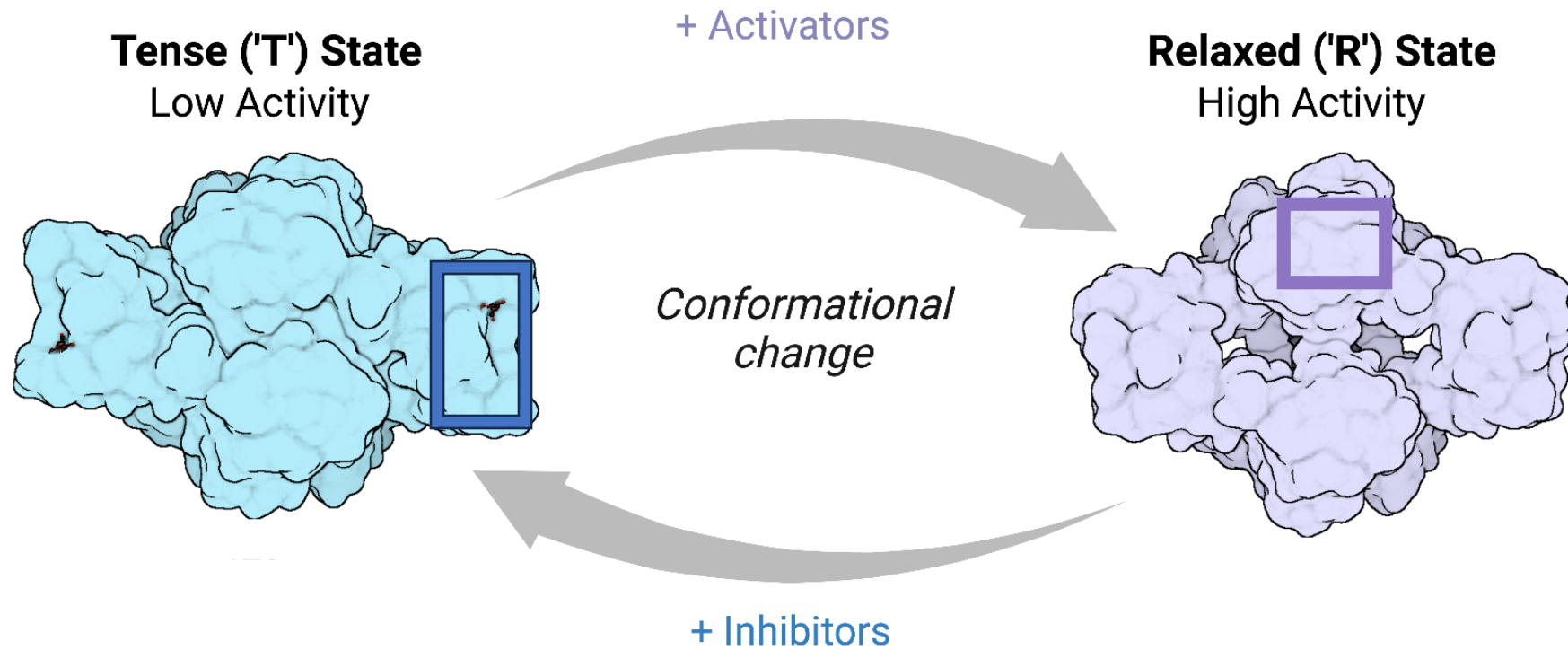
# Introduction

## Thermostable *Geobacillus stearothermophilus* LDH

- A standout biocatalyst in industrial biotechnology due to its unique combination of **thermostability**, **catalytic efficiency**, and **adaptability**.
- Operational Stability and Reusability
- Versatility in Biocatalytic Applications
- Cost-Effective Production

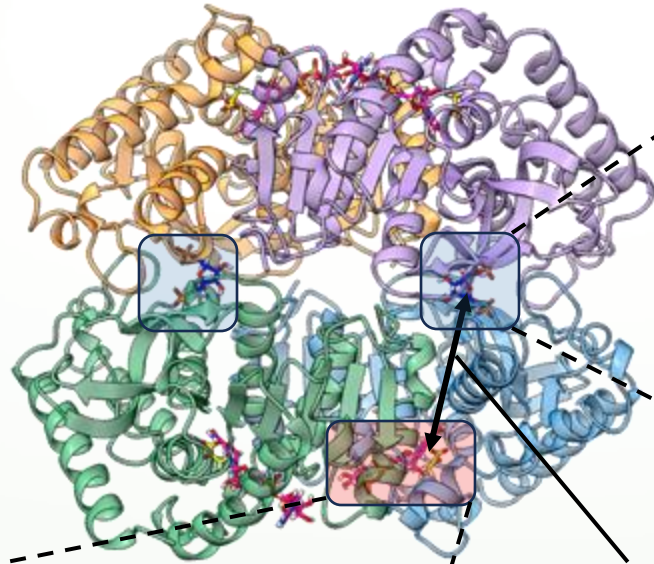


# Allostery

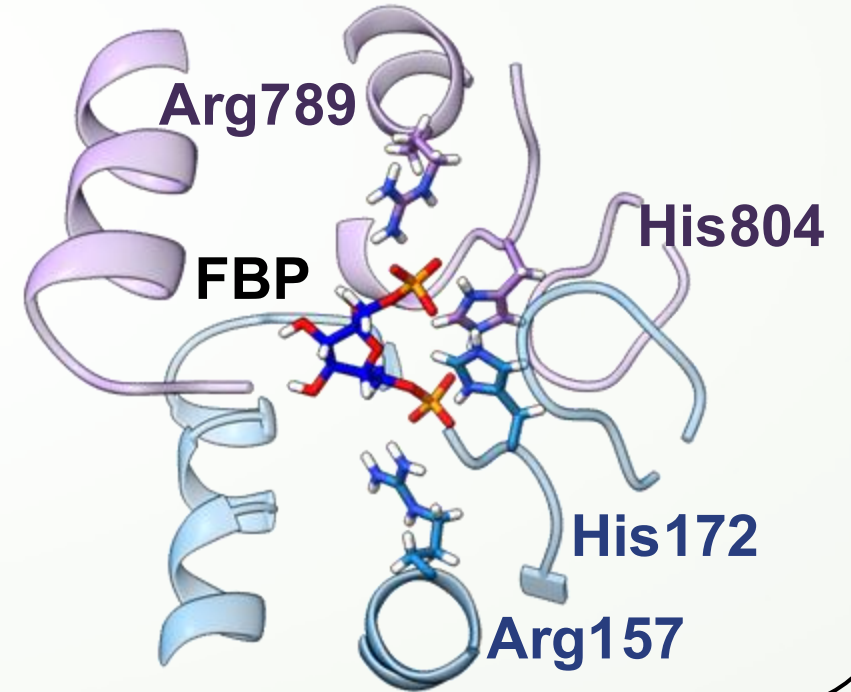


# Lactate Dehydrogenase (LDH)

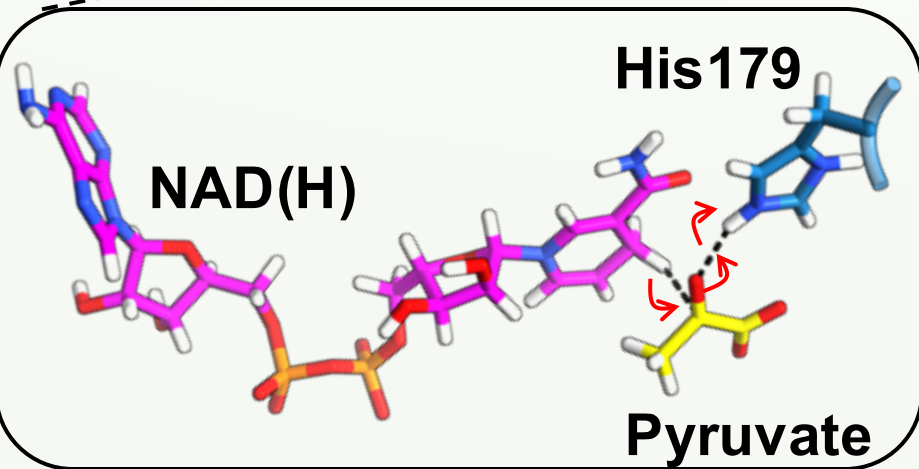
*Geobacillus stearothermophilus* (GsLDH)



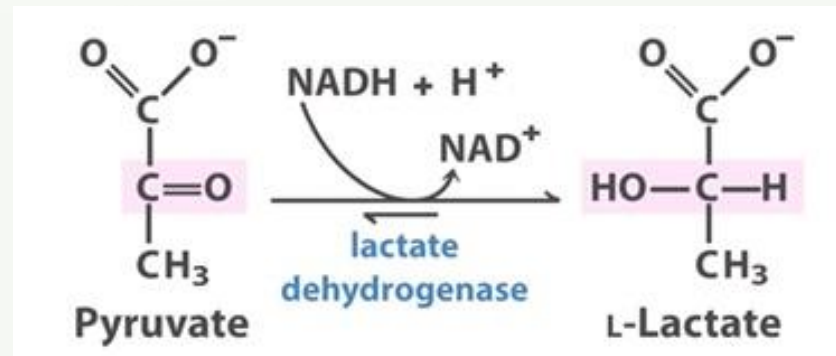
22.9Å



**FBP binding site**  
(Fructose 1,6-bisphosphate)



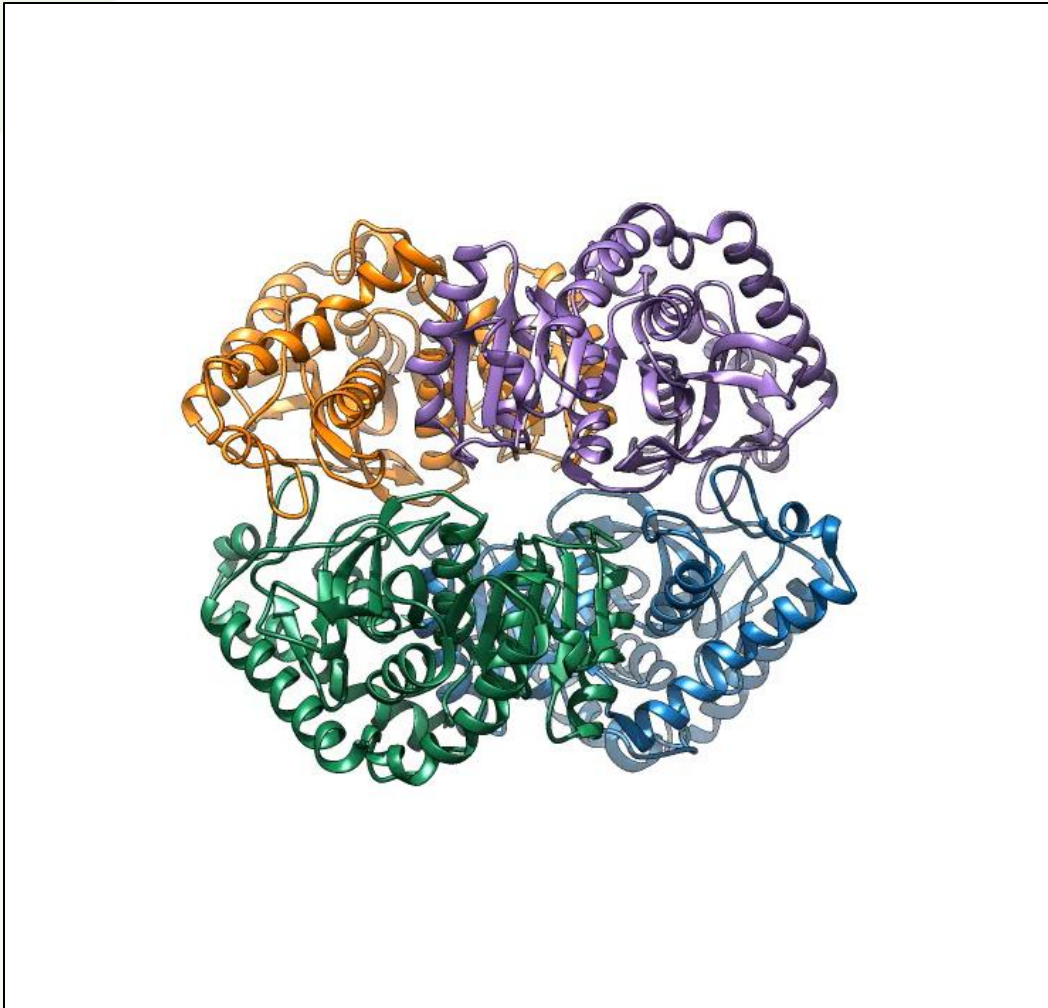
**Catalytic pocket**



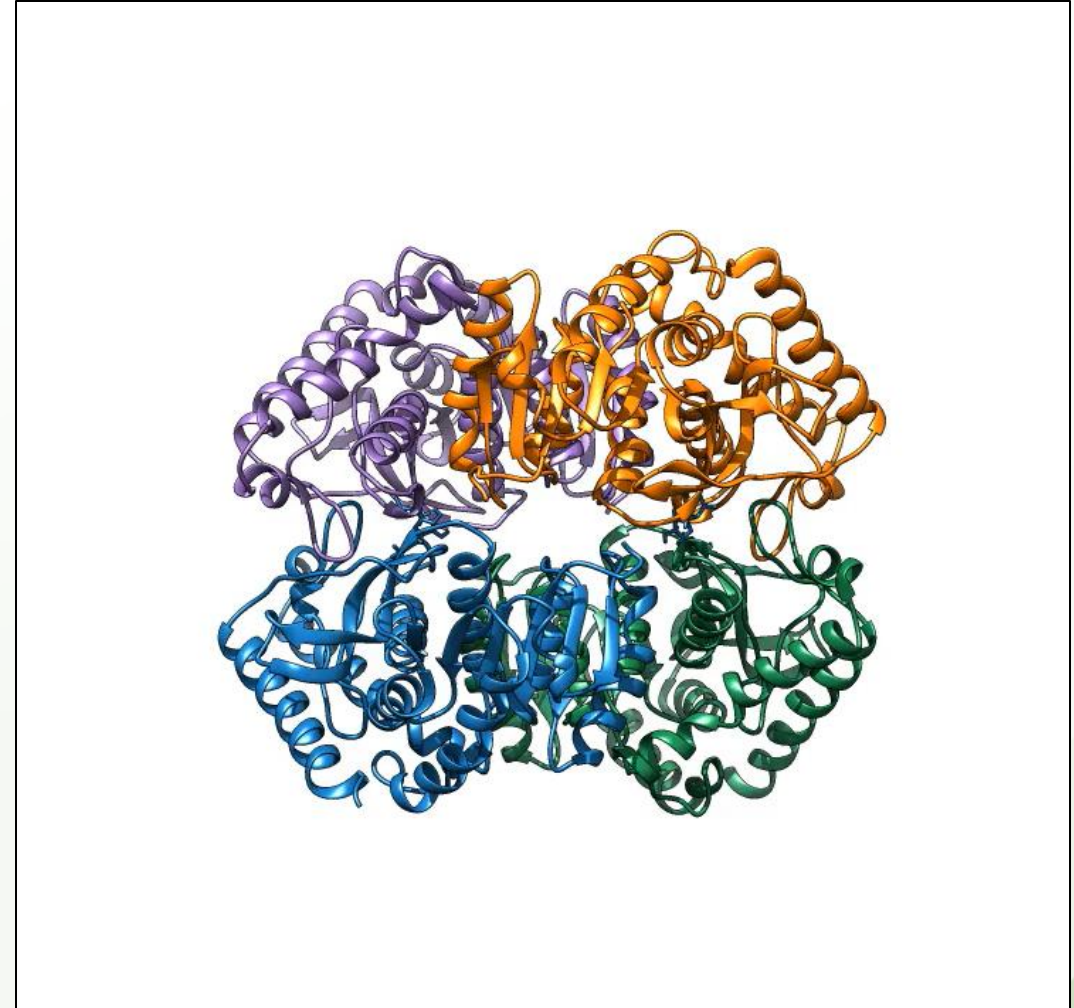


# MD simulation captured oligomeric state change

WT GsLDH without FBP



WT GsLDH with FBP

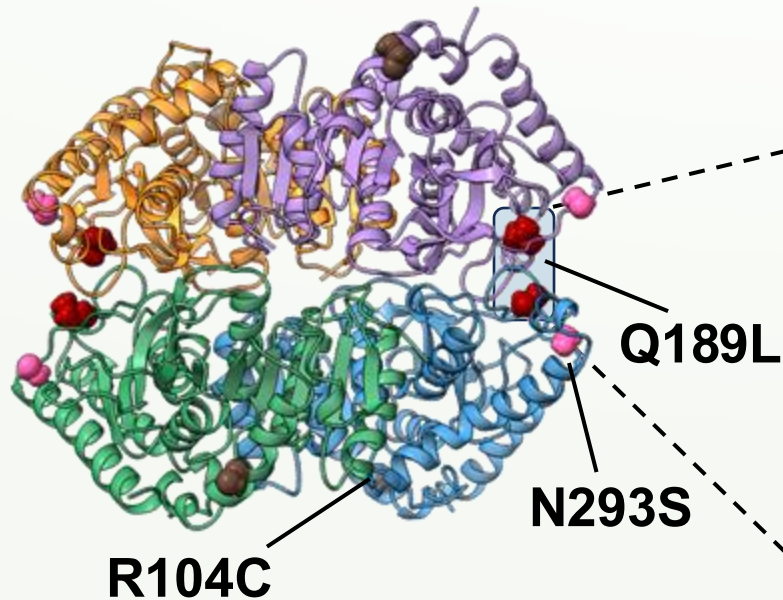




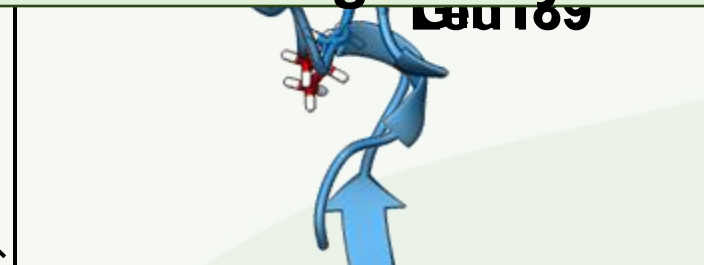
# The dual role of FBP

GsLDHs	Specificity constant $k_{cat}/K_m$ ( $s^{-1}mM^{-1}$ )		Oligomeric state
	-FBP	+FBP	
Wild type	$35 \pm 13$	$144 \pm 67$	Dimer/Tetramer
Triple mutant	$141 \pm 18$	$156 \pm 59$	Tetramer
Q189L	$32 \pm 4$	$106 \pm 19$	Tetramer

R104C/Q189L/N293S ←



**Tetrameric structure is not enough to fully restore catalytic efficiency. The binding of FBP or introduced mutations are needed to induce specific conformational changes to govern the high enzyme activity.**



# **FBP-induced conformational change**

## **Overall structure**

**RMSF**

**Dihedral  
angles**

## **Pyruvate binding**

**H-bond**

**Binding  
affinity**

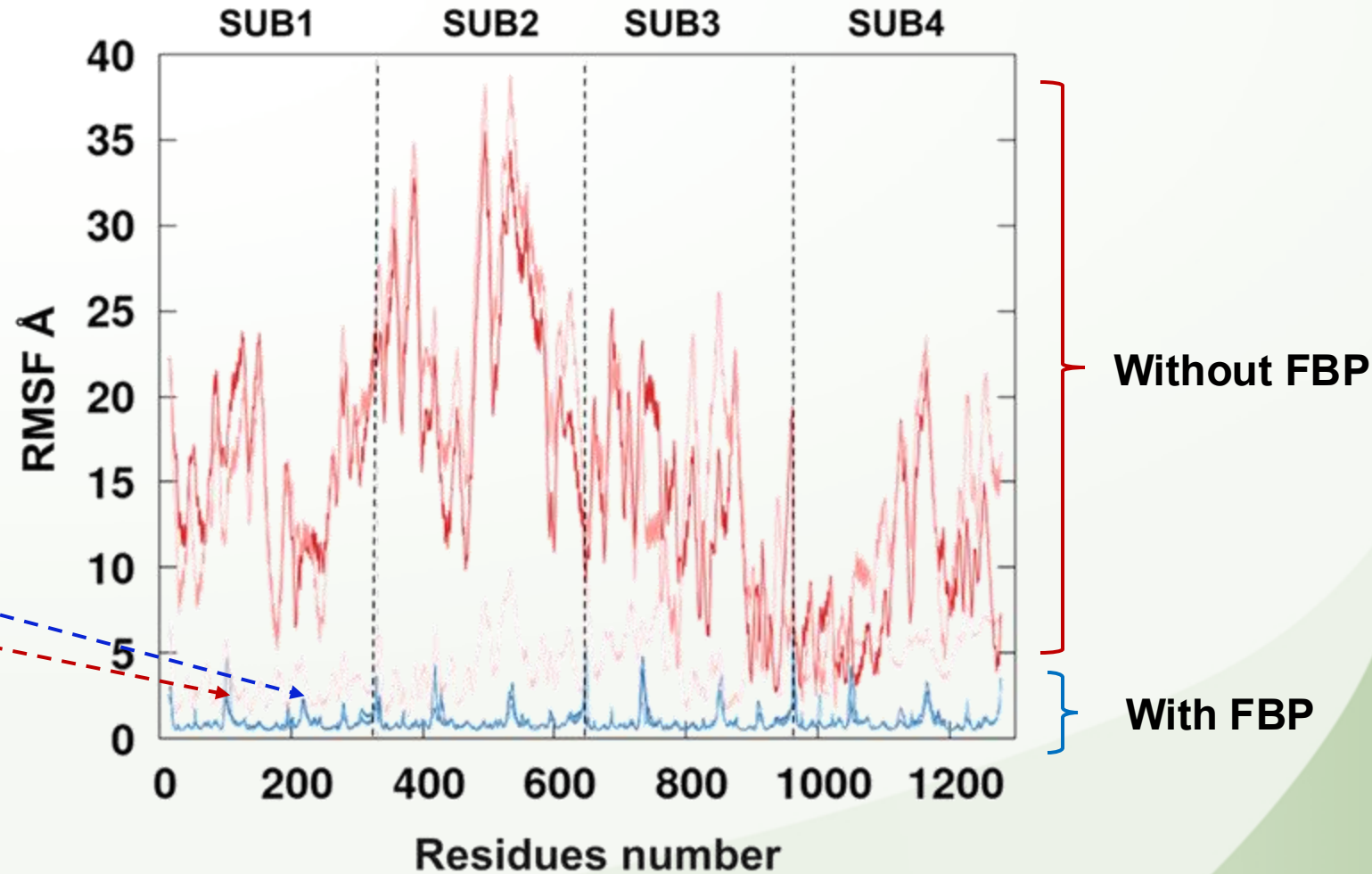
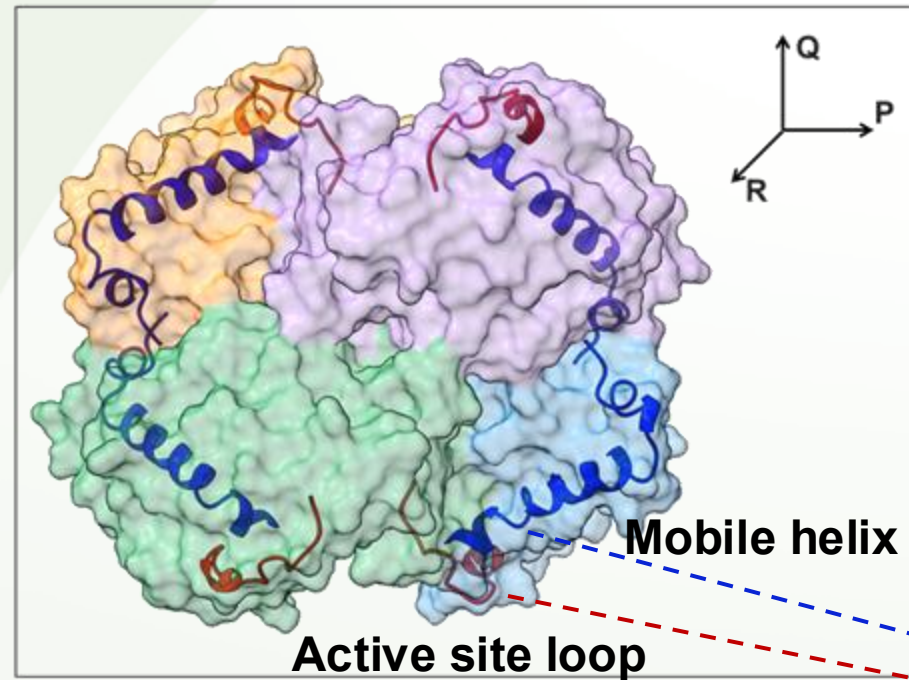
## **Cofactor binding**

**ITC experiment**



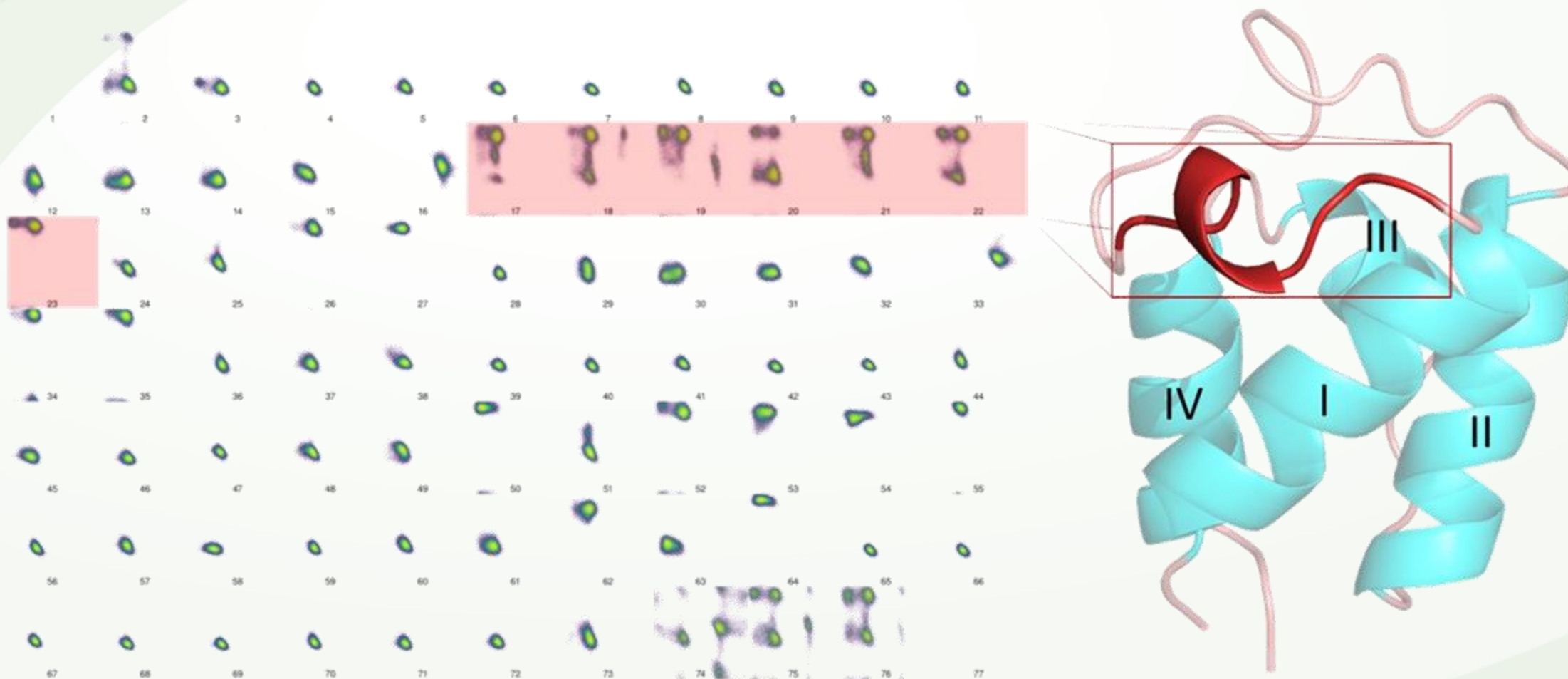
# High residues fluctuations without FBP

Backbone (C $\alpha$ ) Root Mean Square Fluctuation (RMSF)



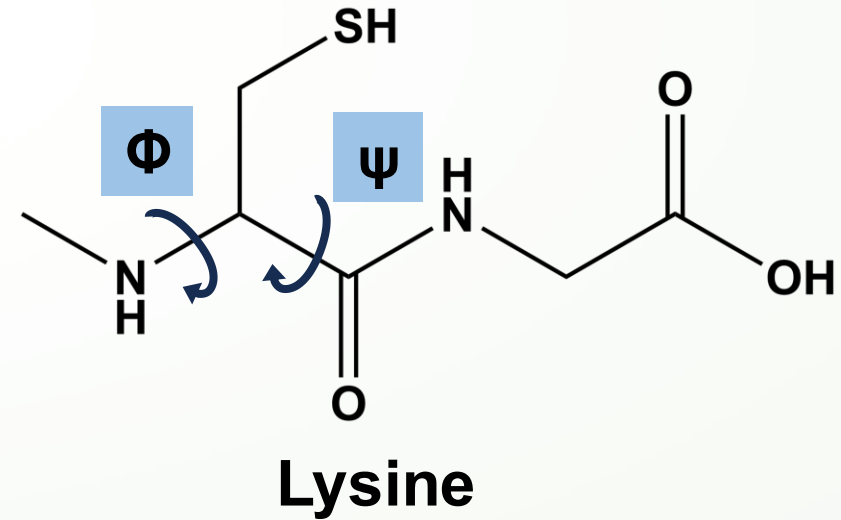
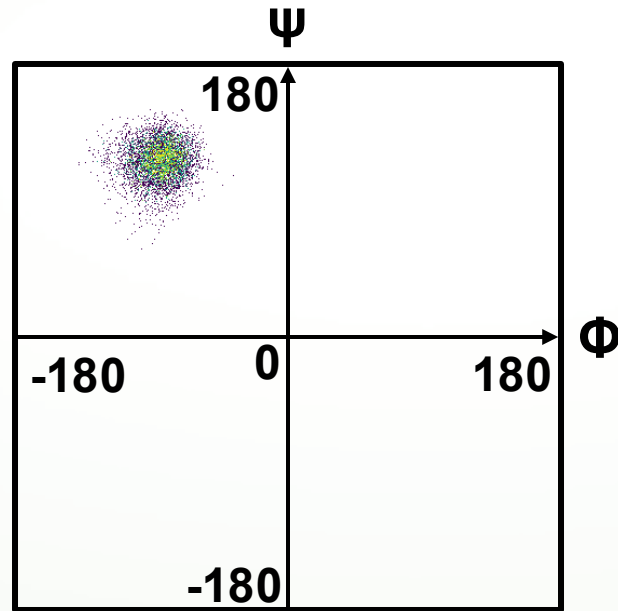
Higher RMSF value  $\Rightarrow$  Higher flexibility

# MDavocado: Fast Screening for Dihedral Angles

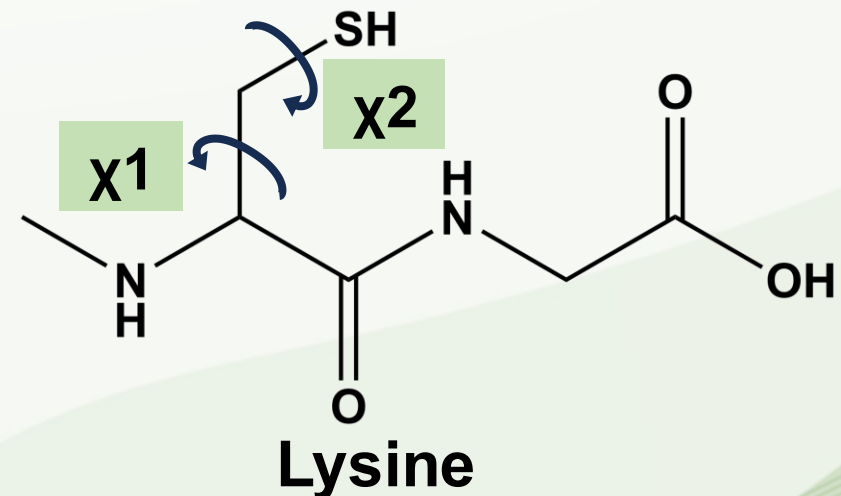
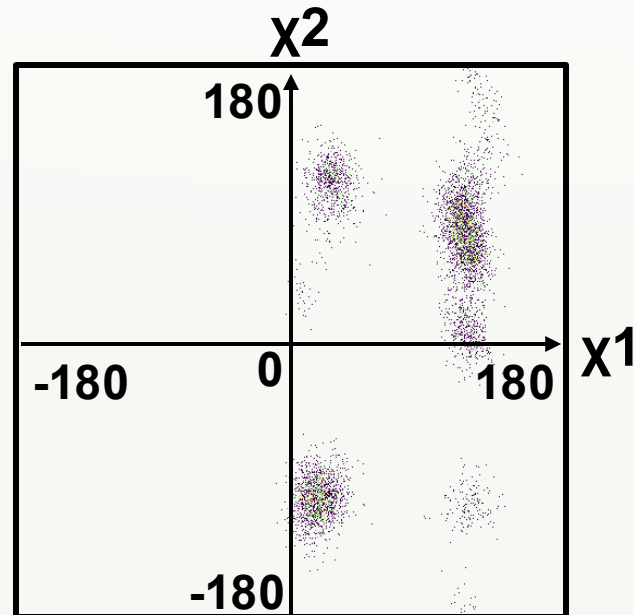


# Dihedral angles (backbone and side chain)

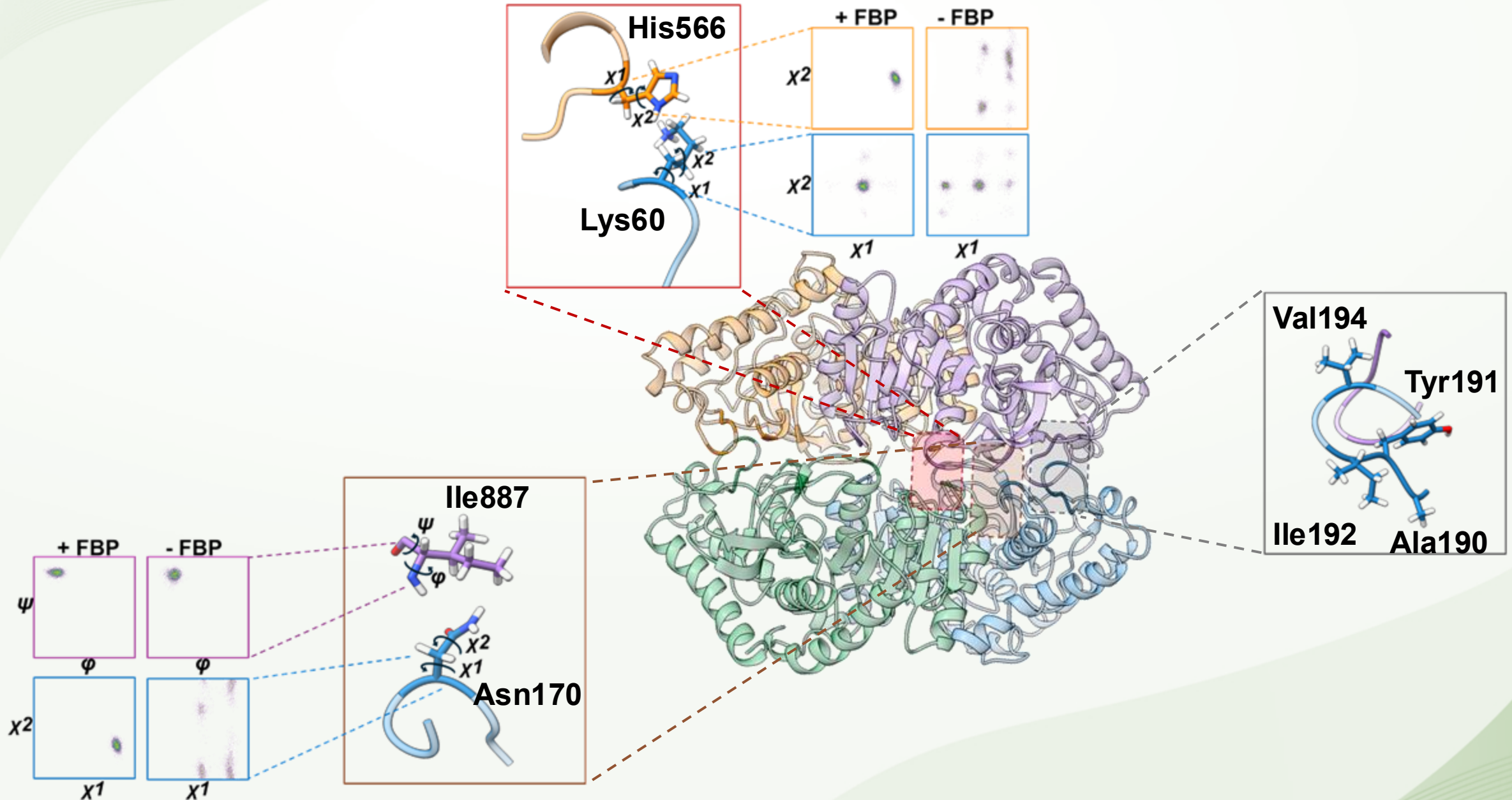
Backbone  
dihedral angles



Side chain  
dihedral angles

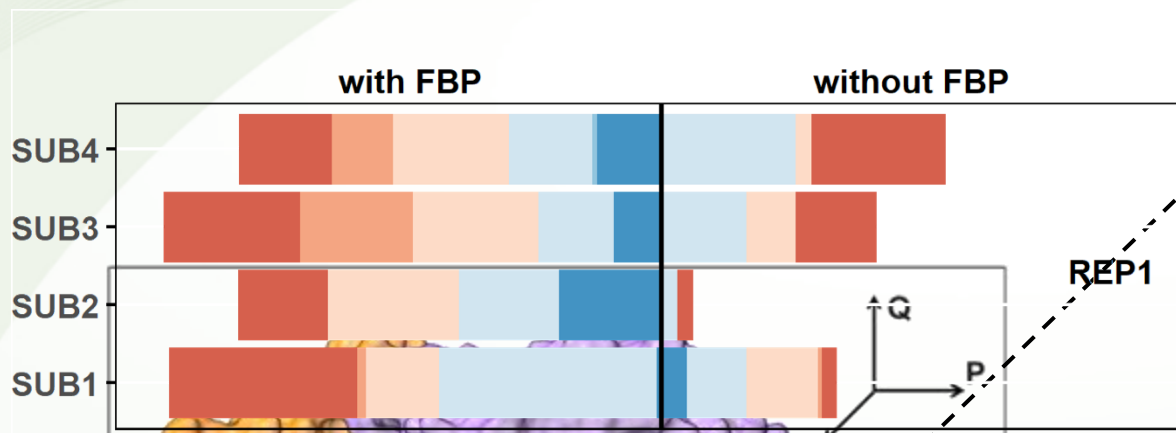


# MDavocado identified three critical regions

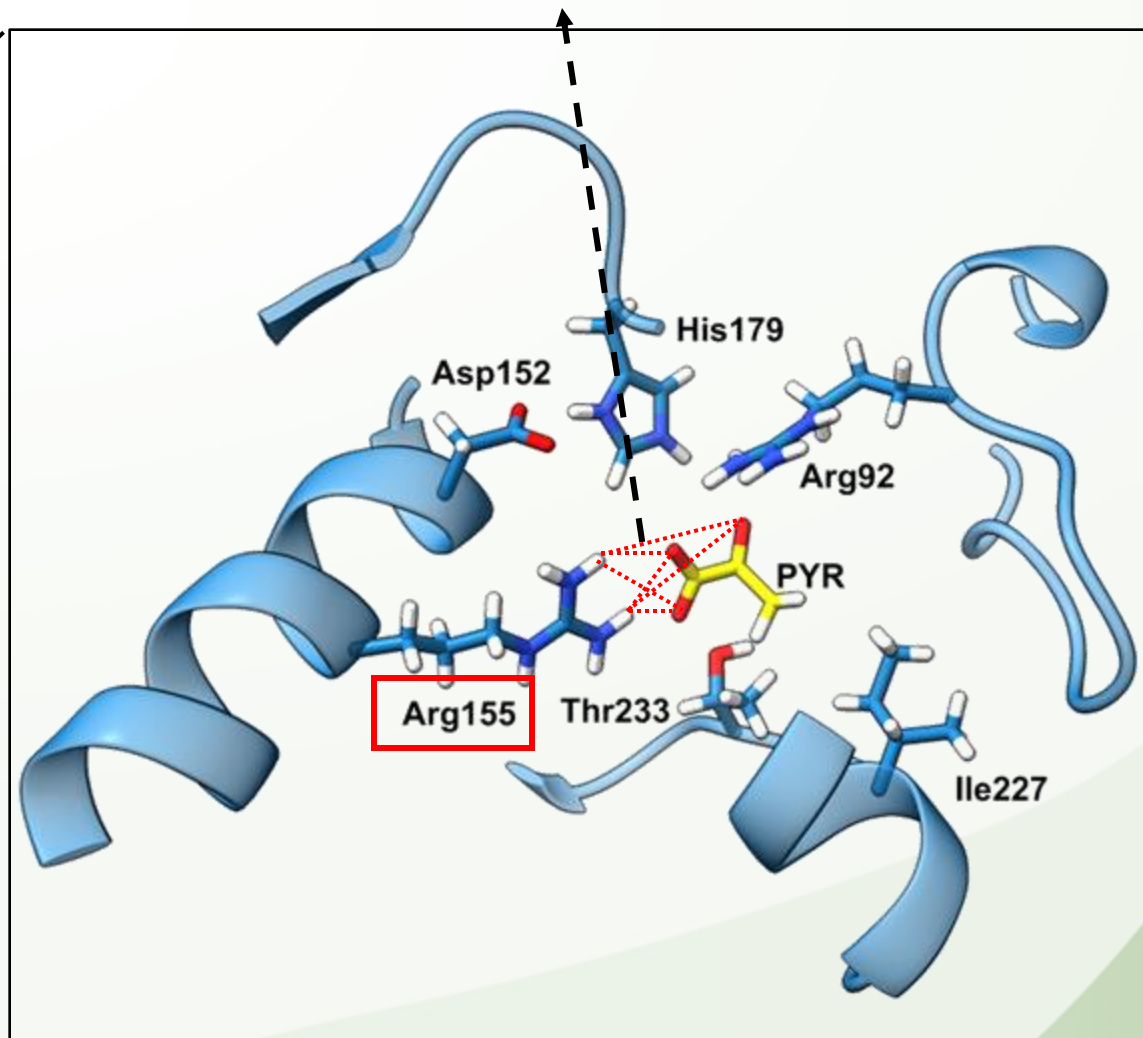




# FBP promotes and stabilizes H-bonds



Six possible H-bonds between Arg155 and PYR shown in different colors :

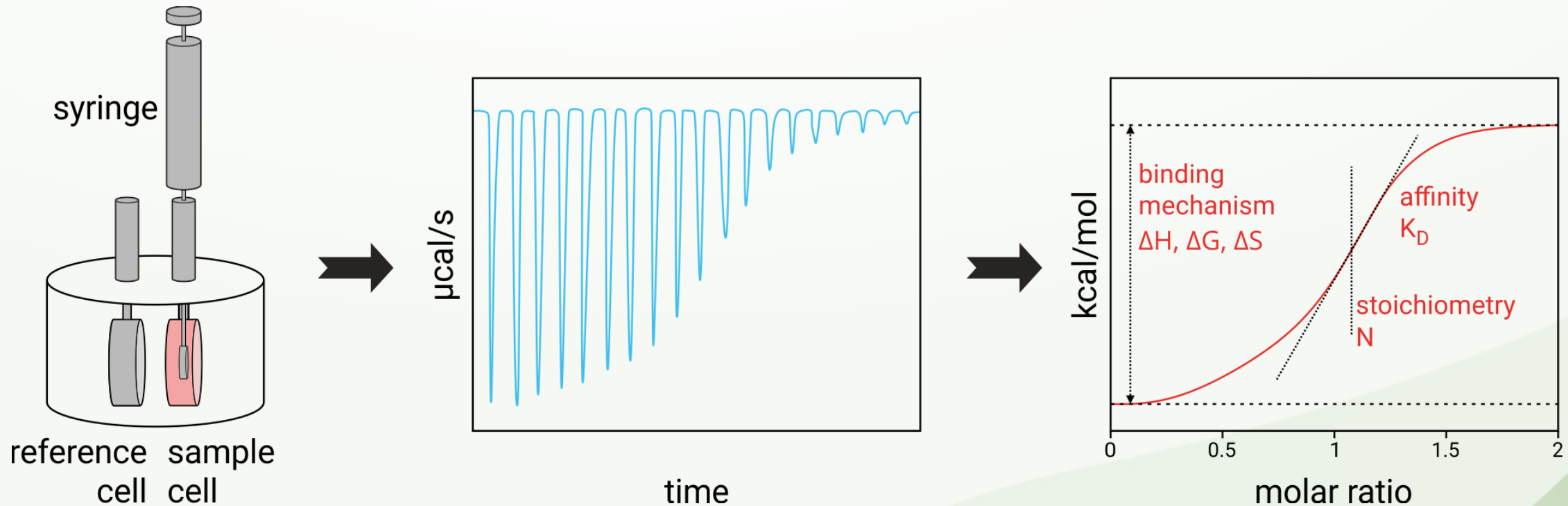




# Microcalorimetry titration studies

## Isothermal titration calorimetry (ITC)

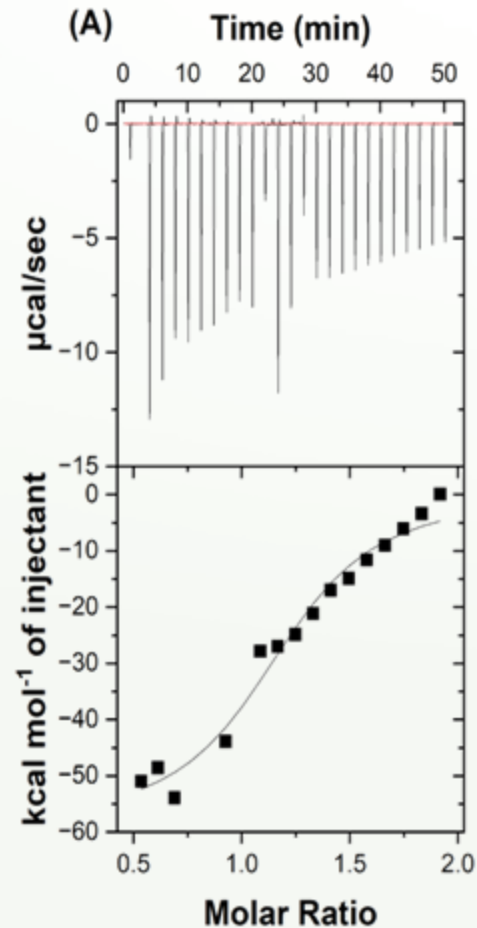
The principle is the direct measurement of the heat change that occurs when two molecules interact.



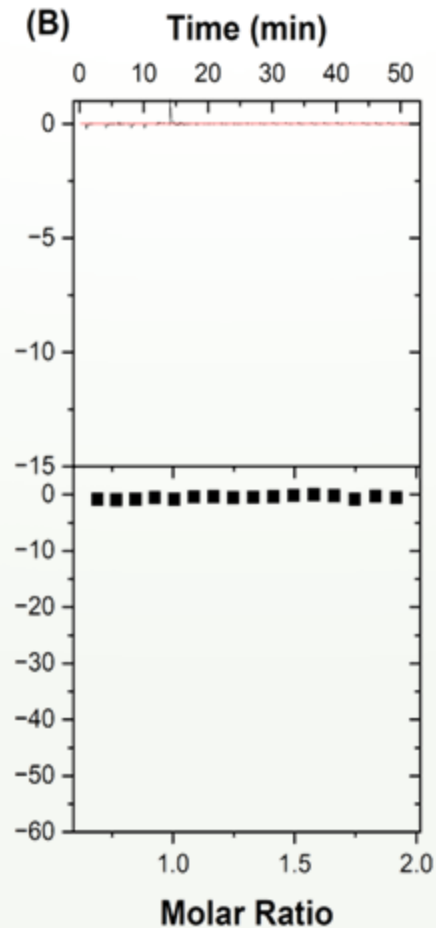
# FBP enhances cofactor binding

GsLDH titrated with NADH

+FBP

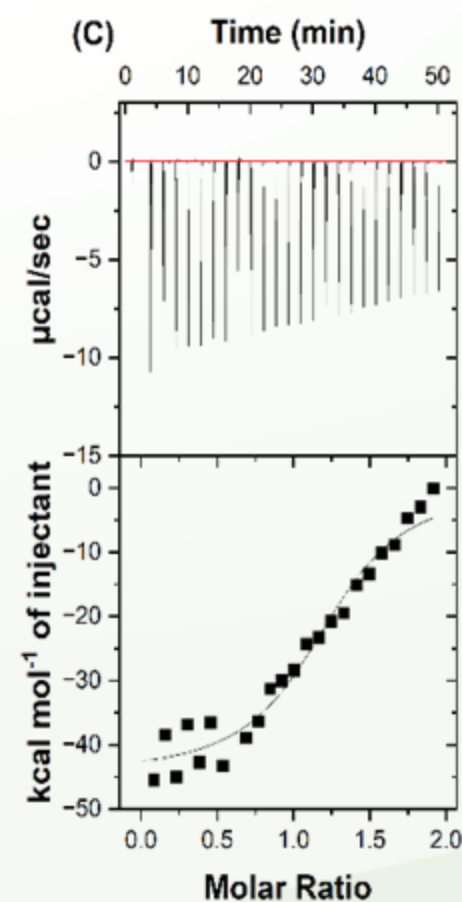


-FBP

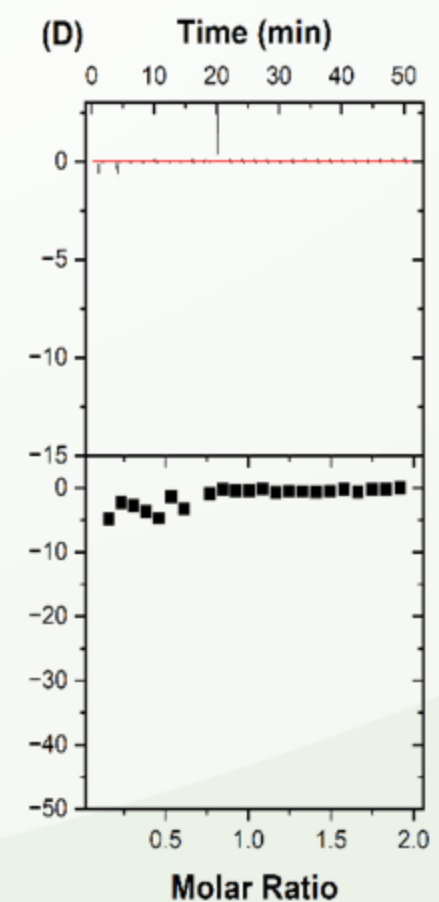


GsLDH titrated with NAD<sup>+</sup>

+FBP

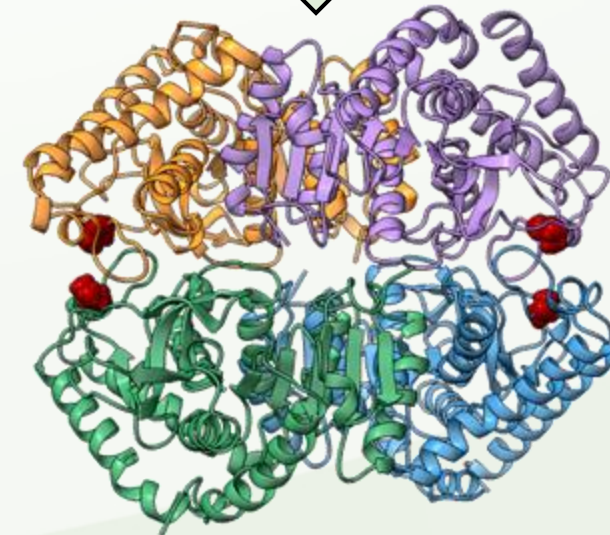


-FBP



# Q189L maintains tetramer without FBP

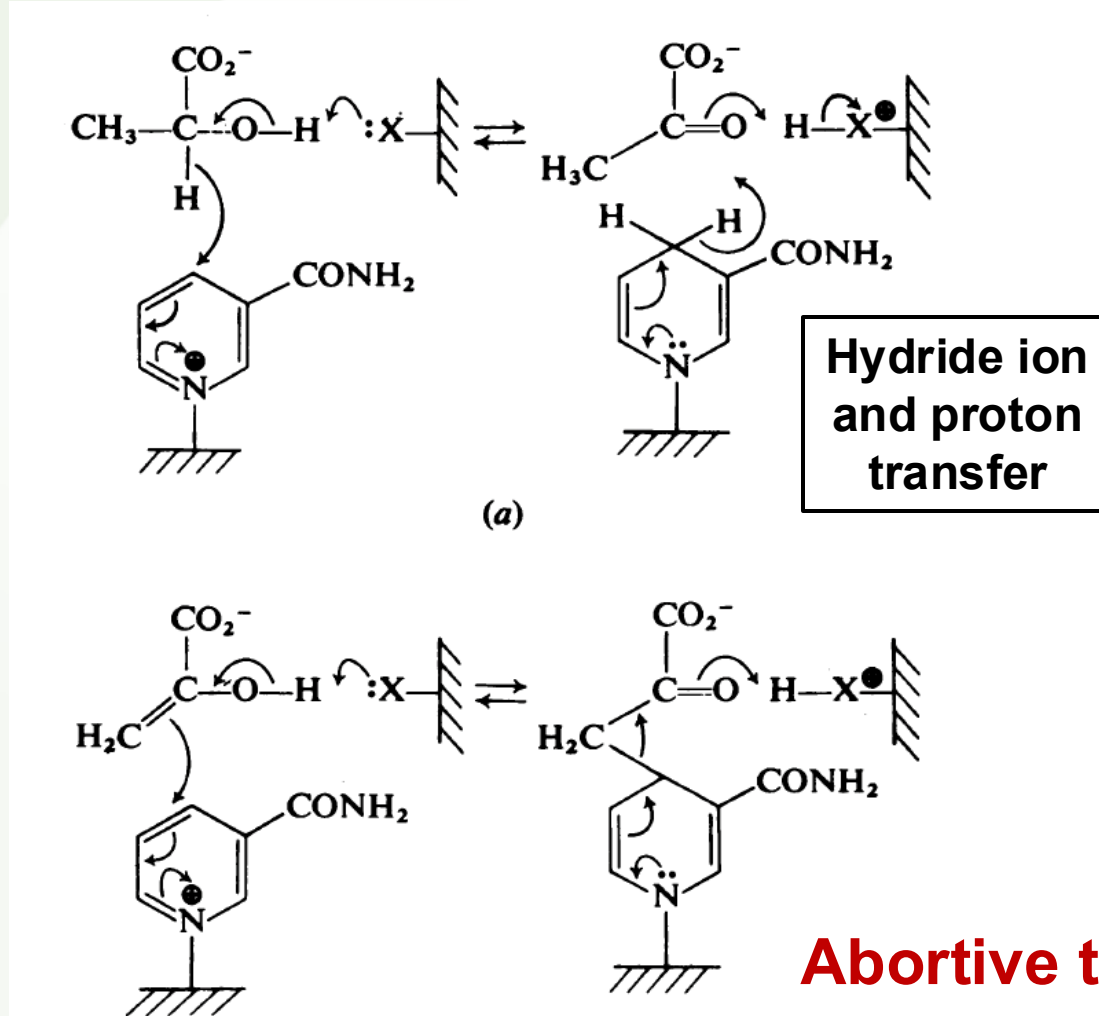
GsLDHs	$k_{cat}/K_m$ ( $s^{-1}mM^{-1}$ )		$K_i$ (mM)		Oligomeric state
	-FBP	+FBP	-FBP	+FBP	
Wild type	$35 \pm 13$	$144 \pm 67$	ND	$3.5 \pm 0.6$	Dimer
Triple mutant	$141 \pm 18$	$156 \pm 59$	ND	$1.4 \pm 0.4$	Tetramer
Q189L	$32 \pm 4$	$106 \pm 19$	ND	$0.3 \pm 0.1$	Tetramer



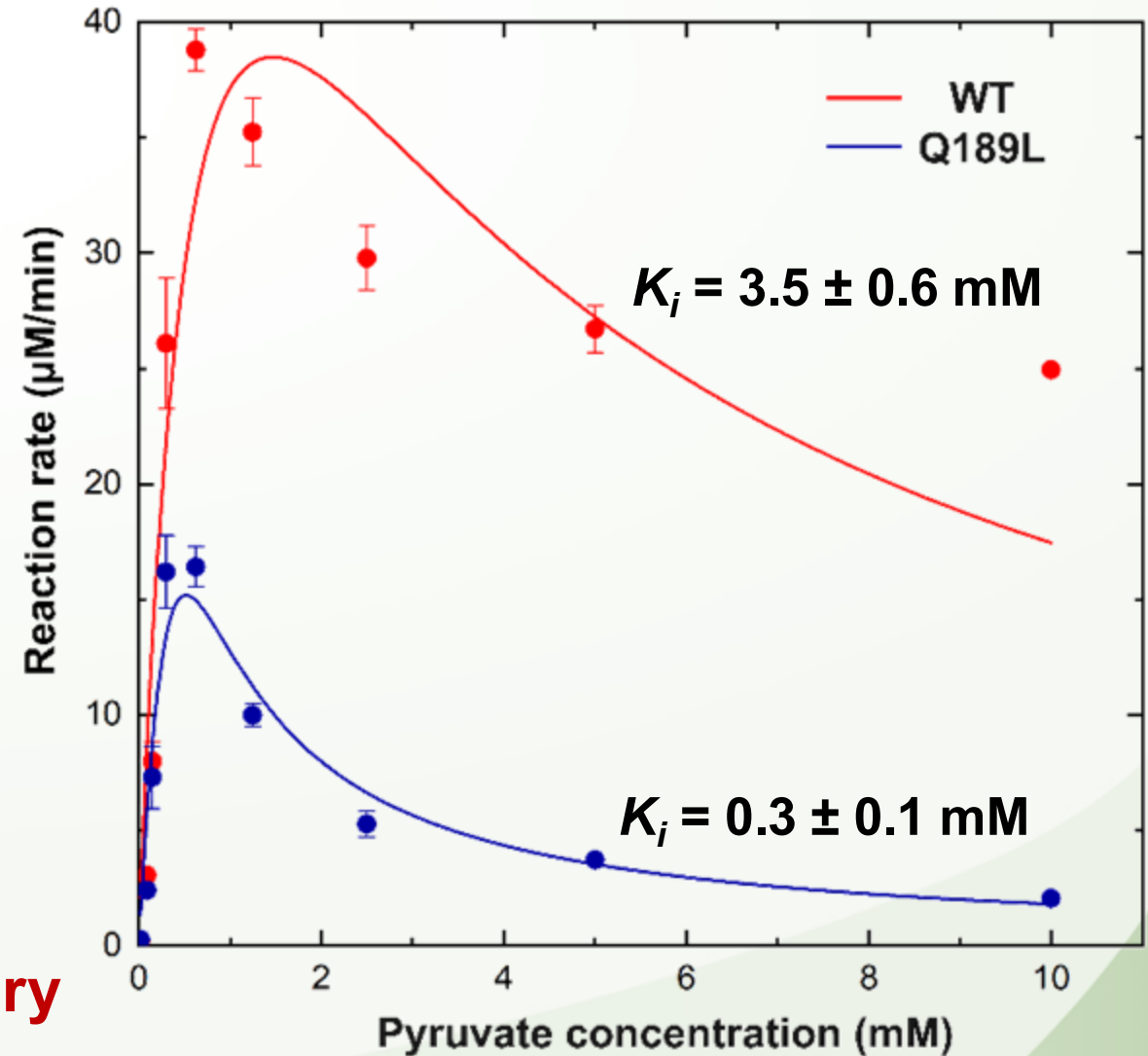
**Q189L**

- No improved activity without FBP.
- Tetramer without FBP.
- Enhanced substrate inhibition in the presence of FBP.

# Q189L enhances substrate inhibition



**Abortive ternary complex**



# Summary

- GsLDH tetramerization alone is insufficient for achieving allosteric regulation. Specific conformational changes initiated by FBP are essential.
- FBP stabilizes key residues within the pyruvate binding site and affects three critical regions on the dimer-dimer interface. It is also crucial for cofactor binding affinity.
- The single-point mutant Q189L can retain the tetrameric structure of GsLDH without FBP but does not exhibit allosteric behavior. Interestingly, the presence of FBP with the Q189L mutation results in high substrate inhibition, which will be further investigated.



# Acknowledgments



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# Integrating Computational IR Spectroscopy and Principal Component Analysis for Monitoring Mechanoenzymatic Transformation of Glycolic Acid

Zrinka Pišonić, Jakov Borovec, Tomica Hrenar and Aleksandra Maršavelski

## Integrating computational IR spectroscopy and principal component analysis for monitoring mechanoenzymatic transformation of glycolic acid

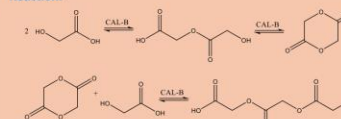
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### Introduction:

Poly(glycolic acid) (PGA) is a biodegradable, biocompatible polymer with significant promise in biomedical and sustainable materials [1]. Conventionally synthesized through high-temperature, metal-catalyzed processes, PGA production typically involves esterification of glycolic acid followed by cyclization to glycolide and ring-opening polymerization [2]. However, these methods pose environmental and scalability concerns. An emerging alternative is mechanoenzymatic synthesis using *Candida antarctica* lipase B (CALB) under solvent-free conditions, offering a greener route.

### Reaction:



### Experimental setup:

- Glycolic acid, immobilized CALB, silica
- Vortex mixing conducted under vacuum
- Silica added for water absorption, driving equilibrium forward



### Monitoring:

The reaction was monitored using attenuated total reflection infrared (ATR-FTIR) spectroscopy. To support spectral interpretation, theoretical IR spectra of glycolic acid and glycolide were calculated using density functional theory (DFT) at the B3LYP-D3BJ/6-311++G(d,p) level. Principal component analysis (PCA) was then applied to the experimental data to distinguish between reactant and product phases. This combined spectroscopic and computational approach enabled enhanced resolution of overlapping bands and clear identification of reaction products.

### Results:

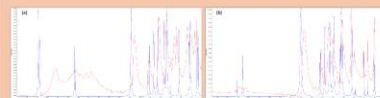


Figure 1. Overlay of experimental ATR-FTIR spectra (red) and DFT-calculated IR spectra (B3LYP-D3BJ/6-311++G(d,p)) (blue) of (a) glycolic acid and (b) glycolide, recorded at room temperature, showing strong agreement between theoretical and experimental data.

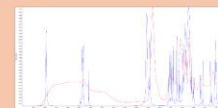


Figure 2. Overlay of experimental ATR-FTIR spectrum of the reaction mixture (red) and DFT-calculated IR spectrum (B3LYP-D3BJ/6-311++G(d,p)) of the linear trimer of glycolic acid ester (blue).

### Additional Analytical Results:



Figure 3. MS mass spectrum (M+H+) mode of the reaction mixture. Samples mixture showing a dominant peak at m/z 133.0342, include glycolic acid (9), glycolide (9), and the reaction corresponding to the deprotonated linear dimer of glycolic acid mixture (95), dissolved in ethyl acetate (EtOAc). The plate ester, and a minor peak at m/z 265.1476, attributed to the well-developed using a 4.5 HPLC-orthod (HOD) mobile deprotonated linear trimer.



### Reference:

- [1] D. J. A. Cameron, M. P. Shaver, Chem. Soc. Rev. 40 (2011) 1761-1776.
- [2] S. W. Duchiron, E. Pollet, S. Givry, L. Avérus, RSC Adv. 5 (2015) 84627-84635.

### Conclusion:

- Good agreement between theoretical and experimental IR spectra confirms the accuracy of computational predictions.
- Key vibrational modes were successfully identified and matched, validating the molecular structure.
- Further optimization of reaction conditions is needed to enhance consistency and scalability.
- Overall, the study demonstrates a strong foundation for future refinement and application.



# Thanks for listening!

