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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Outline

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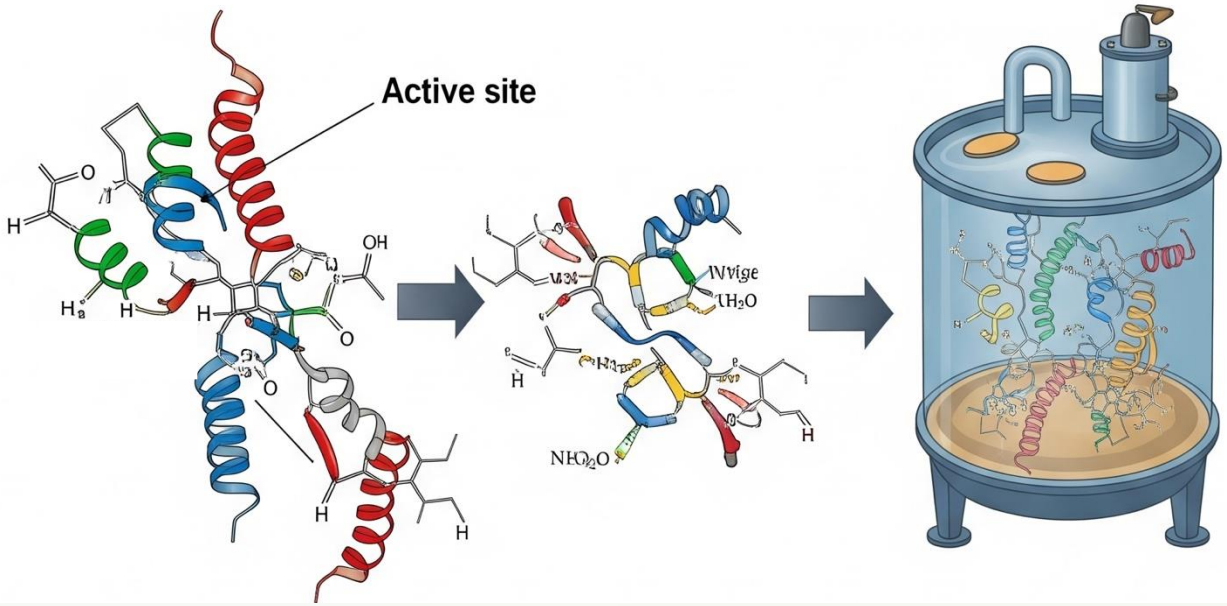
    - Substrate binding (pyruvate)
    - Cofactor binding (NADH)
6. Single point mutant Q189L
7. Summary

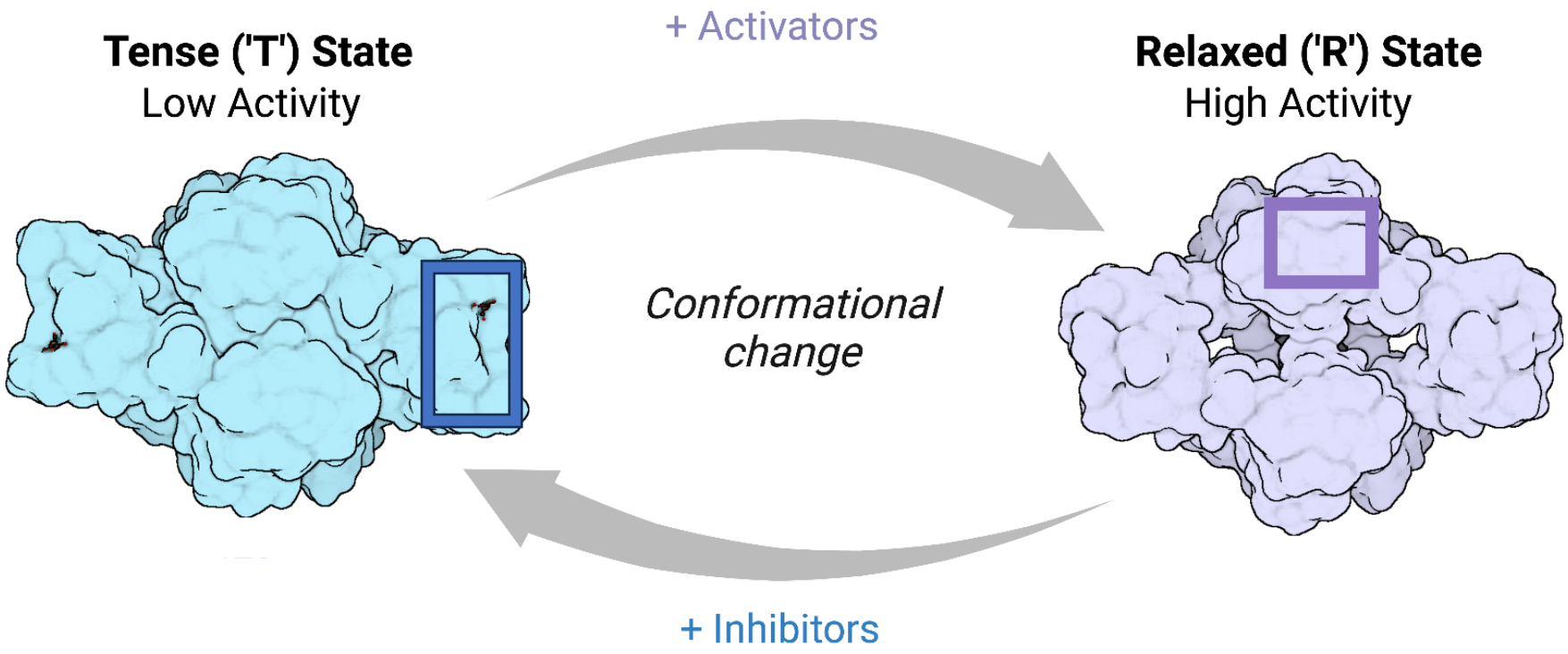
Introduction

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

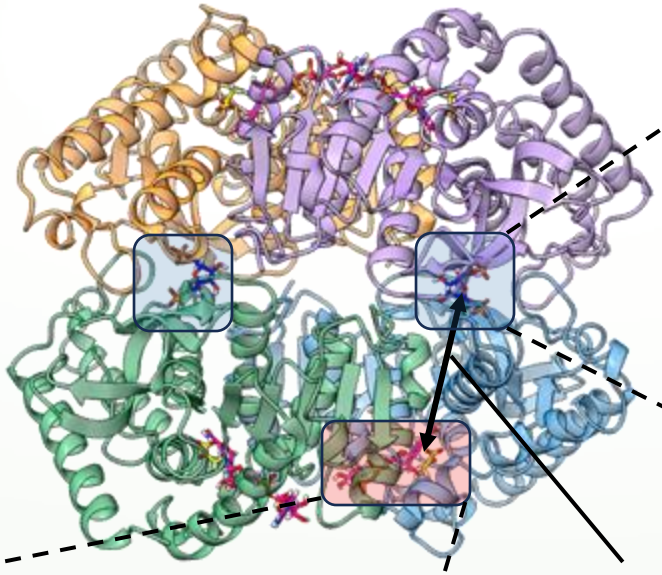


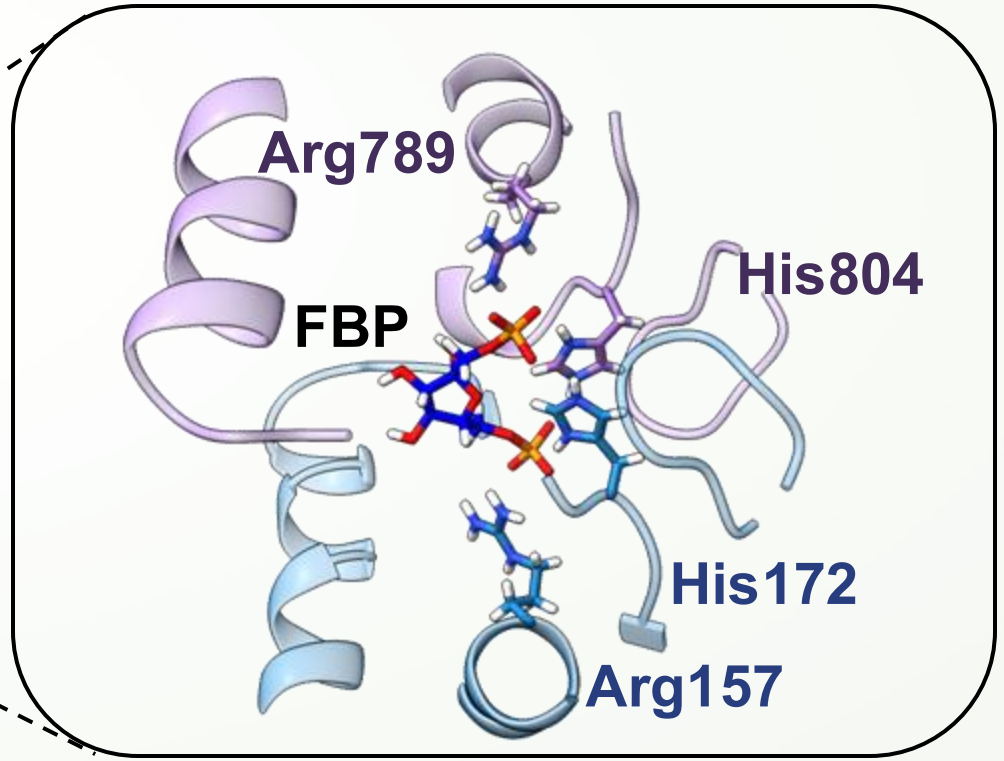
Allostery


Tense ('T') State
Low Activity
+ Activators
Relaxed ('R') State
High Activity
Conformational change
+ Inhibitors

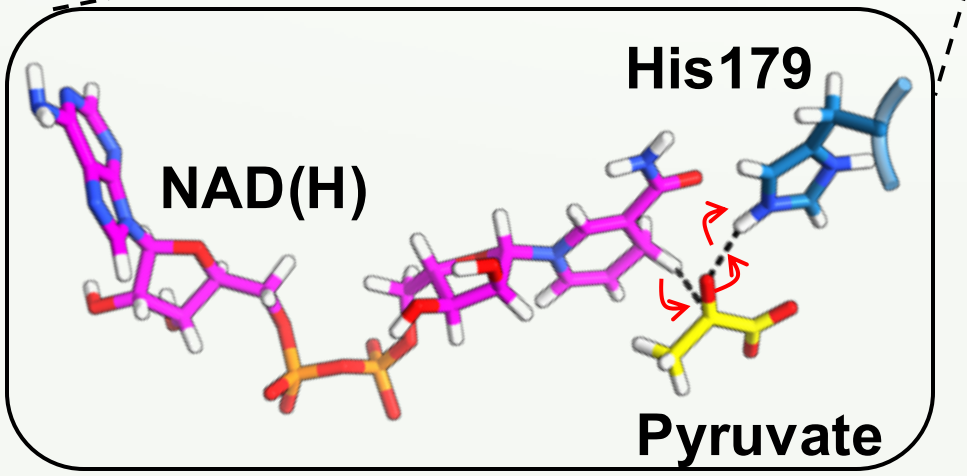
Lactate Dehydrogenase (LDH)

Geobacillus stearothermophilus (GsLDH)

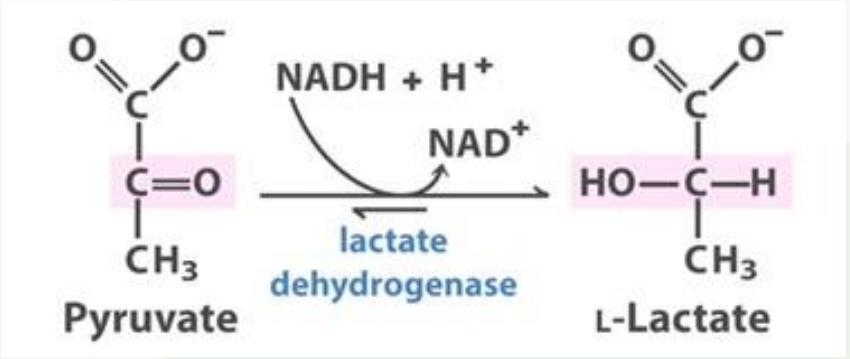




FBP binding site
(Fructose 1,6-bisphosphate)

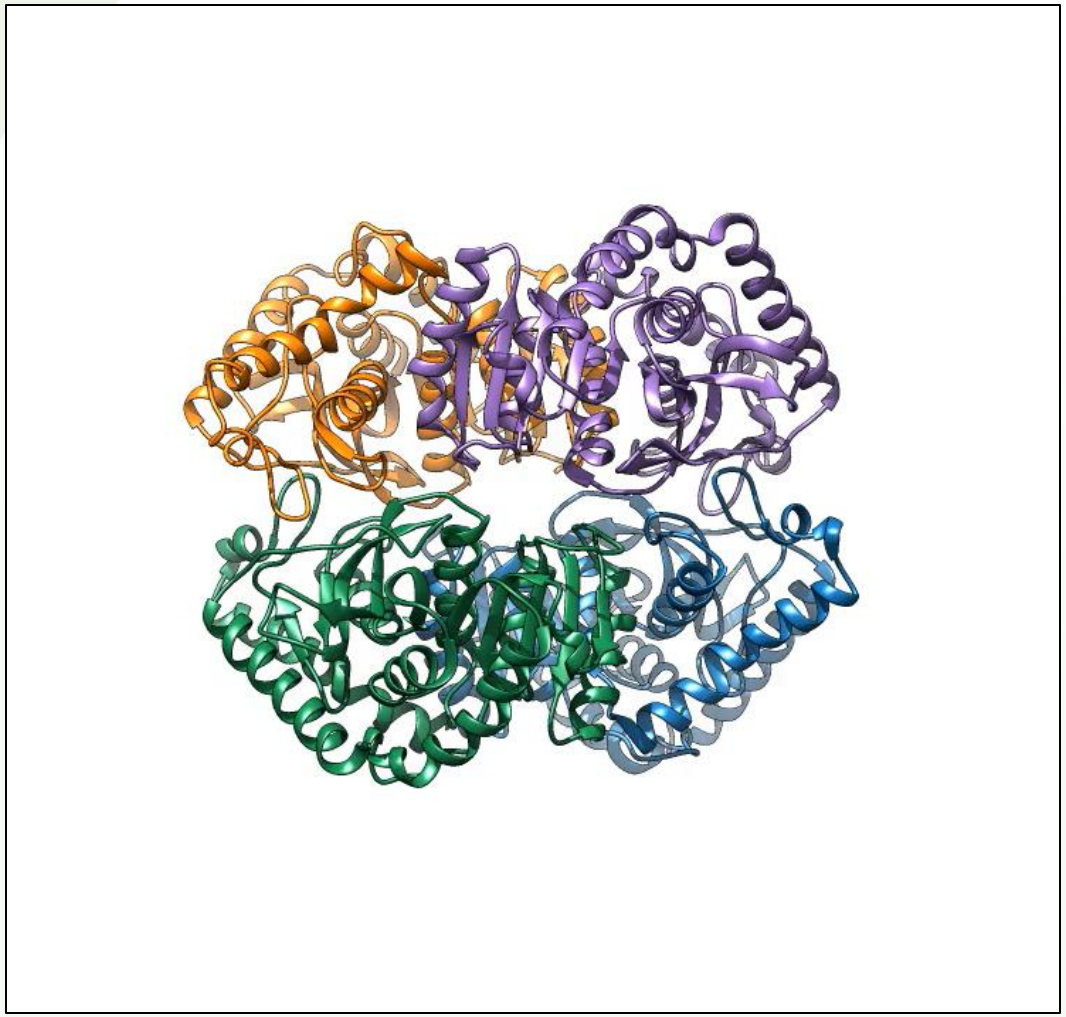


Catalytic pocket

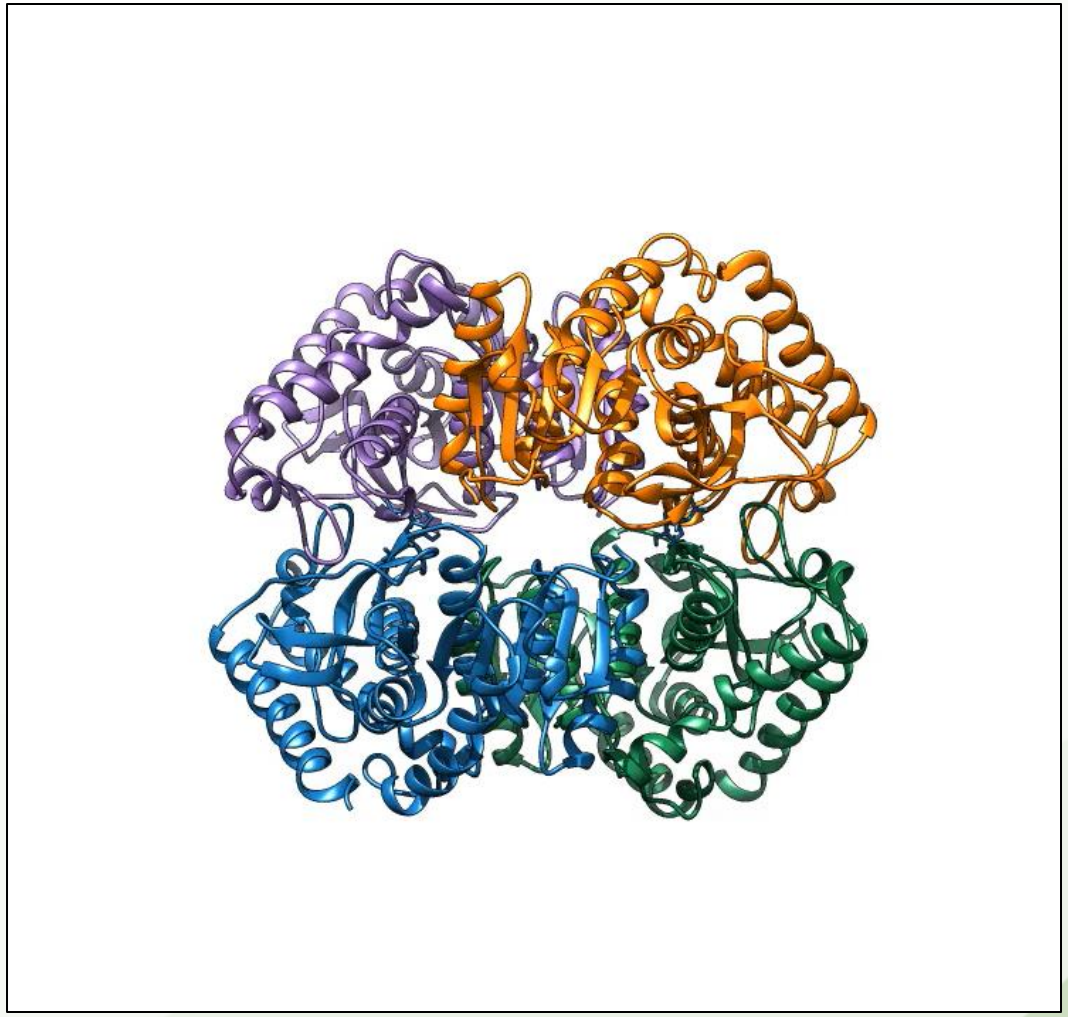


MD simulation captured oligomeric state change

WT GsLDH without FBP



WT GsLDH with FBP



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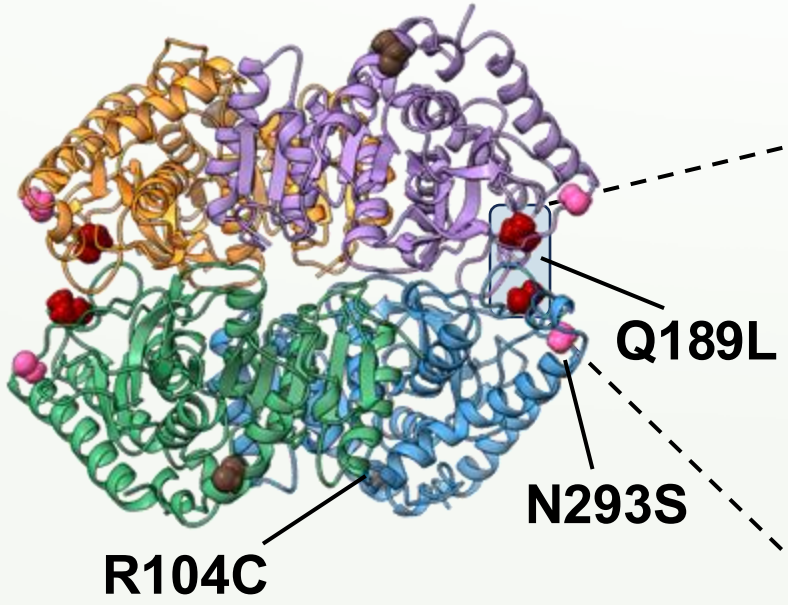
The dual role of FBP

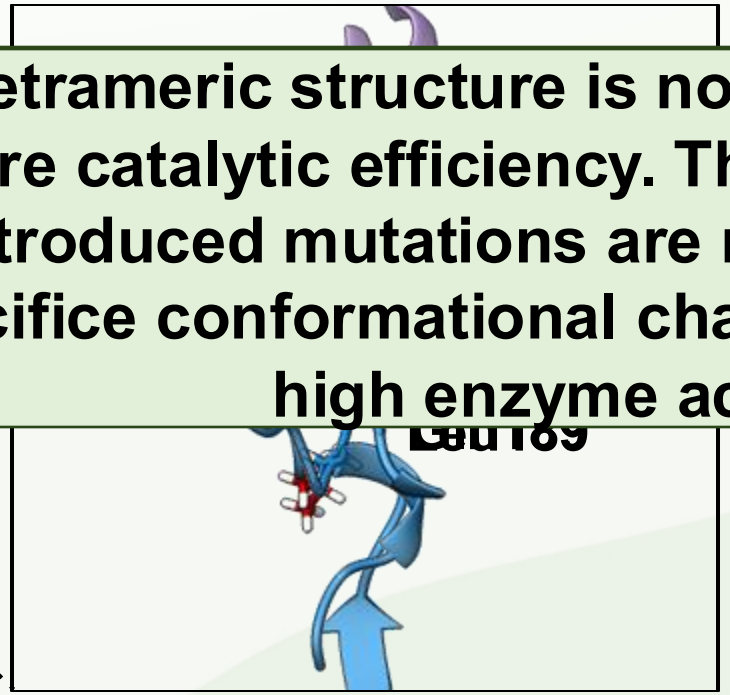
Gel filtration ⇩

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

R104C/Q189L/N293S ⇦

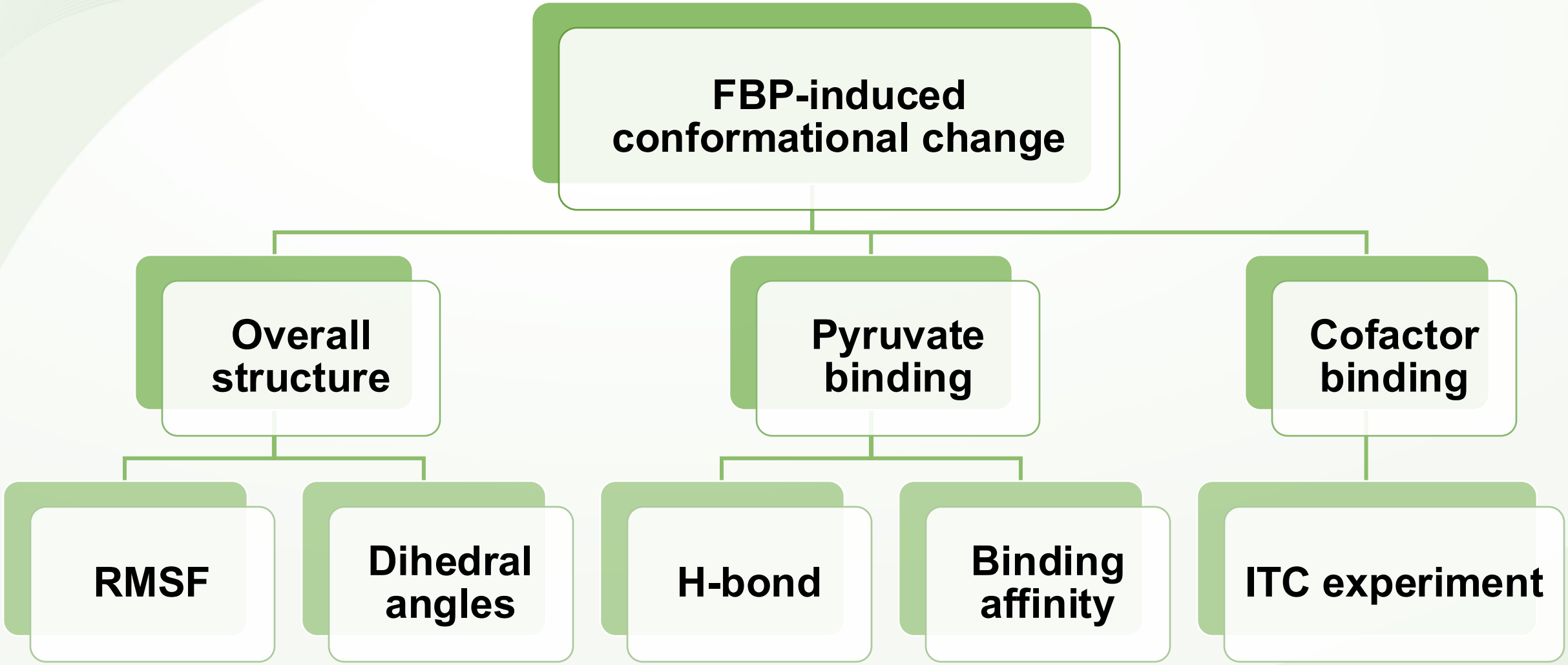
⇩





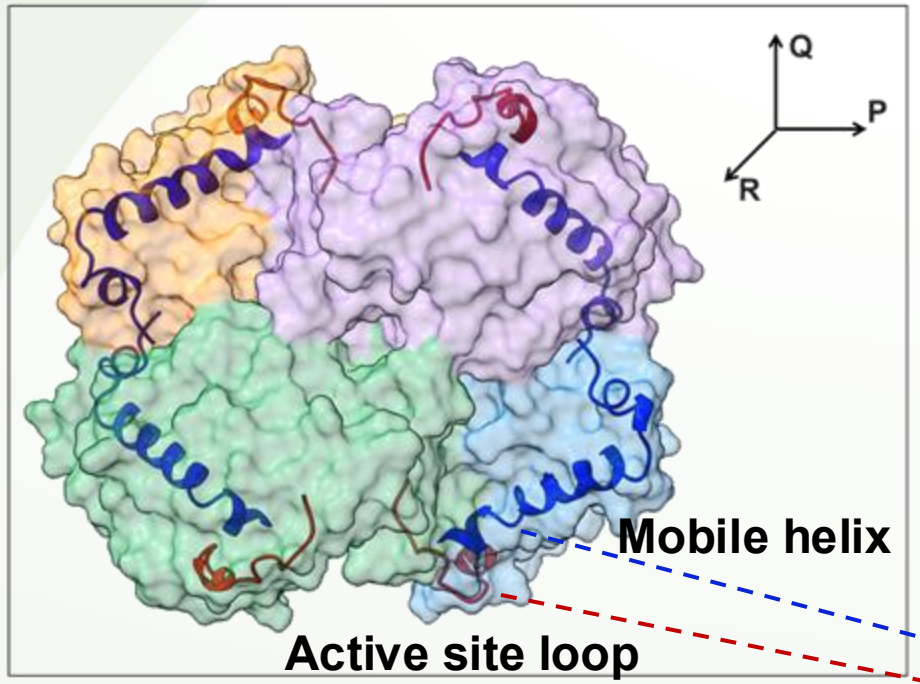
Tetrameric structure is not enough to fully restore catalytic efficiency. The binding of FBP or introduced mutations are needed to induce specifice conformational changes to govern the high enzyme activity.

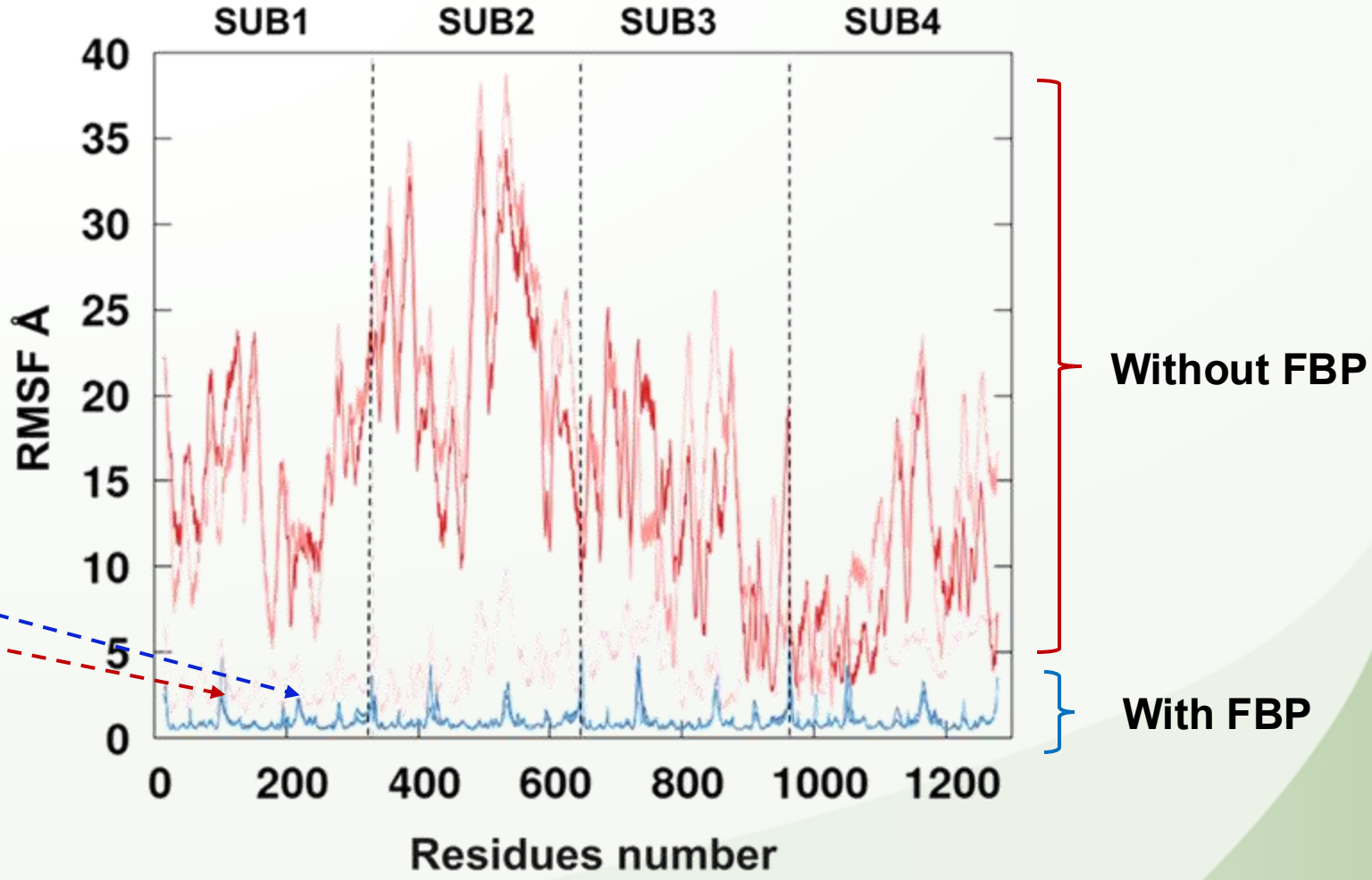
Allen *et al.* (2000) *Protein Engineering, Design and Selection*


FBP-induced conformational change
Overall structure
Pyruvate binding
Cofactor binding
RMSF
Dihedral angles
H-bond
Binding affinity
ITC experiment

High residues fluctuations without FBP

Backbone (Cα) Root Mean Square Fluctuation (RMSF)





Higher RMSF value ⇨ Higher flexibility

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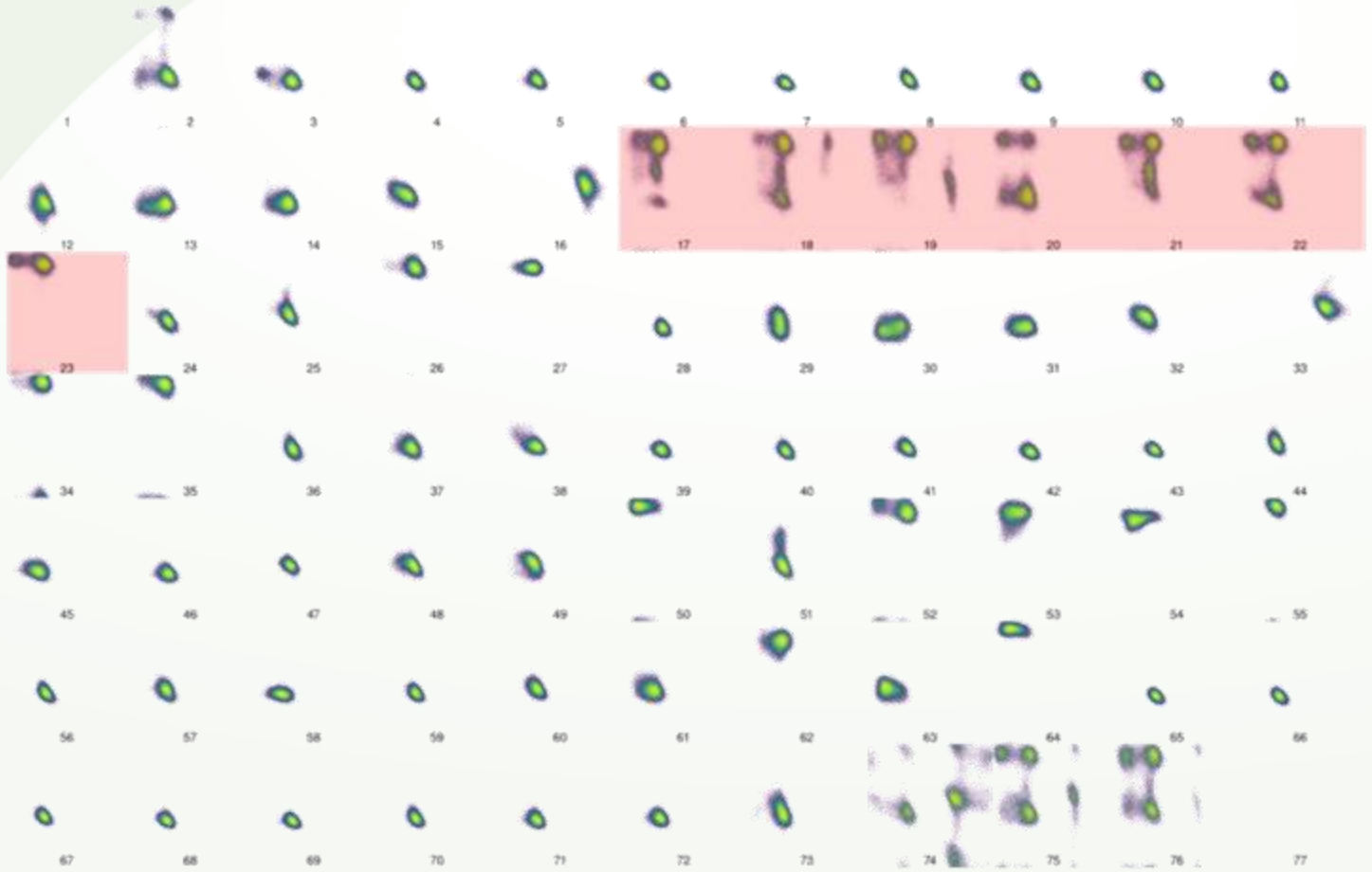
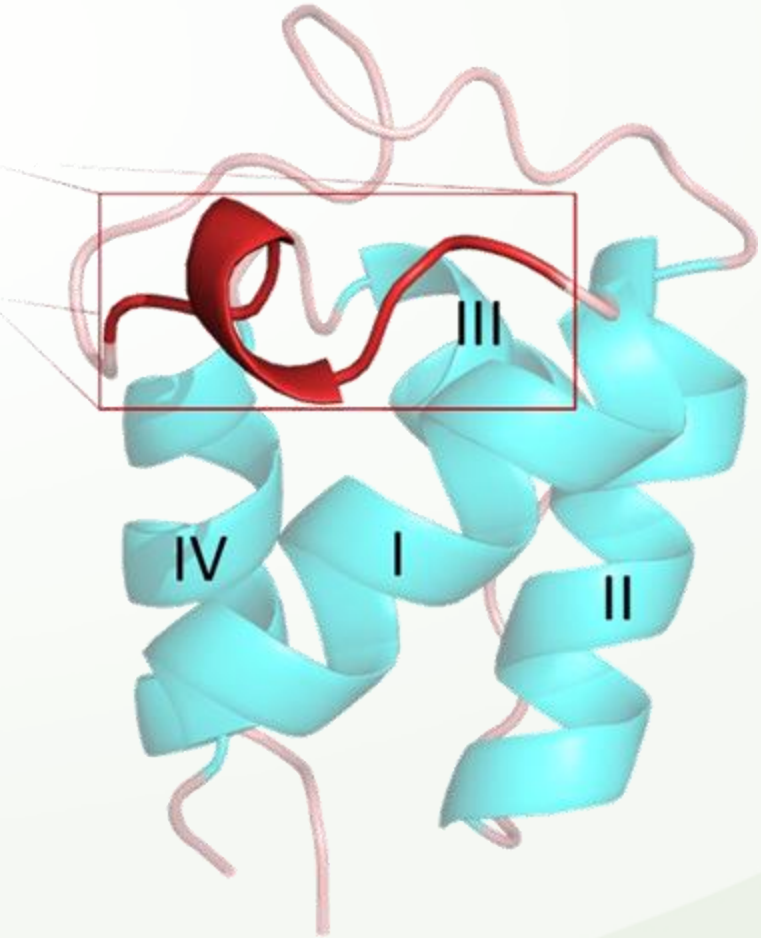
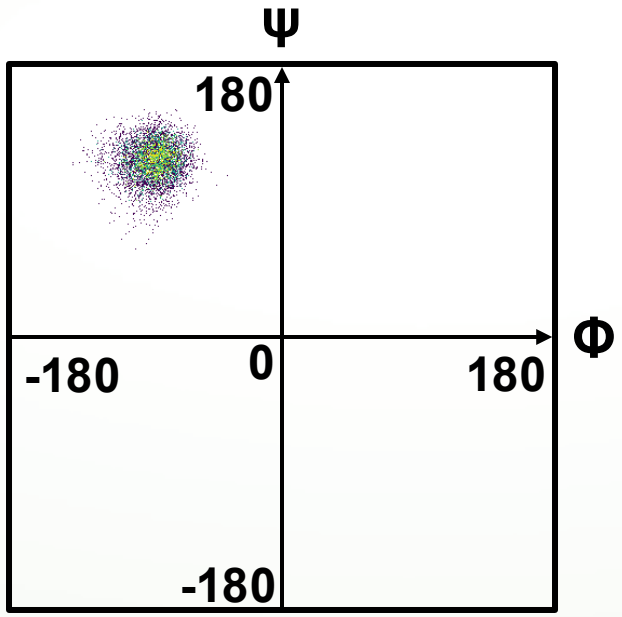
MDavocado: Fast Screening for Dihedral Angles

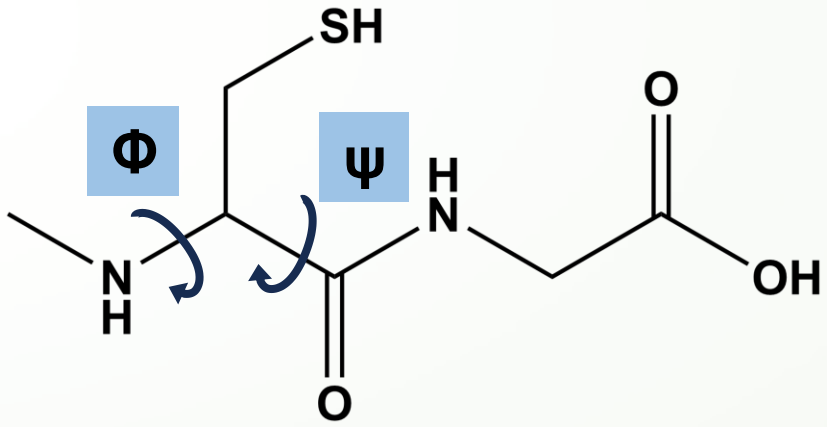




Dihedral angles (backbone and side chain)

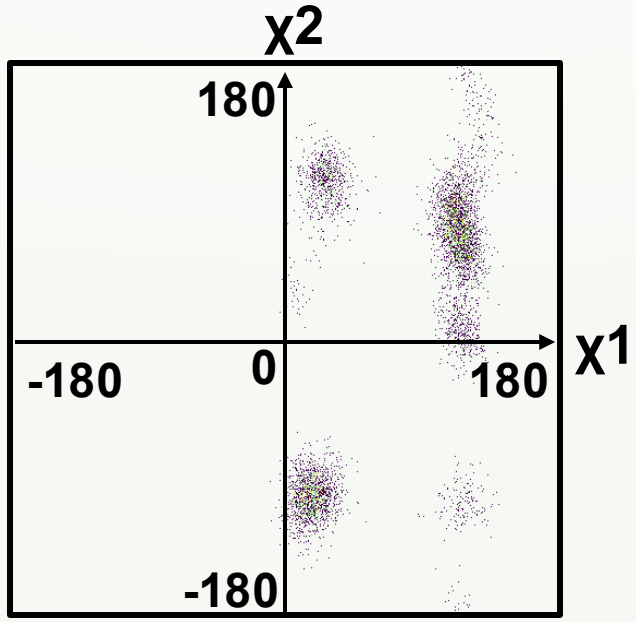
Backbone dihedral angles

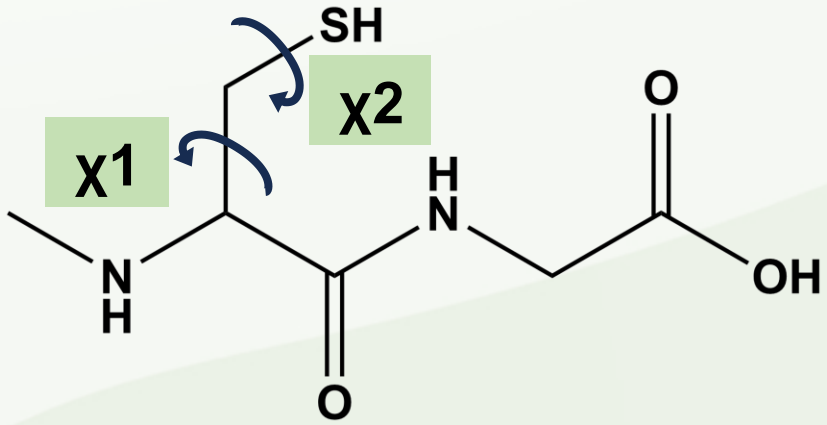




Lysine

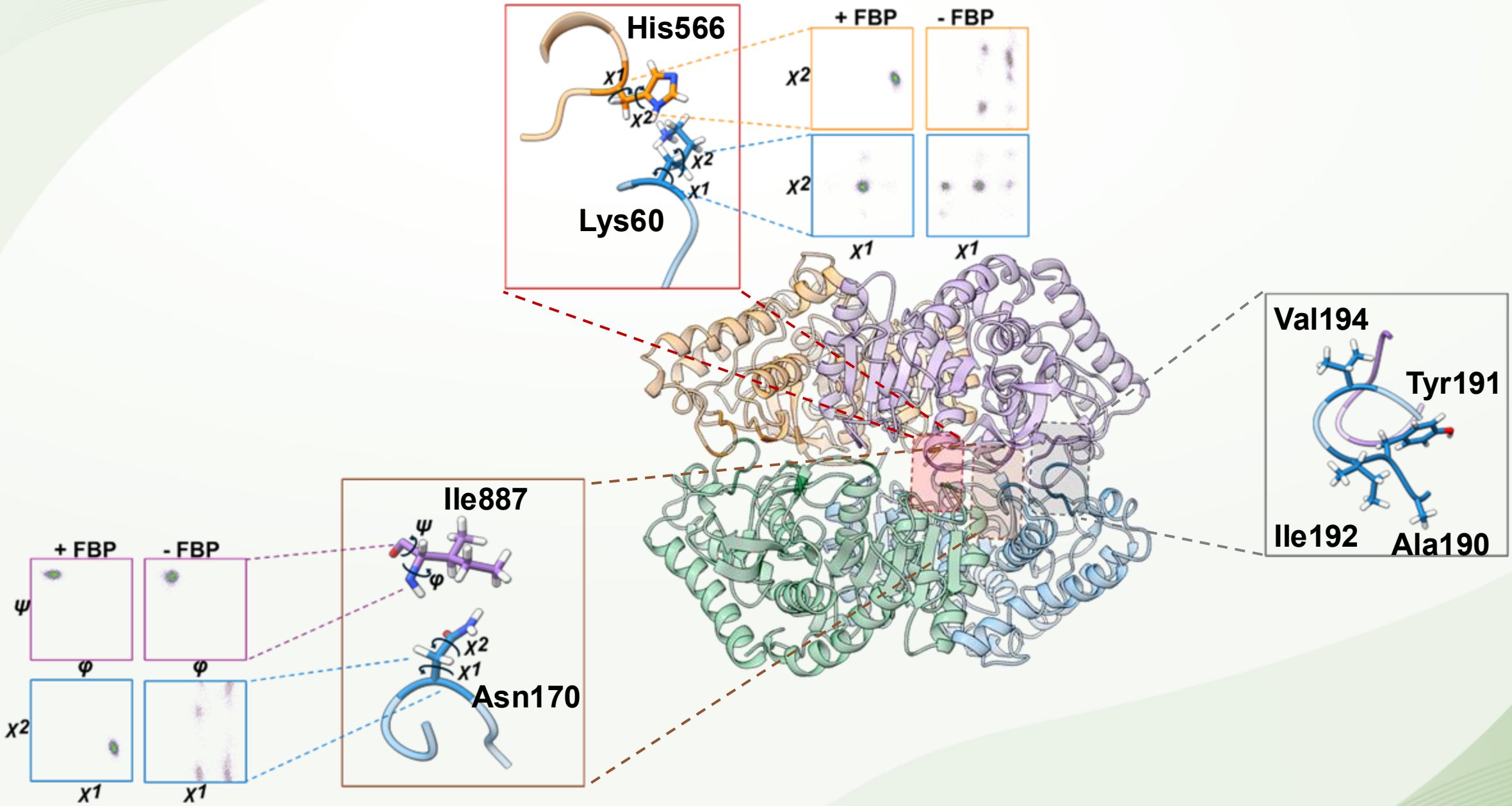
Side chain dihedral angles



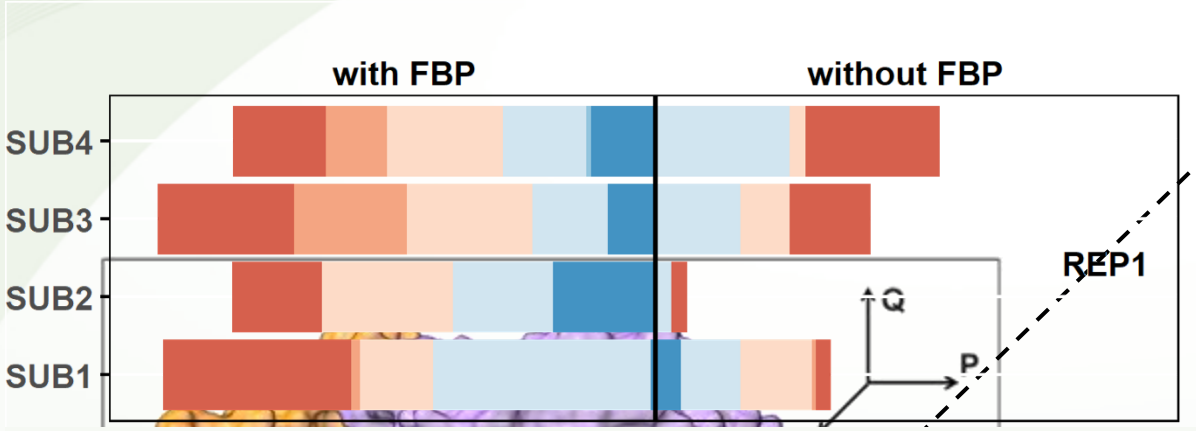


Lysine

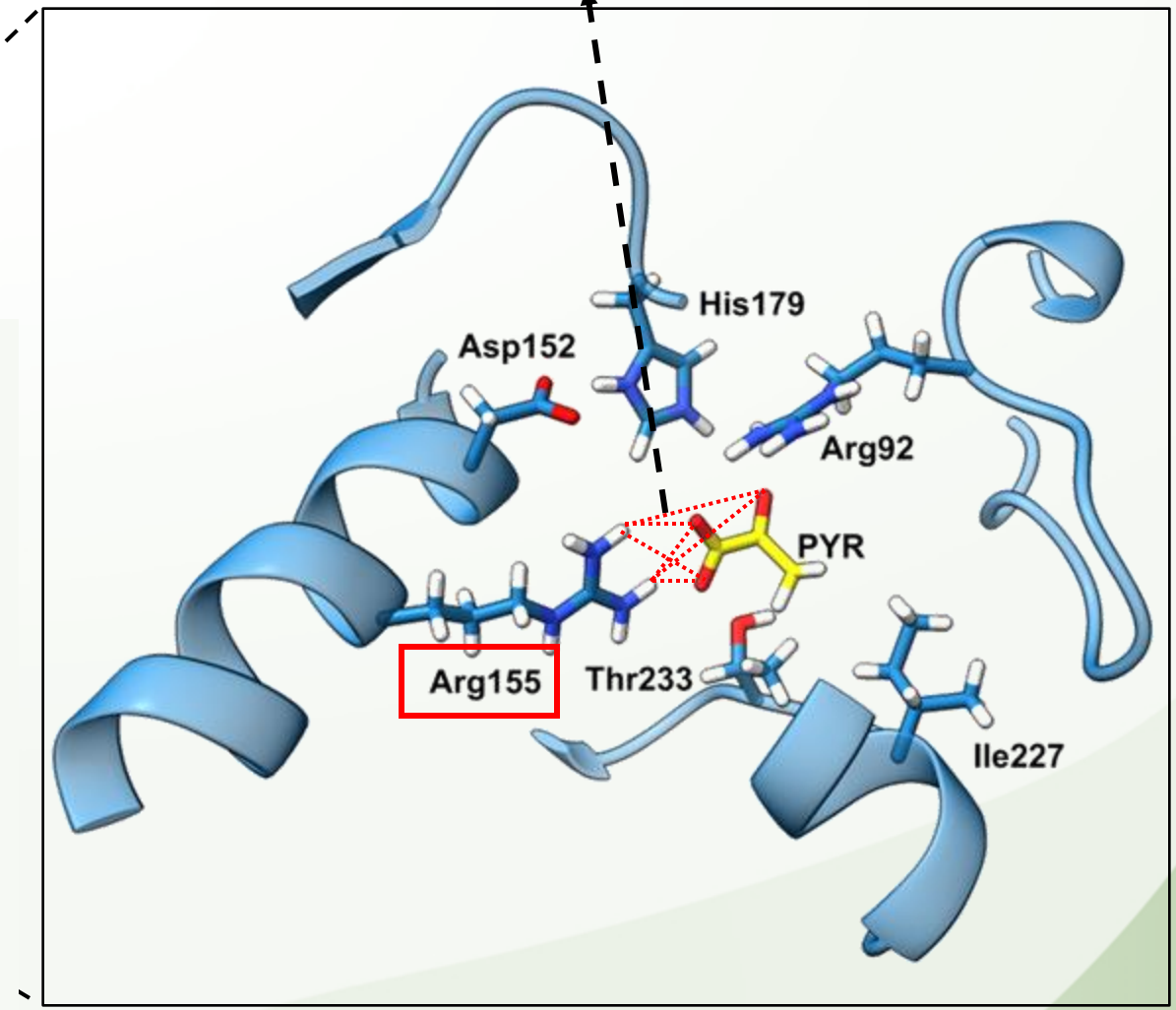
MDavocado identified three critical regions



FBP promotes and stabilizes H-bonds



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



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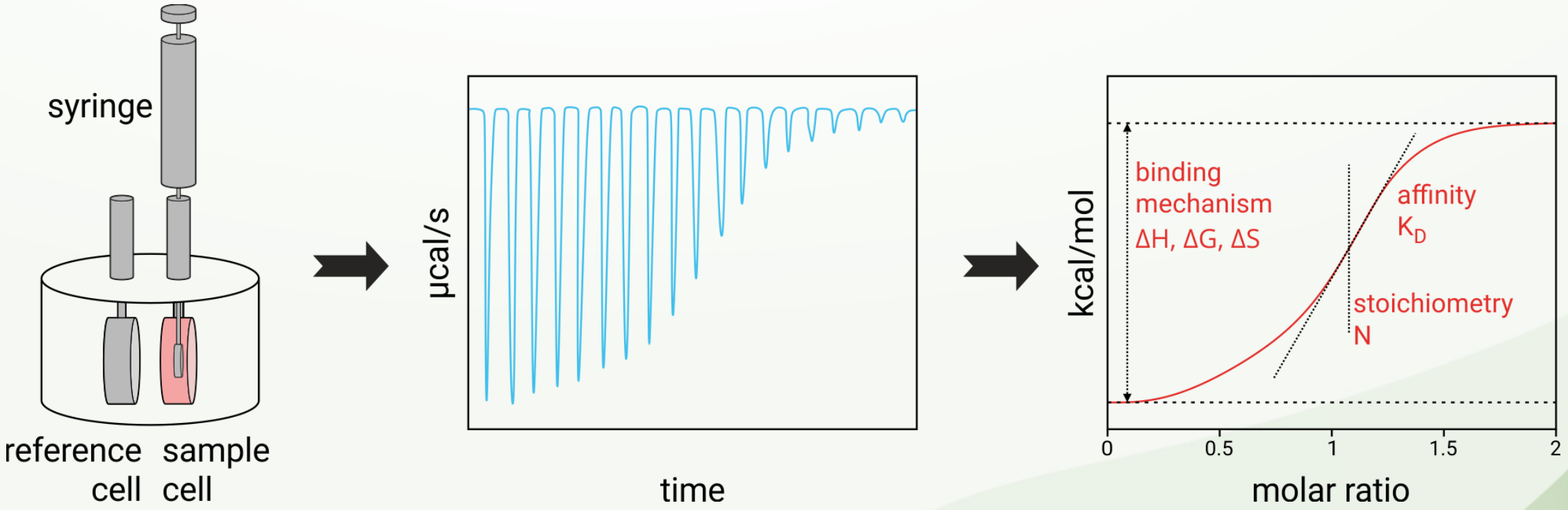
Microcalorimetry titration studies

Isothermal titration calorimetry (ITC)

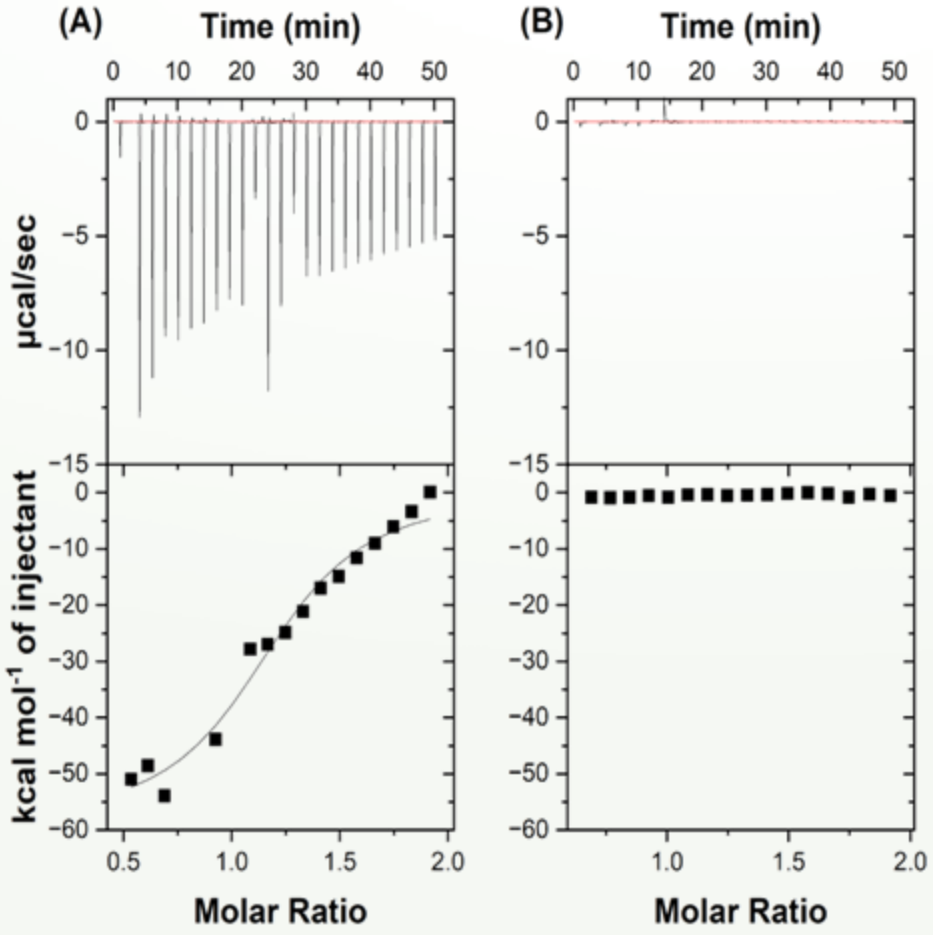
The principle is the direct measurement of the heat change that occurs when two molecules interact.



FBP enhances cofactor binding

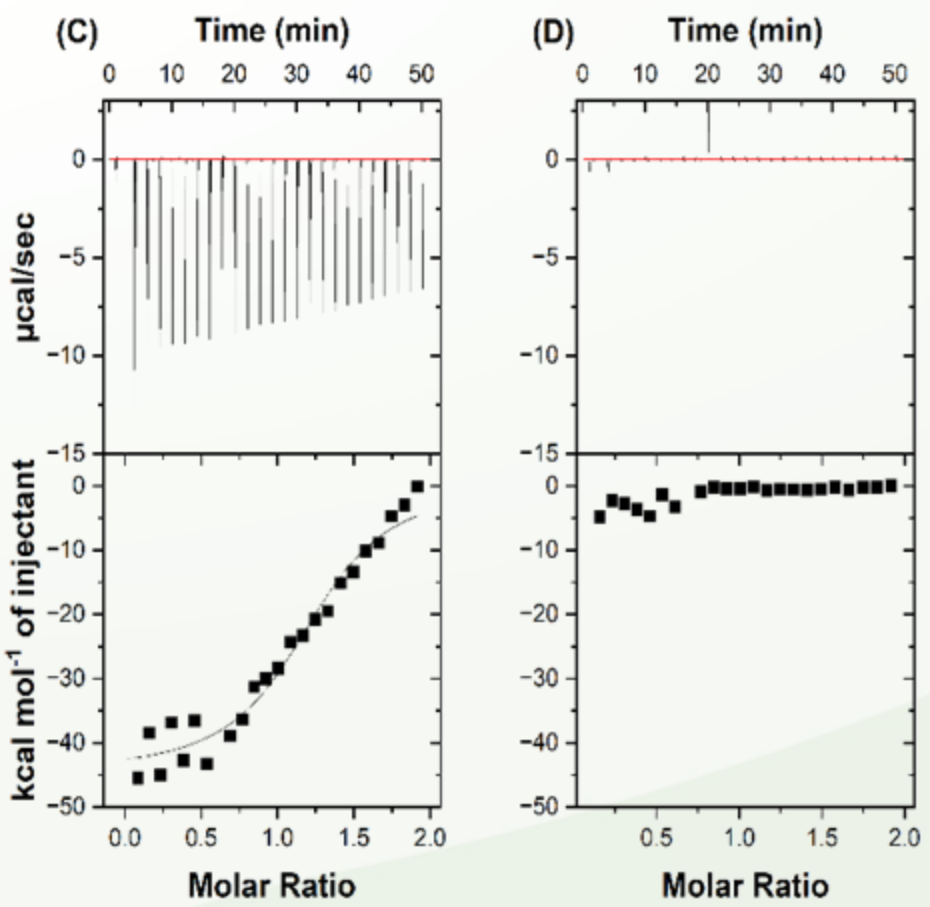
GsLDH titrated with NADH

+FBP -FBP



GsLDH titrated with NAD$^+$

+FBP -FBP



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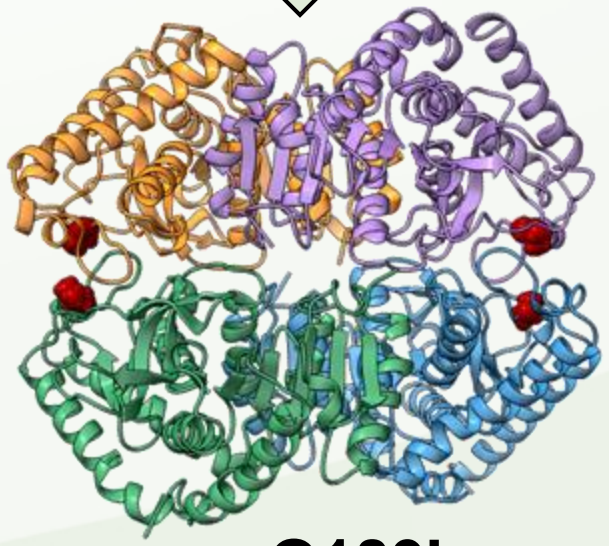
Q189L maintains tetramer without FBP

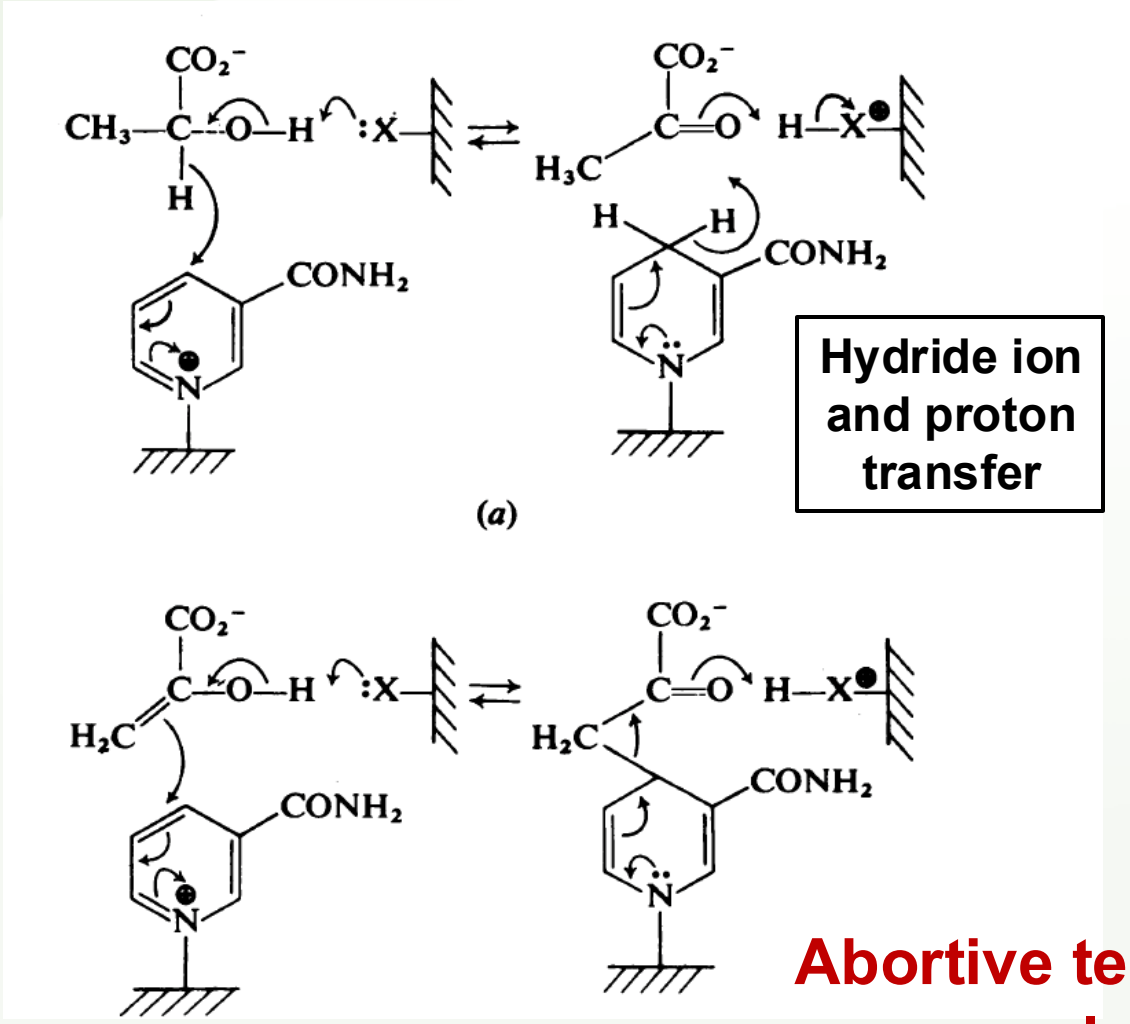
GsLDHs	k_{cat}/K_m ($s^{-1}mM^{-1}$)		K_i (mM)		Oligomeric state
	-FBP	+FBP	-FBP	+FBP	
Wild type	35 ± 13	144 ± 67	ND	3.5 ± 0.6	Dimer
Triple mutant	141 ± 18	156 ± 59	ND	1.4 ± 0.4	Tetramer
Q189L	32 ± 4	106 ± 19	ND	0.3 ± 0.1	Tetramer

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



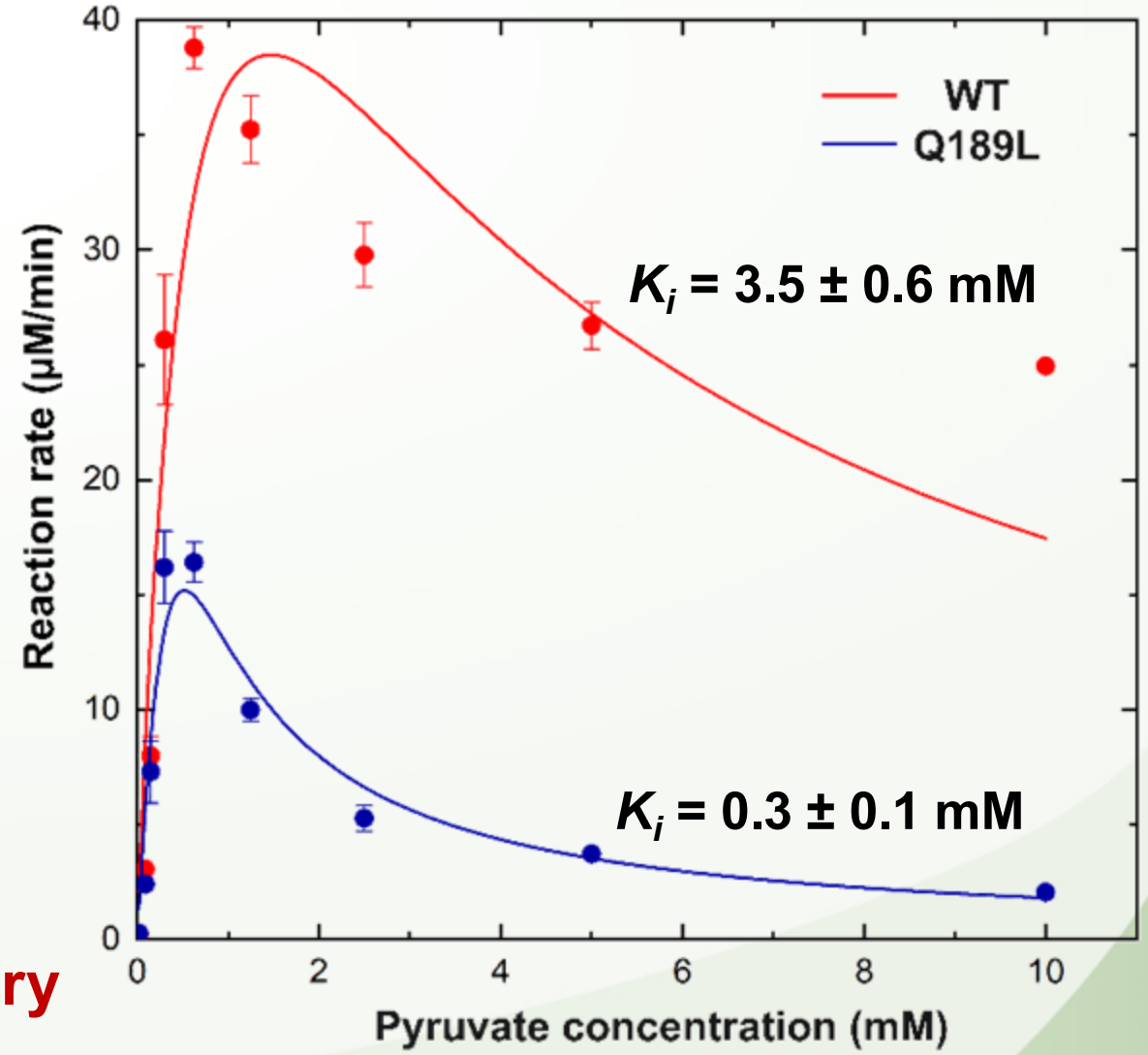
Q189L

Q189L enhances substrate inhibition



Hydride ion and proton transfer

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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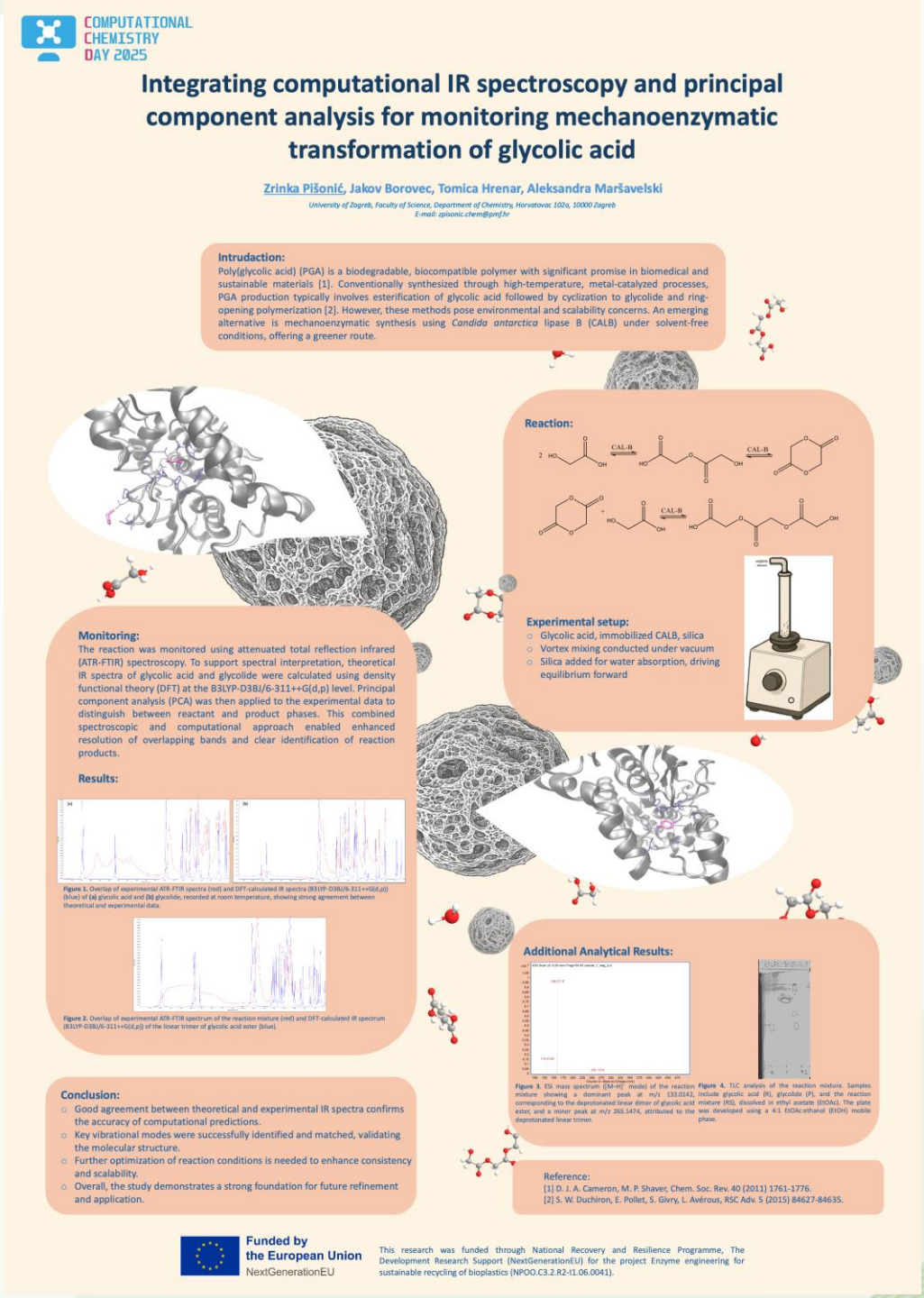

Thanks for listening!