

Effect of an arginine-to-  
isoleucine active site  
mutation on *Escherichia*  
*coli* malate dehydrogenase  
enzymatic activity

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Biochemistry

# Biochemistry Research at Olivet:

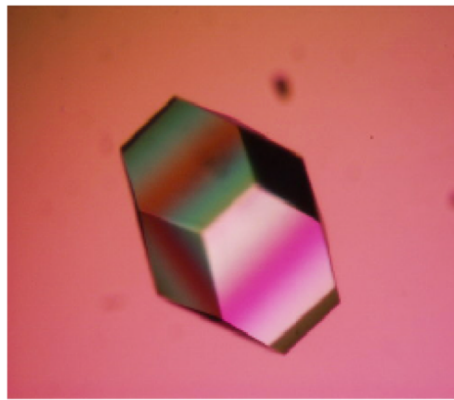
- I. This Talk
  - a. Protein research  
(general)
  - b. Independent Research  
Experience
- II. Next Talk
  - a. Research in Advanced  
Biochemistry Course
  - b. Future Research in the  
Chemistry Department



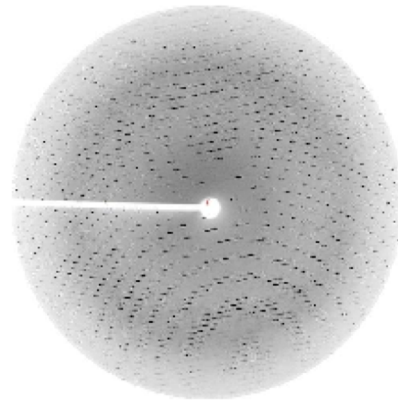
# Protein Review

- are large **biomolecules**, or **macromolecules**,
- consisting of one or more long chains of **amino acid residues**.
- Proteins perform a vast array of functions within **organisms**, including
  - **catalysing metabolic reactions – called enzymes**,
  - **DNA replication**,
  - **responding to stimuli**, and
  - **transporting molecules** from one location to another.
- Proteins differ from one another primarily in their sequence of **amino acids**
- Proteins are produced from **gene expression – central dogma**

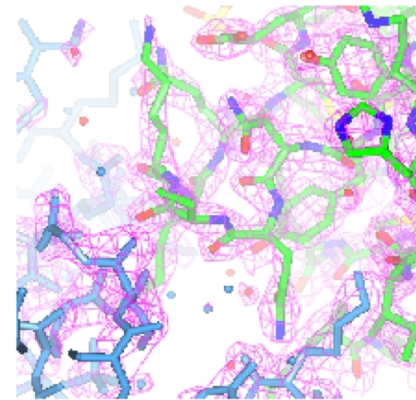
# Protein Research – Structure and Function



Crystal



Diffraction pattern



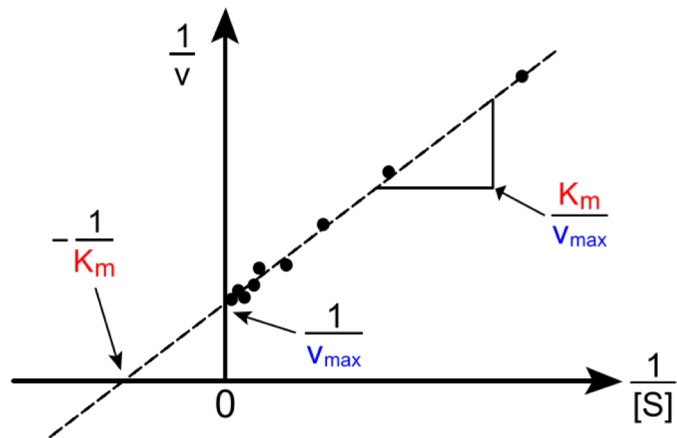
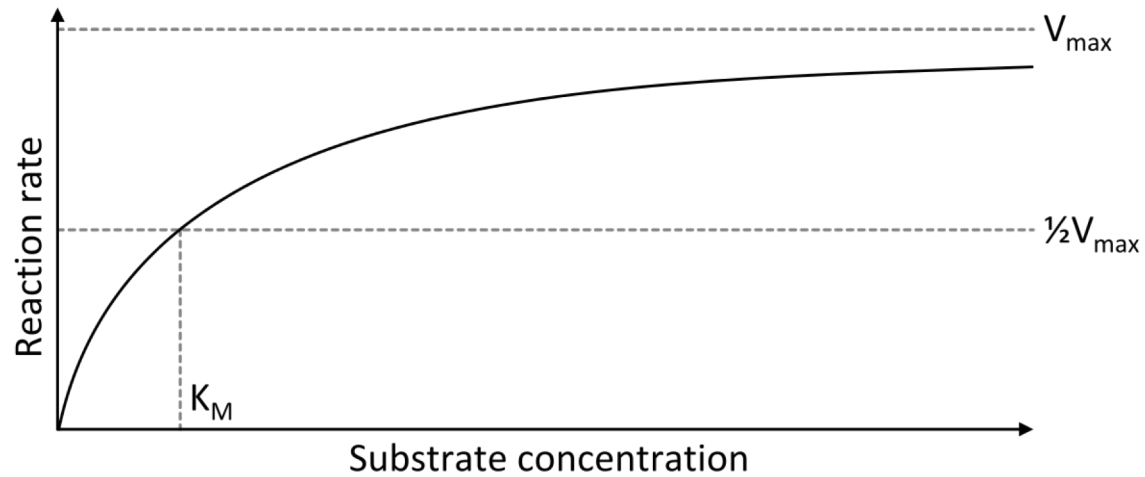
Electron density map



Protein model

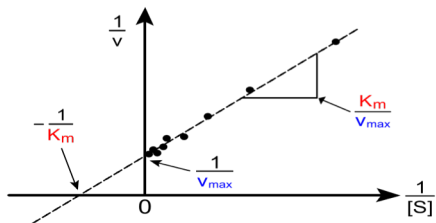
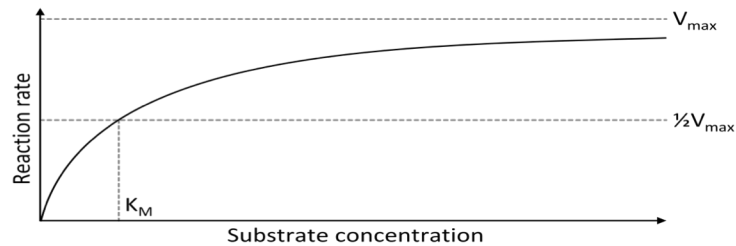
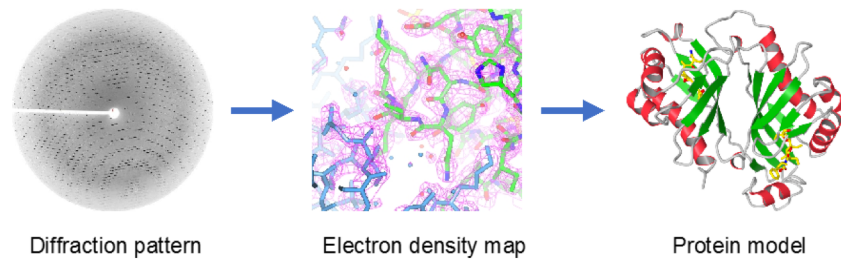


# Protein Research – Structure and Function



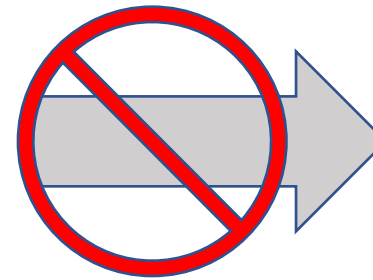
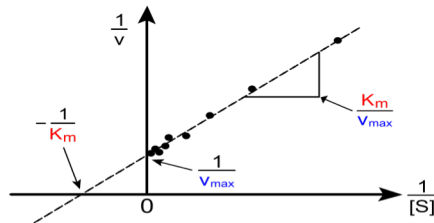
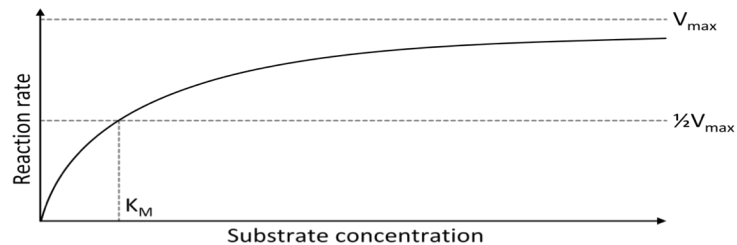
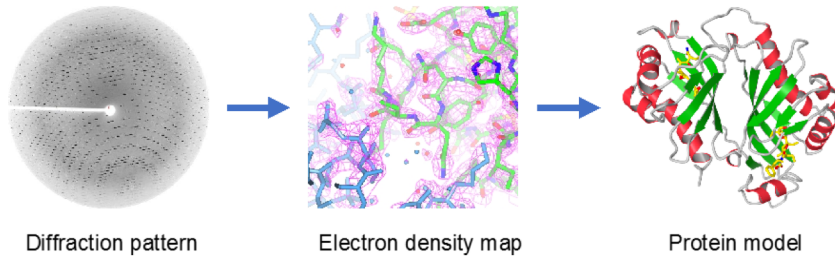
- Substrate Specificity
- Enzyme Promiscuity
- Inhibitors
- Optimal Conditions

# Protein Research – Structure and Function



- General function
- Active site location
- Allosteric site
- Hypothesize a catalytic mechanism

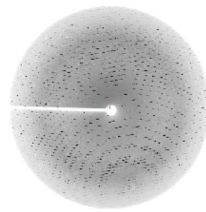
# Protein Research – Structure and Function



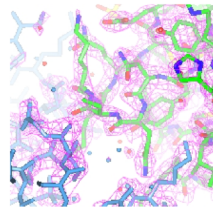
- Uncertain mechanism of action
- Substrate specificity?
- Overall conformational changes

# Protein Research – Mutations

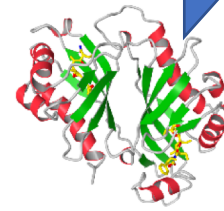
Analyze Mutant Protein



Diffraction pattern

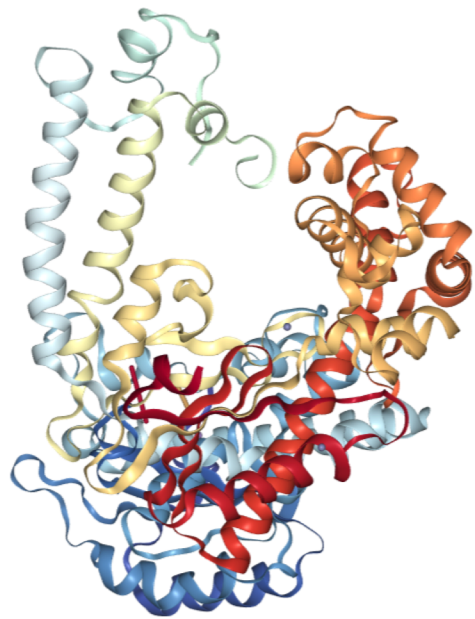
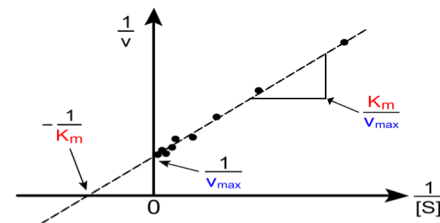
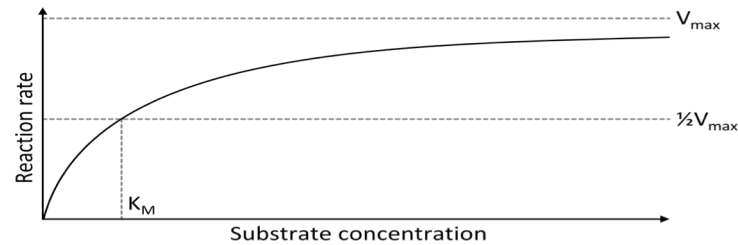


Electron density map



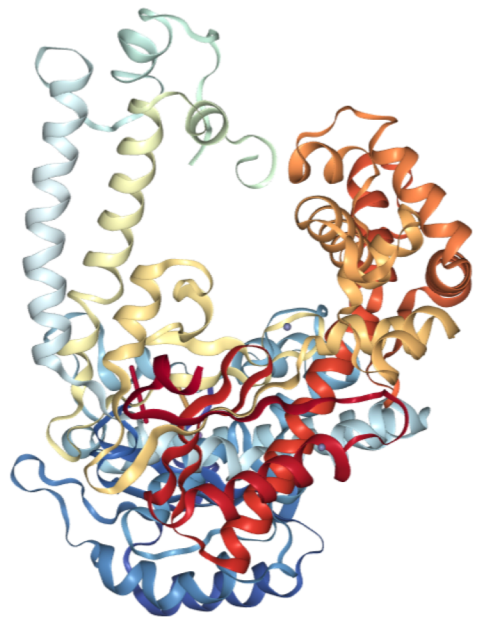
Protein model

Mutation



Known Protein

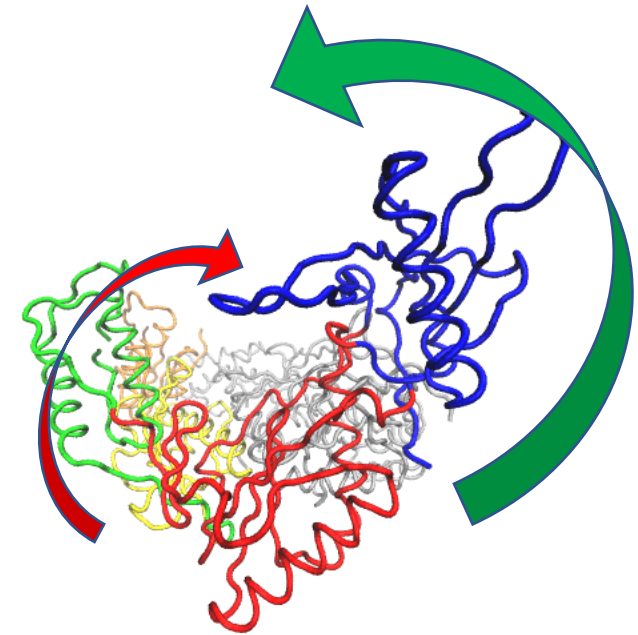
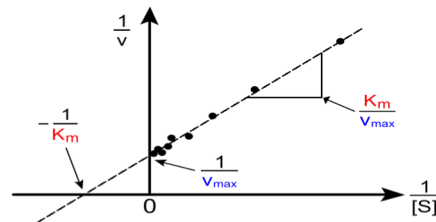
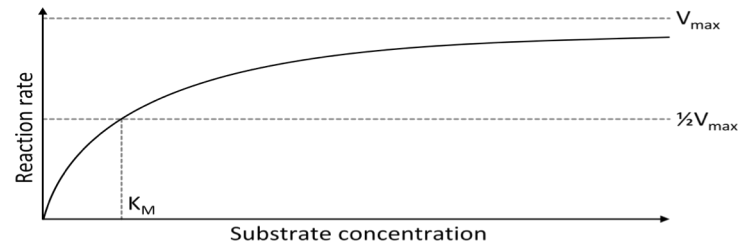
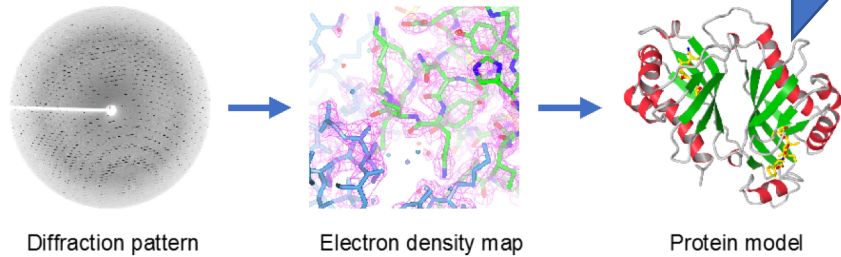
# Protein Research – Mutations



Known Protein

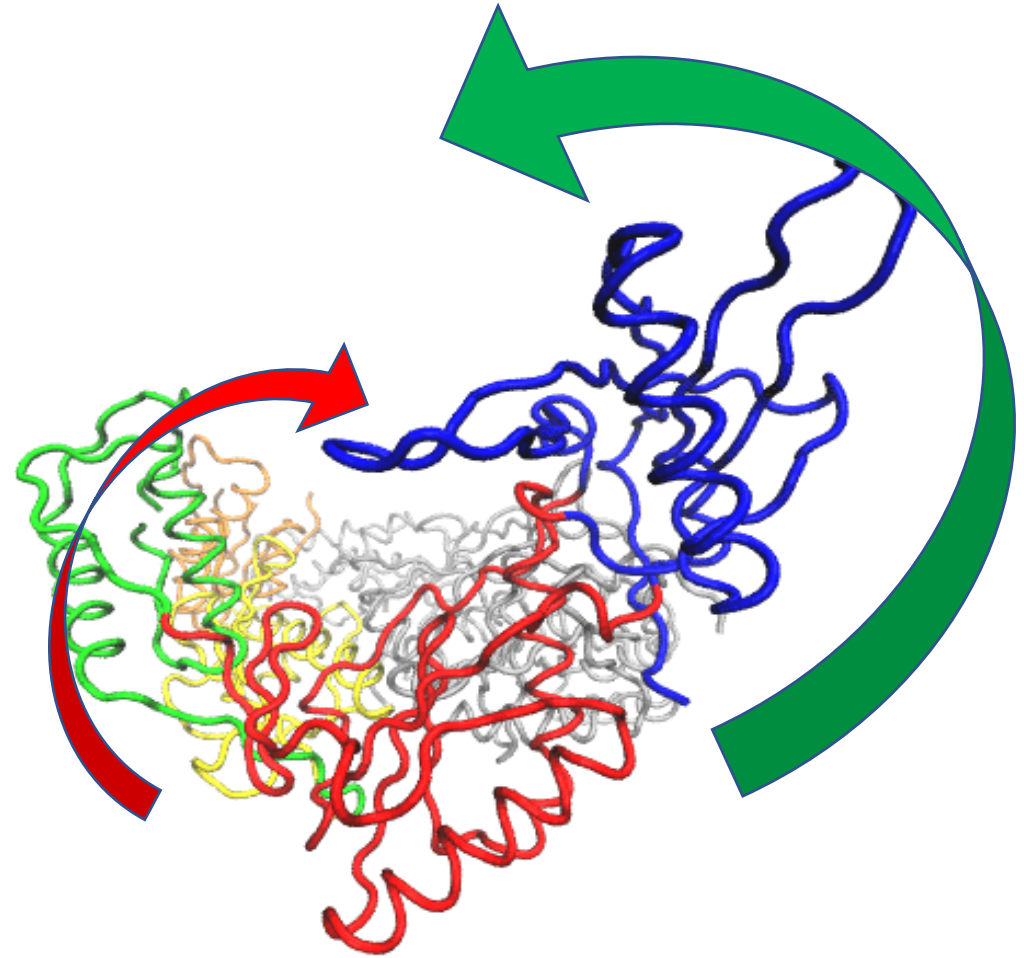
Mutation

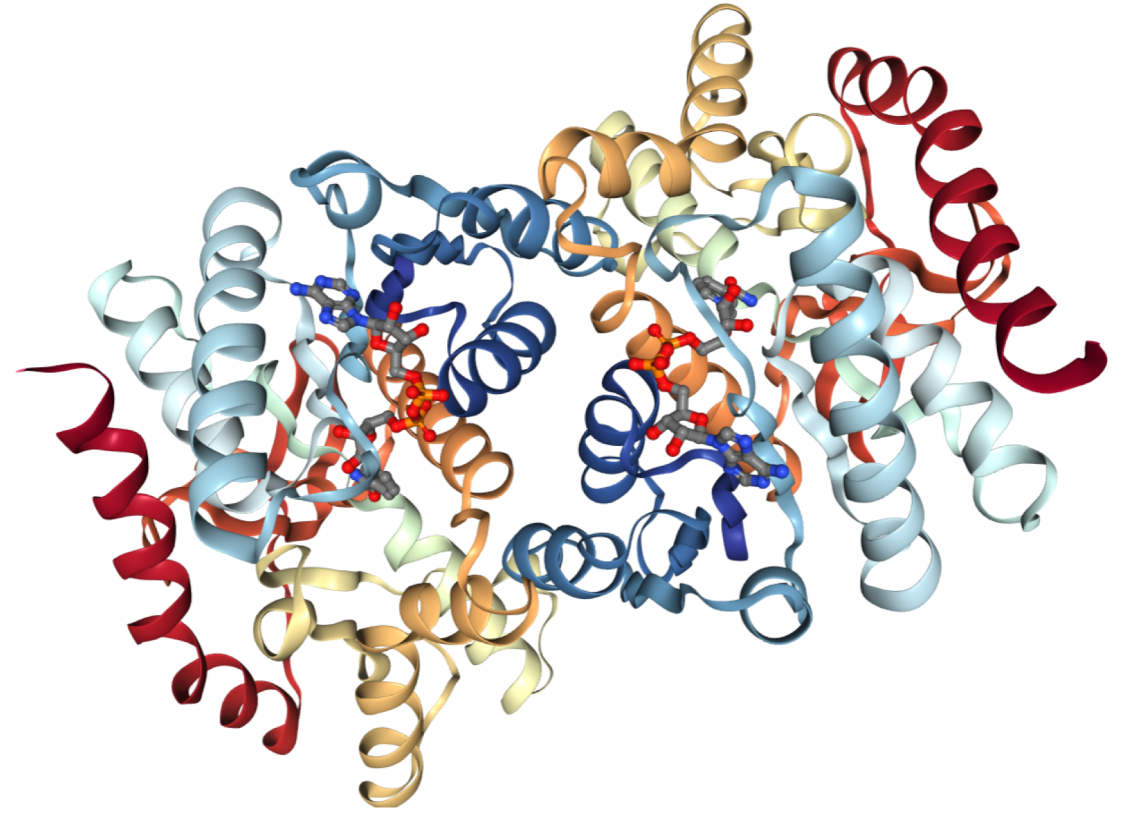
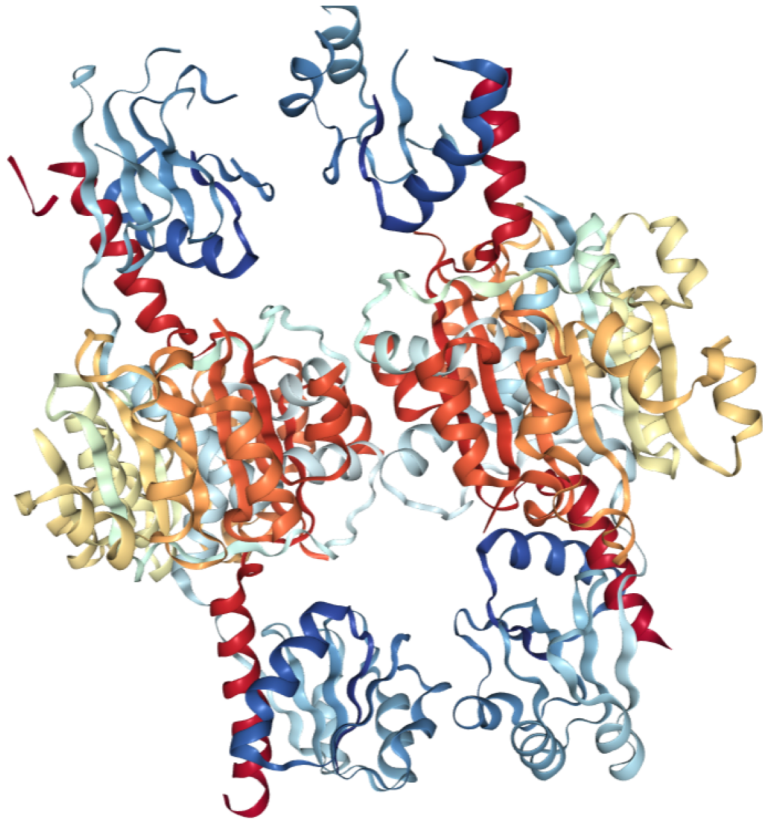
Analyze Mutant Protein



## Purpose of Protein Research:

- a. Understand catalytic mechanisms
- b. Design inhibitors – drug discovery
- c. Gain knowledge of structural motifs within protein families
- d. Evolution of protein families



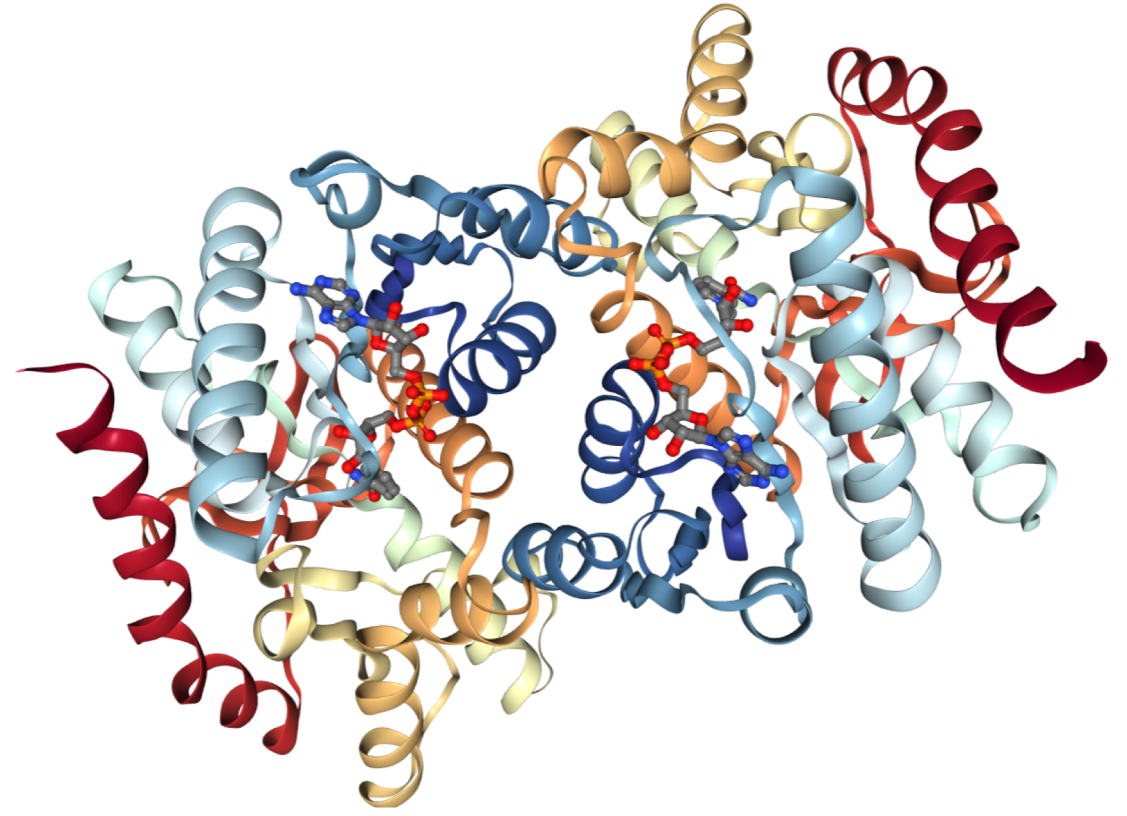


*E. Coli* MDH & LDH: A Peculiar Pair of Proteins

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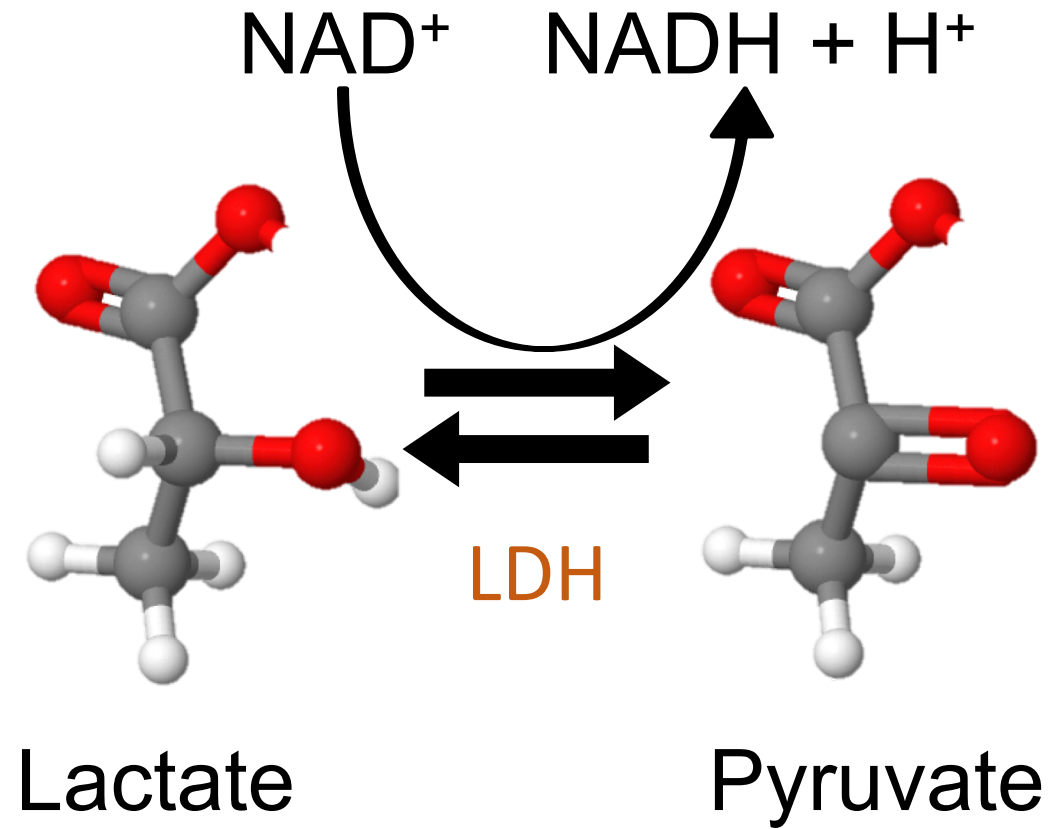
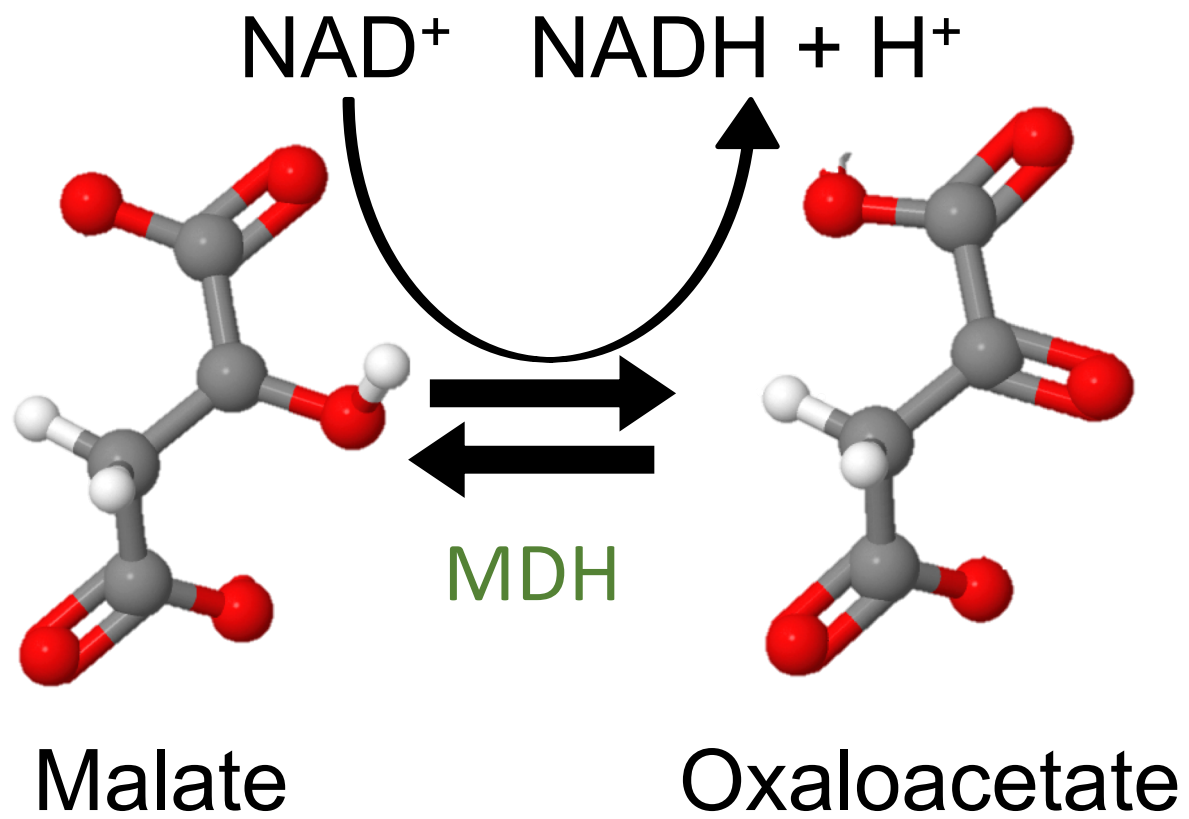


- Model for identification of functional paralog shift mutations in dehydrogenases



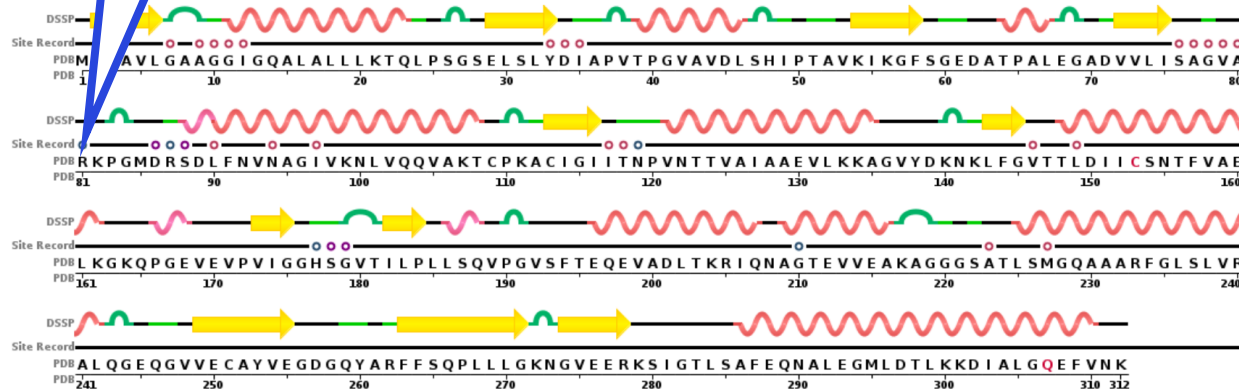
*E. Coli* MDH & LDH: A Peculiar Pair of Proteins





R

## Malate dehydrogenase

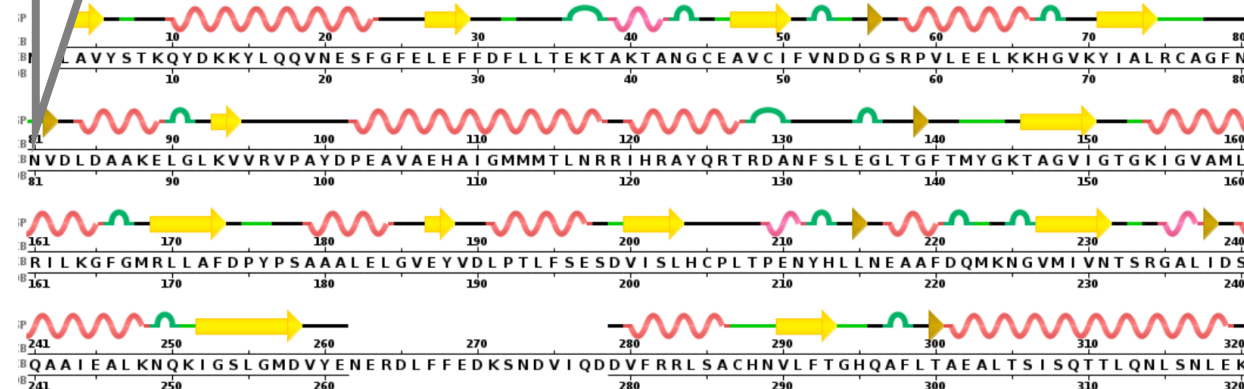


PDB: 1IB6

Bell, J.K., Yennawar, H.P., Wright, S.K.,  
Thompson, J.R., Viola, R.E., Banaszak, L.J. (2001)  
J Biol Chem 276 31156-31162

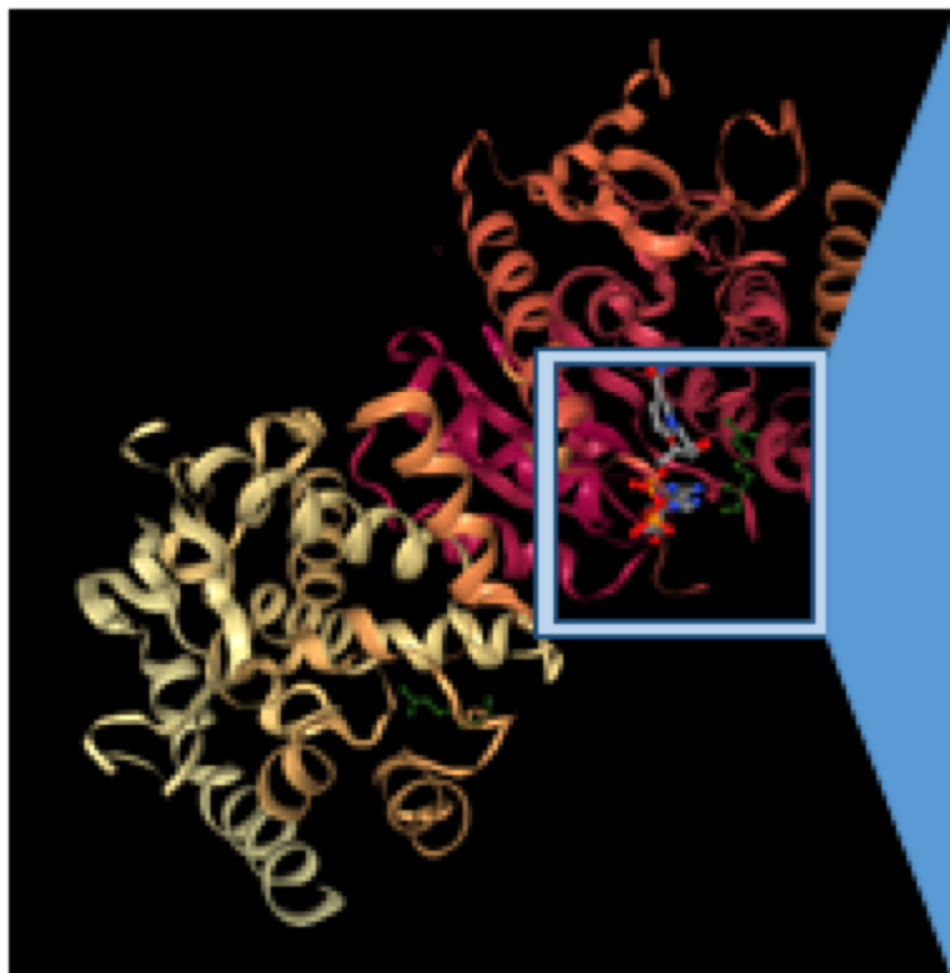
N

## Lactate dehydrogenase

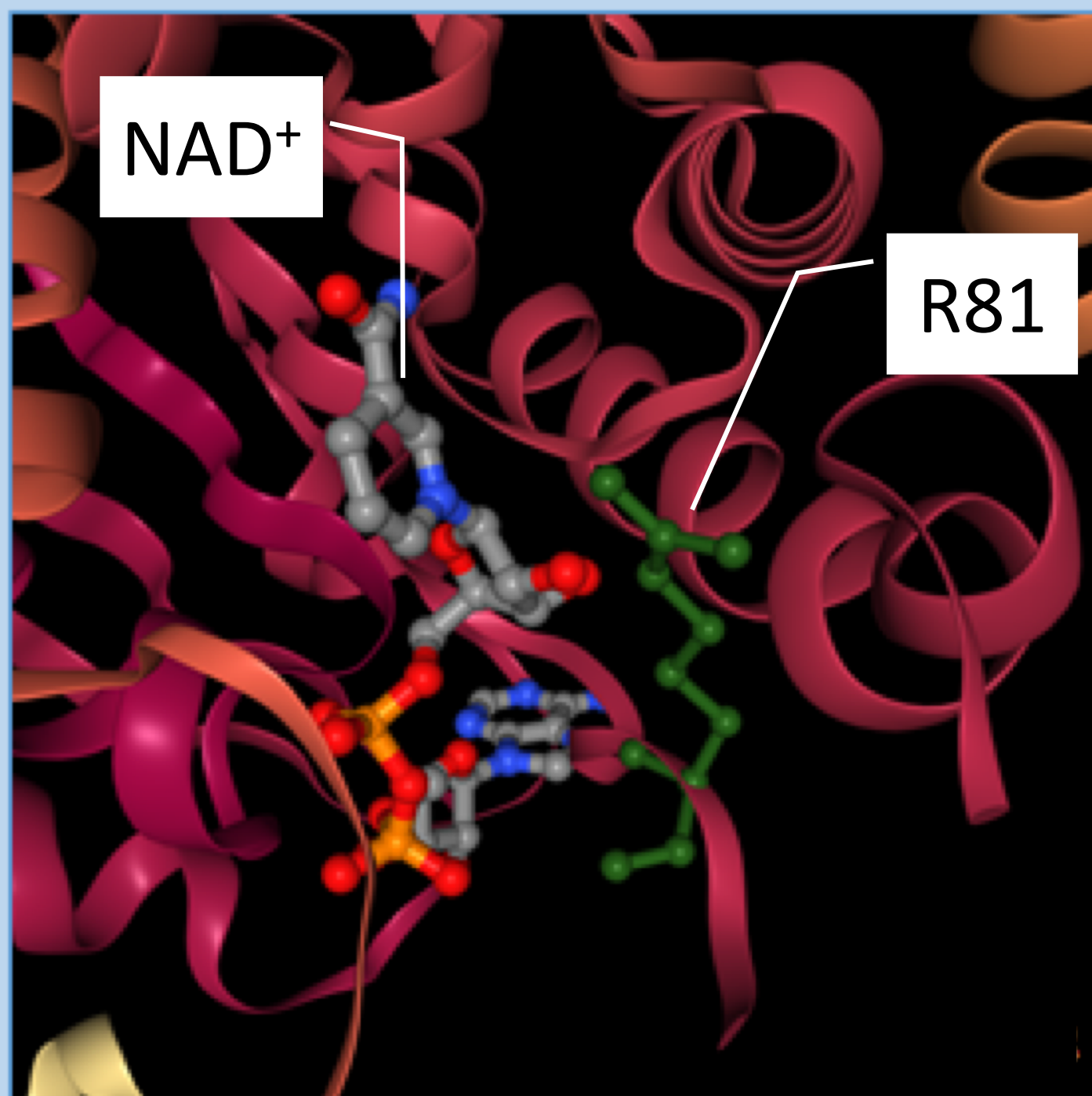


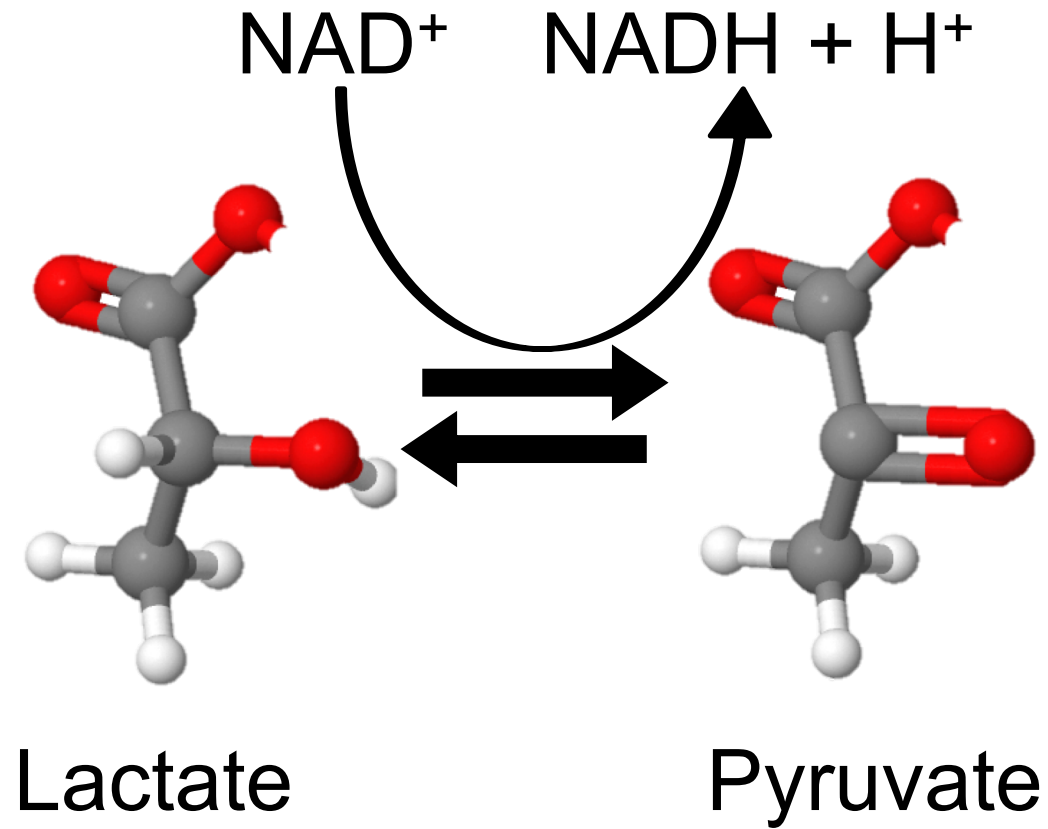
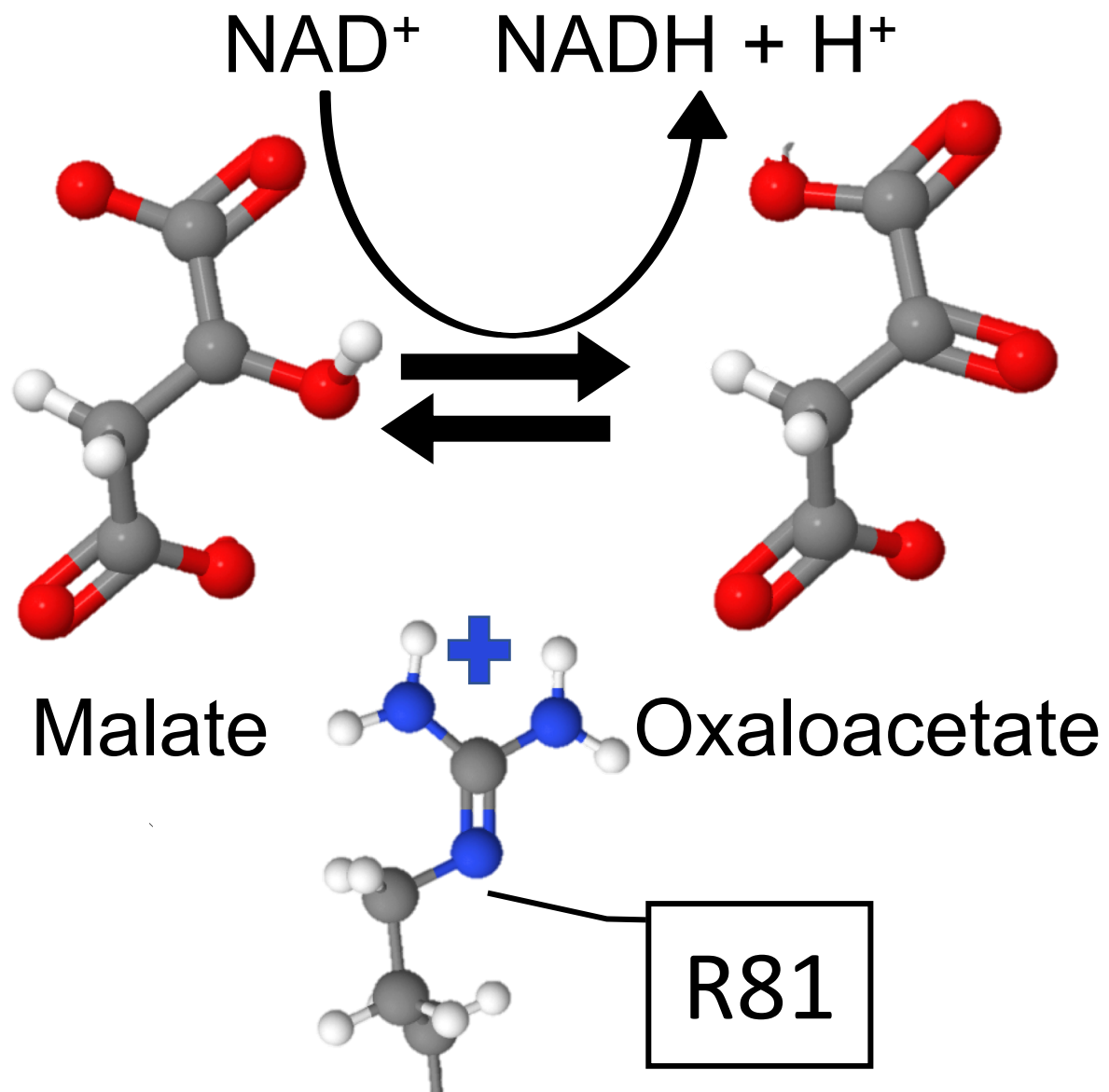
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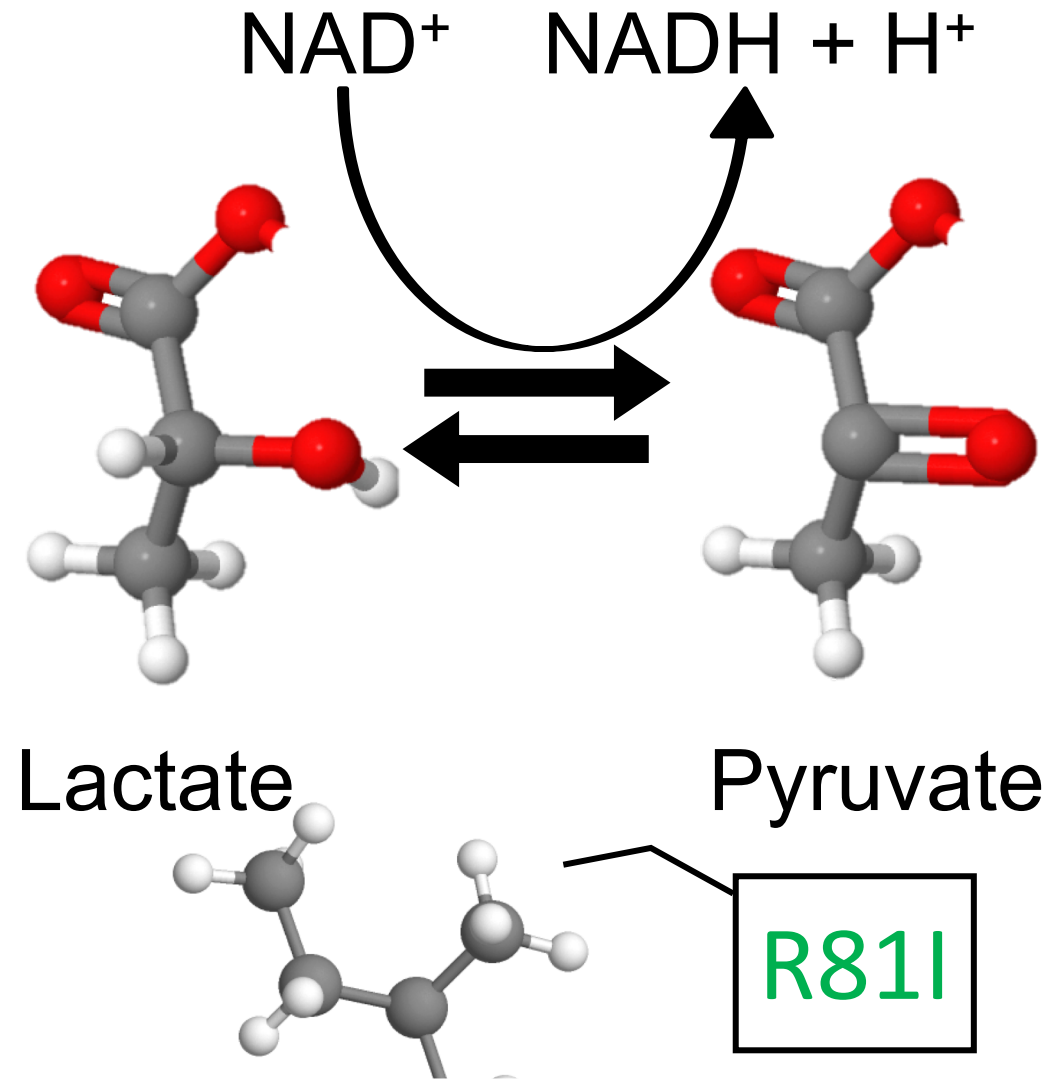
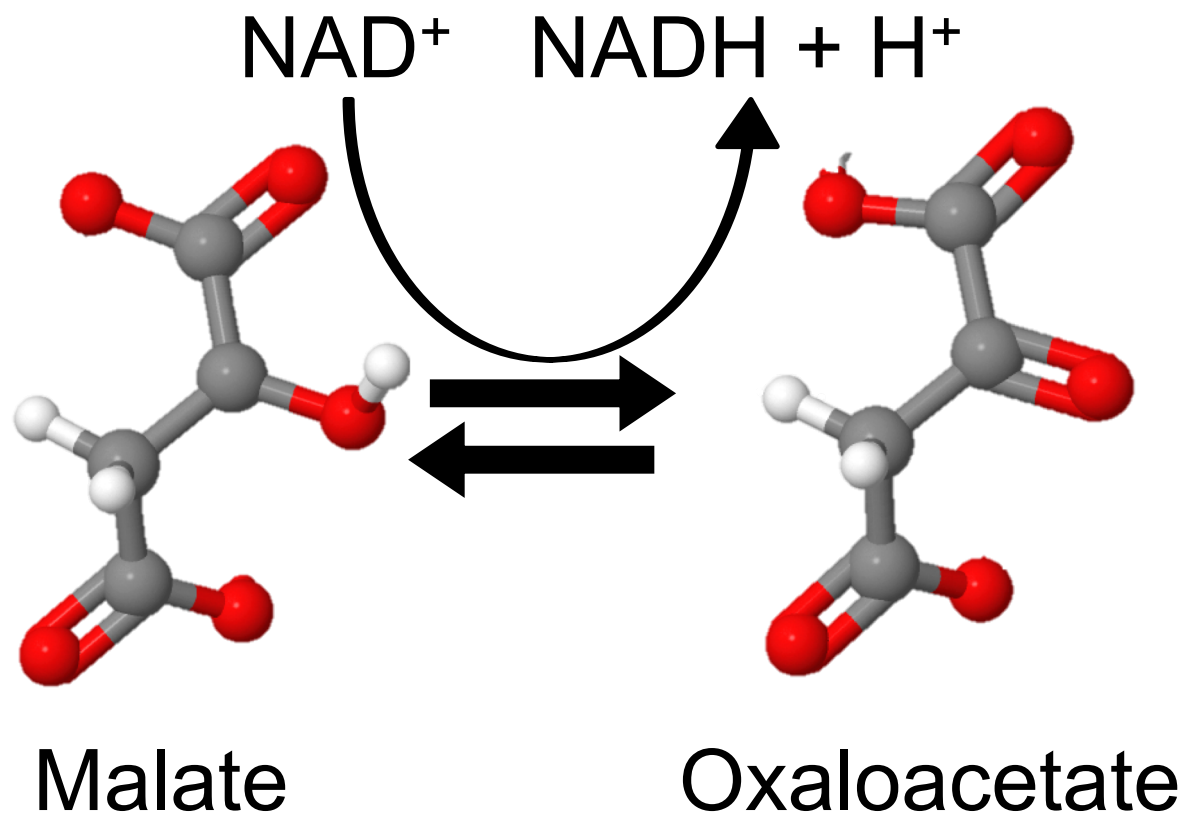
Furukawa, N., Togawa, M., Miyanaga, A.,  
Nakajima, M., Taguchi, H.



PDB: 1IB6



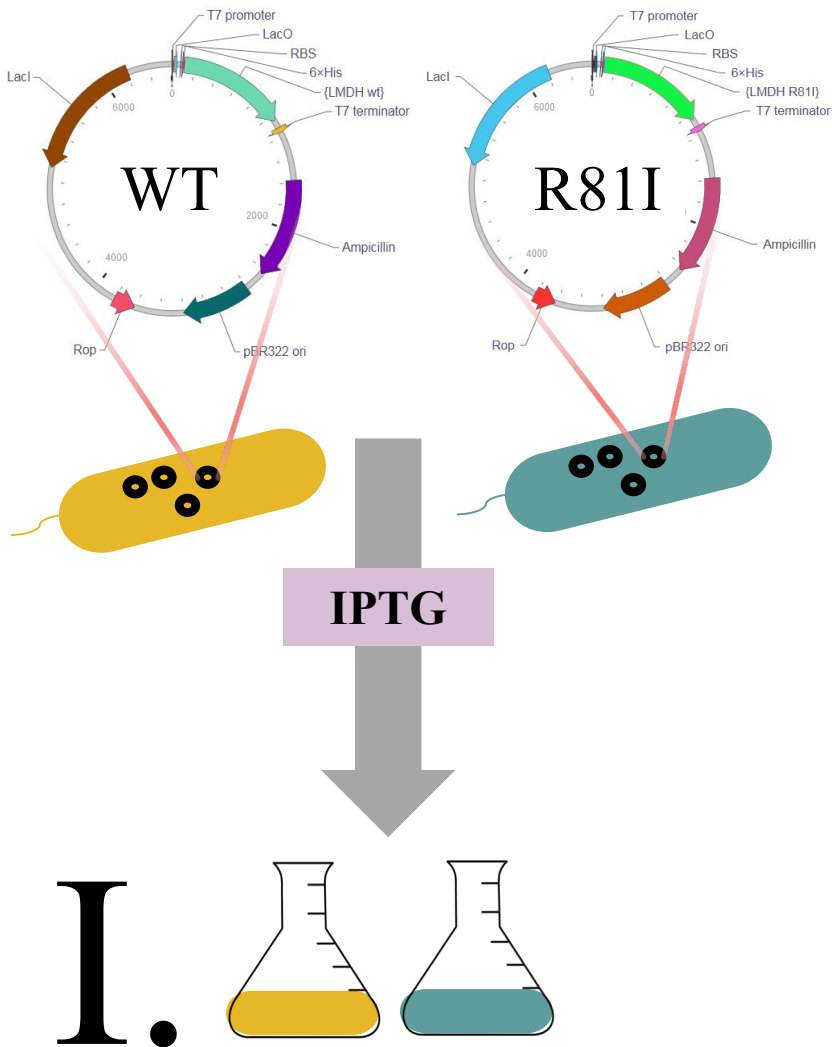




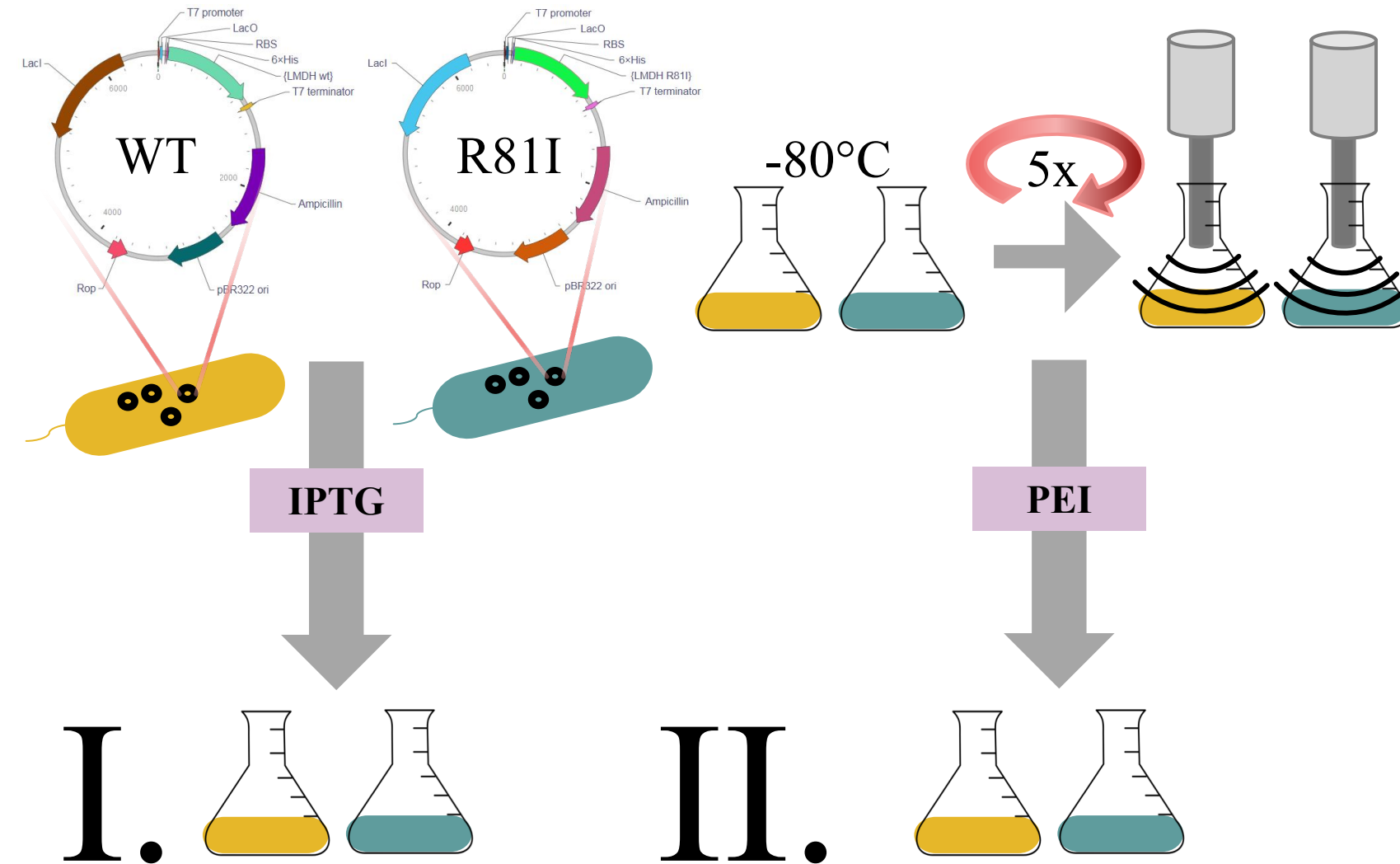
## •Hypothesis

- The arginine-to-isoleucine (R81I) mutation in MDH would alter enzyme activity in a way that favors the lactate-pyruvate reaction because of the methyl group on lactate and pyruvate.

# Method – Expression, Isolation & Purification

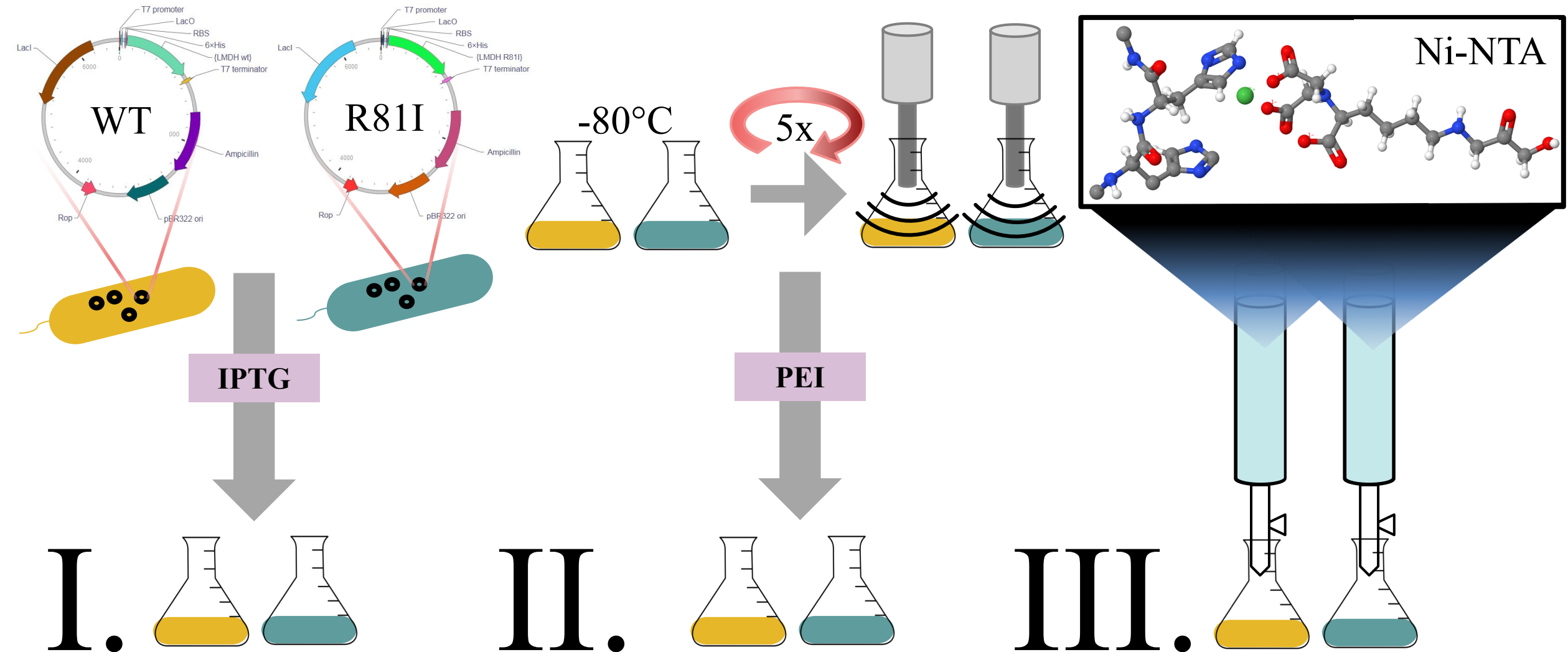


# Method – Expression, Isolation & Purification

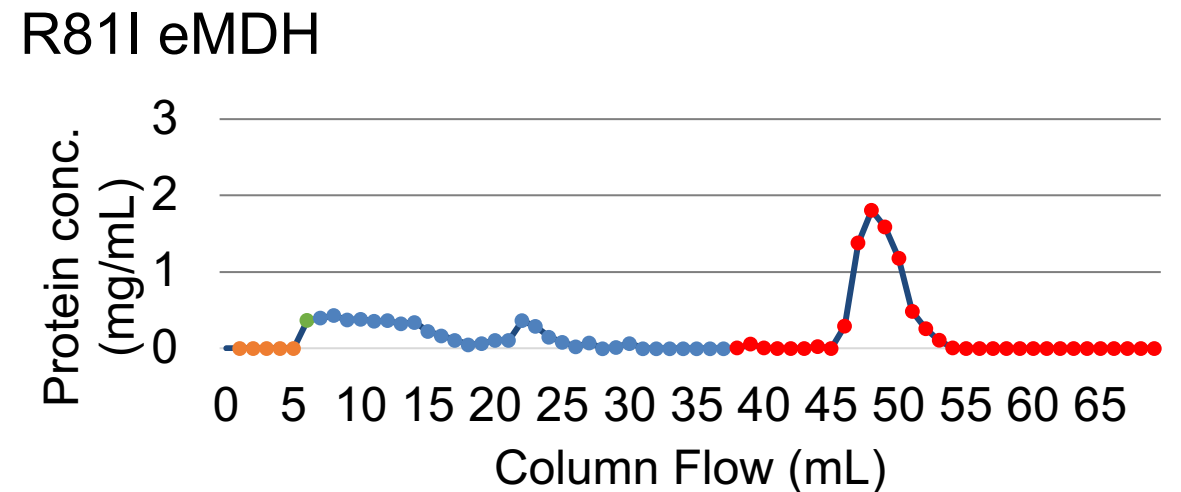
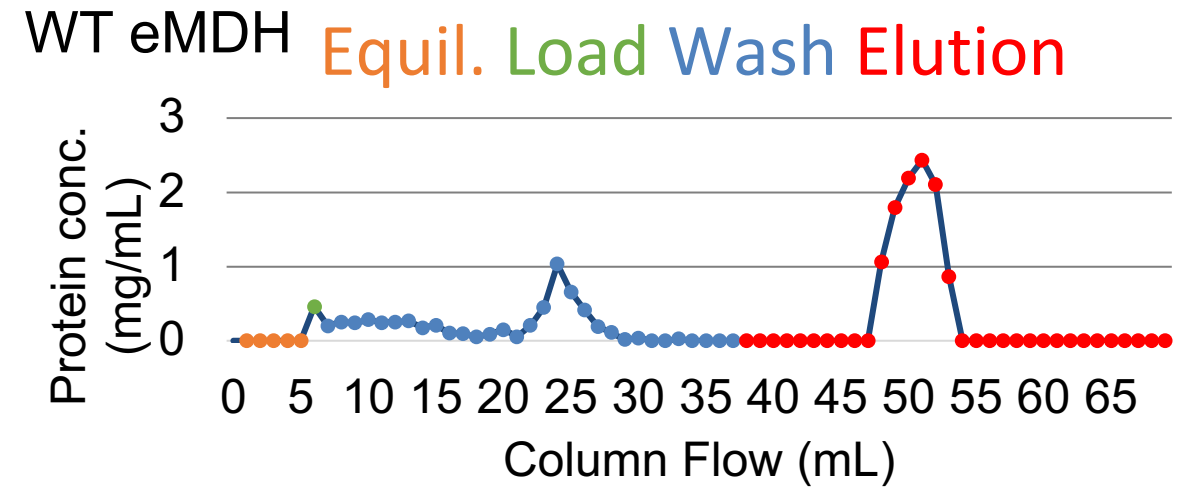
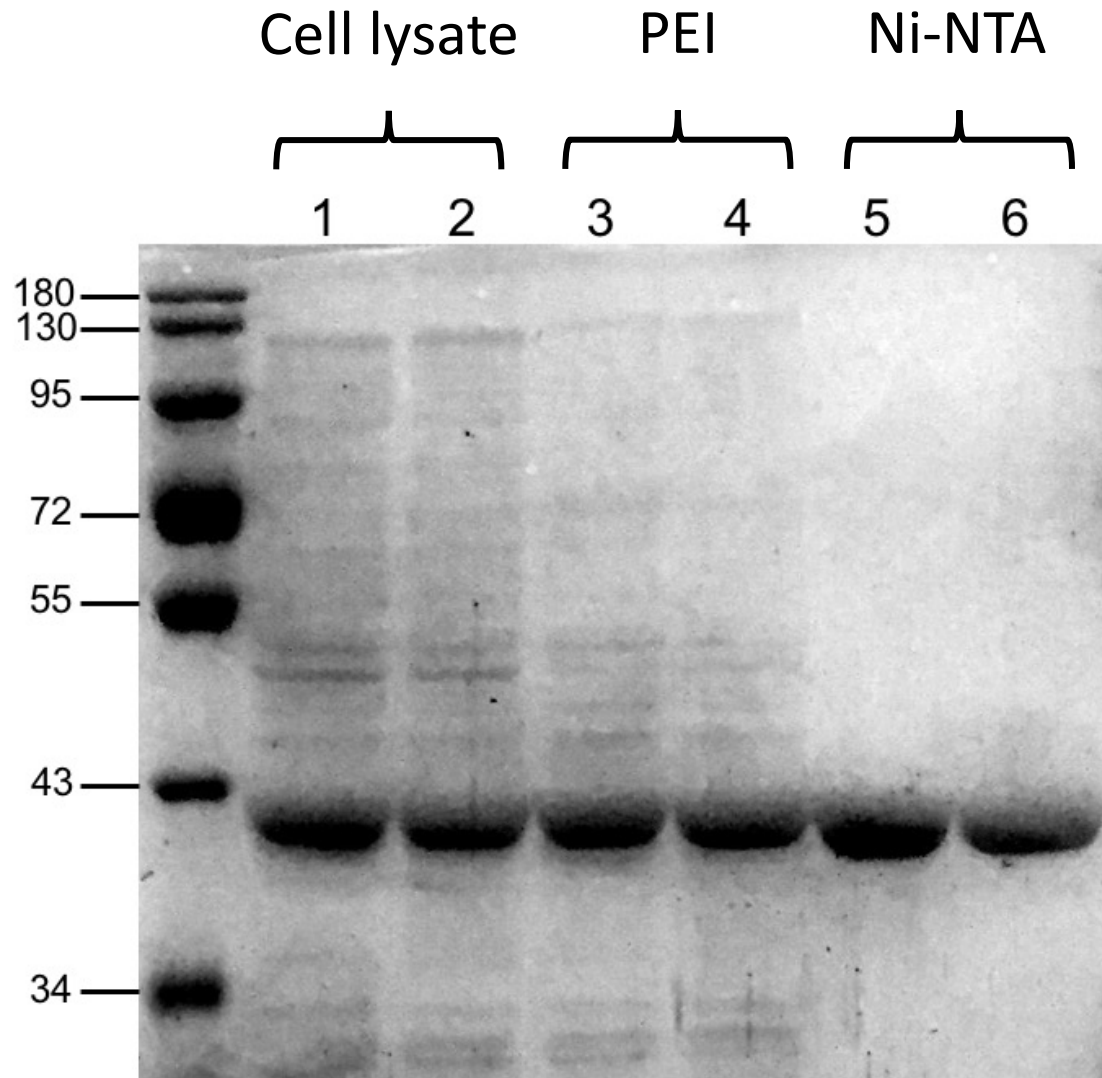




# Method – Expression, Isolation & Purification



# Results – Expression, Isolation & Purification

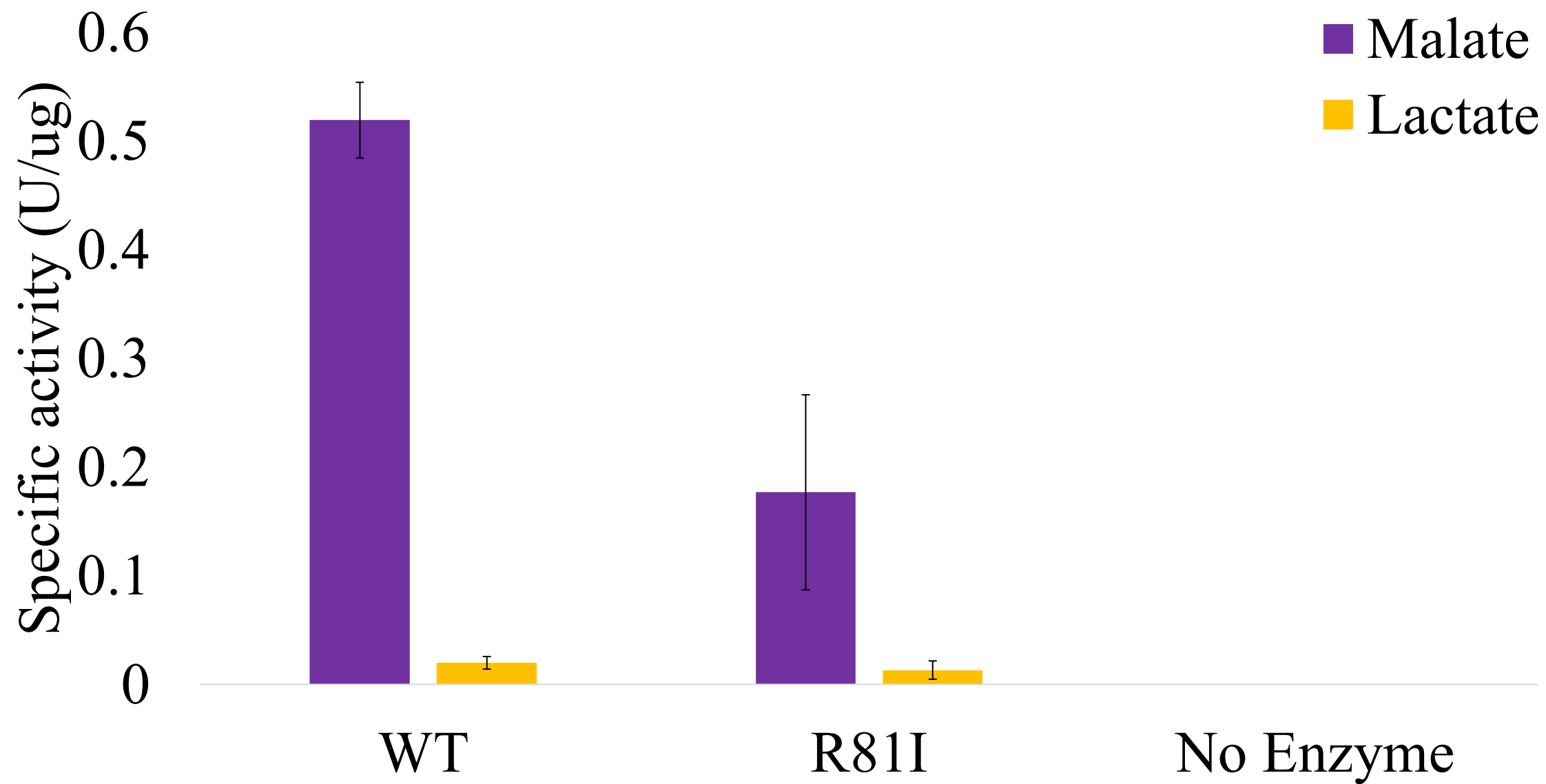


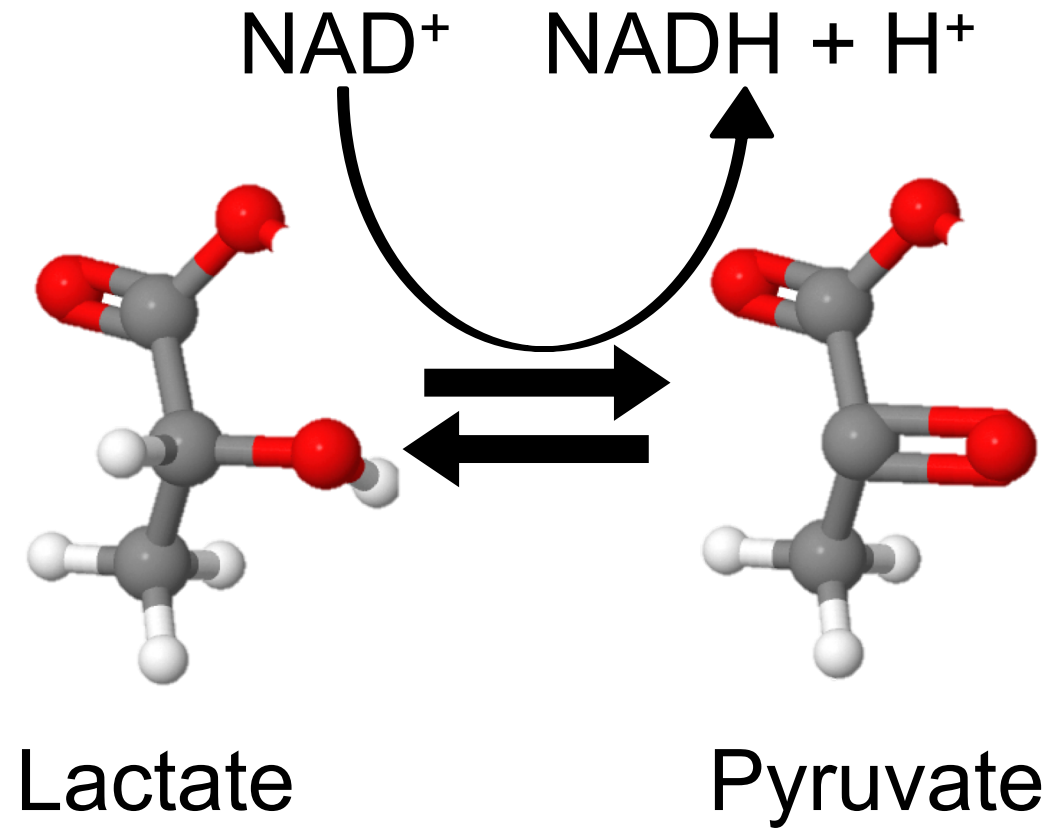
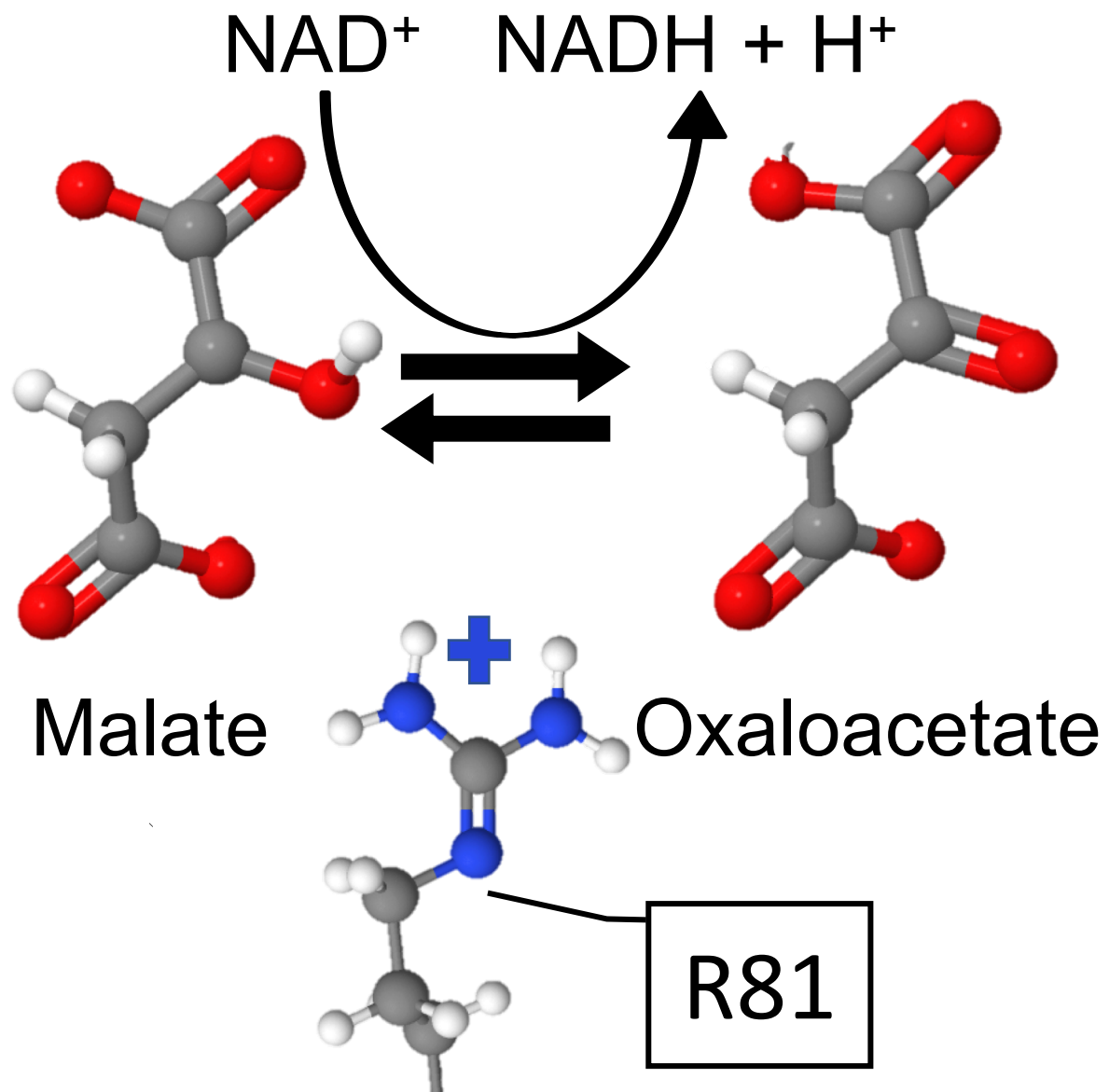
# Method – Characterization

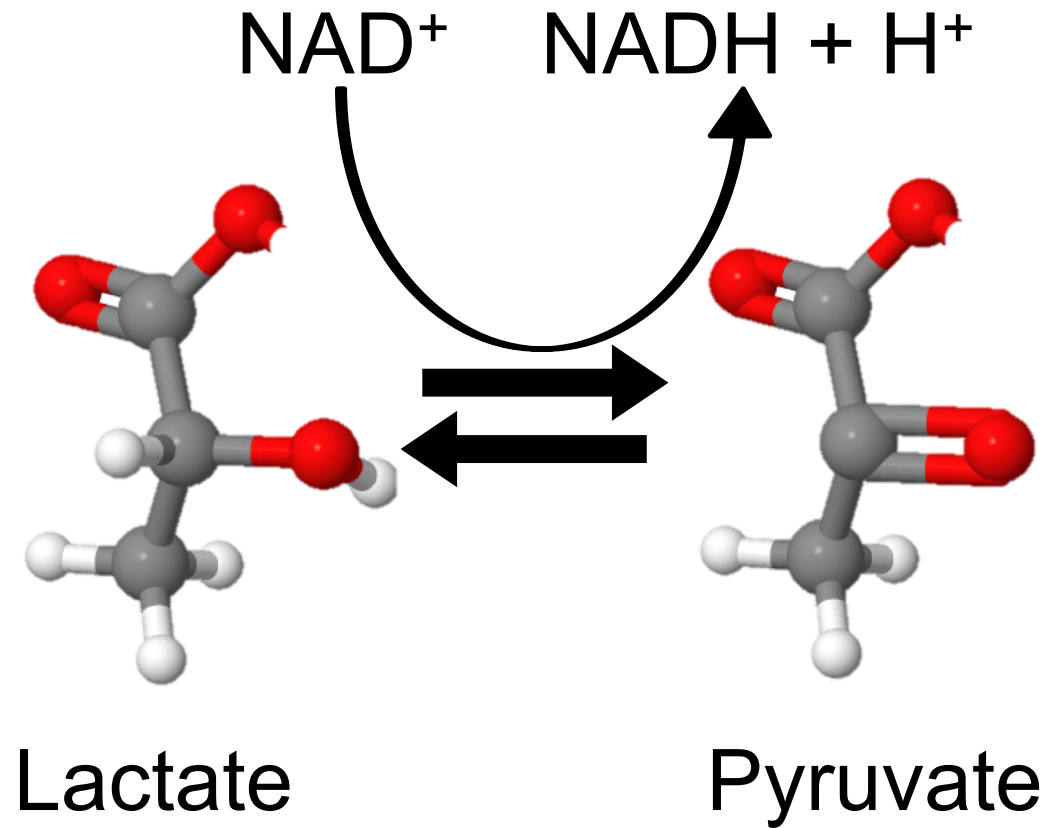
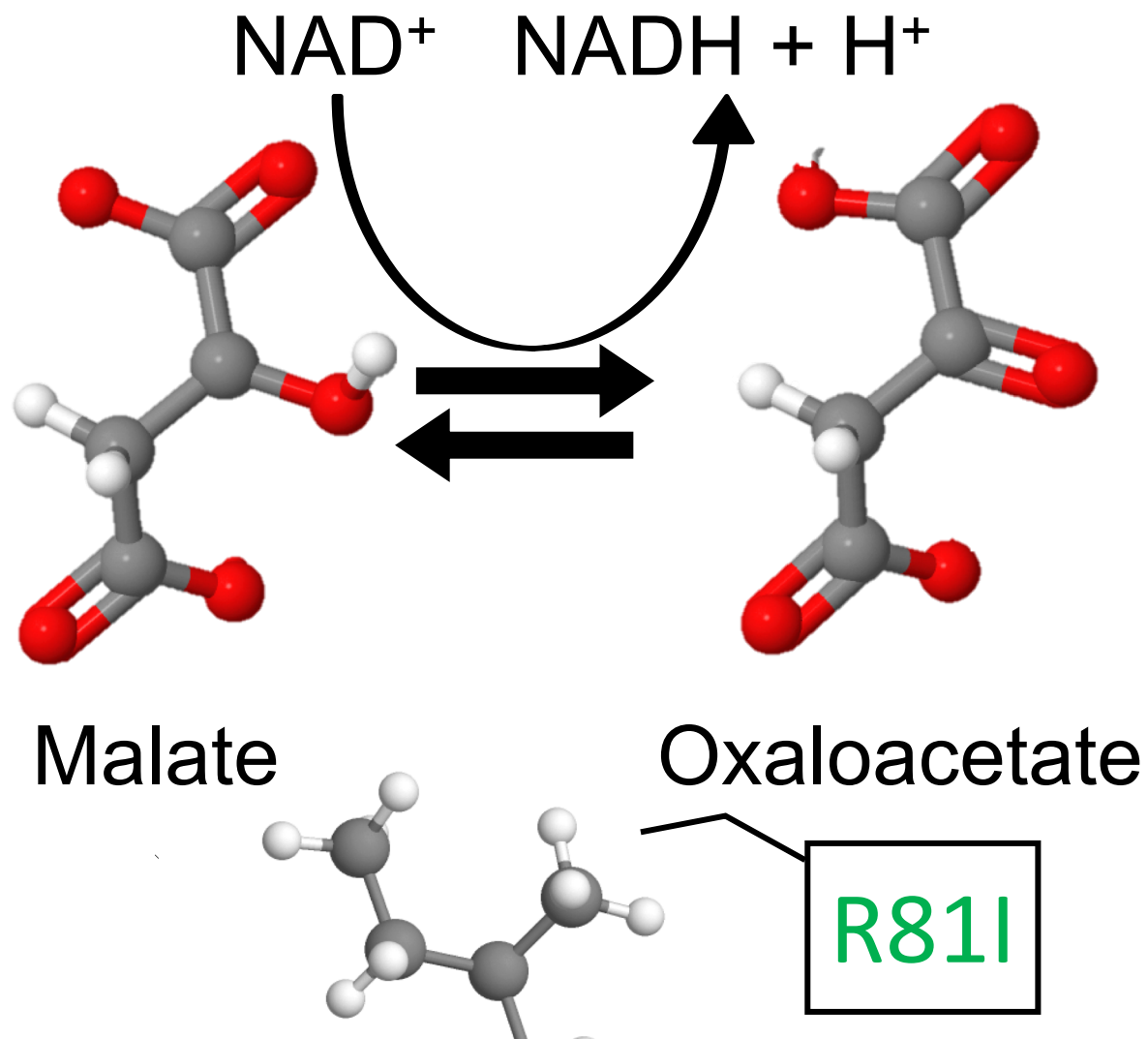
Wild Type  
R81I

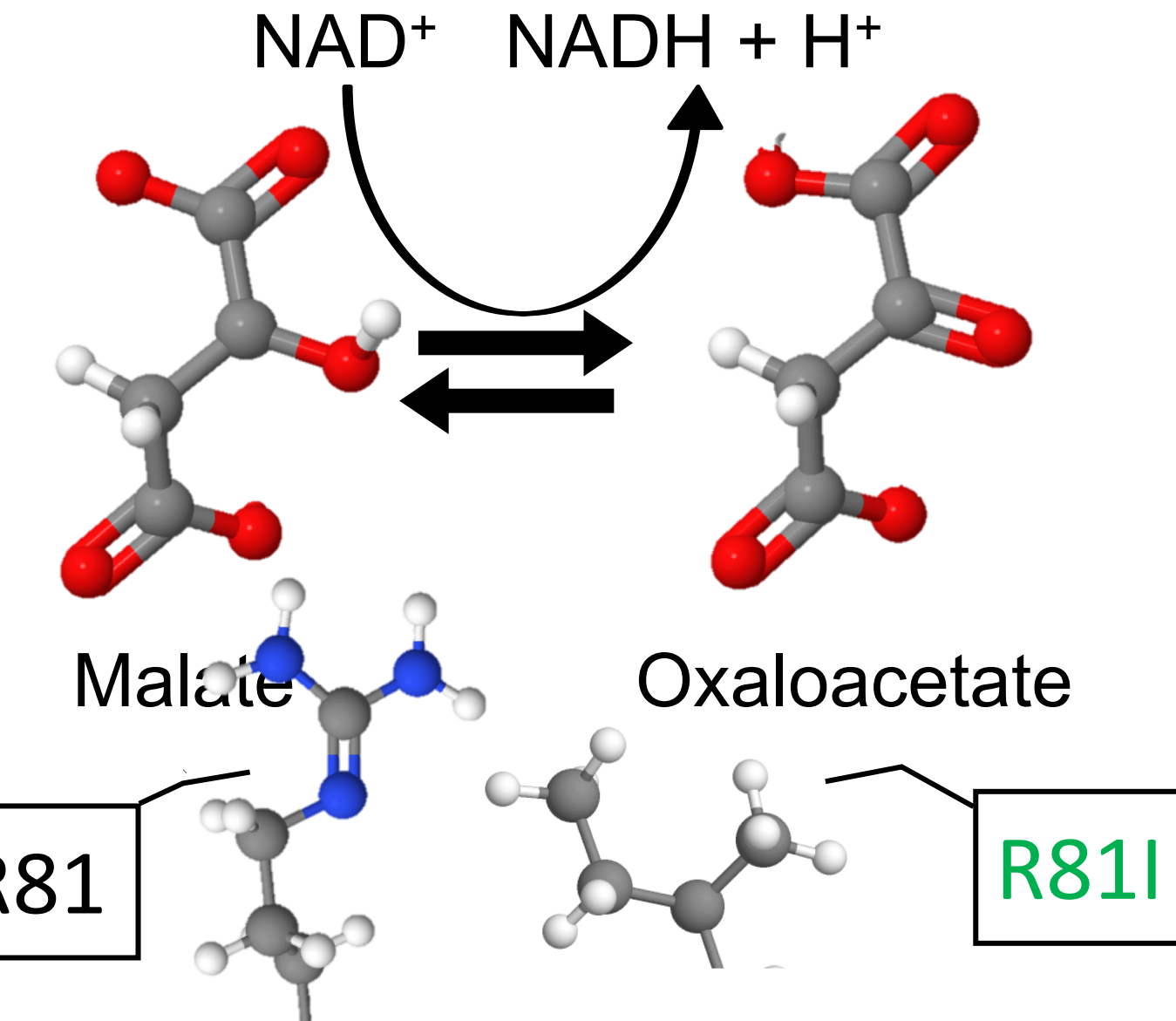
- Bradford assay
- Enzyme assay with **malate**
- Enzyme assay with **lactate**
- Enzyme assay with **pyruvate**

Specific  
activity  
per  
substrate



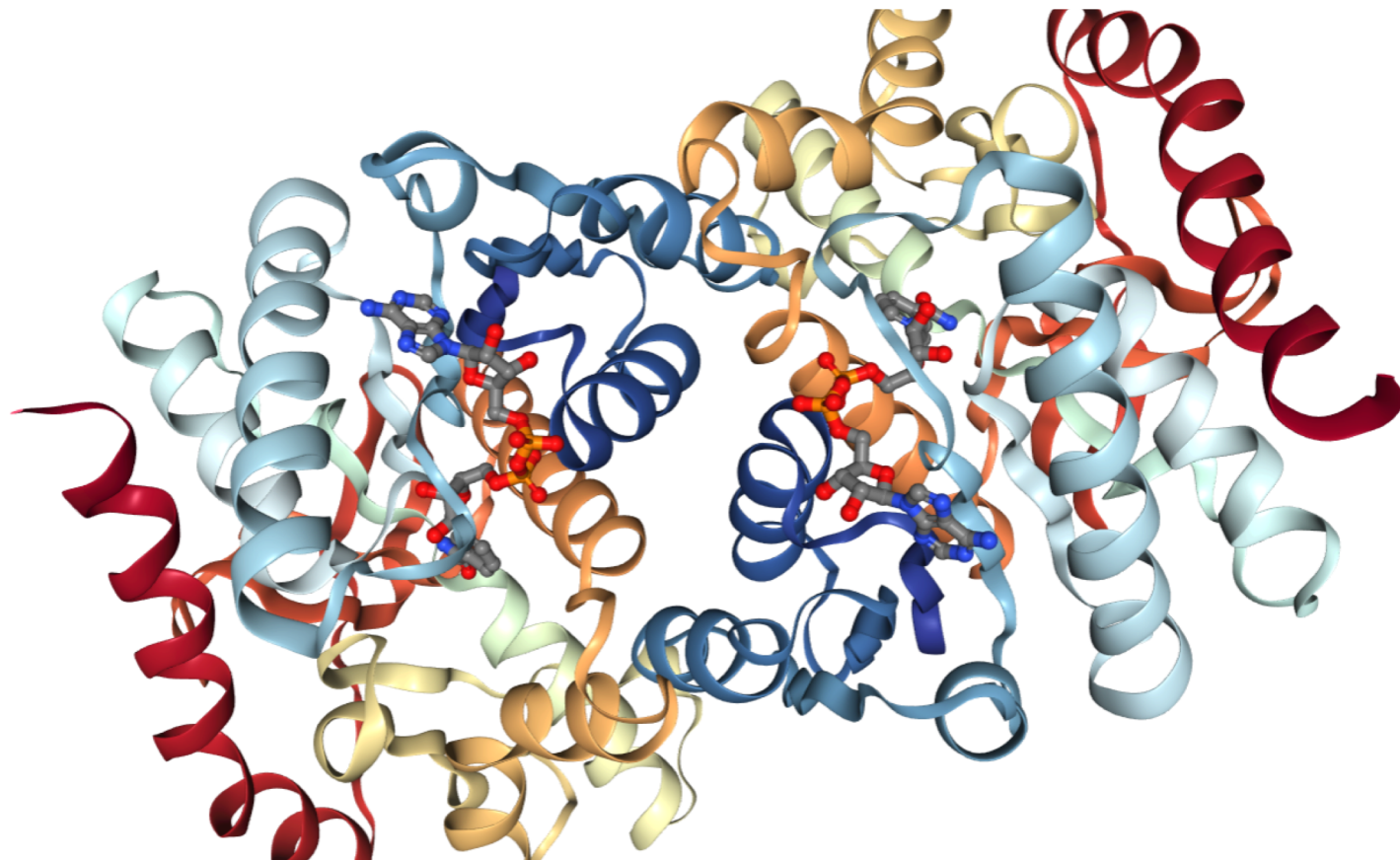






## Questions to Answer:

1. Dehydration of the active site / hydration potential of active site enzymes
2. Steric or electrostatic forces guide the transition?
3. Contribution from neighboring positively charged amino acids?



Thank You

- Pence-Boyce Research Experience
- Dr. Heyen
- Department of Chemistry & Geosciences





Questions?