

# Site-Directed Mutagenesis of Malate Dehydrogenase: A Class Project

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and Andrew Gemmaka

# Challenge:

Make your own mutant protein:

1. Design the mutation
2. You have 8 weeks to create the protein

Order supplies,  
Express the protein  
in a host,  
Purify,  
Characterize.

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# Continuing the Malate dehydrogenase project

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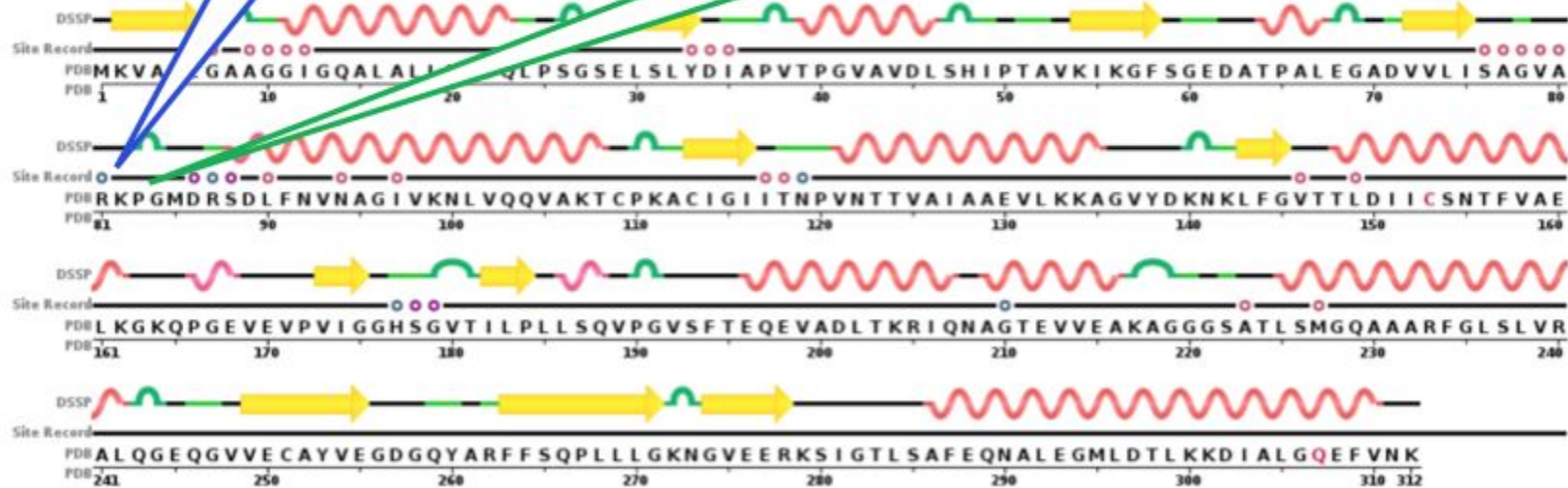
## Goals:

1. To engineer an dehydrogenase enzyme with new substrate specificity towards lactate
2. To propose a catalytic mechanism for *E. coli* malate dehydrogenase

# MDH

R

K

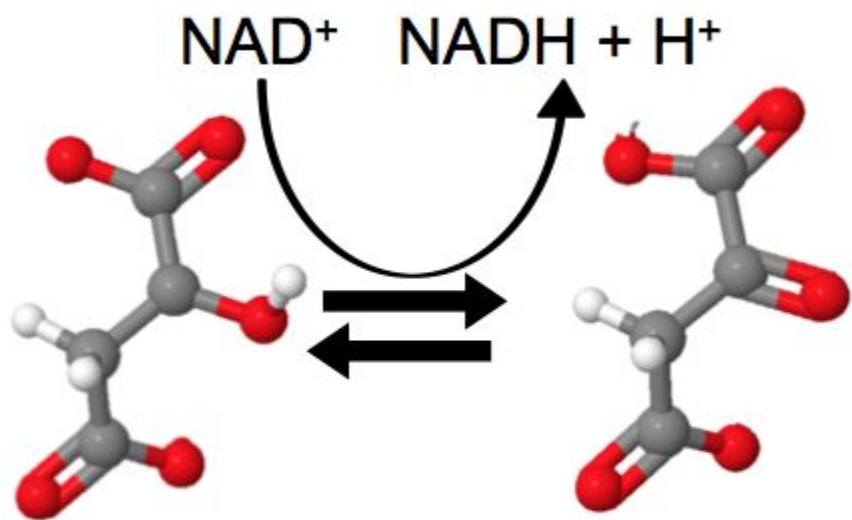


PDB: 1IB6

# Hypothesis:

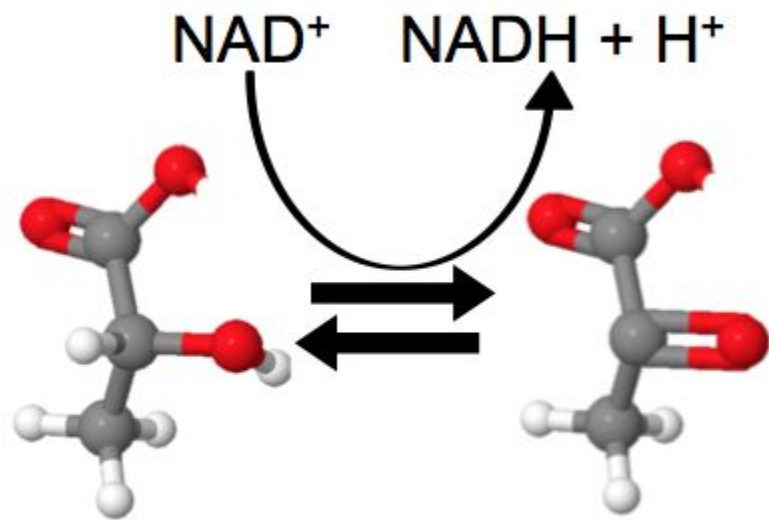
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A R81I/K82I dimutant will **create a hydrophobic pocket** in the active site that will lead to a change in substrate specificity from malate to lactate.



Malate

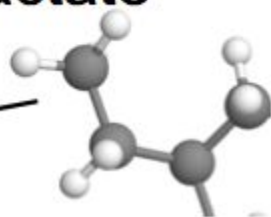
Oxaloacetate



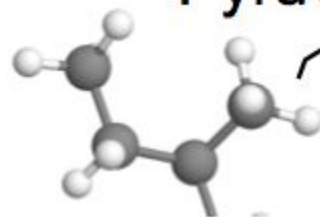
Lactate

Pyruvate

R81I



K82I



# Methods

# Site Directed Mutagenesis

- Mutating the plasmids

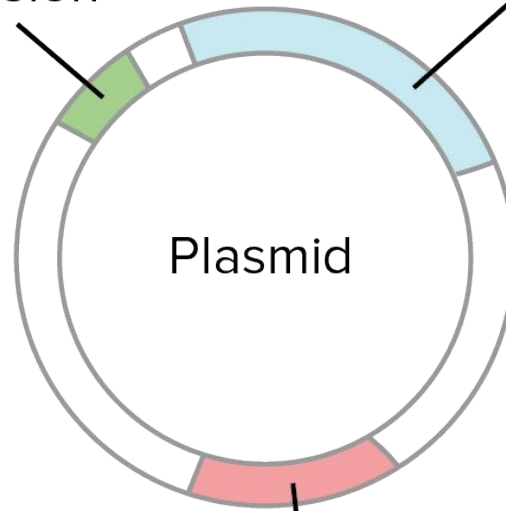




# Site Directed Mutagenesis

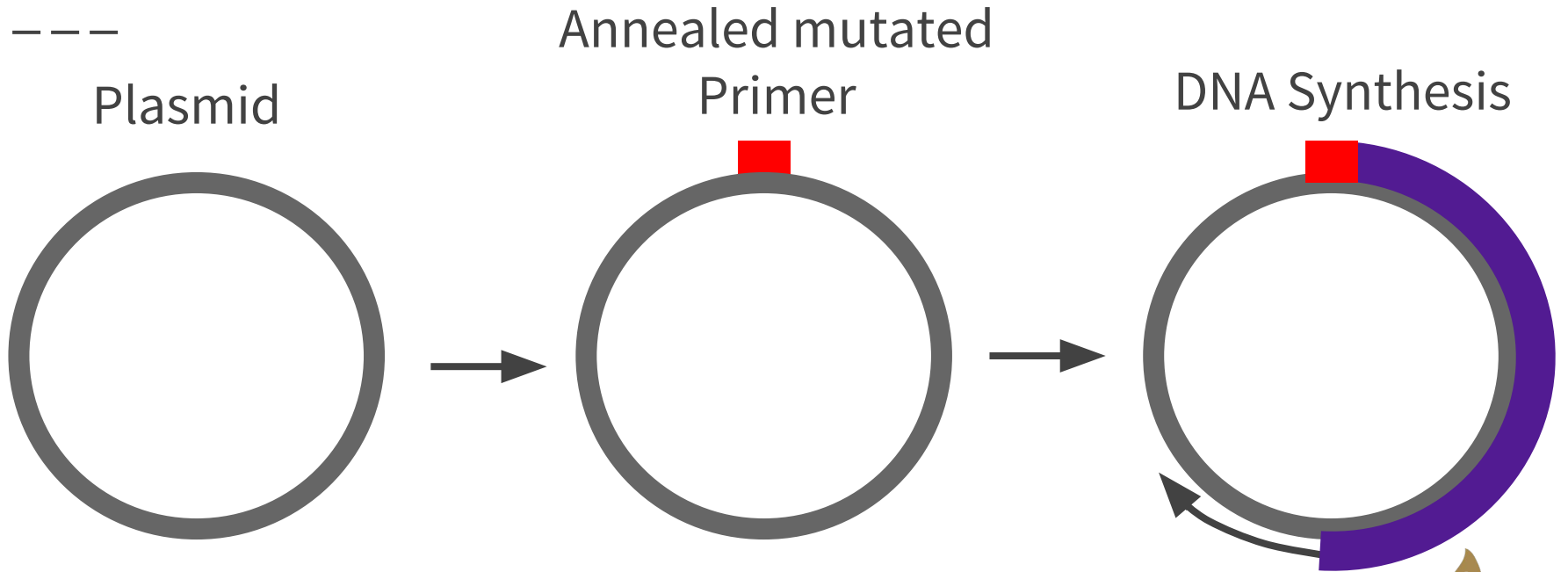
Promoter to drive  
target gene  
expression

WT mdh



ampR

# Site Directed Mutagenesis



# PCR

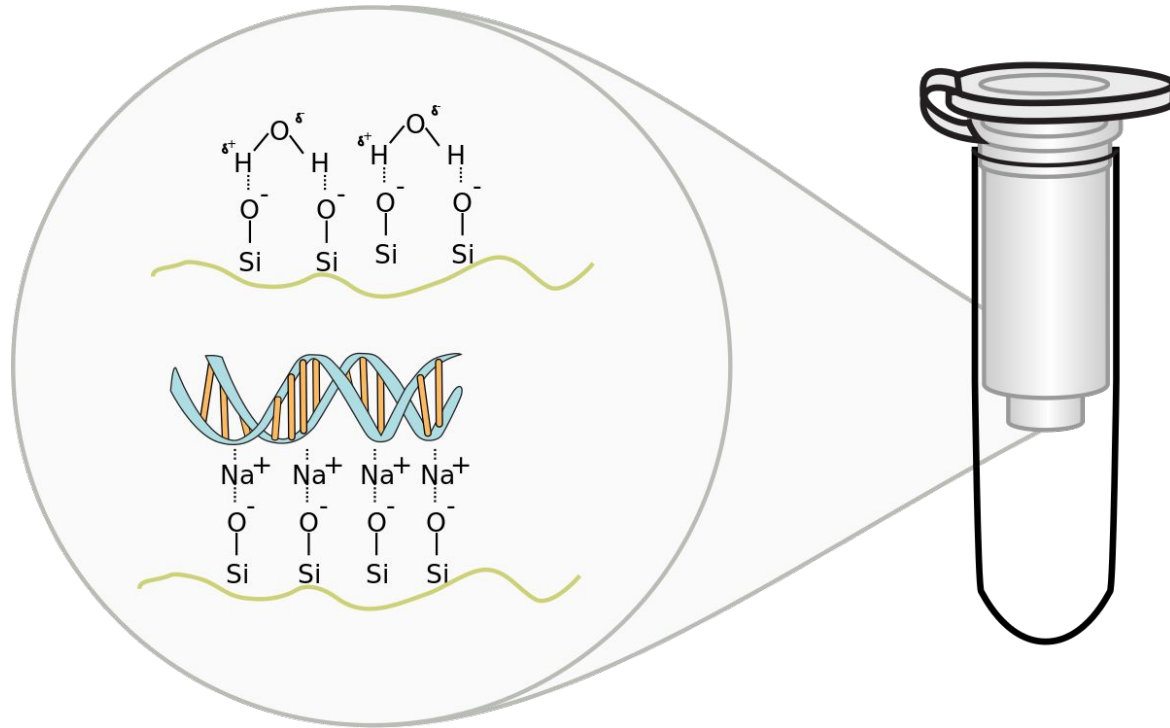


# Purifying the Mutagenic Plasmids

- Spin column

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# Spin Column



# Purifying DNA via a Spin Column

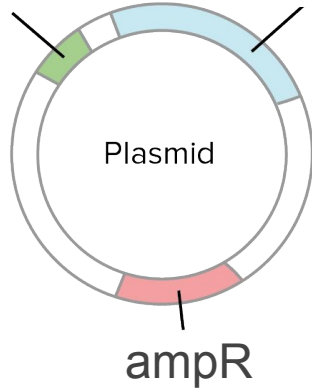


*PCR product*

# Site Directed Mutagenesis

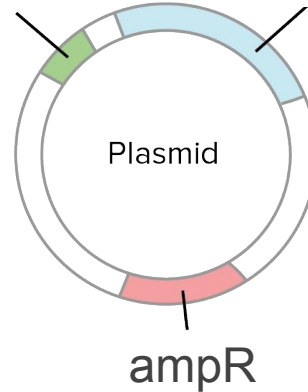
Promoter to drive target gene expression

**R81I Mutant**



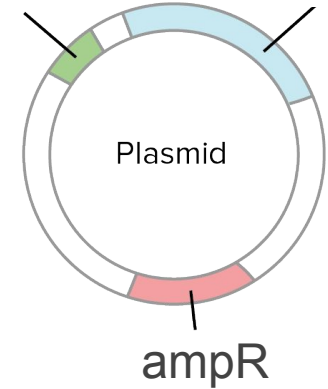
Promoter to drive target gene expression

**K82I Mutant**



Promoter to drive target gene expression

**Dimutant**



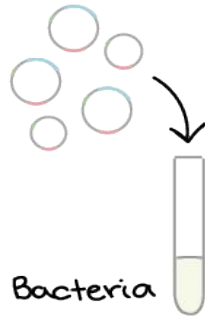
# Transformation

- Inserting the plasmids into the cells

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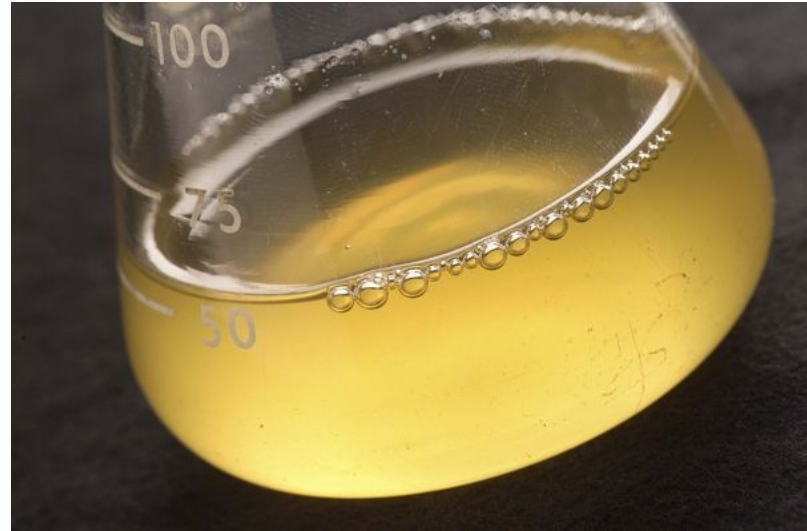


# Transformation



# Cell Growth

- Ampicillin Broth
- Selects for transformed cells



# Expression

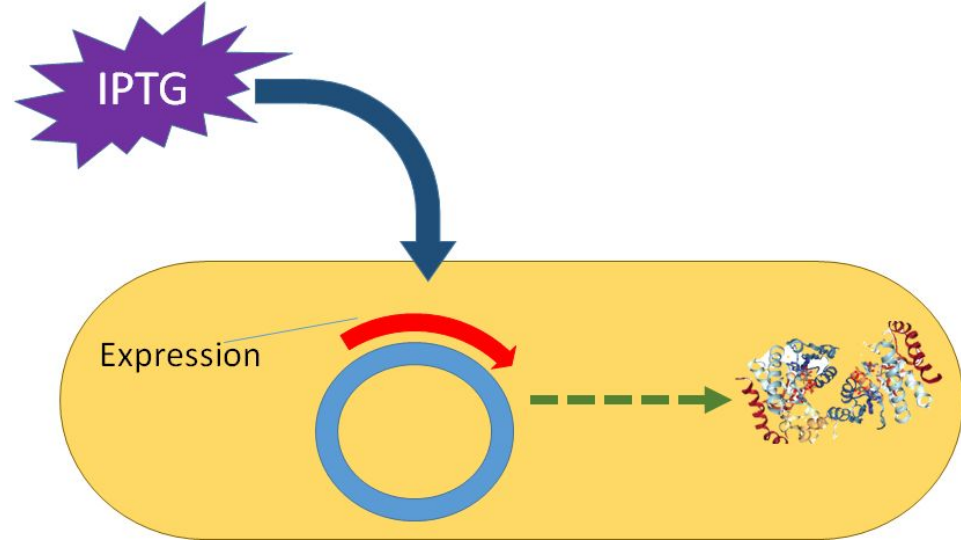
- Expressing MDH



# Protein Expression

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- When lactose is present, the gene is expressed
- IPTG is a lactose analog that binds with higher affinity
- This allows for high gene expression



# Purification

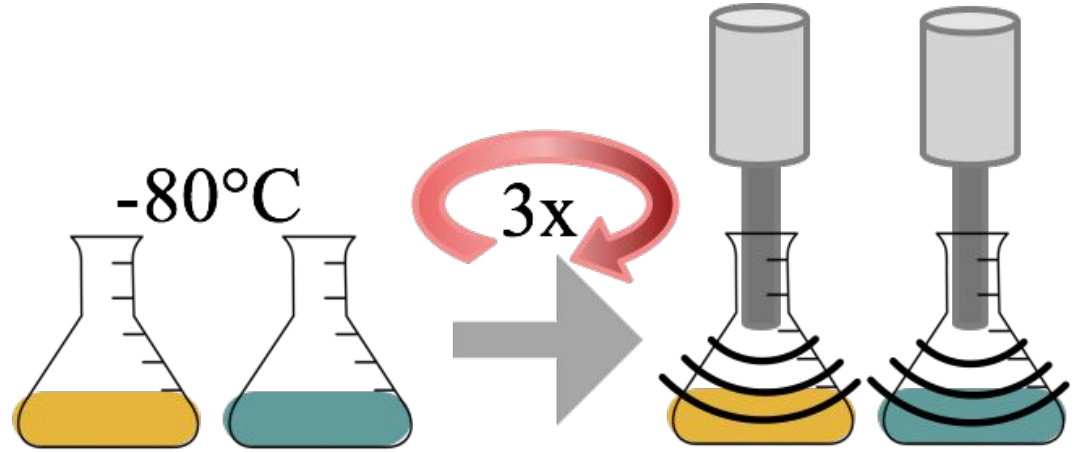
- Isolating protein



# Cell Lysis

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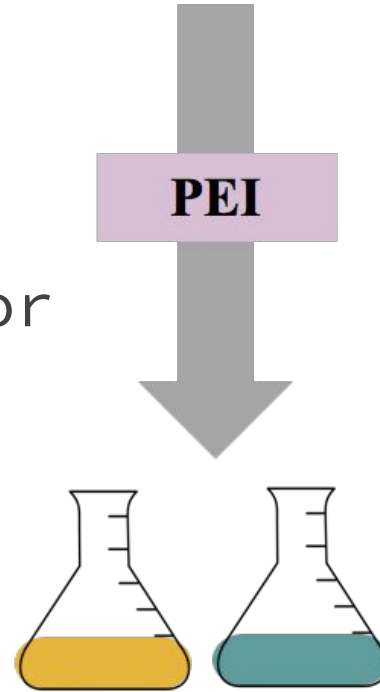
- Freeze
- Thaw
- Sonicate



# Precipitation

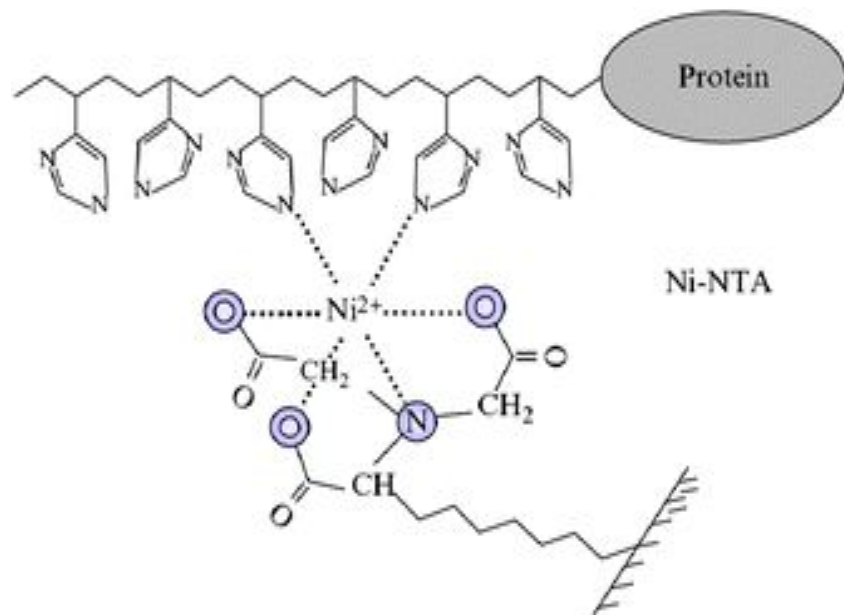
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- Added protease inhibitor
- PEI precipitates DNA



# Purification

- The protein was engineered to have a histidine tag
- Histidine has an imidazole R-group, which is attracted to our Ni-NTA purification column.





# Purification

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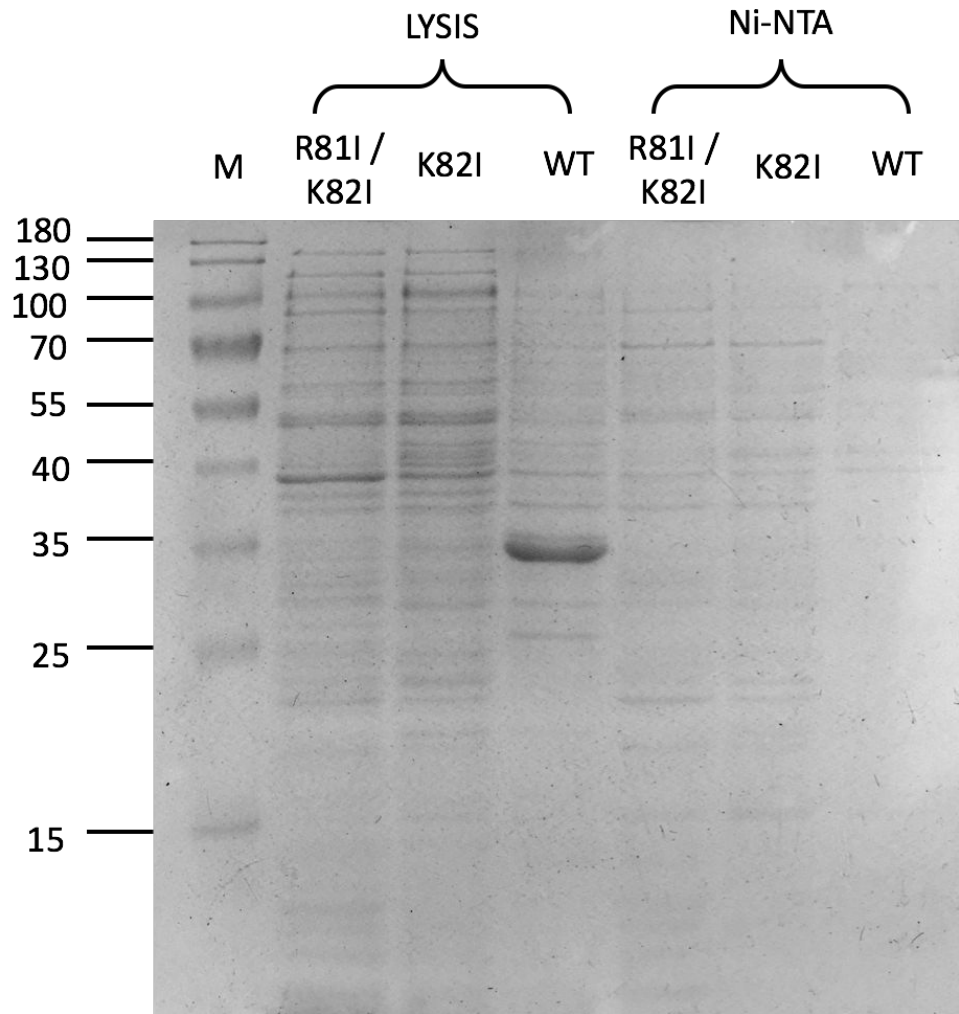
- Combined all fractions from the elution step
- Centrifugal filtration

# Results

# SDS-PAGE

- Protein analysis

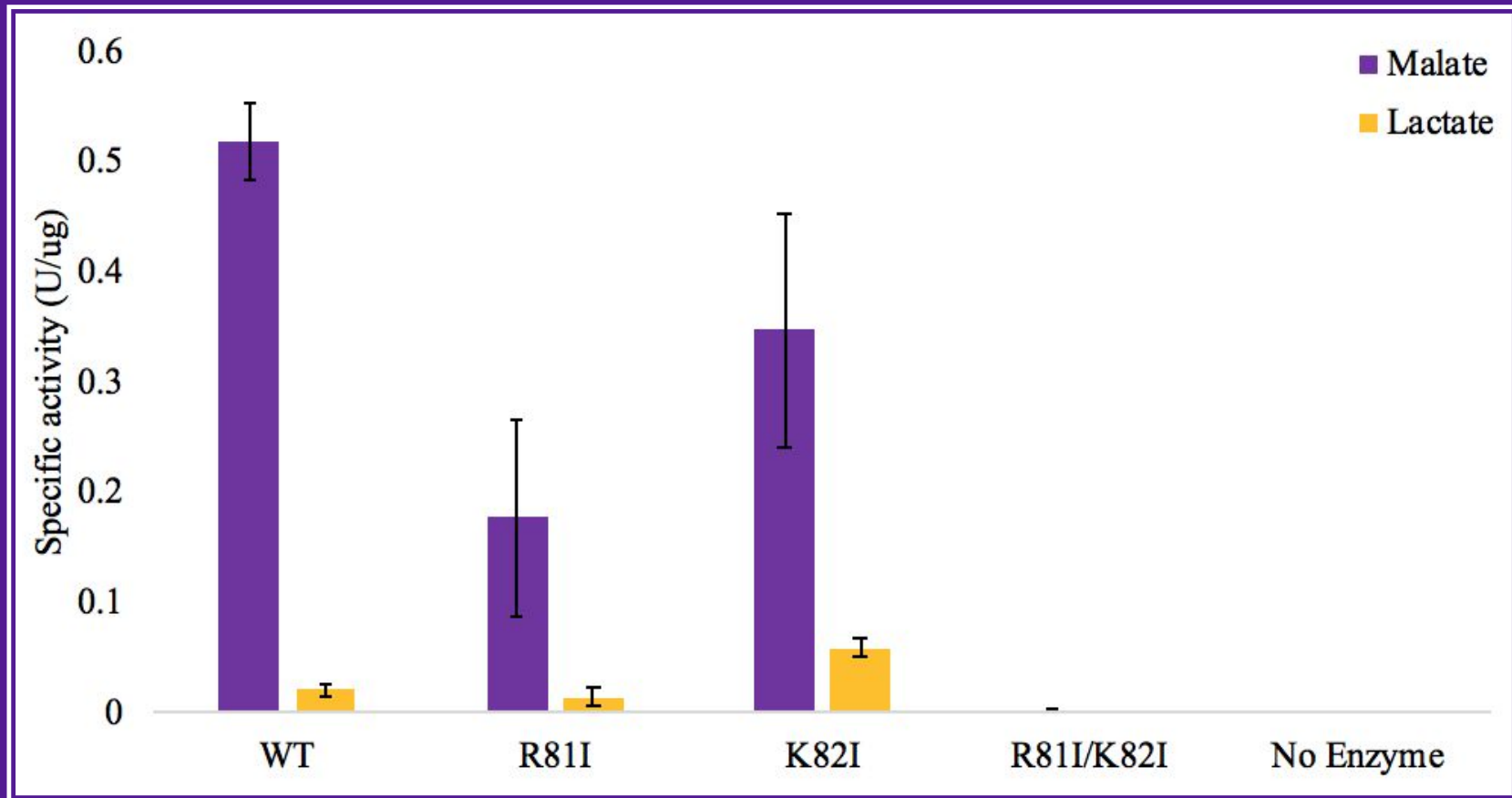




# Enzyme Activity

- Bradford assay
- Comparing malate vs lactate specificity

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# Conclusion

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Hypothesis not supported. Results provide a new insight into the catalytic mechanism of E. coli

- Arginine-81 may have greater influence on malate-oxaloacetate catalysis
- Lysine-82 may have greater influence on specificity
- Arg-81 was previously listed as a functional paralog shift mutation, but Lys-82 was not.



# Near Future - Other methods, other proteins

## Methods

- pH probe enzyme assays
- Bioinformatics
- Structure techniques
  - X-ray
  - Protein NMR

## Proteins

- Any protein
- Any organism
  - CHO cells

# Reflections

- Opportunity for independent research
- Importance of independent research
- Requirement for getting into grad school/jobs
- Class Bonding
- Critical thinking/ problem solving

# Acknowledgements



- Olivet Nazarene University
- Department of Chemistry
- Dr. Bruce J. Heyen

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# Questions?

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