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Ministero dell'Università e della Ricerca

Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

PRODUCTION OF RECOMBINANT ENZYMES IN BACTERIAL HOSTS

Università degli Studi di Urbino

23 maggio 2024

Our Main recombinant enzymes

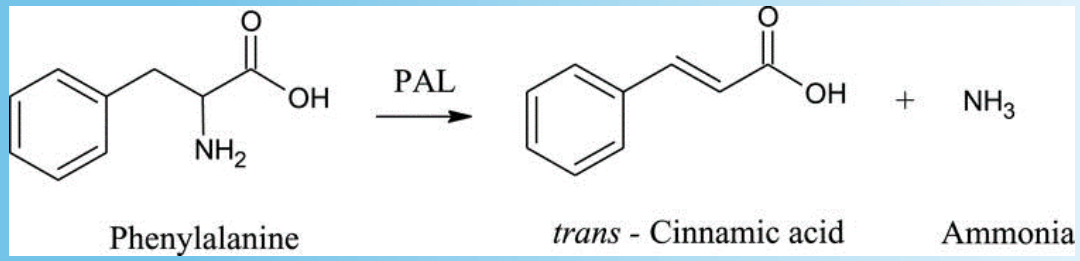
- **PAL: Phenyalanine Ammonia Lyase**
- **GAMT: GuanidinoAcetate MethylTransferase**
- **MAT2A: Methionine AdenosylTransferase 2A**
- **EDCas9: Enhanced Deletion Cas9**

All are expressed in *Escherichia coli* BL21(DE3)

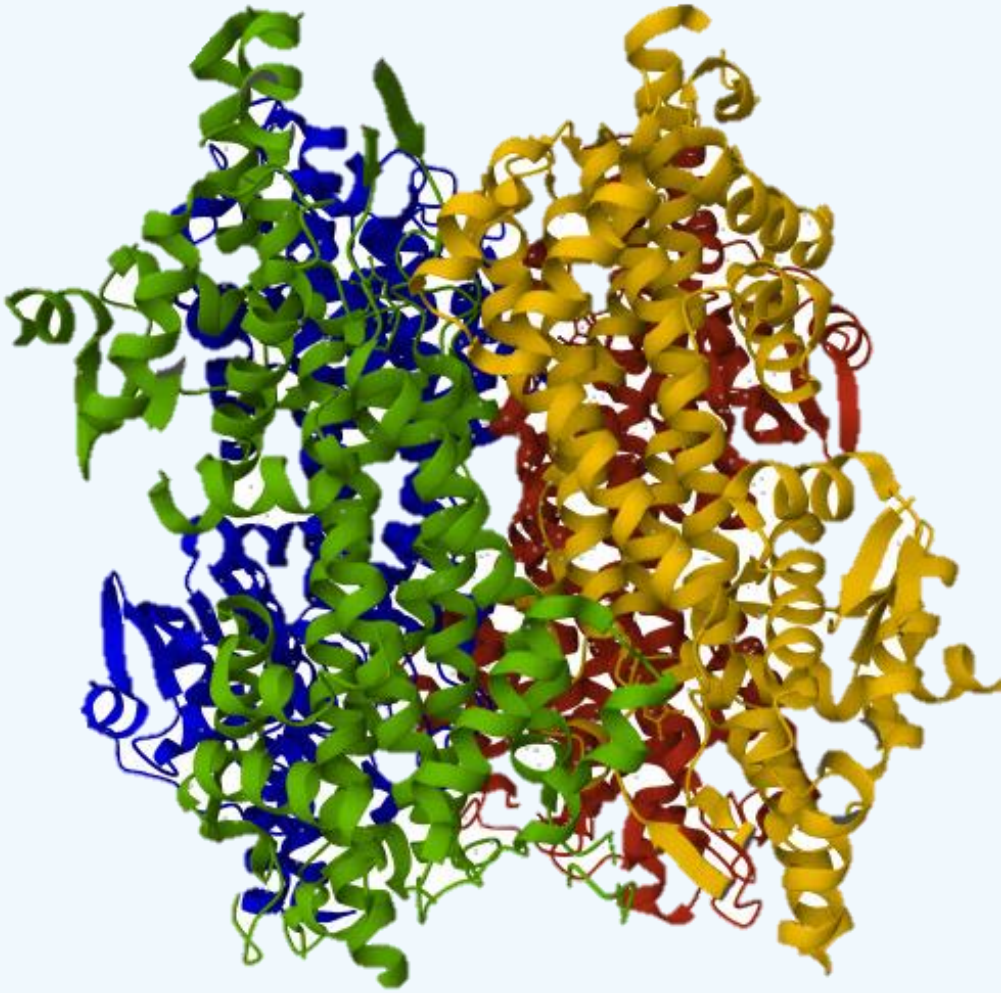
PAL
Phenyalanine Ammonia Lyase

- sequence derived from the cyanobacterium *Anabaena variabilis*

- converts L-Phenylalanine into *trans*-cinnamic acid and ammonia



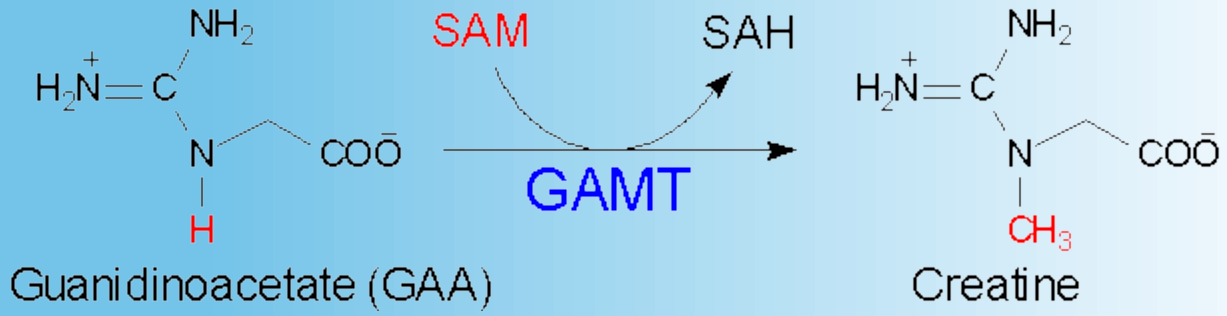
- potential treatment for Phenylketonuria



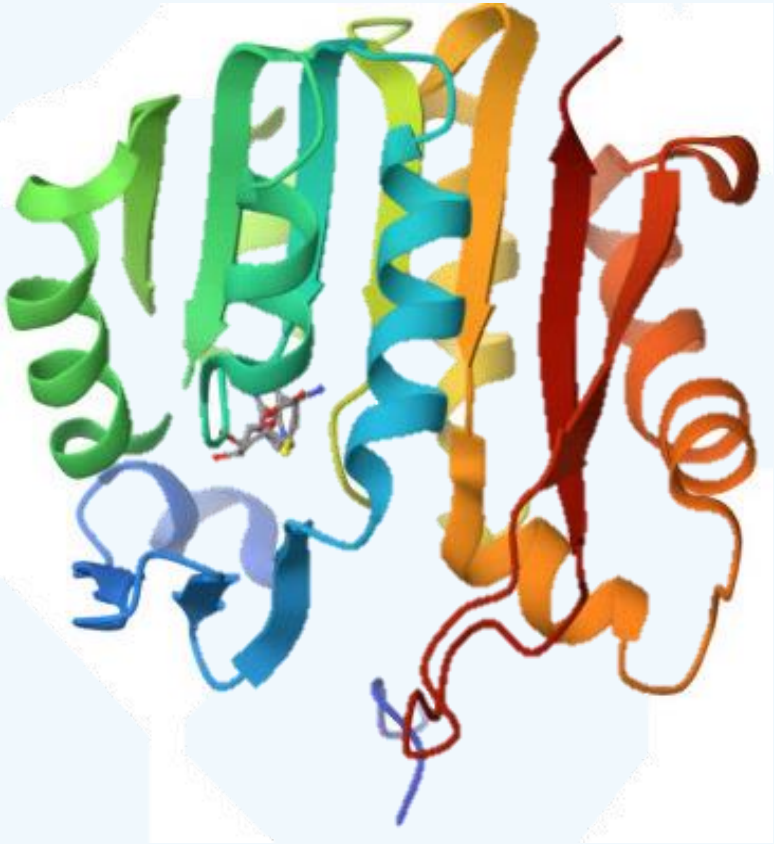
GAMT
GuanidinoAcetate MethylTransferase

- originally derived from the human gene, 4 aminoacids have been mutated to increase stability and solubility

- converts Guanidinoacetate and S-Adenosyl-Methionine into Creatine and S-Adenosyl-Homocysteine



Guanidinoacetate (GAA) Creatine

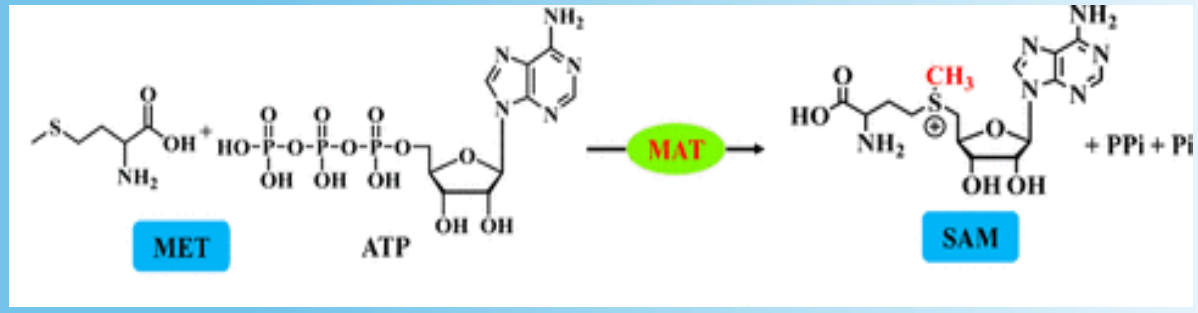


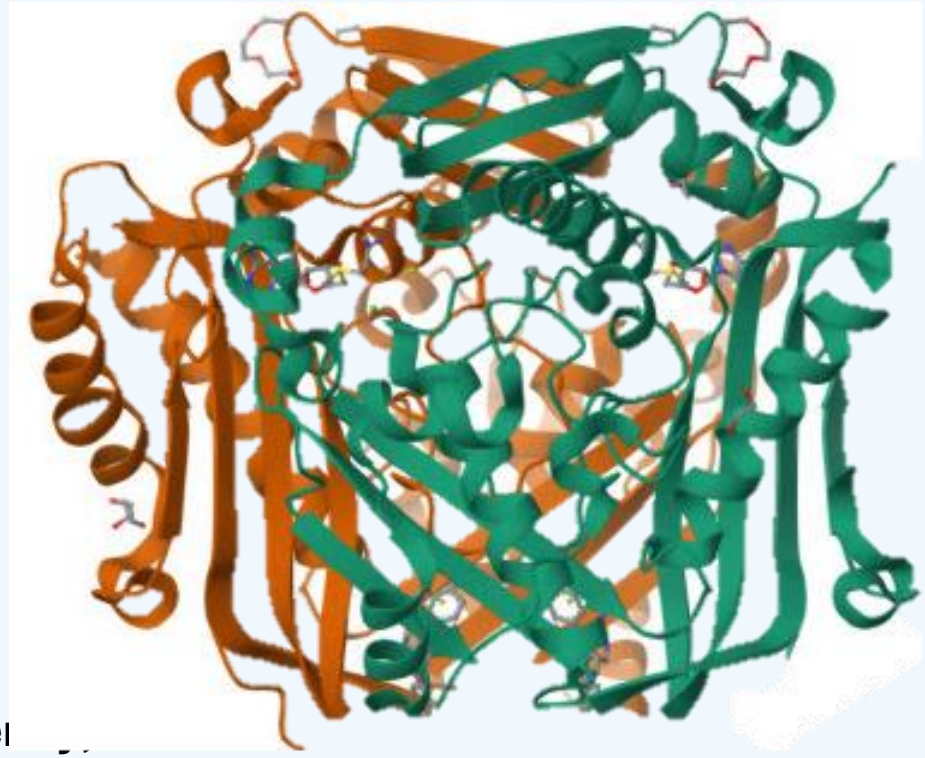
- to be administered in conjunction with MAT2A

MAT2A
Methionine AdenosylTransferase 2A

- derived from the human MAT2A sequence, with no modifications

- Forms S-Adenosyl-Methionine from Methionine and ATP.

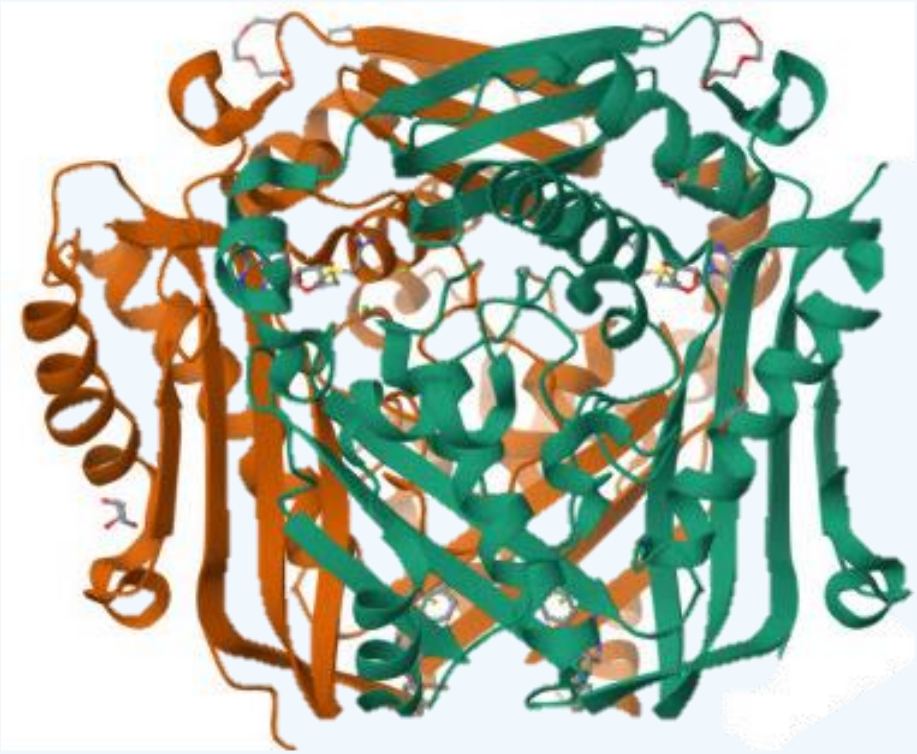




- to be used in conjunction with GAMT as a therapy for GAMT-deficie
MAT2A supplies SAM, which GAMT needs to produce creatine

Guanidinoacetate + S-Adenosyl-Methionine ⟶ Creatine + S-Adenosyl-Homocysteine

As MAT2A is inactive when expressed in bacteria, production has been moved to *Saccaromyces cerevisiae*

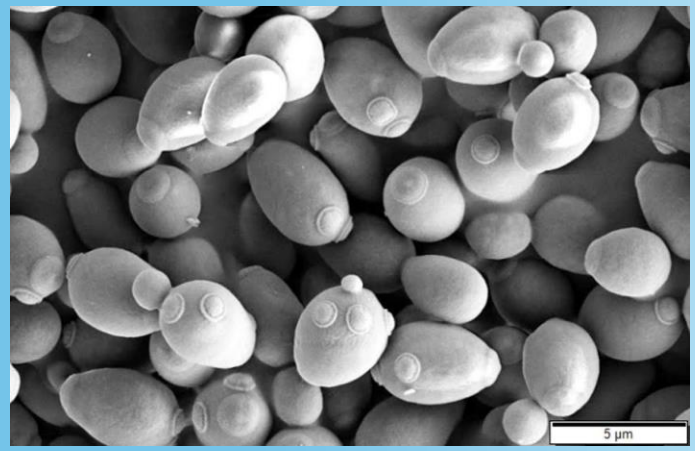


Advantages over Escherichia Coli:

- no contamination from bacterial Endotoxins

- capable of post-translational modifications

- cost-effective and safe



Thanks to:
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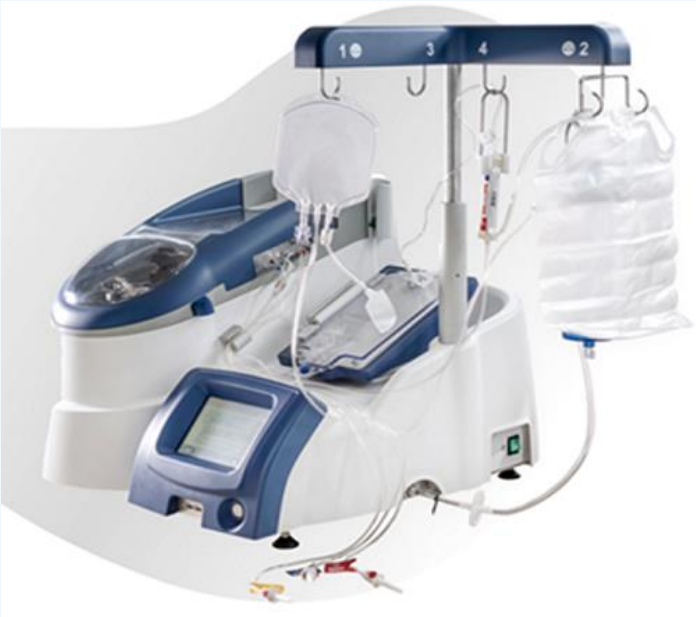
Erythrocytes as preferred delivery method

Enzyme loading into erythrocytes, which are later reinfused into the patient

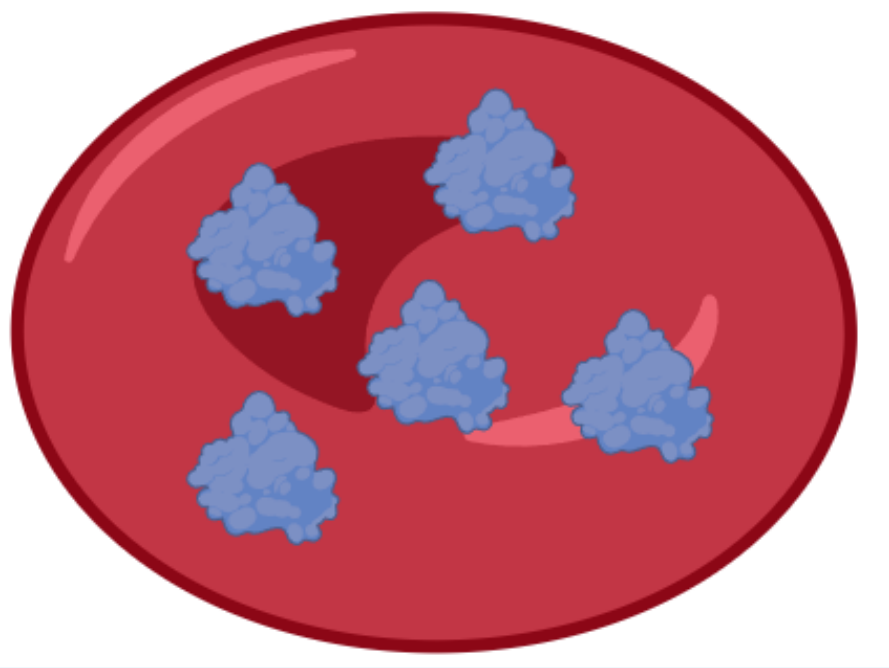


Advantages:

- erythrocytes provide a “safe” environment

- longer half-life, which translates into fewer admistrations

- fewer adverse affects, such as immune activation

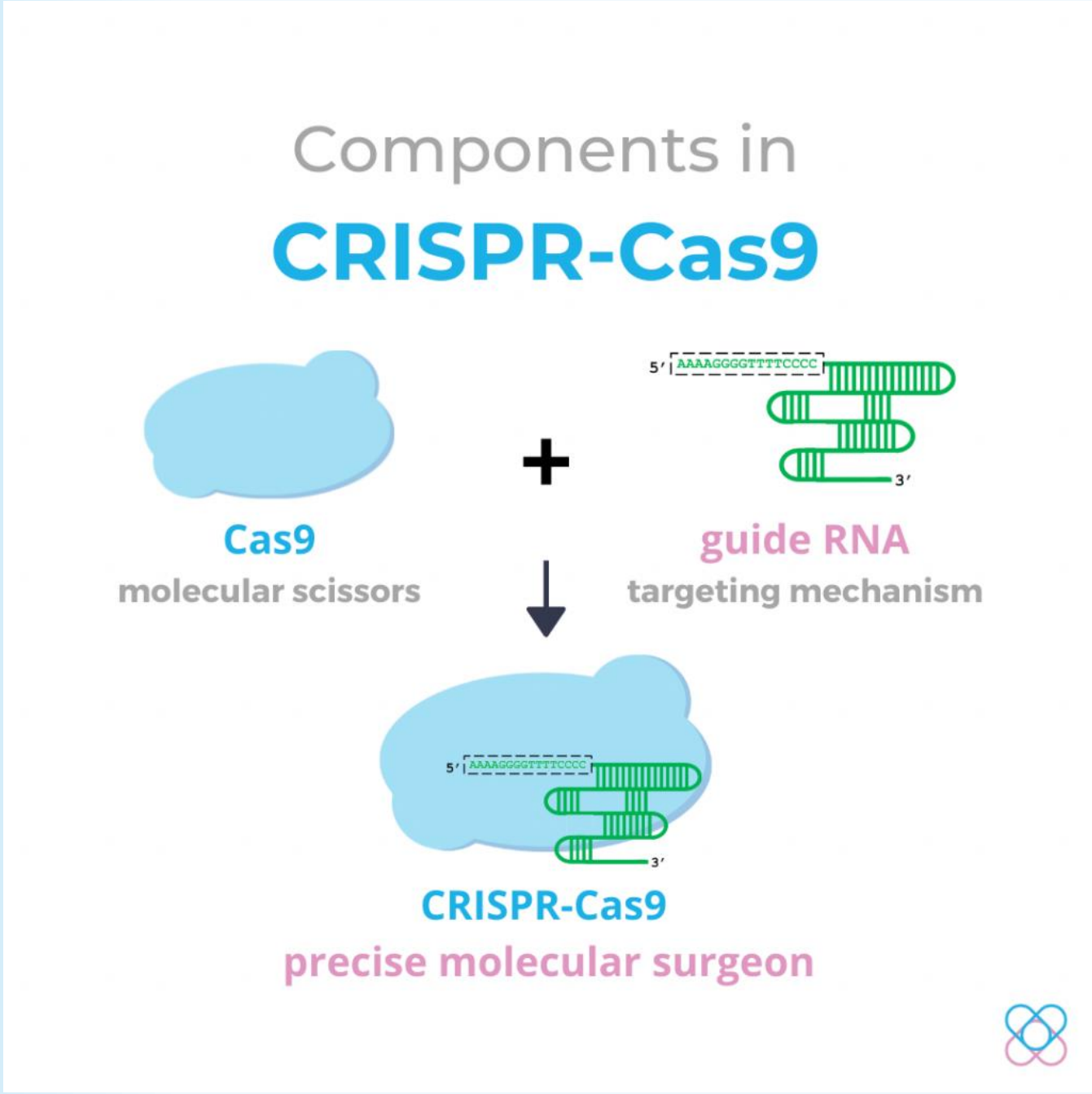


EDCas9
Enhanced Deletion Cas9

- Derived from the acclaimed CRISPR/Cas9 genome editing system

- fusion between Cas9 endonuclease and TREX2 esonuclease

- when coupled with a specific gRNA, the ribocomplex targets a pathologic variant of the USH2A gene, thus correcting the mutation



EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

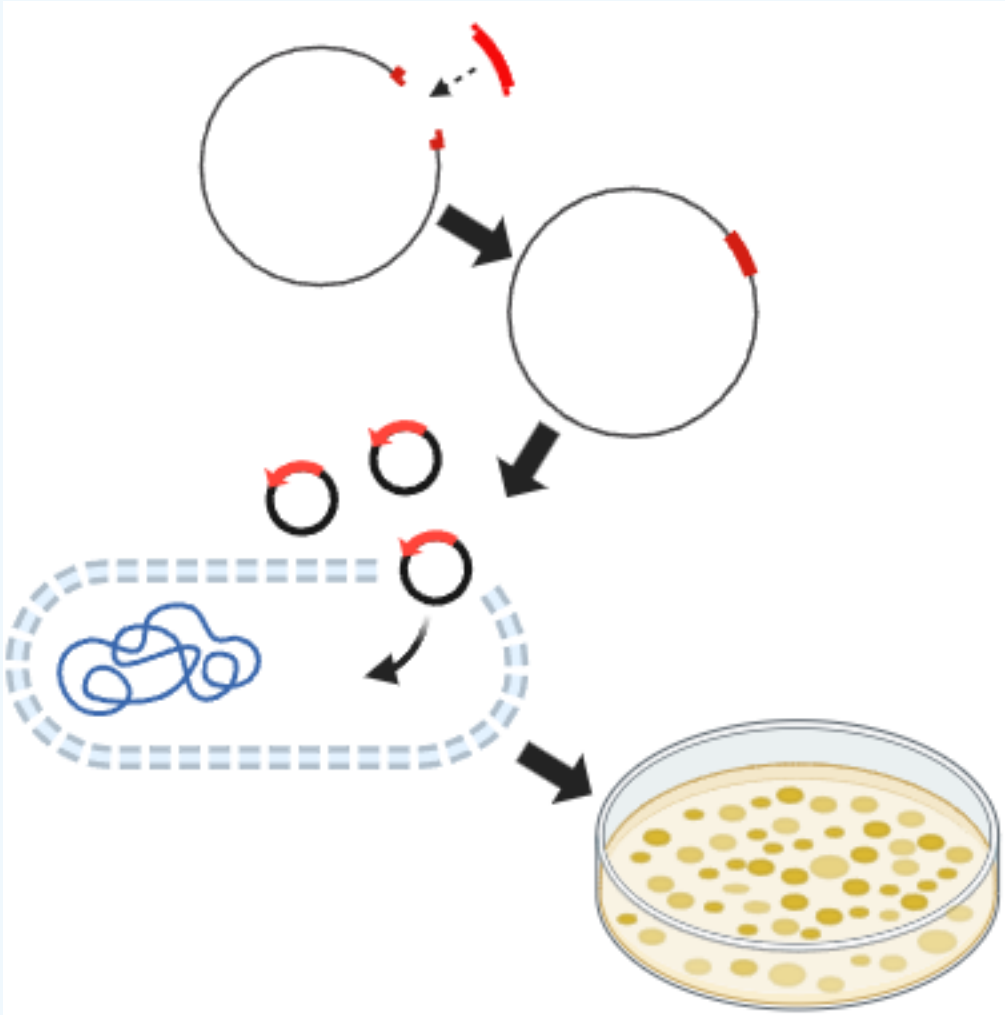
Thanks to:
Pietro de Angeli, Ph.D.

Recombinant protein production workflow

Protein-coding sequence is inserted into a plasmid

The plasmid is transformed into competent bacterial cells

Successfully transformed cells are selected in agar plates



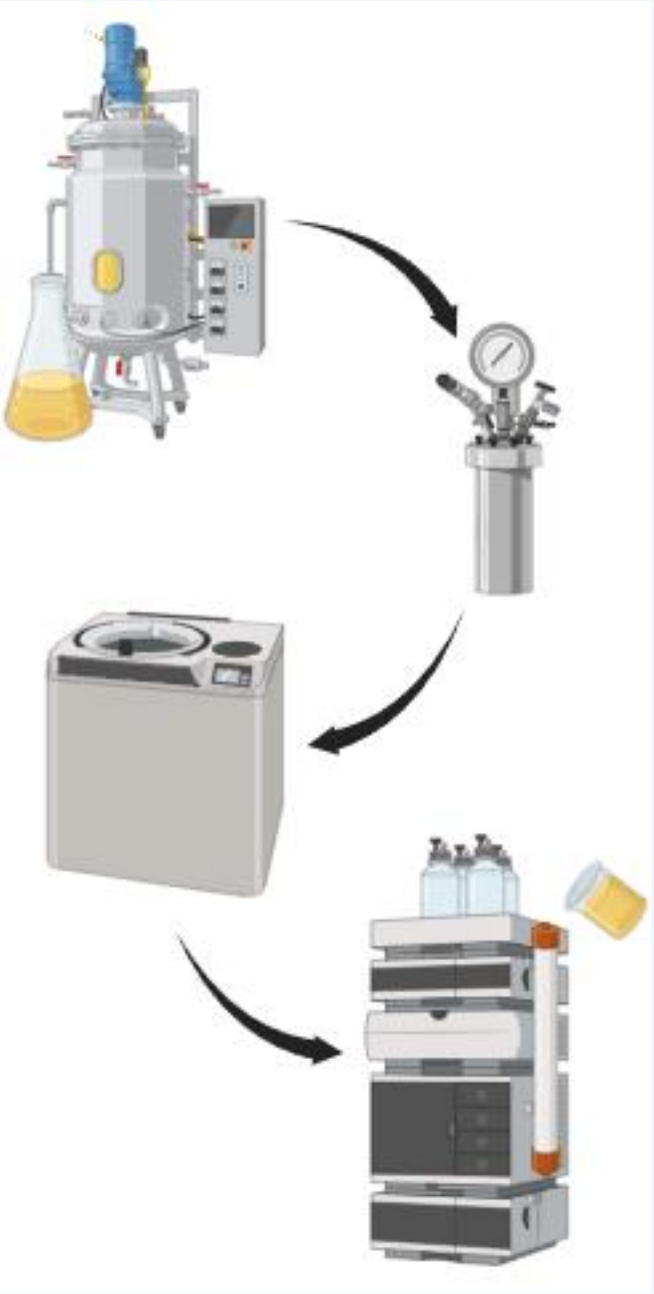
Recombinant protein production workflow: Microbial culture processing

Transformed cells are cultured in a flask or bioreactor

Protein expression is induced through chemicals, such as IPTG

After a set amount of time, cells are harvested, lysed and centrifuged

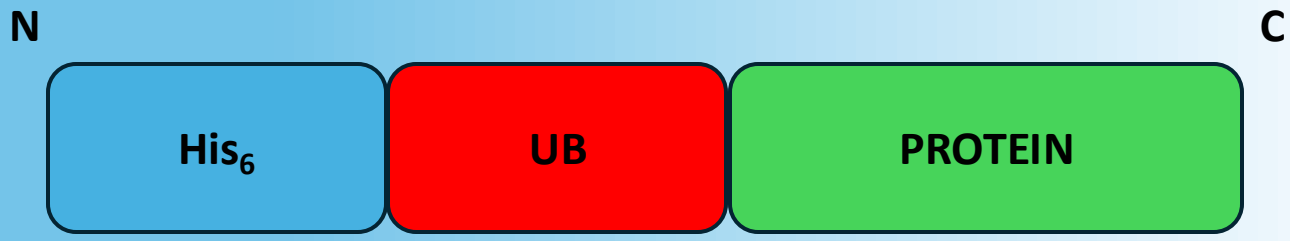
The clarified lysate is loaded onto a FPLC (Fast Protein Liquid Chromatography) column



Recombinant protein production workflow: protein purification

Most of our recombinant proteins are expressed with a **His-Ubiquitin tag** as a fusion partner

The Histidine portion of the tag allows purification of the recombinant protein through IMAC



IMAC: Immobilized Metal Affinity Chromatography

Cell lysate =
His-Ub-Protein + contaminants

HisTrap column

Ni^{++}

Ni^{++}

Ni^{++}

The cell lysate is loaded onto the HisTrap column

IMAC: Immobilized Metal Affinity Chromatography

His-trap column

Ni^{++} **His-Ub-Protein**

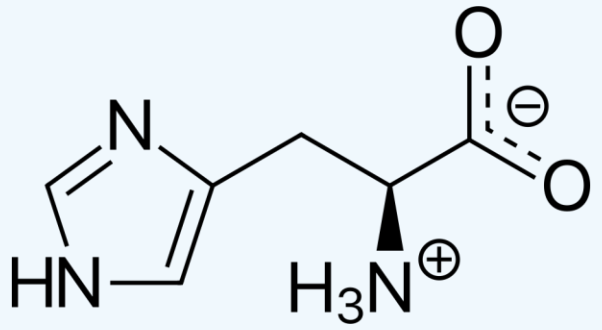
Ni^{++} **His-Ub-Protein**

Ni^{++} **His-Ub-Protein**

Contaminants

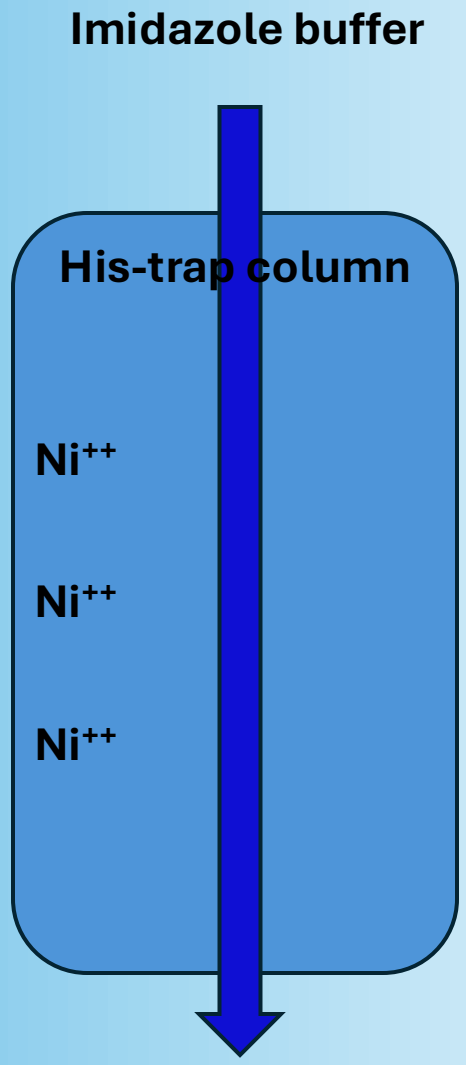
The histidine portion of the recombinant protein binds the nickel ions attacched to the column resin

The contaminants are eliminated

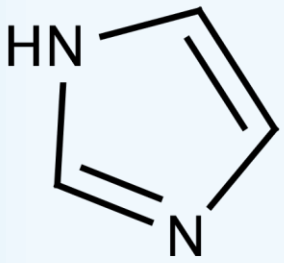


Histidine

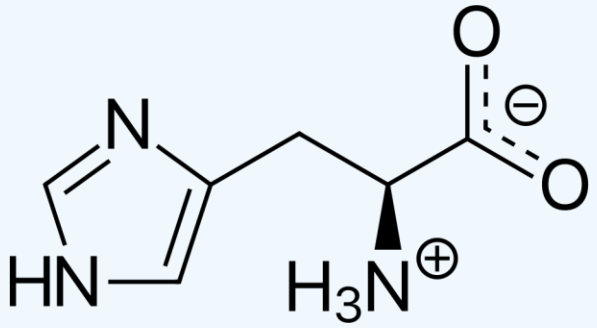
IMAC: Immobilized Metal Affinity Chromatography



The recombinant protein is detached from the resin through an imidazole buffer

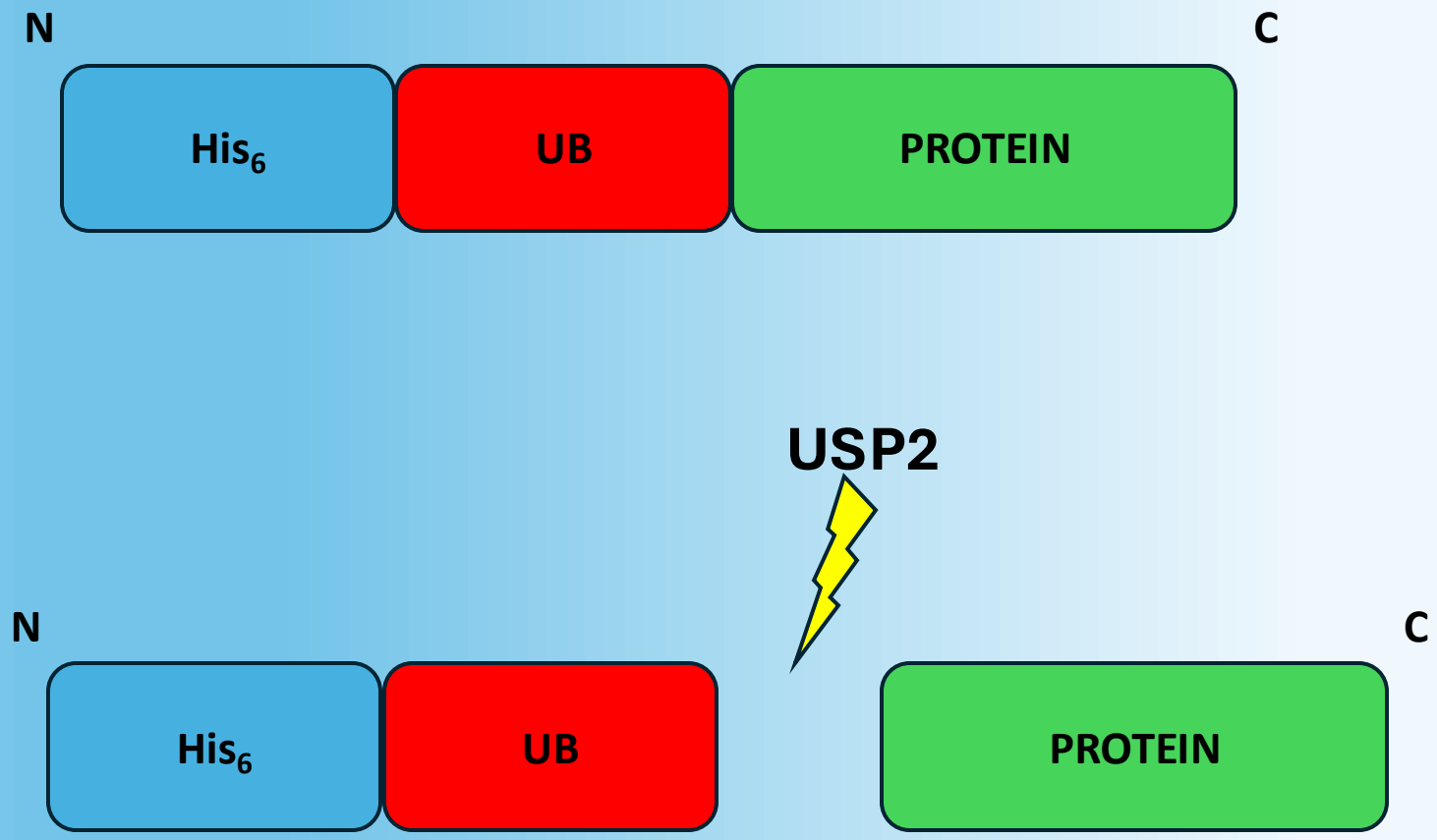


Imidazole



Histidine

His-Ubiquitin tag removal



The ubiquitin portion of the tag allows easy removal of the entire fusion partner

The His-Ubiquitin tag is removed through digestion with USP2

The resulting protein is now free of exogenous portions

Further processing

The recombinant protein can be purified further to eliminate remaining contaminants

To this end, additional chromatography techniques are employed, such as ion exchange and size exclusion chromatography

Thank you for your attention!

Special Thanks to:

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VITALITY