Topic

Enzyme protein engineering

Presented by:

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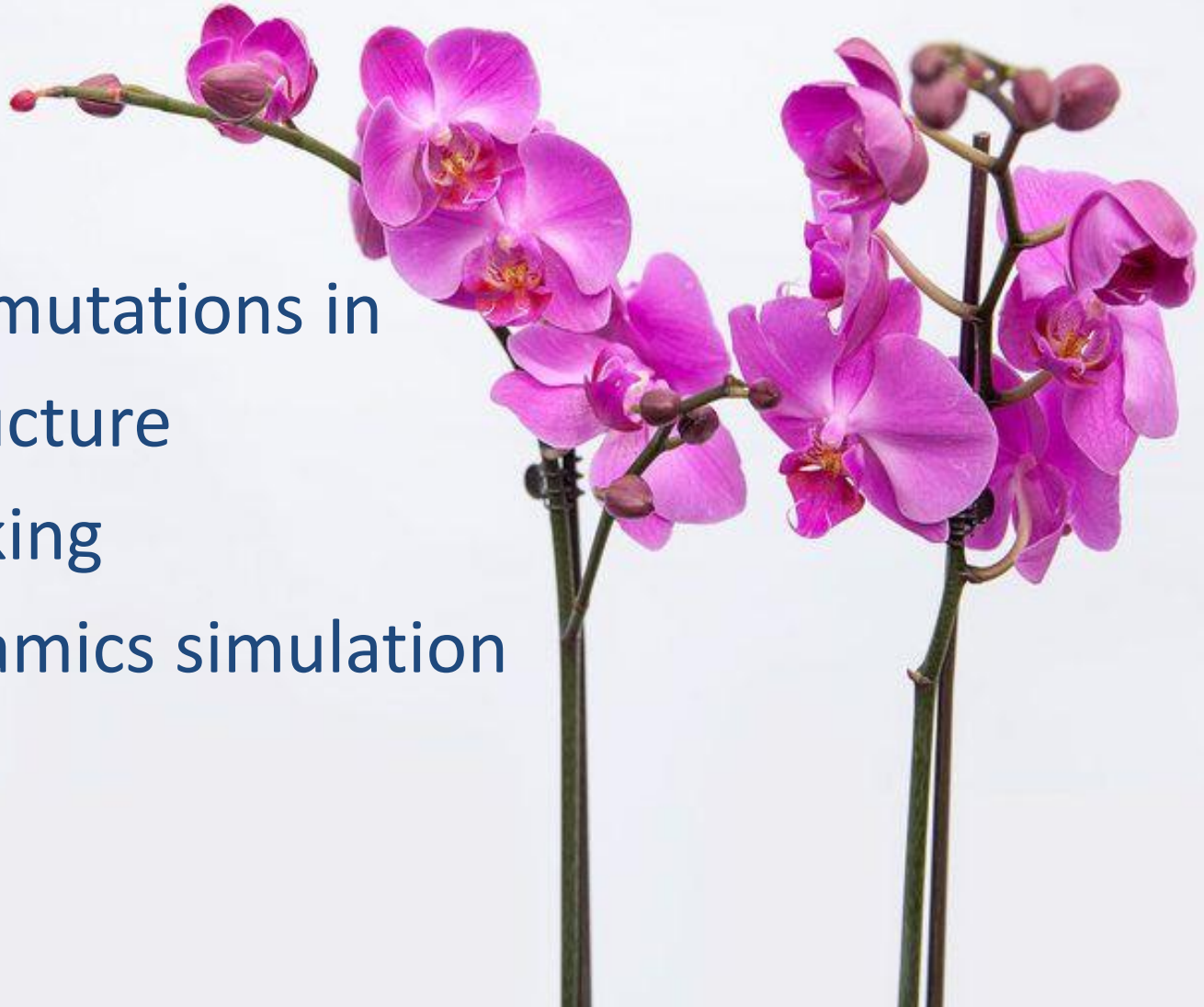
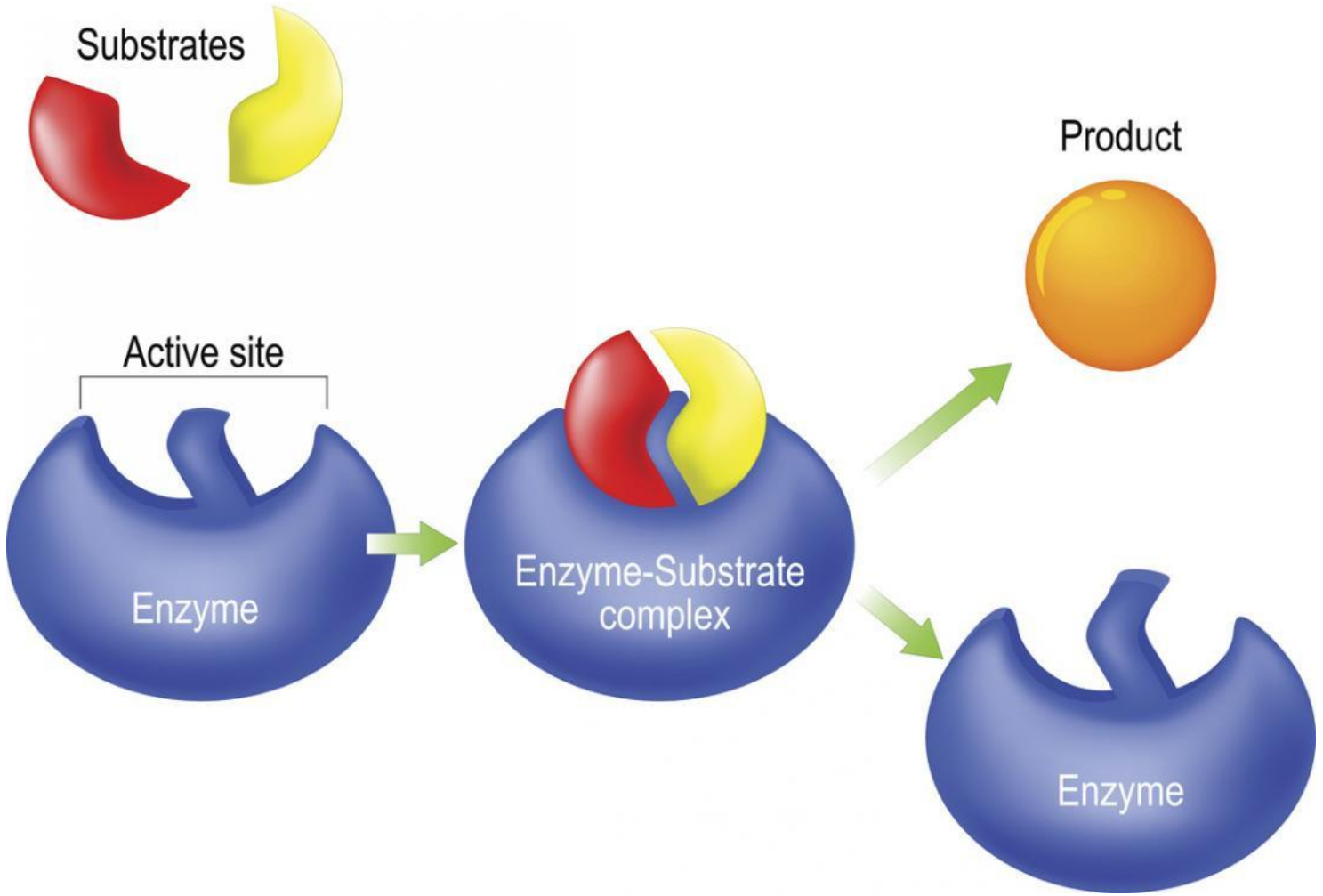
- ✓ Introduction
- ✓ Obtaining nucleotide sequences
- ✓ Predicting protein structure
- ✓ How to create mutations in the protein structure
- ✓ Molecular docking
- ✓ Molecular dynamics simulation
- ✓ Methods



Enzyme


Substrates
Product
Active site
Enzyme
Enzyme-Substrate complex
Enzyme

Enzyme advantage over chemical reactions

Ease of catalyst separation

Obtaining products with high purity

It does not require harsh conditions to react

Enzyme production source

There are three major sources of enzymes :

Plants (≅ 4%)
(papain, bromilain)

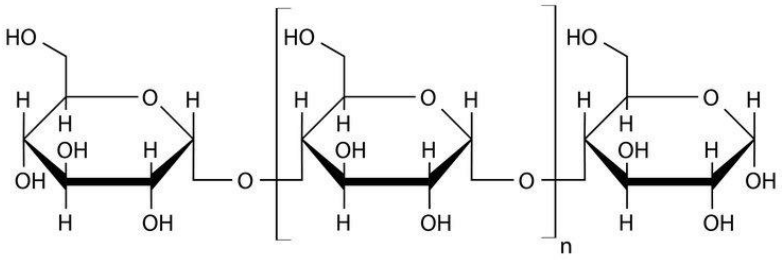
Animals (≅ 8%)
(renet)

Microorganisms
(>80%)
(yeast, fungi and bacteria)

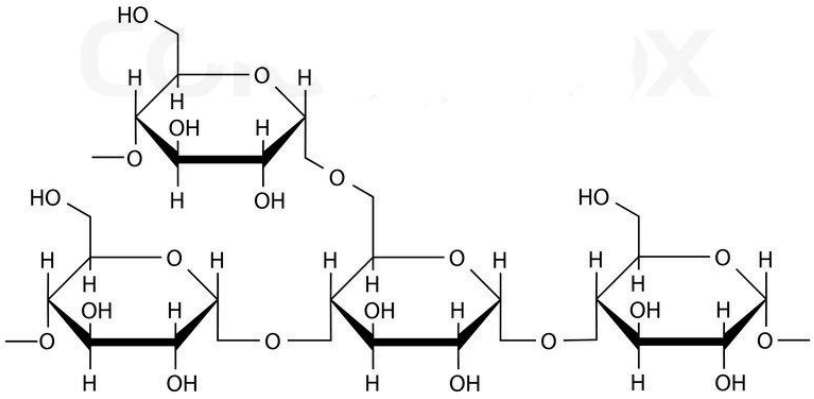
Advantage of microbial enzymes

- Producing large quantity of enzyme
- Easily genetically manipulation
- Novel characteristics like thermos-stability
- Economical production

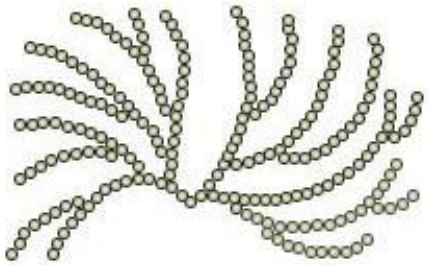
Starch



Amylose



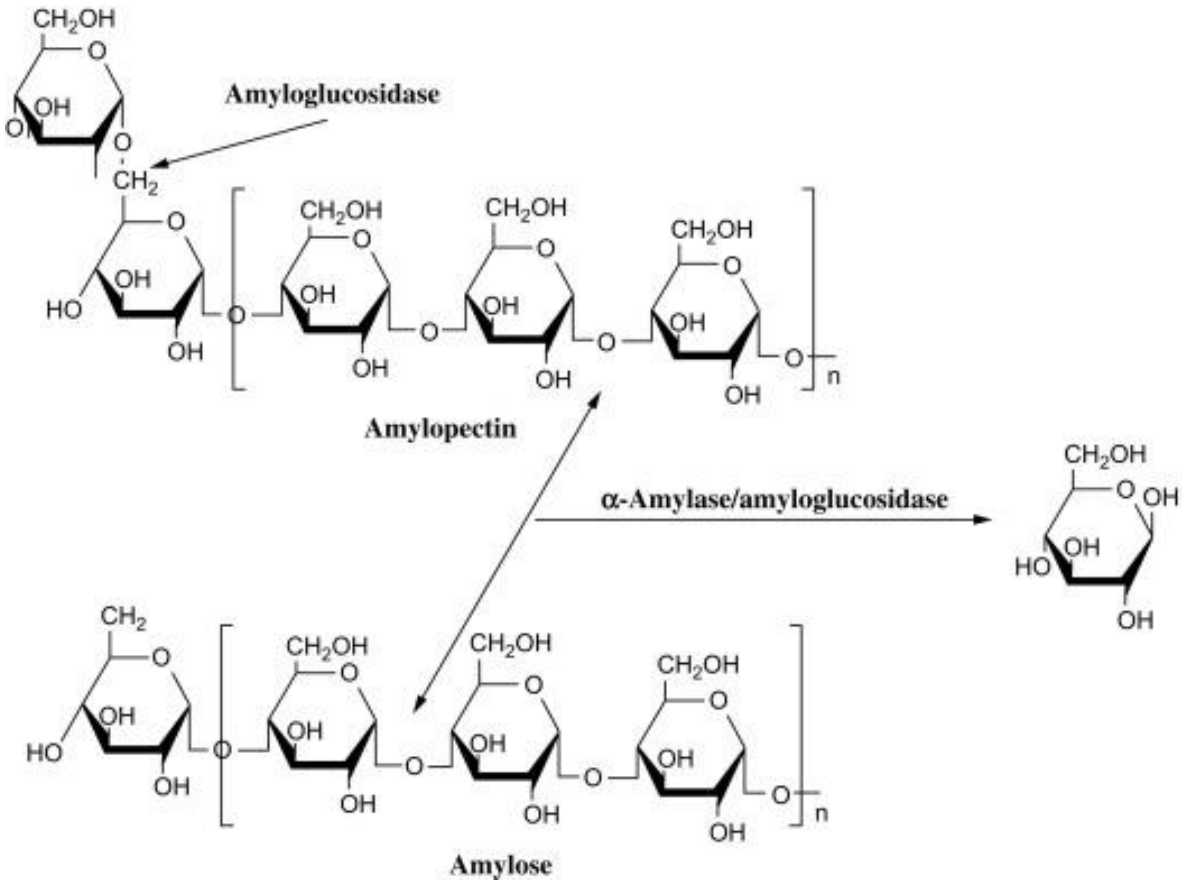
Amylopectin



Starch Modifying Enzymes

- Alpha-amylase
- Glycogen branching enzyme
- Pullulanase

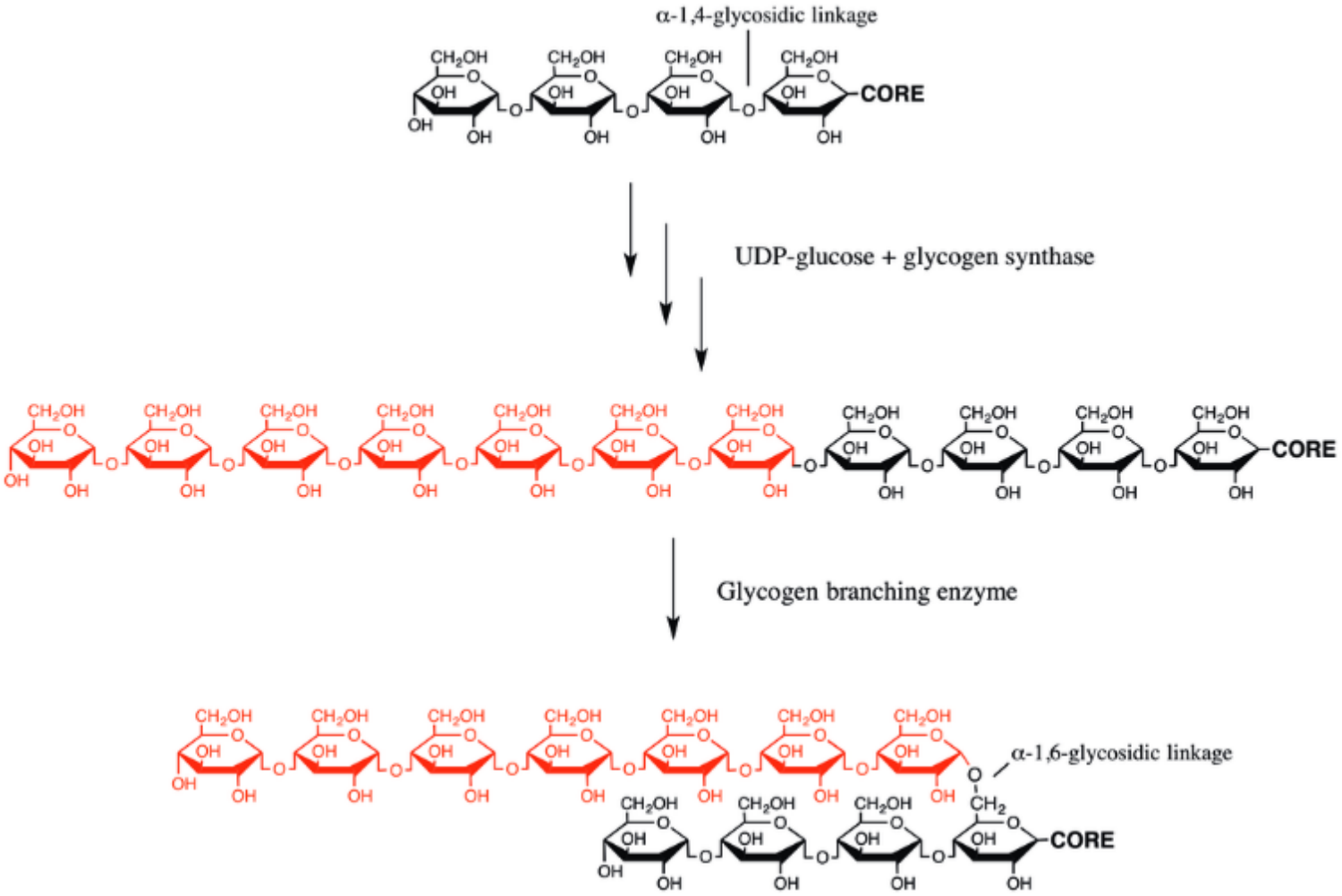
Amylase


Amyloglucosidase
Amylopectin
α-Amylase/amyloglucosidase
Amylose

Application

- Starch processing
- Pharmaceutical
- Food
- Paper
- Textile
- Bakery and anti salting
- Detergent
- Alcohol

Glycogen branching enzyme



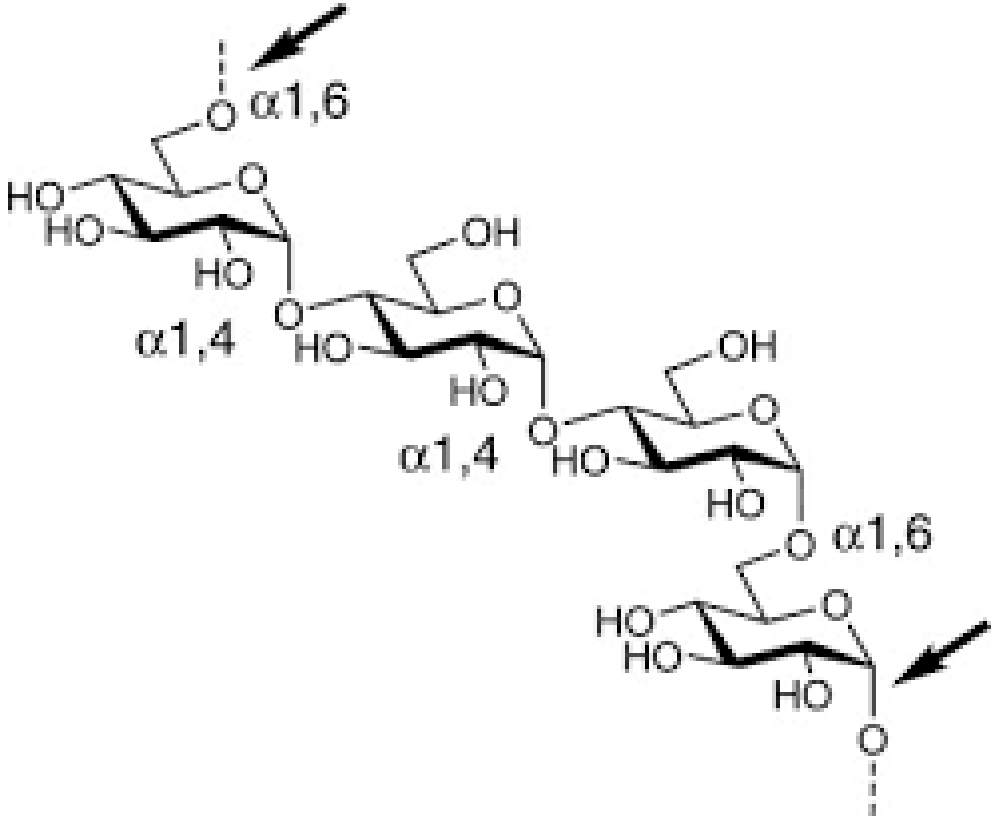
Application

Increasing of starch stability and solubility

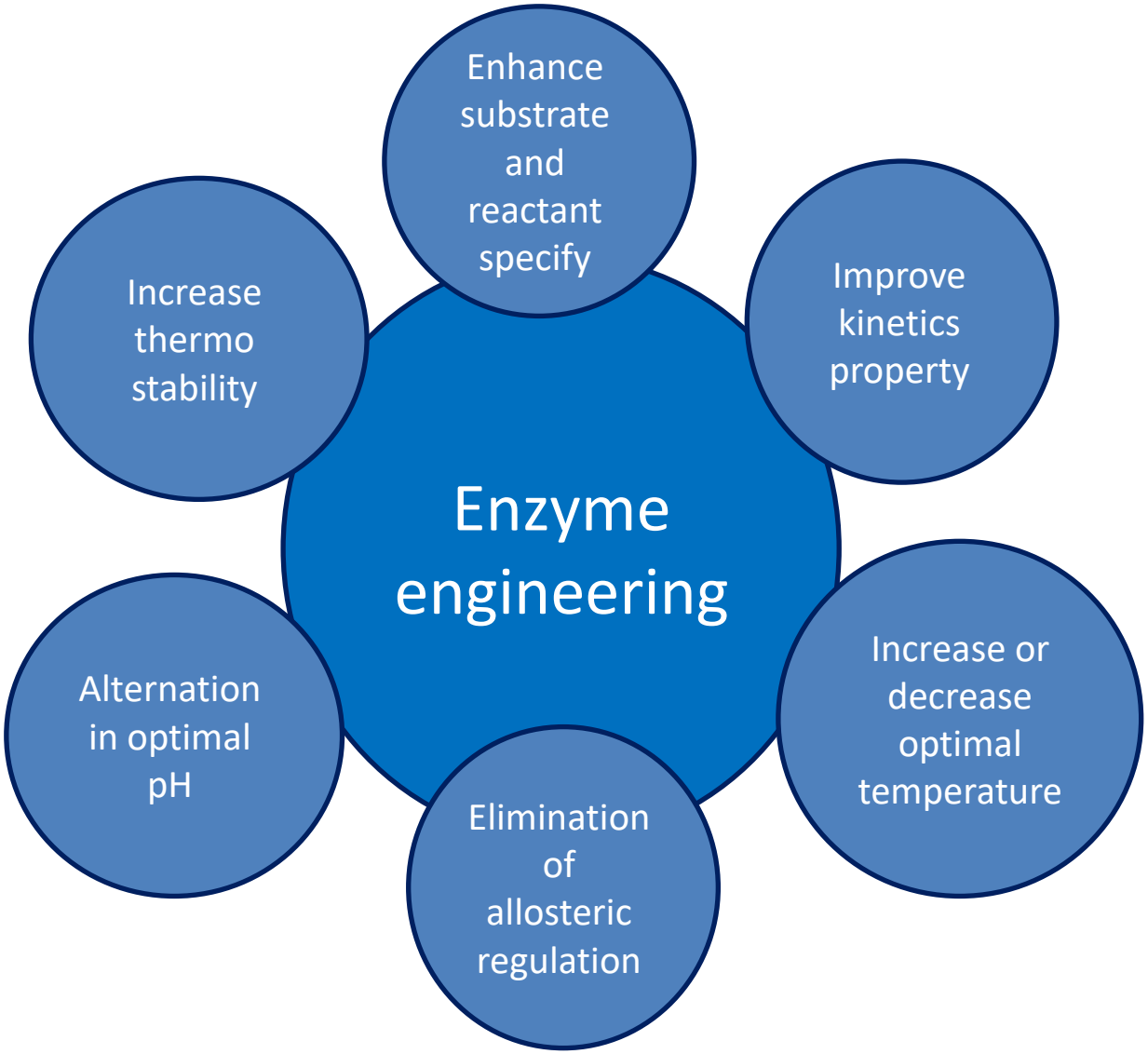
Coating step of paper manufacture

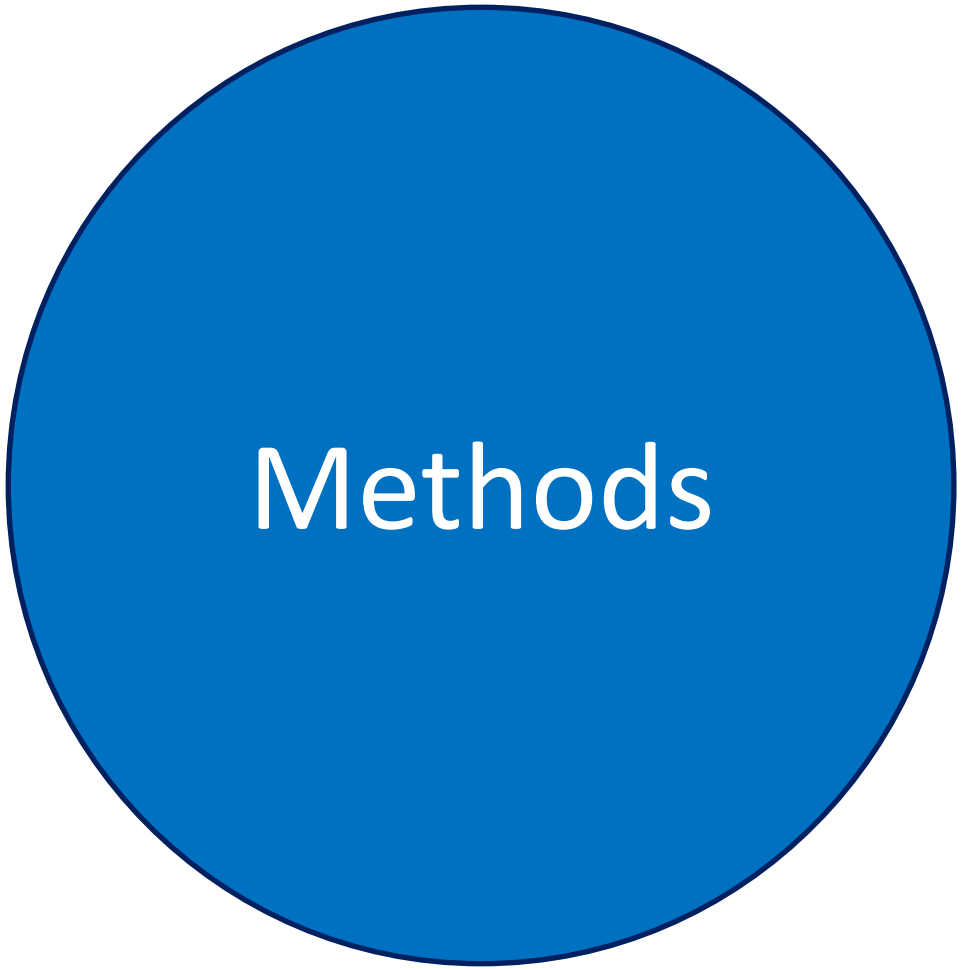
Decrease Retrogradation

Pullulanase

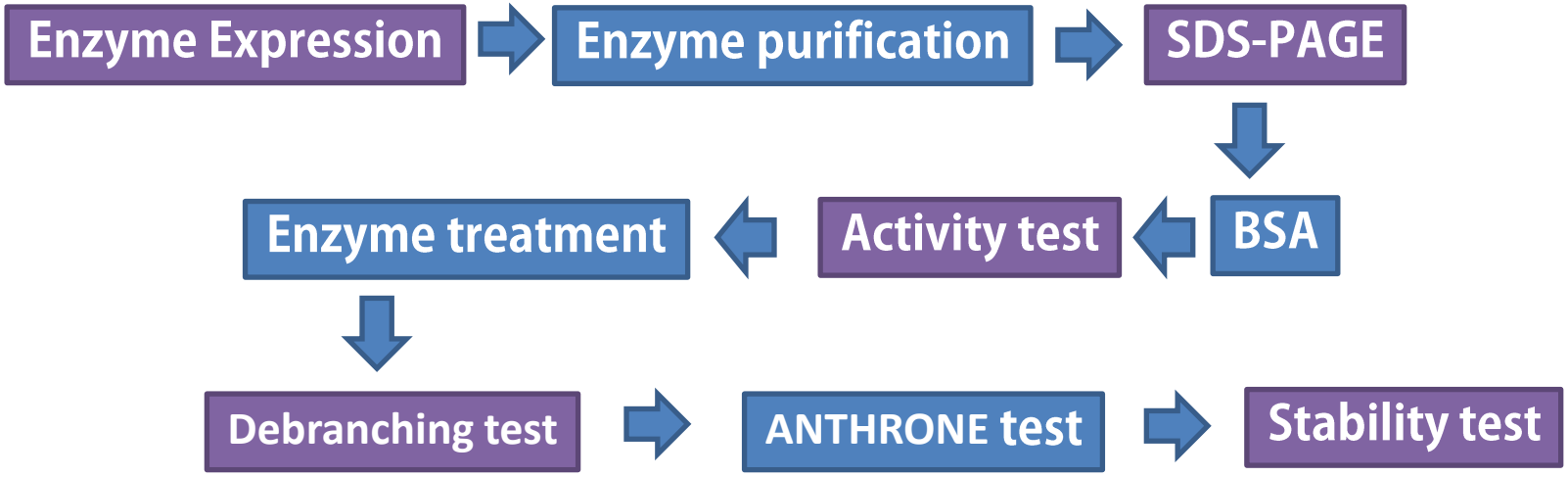


Application of Enzyme engineering




Methods

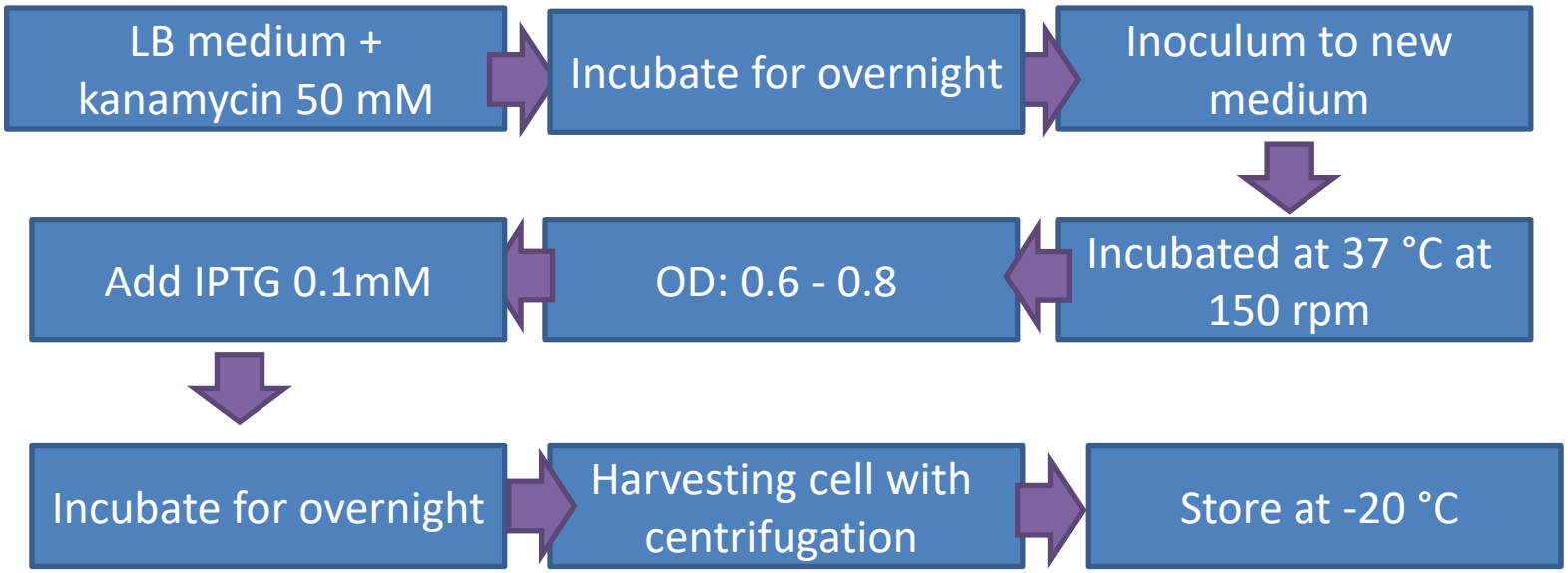
Methods


Enzyme Expression
Enzyme purification
SDS-PAGE
BSA
Activity test
Enzyme treatment
Debranching test
ANTHRONE test
Stability test

Method

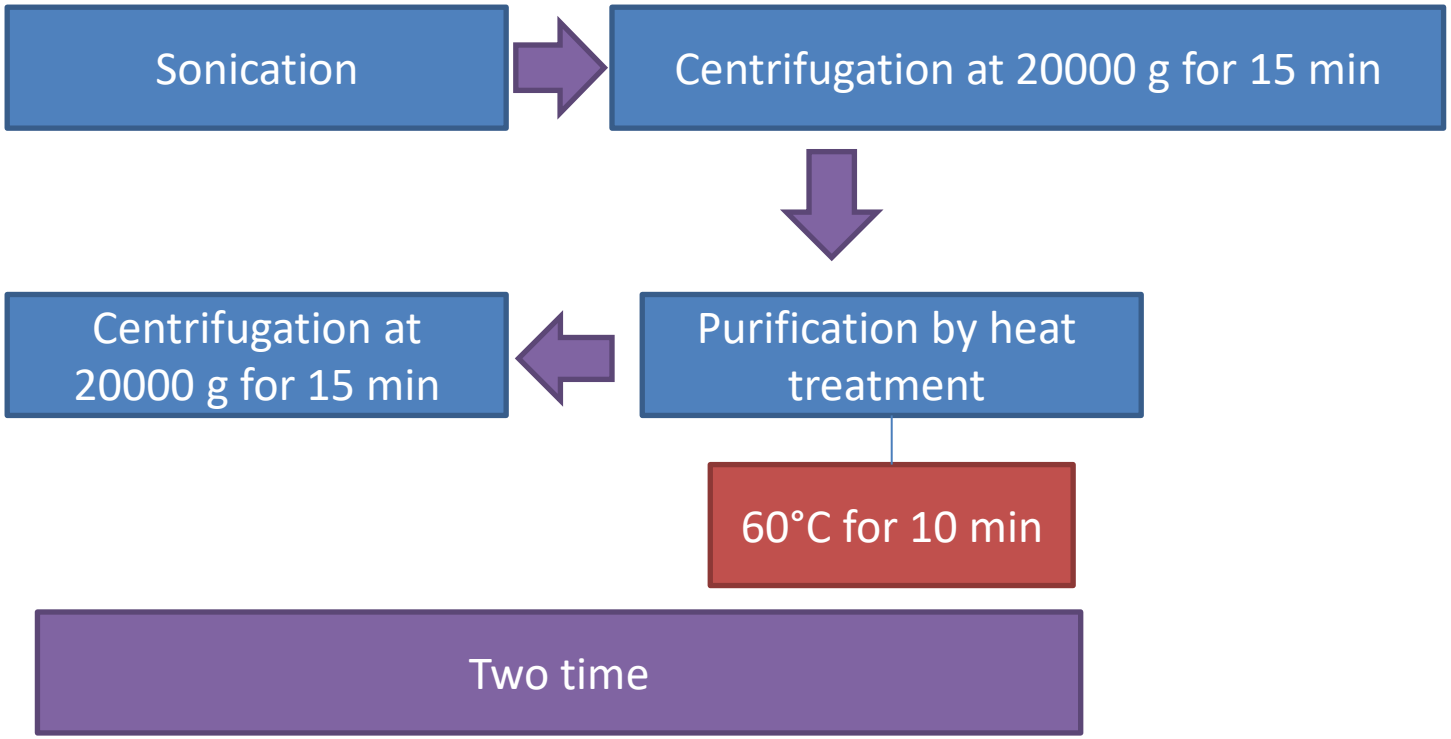
Enzyme Expression

Vector: PET 28 a | Host: *Ecoli21* (DE3)



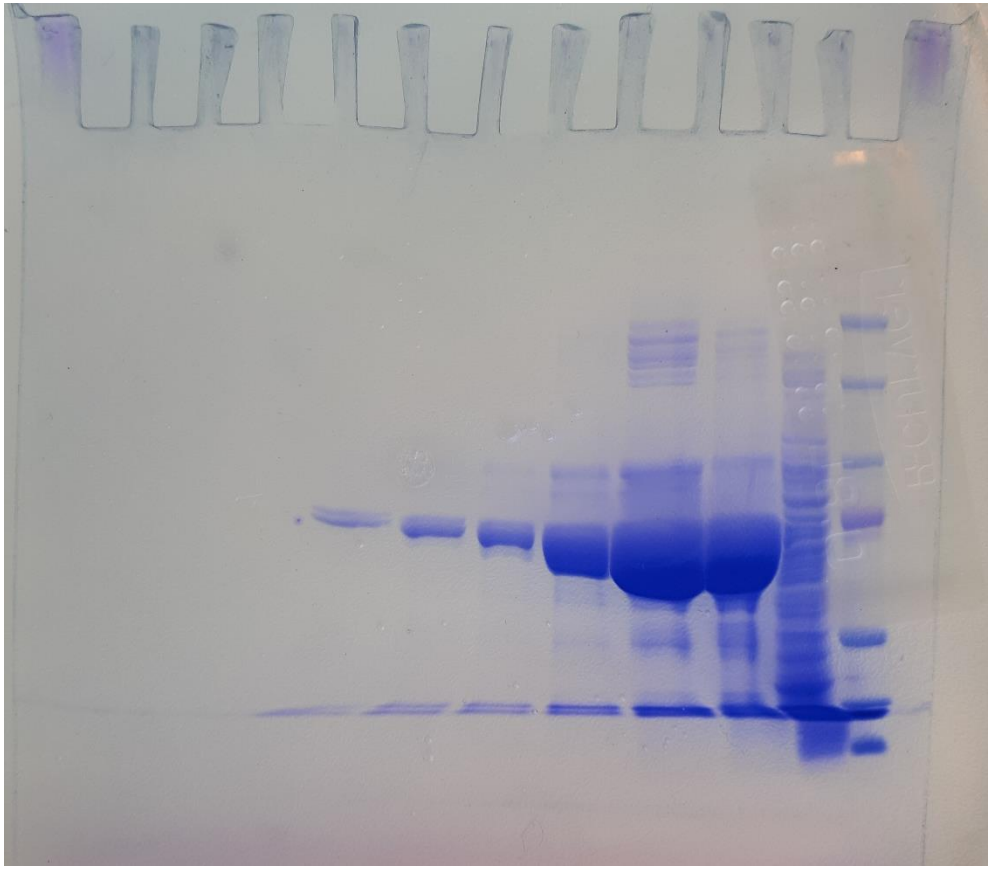
Method

Lyse of cell and purification



Result

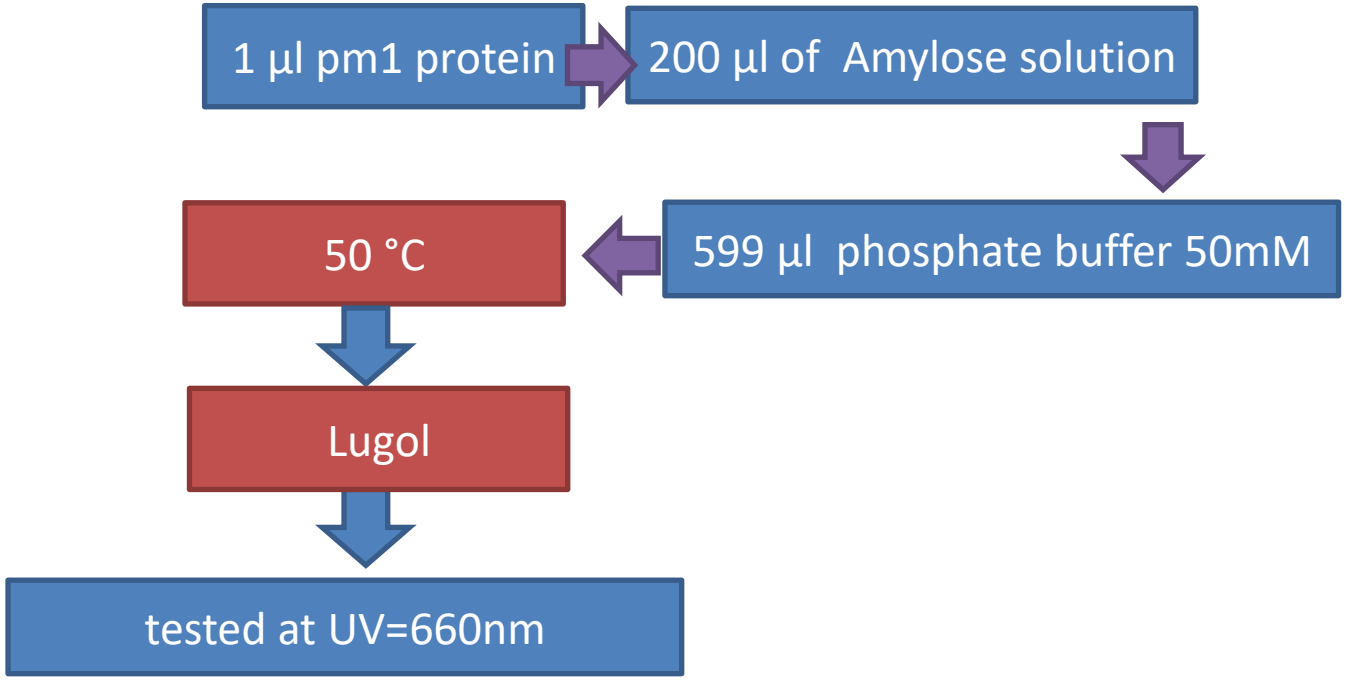
SDS-PAGE and BSA



Method

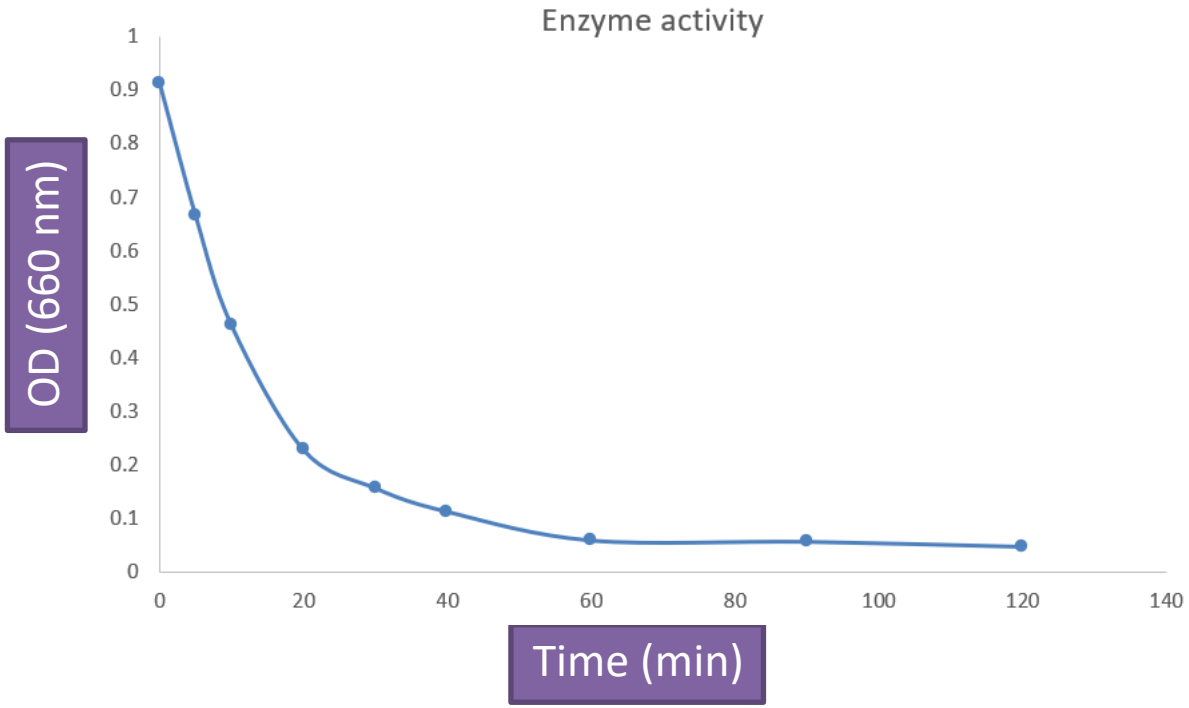
Enzyme activity

Amylose solution: 0.8ml of NaOH 5M+ 3.2ml phosphate buffer 50mM+25 mg Amylose from potato+0.3ml Hcl 12M. then pH was adjusted at 7



Result

Enzyme activity



Method

BSA method

Proportion of several concentrations of protein

mg/ml

0.75 | 0.5 | 0.25 | 0.125

Add 100 µl of protein concentration to 5 ml 1X Dye reagent

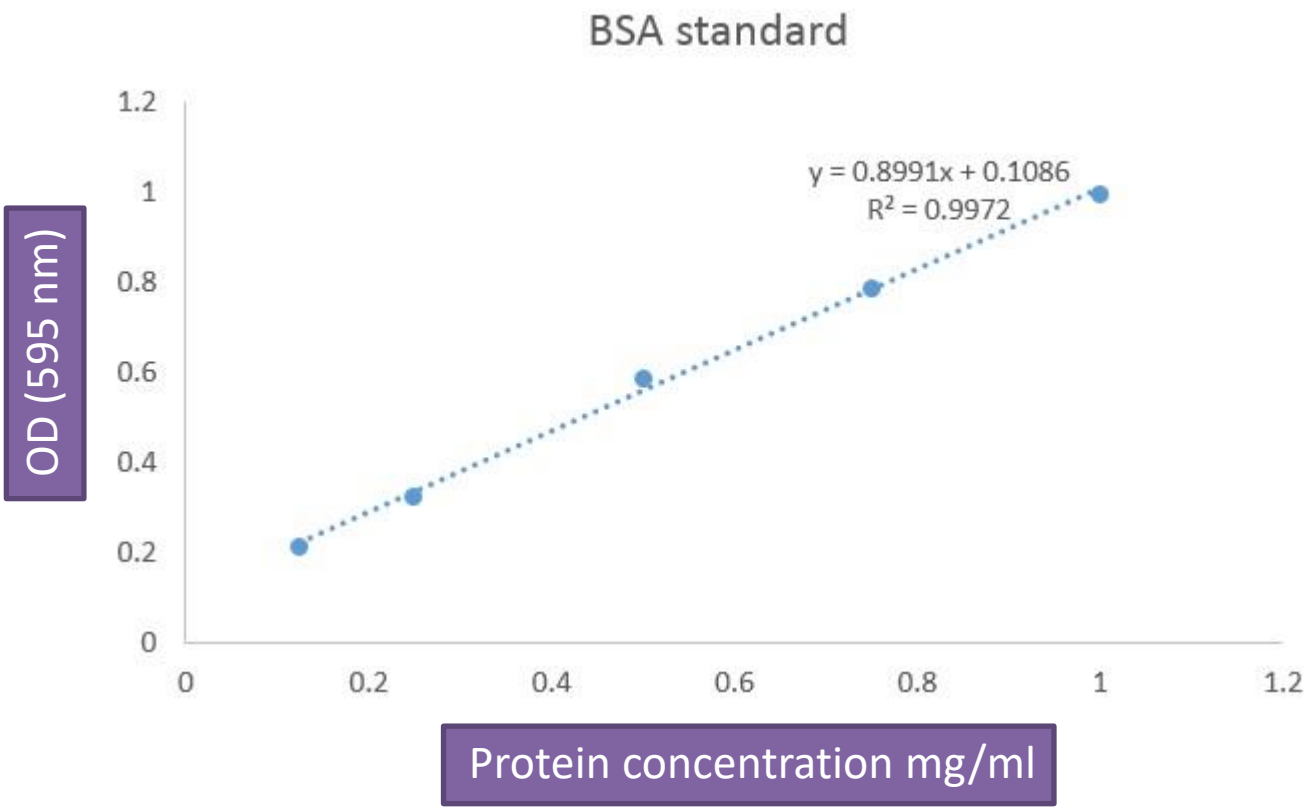
Vortex the complex for 10 second

Read at 595 nm

Enzyme was diluted for 20 times

Result

BSA method



9.64 mg/ ml

Method

Enzyme treatment

Dissolve 5 g potato starch in 500 ml phosphate buffer 5mM at pH 7

Add 1.25 ml glycogen branching enzyme

Incubate that at 50 °C

5 min | 10 min | 15 min | 20 min | 30 min | 40 min | 60 min

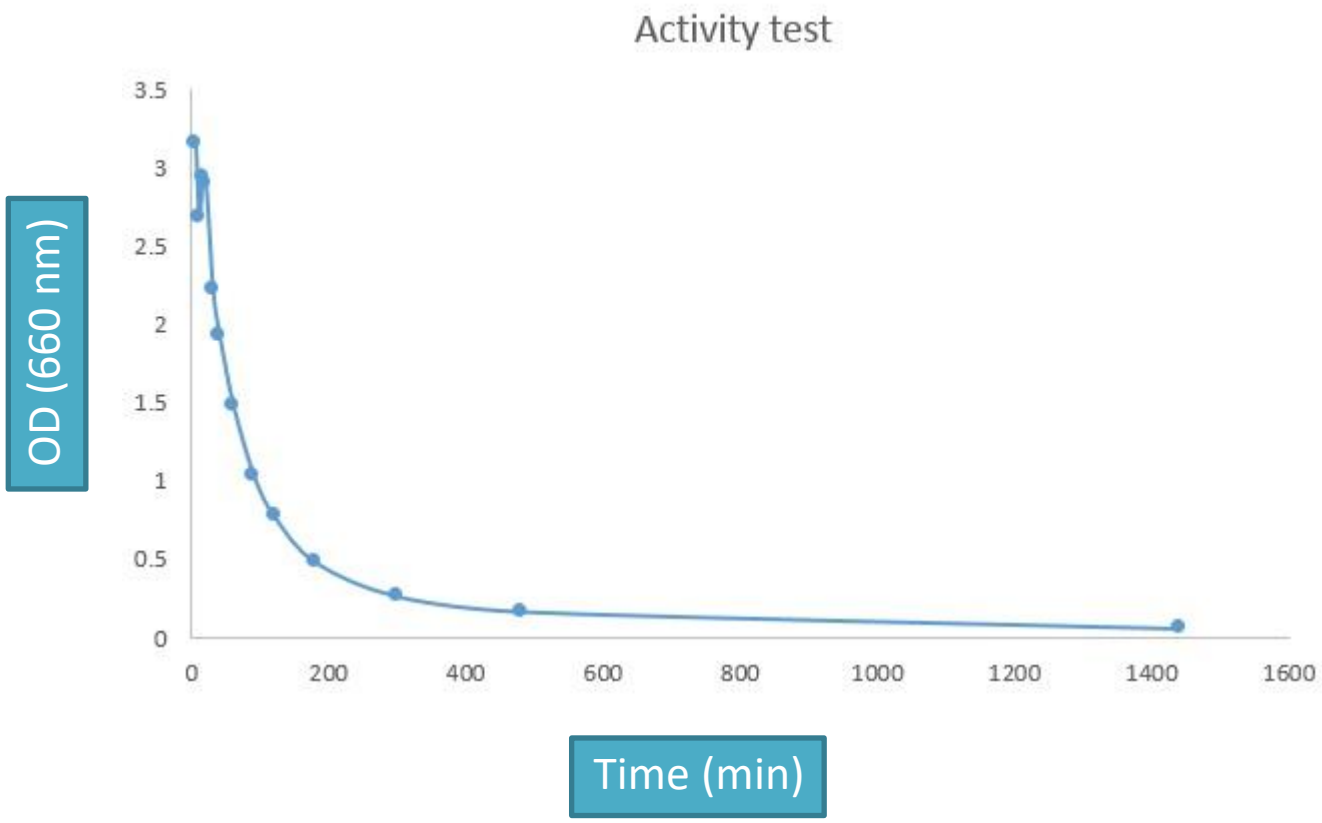
90 min | 120 min | 180 min | 300 min | 480 min | 1440 min

Enzyme activity was stopped by putting in boiling water for 10 min.

Activity test → Freeze Dry

Result

Activity test



Method

Debranching test

3mg dry sample add to 1mL NaAC (1mM ,pH 5)

↓

adding 1µl pullulanase+ 1µl isoamylase

↓

mix for 5 second

↓

incubated at 40 °C for overnight

↓

stop reaction by putting in boiling water for 5 min

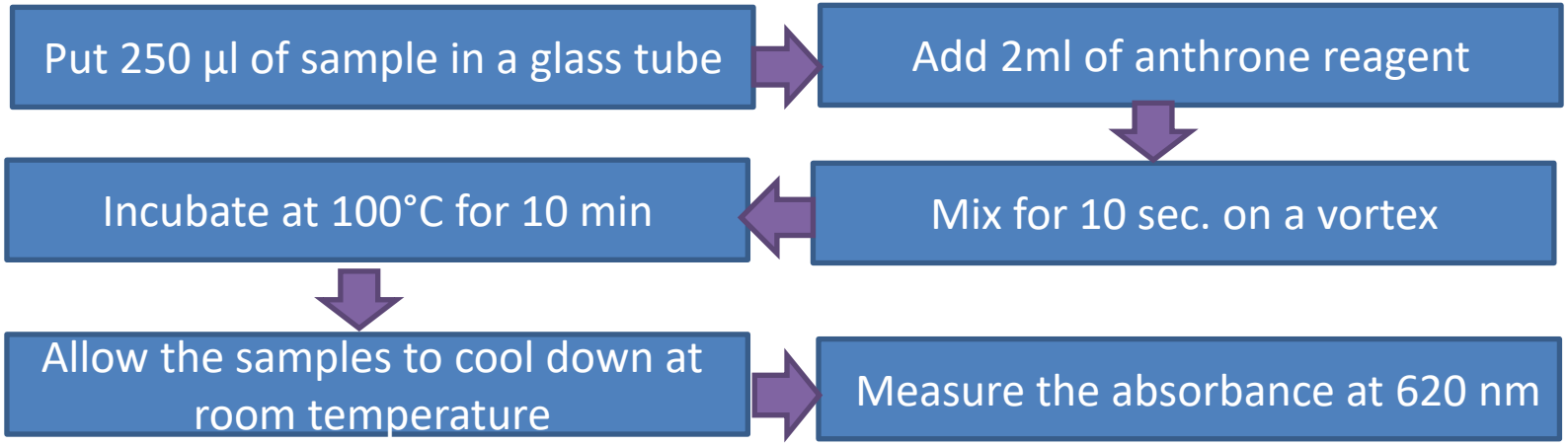
↓

centrifuge at 20000 g for 15 min

ANTHRONE (Total Carbohydrates)

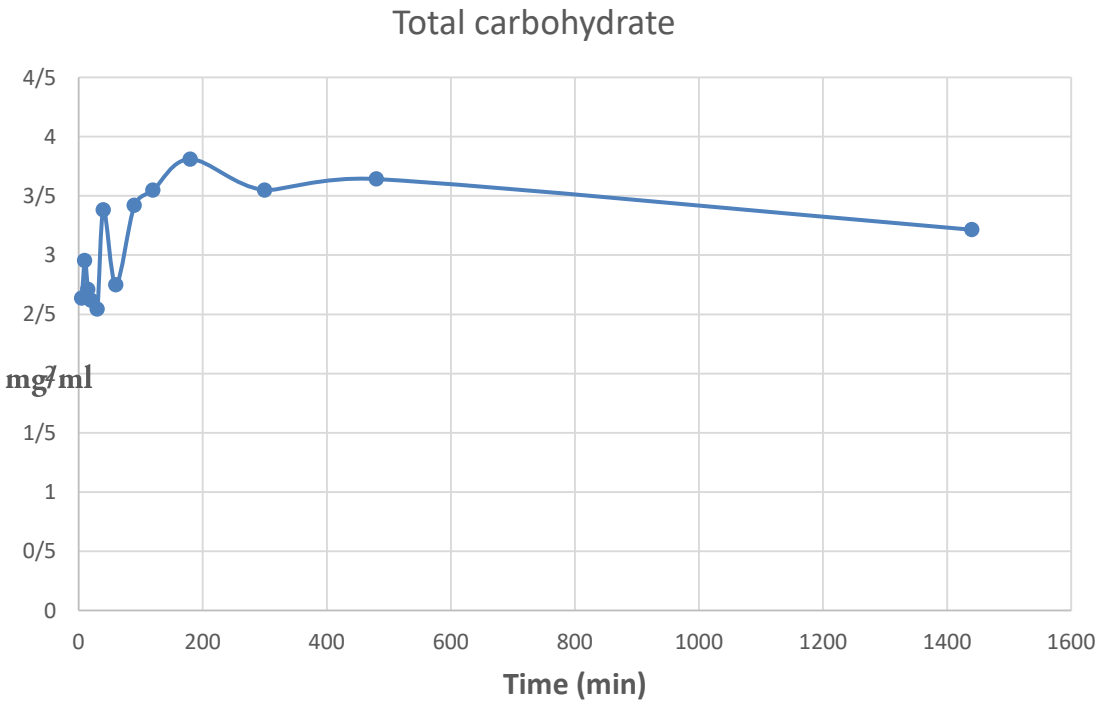
Method

Standard curve



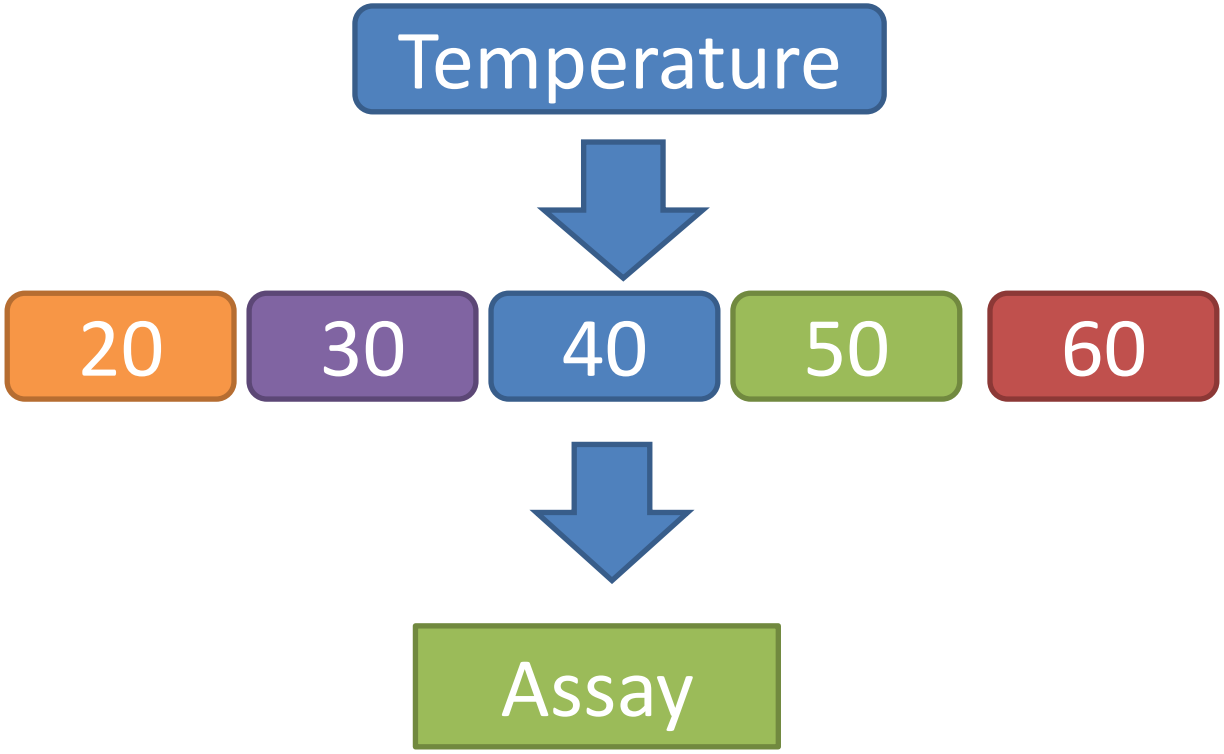
Result

ANTHRONE (Total Carbohydrates)



Stability

Method




If we knew what it was we were doing, it would not be called research, would it ? (Albert Einstein)
Thanks for your nice attention