



Topic

Enzyme protein engineering

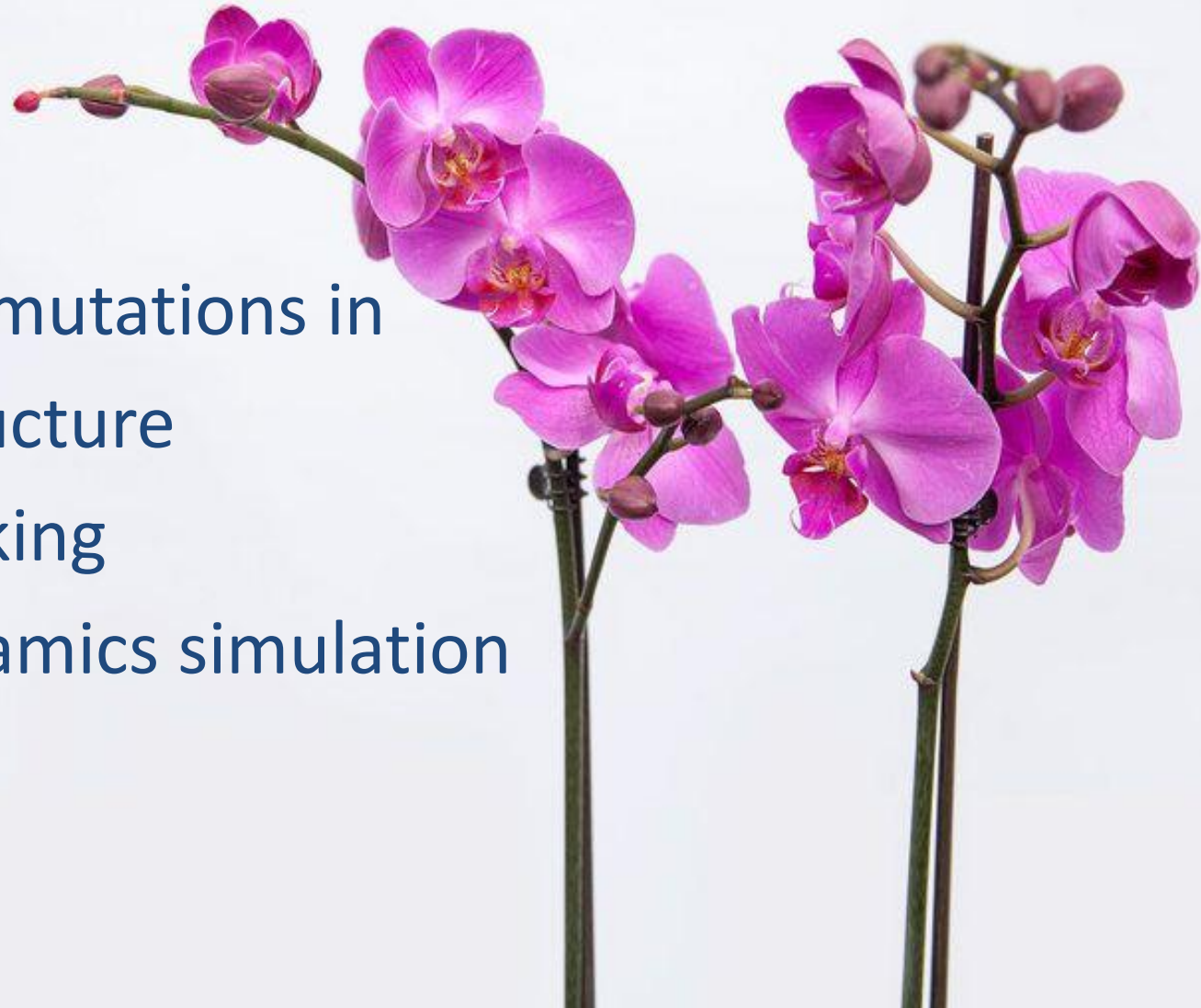
Presented by:

Babak Elysi Far

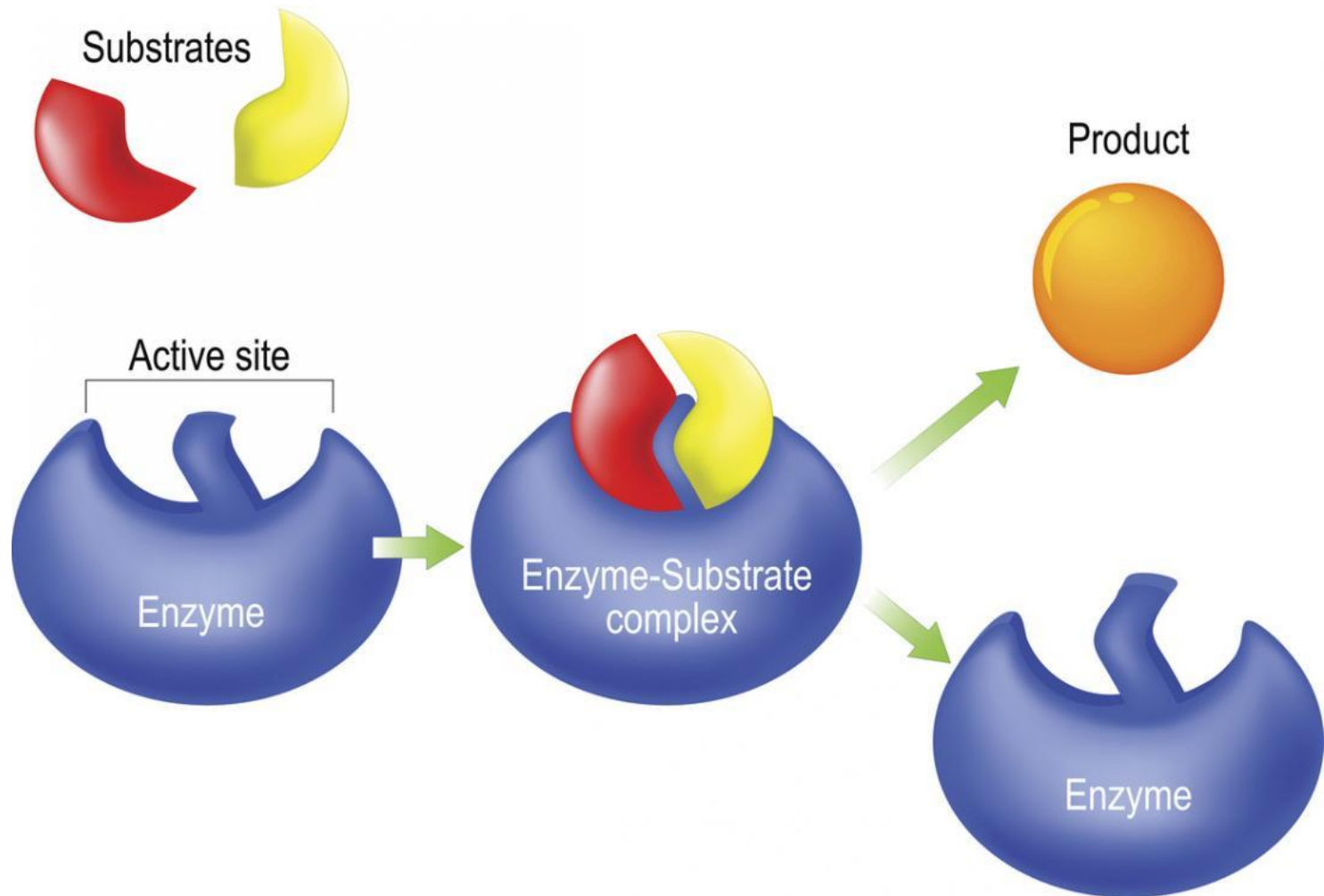
**Department of Physiology and Pharmacology, School of Medicine, Dezful
University of Medical Sciences, Dezful, Iran**

elyasifar.b@dums.ac.ir

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



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 ($\cong 4\%$)**
(papain, bromelain)



Animals ($\cong 8\%$)
(renet)



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

Advantage of microbial enzymes

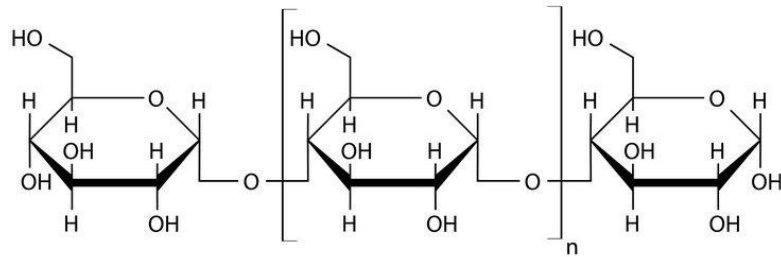
Producing large quantity of enzyme

Easily genetically manipulation

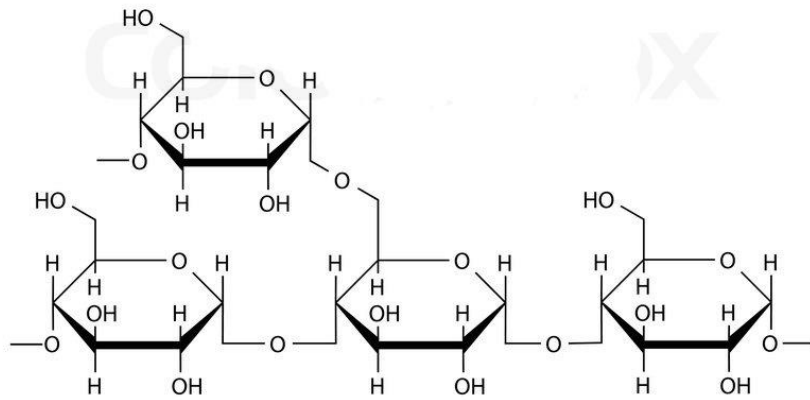
Novel characteristics like thermos-stability

Economical production

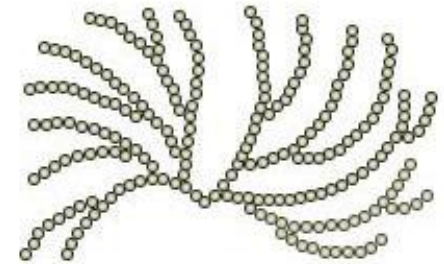
Starch



Amylose



Amylopectin



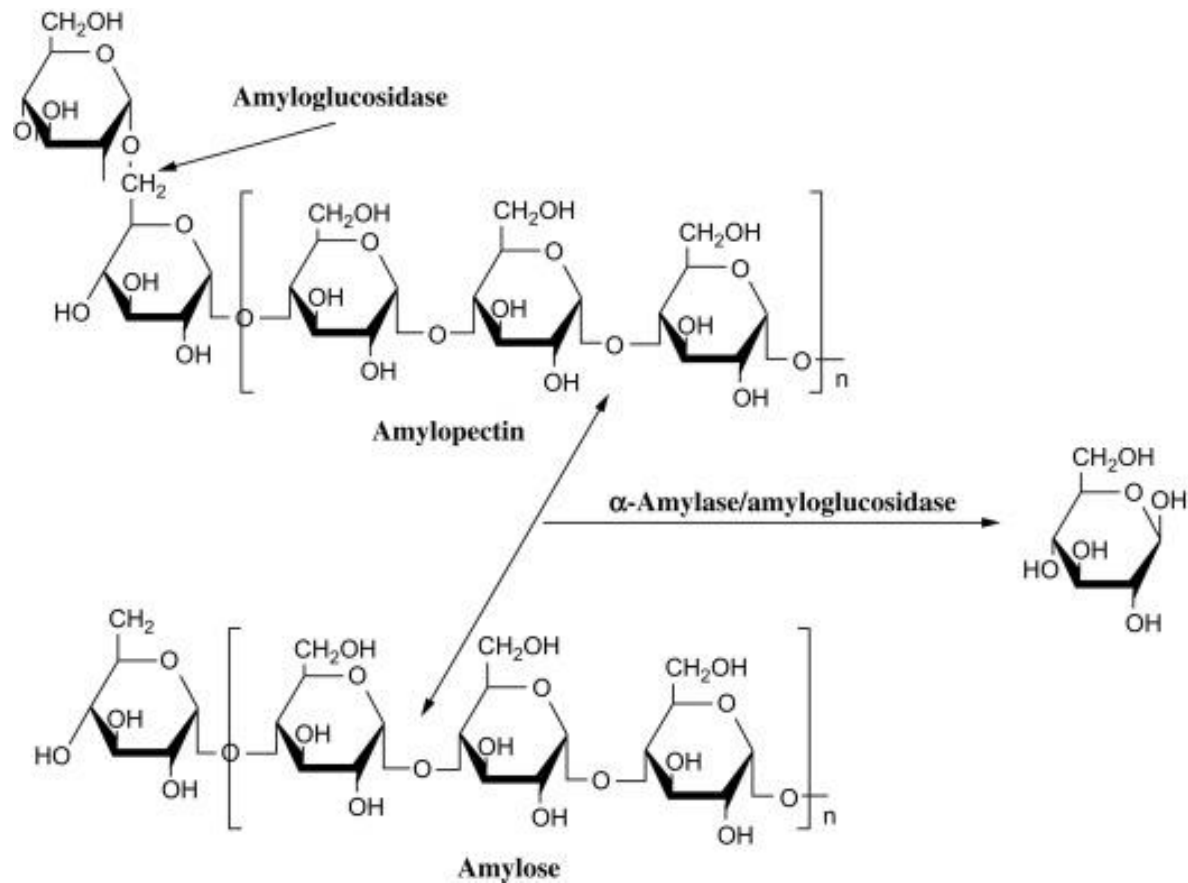
Starch Modifying Enzymes

Alpha-amylase

Glycogen branching enzyme

Pullulanase

Amylase



Application

Starch processing

Textile

Pharmaceutical

Bakery and anti salting

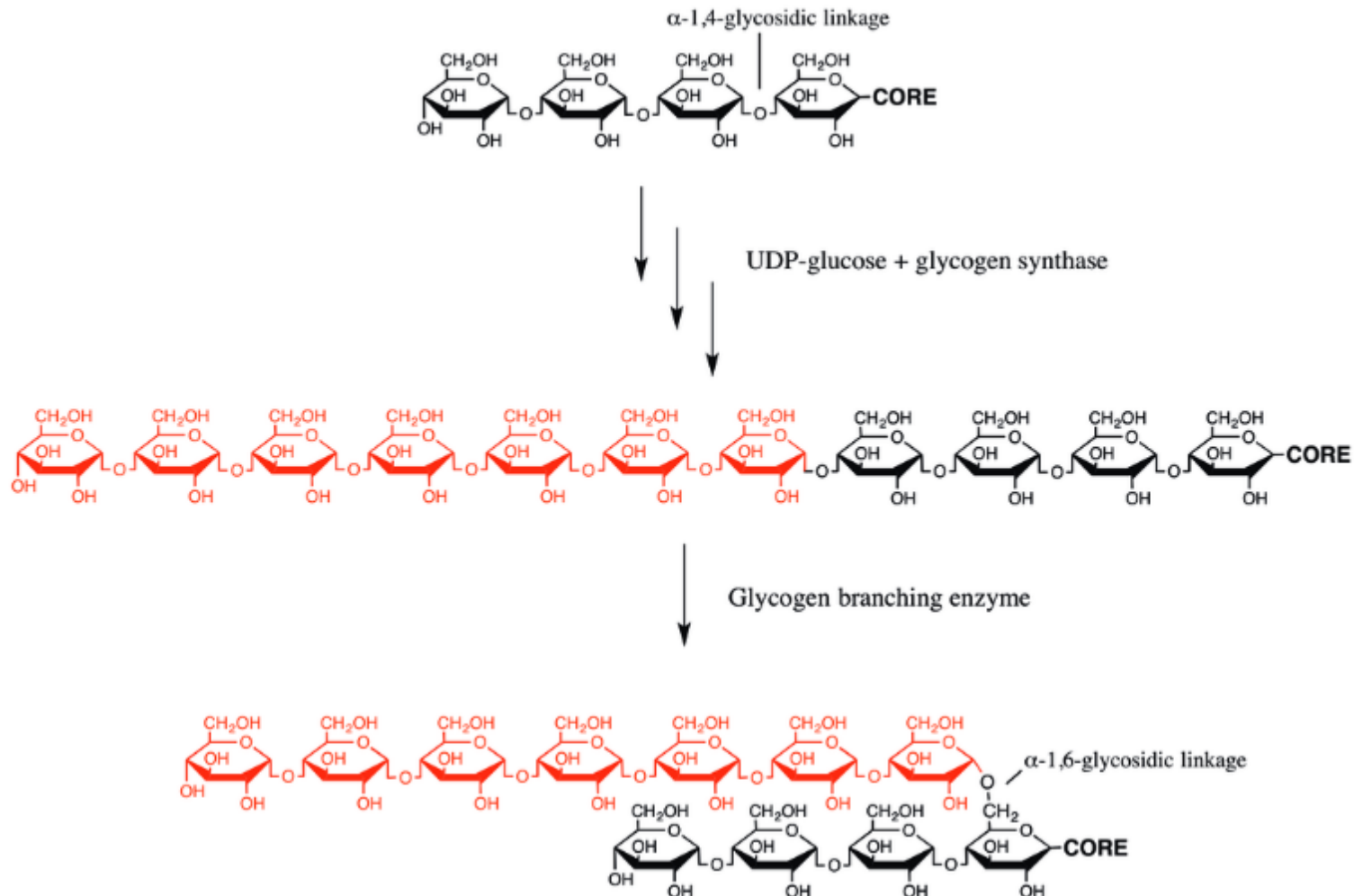
Food

Detergent

Paper

Alcohol

Glycogen branching enzyme



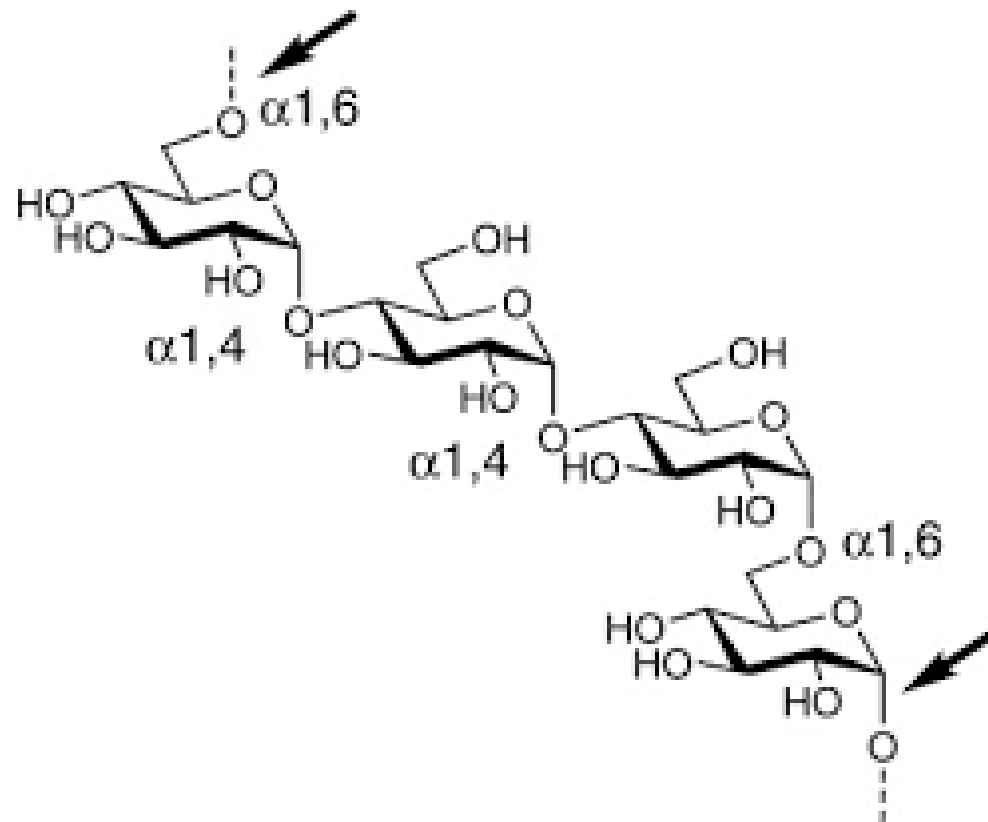
Application

Increasing of starch stability and solubility

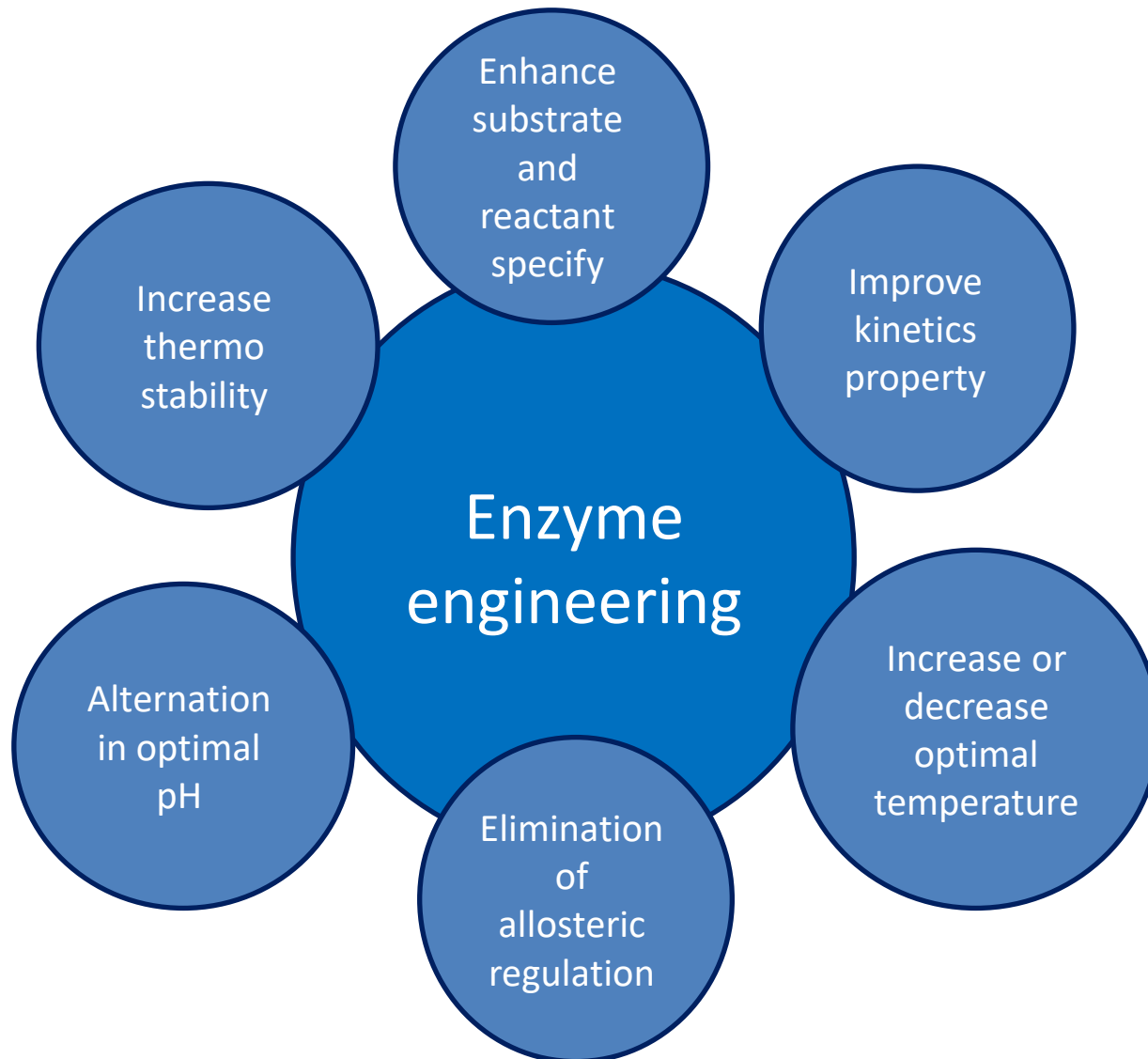
Coating step of paper manufacture

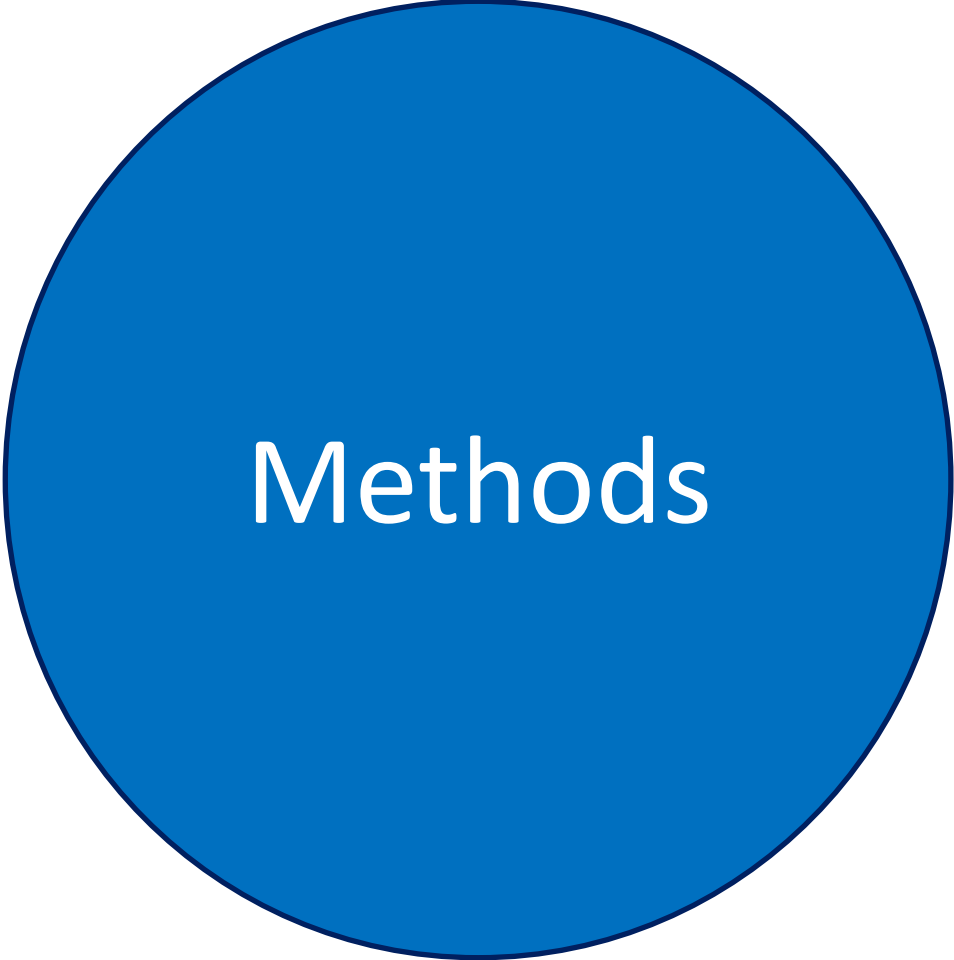
Decrease Retrogradation

Pullulanase



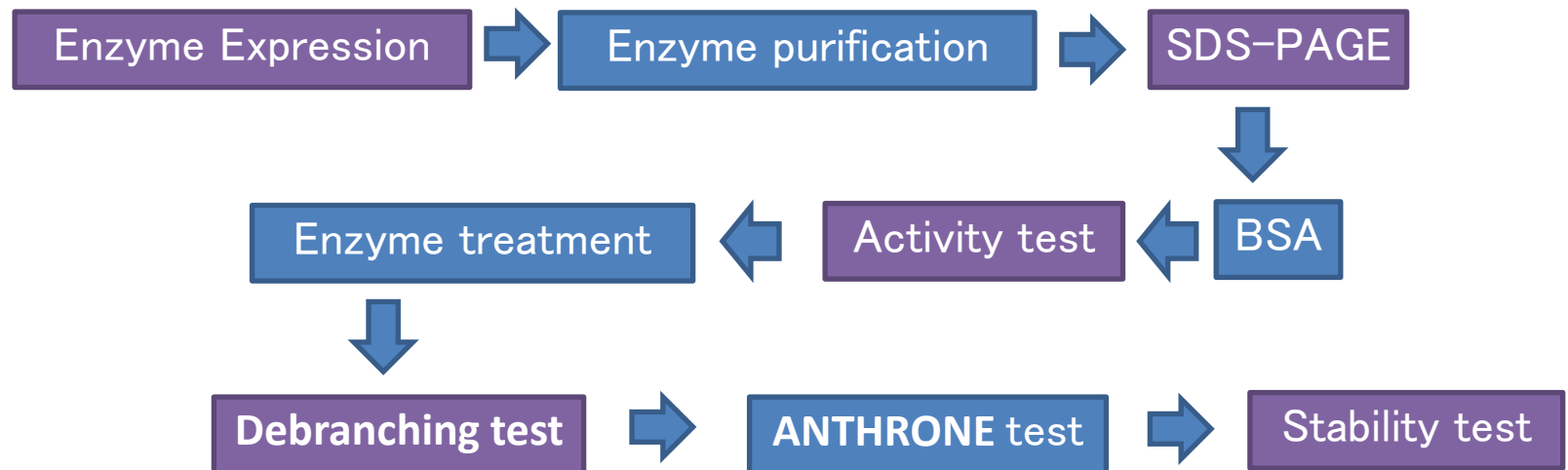
Application of Enzyme engineering





Methods

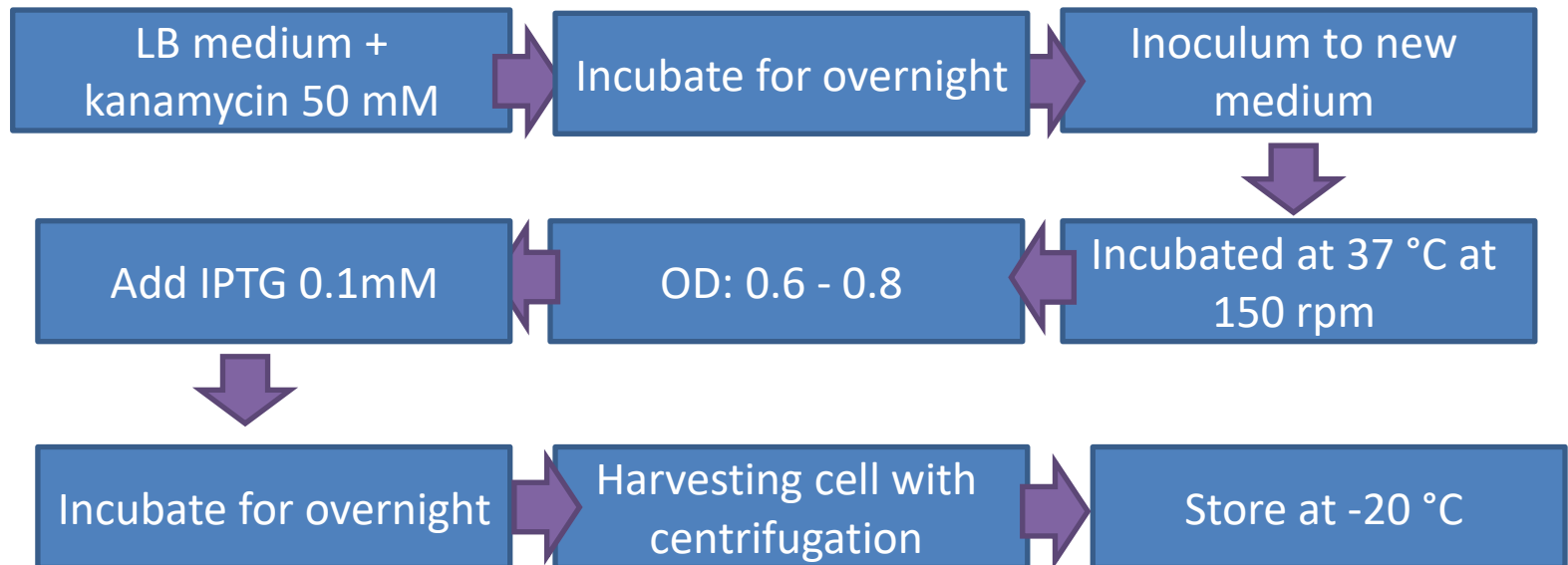
Methods



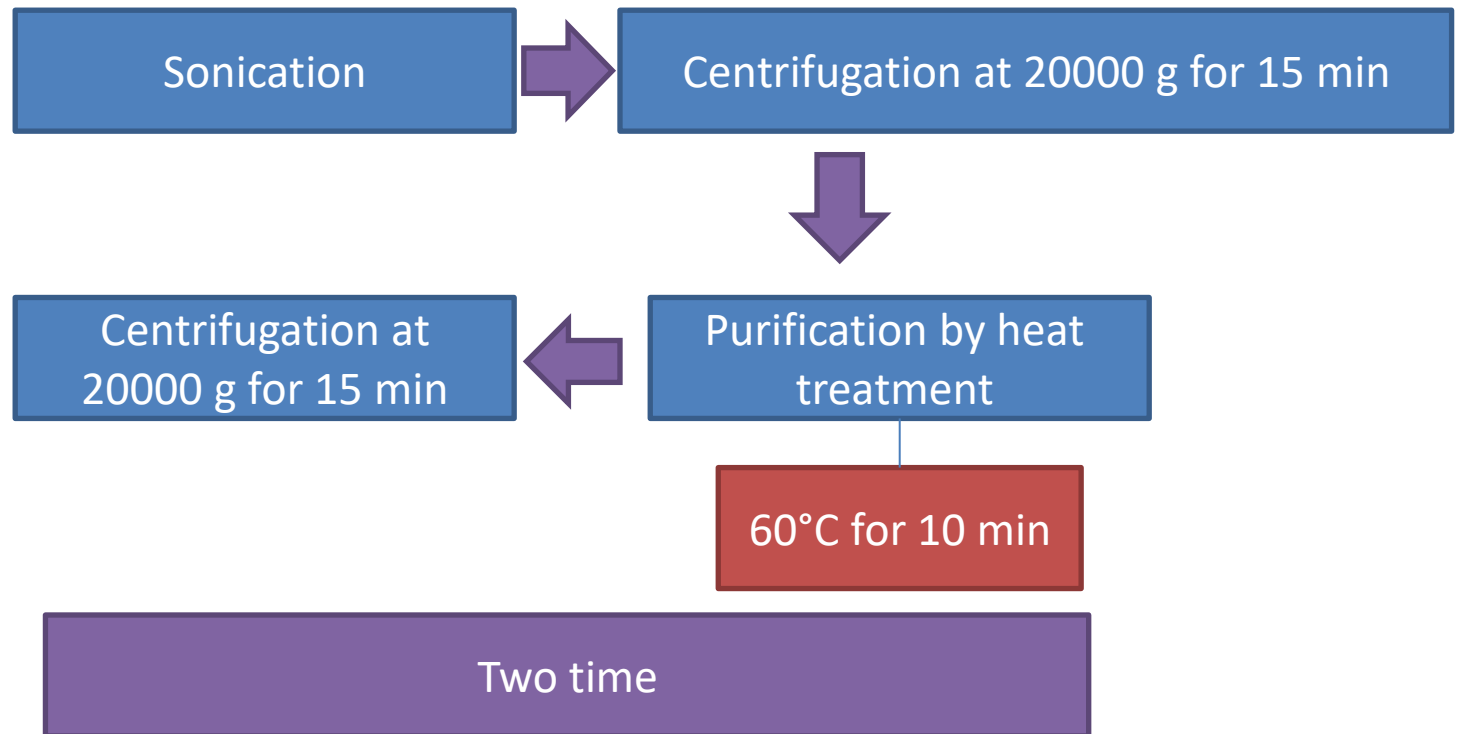
Enzyme Expression

Vector: PET 28 a

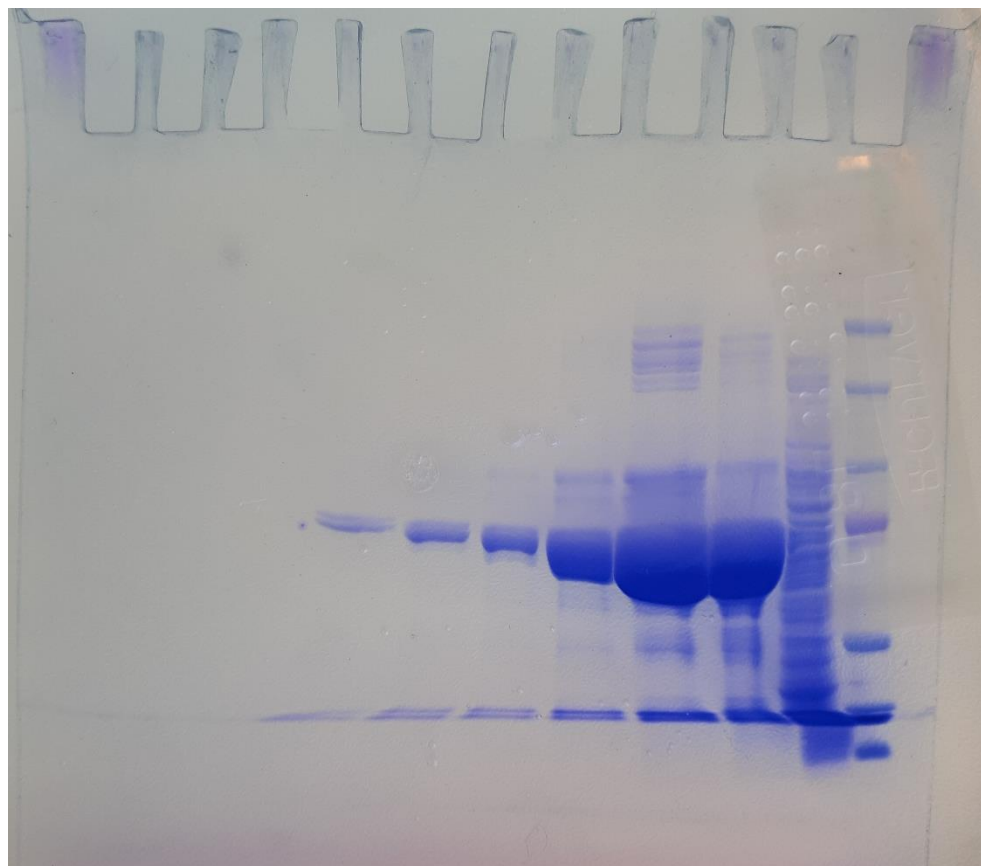
Host: *Ecoli21* (DE3)



Lyse of cell and purification

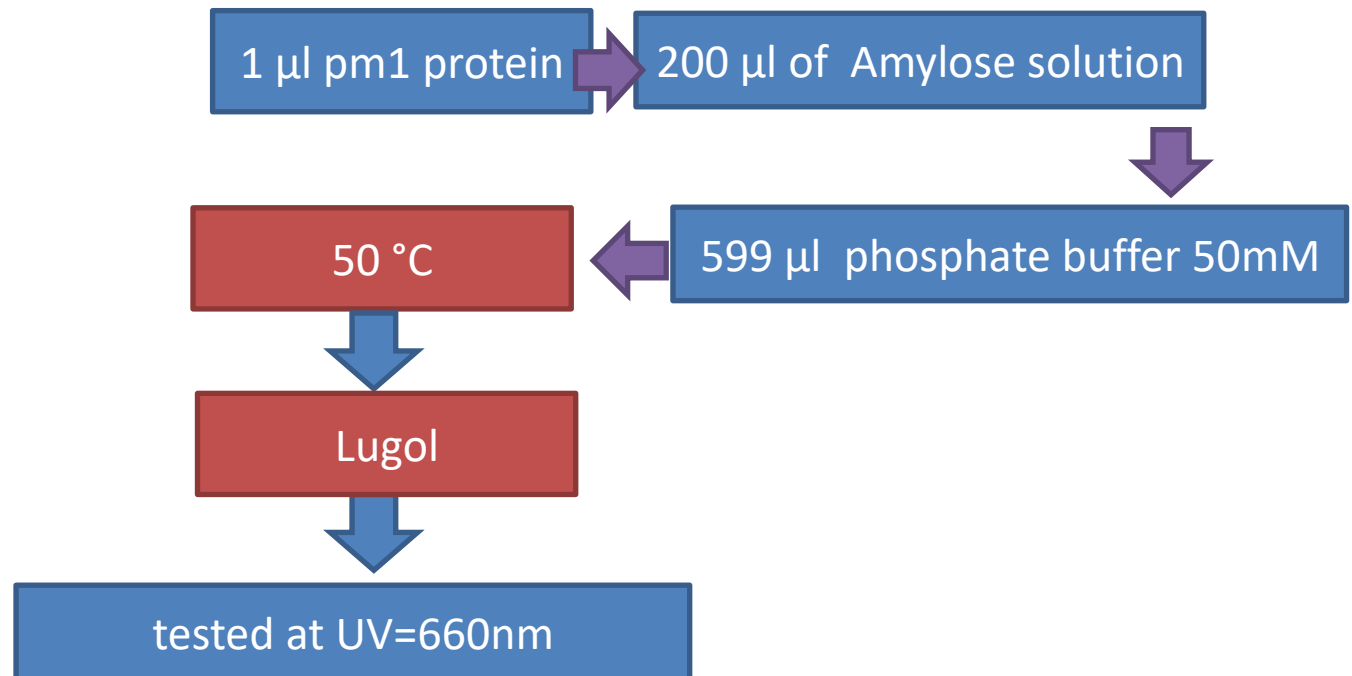


SDS-PAGE and BSA

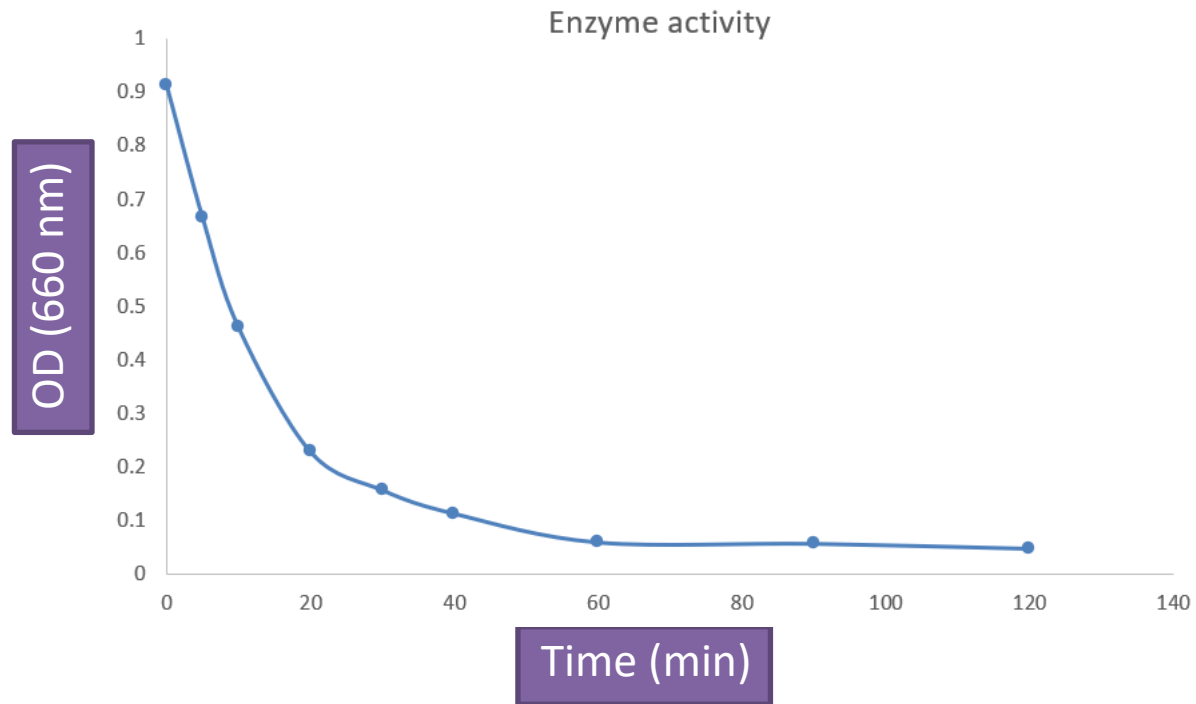


Enzyme activity

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



Enzyme activity



BSA method

Proportion of several concentrations of protein

mg/ml

0.75

0.5

0.25

0.125

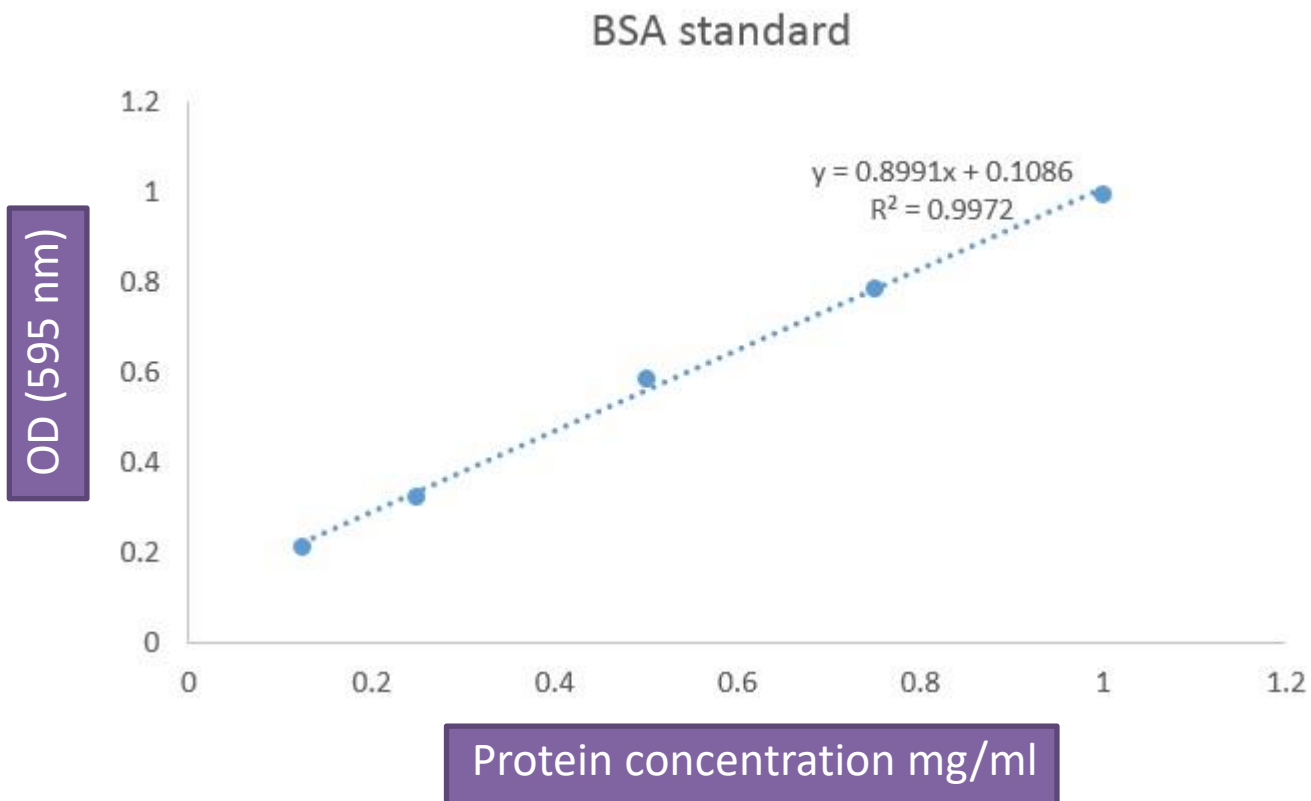
Add 100 μ l of protein concentration to 5 ml 1X Dye reagent

Vortex the complex for 10 second

Read at 595 nm

Enzyme was diluted for 20 times

BSA method



9.64 mg/ ml

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.

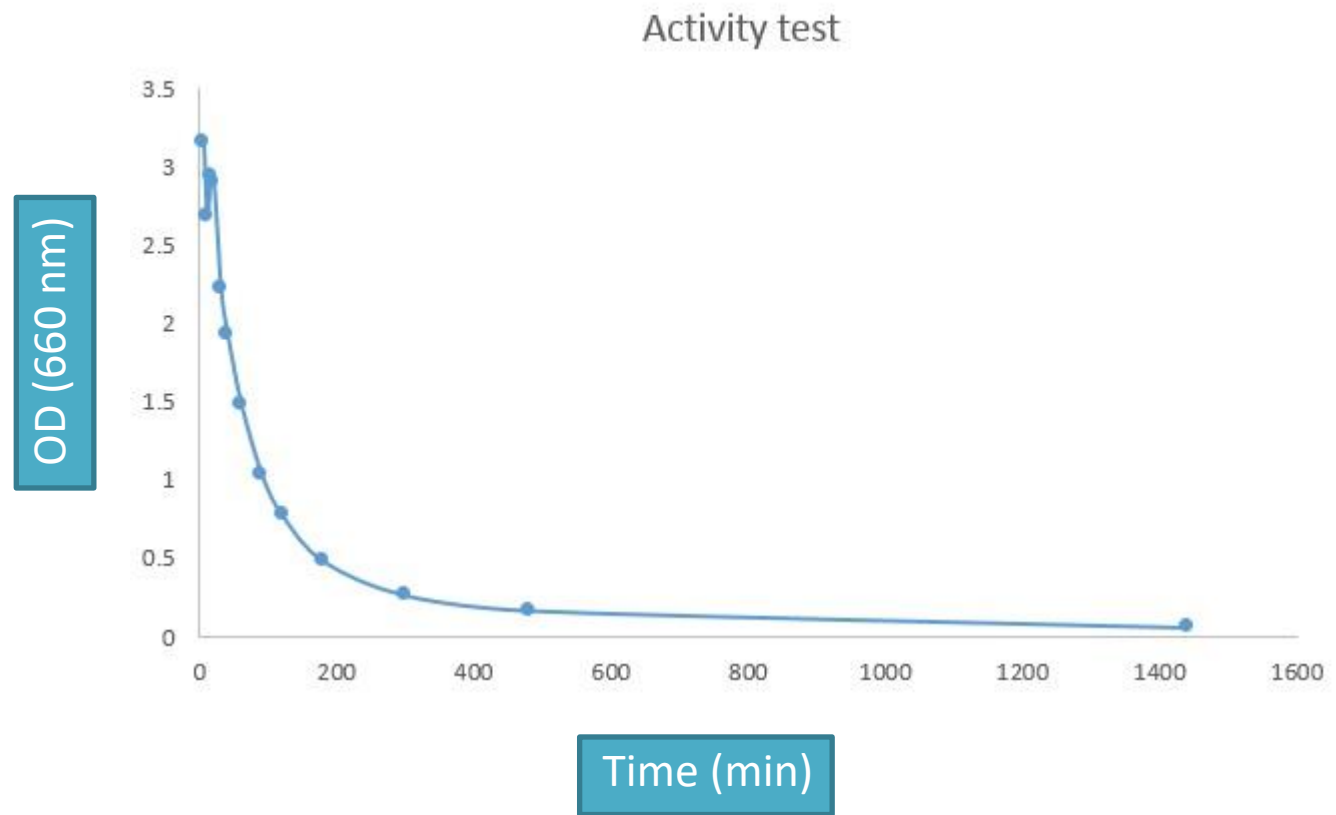


Activity test



Freeze Dry

Activity test



Debranching test

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



adding 1 μ L pullulanase+ 1 μ L isoamylase



mix for 5 second



incubated at 40 °C for overnight



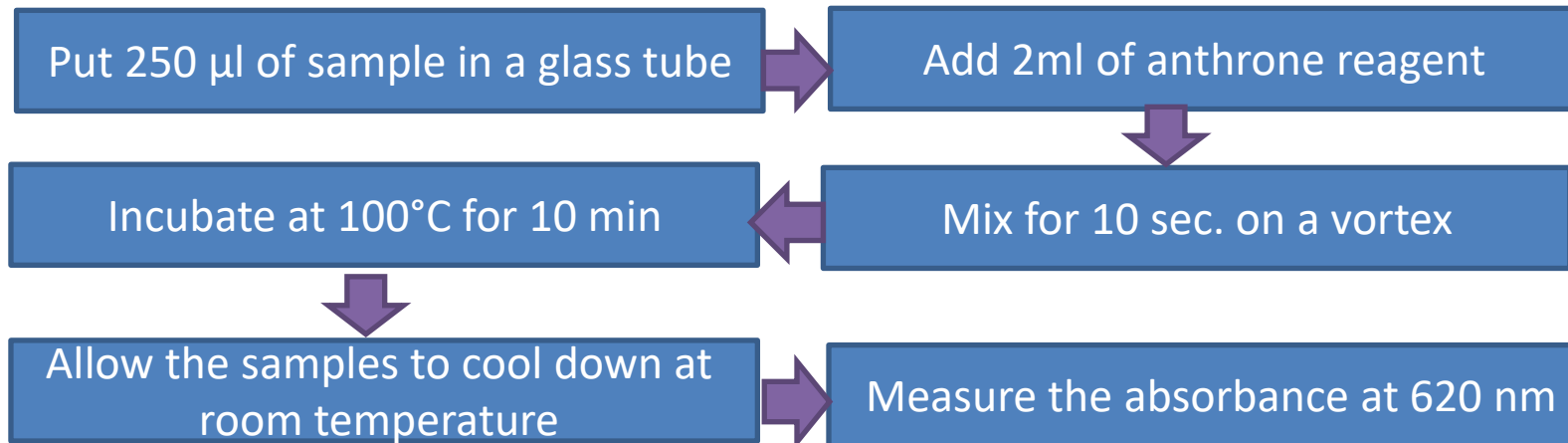
stop reaction by putting in boiling water for 5 min



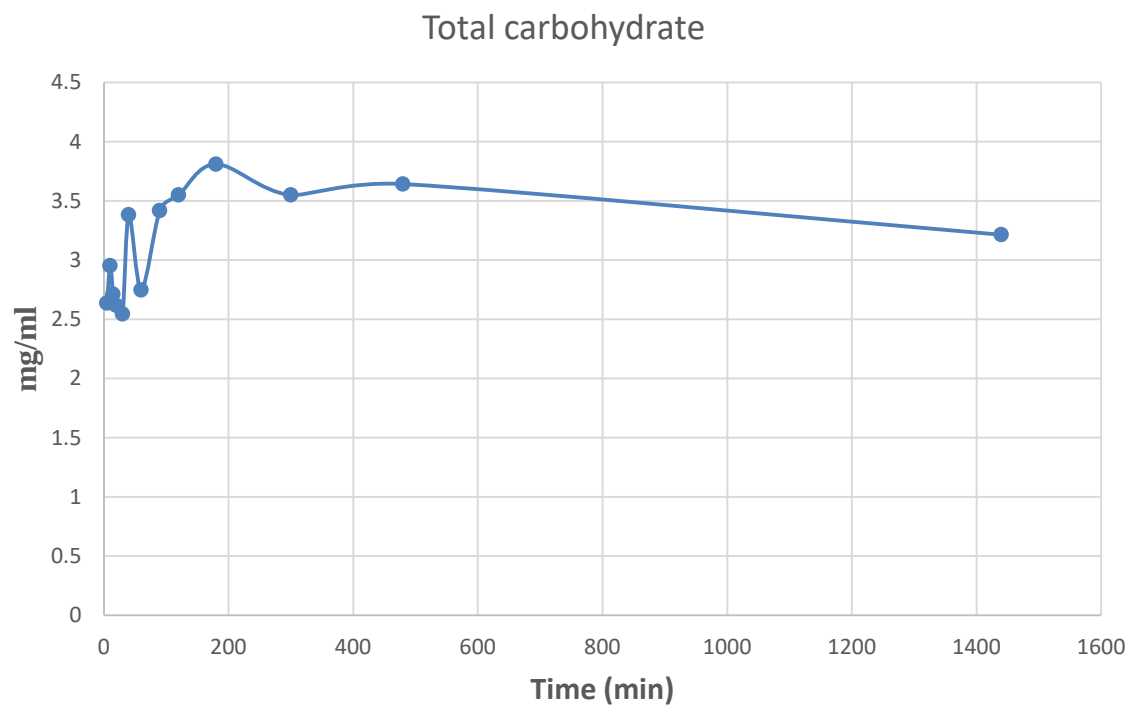
centrifuge at 20000 g for 15 min

ANTHRONE (Total Carbohydrates)

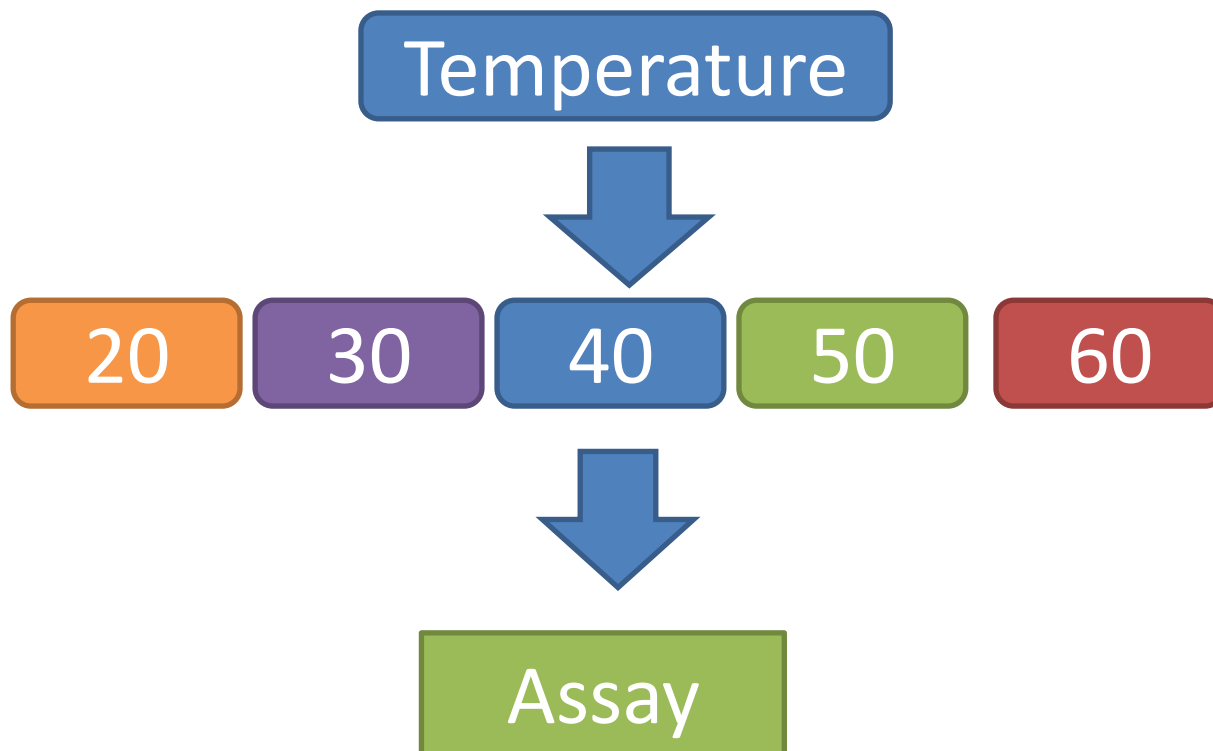
Standard curve



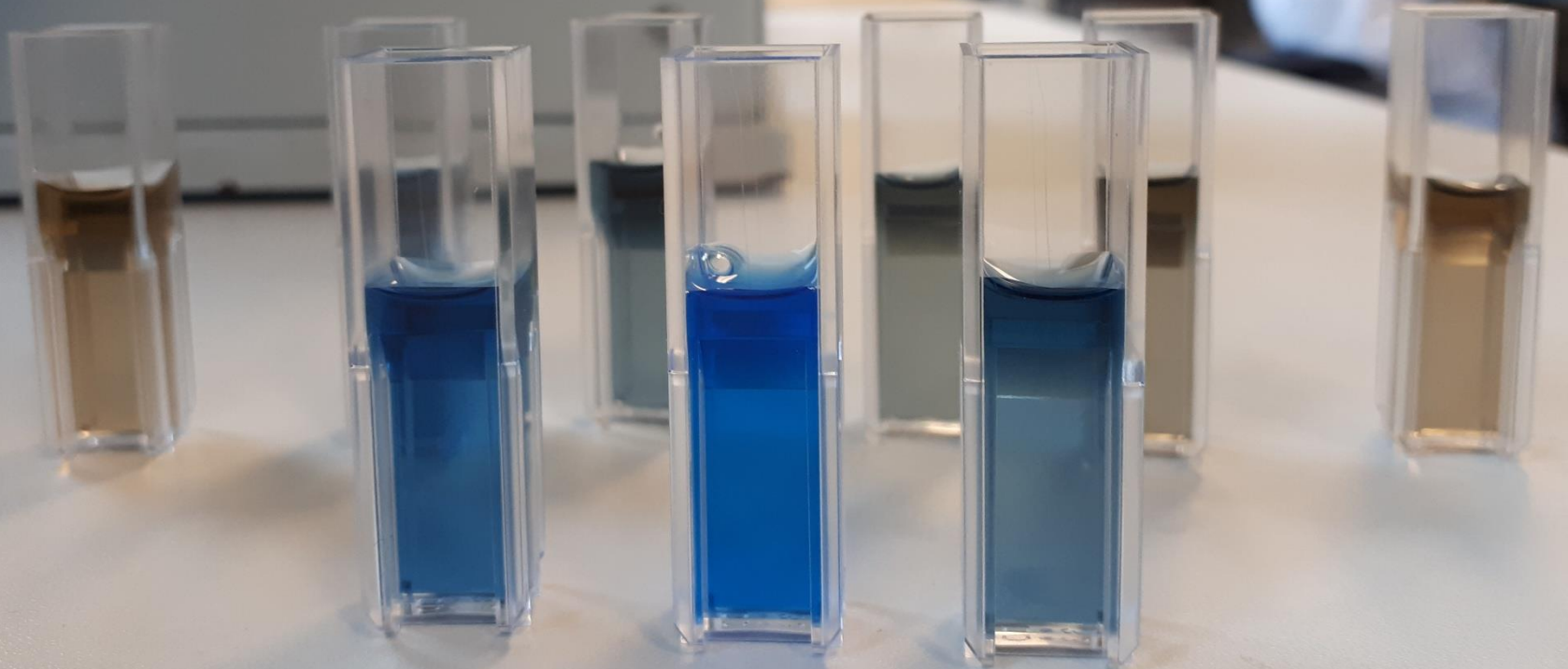
ANTHRONE (Total Carbohydrates)



Stability



If we knew what it was we were doing, it would not be called research, would it ? (Albert Einstein)



Thanks for your nice attention