# Insulin Purification Enzymatic Synthesis: Investigation of Reaction Kinetics

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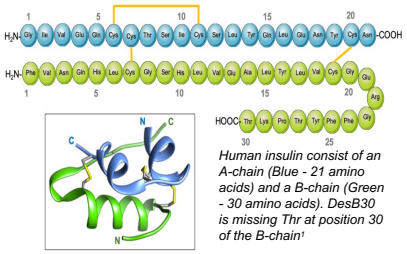
## Introduction

- Production of human insulin is essential to treat diabetes
- Genetically modified *S. Cerevesia* efficiently express and secrete the human insulin precursor: DesB30



*Human insulin consist of an A-chain (Blue - 21 amino acids) and a B-chain (Green - 30 amino acids). DesB30 is missing Thr at position 30 of the B-chain[1]*

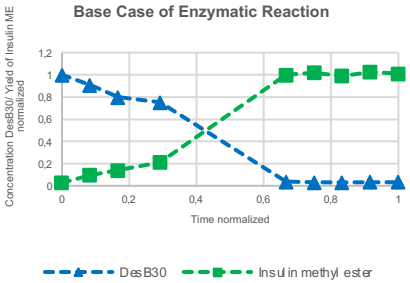
- Lysyl endopeptidase catalyzes DesB30-Thr coupling, aided by organic solvent to shift equilibrium

*DesB30 + L - threonine methyl ester ⇌ Insulin methyl ester + water*

- Parameter varations to improve yield, decrease reaction time and organic solvent used = more green and efficient coupling reaction

## Methods

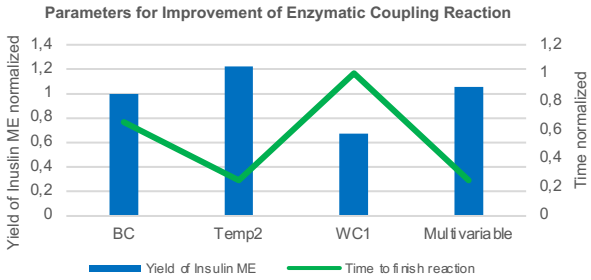
- Progress Curves to establish yield and reaction time
- Steady-State Experiments to determine Michaelis – Menten kinetics
- Reversed-phase UPLC to quantify substrate and product concentrations




*Progress curve using the parameters used in production – this was the Base Case of the study*

## Outcome

- Single variable variations in temperature, pH and water content (WC) – most promising was the temperature variation Temp2 when compared to Base Case (BC)
- BC vs Temp2: Increase in all kinetic parameters ($V_{max}$, $K_m$, $k_{cat}$, $\frac{K_{cat}}{K_m}$)
- Multivariable experiment using pH from BC, Temp2 and WC1




- Reaction completed in 0.42 less normalized time, using 10% less organic solvent, while reaching a 0.06 higher yield, compared to BC.

## Perspective

- Inverstigation of by-products/impurities
- Mutation of enzyme to fit Base Case parameters
- Green solvent alternatives
- Enabling enzyme reusability through immobilization

[1]'Diabetes Mellitus', RCSB PDB-101.