**NTNU Trondheim (Norway)**

**Alginate-Encapsulated Glucose-Sensing Pseudomonas putida**

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**MOTIVATION**

- Diabetes is a group of metabolic diseases caused by a deficiency of the hormone insulin and/or a decreased response to the insulin produced in the body.
- According to WHO, approximately 9% of adults worldwide suffer from diabetes. In 2012 alone, 1.5 million deaths were directly caused by diabetes.
- Over the last decades there has been no substantial improvement in diabetes management.
- We propose to make implantable glucose biosensor devices and benchmark their performances.
- With our novel concept, we aim to expand the horizon of medical treatment of diabetes.

**RESULTS**

Glucose detection by devices

- All devices show the expected increase in fluorescence with increasing glucose concentration in the medium.
- Since glucose is the only carbon source in M9 medium, we can clearly correlate the increase in fluorescence with function of the devices.

**CONCLUSION**

We successfully engineered *P. Putida* to function as a glucose sensor. Characterization of four different devices via various experiments and computational modeling identifies the Zwf-Device as most efficient. Its linear correlation of fluorescence and glucose level and the stability of the measurements over time reveal it as promising glucose detection system. The Zwf-device could be the basis of a novel treatment method for diabetes. We have managed to limit the bacterial leakage of the alginate capsules, which is important for any future biomedical applications.

**FUTURE PERSPECTIVES**

Based on our findings, a variety of innovative techniques can be developed. One of these is about diabetes. Our project is just the first step towards the establishment of a novel, improved medical treatment for diabetic patients. In the future, the reporter gene mCherry can be exchanged by insulin. Encapsulated in alginate and implanted into the body, our engineered bacteria can work as an artificial pancreas. This concept constitutes an extraordinarily sensitive and efficient therapy.

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**BACKGROUND**

Glucose metabolism in *Pseudomonas putida*

HexR:
- Repressor preventing transcription of Edd and Zwf.
- If 2-Keto-3-deoxy-6-phosphogluconate binds to HexR, it is released from the respective promoters.

PtxS:
- Repressor preventing transcription of Kgu and Gad.
- If 2-Keto-gluconate binds to PtxS, it is released from the respective promoters.

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**CONCEPT**

- Semipermeable gel
- Fluorescence (mCherry)

**RESULTS**

Glucose detection by devices

- Limited capsule cell leakage
- Highly predictable performance
- Reliability and stability are Important features of a glucose sensor of the measurement.
- Theoretical modeling and experimental data support that the zwf-device possesses these qualities.

**BIODRICK PARTS**

The devices consist of a double terminator, the respective promoter and the reporter protein mCherry.

**MODELING**

Kinetics

Rates definition

Closed form expression

**SOFTWARE**

iGEM Matchmaker 3.0:
- Automatic matchmaking of iGEM teams using machine learning and keyword extraction.
- Quantitative image analysis:
  - Batch processing of confocal microscopy images.
- Brixells Modeling Tool:
  - Collaboration with Team Warwick

**TEAM**

Students

Mentors

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**Alginate: National molecule of Norway**

The capsules will shield the cells from their surroundings, at the same time as small molecules like glucose and insulin can diffuse in and out.

**CELL ENCAPSULATION**

Alginate: A polysaccharide that can be extracted from marine brown algae (e.g. laminaria hyperborea), or synthesized by some soil bacteria (some Pseudomonas and Alcaligenes species). A linear polymer consisting of α-D-mannuronic (M) and β-D-guluronic (G) residues (Figure 2A). Two or more β-D-mannuronic acid blocks aligned synthetically hold alginate strong and form a gel network (the algigel model, Figure 2B). (Figure) From: B. J. G. Holmsen (source: Comparative Medicine, NTNU).