DISCO

DESIGNING INSULIN TO BE SINGLE CHAIN AND OPEN SOURCE

THE UNIVERSITY OF SYDNEY

iGEM

Insulin replacement therapy is the only effective treatment for **all** Type I and **30%** of Type II diabetics.

But despite insulin being commercially available since 1923...

"Insulin is **only** for the *rich*"

"Paying more than **USD $700** a month for [insulin]"

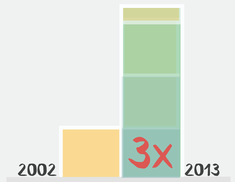
"Some of my friends have **died** from lack of insulin"

"There are many times I **can't afford** my meds so I go without, even knowing it will kill me."

WHY is insulin accessibility a problem?

The incidence of diabetes is rising rapidly

AND



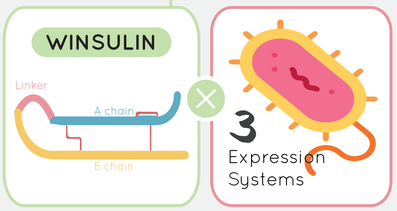
the cost of insulin has more than tripled in a decade

AND

supply to remote areas is usually poor
(particularly without refrigeration)

Our Solution

After consulting experts with start-up, pharmaceutical and legal backgrounds, we concluded that our solution was to create a novel, open source, single chain insulin using 3 optimised expression systems.



AFFORDABLE

Activation of proinsulin requires cleavage of the C-peptide, whereas single chain insulins do not require this additional processing step.

STABLE

Due to the structure of the linker peptide, single chain insulins are more stable than human insulin.

SHORT ACTING

WHO announced in 2013 that short-acting insulin is an "essential medicine", while long-acting is not. So, we designed - using modelling - our single chain insulin to be short-acting.

$$Insulin_{Hexamer} \rightleftharpoons Insulin_{Dimer}$$

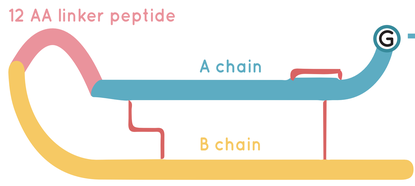
UNPATENTABLE

Our Winsulin was designed to be open-source.

In collaboration with legal experts, we took steps to ensure that our linker region did not infringe existing patents.

HOW DID WE DO THIS ?

Single Chain Insulin: Winsulin



GLYCINE SUBSTITUTION
Arginine to Glycine substitution at position A21.

Increases pI to improve stability.

LINKER DESIGN

DIBASIC RESIDUES
Systematic screening revealed that these dibasic residues are required for insulin receptor binding.

FLEXIBLE LINKER
Generic linker providing flexibility for A and B chain to form the correct disulphide bonds for activity

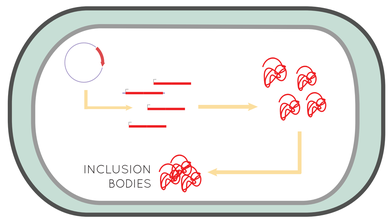
12 AMINO ACIDS LONG
Short linker sequences cause steric hindrance, impairing the ability of insulin to fold properly.

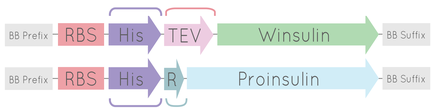
There is no optimal length but high activity has been shown in linkers between 5 - 15 amino acids long.

PROTEASE RESISTANT
The sequence this linker was adapted from was cleavage resistant, so we expect our linker to also have this property.

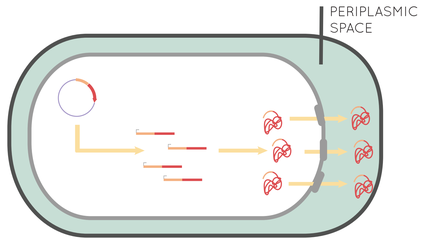
3 Expression Systems

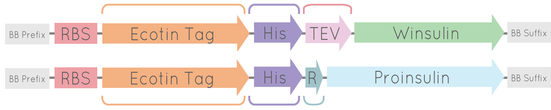
E. coli CYTOPLASMIC EXPRESSION



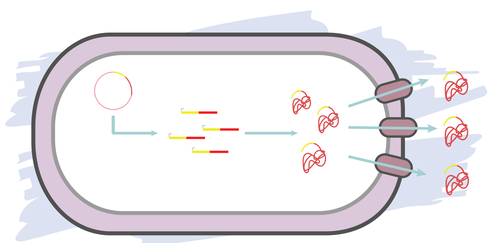


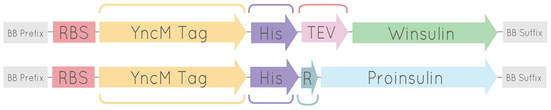
E. coli PERIPLASMIC EXPRESSION





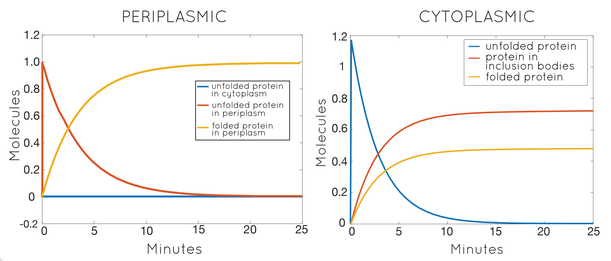
B. subtilis SECRETORY EXPRESSION





Cytoplasmic vs. Periplasmic

Synthetic Circuit Dynamics



Comparative modelling of cytoplasmic and periplasmic expression indicated that an oxidising environment is a key determinant of yield. Therefore, we used SHUFFLE strain in an attempt to match cytoplasmic yield with periplasmic.

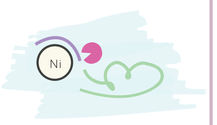
Purification

6x His Tag on our insulin constructs will bind to a nickel column

TRYPSIN for PROINSULIN

to cleave off the **C-peptide** AND the **His-Tag**

TEV protease for WINSULIN



to cleave off the **His-Tag**

Achievements

ELISA Assay

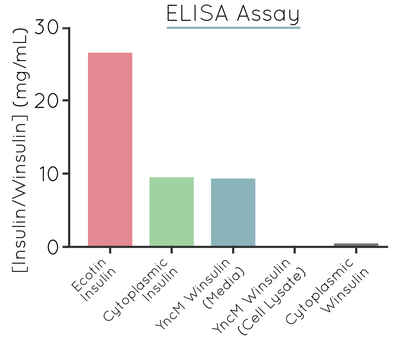


Figure 1: Cells successfully produced correctly folded human insulin and Winsulin.
ELISA demonstrates insulin specific antibody binding to folded insulin proteins within samples.

Did we **make** insulin / Winsulin ?

YES

ELISA demonstrated the expression of correctly folded human insulin / Winsulin, and the successful secretion of YncM-Winsulin into culture media.

HepG2 Assay

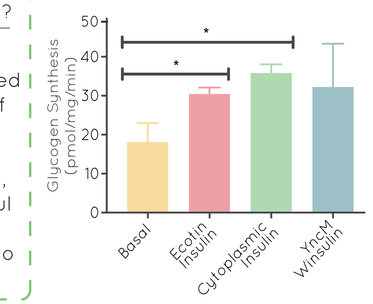


Figure 2: Cells successfully produced biologically functional human insulin and Winsulin. Glucose uptake assay in human HepG2 cells shows increased glycogen synthesis when incubated with samples of human insulin / Winsulin compared to a basal (no insulin added) control. Error bars represent SEM, horizontal bars indicate statistical significance (* = p <0.05) calculated using unpaired 1-tailed T-test (n=3).

And is it **functional?**

YES
- Human insulin definitely
- Winsulin most likely

Glucose uptake assay in human hepatocytes proved that human insulin is functional and Winsulin is highly probably functional *in vitro*.

Attributions & Sponsors

Attributions

PI: Dr Nick Coleman
Secondary PI: Professor Jacqui Matthews & Dr Ed Hancock
Lab Support: Mark Somerville & Yanwei Ma
Expert Advisors: Len Mancini, Neil Donelan, Mike Nicholls, Narcyz Ghinea, Dr David Beran, Dr Andrew Hoy
Special Thanks to Professor Peter Arvan, Professor Sui-Lam Wong, Simon Lewinson & Open Insulin

Sponsors

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