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...

**WHY is insulin accessibility a problem?**

- Paying more than USD 700 a month for insulin
- "Insulin is only for the rich"
- "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.”

**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.

**3X** since 2007

- 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 (particular without refrigeration)

**ACCRUAL**

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.

**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.

**Achievements**

- ELISA Assay: 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: 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.

**HOW DID WE DO THIS?**

**Single Chain Insulin: Winsulin**

GLYCYNE SUBSTITUTION

Arginine to Glycine substitution at position A21

Increases pI to improve stability.

**12 AA linker peptide**

A chain

B chain

**GLYCINE SUBSTITUTION**

Arginine to Glycine substitution at position A21

Increases pI to improve stability.

**FLEXIBLE LINKER**

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

**LINKER DESIGN**

Systematic screening revealed that these disbasic residues are required for insulin receptor binding

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**
- **A. subtilis SECRETORY EXPRESSION**

**Cytoplasmic vs. Periplasmic**

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**

- Trypsin for PROINSULIN
- TEV protease for WINSULIN

- Ax-His Tag on our insulin constructs will bind to a nickel column

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

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

**Figure 2** Cells successfully produced biologically functional human insulin and Winsulin. Glucose uptake assay in human HepG2 cells shows increased glucose 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< 0.005) calculated using unpaired 1-tailed T-test (n=3).

**Attributions & Sponsors**

- **PI:** Dr Nick Coleman
- **Secondary PI:** Professor Peter Arvan
- **PI:** Dr Nick Coleman
- **Sponsors:**
  - **MaxWorks**
  - **Maxwell**
  - **StartGem**
  - **Bioworks**
  - **Open Insulin**
  - **Biome**
  - **Labfall**
  - **Access**
  - **DIBS**
  - **Incubate**
  - **IDT**
  - **Bi Foundry**
  - **Open Insulin**
  - **Newcastle University**

**Molecules**

- Winsulin
- Type II diabetics.

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