**Hyperglycemia: A Daily Danger**

- Post-prandial hyperglycemic spikes (PHSs) are a sharp rise and fall of blood sugar following a meal. PHSs adversely affect patients with insulin resistance such as pre-diabetics and diabetics, an estimated 100 million Americans. Failure to mitigate PHSs results in complications from malaise and headaches to macrovascular disease, retinopathy, and cardiac event.
- Current drugs treatments include alpha-glucosidase inhibitors and amylin, which are expensive and inconvenient to dose, usually requiring an injection.
- We aim to reduce the amplitude of elevated postprandial blood glucose levels (≥7.8 mmol) by a sugar-concentration-dependent microbial system for delayed and reduced absorption of glucose and fructose from the small intestine.

**The Solution: Mitigating Hyperglycemic Spikes with Microorganisms**

- **Sugar Uptake**
  - Free fructose and glucose are taken up by E. coli.
  - Both sugars diffuse freely through the outer membrane of E. coli.
  - Transmembrane protein Enzyme-IIA (EIIA) transports glucose into the cytosol. Fructose stays in the periplasm.

- **Polymerization**
  - In the periplasm (intermembrane space), Levansucrase (SacB) enzyme catalyzes the polymerization of fructose into levansugar.

- **Toxic Kill Switch**
  - After cell lysis, polymerized sugars are released into the lumen of the small intestine.
  - The time required to hydrolyze the polymers slows glucose and fructose absorption into the body, mitigating the amplitude and duration of the hyperglycemic spike.

- **Polymers Released**
  - SacB also confers fructose-sensitive cell lysis of the E. coli.

**Footprint: The Body**

**Modeling: Into the Body**

The model projects the effects of our engineered bacteria if introduced into the human body.

The changing concentration of glucose over time was modeled as a 2-compartment system.

**Looking to the Future**

- Develop a SacB-dependent mechanism to polymerize fructose into levansugar over an optimum range of sugar concentrations.
- Refine the quantitative modeling by better representing the absorption of glucose into the small intestine.
- Address the challenges that genetically-modified probiotics pose to patients such as horizontal gene transfer of antibiotic resistance, colonization of the gut, or competition with existing gut flora.
- Develop another integrated kill-switch into the bacteria that can lyse the cell should SacB mutate to no longer operate as a kill switch.
- Code for a Smartphone App that, after inputting a user’s metabolic profile, will advise the best dosage of this bacteria to mitigate a hyperglycemic spike from a given glycanic intake input.

**Acknowledgements**

- **Faculty**
  - Dean Parrish, PhD - Team Advisor
  - Assistant Professor of Biology, UVA
  - Professor, MD - Team Advisor
  - Associate Professor of Biomedical Engineering, UVA
  - Assistant Professor of Chemical Engineering, UVA
  - Assistant Professor of Engineering, *Computer Science*
  - Student Member: 
    - Michael Tucker, Virginia GEM 2013 & 2014 Team Member
  - beeChittari, PhD - Team Advisor
  - Associate Professor of Biomedical Engineering, UVA
  - Assistant Professor of Biology, UVA
  - Co-Chair, Laboratory Manager
  - Zhao Chab's Laboratory Coordinator

- **iGEM Documentary**
  -賀馬金知, PhD - Diabetes Expertise
  - 石井泰喜, MD - Diabetes Expertise
  - 梶原猛, MD - Diabetes Expertise
  - 塩田一之, PhD - Research Advising
  - 稲垣直樹, PhD - Research Advising

- **Supporting iGEM Members**
  - 金子孝美, PhD - Research Advisor
  - 佐野達也, PhD - Research Advisor
  - 佐藤直之, PhD - Research Advisor

- **Patents and Patents**
  - Dr. Christopher Chittari, PhD - President, Vanderbilt University Library

- **Contact**
  - **University of Virginia iGEM Team 2015 – Charlottesville, VA, USA**
  - Suprajit Chittari, Connor Jahelka*, Issac Li*, Dominic Ritchey*, Sean Sequeira*, Shiran Su*, Rena Yuan, Liam Wolf*, Jingyuan Zhang*

- **Departments of Biomedical Engineering, Biology, *Neuroscience, *Systems Engineering, *Computer Science**

**Synthetic Biology as a Solution**

- **We engineered an Escherichia coli K-12 bacterium to mitigate PHSs by upatching free glucose and fructose, homopolymerizing them, and releasing the polymers (e.g., starch) into the lumen of the small intestine.**

- **This solution exploits the concentrations of simple sugars and their polymers in the gut after meals. The spikes and dips of the sugars have reduced amplitude, or mitigated, after sugar-polymer-rich meals than simple-sugar meals because polymers must be hydrolyzed and re-absorbed slowly, which is an energy- and time-intensive process** (Fig. 1).

**Proof of Principle: Data and Assays**

- **Glucose Polymerization and Uptake** (Fig. 3)
  - The glycogen assay shows an observable difference of % glycogen concentration in BioBrick-transformed cells compared to the nontransformed A15x control.
  - Glycogen + glucose hydrogen peroxide \( \rightarrow \text{H}_{2} \text{O} + \text{AMP} + \text{Redox} \rightarrow \text{Red color} \)
  - Negative control. Negligible difference with fructose demonstrates specificity of GlgC with glycogen synthesis

- **Kill Switch Mechanism by Toxicity** (Fig. 4)
  - A reduction of cell density by 50% was observed at 0.6% fructose after 2 hours. Negative control.

- There was no significant difference between cell density with differing concentrations of glucose, suggests that cell lysis is predominantly due to levansugar and SacB-induced toxicity.

**Policy & Practices: FDA Regulation**

- **Policy and Regulation Study**
  - Developed a video documentary on the process of gaining FDA approval for this potential probiotic, with interviews from experts in the field.

- **Patient Connections**
  - Diabetes diet pamphlet details how to integrate this probiotic into the diabetic lifestyle.
  - Did a pre-project review with Diabetes experts

- **Collaborations**
  - Attended a meetup hosted by iGEM UMD College Park
  - Discussed Policy and Practices with iGEM Vanderbilt