**BACKGROUND**

**ANTIFREEZE PROTEINS**
- Independently evolved in certain species of fish, insects, plants, and bacteria
- Proteins have a flat, rigid ice-binding surface
- The bound AFPs causes curvature on the ice surface, inhibiting ice growth

Our project uses the Type III ocean pout AFP, a moderately active protein.

Ice-binding surface

**PROBLEM**

- Long waitlists for transplants
- Limited availability of organs
- Preservation conditions limit geographical range between donor and recipient

Cryopreservation (subzero) improves the viability of the tissue by slowing cell metabolism

CURRENT PREPARATION TIME
- 4-6 h
- 21-24 h
- ???

**HUMAN PRACTICES**

- Research forums
- Public seminar
- Community exposure
- SHAD high school enrichment synthetic biology workshop
- Intellectual property study
- Second year online molecular biology problem-based learning modules
- First year engineering design project
- Ontario iGEM conferences

**SOLUTION: THE ICE QUEEN**

**DESIGN & MODELING**

E/K coils used to attach AFPs to T3-10 scaffold:
- High affinity
- Engineered coiled coil domains
- Non-covalent with covalent strength

- Used PyRosetta docking software to model favourable parallel versus anti-parallel orientation of the coils
- Constructed, expressed and purified AFP + E coil

**ICEFINITY AFP CIRCULARIZATION**

- AFP use in industry is limited because of harsh processes: Type III denatures at 40°C & measurable activity drops
- Circularized proteins have a greater stability in variable temperatures
- Heat treated at 37°C, 68°C, 90°C, and 100°C
- Purified circularized protein maintained nearly 80% activity at highest temperatures while wild type exhibited only 25% activity

**STABILITY OF CIRCULAR AFP**

Three linkers were designed and tested via molecular dynamic simulation and compared to an unmodified AFP file.

**REFERENCES**


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**BIOBRICKS & ACCOMPLISHMENTS**

- Circularized AFP + promoter BBa_K1831000
- Improved Heidelberg 2014 part BBa_K1362000
- AFP + E coil + His-tag BBa_K1831001
- Scaffold + K coil BBa_K1831002
- AFP + E coil BBa_K1831003

- Investigated intellectual property surrounding our project
- Tested functionality of our circularized AFP
- Public seminar and community exposure
- Problem-based learning for biochemistry and engineering courses

**THANKS & TO OUR SPONSORS**

- BioLabs
- Cubit
- IDT

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