BREAKING THE ICE

IMPROVING ANTIFREEZE PROTEINS FOR PRACTICAL USE

QGEM
Queen's University
NATURAL PHENOMENA
ANTIFREEZE PROTEINS

ANTIFREEZE PROTEINS

Type III
From ocean pout

ice-binding surface
Ice surface

Bound AFP causes curvature

2-10 nm
THERMAL HYSTERESIS

Without AFPs  With AFPs

fp = mp  elevated melting point  depressed freezing point

TH gap
123,851 waitlist patients
29,532 transplants
76% remained on the lists

U.S.A. 2014

SOURCE: Canadian Organ Replacement Register, 2013, Canadian Institute for Health Information.

http://www.organdonor.gov/about/graphdescription.html
CURRENT LIMITATIONS

Amir et al. 2005
Our solution - ICE Queen

1 – increases local concentration

2 – fixes protein orientation

AFP

Connection Motif

Scaffold
E/K COIL SYSTEM

REQUIREMENTS

- Strong
- Specific
- Modular

Calgary 2013
E/K Coil BioBricks

Lindhout et al. 2004
NMR Solution Structure

ANTI-PARALLEL
PARALLEL
PyRosetta
Interactive Molecular Modeling for Proteins

CONCEPT MODELING

QGEM
Queen's University
LAB RESULTS

pSB1C3

AFP + E-coil

Ice affinity purification fractions

MW (kDa)  Sup  Liq 1  Ice 1  Liq 2  Ice 2

AFP + E coil
**LAB RESULTS**

Ni\(^{2+}\) purification of T3-10 units

Size exclusion purification of assembly
IMPLICATIONS

IMPLICATIONS

QUEBEC CITY

OTTAWA

TORONTO

KINGSTON

DETROIT

BOSTON
COULD ANTIFREEZE PROTEINS SOLVE OUR ORGAN SHORTAGE?

"THE SCIENCE, APPLICATIONS, AND SOCIETAL IMPLICATIONS OF CRYOPRESERVING ORGANS WITH ANTIFREEZE PROTEINS"

TOPICS COVERED

BACKGROUND & ABOUT QGEM
THE RESEARCH & PROJECT
ANTIFREEZE PROTEIN APPLICATIONS
CRYOPRESERVATION: SOCIETAL, PUBLIC HEALTH, & COMMERCIAL CONSIDERATIONS

ADMISSION BY DONATION
PROCEEDS GO TOWARDS SUPPORTING QGEM’S RESEARCH

VENUE
Queen’s University, Kinesiology Building, Rm 101
July 28th, 2015
5:30 - 7PM
Reception to follow with refreshments provided

ALL WELCOME
Audiences of a variety of backgrounds encouraged to attend this interdisciplinary seminar & discussion forum

REGISTER AT
http://goo.gl/forms/JmC7hMh6c

REFRESHMENTS COURTESY OF:

COMMUNITY OUTREACH

GROUP EYES GAME CHANGER FOR TRANSPLANT

By Sebastian Leck
Wednesday, July 29, 2015 5:02:06 EDT PM
INDUSTRIALIZATION

NORMAL PROTEIN

LOSS OF BIOLOGICAL ACTIVITY

DENATURED PROTEIN
CIRCULARIZATION - ICEFINITY
CIRCULARIZATION

assembled inteins

extein segment

linker

CIRCULARIZATION
RAUT
the linker-generator

Heidelberg 2014
linker software

DESIGN AND MODELING
RMSD plot of protein core
RESULTS

SDS size comparison of unmodified and circular AFP
RESULTS

Sample?
Tm = -1.46
T = -1.591
RESULTS

Ice affinity purification of circular AFP

MW (kDa)  Sup  Liq 1  Ice 1

34.6
27.0
20.3
14.3
6.5

Ice affinity purification of circular AFP
RESULTS

Activity Test Conditions

No Treatment | 37°C | 68°C | 90°C

Relative TH Activity

- No Treatment: 100%
- 37°C: 85%
- 68°C: 45%
- 90°C: 25%

WT vs. Circular
Verification of promoter insertion

- Improved Heidelberg 2014’s NpuDnaE intein part
  – BBa_K1362000
- Added a T7 promoter to insert – BBa_K1831000
ACADEMIC OUTREACH

BCHM218
Molecular Biology (Online)

APSC100
Engineering Design Course
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Corey Stevens
Koli Basu
Tyler Vance
Department of Biomedical & Molecular Sciences
Faculty of Health Sciences
Department of Chemical Engineering
Dean’s Donation (Engineering)
Student Initiatives Fund
**Slide 2:**

- Ocean Pout: [https://www.tumblr.com/search/ocean%20pout](https://www.tumblr.com/search/ocean%20pout)

**Slide 7 & 33:**

- Ice Cream:

**Slide 8:**


**Slide 10 & 33:**

THANK YOU

QGEM
Ice Crystal Growth Patterns

No AFPs

Optimum AFPs at TH gap

High [AFP]

ICE AFFINITY PURIFICATION
TH ASSAY
LINKER DESIGN

- **RGKCWE**
  - LINKER 1

- **RGKCWEG**
  - LINKER 2

- **RGKCWE GG**
  - LINKER 3

- **GGAEAAKAARRGKCWE**
LINKER MODELING
PROTEIN DOCKING

Initial PDB of 4EGG/1AME

Low Res Docking:
1. Randomized rotation about geometric center
2. Centroid mode docking of protein

10,000 Decoys created

Sorted into:
1. Energetically favorable
2. Close E/K coils

1,407 energetically favorable
224 close E/K coils

M.C. Probability of Low energy

Overlapping Structures not in M.C. prob

Top 3 candidates visually inspected and lowest energy chosen

High Resolution Docking and Side Chain Packing

22 Structures
25 Structures