Lowering Barriers to Membrane Protein Expression and Crystallization

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Outline

I. Improving expression of membrane proteins in E. coli
   Liz Massey—see poster

II. New methods for crystallizing membrane proteins
   i) Bicelles (Salem Faham)
   ii) SAM crystallization module (Sehat Nauli)

III. Screening for compounds that improve membrane protein/stability rigidity.
   Tracy Mitchell and Heedeok Hong
Expression is a major roadblock in the study of membrane proteins

A study from the Cross and Nakamoto labs:

- 276 membrane proteins tested for expression

- Only 20 were well expressed and in membrane

- Only 2 were > 150 residues.

Many steps where membrane protein expression could fail

Solution can not be predicted. We therefore take a genetic approach.
A double selection system

Selectable marker if membrane protein expresses

pMemKan

pMemDfr
The Method Works

No Drug, + Induction

+Drug, No Induction

+Drug, + Induction

Well Expressed

Poorly Expressed
Methods for Improving Expression

Genomic Mutants

Overexpress Genes

Chemical Genetics

Select for MP Expression

Identify Genes / Proteins

Improve Understanding

Develop Strains
Mutants Selected for Improved Expression of TB Protein Rv1337
Testing Expression of a Variety of Targets in Mutant Selected for Improved Expression of TB Protein Rv1337
Our Goal is to Generate A Library of Strains that Improve Expression of Different Membrane Proteins

TB Rv0985
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Crystallization Methods

Detergent

Lipid Cubic Phase

Bicelle
Phase Diagram DMPC/DHPC

PC Mixtures

Radius decreases with decreased c_{lp}

T (°C)

MLV

Lamella

Bicelle

Fig 9(a)

0.0025 0.01 0.05 0.1 0.15 0.25

C_{lp}, (g/mL)

gel

liquid

Mu-Ping Nieh, Charles J. Glinka, Susan Krueger, R. Scott Prosser, John Katsaras  Biophys. J. vol 82. , 2002
“Lamellar” Phase Crystals

- P2₁
- DTPC/Chapso 2.1Å (RT)
- DMPC/Chapso 1.8Å (37°C)

“Bicelle” Phase Crystals

- C222₁
- DMPC/Chapso (RT) 2.2Å
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SAM domains are small polymerization modules

Kim, Bowie JU, *EMBO J.*, 20, 4173-82 (2001)
Polymer

Make mutants that weaken the interface, increasing solubility

Grow crystals... Weakened polymer interface reforms in the crystal.

Strategy for Obtaining Crystals of Insoluble, Heterogenous SAM Polymers
A Special, Crystallizable Mutant of TEL-SAM

WT TEL-SAM

V80E TEL-SAM

High pH

Low pH

TEL-SAM Structure
7/8 Initially tested TEL fusion proteins crystallized

Teltel-Gene V  Teltel-T4 (WT)
Teltel-T4 (DM)  Teltel
Teltel-GST  Teltel-CoaE
Teltel-NuG2
Crystals of TEL fusions to proteins of unknown structures that refused to crystallize separately (1500 trials)

C1-AGP-hRING2

CF1-ZBD
Crystals of two different TEL-Lysozyme Fusions Reveal Similar TEL-SAM Packing and Flexibility in How Fusion Can Be Arranged
Problem: Not So Hard to Get Crystals Maybe, but Quality Not So Great….Yet

Often Crystallize as Thin Needles that are hard to make thicker…. 
Some Open Questions:

Can we produce better/fatter crystals more routinely?

Will the method work for membrane proteins? RNA?
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Major Problems with Membrane Protein Crystallization

**Problem 1:** Many membrane proteins are unstable in detergent

**Problem 2:** Often get membrane protein crystals, but they diffract poorly, possibly due to protein flexibility

**Possible solution:** Find compounds that bind to the folded protein and thereby stabilize it.
Any ligand that binds to the folded state will stabilize the folded protein
Compound screening using rapid stability assays

United States Patent [19]
Bowie et al.

[54] SCREENING METHOD FOR IDENTIFYING LIGANDS FOR TARGET PROTEINS


[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,585,277.

[21] Appl. No.: 263,923
[22] Filed: Jun. 21, 1994
Chemical screening methods to identify ligands that promote protein stability, protein crystallization, and structure determination

Masoud Vedadi*, Frank H. Niesen†, Abdellah Allali-Hassani*, Oleg Y. Fedorov†, Patrick J. Finerty, Jr.*, Gregory A. Wasney*, Ron Yeung*, Cheryl Arrowsmith*, Linda J. Ball†, Helena Berglund‡, Raymond Hui*, Brian D. Marsden†, Pär Nordlund‡, Michael Sundstrom†, Johan Weigelt‡, and Aled M. Edwards*§
Spectroscopic methods for rapidly measuring unfolding in soluble proteins are problematic for membrane proteins

Environment sensitive fluorescent dyes:
Detect hydrophobic surface exposure. Membrane protein already have a lot of exposed hydrophobic surface and dyes will partition into micelles.

Light scattering (aggregation):
Generally requires large quantities of protein. Thermal unfolding irreversible and not clear whether there is a direct correlation with stability.
Preliminary Test of Steric Wedge Method

Stable TM Dimer

Diluted

Concentrated

Biotin

BODIPY

GpA

GpA

Stable TM Dimer

BODIPY
Steric Wedging of Diacylglycerol Kinase Trimer
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- Heedeok Hong

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- Salem Faham

SAM Module Crystallization
- Sehat Nauli
- Salem Faham
- Saman Farr
- Cynthia Lee