Developing Reagents for Membrane Protein Studies

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In collaboration with JCIMPT-TSRI Labs
Detergents Are Necessary for Membrane Protein Sample Preparation

- A solubilized membrane protein must be stable for subsequent characterizations
- Still challenging to prepare stable membrane protein samples
- Limit of useful detergents (OG, NG, Maltosides, C8E4, C12E8, LDAO…)
- Low Resolution structures
Drug Screening Approach to Screen New Detergents?

- Detergents are hard to purify—the purity is critical
- Large amounts of detergents (grams) are required for a single experiment
- No high-throughput screening of a library of detergents
- Many variables need be screened for a single detergent
Can We Design Detergents?

S. Penel et al. *Biochimie* 1998, 80, 543-551
Criteria of A Good Detergent

Protein-detergent complex

- Large integrated protein-detergent complex
- Only hydrophilic protein-protein contacts
- High solvent Content

Ideal detergents

- Non-denaturing
- Stabilize the protein
- Form small protein-detergent complex
- Tight packing
- Facilitate larger area of protein-protein interaction
- Better mimicking membraneous structure?
Strategies / Detergent Classes

1. Tuning the detergent structures
2. Lipid-like phosphodetergents
3. Facial amphiphiles
1. Finely Tuning the Detergent Structures

Detergents with Short Branches

- Smooth wedged-shape monomer
- Pack more hydrophobicity into micelles

Appending short branches
Detergents with Short Branches

Micellar Size (Dynamic Light Scattering)

- Size is not changed by adding one carbon to the branch
-Appending two-carbon or longer branch alters the micellar properties
Protein Stability in Branched Detergents

**ST MsbA** (Andrew Ward/Chang lab)

- ATPase activity / mmolATP min⁻¹ mgMsbA⁻¹

**Connexin26** (Kent Baker/Yeager and Stevens Labs)

- Normalized Fluorescence

**β-2-adrenergic receptor**

(Chris Roth and Michael Hanson/Stevens Lab)
Tuning Polar Heads and Hydrophobic Tails

Various polar groups (Glu, Mal, Fos…)

Dimeric detergents

“Tripod” detergents
2. Lipid-Like Phospho-detergents

- Glycerol spacer group
- Two alkyl chains

Phospholipid
Phosphocholine detergent
Single-chain

a spacer group to mimic the glycerol motif in lipids

Double-chain

short branches to mimic the dialkyl chain structure of lipids while maintaining their solubility
Pre-screen Detergents for OmpX Refolding by SDS-PAGE

Fos-choline detergents perform the best in refolding OmpX
Single-chain Fos-detergents afforded almost complete refolding of OmpX, much more effective than double-chain analogues
Micro-Scale NMR Analysis

A  DHPC
B  138-Fos
C  179-Fos
D  TPC
E  34-Fos
F  185-Fos

Reto Horst/Wüthrich Lab
3. Facial Amphiphiles
Better Membrane Mimics?

Head-to-tail detergents

Facial amphiphiles
Design Steroid-based Facial Amphiphiles

Resembles conventional head-to-tail detergents

Weak facial amphiphilicity

polar head groups

CHAPS

CHAPSO
Design Steroid-based Facial Amphiphiles

Resembles conventional head-to-tail detergents

Weak facial amphiphilicity

Carboxylate removed to leave a short flexible alkyl chain

Polar groups attached to one side
Proposed Model

Length can be adjusted

Low CMC (0.01%, 0.1 mM)

Twice mass of DDM

A relatively flat and large hydrophobic surface

13.4 Å

14.0 Å
Facial Amphiphiles Stabilize Membrane Proteins

ATPase activity of MsbA at rt

Maltose-cholane

β-UDM

Detergent binding number: 37 cholane/MsbA
219 UDM/MsbA
Terminal OH or Other Polar Groups Induce Small Micelles

![Graph showing the relationship between concentration (wt/v) and mean hydrodynamic radius (R_h) in nanometers (nm).](image)

- **Fos**:
  - Estimated AN: >30
  - RH (nm): 6-10

- **Mal**
  - Estimated AN: 20-30
  - RH (nm): 10-14

- **OH**
  - Estimated AN: 6-10
  - RH (nm): 5-10

- **CHAPS**: 14-38 (1.5-2.3 nm)

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**Estimated AN**

- >30
- 20-30
- 10-14
- 6-10
- 5-10
Protein Stability in facial Amphiphiles

bR in Sodium cholate
pH 7.4 PBS buffer, RT

b3 in 231-chol
UV-vis spectra
reduced-oxidized
10/18/2007
21 days after exchange
09/27/2007

Cytochrome ba3 oxidase

Mitch Luna/Stout and Fee Labs
# Crystallization Trials with Facial Amphiphiles

<table>
<thead>
<tr>
<th></th>
<th>Exchanged from</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td>MsbA</td>
<td>UDM</td>
<td>No crystals</td>
</tr>
<tr>
<td>Cytochrome ba3 oxidase</td>
<td>DDM</td>
<td>Crystallized</td>
</tr>
<tr>
<td>Cytochrome ba3 oxidase</td>
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Cytochrome $ba_3$ oxidase

similar to one of the crystal forms of $ba3$ in NG

Mitch Luna/Stout and Fee Labs
Summary

- Synthesized over 250 new amphiphilic molecules (> 99% purity by HPLC)
- Diversified structures have been made
- The branched detergents can have immediate application in 3-D crystallization
- Developed useful phospho-detergents for the NMR study of membrane proteins
- Steroid-based facial amphiphiles represent a new design of detergents, and they have many appealing properties comparing with the conventional head-to-tail detergents
- The facial amphiphiles impart substantial increased protein stability
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