Detergent Compatibility Assay:
A Simple Method for Exploring Detergent Space

James M. Vergis and Michael C. Wiener
Department of Molecular Physiology and Biological Physics, University of Virginia Health System, Charlottesville, VA 22908-0736

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ABSTRACT: Structural studies on membrane proteins by x-ray crystallography and solution NMR spectroscopy are performed on purified protein solubilized in a particular detergent, forming a protein-detergent complex (PDC). The detergent in the PDC tries to mimic the protein's native, lipid-rich environment by maintaining the solubility and fold of the protein in an aqueous environment. Therefore the same protein in different detergents can possess vastly different behaviors, and in many cases the proper detergent may be as essential as the proper protein construct to the success of obtaining a membrane protein structure. Despite the huge importance of finding a suitable detergent for a particular membrane protein, researchers tend to follow lore first and routinely investigate "traditional detergents" such as octyl-β-D-glucopyranoside (OG), dodecyl-β-D-maltopyranoside (DDM), and octyl tetraethylene glycol ether (C8E4). While these detergents have been successfully used in membrane protein experiments, some membrane proteins are not compatible in these "traditional detergents" or fail to yield well-diffracting crystals. Furthermore, there are now a great number of commercially available detergents with varying chemical properties that may be very useful to membrane protein studies and crystallization.

In order to better explore detergent-space, we are developing a simple, robust, and rapid assay to assess the stability of membrane proteins in a large panel of commercially available detergents. The basic protocol is as follows: 1) bind purified protein to an affinity resin (e.g. IMAC, Strep-Tactin, Glutathione, immobilized-substrate, etc.); 2) aliquot resin into an SBS-format filter plate; 3) perform on-resin detergent exchange into a new detergent; 4) elute protein from resin in the new detergent; and 5) analyze eluted protein on SDS-PAGE and high-performance size exclusion chromatography (HPSEC). Compared to other detergent compatibility assays, this method has the advantages of being a true detergent exchange (i.e. not a dilution into the new detergent), screening a large set of detergents, utilizing current protein constructs (as long as they make use of an affinity purification step), and being performed with equipment available in most laboratories. This new method allows us to consider novel detergents for stubborn membrane proteins (i.e. those that are incompatible and/or unstable in the small, traditional set of detergents and thus unamenable for structural studies).

CURRENT METHODS:

- Abs_{320nm}/Abs_{280nm} ratio [1]
- HPSEC profile [1, 2]
- Ultracentrifugation + SDS-PAGE [3]

WHY IS A NEW METHOD NEEDED?:

- False positives highly probable when a protein-detergent complex (PDC) is concentrated since detergent may be concentrated along with the protein
- Depending on number of detergents tested, large amounts of protein may be required
- Current methods can not easily test large numbers of detergents
- HPSEC analysis is time-expensive if every possible detergent is evaluated

OBJECTIVES:

- Develop a rapid, simple, high-throughput assay/kit for screening membrane protein stability
- Utilize small amounts of protein and detergent for this screen
- Investigate stability in a large panel of detergents

OUR METHOD OUTLINE:

1. Purify protein in a stable detergent
2. Bind protein to affinity resin (*e.g.* IMAC, Strep-Tactin, Glutathione, immobilized-substrate, etc.)
3. Wash resin with 20 column volumes of new detergent wash buffer
4. Elute protein in new detergent elution buffer
5. Run protein gel of elutions
6. HPSEC of positive hits from protein gel

METHOD NOTES:

- Based on "old-school" method for detergent exchange (i.e. bind protein to resin and wash extensively with new detergent)
- Affinity resin with bound protein is aliquoted into SBS format filter plate
- Each well is washed with one of 94 commercially available detergents, detergent free buffer (- control), or current detergent buffer (+ control)
- Protein in compatible detergents will be eluted from resin while protein in incompatible detergents will precipitate on the resin and thus be retained
- Elutions are run on a 96-well E-PAGE™ protein gel to minimize HPSEC runs
- The detergent exchange and E-PAGE™ protein gel visualization can be completed in about **1.5hr** and each HPSEC run takes **6min** when the same mobile phase is utilized
  - Same mobile phase has been shown to yield useful information on protein stability [2]
- HPSEC chromatogram profile is final test for detergent "goodness"
- Depending on the HPLC detection method for HPSEC, the total amount of purified protein required varies from 100µg to 5mg
  - UV/VIS detector = 1-5mg protein
  - Fluorescence detector = 100-200µg

MATERIALS USED:

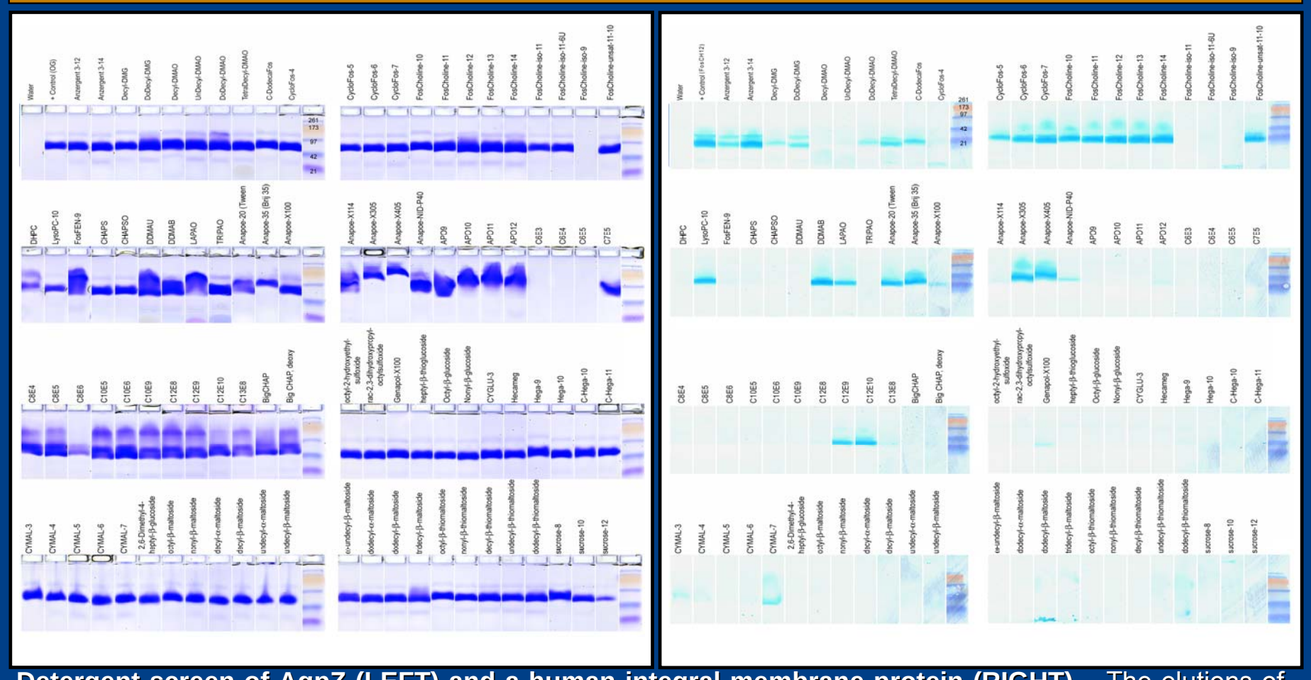
- 384 well 0.45µm PVDF filter plate
- Multi-channel pipette or fluid handling robot
- Vacuum manifold or microplate centrifuge
- E-PAGE™ and iBLOT™ systems
- Superdex™ 200 5/150 gel filtration column

THE DETERGENTS:

Detergent	Num_{struct}
ANZERGENT® 3-12	*12*
ANZERGENT® 3-14	
n-Decyl-N,N-dimethylglycine	
n-Dodecyl-N,N-dimethylglycine	
n-Decyl-N,N-dimethylamine-N-oxide	*10*
n-Undecyl-n,n,-dimethylamine-oxide	
n-Dodecyl-N,N-dimethylamine-N-oxide	*108*
n-Tetradecyl-N,N-dimethylamine-N-oxide	
C-DODECAFOS	
CYCLOFOS-4	
CYCLOFOS-5	
CYCLOFOS-6	
CYCLOFOS-7	
FOS-CHOLINE®-10	
FOS-CHOLINE®-11	
FOS-CHOLINE®-12	*32*
FOS-CHOLINE®-13	
FOS-CHOLINE®-14	*1*
FOS-CHOLINE®-ISO-11	
FOS-CHOLINE®-ISO-11-6U	
FOS-CHOLINE®-ISO-9	
FOS-CHOLINE®-UNSAT-11-10	
DHPC	***6***
LysoPC-10	
FOSFEN-9	
CHAPS	*1*
CHAPSO	*13*
DDMAU	
DDMAB	
LAPAO	*2*
TRIPAO	
ANAPOE®-20 (Tween 20)	
ANAPOE®-35 (Brij 35)	
ANAPOE®-X-100	2
ANAPOE®-X-114	
ANAPOE®-X-305	
ANAPOE®-X-405	2
ANAPOE-NID-P40	
APO9	
APO10	1
APO11	
APO12	
C6E3	
C6E4	
C6E5	
C7E5	
C8E4	52
C8E5	1
C8E6	
C10E5	5
C10E6	1
ANAPOE®-C10E9	
C12E8	
ANAPOE®-C12E9 (Thesit)	19
ANAPOE®-C12E10 (Genapol C-100)	
ANAPOE®-C13E8	19
Big CHAP	
Big CHAP, deoxy	
Octyl-2-hydroxyethyl-sulfoxide	16
Rac-2,3-dihydroxypropyloctylsulfoxide	
Genapol X-100	
n-Heptyl-β-D-thioglucopyranoside	3
n-Octyl-β-D-glucopyranoside	130
n-Nonyl-β-D-glucopyranoside	19
CYGLU®-3	
ANAMEG®-7 (HECAMEG)	
Hega-9	
Hega-10	2
C-Hega-10	
C-Hega-11	
CYMAL®-3	1
CYMAL®-4	1
CYMAL®-5	7
CYMAL®-6	3
CYMAL®-7	
2,6-Dimethyl-4-heptyl-β-D-maltoside	
n-Octyl-β-D-maltopyranoside	2
n-Nonyl-β-D-maltopyranoside	2
n-Decyl-α-D-maltopyranoside	
n-Decyl-β-D-maltopyranoside	56
n-Undecyl-α-D-maltopyranoside	
n-Undecyl-β-D-maltopyranoside	9
ω-Undecylenyl-β-D-maltopyranoside	
n-Dodecyl-α-D-maltopyranoside	1
n-Dodecyl-β-D-maltopyranoside	77
n-Tridecyl-β-D-maltopyranoside	1
n-Octyl-β-D-thiomaltopyranoside	
n-Nonyl-β-D-thiomaltopyranoside	1
n-Decyl-β-D-thiomaltopyranoside	
n-Undecyl-β-D-thiomaltopyranoside	
n-Dodecyl-β-D-thiomaltopyranoside	1
Sucrose8	
Sucrose10	
Sucrose12	

Italicized detergents are zwitterionic, non-italicized detergents are non-ionic. WHITE surfactants are available from Anatrace, GREEN from Avanti Polar Lipids, PURPLE from Calbiochem, and RED from Bachem. The number of non-unique structures obtained using each detergent is given next to each detergent (adapted from Membrane Protein Databank) [4]. All 94 detergents are soluble in water and have CMC values between 0.03mM and 39mM.

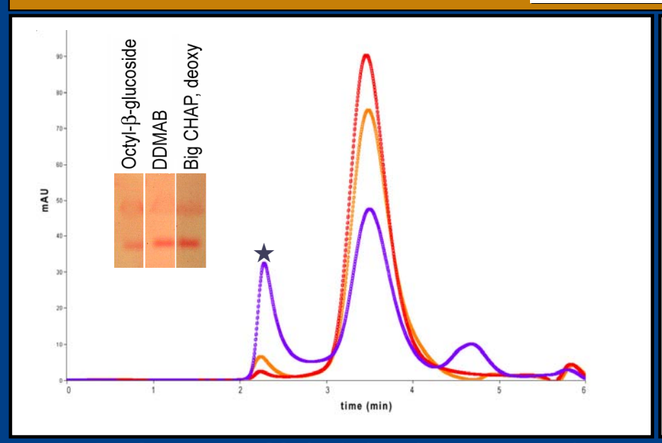
DETERGENT EXCHANGE RESULTS



Detergent screen of AqpZ (LEFT) and a human integral membrane protein (RIGHT) – The elutions of AqpZ and a human IMP after on-resin exchange into 94 different detergents using 10µl Co-TALON™ resin per detergent are shown above . Samples from each of the 96 elutions were run on a 96 well 6% E-PAGE™ gel and visualized with Coomassie staining (LEFT) or blotting to a nitrocellulose membrane and staining with MemCode™ (RIGHT). For AqpZ, only four of the 94 detergents appear to be completely incompatible (signified by the absence of a protein band) and a few other detergents appear to have limited compatibility (signified by a less intense protein band). However, for the human IMP, the stability of this protein is far more dependent on the detergent with zwitterionic detergents more favored than non-ionic detergents. Furthermore, the human IMP is not stable in any of the "traditional detergents".

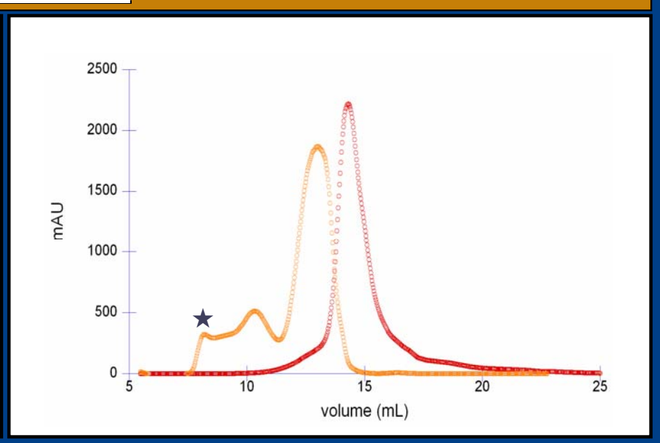
N.B. The differences in protein migration for AqpZ are due to overloading the gel and use of the incorrect power supply/base for the E-PAGE™ gel.

HPSEC RESULTS



HPSEC of AqpZ – 20µl of the detergent exchange elution was injected onto a Superdex™ 200 5/150 gel filtration column with n-octyl-β-D-glucopyranoside (OG) in the mobile phase. The three samples shown above are AqpZ exchanged in OG, DDMAB, and Big CHAP, deoxy. The void-volume for the column is indicated by the star (★). From the chromatograms above, DDMAB seems to be almost as good of a detergent as OG due to the small void volume peak. However, AqpZ exchanged into Big CHAP, deoxy produces a large void volume peak suggesting that this detergent may not be suitable.

(The inset of the chromatogram is from a different AqpZ exchange showing that when the E-PAGE™ gel is not overloaded and the proper gel base is used, these three detergents look similar on a Ponceau S stained nitrocellulose membrane)



HPSEC of a human IMP – a human IMP purified in its original detergent FosCholine-12 and partially purified in $C_{12}E_9$ was injected onto a Superdex™ 200HR 10/30 column with the respective detergent in the mobile phase. The void-volume for the column is indicated by the star (★). FosCholine-12 produces a nice HPSEC profile with no-void peak. The $C_{12}E_9$ chromatogram has a symmetrical main peak but also a void peak and a minor peak (partially due to impurities). Based on this chromatogram, $C_{12}E_9$ seems like a good detergent.

DISCUSSION/CONCLUSIONS:

- Preliminary results with the two proteins presented here show that this simple method works
  - Previous false positives with the human IMP using the Abs_{320nm}/Abs_{280nm} method were negatives using this assay
- Running the gel alone is not sufficient to assess the "goodness" of a particular detergent; an HPSEC step is required
- Despite HPSEC being the "slow step" for this method, eliminating negative samples by 1st running the gel, using a small volume column, and using a constant mobile phase for HPSEC are large timesavers
- Once the best detergents are found, repeating the HPSEC with those detergents in the mobile phase is recommended and when used in conjunction with Refractive Index and Static Light Scattering, the masses of bound detergent, protein, and the PDC can be estimated

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