Supplementary Information for

Combine and Conquer: Surfactants, solvents, and chaotropes for robust mass spectrometry based analyses of membrane proteins

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Supplementary information contains:

1. Methods
2. Table of characteristics of each additive
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Supplementary Methods

Cell Culture

Mouse embryonic fibroblasts (STO cell line) were maintained in Dulbecco’s Modified Eagles Media (DMEM), supplemented with 100 units/mL Penn/Strep, 1X L-Glutamine, 1X Non-essential amino acids, and 15% fetal bovine serum (FBS). All media components were obtained from GIBCO and were filter sterilized (0.2 µm) prior to use. Cells were routinely passaged with 0.05% trypsin/EDTA when 80% confluent and seeded at a cell density of 2x10^6/10 cm plate. Cell count and viability were obtained using a Biorad TC10 Automated Cell Counter.

Cell Lysis, Membrane Protein Preparation, and Protein Digestion

Approximately 1x10^8 cells were washed three times with Phosphate Buffered Saline (PBS) w/ 0.1% FBS and then detached using Enzyme Free Cell Dissociation Solution (Millipore) for 30 min at room temperature. The cells were collected by centrifugation at 500 x g at 4°C for 5 min then resuspended in hypotonic lysis buffer (10 mM Tris pH 7.5 (Quality Biological), 0.5mM MgCl\textsubscript{2} (Quality Biological)) and placed on ice for 10 minutes to swell. The cells were homogenized using a gentleMACS™ Dissociator (Miltenyi Biotec) and the homogenate was centrifuged at 800 x g at 4°C for 10 min to remove nuclei and debris. The supernatant was combined with an equal volume of membrane prep buffer (280 mM Sucrose, 50 mM MES, 450 mM NaCl, 10 mM MgCl\textsubscript{2}) and divided evenly (±.001 g) into six Ultra-clear ultracentrifuge tubes (Beckman) and centrifuged overnight at 210,000 x g at 4°C to pellet the mixed membrane fraction. This preparation was repeated such that twelve sets of six tubes were obtained, wherein all tubes within a single set contained an equivalent amount of protein to allow for comparison of conditions across the tubes within a single set. Each comparison set was performed in duplicate.
Mass Spectrometry

Samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) on an LTQ linear ion trap (Thermo) as well equipped with a Surveyor nano HPLC (Thermo). Peptides were resuspended in 120 µL mobile phase A (5% acetonitrile, 0.1% formic acid) and 2 µL were loaded onto an IntegraFrit ProteoPep™ II column (75 µm ID, 15 cm, C18, 300Å, 5µm particle size) (New Objective; Phase I) or hand-packed Magic C18-AQ (Michrom) column (75um ID, 10cm, 200Å, 3µm particle size; Phase II) and separated using a solvent gradient of mobile phase A and mobile phase B (95% acetonitrile, 0.1% formic acid) at 250 nL/min. The gradient program was: 0–5 min, 100% mobile phase A; 5–50 min, linear gradient to 10% mobile phase B; 50–180 min, linear gradient to 75% mobile phase B; 180-190 min, linear gradient to 100% mobile phase B; 190-195 min, hold at 100% mobile phase B; 195-210 min, back to initial conditions (100% mobile phase A); 210-240 min, re-equilibrate at 100% mobile phase A before the next injection. For each MS scan, the six most intense ions were selected for MS/MS.

Data Analysis

MS data were searched against the mouse UniProt database (downloaded 5/16/2012) using Sorcerer 2™-SEQUEST® (Sage-N Research, Milpitas, CA), using default peak list extraction parameters and post-search analysis performed with the Trans-Proteome Pipeline, implementing PeptideProphet13 and ProteinProphet14 algorithms as described in the supplement. Database search parameters were as follows: full enzyme digest using trypsin (after Lys or Arg) with up to two missed cleavages; monoisotopic precursor mass range of 400–4500 amu; carbamidomethylation (Cys) as a static modification and oxidation (Met) allowed as a differential modification; peptide mass tolerance was set to 1.5 amu; fragment mass type as average. All data from technical replicate analyses of each sample were merged into a single ProteinProphet result file. Default parameters for PeptideProphet and ProteinProphet were used
and consistent among all samples. The ProteinProphet interact-prot.xml result files were imported into ProteinCenter (Thermo) and filtered to contain only proteins corresponding to a false discovery rate (FDR) $\leq 1.5\%$ (FDR calculated by ProteinProphet). Within ProteinCenter, redundancy was removed by grouping indistinguishable proteins to collapse any proteins identified by the same peptide sequence into a single entry. The percent sequence coverage, number of predicted transmembrane domains as calculated by TMAP$^{15}$, and grand average of hydropathy (GRAVY)$^{16}$ scores were provided by ProteinCenter.
Table S-1. Summary of relevant properties of additives tested.

| Additive                        | Cost per sample* | Type                        |
|---------------------------------|------------------|-----------------------------|
| **Organic Solvent**             |                  |                             |
| Acetonitrile                    | $0.02            | Dipole moment: 3.92         |
|                                 |                  | polarity index: 5.8; polar aprotic |
|                                 |                  | δH Hydrogen bonding: 6.1    |
| Methanol                        | $0.01            | Dipole moment: 1.70         |
|                                 |                  | polarity index: 5.1, polar protic |
|                                 |                  | δH Hydrogen bonding: 22.3   |
| **Chaotrophic Agent**           |                  |                             |
| Guanidine-HCl                   | $0.03            |                             |
| Urea                            | $0.01            |                             |
| **Surfactant**                  |                  |                             |
| (Vendor, Catalog #)             |                  |                             |
| Invitrosol                      |                  | Zwitterionic                |
| Life Technologies MS10007       | $2.08            | Dimethylbenzylammonium propane sulfonate |
| PPS Silent Surfactant           |                  | Zwitterionic Acid Labile    |
| Agilent 400500                  | $12.80           | 3-[3-(1,1-Bishexyloxyethyl) pyridin-1-yl] propane-1-sulfonate |
| Progenta AALS I                 |                  | Anionic Acid Labile         |
| Protea Biosciences ALS-100      | $10.72           | sodium 2,2-dihexoxypropyl sulfate |
| Progenta AALS II                |                  | Anionic Acid Labile         |
| Protea Biosciences ALS-110      | $12.50           | sodium 2,2-diheptoxypropyl sulfate |
| Additive                          | Company             | Formula                                                                 | Charge  | Type       | Price  |
|----------------------------------|---------------------|--------------------------------------------------------------------------|---------|------------|--------|
| Progenta CALS I                  | Protea Biosciences  | [Chemical structure of 2,2-dihexoxypropyl ammonium bromide]              | Cationic | Acid Labile | $12.50 |
| ProteaseMAX                      | Promega             | (sodium 3-([(1-(furan-2-yl)undecyloxy)carbonylamino]propane-1-sulfonate) | Anionic  | Acid Labile | $9.04  |
| RapiGest                         | Waters              | (sodium 3-[(2-methyl-2-undecyl-1,3-dioxolan-4-yl)methoxy]-1-propanesulfonate) | Anionic  | Acid Labile | $11.37 |

* added cost per sample for lowest concentration of additive tested
Figure S-1. Summary of results from Phase I (individual additives). A) The total number of TM proteins identified, as distributed by the number of predicted TM domains, and B) the average sequence coverage, as distributed by the length of the protein, are provided.
Figure S-2. Summary of results from Phase II (additive combinations) made possible by PeptideEclipse. A) The total number of TM proteins identified, as distributed by the number of predicted TM domains, B) the average sequence coverage of TM proteins and C) TM domains, as distributed by the number of TM domains, are provided.
Figure S-3. Determined CMC values (top) and raw fluorescence measurements for each surfactant (bottom).

| Surfactant                  | AALS | AALS1 | CALS | CALS1 | PPS Silica Surfactant | ProteaseMax | Terpentin | Flaggert |
|-----------------------------|------|-------|------|-------|------------------------|-------------|-----------|----------|
| 100mM Ammonium Sulfonate    | 25°C | >0.1  | 0.002| 0.023 | >0.1                   | >0.1        | >0.1      | 0.064    | 0.064    |
| 100mM Ammonium Sulfonate, LM Guanidine | 25°C | 0.017| 0.006| 0.080 | >0.1                   | >0.1        | >0.1      | 0.019    | 0.019    |
| 100mM Ammonium Sulfonate, LM Guanidine, 20% Acetanilide | 25°C | 0.016| 0.008| 0.009 | >0.1                   | >0.1        | >0.1      | 0.015    | 0.015    |
| 100mM Ammonium Sulfonate    | 37°C | >0.1  | 0.001| 0.025 | >0.1                   | >0.1        | >0.1      | 0.059    | 0.059    |
| 100mM Ammonium Sulfonate, LM Guanidine | 37°C | 0.018| 0.006| 0.009 | >0.1                   | >0.1        | >0.1      | 0.029    | 0.029    |
| 100mM Ammonium Sulfonate, LM Guanidine, 20% Acetanilide | 37°C | 0.018| 0.009| 0.009 | >0.1                   | >0.1        | >0.1      | 0.016    | 0.018    | 0.162