Proteomics Exercise:

Comparative proteomic profiling of Saponin vs Streptolysisn-O treated Plasmodium falciparum infected erythrocyte cytosol

Introduction:

The objective of this exercise is to identify the proteins present in parasitophorous vacuoles (PV) of Plasmodium falciparum infected erythrocytes by mass spectrometry. This will be achieved by comparative mass spectrometry analysis of fractions obtained from two different membrane rupture methods. The first method essentially involves treatment of infected erythrocytes with Streptolysin O (SLO) which results in the release of the erythrocyte cytosol that can be collected by centrifugation. Whereas the second method employs saponin mediated disintegration of both the erythrocyte membrane and the parasitophorous vacuolar membrane of an infected erythrocyte. The cytosol and PV fractions can then be separated from intact parasites by centrifugation and later processed for mass spectrometry analysis.

Permeabilization of iRBC with Streptolysin O

Sigma provides SLO as a lyophilized powder with a total activity of 25,000 units.

- Dissolve SLO in 2.25 mL PBS.
- Make aliquots of 90 µL and freeze them at -20° C.
- Thaw 90 µl SLO and add 10 µl of 1 M DTT (in water or PBS) - incubating 15 min at RT yields activated SLO (SLO*).
- Use 300 units SLO* for 1 x 10^8 iRBC.
- 10^8 iRBC are dissolved in 70 µL PBS
- Add 30 µL SLO*.
- Incubate 10 min at RT.

Permeabilization of iRBC with Saponin

- Incubate iRBC (in aliquots of 2 x 108 cells) in 200 µL of 0.1% saponin in PBS pH 7.2 on ice for 5 min.
- Centrifuge the samples at 2,500 x g for 5 min
- Remove supernatant (containing host cytosol and vacuolar contents)

In-Solution Digestion Protocol

Chemical Reagents:

- **Trypsin**: Promega sequencing grade modified trypsin (V511A), 20 ug lyophilized powder, can be stored in solution for several weeks at -20°C.
- **ddH2O**: MilliQ water or HPLC water.

Solutions:

• 200 mM Dithiothreitol (DTT) in 100 mM NH_4HCO_3

• 50 mM NH_4HCO_3:

• 1M iodoacetamide in 100 mM NH_4HCO_3

• Buffer A: 50% Acetonitrile (ACN) and 0.1% formic acid in ddH_2O

• Buffer B: 0.1% formic acid in ddH_2O

1. *Sample Preparation*

Bring samples up to 100 ul in 50mM NH_4HCO_3. It is highly recommended that the samples be free of any detergents before digestion (most detergents can be removed by protein precipitation and/or ion exchange chromatography).

2. *Disulfide Reduction*

Reduce the sample by adding 5 ul of DTT stock to the 100 ul sample, vortex the sample, spin it down with a quick burst in the centrifuge and let the sample reduce at room temp for 45 min-1hr.

3. *Sulfhydryl Alkylation*

Alkylate the sample by adding 4 ul of the iodoacetamide stock to the sample and vortex followed by a quick spin to get the sample to the bottom of the tube. Alkylate for 45 min-1hr at room temp.

4. *Trypsin Digest*

1. The ratio of trypsin to sample should between 1:50 to1:20 – 1 mg of trypsin for every 50 to 20 mg of protein.
2. Gently vortex and spin the sample. To allow complete digestion place the rack in the 37°C incubator overnight, or for at least 18 hrs. Gentle or periodic mixing is optional.

Data acquisition on LCMS/MS

(Orbitrap Velos Pro equipped with nano-LC Easy nLC-1000--- Thermofisher Scientific)

The SLO and Saponin treated and trypsin digested samples will be injected into the LCMS/MS system. The loaded peptide mixture will be separated on a C-18 column using increasing concentration of acetonitrile and directly sprayed into the MS. The precursors will be analyzed using Data Dependant mode in Orbitrap with a resolution of 60000 and will be fragmented in Ion trap using Collision Induced Dissociation (CID). The data will be acquired for top 20 precursors.

Data Analysis

The data acquired on LCMS/MS will be searched for proteins identifications using Proteome Discoverer 1.4 (Thermofisher Scientific) software.