This procedure precipitates protein from the polar fraction resulting from CH_3CN-H_2O-$CHCl_3$ (2:1.5:1) extraction. It is an additional "clean-up" step to enhance NMR analysis.

Tissue polar extract Extraction

(03/19/15)

1. Thaw one lyophilized NMR aliquot of the polar fraction resulting from CH_3CN-H_2O-$CHCl_3$ extraction of tissue sample on ice.
2. Dissolve the dry sample in 100 µl of ice-cold nanopure water and vortex to mix.
3. Add 400 µl ice cold 100% pure ACETONE (final conc is 80% ACETONE), which is critical for efficient protein precipitation. Acetone volume can be adjusted according to how much sample you have.
4. Vortex to mix.
5. Put samples into -80°C freezer for 30 minutes to facilitate protein precipitation.
6. Centrifuge samples for 20 minutes @ 14,000 rpm, 4°C.
7. Transfer the supernatant to a 2 ml screw top tube. Save the tip for use in step 10.
8. Extract the protein pellet with 100 µl of 60% acetonitrile (in water). Grind the pellet with a micro-pestle and rinse the pestle with 50 µl of 60% acetonitrile.
9. Repeat step 6.
10. Transfer the supernatant, combining with the supernatant collected in step 7 and using the same pipet tip.
11. Freeze the samples in liquid nitrogen and lyophilize using a liquid nitrogen trap. The transfer of samples to the lyophilizer can and applying vacuum must be very quick, as the acetone solvent thaws much more easily than aqueous samples.
12. Dissolve dry samples in 55 µl of 50% D2O with 50 nmoles DSS **(With EDTA)** per 0.1 mL and vortex to mix.
13. <u>If the extracts have been run via NMR before</u>, Dissolve dry samples in 55 µl of 50% D2O WITHOUT DSS and vortex to mix.
14. Vortex to mix the buffer with the extract and centrifuge for 20 minutes @ 14,000 rpm, 4°C before loading into 1.7 mm NMR tubes to attain a minimum column height of 36 mm.