## Overview and Considerations:

We typically use three methods for protein precipitation, each have advantages and disadvantages. 1) Acetone is reasonably fast and will precipitate free GlcNAc; 2) Methanol is slow, and does not precipitate GlcNAc; 2) Methanol Chloroform is reasonable fast, but does not work well in the presence of free peptide. Your choice will depend on the volume of your sample, the time available, and the presence of free GlcNAc/peptide.

**IMPORTANT:** Organic solvents are not compatible with all of the centrifuge tubes in the laboratory. Check with the manufacturer before using for the first time.

**IMPROTANT:** This technique works best in 15ml conical tubes.

## Reagents:

1. Ice Cold Methanol
2. Microcentrifuge, 4oC
3. Speed-vac

## Protocol:

1. Add 10 volumes of ice cold methanol to your samples (to 100ul of sample add 1ml of methanol);
2. Vortex to mix;
3. Incubate overnight at -20oC;
4. Pellet protein at full-speed in a microfuge at 4oC for 30min;
5. Decant supernatant;
6. Dry samples briefly in a speedvac, ~5-10min.
7. Do NOT overdry…
8. Resuspend samples in sample buffer (I usually put them on the vortex for 5-10min)