Sample Preparation for Mass Spectroscopy using ZipTip$_{C18}$

1. Make solutions.
   Sample preparation solution (2.5% trifluoroacetic acid)
   Combine: 25 µl trifluoroacetic acid
   475 µl nanopure water
   Wetting solution (50% acetonitrile)
   Combine: 500 µl acetonitrile
   500 µl nanopure water
   Equilibration solution (0.1% trifluoroacetic acid)
   Combine: 1 µl trifluoroacetic acid
   999 µl nanopure water
   Wash solution (0.1% trifluoroacetic acid)
   Combine: 1 µl trifluoroacetic acid
   999 µl nanopure water
   Elution solution (50% acetonitrile, 0.1% trifluoroacetic acid)
   Combine: 500 µl acetonitrile
   499 µl nanopure water
   1 µl trifluoroacetic acid

2. Dilute the purified protein in water*, **final amount: 20 µl of a 50 µM** solution. This is a higher concentration than the facility requests, however I have observed that our proteins do not bind well to the ZipTip matrix. Therefore, I overload the tip to compensate and that has produced good Mass Spec data.
   *Maximum binding to the ZipTip$_{C18}$ is achieved in the presence of TFA or other ion-pairing agents. To maximize analyte binding, the **final TFA concentration should be between 0.1% and 1% at a pH of <4.0.**

3. Prewet the ZipTip$_{C18}$ by slowly aspirating 10 µl of Wetting solution into the ZipTip$_{C18}$. Dispense to waste. Repeat.

4. Equilibrate the tip for binding by washing it twice with 10 µl of Equilibration solution.

5. Bind the protein to the ZipTip$_{C18}$ by slowly aspirating and dispensing the sample 10 times.

6. Wash the bound protein three times with Wash solution by slowly aspirating and dispensing to waste.

7. Dispense 10 µl of Elution solution into a clean tube using a standard pipet tip.

8. Carefully, aspirate and dispense the 10 µl of Elution solution through the ZipTip$_{C18}$ five times, **without introducing air into the sample**.

9. Submit for Mass Spec!