**Trypsin In-solution Digestion**

Your protein is in a volume ~20 ul (~ 100 nmole/ ~ 4 ug total protein)

Add 2 ul 100 mM DTT (final 10 mM), incubate 30 min hour at 60 C,

Add 2 ul iodoacetamide (0.15 M in 200 mM Ammonium Bicarbonate). Incubate 20 min at room temperature in the dark

Add 100 ul of 200 mM Ammonium Bicarbonate, and 0.1 ug trypsin (Promega Gold or seq. grade Porcin modified trypsin). Incubate overnight at 37 C

SpeedVac, add 200 ul water and SpeedVac again. Repeat 3 times. Resuspend in 10 ul 0.1% TFA

Prime ZipTip: pipette 10 ul of 100% Acetonytril (0.1% TFA), followed by 10 ul 0.1% TFA (2x) Load sample to Zip Tip by pipetting up and down 10 times.
Wash ZipTip with 0.1% TFA (10 ul, 3 times).
Elute sample to 30 ul 80% Acetonytril, 0.1% TFA

SpeedVac. Resuspend in 0.1% formic acid.

Freshly prepared:
200 mM Ammonium Bicarbonate: 15.8 mg/ml (check pH, should be 8)
0.25M iodoacetamine: 25 mg/ml (dilute in water).

**Chemotrypsin In-solution Digestion**

Reconstitute 100 ug (Sigma, sequencing grade) in 200 ul 1 mM HCl, keep at -20C

Dissolve you protein in 20 ul, 100 mM Tris-HCL (pH 8.0), 10 mM CaCl2.
Add 2 ul 100 mM DTT (final 10 mM), incubate 30 min at 60 C,

Add 2 ul iodoacetamide (0.15 M in 200 mM Ammonium Bicarbonate). Incubate 20 min at room temperature in the dark

Add 1 ul (~1:200 – 1:20 ratio, ug/ug) chemotrypsin. Incubate overnight at room temperature.
Add 2 ul 10% TFA to stop chemotrypsin.
Purify on Zip-tip (or desalt and then Zip-tip).