MALDI protein identification protocol for *NPS* RP HPLC 2D Fractions

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1. To collect the fractions, wear powder free gloves. Wash the gloves with soap and rinse under water. Dry the gloves with paper.

2. Rinse the Eppendorf tubes or 96 well plates with 70% ethanol to minimize keratin contamination.

3. Collect RP HPLC fractions (in a laminar flow hood preferably) and store the fractions at -20°C after collection to reduce protein sticking.

4. Reduce the volume of RP fractions using Speedvac concentrator. The final volume should be 20-30 microliters.

5. Preparation of the protein digests should be made under a laminar flux hood to avoid contaminations.

6. Add 1 M NH_4HCO_3 and 10mM DTT to each fraction. The final concentrations of NH_4HCO_3 and DTT are 100mM and 1mM respectively. The resulting fractions are incubated at 60°C for 10 min in the oven.

7. Add trypsin as an enzyme to substrate ratio of 1:50 and vortex gently.

8. Put the fractions on a shaker at medium speed and incubate them at 37°C for 24 hrs.

9. If you have to store the peptides: Add 2% TFA (v/v) to end the digestion.

10. Store the peptide mixtures at 4°C (short term) or -80°C (long term) until further use.

11. Evaporate the fractions to dryness using Speedvac concentrator and add 3 microliters of water.

12. Equilibrate a C-18 ZipTip with ACN 80% 0.1% TFA.

13. Equilibrate the same ZipTip with H_2O 0.1% TFA.

14. Bind the peptides on the ZipTip: aspirate and dispense the sample 10 times.

15. Aspirate wash solution (H2O 0.1% TFA) into tip and dispense to waste 5 times.

16. Peptide elution: dispense 1 microliter of elution solution (ACN 80% 0.1% TFA) into a clean vial.

17. Aspirate and dispense the sample 10 times through the ZipTip.

18. Dispense the sample on the MALDI plate directly from the ZipTip.

19. Dispense 1 microliter of MALDI matrix (alpha-cyano) on the MALDI plate. Aspirate and dispense 2 times to mix the matrix and the sample.

20. Let the matrix dry 2 hours before MALDI analysis.

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