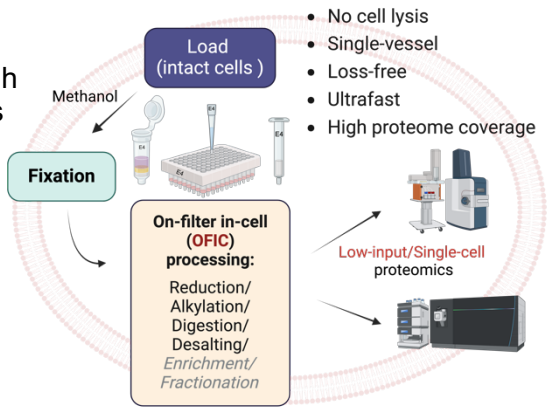
UNIVERSITY OF DELAWARE MASS SPECTROMETRY FACILITY

On-filter in-cell (OFIC) digestion of intact *C. elegans* with E4technology

Summary

This SOP pertains to processing biological samples for high resolution mass spectrometry (MS) based proteomics analysis. The protocol describes digesting proteins directly in the intact C. elegans, without cell lysis and protein extraction, thus it significantly simplifies the entire workflow, and drastically reduces sample loss. This method could be used for low-input, and single-worm/single-cell proteomics.



Note: ready-to-go E4 filter devices are available here.

1. **Worm treatment**
   Rinse live *C. elegans* three times with M9 buffer, then one time with water, to remove *E. coli* food source.
2. **Sample loading**
   Pick worm manually, and transfer directly into E4filters that are pre-filled with 200 µl of pure methanol. Visually inspect worm pick to confirm transfer.
   Note: Estimated capacity for E4tip, < 100 worms, and E4 spin columns, 100-2,000 worms.
3. **Worm fixation**
   Incubate E4filters at room temperature for 15 min. Centrifuge at 1,500 x g for two min, discard flow through. Add 200 µl of methanol, repeat this step one more time.
   Note: Flow through may be collected here for metabolomics analysis.
4. **Reduction and alkylation**
   Add 100 µl of 50 mM triethylammonium bicarbonate (TEAB) and final concentration of 10 mM Tris(2-carboxyethyl)phosphine (TCEP) and 40mM chloroacetamide (CAA), incubate at 45°C for 10 min with gentle shaking.
5. **Wash**
   Add 200 µl of 50 mM TEAB solution, centrifuge at 1,500 x g for two min, discard flow through.
6. **Digestion**
   Add 100 µl 50 mM TEAB, desired enzyme (Trypsin or Trypsin/Lys-C mix) at 1:50 ratio. Incubate at 37°C for 16-18 hours with gentle shaking.
   Note: no cap is required for E4tips.
7. **Acidification and desalting**
   After digestion, add formic acid to final concentration of 1%, centrifuge at 500 x g for 10 min. Add 200 µl 0.5% acetic acid in water, centrifuge at 1,500 x g for 2 min, discard flow through.
   Note: here, E4tips (E4tip ultra) can be transferred to Evosep ONE LC for direct LCMS acquisition.
8. **Elution**
   Transfer E4filters to clean collection tubes, do two sequential elution by adding 200 µl 60% acetonitrile/0.5% acetic acid in water (elution I), and 80% acetonitrile/0.5% acetic acid in water (elution II), centrifuge at 1,500 x g for 2 min to collect elution to the same tube. Dry samples in SpeedVac, and store at -80°C. The peptides are now clean and ready for LCMS analysis.