Development and Application of a Simple Nano-Proteomic Platform (SNaPP) for Effective Analysis of Sub-microgram Proteome Samples

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Overview

• We have successfully applied this platform to a variety of cell types.
• We have developed a Simple Nano-Proteomic Platform (SNaPP) that provides quick, reproducible, semi-automated analysis of sub-microgram protein samples.

Methods

• Samples are prepared by adding 10 µl of 8M urea, 5 mM DTT to 50 mM ammonium bicarbonate, sonicated and incubated at 37°C for 30 min to extract and denature proteins.
• The system includes the following fluidic components: two Agilent Nanoflow 1200 pumps (Agilent Technologies, Santa Clara, CA), one capillary 110 pump (Agilent Technologies, Santa Clara, CA), a six-port injection valve with 50–2000 ng total protein.
• The trypsin-based immobilized enzyme reactor consists of Poroszyme® immobilized trypsin beads (Grand Island, NY) in a 150 µm i.d. capillary. The IMR is infused in a butterfly portfolio heater set at 37°C.
• The bioprocess is 7 min with an optimal flow rate set at 500 nL/min of 5% acetonitrile in 95% 50 mM Tris (pH 8) and 5 mM CaCl₂ at pressure less than 70 bar.
• The digestion column is conditioned with 25 injections of 250 ng humaneraeovirus 16 cellular cysteine to remove excess trypsin peptides from autolysis and passivate non-specific binding sites.
• After online digestion, the peptides are trapped and desalted via on-line SPE and protein identifications measured using a LTQ Orbitrap or Q Exactive mass spectrometer (Thermo Scientific, San Jose, CA) outfitted with a custom, electrospayed ionization (ESI) interface.

Results

System Characterization Proteome Coverage

Figure 1

• Figure 1: Peptide and protein annotations identified using a single pot with diverse methods. Shown is a global FDR. Four are standard deviations from 4 replicate injections. Protein sequences can be downloaded from Databank, www.pnnl.gov.

System Applications

Comparison to “Single Pot” digest

Figure 6

Conclusions

• We have developed a simple, automated, online sample processing platform capable of global proteomic analysis of sub-microgram protein quantities as low as 50 ng.
• Platform characterization shows SNaPP has the required sensitivity (>7000 peptides & 1100 proteins at 200 ng loading) and reproducibility (median CV > 20%) to produce quantitative label-free data from limited samples.
• The platform has been successfully applied to a wide variety of sample types previously unattainable in our laboratory including: blastocysts collected from a single mouse, β-cells and T-cells enriched by FACS.
• Future improvements to the platform include, increasing separation precision and decreasing analytical column diameter to further increase the proteome coverage of the SNaPP platform.

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References


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