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

Paul D. Piehowski[1], Eric L. Huang[1], Daniel J. Orton[1], Ronald J. Moore[1], William B. Chrisler[1], Rosalie K. Chu[1], Kristin E. Burnum-Johnson[1], Xiaofei Sun[2], Sudhansa K. Dey[2], Tao Liu[1], Wei-Jun Qian[1], and Richard D. Smith[1]
[1]Pacific Northwest National Lab, Richland, WA; [2]Cincinnati Children's Hospital Medical Center, Cincinnati, OH

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Overview

- Analyzing only the cell or tissue feature of interest has great promise for increasing the specificity of proteomic measurements by increasing the homogeneity of the sampled cell populations.
- Cell-type-specific samples (created using laser capture micro-dissection or fluorescence activated cell sorting), pose a significant challenge for global proteomics due to their limited protein contents.
- We have developed a Simple Nano-Proteomics Platform (SNaPP) that provides quick, reproducible, semi-automated analysis of sub-microgram protein samples.

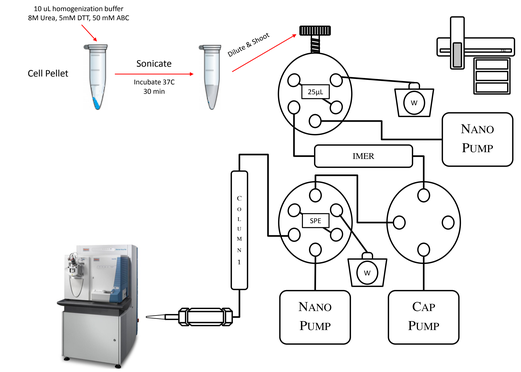
Introduction

- It has been broadly established that efficient and reproducible sample preparation workflows are crucial to successful quantitative comparisons, especially when applying label free methods.[1,2,3]
- Reproducible sample handling becomes increasingly challenging when protein amounts are limited due to decreased volumes and non-specific losses.[4]
- In a standard proteomics experiment, bulk homogenization is applied to generate the required protein quantities. This blends the proteomes of many different cell types and disparate tissue regions resulting in an "average" proteome, diluting and distorting the proteome changes of interest.
- We have developed a simple online platform for semi-automated processing and analysis of nano-scale proteome samples.
- This platform is sensitive, robust, and reproducible for analyzing nano-scale samples (50 – 2000 ng total protein).
- We have successfully applied this platform to a variety of cell-specific, protein-limited sample types created FACS and LCM.

Methods

- Samples are prepared by adding 10 uL of 8M urea, 5 mM DTT in 50 mM ammonium bicarbonate, sonicated and incubated at 37C 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 25 µl sample loop, two six-port valves (VICI Valco, Houston, TX) and a PAL autosampler (Leap Technologies, Carrboro, NC), **FIGURE 1**.
- The analytical column is 40 cm long 75 µm i.d packed in-house with 3 µm diameter C18 bonded particles (Phenomenex, Torrence, CA).
- The trypsin-based immobilized enzyme reactor consists of Poroszyme® immobilized trypsin beads (Grand Island, NY) in a 150 µm i.d. capillary. The IMER is incubated in a butterfly portfolio heater set at 37°C.
- A typical digestion is 75 min at a flow rate is set at 500 nL/min of 5% acetonitrile in 95% 50 mM Tris (pH 8) and 5 mM $CaCl_2$ at pressure less than 70 bar.
- The digestion column is conditioned with 15 injections of 250 ng *Shewanella oneidensis* cell lysate 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 consisting of 4 cm 150 µm i.d. 5-µm Jupiter C18 (Phenomenex, Torrence, CA).
- MS analysis is performed using either a LTQ Orbitrap or Q Exactive mass spectrometer (Thermo Scientific, San Jose, CA) outfitted with a custom, electrospray ionization (ESI) interface.

Figure 1



Results

System Characterization

Proteome Coverage

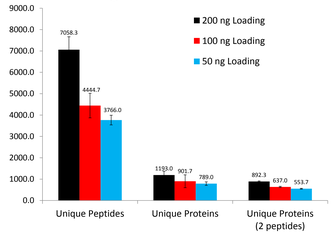


Figure 2. Confident peptide and protein identifications using MSGF search with dynamic M oxidation, filtered < 1% global FDR. Error bars are standard deviations from 3 replicate SNaPP injections. Protein samples are bulk Shewanella Oneidensis whole cell lysate serially diluted to create desired injection volume.

Reproducibility

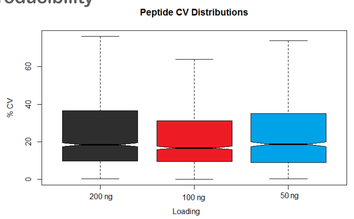


Figure 3. Coefficient of Variance (**CV**) of peptide AUC intensity measurements from 3 replicate injections. Peptides were required to appear across all 3 runs.

Digestion Column Stability

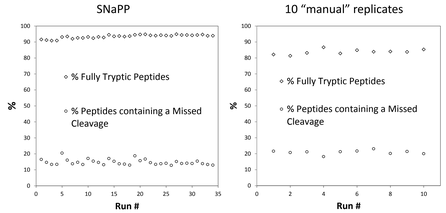


Figure 4. To test the stability of our online digestion, 35 replicates of Shewanella oneidensis cell lysate were ran in serial. Percentage of peptides containing a missed cleavage and percentage fully tryptic peptides were used to monitor digest quality. Injections were 500 ng Shewanella cell lysate.

System Applications

Comparison to "Single Pot" digest

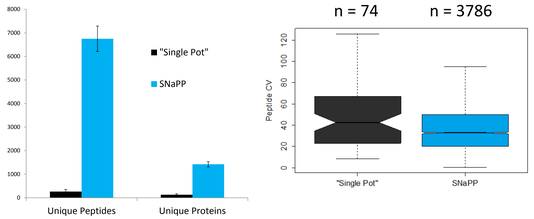


Figure 5. T-Cell samples isolated from mouse plasma by FACS. Samples were split and half were analyzed using a "single pot" in-solution digest method. Estimated protein amount was 500 ng per sample. Biological replicates yielded a median peptide CV of 32%.

T-cells isolated from a Diabetic Mouse Model

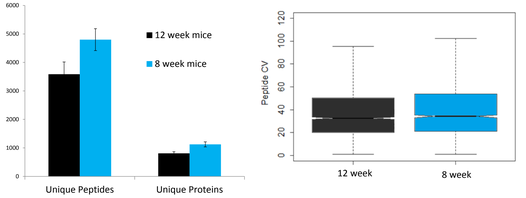


Figure 6. Study of FACS isolated T-cells at 8 and 12 weeks in a diabetic mouse model. **Left** Peptide and Protein identifications **Right** Coefficient of Variance (**CV**) of peptide AUC intensity measurements from 3 replicate injections.

Blastocyst Implantation in Pregnant Mouse Model

Preimplantation Blastocyst Proteins	Percent Sequence Coverage
Developmental pluripotency-associated protein 3	8
Developmental pluripotency-associated protein 5A	71.186
Egg and embryo abundant Peptidylarginine deiminase	78.592
Embryonic alkaline phosphatase	23.629
Factor located in oocytes permitting embryonic development	40.854
Fertilization antigen 1	51.562
Germ cell-specific leucine-rich repeat NTPase	26.788
Germ cell-specific Y-box-binding protein	5
Male-enhanced antigen 1	10.345
Maternal antigen that embryos require	24.764
Membrane-associated progesterone receptor component 1	7.179
Nuclear autoantigenic sperm protein	9.444
Nucleoplasmin-2	65.7
Oocyte astacin	21.149
Oocyte-secreted protein 1	8.911
Oviduct-specific glycoprotein	34.397
Spindlin-1	37.023
Zona pellucida sperm-binding protein 1	24.238
Zona pellucida sperm-binding protein 2	51.753
Zona pellucida sperm-binding protein 3	30.896

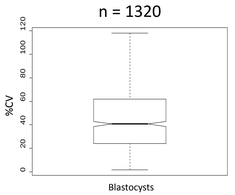


Figure 7. Day 8 blastocysts were isolated from individual model mice prior to implantation. Estimated about 50 ng protein mass for analysis. Were able to quantify over 1300 peptides matching to 400 proteins found in all 3 replicates. Median peptide CV was 40% for biological replicates. Table shows preimplantation blastocyst proteins not identified from in-depth bulk analyses.

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 untenable 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 pressure and decreasing analytical column diameter to further increase the proteome coverage of the SNaPP platform.

Acknowledgements

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CONTACT: Paul Piehowski, Ph.D.
Biological Sciences Division
Pacific Northwest National Laboratory
E-mail: paul.piehowski@pnnl.gov

www.omics.pnl.gov