Identification of 2D Gel Fractionated Intact Proteins Using FT-ICR Mass Spectrometry

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High Throughput "Top-Down" Protein Identification and Characterization

The Kelleher Laboratory at the University of Illinois at Urbana-Champaign has demonstrated use of the NanoMate™ 100 system for the automated analysis of 2D fractionated intact protein samples by nanoelectrospray FT-MS. The NanoMate 100 is an automated sample-handling system coupled with the ESI Chip™ (an array of nano-electrospray nozzles etched in silicon).

A growing alternative to proteomics using trypic digests is the detection and characterization of intact proteins using high resolution Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR MS). One reason for this is the inherent lack of protein information (i.e., sequence coverage) provided by the peptide approach--a "bottom-up" philosophy. While the traditional approach of obtaining protein indentifications is highly automated, the "top-down" strategy is currently more user-intensive. The NanoMate 100 controlled by Tcl scripts in conjunction with a MIDAS data station, expedites and automates this process.

Method

Cells of *Saccharomyces cerevisiae* are lysed using a French press, and cellular debris is removed via centrifugation. The collected 2D fractions are loaded into a 96-well plate of the NanoMate 100 for infusion to an 8.5 T magnet FT-ICR mass spectrometer.

Analysis of intact spectra is accomplished on-the-fly using a custom deconvolution algorithm, and the most abundant protein in the fraction is isolated using a stored waveform inverse Fourier transform (SWIFT). Fragmentation of the isolated protein is then preformed by infrared multiphoton dissociation (IRMPD).

Fragment spectra analysis is performed by THRASH, and the resulting peak lists are sent to the custom ProSight PTM software package for identification and characterization of the protein from the S. cerevisiae database.

Sample Preparation for Nanoelectrospray

- Samples are lysed using a French press, and cellular debris is removed via centrifugation.
- The soluable portion is dialyzed against 50mM Tris, pH 8.0 overnight.
- The lysate is fractionated by size with preparative gel electrophoresis using acid labile surfactant (ALS) using a Prep Cell from BioRad.
- Fractions from the separation are treated to remove the ALS by performing an acetone precipitation followed by re-suspension in pH 2.0 solution
- Samples are further fractionated using reversed-phase HPLC using ODS-I non-porous silica (NPS) columns from Eichrom.

Results

A sample size of 10 µL is sufficient for maintaining stable nanoelectrospray for up to 45 minutes using nanoelectrospray chips with a 10 µm i.d. The sensitivity with the Nanomate 100 is typically a factor of two to three better than with microspray ESI. Automated tandem MS analysis of the samples yields identification of proteins in about 70% of these samples. To date, over 70 distinct proteins from both *M. jannaschii* and *S.cerevisiae* have been identified and completely characterized with this system for intact protein analysis.

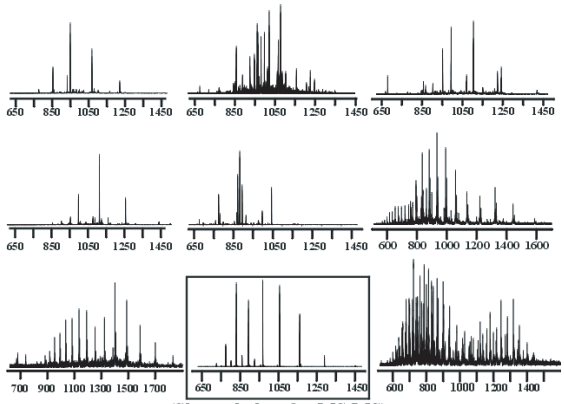
Advantages

- No carryover
- Low sample volume consumption, 2-5µl
- Good desolvation for intact protein ions
- Automated characterization of recombinant proteins and large molecule therapeutics

Figure 1

Automated MS/MS-Processing of Intact Proteins Using The NanoMate 100 and ESI/Q-FTMS

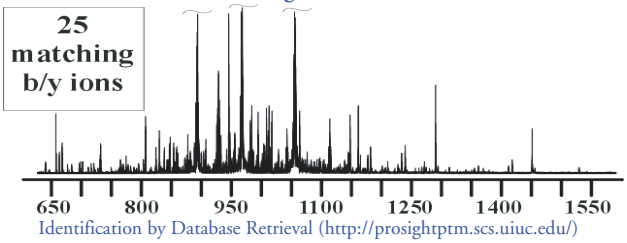
40 Mixtures created by 2D Proteome Fractionation (ALS-PAGE/RPLC)



(Shown below for MS/MS)

Figure 2

Automated Isolation and Fragmentation of a 11.6 kDa Protein



Identification by Database Retrieval (http://prosightptm.scs.uiuc.edu/)

Probability of this search process being random:
P Score = 4.548432e-10
>NR_SC:SW-HS12_YEAST SW:HS12_YEAST P22943 saccharomyces cerevisiae (baker's yeast).
12 kda heat shock protein (glucose and lipid-regulated protein).
5/2000; PIR:HHBY12 heat shock protein 12 - yeast (Sacchar
With Met Removed. With N-terminal Acetylation.
sequence length = 108, MW = 11596.628240, deltaMW = 7.907927
score = 131135, numMatch = 25

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