Application Note

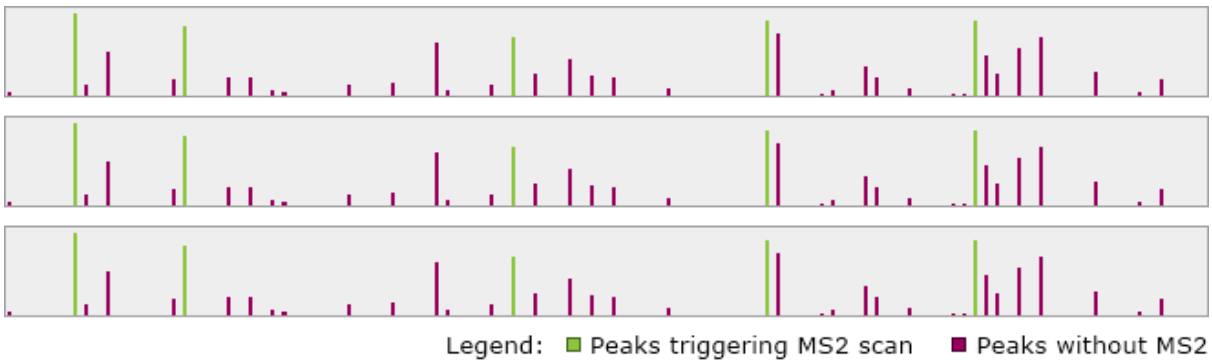
Increased protein and proteome coverage using informatics-based gas-phase fractionation

Overview

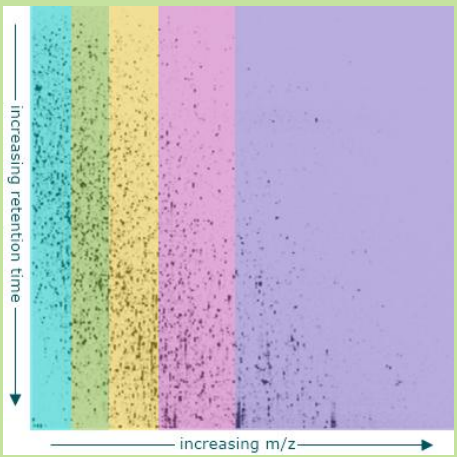
- Generating reliable quantification data alongside identification data, which can cover the wide dynamic range of proteins within a complex sample, is a challenge for MS-based proteomics.
- The quantify-then-identify approach provided by Progenesis LC-MS ideally complements informatics-based gas-phase fractionation (iGPF) to increase protein/proteome coverage when using replicate analysis of label-free samples.
- Here we provide an example† of iGPF applied to quantitative proteomic analysis of *Schizosaccharomyces pombe* **doubling the number of identified proteins** without increasing sample or instrument time required.

Introduction

Even with high resolving power and fast scan speeds in current MS instruments compromises in duty-cycle for optimal quantification or identification cannot survey scan and fragment every peptide ion[1,2,3]. Data-dependent acquisition, where the most abundant ionized species from each MS survey scan is selected for subsequent fragmentation (MS/MS) analysis, is used to address this issue. However, this tends to result in the same set of strong peptide signals being targeted within your replicate runs[1-5] as illustrated below.



Fractionation, to reduce sample complexity and increase detection of lower abundance features, can also be applied. But this approach suffers from sample loss during fractionation and ~4-fold resampling due to overlap of peptides between fractions, increasing demand on sample, instrument time and data analysis[1,2,3].



Informatics Gas-Phase Fractionation (iGPF)

- FTMS scan interval can be optimised for MS1 quantification for label-free experiments but this can compromise the time window over which precursor ions are selected for fragmentation and identification.
- To maintain data quality for quantitative analysis you can limit MS/MS capture to a specific m/z range for each replicate in your label-free quantification but capture all MS1 data across the entire m/z range in each replicate.
- **Progenesis LC-MS** allows you to recombine MS1 data with MS/MS spectra collected across the different m/z ranges to build up a comprehensive set of protein identifications for the whole sample.

†Data provided by Dr Duncan Smith, Paterson Institute for Cancer Research, University of Manchester, UK

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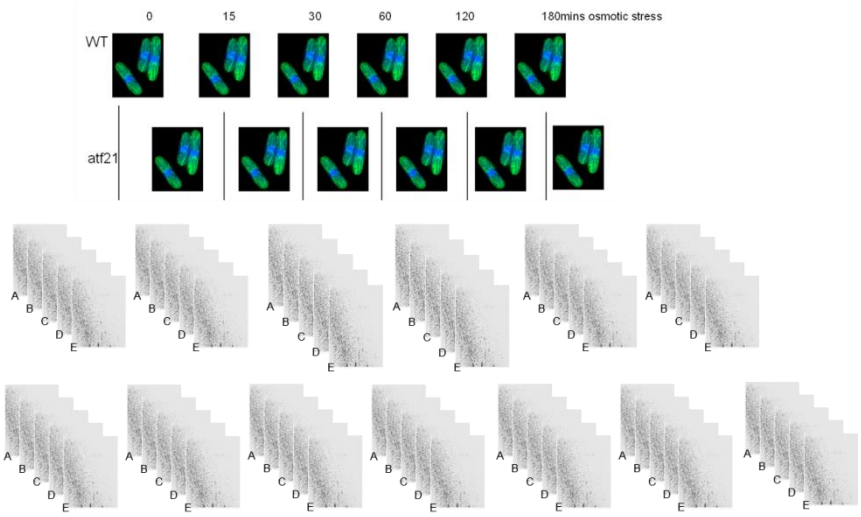
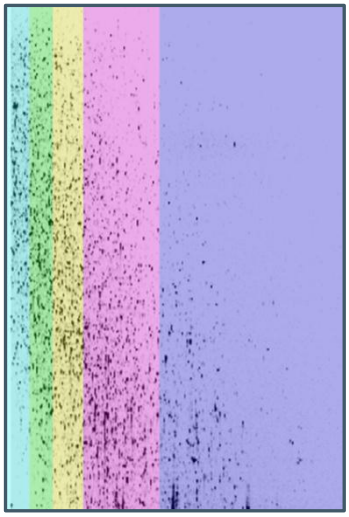
www.nonlinear.com/lc-ms

Method

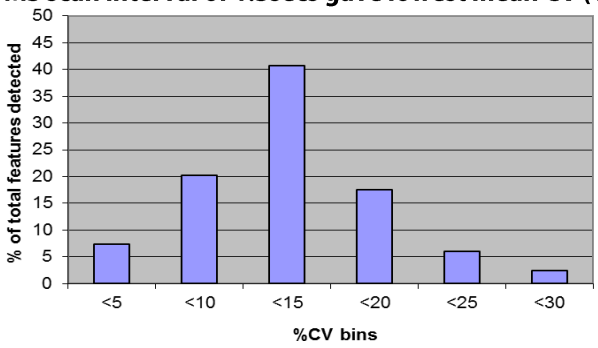
A. Label-free experiment based on quantifying MS1 features…



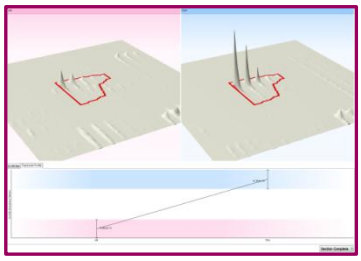
B. Optimise precursor selection for identification



…where FTMS scan interval of 1.3secs gave lowest mean CV (15%)



C. Recombine replicate MS1 quantification data with iGPF identification data



A) Interrogating response to osmotic stress in *S. pombe* using quintriplcate nLC-MS runs/sample: 41,482 multiply charged ions quantified from MS1 data with average CV 14.3% using optimal FTMS scan interval of 1.3 sec. **B)** Five different iGPF m/z ranges were created and each one applied to one of the replicates: The result is 5 different MS/MS experiments per sample while MS data is captured for the entire m/z range. The m/z ranges were calculated to generated equal numbers of peptides/fraction so lower masses require narrower ranges **C)** Progenesis LC-MS data analysis: The software allows you to recombine the 5 different MS/MS results from each sample with replicate MS1 data aligned and co-detected across all 5 runs per sample. More information available on our website at : http://nonlineardynamics.wordpress.com/2011/07/13/gas-phase-fractionation/

Results

- ✓ 5.5 day experiment and only ~4ug Pombe lysate digest/condition (750ng/inj)
- ✓ Quantified 41,482 multiply charged ions in MS1 data with mean CV 14.3%
- ✓ Identified 16,285 peptides (1% FDR) compared to 4,522 with no GPF
- ✓ Quantified and identified 3,427 proteins (1% FDR) compared to 1,871 with no GPF and 75% of all proteins have >3 peptides

Conclusion

iGPF can compensate for limited MS/MS fragmentation when optimising data quality for MS quantification
It provides increased proteome coverage with no extra demand on sample or instrument time when applied to replicates used in label-free LC-MS analysis but requires software such as Progenesis LC-MS to link detected features with fragmentation data generated in this way. Further increases in proteome coverage are possible by fractionating a pooled mix of all runs and applying MS/MS data from it to existing quantified MS1 features in the initial experiment.

References:

1. Kennedy J, Yi EC. Methods Mol Biol. 2008;432:217-28.
2. Ahn NG, Shabb JB, Old WM, Resing KA. ACS Chem Biol. 2007 Jan 23;2(1):39-52.
3. Scherl A, Shaffer SA, Taylor GK, Kulasekara HD, Miller SI, Goodlett DR. Anal Chem. 2008 Feb 15;80(4):1182-91..
4. Mol Schmidt A, Gehlenborg N, Bodenmiller B, Mueller LN, Campbell D, Mueller M, Aebersold R, Domon B. Cell Proteomics. 2008 Nov;7(11):2138-50.
5. Panchaud A, Scherl A, Shaffer SA, von Haller PD, Kulasekara HD, Miller SI, Goodlett DR Anal Chem. 2009 Aug 1;81(15):6481-8.

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