Studying Kinetics of Chromatin Assembly with SWATH™ MS

Andreas Schmidt
Chromatin Proteomics
Biomedical Center – LMU Munich
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Outline

• What is chromatin?

• “In vitro” reproduction of chromatin assembly

• Proteome analysis with SWATH™-MS

• Proteins involved in chromatin assembly & maturation
What is chromatin?

DNA methylation

DNA double helix

Histones

Nucleosome

Histone tails

Open chromatin

Closed chromatin

Epigenetic factor

23 pairs of chromosomes packed into the nucleus

Marx V., Nature 2012
Aim of the Study

- Which proteins are involved in chromatin assembly and maturation processes?
- What are the binding kinetics of chromatin-interacting proteins?
"In vitro" chromatin assembly

Immobilisation of DNA

Addition of DREX

Chromatin assembly

Elution

Isolation of chromatin
Mass spectrometry
Mass spectrometry-based proteomics

nucleosomes & proteins

Proteolytic Cleavage
1. LysC
2. Trypsin

peptides

C18-HPLC nESI

RP-C18 separation

Time

nESI Mass Spectrometer (m/z)

www.3dmoleculardesigns.com

MS detection
Data acquisition conditions

**HPLC – MS/MS system**
Ultimate 3000 nanoLC equipped with *in house*-packed nanoRP-C18 column (150x0.075 mm, Reprosil-AQ, 2.4 um, Dr. Maisch) and trapping column elution gradient from 3-32% ACN

**TripleTOF 6600 mass spectrometer**

**library generation**
information-dependent acquisition up to 40 precursors (charge 2-5) / cycle

**SWATH – MS** acquisition with SWATH 2.0
40 selected mass windows covering from (300-1200 m/z) collision energy optimized for 2+ prec. (spread 7)
SWATH™ 2.0 technology

data-independent acquisition of composite MS/MS spectra of wide Q1 mass ranges

extract peptide signals from fragment ion abundance/RT

optimized distribution of SWATH window size based on the abundance of precursor ions

variable SWATH window size
Why SWATH-DIA mass spectrometry?

Data quality

SWATH™: Data Independent Acquisition
BEST OF ALL STRATEGIES
Exceptional multiplexing, with completeness that rivals MRM

Targeted (MRM): Narrow focus, with excellent data completeness

Conventional: Data Dependent Acquisition (DDA)
Broad coverage, large variability

Data analysis tree

LC-MS samples

Information-Dependent Acquisition (IDA)

spectral library

window size

Feature detection

Statistical Analysis & Biological Interpretation

Analysis Tree

Sciex
Proteomics of *in-vitro* assembled chromatin

Völker-Albert & Pusch et al. MCP, 2016
Finding chromatin-specific proteins

-Log10 (p-value(t-test)) vs. t-test Difference (Chromatin/control)

chromatin-interacting proteins

s0 = 3, FDR value = 0.005
Determining binding kinetics
Evaluation of results

1. Western blot of selected target proteins

2. Comparison to published “in vivo” data from nascent chromatin capture - MS
Summary

• *In vitro* assay reliably reconstitutes chromatin assembly and is a valuable tool to address further questions by challenging the system

• SWATH™-MS is a versatile tool to obtain reproducible results
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