Solutions for the Core and Protein Laboratory: MassHunter Walkup

Jade C. Byrd
MassHunter Product Manager
Common Challenges in Characterizing Biomolecules

Molecular size (insulin is 5 kDa, mAbs are > 100 kDa)

Non-enzymatic Post-Translational Modifications can occur during storage or degradation

Optical (Circular Dichroism, fluorescence) and Separation (SEC, Analytical Ultracentrifugation) techniques do not provide detailed information on higher-level structure

Gels and Western blots may not resolve differences well
Benefits of LC/MS for Core and Protein Laboratories

MS provides molecular weight confirmation with a high degree of accuracy
MS can validate protein sequences
MS identifies post-translational modification (PTM) and its location via MS/MS
MS can be automated for higher productivity and accessibility

LC/MS offers selectivity and specificity!
Intact Protein Analysis – the Manual Process

- **Data Acquisition**
- **MS Spectrum Deconvolution**
- **Zero-charge Mass Spectrum**
- **Compare Observed Mass with Expected**
- **Determine Post Translational Modifications**

**Example Calculations:**
- \(2^*G0F \text{ (NGA2F)} (+1445.3580)\)
- \(2^*G1F (+1607.5013)\)
- \(1^*G1F/G2F (+3377.1458)\)

**Sample Preparation and Submission**

**Reporting**

![Image of protein analysis process](image-url)
Intact Protein Analysis – the Automated Process

Data Acquisition

MS Spectrum Deconvolution

Zero-charge Mass Determination

Sequence Matching

Determine Post Translational Modifications

2*G0F (NGA2F)
(+1445.3580)

2*G1F
(+1607.5013)

1*G1F/G2F
(+3377.1458)

Upload Sequence

Sample Preparation

Sequence Matching

Reporting

Delivery of Results
Automation for Peptide Mapping

Data Acquisition

Compound Extraction

Sequence Matching

Sequence Coverage

Upload Sequence

Sample Preparation

Enzymatic Digestion

2*G0F (NGA2F) (+1445.3580)

2*G1F (+1607.5013)

1*G1F/G2F (+3377.1458)

Determine Post-Translational Modifications

Reporting

Delivery of Results
“…offered the possibility to accept [protein] sequences by the end user at the time of sample submission, run through the data analysis and produce an annotated report automatically…we produce hundreds of [protein] sequences a year and that really removes a potential point-of-failure and stress point in our analytical workflow…huge time savings…usage has exploded! We [saved] more than 10 FTE weeks a year.”

...changes intact protein analysis from a service to a tool…you become an enabler for their work instead of a gate-keeper, people really appreciate that…it changes the relationship to them routinely.”

http://cnpg.companenetworks.com/163768-Open-Access-Intact-Protein-LC-MS-in-a-Recombinant-Protein-Laboratory/
3 Step Sample Submission

Enter username and number samples to be run.
Workflows eliminate unnecessary fields and mouse clicks

Page 1: Choose Workflow

Page 2: Fields change to reflect workflow selected
- Examples: Formula, Protein Sequence, Modifications, Dilution Factor
New Protein Sequence Editor and Import Formats

- A Protein Sequence Editor program that users can freely install on their desktops and laptops
- Import of FASTA and TXT sequence formats
- Included in Sample Submission in Walkup
3 Step Sample Submission

1. **Login**
   - Enter username and number samples to be run.

2. **Choose Method**
   - Enter sample information and select from a list of available methods.
3 Step Sample Submission

1. **Login**
   - Enter username and number samples to be run.

2. **Choose Method**
   - Enter sample information and select from a list of available methods.

3. **Place Sample**
   - Place sample in the position as directed by the software.
3 Step Sample Submission

1. **Login**
Enter username and number samples to be run.

2. **Choose Method**
Enter sample information and select from a list of available methods.

3. **Place Sample**
Place sample in the position as directed by the software.

4. **Receive Report**
BioConfirm Reports from MassHunter Walkup
Sample Reprocessing

Common problem is incorrect formula or protein sequence entered, user wants to reprocess without rerunning

1. Select Reprocess
2. Enter search Criteria
3. Select Samples and modify input
Benefits of MassHunter Walkup for the Sample Submitter

- Access to LC/MS capabilities without needing to be an expert in LC/MS
- Control over sample preparation and treatment and storage
- Many applications possible
  - Clone selection
  - Formulation optimization
  - Covalent inhibitor studies
  - Protein ID by Peptide LC-MS/MS
  - Comparing unmodified and modified samples (without knowing the sequence)
- Consistent data analysis and spectral deconvolution allows for greater confidence during comparative studies
  - Example: pseudo 1\textsuperscript{st} order rate constants
Benefits of MassHunter Walkup for the Administrator

- View Instrument status
- Add/remove/modify users and update methods from any network computer
- Automatic notification of instrument errors via email or text (SMS)
- Remotely take an instrument offline (e.g., for maintenance)
  - Existing queue finishes
  - No new samples can be submitted
- Facilitated by OpenLAB Shared Services
OpenLAB Shared Services
Manage MassHunter and OpenLAB-controlled instruments
Where to Find More Information

Walkup Brochure 5991-3836
Walkup Method Development 5991-2868
Walkup Technical Overview 5991-2868

Application Notes
- Intact Protein Analysis Using an Agilent 6560 Q-TOF Mass Spectrometer 5991-2116EN
- Analysis of Polyethylene Glycol (PEG) and a Mono and Di-PEGylated Therapeutic Protein Using HPLC and Q-TOF Mass Spectrometry
- Reverse Phase Separation of Intact Monoclonal Antibodies Using Agilent ZORBAX Rapid Resolution High Definition 300SB-C3 Column 5900-9667EN
- Reverse-Phase Optimization for Ultra-Fast Profiling of Intact and Reduced Monoclonal Antibodies Using ZORBAX Rapid Resolution High Definition 300SB-C3 Column 5990-9667EN
- Fast Separation of Recombinant Human Erythropoietin Using Reversed Phased Agilent ZORBAX RRHD 300SB-C18, 1.8 μm 5990-9248EN
- Analysis of Oxidized Insulin Chains using Reversed Phase Agilent ZORBAX RRHD 300SB-C18 5990-7988EN
- Peptide Mapping of Glycoprotein Erythropoietin by HILIC/MS and RP-LC/MS 5991-2085EN
- Rapid UHPLC Analysis of Reduced Monoclonal Antibodies using an Agilent ZORBAX Rapid Resolution High Definition (RRHD) 300SB-C8 Column 5990-9631EN
- High Resolution Glycopeptide Mapping of EPO Using an Agilent AvanceBio Peptide Mapping Column 5991-1813EN
- Reversed-Phase Separation of Intact Monoclonal Antibodies Using Agilent ZORBAX Rapid Resolution High Definition 300SB-C8 1.8 μm Column 5990-9016EN
- Reversed-Phase Optimization for Ultra-Fast Profiling of Intact and Reduced Monoclonal Antibodies Using ZORBAX Rapid Resolution High Definition 300SB-C3 Column.
- Ultra High Speed and High Resolution Separations of Reduced and Intact Monoclonal Antibodies with Agilent ZORBAX RRHD Sub-2 μm 300 Diphenyl UHPLC Column 5990-9668EN
- Fast Separation of Recombinant Human Erythropoietin Using Reversed Phased Agilent ZORBAX RRHD 300SB-C18, 1.8 μm 5990-9248EN
- Analysis of Oxidized Insulin Chains using Reversed Phase Agilent ZORBAX RRHD300SB-C18 5990-7988EN
More on Information pMod Deconvolution

- Reduction of artifacts
- Spectral data that does not fit the model is rejected as noise
- Improved mass resolution in mixtures
- Uncertainty is represented as peak width
New Walkup Administration

Import/Export Utilities

Common Tasks on Toolbar

Logical navigation for system setup
Brings up ChemStation (and ASR) method for reviewing/editing – no need to exit and go to ChemStation
Administration - Validation of all Changes

Double clicking error takes you to area needing editing