Protein & PTM Profiling and Identification Core

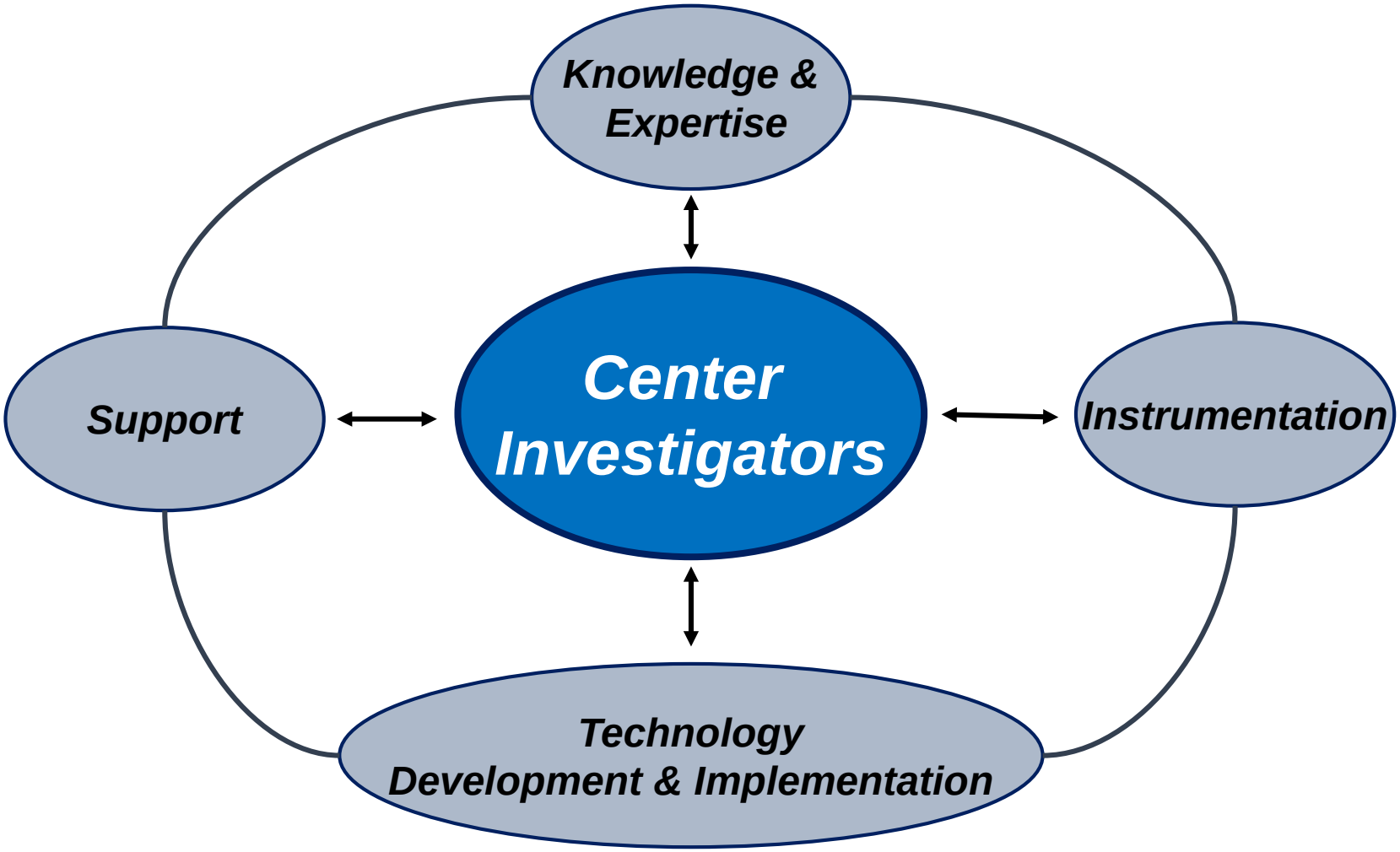
Yale/NIDA Neuroproteomics Center EAB Meeting

TuKiet T. Lam, PhD

May 1st, 2019

www.yale.edu

Mission and Operating Principle



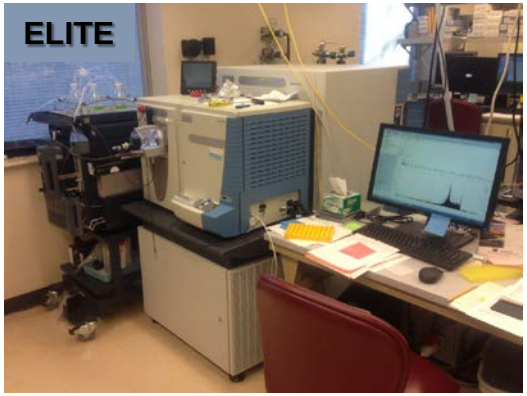
Cost recovery

Center Investigators Projects (90% Center funds, 10% Investigator funds)

Pilot Projects (100% Center funds)

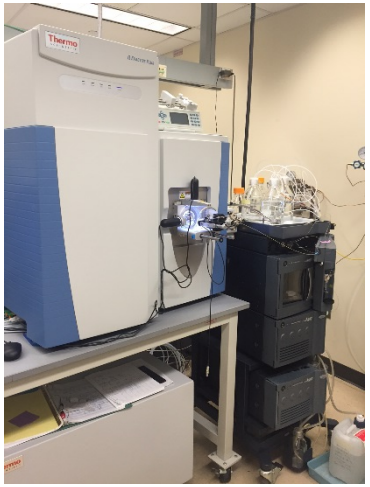
Mass Spectrometers currently located within the Core

For Protein ID

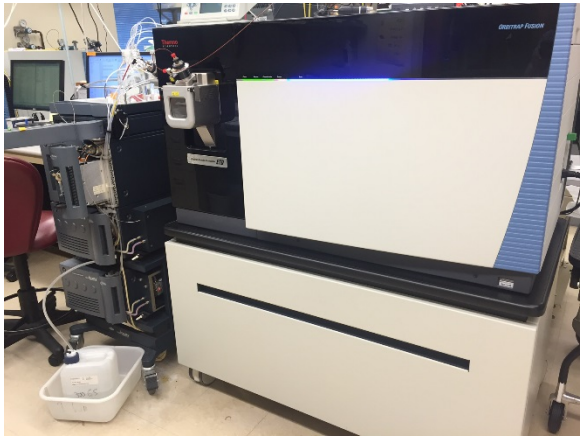


Thermo Fisher Scientific nano-UPLC ESI ***LTQ-Orbitrap ELITE*** MS systems

For Protein PTM, Profiling, & Quantitation

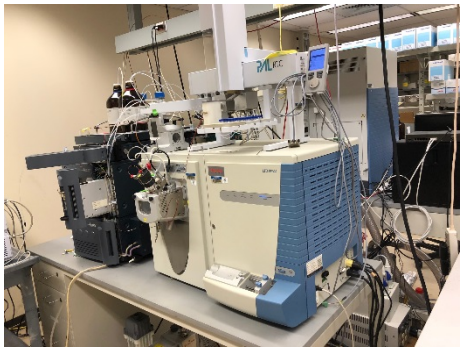


Thermo Fisher Scientific nano-UPLC ESI ***Q-Exactive Plus*** MS systems



Thermo Fisher Scientific nano-UPLC ESI ***Orbitrap Fusion*** MS systems

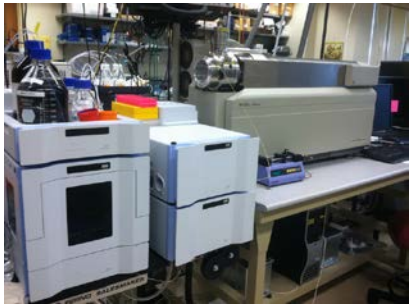
For Open Access Usage



Thermo Fisher Scientific nano-UPLC ESI ***LTQ-Velos*** MS systems

Waters UPLC (H-Class)

For Metabolism Separation & Quantitation



Agilent 1200 UPLC
AB Sciex ***4000 QTRAP*** MS system

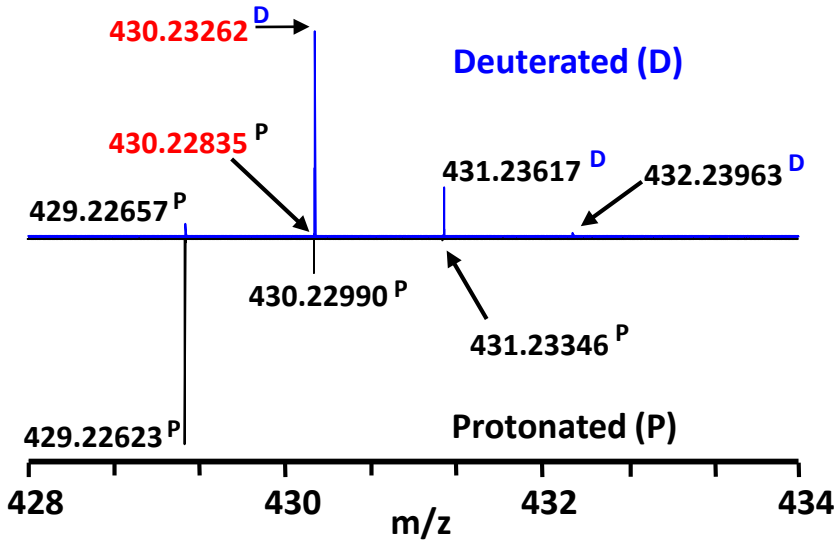
New Instrumentation

Q-Exactive HF-X mass spectrometer with ACQUITY UPLC M-Class.

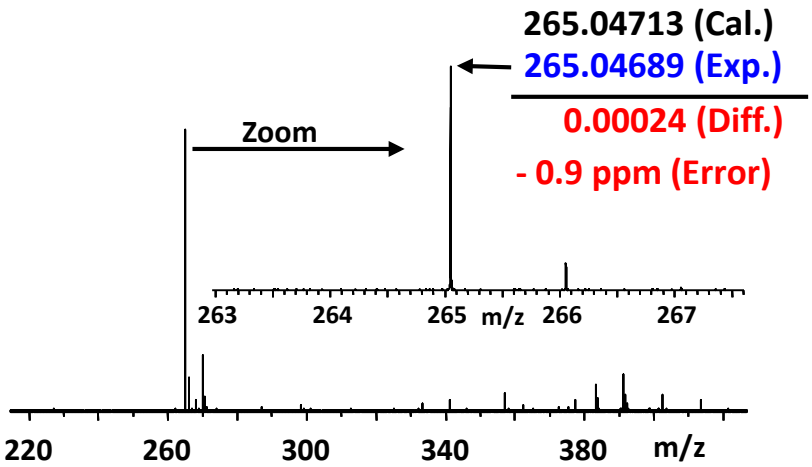
Instrumentation

Mass Spectrometer Capabilities

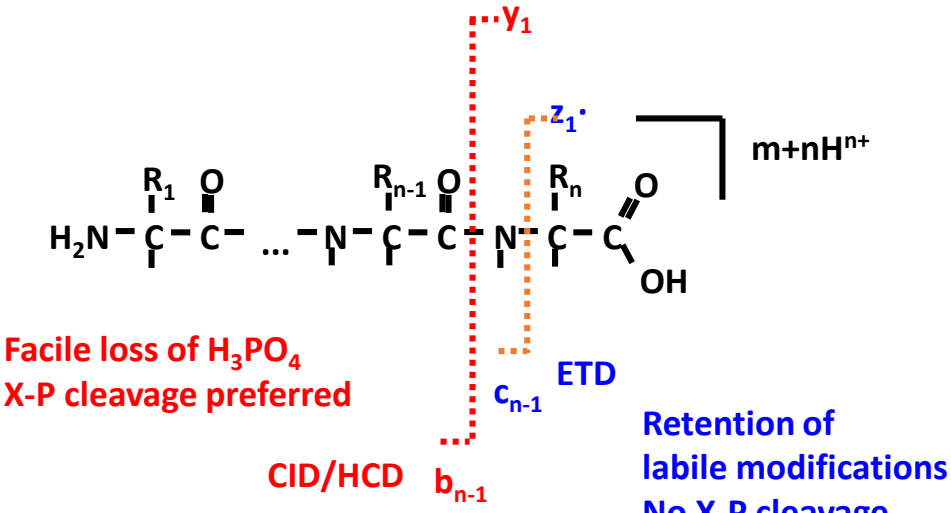
Resolution



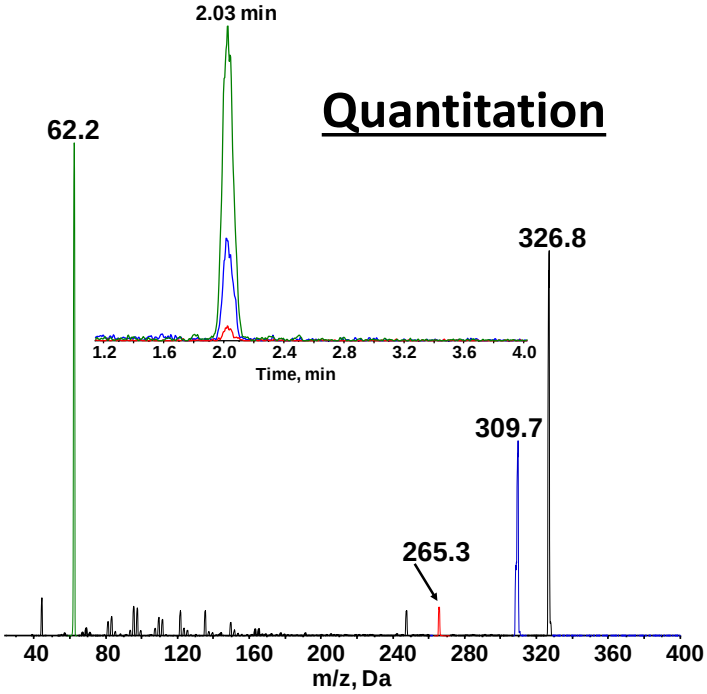
Mass Accuracy



Fragmentation Capabilities



Quantitation

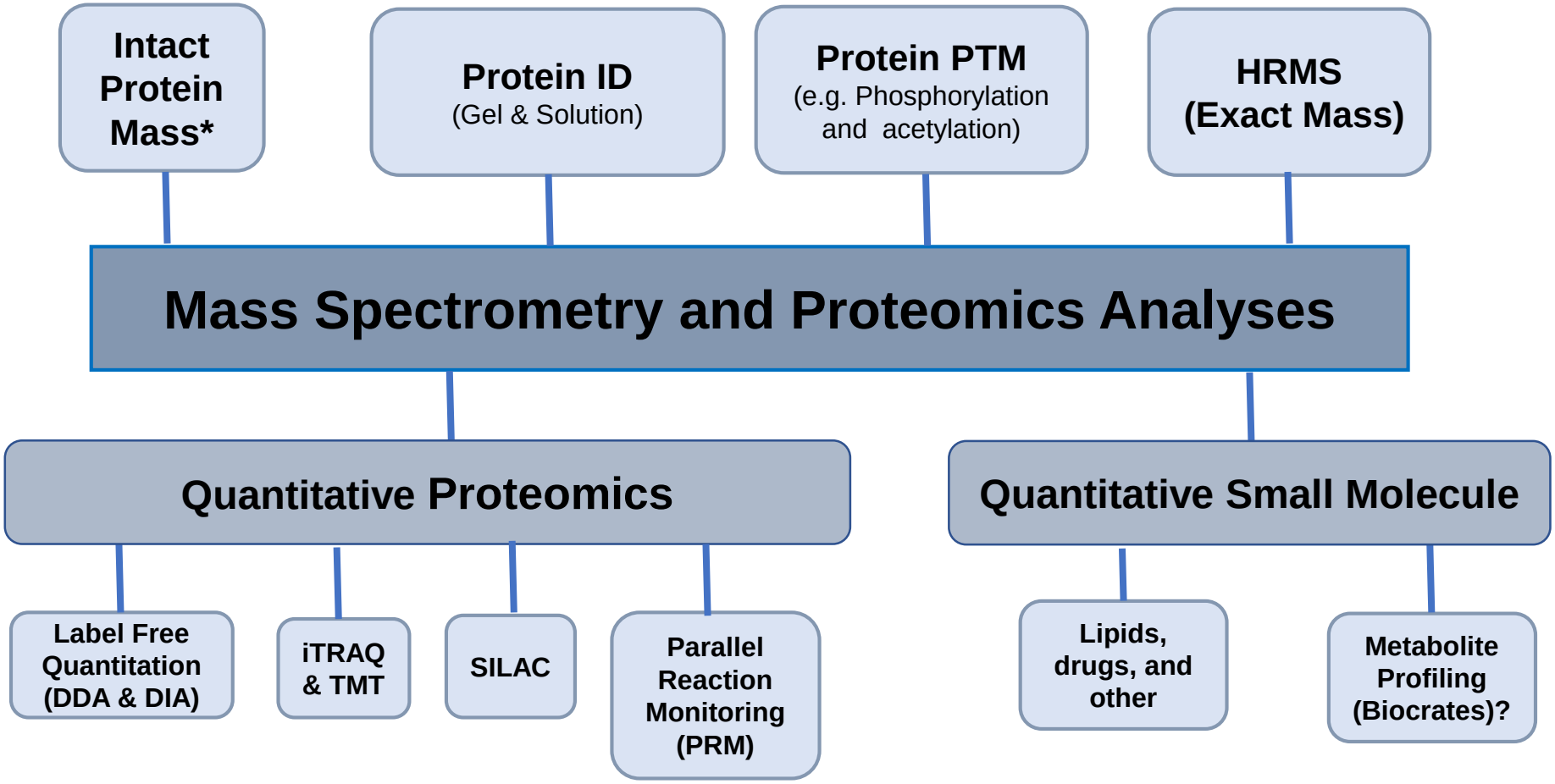


Comparison of Thermo MS (QE and Orbitrap)

Insturment	QE-Focus	QE	QE-Plus	QE-HF	QE-HFX	QE-UHMR	O-Fusion	O-Fusion Lumos	O-ID-X
Max resolution (FWHM) @*m/z* 200	70k	140k	140k (Opt. 280k)	240k	240	200k at m/z 400	500k	1,000k	7.5-500k
Mass accuracy, (internal)	<1 ppm								
Mass accuracy (external)	<3 ppm								
Mass range	50 to 3,000 *m/z*	50 to 6,000 *m/z*		50 to 8,000 *m/z*		350 to 80,000 *m/z*			50-2,000 m/z
Dynamic Range	>5000:1						-	-	-
Scan rates	Up to 12 Hz			Up to 18 Hz	Up to 40 Hz	Up to 12 Hz	20Hz	40Hz	30Hz Orbitrap 40Hz ion trap
Polarity switch	<1 sec						<1.1sec	<1.1sec	1.1 Sec
PRM	-	Yes							
Multiplex (precursor/ scan)	-	10					10	20	10
Decision-tree (CID/HCD/ ETD)							Yes		
ETD option							Yes		

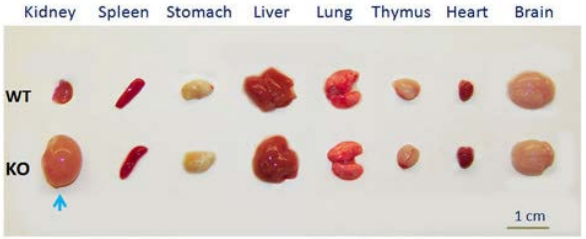
From Thermo Fisher Scientific Brochure

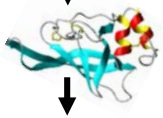
Overview of the Services available to Center Investigators

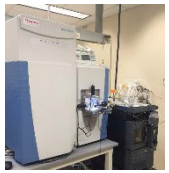


*Upper mass limitation.

Identification of Protein and Protein Posttranslational Modifications







Start

Sample Preparation

Data Acquisition

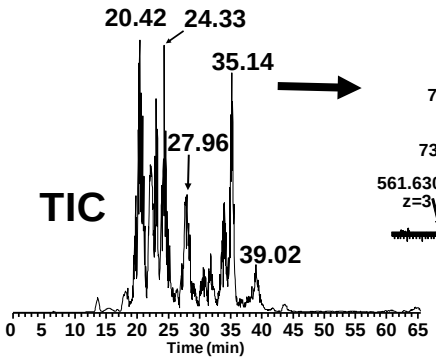
Data Processing

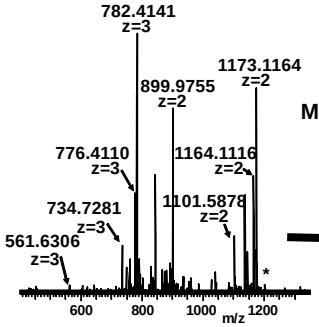
End

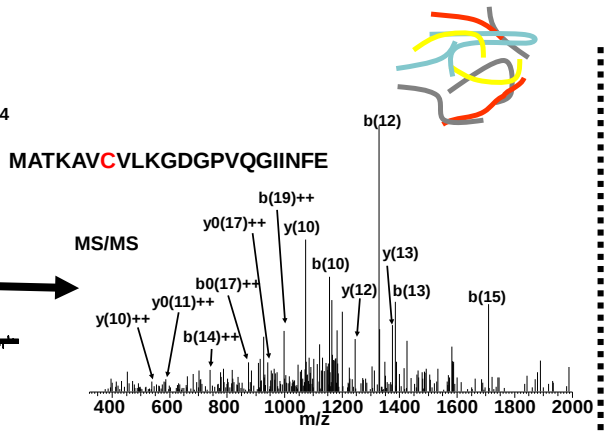
SOP

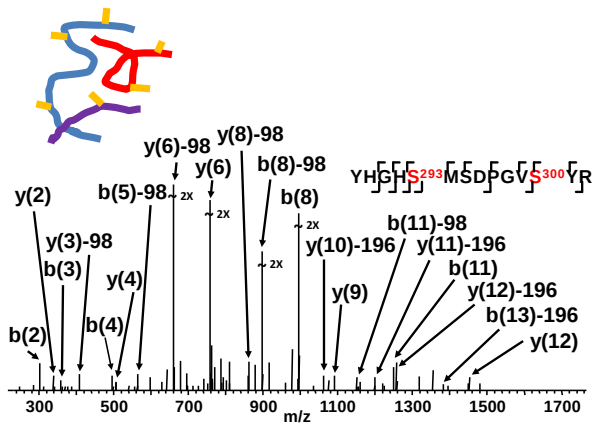
Cell Lysate
Tissue
Biological Fluid
Cellular Fractionation
Subcellular Fractionation
Protein Extraction
Protein Fractionation
Protein enrichment
Enzymatic Digestion
Peptide Fractionation
Peptide enrichment
1st Dimension Chromatography
2nd Dimension Chromatography
Mass Spectrometry
Data analyses
Results Interpretation

Resource
Users
Optional



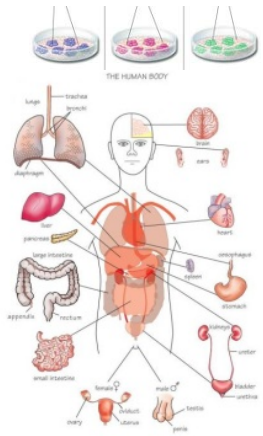




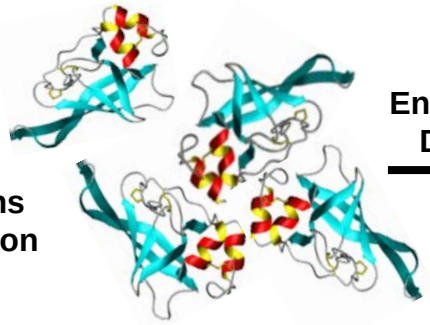


Quantitative Proteomics Workflow

Biological Source



proteins extraction



Enzymatic Digest

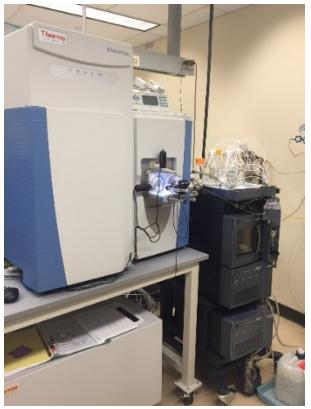
PTM Peptide enrichment

+

Flow through (FT) fraction

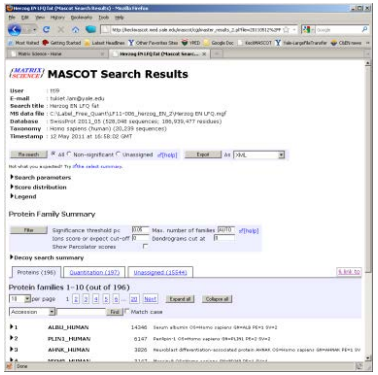
Enriched (EN) fraction

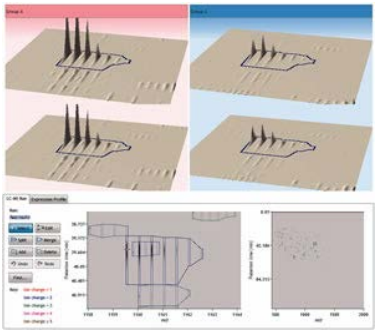
SOP



on-line LC MS and LC MS/MS **separation** and **data acquisition**

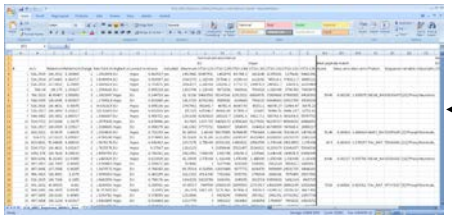
Acquired raw Spectral data are **processed and detected** features are searched against selected Protein Database using MASCOT/Sequest Search Engine





Quantitation carried out by additional software:

- Progenesis QI Software
- Proteome Discoverer
- Scaffold Software
- Skyline
- MASCOT Quantitation Tool Box



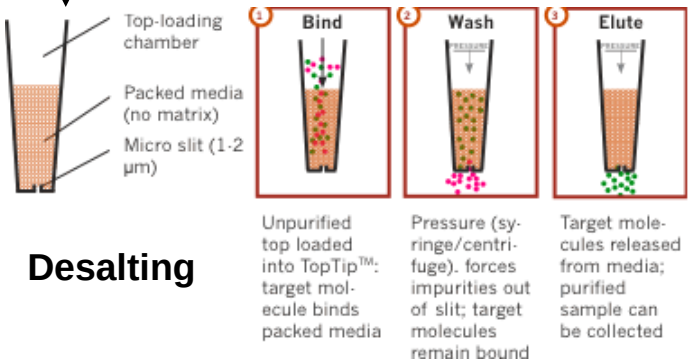
Reporting

Tabulated protein list with calculated abundances and expression changes

Robustness

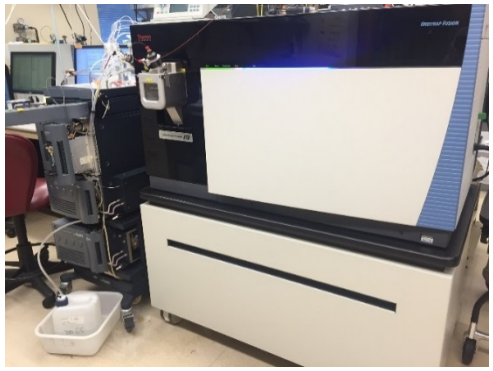
Standardized Thoroughness

Intact Protein Analysis Workflow: Determination of Intact Protein MW

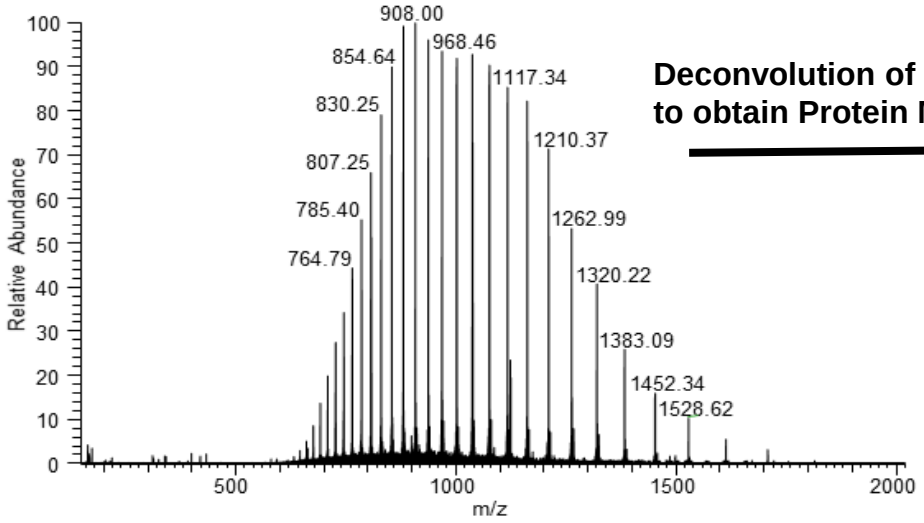


Desalting

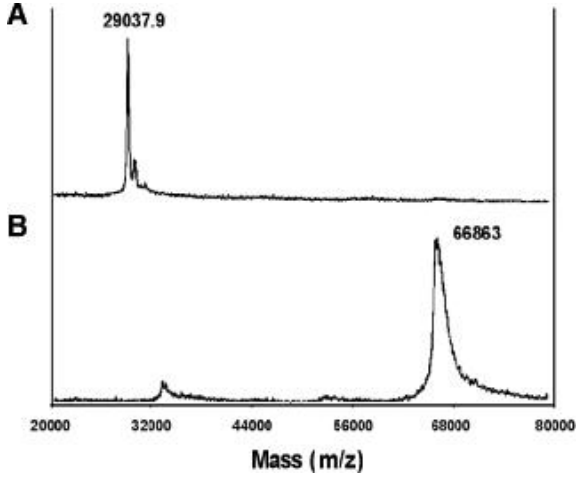
Direct Injection ESI MS for data acquisition



MS spectral data are processed and analyzed using Deconvolution Software to obtain accurate high resolution protein mass

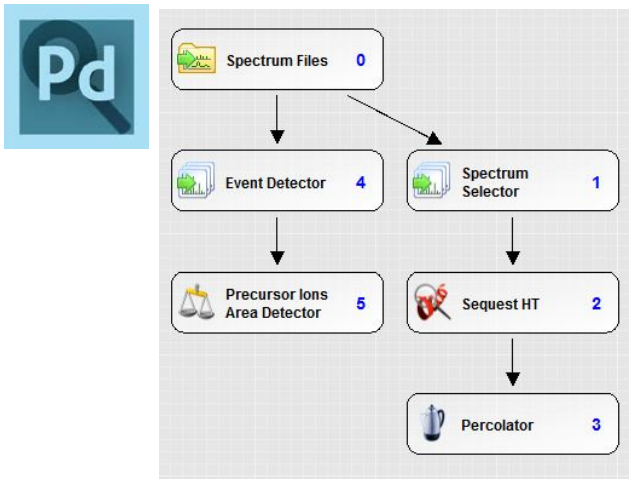


Deconvolution of MS to obtain Protein MW



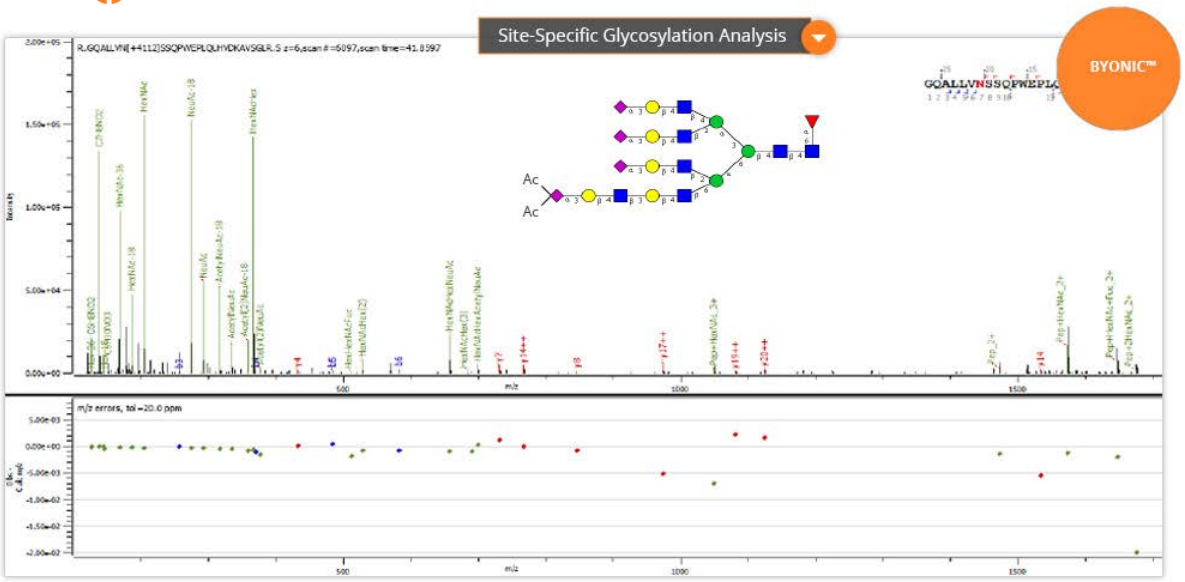
Desalting image from: http://www.glysci.com/products/Protein%7B47%7DPeptide-Desalting-TopTip.html

Software for analysis and visualization

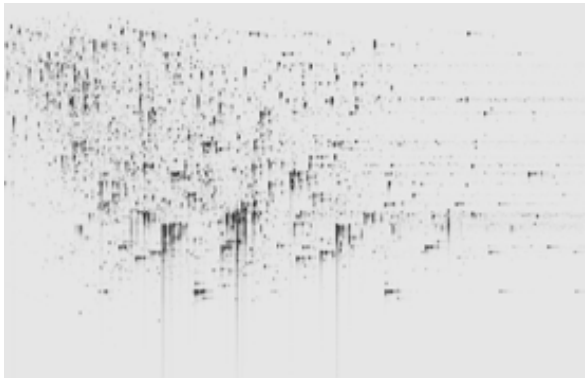


Skyline

PROTEIN METRICS
Boldly Advancing Protein Characterization



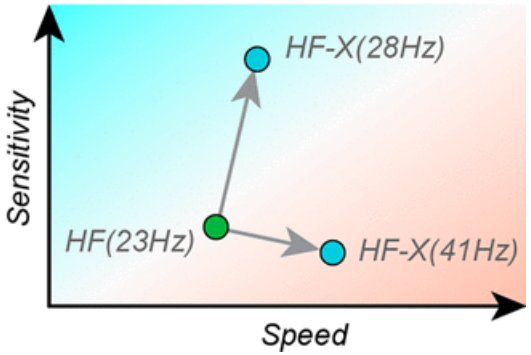
Progenesis QI



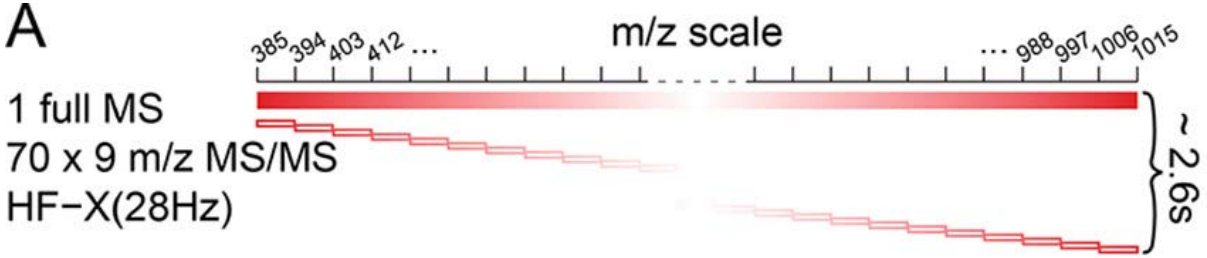
New Services coming in 2019…

Technology Implementation

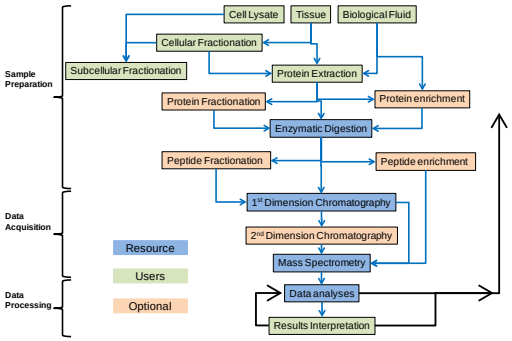
- Improved LFQ (higher throughput with increased robustness)

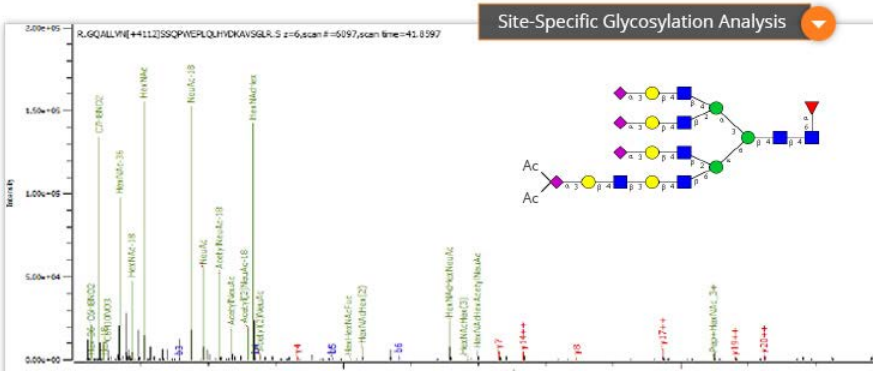


- Data Independent Acquisition (improved # of protein identifications)



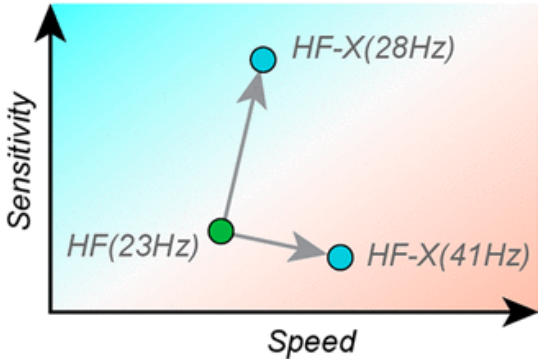
- Glycosylation workflow.

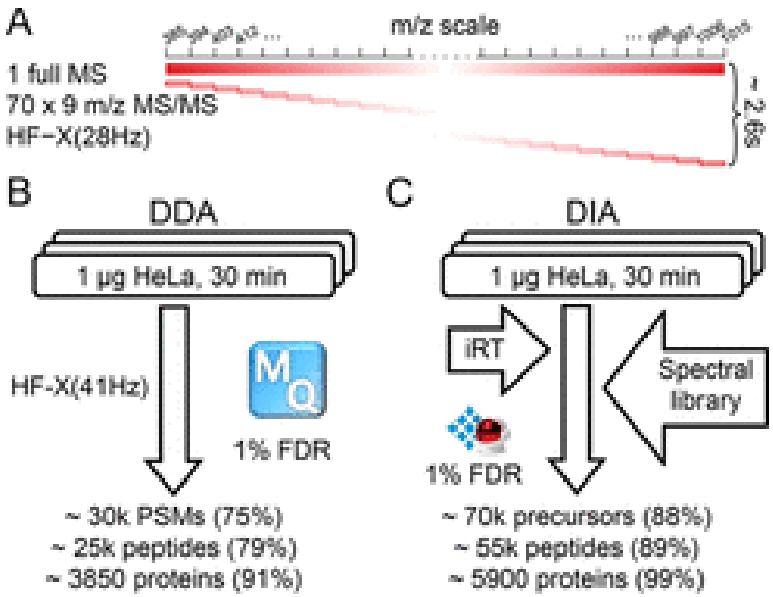


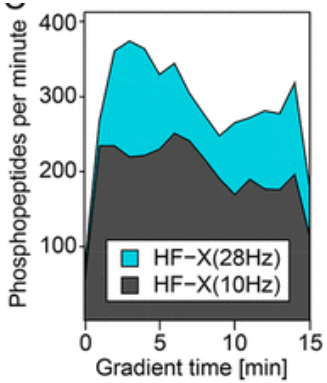


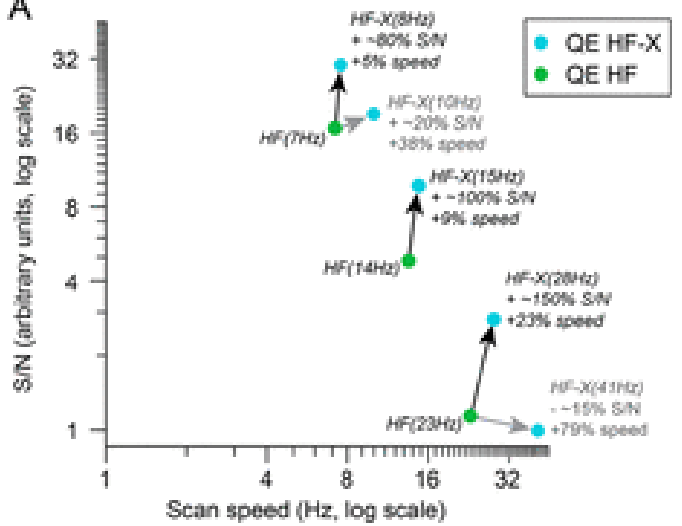
New Instrumentations: Improvement in Sensitivity and Throughput

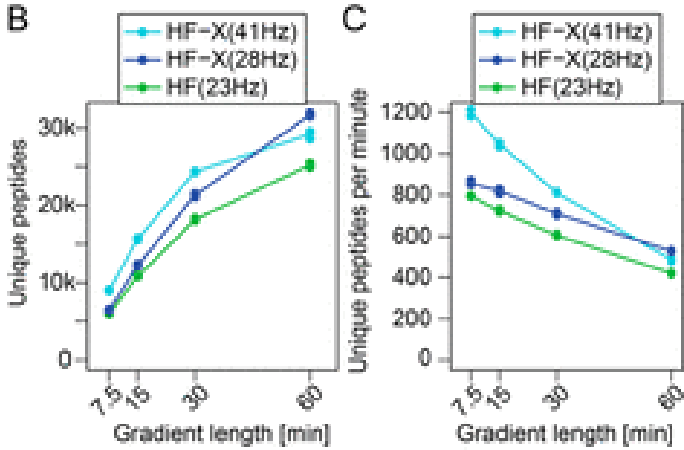
Figure 1: Physical appearance of Q-Exactive HF-X mass spectrometer with ACQUITY UPLC M-Class.

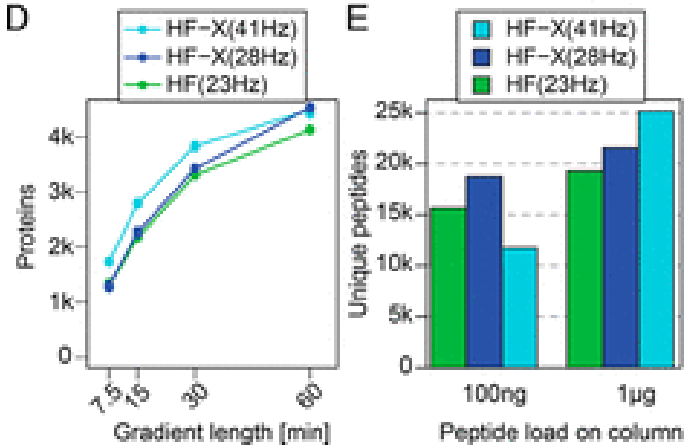






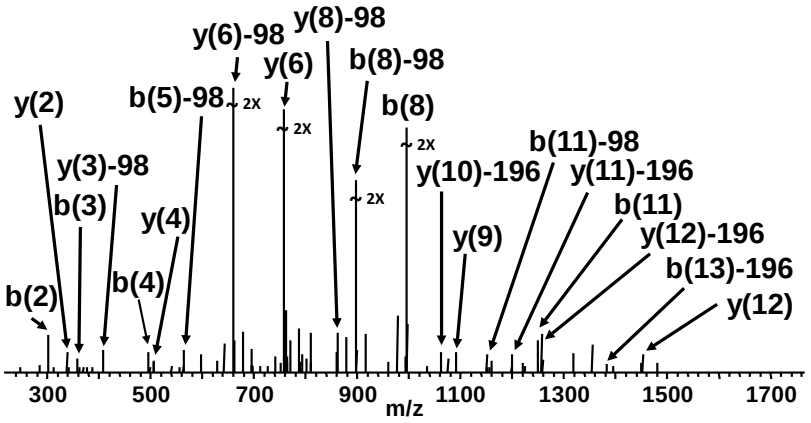


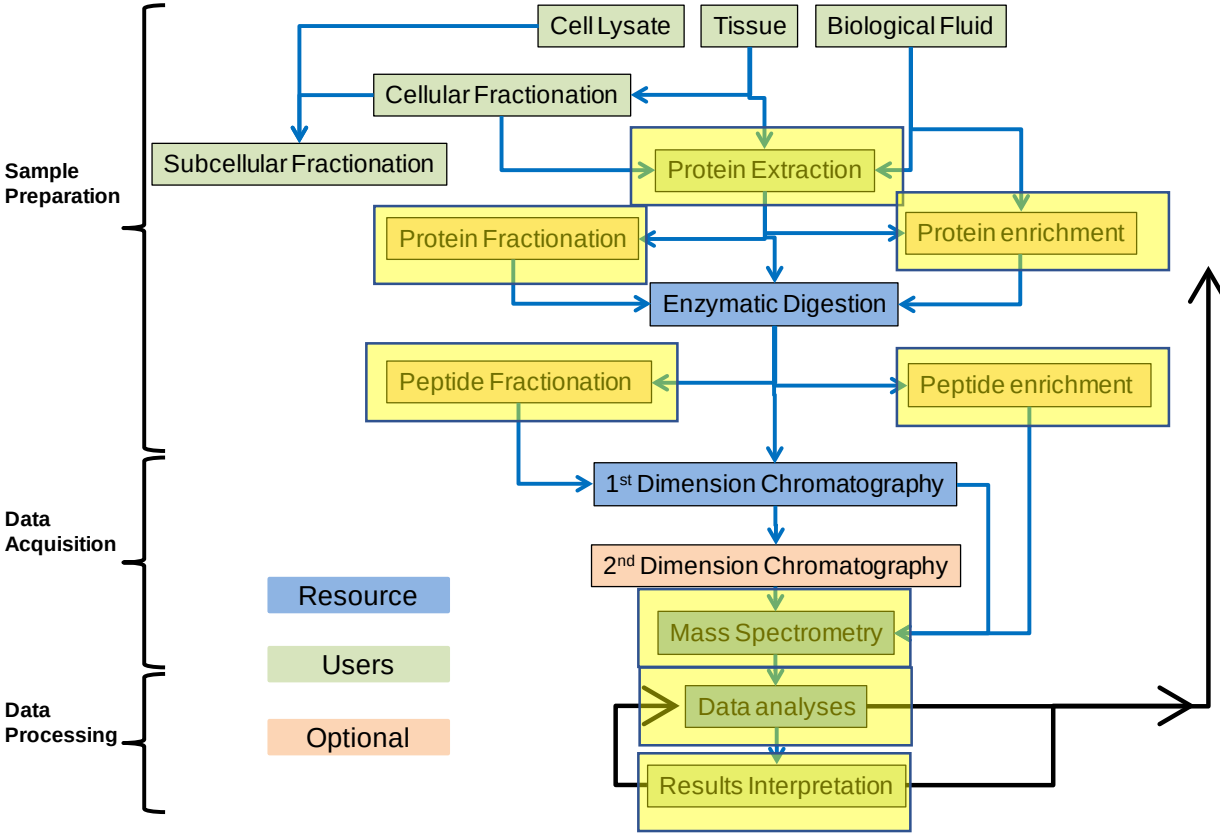


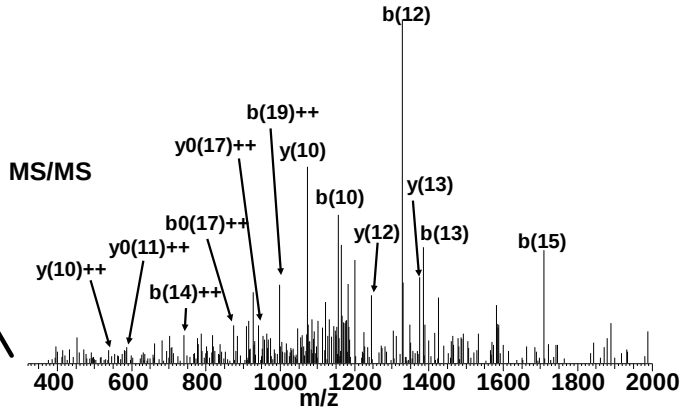


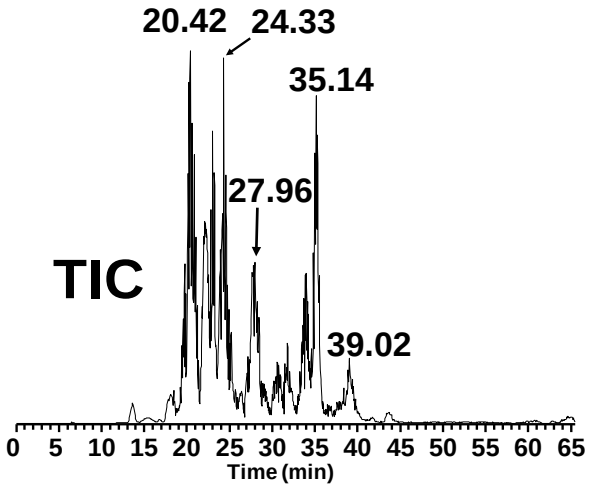
Performance Evaluation of the Q Exactive HF-X for Shotgun Proteomics
Christian D. Kelstrup, Dorte B. Bekker-Jensen, Tabiwang N. Arrey, Alexander Hogrebe, Alexander Harder, and Jesper V. Olsen: *Journal of Proteome Research* **2018** *17* (1), 727-738 DOI: 10.1021/acs.jproteome.7b00602

Future Workflow enhancement for Protein and Protein Posttranslational Modification Identification & Quantitation





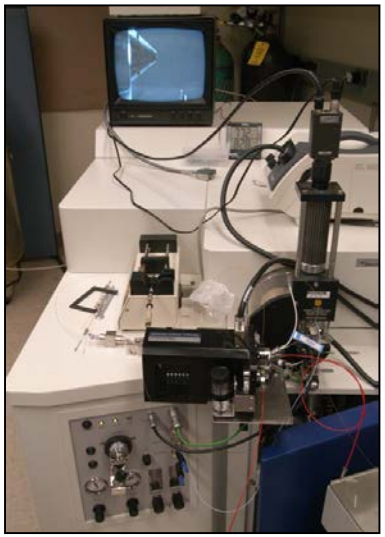




Progress…

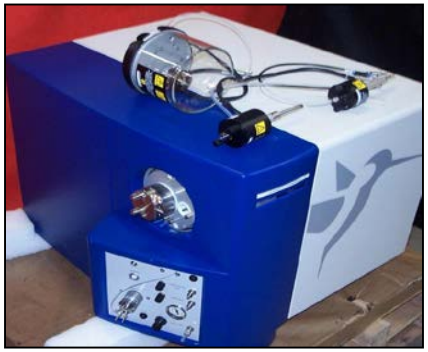
MS and Protein Profiling Core: Instrumentations (2006)

AB QSTAR XL
ESI QTOF
System
ICAT, MudPIT



Micromass QTOF API
MS System
Phosphoprotein
Profiling

Micromass
MALDI TOF MS
Serum
Biomarkers, QC



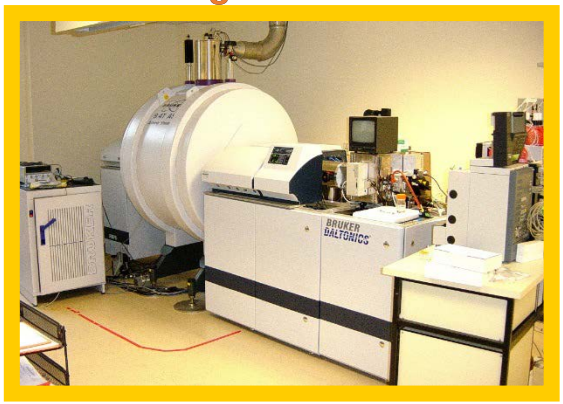
Micromass QTOF
Micro MS System

Lipid Profiling
Intact Protein MW

Beckman-Coulter PF2D

Chromatofocusing & NPS-RPLC

2D Protein Separation System

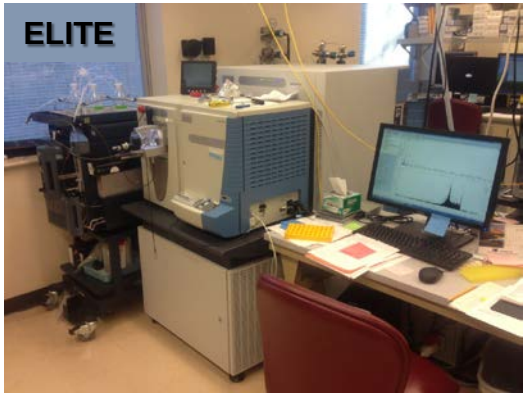


Bruker APEX Qe 9.4Tesla
FT-ICR MS System
Accurate mass, Top Down, PTM

AB 4700
MALDI TOF/TOF MS System
DIGE, 1D SDS PAGE, LC-MALDI

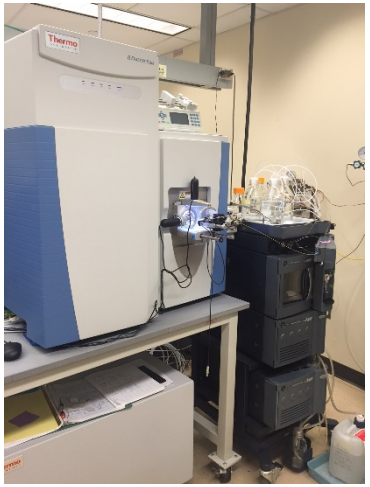
Mass Spectrometers located within the MS & Proteomics Resource (2019)

For Protein ID

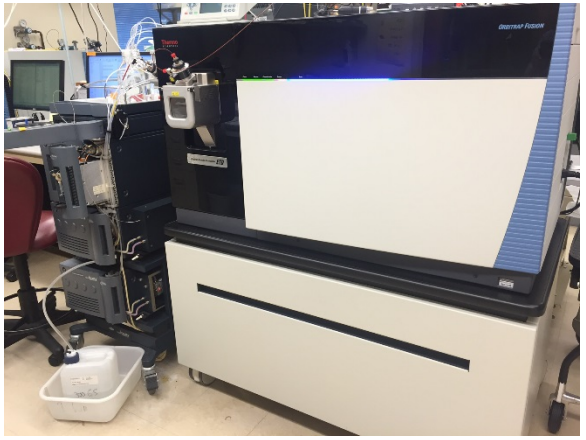


Thermo Fisher Scientific nano-UPLC ESI ***LTQ-Orbitrap ELITE*** MS systems

For Protein PTM, Profiling, & Quantitation



Thermo Fisher Scientific nano-UPLC ESI ***Q-Exactive Plus*** MS systems

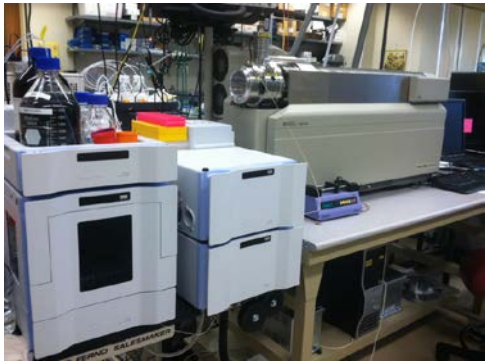


Thermo Fisher Scientific nano-UPLC ESI ***Orbitrap Fusion*** MS systems

For Open Access Usage

Thermo Fisher Scientific nano-UPLC ESI ***LTQ-Velos*** MS systems

For Metabolism Separation & Quantitation



Agilent 1200 UPLC
AB Sciex ***4000 QTRAP*** MS system

Waters UPLC (H-Class)

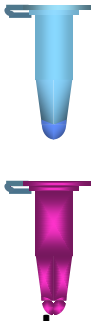
New Instrumentation

Q-Exactive HF-X mass spectrometer with ACQUITY UPLC M-Class.

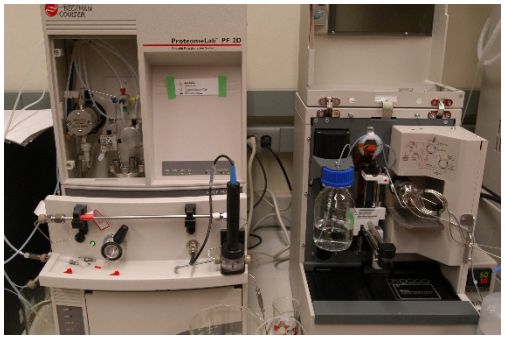
Instrumentation

ProteomeLab PF2D (Beckman-Coulter): 2D Chromatofocusing & non-porous RP-HPLC

0.2 – 1 mg needed per sample condition



Samples are run serially

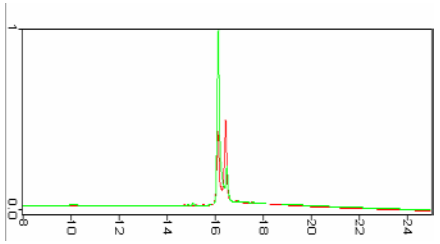


1st Dimension:
Chromatofocusing
(pH 8.5 to 4.0) (UV 280nm)

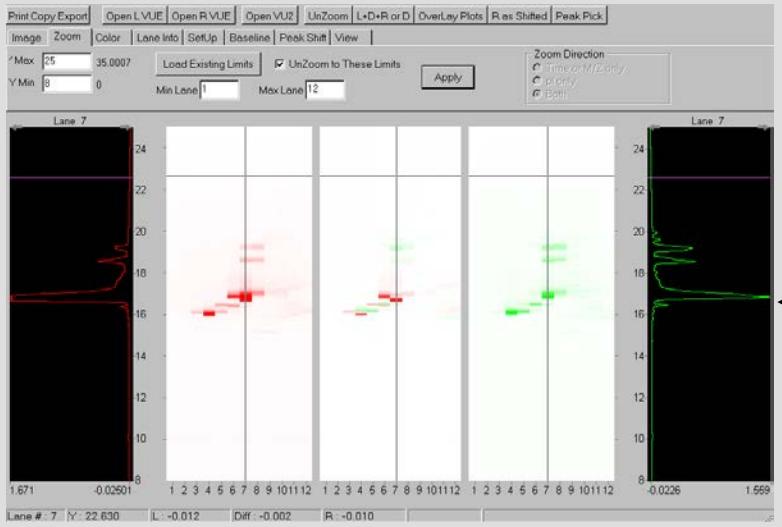
Fractions collected every 0.3 pH unit change

2nd Dimension: Non-Porous-Silica Reversed Phase Chromatography

Each pH fraction is run for 20-24 min. with 1 minute fraction collection (UV 214nm);

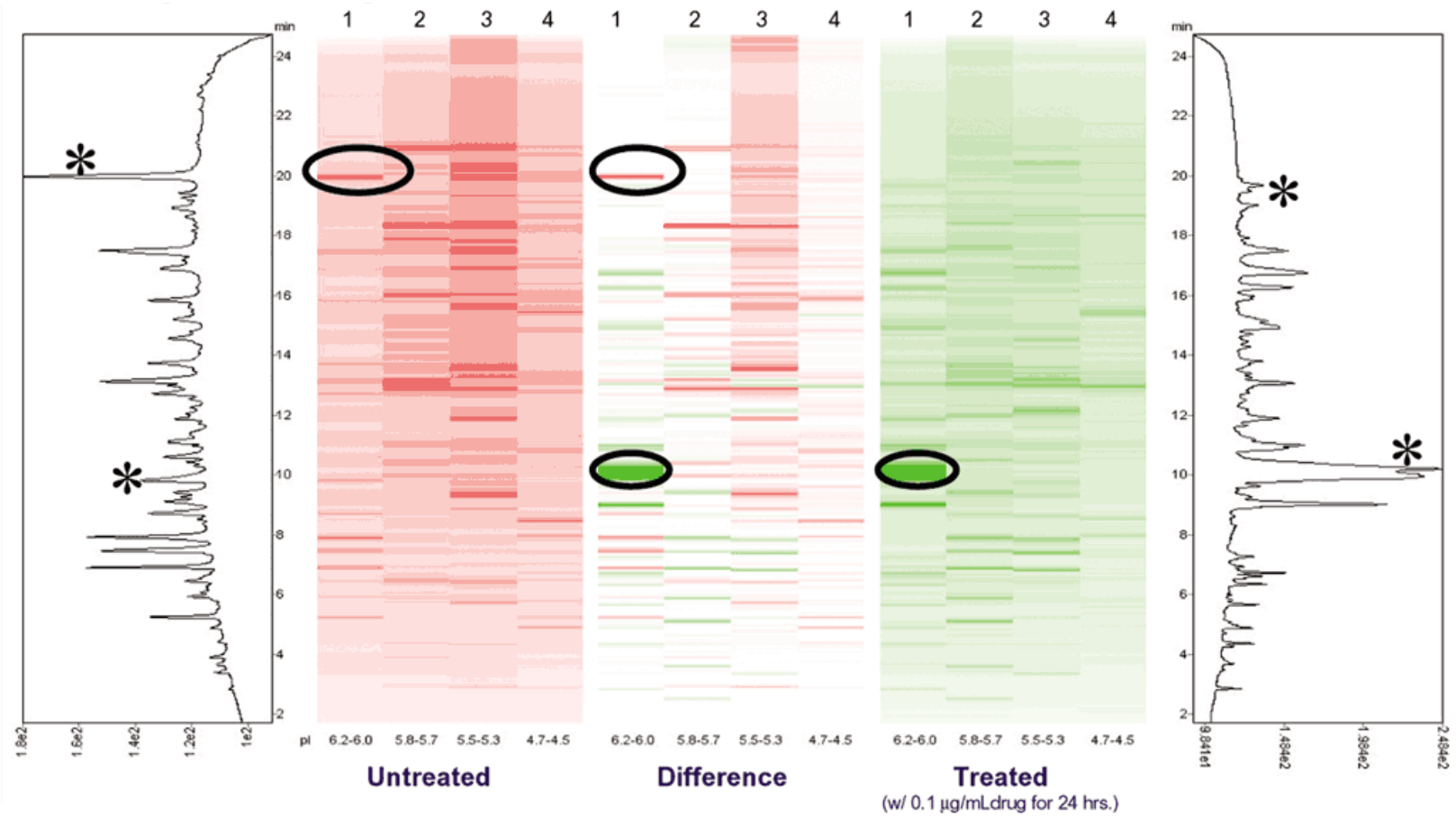


Comparative "gel" and UV view of samples 1 and 2



PI: Geoffrey Chupp,
Yale University, Int. Med.

Comparative pI – LC (UV) Protein Profiling before/after Drug Treatment



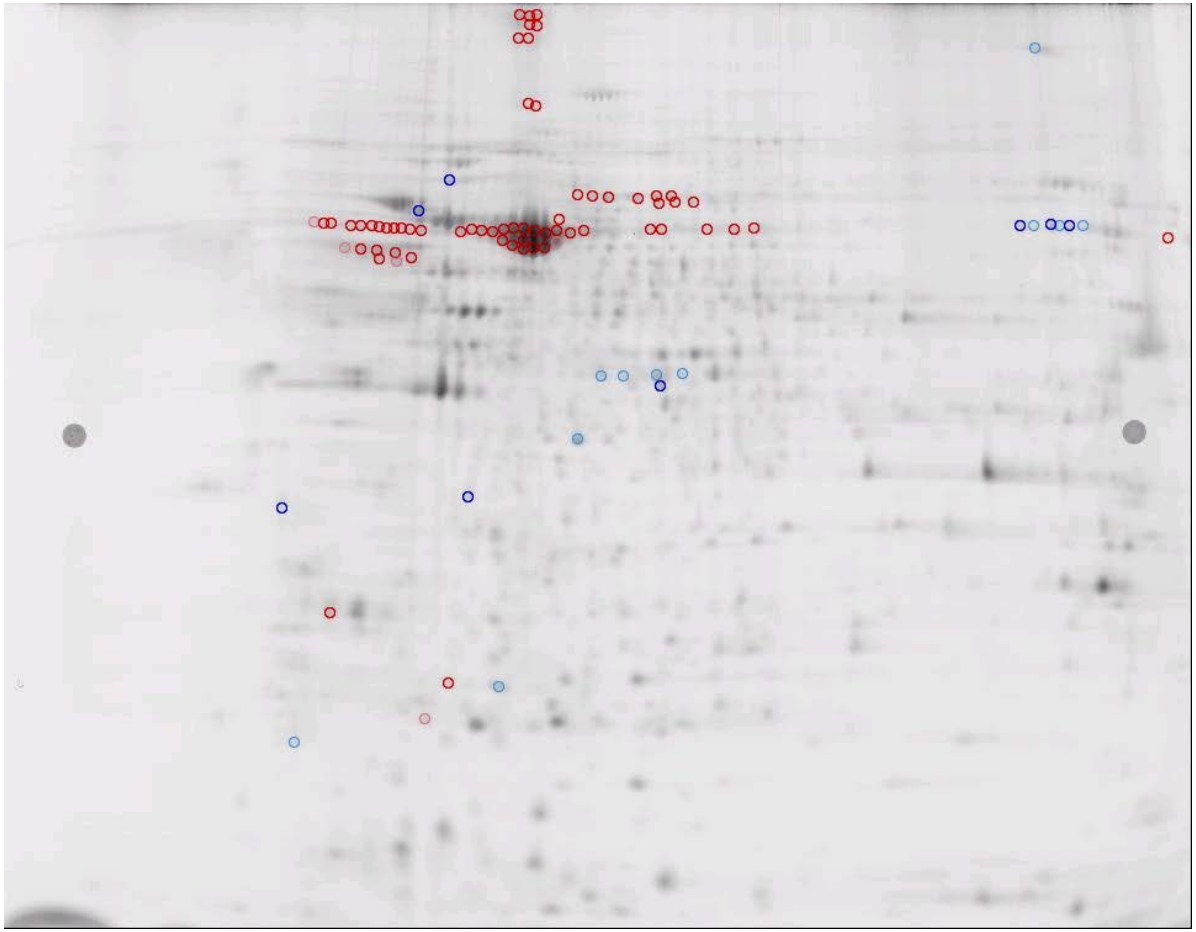
- **Partial pI/UV map**
- **The RP-HPLC profiles illustrate differences in the pI 6.0-6.2 fraction**
- **Both the UV and color coded band depictions of these RP-HPLC profiles (from Beckman-Coulter)**

YPED

DIGE Results for Sample: DIGE_GEL409 (12092005)

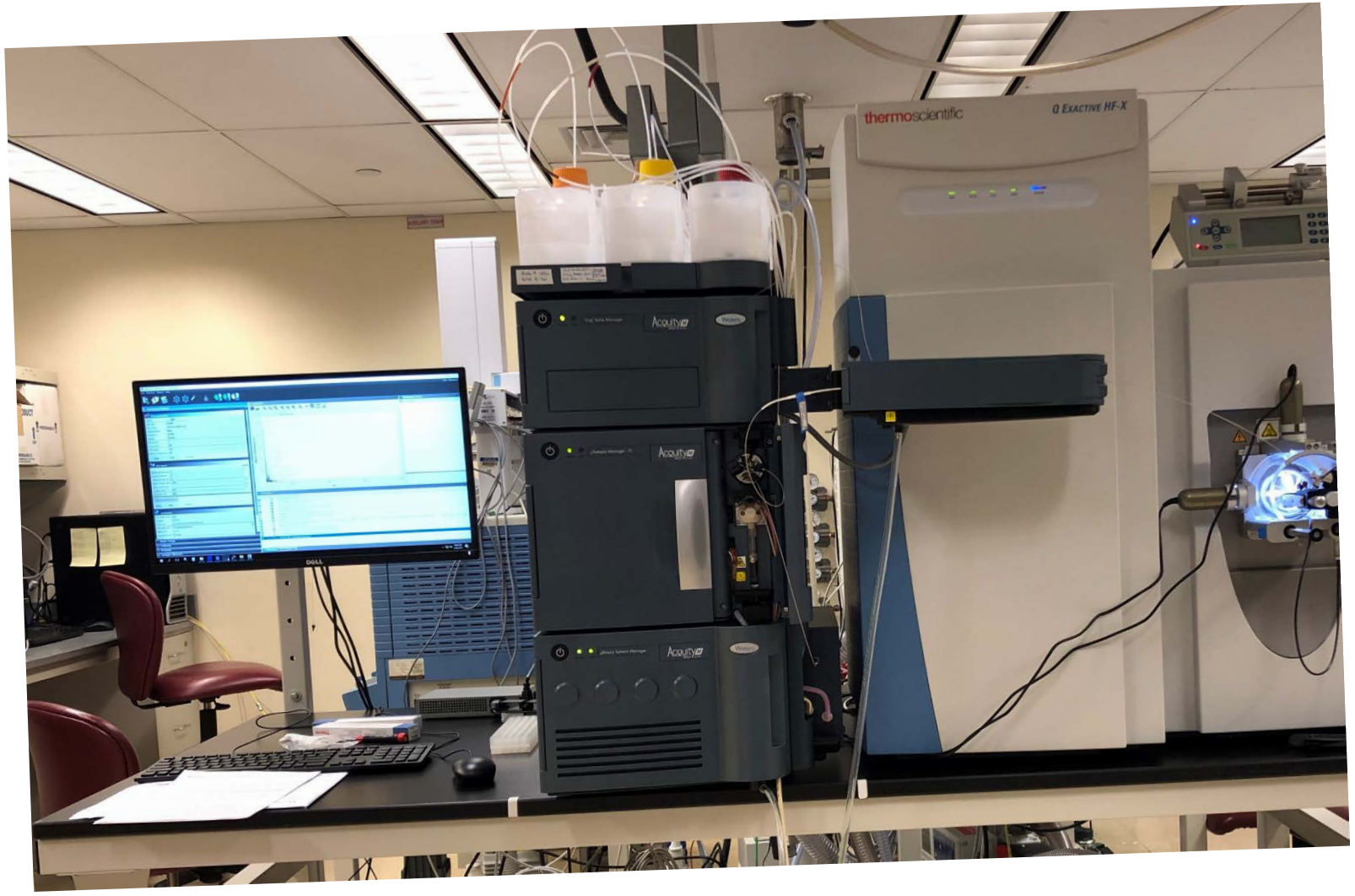
Database – NCBInr
Search Engine - MASCOT

#spots picked/analyzed	85
#spots with proteins id'd	69
#proteins id'd with 1 peptide	0
#spots with C5/C3 >= 2 fold difference (all spots)	22
#spots with C5/C3 >= 2 fold difference (proteins Id'd)	18



Red – Down regulated
Blue – Up regulated
Dark Red/Blue – Protein Id'd
Light Red/Blue – Protein not Id'd

March 20th, 2019: Q-Exactive HFX with ACQUITY M-Class



Supporting Users

- **Provide support letter for grant applications**
- **Help with grant write-up materials (budgeting, methodology, aims)**
- **Training and education (from Sample preparation to software use)**
- **In person initial consultation**
- **Free access to licensed Proteomics Software**
  - **Within the MS & Proteomics Resource**
  - **At Cushing/Whitney Medical Library (contact Rolando Garcia-Milian)**
- **Write our own grants as a PI (NIH SIG, Pilot, NIH R21)**
- **Developing Technology catering to Users experimental needs**

Funding

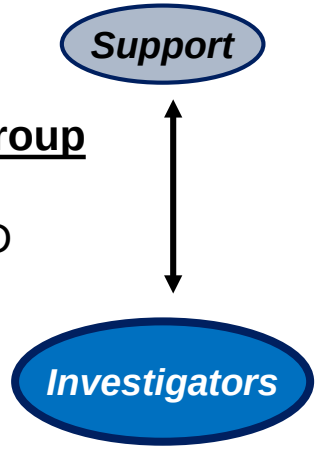
Instrumentations:

NIH SIG S10OD023651-01A1: QE-HFX (PI: Lam)
NIH SIG 1S10OD019967-01: UPLC (PI: Lam)
NIH SIG 1S10OD018034-01 + YSM: Orbitrap Fusion & Q-Exactive Plus
NIH CTSA,UL1 RR024139: 4000 QTRAP
NIH SIG, RR031795: LTQ Orbitrap ELITE
Donation: LTQ Velos

Collaborations and other support:

R01 NS109358-01 (2018; PI:Kahle, Collaborator: Lam)
R01 AG057912-02 (2018; PI:Levine, Collaborator: Lam)
R01 MH115939-01 (2018; PI:Koleske, Collaborator: Lam)
R01 GM102262-01 (2017; PI:Turk, Collaborator: Lam)
P30DA018343 (2016; PI's:Narin & Williams; Discovery Core Director: Lam)

Acknowledgement



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