

Protein & PTM Profiling and Identification Core

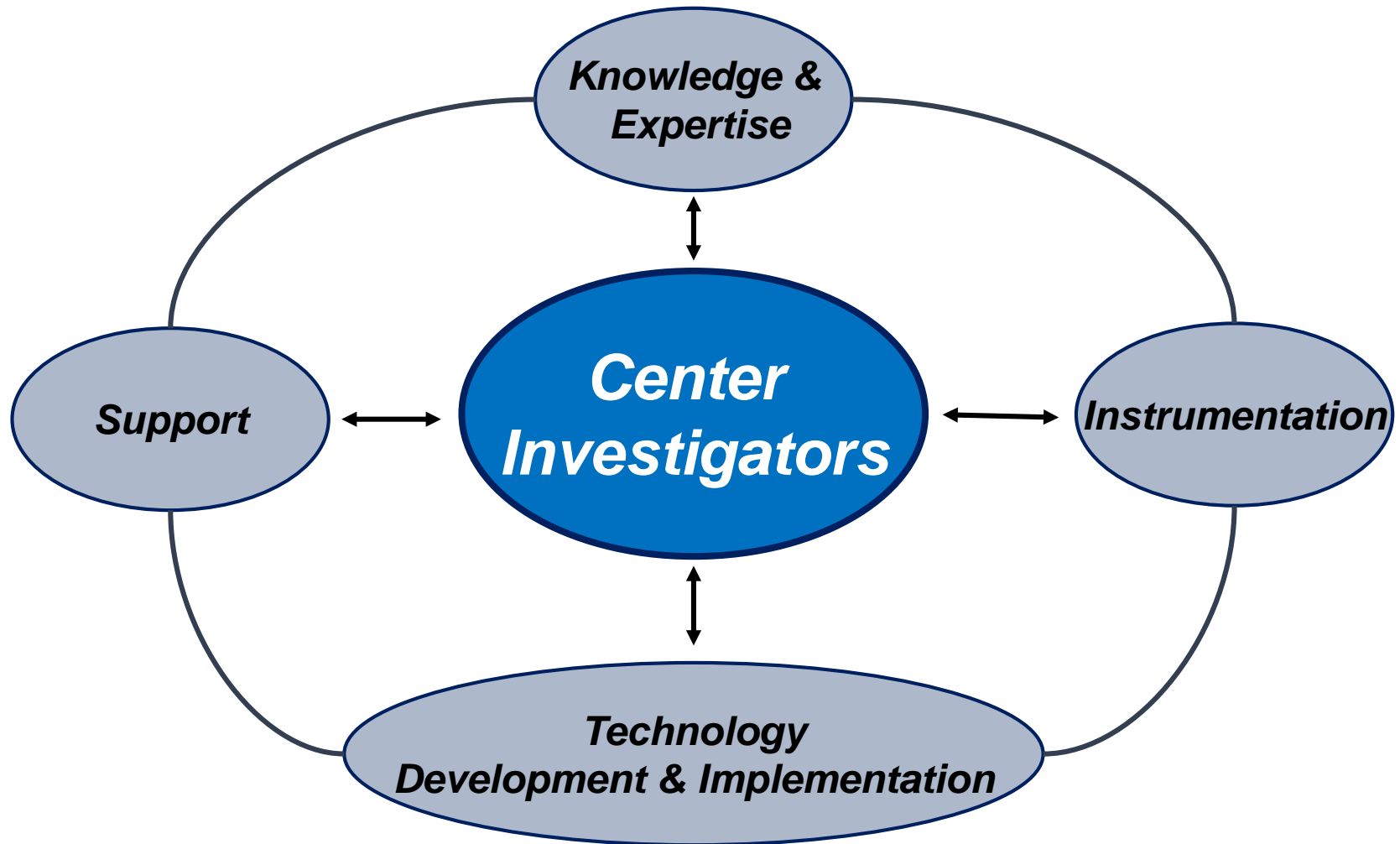
Yale/NIDA Neuroproteomics Center EAB Meeting

TuKiet T. Lam, PhD

May 1st, 2019



Mission and Operating Principle



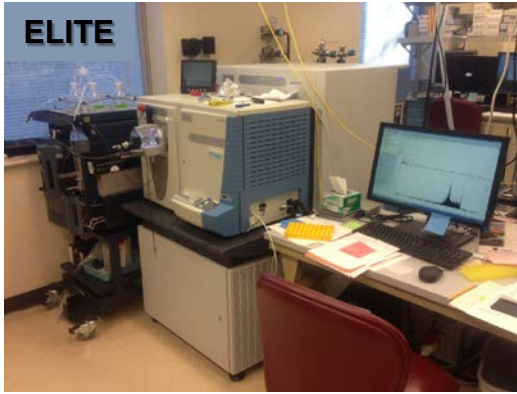
Cost recovery

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

Pilot Projects (100% Center funds)

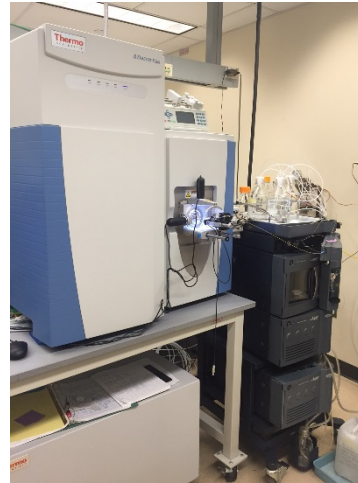
Mass Spectrometers currently located within the Core

For Protein ID

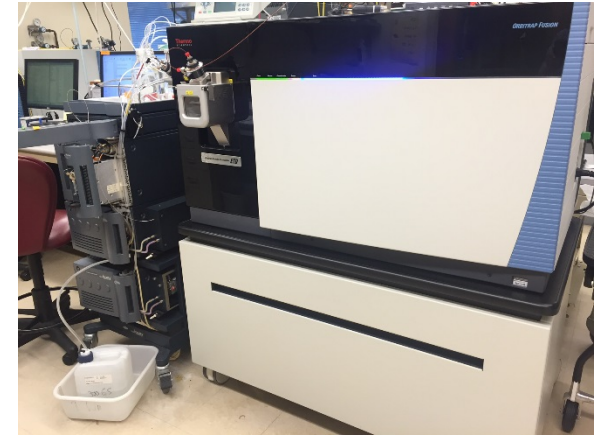


Thermo Fisher Scientific nano-UPLC ESI **LTQ-Orbitrap ELITE** MS systems

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

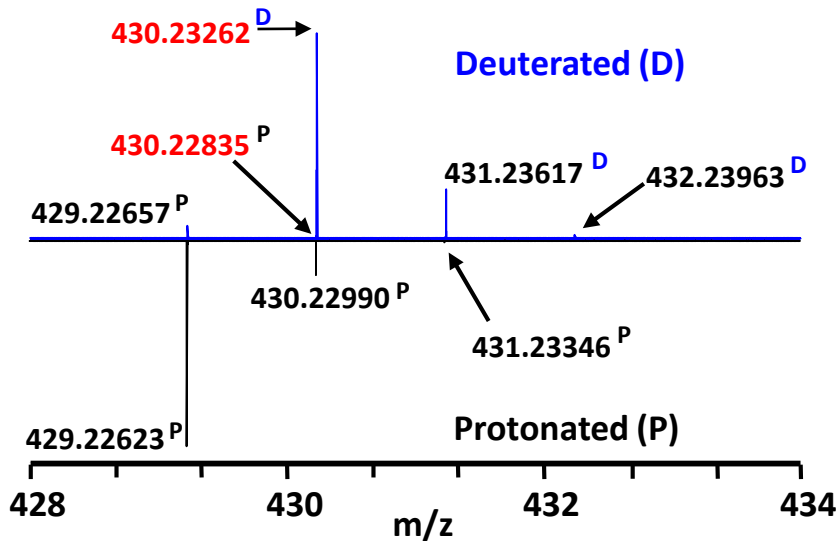


Q-Exactive HF-X mass spectrometer with ACQUITY UPLC M-Class.

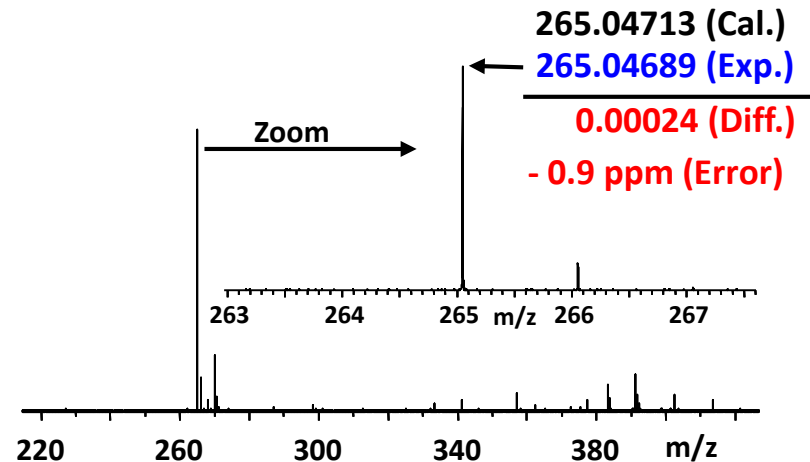
Instrumentation

Mass Spectrometer Capabilities

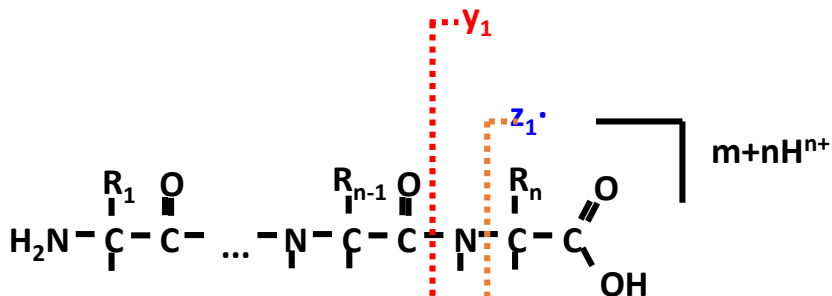
Resolution



Mass Accuracy



Fragmentation Capabilities



Facile loss of H_3PO_4
X-P cleavage preferred

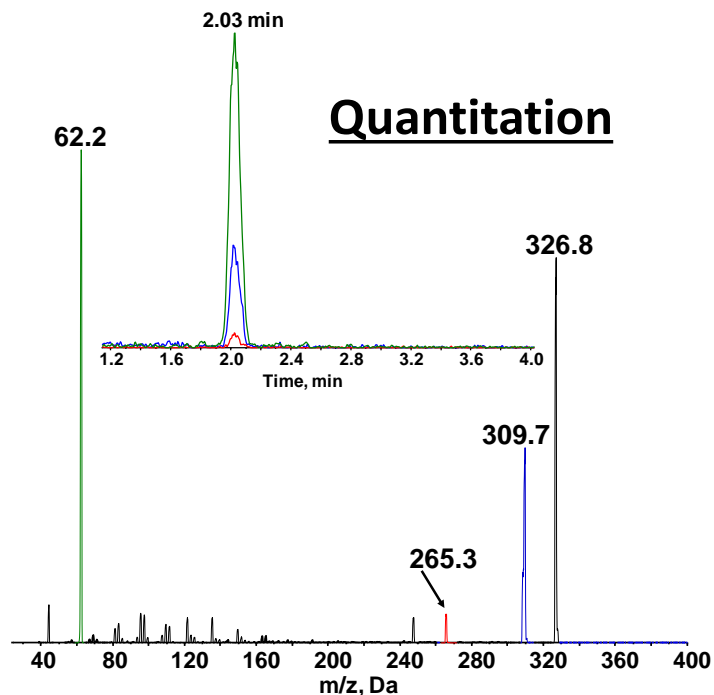
CID/HCD

b_{n-1}

ETD

Retention of labile modifications
No X-P cleavage

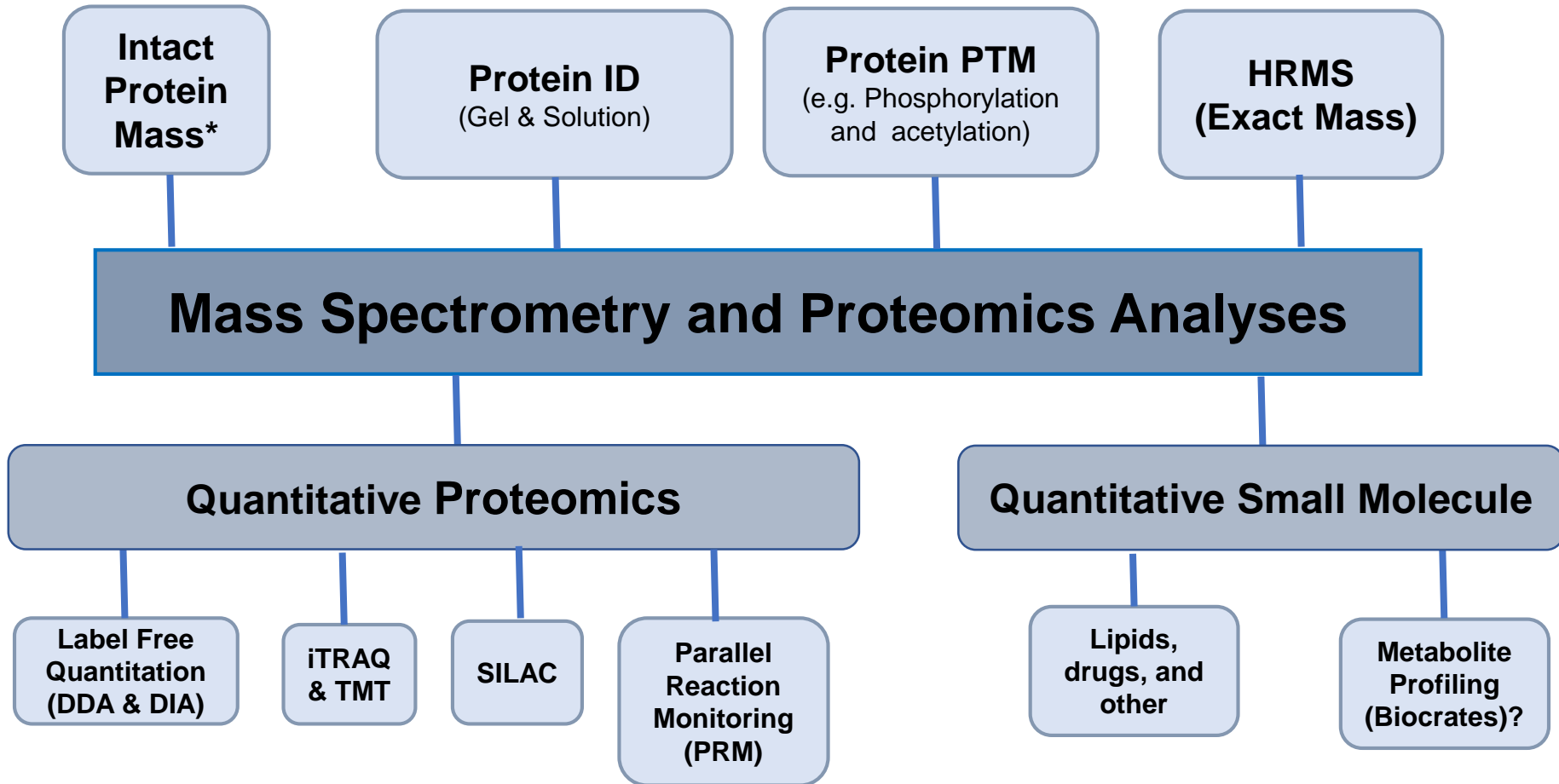
Quantitation



Comparison of Thermo MS (QE and Orbitrap)

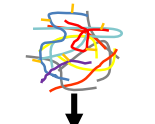
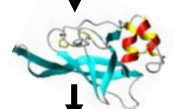
Instrument	QE-Focus	QE	QE-Plus	QE-HF	QE-HFX	QE-UHMR	O-Fusion	O-Fusion Lumos	O-ID-X
Max resolution (FWHM) @ <i>m/z</i> 200	70k	140k	140k (Opt. 280k)	240k	240	200k at <i>m/z</i> 400	500k	1,000k	7.5-500k
Mass accuracy, (internal)	<1 ppm								
Mass accuracy (external)	<3 ppm								
Mass range	50 to 3,000 <i>m/z</i>	50 to 6,000 <i>m/z</i>		50 to 8,000 <i>m/z</i>		350 to 80,000 <i>m/z</i>			50-2,000 <i>m/z</i>
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		

Overview of the Services available to Center Investigators



*Upper mass limitation.

Identification of Protein and Protein Posttranslational Modifications

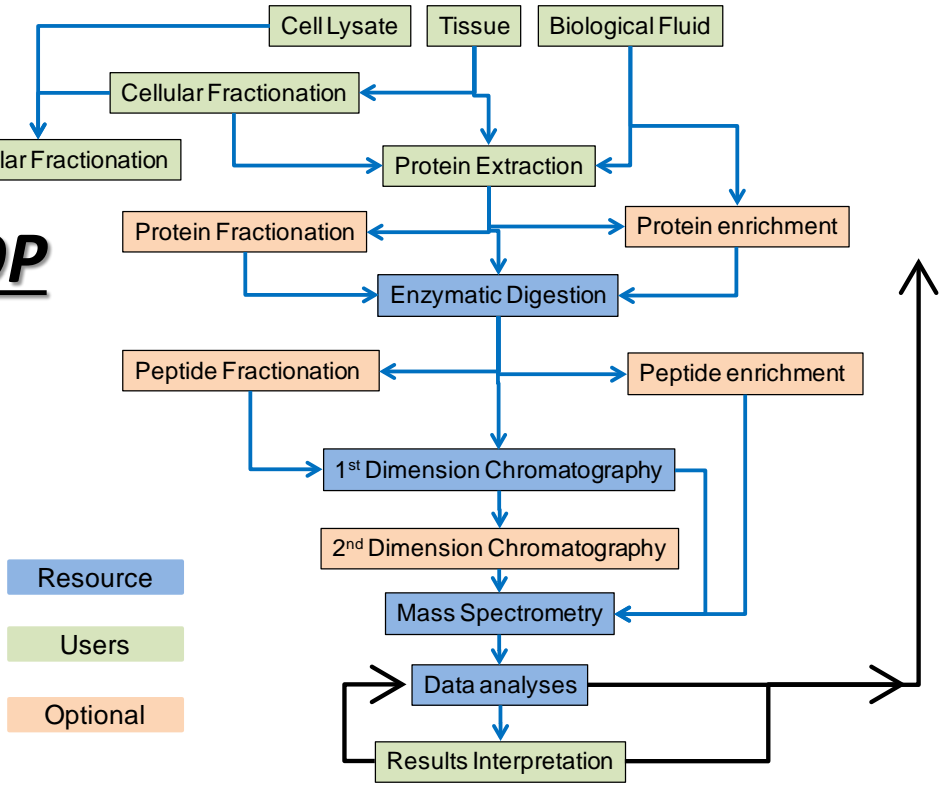


Start

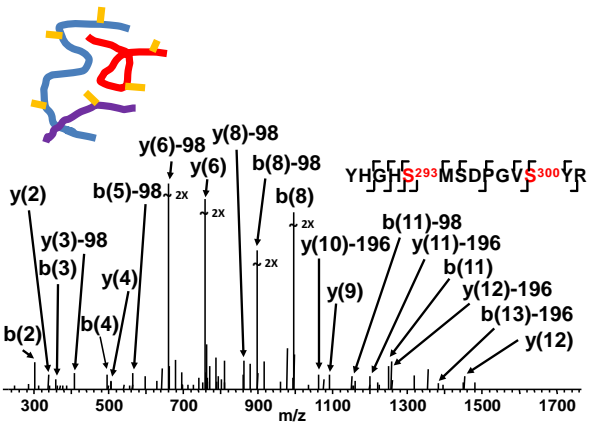
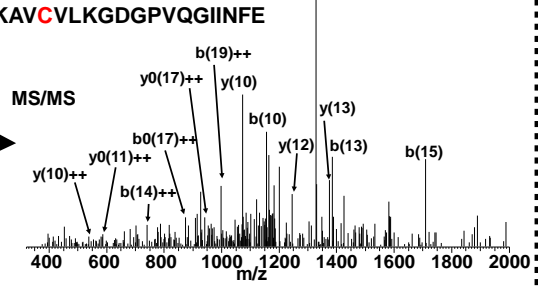
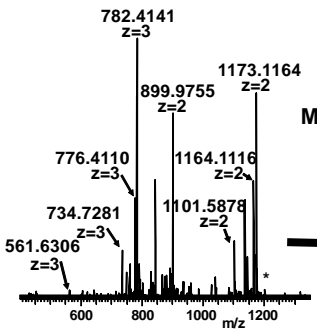
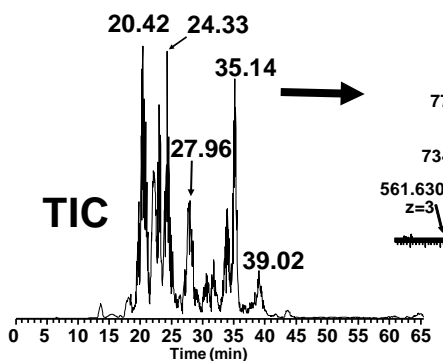
End

Sample Preparation
Data Acquisition
Data Processing

SOP

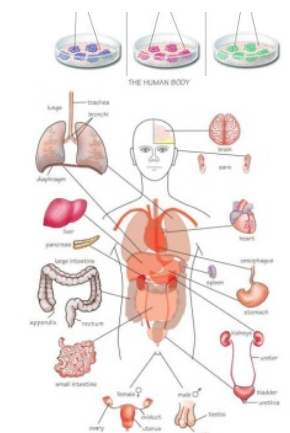


Resource
Users
Optional

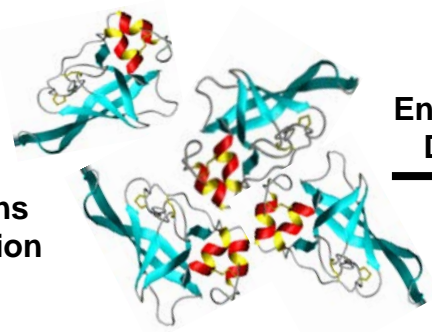


Quantitative Proteomics Workflow

Biological Source



proteins extraction



Enzymatic Digest

SOP

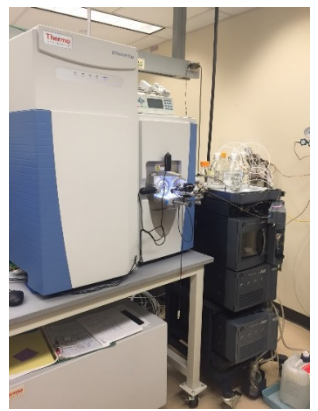


PTM Peptide enrichment



Flow through (FT) fraction

Enriched (EN) fraction

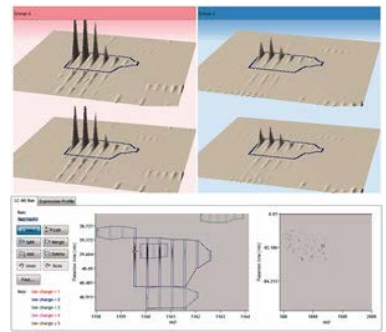


on-line LC MS and LC MS/MS separation and data acquisition

Robustness



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:

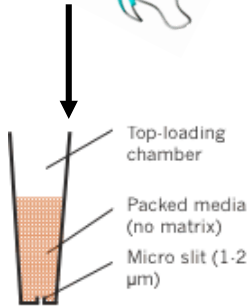
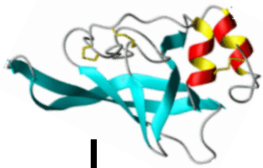
- Progenesis QI Software
- Proteome Discoverer
- Scaffold Software
- Skyline
- MASCOT Quantitation Tool Box

Reporting
Tabulated protein list with calculated abundances and expression changes

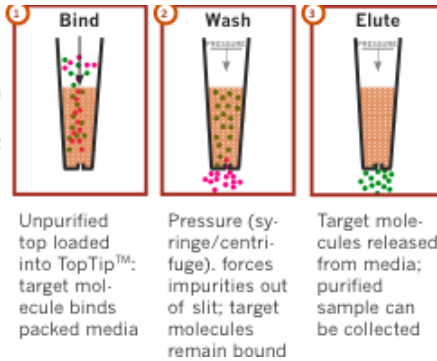
Accession	Protein Name	Score	Significance
P1	ALBU_HUMAN	1436	1.0e-100
P2	PCSK2_HUMAN	6247	1.0e-100
P3	APPK1_HUMAN	3020	1.0e-100
P4	APPK1_HUMAN	1117	1.0e-100

Standardized Thoroughness

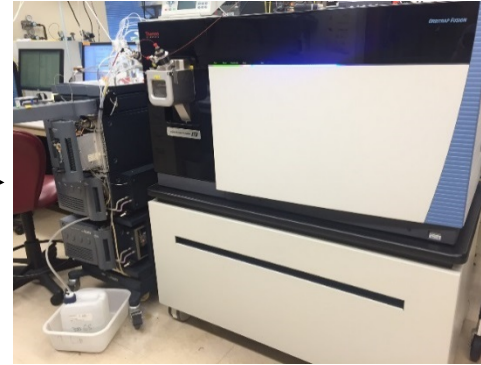
Intact Protein Analysis Workflow: Determination of Intact Protein MW



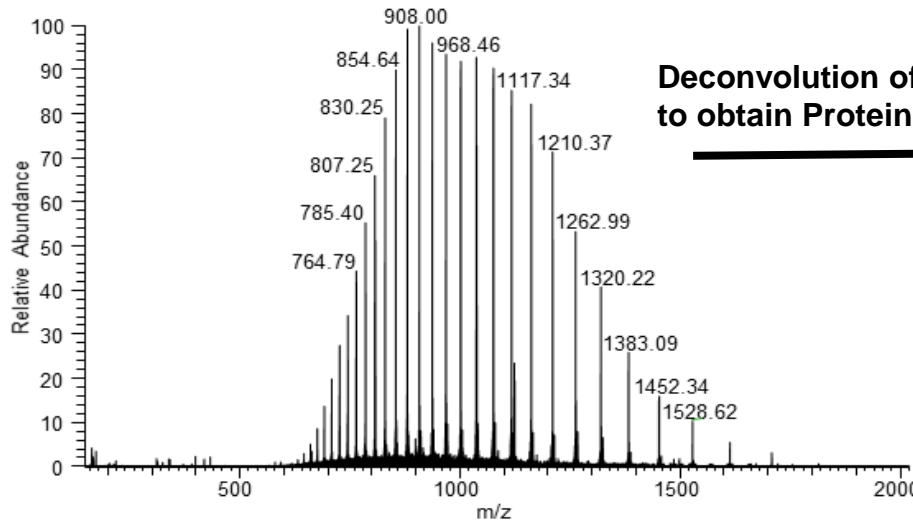
Desalting



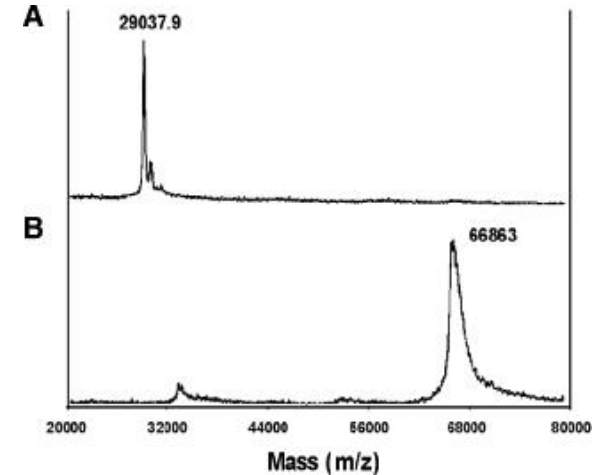
Direct Injection ESI MS for data acquisition



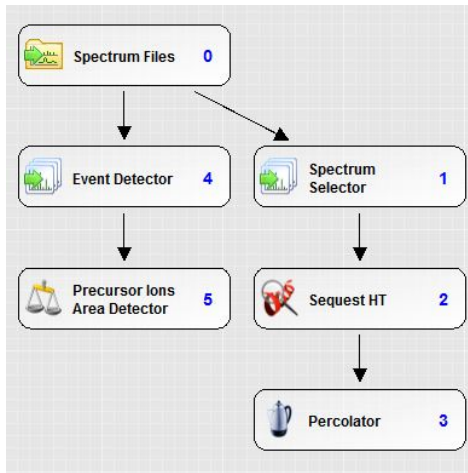
MS spectral data are **processed and analyzed** using **Deconvolution Software** to obtain accurate high resolution protein mass



Deconvolution of MS to obtain Protein MW



Software for analysis and visualization

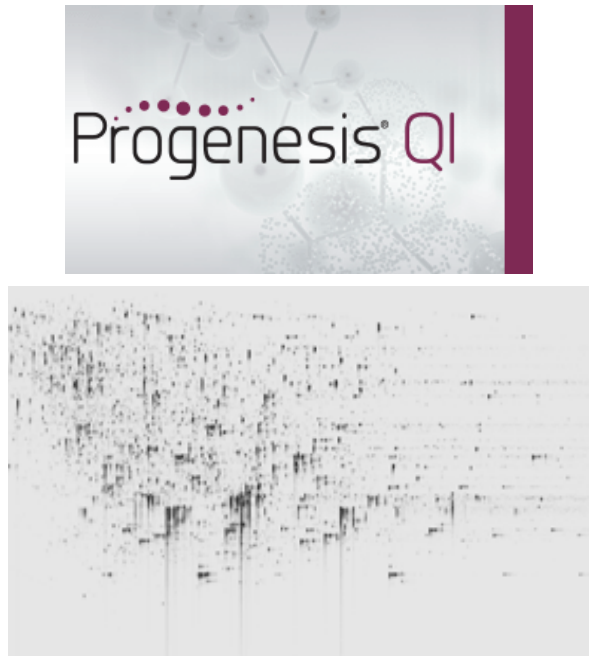
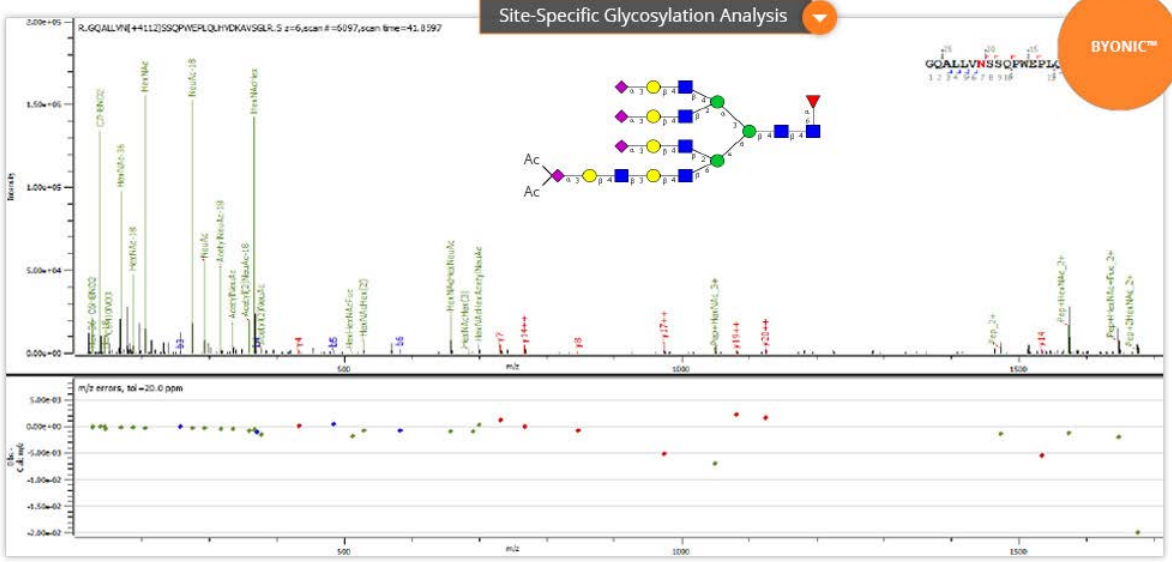


Protein Name	Accession Number	Molecular Weight	Biological Process	Cellular Component	Molecular Function	Probability
(P02410) Alpha crystallin A chain	CRYAB_BOV	20 kDa	cellular process	cytoplasm	protein folding	100%
(P07318) Beta crystallin B1	CRBB1_BOV	28 kDa	cellular process	cytoplasm	protein folding	100%
(P19141) Beta crystallin B3 (Beta-2)	CRBB3_BOV	24 kDa	cellular process	cytoplasm	protein folding	100%
(P11843) Beta crystallin A3 (Beta-1)	CRBA3_BOV	25 kDa	cellular process	cytoplasm	protein folding	100%
(P11842) Beta crystallin A4 (Beta-2)	CRBA4_BOV	24 kDa	cellular process	cytoplasm	protein folding	100%
(P02510) Alpha crystallin B chain	CRYAB_BOV	20 kDa	cellular process	cytoplasm	protein folding	100%
(P02522) Beta crystallin B2 (Beta-1)	CRBB2_BOV	23 kDa	cellular process	cytoplasm	protein folding	100%
(P26444) Beta crystallin A2 (Beta-1)	CRBA2_BOV	22 kDa	cellular process	cytoplasm	protein folding	100%
(P02526) Gamma crystallin B (Gamma-1)	CRGB_BOV	21 kDa	cellular process	cytoplasm	protein folding	100%
(P48644) Gamma crystallin B (Gamma-2)	CRGB_BOV	21 kDa	cellular process	cytoplasm	protein folding	100%
(P00727) Cytosol aminopeptidase	AMPE_BOV	53 kDa	metabolic process	cytoplasm	peptidase activity	100%
(P48616) Vinculin	VH1_BOV	54 kDa	cellular process	cytoplasm	protein binding	100%
(P00712) Actin, cytoplasmic 1 (Beta-1)	ACTB_BOV	42 kDa	cellular process	cytoplasm	protein binding	100%
(P08209) Gamma crystallin D (Gamma-1)	CRGD_BOV	21 kDa	cellular process	cytoplasm	protein folding	100%
(Q02634) Alpha enolase (EC 4.2.1.3)	ENO1_BOV	47 kDa	metabolic process	cytoplasm	enolase activity	100%
(Q28127) Phalloidin (Blebbistatin)-binding protein 1	BP1_BOV	46 kDa	cellular process	cytoplasm	protein binding	100%
(P10096) Glyceroldehyde-3-phosphate dehydrogenase	G3P_BOV	34 kDa	metabolic process	cytoplasm	oxidoreductase activity	100%
(P06504) Beta crystallin 5 (Gamma-2)	CRB5_BOV	21 kDa	cellular process	cytoplasm	protein folding	100%
(Q01764) Zeta-crystallin	ZCR_BOV	35 kDa	cellular process	cytoplasm	protein folding	100%
TRYP5B PRECURSOR	CONT[pt]L	24 kDa	unknown	unknown	unknown	100%
(P55052) Fatty acid-binding protein 1	FABP1_BOV	15 kDa	cellular process	cytoplasm	protein binding	100%
(Q08088) Gamma crystallin C (Gamma-1)	CRGC_BOV	21 kDa	cellular process	cytoplasm	protein folding	100%
(P08208) gamma E crystallin adduct	GRGE_BOV	21 kDa	unknown	unknown	unknown	98%
(P13696) Phosphatylethanolamine phosphotransferase	PEEP_BOV	21 kDa	cellular process	cytoplasm	transferase activity	100%
(P16164) Aldose reductase (EC 1.1.1.21)	ALDR_BOV	36 kDa	metabolic process	cytoplasm	oxidoreductase activity	100%
(P02584) Profilin-1 (Profilin 1)	PROF1_BOV	15 kDa	cellular process	cytoplasm	protein binding	100%
(Q06062) Hensen (Bleedless) fibronectin type-1	FN1_BOV	83 kDa	cellular process	cytoplasm	protein binding	100%
(P04272) Anemone A2 (Anemone II)	AN2_BOV	38 kDa	cellular process	cytoplasm	protein binding	100%
(P68103) Elongation factor 1-alpha	EF1A1_BOV	50 kDa	cellular process	cytoplasm	protein binding	100%
(P62935) Peptidyl prolyl cis-trans isomerase	PP1A_BOV	18 kDa	cellular process	cytoplasm	isomerase activity	100%
(P11013) 14-3-3 protein zeta/delta	143Z_BOV	28 kDa	cellular process	cytoplasm	protein binding	100%
(Q29450) Adenylate cyclase, type-ADCY7	ADCY7_BOV	121 kDa	cellular process	cytoplasm	enzyme activity	100%



PROTEIN METRICS

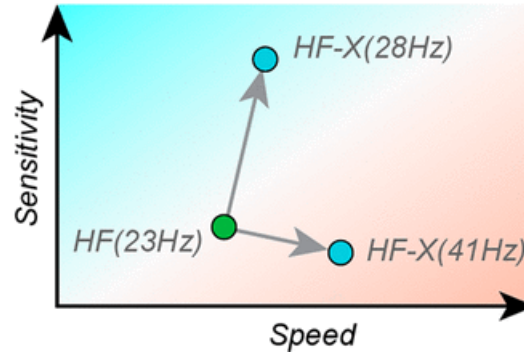
Boldly Advancing Protein Characterization



New Services coming in 2019...

Technology Implementation

- Improved LFQ (higher throughput with increased robustness)

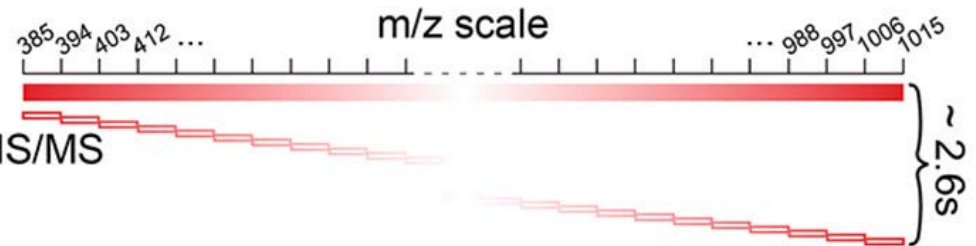


- Data Independent Acquisition (improved # of protein identifications)

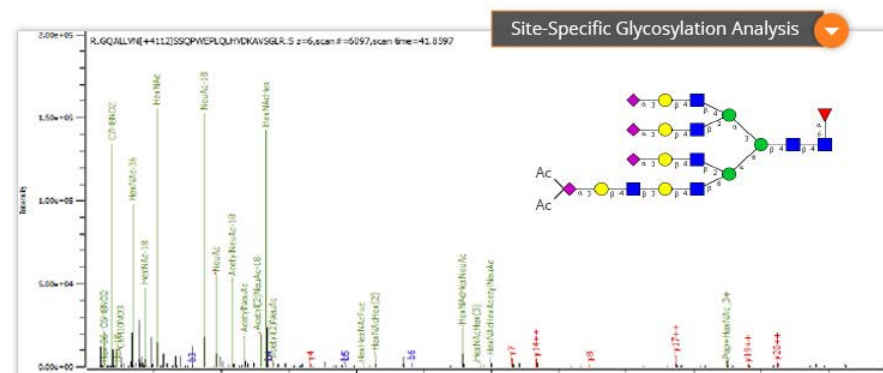
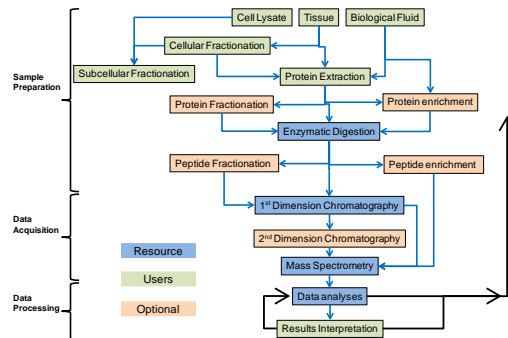


A

1 full MS
70 x 9 m/z MS/MS
HF-X(28Hz)



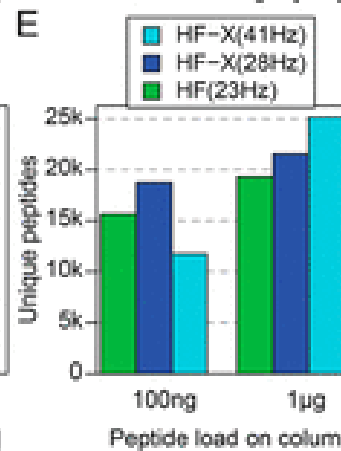
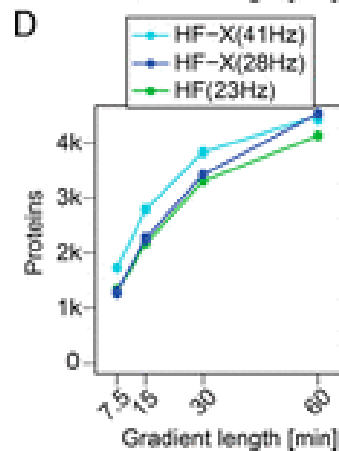
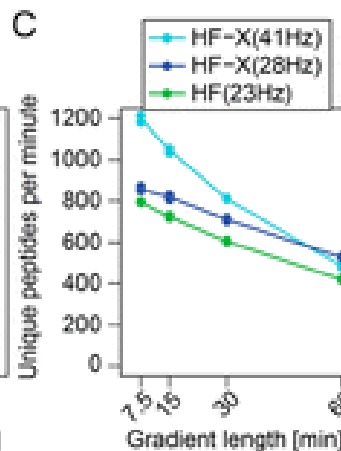
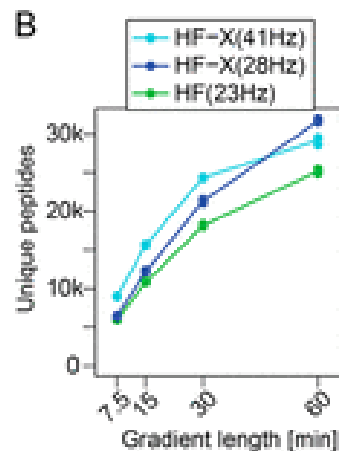
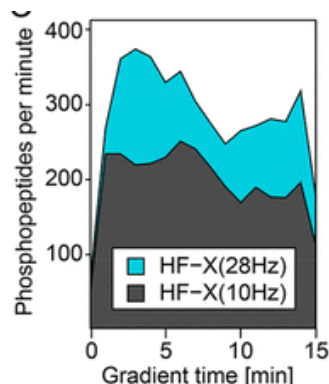
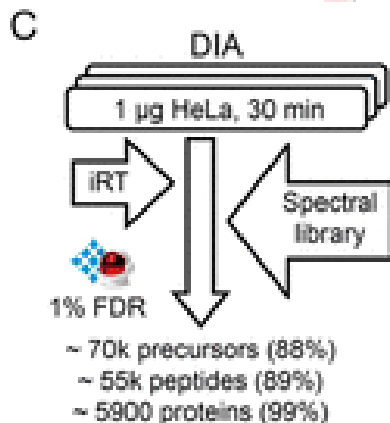
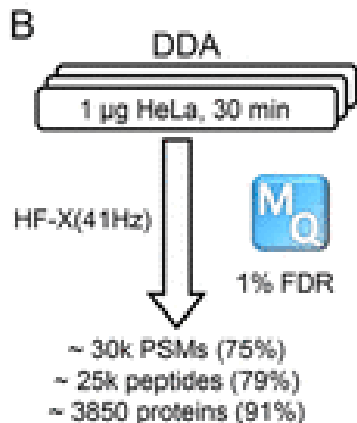
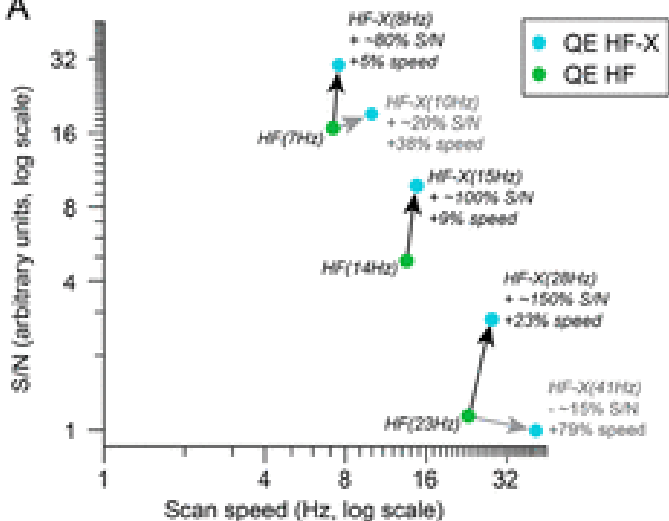
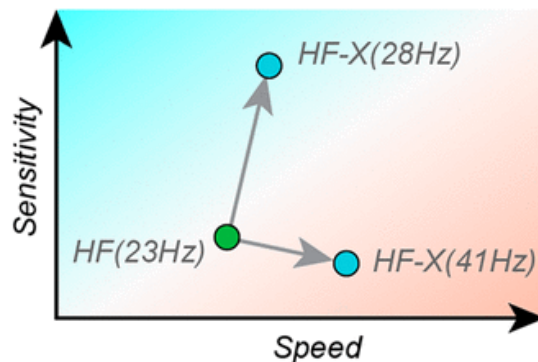
- Glycosylation workflow.



New Instrumentations: Improvement in Sensitivity and Throughput



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

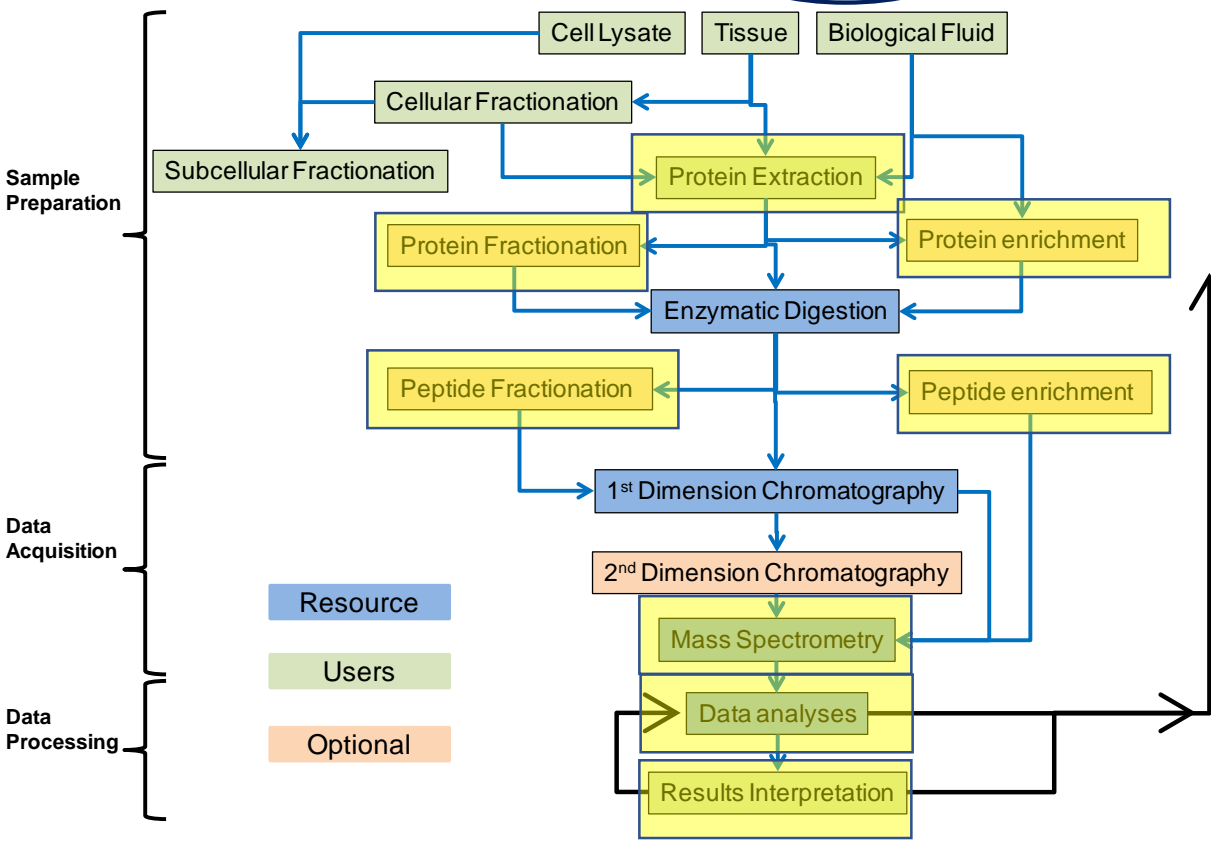
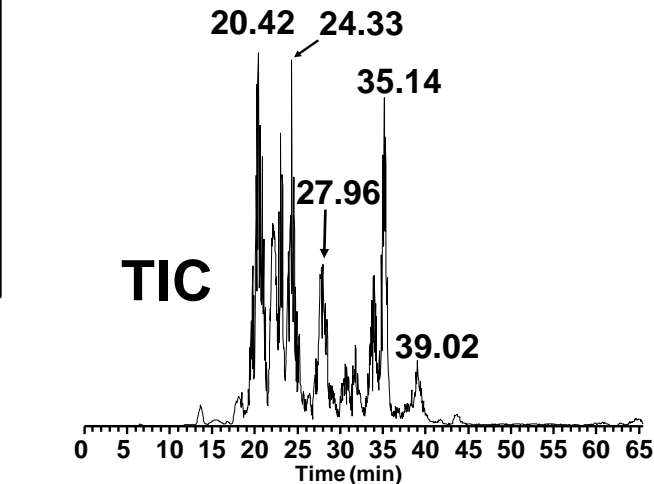
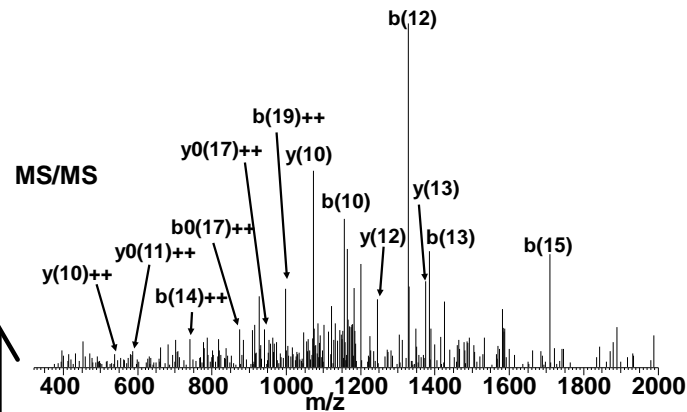
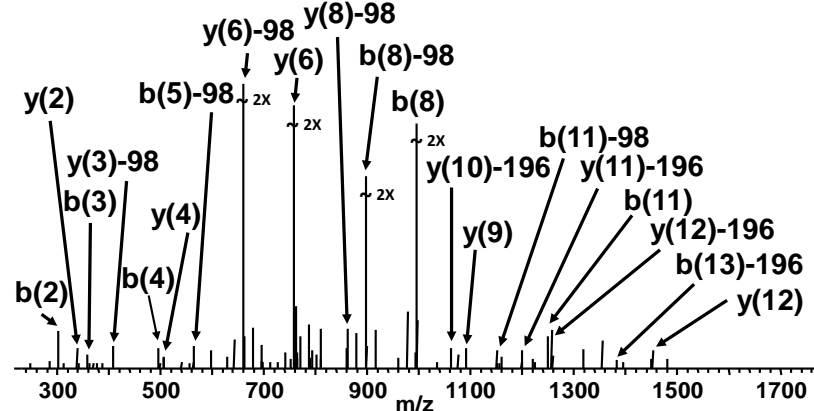


Performance Evaluation of the Q Exactive HF-X for Shotgun Proteomics

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

Technology Development



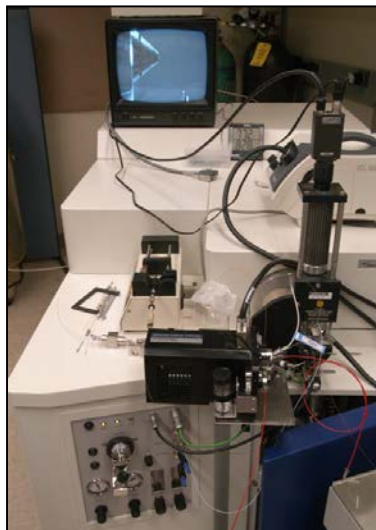
Progress...



MS and Protein Profiling Core: Instrumentations (2006)



**AB QSTAR XL
ESI QTOF
System**
ICAT, MudPIT



**Micromass QTOF API
MS System**
Phosphoprotein
Profiling



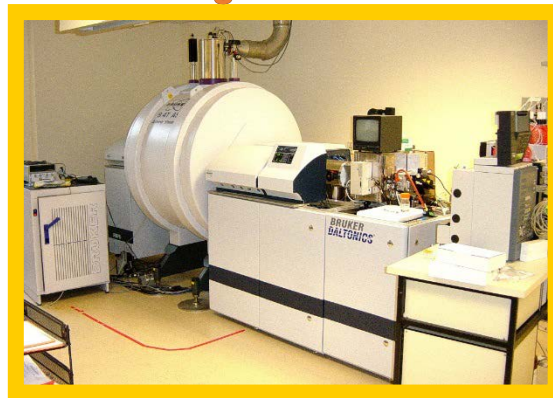
**Micromass
MALDI TOF MS**
Serum
Biomarkers, QC



**Micromass QTOF
Micro MS System**
Lipid Profiling
Intact Protein MW



Beckman-Coulter PF2D
Chromatofocusing & NPS-RPLC
2D Protein Separation System



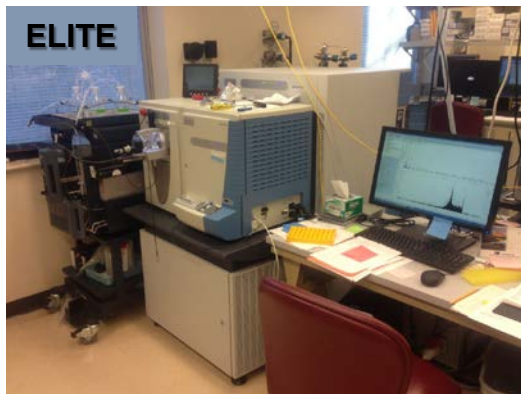
**Bruker APEX Qe 9.4Tesla
FT-ICR MS System**
Accurate mass, Top Down, PTM



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

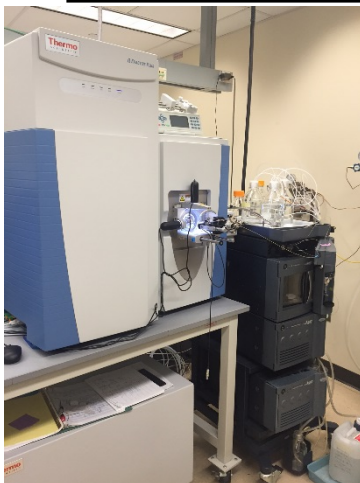
Mass Spectrometers located within the MS & Proteomics Resource (2019)

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



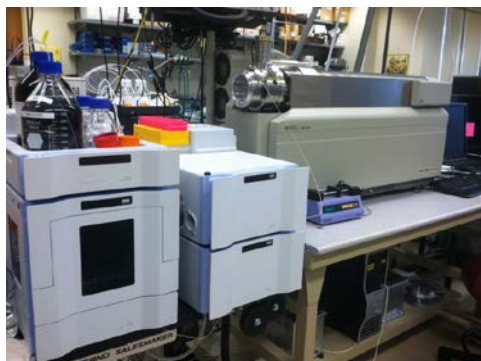
Thermo Fisher Scientific nano-UPLC ESI **Orbitrap Fusion** MS systems

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

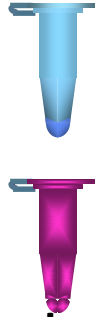


Q-Exactive HF-X mass spectrometer with ACQUITY UPLC M-Class.

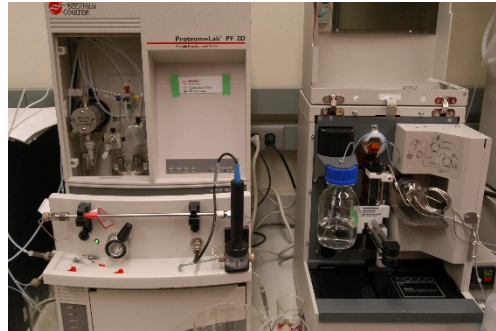


ProteomeLab PF2D (Beckman-Coulter): 2D Chromatofocusing & non-porous RP-HPLC

0.2 – 1 mg needed per sample condition



Samples are run serially

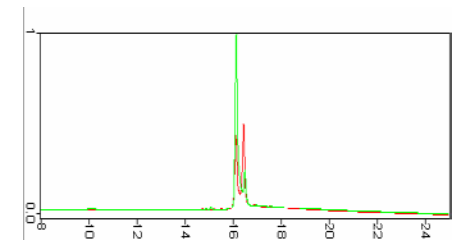


1st Dimension:
Chromatofocusing
(pH 8.5 to 4.0) (UV 280nm)

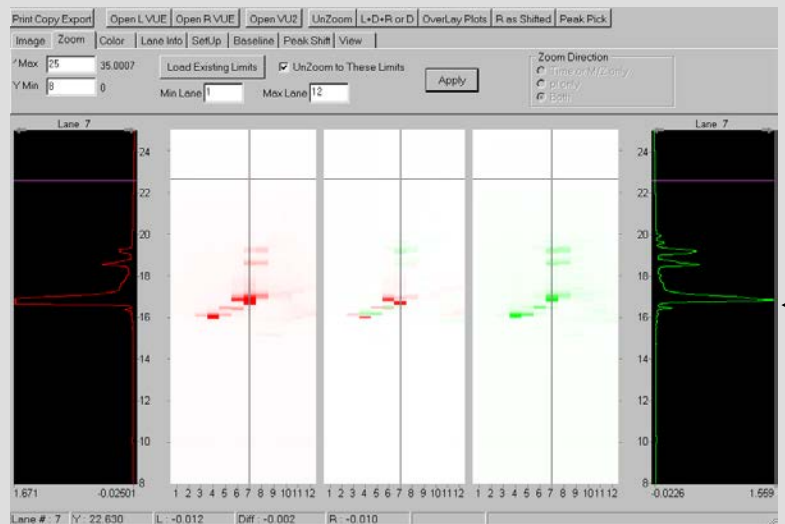
Fractions collected every 0.3
pH unit change

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

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

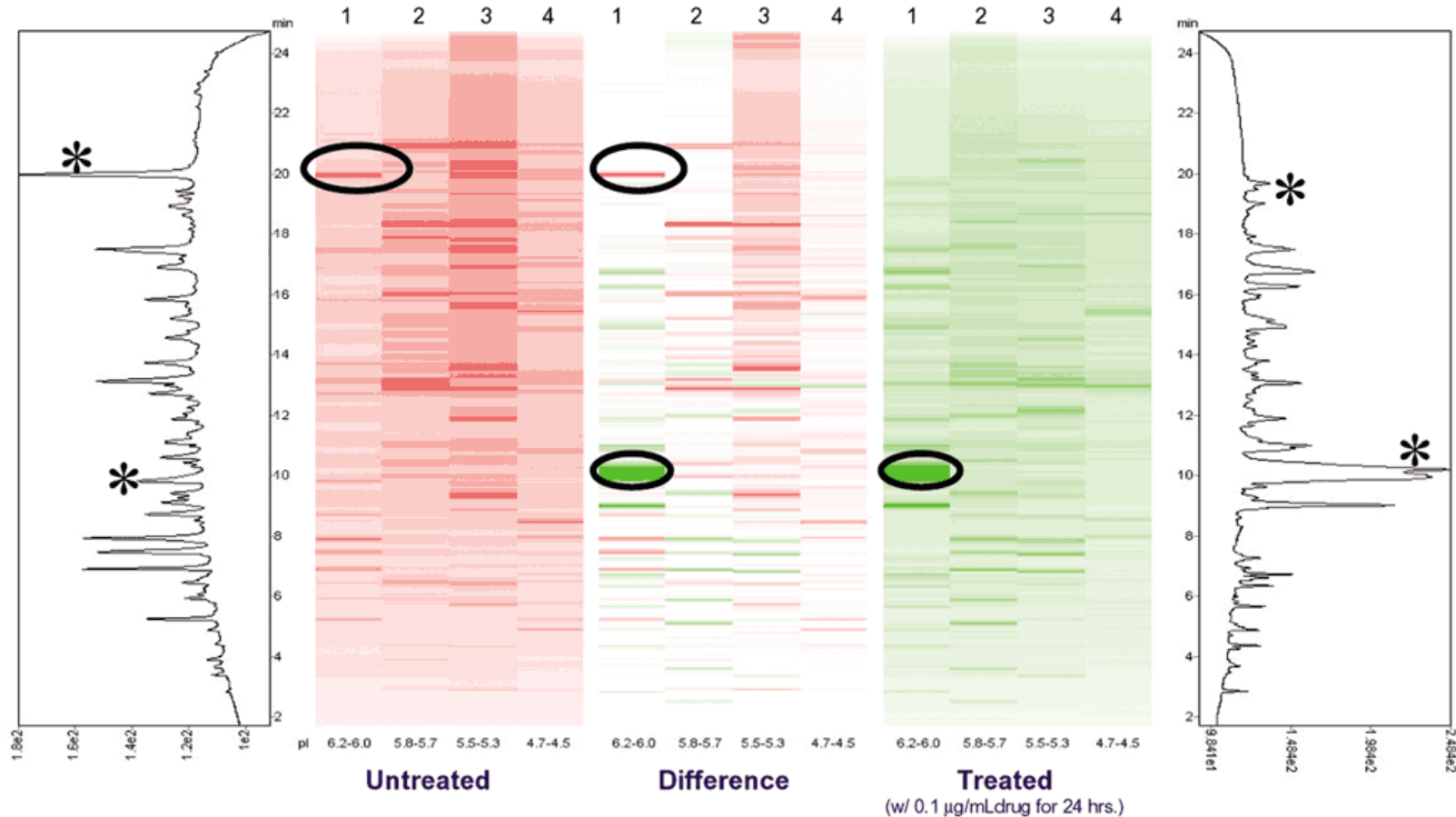


Comparative
“gel” and UV
view of samples
1 and 2



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 – NCBI nr

Search Engine - MASCOT

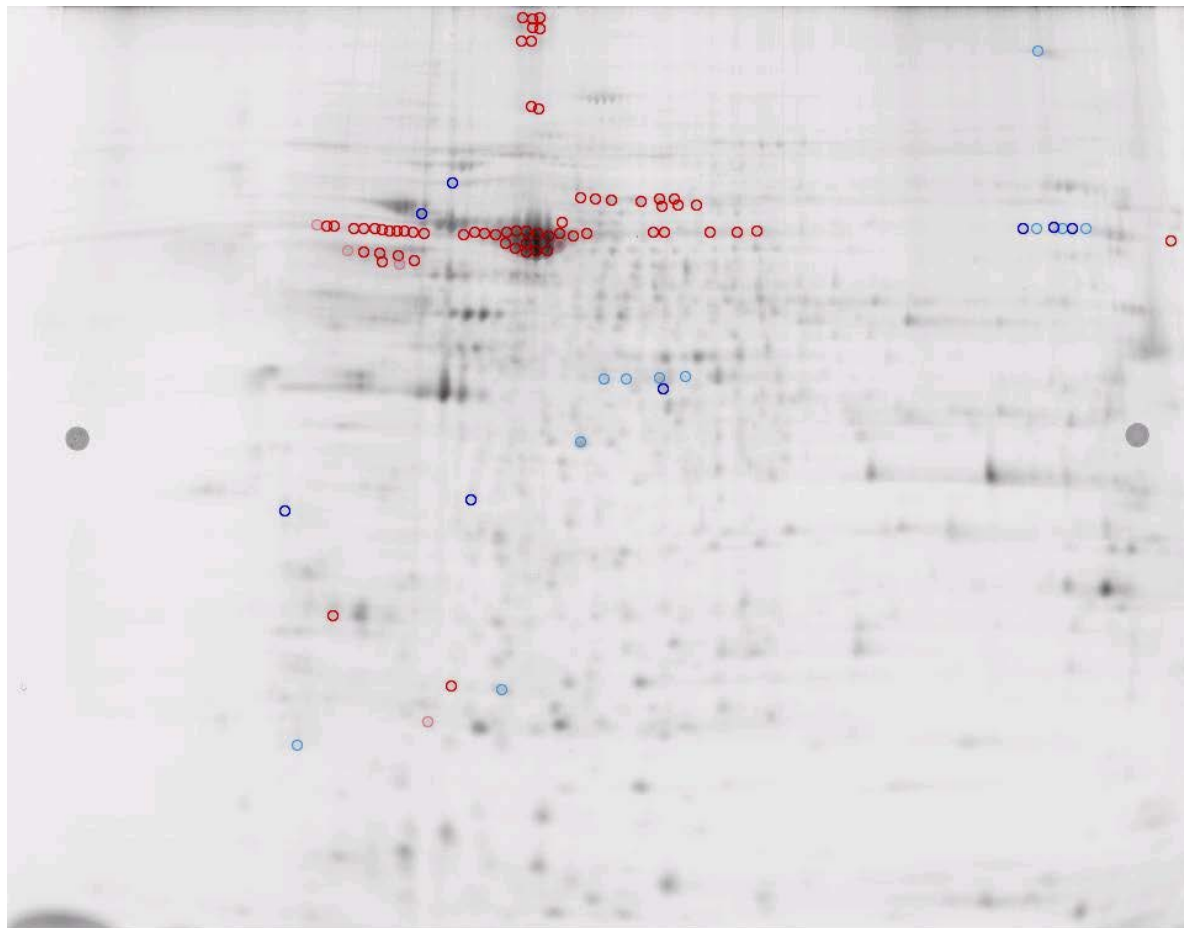
#spots picked/analyzed	85
#spots with proteins id'd	69
#proteins id'd with 1 peptide	0
#spots with C5/C3 \geq 2 fold difference (all spots)	22
#spots with C5/C3 \geq 2 fold difference (proteins Id'd)	18

Red – Down regulated

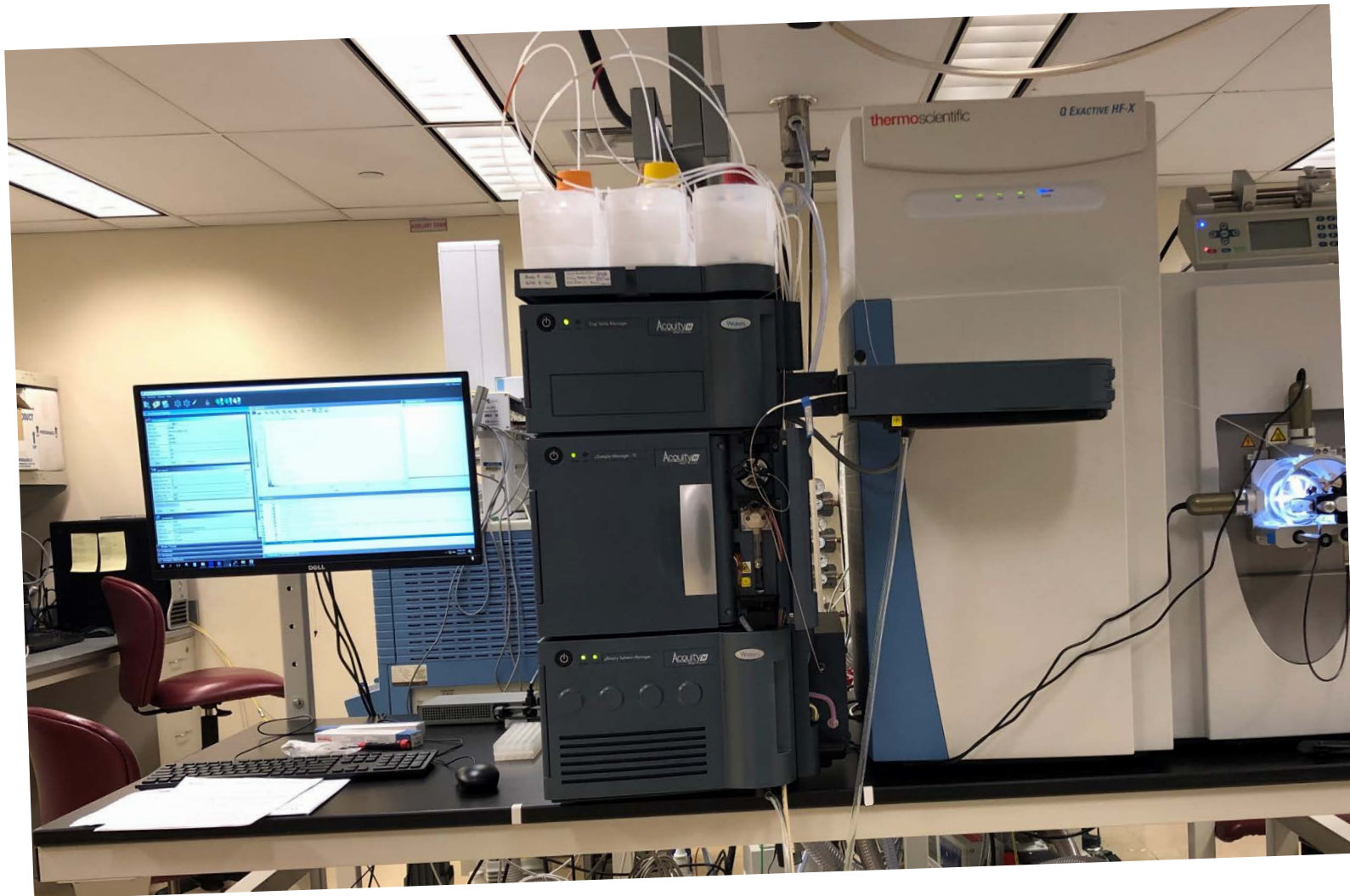
Blue – Up regulated

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

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[See my publications in PubMed](#)

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Yale CANCER
CENTER

A Comprehensive Cancer Center Designated
by the National Cancer Institute



Yale/NIDA NEUROPROTEOMICS CENTER

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