

Mass Spectrometry as a Leading Technique in Life sciences

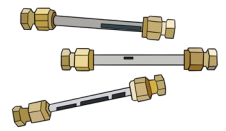
PRESENTED BY

Pongsagon Pothavorn

Sci **Spec** Co., Ltd.

Lifomics Co., Ltd.

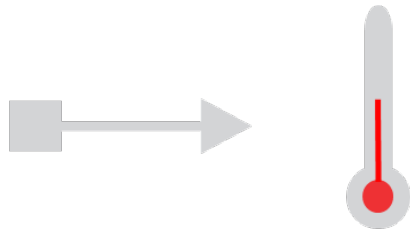




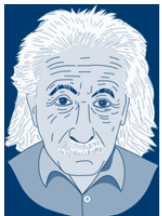
The TRUTH!!



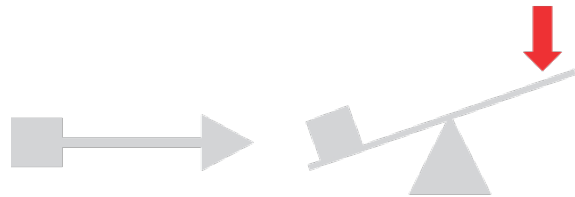
CHEMIST



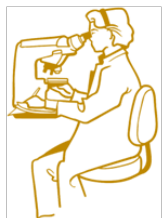
สามารถคำนวณการเพิ่มขึ้นของอุณหภูมิได้อย่างแม่นยำระหว่างการเกิดปฏิกิริยา



PHYSICIST



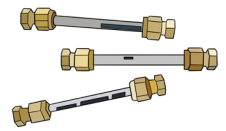
สามารถคำนวณแรงที่จะสามารถทำให้กล่องบนคานสมดุลได้อย่างถูกต้อง



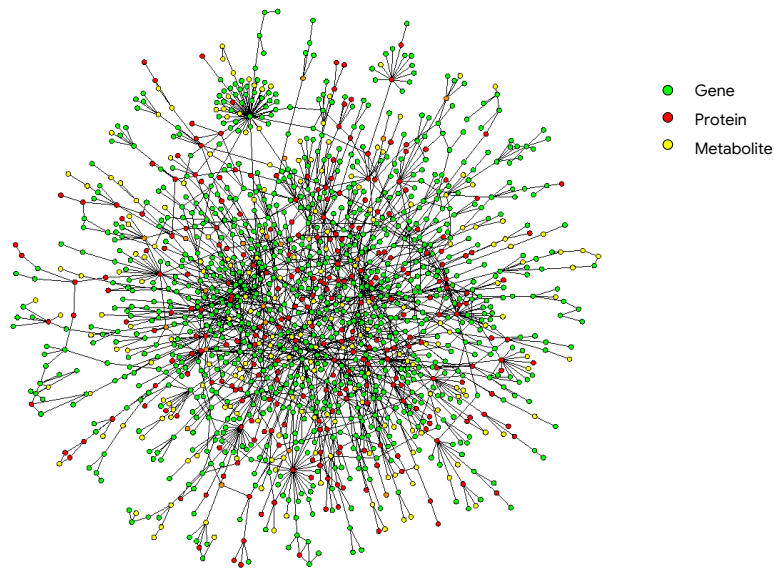
BIOLOGIST



สามารถจัดบันทึกพีโนไทป์ที่สังเกตเห็นได้อย่างเชี่ยวชาญ แต่ไม่สามารถทำนายกลไกภายในเซลล์ได้

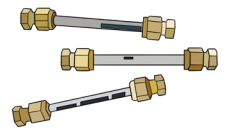


ชีววิทยาเชิงระบบ - System Biology

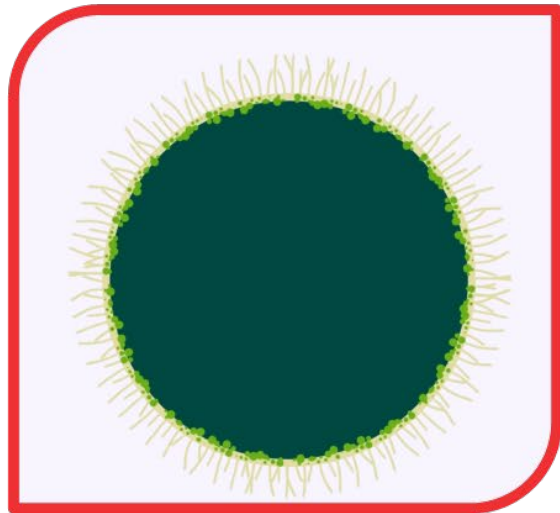


อาศัยความรู้หลายด้านเช่น ด้านชีววิทยา คณิตศาสตร์ขั้นสูง วิทยาการคอมพิวเตอร์และชีวสารสนเทศศาสตร์ เพื่อศึกษาความสัมพันธ์ระหว่างส่วนประกอบย่อยๆ ของสิ่งมีชีวิตในภาพรวม

- สามารถทำความเข้าใจตั้งแต่ระดับโมเลกุลจนถึงระดับเซลล์สิ่งมีชีวิต
- เน้นการสร้างแบบจำลองเพื่อแสดงถึงปรากฏการณ์ภายในเซลล์บนคอมพิวเตอร์ โดยอาศัยข้อมูลจำนวนมากและการคำนวณเป็นพื้นฐาน
- จำลองพฤติกรรมของเซลล์ภายใต้สภาพแวดล้อมต่างๆ โดยอาศัยฐานข้อมูลทางจีโนม ชีวเคมีร่วมเป็นองค์ประกอบ
- ตัวอย่างเช่นการเน้นการวิเคราะห์เชิงระบบของวิศวกรรมเมตาบอลิกเพื่อปรับปรุงสายพันธุ์ หรือนำไปใช้ในอุตสาหกรรมด้านต่างๆ

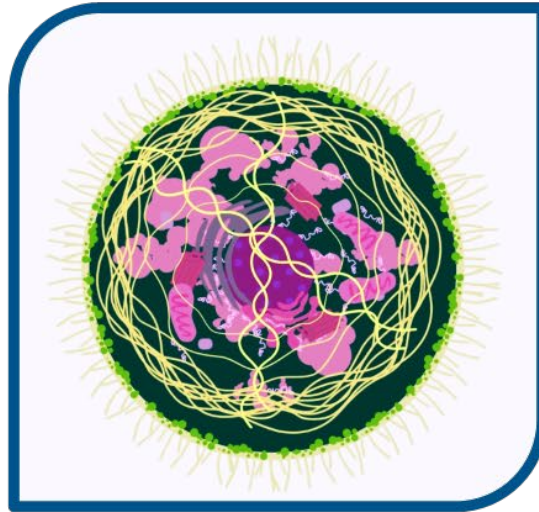


Challenges Predicting Outcome in Biological System



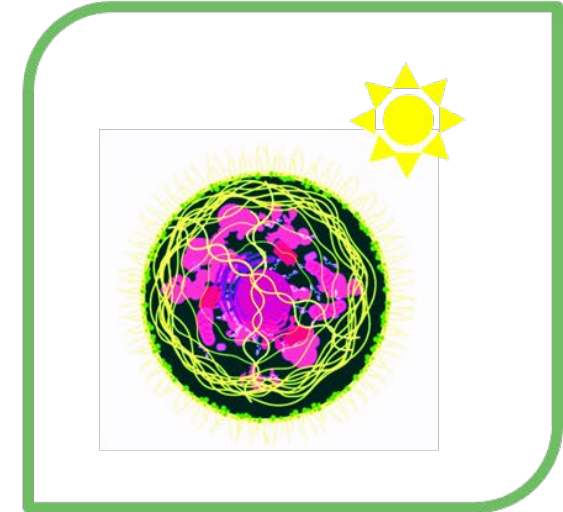
COMPLEXITY

Huge number of molecules inside them, and highly diverse



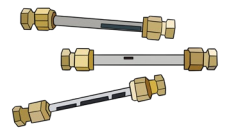
SELF-ORGANIZED

Very well organized in a very dense space. Every part must obey the rules to which it belongs

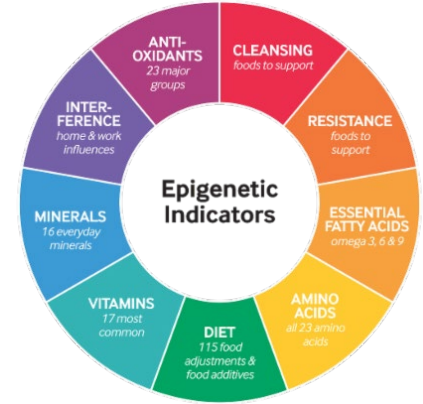
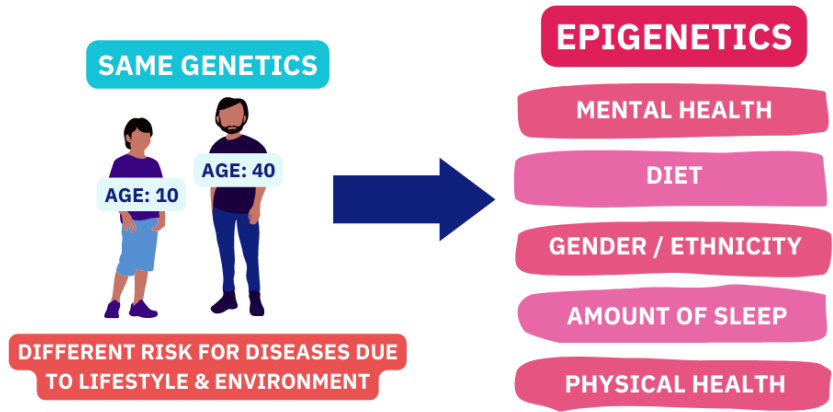
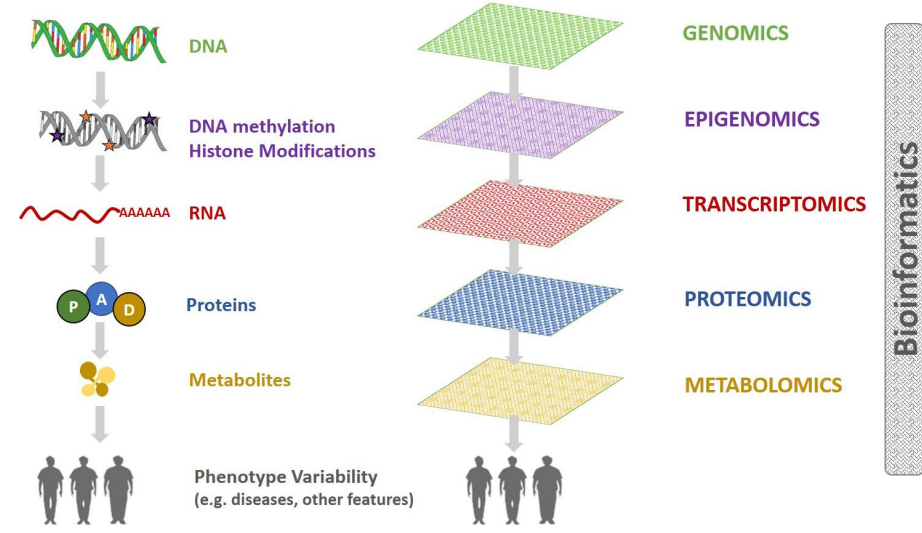
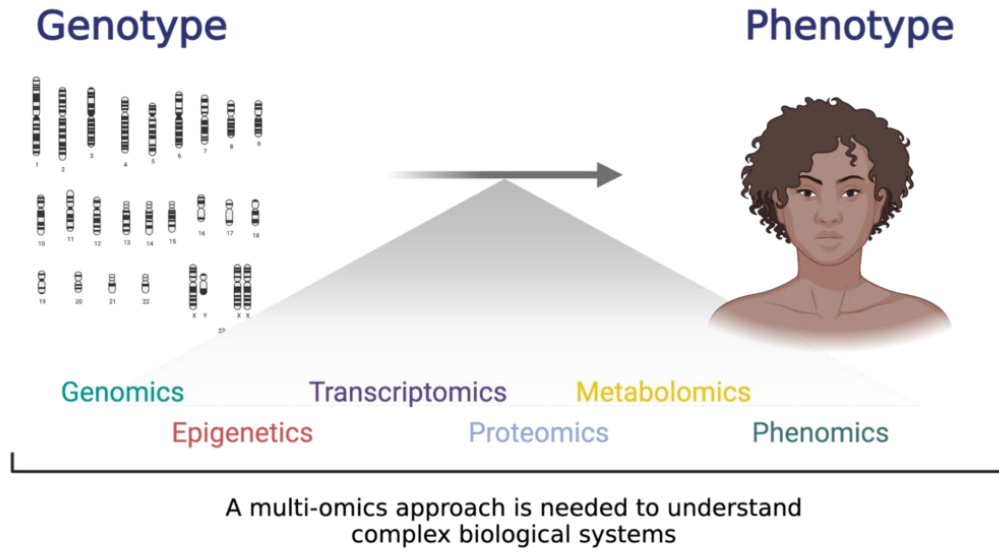


OPEN SYSTEM

The external environment can interfere with the system. The system may be regulated



Integration of Multi-Omics Data



14.1M

คือจำนวนเคสใหม่
ของผู้ป่วยโรคมะเร็ง¹

8.2M

คือจำนวนผู้ป่วยที่
ตายด้วยโรคมะเร็ง¹

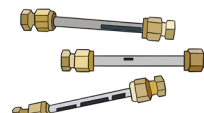
440,800

คือจำนวนผู้ป่วยที่
ตายเนื่องจากอาการ
ไม่พึงประสงค์จากยา²

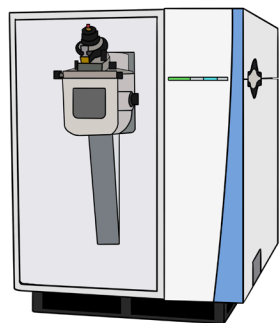
PER YEAR

¹Global Cancer Facts and Figures from www.cancer.org

²Extrapolated from "Identifying and reporting adverse drug events in oncology," Aleta J. Hong, Matthew J. Fisher, Christina H. Georgopoulos BS, and Charles L. Bennett, MD.

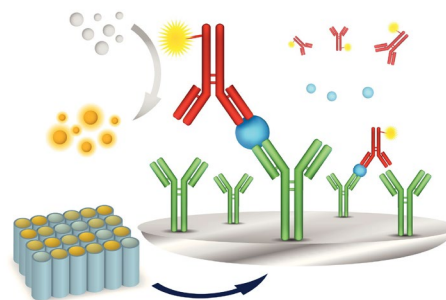


Mass Spectrometry VS. Classical Chemistry Approaches



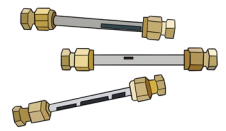
Mass Spectrometry-based

- Rapidly growth
- More accurate
- Less expense in single run
- Online sample preparation
- High throughput



Classical Chemistry

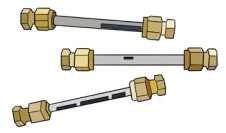
- Dominate in toxicology/clinical lab
- Specificity and sensitivity are compromised
- More expensive
- Need own enzyme conjugated
- Time-consuming



Emerging Technology and Guidance Responses

Regulatory Oversight

- 11,000 Laboratories Developed Test (LDTs) offered by 2,000 labs
- In vitro diagnostic devices (IVDs) compliance
- MS technology is growing and advancing, continuing performance improvement
- Complex biological samples can be used in automated sample preparation



Genomics Vs Proteomics Approaches

MALDI-ToF on Genomics Analysis

เทคนิค MALDI-ToF ได้รับการพัฒนาเพื่อใช้ในการตรวจวัดมวลของนิวคลีโอไทด์ โดยการแปลผลจะอาศัยมวลที่ต่างกันของทั้งสี่นิวคลีโอไทด์

Nc1ncnc2n(cnc12)C(=O)N
 Adenine
Purines

Nc1nc2[nH]cnc2c(=O)[nH]1
 Guanine
Purines

Nc1cc[nH]c1=O
 Cytosine
Pyrimidines

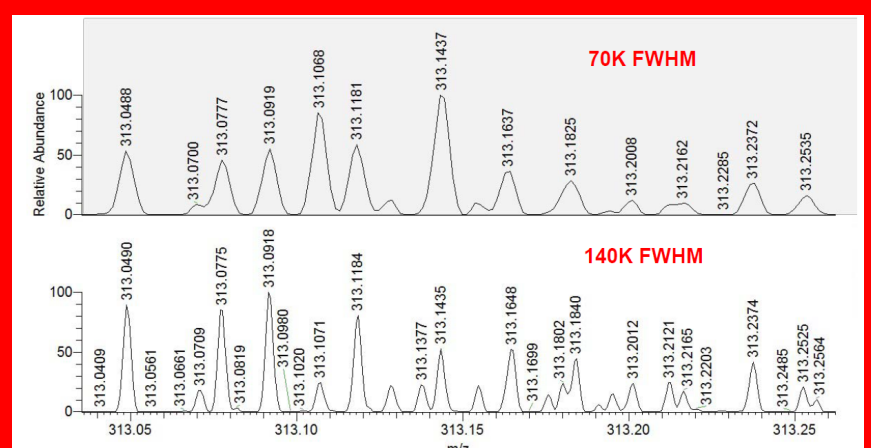
Cc1c[nH]c(=O)[nH]c1=O
 Thymine (DNA)
Pyrimidines

O=c1cc[nH]c1=O
 Uracil (RNA)
Pyrimidines

Cytosine = 247.2 Da
 Adenosine = 271.2 Da
 Guanine = 287.2 Da
 Thymine = 327.1 Da

Orbitrap™ Technology on Proteomics Analysis

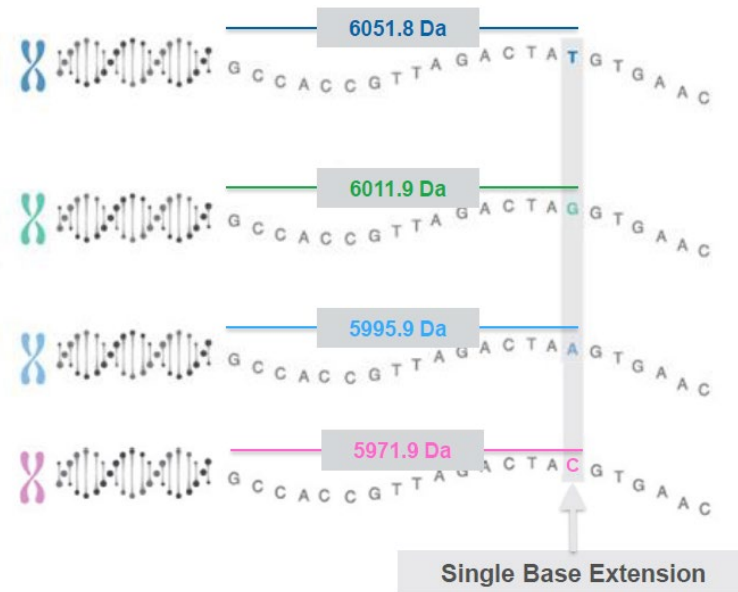
เทคโนโลยี Orbitrap™ ถูกพัฒนาเพื่อใช้ได้อย่างจริงจังในห้องปฏิบัติการเมื่อปี 2006 โดยนักฟิสิกส์ชาวรัสเซีย จุดเด่นของเทคนิคนี้คือความละเอียดของการอ่านมวลที่ดีที่สุดในปัจจุบัน



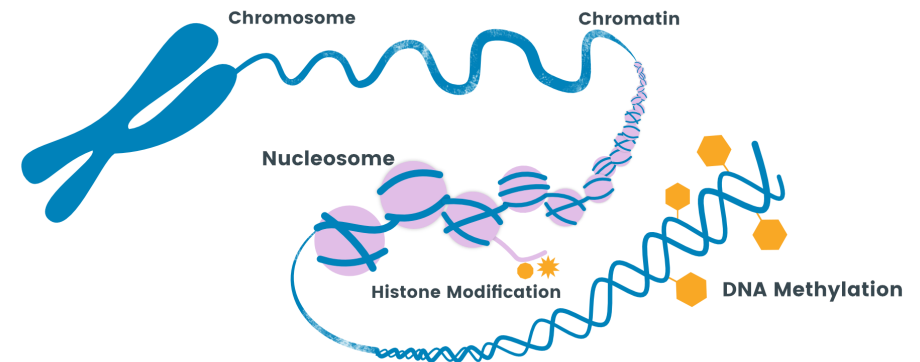
Advance & Precision Translational Genomics



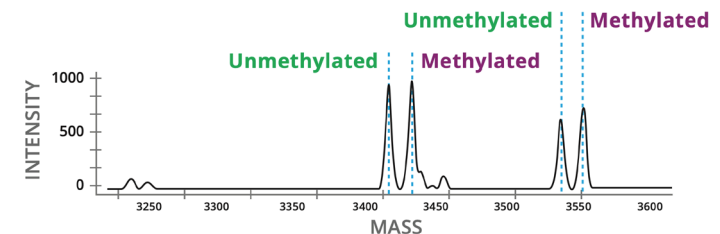
MassArray®



บทบาทสำคัญในการแสดงออกของยีน



การเกิด DNA methylation โดยการเร่งของเอนไซม์ DNMTs การเกิด Hypermethylation จะพบมากในเซลล์มะเร็ง โดยจะปิดการทำงานของ Tumor suppressor genes

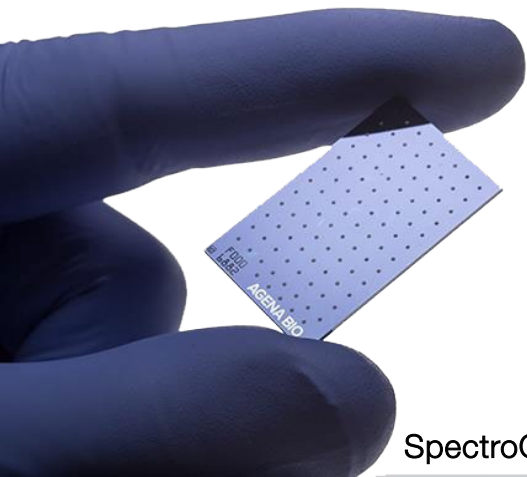


Target Detection

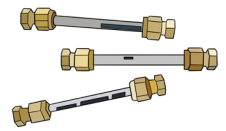
- Single Nucleotide Polymorphism (SNPs)
- Insertions / Deletions
- Copy Number Variation (CNV)
- Somatic mutation
- Methylation (Epigenetics)

Applied Application

- Pharmacogenetics
- Oncology
- Genetic disorders
- Infectious disease
- Drug resistance



SpectroCHIP® Arrays



MassArray® System Workflow

PCR/SAP/Extension



1

Seq Detection

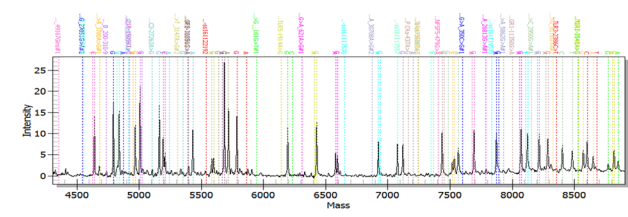
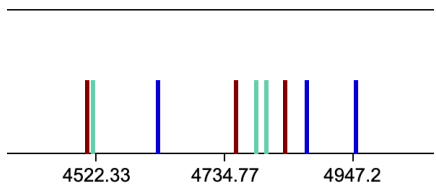


2

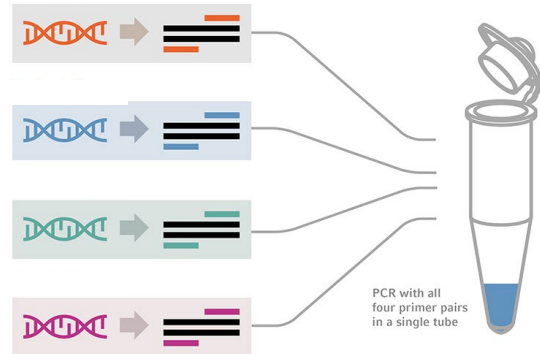
Data Analysis



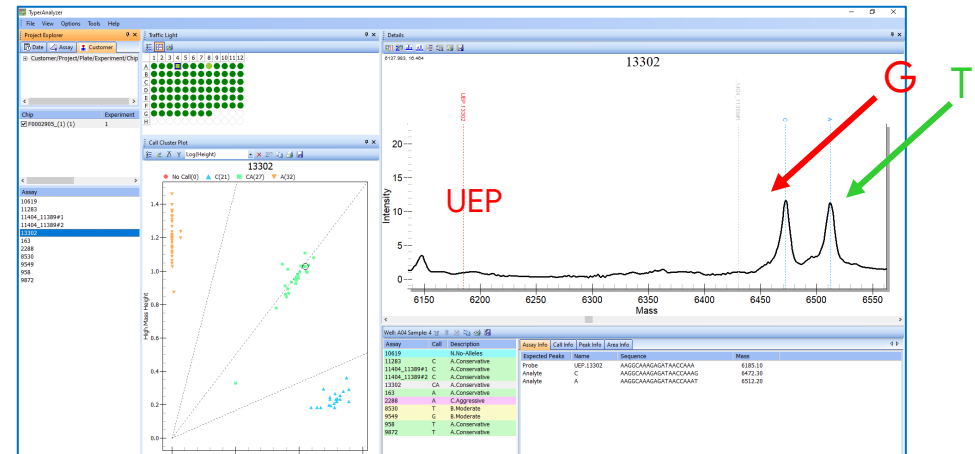
3



เลือกตำแหน่ง SNPs ที่สนใจ พร้อมการออกแบบไพรเมอร์ ผ่านระบบอัลกอริทึมอัจฉริยะ “Agena CX” จากนั้นทำ PCR (Multiplexing) และตามด้วยการทำ Single Base Extension

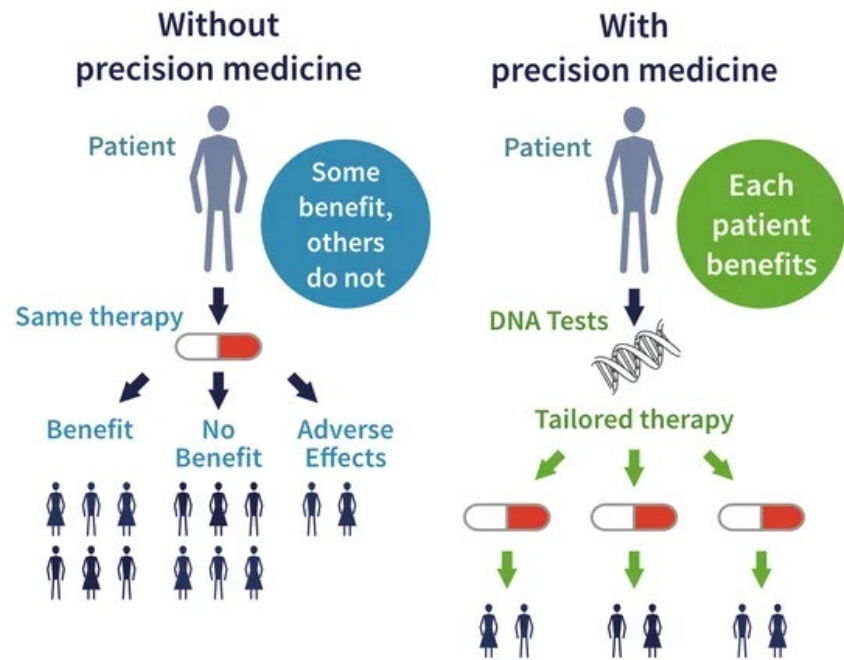


Resin Pretreatment และระบบ Automated Dispenser จะทำหน้าที่ ดูดจ่ายตัวอย่างลงบน SpectroCHIP®



Expected Peaks	Name	Sequence	Mass
Probe	UEP.13302	AAGGCAAAGAGATAACCAA	6185.10
Analyte	C	AAGGCAAAGAGATAACCAA G	6472.30
Analyte	A	AAGGCAAAGAGATAACCAA T	6512.20

Precision Medicine : Forthcoming Trend



- Up to 50% of patients do not benefit from first drug offered
- Differences in response in genes that coded for drug-metabolite enzyme

OPEN ACCESS Freely available online

PLOS ONE

Multidrug Resistant *Mycobacterium tuberculosis*: A Retrospective *katG* and *rpoB* Mutation Profile Analysis in Isolates from a Reference Center in Brazil

Flávia A. D. de Freitas¹, Vagner Bernardo¹, Michel K. Gomgnimbo², Christophe Sola², Hélio R. Siqueira³, Márcia A. S. Pereira⁴, Fátima C. O. Fandinho⁴, Harrison M. Gomes⁵, Marcelo E. I. Araújo⁵, Philip N. Suffys⁵, Elizabeth A. Marques³, Rodolpho M. Albano^{1*}

¹Departamento de Bioquímica, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil, ²CNRS-Université Paris-Sud, Institut de Génétique et Microbiologie-Infection Genetics Emerging Pathogens Evolution Team, Orsay, France, ³Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil, ⁴Centro de Referência Professor Hélio Fraga, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, ⁵Laboratório de Biologia Molecular Aplicada a Micobacterias, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Abstract

Background: Multidrug resistance is a critical factor in tuberculosis control. To gain better understanding of multidrug resistant tuberculosis in Brazil, a retrospective study was performed to compare genotypic diversity and drug resistance associated mutations in *Mycobacterium tuberculosis* isolates from a national reference center.

Methods and Findings: Ninety-nine multidrug resistant isolates from 12 Brazilian states were studied. Drug-resistance patterns were determined and the *rpoB* and *katG* genes were screened for mutations. Genotypic diversity was investigated by IS6110-RFLP and Luminex 47 spoligotyping. Mutations in *rpoB* and *katG* were seen in 91% and 93% of the isolates, respectively. Codon 315 *katG* mutations occurred in 82.8% of the isolates with a predominance of the Ser315Thr substitution. Twenty-five isolates were clustered in 11 groups with identical IS6110-RFLP patterns while 74 showed unique patterns with no association between mutation frequencies or susceptibility profiles. The most prevalent spoligotyping lineages were LAM (47%), T (17%) and Haerlen (12%). The Haerlen lineage showed a higher frequency of codon 516 *rpoB* mutations while codon 531 mutations prevailed in the other isolates.

Conclusions: Our data suggest that there were no major multidrug resistant *M. tuberculosis* strains transmitted among patients referred to the reference center, indicating an independent acquisition of resistance. In addition, drug resistance associated mutation profiles were well established among the main spoligotyping lineages found in these Brazilian multidrug resistant isolates, providing useful data for patient management and treatment.

Citation: de Freitas FAD, Bernardo V, Gomgnimbo MK, Sola C, Siqueira HR, et al. (2014) Multidrug Resistant *Mycobacterium tuberculosis*: A Retrospective *katG* and *rpoB* Mutation Profile Analysis in Isolates from a Reference Center in Brazil. PLoS ONE 9(8): e104100. doi:10.1371/journal.pone.0104100

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. Data is found in the body of the paper and in the supplementary information.

Funding: Some of the authors received funding for this work. RMA received funding from the following Brazilian Agencies: Fundação Carlos Chagas de Amparo a Pesquisa (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: albano@uerj.br

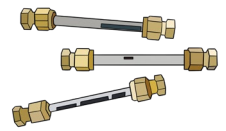
Rifampin¹ resistance

Table 2. Mutation patterns for the *rpoB* and *katG* genes found in 99 MDR isolates.

Gene	Mutation			n
	Mutated codon	Specific Mutation	Amino acid change	
<i>rpoB</i>	531	TCG - TTG	Ser - Leu	44
		TCG - TGG	Ser - Trp	7
	513	CAA - CCA	Gln - Pro	2
	516	GAC - GTC	Asp - Val	3
		GAC - TAC	Asp - Tyr	8
	526	CAC - GAC	His - Asp	9
		CAC - TAC	His - Tyr	8
		CAC - CGG	His - Arg	2
		CAC - TGC	His - Cys	2
		CAC - AAC	His - Asn	2
	511	CTG - CCG	Leu - Pro	4
	475*	GTG - GGG	Val - Gly	1
	522*	TCG - TTC	Ser - Phe	1
	539*	TCA - TTC	Ser - Phe	1
533	CTG - CCG	Leu - Pro	1	
545	CTG - CCG	Leu - Pro	2	
508*	ACC - CCC	Thr - Pro	1	
471*	ATG - ATT	Met - Ile	1	
<i>katG</i>	4	A deletion at position 60		26
	65*	G deletion at position 241		2
	17*	AGC - ACC	Ser - Thr	1
	92-93*	T insertion at position 325		1
	2*	C deletion at position 54		1
	11*	C deletion at position 81		1
	26*	G deletion at position 126		1
	107	G deletion at position 368		1
	67	G deletion at position 249		1
	115*	T insertion at position 392		1
	1*	GTG - CCG	Val - Ala	1
	93	GCC - ACC	Ala - Thr	1
	<i>katG2</i>	315	AGC - ACC	Ser - Thr
		AGC - AAC	Ser - Asn	3
		AGC - ACA	Ser - Thr	3
		AGC - ATC	Ser - Ile	1
463		CGG - CTG	Arg - Leu	2
431		G deletion at position 1293		1
399*		GAA - GAG	Glu - Glu	2
493*		A deletion at position 1525		1
439		G insertion at position 1365		1
485*		G deletion at position 1501		1

*associated with another mutated codon. doi:10.1371/journal.pone.0104100.t002

¹ Rifampin can destroy 99% of tuberculosis bacilli.



Core Applications



ONCOLOGY (CANCER)

LUNG

BREAST

HPV CERVIX

MELANOMA

COLON

PHARMACOGENOMICS (PRECISION MED)

PGX 74

CYP2D6

EPIGENETIC
(METHYLATION)

GENETIC DISORDERS

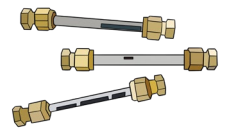
HEARING LOSS

NIPT

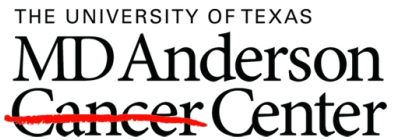
INFECTIOUS APPLICATIONS

COVID-19

“ASSAY BY DESIGN : CUSTOMIZATION”



Trusted by Leading Clinical Labs



Memorial Sloan Kettering Cancer Center...



Intermountain Precision Genomics Cancer



HOUSTON Methodist LEADING MEDICINE



SOUTH BEND MEDICAL FOUNDATION



ศูนย์จีโนมทางการแพทย์ Center for Medical Genomics

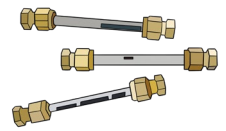


American Red Cross

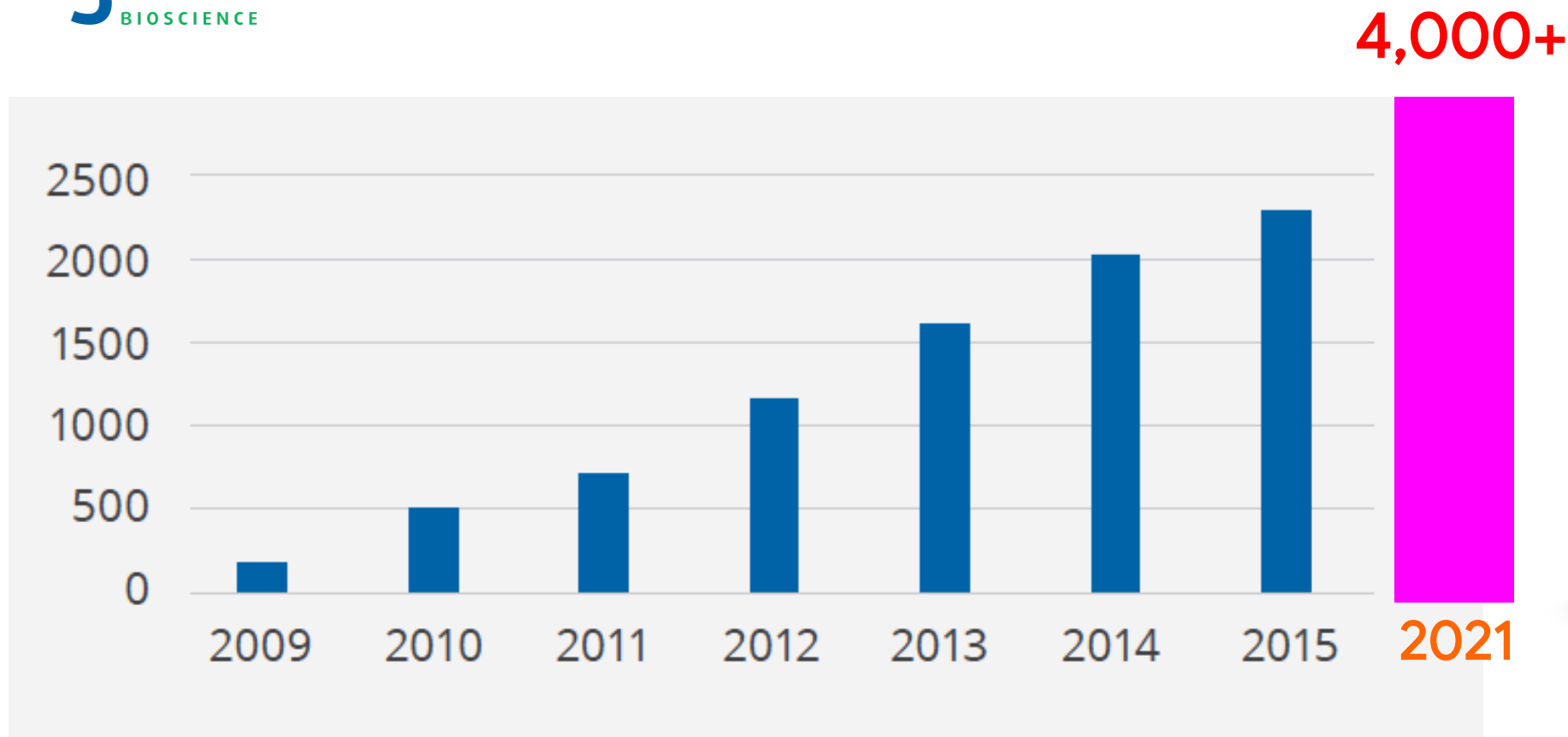


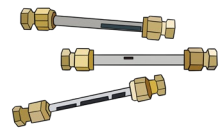
saint mary's hospital charity





International Publications

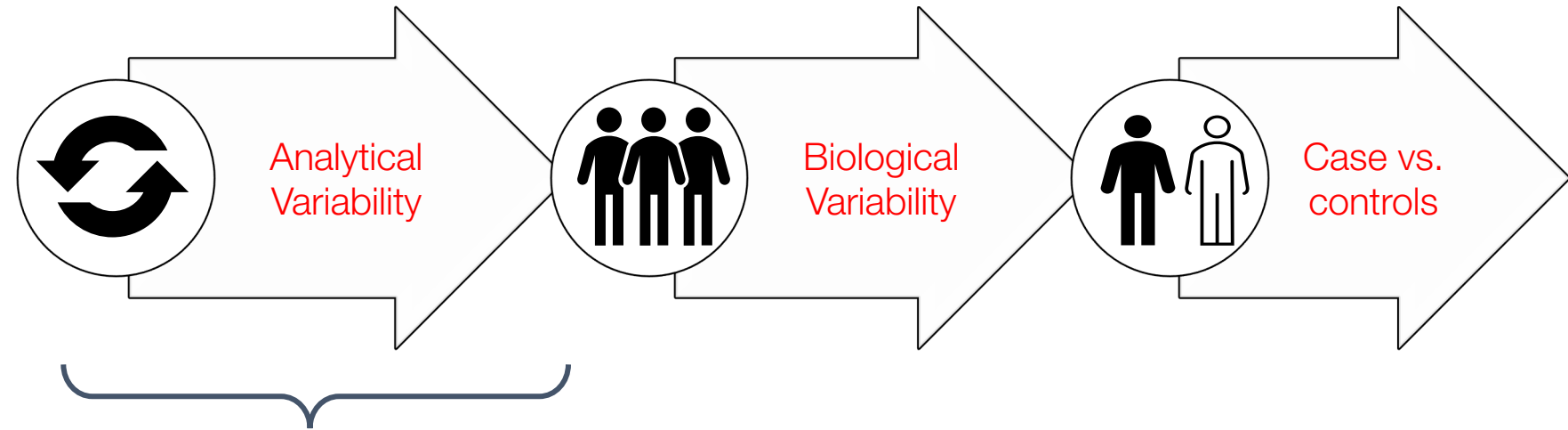




Proteomics Precision and Accuracy for High-throughput

Challenges of large-scale precision medicine studies

- Multiple sample collection sites
- Thousands of samples
- LCMS laboratories across the world



Within one series of experiments

Between series of experiments in one lab

Between laboratories, operators, continents



Study Design and Results

- Standardized high-throughput capillary-flow LC-MS methods
- Robustness of high-throughput (HT) capLC-MS separations
- Reproducibility within one laboratory and multiple series of experiments
- Multi-site reproducibility of proteome profiling: cell lysate and crude plasma
- Deeper single-shot proteome profiling with AI data processing

Test samples



HeLa protein digest



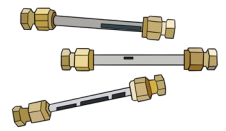
Plasma protein digest

Low-flow LC-MS setup



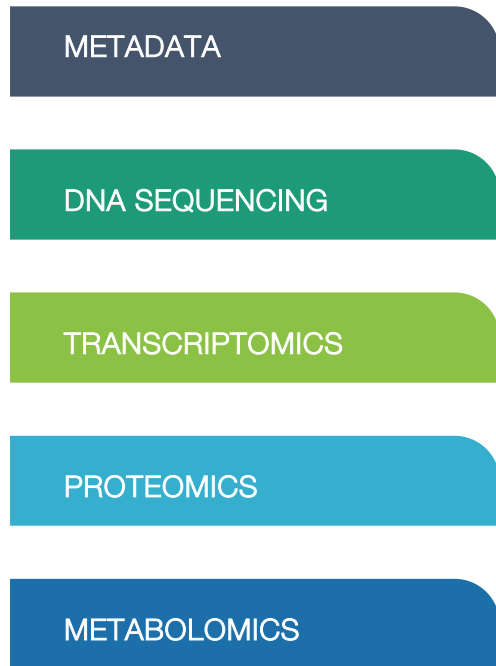
Data Analysis





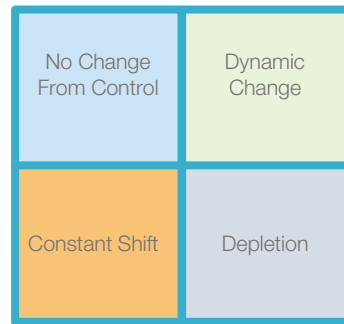
Multi-OMICs Workflow

MULTI-SCALE DATA ACQUISITION



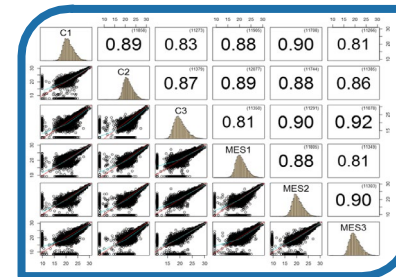
STAGE 1

UNDERSTANDING BASIC DIFFERENCES



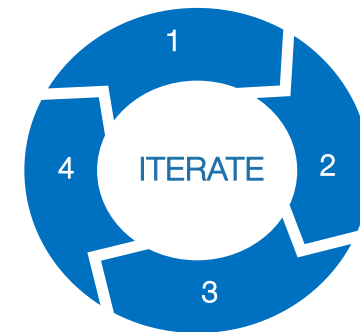
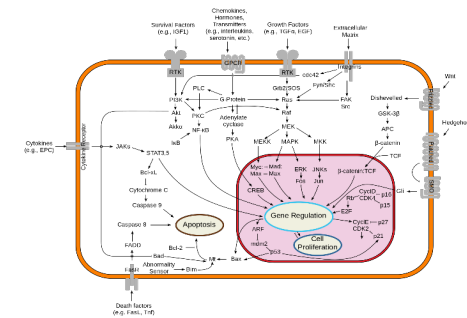
STAGE 2

UNDERSTANDING CORRELATION IN DATA

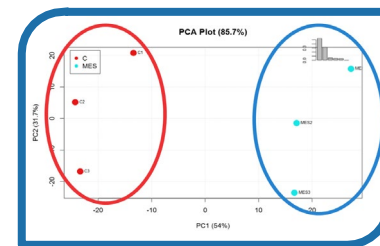
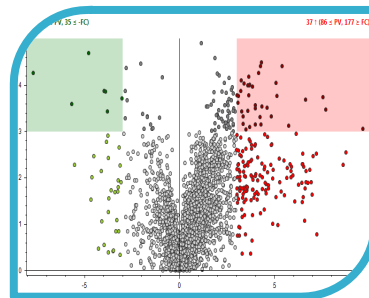


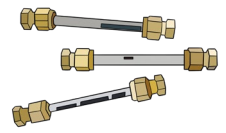
STAGE 3

UNDERSTANDING METABOLIC OR SIGNALING MECHANISMS



STAGE 4



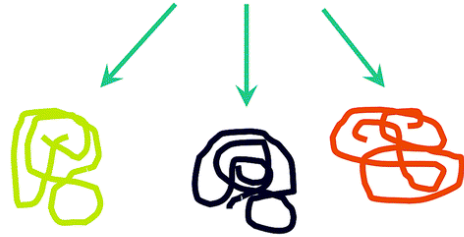


Understanding Proteomics

Prokaryotic

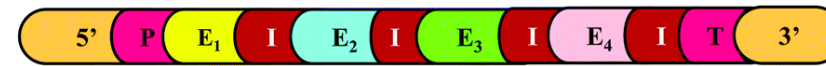


Polycistronic mRNA

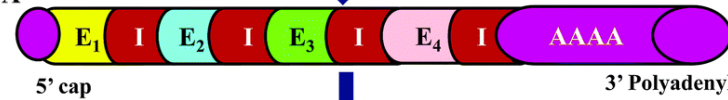


Translate from Coding Sequence

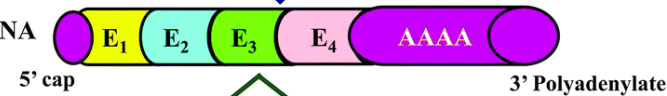
Eukaryotic



mRNA



Mature mRNA



Normal Splicing

Alternate Splicing



Protein A

Protein B

Protein C

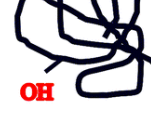
Protein D

PTMs

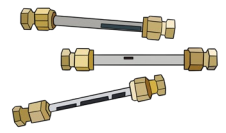
PTMs

PTMs

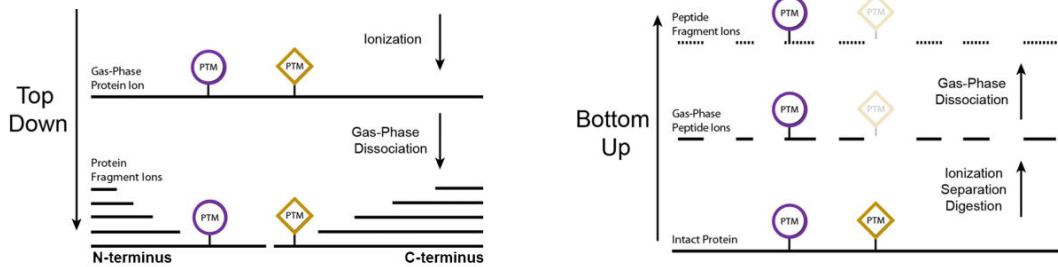
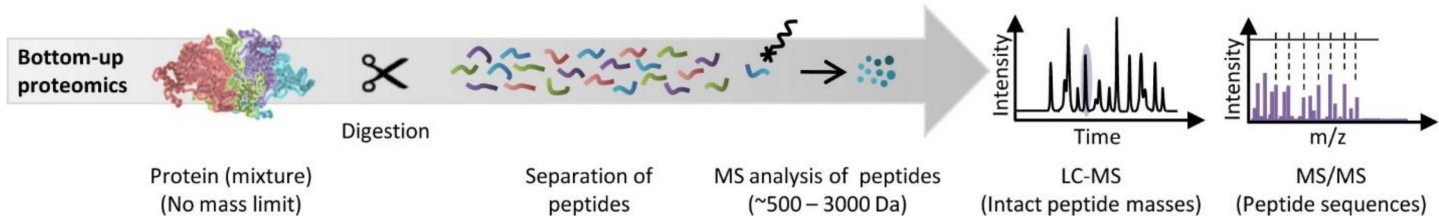
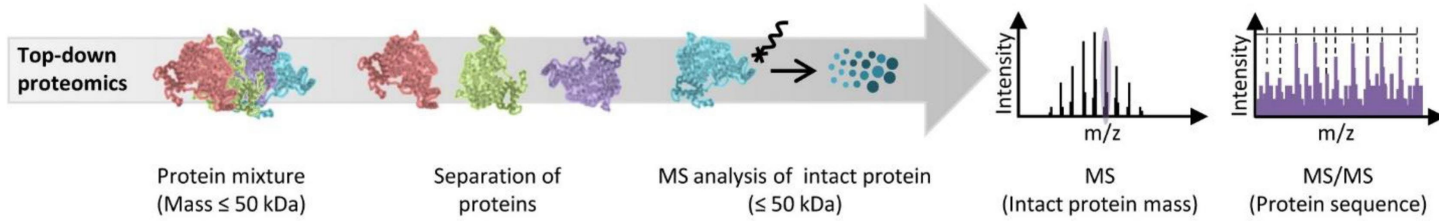
PTMs



P = Promotor
E = Exon
I = Intron
T = Terminator
G = Gene
O = Operon
C = coding sequences
PTMs = Post Translational Modifications

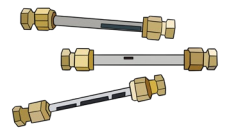


Type of Proteomics Approaches

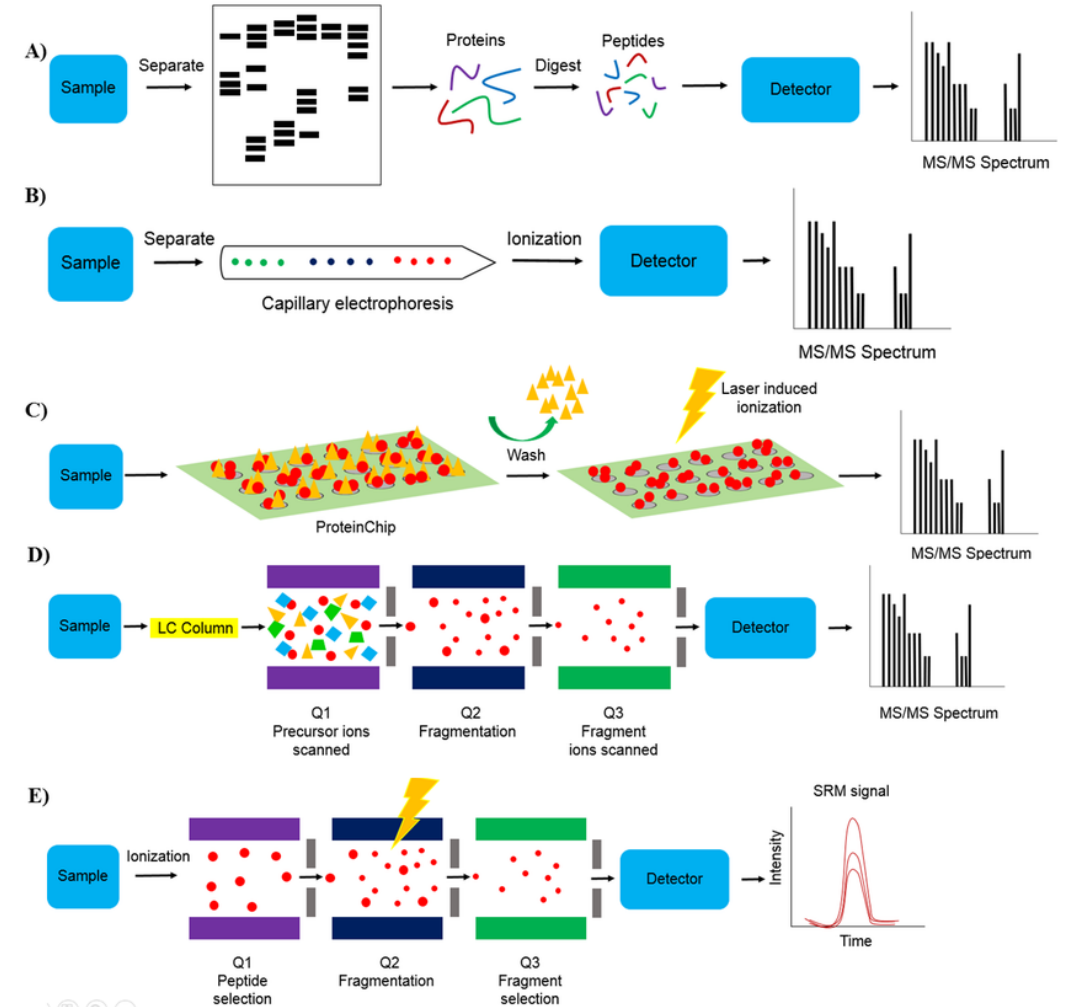
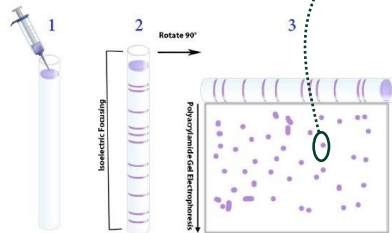
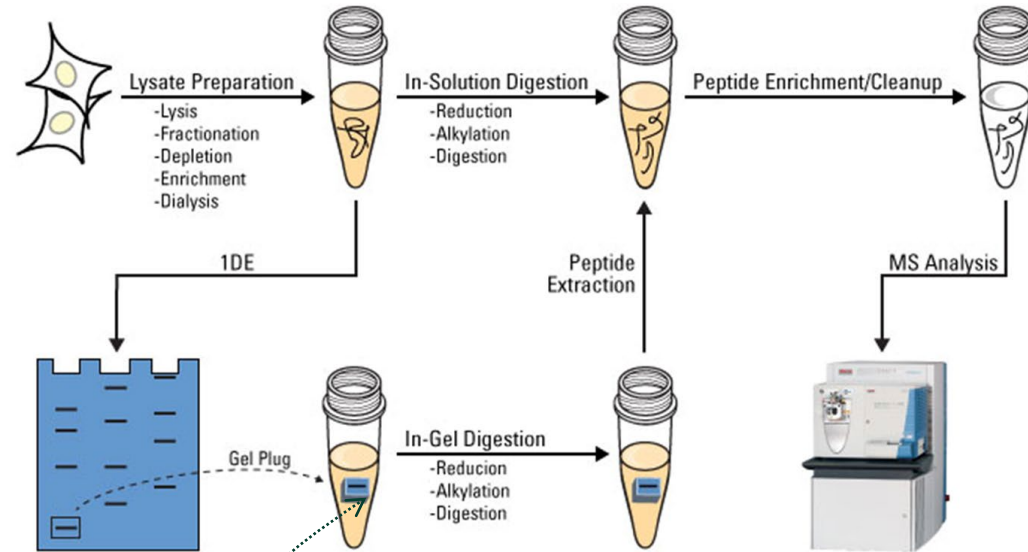


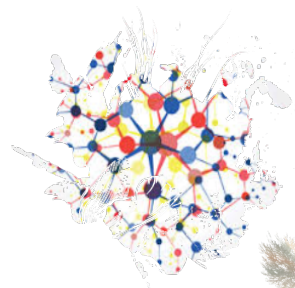
	Bottom-up	Top-down
Peptide Identification	★★★★	★★★☆☆
Protein Modification	★★★★☆	★★★★★
Protein Quantitation	★★★★★	★★★★☆

1. Bottom up หรือ Short Gun จะใช้การเทียบข้อมูลที่ได้ กับฐานข้อมูลขนาดใหญ่ซึ่งมีการอัปเดตมาอย่างยาวนาน จึงสามารถระบุ Seq ของเปปไทด์ที่ตรง รวมถึงสามารถระบุถึง Species ของตัวอย่างได้อีกด้วย
2. Top Down หรือ Intact Protein จะใช้การวิเคราะห์ Seq ของโปรตีน จึงใช้ในการ Characterization โปรตีนหรือแอนติบอดี
3. การเกิด Post Translation Modification (PTM) จะเป็นการเติมโมเลกุลอื่นๆเข้าไปในโปรตีน เช่น การเติมหมู่น้ำตาล การเติมหมู่ฟอสเฟต เป็นต้น ซึ่งจะสัมพันธ์กับการแสดงออกของโปรตีน



Proteomics Workflow





System Biology – Pathway, Network, Complex interaction



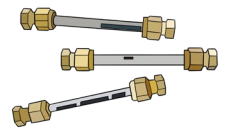
Biological Processes – Protein expression, Cellular protein



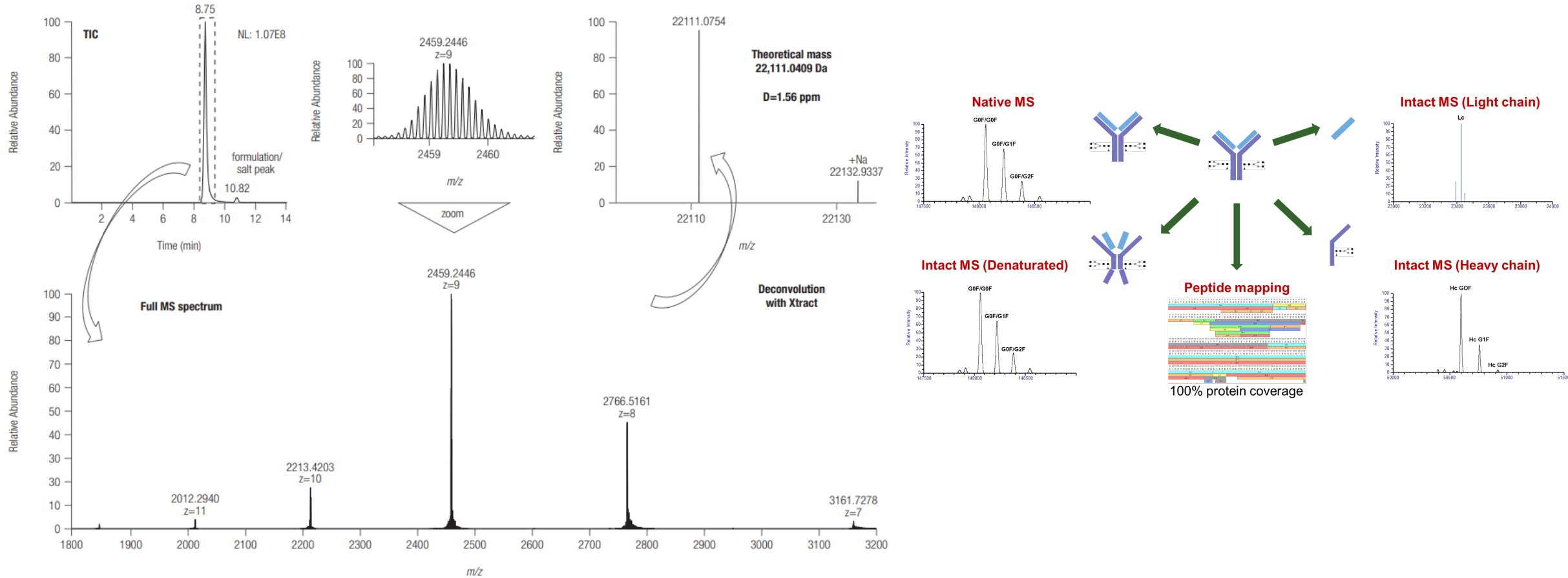
Bio-Markers – Discovery of disease, diagnostics, treatment, monitoring

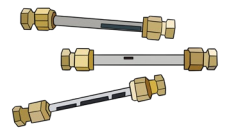


Drug Targets – Evaluate biological processes with drug treatment, toxicity

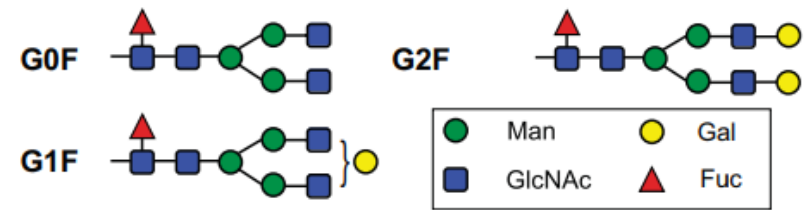
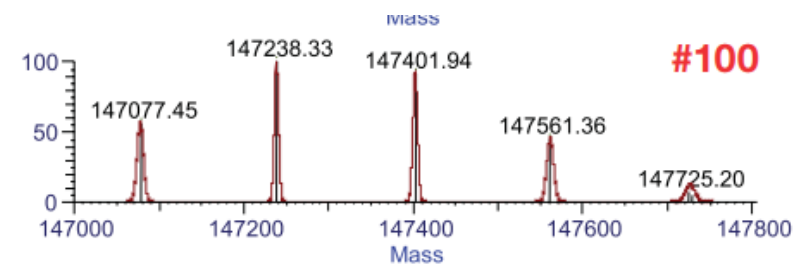
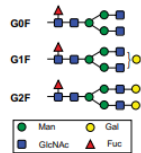
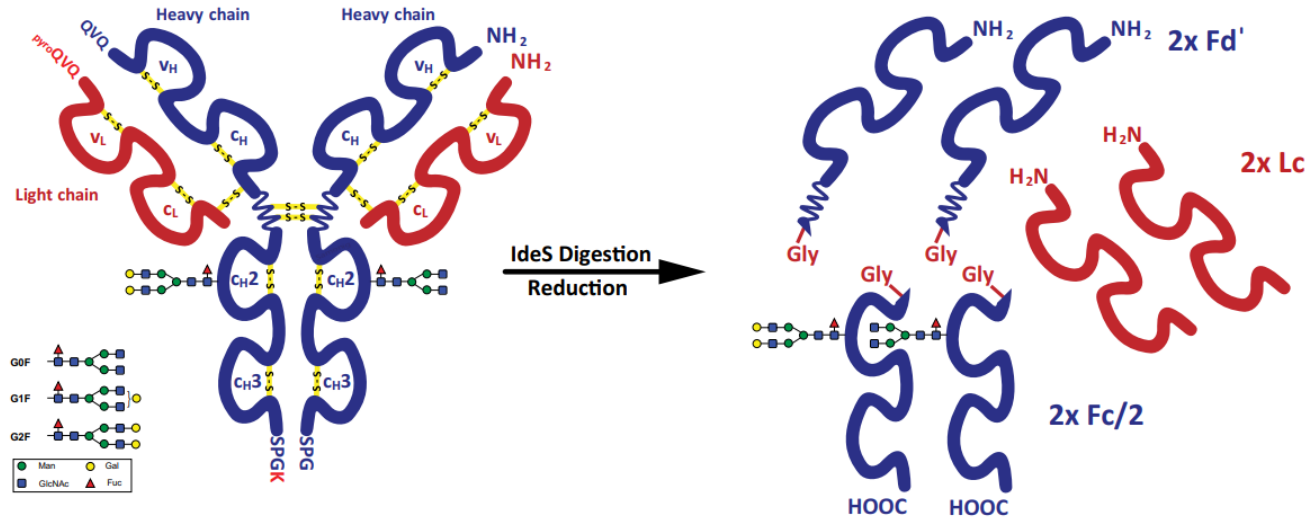


Intact Protein Analysis (Biopharmaceutical)





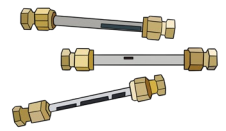
Protein Mapping: Glycosylation



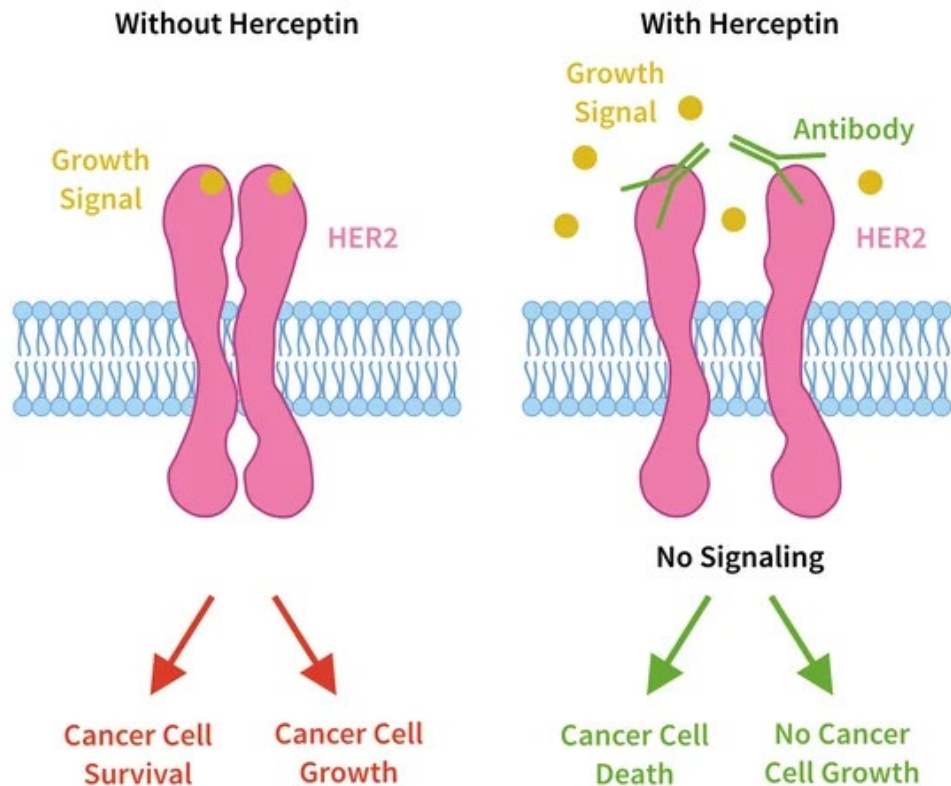
Peak (Figure 1)	Compound structure (ZAB labeling is not shown)	Peak (Figure 1)	Compound structure (ZAB labeling is not shown)
1		8	
2		9	
3		10a	
4		10b	
5		10c	
6		11a	
7		11b	



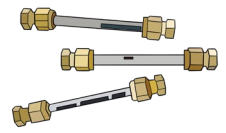
12		19	
13		20	
14		21	
15		22	
16		23	
17		24	
18		25	



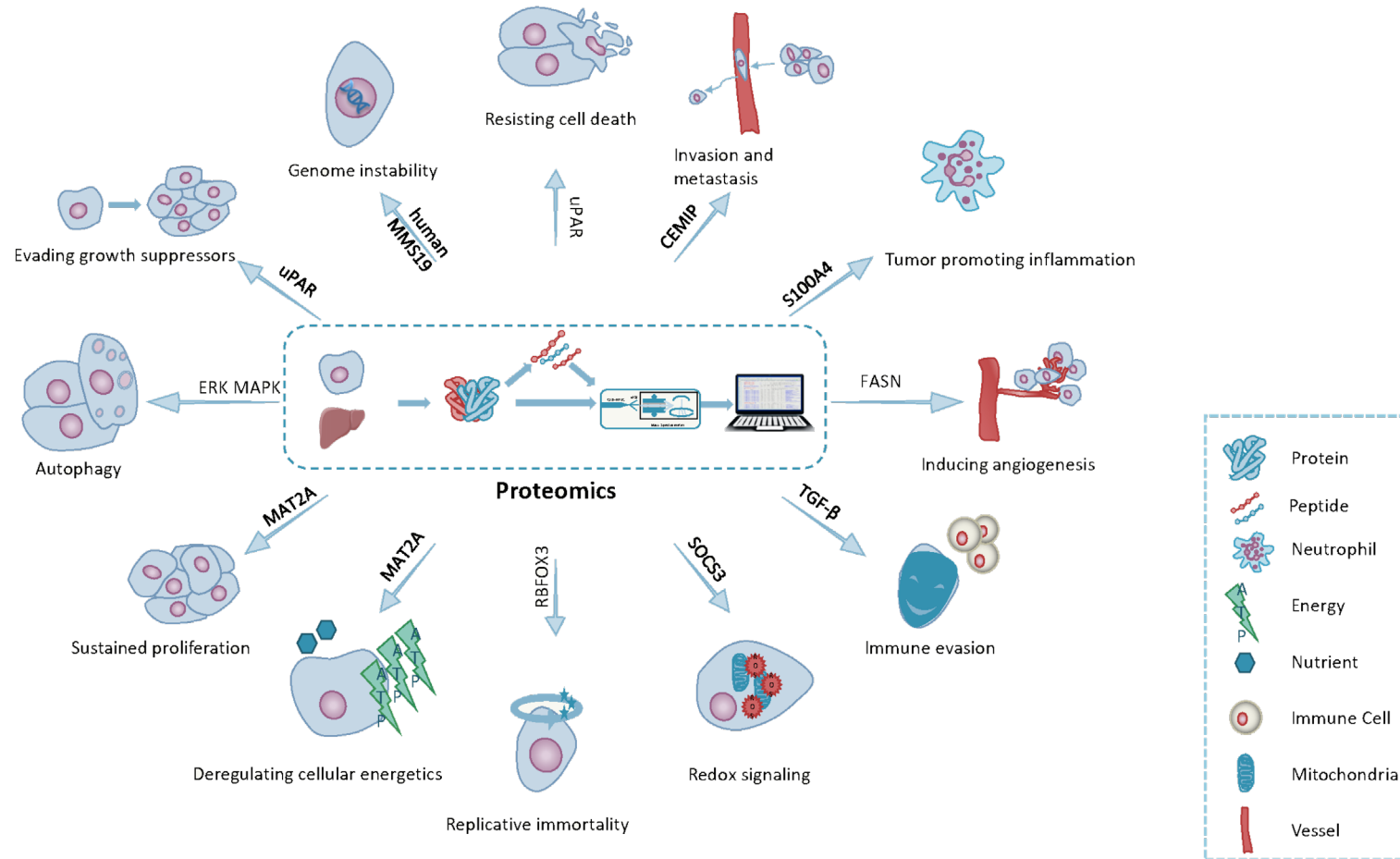
Proteomics in Precision Medicine

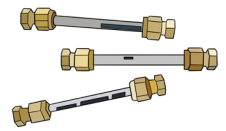


- Up to 50% of patients do not benefit from first drug offered
- Differences in response to the differences in genes that code for drug-metabolite enzyme
- 30% of breast cancer case found an over expression of HER2
- Antibody drug names **Herceptin® (trastuzumab)** can half the tumor when used with chemotherapy
- Characterization of antibody need a proteomics

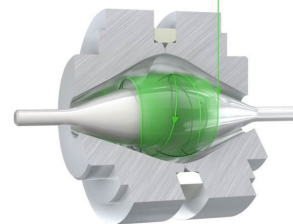


Proteomics Helps Advance the Understanding Cancer

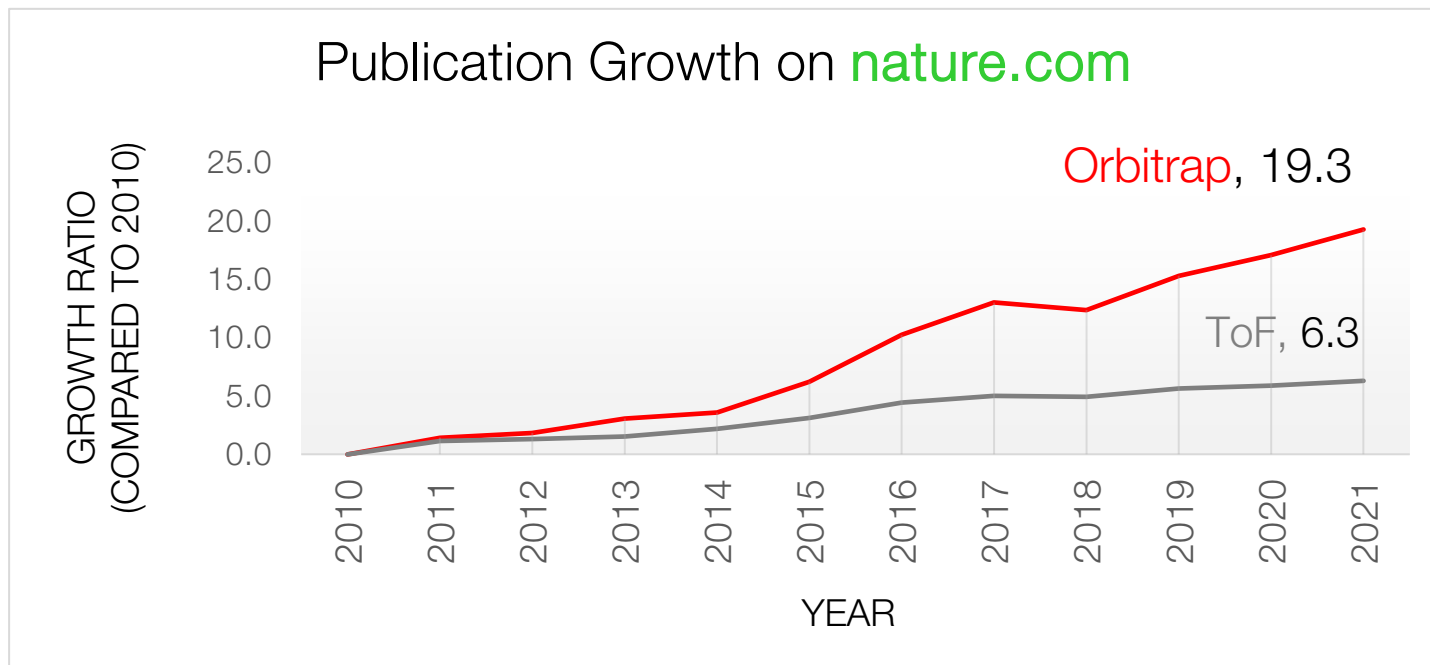




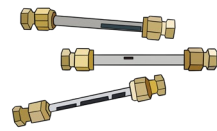
Orbitrap™ Trend Nowadays



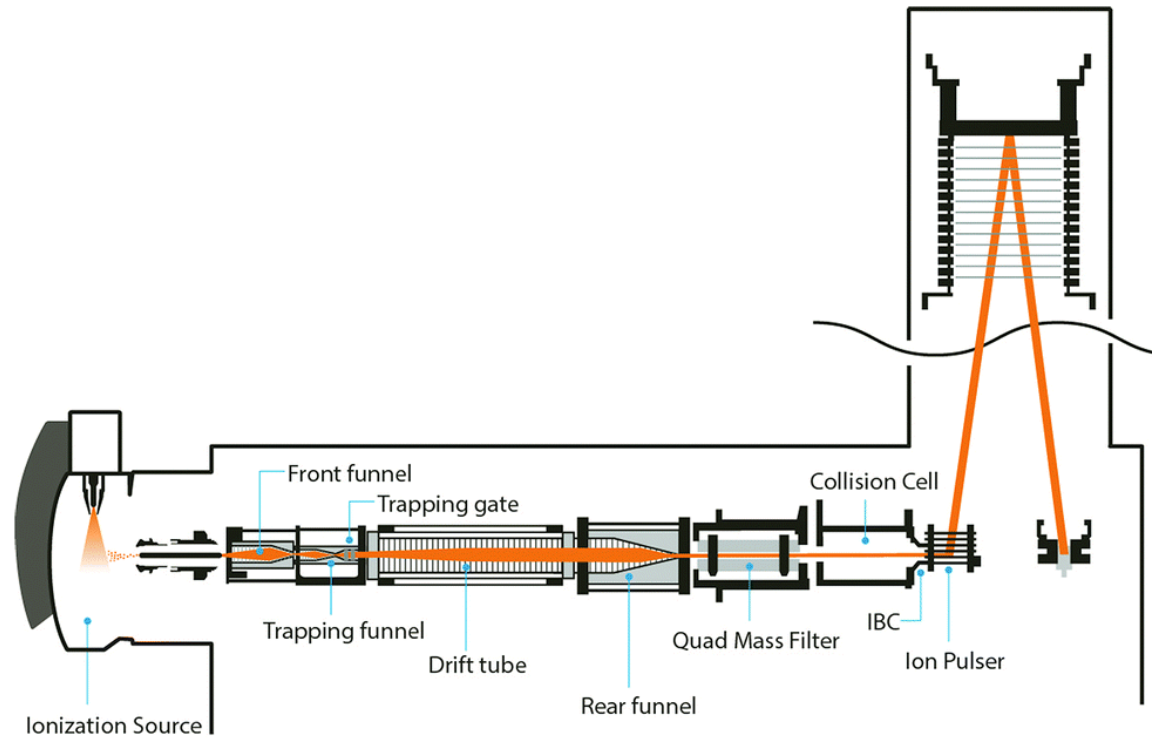
Year	Orbitrap	ToF
2010	61	165
2011	87	189
2012	112	219
2013	188	255
2014	219	361
2015	380	516
2016	625	734
2017	794	830
2018	755	813
2019	934	934
2020	1042	971
2021	1176	1042
Total	6373	7029



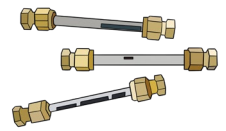
By the end of 2021, the publication ratios in each years, compared to those of 2010, show superiority of the publication growth from Orbitrap technology 3 times higher than that of TOF.



Hi-end Q-ToF Technology



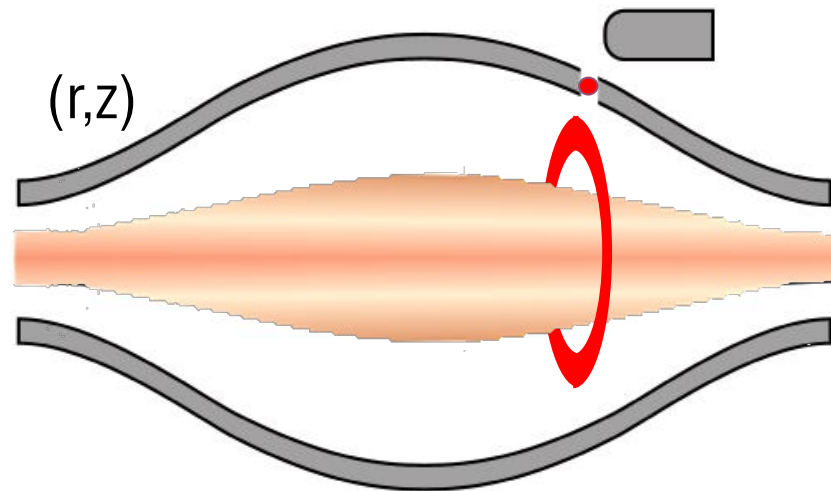
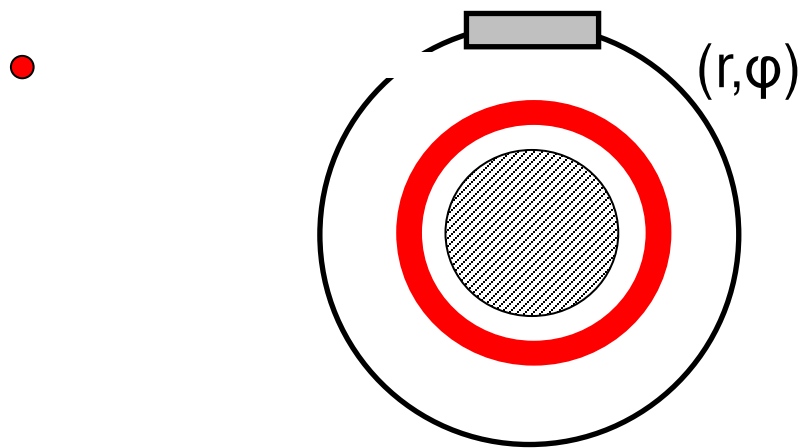
Ion Mobility Q-ToF

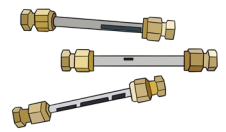


Orbitrap™ Technology



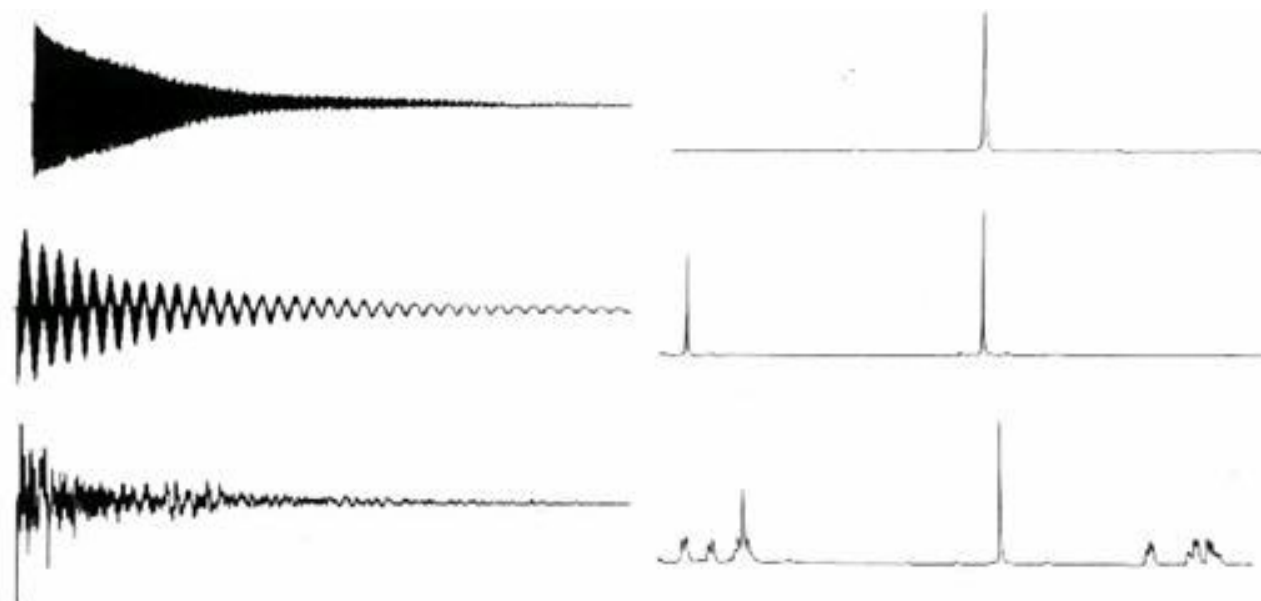
- An ion packet of a selected m/z enters the field
- Increasing voltage squeezes ions
- Voltage stabilises and ion trajectories are also stabilized
- Angular spreading forms a ROTATING RING





Orbitrap™ Powered by Fourier Transform

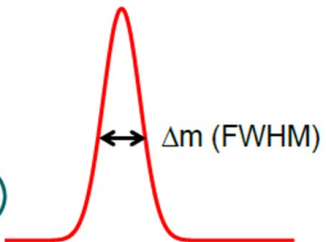
- Free Induction Decay (FID)



Time Domain -> Fourier Transform -> Spectrum (Frequency Domain)

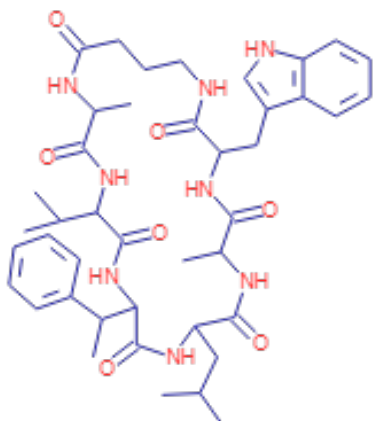


Mass Resolution

$$R = \frac{m}{\Delta m}$$


m - measured mass

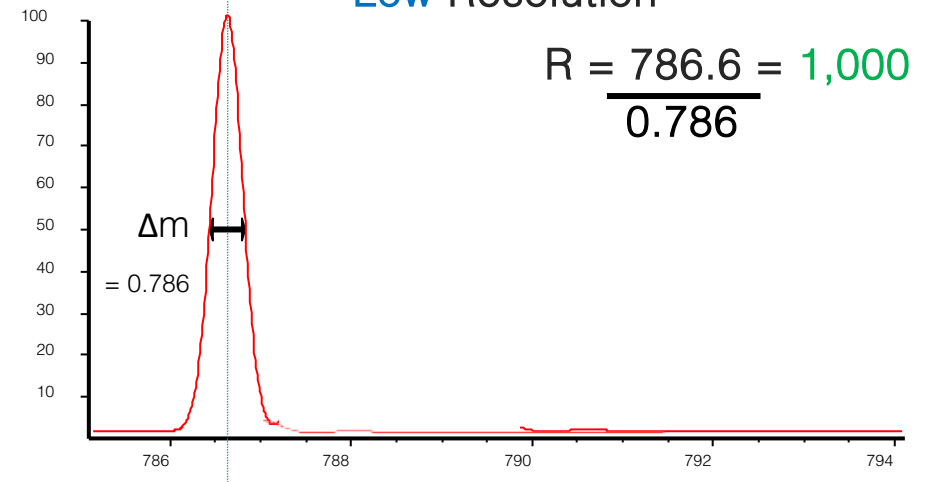
Δm - peak width measured at 50% peak intensity (Full Width Half Maximum)



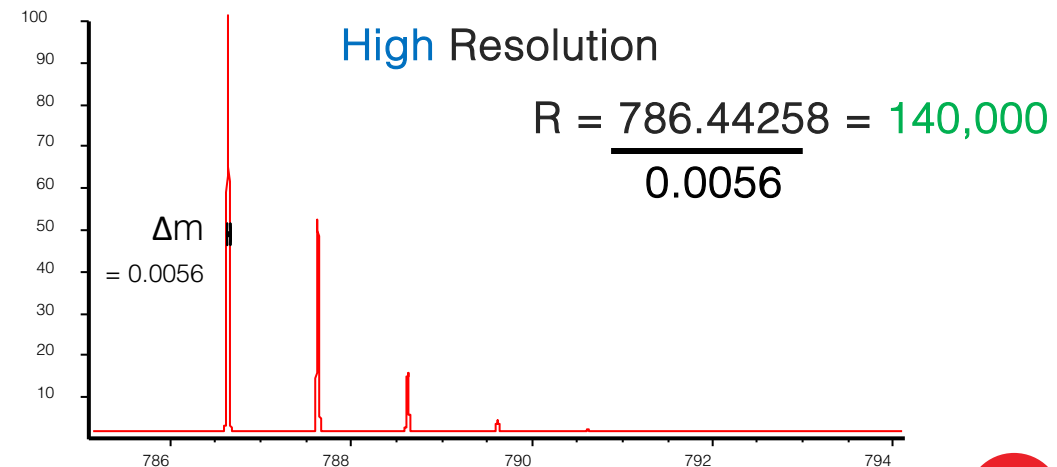
Formular : $C_{42}H_{58}N_8O_7$
 Exact Mass: 786.4428

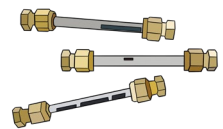
3-[(1H-indol-3-yl)methyl]-6,18-dimethyl-9-(2-methylpropyl)-12-(1-phenylethyl)-15-(propan-2-yl)-1,4,7,10,13,16,19-heptaazacyclotricosane-2,5,8,11,14,17,20-heptone

Low Resolution

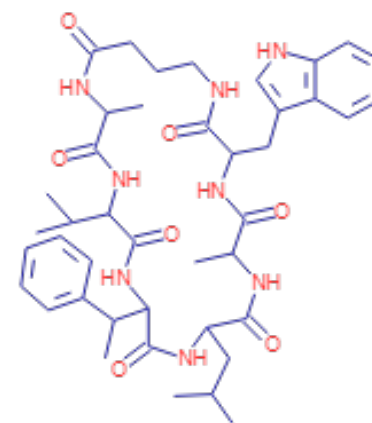
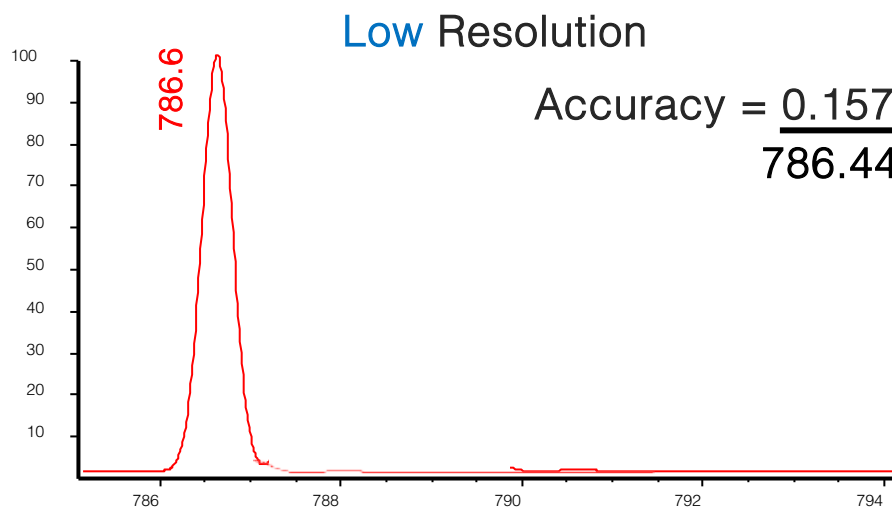


High Resolution

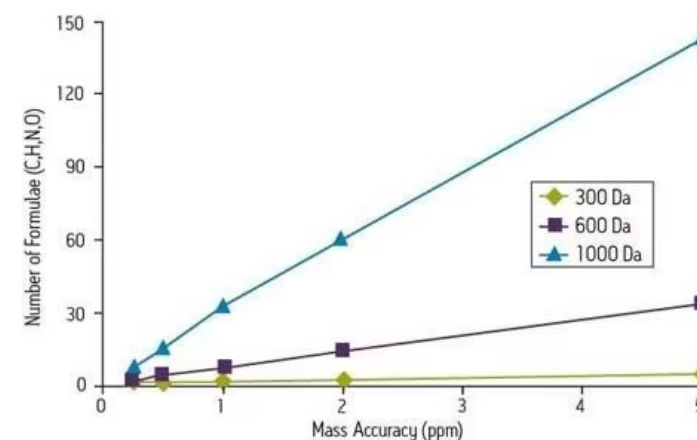
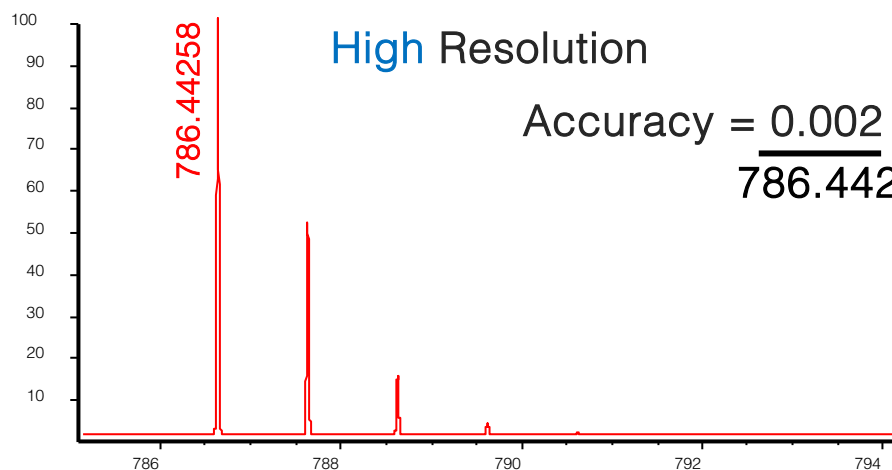




Mass Accuracy – Elemental Composition



Formular : $C_{42}H_{58}N_8O_7$
 Exact Mass: 786.4428





Mass Measurement by Q-ToF

Exact mass (1 ppm RMS)

$$f_{\text{rms}} = \sqrt{\frac{1}{T_2 - T_1} \int_{T_1}^{T_2} [f(t)]^2 dt}$$

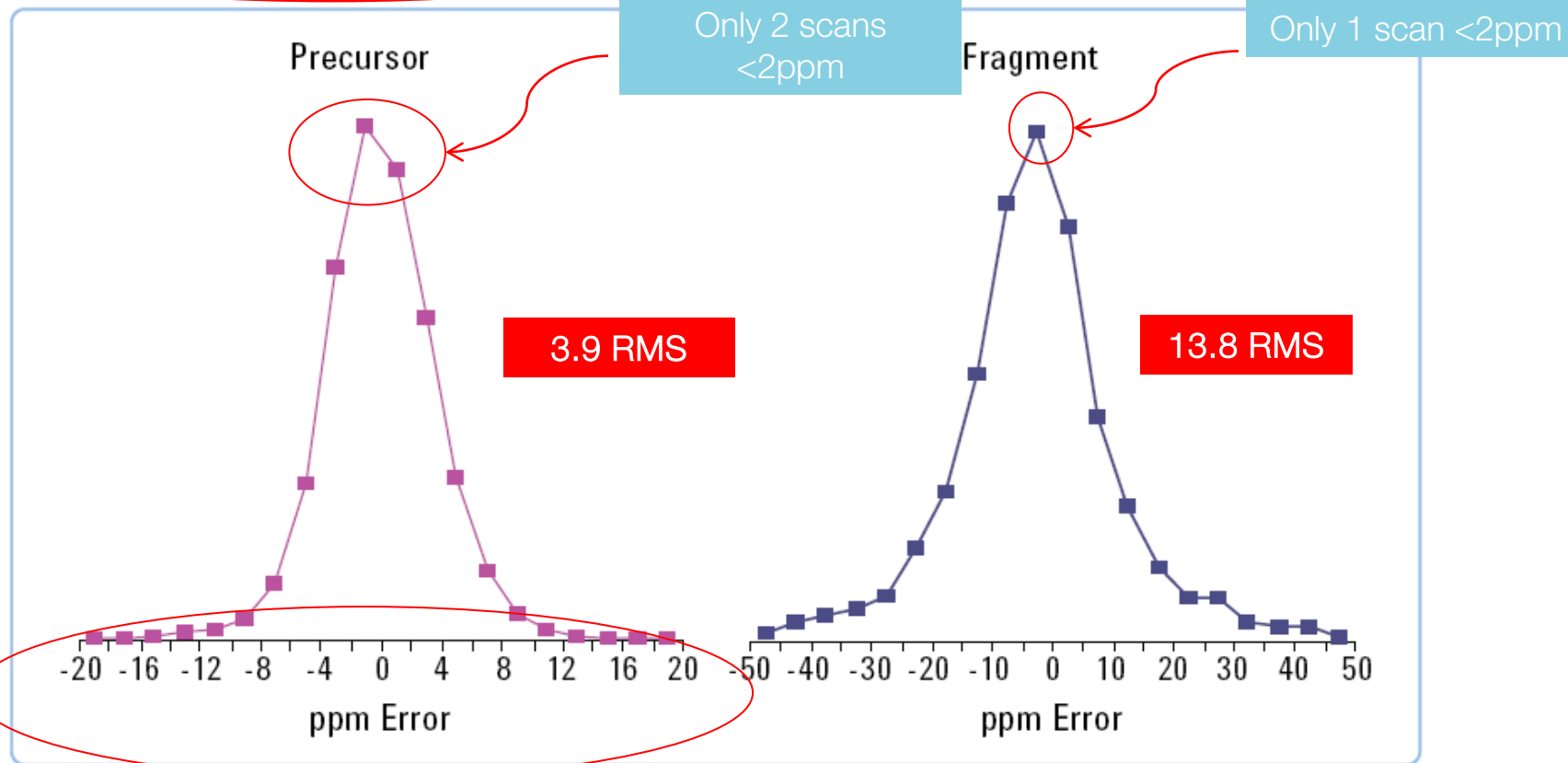
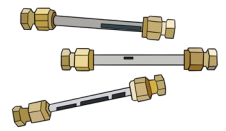
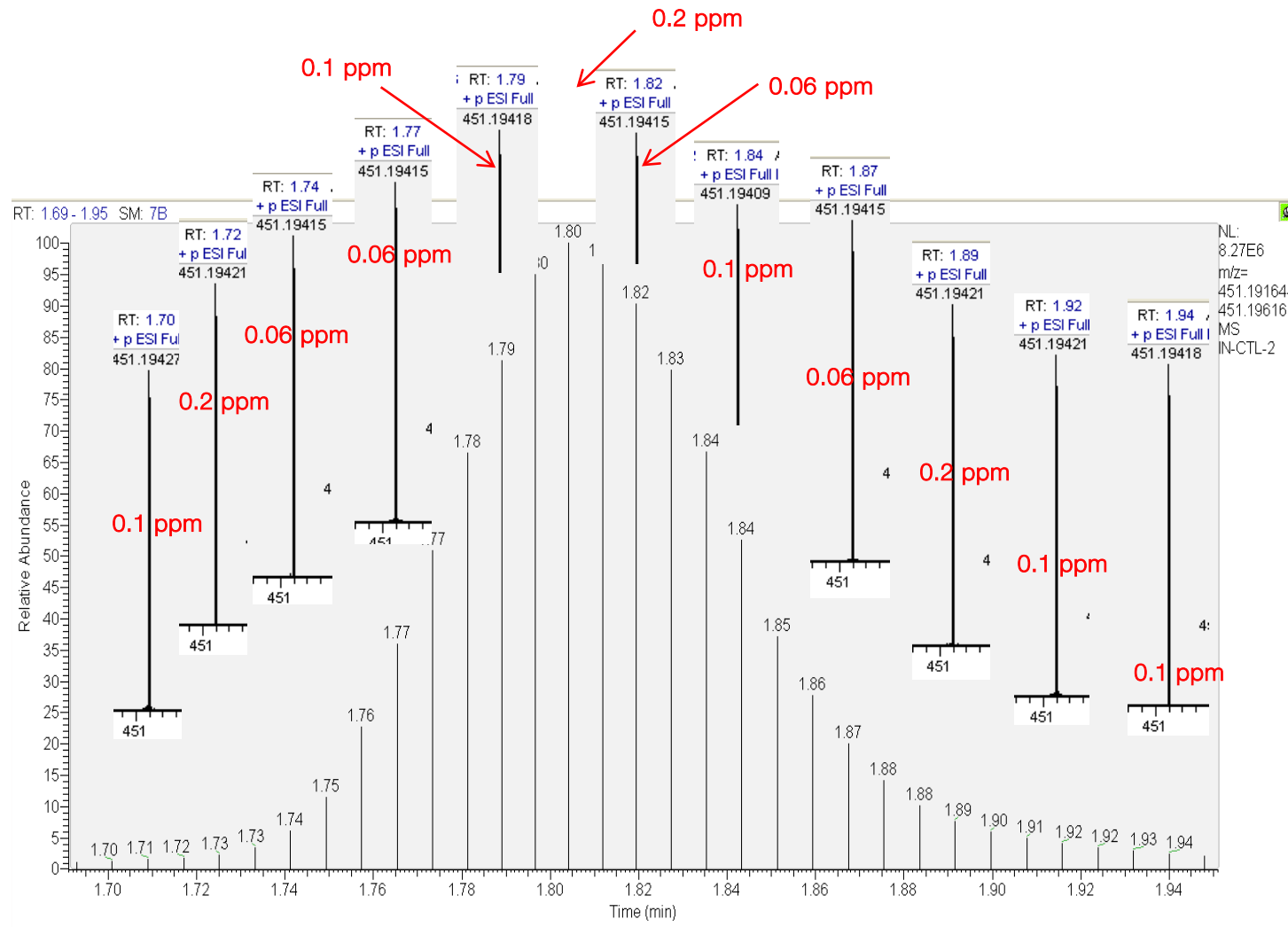


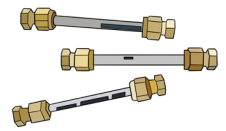
Figure 2. Mass measurement deviation distributions for precursor (3.9 rms ppm) and fragment ions (13.8 rms ppm) using the 6510 Q-ToF.



Mass Measurement by Orbitrap™



< 2 RMS

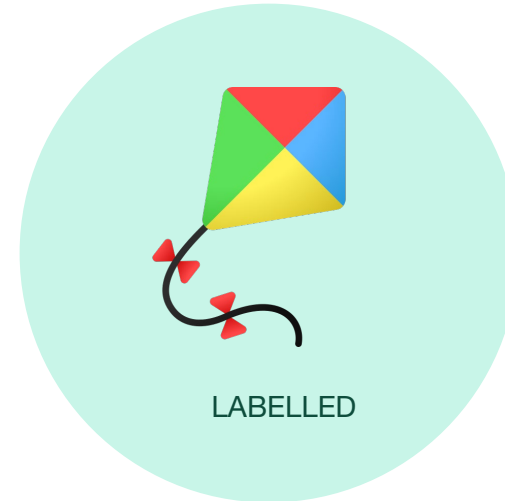


Comparative (Quantitative) Proteomics



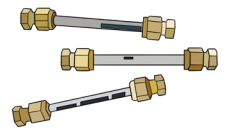
LABEL-FREE

APEX
emPAI
IBAQ
NSAF
ALF
Label-free

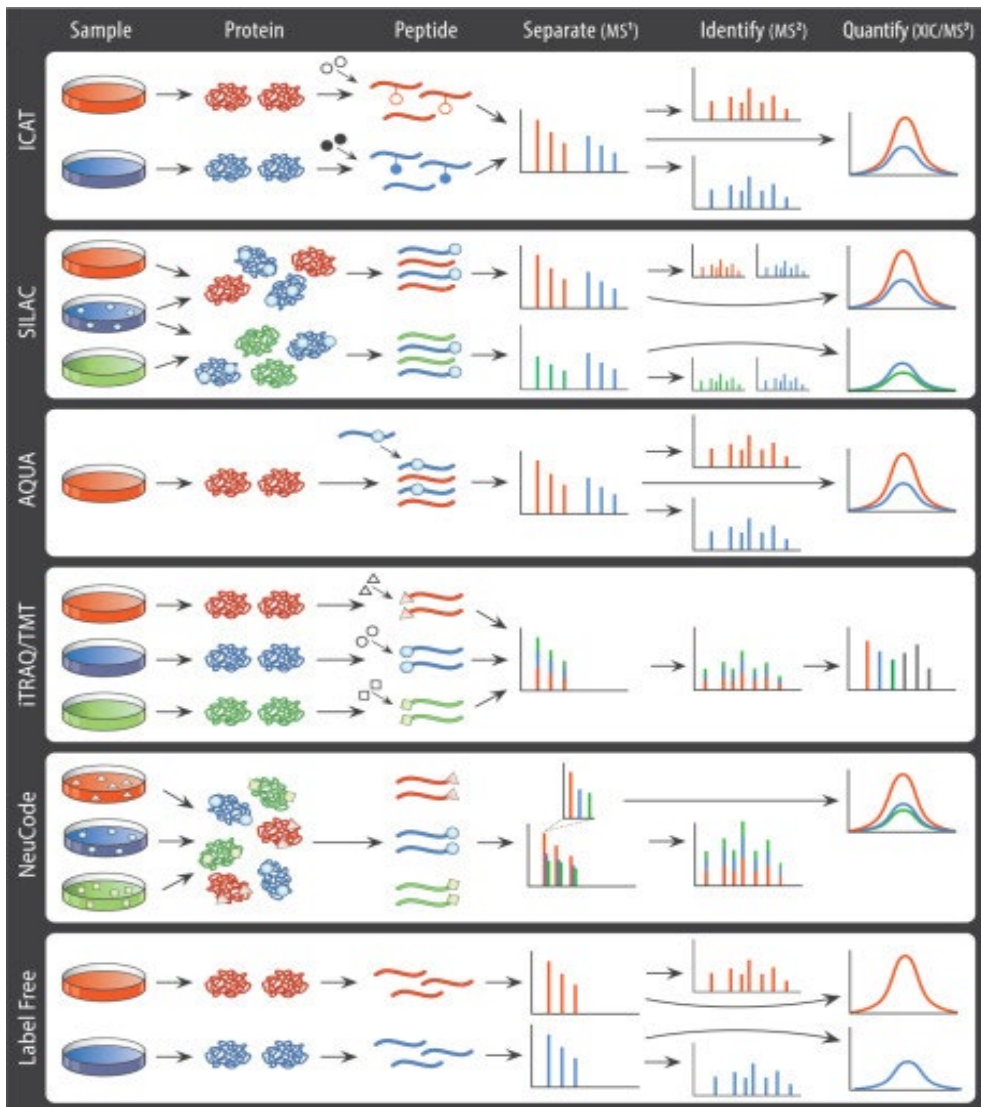


LABELLED

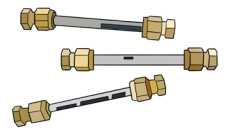
SpikeTides
ICAT
AQUA
TMT
iTRAQ
Dimethyl
SILAC
¹⁵N
¹⁸O
QconCAT



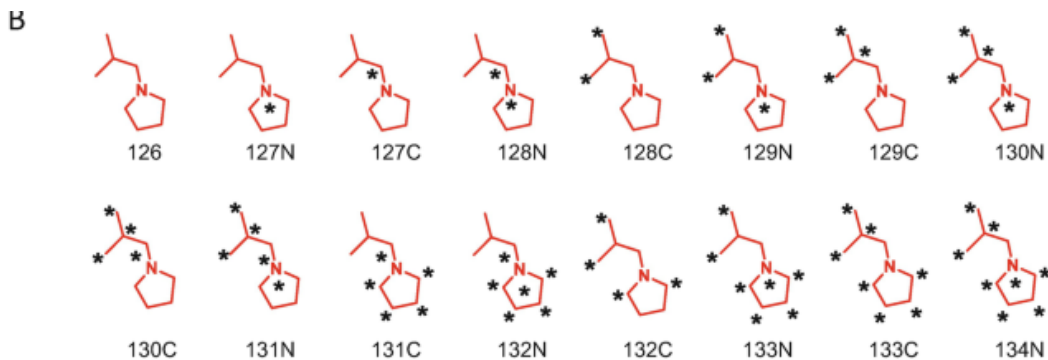
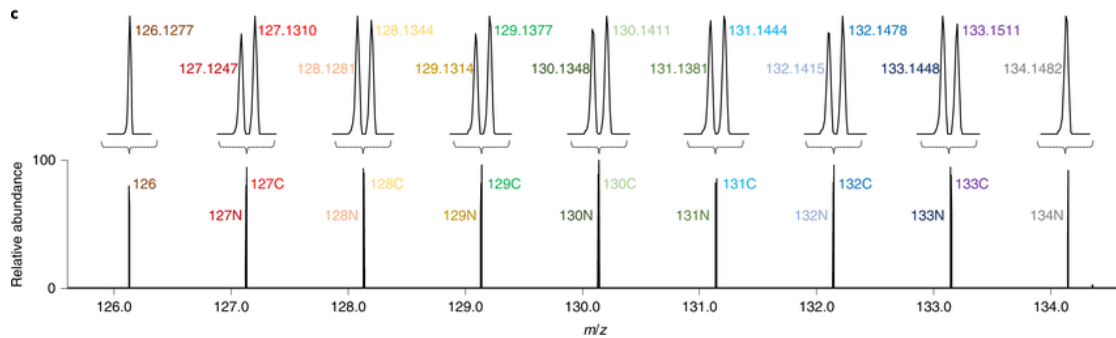
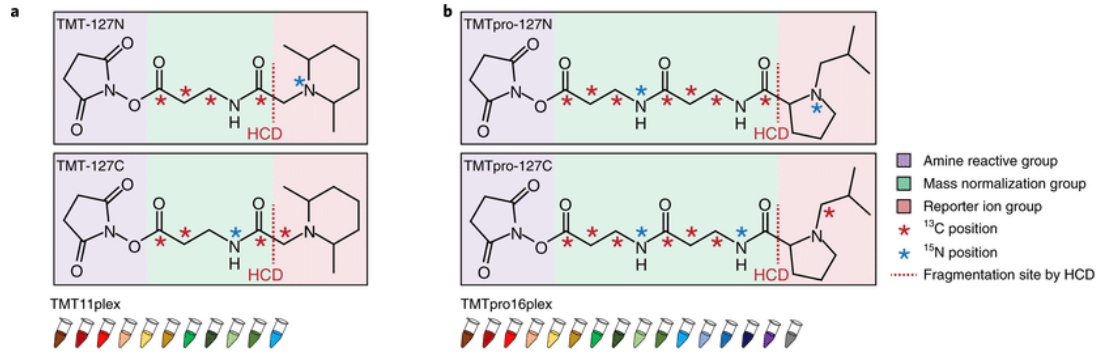
Labelled Vs Label-free



Categories	LABEL-FREE	LABELLED
Time	Sequential	Pooled
Sample Prep	Pre-treatment	Labelled
Comparability	Difficult	Easy
Bio-informatics	Need Software/Labor	
Cost	Cheap	Expensive

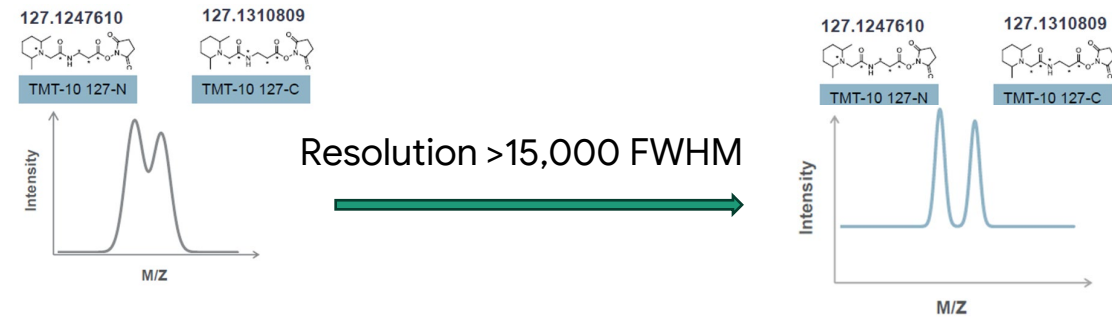


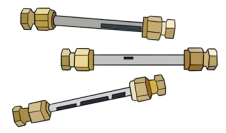
Tandem Mass Tag (TMT)



TANDEM MASS TAG (TMT) เป็นเทคนิคการติดฉลากไอโซบาร์ริก โดยมีวัตถุประสงค์ในการระบุชนิดและปริมาณของโปรตีนในตัวอย่างที่คอนดิชันต่าง ๆ กัน

- ออกแบบครั้งแรกโดยบริษัท Thermo Fisher Scientific
- แต่ละ Plex ต่างกันที่การแทนที่ด้วยไอโซโทป
- ประกอบด้วย NHS-Ester (R), Spacer (N) และ MS/MS reporter (M)
- เกิดพันธะโควาเลนต์กับหมู่เอมิโนของเปปไทด์ที่ปลาย N และไลซีน

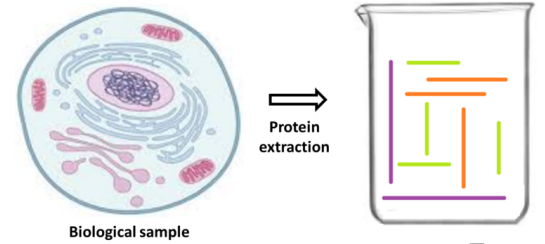
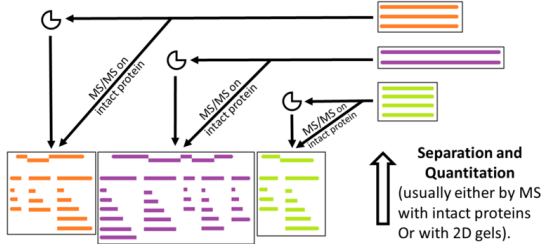




TMT-Based Approach to Quantify

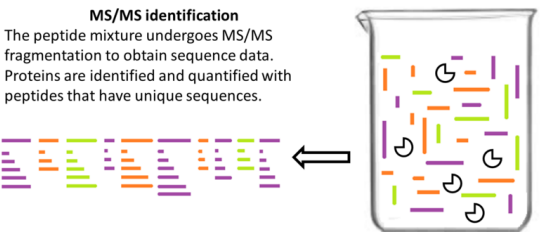
Top-down proteomics

MS/MS for identification
Purified proteins are either enzymatically digested (2D gel spots) for MS/MS fragmentation or intact proteins are just MS/MS fragmented to obtain sequence data for identification.



Bottom-up proteomics

MS/MS identification
The peptide mixture undergoes MS/MS fragmentation to obtain sequence data. Proteins are identified and quantified with peptides that have unique sequences.



PROSIGHTPD



PROTEIN DECONVOLUTION



MASH EXPLORER



BIOPHARMA FINDER

T
TOPIC SUITE/ TOPMG



PROTEOME DISCOVERER + SEQUEST® + MASCOT®

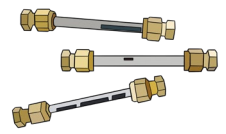


MAXQUANT + ANDROMEDA®

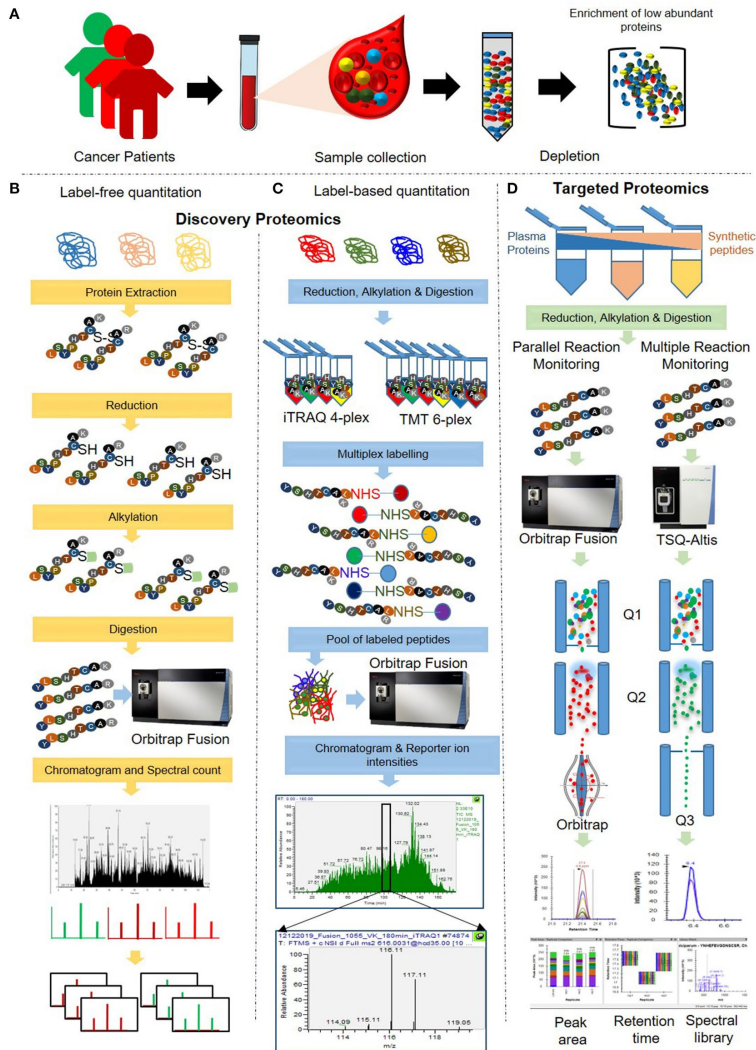


PERSEUS





Quantitative Proteomics for Cancer Biomarkers Discovery

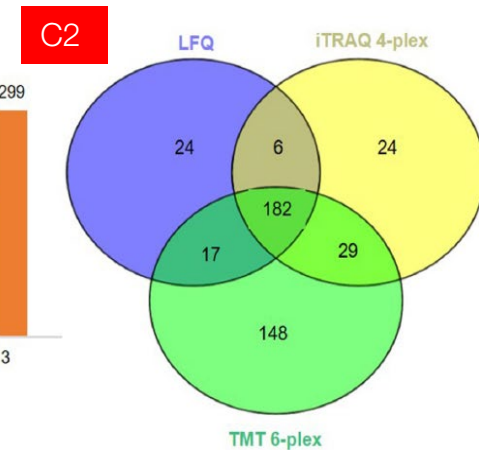
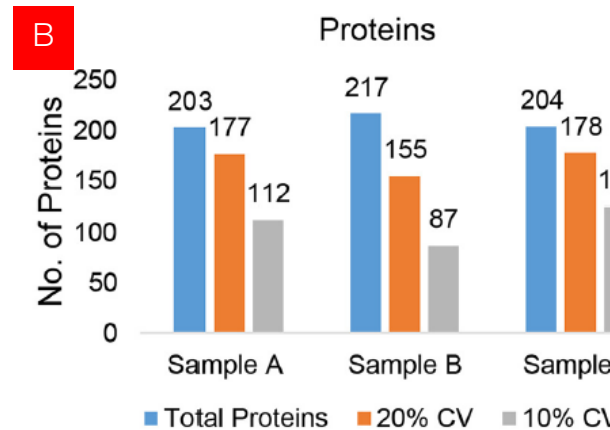


A : Remove high abundance protein from crude plasma

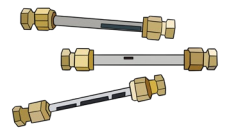
B: Label-free approach after in-solution digestion

C: Label-base quantitation

D: MS/MS experiment with labelled synthetic peptide.



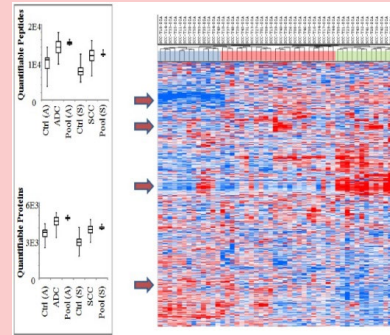
An Integrated Quantitative Proteomics Workflow for Cancer Biomarker Discovery and Validation in Plasma, Kumar V, et al.



Stage 2: Understanding the Basic Differences

Complex studies/Large datasets

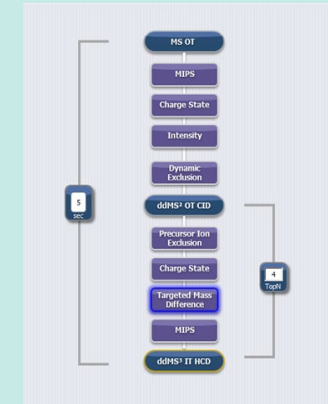
- 100's of raw files
- Results need to be presented by sample, not raw file
- Statistics and proper study design are required



Study management

Complex acquisition methods

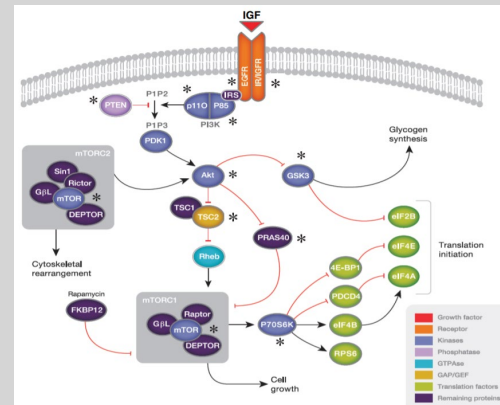
- TMT – SPS MS³
- Glycopeptides – HCD-triggered-> CID->EThcD
- Cross-linking – MS2/MS2/MS3
- Top down – CID, ETD, HCD, EThcD, UVPD



Customizable workflows

Complex biology

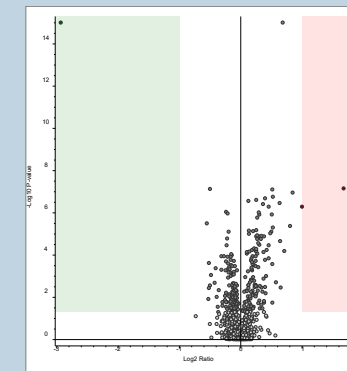
- >10,000 protein IDs
- Microbiome
- PTMs
- Pathway analysis
- Proteoform analysis
- Protein structure



Biological annotation

Results interpretation

- How to denote significantly changing proteins/peptides?
- What is already known about proteins of interest?
- How do we make biological conclusions?

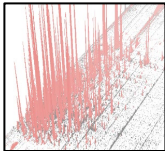


Powerful visualization tools

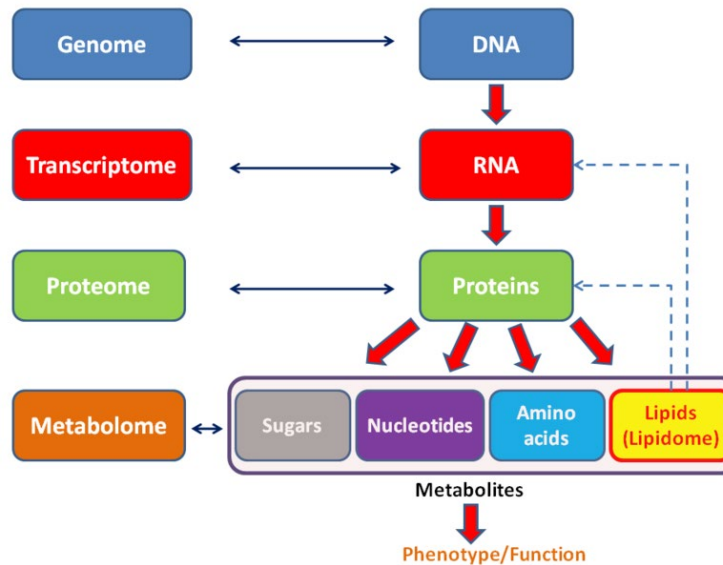


Two Basic Approaches

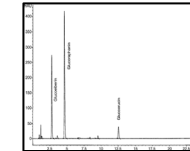
UNTARGETED : SHOTGUN PROFILING



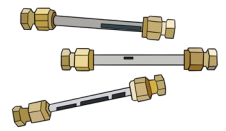
- Many known & more Unknown
- Relative abundances of hundreds compounds
- Potentially a powerful phenotyping
- Analysis is tough



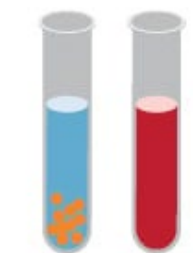
TARGETED : ANALYTICAL BIOCHEMISTRY



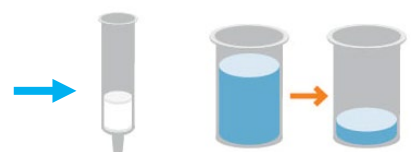
- Biological Pathway
- Flavonoids, Hormones
- Quantifiable
- Analysis is easy



Metabolomics Workflow



Sample Collection

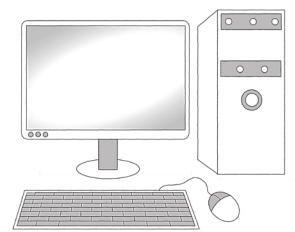


Sample Preparation

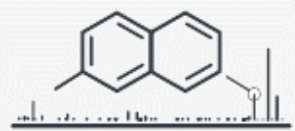


Data Acquisition

Data Processing



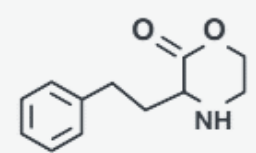
Spectral Interpretation



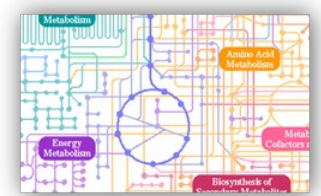
MSⁿ Spectral Tree



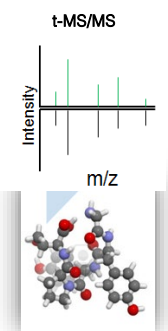
Pathways Analysis



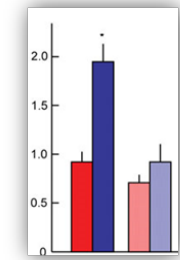
Compound Identification



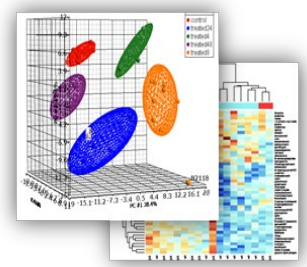
Pathways



Metabolites



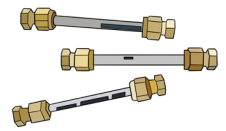
Trends



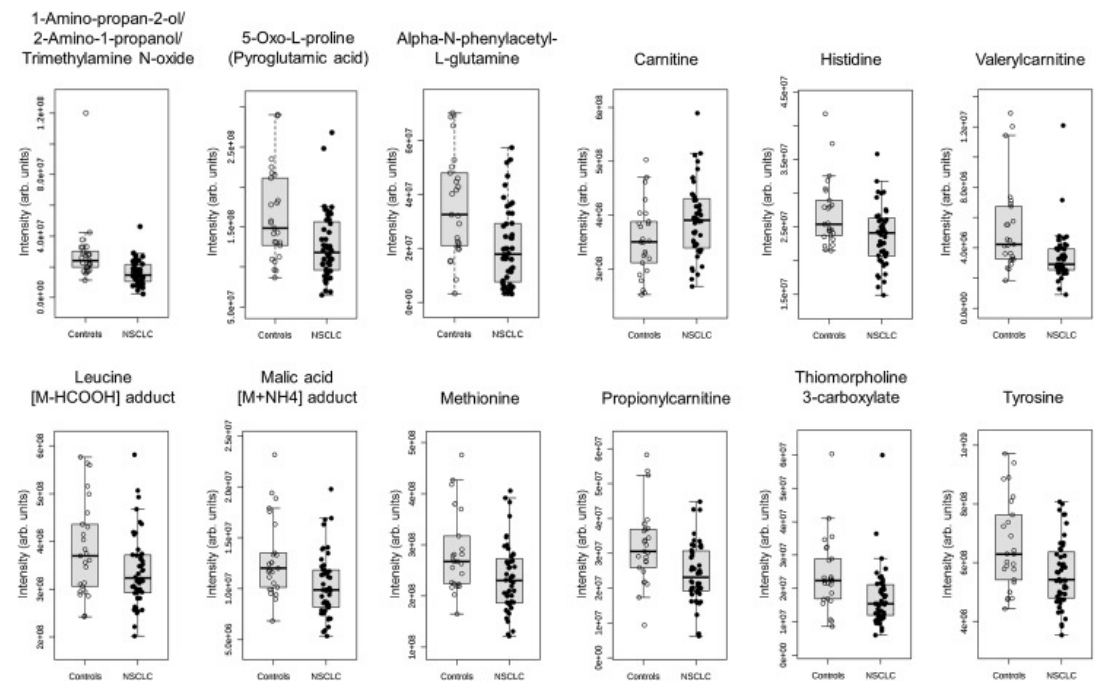
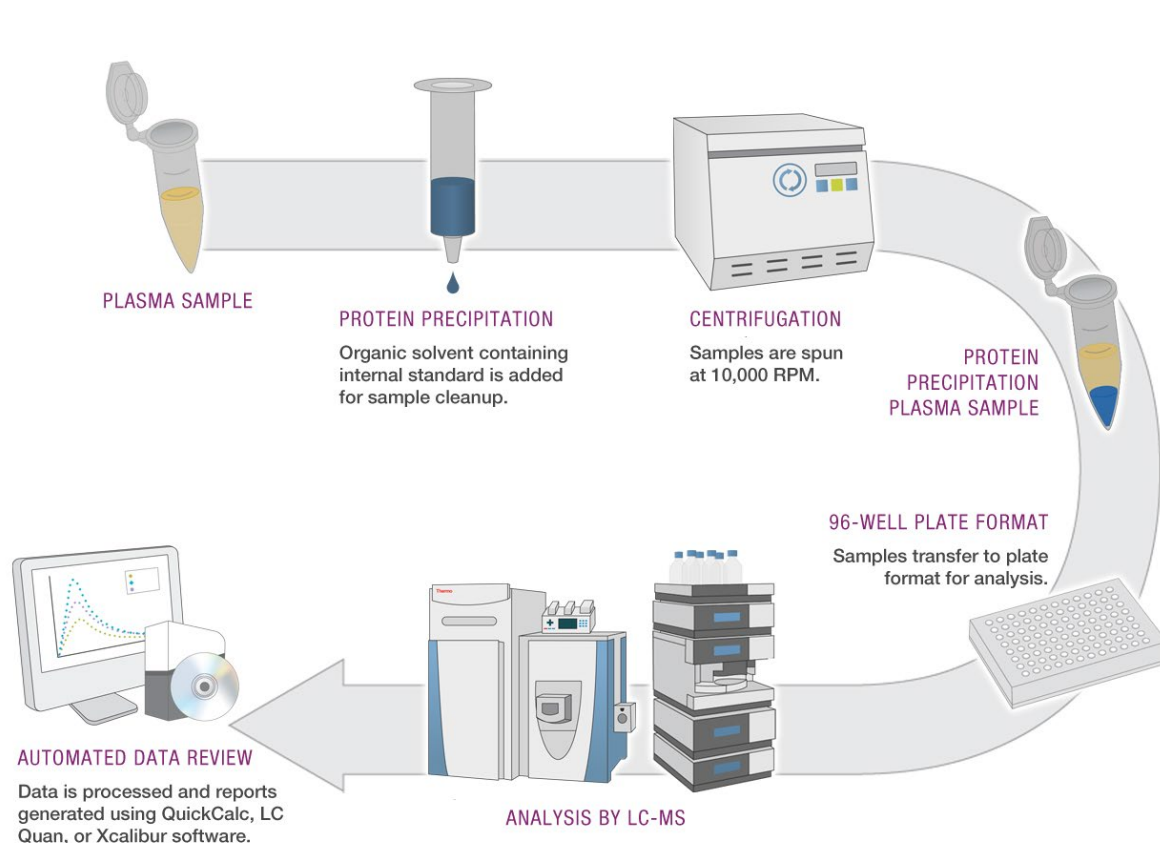
Statistical Analysis



Lists of Compounds



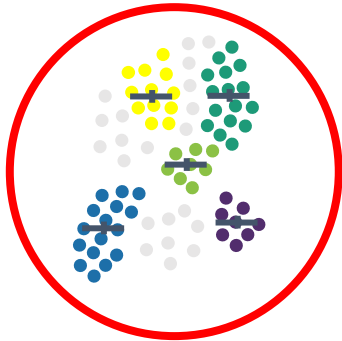
Orbitrap™ Identifies Biomarker for Early Cancer



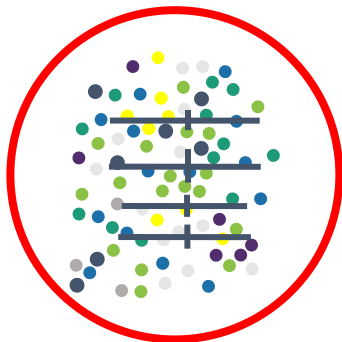
25 Control
14% Male
Age 50-78

50 Early Lung Cancer (IA-IIB)
28% Male
Age 53-86

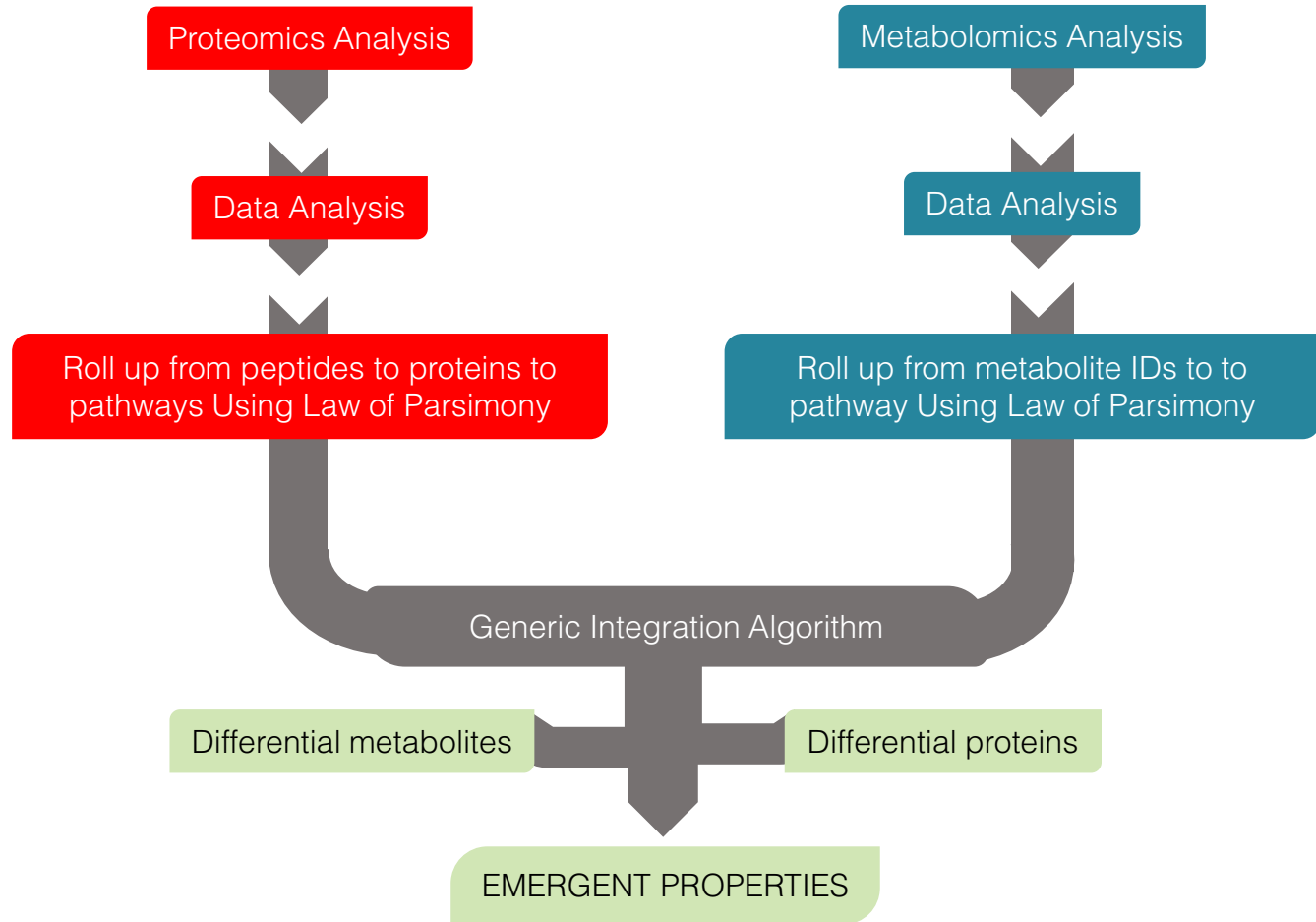
Stage 3: Understand the Correlation in Data

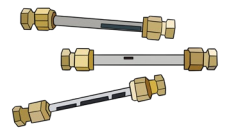


Completely coordinated (ideal) behavior

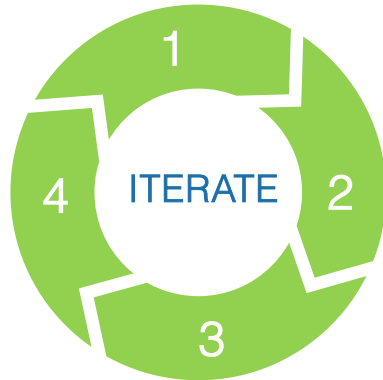
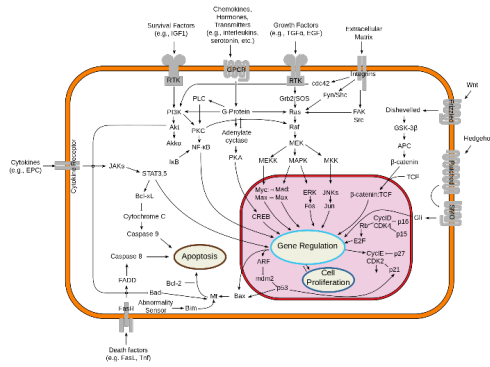


Non coordinated behavior





Stage 4: Understand the Pathway and Metabolism



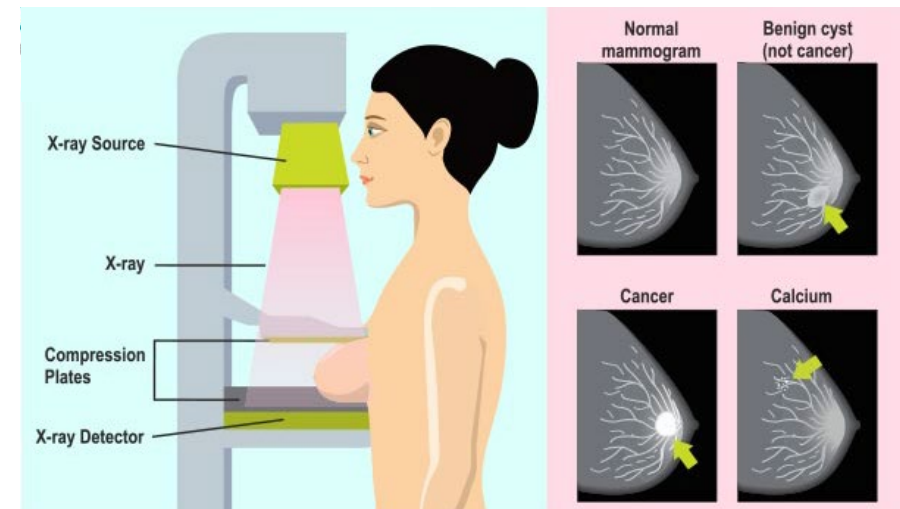
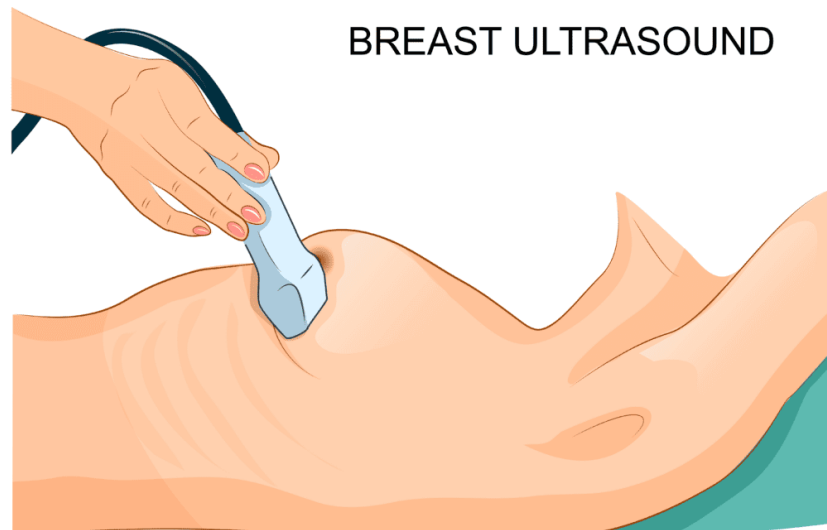
STAGE 4

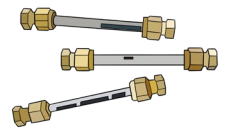
MODULE	INSTITUTION
2DGrapher	SRI
Add Column to Timeseries	UTK
Biodata Viewer	SRI
BioGrid	UTK
BioMat Bridge	SRI
BioNets	UNC
BioPack	VT
BioSens	UCSB
BioSketchPad	U Penn/BBN
BioSmokey	UTK
BioSpreadsheet	UTK
BioWarehouse	SRI
BioWarehouse Query	SRI
BioWarehouse2SBML	Harvard
BioWave	NYU
Cellx	Indiana
Charon	U Penn
Clone Updater	TJU
CoBi	CFDRC
Convert Data to Graph	UTK
DBAgent	SRI
ESS	UTK
Fluxor Computational Analyzer	Harvard
Fluxor Spreadsheet	Harvard
FTF	
GCMConverter	
GCMMerger	
GeneCite	WRAIR
GeneScreen	UCLA
Geneways	Columbia
Get Column from Timeseries	UTK
Get Rows from Timeseries	UTK
Graphviz	LBL
Graph Viewer	UTK
Homologue Finder	LBL
Hybrid Automata Symbolic Reachability Tool	Stanford
IcDNA	UCLA
Jdesigner	KGI



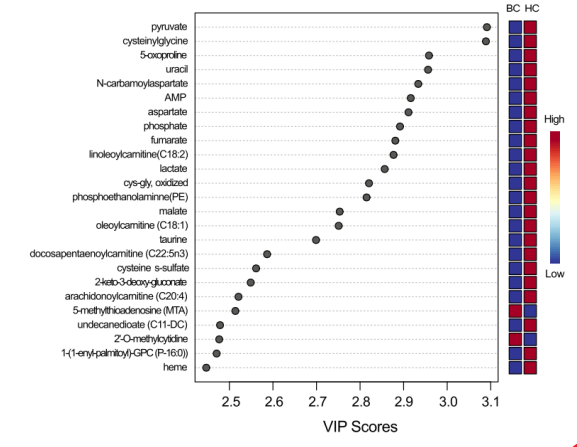
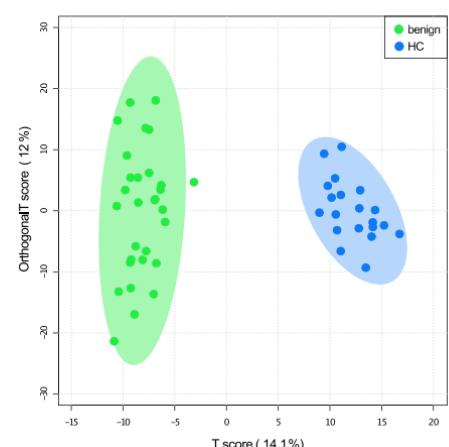
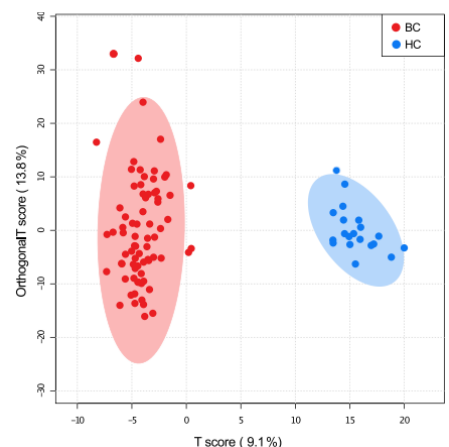
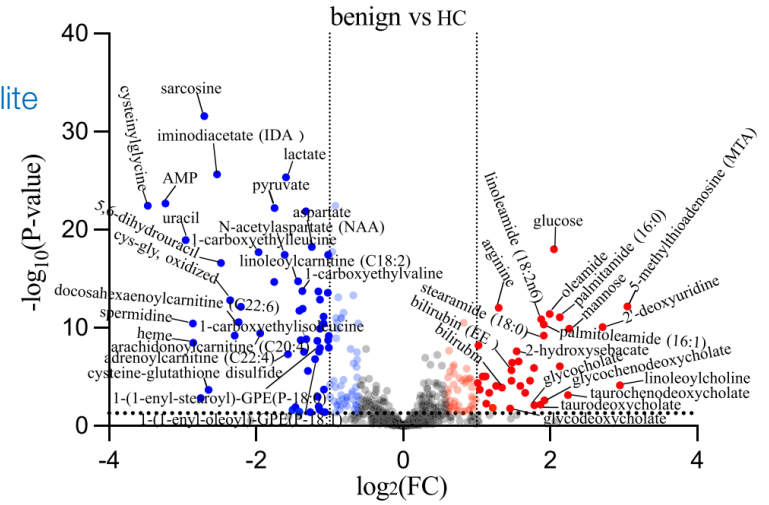
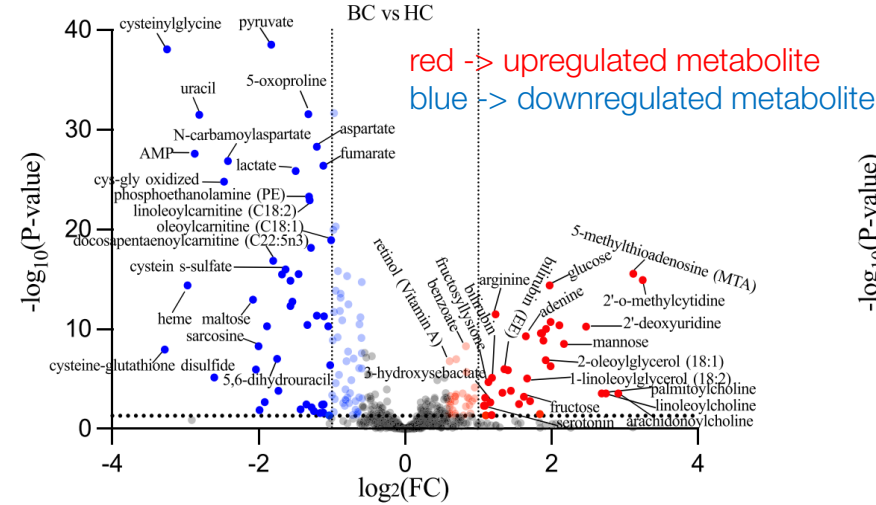
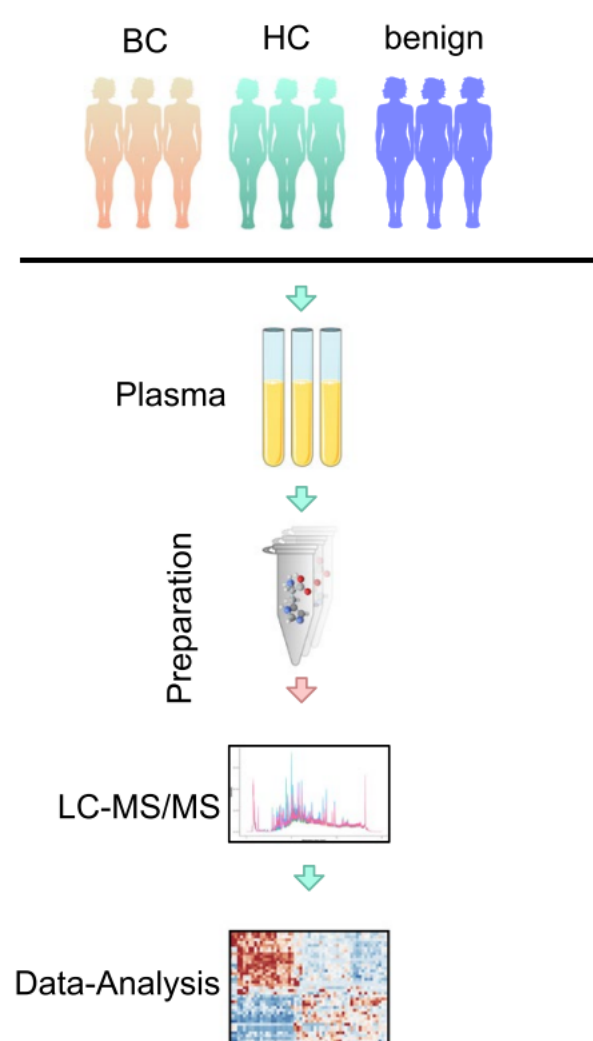
Case Study : Breast Cancer

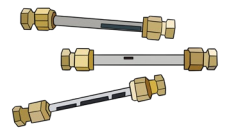
- **Background** : Breast Cancer (BC) is the most commonly cancer. Mammography and ultrasonography are main clinical screening
- **Experiment** : 216 participants, including BC, benign and healthy control (HC). Plasma samples were collected to perform untargeted metabolomics and proteomics
- **Goal** : Find alternative and explore the possibility of specific metabolic signature.





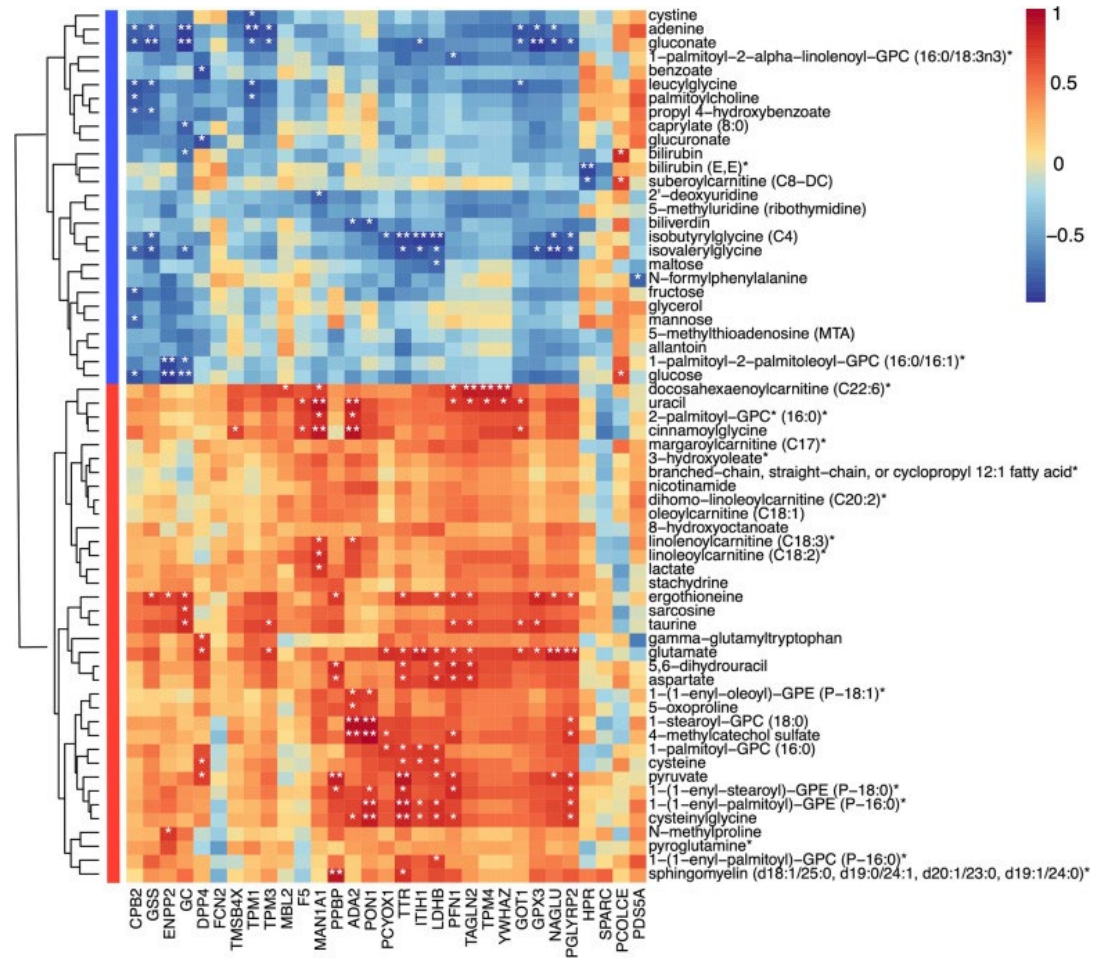
Metabolomics Pipeline & Result

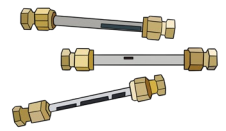




Proteomics – Metabolomics Networking

- A total of 2103 proteins encoded by 1538 genes were identified, of which 1934 proteins encoded by 1407 genes were quantified
- Potentially biomarkers





Summary

- The alanine, aspartate and glutamate pathways, glutamine and glutamate metabolic pathways, and arginine biosynthesis pathways were the critical biological metabolic pathways in BC
- Proteomics identified 29 upregulated and 2 downregulated proteins in BC
- Aspartate aminotransferase (GOT1), L-lactate dehydrogenase B chain (LDHB), glutathione synthetase (GSS), and glutathione peroxidase 3 (GPX3) were closely involved in these metabolic pathways