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Orbitrapを含めた質量分析とプロテオーム解析の基礎と最新トピックス

サーモフィッシャーサイエンティフィック
肥後 大輔

The world leader in serving science

AREA of DETAIL
Site of Aggregation
Adduct formation
N-terminal pyroE formation
Conjugation Site (ADC)

LC-MS: 液体クロマトグラフィー、質量分析計

Sample Prep. HPLC Column **Mass spec.** Software

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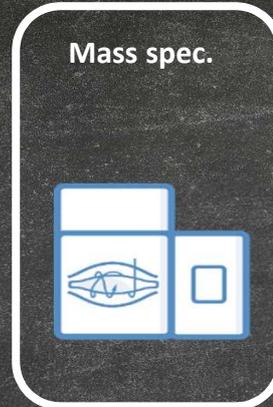
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Mass spectrometer, Mass spec., MS : 質量分析計

分子の質量を計測する“はかり”

- × 分子 → 質量分析
- 分子 → **分子イオン** → 質量分析

高真空の質量分析計に分子イオンを取込み
 分析計内部は電場 and/or 磁場を利用
 分子イオンを分析計内部で移動、飛行、運動
 分子イオンを質量と電荷比(m/z)で分離/検出

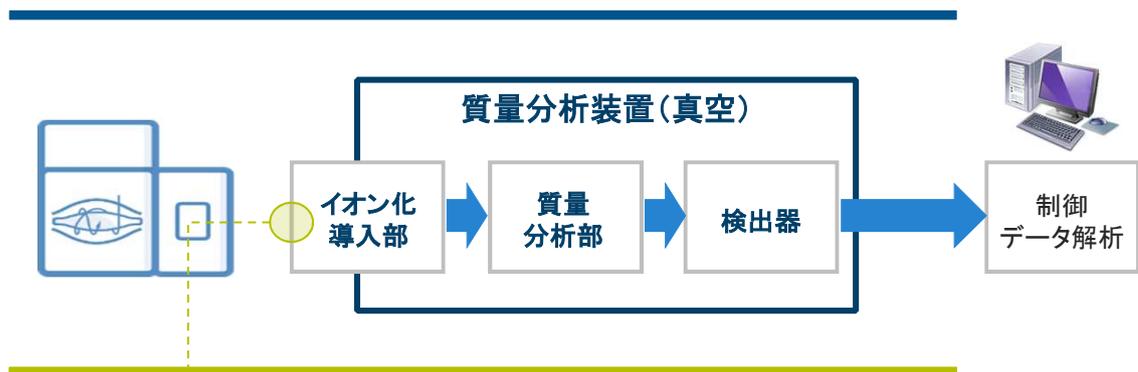


質量分析計で得られた結果 → 構造解析、定量解析

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質量分析計: イオン化法

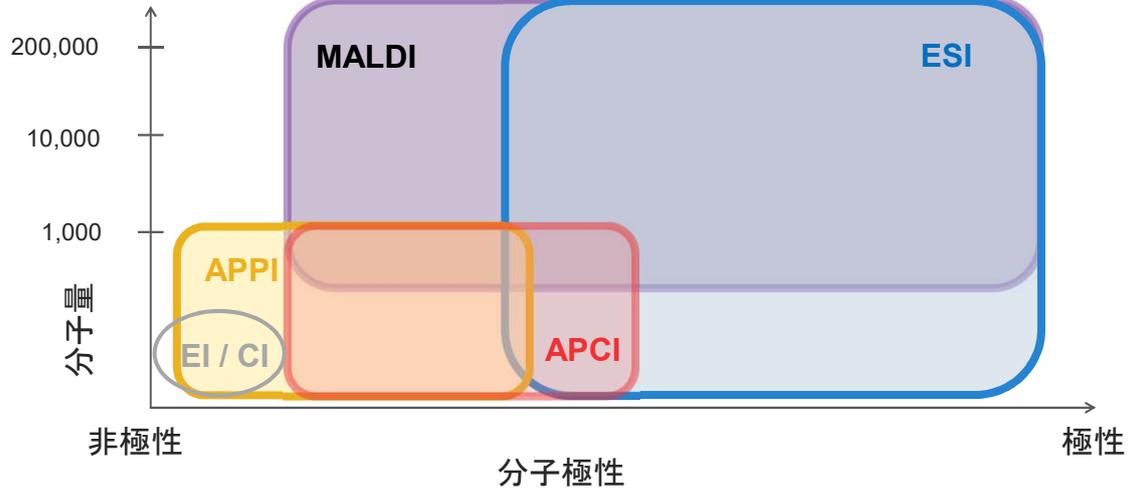


サンプルの形状	液体	気体	固体 (結晶)
イオン化法	ESI APCI APPI	EI, CI	MALDI

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質量分析計：イオン化法

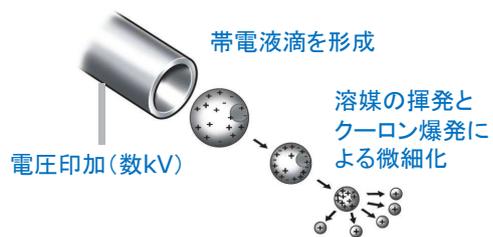


5

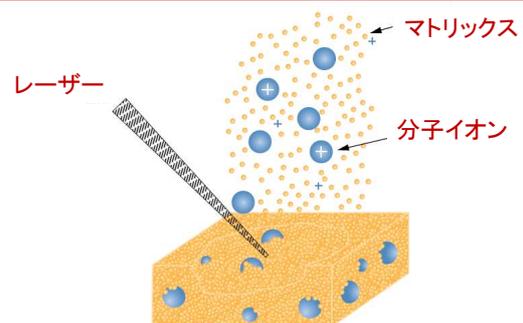
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質量分析計：イオン化法 ESI & MALDI

ESI (Electro Spray Ionization)



MALDI (Matrix Assisted Laser Desorption Ionization)



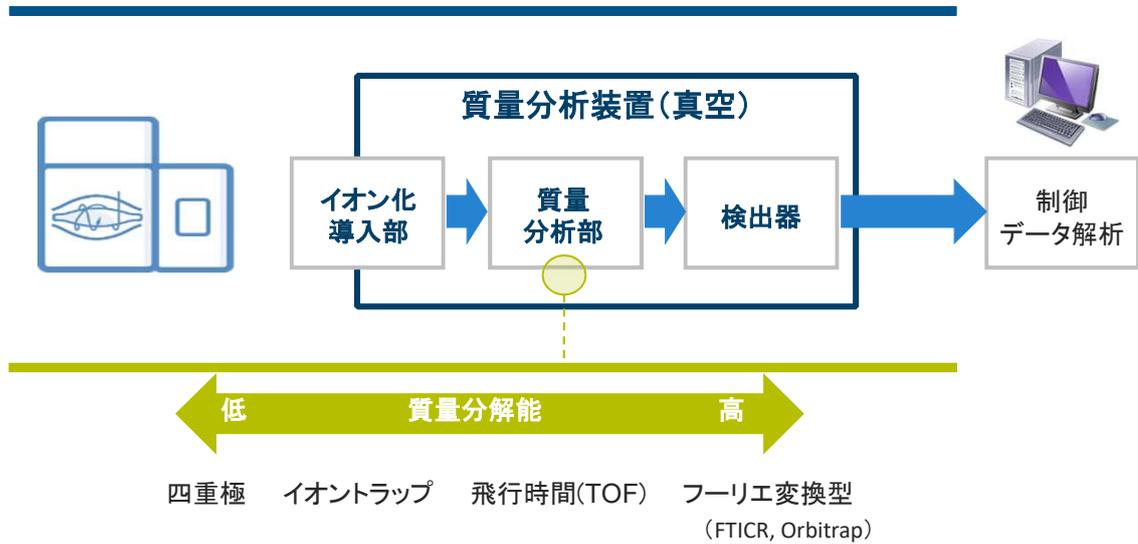
マトリックスはレーザー光を吸収、加熱されサンプルとともに気化、このときに真空中でイオン化



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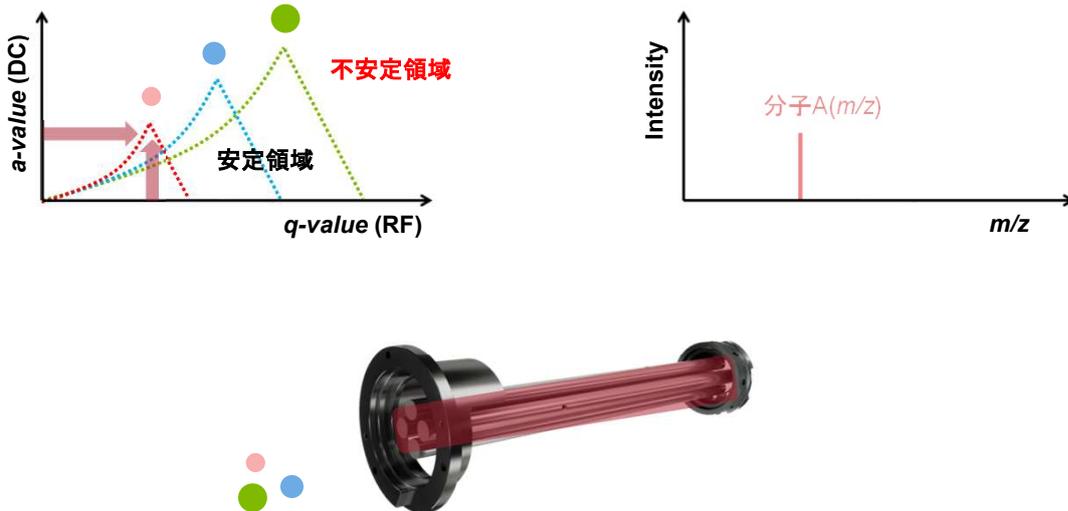
質量分析計：質量分析部



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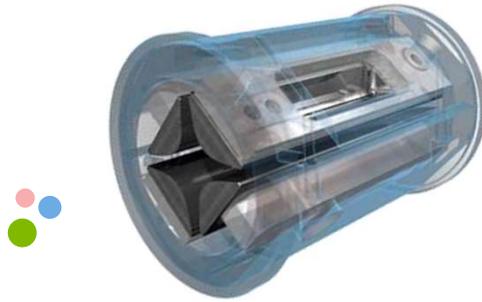
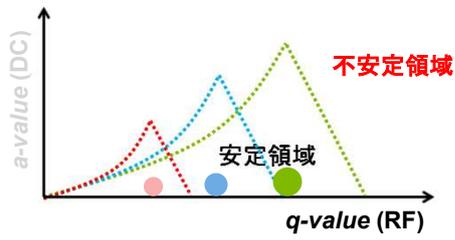
質量分析計：質量分析部：四重極



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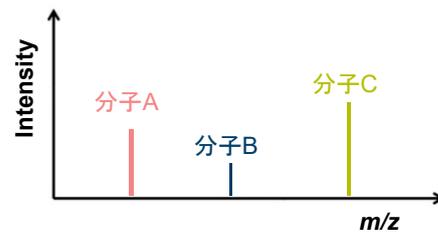
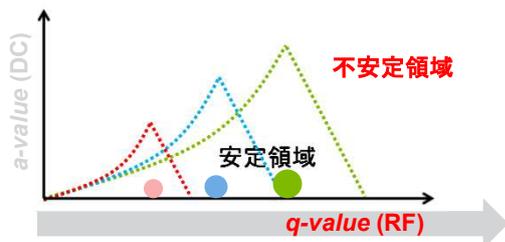
質量分析計：質量分析部：イオントラップ



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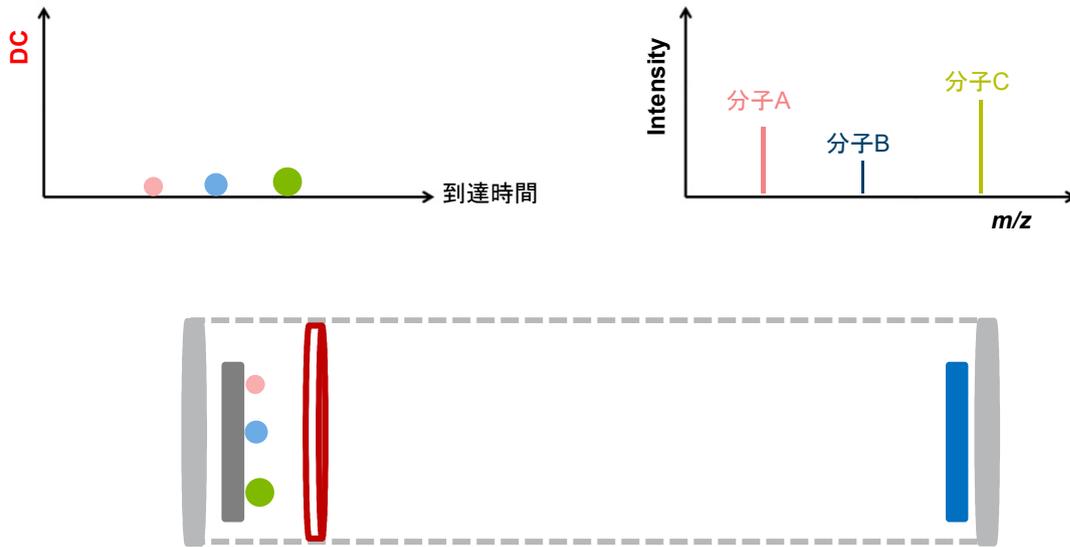
質量分析計：質量分析部：イオントラップ



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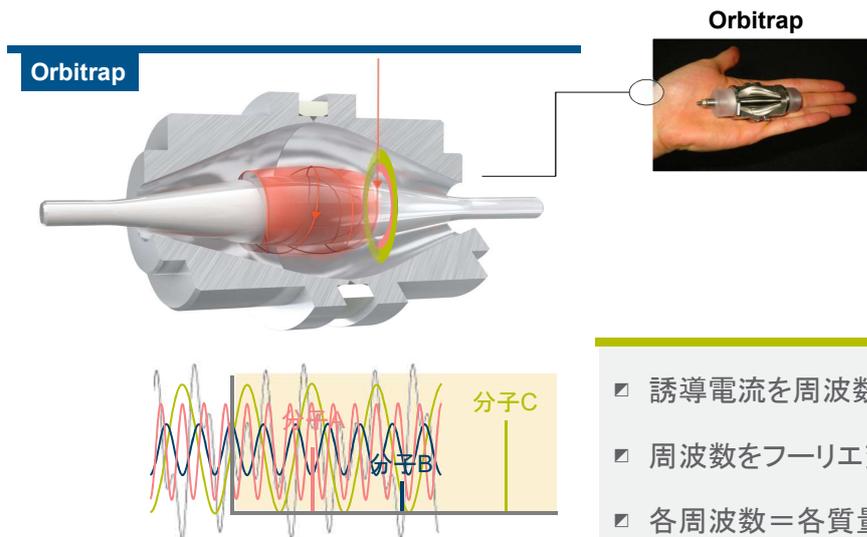
質量分析計：質量分析部：TOF (Time of flight)



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質量分析計：質量分析部：オービトラップ



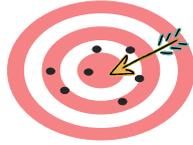
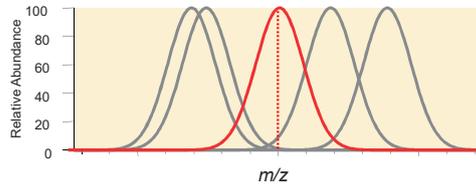
- ❑ 誘導電流を周波数として記録
- ❑ 周波数をフーリエ変換で分解
- ❑ 各周波数=各質量成分 (m/z)

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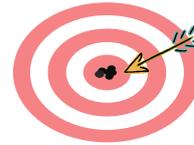
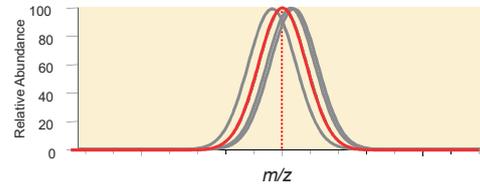
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Orbitrapの特徴: 高い質量精度

質量精度: poor



質量精度: good

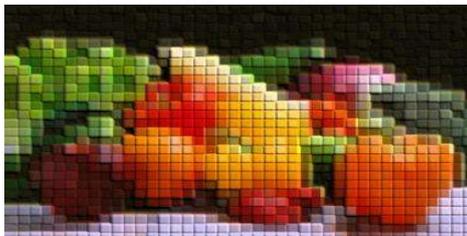
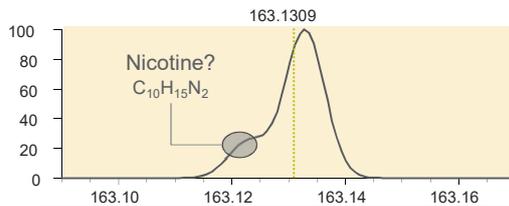
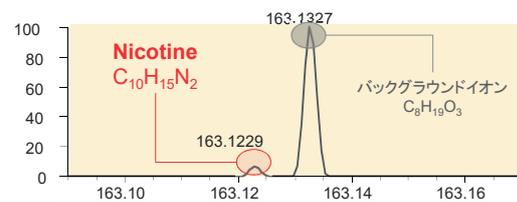


- 観測される m/z のばらつき。測定値間誤差
- 装置の測定原理に依存

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Orbitrapの特徴: 高い質量分解能

低分解能 ($R=20,000$)高分解能 ($R=50,000$)

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Single MS / Hybrid (Tandem) MS

Full MS Scan

Single MS

Hybrid MS

Isolation

Fragmentation

MS/MS Scan

Data dependent

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MS/MS : Fragmentation : Collision Induced Dissociation

CID

Fragmentation

CC(C)NC(=O)C(C)NC(=O)C(C)NC(=O)C(C)N

a b c

x y z

D A I L P E N L P P L T J A D F A E D K D V c K

Intensity (Counts)

m/z

147.1128 227.1010 341.1431 464.2295 685.3055 753.2711 852.8938 1081.0051 1230.5801 1390.6240 1608.7334 1707.7836 1820.8589 2160.8981

Isolation

Fragmentation

MS/MS Scan

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Single MS / Hybrid (Tandem) MS

MALDI-TOF

Qq-TOF

Qq-Iontrap-FT

MALDI-TOF/TOF

Qq-FT

Tribrid MS

QqQ

ターゲット定量装置

ESI MALDI

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Single MS / Hybrid (Tandem) MS : Proteomics

MALDI-TOF

MALDI-TOF PMF search

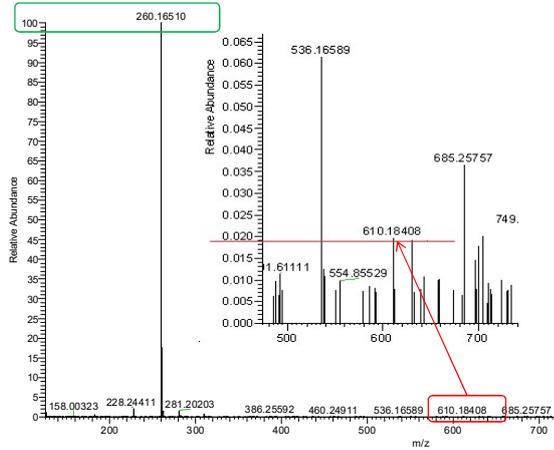
MALDI-TOF/TOF

MALDI-TOF/TOF MS/MS search

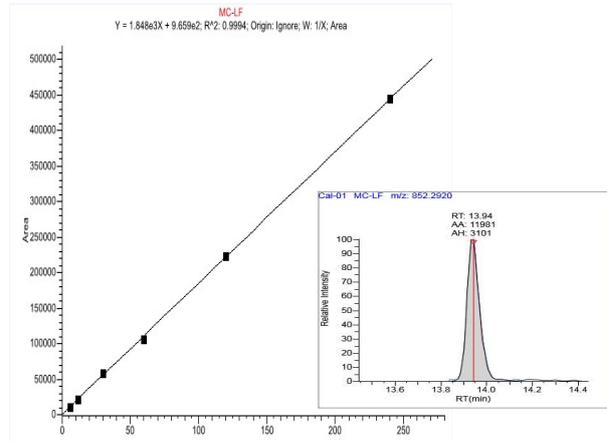
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質量分析計のダイナミックレンジ

スペクトル内ダイナミックレンジ



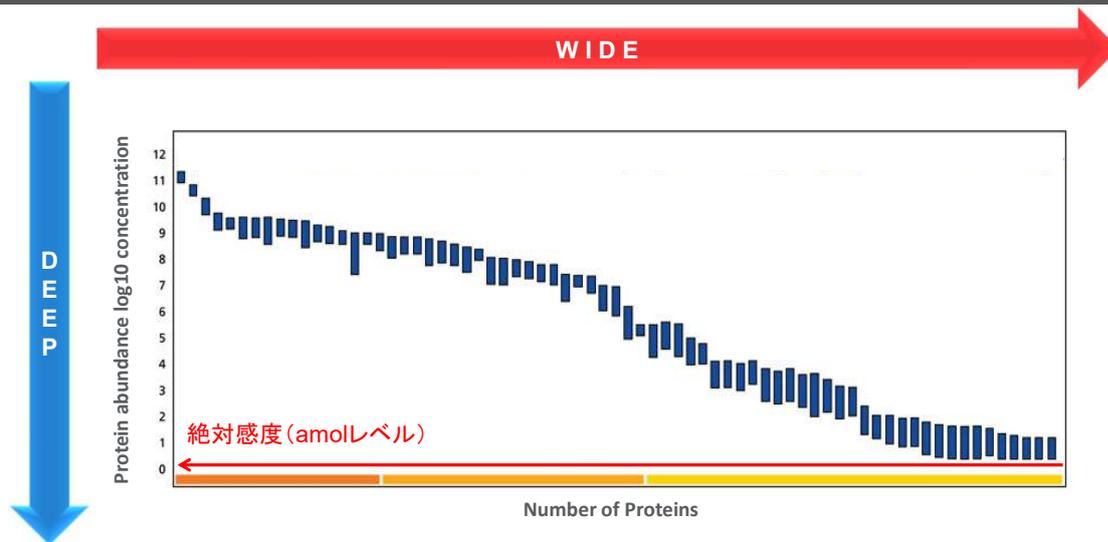
リニアダイナミックレンジ



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質量分析計のダイナミックレンジ: プロテオームサンプル



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質量分析計のダイナミックレンジ: プロテオームサンプル

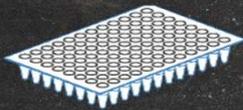


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Proteomics : Hybrid (Tandem) MS

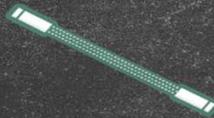
Sample Prep.



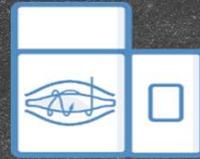
HPLC



Column



Mass spec.



Software



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HPLC & Column

HPLC

混合成分 → ポンプ
オートサンプラー
カラムオープン

各成分

カラム

- 逆相: 疎水性相互作用
- 順相: 親水性相互作用
- イオン交換: 電気親和力
- サイズ排除: 浸透・排除
- ミックスモード: 上記複数

細孔径 (ポアサイズ) 8-30nm

2-10 μm 直径

- ▶ 固定相支持体
 - シリカゲル、ポリマー樹脂 など
- ▶ 化学結合相
 - 固定相支持体に化学的に結合させた官能基

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HPLC vs nanoLC

nanoLC
0.1 ~ 0.5 μL/min

1 ug sample

ポンプ

移動相A: 水+0.1%ギ酸
移動相B: アセニト+0.1%ギ酸

Column

逆相: 疎水性相互作用

HPLC
200 ~ 500 μL/min

0.075 mm ID

↑ x 711 濃縮比(内径²に反比例)=感度の倍率

2.0 mm ID

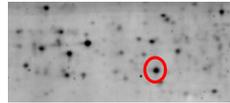
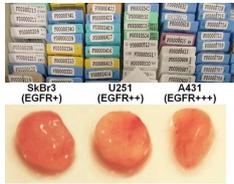
TIC

TIC

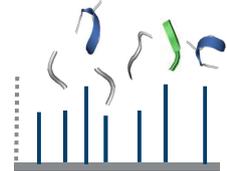
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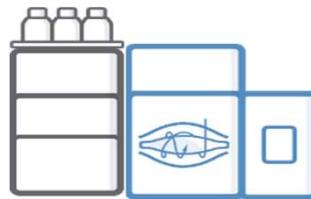
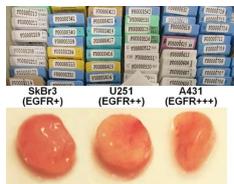
MALDI-TOF(/TOF)



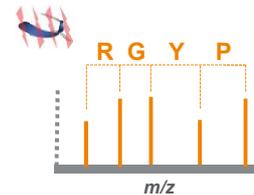
Full MS scan



nanoLC-MS/MS



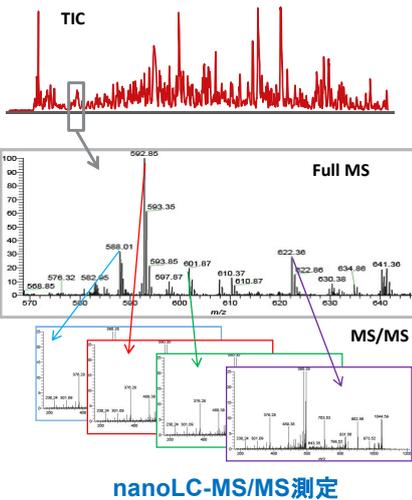
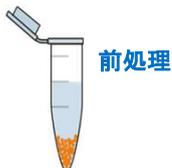
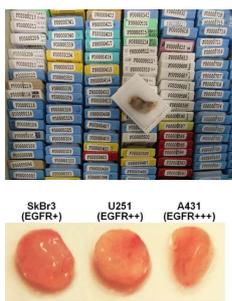
MS/MS scan



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Proteomics : Hybrid (Tandem) MS



同定

Protein	Protein ID	Master	Accession	Description	Exp. q-value	Score	PEP Score	Coverage [%]	Input F
✓	P23533	✓	P23533	Filamin-B [Homo sapiens]	0.000	79.897	1%		
✓	P02818	✓	P02818	Heat shock 70 kDa protein 1A [Homo sapiens]	0.000	61.259	28%		
✓	P11142-1	✓	P11142-1	Heat shock cognate 71 kDa protein [Homo sapiens]	0.000	60.845	28%		
✓	P04792	✓	P04792	Heat shock protein beta-1 [Homo sapiens]	0.000	40.820	25%		
✓	Q23177	✓	Q23177	Spliceosome protein kinase SHAK 2 [Homo sapiens]	0.000	25.581	33%		
✓	P17935	✓	P17935	Transcription factor XII [Homo sapiens]	0.000	20.809	32%		
✓	P05533	✓	P05533	epidermal growth factor receptor [Homo sapiens]	0.000	18.251	3%		
✓	P4712	✓	P4712	Cytosolic phosphodiesterase 82 [Homo sapiens]	0.000	16.680	2%		
✓	P36507	✓	P36507	Mitogen-activated protein kinase kinase kinase 2 [Homo sapiens]	0.000	12.447	3%		
✓	Q16529	✓	Q16529	Dual specificity protein phosphatase 7 [Homo sapiens]	0.000	11.932	4%		
✓	P04637	✓	P04637	Cellular tumor antigen p53 [Homo sapiens]	0.000	9.179	6%		
✓	P51812	✓	P51812	Ribosomal protein S6 kinase alpha-3 [Homo sapiens]	0.000	6.566	3%		
✓	Q0912	✓	Q0912	Mitogen-activated protein kinase kinase kinase MLT [Homo sapiens]	0.001	6.607	3%		
✓	Q23763	✓	Q23763	Spliceosome protein kinase TSC1 [Homo sapiens]	0.001	6.556	2%		
✓	P11831	✓	P11831	Serum response factor [Homo sapiens]	0.001	6.299	5%		
✓	P21359-1	✓	P21359-1	Neurokinin [Homo sapiens]	0.001	6.059	1%		
✓	Q16525-1	✓	Q16525-1	Inhibitor of nuclear factor kappa-B kinase subunit beta [Homo sapiens]	0.001	4.316	2%		
✓	R13143	✓	R13143	ribosomal protein kinase L17 [Homo sapiens]	0.001	4.151	6%		

MS/MS検索

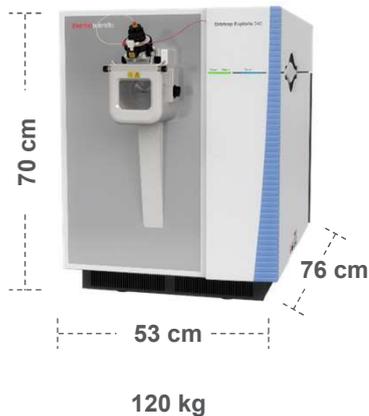


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Orbitrap Exploris 240

Orbitrap Exploris 240



コンパクトな設計

最大分解能: 240,000@m/z 200

最大スキャンスピード: 22Hz

高質量精度: 外部標準<3ppm, 内部標準<1ppm
EASY-IC <sub 1ppm (5日間以上)

Pos/Neg極性切り替え測定: 切替時間 約185ms(Full MS)

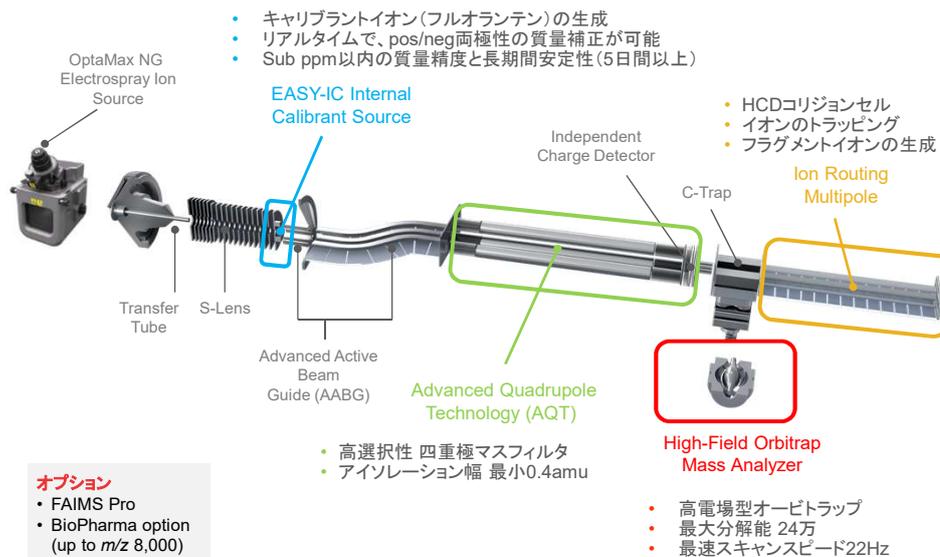
コリジョンセルによるHCD-MS/MS

オプション: FAIMS Pro, BioPharma

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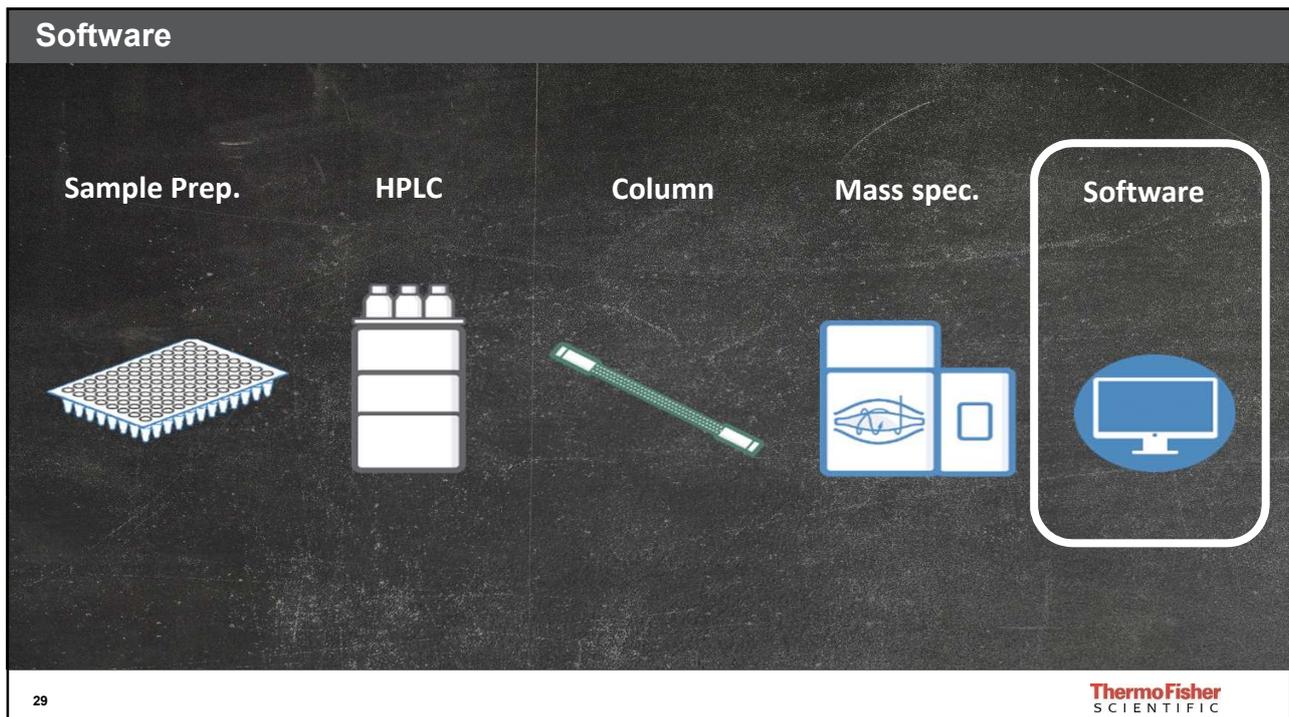
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Orbitrap Exploris 240の装置構成



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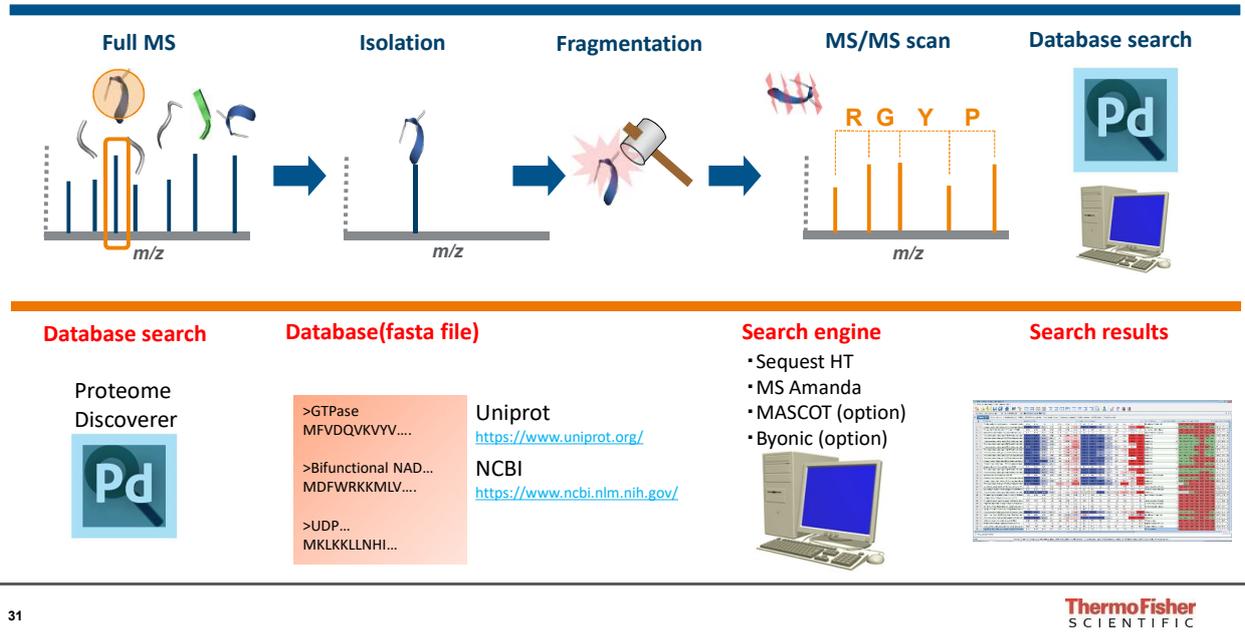


Proteome Discoverer software

- ・プロテオーム解析専用ソフトウェア
 - 公的なタンパク質データベースを利用したタンパク質の同定
 - リン酸化など翻訳後修飾解析に対応
- ・定量プロテオーム解析に対応
 - ケミカルラベル(TMT法)による同定/定量解析に対応
 - 安定同位体ラベル(SILAC法)による同定/定量解析に対応
 - ラベルフリーによる比較定量解析に対応
 - 同定/定量結果は統計解析(ANOVA, PCA, p-value, Heat map)
- ・糖鎖修飾ペプチド解析プログラム(オプション)
 - Byonicアルゴリズムを追加することによってN/O型糖ペプチドの同定に対応
- ・クロスリンク試薬によるタンパク質相互作用解析(オプション)
 - XlinkXアルゴリズムを追加することによって相互作用したタンパク質を同定

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Software work flow

- 定量解析メソッドの選択 (SILAC, TMT or LFQ)
- 測定データの選択 (Rawファイル)
- 定量解析と測定データの紐付け (コントロールの選択など)
- 検索 & 定量解析条件の設定 (データベースや翻訳後修飾の選択など)
- 定量結果の表示方法の指定 (比較定量の計算設定)
- 解析実行

The screenshot shows the Proteome Discoverer software interface. The 'Study Definition' window displays a list of samples (F1-F4) with IDs and names. The 'Analysis' window shows a workflow with 'Consensus Step' and 'Processing Step'.

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MSによる定量解析法

LC-MS/MS

Database search

- Peptide ID
- RT
- m/z, z

XIC, Peak area

Label Free Quan (LFQ)

SILAC labeling

K0, R0 K6, R10

Mix 1:1

Trypsin digest

K containing peptide R containing peptide

SILAC
(Stable Isotope Labeling by Amino Acids in Cell Culture)

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MS/MSによる定量解析法

DIA-MS/MS

Precursor m/z

Time

Duty cycle

Spectrum Library

Data Independent Acquisition (DIA)

Reporter Normalizer

126-131 103-97

N-term and K

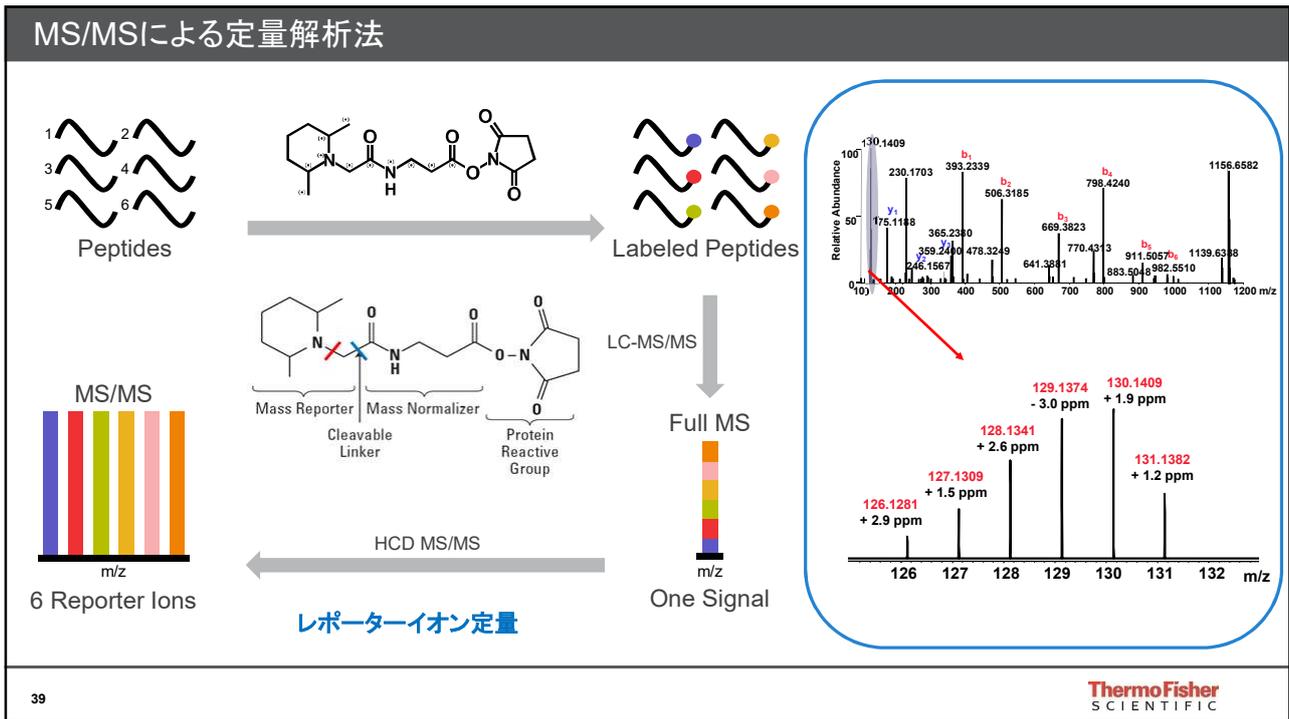
TMT-labeled Peptides

MS/MS

Reporter Ion Quan.
(Tandem Mass Tag, iTRAQ etc...)

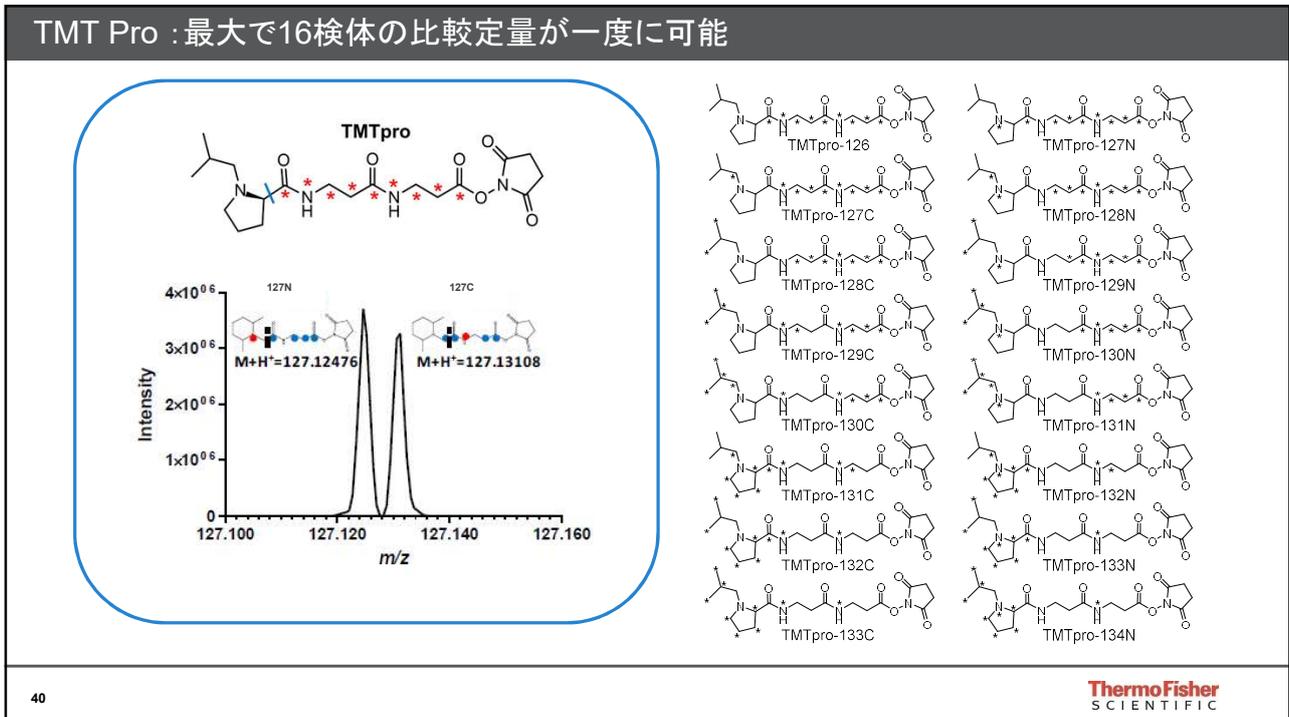
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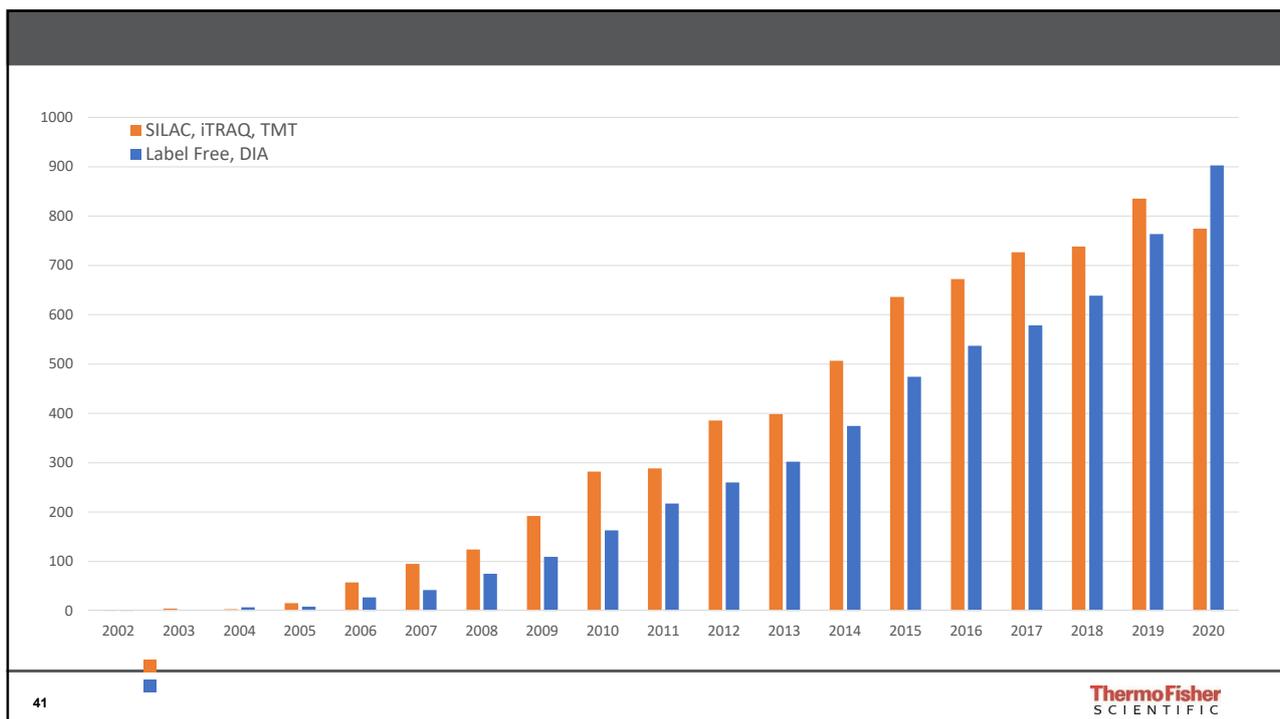
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	SILAC	iTRAQ/TMT	LFQ	DIA
Quantify with	MS1 Precursor	MS2 Reporter	MS1 Precursor	MS2 Fragment
Coverage (1shot)	++	+++	++++	+++++
Coverage (Extend)	+++++ (Fraction)	+++++ (Fraction)		
Accuracy	+++++	++* (Ratio Compression)	+++	++++
Precision	++++	+++++	+++	++++
Throughput	+++ (Up to 3plex)	+++++ (Up to 16plex)	++ (1 by 1)	++ (1 by 1)
Easy to start	+++ (Cost)	+++ (Cost)	+++++	++++ (Processing tool)
Notes	Cell line only.	Compatible with any samples (tissue, fluid etc.) *with mobility or MS3 method, accuracy will be improved to +++ or ++++	Most simple method same as standard shotgun proteomics	PTM is not supported in library prediction

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Proteome Discoverer software : Quan Proteomics

The screenshot displays the Proteome Discoverer interface with a table of protein identifications. Two red callout boxes highlight specific features:

- Ratios based on median values from replicates:** Points to the 'Median (Median)' column in the table.
- p-values can be calculated due to replicate measurements:** Points to the 'p-Value' column, which contains values like 1.76e-1.

Checked	Protein FDR	Master	Gene	Accession	Protein Name	Median (Median)	p-Value	Abundance	...
1	High	✓	ADP1	P27188	Argininosuccinate synthase [OS=Saccharomyces cerevisiae (strain ATCC 22016 / DSM 15204 / EC 4.1.3.15)]	3.223	1.76e-1	69.1	...
2	High	✓	TDH1	P03260	Glyoxaldehyde 3-phosphate dehydrogenase 1 [OS=Saccharo...	0.857		72.2	...
3	High	✓	GDV2	P49095	Glyoxal dehydrogenase (scapaboylating), mitochondrial [OS=...	1.454		88.6	...



解析結果を様々な図表として出力が可能

The collage illustrates various data visualization methods available in the software:

- PCA:** A scatter plot showing the separation of different experimental conditions.
- Volcano plots:** A plot of log2 fold change versus -log10 p-value, highlighting significant differentially expressed proteins.
- Pathway maps:** Network diagrams showing the biological context of identified proteins, such as mitochondrial membranes and organelle envelopes.
- Heat maps:** A heatmap with dendrogram showing relative protein abundance across multiple samples.
- Enrichment analysis:** A table showing the enrichment of specific gene ontology terms.

Accession	Description	Size	Overlap	Expected	Fold Enrichment	p-Value	-log2 (p-Value)
GO:0019888	organelle inner membrane	47	24	8.3	2.9	0.000105	23.26
GO:000743	mitochondrial inner membrane	47	24	8.1	2.9	0.000105	23.26
GO:0019866	mitochondrial membrane	68	29	12	2.4	0.000105	20.71



プロテオーム解析手法のトレンド

Peptide ID	TMT SPSMS3	LFQ	Phosphorylation	Glycoproteomics	Crosslinking	TMT MS2
SILAC	Spectral Counting	Lipidomics	IBAQ	Intact Profiling	Middle-Down	DIA
Top Down	Denovo Sequencing	δ -carboxylation	Neu Code	Metabolic Labeling (SL)	Oxidation	AIM
PRM	Other PTMs	Combinatorial Isotopic Mass Tags	δ -Carboxylation	δ -glutamylation	δ -sulfonylation	Penylation
Ubiquitylation	ADP Ribosylation	SUMOylation	SUMOylation	SUMOylation	SUMOylation	SLIM
Metabolomics	Glycation	Glycation	Glycation	Glycation	Glycation	SLIM
ITRAQ MS2	WiSIM-DIA	Lasso peptides	SIM	Acylation	Acylation	DipyrO
Dimethyl Labeling	S-nitrosylation	Disulfide Structures	Limited Proteolysis	Pegylation	Disulfide Mapping	Dileu Labeling
Glycomics	iTRAQ SPSMS3	Peptide Epimer Analysis	iodoTMT	Cysteine Residue	S-Sulfination	Thiol Modifications
Acetylation	Peptide Mapping	S-Palmitoylation	Hydroxylation	Thiol Modifications	Thiol Modifications	Hydroxylation

- Peptide ID
- Quan Proteomics
- Phosphorylation

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Proteomics : Deep : Phosphopeptide Enrichment

DEEP

Fe-NTA

3,378 Proteins

12,465 Phosphopeptides

10,436 Quantified

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