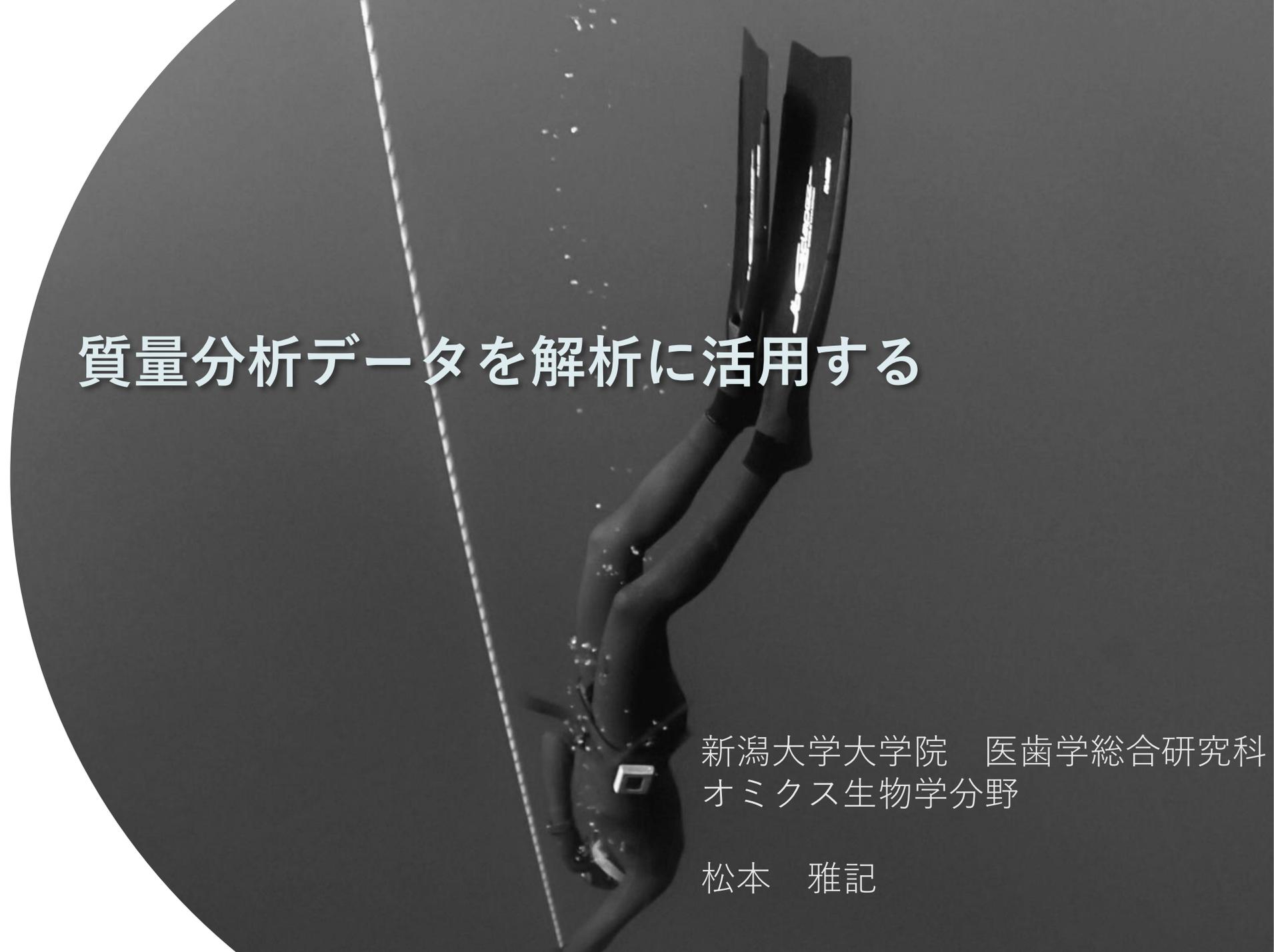


2023.8.30

AJACSオンライン17

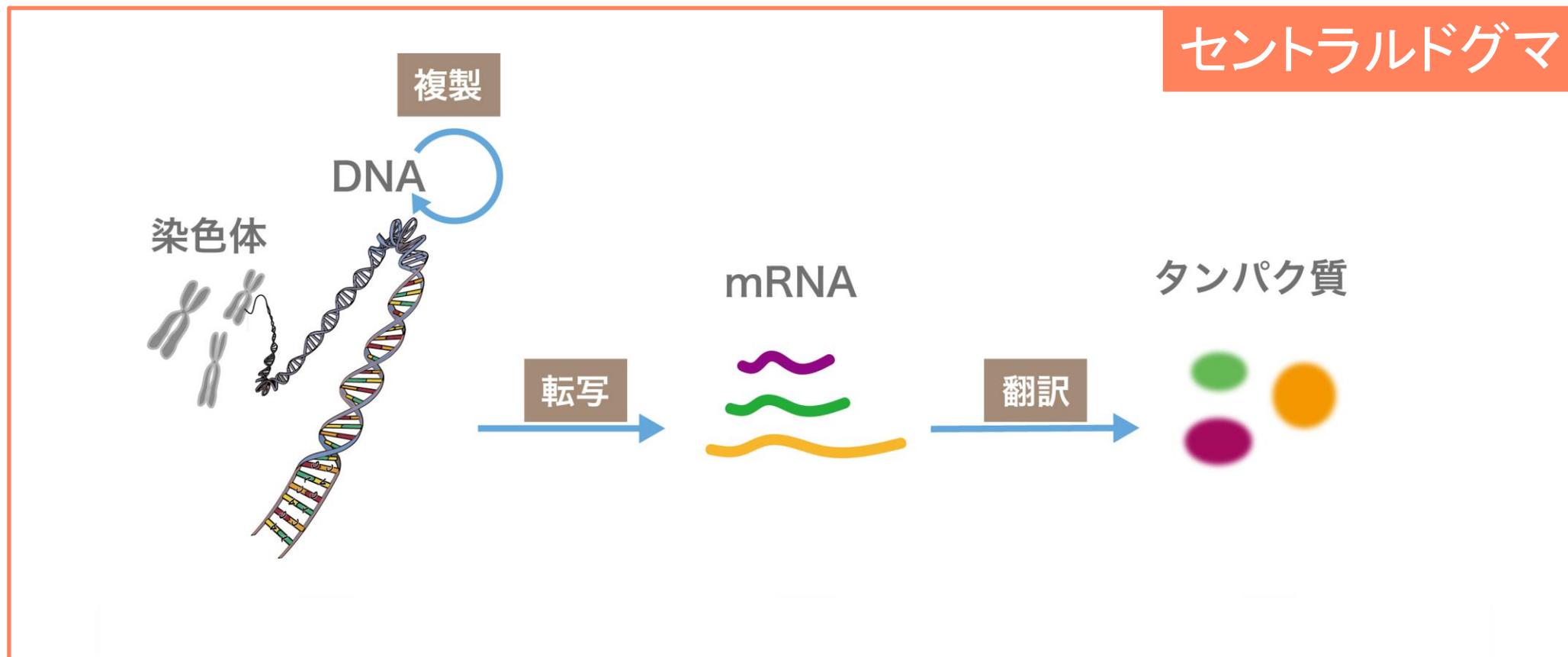
質量分析データを解析に活用する



新潟大学大学院 医歯学総合研究科
オミクス生物学分野

松本 雅記

遺伝子はタンパク質の設計図である



ゲノム

トランスクリプトーム

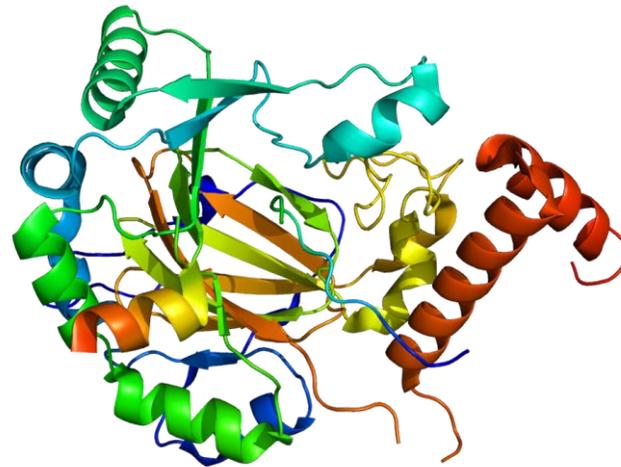
プロテオーム

ゲノムを基盤とする生命システムの成り立ち

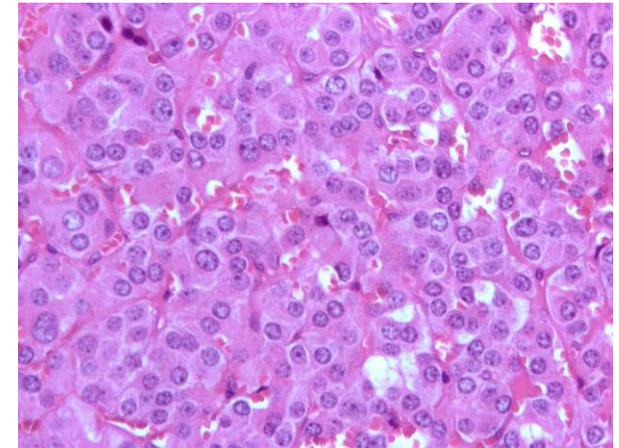
遺伝子 (DNA→mRNA)



タンパク質



細胞・組織 などの生命システム



その他の物質
(核酸・脂質・糖質・金属など)

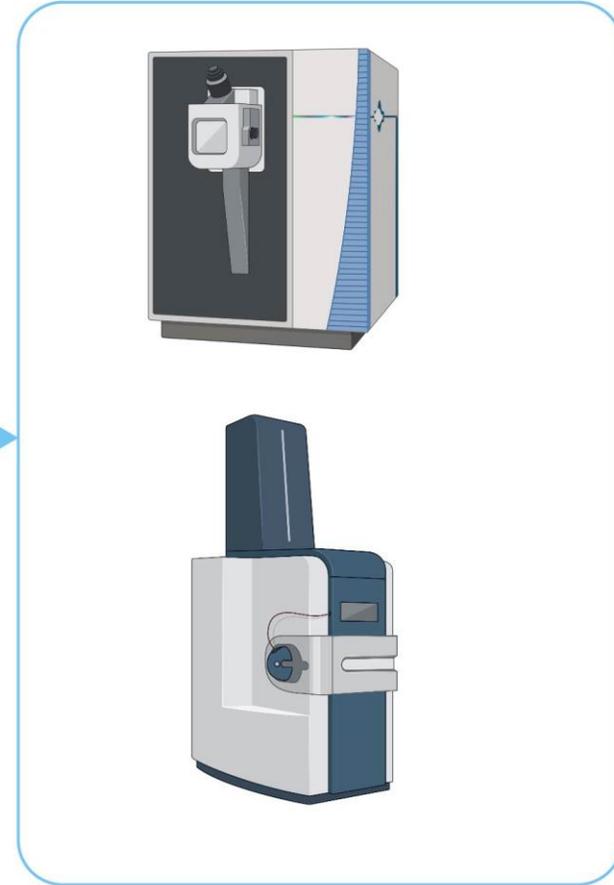
ゲノム情報と分子計測技術の組み合わせが 可能にしたポストゲノム研究

Next-generation sequencer



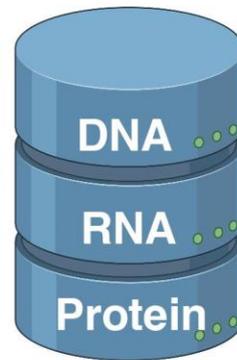
核酸

Mass spectrometry



タンパク質

Sequence DB



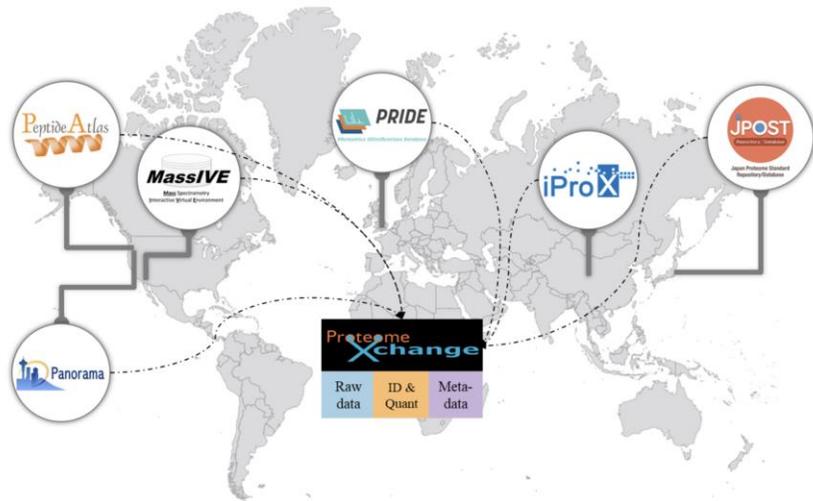
蓄積するプロテオームデータ



Mission

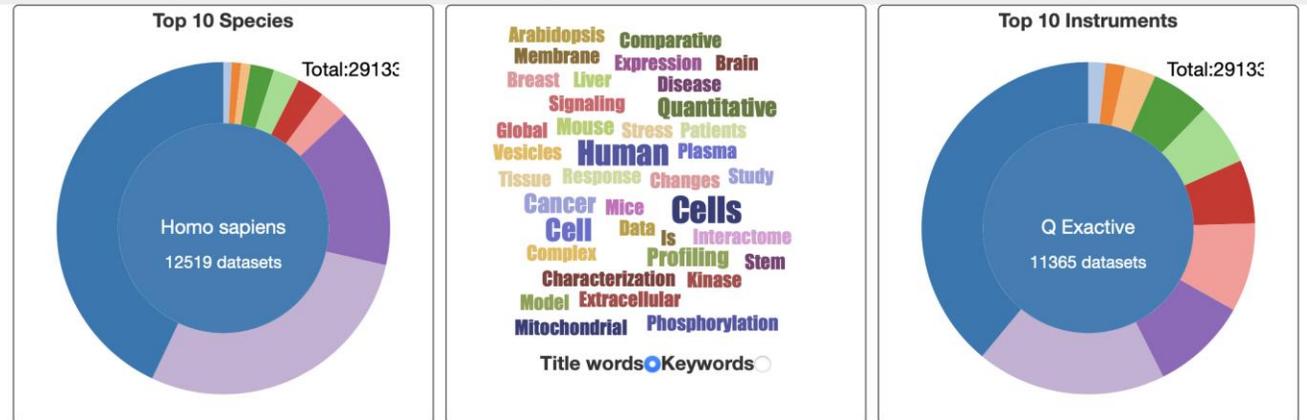
The ProteomeXchange Consortium was established to provide globally coordinated standard data submission and dissemination pipelines involving the main proteomics repositories, and to encourage open data policies in the field. Please review our [Data Submission Guidelines](#), [Guidelines for Reprocessed datasets](#) and [PX Membership Agreement](#).

See also the original Nature Biotechnology publication and the 2017 and 2020 update papers.



ProteomeXchange Datasets Overview

Below is a listing of publicly accessible ProteomeXchange datasets. You can use the search box or interactive graphics to filter the list.



Need to access individual spectra from a ProteomeXchange dataset?





Japan Proteome Standard
Repository/Database

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other

JCompMS 8th workshop

🕒 2023-04-7 👤 jpost

Workshop details in Japanese

other

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🕒 2022-12-19 👤 jpost

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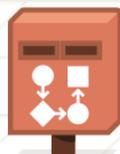
Repository [Help](#)

jPOSTrepo is a raw and processed data repository of mass-based proteomics.



Database [Help](#)

jPOSTdb is a database containing re-analysis results with unified criteria for proteome data from jPOSTrepo.



Workflow [Help](#)

This provides the re-analysis workflow used in jPOST.



Gadgets [Help](#)

jPOST gadget is a collection of tools we have developed as well as other commonly used tools for mass spectrometry-based proteomics.



COVID-19 [Help](#)

This is a special page on coronavirus (SARS-CoV-2) and the COVID-19 disease data, where mass-based proteomics data in ProteomeXchange resources are analyzed by the jPOST re-analysis workflow.



Help

A guide to using the jPOST resources.

jPOSTdb (Japan ProteOme SStandard DataBase) is a database containing re-analysis results with unified criteria for proteome data from **jPOSTrepo**. It provides viewers showing the frequency of detected post-translational modifications, the co-occurrence of phosphorylation sites on a peptide and peptide sharing among proteoforms.

Filter ▲

Reset

Species



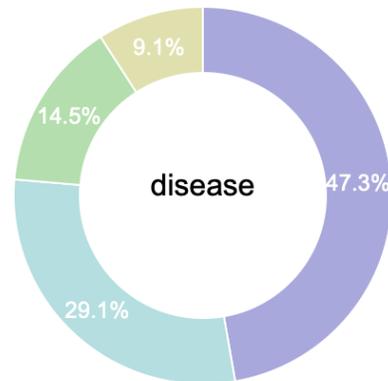
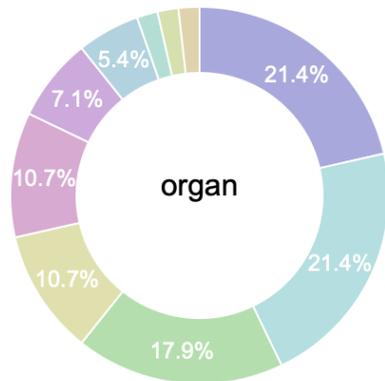
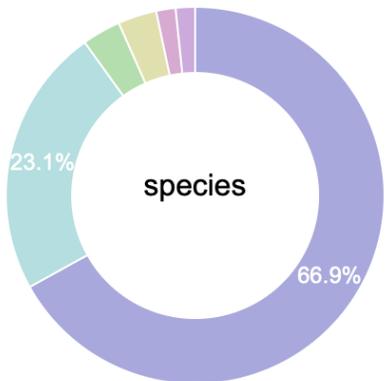
Organ



Disease



Add filter



個々のタンパク質情報の検索1

Protein: **O14639**

Protein Name Actin-binding LIM protein 1

Protein ID ABLM1_HUMAN

Gene Name ABLIM1

Accession [O14639](#)

Length 778 aa

Sequence
MPAFLGKLCGLKCSSEKSKVTSSEKTSARGSNRRLIVEDRRVSGTSFTAHRRAITHTLLYLCPKDYCPGRVCSNDPFAHPQDPHPSEKPIVHCHKCEPKCKEVLRVQTKHFHIKCTCKVCGCDLAQGGFFIKNGEYLCTLDYQRMYGTRCHG
GEFVEGEVVTALGKTYHPNCFACITCKRPFPPGDRVTFNGRDCLCQLCAQPMSSPKETTFSSNCAGCGRDIKNGQALLALDKQWHLGCFKCKSCGKVLTEYISKDGAPYCEKDYQGLFGVKCEACHQFITGKVLKAGDKHYHPSCARCSRNMFTGE
EMYLQGSTVWHPDCKQSTKTEELRPTRTSSSEIYSRPGSSIPGSPGHTIYAKVDNEILDYKDLAAIPKVKAIYDIERPDLITYEFPYTSGYDDKQERQSLGESPRTLSPTPSAEGYQDVRDRMIHRSTSQGSINSPVYSRHSYPTTTSRSPQHFHRPGNE
PSSGRNSPLPYRPDSRPLTPTYAQAQKHFHVPDQGINIYKPPYKQHAALAAQSKSSEDIKFSKFAAQAPDPSETPKIETDHWPGPPSFVAVGPMKRRSSGREEDDEELLRRRQLQEEQLMKLNSGLGQLILKEEMEKESTRERSLLASRYDSPINS
ASHIPSSKTASLPGYGRNGLHRPVSTDFAQYNSYGDVSGGVRDYQTLPDGHMPAMRMDRGVSMNMLEPKIFPYEMLMVTNRGRNKILREVDRTRLERHLAPEVFEIFGMSIQEFDRLPLWRRNDMKKKAKLF

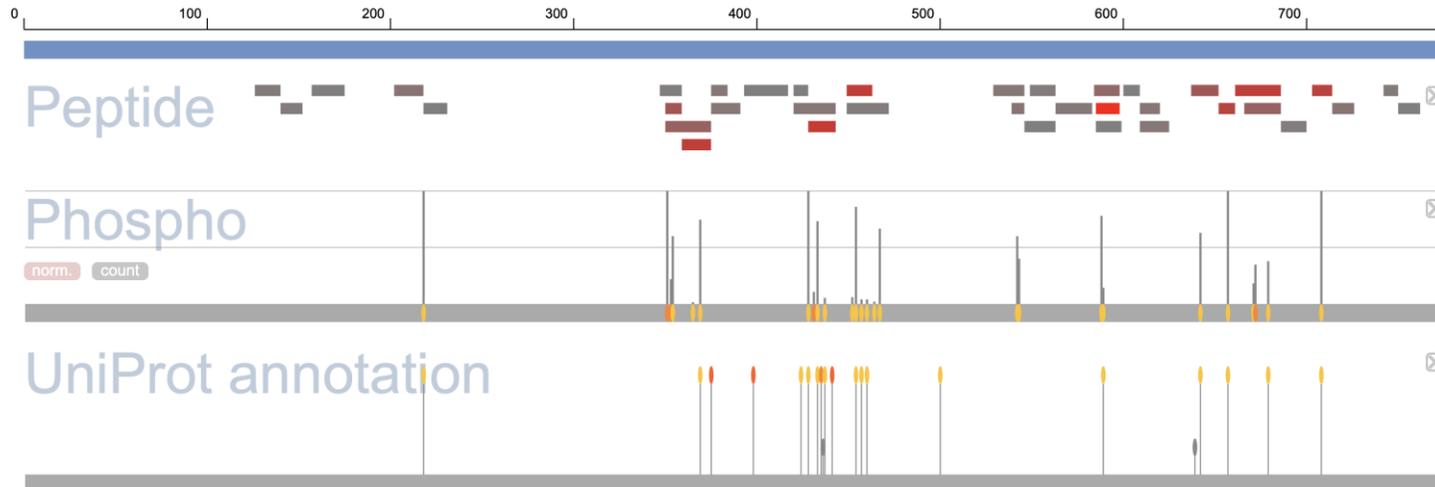
Location Chromosome 10

Statistics
peptides: 37
spectra: 542
unique peptides (UniProt entry level): 36
unique peptides (gene name level): 37

GenelD: 3983

for Absolute
Quantification

Protein Browser



個々のタンパク質情報の検索2

Protein Name	Tyrosine-protein kinase ABL1	
Protein ID	ABL1_HUMAN	
Gene Name	ABL1	
Accession	P00519	
Length	1130 aa	
Sequence	<pre> MLEICLKLVGCKSKKGLSSSSCYLEEALQRPVASFEPQGLSEARWNSKENLLAGPSENDPNLFVALYDFVASGDNTLSITKGEKLRVLGYNHNGEWCEAQTNGQGWVPSNYITPVNSLEKHSWYHGPVSRNAEYLLSSGINGSFLVRESESSPGQR SISLRYEGRVYHYRINTASDGKLYVSSSRFNTLAEVLVHHHSTVADGLITTLHYPAKRNKPTVYGVSPNYDKWEMERTDITMKHKLGGQYGEVYEGVWKYSLTVAVKTLKEDTMEVEEFLKEAAMVKEIKHPNLVQLLGVCTREPPFYIITEFMTYGN LLDYLRECNRQEVNAVLLYMATQISSAMEYLEKKNFIHRDLAARNCLVGENHLVKVADFGLSRLMTGDTYTAHAGAKFPIKWTAPESLAYNKFSIKSDVWAFGLLWEIATYGMSPYPGIDLQVYELLEKDYRMERPEGCEKVYELMRACWQWNPSSDR PSFAEIQAFETMFQESSISDEVEKELGKQGVRGAVSTLLQAPELPTKTRTSRRAAEHRDITDVPMPHSKGGQGESDPLDHEPAVSPLPRKERGPPPEGGLNEDERLLPKDKKTNLFSALIKKKKKTAPTTPKRSSSFREMDGQPERRGAGEEEGRDISNG ALAFPLDTADPAKSPKPSNGAGVPNGALRESGGSGFRSPHLWKKSSTLTSRRLATGEEEGGSSSRFLRSCASCPHGAKDTEWRSVTLPRDLQSTGRQFDSSTFGGHKSEKALPRKRAGENRSDQVTRGTVTPTPPRLVKNEEADEVFKDIMESS PGSSPPNLTPKPLRRQVTVAPASGLPHKEEAGKGSALGTPAAAEPVTPTSKAGSGAPGGTSGKPAEESRVRHHKHSSESPGRDKKLSRLKPAPPPAAASAGKAGGKPSQSPSQEAAGEAVLGAKTATSLVDVNSDAAKPSQPGEGLKPKVLPATPKP QSAKPSGTPISPAPVPSTLPSASSALAGDQPSSTAFIPLISTRVSLRKTRQPPERIASGAIITGVVLDSTEALCLAIRNSEQMASHAVLEAGKNLYTFCVSYVDSIQMRNKFARAINKLENNLRELQICPATAGSGPAATQDFSKLLSSVKEISDI VQR </pre>	
Location	Chromosome 9	
Statistics	# peptides:	28
	# spectra:	428
	# unique peptides (UniProt entry level):	20
	# unique peptides (gene name level):	20
	GeneID: 25	

Isoform 1

MLEICLKLVGCKSKKGLSSSSCYLEEALQRPVASFEPQGLSEARWNSKENLLAGPSENDPNLFVALYDFVASGDNTLSITKGEKLRVLGYNHNGEWCEAQTNGQGWVPSNYITPVNSLEKHSWYHGPVSRNAEYLLSSGINGSFLVRESESSPGQR

1

Isoform 2

MGQQPGKVLGDQRRPSLPALHFVFKGAGKKESSRHGGPHCNVVFVEHEALQRPVASFEPQGLSEARWNSKENLLAGPSENDPNLFVALYDFVASGDNTLSITKGEKLRVLGYNHNGEWCEAQTNGQGWVPSNYITPVNSLEKHSWYHGPVSRNAEYLLSSGINGSFLVRESESSPGQR

1

1

name color trypsin exon guide edit

実験グループ(slice)間の比較: sliceの作成

New Slice

Filter ▲

Page size: 10
Showing 1 to 9 of 9 entries (filtered from 121 entries)

<input checked="" type="checkbox"/>	Dataset ID	Project ID	Project Title
<input checked="" type="checkbox"/>	DS815_1	JPST000815	Human Fibroblast cell_aHDF1388-P9
<input checked="" type="checkbox"/>	DS815_2	JPST000815	Human Fibroblast cell_aHDF1388-P9
<input checked="" type="checkbox"/>	DS815_3	JPST000815	Human Fibroblast cell_aHDF1388-P9
<input checked="" type="checkbox"/>	DS816_1	JPST000816	Human Fibroblast cell_aHDF1419-P10
<input checked="" type="checkbox"/>	DS816_2	JPST000816	Human Fibroblast cell_aHDF1419-P10
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<input checked="" type="checkbox"/>	DS817_1	JPST000817	Human Fibroblast cell_Tig120slc-P8
<input checked="" type="checkbox"/>	DS817_2	JPST000817	Human Fibroblast cell_Tig120slc-P8
<input checked="" type="checkbox"/>	DS817_3	JPST000817	Human Fibroblast cell_Tig120slc-P8

Page: 1

New Slice

Filter ▲

Page size: 25
Showing 1 to 25 of 27 entries (filtered from 121 entries)

<input checked="" type="checkbox"/>	Dataset ID	Project ID	Project Title
<input checked="" type="checkbox"/>	DS810_1	JPST000810	Human iPS cell_201B7-P32
<input checked="" type="checkbox"/>	DS810_2	JPST000810	Human iPS cell_201B7-P32
<input checked="" type="checkbox"/>	DS810_3	JPST000810	Human iPS cell_201B7-P32
<input checked="" type="checkbox"/>	DS811_1	JPST000811	Human iPS cell_32R1-P32
<input checked="" type="checkbox"/>	DS811_2	JPST000811	Human iPS cell_32R1-P32
<input checked="" type="checkbox"/>	DS811_3	JPST000811	Human iPS cell_32R1-P32
<input checked="" type="checkbox"/>	DS812_1	JPST000812	Human iPS cell_414C2-P43
<input checked="" type="checkbox"/>	DS812_2	JPST000812	Human iPS cell_414C2-P43
<input checked="" type="checkbox"/>	DS812_3	JPST000812	Human iPS cell_414C2-P43
<input checked="" type="checkbox"/>	DS813_1	JPST000813	Human iPS cell_585A1-P55
<input checked="" type="checkbox"/>	DS813_2	JPST000813	Human iPS cell_585A1-P55
<input checked="" type="checkbox"/>	DS813_3	JPST000813	Human iPS cell_585A1-P55

サンプル群間で発現変化するタンパク質の探索

Spectral counting-based quantification between fibroblasts and iPS cells

	slice: Fibroblasts		share	slice: iPS	
	total	unique		total	unique
# datasets	9			15	
# proteins	5,160	1,101	4,059	6,372	2,313
# peptides	37,999	12,233	25,766	50,307	24,541

Differential Expression Analysis

- The quantification is based on spectral counting.
- Some methods need at least 2 datasets in either slice.

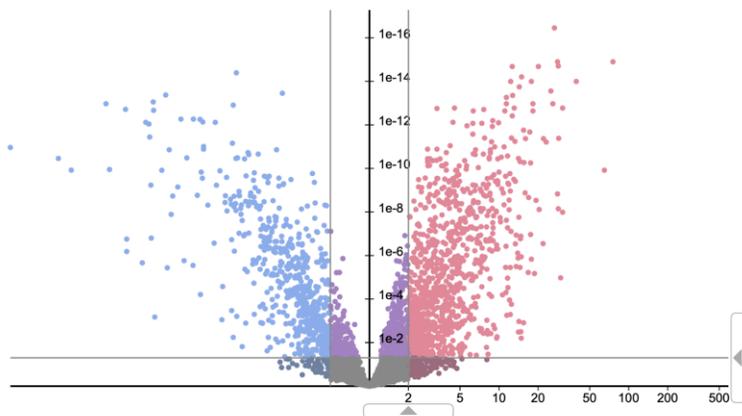
-- Select method --

Fold change >= 2

p-value <= 5e-2

Proteins: 1534

both



Page Size: 25

search protein

Showing 1 to 25 of 1534 entries

Protein name	Accession	ID	Fold change	log(fc)	p-value
Fibronectin	P02751	FINC_HUMAN	1 / 590	-9.2	1.09e-11
Collagen alpha-3(VI) chain	P12111	CO6A3_HUMAN	1 / 252	-7.97	3.48e-11
Major vault protein	Q14764	MVP_HUMAN	1 / 200	-7.64	1.20e-10
Basement membrane-specific...	P98160	PGBM_HUMAN	1 / 108	-6.75	1.07e-13
Collagen alpha-2(VI) chain	P12110	CO6A2_HUMAN	1 / 101	-6.66	1.12e-10
Neuroblast differentiation-ass...	Q09666	AHNM_HUMAN	1 / 76.3	-6.25	1.93e-13
Alpha-aminoadipic semialdeh...	Q9UDR5	AASS_HUMAN	75.4	6.24	1.26e-15
Nephrilysin	P08473	NEP_HUMAN	1 / 74.4	-6.22	1.76e-07
LIM domain only protein 7	Q8WWI1	LMO7_HUMAN	1 / 74.5	-6.22	6.57e-07
Keratin, type I cytoskeletal 18	P05783	K1C18_HUMAN	65.1	6.02	1.21e-10
Collagen alpha-2(I) chain	P08123	CO1A2_HUMAN	1 / 56.5	-5.82	2.17e-06
Vimentin	P08670	VIME_HUMAN	1 / 53.1	-5.73	7.66e-13
Collagen alpha-1(VI) chain	P12109	CO6A1_HUMAN	1 / 50	-5.65	9.13e-13
Unconventional myosin-1c	O00159	MYO1C_HUMAN	1 / 49.5	-5.63	3.60e-12
EH domain-containing protein 2	Q9NZN4	EHD2_HUMAN	1 / 48.4	-5.6	5.88e-10
Sulfide:quinone oxidoreducta...	Q9Y6N5	SQOR_HUMAN	1 / 48.1	-5.59	1.59e-07
Calpain-2 catalytic subunit	P17655	CAN2_HUMAN	1 / 46.5	-5.54	8.84e-14
Annexin A1	P04083	ANXA1_HUMAN	1 / 46.1	-5.53	2.15e-13
Chondroitin sulfate proteoglyc...	Q6UVK1	CSPG4_HUMAN	1 / 45.2	-5.5	6.71e-04
Reticulocalbin-3	Q96D15	RCN3_HUMAN	1 / 39.8	-5.32	1.22e-10
Protein RCC2	Q9P258	RCC2_HUMAN	39.5	5.3	1.01e-14
1,4-alpha-glucan-branching e...	Q04446	GLGB_HUMAN	1 / 37.4	-5.22	4.24e-14
Peptidyl-prolyl cis-trans isome...	O95302	FKBP9_HUMAN	1 / 36.2	-5.18	3.68e-06
Caveolae-associated protein 1	Q6NZI2	CAVN1_HUMAN	1 / 35	-5.13	1.39e-11
Unconventional myosin-1d	O94832	MYO1D_HUMAN	1 / 33.8	-5.08	1.29e-08

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🕒 2023-04-7 👤 jpost

Workshop details in Japanese

other

PXC has been selected as one of Global Core Biodata Resources.

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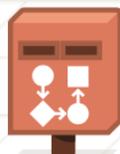
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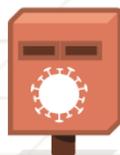
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Help

A guide to using the jPOST resources.

リポジトリにあるrawデータを活用するには？

こんな場合に有効

- 検索対象データベースを変えたい (mutation, riboseqから作成したfastaなど)
- 自分のデータや異なるプロジェクトのデータを同一条件で比較したい
- 異なる解析プラットフォームを使いたい

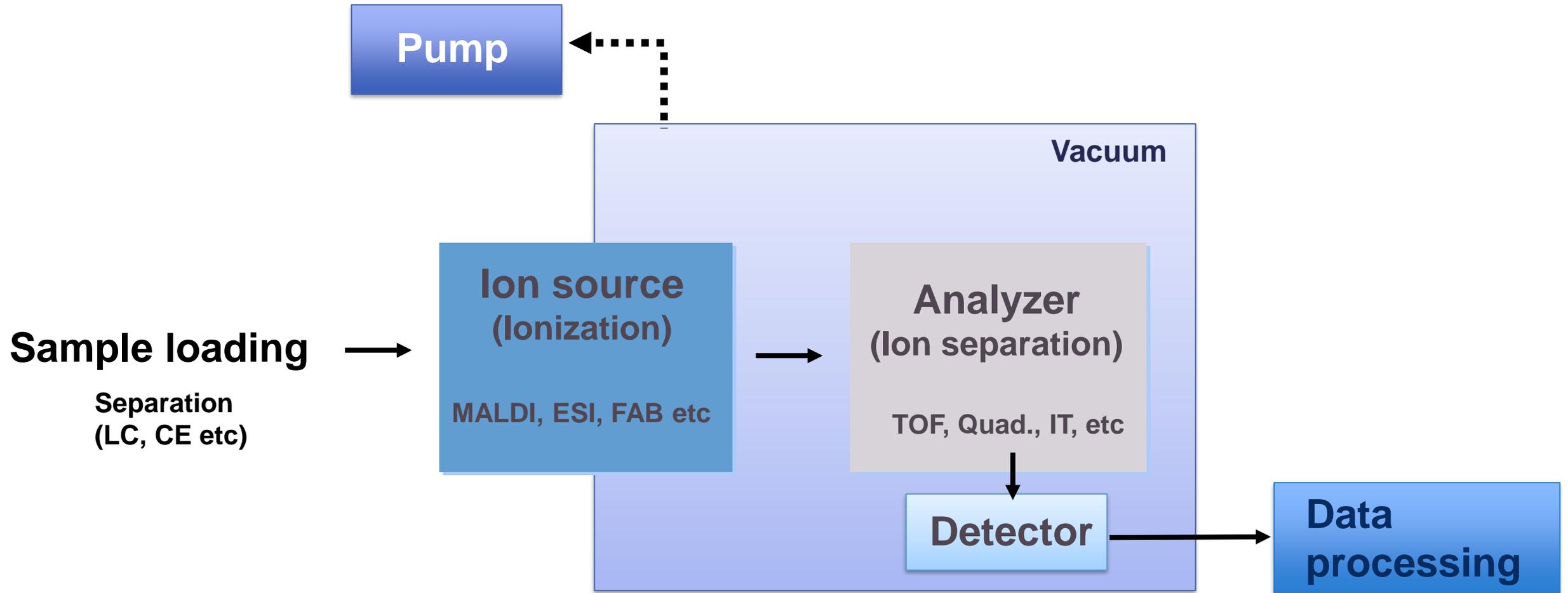
- 装置や測定手法によって得られるデータは多様。
- 実験的な前処理法との組み合わせによる複雑化。
- リポジトリにおけるメタデータの不足や誤りが多い



- 質量分析計によるプロテオーム解析の原理の理解
- データの種類やデータ解析手法の理解

質量分析計によるプロテオーム解析の原理

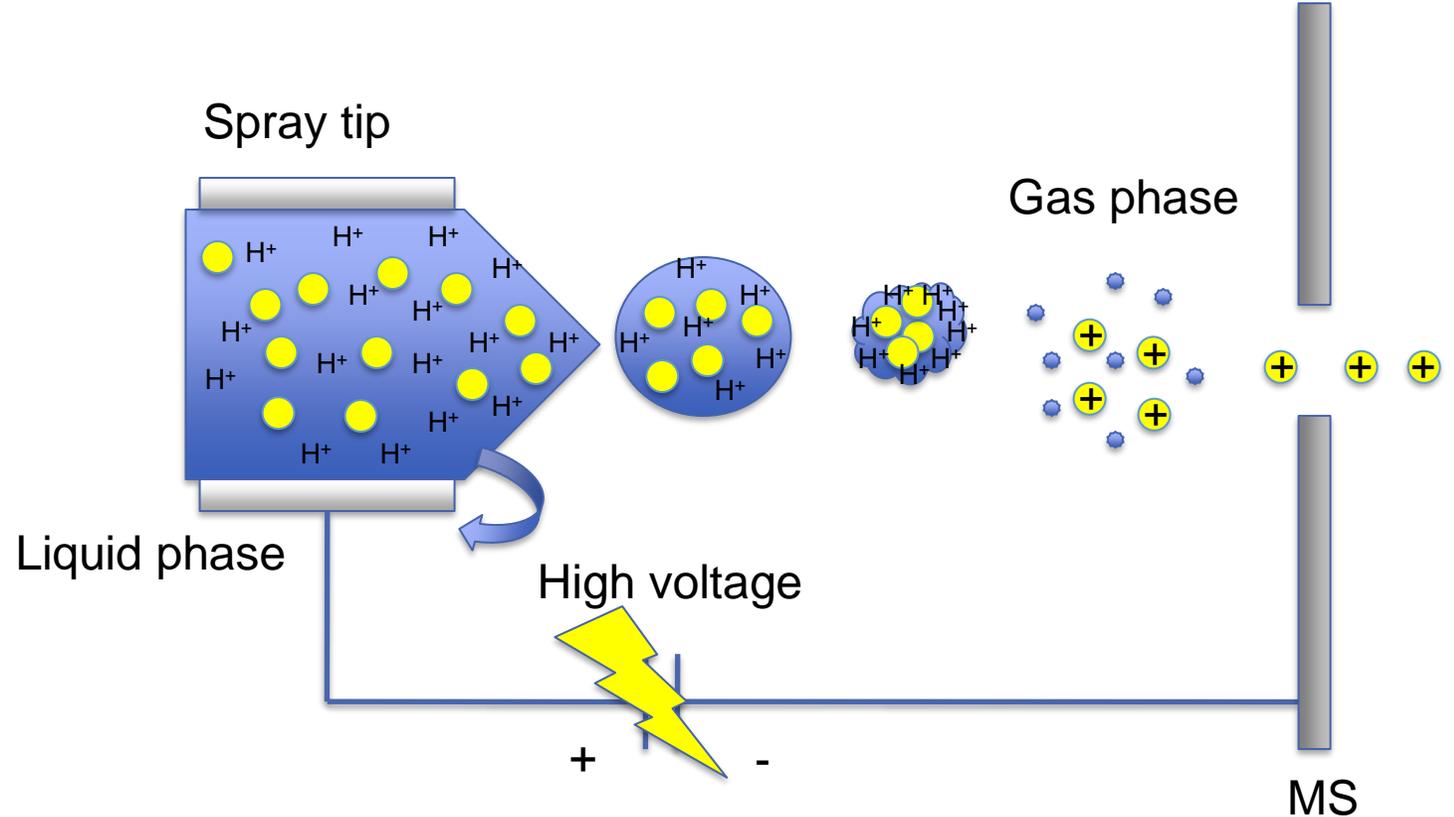
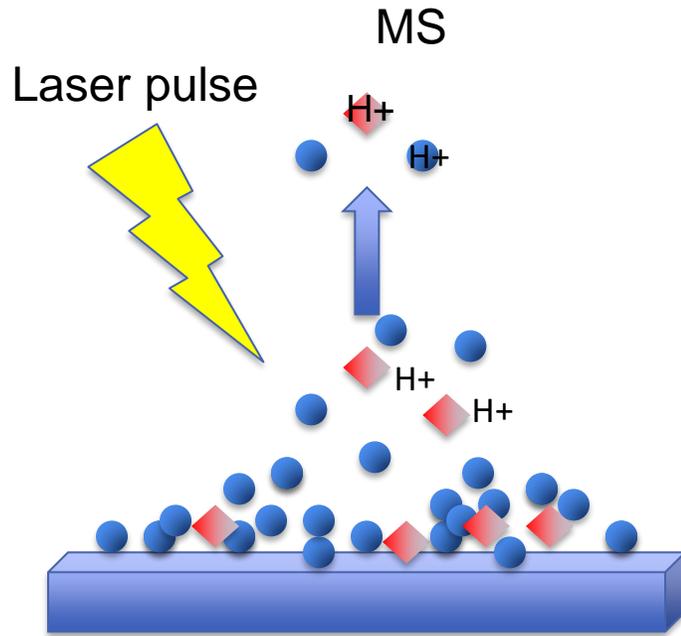
質量分析計の概要



ソフトイオン化法

Matrix Assisted Laser Desorption Ionization (MALDI)

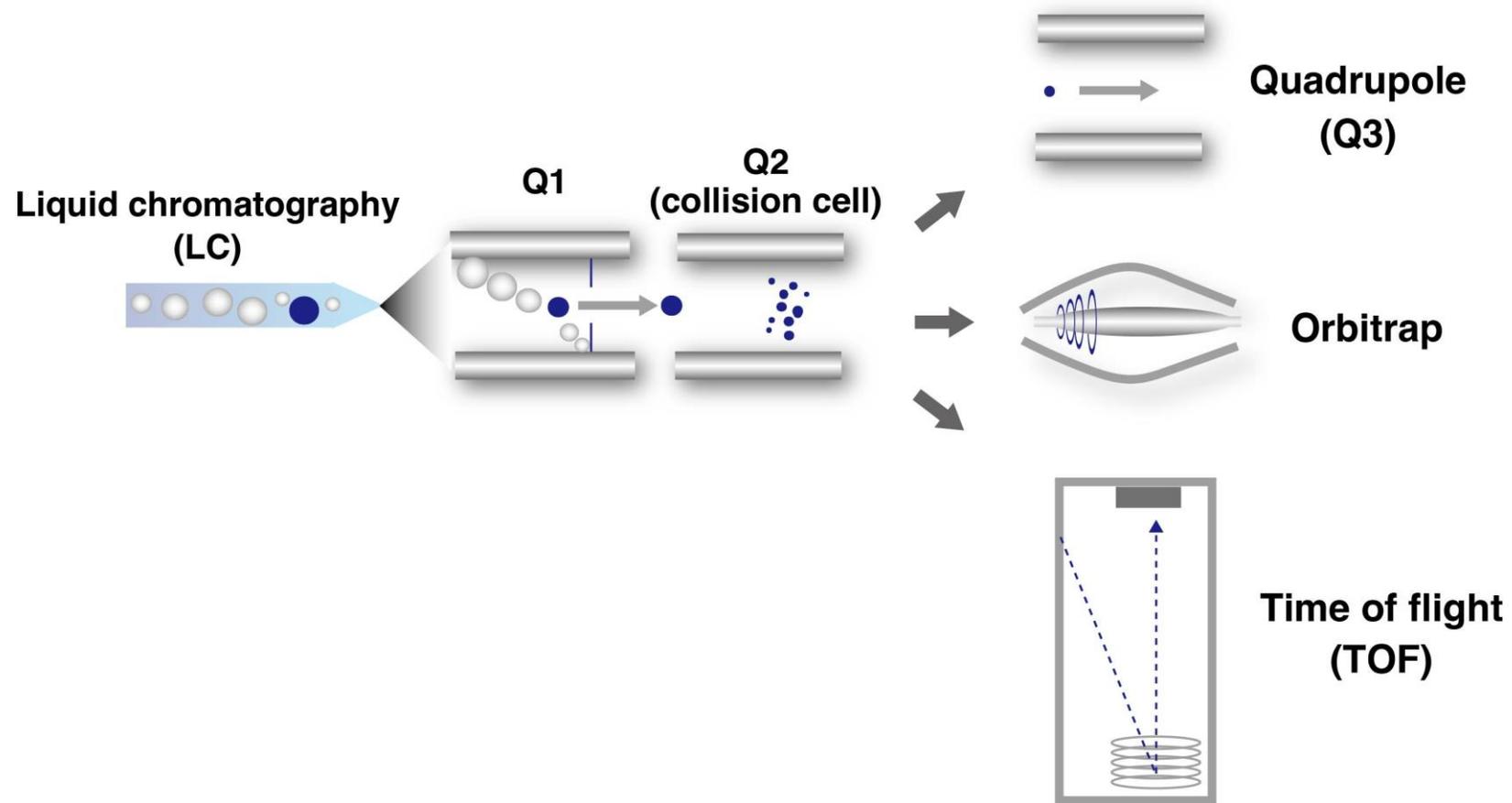
Electro Spray Ionization (ESI)



- 成分数が少ない場合に有効
- 1サンプルあたりの計測時間が短い
- 多検体の解析が可能

- HPLC等とのオンライン化が容易である。
- 装置の中に溶媒が入るため、装置の汚染を招きやすい。

タンデム質量分析計



複数のイオン分離系を組み合わせることでMS/MSを取得可能

質量分析計の横軸: m/z (m over z)

ある質量Mを持った物質に水素等が付加してイオン化した場合 = ポジティブイオン

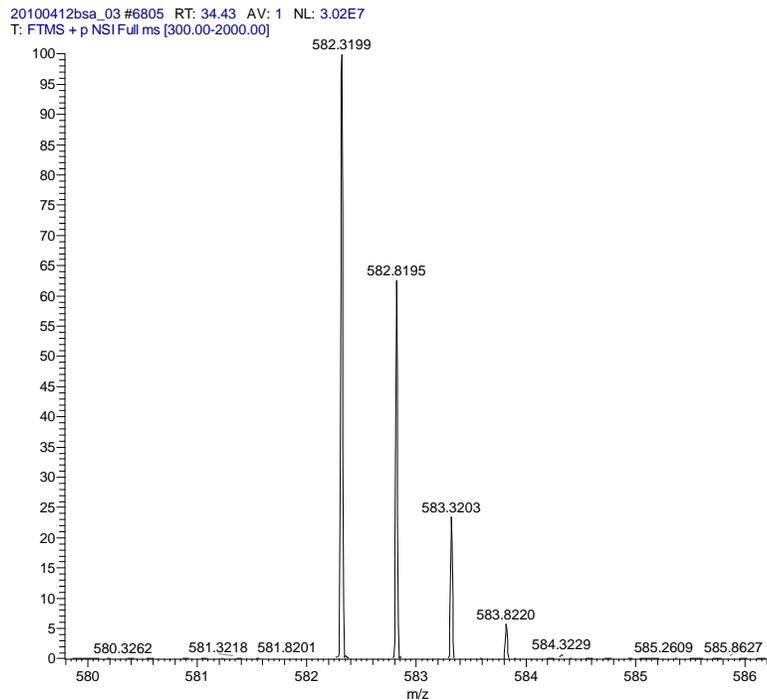
プロトン化イオンの 一般式: $[M+nH]^{n+}$	$[M+H]^{1+}$	1価イオン
	$[M+2H]^{2+}$	2価イオン
	$[M+3H]^{3+}$	3価イオン

$$m/z = \frac{[M+nH]}{n} \quad {}^1\text{Hの質量数} = 1.007825$$

例えば分子質量数500の分子に2つのプロトンが付加した場合
 $m/z = [500 + 2 * 1.007825] / 2 = 251.0078$

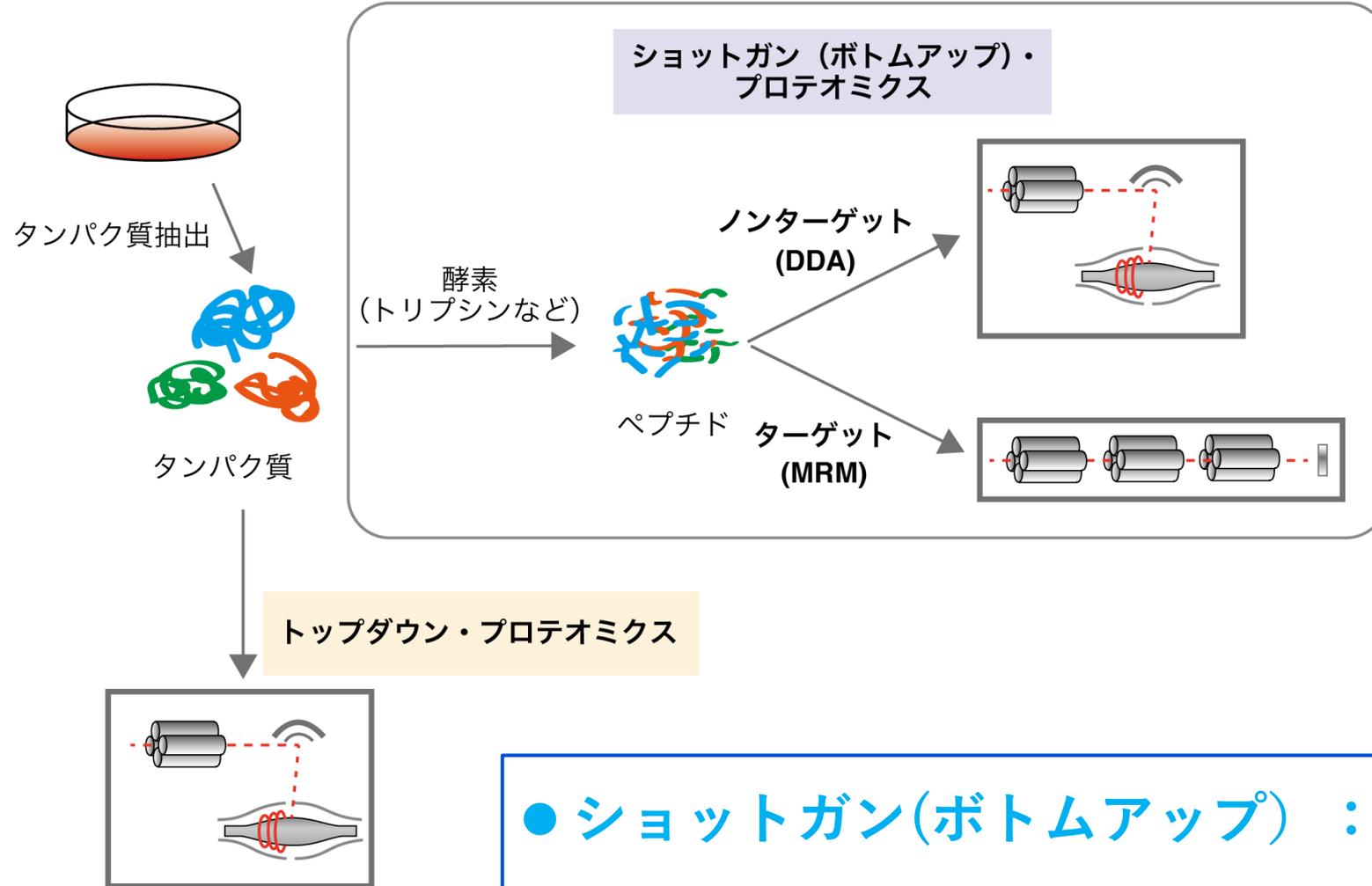
多価イオン

- ESI法では多くの場合、多価イオンを生じ傾向がある。
- プロテオミクスにおいて解析するペプチドはトリプシン消化によって生じているものが多く、N末端のアミノ基とC末端のリジンあるいはアルギニンの側鎖に存在するアミノ基の影響で2価や3価イオンを生じやすい。



- 天然の物質は同位体を一定の割合で含んでいる。
- そのため、元素の同位体分布に従って質量が1Daのずれを持つ同位体クラスターが観測される。
- この同位体クラスターの間隔は1価イオンなら1.0 m/zであるが、2価イオンなら0.5 m/zであり、3価イオンなら0.33 m/zである。
- 従って、この同位体ピーク間の幅からイオンの価数可能。逆に言えば価数判定ができないと、実測されるm/zから質量を算出できない。

質量分析計を基盤とするプロテオミクスの概要

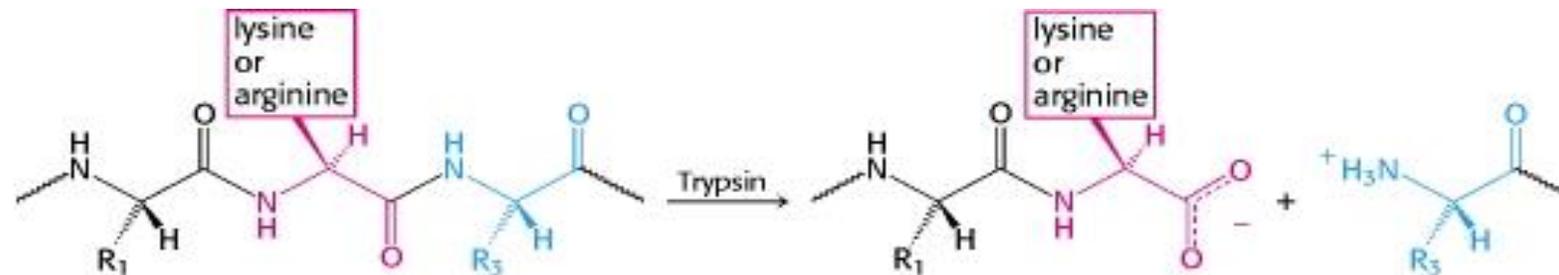


- ショットガン(ボトムアップ) : ペプチドにして解析
- トップダウン : タンパク質のまま解析

プロテオミクスにはボトムアップとトップダウンの二種類の戦略がある

ショットガンプロテオミクスにはタンパク質の断片化 (ペプチド化が必須)

Trypsin



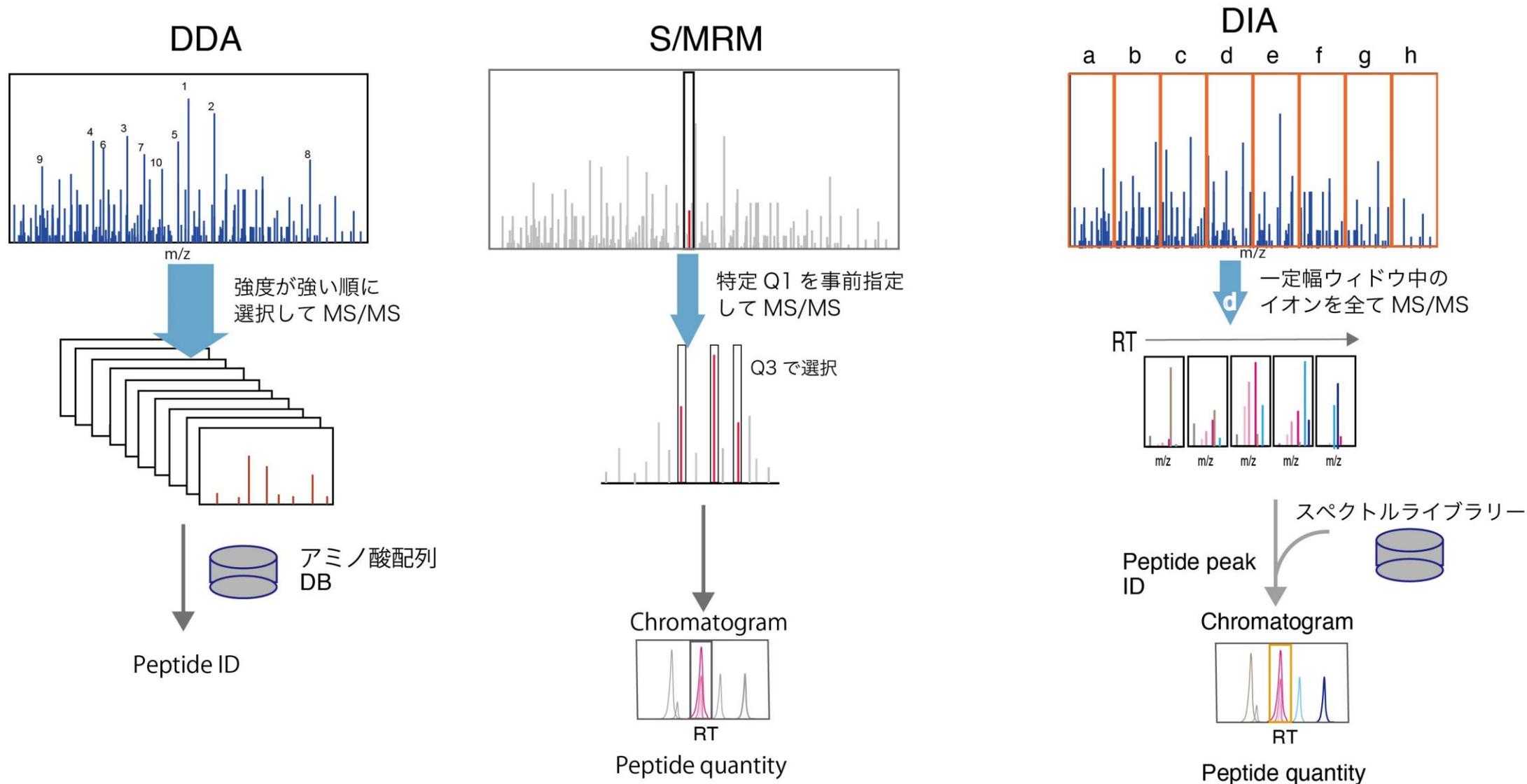
特定のアミノ酸で切断されることが重要！

----KXXXXXXXXKXXXXXXXXXXXXRXXXXXXXX----

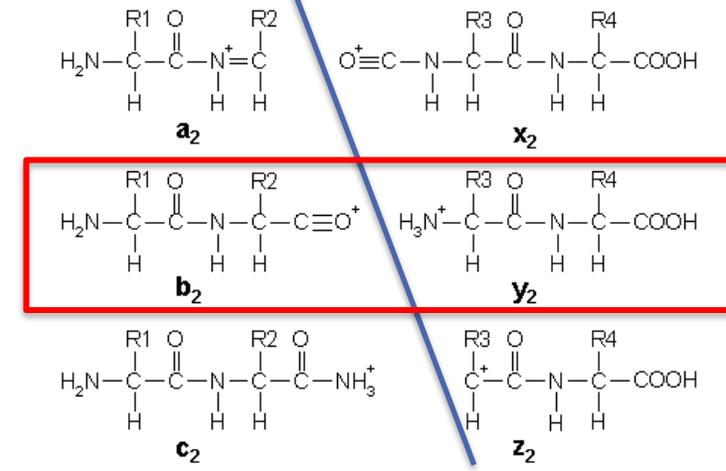
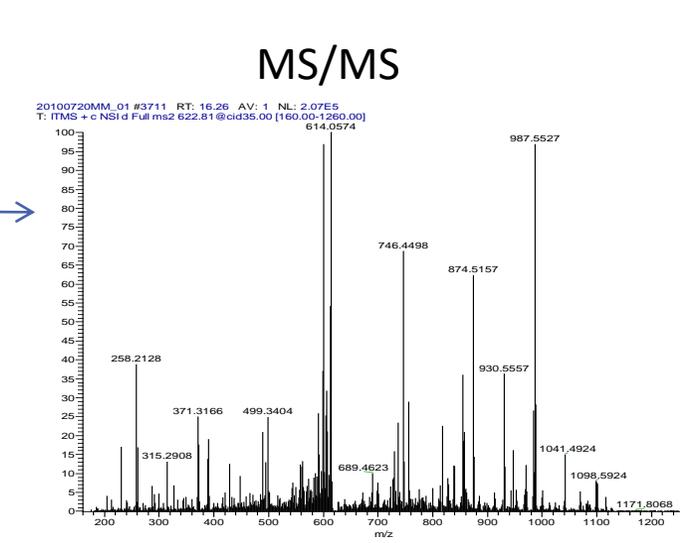
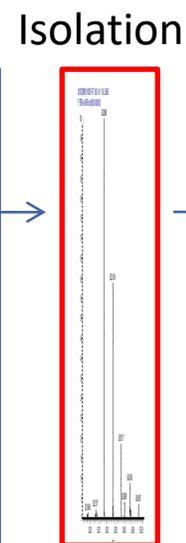
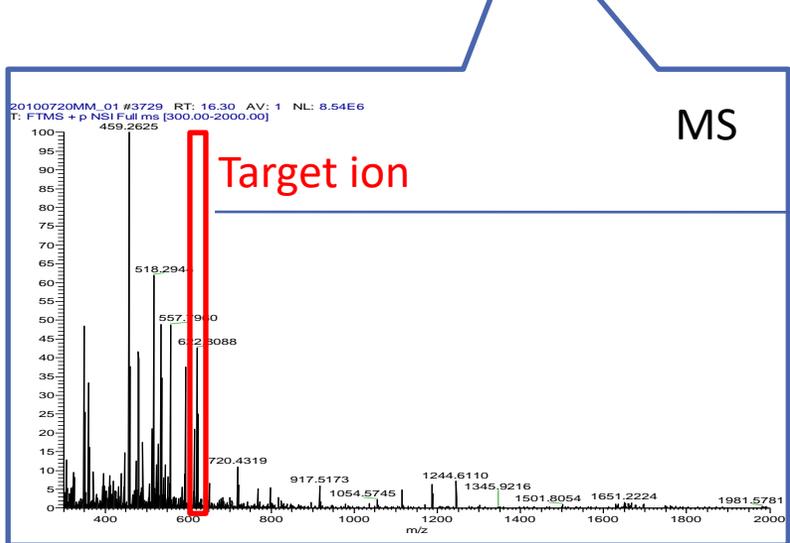
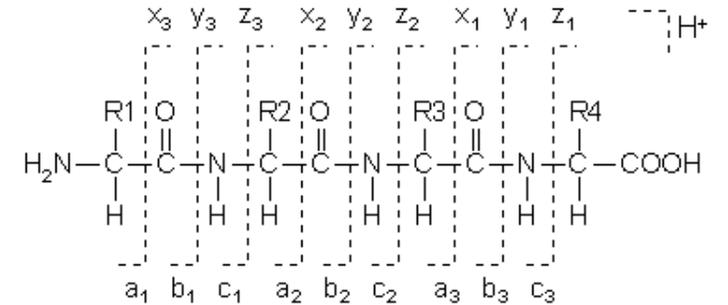
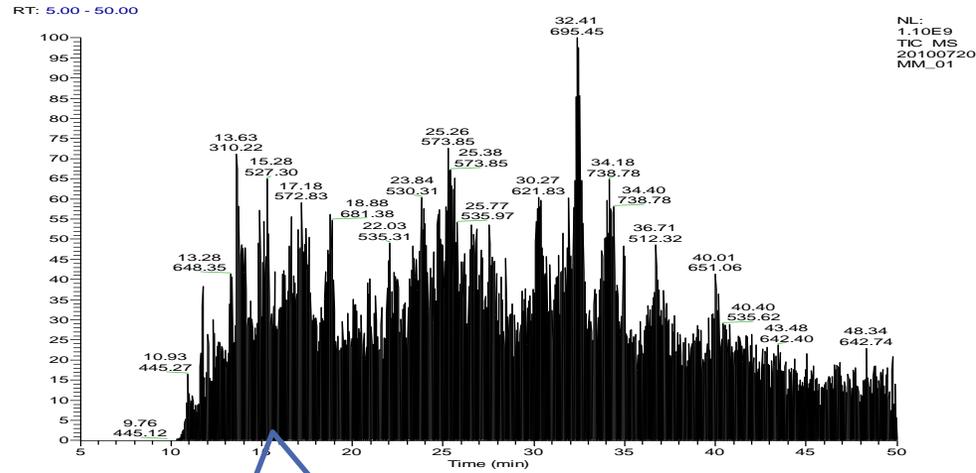
必然的に末端のアミノ酸配列が決められる

Trypsinの場合はC末端がKかR

LC-MS/MSにおける測定モードの多様化



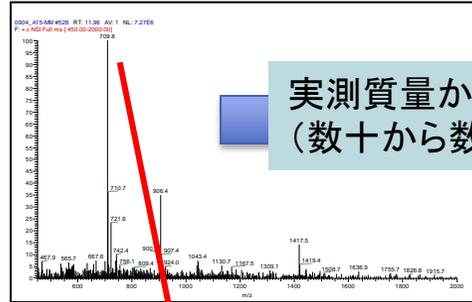
Data-dependent acquisition (DDA)モードによるLC-MS/MS測定



DDAによるタンパク質同定の原理

In silico (database)

Precursor ion spectra (MS)



実測質量から候補ペプチドの選定
(数十から数千の候補)

Precursor ion
SAVGHEYVAEVEK

Isolation

CID

Database search
(MASCOT etc)

Fragment ions

b-ion

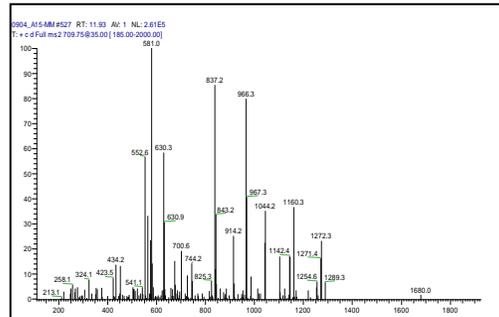
S AVGHEYVAEVEK
SA VGHEYVAEVEK
SAV GHEYVAEVEK
⋮
⋮
⋮
⋮

y-ion

フラグメントの質量で照合

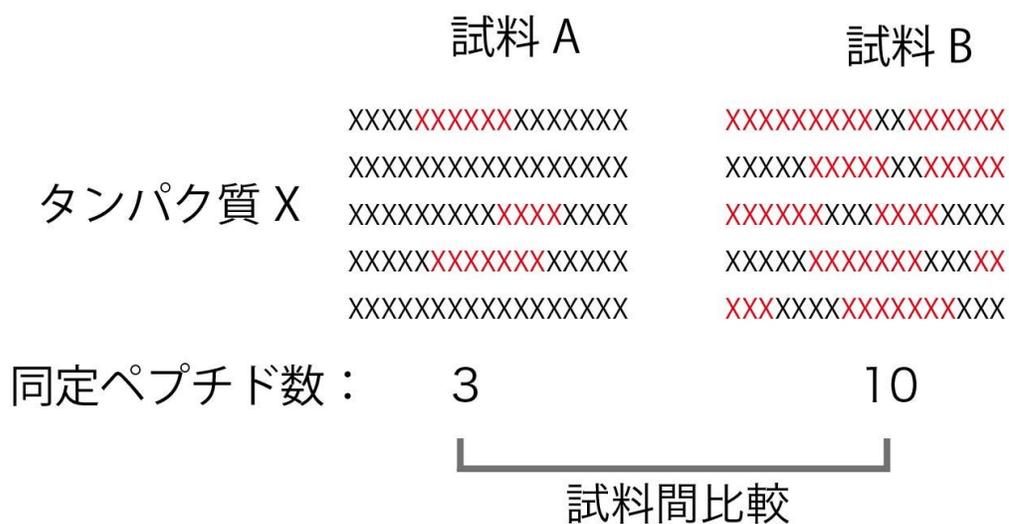
Identify

Product ion spectra (MS/MS)

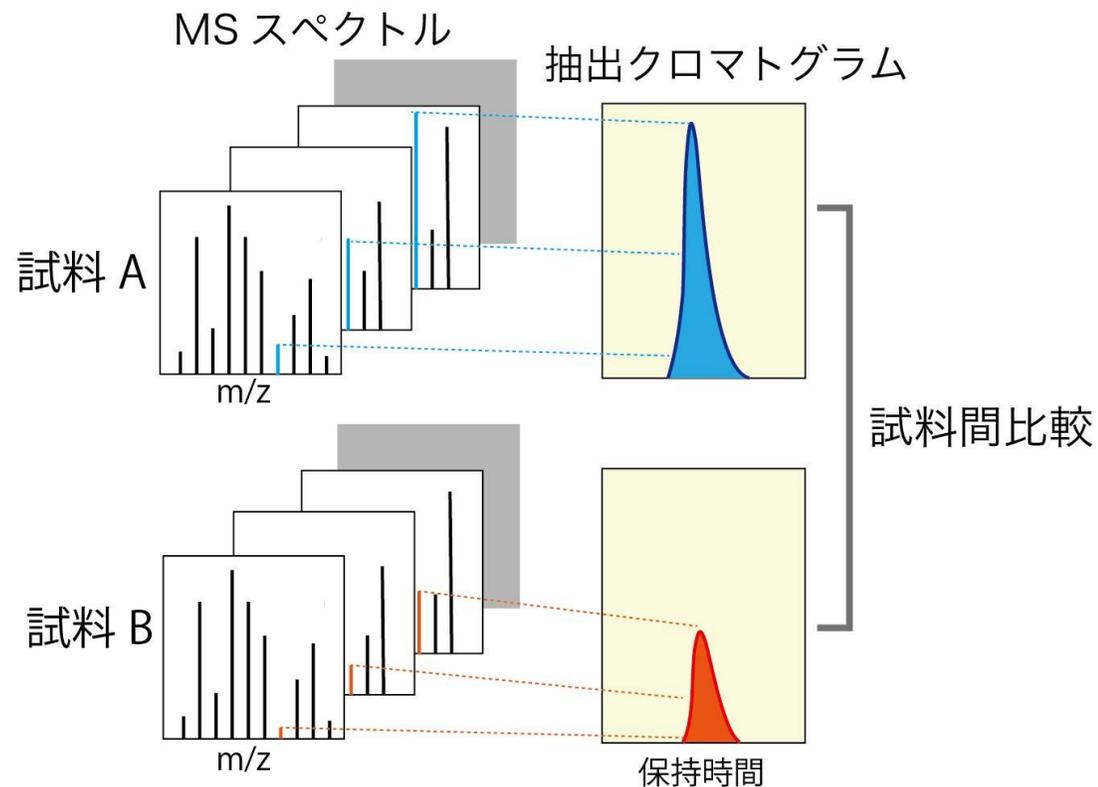


ラベル化しない定量法

スペクトルカウント

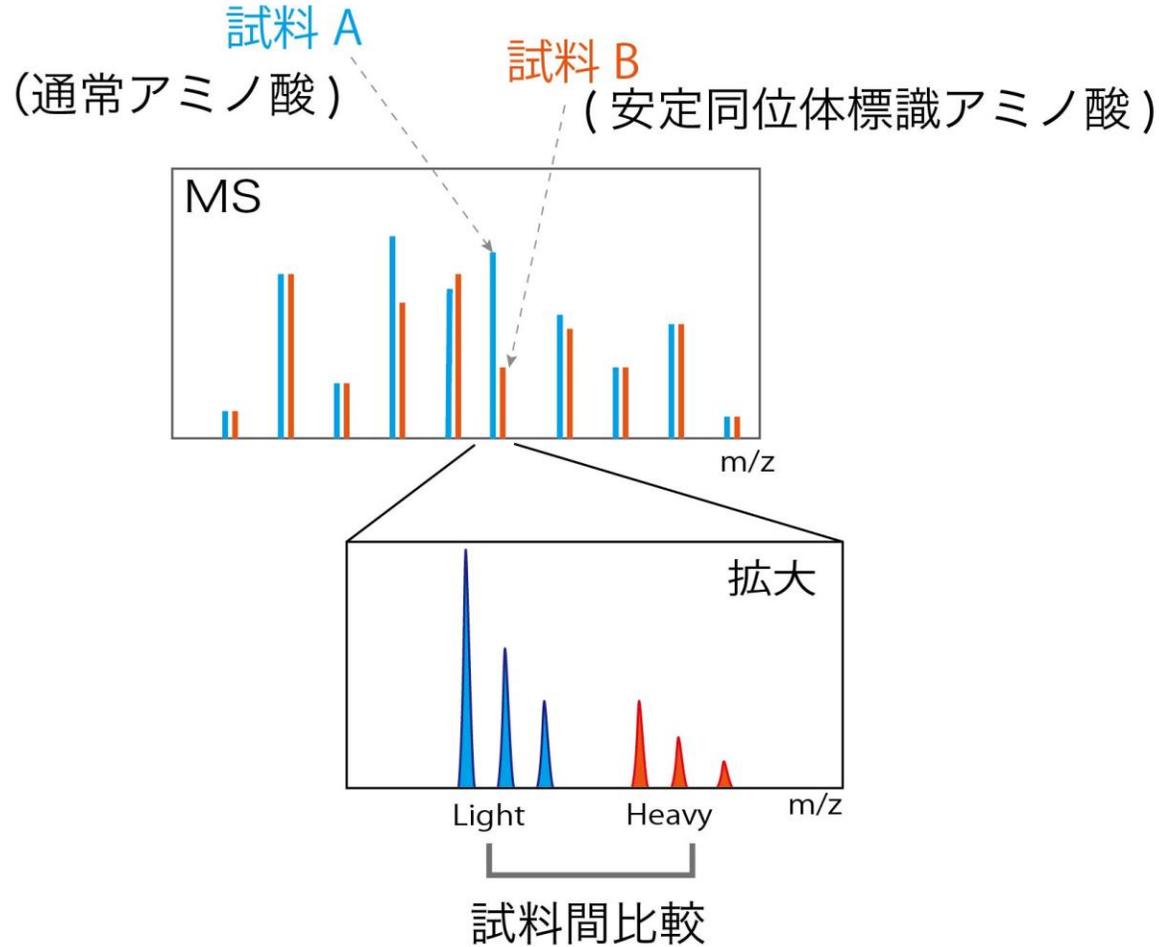


シグナル強度定量

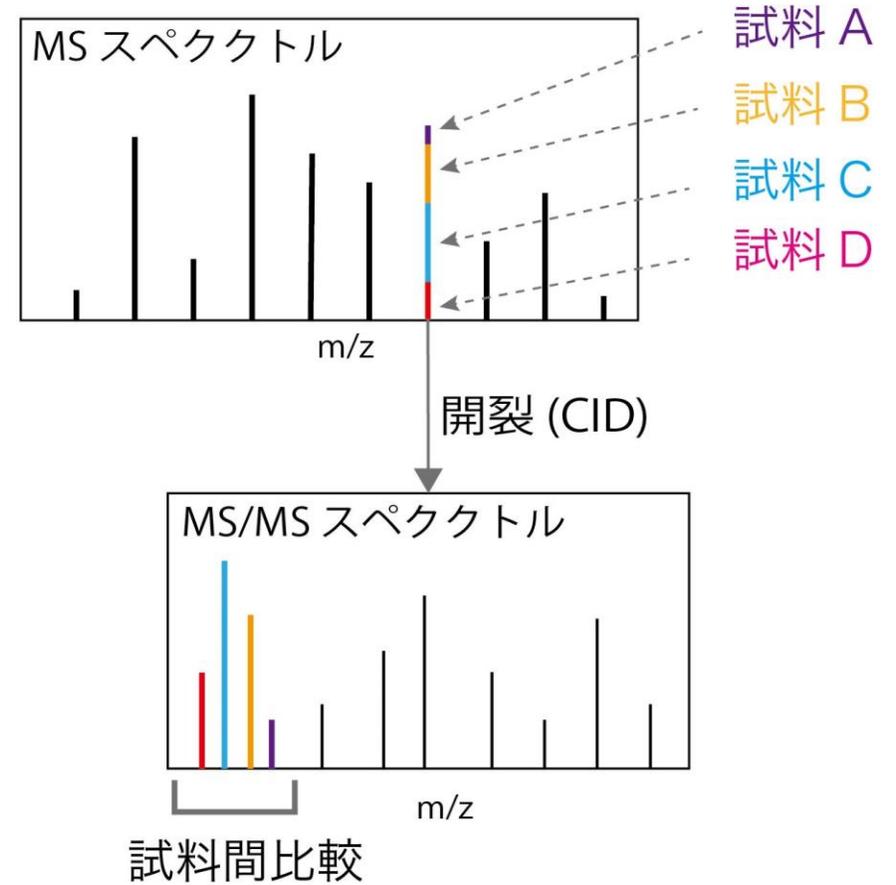


ラベル化による定量法

Isotopic ラベル定量 (SILAC や dimethyl 化など)



Isobaric ラベル定量 (iTRAQ や TMT など)



Public dataの活用：jPOST Repository

About jPOSTrepo

jPOSTrepo (Japan ProteOme STandard Repository) is a new data repository of sharing MS raw/processed data. It consists of a newly-developed, high-speed file upload process, flexible file management system and easy-to-use interfaces. Users can release their "raw/processed" data via this site with a unique identifier number for the paper publication. Users also can suspend (or "embargo") their data until their paper is published. The file transfer from users' computer to our repository server is very fast (roughly ten times faster than usual file transfer) and uses only web browsers – it does not require installing any additional software.

jPOST is a certified member of [ProteomeXchange Consortium](#) and jPOSTrepo provides official ProteomeXchange Identifiers to projects stored in our repository.



Reference

Please cite the following article when using jPOSTrepo:

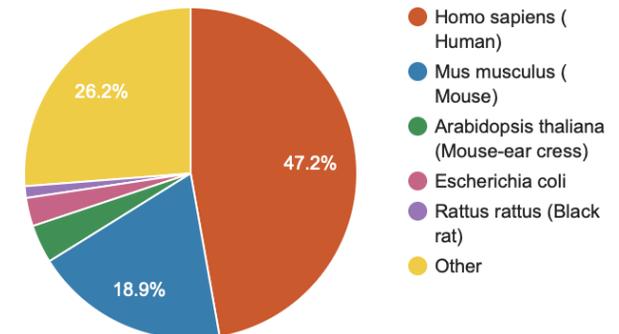
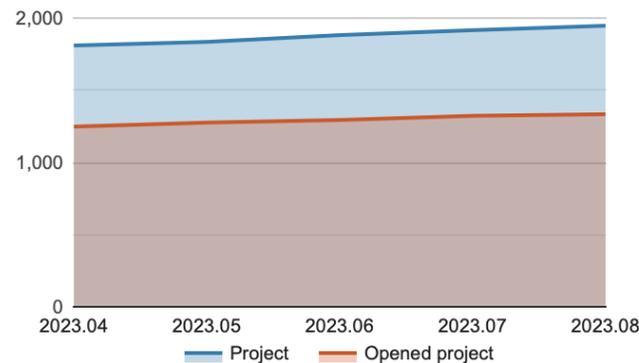
Okuda, S. et al. jPOSTrepo: an international standard data repository for proteomes. Nucl. Acids Res. 45 (D1): D1107-D1111 (2017). doi: [10.1093/nar/gkw1080](https://doi.org/10.1093/nar/gkw1080) [pubmed]

Statistics

1947 projects are registered. **1334** are opened.

129672 files amount to **72.5 TB**.

268 species.



Data list

Free word

Ontology keyword

Search by free word

Project type

All Mass spectrometry Gel electrophoresis Antibody

Search

Reset

1 - 20 / 1334

1 2 3 ... 67

JPOST ID	PXID	Project title	Description	Complete / Partial	Publication	Principal investigator	Announcement date	
JPOST002070	PXD040569	 TurboID-EV: a proximity-labeling proteomic approach to dissect the uptake mechanism of extracellular vesicles	Extracellular vesicles (EVs), including exosomes, ...	Partial	Pre-publication	Yasushi Ishihama Kyoto university	2023-08-21	Detail page Quick view
JPOST001816	PXD036054	 Absolute quantification of ASCC3	We measured the ASCC molecules in the complex of s ...	Partial	Pre-publication	Keiichi I Nakayama Kyushu University	2023-08-15	Detail page Quick view
JPOST001967	PXD039097	 Interactome analysis for Kdm4B	In this project, to identify the proteins interact ...	Complete	Pre-publication	Yukinori Hirano The Hong Kong University of Science and Technology	2023-08-12	Detail page Quick view
JPOST001814	PXD035997	 Brain phosphoproteomics in aging without AD	(Phospho)proteomic studies in the human aging brai ...	Partial	Pre-publication	isidro ferrer IDIBELL- Barcelona, Spain	2023-08-11	Detail page Quick view
JPOST001811	PXD035906	 Serum peptidome profiles in metastatic and non-metastatic feline mammary carcinoma	Feline mammary carcinoma (FMC) is a common cancer ...	Partial	Pre-publication	Sittiruk Roytrakul National Center for Genetic Engineering and Biotechnology	2023-08-09	Detail page Quick view
JPOST002279	PXD044435	 Insulin-administrated phosphorylation of the CNS proteins in Lymnaea	In the gastropod mollusc Lymnaea stagnalis, insuli ...	Partial	Pre-publication	Junko Nakai Department of Biology, Waseda University	2023-08-08	Detail page Quick view
JPOST001808	PXD035830	 SILAC Bovine Aortic Endothelial Cells	This is a pilot SILAC study wherein bovine aortic ...	Partial	Pre-publication	Luciana Hannibal Medical Center, University of Freiburg	2023-08-06	Detail page Quick view
JPOST001807	PXD035832	 Proteome analysis of muscle, liver, and blood samples associated with cancer cachexia	We analyzed samples from multiple mouse cancer cac ...	Partial	Pre-publication	Masahiro Aoki, Yasushi Kojima Aichi Cancer Center	2023-08-04	Detail page Quick view
JPOST002183	PXD042807	 Proteome analysis of liver samples associated with cancer cachexia_sex	We analyzed samples from multiple mouse cancer cac ...	Partial	Pre-publication	Masahiro Aoki, Yasushi Kojima Aichi Cancer Center	2023-08-04	Detail page Quick view

測定方法に応じた検体-raw file対応

Single shot/label-free:

1 検体/1 raw file

Fractionation/label-free:

1 raw fileには単一検体のみ

ただし、複数raw fileが発生 (fractionationの数だけ)

Fractionation/Multiplex (labeled samples) :

1 raw fileに複数検体が含まれる

複数raw fileが存在

残念ながらpublic repositoryのメタデータはあまり信用できない(適当な登録が多い)。
論文からメタデータを引っ張り出した方が正しい。

→ [データジャーナルの活用\(Journal of Proteome Data and Methods\)](#)

プロジェクト検索とメタデータの確認

Data list

Free word Ontology keyword

Project type

All Mass spectrometry Gel electrophoresis Antibody

[Search](#) [Reset](#)

Search result 016259 1 - 1 / 1 **1**

JPOST ID	PXID	Project title	Description	Complete / Partial	Publication	Principal investigator	Announcement date	
JPST000697	PXD016259	Label-free quantification of proteins by LC-MS/MS analysis	The P1 and P2 fractions were subjected to label-fr ...	Partial	Pre-publication	Keiichi I Nakayama Kyushu university	2020-11-13	Detail page Quick view

JPST000697 summary

Species	Homo sapiens (Human) [9606]
Tissue	lung [BTO:0000763]
Cell type	fibroblast [CL:0000057]
Subcellular	Nucleus
Enzyme	Trypsin [MS:1001251]
Fixed modification	Carbamidomethyl (C) [UNIMOD:4#C]
Variable modification	Oxidation (M) [UNIMOD:35#M]
Taxonomy	Homo sapiens (Human) [9606]
Instrument	Q Exactive [MS:1001911]
Instrument mode	DDA-high res.
Purpose	Relative quantification
Quantification platform	Precursor ion label free
File format	other
Software	MaxQuant

JPST000697	
Project title	Label-free quantification of proteins by LC-MS/MS analysis
Keywords	label-free, cytosol, nuclear
Project description	The P1 and P2 fractions were subjected to label-free proteomics analysis.
PubMed ID(s)	Pre-publication
Created date	2019-11-06
Announcement date	2020-11-13
PXID	PXD016259
Note	
Principal investigator	Keiichi I Nakayama Kyushu university
Submitter	Yuki Kito Kyushu university
XML file	JPST000697.0.xml
Revision	0
Submission type	Partial submission

[Project list](#)

File Name	Experimental presets (Sample / Fractionation / Enzyme/Mod. / MS mode)	File Size	Detail
20170929_TIG3WT_P1_17G05.raw	<ul style="list-style-type: none"> TIG-3 P1 fraction [1] In solution digestion with trypsin DDA analysis using QE 	847.4 MB	Detail

Experimental presets

Sample	Fractionation	Enzyme/Mod.	MS mode
<p>Title: TIG-3</p> <p>Species: Homo sapiens (Human)</p> <p>Tissue: lung</p> <p>Cell type: fibroblast</p> <p>Disease:</p> <p>Note: TIG-3 infected with retroviruses encoding human telomerase reverse transcriptase (hTERT), the early region (ER) proteins of SV40 and c-Myc.</p>	<p>Title: P1 fraction</p> <p>Subcellular: Nucleus Replicate:1/3</p> <p>Protein:</p> <p>Peptide:</p> <p>Note:</p>	<p>Title: In solution digestion with trypsin</p> <p>Enzyme: Trypsin</p> <p>Fixed modification: Carbamidomethyl (C)</p> <p>Variable modification: Oxidation (M)</p> <p>Taxonomy: Homo sapiens (Human)</p> <p>Note:</p>	<p>Title: DDA analysis using QE</p> <p>Instrument: Q Exactive</p> <p>Instrument mode: DDA-high res.</p> <p>Purpose: Relative quantification</p> <p>Quantification platform: Precursor ion label free</p> <p>Plex: -</p> <p>Label:</p> <p>Note:</p>

Isobaric tag定量のメタデータ

↳ JPST000144 summary

Species	Mus musculus (Mouse) [10090]
Tissue	T-lymphocyte [BTO:0000782]
Cell type	T cell [CL:0000084]
Subcellular	Whole
Peptide	Fe-IMAC
Enzyme	Trypsin [MS:1001251]
Fixed modification	Carbamidomethyl (C) [UNIMOD:4#C], ITRAQ4plex (K) [UNIMOD:214#K], ITRAQ4plex (N-term) [UNIMOD:214#N-term]
Variable modification	Phospho (S) [UNIMOD:21#S], Phospho (T) [UNIMOD:21#T], Phospho (Y) [UNIMOD:21#Y], Gln->pyro-Glu (Q) [UNIMOD:28#Q], Glu->pyro-Glu (E) [UNIMOD:27#E], ITRAQ4plex (Y) [UNIMOD:214#Y]
Taxonomy	Mus musculus (Mouse) [10090]
Instrument	QSTAR [MS:1000190]
Instrument mode	DDA-high res.
Purpose	Relative quantification
Quantification platform	Product ion (reporter quantification)
Label	114, 115, 116, 117
File format	, MGF, mzIdentML
Software	, Mascot

Files

Download all (1.57 GB)

File Name	Experimental presets (Sample / Fractionation / Enzyme/Mod. / MS mode)	File Size	Detail
20131026_CD3_thymocyte.wiff Raw	<ul style="list-style-type: none"> S Mouse thymocyte antibodies to CD3e stimulation 0 min F Phosphopeptide Fe-IMAC [1,1] E trypsin Fe-IMAC ITRAQ M QSTAR elite ITRAQ [114] 	7.3 MB	Detail
	<ul style="list-style-type: none"> S Mouse thymocyte antibodies to CD3e stimulation 2.5 min F Phosphopeptide Fe-IMAC [1,1] E trypsin Fe-IMAC ITRAQ M QSTAR elite ITRAQ [115] 		
	<ul style="list-style-type: none"> S Mouse thymocyte antibodies to CD3e stimulation 5 min F Phosphopeptide Fe-IMAC [1,1] E trypsin Fe-IMAC ITRAQ M QSTAR elite ITRAQ [116] 		
	<ul style="list-style-type: none"> S Mouse thymocyte antibodies to CD3e stimulation 10 min F Phosphopeptide Fe-IMAC [1,1] E trypsin Fe-IMAC ITRAQ M QSTAR elite ITRAQ [117] 		

114	115	116	117
Sample	Fractionation	Enzyme/Mod.	MS mode
Title: Mouse thymocyte antibodies to CD3e stimulation 0 min Species: Mus musculus (Mouse) Tissue: T-lymphocyte Cell type: T cell Disease: Note:	Title: Phosphopeptide Fe-IMAC Subcellular: Whole Replicate:1/1 Protein: Peptide: Fe-IMAC Fraction:1 Replicate:1/1 Note:	Title: trypsin Fe-IMAC ITRAQ Enzyme: Trypsin Fixed modification: Carbamidomethyl (C), ITRAQ4plex (K), ITRAQ4plex (N-term) Variable modification: Phospho (S), Phospho (T), Phospho (Y), Gln->pyro-Glu (Q), Glu->pyro-Glu (E), ITRAQ4plex (Y) Taxonomy: Mus musculus (Mouse) Note:	Title: QSTAR elite ITRAQ Instrument: QSTAR Instrument mode: DDA-high res. Purpose: Relative quantification Quantification platform: Product ion (reporter quantification) Plex: 4 Label: 114, 115, 116, 117 Note:

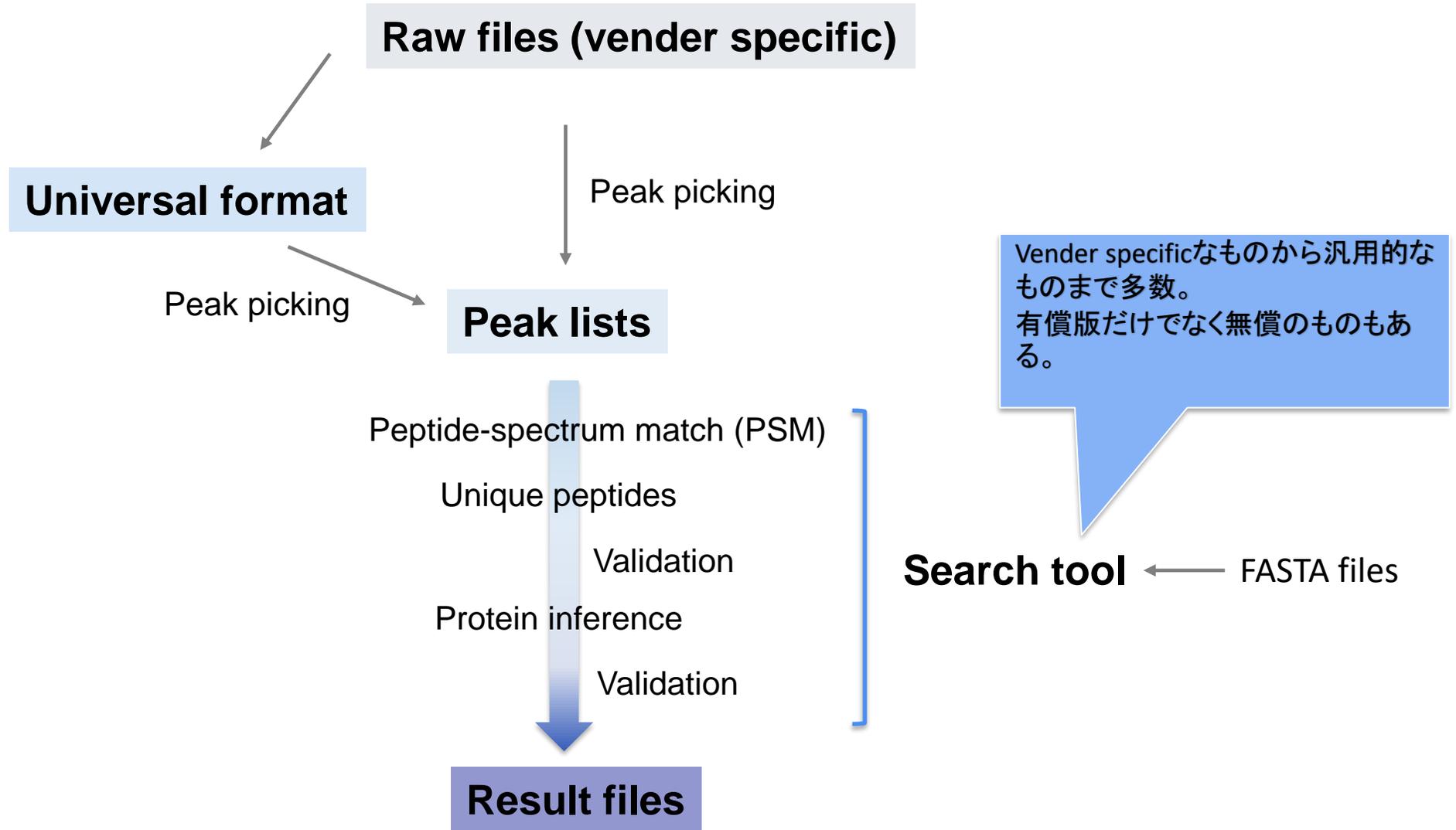
114	115	116	117
Sample	Fractionation	Enzyme/Mod.	MS mode
Title: Mouse thymocyte antibodies to CD3e stimulation 2.5 min Species: Mus musculus (Mouse) Tissue: T-lymphocyte Cell type: T cell Disease: Note:	Title: Phosphopeptide Fe-IMAC Subcellular: Whole Replicate:1/1 Protein: Peptide: Fe-IMAC Fraction:1 Replicate:1/1 Note:	Title: trypsin Fe-IMAC ITRAQ Enzyme: Trypsin Fixed modification: Carbamidomethyl (C), ITRAQ4plex (K), ITRAQ4plex (N-term) Variable modification: Phospho (S), Phospho (T), Phospho (Y), Gln->pyro-Glu (Q), Glu->pyro-Glu (E), ITRAQ4plex (Y) Taxonomy: Mus musculus (Mouse) Note:	Title: QSTAR elite ITRAQ Instrument: QSTAR Instrument mode: DDA-high res. Purpose: Relative quantification Quantification platform: Product ion (reporter quantification) Plex: 4 Label: 114, 115, 116, 117 Note:

114	115	116	117
Sample	Fractionation	Enzyme/Mod.	MS mode
Title: Mouse thymocyte antibodies to CD3e stimulation 5 min Species: Mus musculus (Mouse) Tissue: T-lymphocyte Cell type: T cell Disease: Note:	Title: Phosphopeptide Fe-IMAC Subcellular: Whole Replicate:1/1 Protein: Peptide: Fe-IMAC Fraction:1 Replicate:1/1 Note:	Title: trypsin Fe-IMAC ITRAQ Enzyme: Trypsin Fixed modification: Carbamidomethyl (C), ITRAQ4plex (K), ITRAQ4plex (N-term) Variable modification: Phospho (S), Phospho (T), Phospho (Y), Gln->pyro-Glu (Q), Glu->pyro-Glu (E), ITRAQ4plex (Y) Taxonomy: Mus musculus (Mouse) Note:	Title: QSTAR elite ITRAQ Instrument: QSTAR Instrument mode: DDA-high res. Purpose: Relative quantification Quantification platform: Product ion (reporter quantification) Plex: 4 Label: 114, 115, 116, 117 Note:

114	115	116	117
Sample	Fractionation	Enzyme/Mod.	MS mode
Title: Mouse thymocyte antibodies to CD3e stimulation 10 min Species: Mus musculus (Mouse) Tissue: T-lymphocyte Cell type: T cell Disease: Note:	Title: Phosphopeptide Fe-IMAC Subcellular: Whole Replicate:1/1 Protein: Peptide: Fe-IMAC Fraction:1 Replicate:1/1 Note:	Title: trypsin Fe-IMAC ITRAQ Enzyme: Trypsin Fixed modification: Carbamidomethyl (C), ITRAQ4plex (K), ITRAQ4plex (N-term) Variable modification: Phospho (S), Phospho (T), Phospho (Y), Gln->pyro-Glu (Q), Glu->pyro-Glu (E), ITRAQ4plex (Y) Taxonomy: Mus musculus (Mouse) Note:	Title: QSTAR elite ITRAQ Instrument: QSTAR Instrument mode: DDA-high res. Purpose: Relative quantification Quantification platform: Product ion (reporter quantification) Plex: 4 Label: 114, 115, 116, 117 Note:

DDAのデータ解析

DDA data の解析ワークフロー



広く使われているMS/MSデータ解析ソフト

Mascot

- 最もポピュラーな検索エンジン(有償)
- 操作がシンプルで使いやすい
- スペクトル帰属図などが見やすい
- 結果の統合や定量は別ソフトが必要

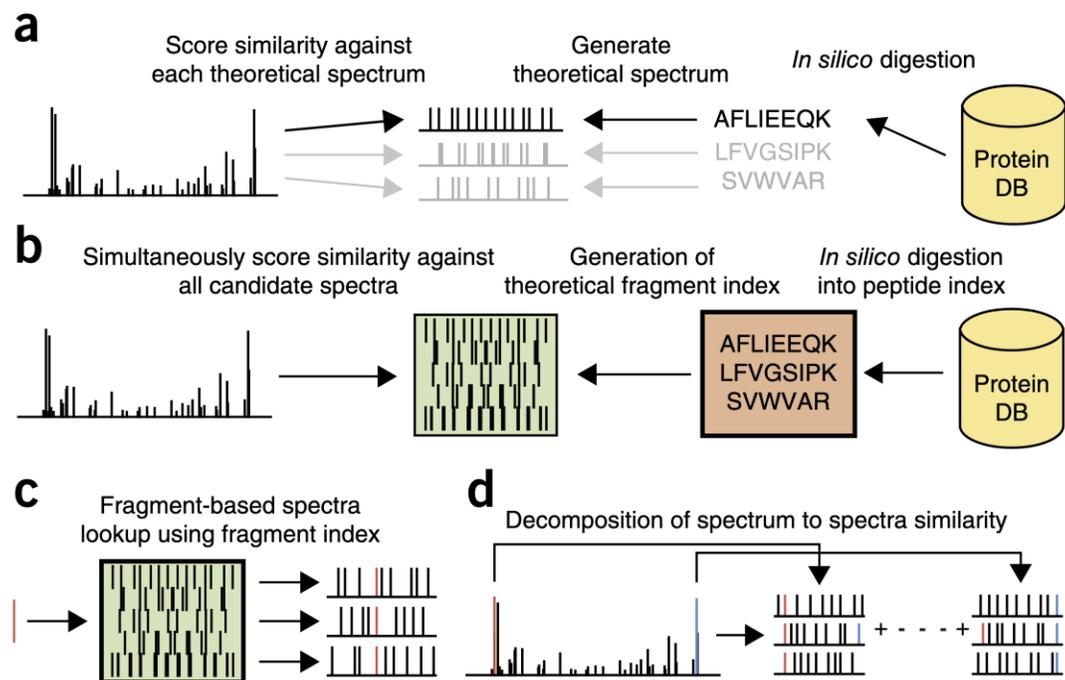
MaxQuant

- フリーソフト
- タンパク質同定(Andromeda)から定量まで実施可能
- スペクトルの可視化などが重い

Vender specificなソフトの利用も一般的
(ProteomeDiscover, ProteinPilot など)

MSFragger: ultrafast and comprehensive peptide identification in mass spectrometry-based proteomics

Andy T Kong^{1,2}, Felipe V Leprevost² , Dmitry M Avtonomov², Dattatreya Mellacheruvu²  & Alexey I Nesvizhskii^{1,2} 



- フリーソフトウェア
- 検索が早い
- Open search が現実的に可能 → 網羅的な翻訳後修飾の解析が可能
- 他のツールとFragPipe のプラットフォーム内で連携

Figure 1 | Database-search strategies and the MSFragger algorithm.

FragPipeの設定

System OS: Windows 10, Architecture: AMD64
Java Info: 11.0.9.1, OpenJDK 64-Bit Server VM, AdoptOpenJDK
NET Core Info: N/A
FragPipe: v20.0

Main tools configuration
More info and docs: [DIA-Umpire](#), [Crystal-C](#), [MSBooster](#), [Percolator](#), [PTMProphet](#), [PTM-Shepherd](#), [Q-Pair](#), [IonQuant](#), [TMT-Integrator](#), [EasyPQP](#), [DIA-NN](#), [FragPipe-PDV](#), [SAINT](#)

MSFragger
C:\FragPipe\fragpipe-jre-20.0\tools\MSFragger-3.8\MSFragger-3.8.jar
MSFragger version: 3.8 More info and docs: [MSFragger](#)

IonQuant
C:\FragPipe\fragpipe-jre-20.0\tools\IonQuant-1.9.8.jar
IonQuant version: 1.9.8 More info and docs: [IonQuant](#)

Philosopher
C:\FragPipe\fragpipe-jre-20.0\tools\philosopher_v5.0.0_windows_amd64\philosopher.exe
Philosopher version: 5.0.0 More info and docs: [Philosopher](#)

Python
C:\Anaconda3\python
Python version: Python 3.9.12

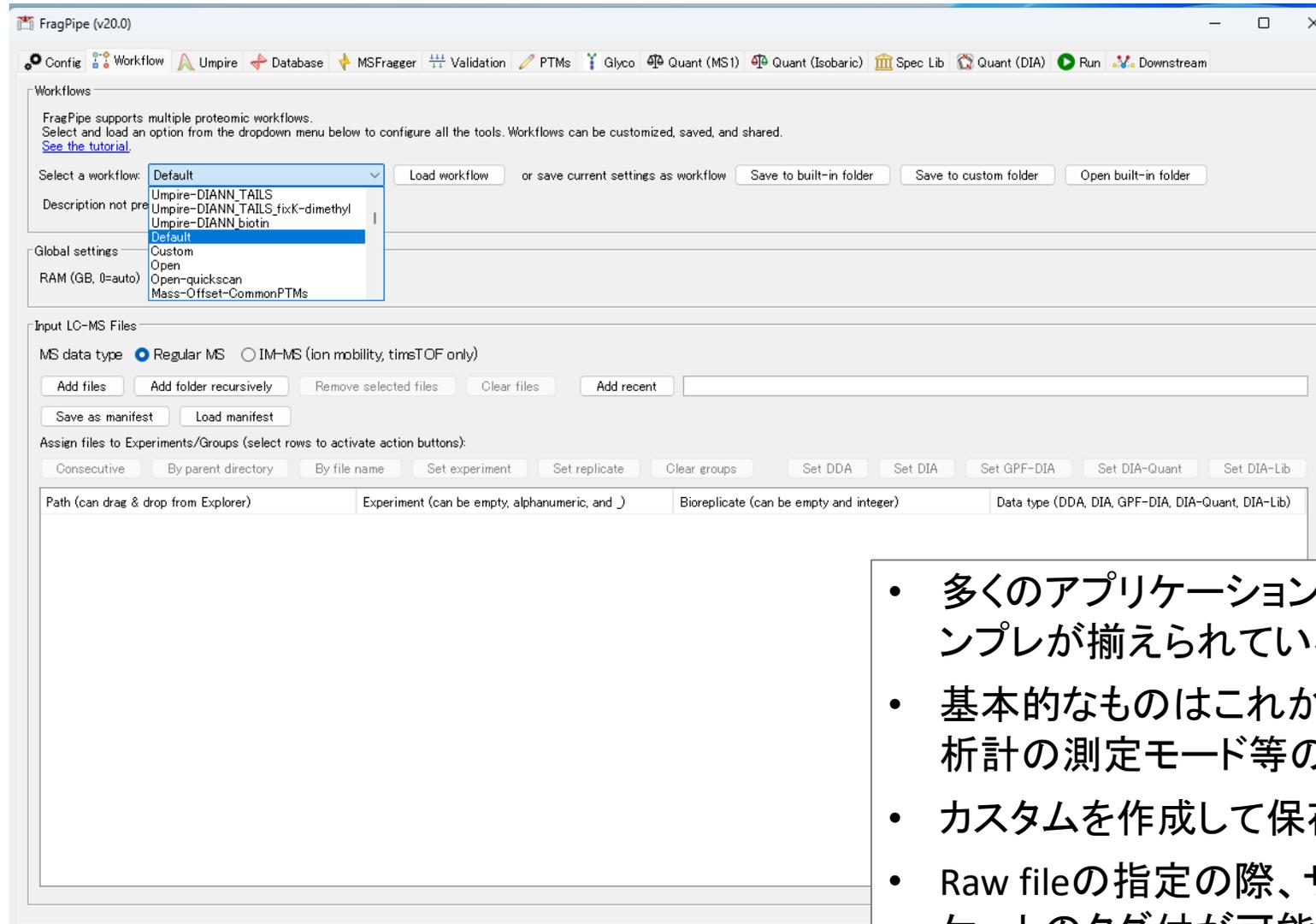
Database Splitting
Database Splitting: **Available**. Used for searching very large databases by splitting into smaller chunks.

Spectral Library Generation
EasyPQP: **Available**. Version: 0.1.36
There is a new version (0.1.39). Please upgrade it by clicking the button below and waiting.

Tabs at the top represent processing steps that will be performed sequentially if enabled.
Load a workflow from the dropdown menu on the 'Workflow' tab to get started.
[Configuration Help](#)

- MSfragerは基本FragPipeの中で動く。
- 定量やバリデーションなどで働くツールをFragPipeの中で動かす。

Workflowの呼び出しとrawデータの指定



- 多くのアプリケーションに対応したワークフローのテンプレートが揃えられている。
- 基本的なものはこれから選べばOK(もちろん質量分析計の測定モード等の条件依存)。
- カスタムを作成して保存も可能。
- Raw fileの指定の際、サンプルグループやレプリケートのタグ付が可能。

検索条件の設定

FragPipe (v20.0)

Config Workflow Umpire Database MSFragger Validation PTMs Glyco Quant (MS1) Quant (Isobaric) Spec Lib Quant (DIA) Run Downstream

Run MSFragger

Save Config Load : Custom MSFragger parameter file from di...

MSFRAGGER

Common Options (Advanced Options are at the end of the page)

Peak Matching

Precursor mass tolerance PP... -20 - 20 Fragment mass tolerance PP... 20

Calibration and Optimization Mass calibration, parameter optimizati... Isotope error 0/1/2

Protein Digestion

Cleavage ENZYMATIC Clip N-term M

Enzyme name 1 trypsin Load rules trypsin Cuts 1 KR No cuts 1 P Missed cleavages 1 2 Sense 1 ...

Enzyme name 2 null Load rules null Cuts 2 No cuts 2 Missed cleavages 2 2 Sense 2 ...

Peptide length 7 - 50 Peptide mass range 500 - 5,000 Split database 1

Modifications

Variable modifications

Max variable mods on a peptide 3 Max combinations 5,000 Use all mods in first search

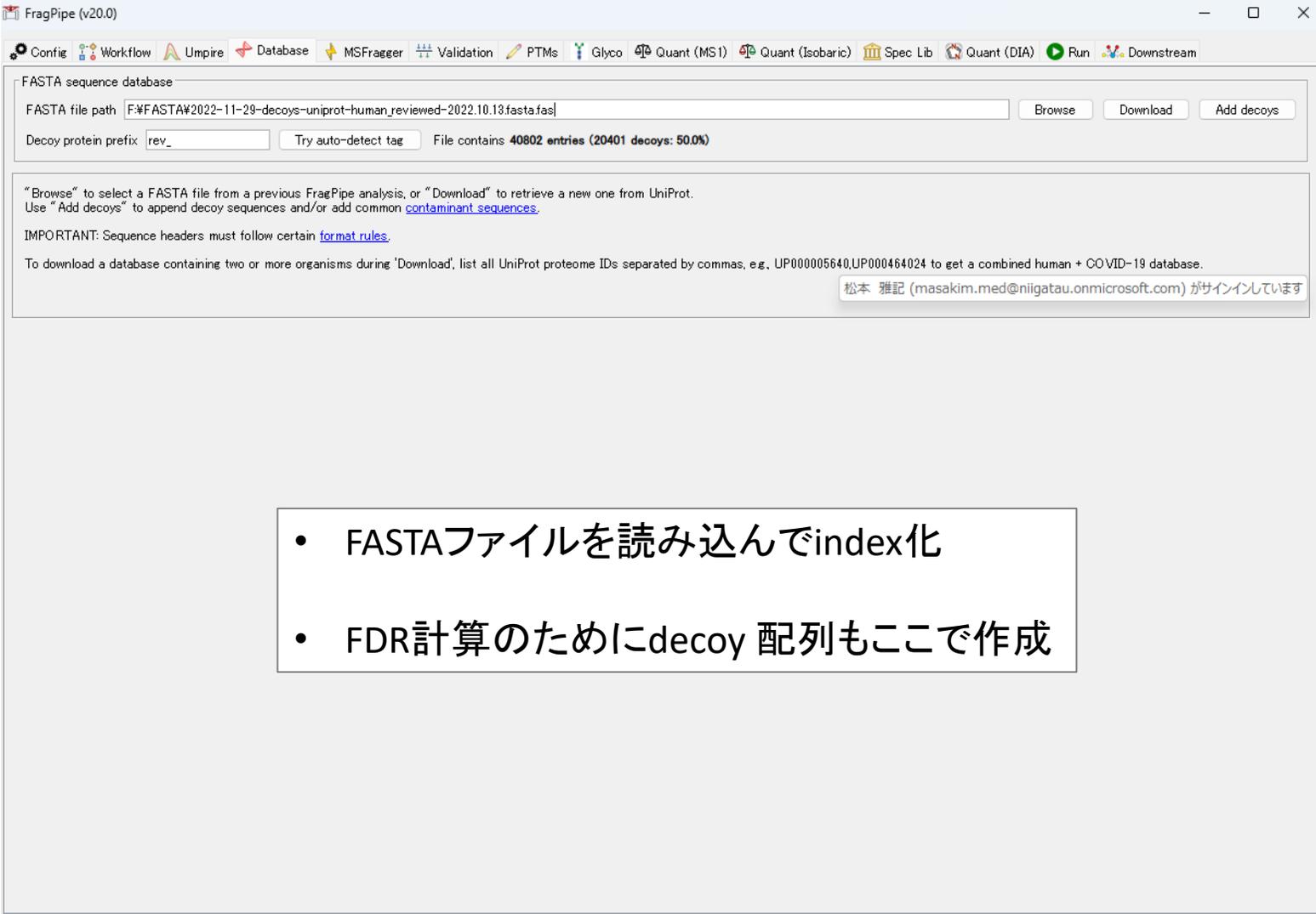
Enabled	Site (editable)	Mass Delta (edita...	Max occurrences ...
<input checked="" type="checkbox"/>	M	15.9949	3
<input checked="" type="checkbox"/>	C	42.0106	1
<input type="checkbox"/>	STY	79.96633	3
<input checked="" type="checkbox"/>	nQnC	-17.0265	1
<input checked="" type="checkbox"/>	nE	-18.0106	1
<input type="checkbox"/>	site_06	0.0	1
<input type="checkbox"/>	site_07	0.0	1
<input type="checkbox"/>	site_08	0.0	1

Fixed modifications

Enabled	Site	Mass Delta (editable)
<input checked="" type="checkbox"/>	C-Term Peptide	0.0
<input checked="" type="checkbox"/>	N-Term Peptide	0.0
<input checked="" type="checkbox"/>	C-Term Protein	0.0
<input checked="" type="checkbox"/>	N-Term Protein	0.0
<input checked="" type="checkbox"/>	C (phospho)	0.0

- 酵素の選定
- 翻訳後修飾などを入力
- 装置の質量精度に応じてトレランスを設定

FASTAの準備



The screenshot shows the FragPipe (v20.0) software interface. The main window is titled "FragPipe (v20.0)" and has a menu bar with options: Config, Workflow, Umpire, Database, MSFragger, Validation, PTMs, Glyco, Quant (MS1), Quant (Isobaric), Spec Lib, Quant (DIA), Run, and Downstream. The "FASTA sequence database" section is active, showing a text input field for the "FASTA file path" containing "F:\FASTA\2022-11-29-decoys-uniprot-human_reviewed-2022.10.13.fasta.fas". To the right of this field are buttons for "Browse", "Download", and "Add decoys". Below the file path is a "Decoy protein prefix" field with "rev_" and a "Try auto-detect tag" button. A status line indicates "File contains 40802 entries (20401 decoys: 50.0%)".

“Browse” to select a FASTA file from a previous FragPipe analysis, or “Download” to retrieve a new one from UniProt. Use “Add decoys” to append decoy sequences and/or add common [contaminant sequences](#).

IMPORTANT: Sequence headers must follow certain [format rules](#).

To download a database containing two or more organisms during 'Download', list all UniProt proteome IDs separated by commas, e.g., UP000005640,UP000464024 to get a combined human + COVID-19 database.

松本 雅記 (masakim.med@niigatau.onmicrosoft.com) がサインインしています

- FASTAファイルを読み込んでindex化
- FDR計算のためにdecoy 配列もここで作成

Validation

FragPipe (v20.0)

Config Workflow Umpire Database MSFragger Validation PTMs Glyco Quant (MS1) Quant (Isobaric) Spec Lib Quant (DIA) Run Downstream

Run Validation Tools

Crystal-C

Run Crystal-C Crystal-C performs additional search results cleanup. Recommended for Open Searches only.

Rescoring Using Deep Learning Prediction

Run MSBooster Rescoring using deep learning prediction. Require **Run Percolator** in PSM validation panel.

Predict RT Predict spectra Use correlated features

PSM Validation

Run PSM Validation

Run PeptideProphet Defaults for: Closed Search Load Single combined pepxml file per experiment / group

Cmd line opts: --decoyprobs --ppm --accmass --nonparam --expectscore

Run Percolator Keep intermediate files Min probability 0.5

Cmd line opts: --only-psms --no-terminate --post-processing-tdc

PTM Site Localization

Run PTMProphet Load defaults Not for open searches. Mods format example: STY:79.966331,M:15.9949

Cmd line opts: KEEPOLD STATIC EM=1 NIONS=b M:15.9949;n:42.0106 MINPROB=0.5

Protein Inference

Run ProteinProphet

Cmd line opts: --maxppmdiff 2000000

FDR Filter and Report

Generate reports

Filter --sequential --picked --prot 0.01

Do not use ProteinProphet file



- Validation toolの選択
- FDRフィルターの設定
- PTMのvalidationも可能

定量モード

Label-free or isotopic-label 定量

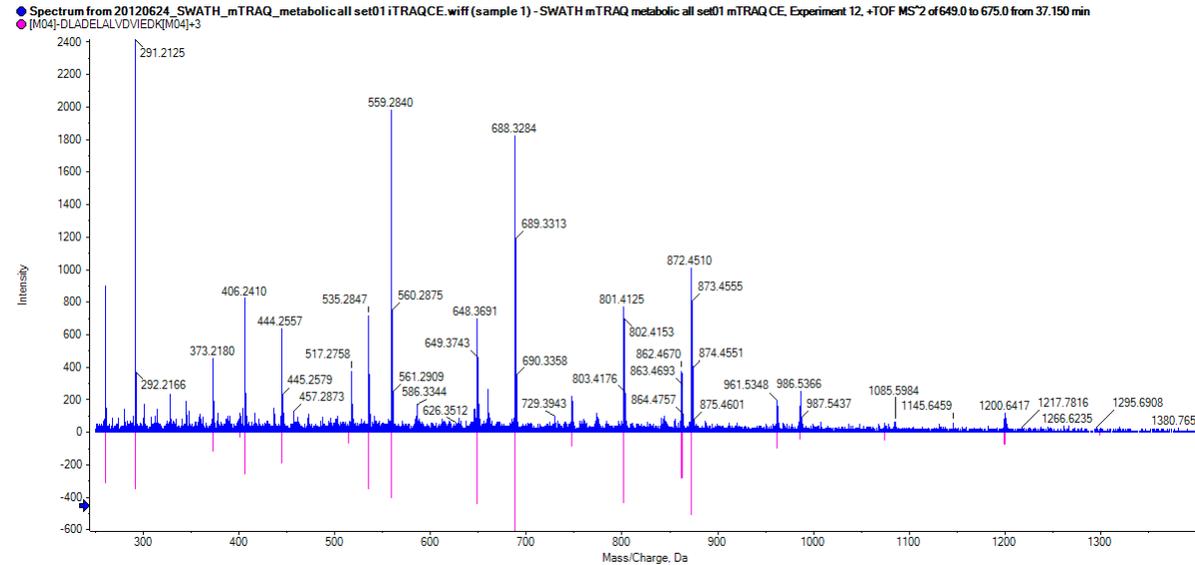
The screenshot shows the IonQuant software interface in MS1 Quantification mode. The main window title is "FragPipe (v20.0)". The top menu bar includes options like Config, Workflow, Umpire, Database, MSFragger, Validation, PTMs, Glyco, Quant (MS1), Quant (Isobaric), Spec Lib, Quant (DIA), Run, and Downstream. The "MS1 Quantification" section is active, with "Run MS1 quant" selected. The "Basic options" section includes "LFG" (selected), "Add MaxLFG" (checked), "MaxLFG min ions" (1), "Labeling" (Light, Medium, Heavy), and "Re-quantify" (checked). The "Common" section has "Match between runs (MBR)" (unchecked), "Normalize intensity across runs" (checked), "MBR ion FDR" (0.01), and "Peptide-protein uniqueness" (unique+raz...). The "Advanced options" section includes "Feature detection and peak tracing" (Min scans: 3, Min isotopes: -, m/z tolerance: 10, RT tolerance: 0.4, IM tolerance: 0.05), "Match between runs (MBR)" (MBR RT tolerance: 1, MBR IM tolerance: 0.05, MBR peptide FDR: 1, MBR min correlation: 0, MBR top runs: 10, MBR protein FDR: 1), "Intensity" (Top N ions: 0, Min freq: 0), and "Other" (Excluded mods, Min site localization probability: 0.75, Keep index on disk: unchecked). At the bottom, there are options for "FreeQuant (alternative tool)", "RT Window (minutes): 0.4", and "m/z Window (ppm): 10".

Isobaric-label定量 (TMT/iTRAQ)

The screenshot shows the TMT Integrator software interface in Isobaric Labeling-Based Quantification mode. The main window title is "FragPipe (v20.0)". The top menu bar is identical to the IonQuant interface. The "Isobaric Labeling-Based Quantification" section is active, with "Run TMT-Integrator" selected. The "Basic Options" section includes "Label type" (TMT-16), "Quant level" (-), "Mass tolerance (ppm)" (20), "Define reference" (Reference samp...), "Ref sample tag" (Bridge), "Group by" (Gene level), and "Normalization" (None). The "PTMs" section has "Mod tag" (none), "Min site probability" (-1), "Use Glycan Compositions" (unchecked), and "Glycan FDR filter" (-1). The "Advanced Options" section includes "Filtering and normalization" (Peptide-Gene uniqueness: Keep all PSMs, Peptide-Protein uniqueness: Unique+Raz...), "Min PSM probability" (0.9), "Min purity" (0.5), "Min Intensity (percent)" (0.05), "Min best peptide probability" (0), "Min NTT" (0), "Aggregation method" (Median), "Best PSM" (checked), "PSM norm" (unchecked), "Allow overlabel" (checked), "Allow unlabeled" (checked), "Outlier removal" (checked), "Exclude proteins" (none), and "Ratio to Abundance conversion" (Use MS1 intensity: checked, Top 3 ions: checked, Print reference intensity: unchecked, Log2 transform the intensity: checked). A "Sample/Channel Annotation" table is present, with columns for "Experiment" and "Annotation file path".

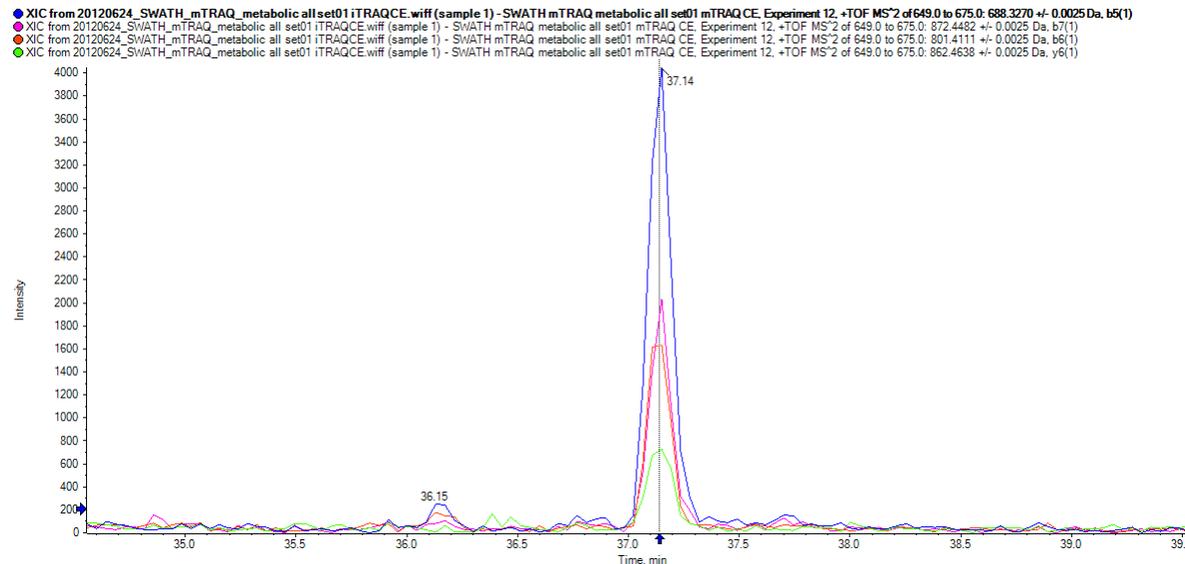
DIAのデータ解析

DIAデータ解析の基本



DIA spectra
(混合MS/MS)

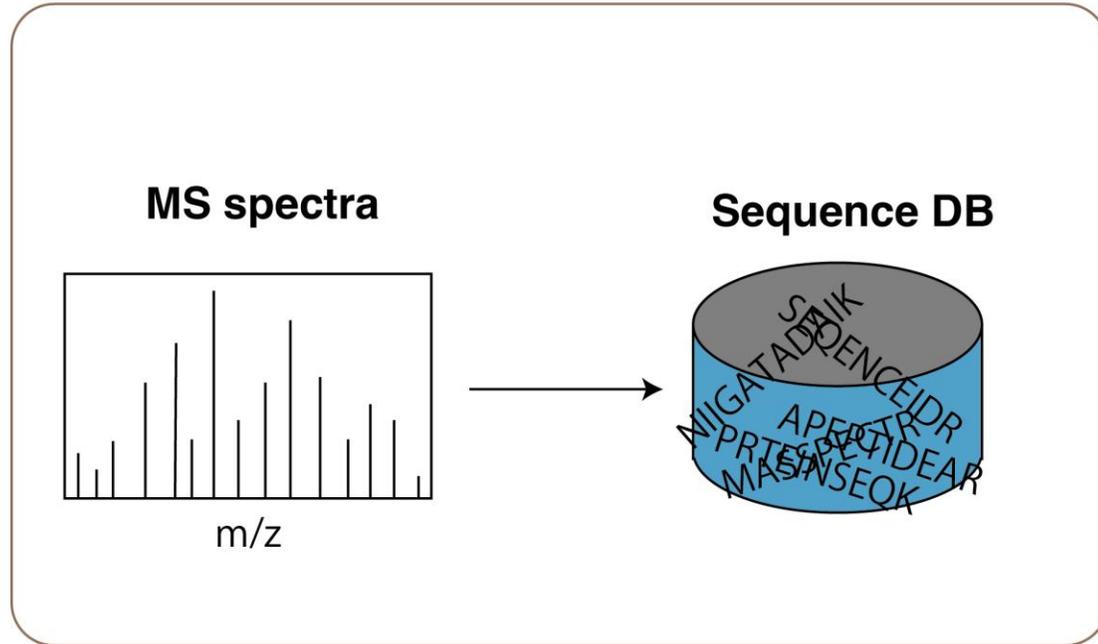
Spectra library



Fragment ion extracted
chromatography

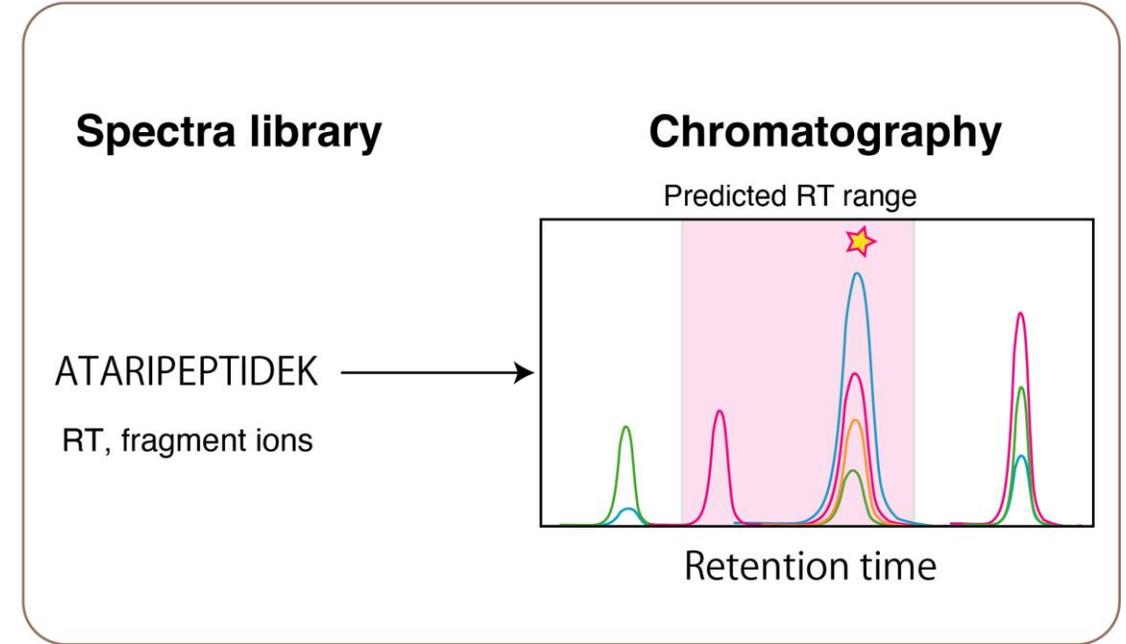
質量分析プロテオームデータ解析のアプローチ

Spectra-centric approach



- Suitable for DDA analysis
- Applicable to PTMs search
- Accumulation of data in public repository
- Stochastic identification

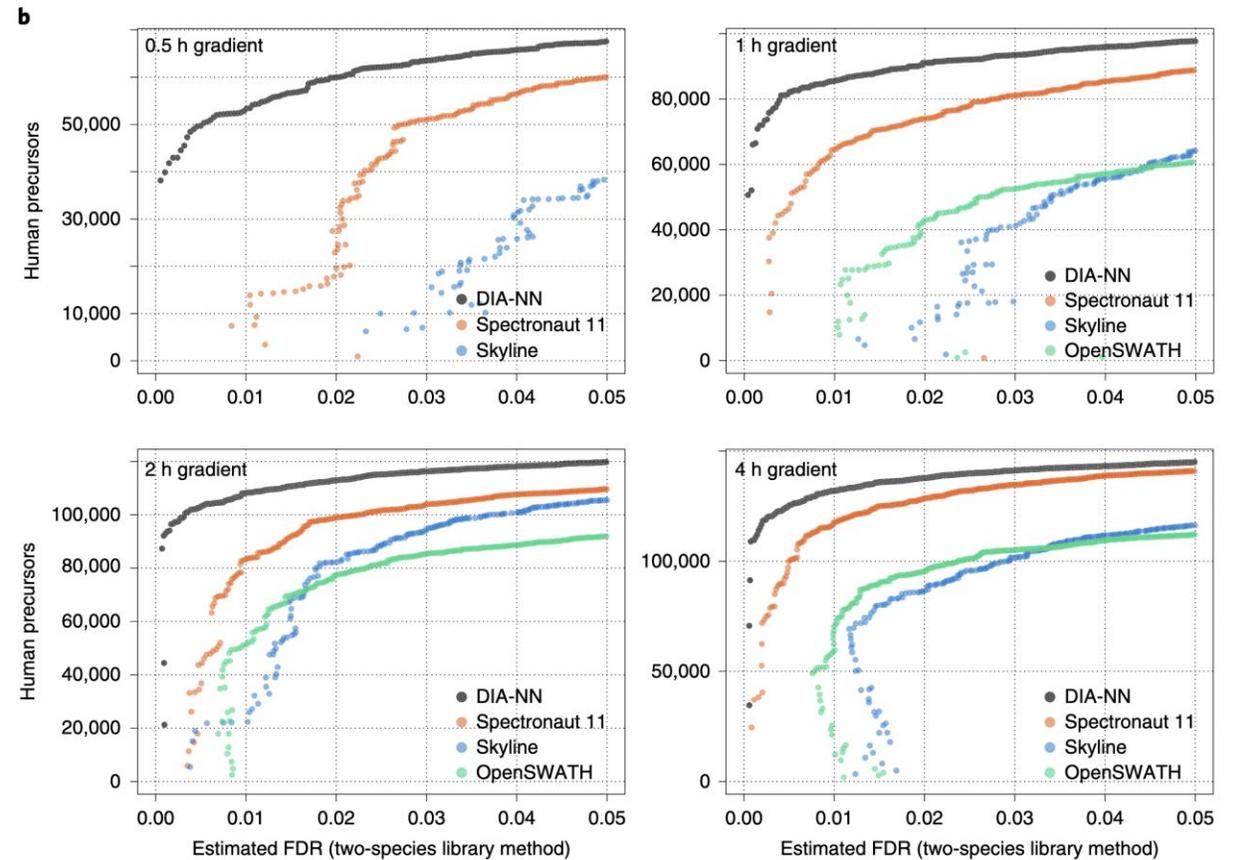
Peptide-centric approach



- Suitable for DIA analysis
- More efficient peptide identification
- RT constraint enhances reliability
- High data consistency

DIA解析ソフトの発展

	Library search	Library free-search	
		Spectra-centric	Peptide-centric
● OpenSWATH	○		
● DIA-Umpire	○	○	
● PECAN	○		○
● Skyline	○		
● EncyclopeDIA	○		○
● DIA-NN	○		○
● DIAMeter	○	○	
● MaxDIA	○	○	
■ Spectronaut	○	○	
■ Scaffold-DIA	○		○
■ PEAKS	○		?



Demichev, V et al. *Nat. Methods* 17, 41–44 (2020).

DIA-NNによるライブラリーフリーサーチ

The screenshot displays the DIA-NN software interface with the following settings:

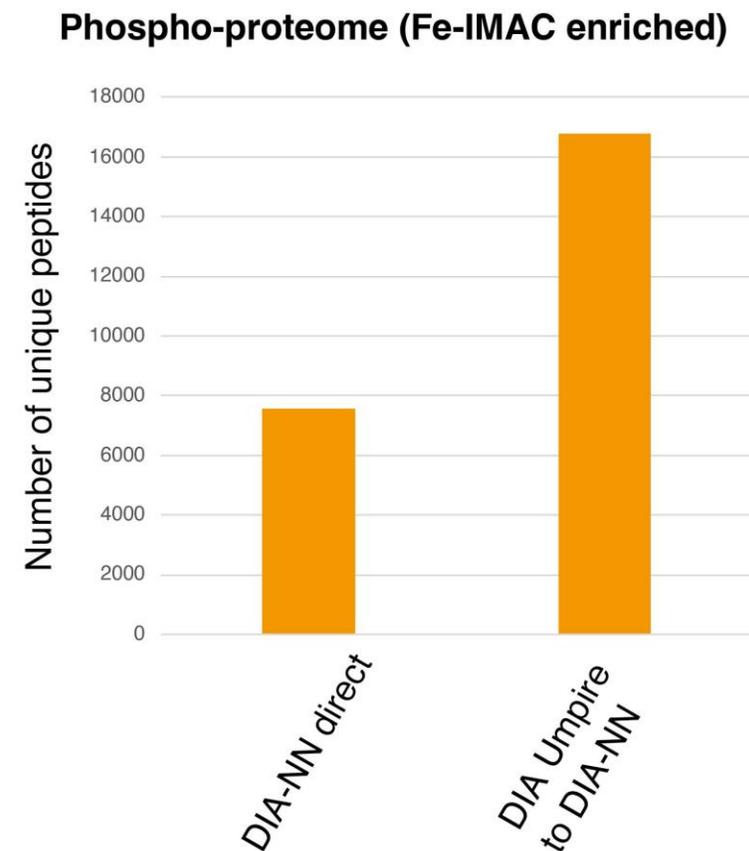
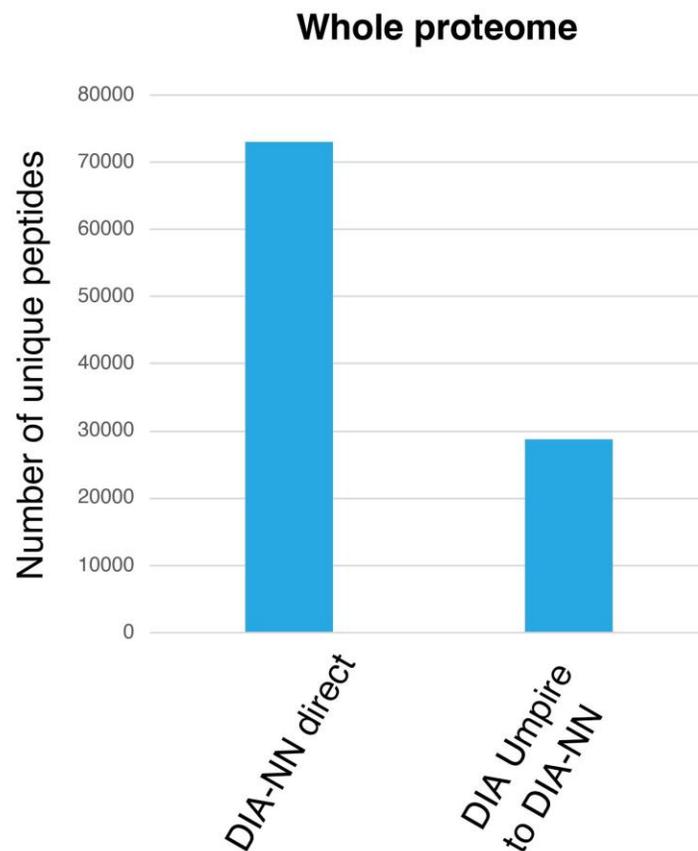
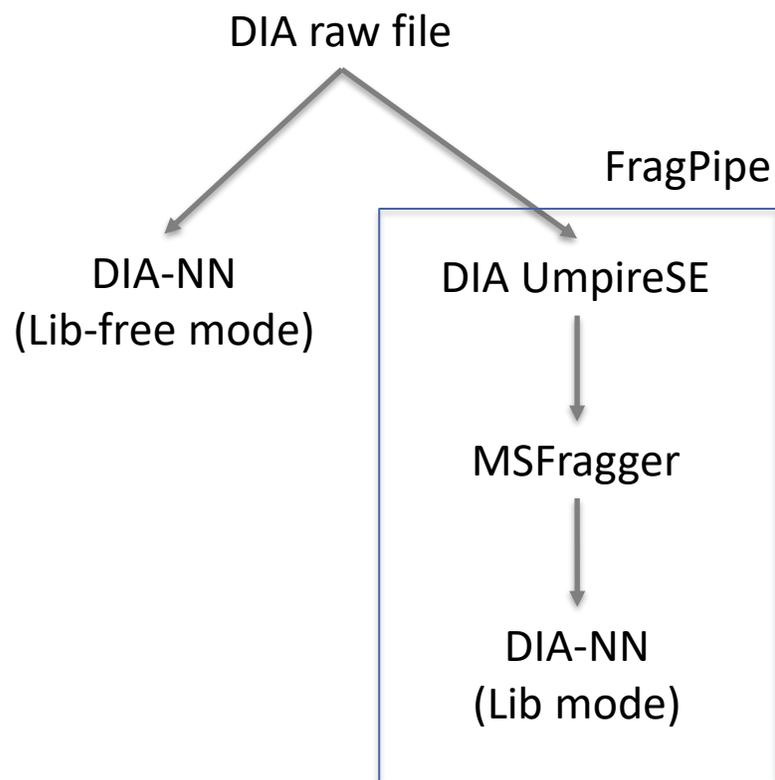
- Input:** Raw files are listed in the left pane. The 'Spectral library' section is empty, with 'Add FASTA' and 'Clear list' buttons available.
- Output:** 'Generate spectral library' and 'Generate PDF report' are checked. 'Precursor FDR (%)' is set to 1.0 and 'Threads' to 8.
- Precursor ion generation:** 'FASTA digest for library-free search / library gener' and 'Deep learning-based spectra, RTs and IMs prediction' are checked. Protease is set to 'Trypsin/P' and missed cleavages to 1.
- Algorithm:** 'Mass accuracy' and 'MS1 accuracy' are set to 0.0. 'Use isotopologues' and 'MBR' are checked. 'Protein inference' is set to 'Genes'.
- Peptide length range:** 7 - 30
- Precursor charge range:** 1 - 4
- Precursor m/z range:** 450 - 1200
- Fragment ion m/z range:** 200 - 1600

ライブラリーフリーサーチとは？

FASTAファイルから予測スペクトルライブラリーを直接構築して解析

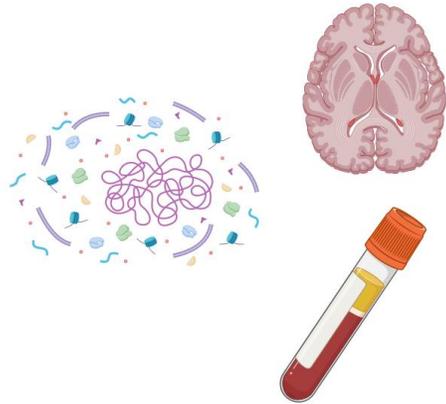
- Raw fileとFastaファイルを指定して、Precursor ion generationにチェックを入れてスタートするだけ。
- 修飾はリン酸化とユビキチン化に対応済み
- 一旦作った予測ライブラリーは使い回し可能
- 1ペプチドあたりの修飾部位数やミス切断数を増やすと終わらないので注意が必要。

DIA Umpire SE effectively enhances identification of phosphopeptides

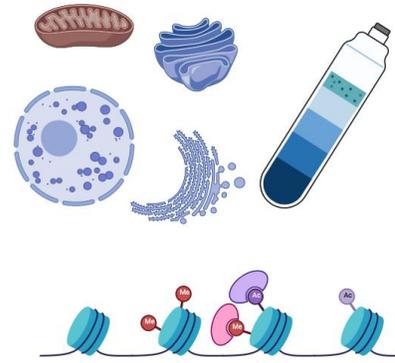


多様化するプロテオーム研究

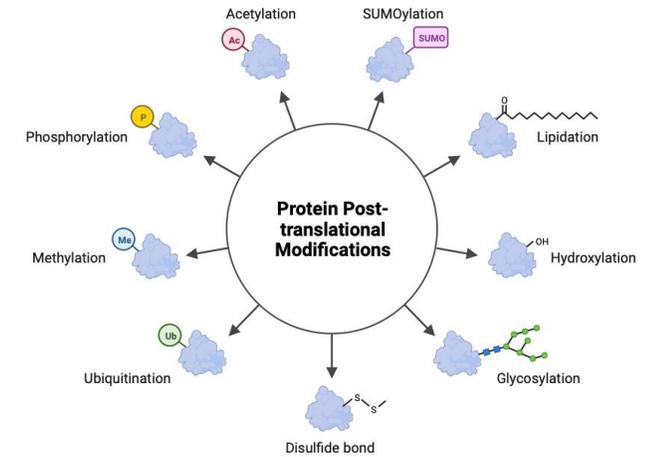
Whole proteome



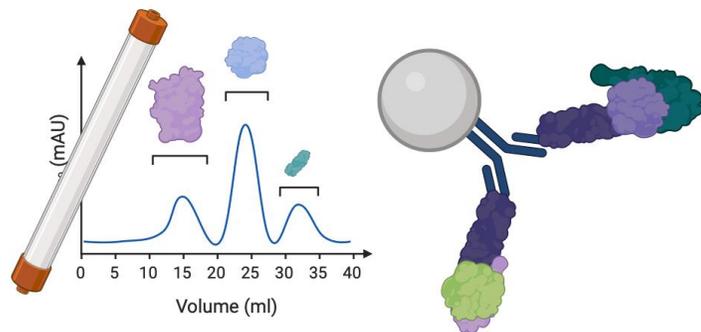
Organelle proteome



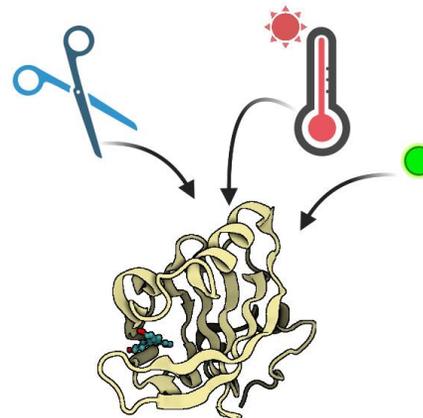
Modificome



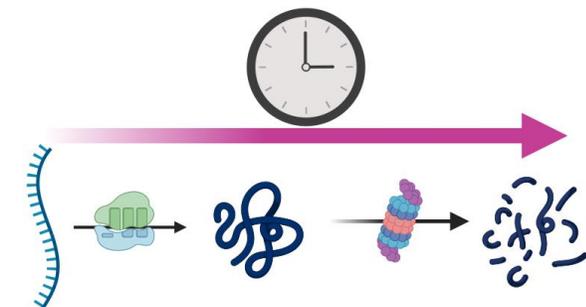
Interactome



Structural proteome



Proteostasis





Take home message

まとめ

- 単純な発現比較や個々のタンパク質情報の収集にはRepositoryと連動したプロテオームデータベースの活用が有効
- Repositoryにあるデータを再解析等をするには実験方法のある程度の理解が必要
- フリーソフトも多数あるので、誰でも再解析は可能

展望

- 今後ますます大規模化が促進（1データセット数百検体時代に）
- 他のオミクスデータも充実し統合が可能
- データサイエンスもChatGPTなどを活用すれば誰でも可能