2026 ASMS Short Course (May 30 and May 31): Top-Down Proteomics (Saturday) AND Top-Down MS Data Analysis and Visualization (Sunday)

Saturday Instructors

Ying Ge
University Of Wisconsin

Joe Loo
UCLA

Neil Kelleher
Northwestern University

Ljiljiana Pasa-Tolic
PNNL

Si Wu
University Of Alabama

Jeff Agar
Northeastern University

Yury Tsybin
Spectroswiss

Sunday Instructors

Xiaowen Liu
Tulane University

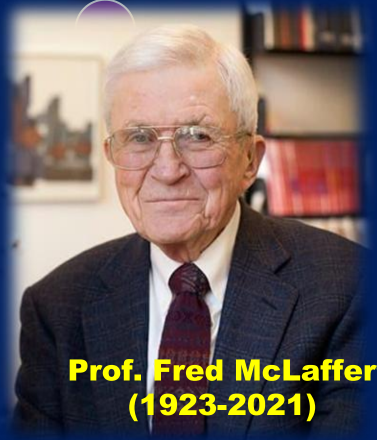
Kyowon Jeong
University Of Tubingen

Boris Krichel
University of Lübeck

Topics to be covered:

- History and Future Outlook
- Sample Preparation
- Intact Protein Separation
- Instrumentation for Top-Down Proteomics
- Quantitative Top-Down Proteomics
- Applications: Biomedical, Clinical, Biopharmaceutical, etc.
- Software Tool Introduction and Hands-on Data Analysis Training

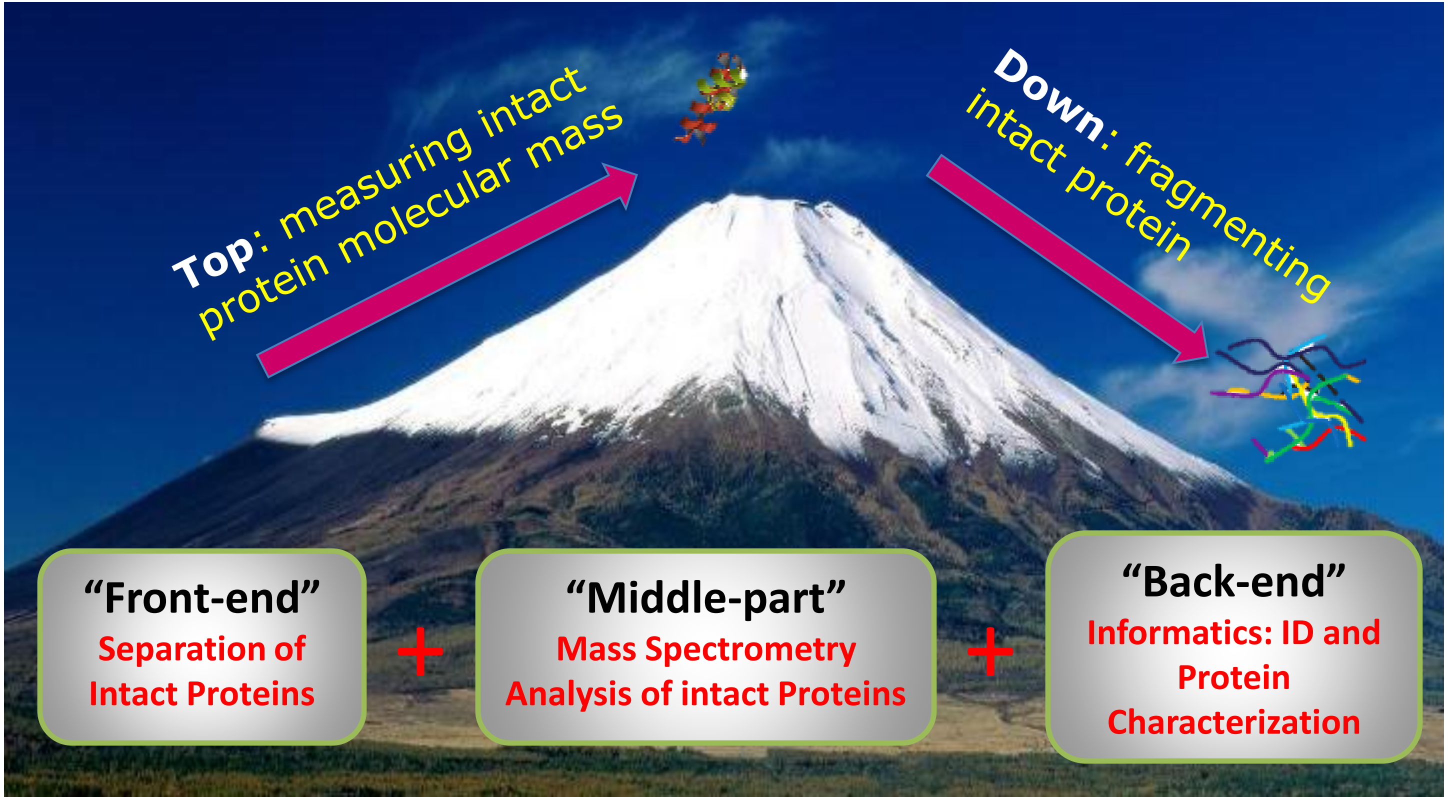
Top-Down Mass Spectrometry: Why Everyone Should Be Doing It!



Prof. Fred McLafferty (1923-2021)

2025 ASMS Short Course on Top-Down Proteomics

Day 1 Instructors: Ying Ge, Neil Kelleher, Joseph Loo, Ljiljana Paša-Tolić, Jeff Agar, Yury Tsybin, Si Wu



Top: measuring intact protein molecular mass

Down: fragmenting intact protein

"Front-end"
Separation of Intact Proteins

+

"Middle-part"
Mass Spectrometry Analysis of intact Proteins

+

"Back-end"
Informatics: ID and Protein Characterization

2025 ASMS Short Course on Top-Down Proteomics

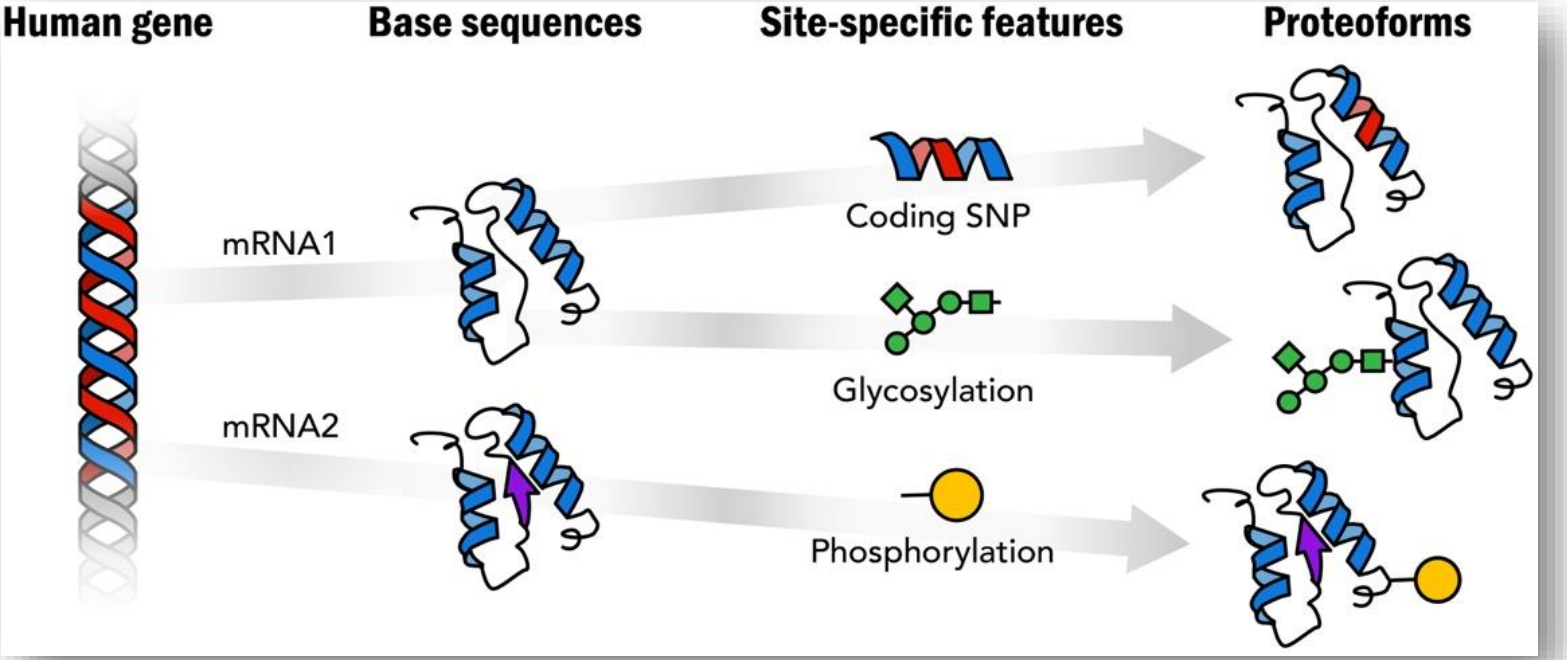
- 1. Introduction – History, concept of proteoforms (9-9:45am)
- 2. Instrumentation, dissociation methods (9:45-10:15am)
    - Coffee (10:15-10:45am)
- 3. Sample preparation (10:45-11:20am)
- 4. Intact protein separation (11:20am-noon)
    - Lunch (noon-1pm)
- 5. Data interpretation and software overview (1-1:45pm)
- 6. Top-down quantitative proteomics (1:45-2:30pm)
    - Coffee (2:30-3:00pm)
- 7. Biomedical/clinical applications (3-3:40pm)
- 8. Biopharmaceutical applications (3:40-4:20pm)
- 9. Future outlook (4:20-5:00pm)

DAY 1

Proteoforms: Distinct protein forms arising from a single gene

- Introduction
- Instrumentation
- Sample Prep
- Separations
- Software
- Quantitative
- Clinical
- BioPharma

Joe Loo
Lecture 1



All of the different molecular forms in which the protein product of a single gene can be found

“How many proteoforms are there?” Nature Chem Biol (2018)

Activation & Dissociation Methods for Top-Down MS

Introduction

Instrumentation

Sample Prep

Separations

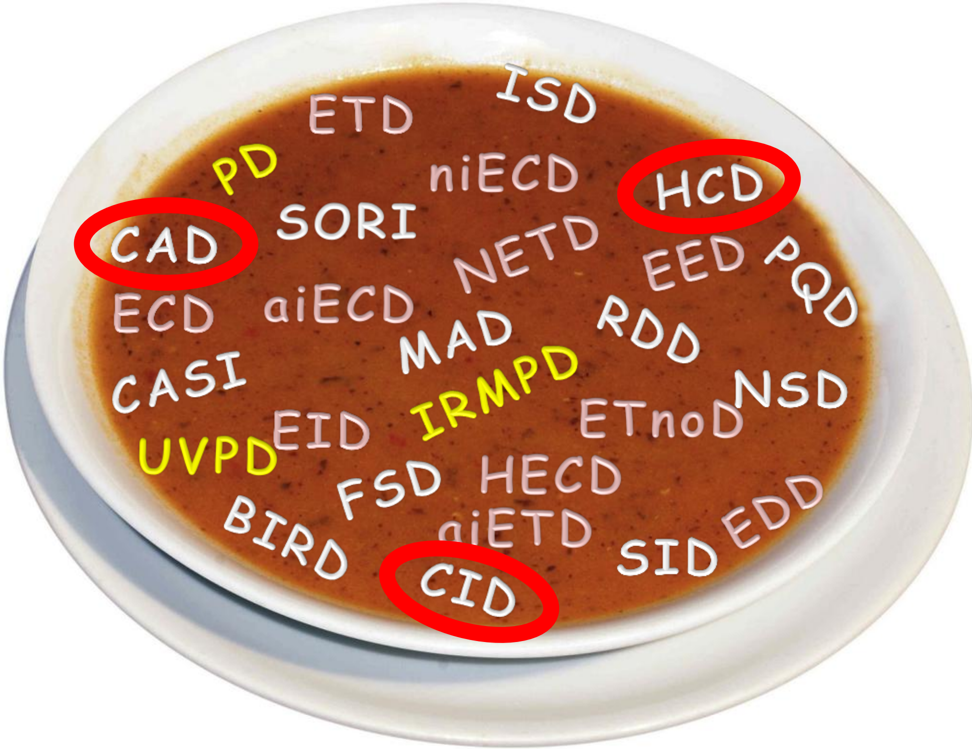
Software

Quantitative

Clinical

BioPharma

Joe Loo
Lecture 2



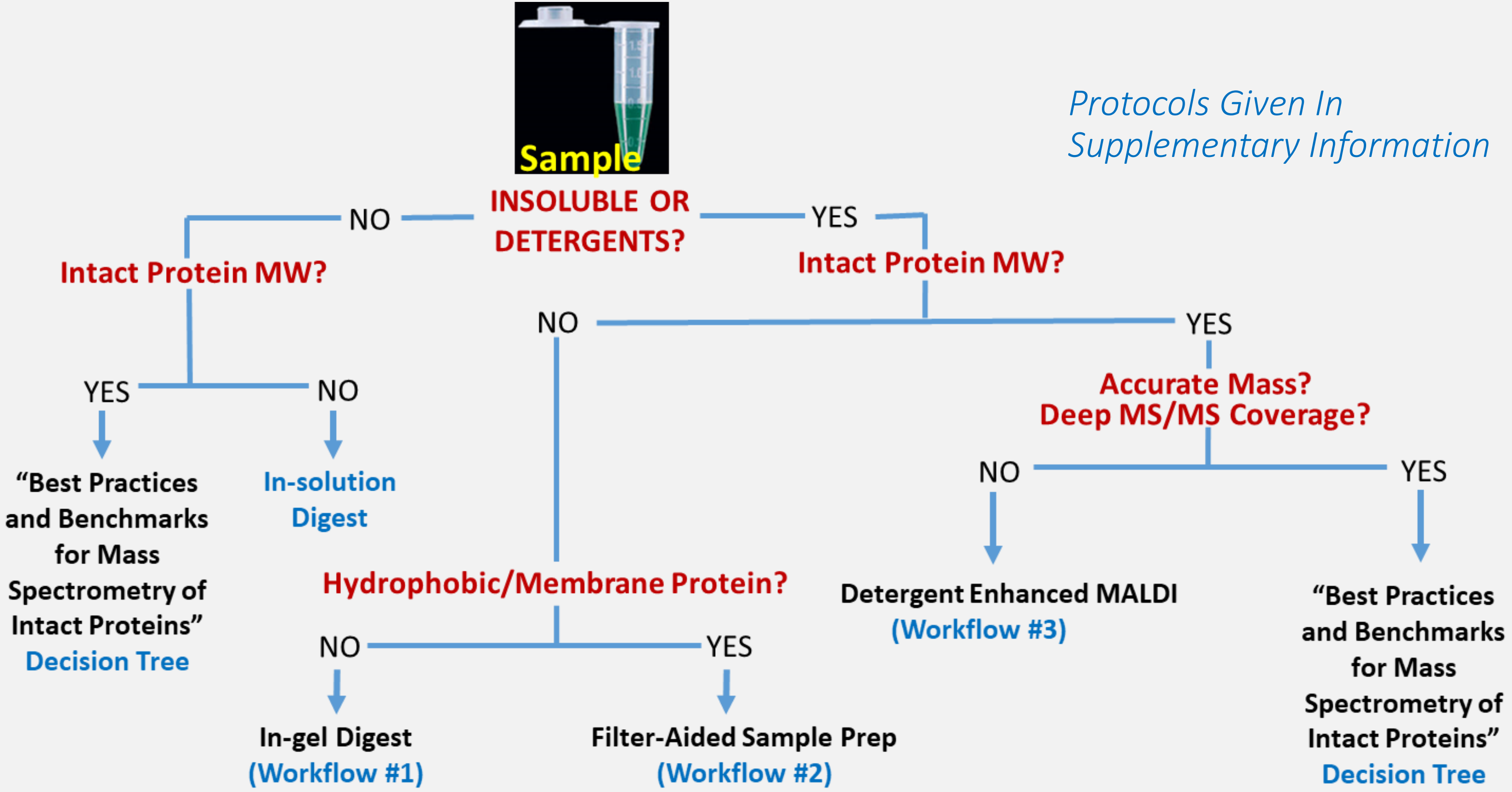
Complementary methods for MS/MS and structure elucidation

Alphabet Soup of Methods

Sample Preparation for Your Top-down Experiment

Jeff Agar
Lecture 3

Protocols Given In Supplementary Information



Sample

INSOLUBLE OR DETERGENTS?

NO

Intact Protein MW?

YES → “Best Practices and Benchmarks for Mass Spectrometry of Intact Proteins” Decision Tree

NO → In-solution Digest

YES

Intact Protein MW?

NO

Hydrophobic/Membrane Protein?

NO → In-gel Digest (Workflow #1)

YES → Filter-Aided Sample Prep (Workflow #2)

YES

Accurate Mass? Deep MS/MS Coverage?

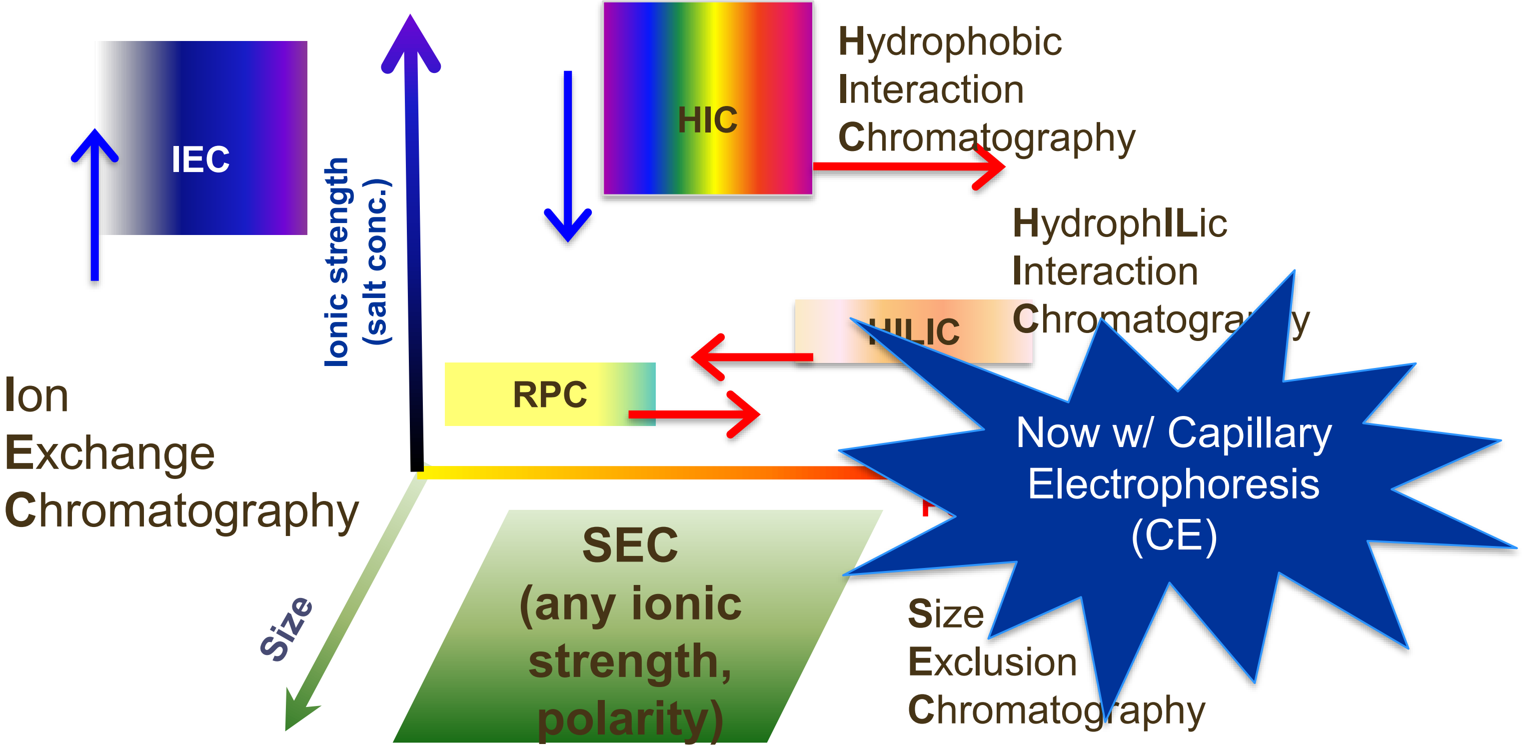
NO → Detergent Enhanced MALDI (Workflow #3)

YES → “Best Practices and Benchmarks for Mass Spectrometry of Intact Proteins” Decision Tree

Multi-dimensional Chromatography for Intact Protein Separation

Introduction
Instrumentation
Sample Prep
Separations
Software
Quantitative
Clinical
BioPharma

Unlike peptides, proteins have a much more diverse range of physiochemical properties -> multiple chromatographic modes are available for intact protein separation.



Ion
Exchange
Chromatography

Hydrophobic
Interaction
Chromatography

Hydroph**IL**ic
Interaction
Chromatogra y

SEC
(any ionic strength, polarity)

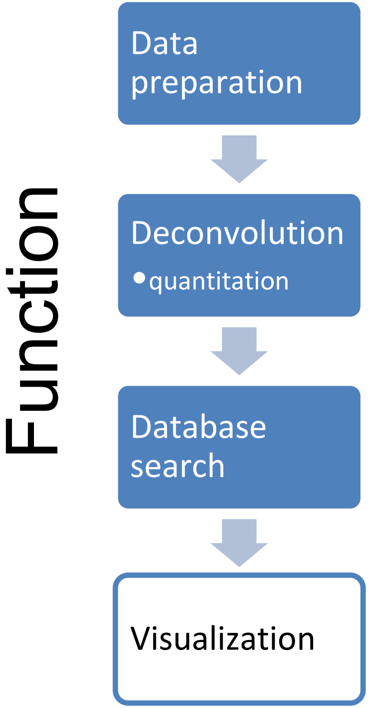
Size
Exclusion
Chromatography

Now w/ Capillary
Electrophoresis
(CE)

Top-down Proteomics Data Analysis Overview

Introduction
Instrumentation
Sample Prep
Separations
Software
Quantitative
Clinical
BioPharma

Ljiljana Paša-Tolić
Lecture 5



Many tools support input of raw instrument data, some require conversion to mzML, etc.

Raw m/z data is deconvoluted to mass.
Most tools also report intensity (abundance) allowing for proteoform quantitation.

MS1 level: mass profiles are matched to expected protein sequences.
MS2 level: fragmentation spectra are mapped to protein sequences

Most software includes visualization modules for manual validation and report generation.

Quantitative Top-Down Proteomics

Introduction

Instrumentation

Sample Prep

Separations

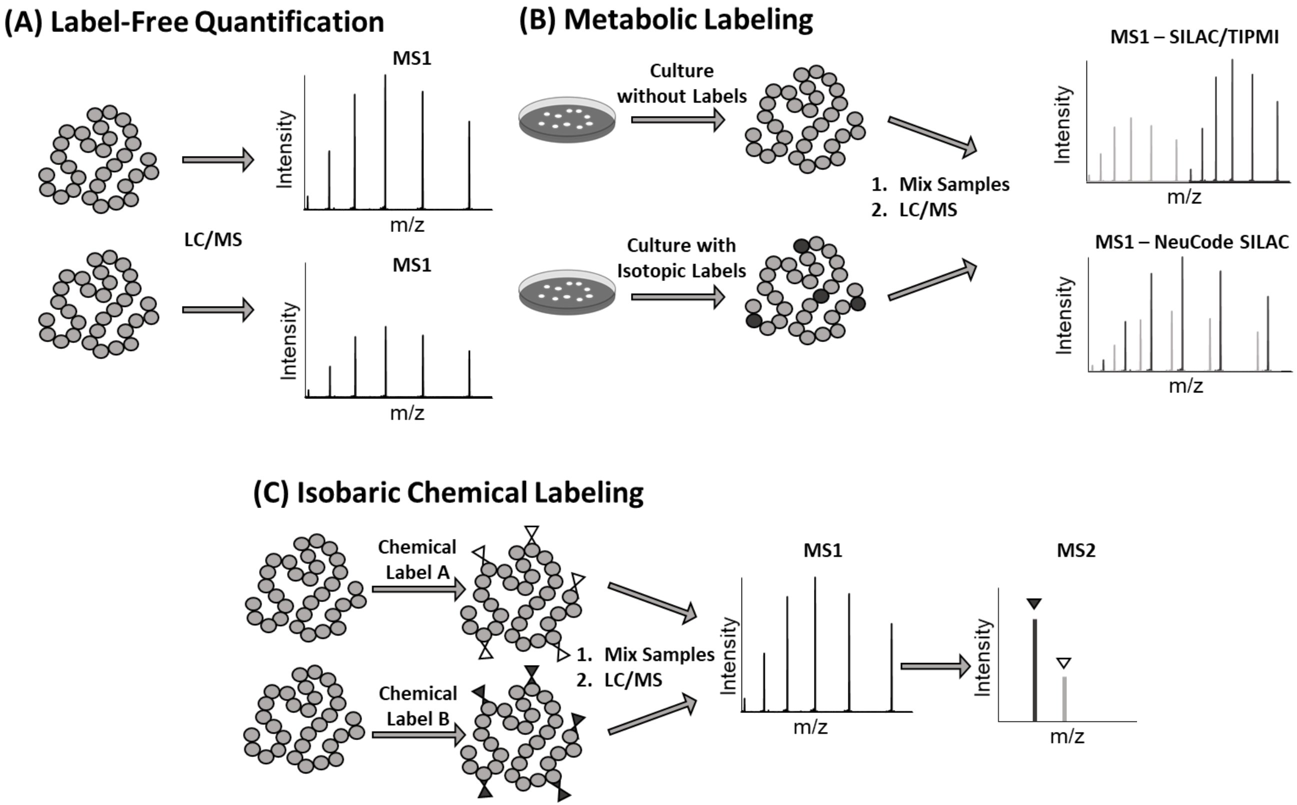
Software

Quantitative

Clinical

BioPharma

Si Wu
Lecture 6



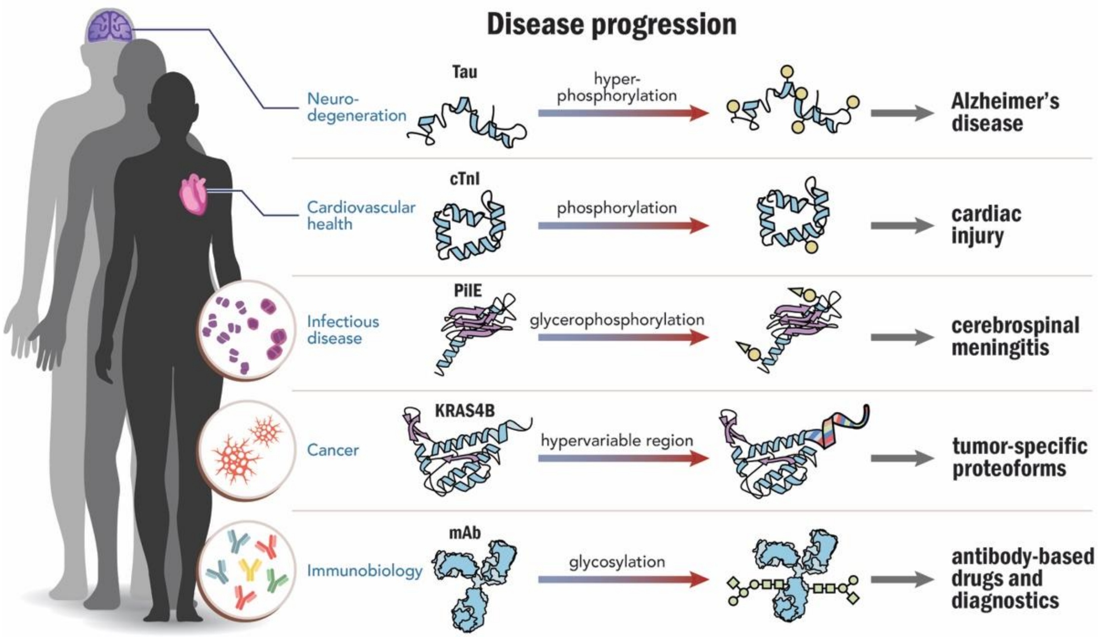
Cupp-Sutton, *et al.* Molecular Omics 2020 , Apr 1;16(2):91-99.

Application of Top-down Proteomics to Biomedical Research

- Introduction
- Instrumentation
- Sample Prep
- Separations
- Software
- Quantitative
- Clinical
- BioPharma

Ying Ge
Lecture 7

The Human Proteoform Project: A Plan to Define the Human Proteome



Lloyd M. Smith[1], Jeffrey N. Agar[2], Julia Chamot-Rooke[3], Paul O. Danis[4], Ying Ge[5], Joseph A. Loo[6], Ljiljana Paša-Tolić[7], Yury O. Tsybin[8], Neil L. Kelleher[*9] & The Consortium for Top-Down Proteomics, *Sci. Adv. 2021*

https://www.preprints.org/manuscript/202010.0368/v1

Five important disease areas are depicted where proteoforms have been identified and linked to the progression of human disease.

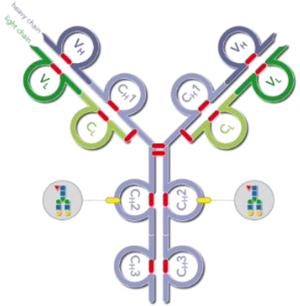
Consortium for Top-Down Proteomics

BioPharma Application: Monoclonal Antibodies & Beyond

Introduction
Instrumentation
Sample Prep
Separations
Software
Quantitative
Clinical
BioPharma

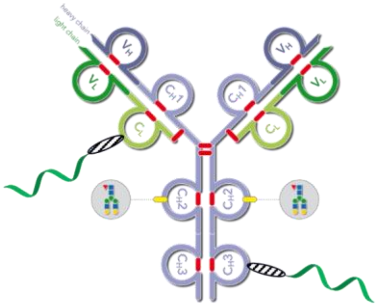
- mAbs and ADCs are exceptional biotherapeutics, particularly anti-cancer
- crucial elements in many biotechnological processes (binding properties)
- mAb properties: ~150 kDa size, (any) quantity, purification/isolation is possible

mAb



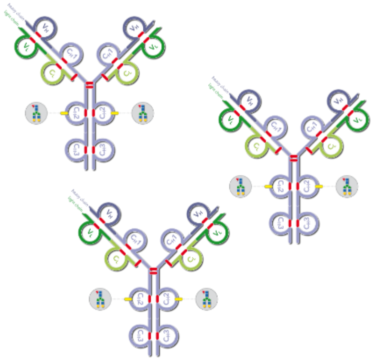
single-specific
multi-specific
glycoengineered

ADC



antibody-drug conjugate
antibody-oligonucleotide
subunit-drug conjugate

mAb cocktail



3-5 mAbs

Ab repertoire

circulating Abs

An Overview: DOI: 10.1007/978-1-0716-1450-1_1

mAbs/ADCs are a perfect match for top-down MS application and development

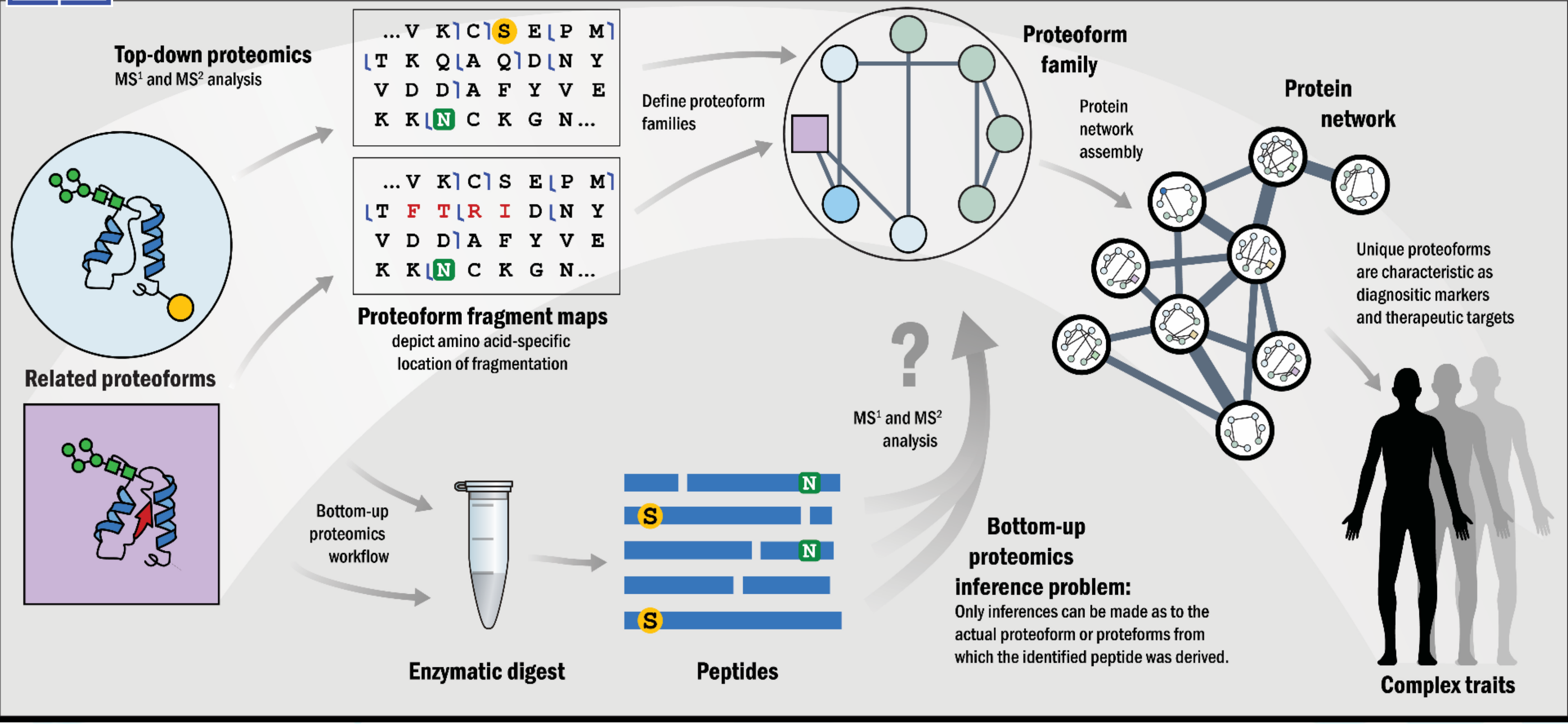
Lecture 9: Neil Kelleher

The Future of Top-down Proteomics

The Human Proteoform Project

A Fundamental Next-Step for Human Biology

Proteoforms: The Next “Currency” in Proteomics



Top-down proteomics
MS^1 and MS^2 analysis

Related proteoforms

Proteoform fragment maps
depict amino acid-specific
location of fragmentation

Define proteoform families

Proteoform family

Protein network assembly

Protein network

Unique proteoforms are characteristic as diagnositic markers and therapeutic targets

Complex traits

Bottom-up proteomics workflow

Enzymatic digest

Peptides

MS^1 and MS^2 analysis

Bottom-up proteomics inference problem:
Only inferences can be made as to the actual proteoform or protefoms from which the identified peptide was derived.

Smith and Kelleher, *Science*, 2018, 359 (6380), 1106-7

Consortium for Top-down Proteomics


Paul Danis
(CEO)


Neil Kelleher
(President)


Lloyd Smith
(Treasurer)


Ying Ge
(Secretary)


Joseph Loo
(Member at-large)


Ljiljana Pasa-Tolic
(Member at-large)


Jeff Agar
(Member at-large)


Julia Chamot-Rooke
(Member at-large)


Yuri Tsybin
(Member at-large)

It takes a village!

FALL WORKSHOP
Top-Down Proteomics

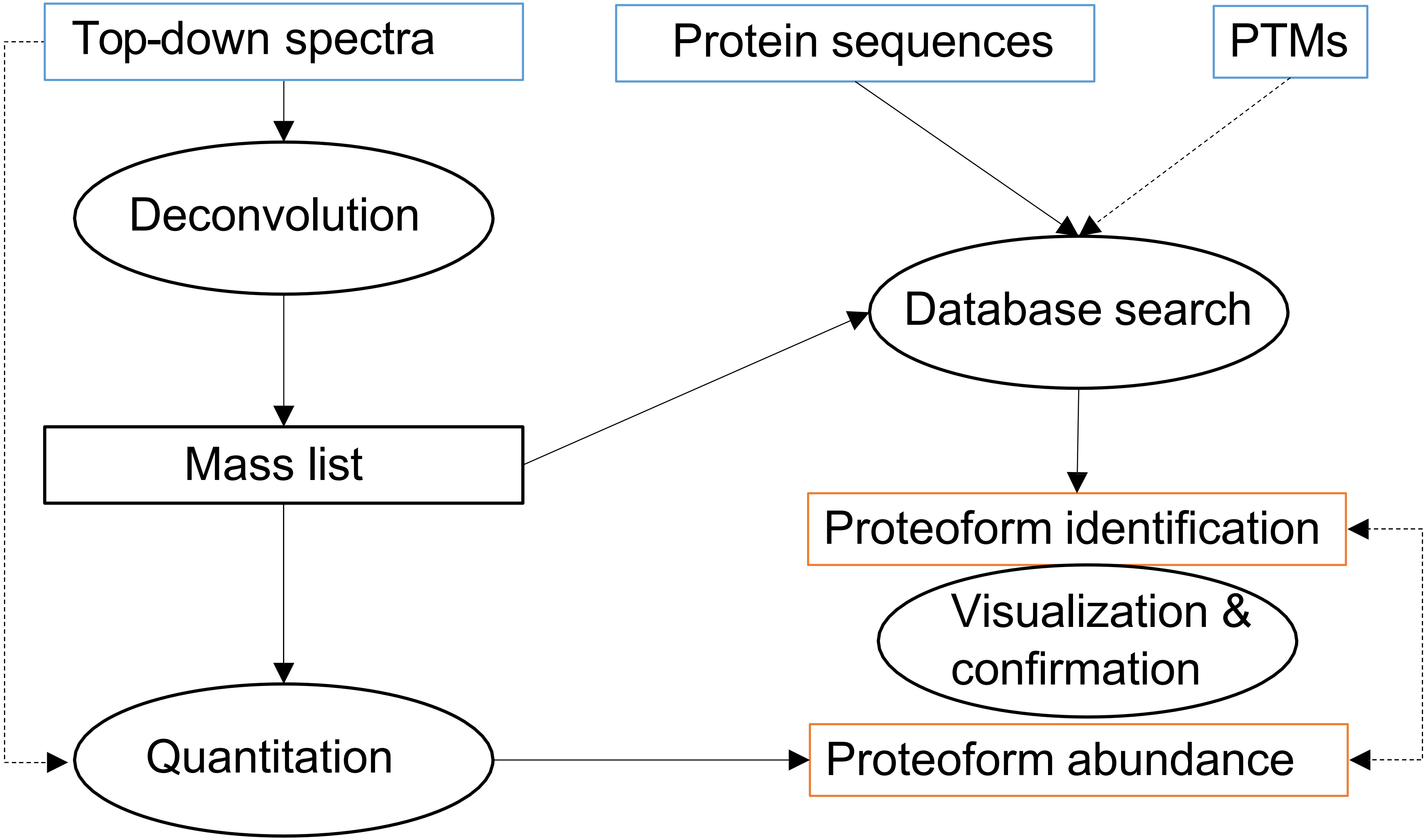
https://www.topdownproteomics.org/

ASMS Short Course

Top-down Mass Spectrometry Data Analysis and Visualization

Xiaowen (Kevin) Liu, Kyowon Jeong, and Boris Krichel

Top-Down MS Data Analysis workflow



Top-down spectra

Protein sequences

PTMs

Deconvolution

Database search

Mass list

Proteoform identification

Visualization & confirmation

Quantitation

Proteoform abundance

Top-down MS software

Spectral Deconvolution

- **Thrash** [Horn *et al*., JASMS 2000]
- **Thrash/Xtract** [Horn *et al*. JASMS 2000,Zabrouskov et al., JASMS 2005]
- **RAPID** [Park *et al*., Anal. Chem. 2008]
- **Decon2LS** [Jaitly *et al*., BMC Bioinformatics, 2009]
- **Hardklör** [Hoopmann *et al*., Anal. Chem. 2007]
- **MS-Deconv** [Liu *et al*. MCP, 2010]
- **MS-Deconv+/TopFD** [Kou et al., BMC Bioinformatics 2014]
- **UniDec** [Marty et al, AC, 2015]
- **pParseTD** [Sun et al, AC, 2016]
- **ProMex** [Park et al., Nature Methods 2017]
- **Intact** [ProteinMetrics, 2018]
- **ProteinDeconvolution** [Thermo]
- **FLASHDeconv** [Jeong et al., Cell Systems 2020]

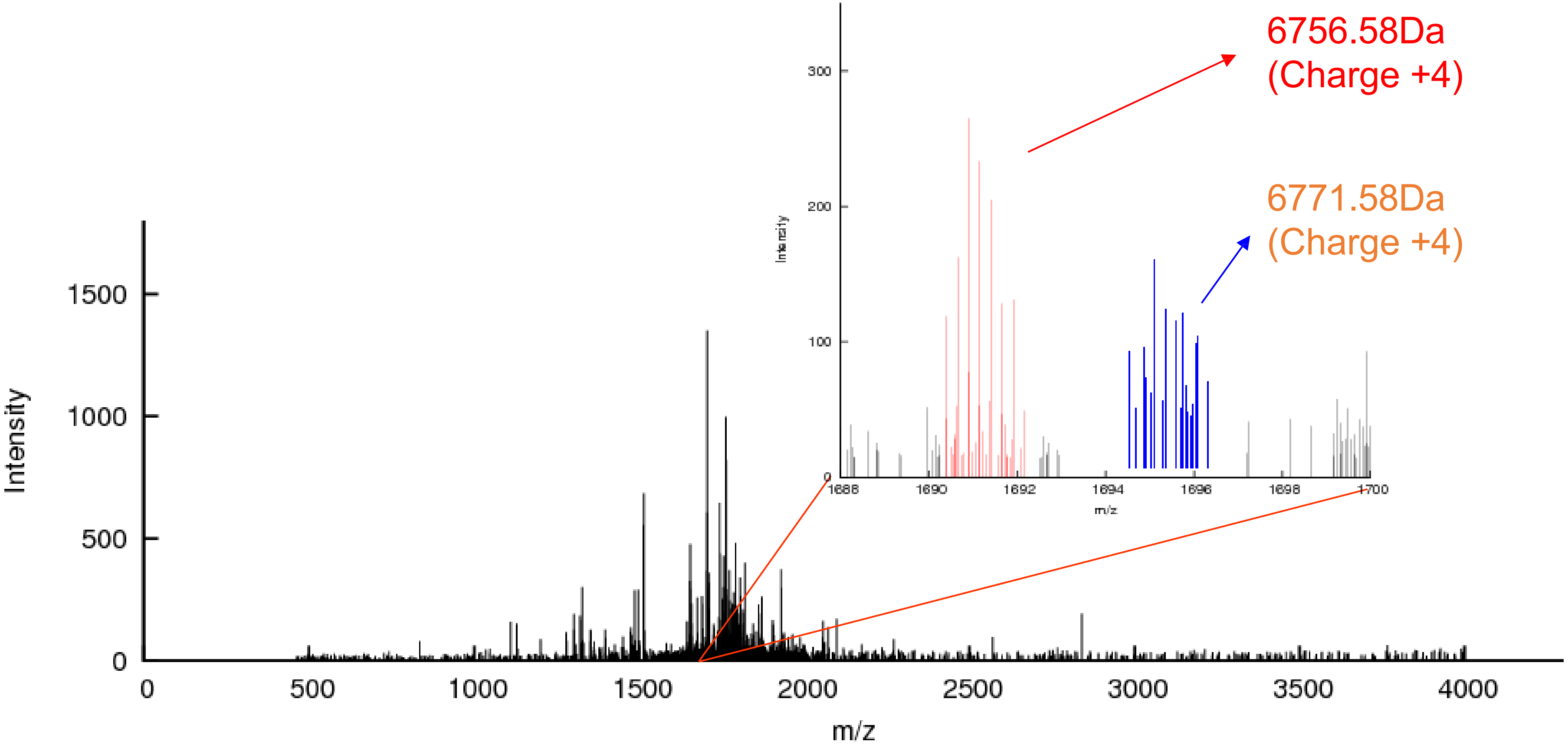
Database Search

- **ProSightPC** [Zamdborg et al., Nucleic Acids Res., 2007]
- **PIITA** [Tsai et al., JASMS., 2009]
- **USTag** [Shen et al., Anal. Chem., 2008]
- **MS-TopDown** [Frank et al., Anal. Chem., 2008]
- **MS-Align+** [Liu et al., MCP 2011]
- **MS-Align-E** [Liu et al., JPR 2013]
- **pTop** [Sun et al., AC, 2016]
- **TopPIC** [Kou et al. Bioinformatics 2016]
- **ProteinGoggle** [Xiao et al. Scientific Reports, 2016]
- **Proteoform Suite** [Shortreed et al., JPR, 2016]
- **TopMG** [Kou et al. Bioinformatics 2017]
- **MSPathFinder** [Park et al., Nature Methods 2017]
- **TDPortal** [Northwestern, ~2017]
- **PERCEPTRON** [Khalid et al., Nucleic Acid Res 2021]

Several packages with complete solutions for top-down proteomics applications

- TDPortal
  http://nrtdp.northwestern.edu/tdportal-request/
- Mash Explorer
  https://labs.wisc.edu/gelab/MASH_Explorer/index.php
- FlashDeconv
  https://openms.de/FLASHDeconv
- TopPIC
  http://www.toppic.org/

Deconvolution of top-down mass spectra



6756.58Da
(Charge +4)

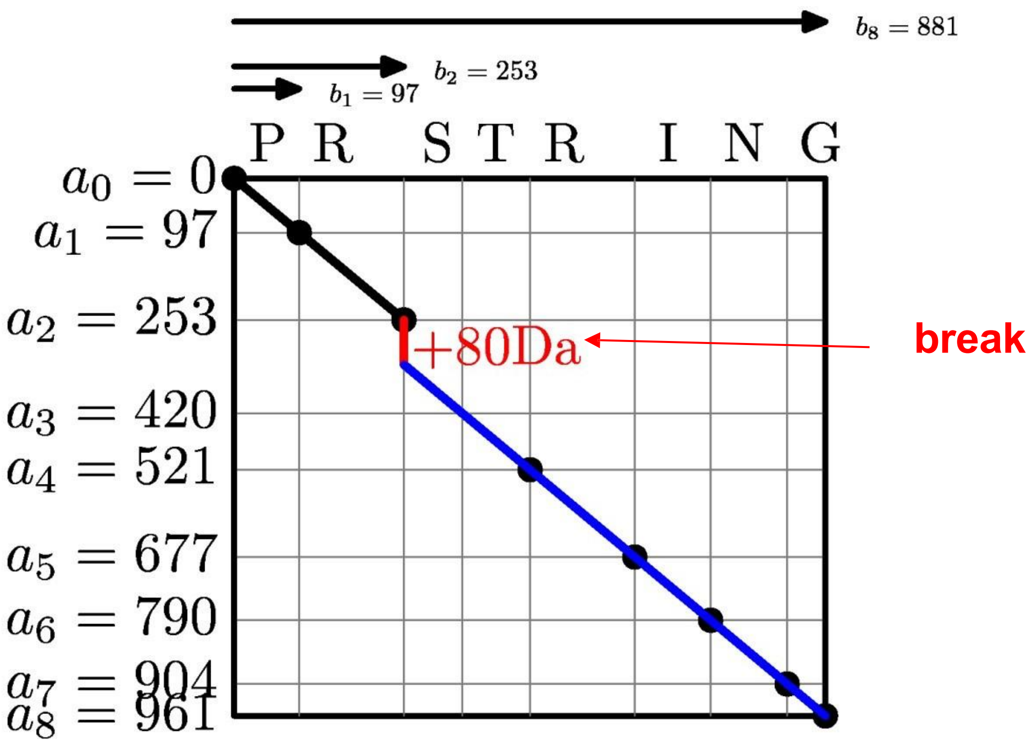
6771.58Da
(Charge +4)

Top-down mass spectra usually have many peaks and complex patterns of **isotopic envelopes.** This spectrum has about 19,000 peaks.

Spectral alignment for blind PTM search

Spectrum of prefix ions for **PRS^{+80}TRING**
{0, 97, 253, 420, 521, 677, 790, 904, 961}

Database protein: **PRSTRING**



Spectral alignment

Spectral alignment with *F* modifications is a diagonal path from the top left node to the bottom right node with at most *F* **breaks**.

Spectral alignment score

Number of 2-D points (a_i, b_j) that the path passes through.

Ultramodified proteoforms

- Histone H4 has billions of possible proteoforms

- Histone H4 proteoform identified by top-down MS