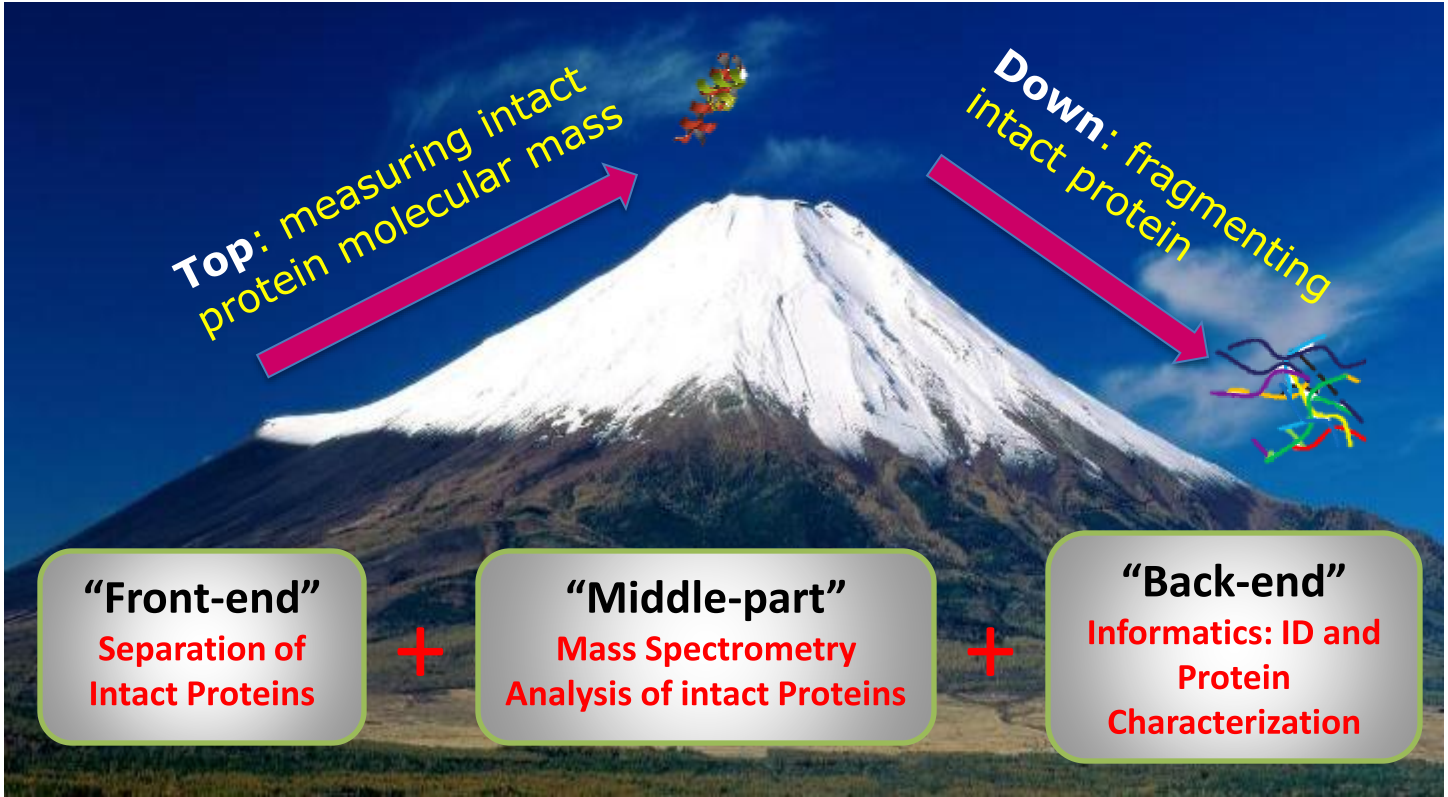
2025 ASMS Short Course on Top-Down Proteomics

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

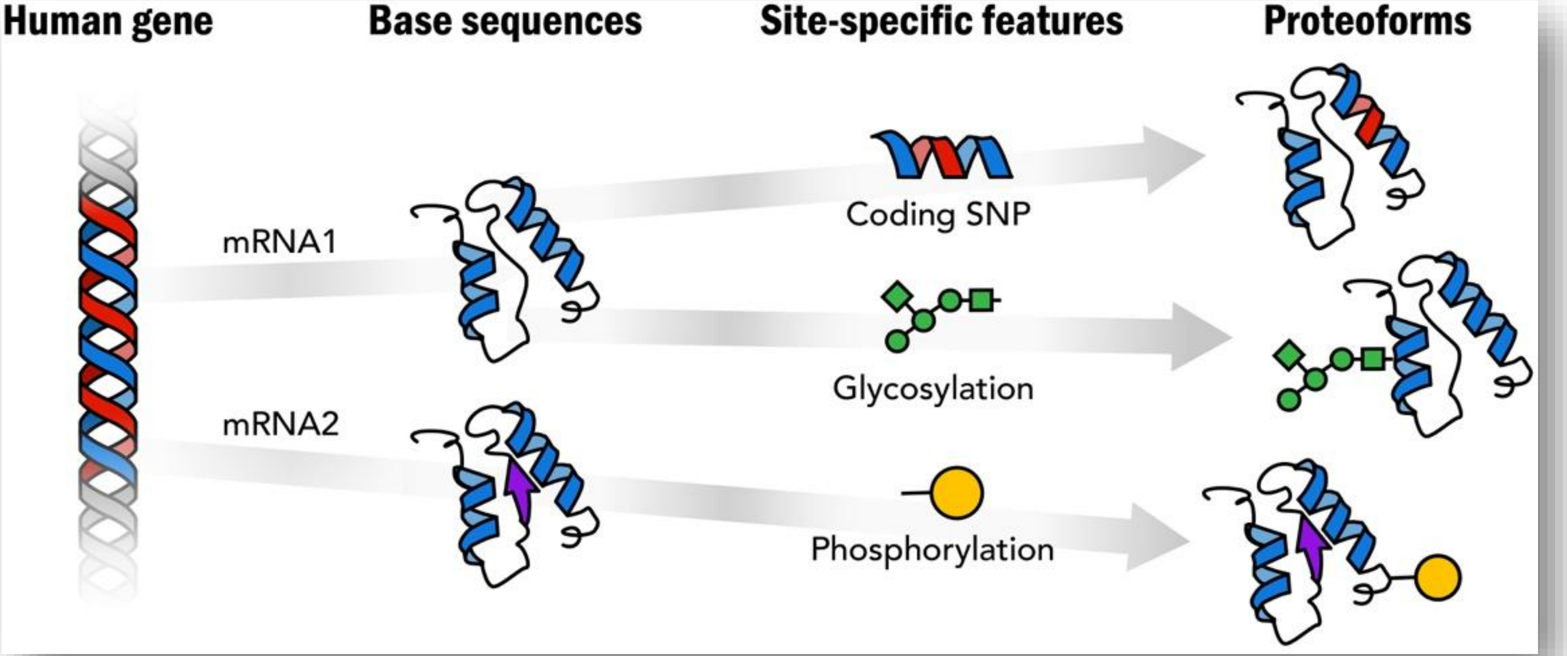
2025 ASMS Short Course on Top-Down Proteomics

- ❑ 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

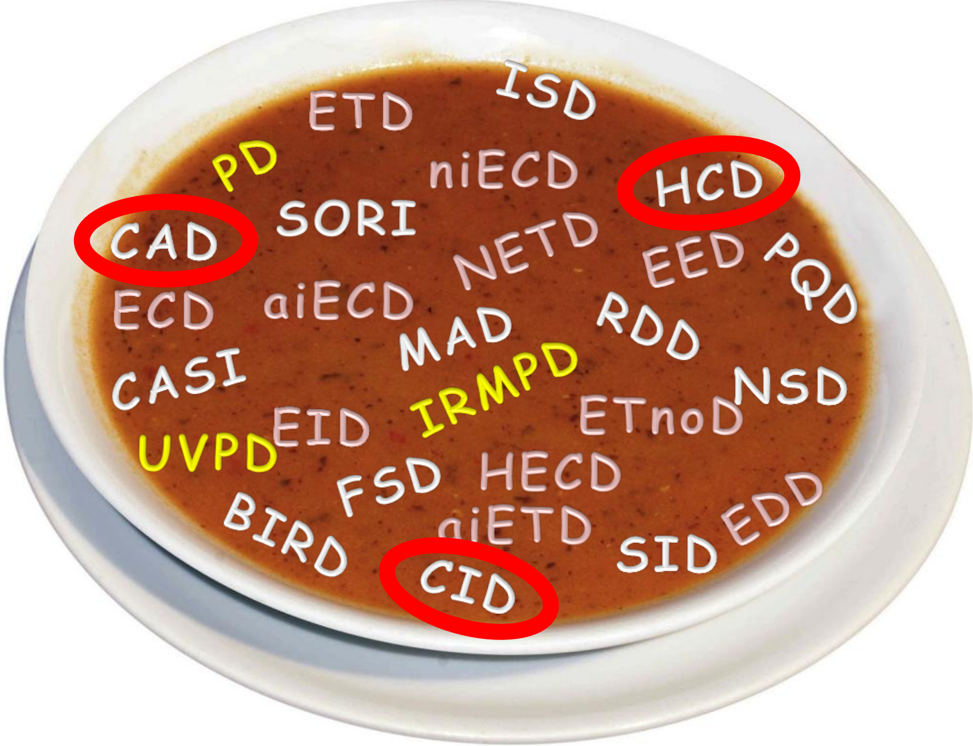
Joe Loo
Lecture 1



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



Complementary methods for MS/MS and structure elucidation

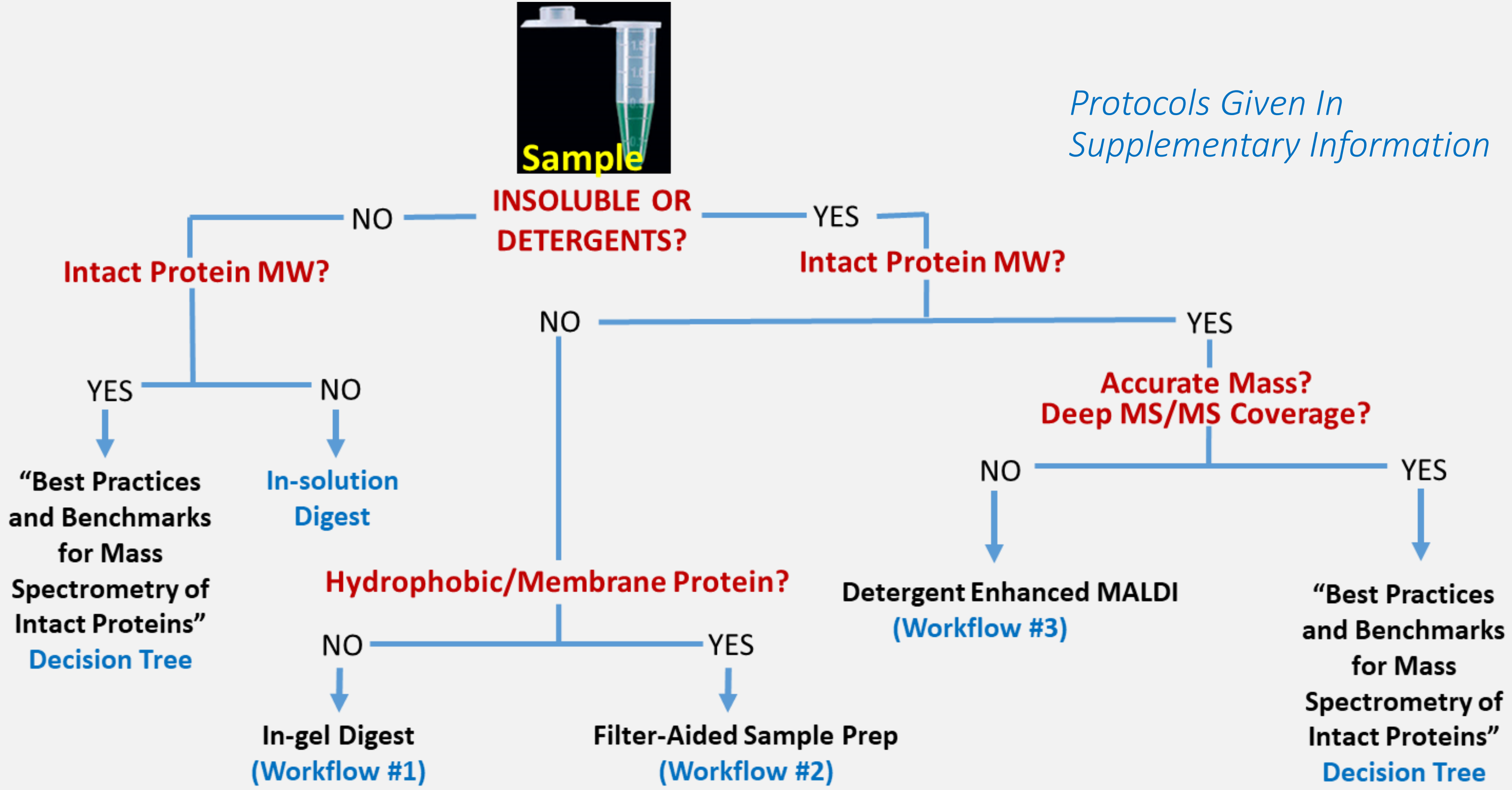
Alphabet Soup of Methods

Sample Preparation for Your Top-down Experiment

Introduction
Instrumentation
Sample Prep
Separations
Software
Quantitative
Clinical
BioPharma

Jeff Agar
Lecture 3

Protocols Given In Supplementary Information



Sample

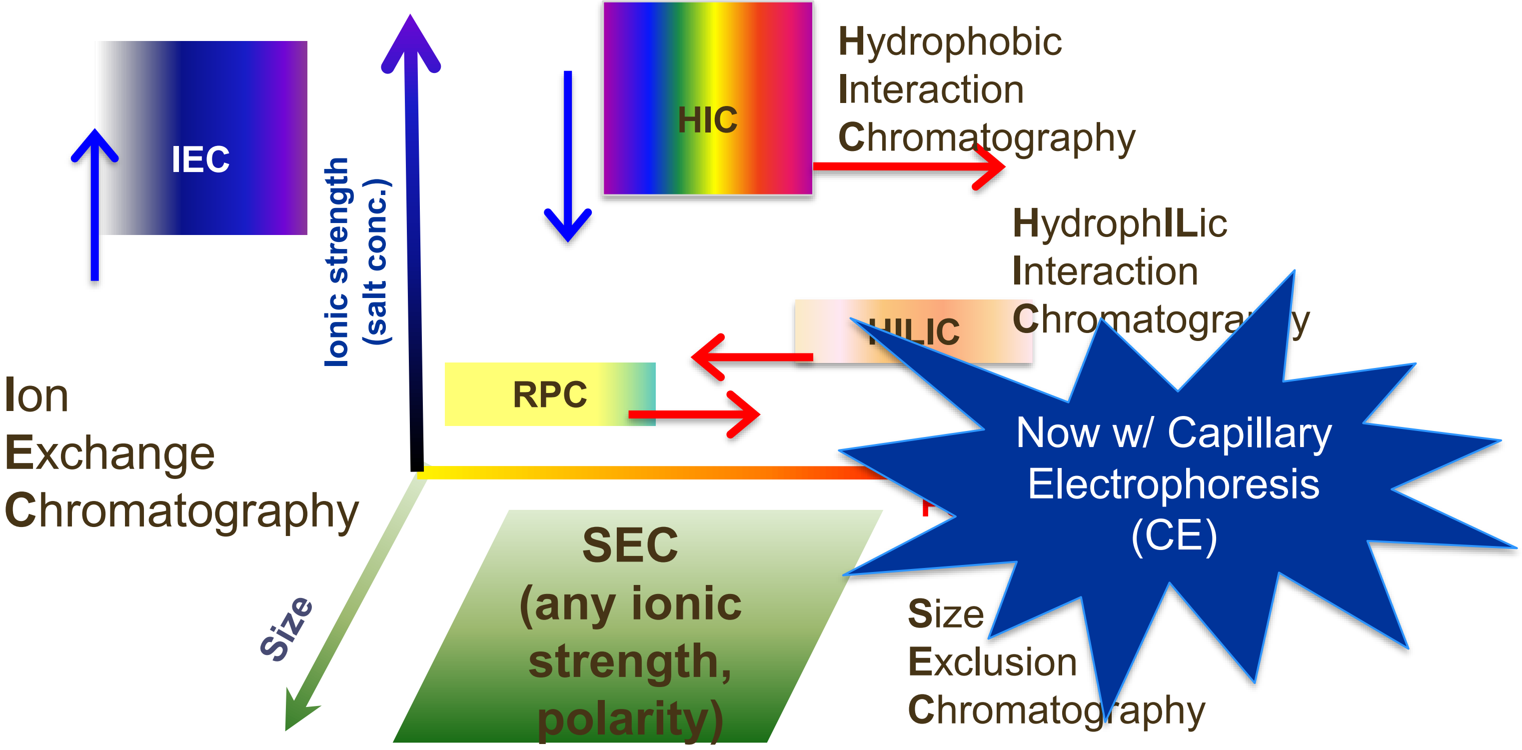
INSOLUBLE OR DETERGENTS?

- 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.



Hydrophobic
Interaction
Chromatography

Hydroph**IL**ic
Interaction
Chromatogra y

Ion
Exchange
Chromatography

SEC
(any ionic strength, polarity)

Size
Exclusion
Chromatography

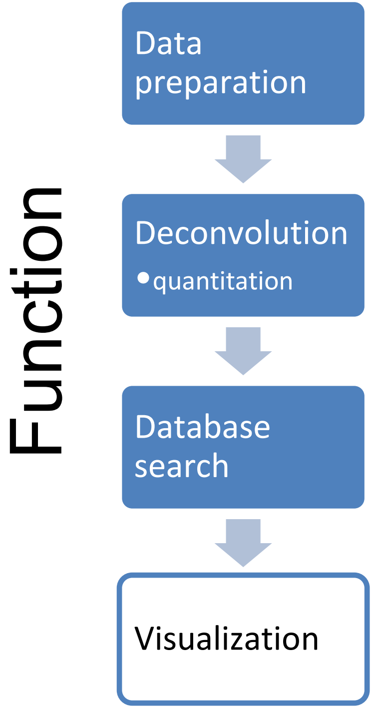
Now w/ Capillary
Electrophoresis
(CE)

Ying Ge
Lecture 4

Top-down Proteomics Data Analysis Overview

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

Ljiljana Paša-Tolić
Lecture 5



Function

Data preparation

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

Deconvolution
- quantitation

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

Database search

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

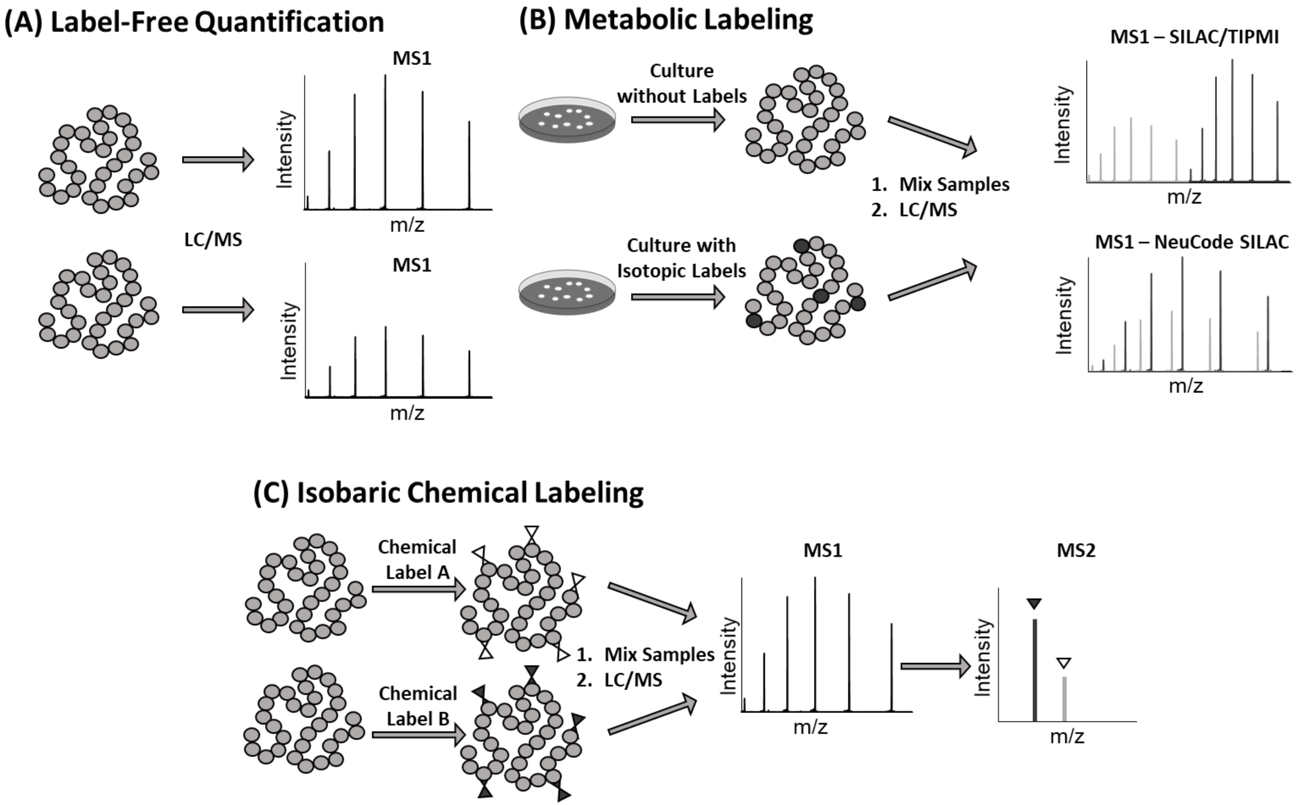
Visualization

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



(A) Label-Free Quantification

(B) Metabolic Labeling

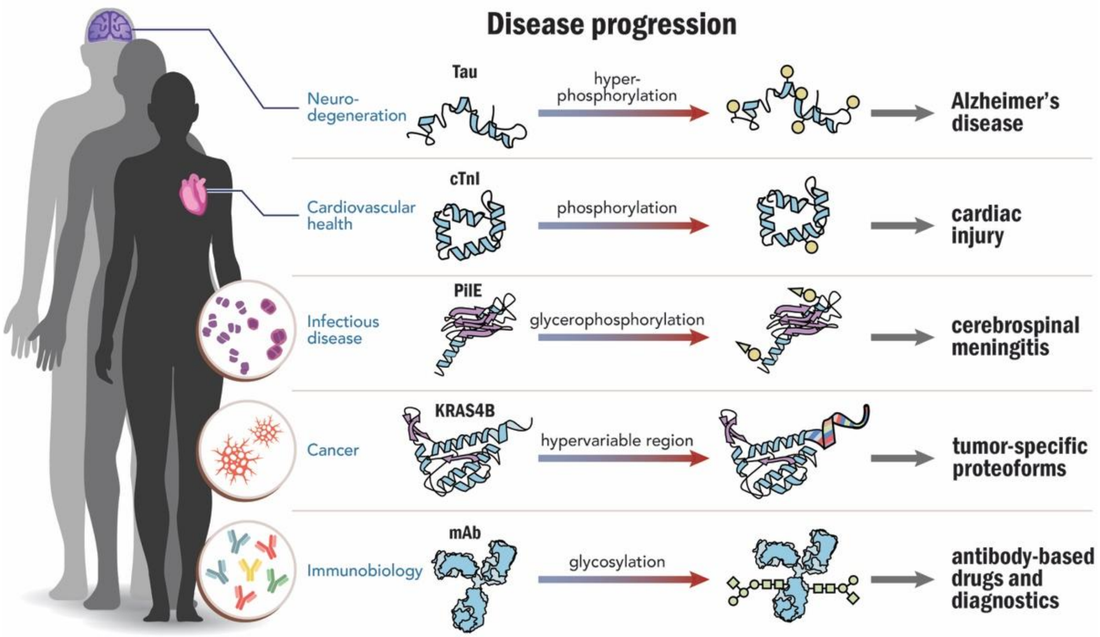
(C) Isobaric Chemical Labeling

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

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

CTDP
Consortium for Top-Down Proteomics

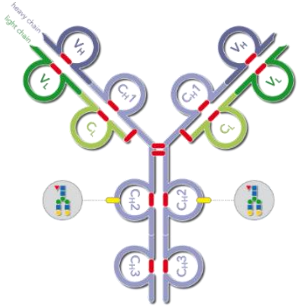
Five important disease areas are depicted where proteoforms have been identified and linked to the progression of human disease.

BioPharma Application: Monoclonal Antibodies & Beyond

Introduction
Instrumentation
Sample Prep
Separations
Software
Quantitative
Clinical
BioPharma

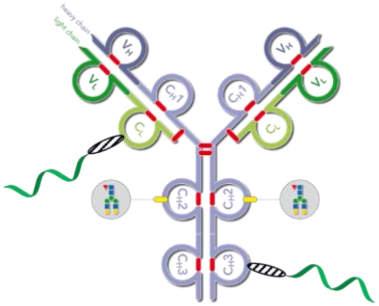
- mAbs and ADCs are exceptional biotherapeutics, particularly anti-cancer
- crucial elements in many biotechnological processes (binding properties)
- mAb properties: ~150 kDa size, (any) quantity, purification/isolation is possible

mAb



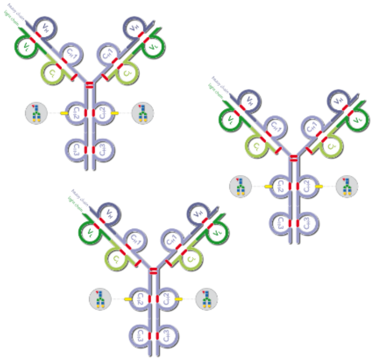
single-specific
multi-specific
glycoengineered

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

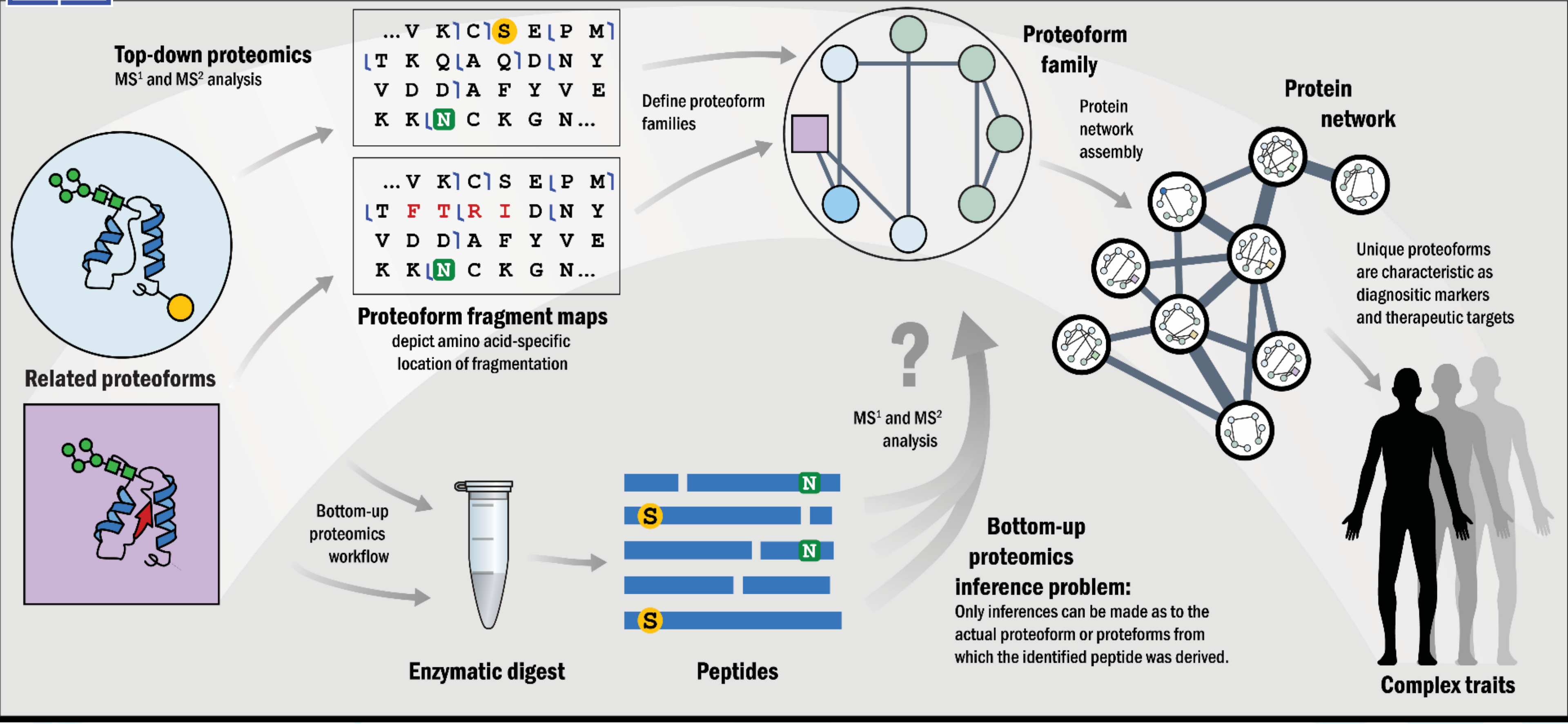
Lecture 9: Neil Kelleher

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
diagnositc markers
and therapeutic targets

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 proteforms from which the identified peptide was derived.

Complex traits

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

Consortium for Top-down Proteomics

Paul Danis
(CEO)

Neil Kelleher
(President)

Lloyd Smith
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Ying Ge
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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/