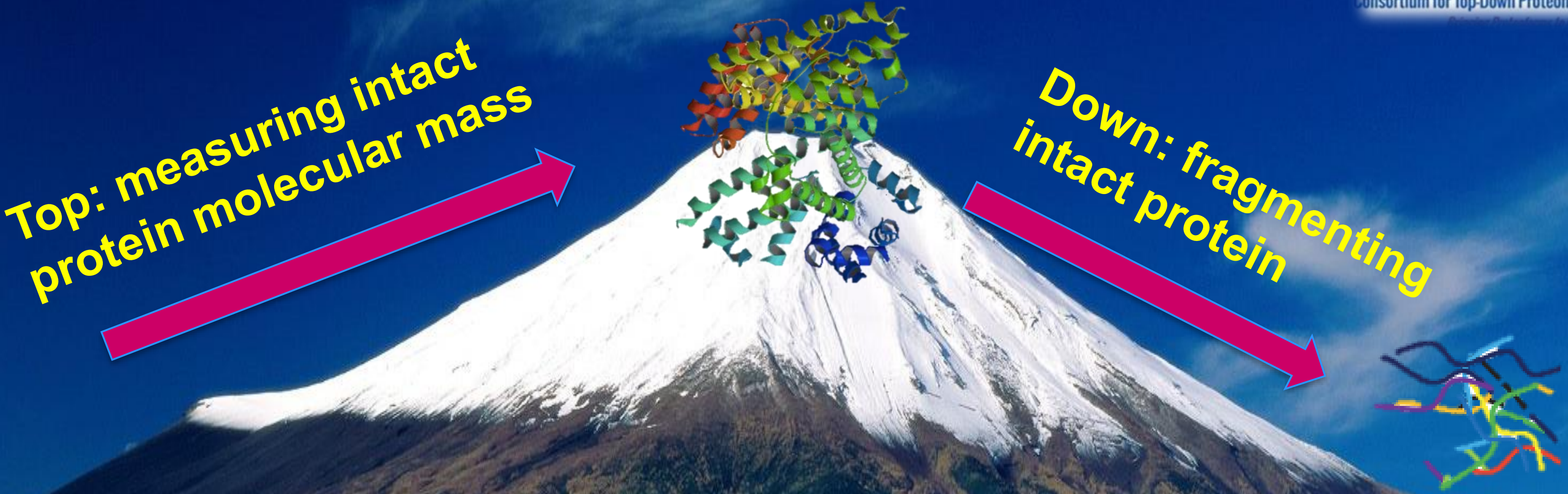
ASMS Short Course

Top-Down Proteomics

Instructors: Ying Ge, Neil Kelleher, Joseph Loo, Ljiljana Paša-Tolić

CTDP Consortium for Top-Down Proteomics



"Front-end"
Separation of Intact Proteins

\+

"Middle-part"
Mass Spectrometry Analysis of intact Proteins

\+

"Back-end"
Informatics for Identification and Characterization of Intact proteins

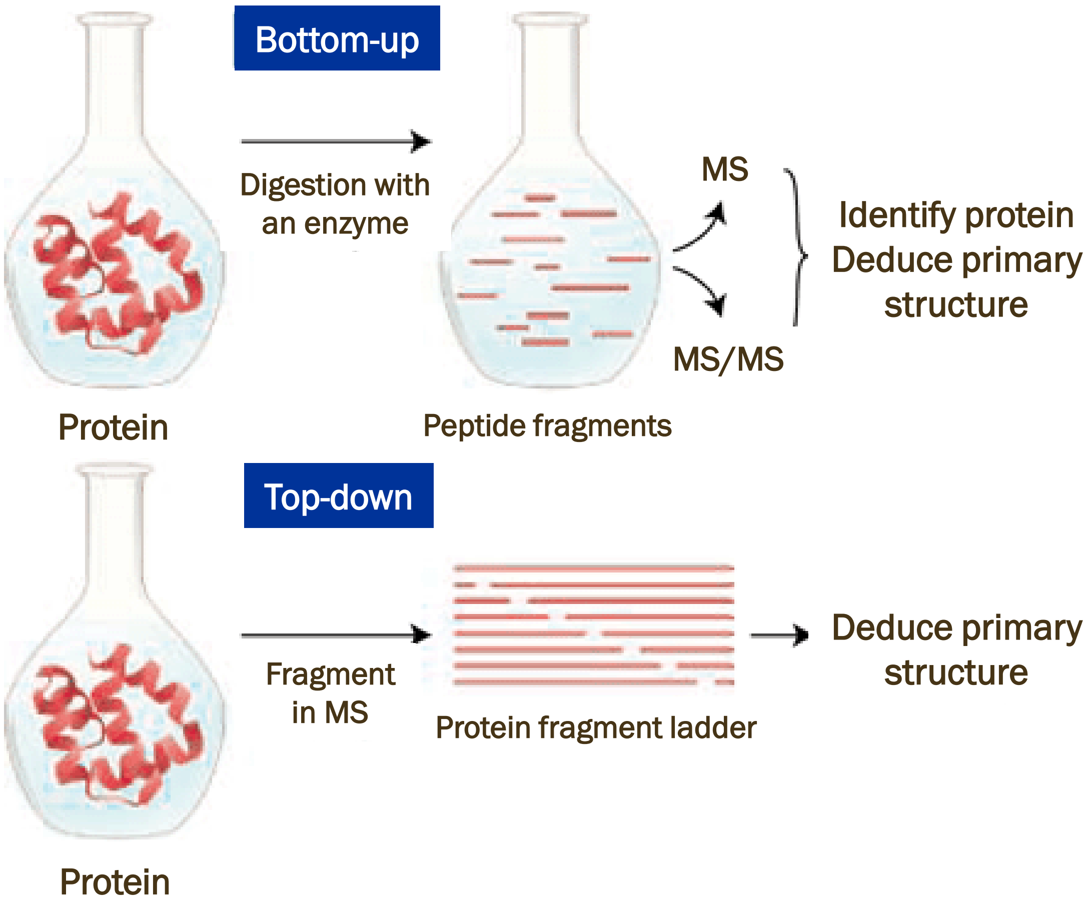
Topics to Be Covered

- Introduction
    - History of top-down proteomics
    - Concept of proteoforms
    - Measurement of Proteoforms and their complexes
    - Denatured vs. native mode
- Sample preparation
- Intact protein separation
- Instrumentation, activation and dissociation methods for top-down MS
- Comparison of bottom-up and top-down – pros and cons
- Data interpretation and software tools for top-down proteomics
- Top-down quantitative proteomics – including experimental design
- Biomedical and biopharmaceutical applications of top-down MS
- Future outlook

SCIENCE VOL 314 6 OCTOBER 2006

Mass Spectrometry: Bottom-Up or Top-Down?

Brian T. Chait

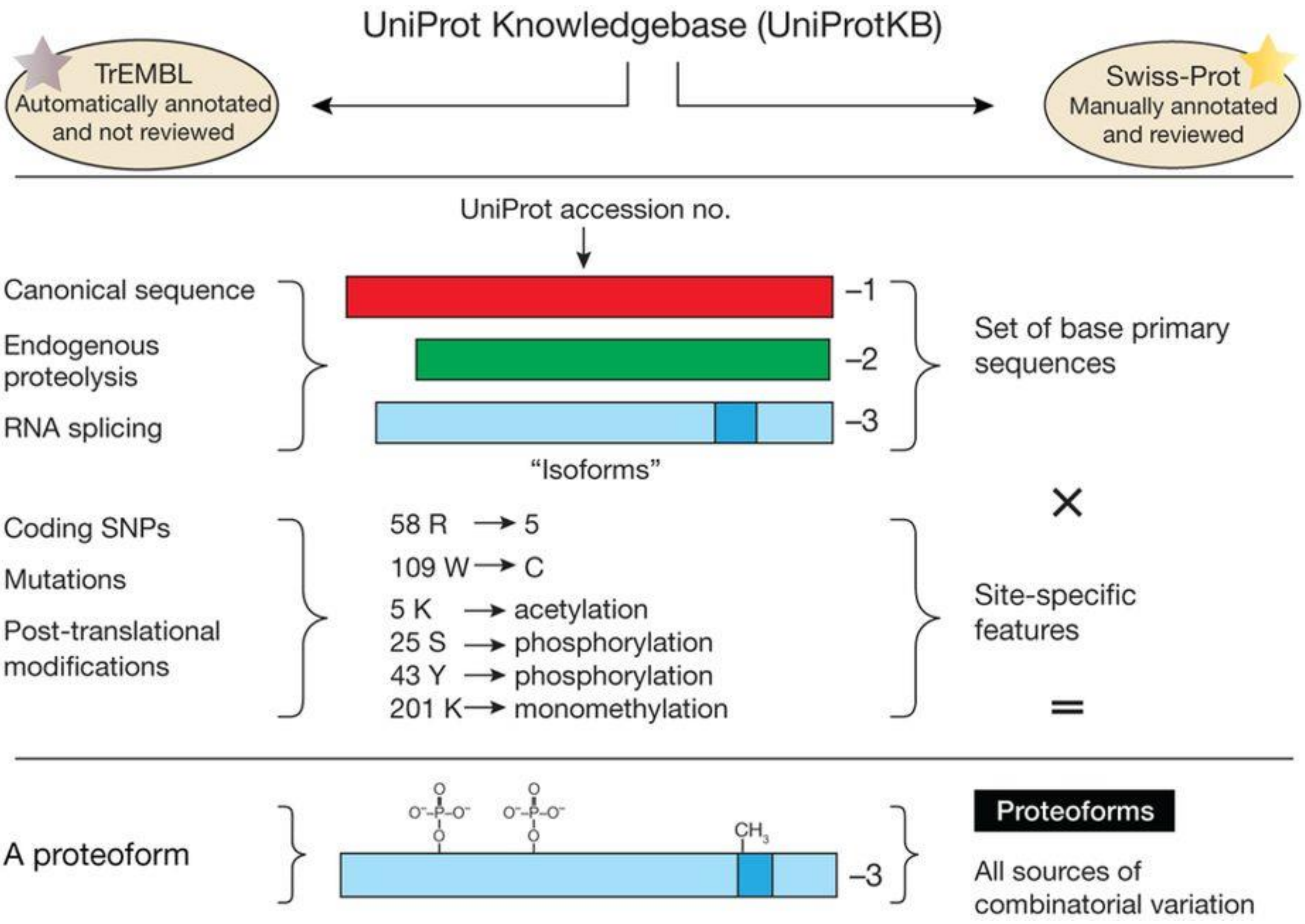


The bottom-up approach is (therefore) *suboptimal for determining modifications* and alternative splice variants.

In the top-down approach…if a sufficient number of informative fragment ions are observed, this analysis *can provide a complete description of the primary structure of the protein and reveal all of its modifications*, as well as any correlations that exist between these modifications.

Concept of Proteoforms

Proteoform: a single term describing protein complexity



UniProt Knowledgebase (UniProtKB)

TrEMBL
Automatically annotated
and not reviewed

Swiss-Prot
Manually annotated
and reviewed

UniProt accession no.

Canonical sequence
Endogenous proteolysis
RNA splicing

–1
–2
–3

"Isoforms"

Set of base primary sequences

×

Coding SNPs
Mutations
Post-translational modifications

58 R → 5
109 W → C
5 K → acetylation
25 S → phosphorylation
43 Y → phosphorylation
201 K → monomethylation

Site-specific features

=

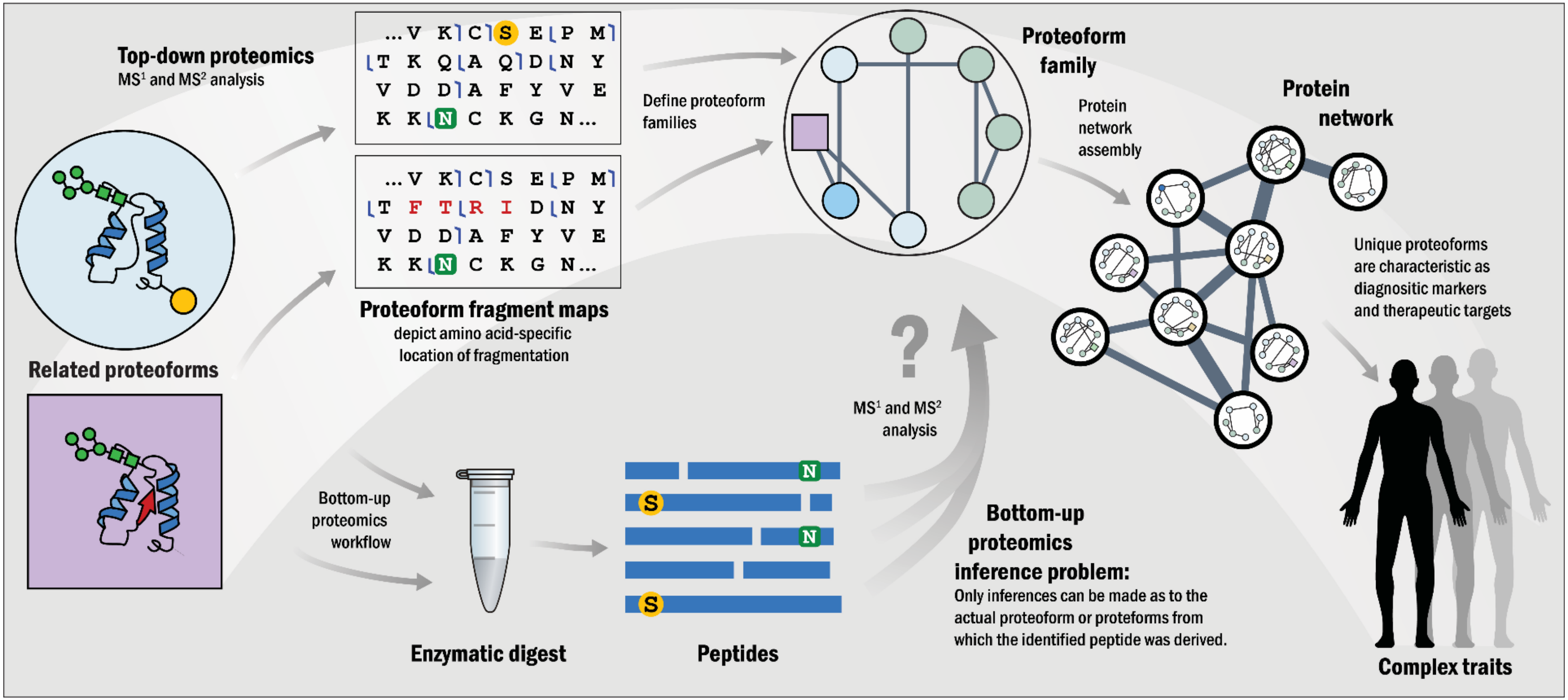
A proteoform

Proteoforms

All sources of combinatorial variation

Smith, Kelleher & Consortium for Top-Down Proteomics. *Nat Methods* 2013, *10* (3), 186-7.

Proteoforms as a New “Currency” in Proteomics



Top-down proteomics
MS¹ and MS² 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

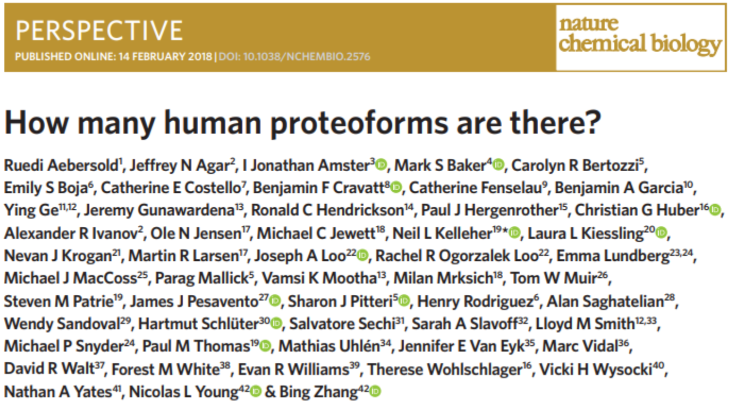
Enzymatic digest

Peptides

MS¹ and MS²
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.

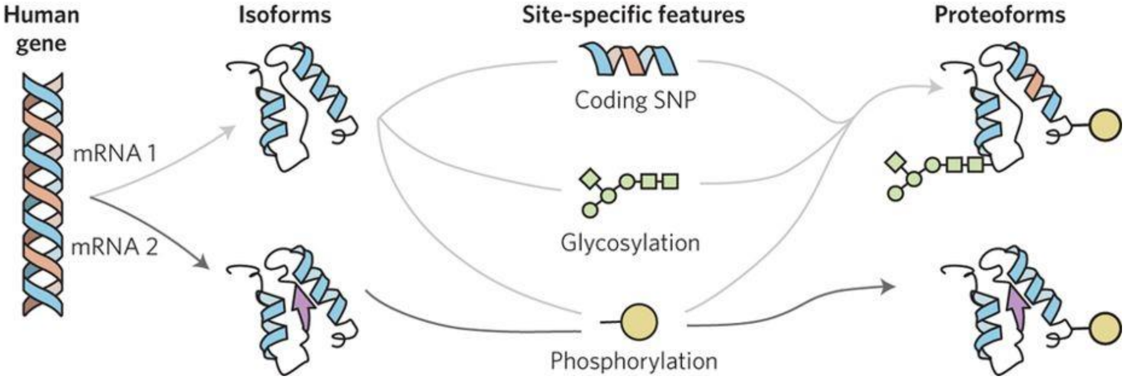
Smith and Kelleher, Science 2018, *359* (6380), 1106-1107.



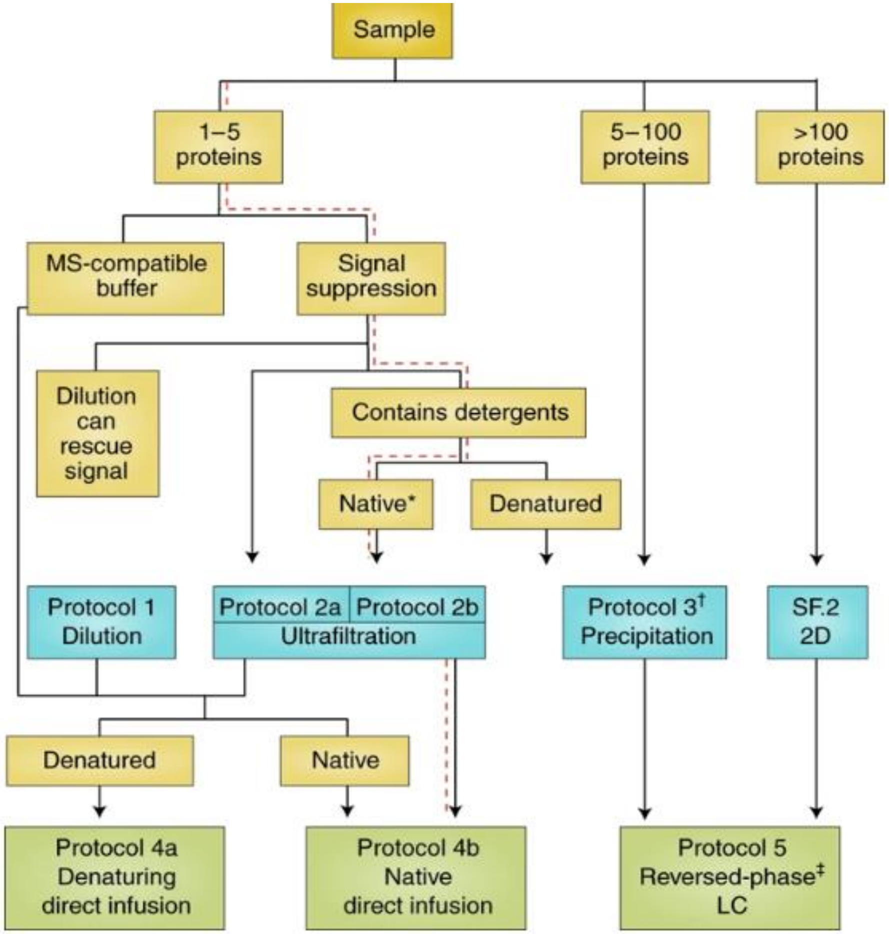
PERSPECTIVE
nature chemical biology
PUBLISHED ONLINE: 14 FEBRUARY 2018 | DOI: 10.1038/NCHEMBIO.2576

How many human proteoforms are there?

Ruedi Aebersold[1], Jeffrey N Agar[2], I Jonathan Amster[3], Mark S Baker[4], Carolyn R Bertozzi[5], Emily S Boja[6], Catherine E Costello[7], Benjamin F Cravatt[8], Catherine Fenselau[9], Benjamin A Garcia[10], Ying Ge[11,12], Jeremy Gunawardena[13], Ronald C Hendrickson[14], Paul J Hergenrother[15], Christian G Huber[16], Alexander R Ivanov[2], Ole N Jensen[17], Michael C Jewett[18], Neil L Kelleher[19]*, Laura L Kiessling[20], Nevan J Krogan[21], Martin R Larsen[17], Joseph A Loo[22], Rachel R Ogorzalek Loo[22], Emma Lundberg[23,24], Michael J MacCoss[25], Parag Mallick[5], Vamsi K Mootha[13], Milan Mrksich[18], Tom W Muir[26], Steven M Patrie[19], James J Pesavento[27], Sharon J Pitteri[5], Henry Rodriguez[6], Alan Saghatelian[28], Wendy Sandoval[29], Hartmut Schlüter[30], Salvatore Sechi[31], Sarah A Slavoff[32], Lloyd M Smith[12,33], Michael P Snyder[24], Paul M Thomas[19], Mathias Uhlén[34], Jennifer E Van Eyk[35], Marc Vidal[36], David R Walt[37], Forest M White[38], Evan R Williams[39], Therese Wohlschlager[16], Vicki H Wysocki[40], Nathan A Yates[41], Nicolas L Young[42] & Bing Zhang[42]



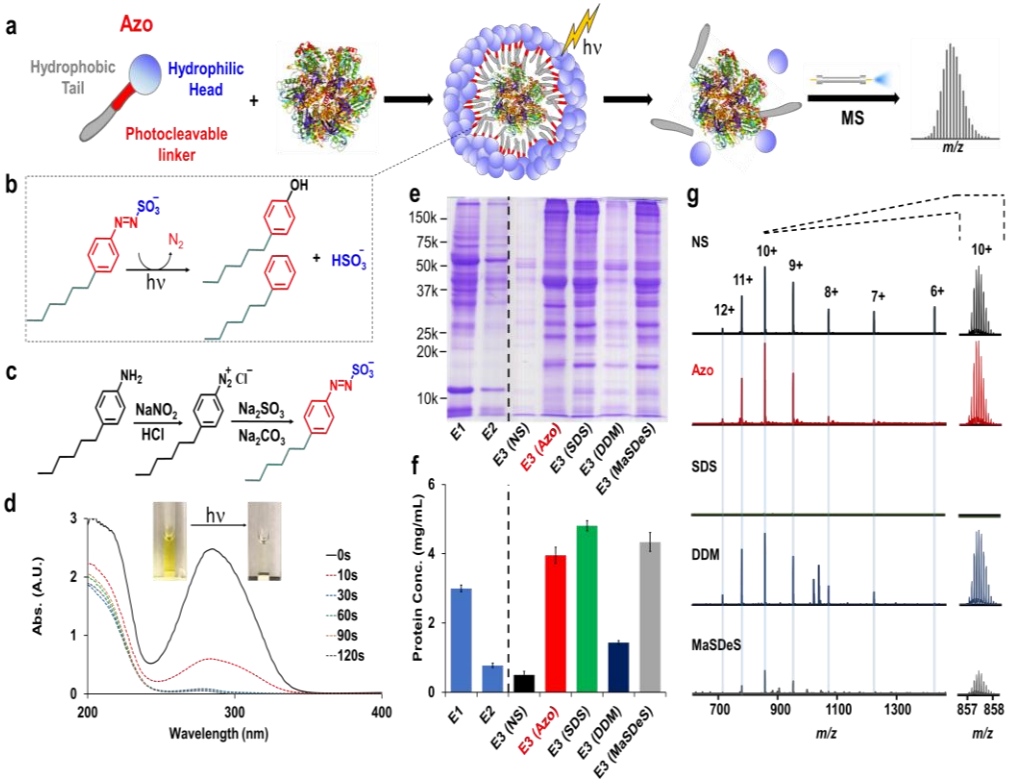
Sample Preparation in Top-down Proteomics



Best Practices and Benchmarks for Intact protein Analysis for Top-down Mass Spectrometry

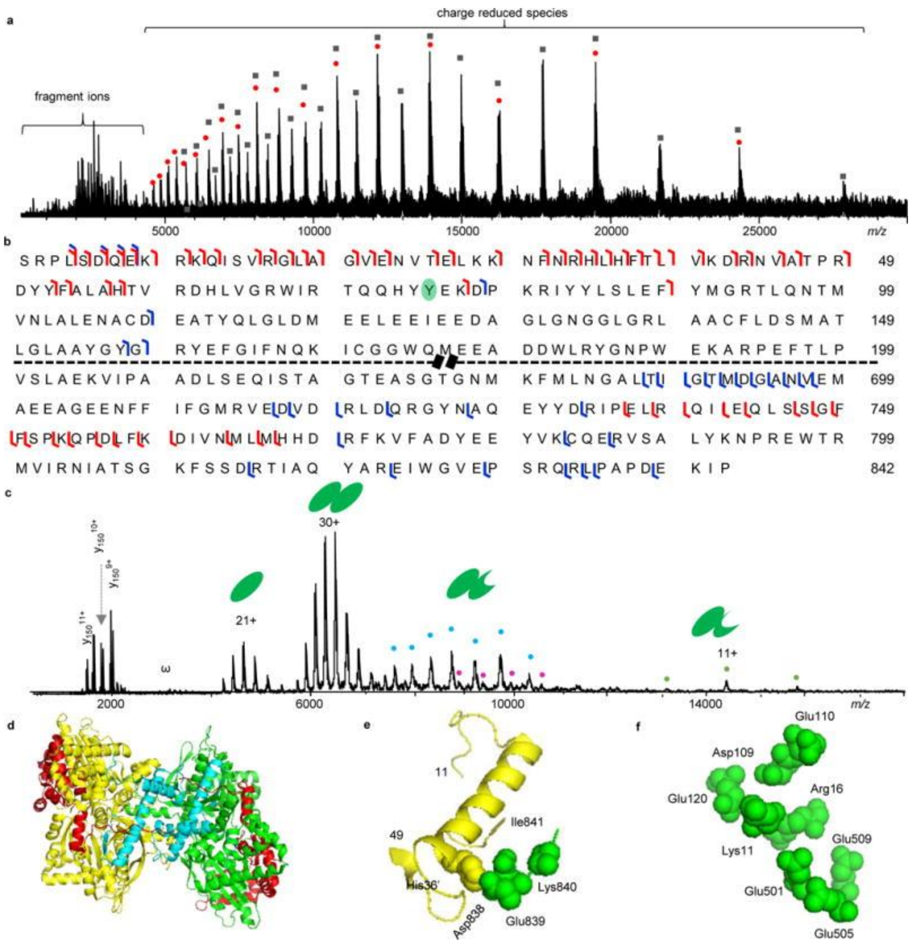
Donnelly et al. *Nature Methods.* 2019, 16, 587-594

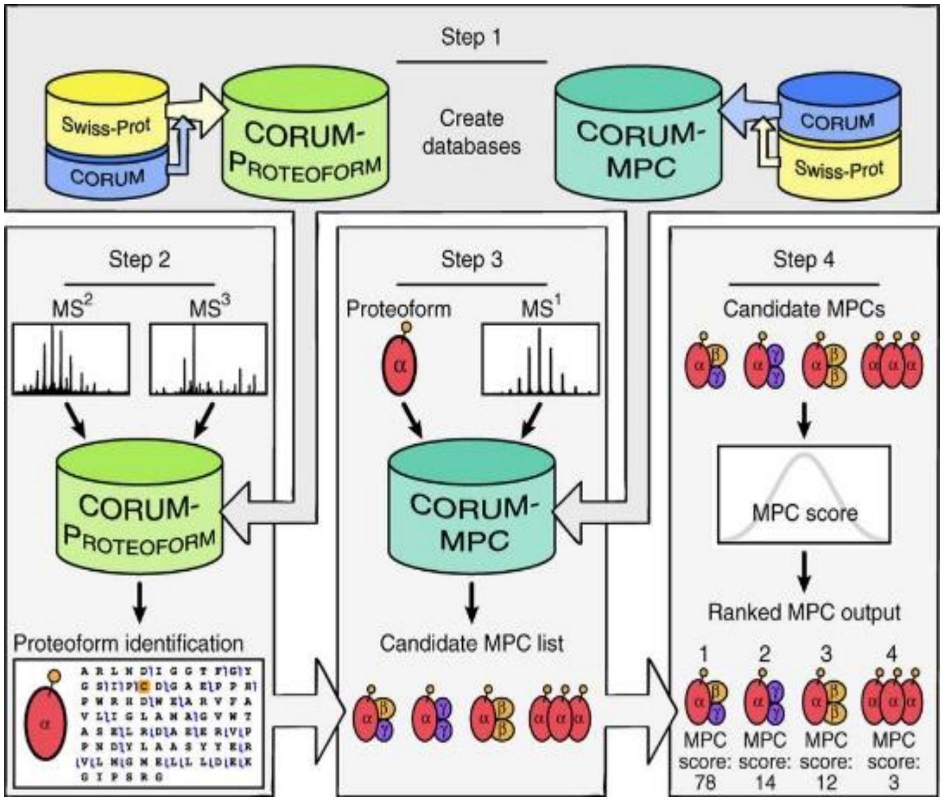
A Photo-cleavable Surfactant for Top-down Proteomics



Brown et al. *Nature Methods* 2019, 16, 417-420

Native Top-down Proteomics





Skinner et al. Nat. Methods 2016, 13, 237

Li et al. Nature Chemistry, 2018, 10, 139

Integrated Native Mass Spectrometry and Top-Down Proteomics
-Connect Sequence to Structure and Function of Macromolecular Complexes

Software Tools Available for Top-down Proteomics

ProSight PTM https://prosightptm2.northwestern.edu/

ProSightPC™ https://www.thermofisher.com/order/catalog/product/PROSIGHTPC10

Mash Suite Pro: http://crb.wisc.edu/yinglab/software.html

MASH Explorer: http://ge.crb.wisc.edu/MASH_Explorer/index.htm

MS-Align+: http://bix.ucsd.edu/projects/msalign/

TopPIC: http://proteomics.informatics.iupui.edu/software/toppic

MSPathFinder: https://omics.pnl.gov/software/mspathfinder

Informed Proteomics: https://github.com/PNNL-Comp-Mass-Spec/Informed-Proteomics

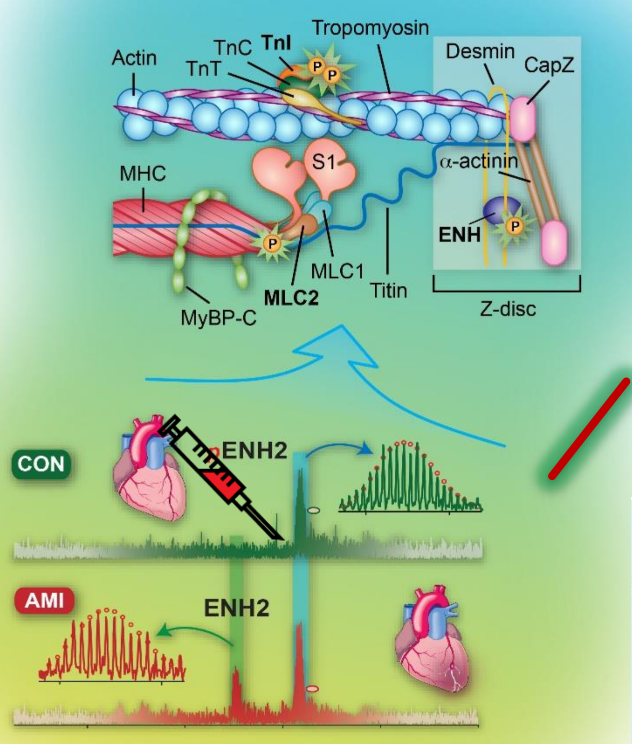
Proteoform Suite: https://github.com/smith-chem-wisc/Proteof

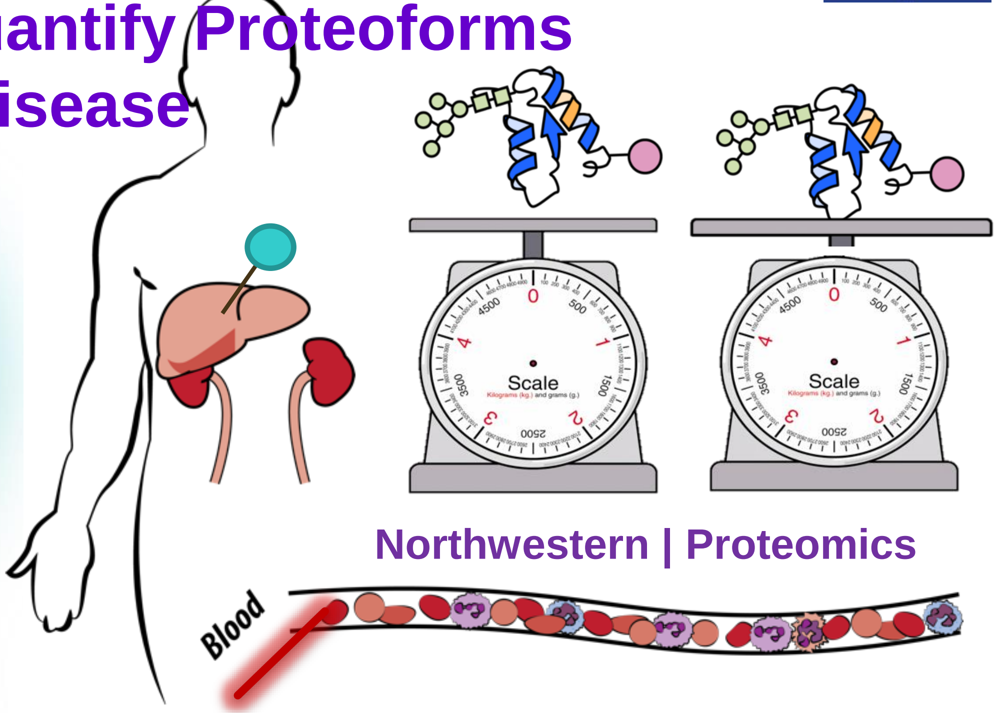
pTop: http://pfind.net/software/pTop/index.html

http://www.topdownproteomics.org/resources/software/

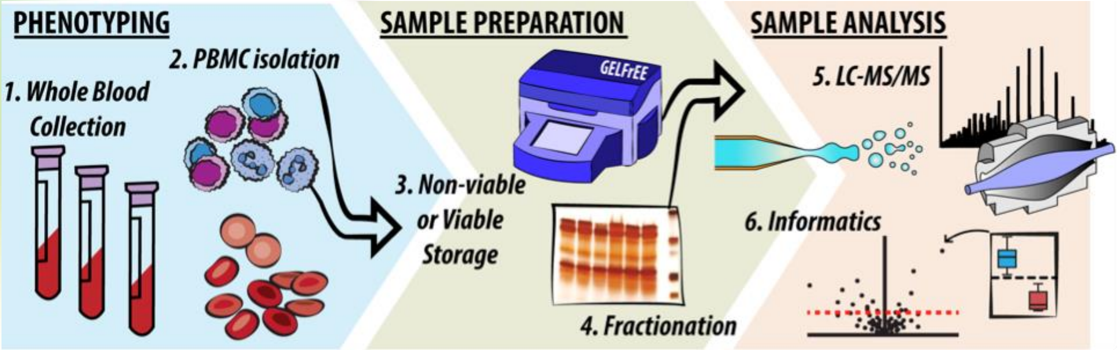
Biomedical and Biopharmaceutical Applications

Discover, Characterize & Quantify Proteoforms In Health and Disease





Northwestern | Proteomics



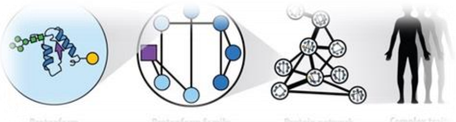
Toby, *et al.*, *Am. J. Transplant*, 2017, 17(9) p. 2458-67.

Peng, et al. Mol. Cell. Proteomics, 2014, 13, 2752-2764.

CTDP
Consortium for Top-Down Proteomics

Conclusions & Outlook

- Measuring proteoforms directly: a major step in the evolution of mass spectrometry-based proteomics



- Top-down proteomics closes knowledge gaps by providing complete molecular specificity for proteins in wellness and disease
- Proteoform-resolved biology will increase efficiency of basic and translational research

It takes a village!