Proteomics & Protein-Protein Interactions

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Biomedical Data Science: Mining & Modeling

CBB 752, Spring 2022

Cellular & Molecular Physiology
Yale University School of Medicine

DNA → RNA → PROTEIN

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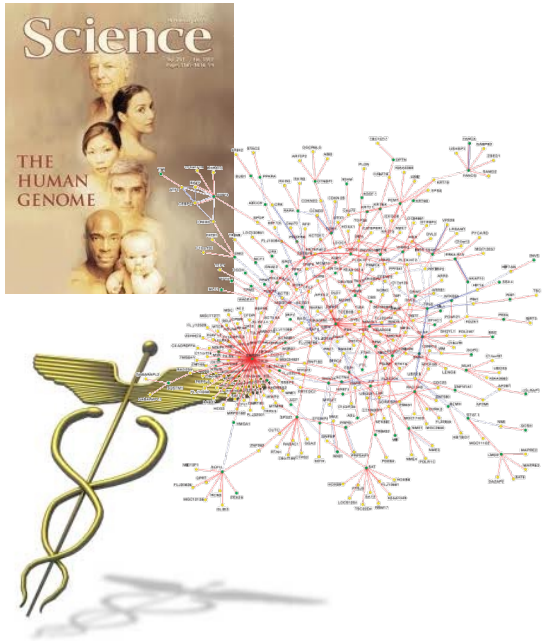
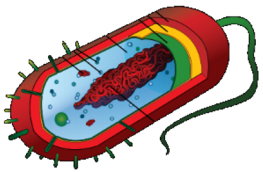
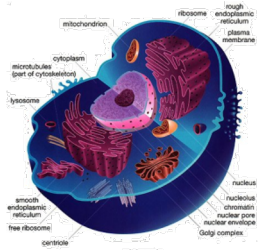
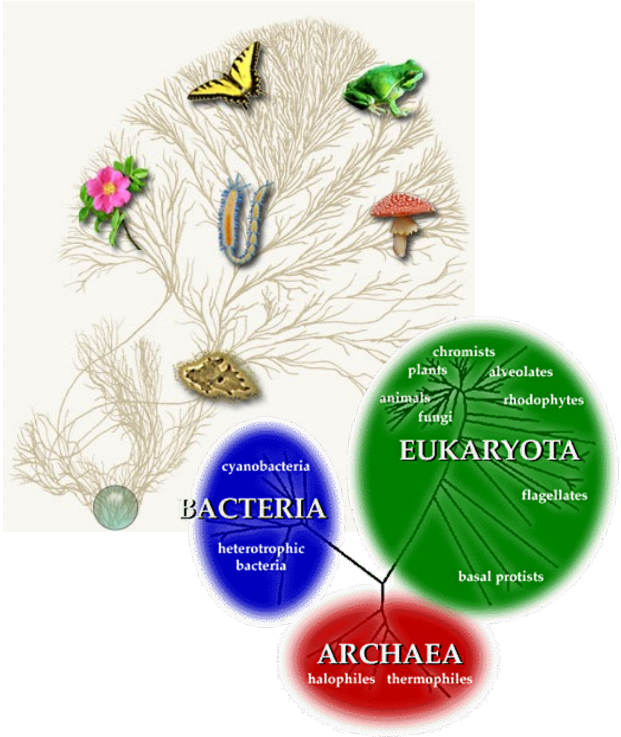
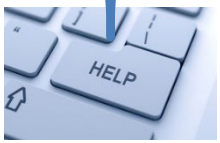


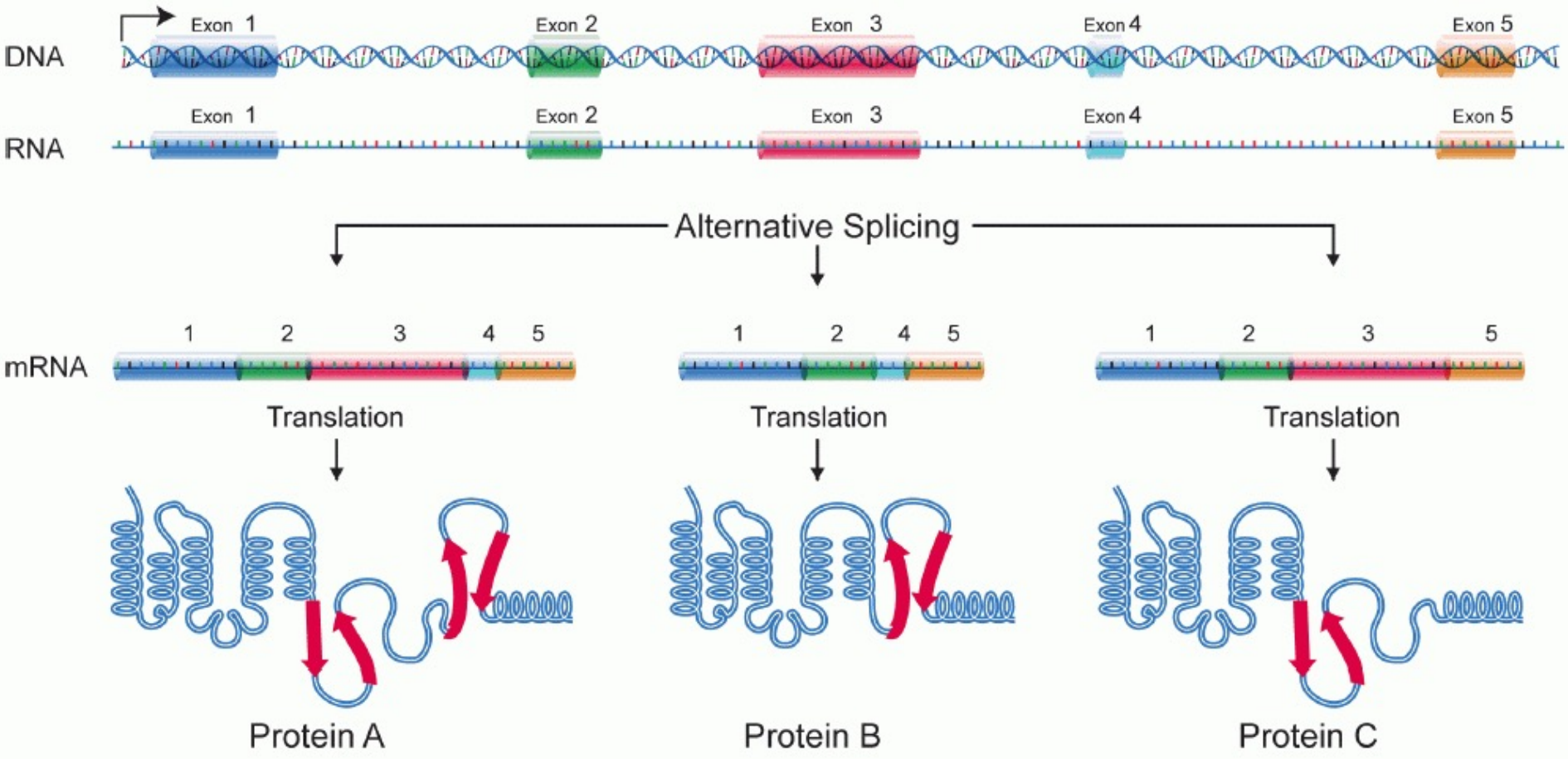






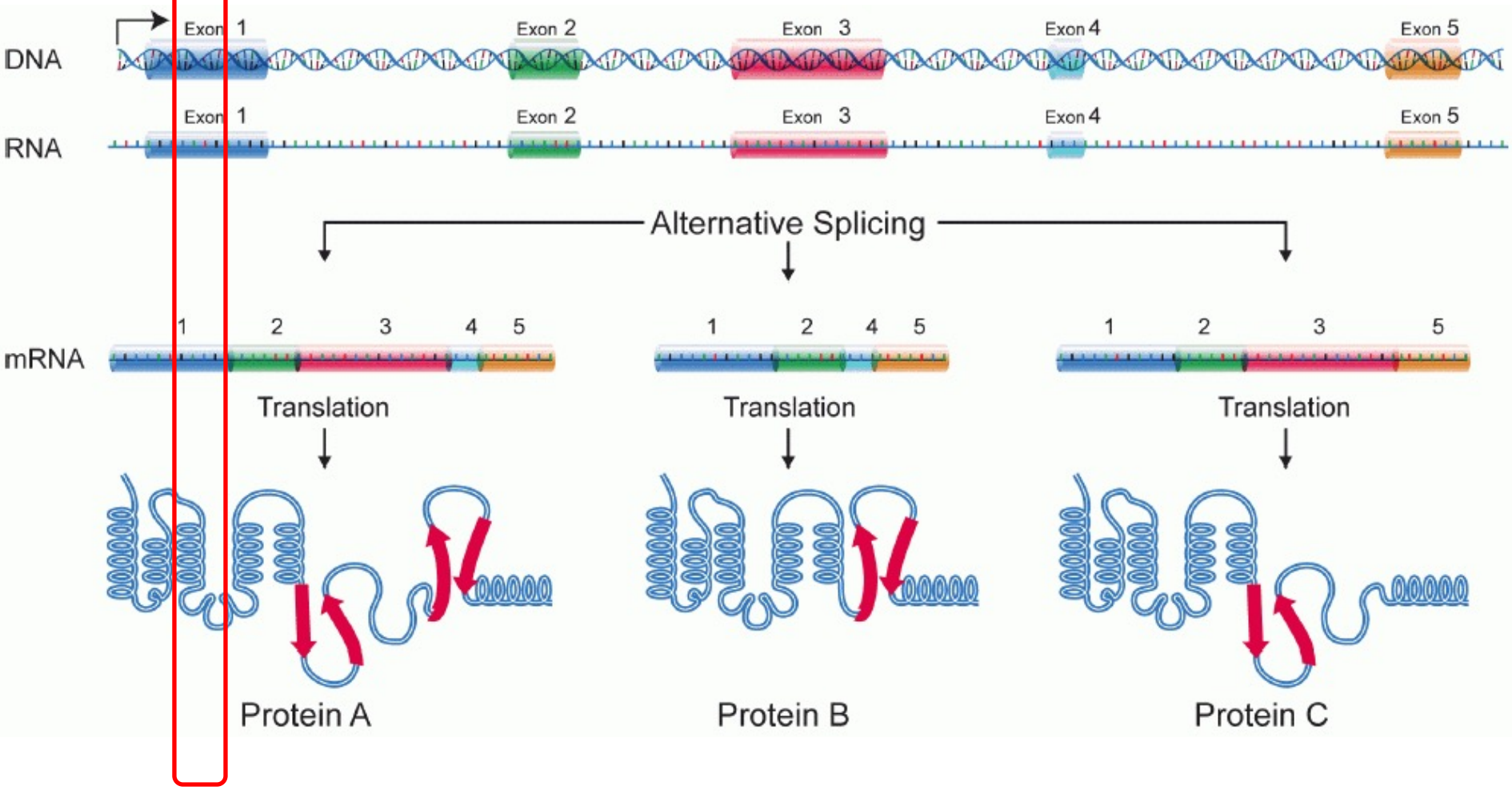


DNA → RNA → PROTEIN



https://en.wikipedia.org/wiki/Alternative_splicing

Data capture here



https://en.wikipedia.org/wiki/Alternative_splicing

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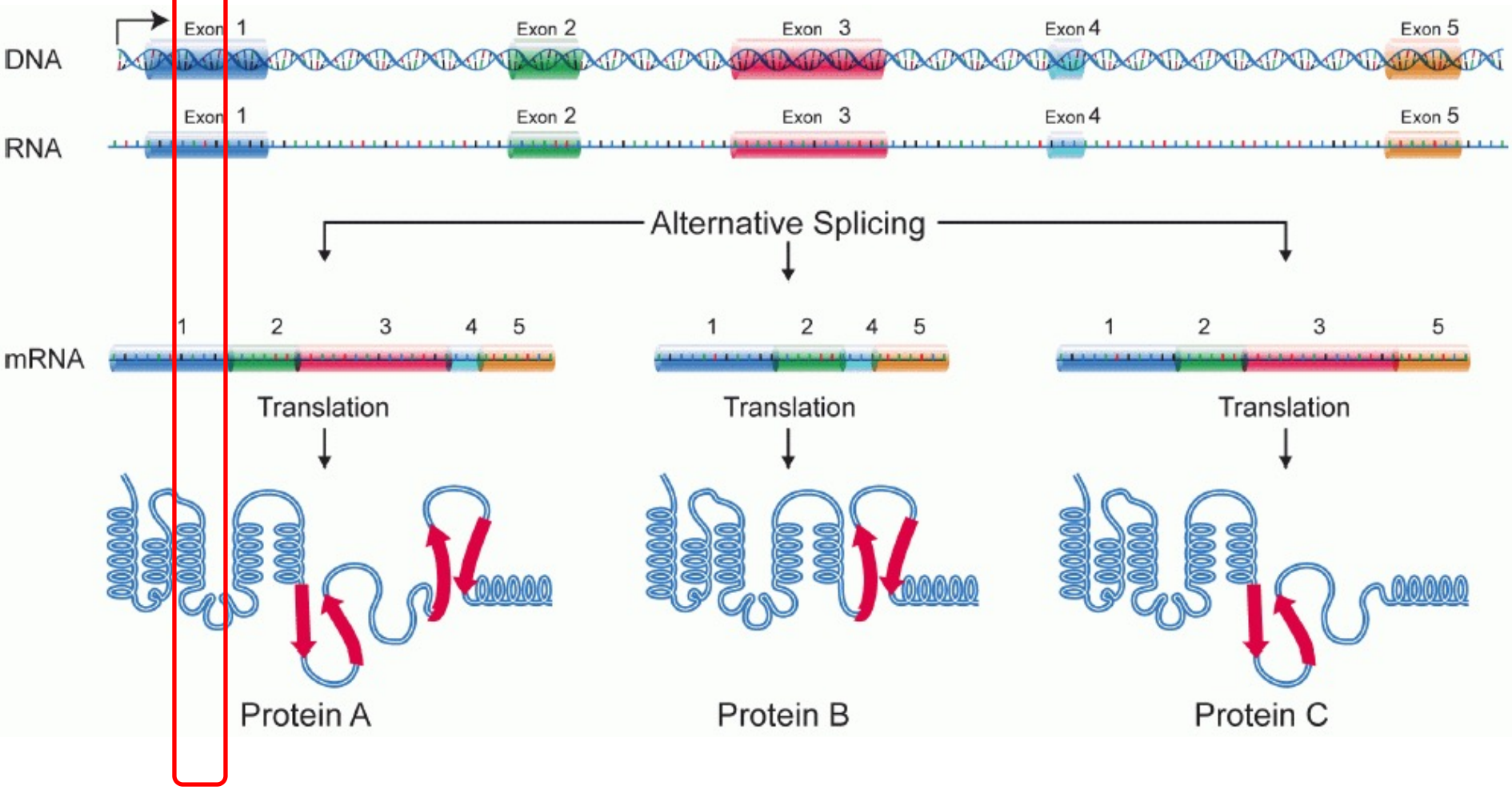
Missing information



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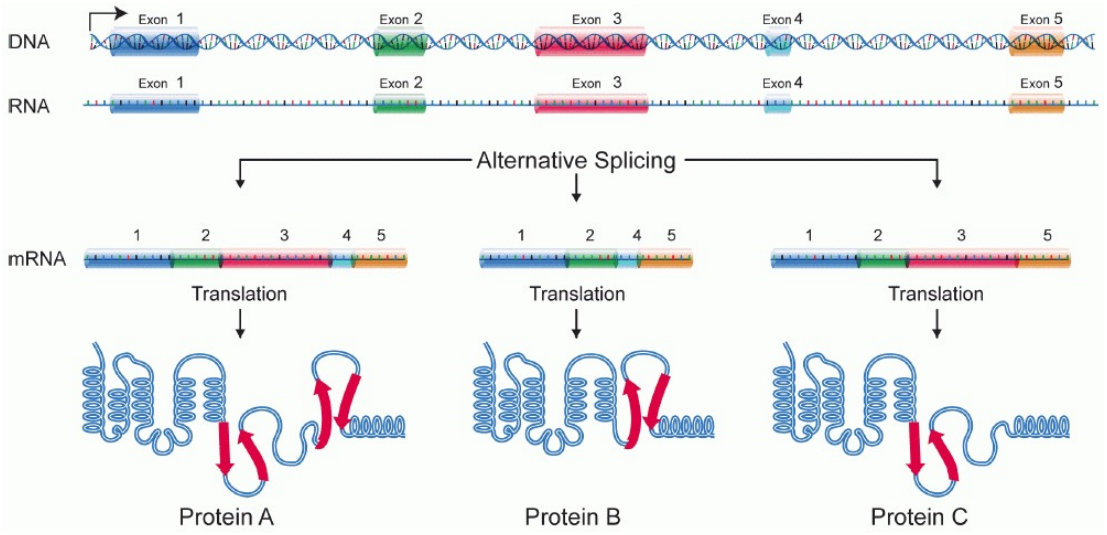


Solutions are coming that will completely change databases

- **Multiple fields developing methods and technology**
- **Extend read length**
- **Measure intact molecules**
- **Push towards single molecule measurements**

Major challenges prevent complete proteome analysis

- **Proteomics is sample limited**
  - Recombinant DNA polymerases revolutionized genome sequencing by allowing for amplification of DNA samples
  - Proteomics has no “polymerase” or amplification method and must contend with natural abundancies
- **Mass spectrometry has limitations**
  - No mass spectrometer, or method, can yet provide full amino-acid resolution of a proteome

Proteomics

The study of the expression, location, modification, interaction, function, and structure of all the proteins in a given cell, organelle, tissue, organ, or whole organism.

Proteomics & Protein-Protein Interactions

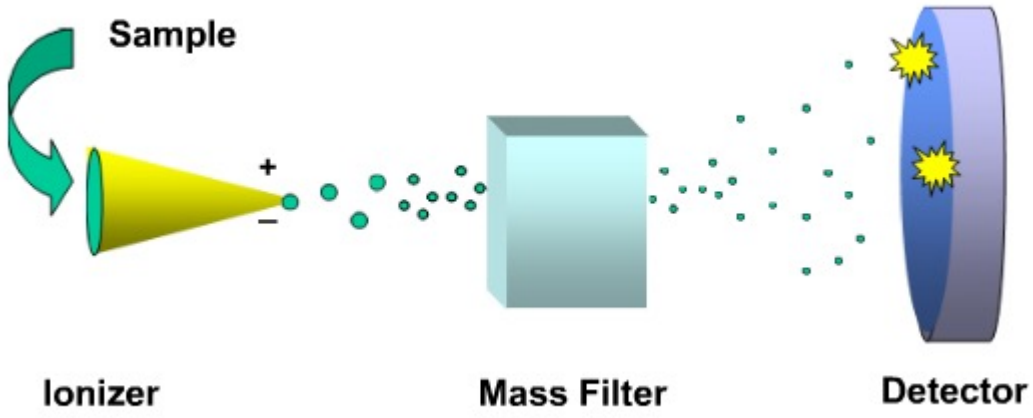
Overview

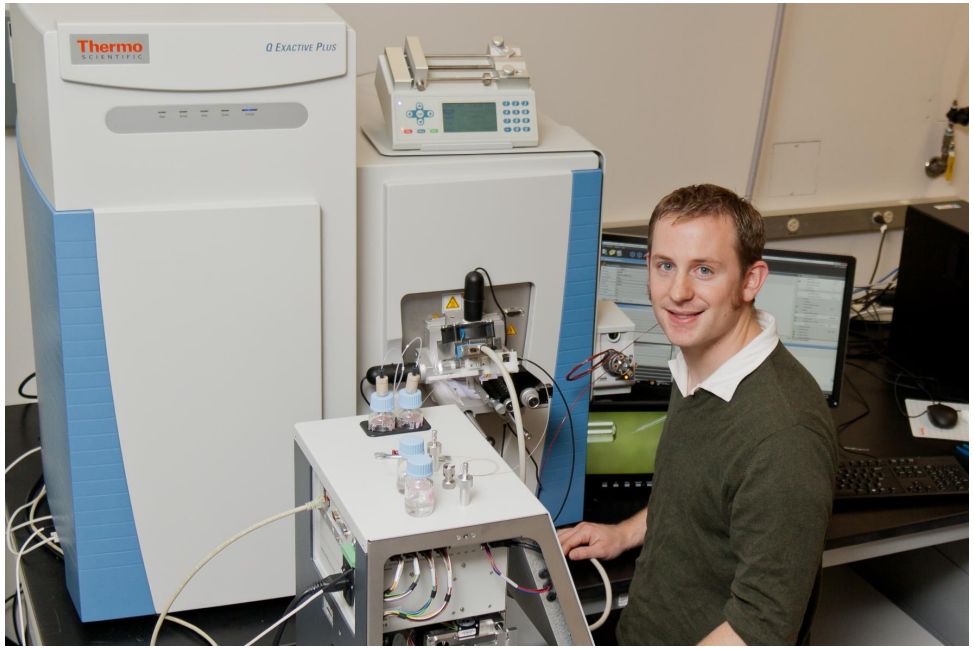
- **Techniques & Technologies**
  - Mass Spectrometry
  - Protein-Protein Interactions
  - Quantitative Proteomics
- **Applications**
  - Representative Studies
- **Putting it all together….**
  - Databases & Pathways

Principles of Mass Spectrometry (MS)

- In a mass spectrum we measure m/z (mass-to-charge)
- For proteins we measure peptide m/z
- A sample must be ionizable in order to be analyzed

Basic Components of a Mass Spectrometer





Two major ionization techniques enabled the success of mass spectrometry in the life sciences.

- Electrospray Ionization (ESI)
  Fenn JB, *Mann M, Meng CK, Wong SF, Whitehouse CM. *Science*. 1989

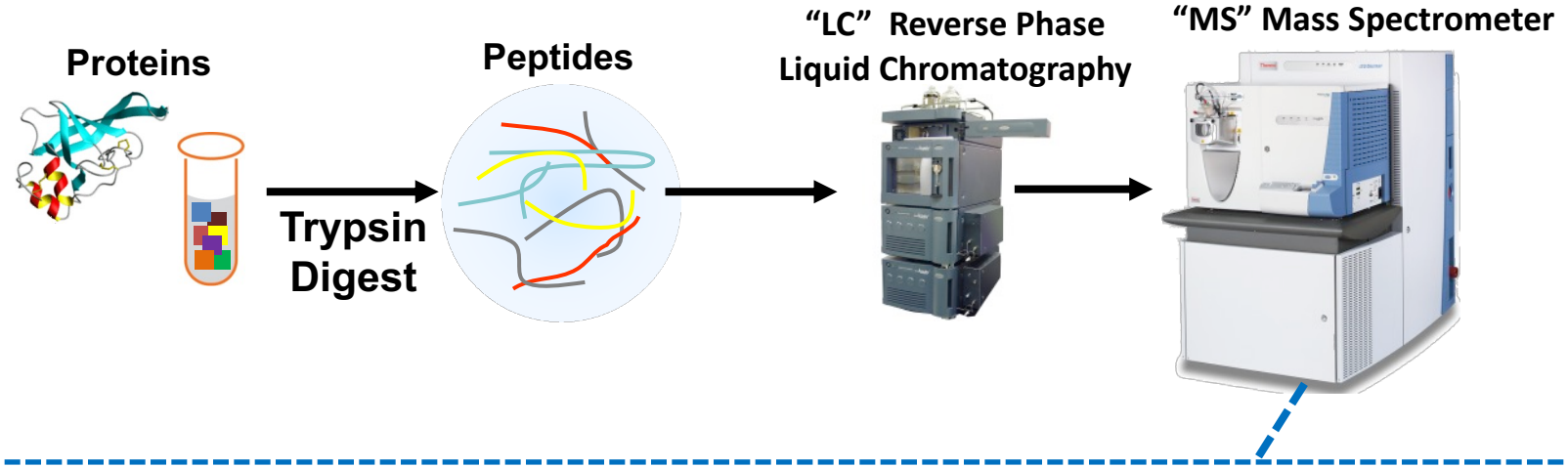
- Matrix Assisted Laser Desorption Ionization (MALDI)
  Tanaka K, Waki H, Ido Y, et al. *Rapid Commun Mass Spectrom* 1988

- 2002 Nobel Prize in Chemistry awarded to
  John B. Fenn & Koichi Tanaka

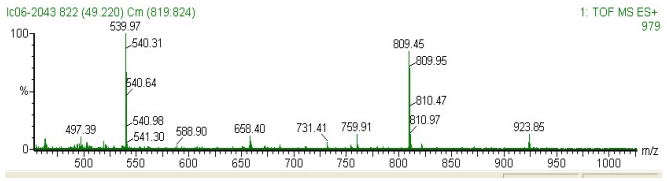
- **Enabled direct measurement and "sequencing" of intact peptides & MS based Proteomics is born**

* Matthias Mann (Yale University; Ph.D.; 1988; Chemical Engineering) trained with John Fenn during some of the breakthrough work at Yale

Typical work-flow for LC-MS "shotgun proteomics"



MS



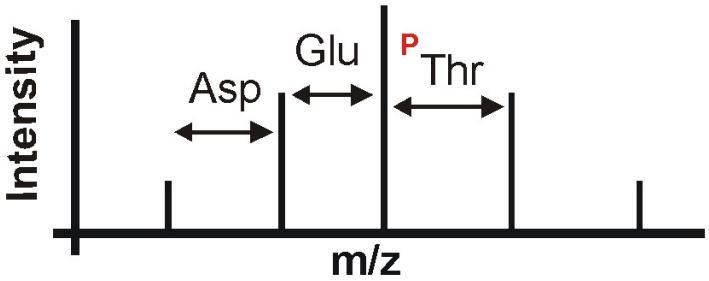
peptide

peptide

isolate & fragment

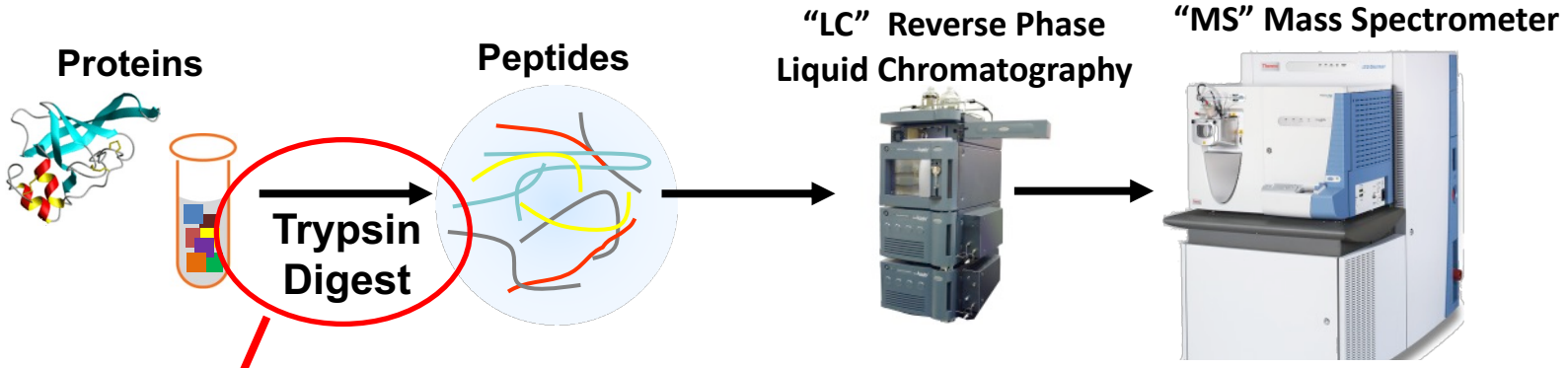
peptide

MS/MS

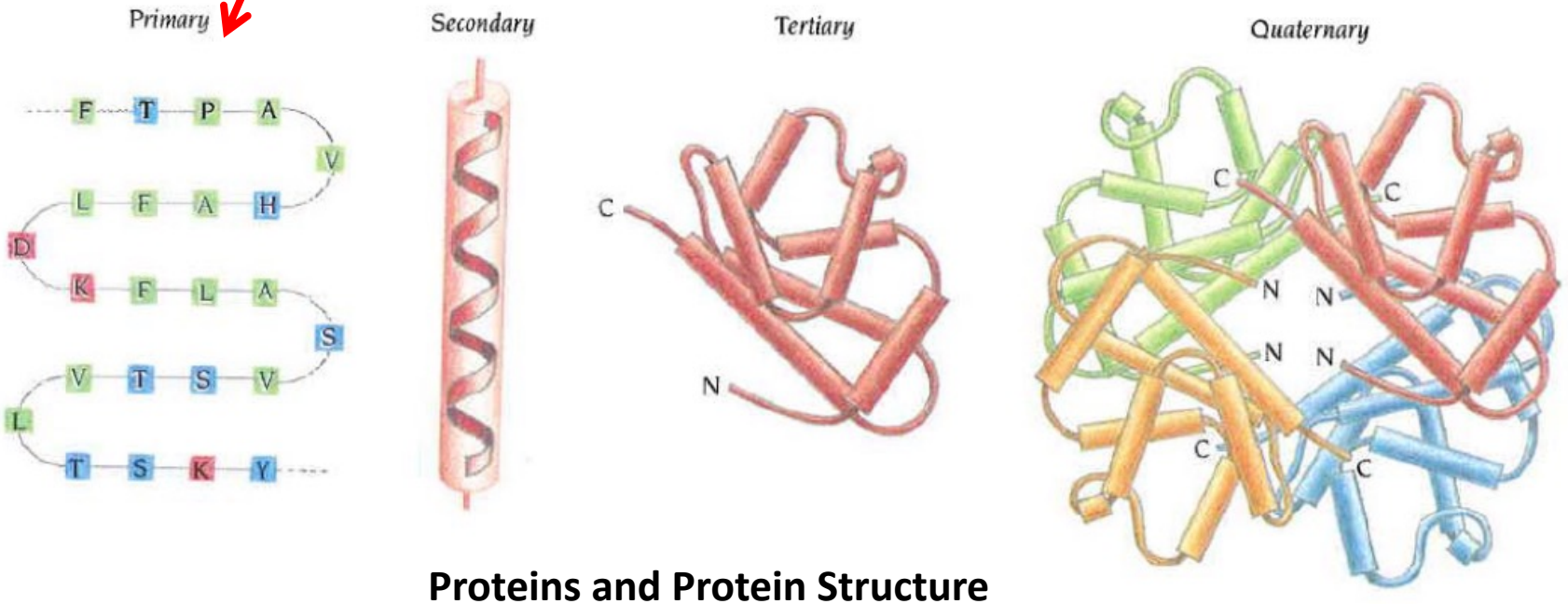


peptide fragments

Typical work-flow for LC-MS "shotgun proteomics"



Trypsin cuts after Lys (K) & Arg (R)



Proteins and Protein Structure

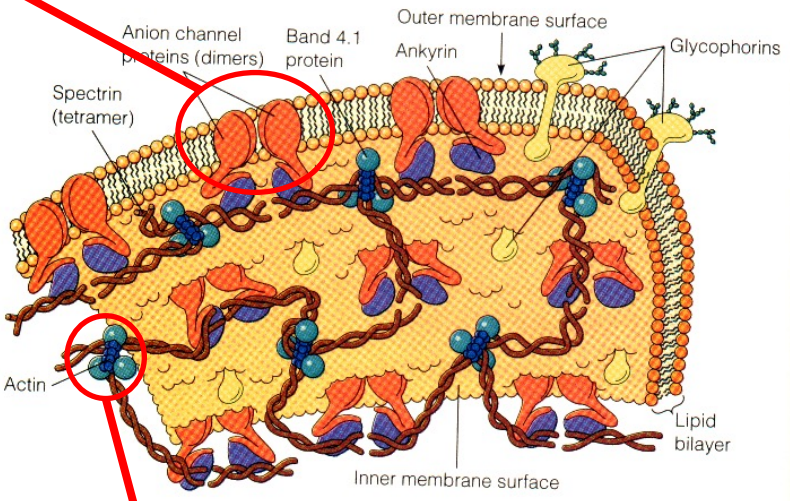
(Branden, C. and Tooze, J. ***Introduction to Protein Structure***)

Trypsin digest followed by LC-MS: Examples of "***Sequence Coverage***"

Band 3 Anion Transporter

Matched peptides shown in **Bold Red**

```
   1 MEELQDDYED MMEENLEQEE YEDPDIPESQ MEEPAAHDTE ATATDYHTTS
  51 HPGTHKVYVE LQELVMDEKN QELRWMEAAR WVQLEENLGE NGAWGRPHLS
 101 HLTFWSLLEL RRVFTKGTVL LDLQETSLAG VANQLLDRFI FEDQIRPQDR
 151 EELLRALLLK HSHAGELEAL GGVKPAVLTR SGDPSQPLLP QHSSLETQLF
 201 CEQGDGGTEG HSPSGILEKI PPDSEATLVL VGRADFLEQP VLGFVRLQEA
 251 AELEAVELPV PIRFLFVLLG PEAPHIDYTQ LGRAAATLMS ERVFRIDAYM
 301 AQSRGELLHS LEGFLDCSLV LPPTDAPSEQ ALLSLVPVQR ELLRRRYQSS
 351 PAKPDSSFYK GLDLNGGPDD PLQQTGQLFG GLVRDIRRRY PYYLSDITDA
 401 FSPQVLAAVI FIYFAALSPA ITFGGLLGEK TRNQMGVSEL LISTAVQGIL
 451 FALLGAQPLL VVGFSGPLLV FEEAFFSFCE TNGLEYIVGR VWIGFWLILL
 501 VVLVVAFEGS FLVRFISRYT QEIFSFLISL IFIYETFSKL IKIFQDHPLQ
 551 KTYNYNVLMV PKPQGPLPNT ALLSLVLMAG TFFFAMMLRK FKNSSYFPGK
 601 LRRVIGDFGV PISILIMVLV DFFIQDTYTQ KLSVPDGFKV SNSSARGWVI
 651 HPLGLRSEFP IWMMFASALP ALLVFILIFL ESQITTLIVS KPERKMVKGS
 701 GFHLDLLLVV GMGGVAALFG MPWLSATTVR SVTHANALTV MGKASTPGAA
 751 AQIQEVKEQR ISGLLVAVLV GLSILMEPIL SRIPLAVLFG IFLYMGVTSL
 801 SGIQLFDRIL LLFKPPKYHP DVPYVKRVKT WRMHLFTGIQ IICLAVLWVV
 851 KSTPASLALP FVLILTVPLR RVLLPLIFRN VELQCLDADD AKATFDEEEG
 901 RDEYDEVAMP V
```

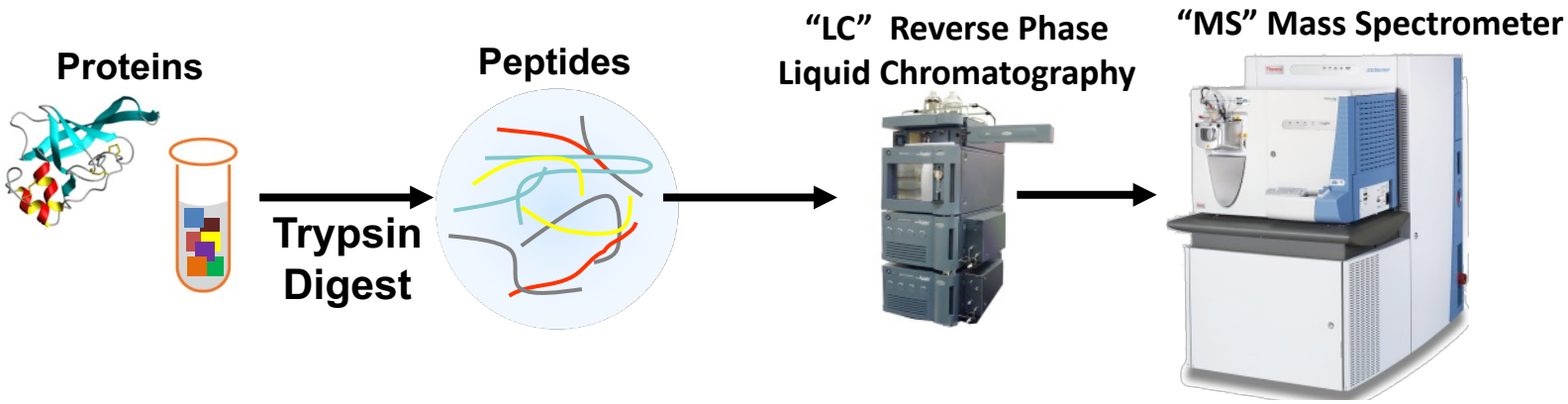


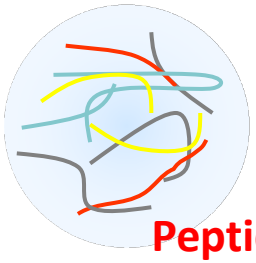
β-actin

Matched peptides shown in **Bold Red**

```
   1 MDDDIAALVV DNGSGMCKAG FAGDDAPRAV FPSIVGRPRH QGVMVGMGQK
  51 DSYVGDEAQS KRGILTLKYP IEHGIVTNWD DMEKIWHHTF YNELRVAPEE
 101 HPVLLTEAPL NPKANREKMT QIMFETFNTP AMYVAIQAVL SLYASGRTTG
 151 IVMDSGDGVT HTVPIYEGYA LPHAILRLDL AGRDLTDYLM KILTERGYSF
 201 TTTAEREIVR DIKEKLCYVA LDFEQEMATA ASSSSLEKSY ELPDGQVITI
 251 GNERFRCPEA LFQPSFLGME SCGIHETTFN SIMKCDVDIR KDLYANTVLS
 301 GGTTMYPGIA DRMQKEITAL APSTMKIKII APPERKYSVW IGGSILASLS
 351 TFQQMWISKQ EYDESGPSIV HRKCF
```

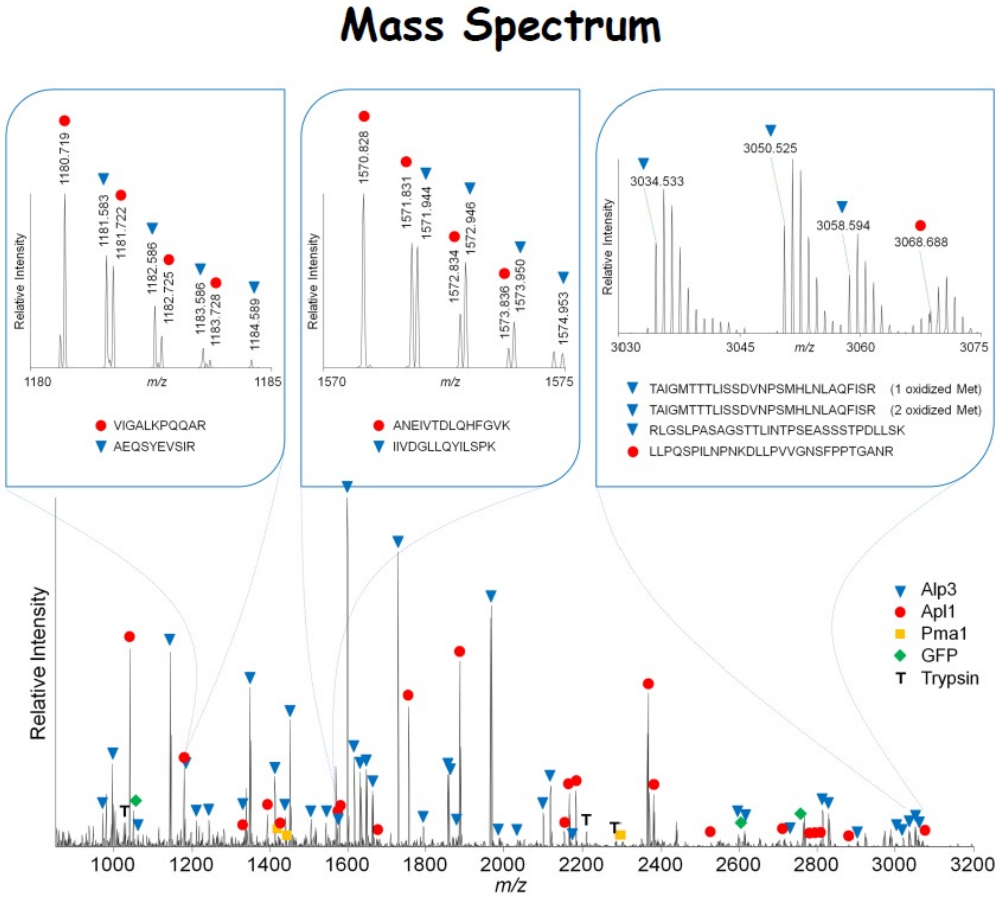
The mass spectra of peptide mixtures are complex



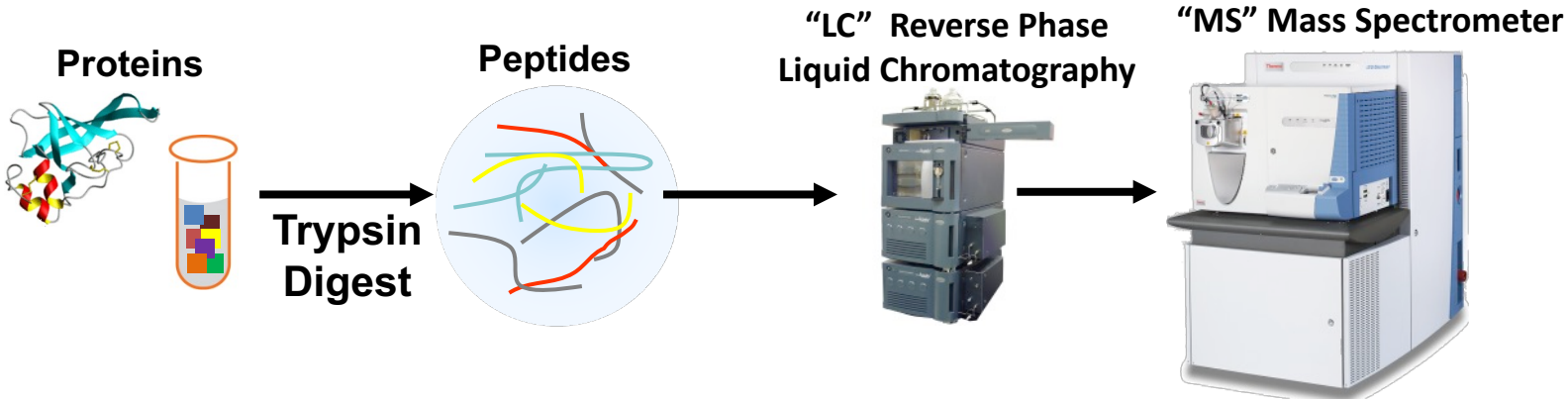


Peptide ions have a mass (m) and a charge (z).

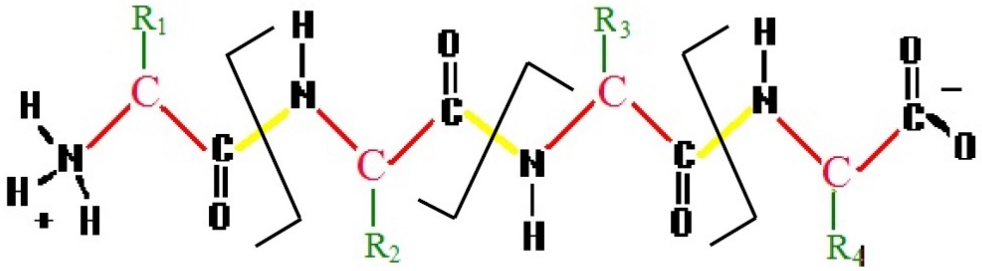
100 Da peptide:
+1 = 100 m/z
+2 = 50 m/z
+3 = 33.3 m/z



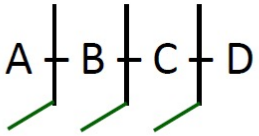
Peptide ions are isolated, fragmented, and “sequenced”



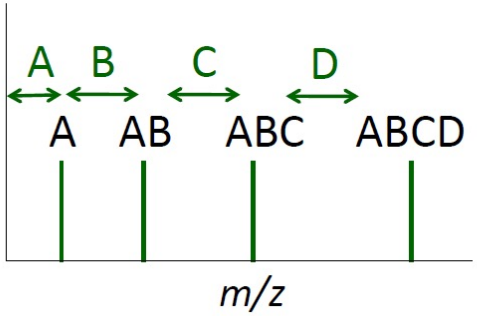
Peptide sequencing



Simplified concept of peptide fragmentation



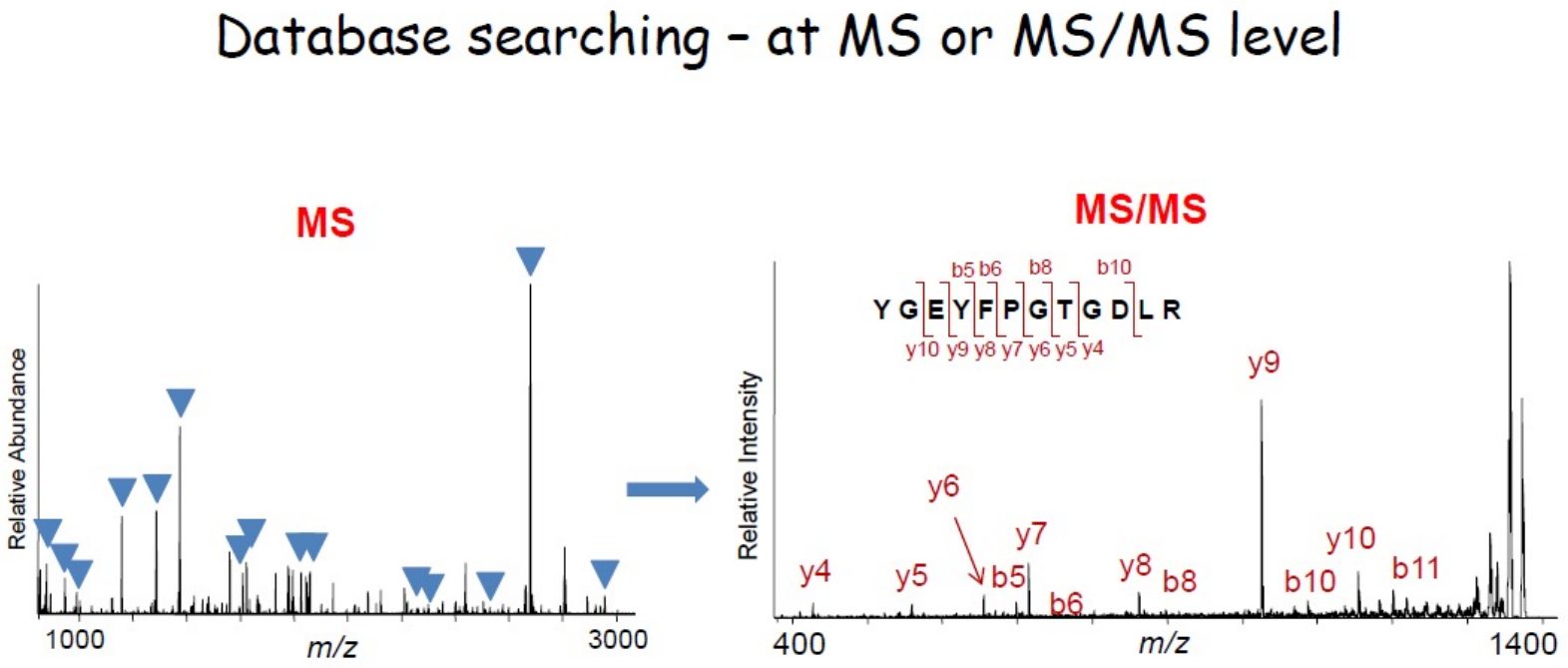
Peptide “ABCD”



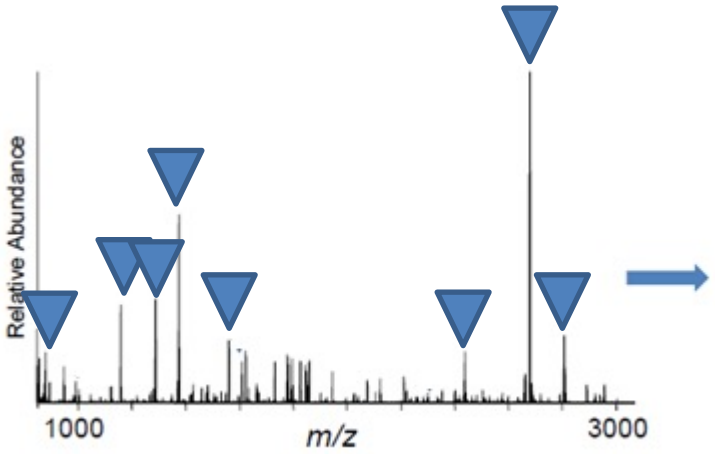
Fragment Spectra of Peptide “ABCD”

Computational Steps:

- Massive amounts of MS and MS/MS data need interpretation
- Genome databases define proteome
- Proteome database used to “match” peptide sequence data

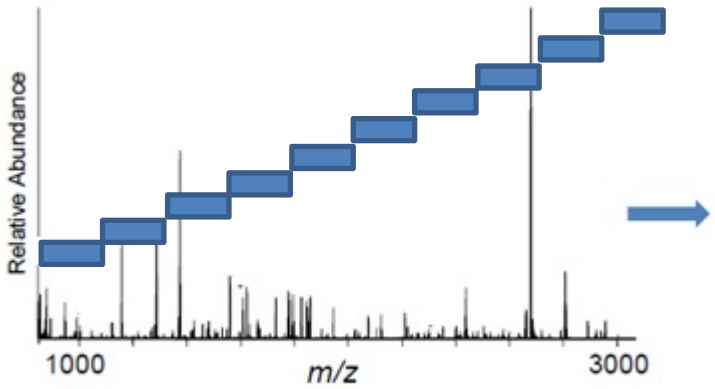


DIA (Data-independent Acquisition) **vs. DDA** (Data-dependent Acquisition)



DDA (Data-dependent Acquisition)

▼ The ***most intense/"abundant"*** ions are selected for MS/MS sequencing



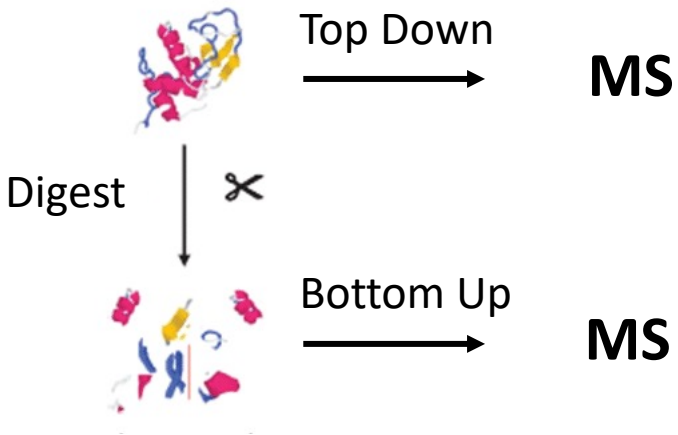
DIA (Data-independent Acquisition)

▬ ***All ions*** in small M/Z windows are selected for MS/MS sequencing

Further Reading: PMID27092249; PMID30104418

Top Down Proteomics to Advance Proteoform Measurement & Biology

***Neil Kelleher, Ph.D.**



ProtIG Lecture Thursday, February 10th, 2022

Seminar: 12 pm - 1:00pm Eastern Time

Chat with the speaker: 1:00 pm – 2:00 pm

*Walter and Mary Elizabeth Glass Professor of Molecular Biosciences, Professor of Chemistry, Director, Proteomics Center of Excellence
Director, Chemistry of Life Processes Institute
Northwestern University, Evanston, IL 60208-3113

Zoom lecture @NIH: email Dr. Rinehart for zoom link. ***jesse.rinehart@yale.edu***

The *pace of proteomics is set by a combination of techniques and technological advances.

*orders of magnitude behind genome technologies (*sequencing*)

Yeast proteome reported in Washburn et al. *Nature Biotech* 2001:

~82 hours* = 1,484 proteins ~0.3 proteins/ min

***estimates from paper: 3 fractions @ 15 X 110 minute "runs" for each fraction**

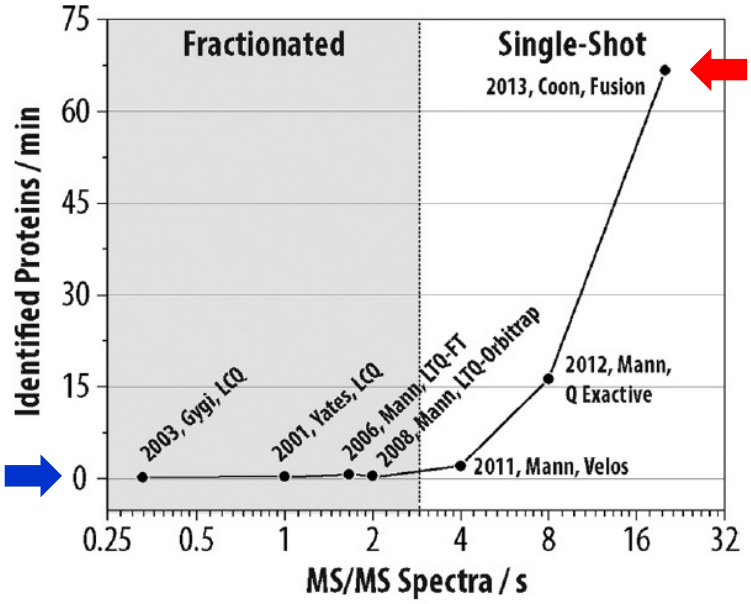


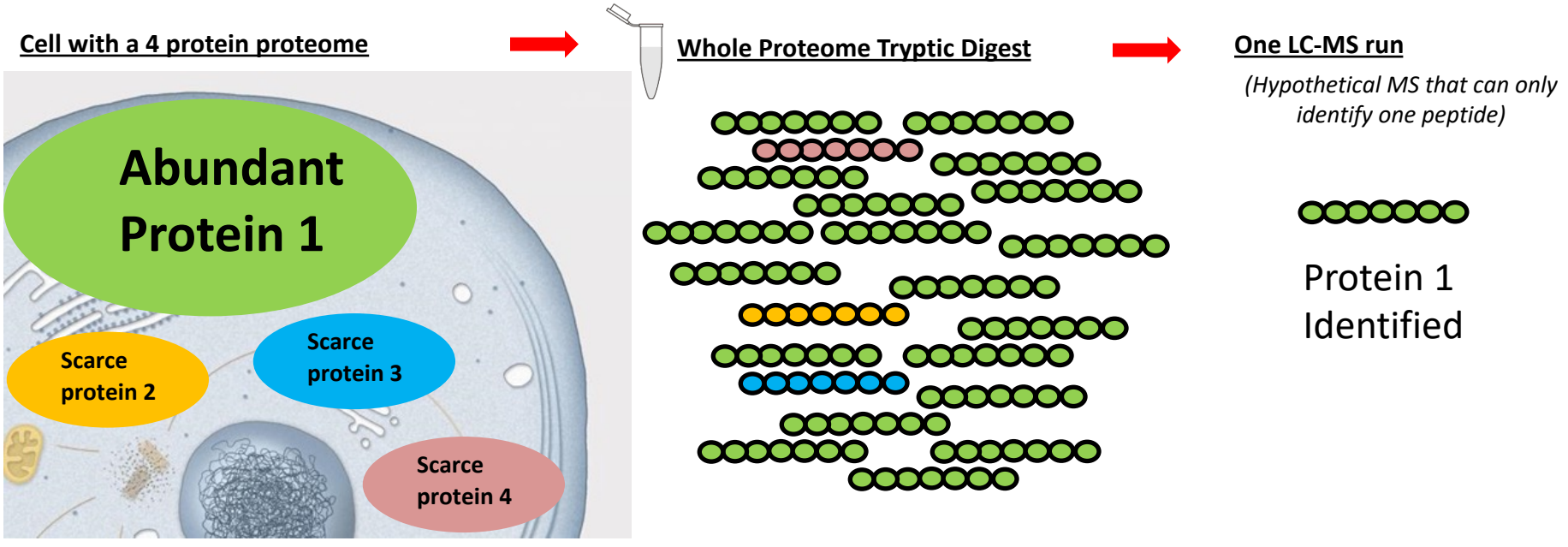
FIG. 5. **Rate of protein identifications as a function of mass spectrometer scan rate for selected large-scale yeast proteome analyses over the past decade.** Each data point is annotated with the year, corresponding author, type of MS system used, and reference number.

"each **one hour** analysis achieved detection of **3,977 proteins"**

The one hour yeast proteome. Hebert et al *Mol Cell Proteomics*. 2014

The one hour yeast proteome. Hebert AS, et a, Coon JJ.
Mol Cell Proteomics. 2014 PMID: 24143002 & *Nat Protoc.* 2015. PMID: 25855955

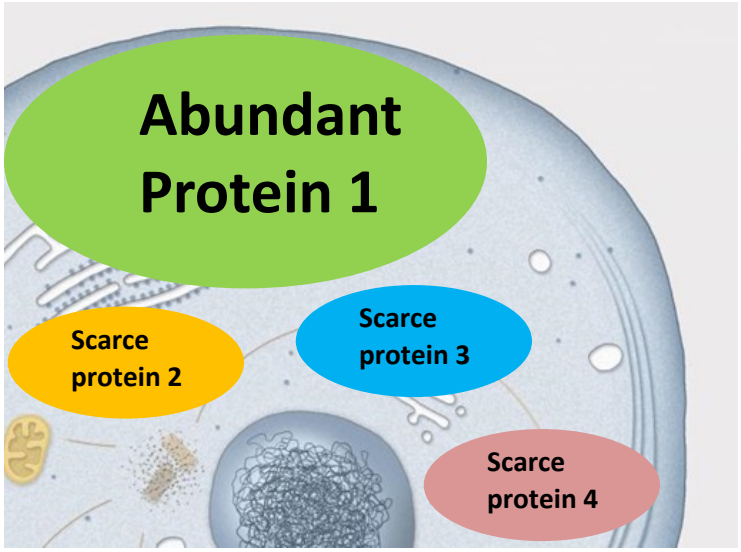
Challenge Question:



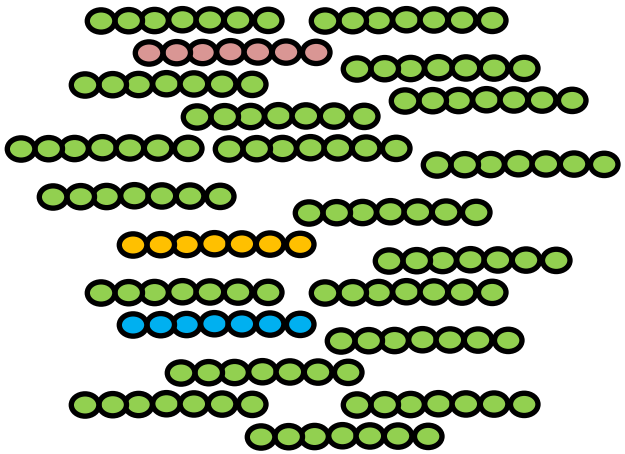
Challenge Question:

How would you detect all four proteins in this cell using a mass spectrometer that can only identify one peptide?

Cell with a 4 protein proteome



Whole Proteome Tryptic Digest

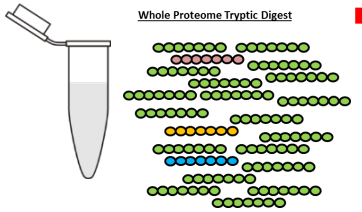


One LC-MS run

(Hypothetical MS that can only identify one peptide)

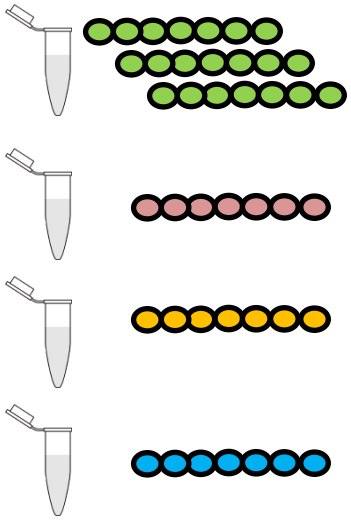
Protein 1 Identified

Option #1: Peptide Fractionation



Chromatography + fractionation

4 separate LC-MS runs



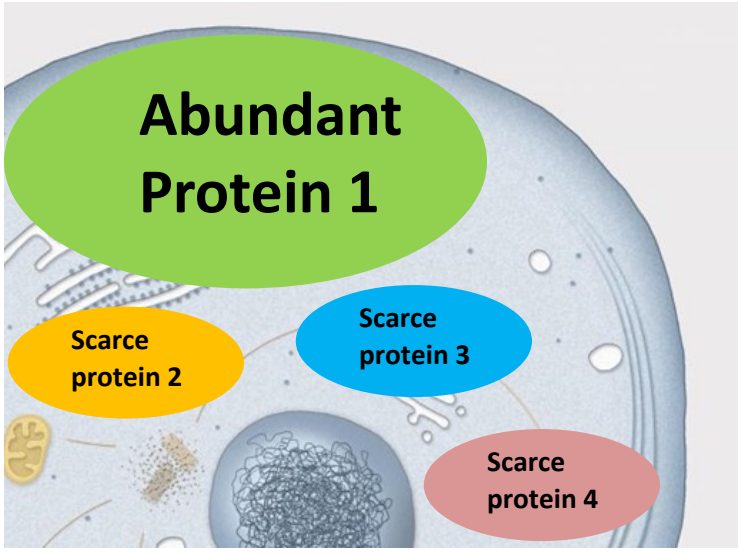
Protein 1 Identified

Protein 2 Identified

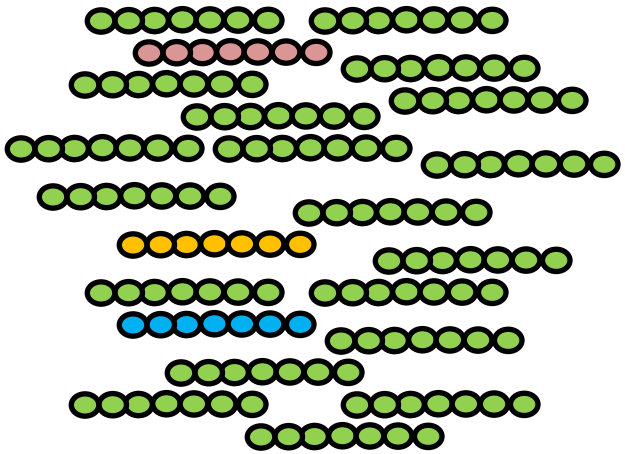
Protein 3 Identified

Protein 4 Identified

Cell with a 4 protein proteome



Whole Proteome Tryptic Digest

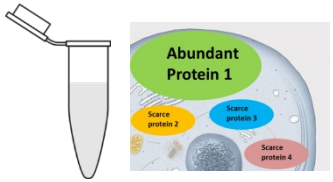


One LC-MS run

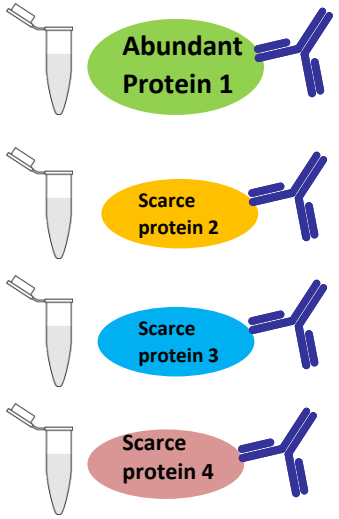
(Hypothetical MS that can only identify one peptide)

Protein 1 Identified

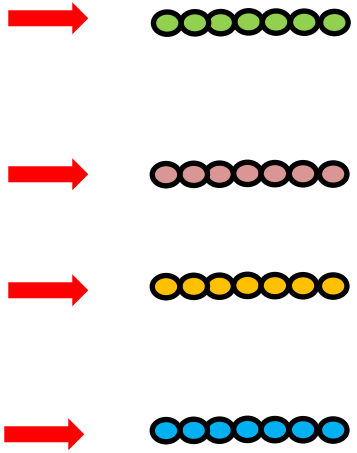
Option #2: Proteome Fractionation (e.g. Immunoprecipitation)



Separate IP Tryptic Digest



4 separate LC-MS runs



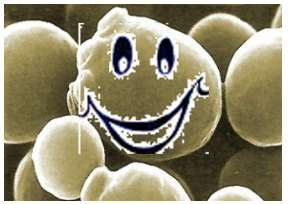
Protein 1 Identified

Protein 2 Identified

Protein 3 Identified

Protein 4 Identified

A tour of proteomics: Studies with the budding yeast *Saccharomyces cerevisiae*



2000 & 2001

Uetz et al, A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. *Nature* .
& Ito et al, A comprehensive two-hybrid analysis to explore the yeast protein interactome . *PNAS.*

➲ **Large scale yeast two hybrid screens to map proteome wide interactions.**

2001

Washburn, et al. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nature Biotechnol*.

➲ **Established the 'shotgun' technology by showing that many proteins in a yeast-cell lysate could be identified in a single experiment.**

2002

Ho, Y. *et al*. Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature*.
& Gavin, A. C. *et al*. Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* .

➲ **Protein–protein interaction maps can be obtained by MS; the yeast cell is organized into protein complexes.**

2003

Ghaemmaghami, S. et al. Global analysis of protein expression in yeast. *Nature*. **&** Huh, W. K. et al. Global analysis of protein localization in budding yeast. *Nature*.

➲ **TAP-Tag and expression studies & GFP-Tag and localization studies**

2006

Krogan NJ, et al. Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. *Nature*.

➲ **TAP-Tag and Protein-Protein Interaction**

2008

de Godoy LM, et al. Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast. *Nature*.

➲ **SILAC based quantitation of an entire proteome.**

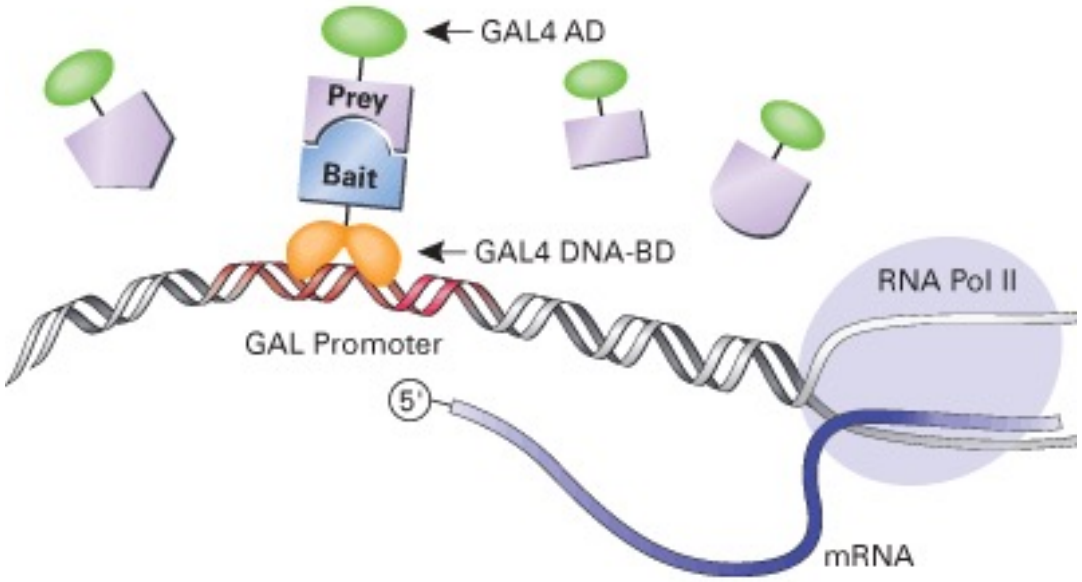
2009

Picotti P, et al. Full dynamic range proteome analysis of S. cerevisiae by targeted proteomics. *Cell.*

➲ **Towards proteome wide targeted proteomics.**

A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae.

Uetz et al, Nature 2000
Ito et al, PNAS 2001



Yeast Two Hybrid Assay

Advantages:

- ***In vivo* assay**
- **Simple**

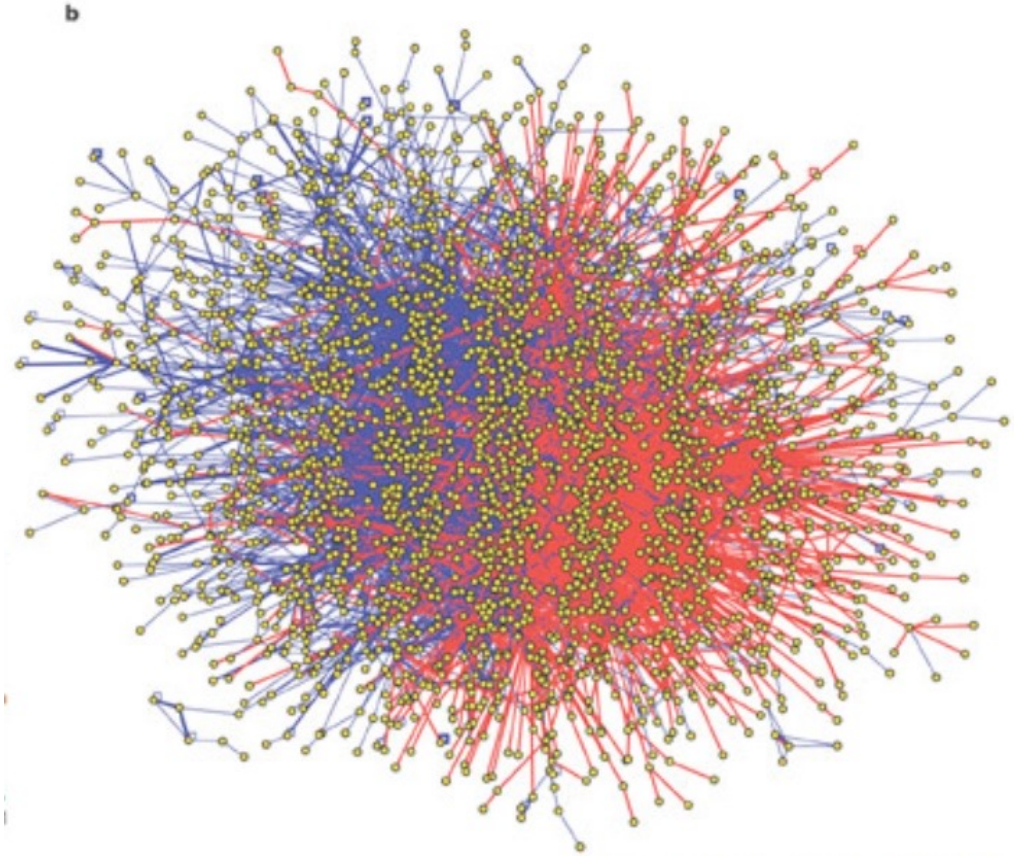
Some Disadvantages

- **Hard to execute on large scale**
- **False positives: a real interaction or "possible" interaction**
- **Interaction in nucleus (required for GAL system)**
- **Clones are fusion proteins and sometimes "partial" proteins**
- **Multiple protein complexes not "captured"**

Human Two Hybrid Map

8,100 ORFs (~7,200 genes)

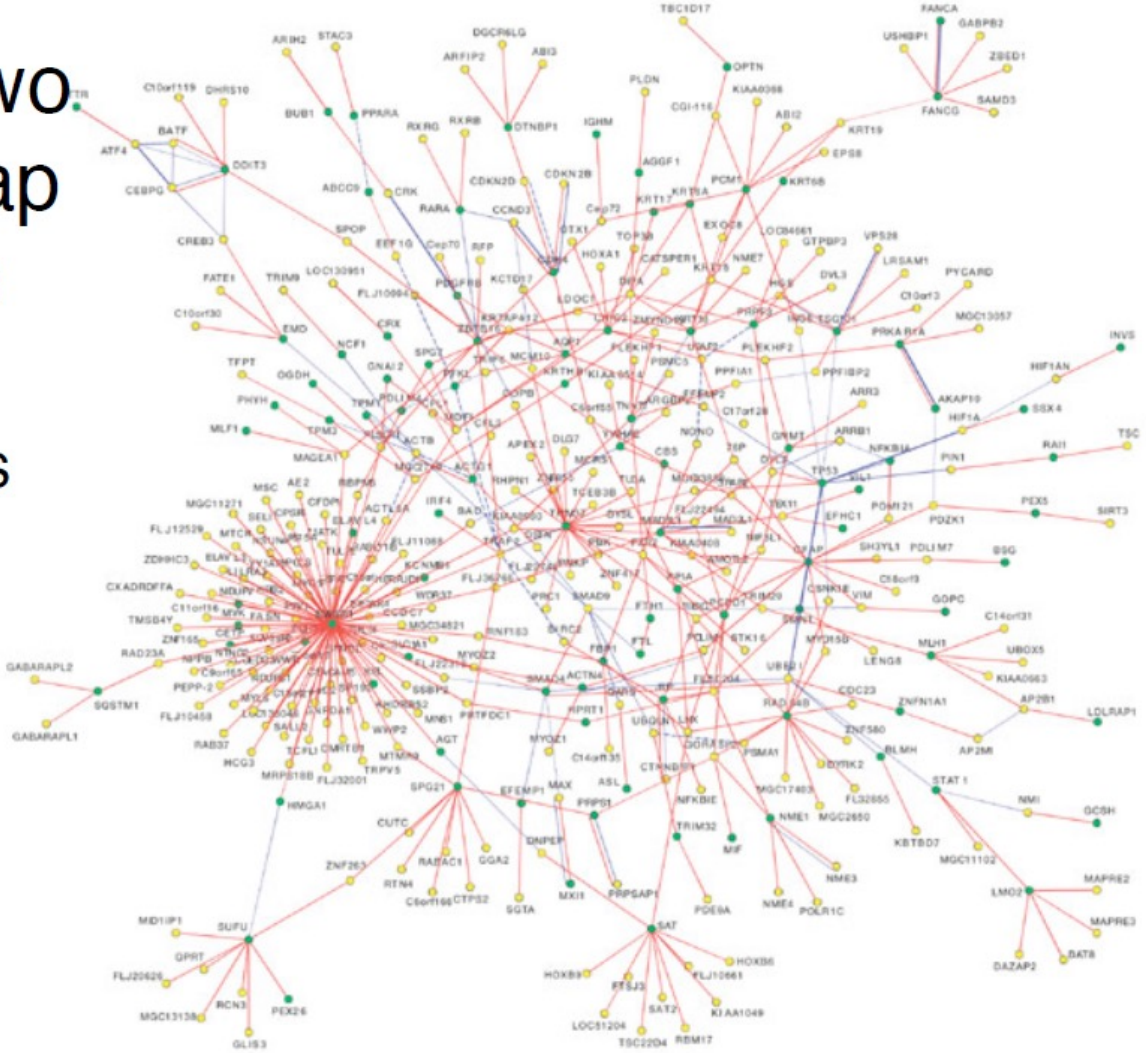
10,597 interactions



Rual et al. Nature 2005

Human Two Hybrid Map Disease Genes

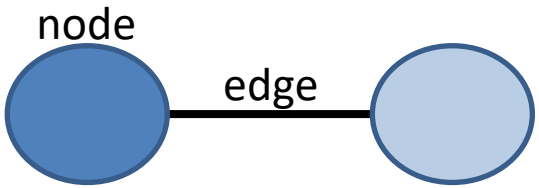
(121 genes (green))

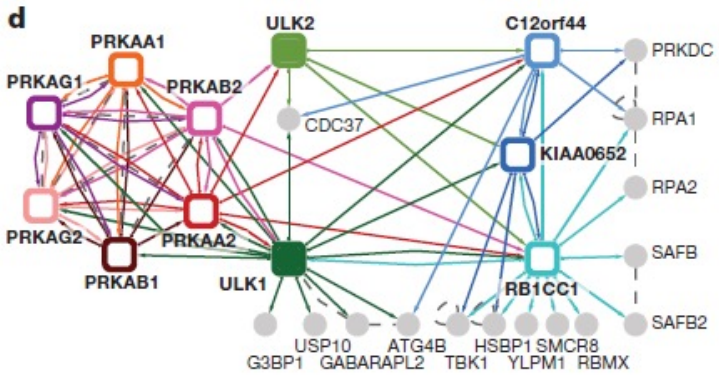


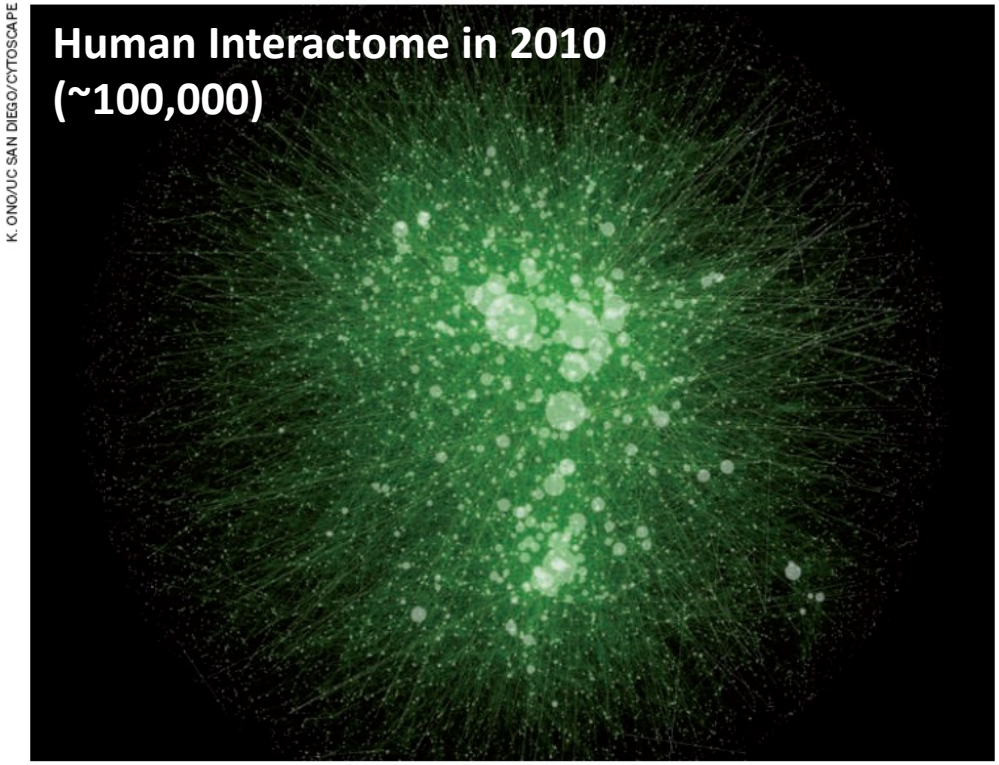
Rual et al. Nature 2005 Vol 437

Protein-Protein interaction maps:

Proteins are represented by **nodes** and interactions are represented by **edges** between nodes.

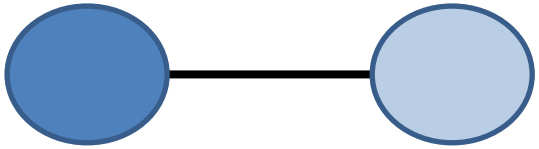






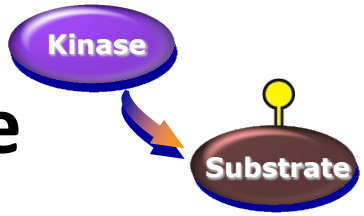
Bonetta, *Nature* 2010

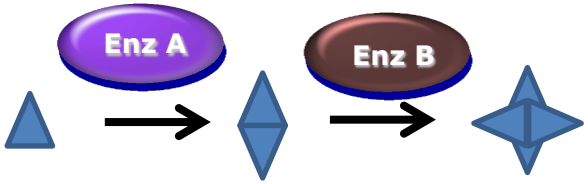
Protein-Protein interactions:



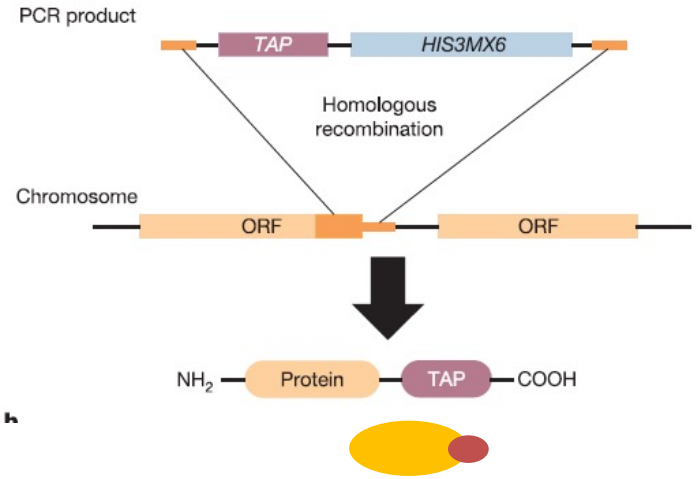
Some examples:

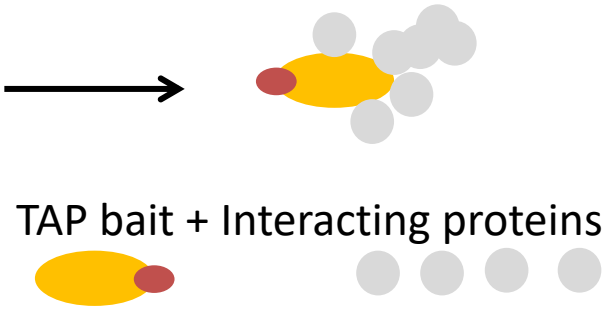
- Physical and direct
- Physical and indirect
  - Multi-protein complexes
  - Scaffolds
- Transient
  - Kinase & substrate
- Metabolic





Adding common molecular handles to every yeast protein: "TAP" tag





Collection of tagged "bait" expression strains

2003

Ghaemmaghami, S. et al. Global analysis of protein expression in yeast. *Nature*. **&** Huh, W. K. et al. Global analysis of protein localization in budding yeast. *Nature*.

➲ **TAP-Tag and expression studies & GFP-Tag and localization studies**

2002

Ho, Y. *et al*. Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature*.
& Gavin, A. C. *et al*. Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* .

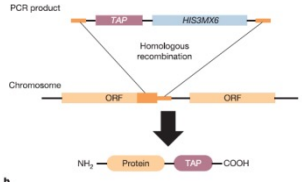
➲ Protein–protein interaction maps can be obtained by MS; the yeast cell is organized into protein complexes.

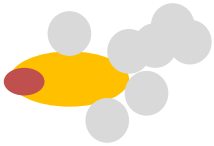
2006

Krogan NJ, et al. Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. *Nature*.

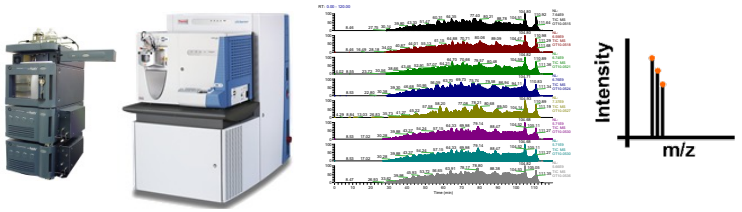
➲ TAP-Tag and Protein-Protein Interaction

Collection of tagged "bait" expression strains





Multiple runs of "shot gun" MS
& SDS-PAGE with MS on individual proteins



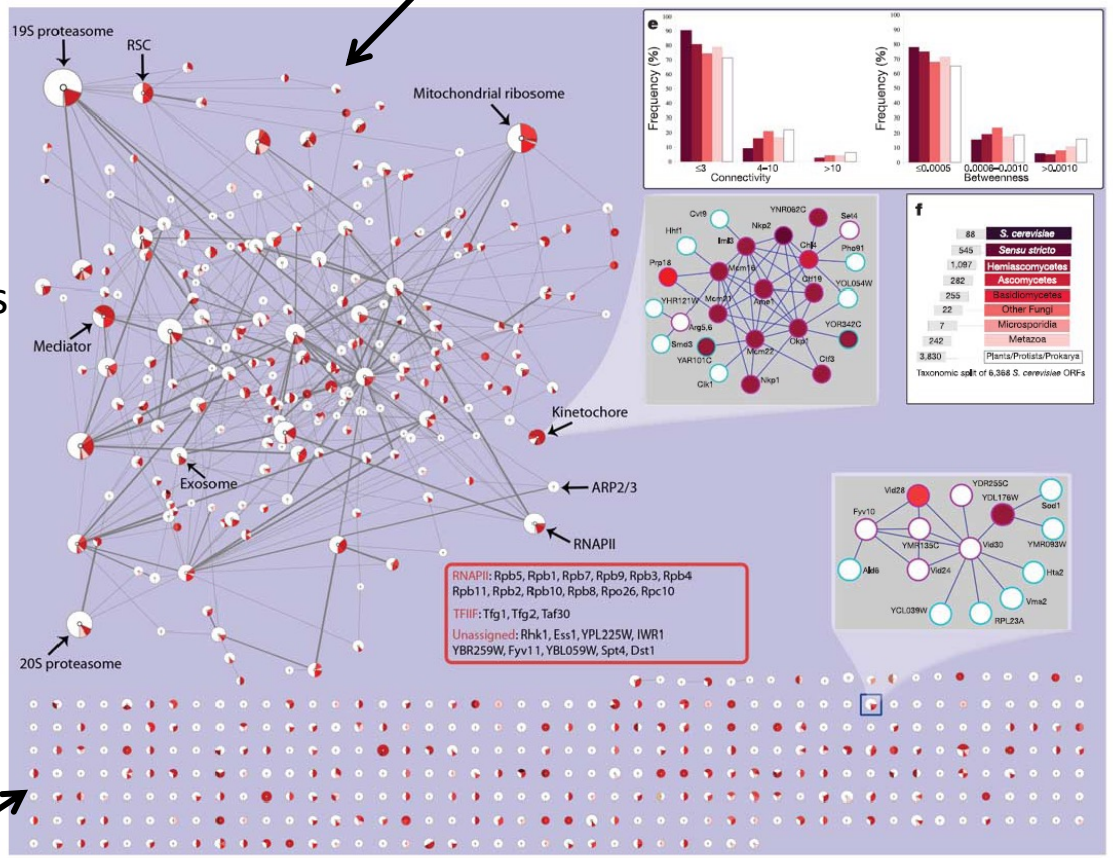
Krogan et al. observed 7,123 protein–protein interactions:

<u>Important aspects:</u>

- **Tagged the native genes and did not overexpress the fusion proteins**
- **Could immediately validate partners (reciprocal purification in data set)**
- **Complementary MS techniques, deeper coverage of complexes**
- **Authors state, "…rigorous computational procedures to assign confidence values to our predictions…"**

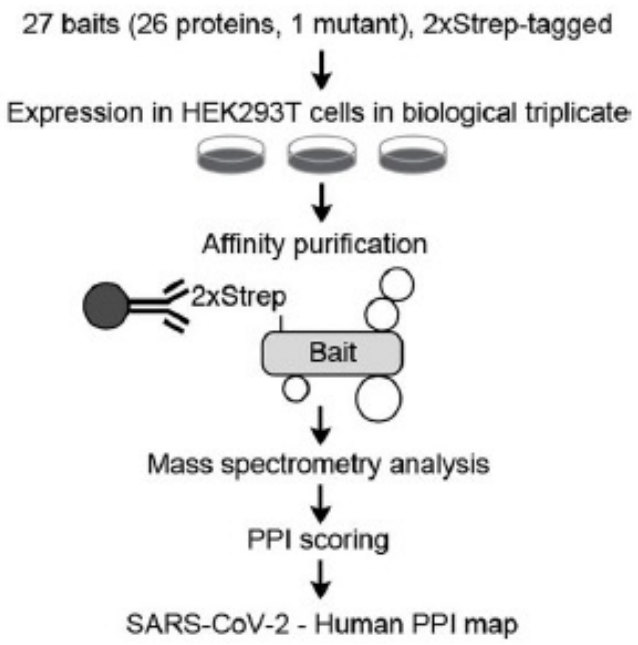
Cellular proteins are organized into complexes

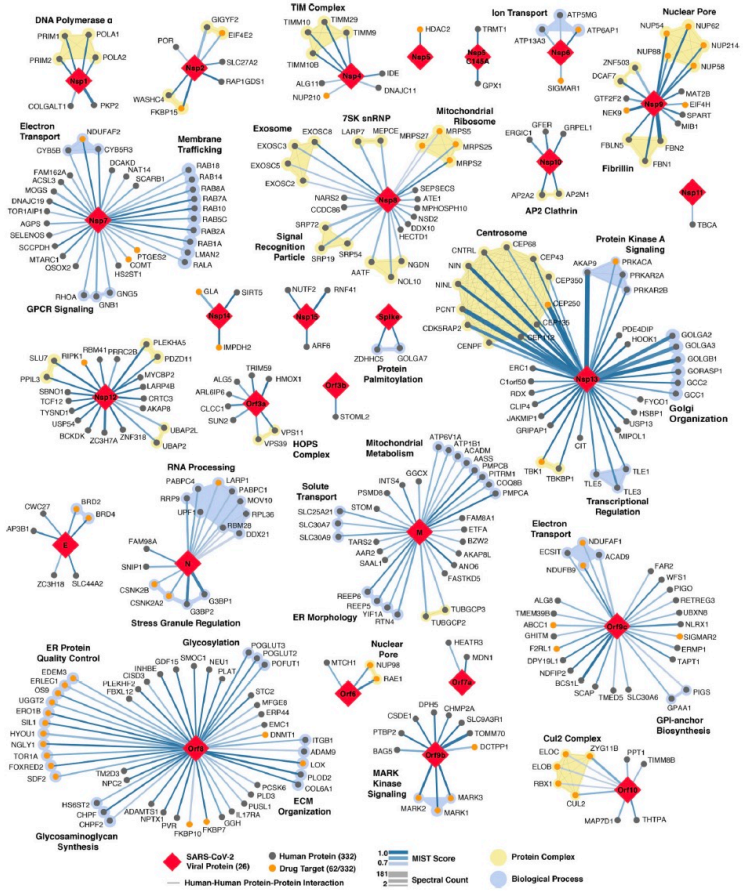
- 4,562 tagged proteins
- 2,357 successful purifications
- Identified 4,087 interacting proteins ~72 % proteome
- Majority of the yeast proteome is organized into complexes
- Many complexes are conserved in other species



Krogan NJ, et al. *Nature.* 2006

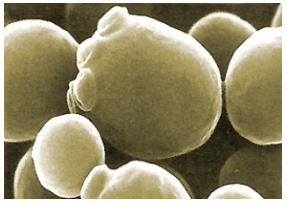
A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug-Repurposing





Krogan lab PMID: 32353859, Gordon et al. Nature. 2020

How do we learn more about the organization of the human proteome?

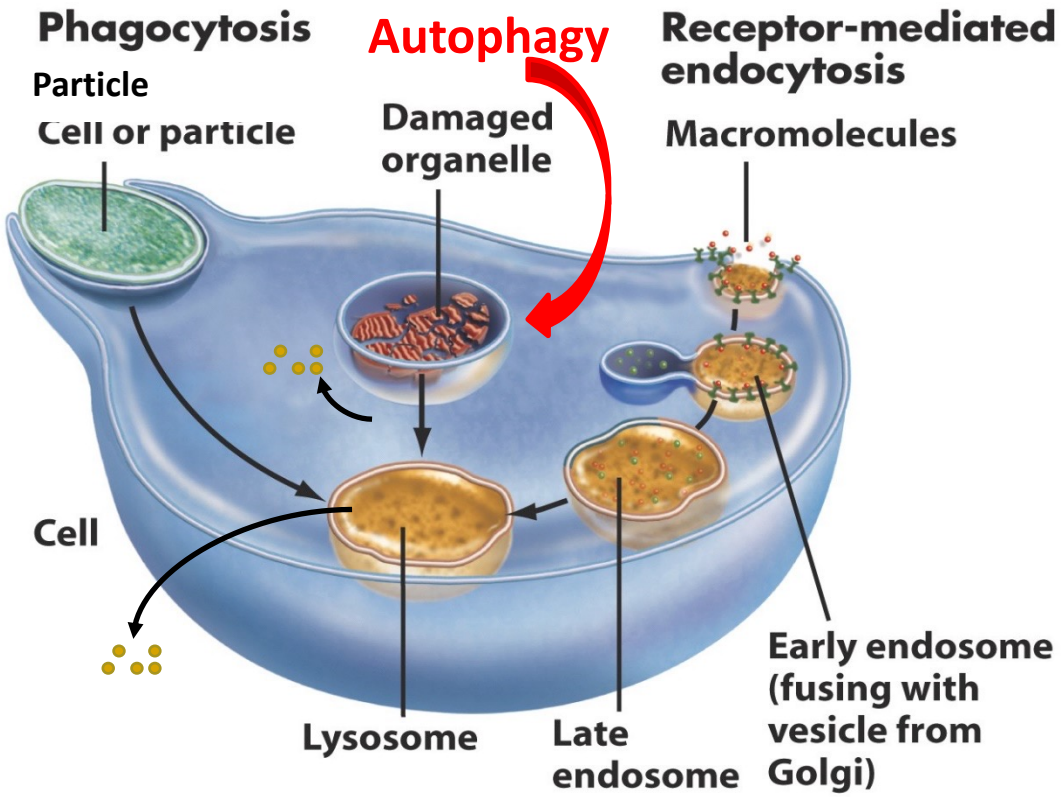


nature Vol 466|1 July 2010|doi:10.1038/nature09204

ARTICLES

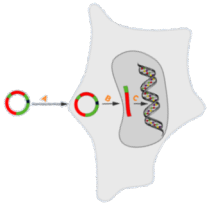
Network organization of the human autophagy system

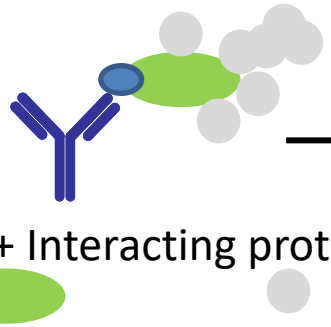
Christian Behrends[1], Mathew E. Sowa[1], Steven P. Gygi[2] & J. Wade Harper[1]



Pearson Prentice Hall, Inc. 2005
www.stolaf.edu/people/giannini/cell/lys.htm

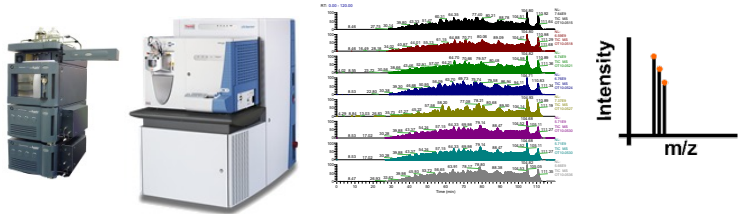
Transfect tagged "bait"





IP Bait + Interacting proteins

Multiple runs of "shot gun" LC-MS/MS



~65 bait proteins
LC-MS/MS identifies
2,553 proteins

Data analysis to sort out real interaction from background

Authors use CompPASS to identify High-Confidence Interacting Proteins (HCIP)

763 HCIPs identified that compose The Autophagy Interaction Network

Behreands et al, Nature 2010

Autophagy Interaction Network

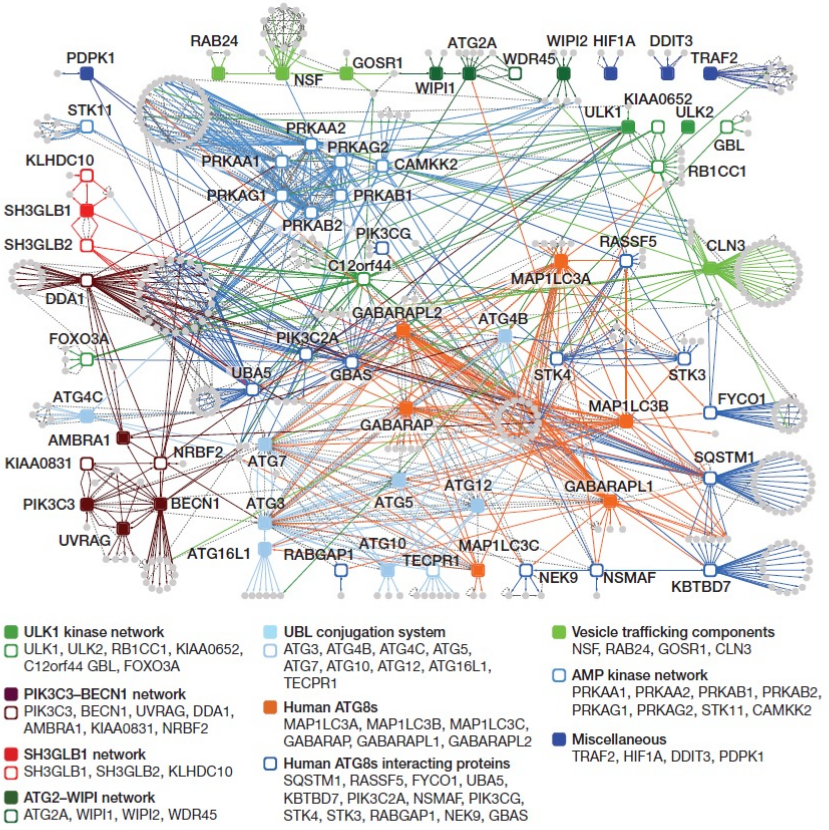


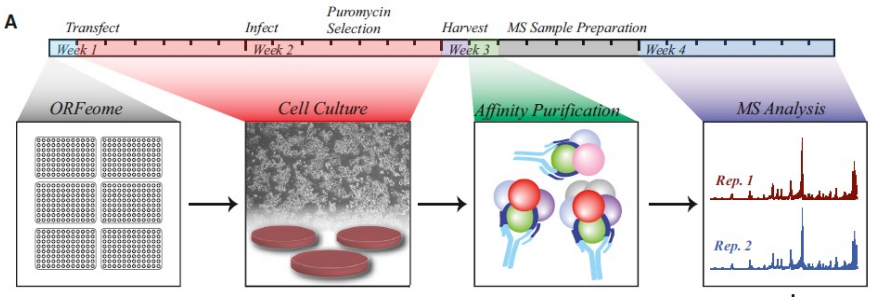
Figure 1 | Overview of the autophagy interaction network (AIN). HCIPs within the autophagy network are shown for 32 primary baits (filled squares) and 33 secondary baits (open squares). Subnetworks are colour-coded. Interacting proteins are indicated by grey circles.

BioPlex (Biophysical Interactions of ORFeome-derived complexes)

~25% of human genes used as baits

5,891 IP-MS experiments

56,553 interactions from 10,961 proteins



http://wren.hms.harvard.edu/bioplex/

The BioPlex Network:
A Systematic Exploration of the Human Interactome

Edward L. Huttlin,[1] Lily Ting,[1] Raphael J. Bruckner,[1] Fana Gebreab,[1] Melanie P. Gygi,[1] John Szpyt,[1] Stanley Tam,[1]

BioPlex 1.0 Huttlin et al, *Cell*. 2015, PMID: 26186194

Architecture of the human interactome defines protein communities and disease networks

Edward L. Huttlin[1], Raphael J. Bruckner[1], Joao A. Paulo[1], Joe R. Cannon[1], Lily Ting[1], Kurt Baltier[1], Greg Colby[1], Fana Gebreab[1],

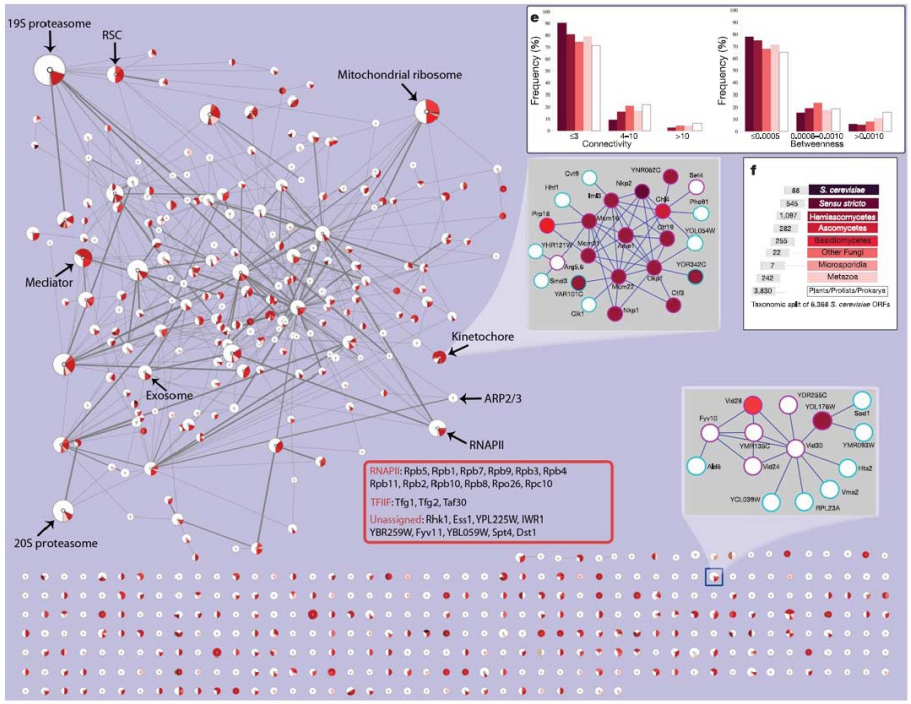
BioPlex 2.0 Huttlin et al, *Nature*. 2017 PMID: 28514442

BioPlex 3.0 Huttlin et al, *Cell* 2021 PMID: 33961781

This dataset contains ~120,000 interactions detected in HEK293T cells using 10128 baits. https://bioplex.hms.harvard.edu/interactions.php.

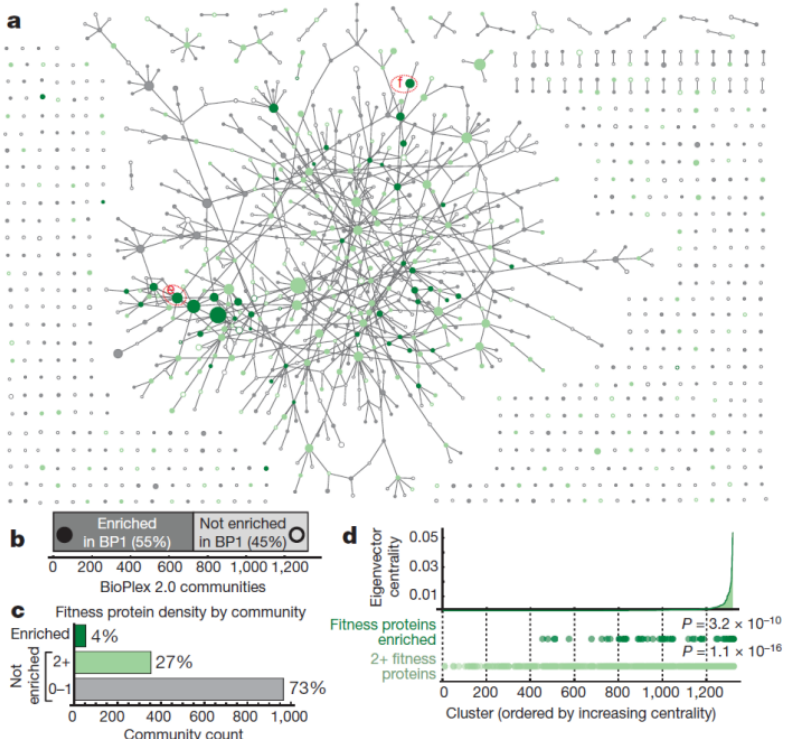
Cellular proteins are organized into complexes and this proteome organization is conserved

Yeast: Interaction Network of Complexes



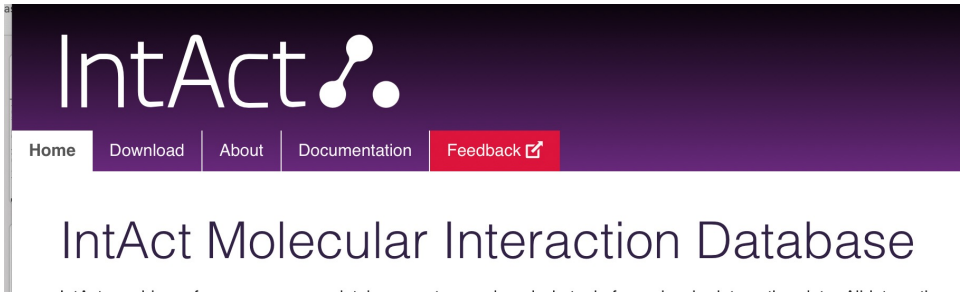
Krogan NJ, et al. *Nature.* 2006 PMID: 16554755

Human: Protein Complex “Communities”

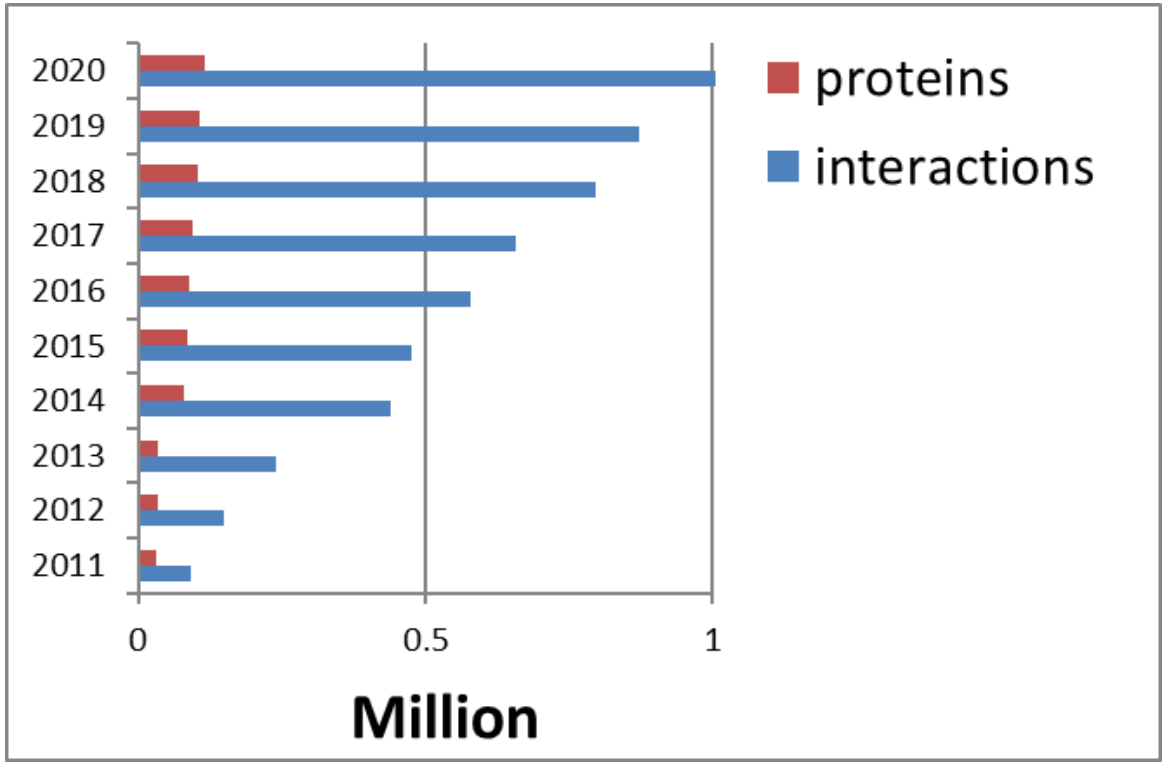


Huttlin et al, *Nature*. 2017 PMID: 28514442

Protein-Protein Interaction Databases



https://www.ebi.ac.uk/intact/home



2022

- Interactors: 118,213
- Interactions: 1,155,201

+ 24,605 interactions
- *1,068 proteins*

2021 Data Content

- Publications: **22037**
- Interactions: **1130596**
- Interactors: **119281**

+ 94,927 interactions
+ 3,902 proteins

2020 Data Content

- Publications: **21086**
- Interactions: **1035669**
- Interactors: **115379**

+ 162,823 interactions
+ 6,887 proteins

Proteomics & Protein-Protein Interactions

Overview

- **Techniques & Technologies**
  - Mass Spectrometry
  - Protein-Protein Interactions
  - Quantitative Proteomics
- **Applications**
  - Representative Studies
- **Putting it all together….**
  - Databases & Pathways

Protein interaction networks:

Some of the many important aspects:

- Parts List
- Organization and assembly
- Biological function can be inferred

However:

- Interaction data is largely static

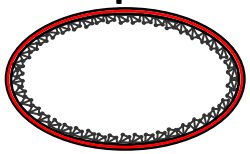
Next Step:

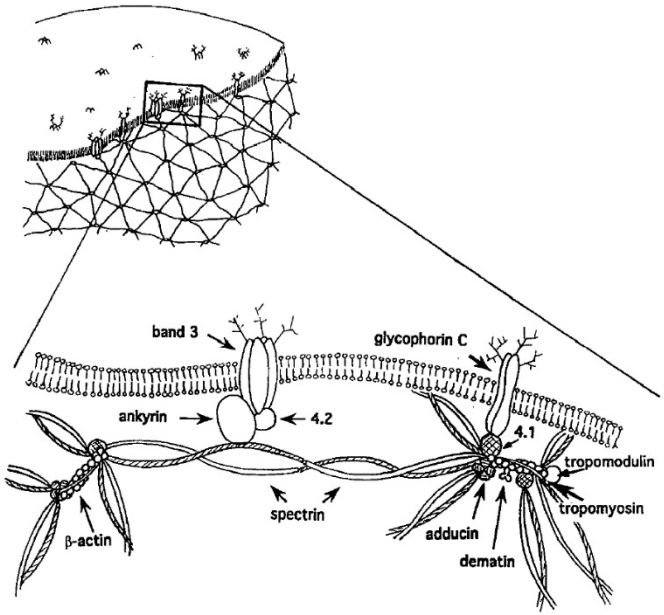
- How do protein interaction networks change over time?

MS Data is not inherently quantitative

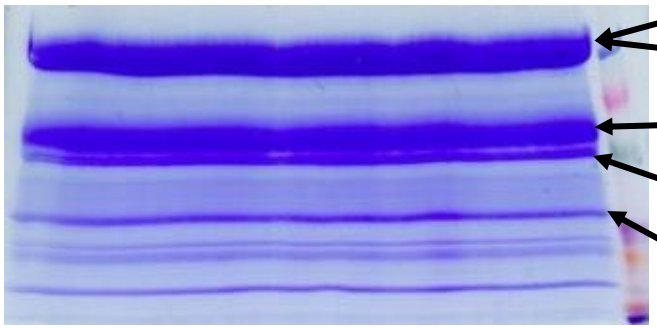
Red Blood Cell

RBC membrane:
a native multi-protein complex





RBC membrane proteome
Coomassie Stained
SDS-PAGE (250 ug Protein)
~16 bands

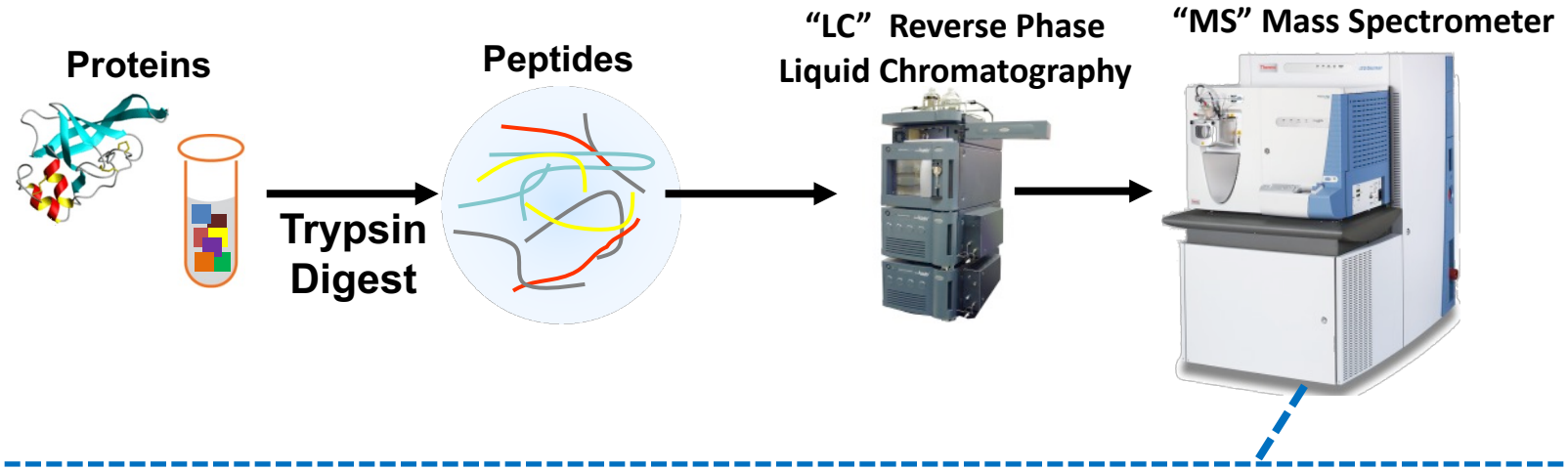


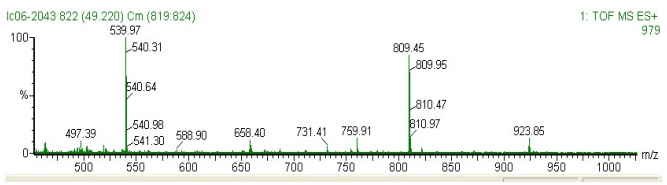
RBC membrane proteome
Shotgun Proteomics
1ug Peptides (242 Proteins)

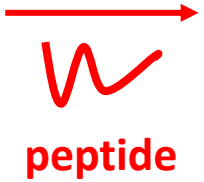
# peptides (unique)	
352 (291)	Spectrin alpha chain, erythrocyte OS=Homo sapiens GN=SPTA1 PE=1 SV=5
291 (233)	Spectrin beta chain, erythrocyte OS=Homo sapiens GN=SPTB PE=1 SV=5
172 (134)	Ankyrin-1 OS=Homo sapiens GN=ANK1 PE=1 SV=3
57 (46)	Band 3 anion transport protein OS=Homo sapiens GN=SLC4A1 PE=1 SV=3
52 (39)	Erythrocyte membrane protein band 4.2 OS=Homo sapiens GN=EPB42 PE=1 SV=3
43 (34)	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
30 (20)	Actin, alpha cardiac muscle 1 OS=Homo sapiens GN=ACTC1 PE=1 SV=1
22 (9)	Beta-actin-like protein 2 OS=Homo sapiens GN=ACTBL2 PE=1 SV=2
28 (6)	POTE ankyrin domain family member J OS=Homo sapiens GN=POTEJ PE=3 SV=1
68 (49)	Protein 4.1 OS=Homo sapiens GN=EPB41 PE=1 SV=4

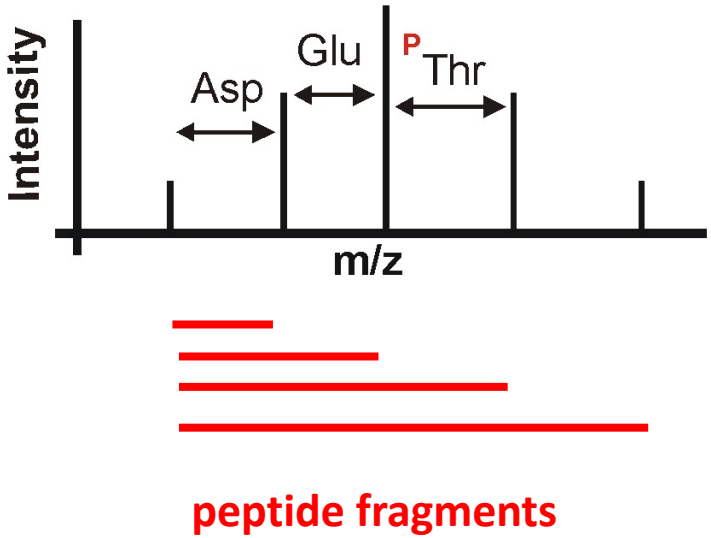
Rinehart et al., *unpublished*

Typical work flow for LC-MS "shotgun proteomics"



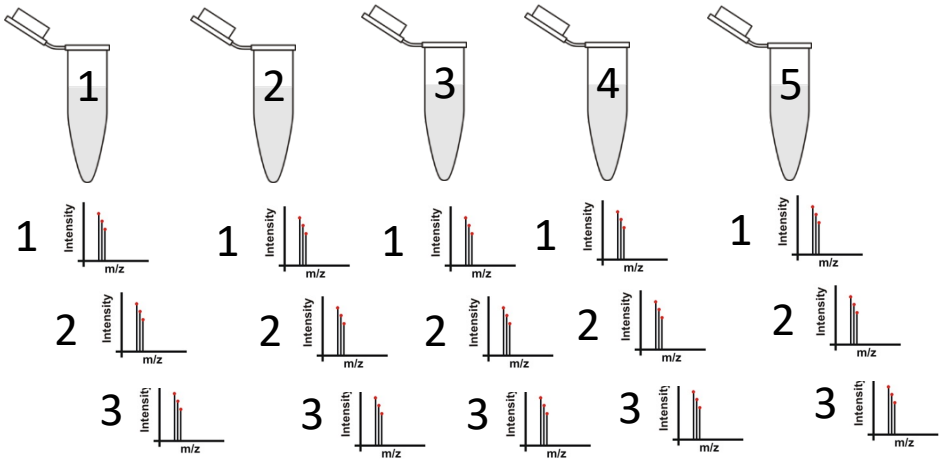




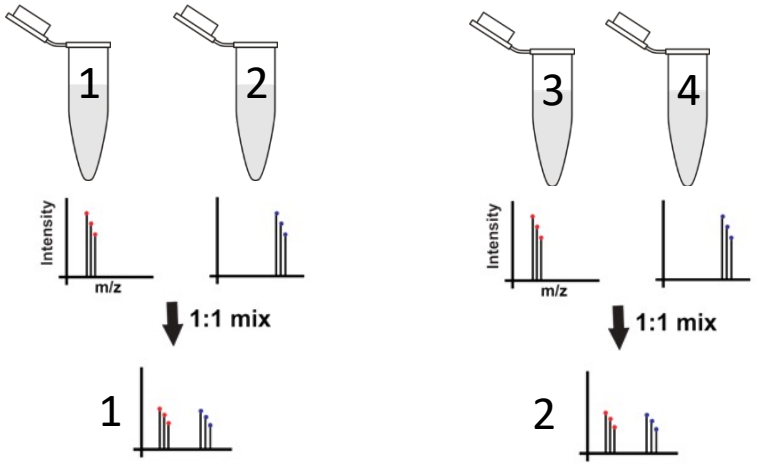


Multiple Techniques Enable Quantitative Proteomics

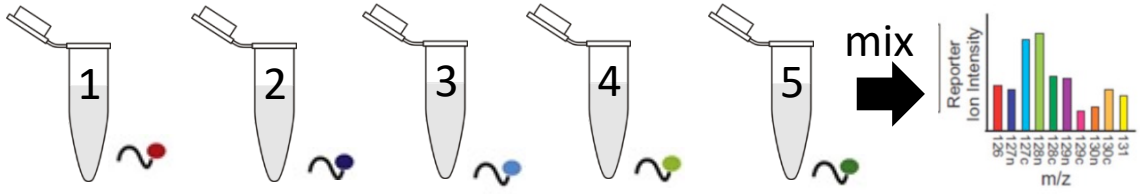
Label Free
-many, many replicates
-indirect quant



“Metabolic” Labeling
-fewer replicates
-multiplex
-direct quant

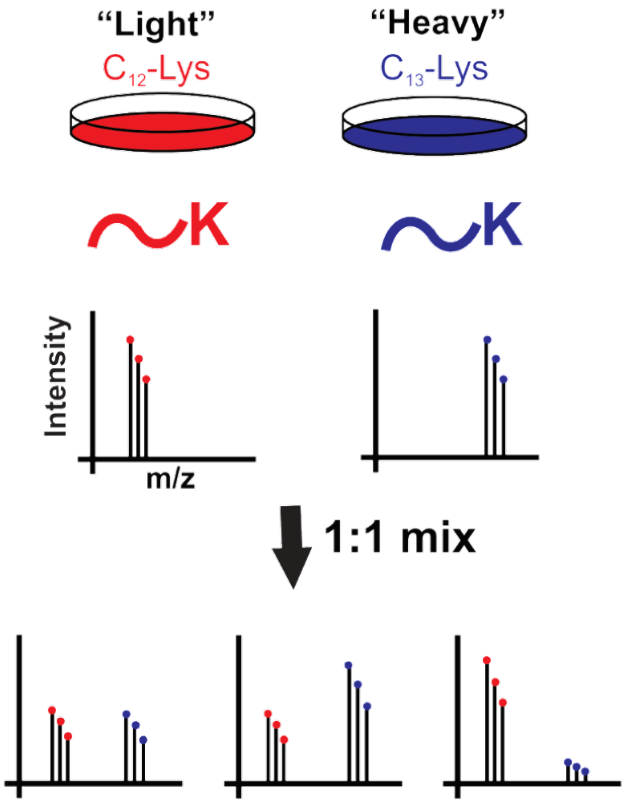


Barcoding
-increased multiplex
-direct quant

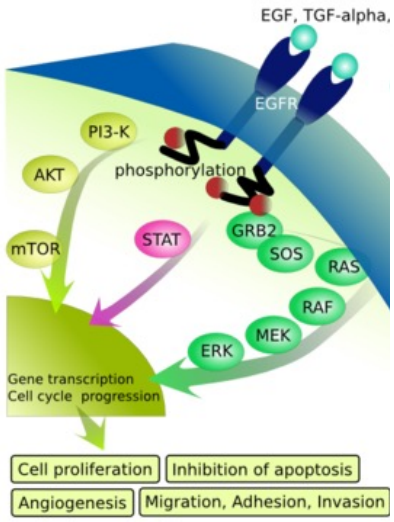


“Metabolic” Labeling: proteins and peptides heavy labeled in cell culture

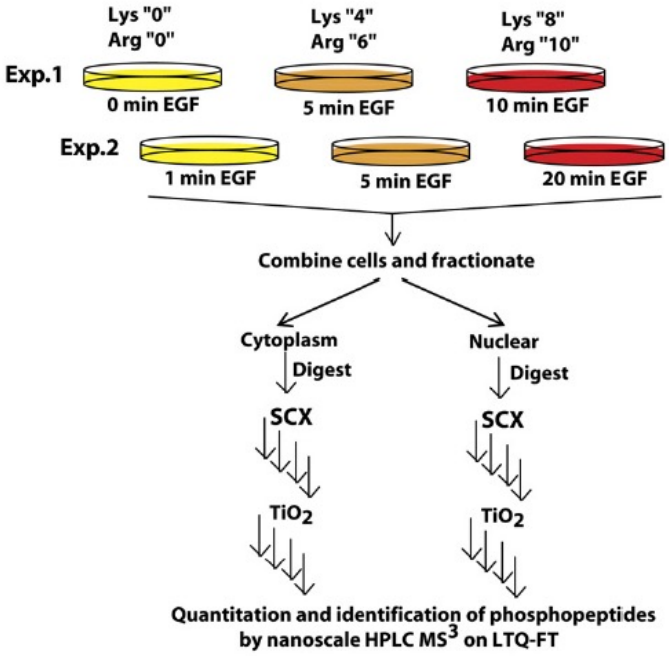
- SILAC approach enables dynamic analysis

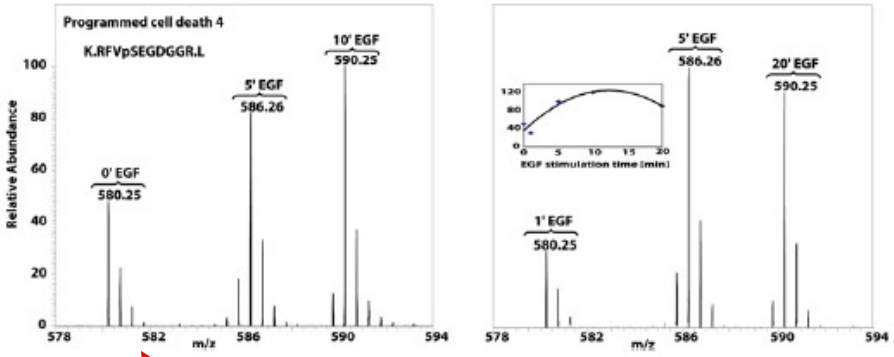


- **Olsen, et al. *Cell*, 2006:** Phosphorylation dynamics after EGF stimulation



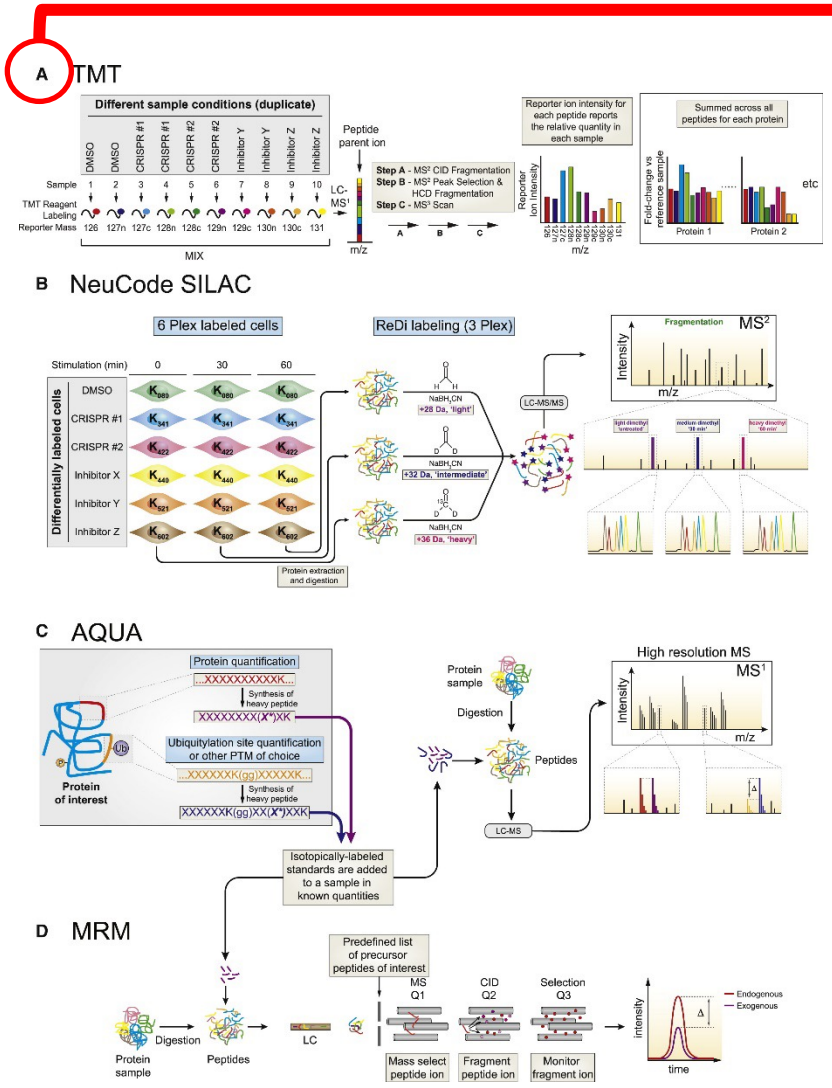
wikimedia.org

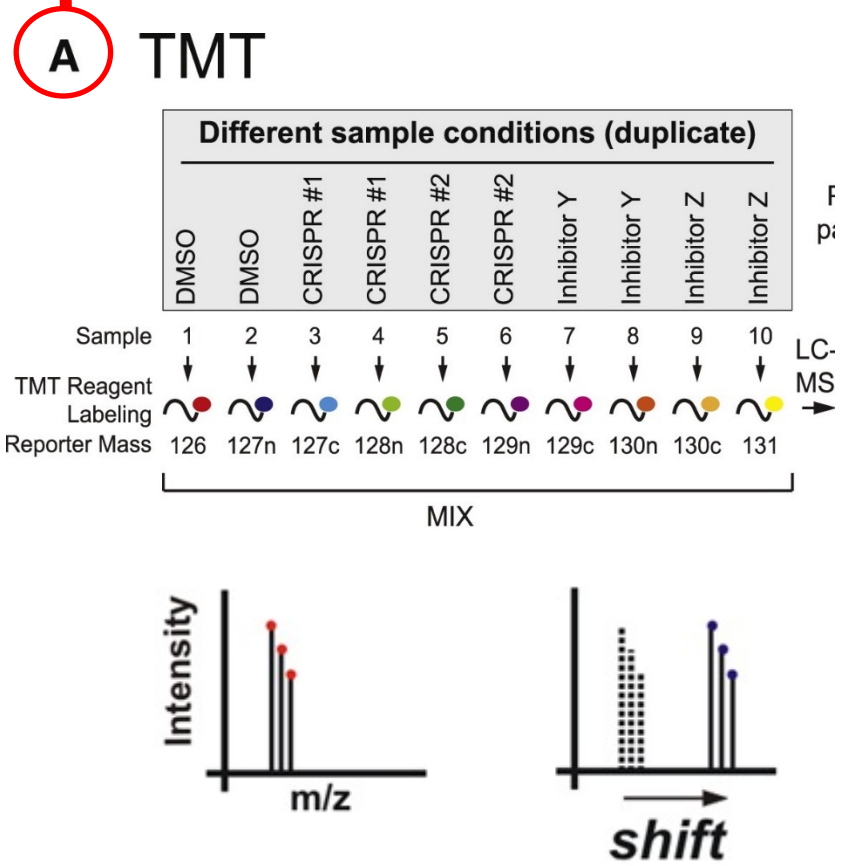




MS spectra triplets

Barcoding: Heavy labels labels can be used for “barcoding” proteomes



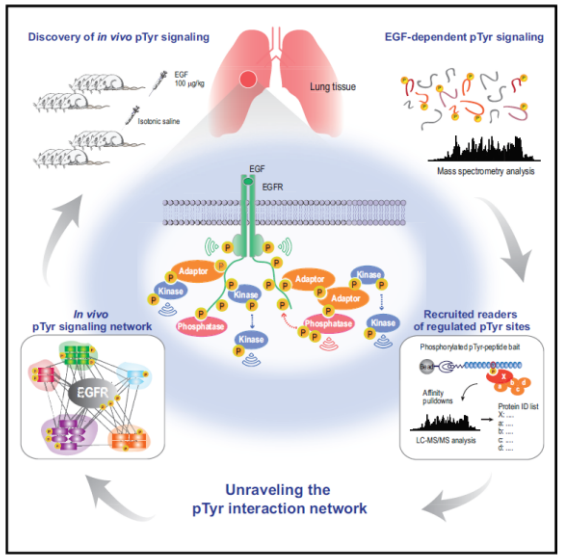


Quantifying ubiquitin signaling; Ordureau A, Münch C, Harper JW. 2015 **PMID: 26000850**

Combinations of technologies are enabling quantitative proteomics at increasing complexity and with greater biological insight

Oncogenic Mutations Rewire Signaling Pathways by Switching Protein Recruitment to Phosphotyrosine Sites

Graphical Abstract



Authors

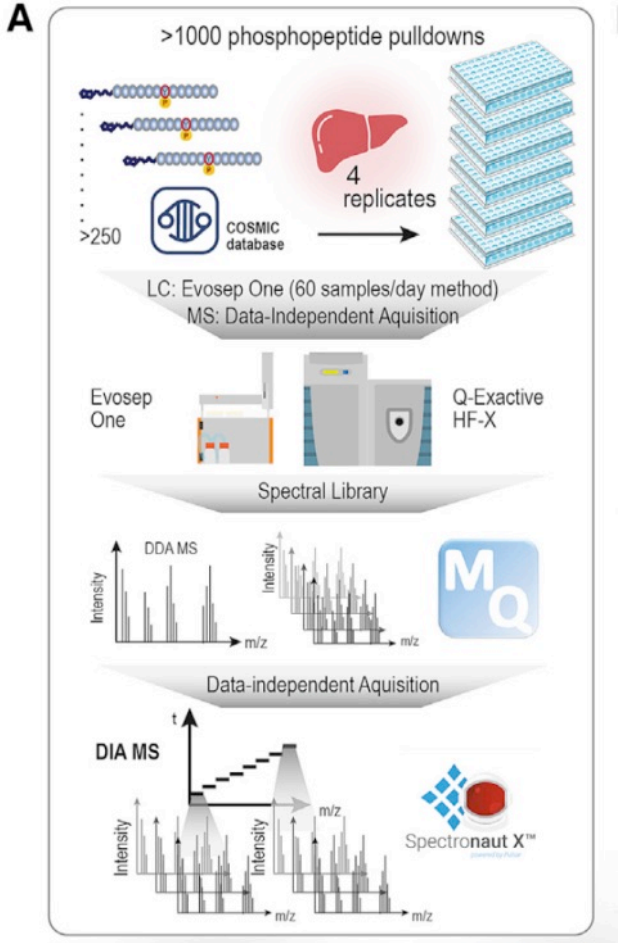
Alicia Lundby, Giulia Franciosa, Kristina B. Emdal, ..., Guillermo Montoya, Lars J. Jensen, Jesper V. Olsen

Correspondence

alicia.lundby@sund.ku.dk (A.L.), jesper.olsen@cpr.ku.dk (J.V.O.)

In Brief

Lundby et al. used mass-spectrometry-based proteomics to analyze *in vivo* EGF signaling in lung tissue. They analyzed changes in the EGF-regulated phosphotyrosine interactome and found that cancer mutations in the vicinity of phosphotyrosine sites induce molecular switches that alter protein signaling networks.



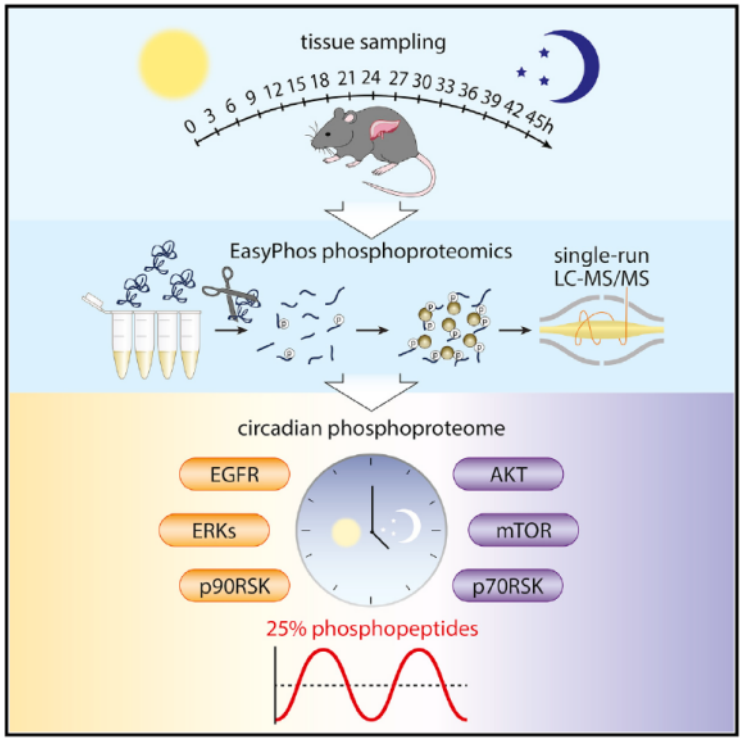
Lundby et al., & Olsen, Cell 2019 PMID: 31585087

Combinations of technologies are enabling quantitative proteomics at increasing complexity and with greater biological insight

Cell Metabolism

Phosphorylation Is a Central Mechanism for Circadian Control of Metabolism and Physiology

Graphical Abstract



Authors

Maria S. Robles, Sean J. Humphrey, Matthias Mann

Correspondence

mmann@biochem.mpg.de

In Brief

Robles et al. profile the global in vivo circadian phosphoproteome of the mouse liver and reveal that 25% of the quantified phosphopeptides oscillate with very high amplitudes compared to the transcriptome and proteome. Phosphorylation-dependent tuning of signaling pathways is a key circadian mechanism for metabolic regulation.

PMID:27818261

Proteomics & Protein-Protein Interactions

Overview

- **Techniques & Technologies**
  - Mass Spectrometry
  - Protein-Protein Interactions
  - Quantitative Proteomics
- **Applications**
  - Representative Studies
- **Putting it all together….**
  - Databases & Pathways

DNA → RNA → PROTEIN

2001

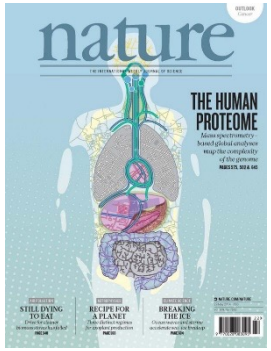
The Sequence of the Human Genome

J. Craig Venter,[1]* Mark D. Adams,[1] Eugene W. Myers,[1] Peter W. Li,[1] Richard J. Mural,[1] Granger G. Sutton,[1] Hamilton O. Smith,[1] Mark Yandell,[1] Cheryl A. Evans,[1] Robert A. Holt,[1]

articles

Initial sequencing and analysis of the human genome

International Human Genome Sequencing Consortium*



2014

ARTICLE

doi:10.1038/nature13319

Mass-spectrometry-based draft of the human proteome

Mathias Wilhelm[1,2]*, Judith Schlegl[2]*, Hannes Hahne[1]*, Amin Moghaddas Gholami[1]*, Marcus Lieberenz[2], Mikhail M. Savitski[3], Emanuel Ziegler[2], Lars Butzmann[2], Siegfried Gessulat[2], Harald Marx[1], Toby Mathieson[3], Simone Lemeer[1], Karsten Schnatbaum[4], Ulf Reimer[4], Holger Wenschuh[4], Martin Mollenhauer[5], Julia Slotta-Huspenina[5], Joos-Hendrik Boese[2], Marcus Bantscheff[3], Anja Gerstmair[2], Franz Faerber[2] & Bernhard Kuster[1,6]

ARTICLE

doi:10.1038/nature13302

A draft map of the human proteome

Min-Sik Kim[1,2], Sneha M. Pinto[3], Derese Getnet[1,4], Raja Sekhar Nirujogi[3], Srikanth S. Manda[3], Raghothama Chaerkady[1,2], Anil K. Madugundu[3], Dhanashree S. Kelkar[3], Ruth Isserlin[5], Shobhit Jain[5], Joji K. Thomas[3], Babylakshmi Muthusamy[3], Pamela Leal-Rojas[1,6], Praveen Kumar[1], Nandini A. Sahasrabuddhe[3], Lavanya Balakrishnan[3], Jayshree Advani[3], Bijesh George[3], Santosh Renuse[3], Lakshmi Dhevi N. Selvan[3], Arun H. Patil[3], Vishalakshi Nanjappa[3], Aneesha Radhakrishnan[3], Samarjeet Prasad[1],

The Sequence of the Human Genome. PMID: 11181995

Initial sequencing and analysis of the human genome. PMID: 11237011

A draft map of the human proteome. PMID: 24870542

Mass-spectrometry-based draft of the human proteome. PMID: 24870543

ARTICLE

doi:10.1038/nature13319

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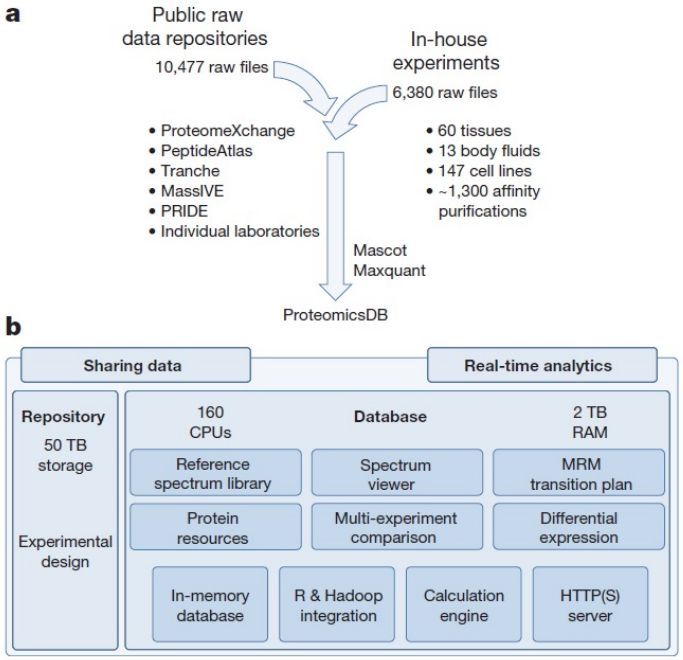
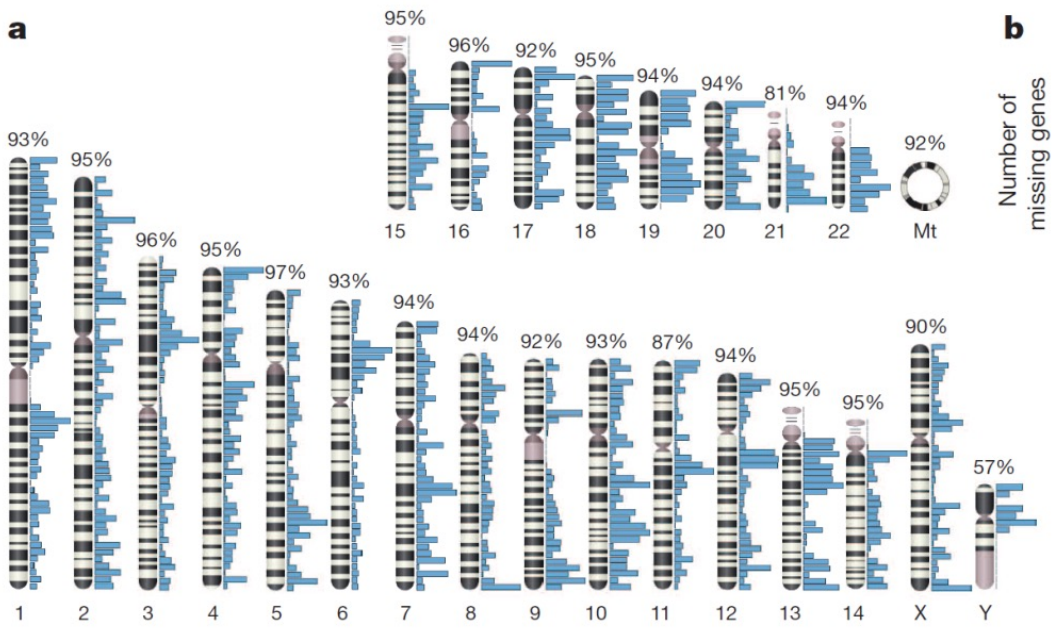
- Large Assembly of new and existing data:
- ProteomicsDB, database designed for the real-time analysis of big data
  https://www.proteomicsdb.org





Mass-spectrometry-based draft of the human proteome; Wilhelm & Bernhard Kuster et al., **PMID: 24870543**

ARTICLE

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Mathias Wilhelm[1,2]*, Judith Schlegl[2]*, Hannes Hahne[1]*, Amin Moghaddas Gholami[1]*, Marcus Lieberenz[2], Mikhail M. Savitski[3], Emanuel Ziegler[2], Lars Butzmann[2], Siegfried Gessulat[2], Harald Marx[1], Toby Mathieson[3], Simone Lemeer[1], Karsten Schnatbaum[4], Ulf Reimer[4], Holger Wenschuh[4], Martin Mollenhauer[5], Julia Slotta-Huspenina[5], Joos-Hendrik Boese[2], Marcus Bantscheff[3], Anja Gerstmair[2], Franz Faerber[2] & Bernhard Kuster[1,6]

- Large Assembly of new and existing data:
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Wilhelm *et al.* carried out 6,380 LC-MS experiments (or runs):

How long would it take to get the same data?

In 2001? ~61 years

In 2014? ~265 Days

Mass-spectrometry-based draft of the human proteome; Wilhelm & Bernhard Kuster et al., **PMID: 24870543**

ARTICLE

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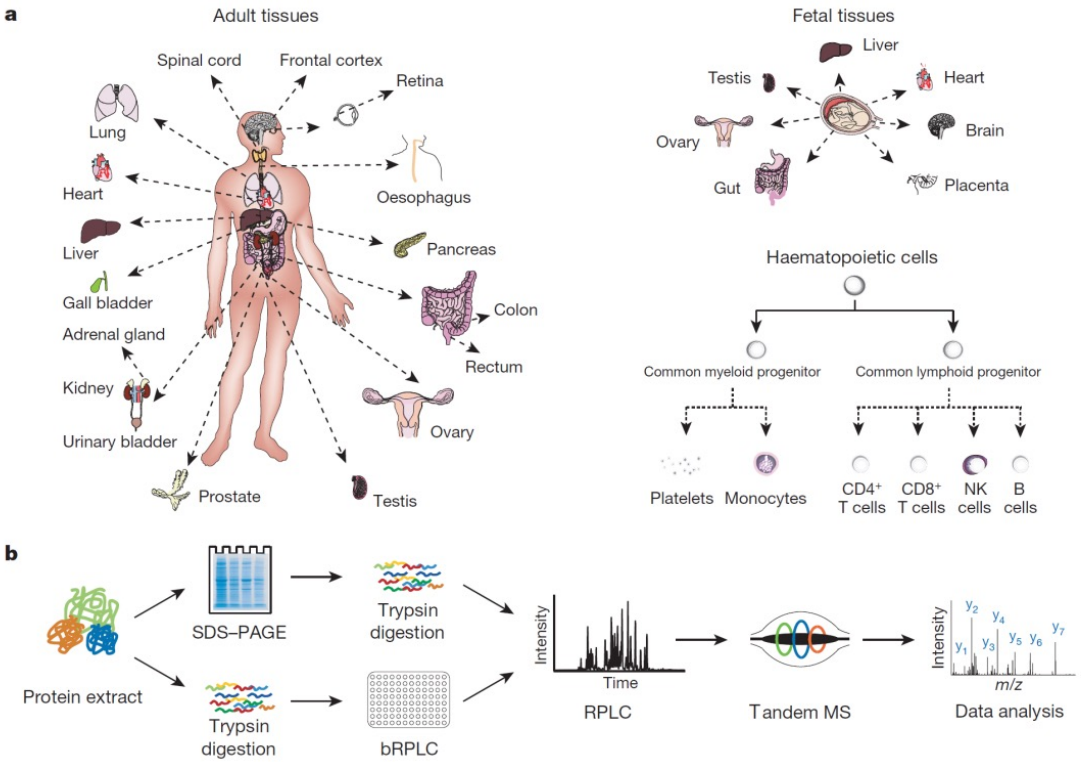
A draft map of the human proteome

Min-Sik Kim[1,2], Sneha M. Pinto[3], Derese Getnet[1,4], Raja Sekhar Nirujogi[3], Srikanth S. Manda[3], Raghothama Chaerkady[1,2], Anil K. Madugundu[3], Dhanashree S. Kelkar[3], Ruth Isserlin[5], Shobhit Jain[5], Joji K. Thomas[3], Babylakshmi Muthusamy[3], Pamela Leal-Rojas[1,6], Praveen Kumar[3], Nandini A. Sahasrabuddhe[3], Lavanya Balakrishnan[3], Jayshree Advani[3], Bijesh George[3], Santosh Renuse[3], Lakshmi Dhevi N. Selvan[3], Arun H. Patil[3], Vishalakshi Nanjappa[3], Aneesha Radhakrishnan[3], Samarjeet Prasad[1],

- New, large collection of proteomics data
  - 30 histologically normal human samples
  - 17 adult tissues,
  - 7 fetal tissues
  - 6 purified primary haematopoietic cells
- 17,294 genes accounting for approximately 84% of the total annotated protein-coding genes in humans.



A draft map of the human proteome; Kim & Akhilesh Pandey et al., PMID: 24870542

Proteomics Databases: Peptide depositories

http://www.peptideatlas.org/builds/

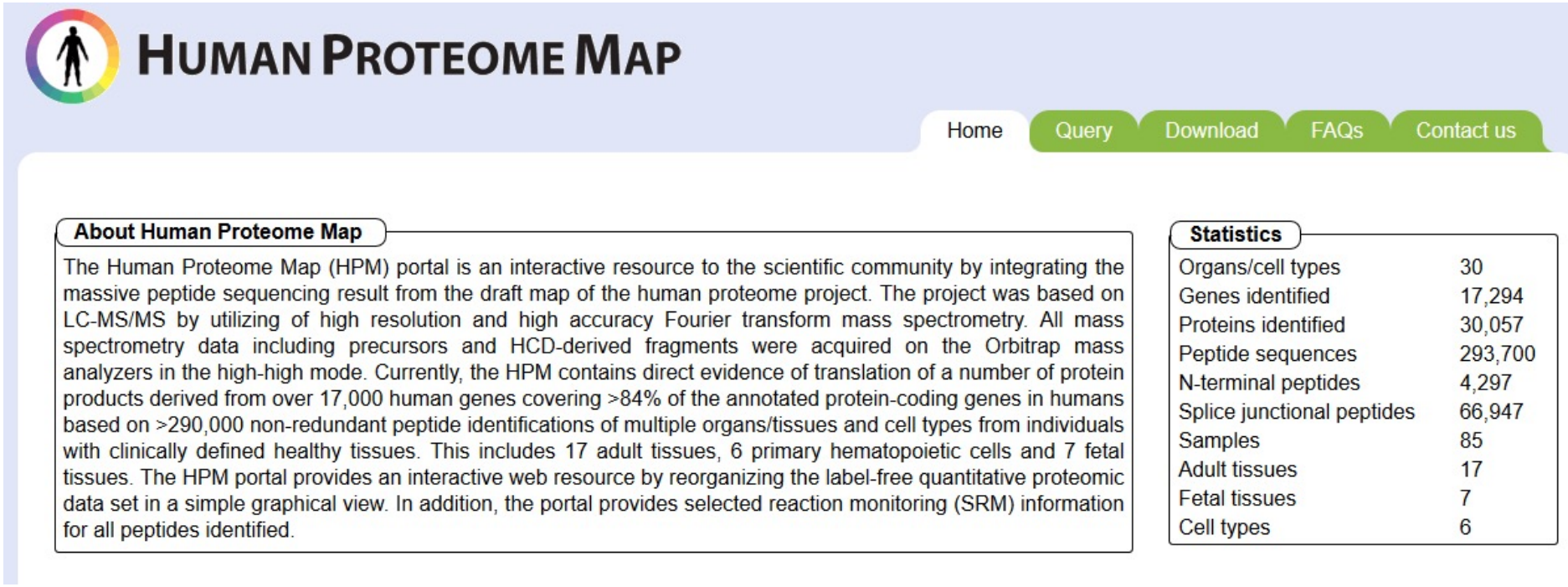
TaxID	Date	Number of Samples	Peptide Inclusion Cutoff	Number of Peptide-Spectrum Matches (PSMs)	Number of Distinct Peptides	Reference Database	Peptide Sequences	Peptide CDS Coordinates	Peptide CDS and Chromosomal Coordinates	Database Tables
9606	Mar 2015	1011	PSM FDR = 0.0002	133,638,335	1,025,698	Ensembl v78+UPSP+Trembl201412+14IPI 3.87+cRAP+nextprotSNP	APD_Hs_all.fasta	prot_map	chrom_map	MYSQL,XML

Protein Identification Terminology used in PeptideAtlas

http://www.peptideatlas.org/docs/protein_ident_terms.php

- Each PeptideAtlas build is associated with a reference database usually a combination of several protein sequence databases (Swiss-Prot, IPI, Ensembl ...)
- From the reference database, any protein that contains any observed peptide is considered to be a member of the Atlas.
- It is easy to see that the entire list of proteins in an Atlas is going to be highly redundant. Thus, we label each Atlas protein using the terminology below.
  - The term '''observed peptides''' in this context refers to the set of peptides in the PeptideAtlas build.
  - These peptides are selected using a PSM (peptide spectrum match)

Proteomics Databases: Peptide depositories



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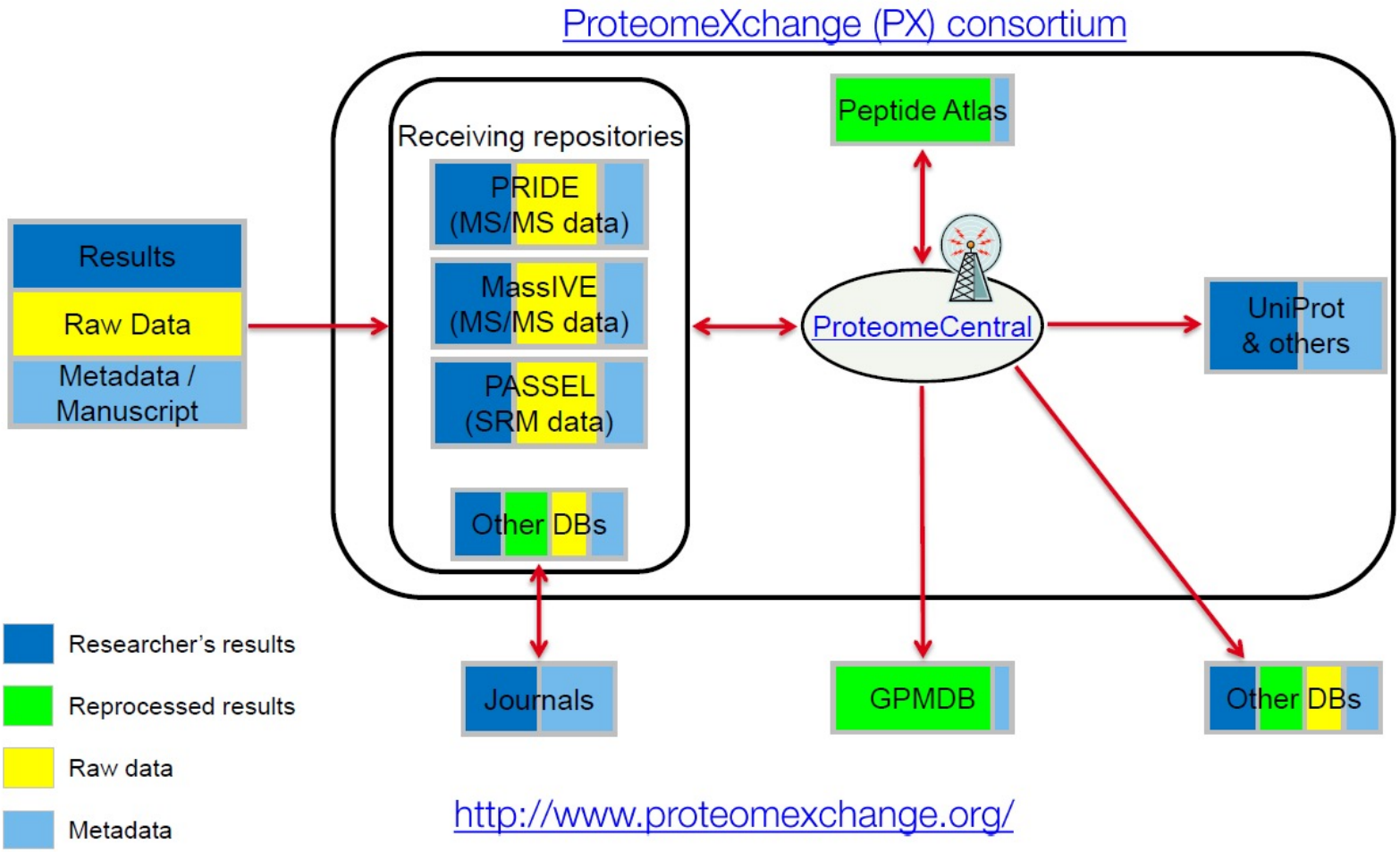
Kim & Akhilesh Pandey et al., ***Nature*** , 2014. PMID: 24870542

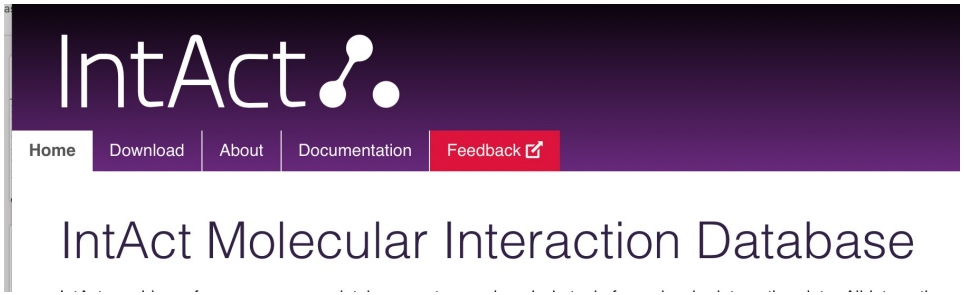
Proteomics Databases: Integrated Resources

http://www.proteomexchange.org/

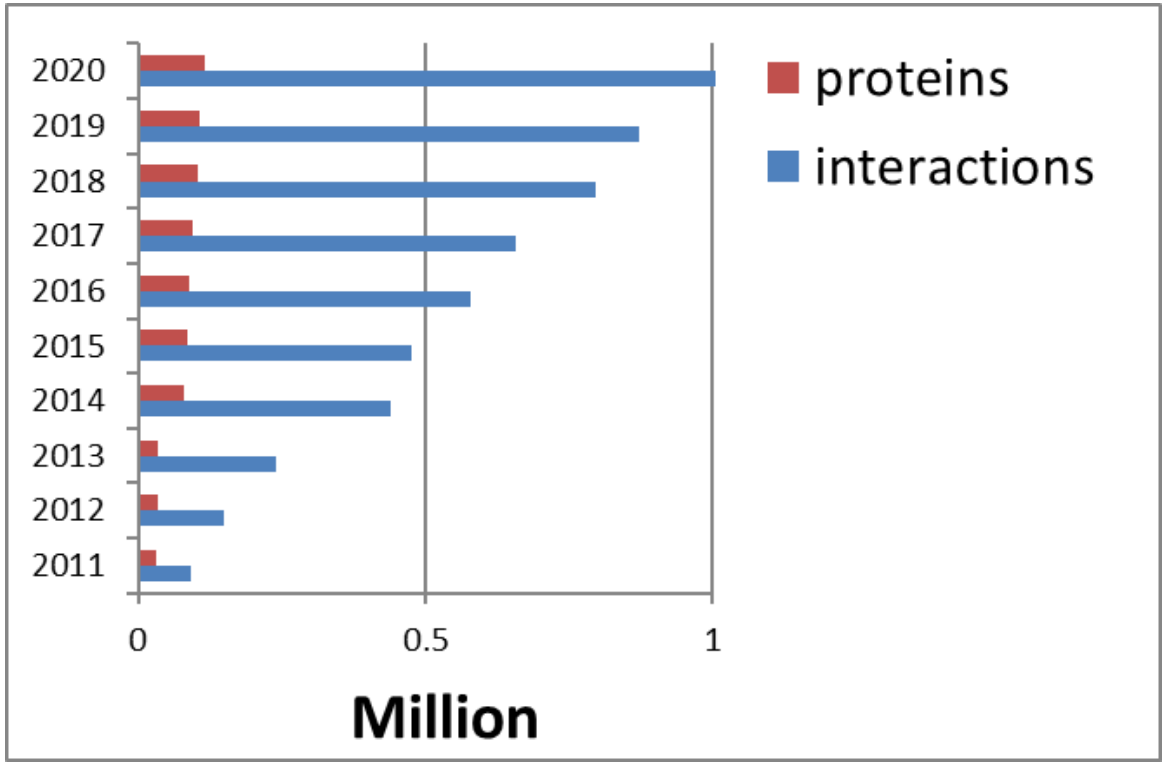


Slide modified from "Computational Mass Spectrometry-Based Proteomics 6th Maxquant Summer School" 21-25 July 2014
Emanuele Alpi, UniProt and PRIDE Development

Protein-Protein Interaction Databases



https://www.ebi.ac.uk/intact/home



2022

- Interactors: 118,213
- Interactions: 1,155,201

+ 24,605 interactions
- *1,068 proteins*

2021 Data Content

- Publications: **22037**
- Interactions: **1130596**
- Interactors: **119281**

+ 94,927 interactions
+ 3,902 proteins

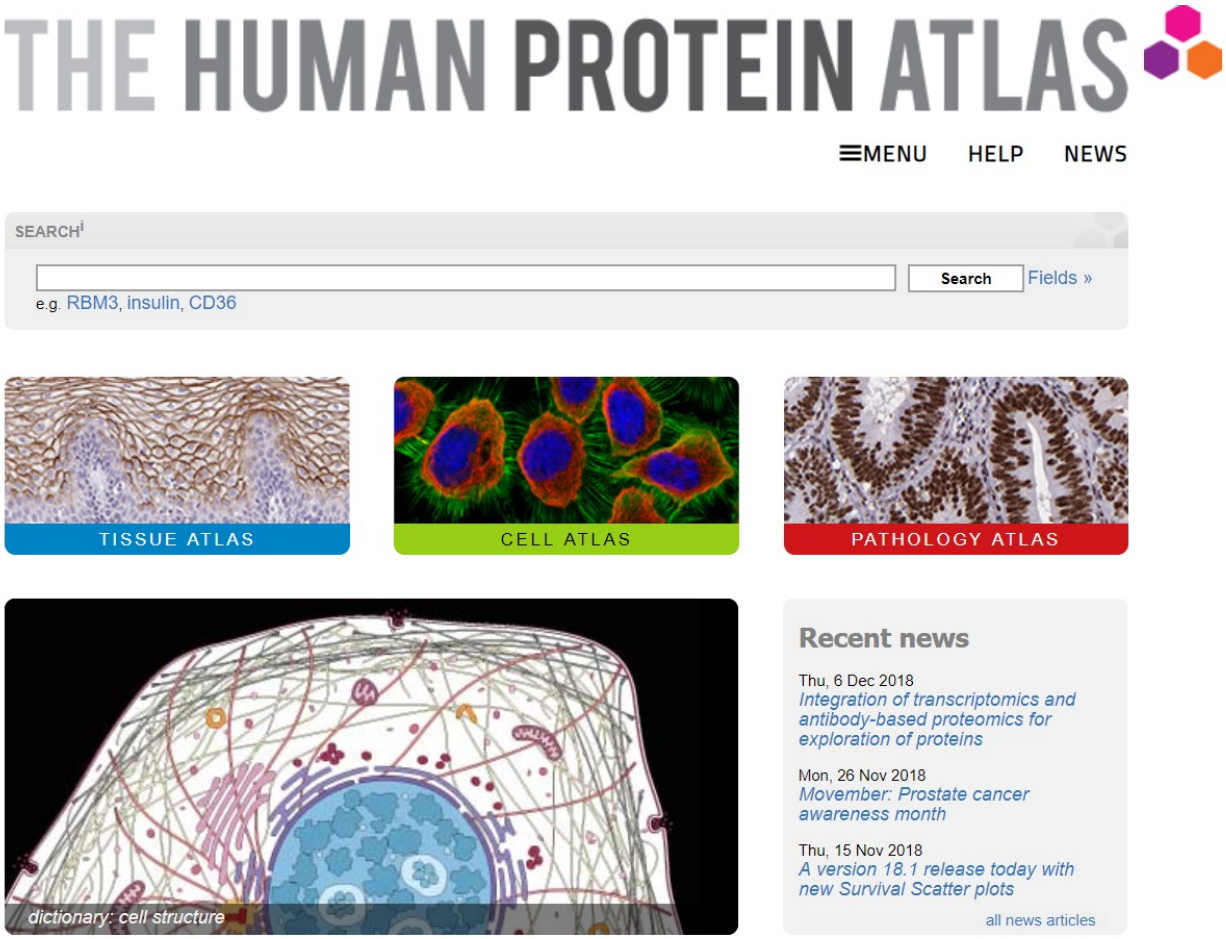
2020 Data Content

- Publications: **21086**
- Interactions: **1035669**
- Interactors: **115379**

+ 162,823 interactions
+ 6,887 proteins

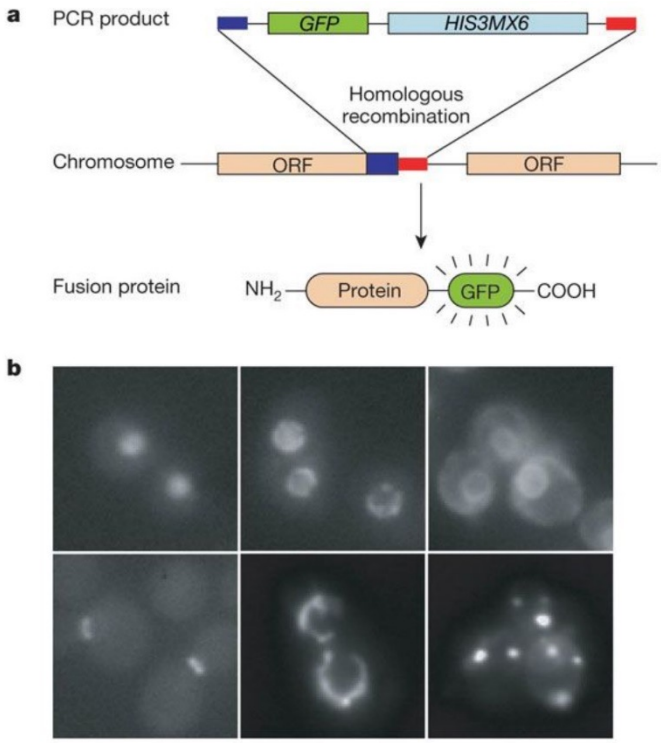
Proteomics Databases: Integrated Resources Beyond Mass Spectrometry

http://www.proteinatlas.org/



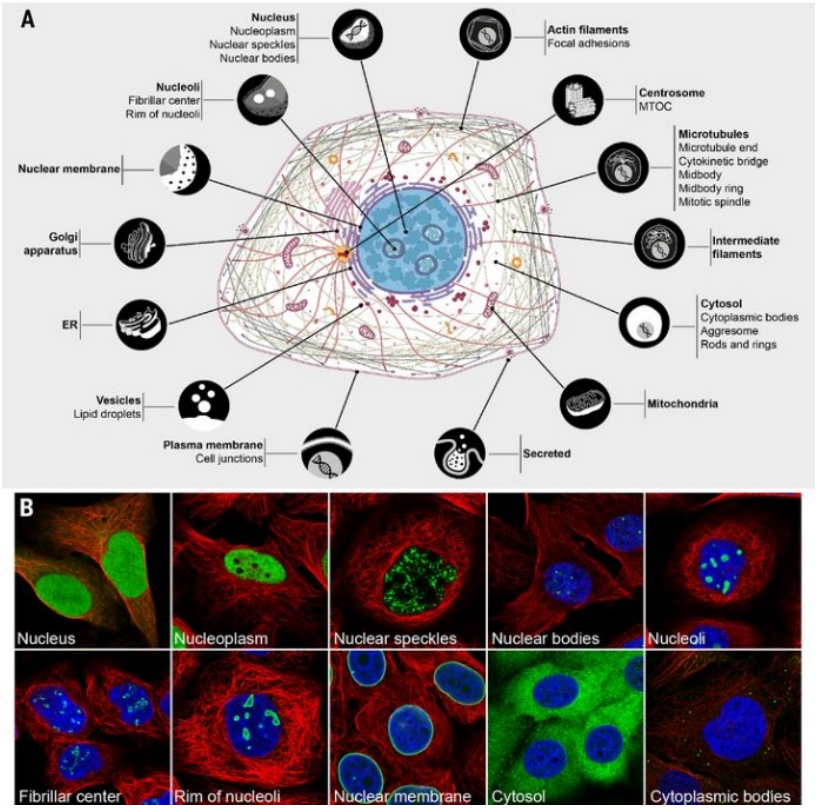
Proteomics Databases: Integrated Resources Beyond Mass Spectrometry

>4,000 GFP-Gene Fusions



Huh et al., Global analysis of protein localization in budding yeast. Nature. 2003 PubMed:14562095

>13,000 Antibodies



Thul PJ, et al. A subcellular map of the human proteome. Science. 2017. PubMed:28495876

Proteomics at single cell resolution

nature

Vol 441|15 June 2006|doi:10.1038/nature04785

ARTICLES

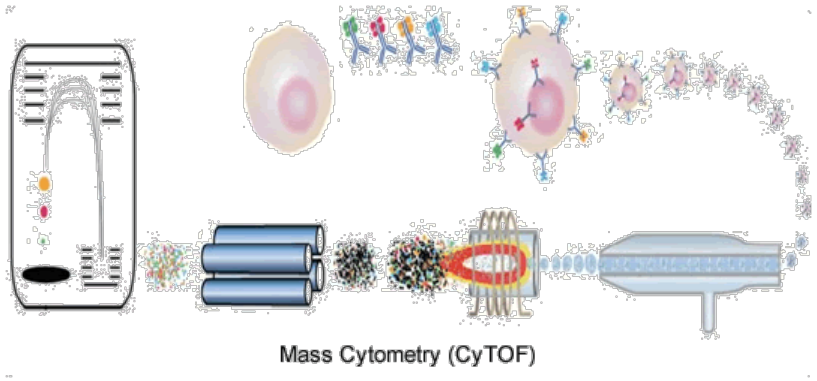
Single-cell proteomic analysis of *S. cerevisiae* reveals the architecture of biological noise

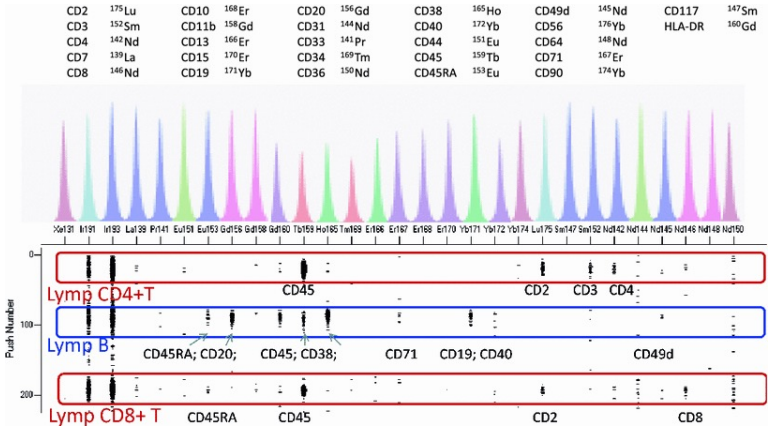
John R. S. Newman[1,2], Sina Ghaemmaghami[1,2]†, Jan Ihmels[1,2], David K. Breslow[1,2], Matthew Noble[1], Joseph L. DeRisi[1,3] & Jonathan S. Weissman[1,2]

A major goal of biology is to provide a quantitative description of cellular behaviour. This task, however, has been hampered by the difficulty in measuring protein abundances and their variation. Here we present a strategy that pairs high-throughput flow cytometry and a library of GFP-tagged yeast strains to monitor rapidly and precisely protein levels at single-cell resolution. Bulk protein abundance measurements of >2,500 proteins in rich and minimal media provide a detailed view of the cellular response to these conditions, and capture many changes not observed by DNA microarray analyses. Our single-cell data argue that noise in protein expression is dominated by the stochastic production/destruction of messenger RNAs. Beyond this global trend, there are dramatic protein-specific differences in noise that are strongly correlated with a protein's mode of transcription and its function. For example, proteins that respond to environmental changes are noisy whereas those involved in protein synthesis are quiet. Thus, these studies reveal a remarkable structure to biological noise and suggest that protein noise levels have been selected to reflect the costs and potential benefits of this variation.

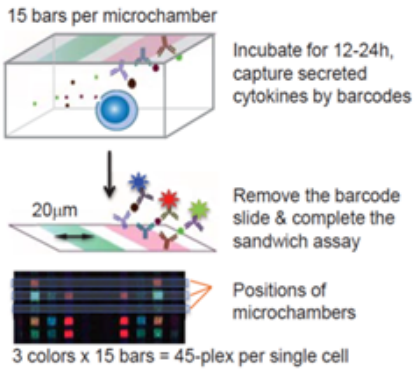
Proteomics at single cell resolution

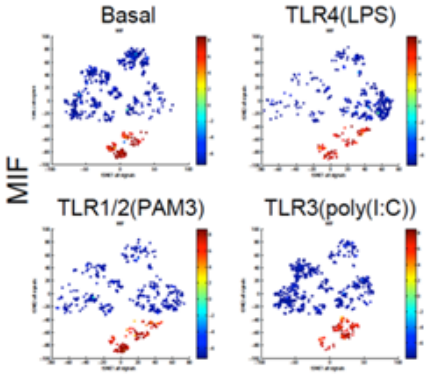
CyTOF





Single cell protein “capture” technology





Lu Y #, Xue Q #, Eisele MR, Sulistijo E, Brower K, Han L, Amir ED, Pe’er D, Miller-Jensen K *, and Fan R *, Highly multiplexed profiling of single-cell effector functions reveals deep functional heterogeneity in response to pathogenic ligands, ***Proc. Natl. Acad. Sci. U.S.A.***,112(7), 607-615 (2015).

Major challenges prevent complete proteome analysis

- **Proteomics is sample limited**
  - Recombinant DNA polymerases revolutionized genome sequencing by allowing for amplification of DNA samples
  - Proteomics has no “polymerase” or amplification method and must contend with natural abundancies
- **Mass spectrometry has limitations**
  - No mass spectrometer, or method, can yet provide full amino-acid resolution of a proteome

Transformative Opportunities for Single-Cell Proteomics

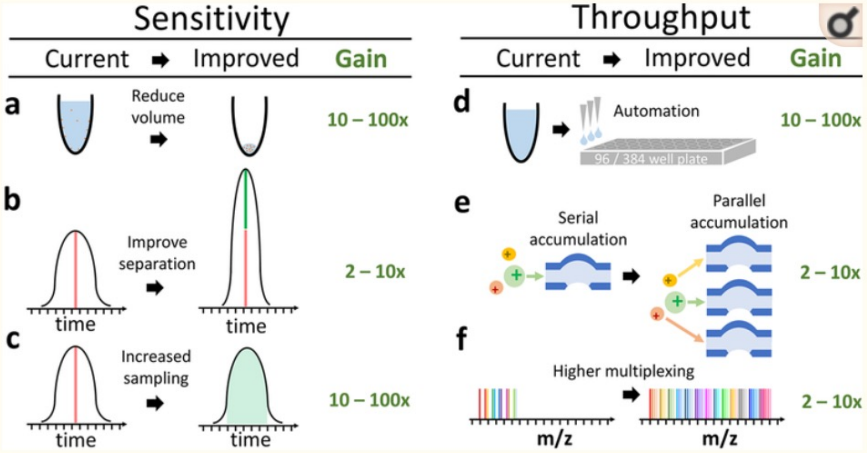


Figure 3.

Transformative opportunities for improving the quantification of single-cell proteomes. (a) Most bulk samples prepared for MS have volume of 10−100 μL.[11,12,17] Reducing the volume for sample preparation to 1 to 2 nL[13] can significantly reduce protein losses from surface adsorption. (b) The sharper the separation peaks, the larger the fraction of the ions can be analyzed for a fixed sampling (injection) time. Sharper peaks can be achieved by reducing the bore of LC columns, using monolithic columns, PLOT columns,[27] or capillary electrophoresis.[25] (c) Typically elution peaks have a full width at the base of ∼60 s and about 10−15 s at midheight, whereas ions for MS2 are sampled for mere milliseconds. These settings are typical for bulk proteomics and result in sampling <1% of the ions delivered to the instruments. Thus increasing the sampling time 100× can substantially increase the ions analyzed by MS, the sensitivity, and the accuracy of quantification. While, the panel displays sampling during the apex of the peak, this cannot always be achieved for all ions. (d) Automated liquid handling and 96/384-well plates can increase the consistency of sample preparation, decrease volumes to the nanoliter range, and increase throughput. (e) Parallel accumulation and serial injection of ions can afford increased ion sampling without reducing throughput. (f) A larger number of barcodes will increase the number cellular proteomes quantified per run without reducing proteome coverage or ion sampling.

Achieving high chromatographic resolution and quantifying thousands of proteins requires an hour of LC−MS/MS time or more. Thus to quantify the proteomes of thousands of single cells within hours, we need to quantify many cells per LC− MS/MS run. Such multiplexing can be achieved by isobaric chemical barcoding.[37,38] These barcodes are chemically identical but distinguishable by MS due to their different isotopic compositions.

Harrison Specht & Nikolai Slavov. Transformative Opportunities for Single-Cell Proteomics. J Proteome Res. 2018 Aug 3 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6089608/

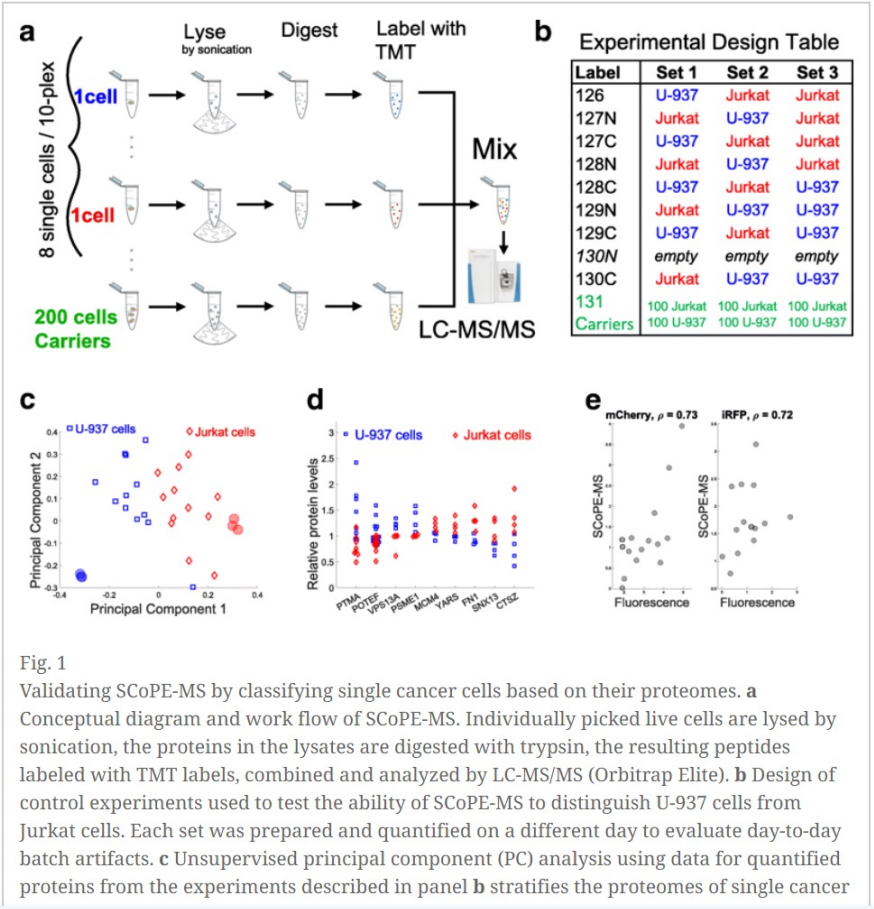
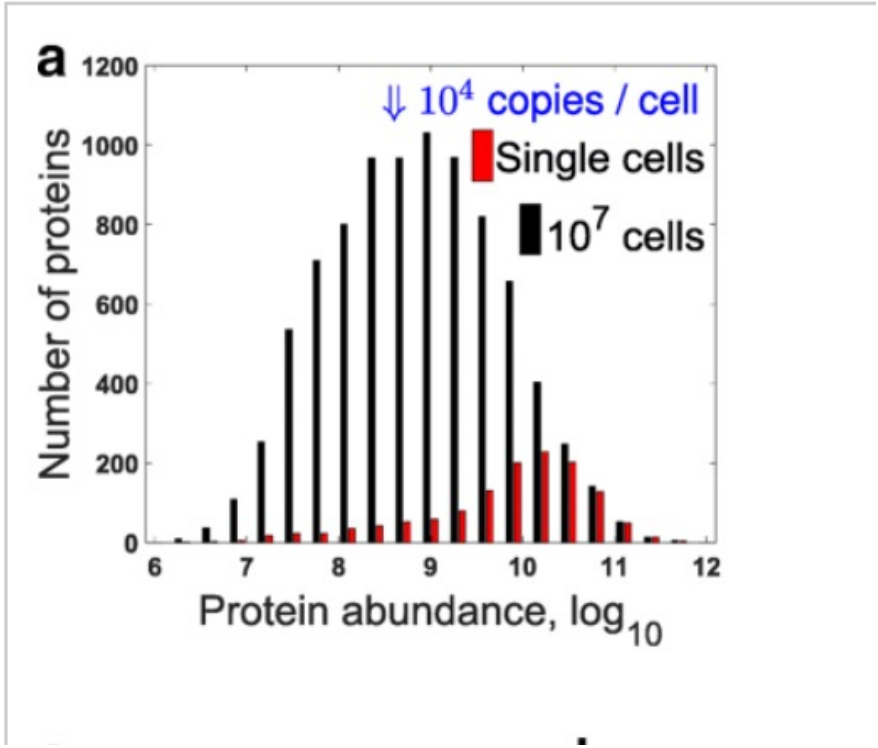


Fig. 1
Validating SCoPE-MS by classifying single cancer cells based on their proteomes. **a** Conceptual diagram and work flow of SCoPE-MS. Individually picked live cells are lysed by sonication, the proteins in the lysates are digested with trypsin, the resulting peptides labeled with TMT labels, combined and analyzed by LC-MS/MS (Orbitrap Elite). **b** Design of control experiments used to test the ability of SCoPE-MS to distinguish U-937 cells from Jurkat cells. Each set was prepared and quantified on a different day to evaluate day-to-day batch artifacts. **c** Unsupervised principal component (PC) analysis using data for quantified proteins from the experiments described in panel **b** stratifies the proteomes of single cancer



Method | Open Access

SCoPE-MS: mass spectrometry of single mammalian cells quantifies proteome heterogeneity during cell differentiation

Bogdan Budnik, Ezra Levy, Guillaume Harmange and Nikolai Slavov

Genome Biology 2018 **19**:161
https://doi.org/10.1186/s13059-018-1547-5
Received: 20 February 2018 | **Accepted:** 19 September 2018 | **Published:** 22 October 2018