# Proteomics & Protein-Protein Interactions

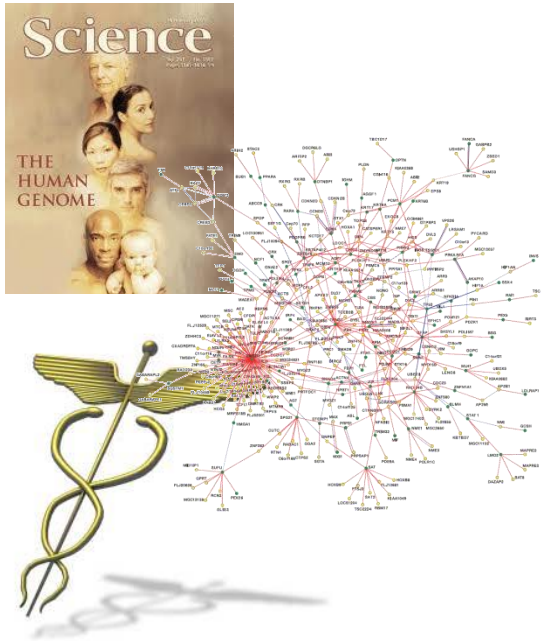

## Jesse Rinehart, PhD

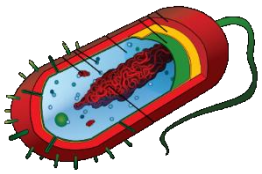

### Biomedical Data Science: Mining & Modeling

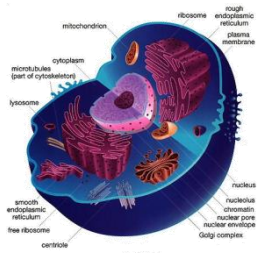
### CBB 752, Spring 2021

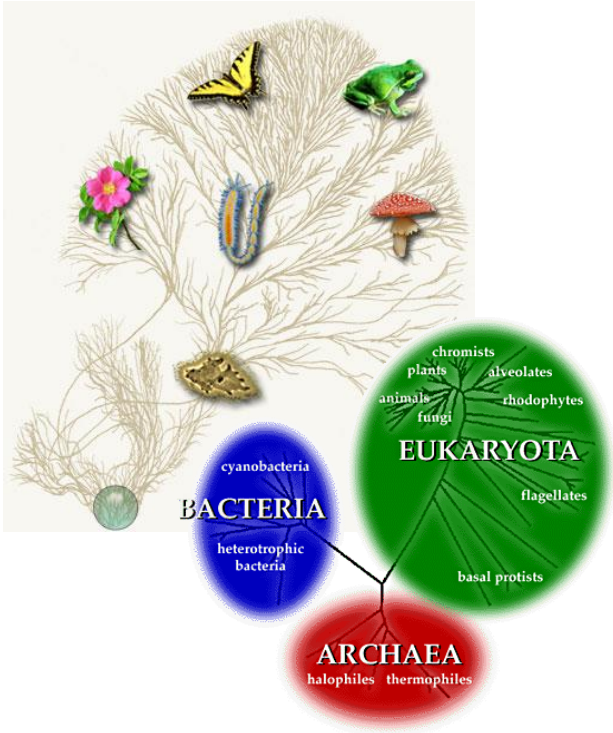

Cellular & Molecular Physiology
Yale University School of Medicine

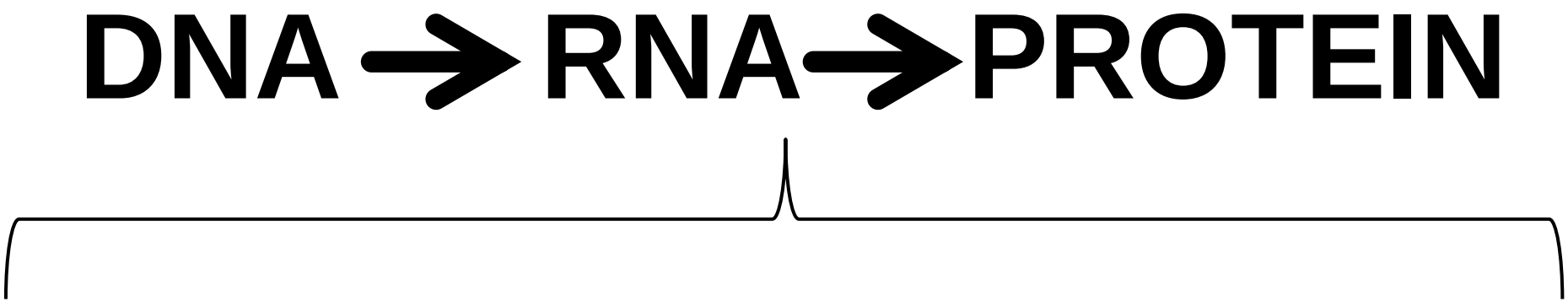

# DNA → RNA → PROTEIN







DNA → RNA → PROTEIN
GENOME EDITING

# DNA → RNA → PROTEIN

## RNA-Guided Human Genome Engineering via Cas9

2013

Prashant Mali,[1]* Luhan Yang,[1,3]* Kevin M. Esvelt,[2] John Aach,[1] Marc Guell,[1] James E. DiCarlo,[4] Julie E. Norville,[1] George M. Church[1,2]†

## Multiplex Genome Engineering Using CRISPR/Cas Systems

2013

Le Cong,[1,2]* F. Ann Ran,[1,4]* David Cox,[1,3] Shuailiang Lin,[1,5] Robert Barretto,[6] Naomi Habib,[1] Patrick D. Hsu,[1,4] Xuebing Wu,[7] Wenyan Jiang,[8] Luciano A. Marraffini,[8] Feng Zhang[1]†

Home ▸ About Us ▸ Archives ▸ January 2016 ▸

## U.S. Summit Draws Attention to Technology with Potential, Peril

By Karen Pallarito (HealthDay News)
Uploaded on December 21, 2015

Dec 2015

## *Chinese Scientist Claims to Use Crispr to Make First Genetically Edited Babies*

The New York Times

Nov. 2018

THE CRISPR REVOLUTION

## Gene-Edited 'Supercells' Make Progress In Fight Against Sickle Cell Disease

November 19, 2019 · 7:01 AM ET
Heard on Morning Edition

Nov. 2019

NEWS · 07 OCTOBER 2020

## Pioneers of revolutionary CRISPR gene editing win chemistry Nobel

Aug. 2020

Emmanuelle Charpentier and Jennifer Doudna share the award for developing the precise genome-editing technology.

# 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

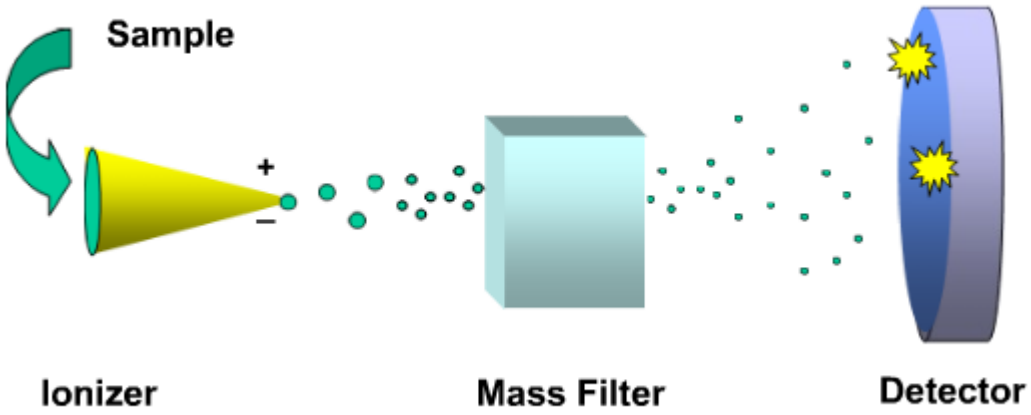
**Overview**

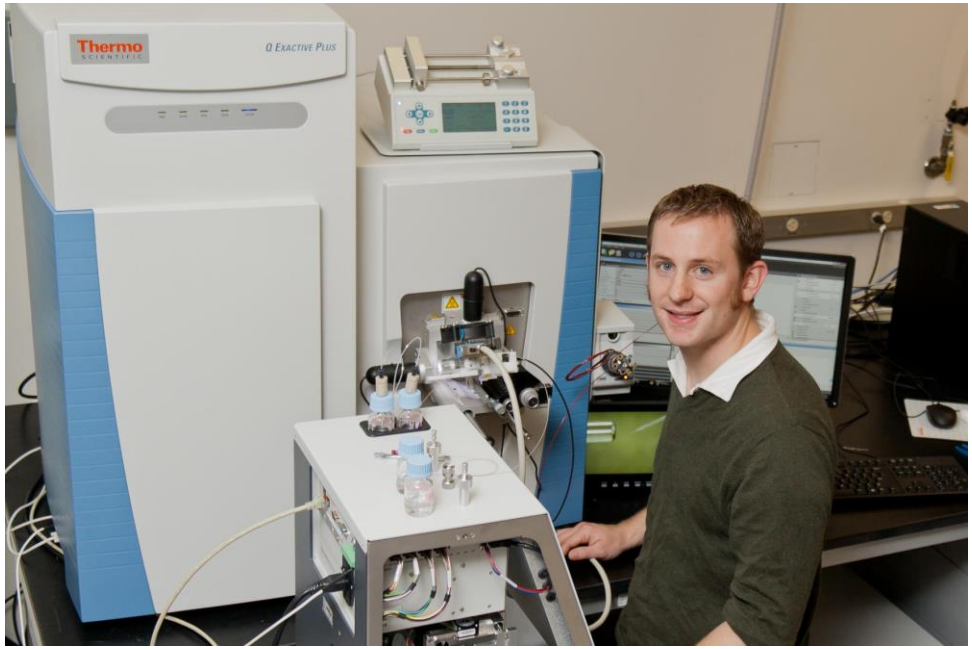
- **Techniques & Technologies**
  - Mass Spectrometry
  - Protein-Protein Interactions
  - Quantitative Proteomics
- **Applications**
  - Representative Studies
- **Putting it all together….**
  - Databases & Pathways

# 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 has to 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

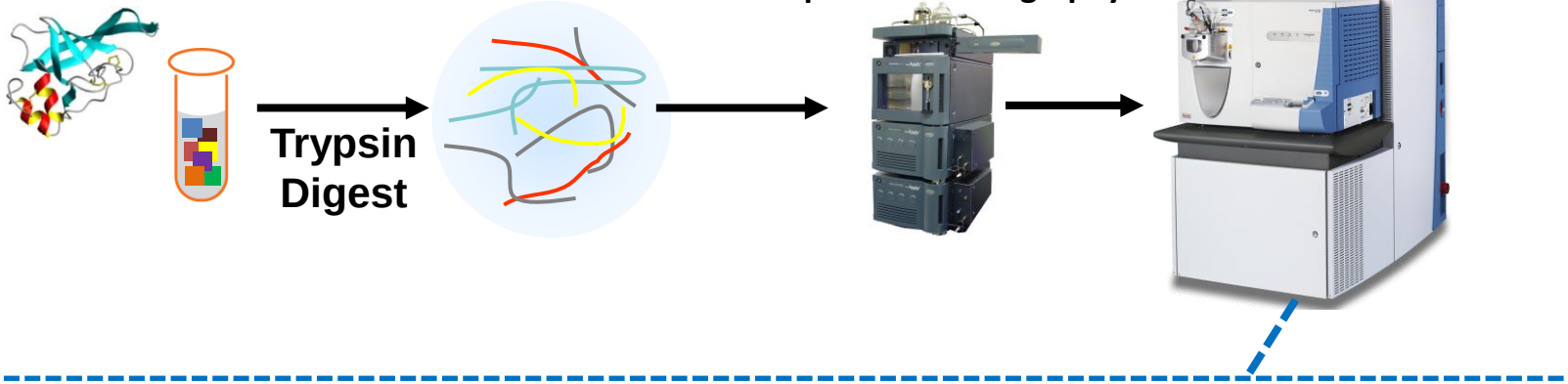
- Matrix Assisted Laser Desorption Ionization (MALDI)
  Tanaka K, Waki H, Ido Y, et al. *Rapid Commun Mass Spectrom* 1988

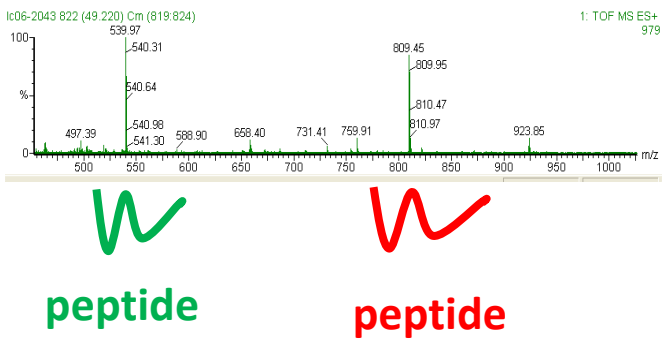
- 2002 Nobel Prize in Chemistry awarded to
  John B. Fenn & Koichi Tanaka

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

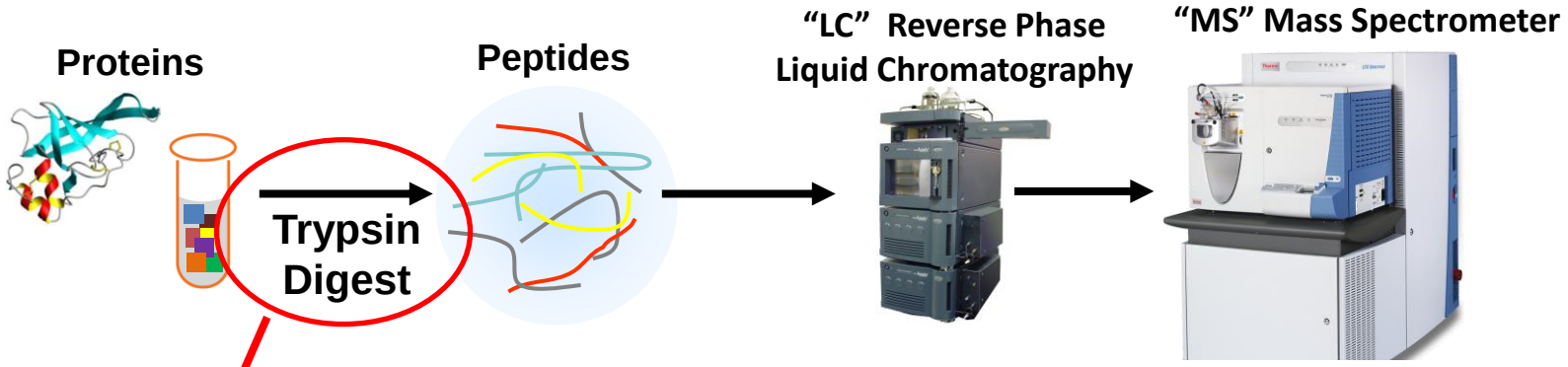
*Matthias Mann (Yale University; Ph.D.; 1988; Chemical Engineering) trained with John Fenn during some of the breakthrough work at Yale

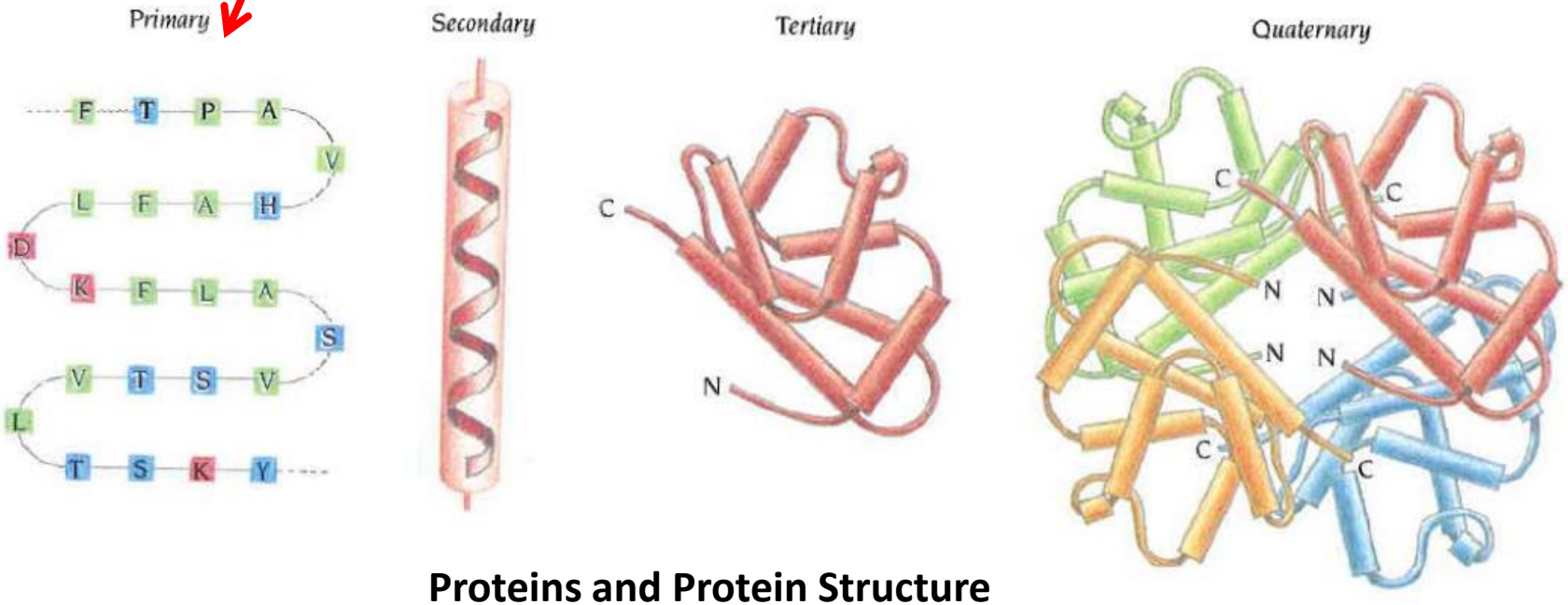
# Typical work flow for LC-MS "shotgun proteomics"



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

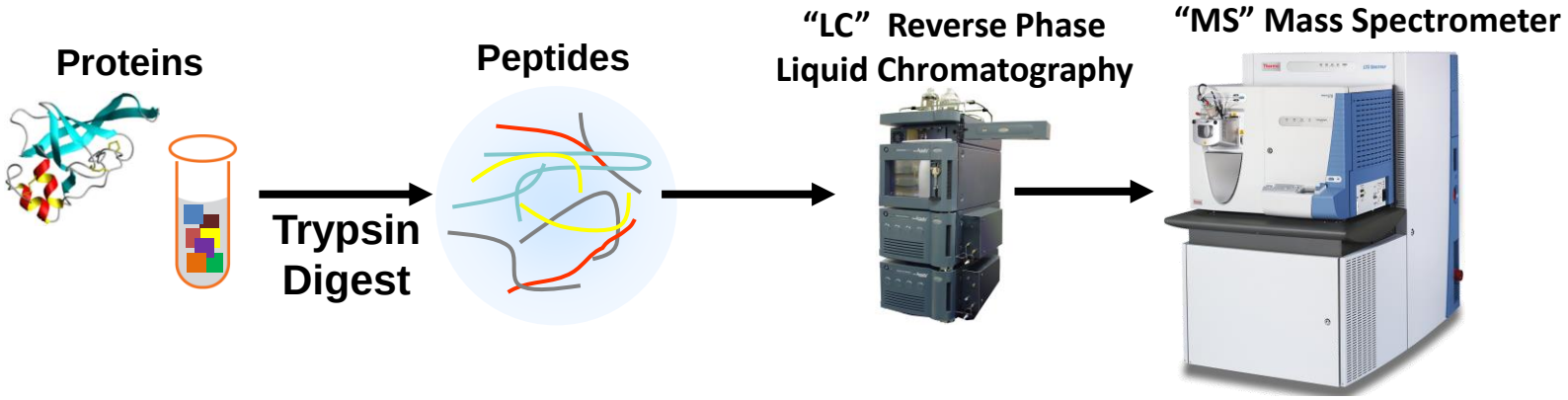


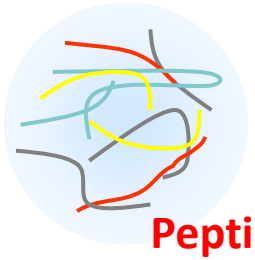




**Proteins and Protein Structure**

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

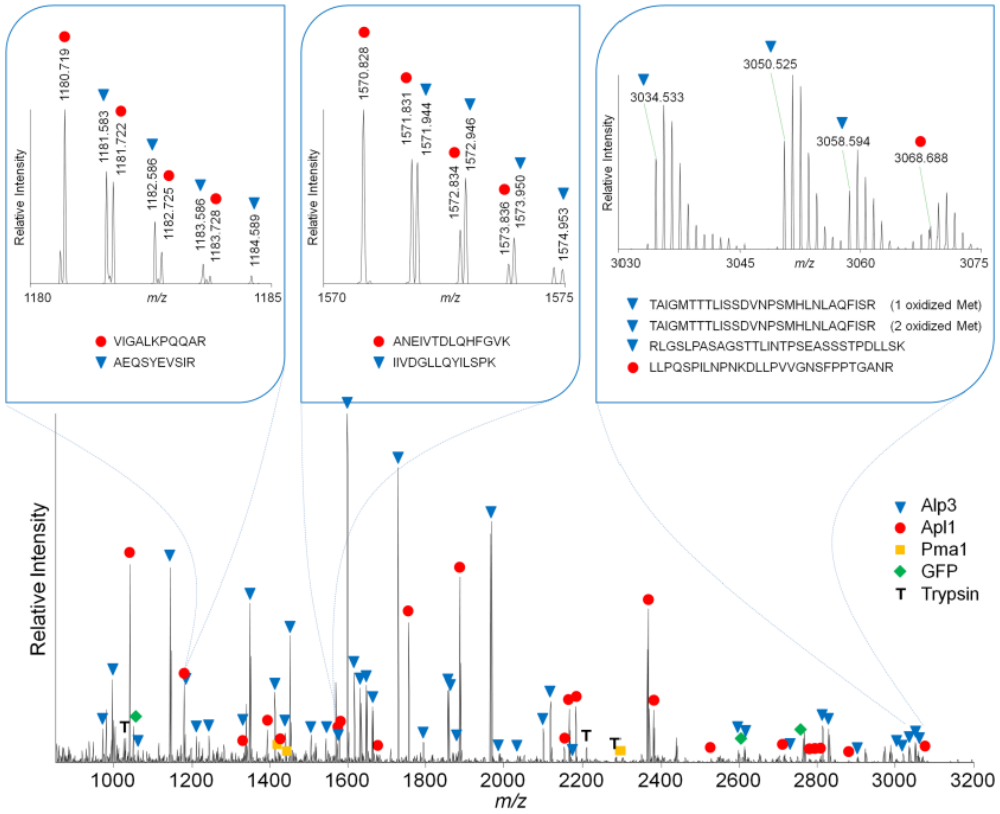
# The mass spectra of peptide mixtures are complex



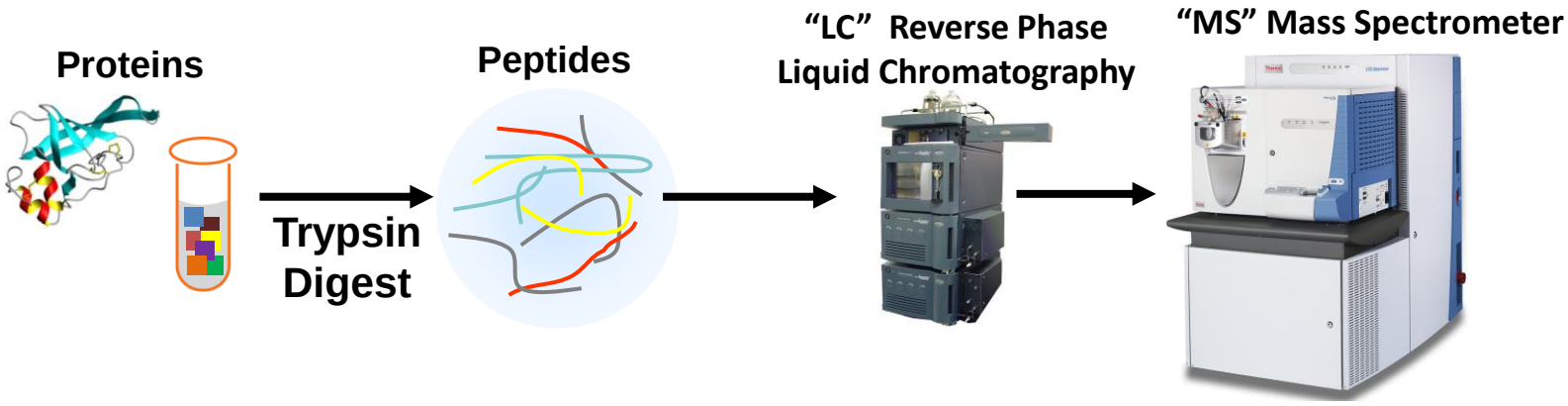



**Peptide ions have a mass (m) and a charge (z).**

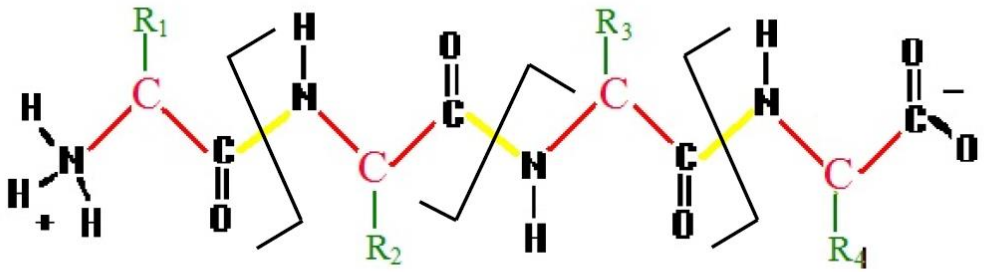
**100 Da peptide:**
**+1 = 100 m/z**
**+2 = 50 m/z**
**+3 = 33.3 m/z**



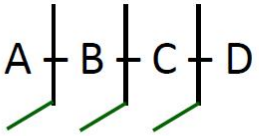
# Peptide ions are isolated, fragmented, and “sequenced”



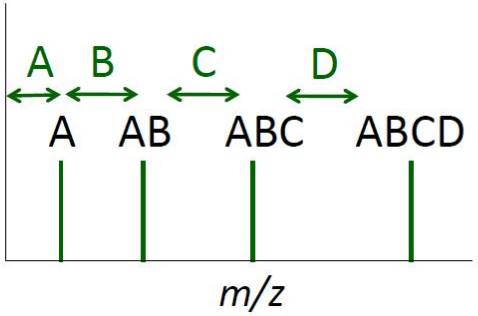

Peptide sequencing



Simplified concept of peptide fragmentation




**Peptide “ABCD”**



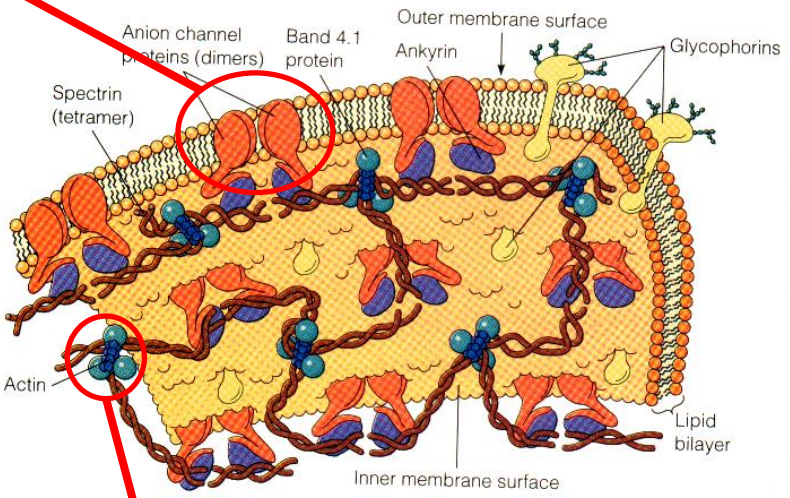

**Fragment Spectra of Peptide “ABCD”**

# 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
```



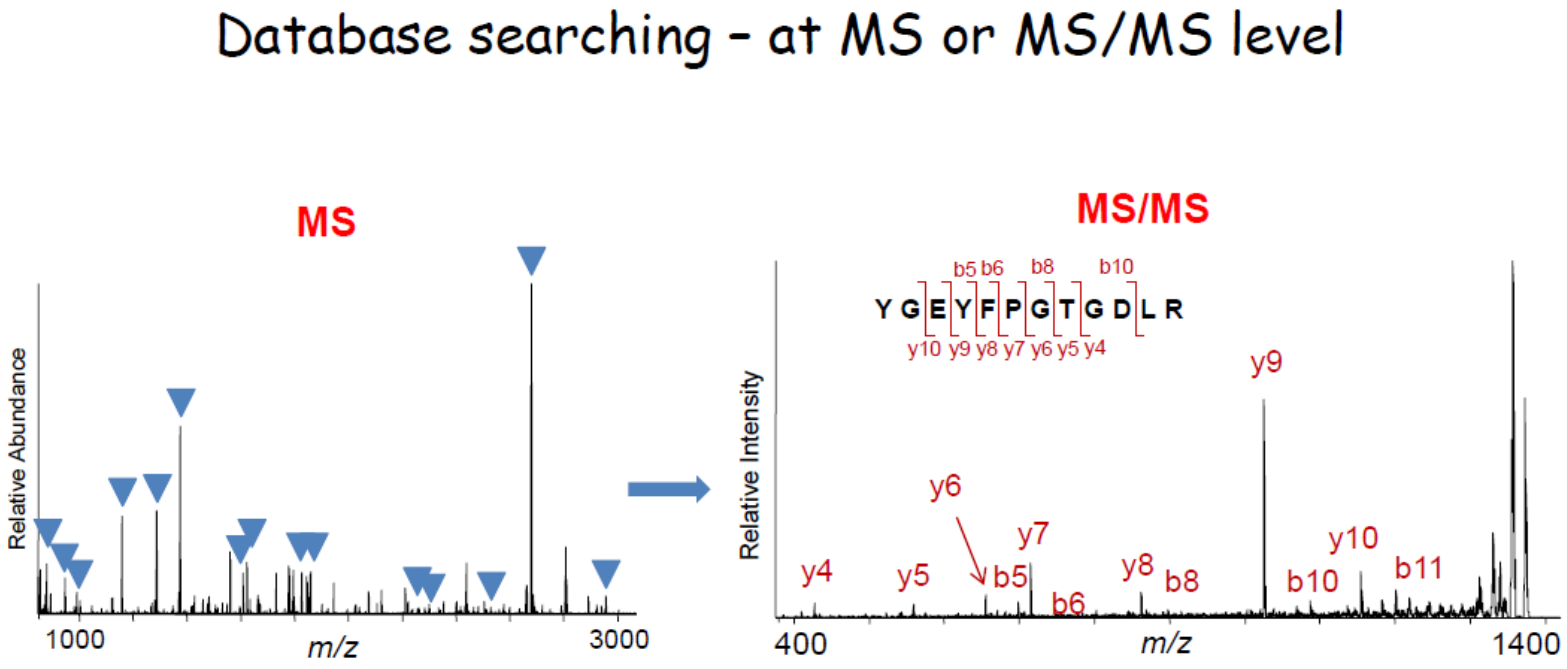

## β-actin

Matched peptides shown in Bold Red

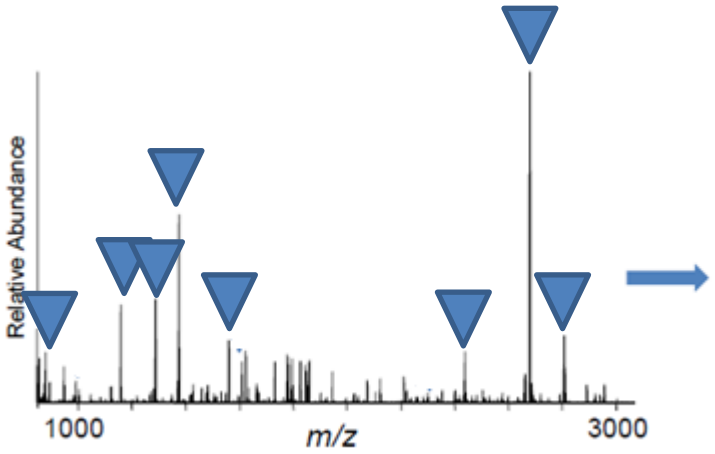
```
  1 MDDDIAALVV DNGSGMCKAG FAGDDAPRAV FPSIVGRPRH QGVMVGMGQK
 51 DSYVGDEAQS KRGILTLKYP IEHGIVTNWD DMEKIWHHTF YNELRVAPEE
101 HPVLLTEAPL NPKANREKMT QIMFETFNTP AMYVAIQAVL SLYASGRTTG
151 IVMDSGDGVT HTVPIYEGYA LPHAILRLDL AGRDLTDYLM KILTERGYSF
201 TTTAEREIVR DIKEKLCYVA LDFEQEMATA ASSSSLEKSY ELPDGQVITI
251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

# Computational Steps:

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

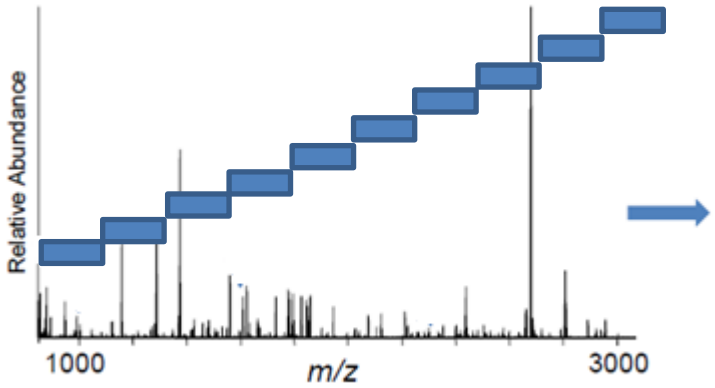


# **DIA** (Data-independent Acquisition) **vs. DDA** (Data-dependent Acquisition)




**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

**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**

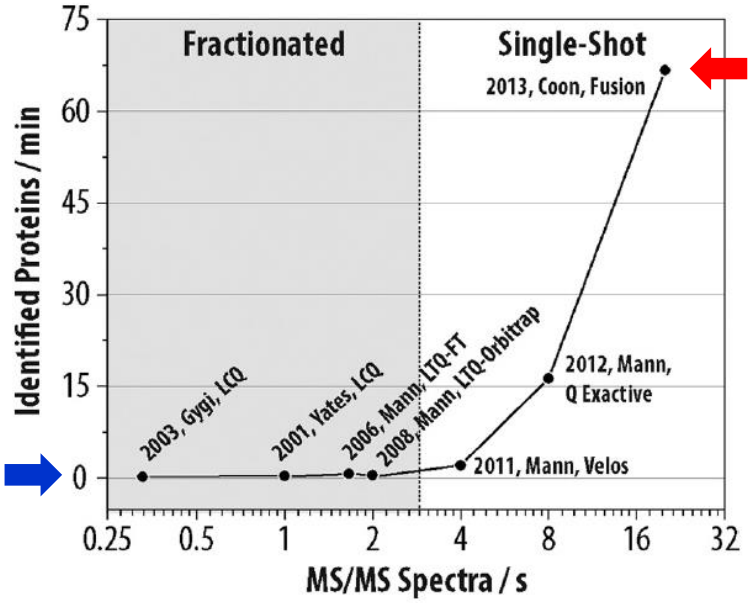



FIG. 5. **Rate of protein identifications as a function of mass spectrometer scan rate for selected large-scale yeast proteome analyses over the past decade.** Each data point is annotated with the year, corresponding author, type of MS system used, and reference number.

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

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

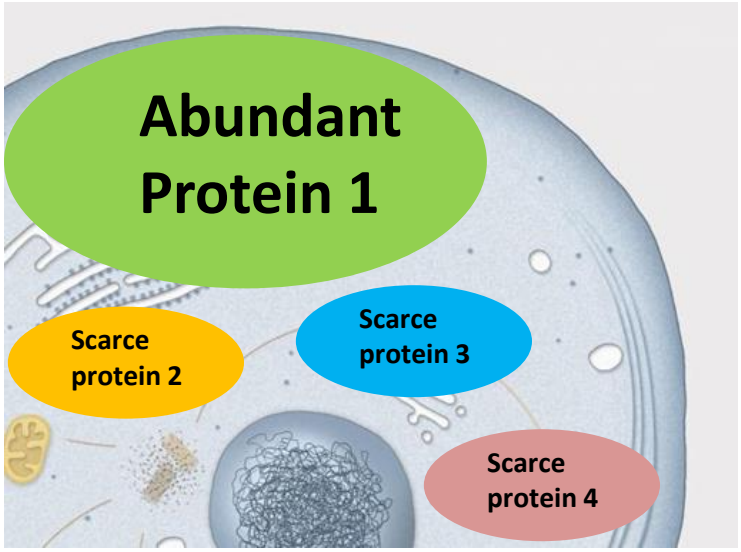
The one hour yeast proteome. Hebert AS, et a, Coon JJ.
*Mol Cell Proteomics*. 2014 PMID: 24143002 & *Nat Protoc.* 2015. PMID: 25855955

# Major challenges prevent complete proteome analysis

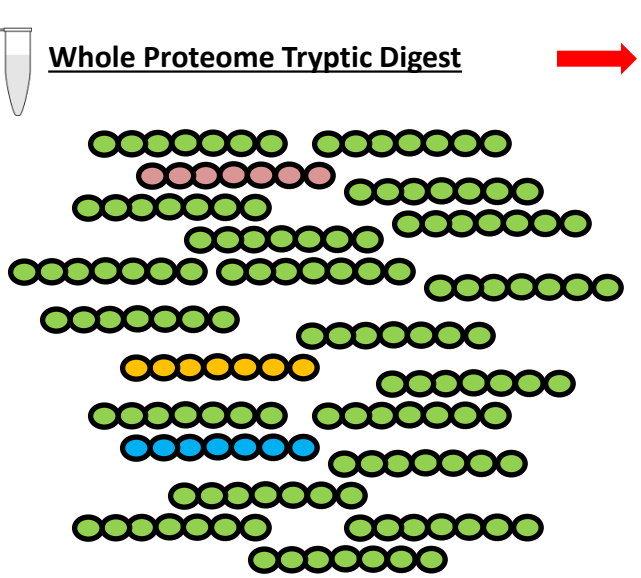
- **Proteomics is sample limited**
  - Recombinant DNA polymerases revolutionized genome sequencing by allowing for amplification of DNA samples
  - Proteomics has no “polymerase” or amplification method and must contend with natural abundancies
- **Mass spectrometry has limitations**
  - No mass spectrometer,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

# Challenge Question:

Cell with a 4 protein proteome




Whole Proteome Tryptic Digest



One LC-MS run

*(Hypothetical MS that can only identify one peptide)*

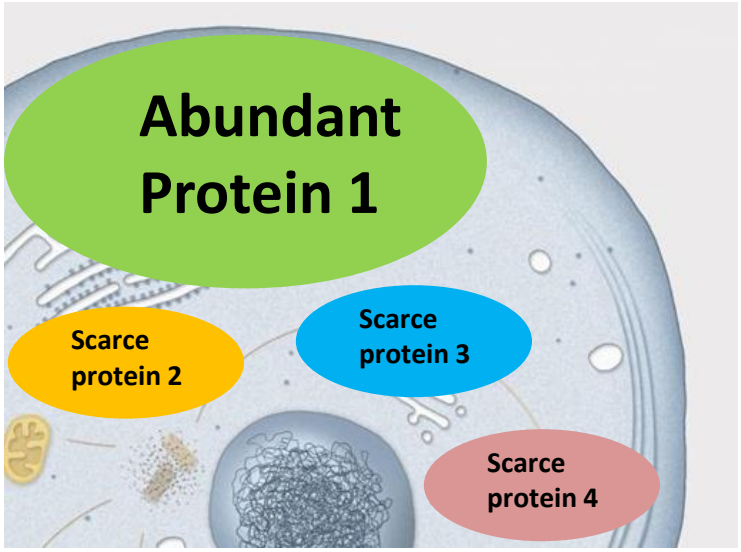
Protein 1 Identified

---

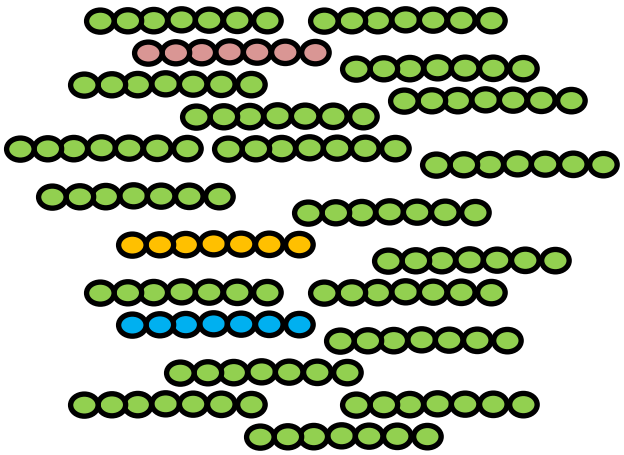
**Challenge Question:**

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

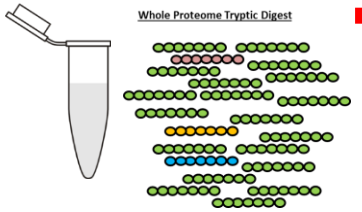


One LC-MS run

*(Hypothetical MS that can only identify one peptide)*

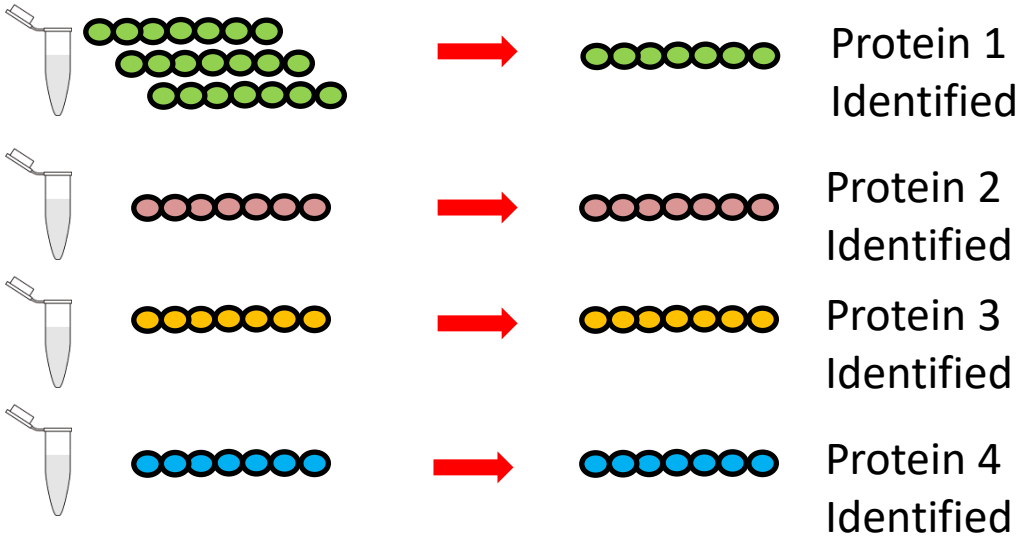
Protein 1 Identified

## Option #1: Peptide Fractionation




Chromatography + fractionation

4 separate LC-MS runs



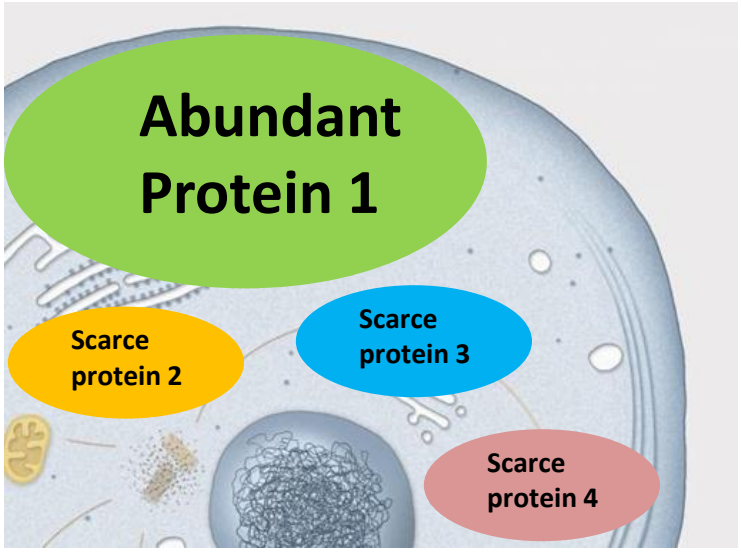
Protein 1 Identified

Protein 2 Identified

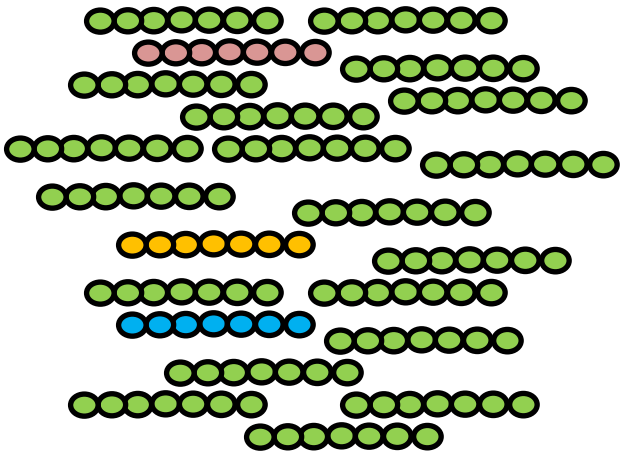
Protein 3 Identified

Protein 4 Identified

Cell with a 4 protein proteome




Whole Proteome Tryptic Digest



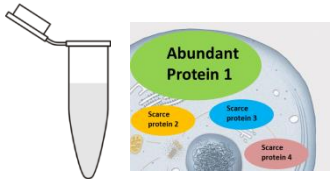
One LC-MS run

*(Hypothetical MS that can only identify one peptide)*

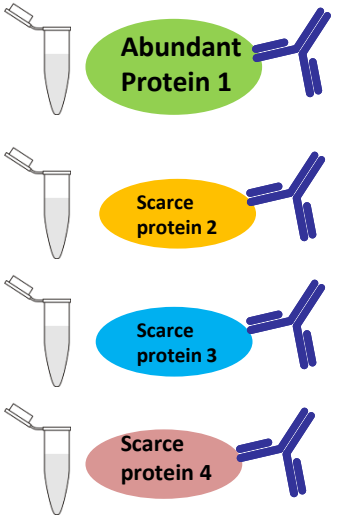
Protein 1 Identified

---

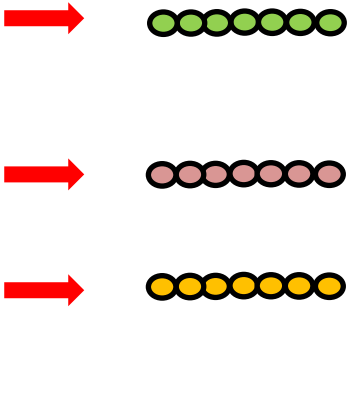
## Option #2: Proteome Fractionation (e.g. Immunoprecipitation)




Separate IP Tryptic Digest




4 separate LC-MS runs



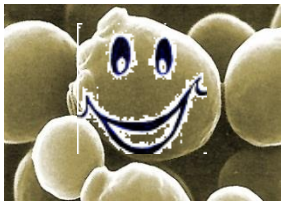
Protein 1 Identified

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.**

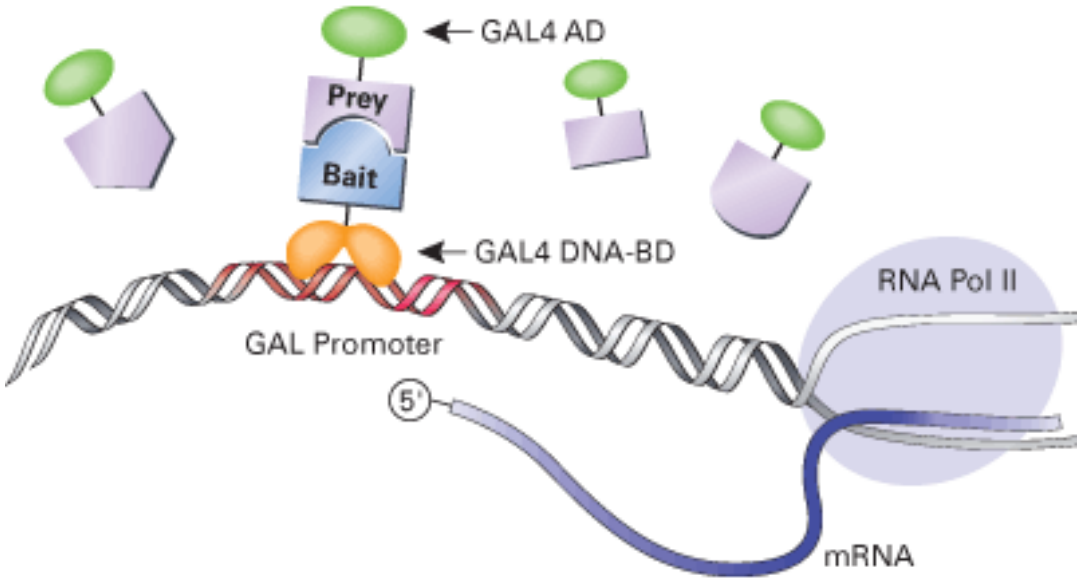
## 2009

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**

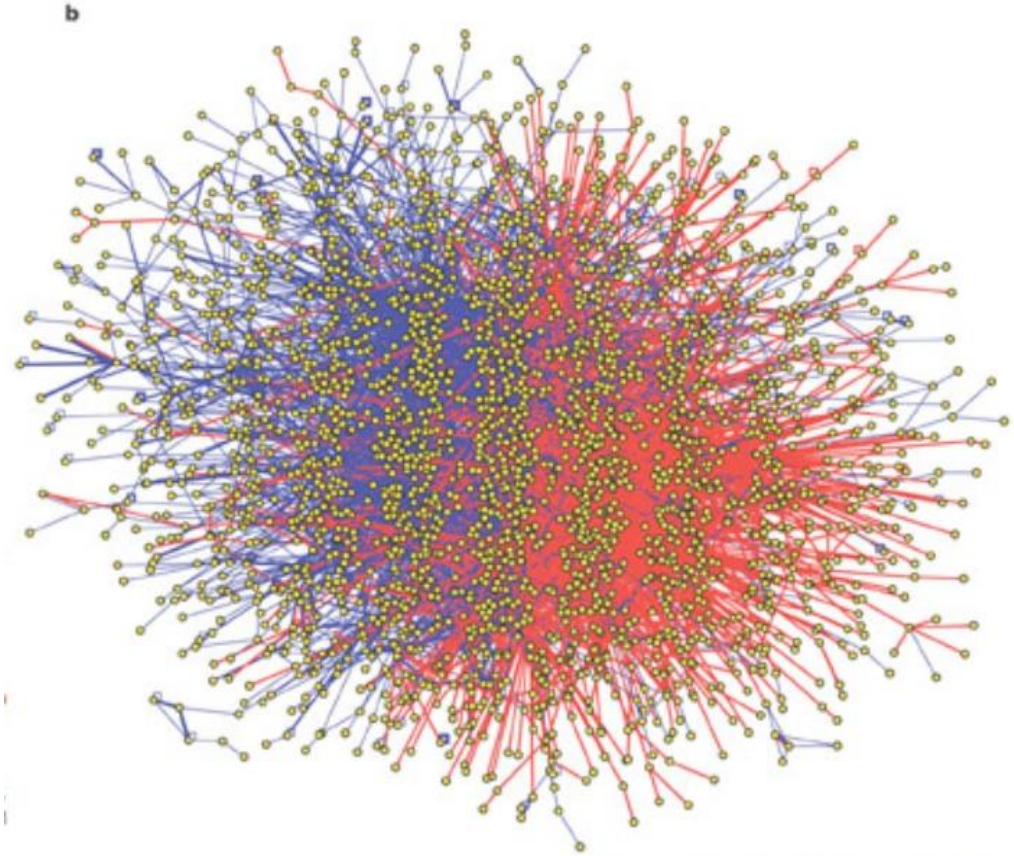
**Some Disadvantages**

- **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)

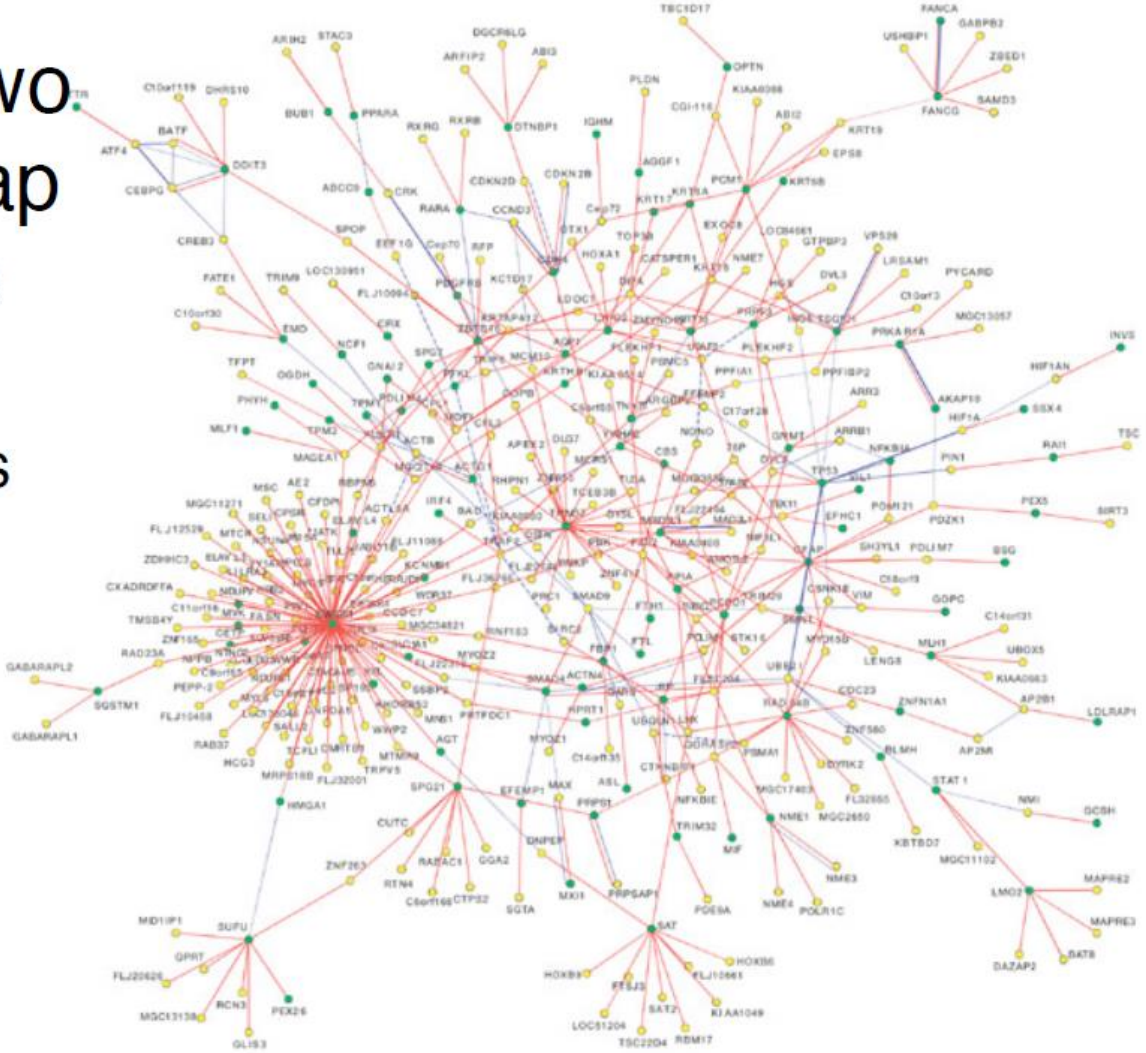
## 10,597 interactions




Rual et al. Nature 2005

# Human Two Hybrid Map Disease Genes

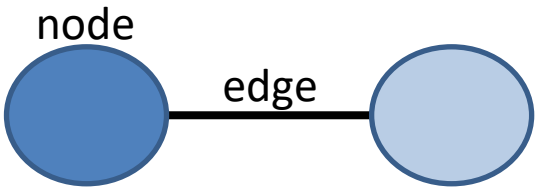
(121 genes (green))

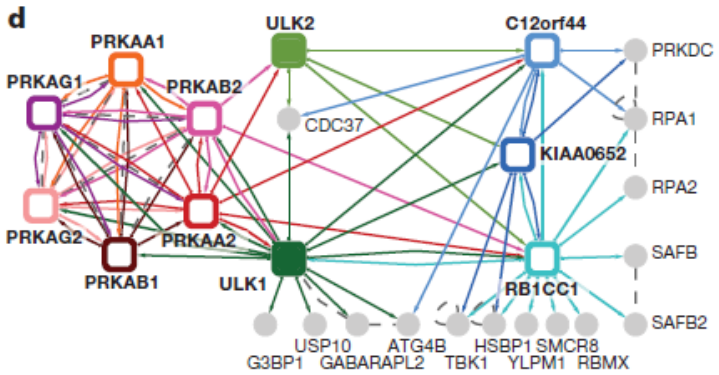


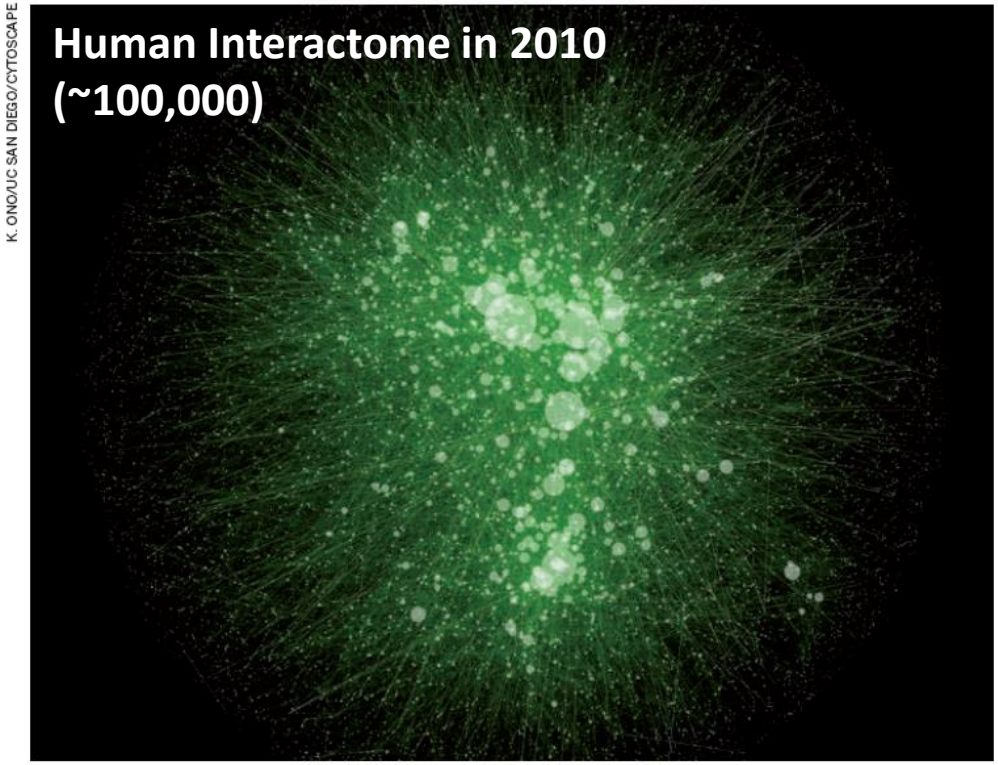
Rual et al. Nature 2005 Vol 437

Protein-Protein interaction maps:

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

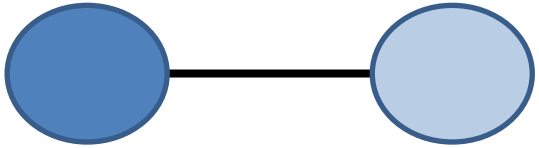


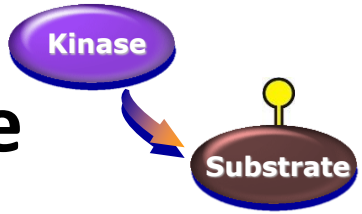

Bonetta, *Nature* 2010

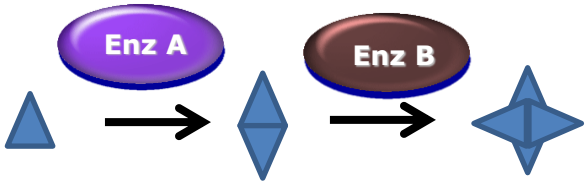
# Protein-Protein interactions:



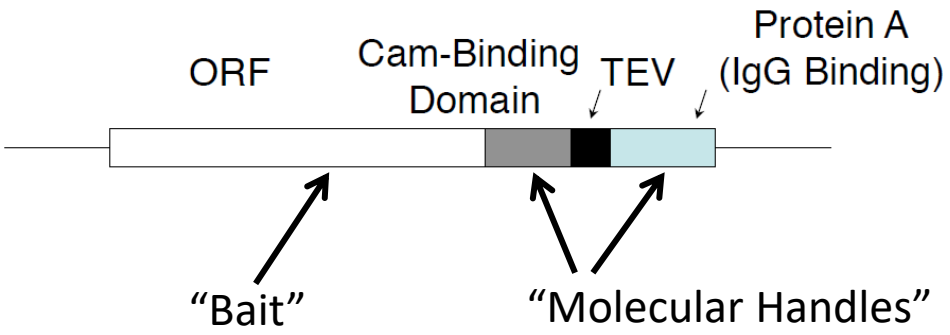
Some examples:

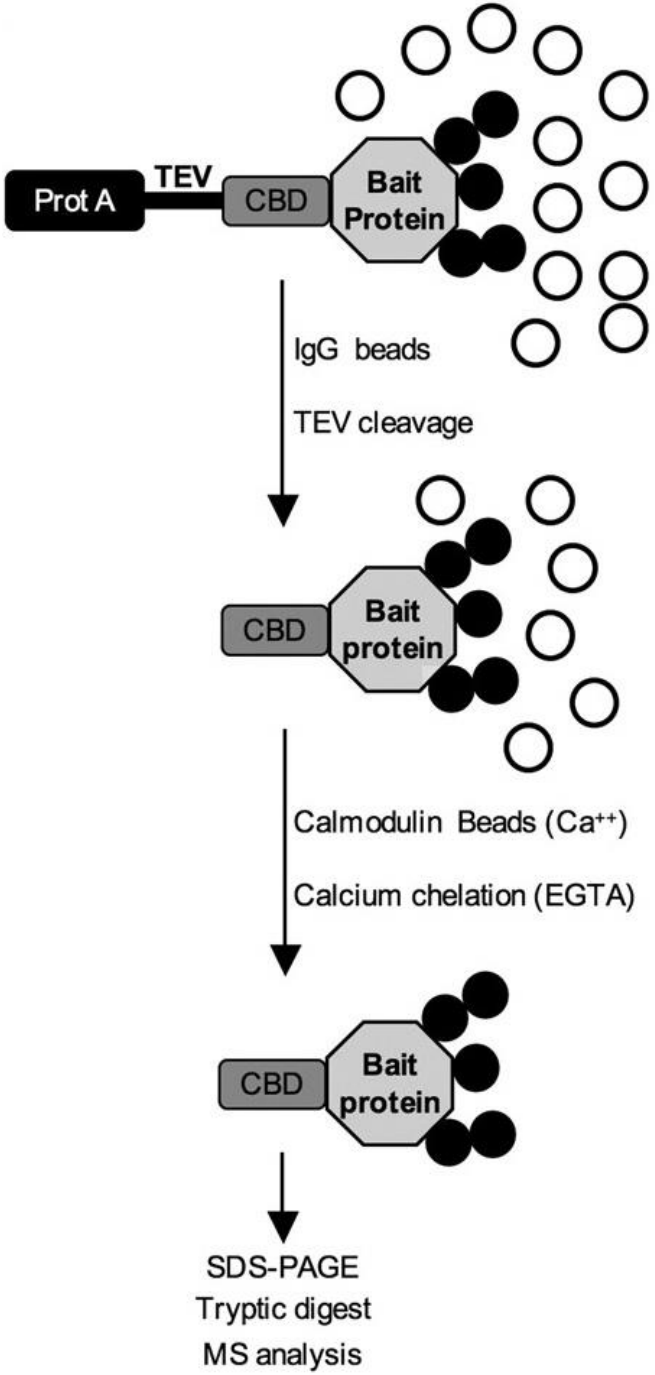
- Physical and direct
- Physical and indirect
  - Multi-protein complexes
  - Scaffolds
- Transient
  - Kinase & substrate
- Metabolic



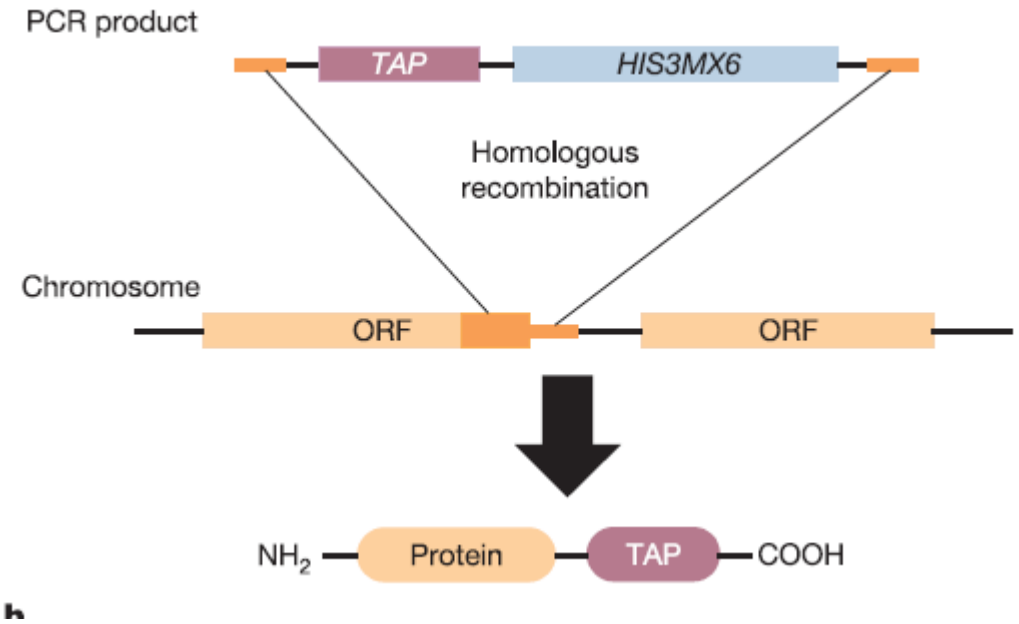
# Tandem Affinity Purification (TAP) Tagging




**Cannavo E et al. J. Biol. Chem. 2007**

# Global TAP Tagging in yeast




**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* .

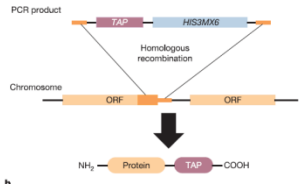
➲ Protein–protein interaction maps can be obtained by MS; the yeast cell is organized into protein complexes.

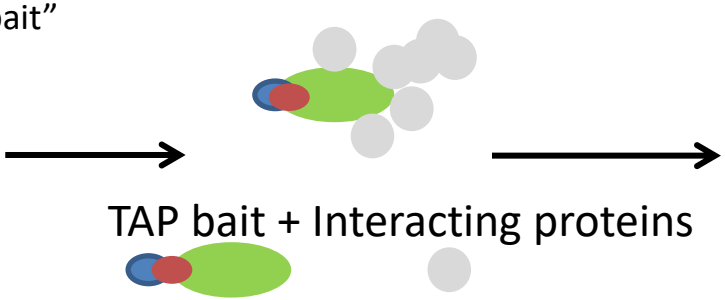
## 2006

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

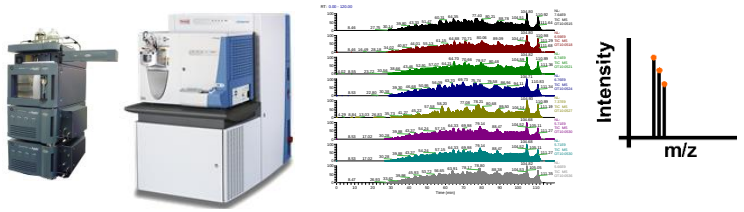
**➲ TAP-Tag and Protein-Protein Interaction**

Collection of tagged "bait" expression strains




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



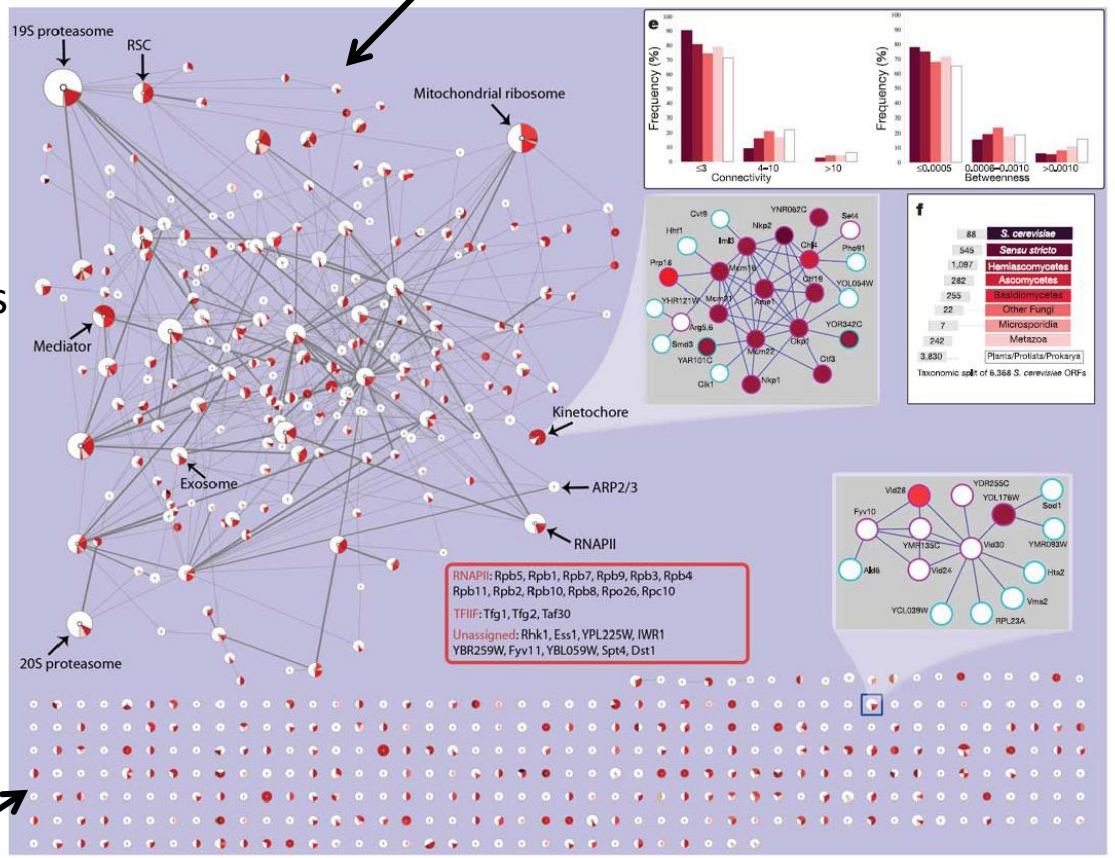

**Krogan et al. observed 7,123 protein–protein interactions:**

**<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

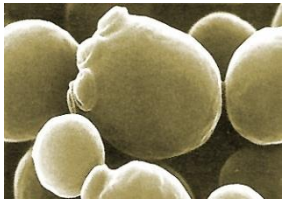
- 4,562 tagged proteins
- 2,357 successful purifications
- Identified 4,087 interacting proteins ~72 % proteome
- Majority of the yeast proteome is organized into complexes
- Many complexes are conserved in other species




Complexes with little or no interconnectivity

Krogan NJ, et al. *Nature.* 2006

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

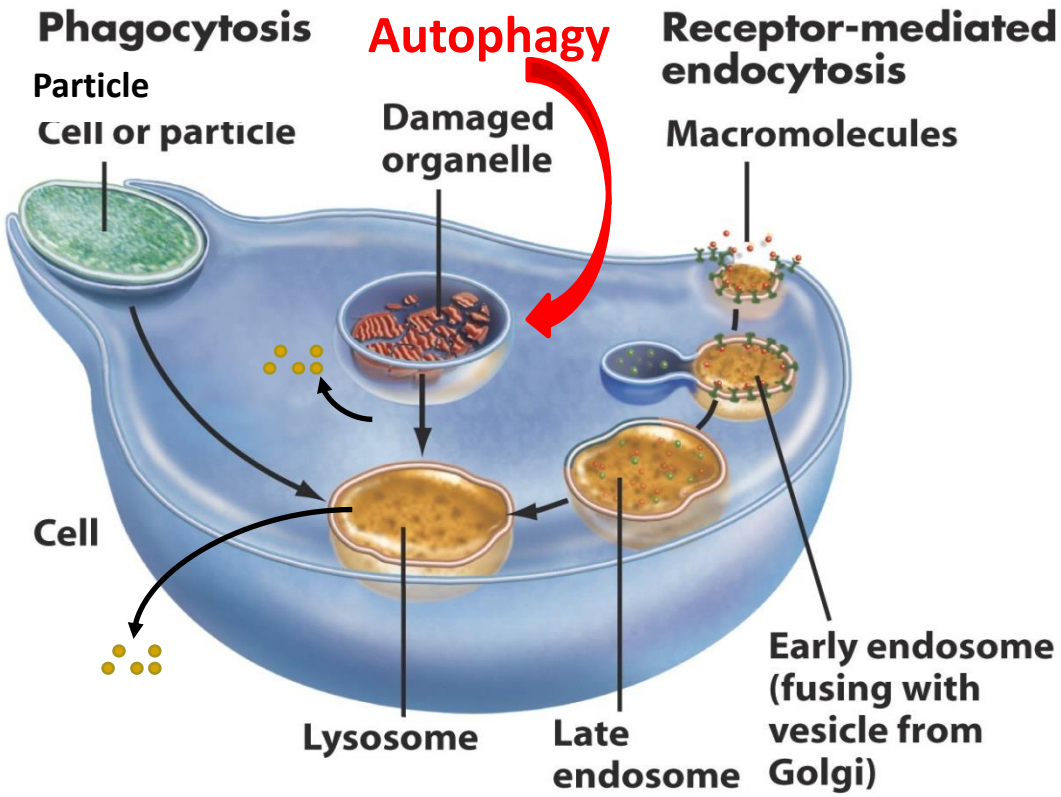


nature Vol 466|1 July 2010|doi:10.1038/nature09204

ARTICLES

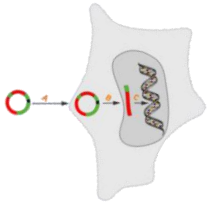
# Network organization of the human autophagy system

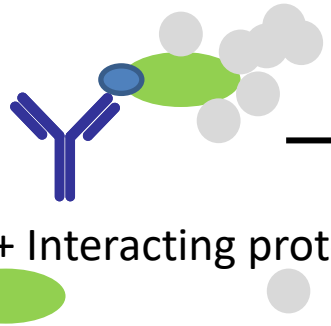
Christian Behrends[1], Mathew E. Sowa[1], Steven P. Gygi[2] & J. Wade Harper[1]




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

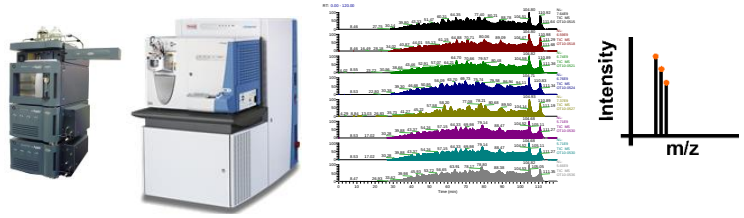
Transfect tagged "bait"





IP Bait + Interacting proteins

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



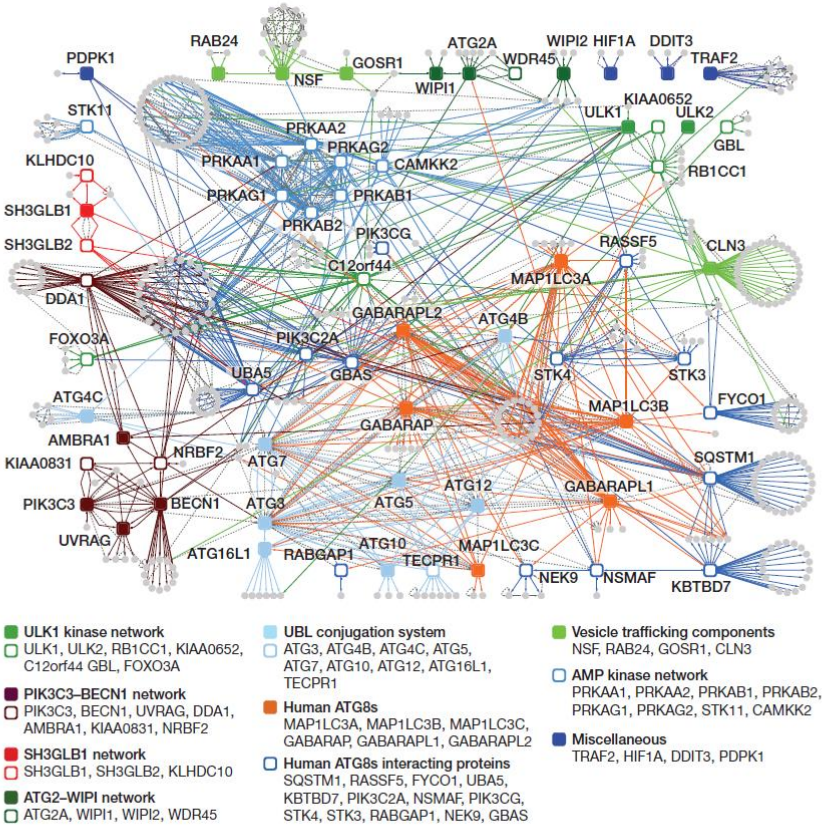

**~65 bait proteins**
**LC-MS/MS identifies**
**2553 proteins**

**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**

## Autophagy Interaction Network




**Behreands et al, Nature 2010**

**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.

# The Hippo Signaling Pathway Interactome

Young Kwon,[1] Arunachalam Vinayagam,[1]* Xiaoyun Sun,[3]* Noah Dephoure,[4] Steven P. Gygi,[4] Pengyu Hong,[3] Norbert Perrimon[1,2]†

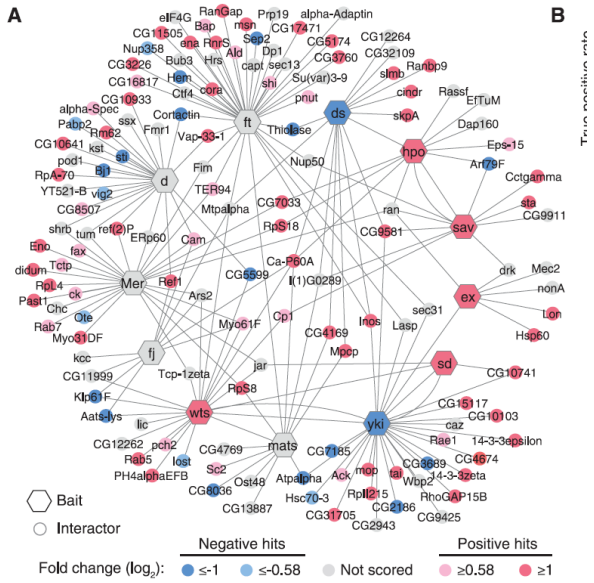
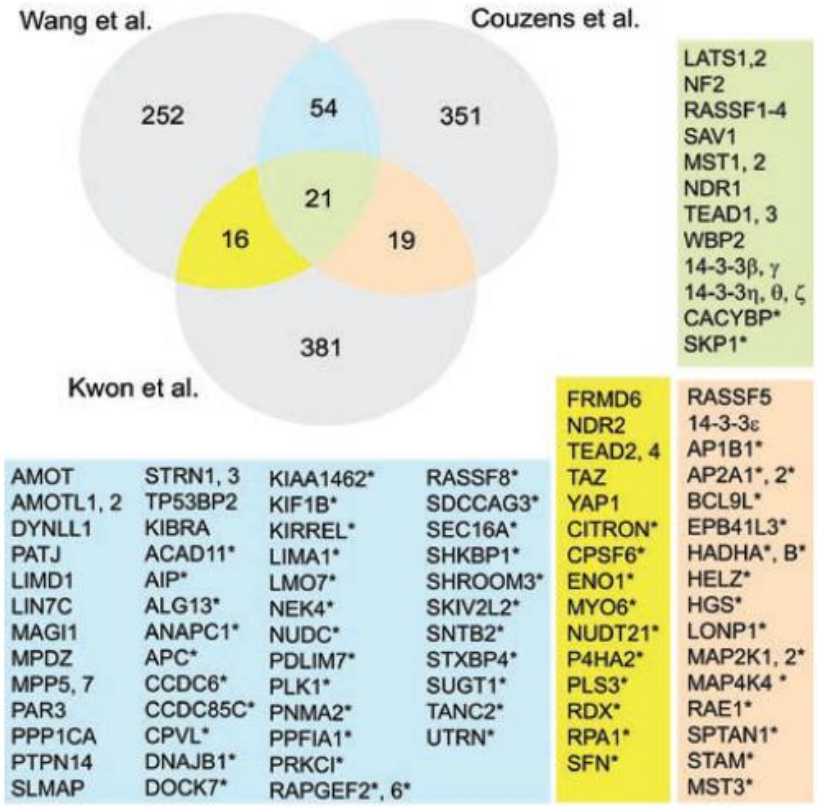



**Fig. 2. Validation of Hippo-PPIN with functional RNAi screen and co-IP.** (**A**) Distribution of Yki-reporter values for individual double-stranded RNAs (dsRNAs) in our focused RNAi screen. About 70% of genes are covered by two dsRNAs. (**B**) Recovery of Hippo pathway components from RNAi screen [fold-change ($log_2$) cutoff ± 1]. (**C**) The positive




Cell Research (2014) 24:137-138.

www.nature.com/cr

RESEARCH HIGHLIGHT

## Discovering the Hippo pathway protein-protein interactome

*Cell Research* (2014) **24**:137-138. doi:10.1038/cr.2014.6; published online 14 January 2014

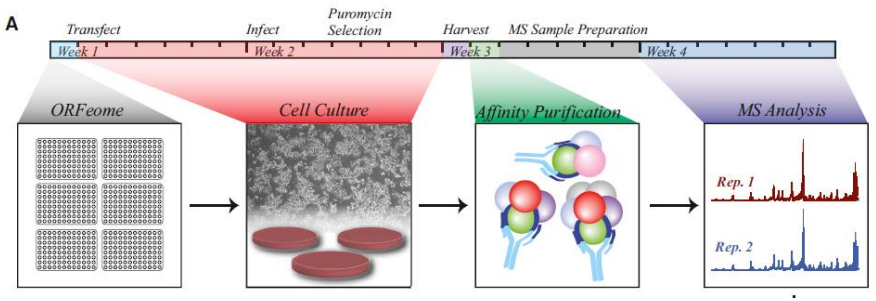
**Kwon Y, *et al*. *Science* 2013; Couzens AL, *et al*. *Sci Signal* 2013; Wang W, *et al*. *Mol Cell Proteomics* 2014**

# 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] Gabriela Zarraga,[1] Greg Colby,[1] Kurt Baltier,[1] Rui Dong,[2] Virginia Guarani,[1] Laura Pontano Vaites,[1] Alban Ordureau,[1] Ramin Rad,[1] Brian K. Erickson,[1] Martin Wühr,[1] Joel Chick,[1] Bo Zhai,[1] Deepak Kolippakkam,[1] Julian Mintseris,[1] Robert A. Obar,[1,3] Tim Harris,[3] Spyros Artavanis-Tsakonas,[1,3] Mathew E. Sowa,[1] Pietro De Camilli,[2] Joao A. Paulo,[1] J. Wade Harper,[1,*] and Steven P. Gygi[1,*]

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

**Architecture of the human interactome defines protein communities and disease networks**

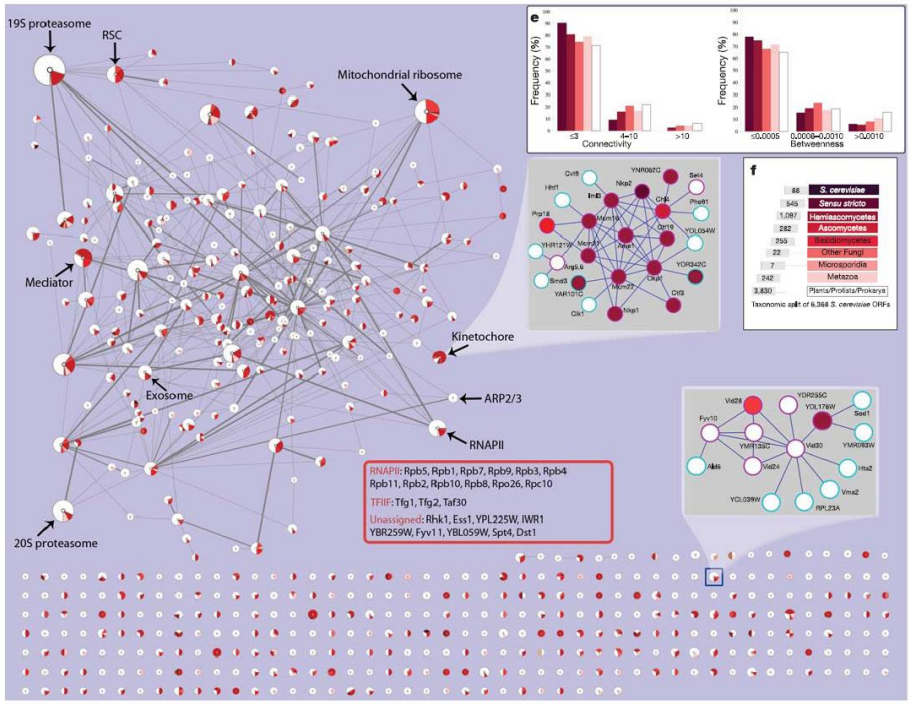
Edward L. Huttlin[1], Raphael J. Bruckner[1], Joao A. Paulo[1], Joe R. Cannon[1], Lily Ting[1], Kurt Baltier[1], Greg Colby[1], Fana Gebreab[1], Melanie P. Gygi[1], Hannah Parzen[1], John Szpyt[1], Stanley Tam[1], Gabriela Zarraga[1], Laura Pontano-Vaites[1], Sharan Swarup[1], Anne E. White[1], Devin K. Schweppe[1], Ramin Rad[1], Brian K. Erickson[1], Robert A. Obar[1,2], K. G. Guruharsha[2], Kejie Li[2], Spyros Artavanis-Tsakonas[1,2], Steven P. Gygi[1] & J. Wade Harper[1]

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

BioPlex 3.0 Dual Proteome-scale Networks Reveal Cell-specific Remodeling of the Human Interactome. bioRxiv doi:10.1101/2020.01.19.905109

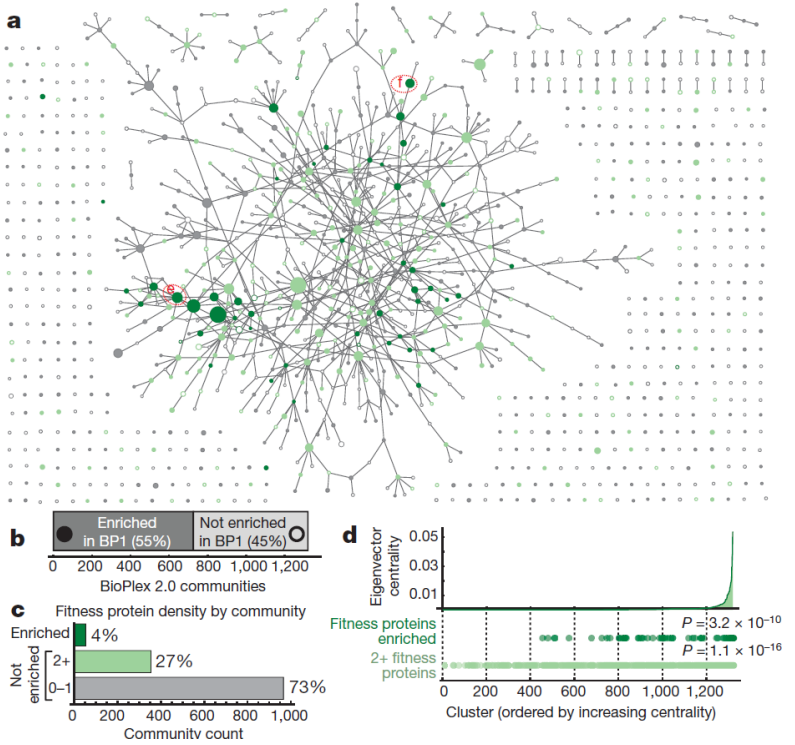
# Cellular proteins are organized into complexes and this proteome organization is conserved

**Yeast:** Interaction Network of Complexes




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

**Human:** Protein Complex "Communities"



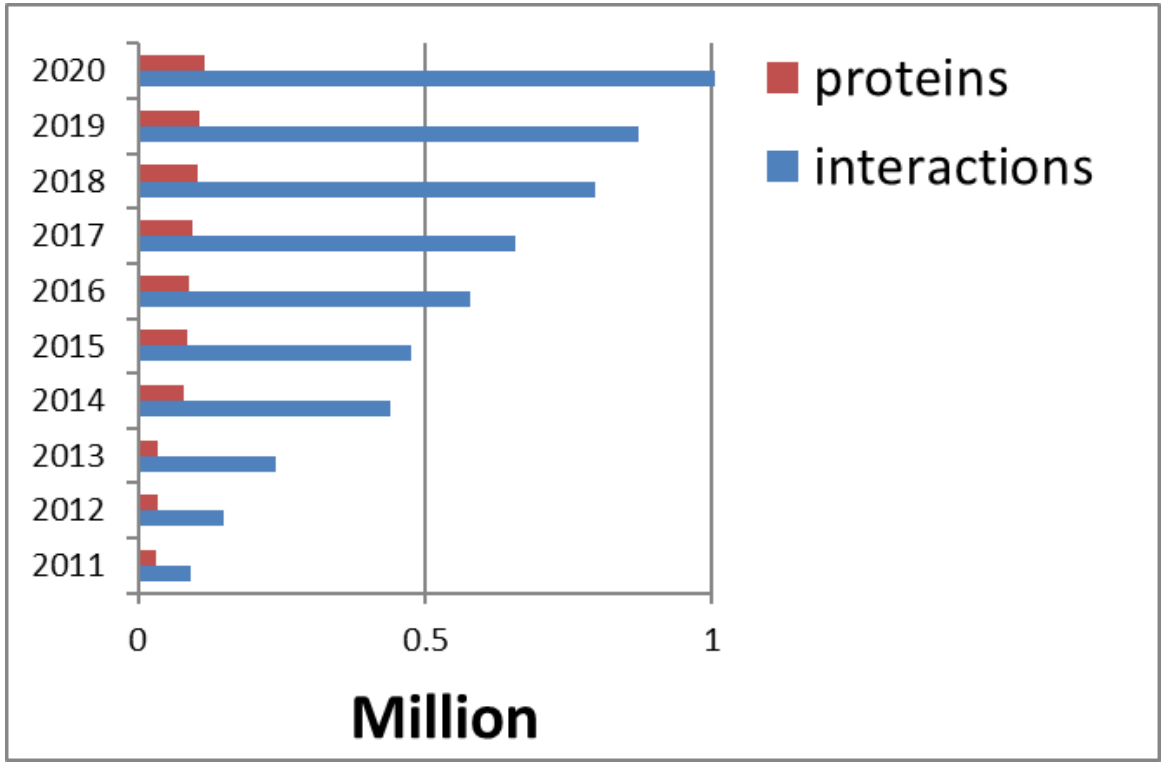

Huttlin et al, *Nature*. 2017 PMID: 28514442

# Protein-Protein Interaction Databases

EMBL-EBI

IntAct

http://www.ebi.ac.uk/intact/




**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**

**2019** Data Content

- Publications: **20429**
- Interactions: **872946**
- Interactors: **108492**

**+ 78,024 interactions**
**+ 3,982 proteins**

# Protein-Protein Interaction Databases

EMBL-EBI

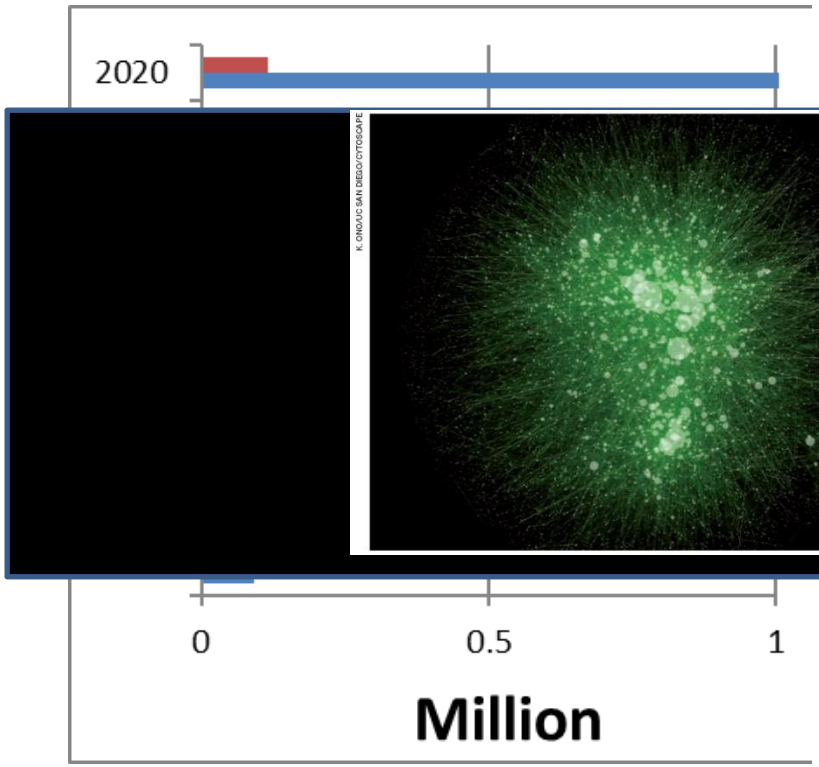
IntAct

**http://www.ebi.ac.uk/intact/**

Data Content

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

**2020**






**Human Interactome in 2010 (~100,000)**

Bonetta, *Nature* 2010

# 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

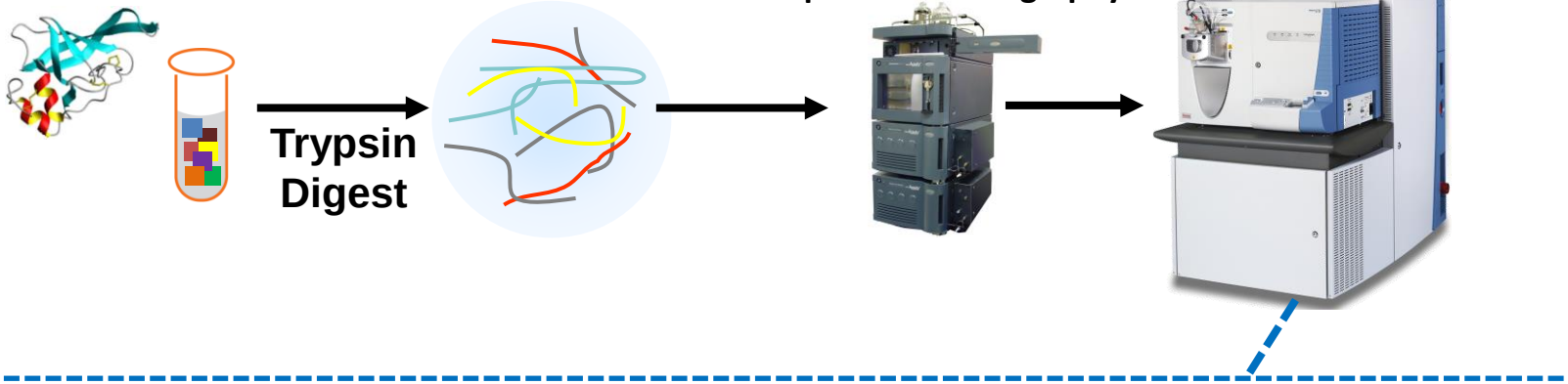
However:

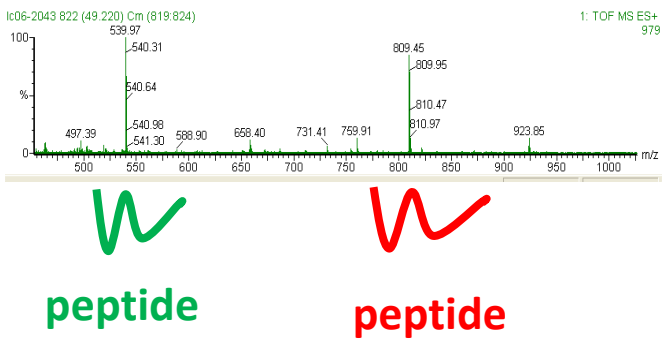
- Interaction data is largely static

**Next Step:**

**- How do protein interaction networks change over time?**

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

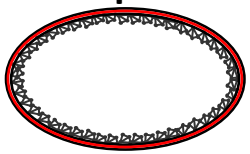


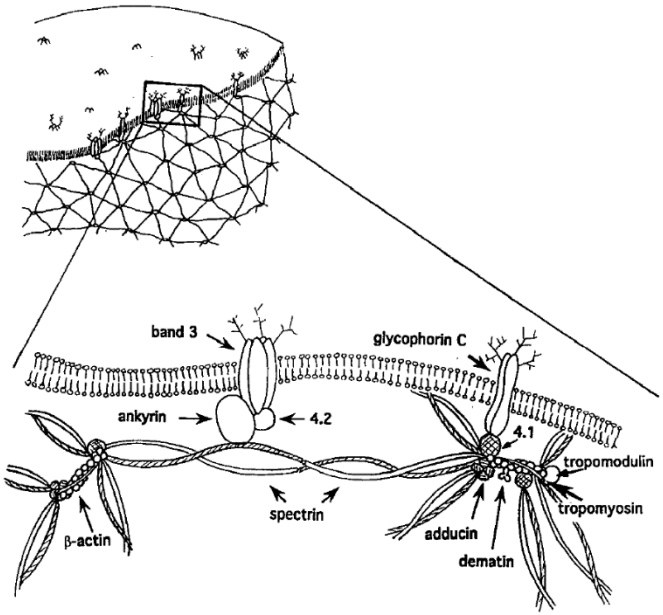



# MS Data is not inherently quantitative

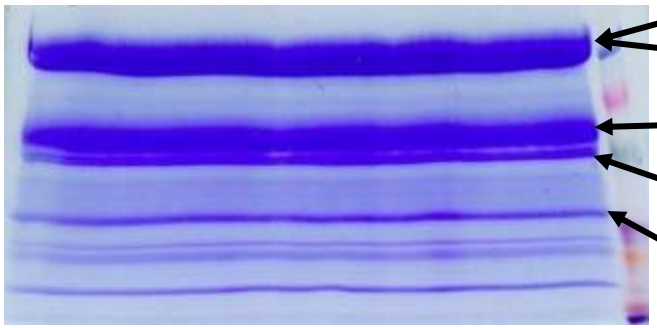
**Red Blood Cell**

**RBC membrane:**
**a native multi-protein complex**






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



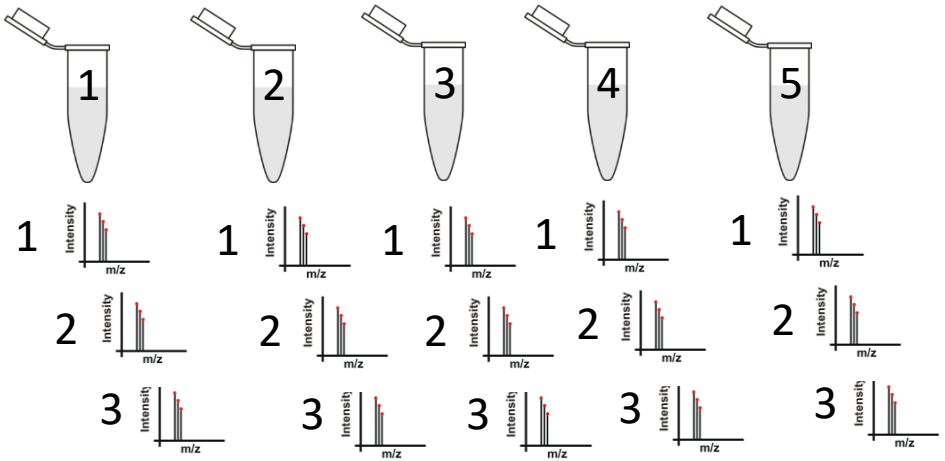

**RBC membrane proteome**
***Shotgun Proteomics***
***1ug Peptides (242 Proteins)***

| # 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 |

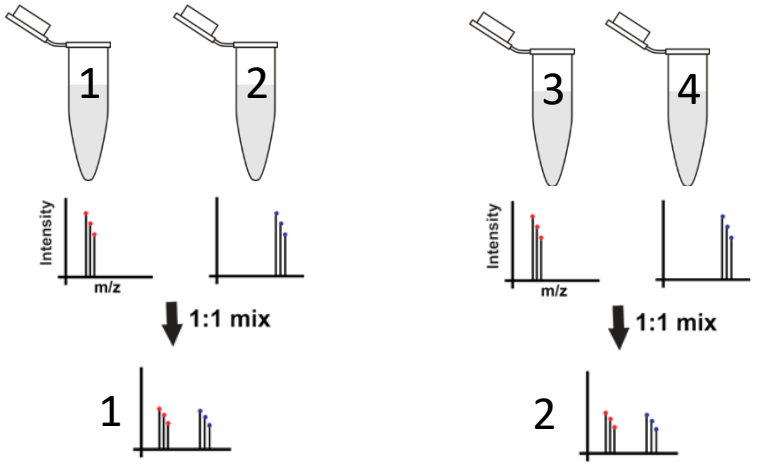
Rinehart et al., *unpublished*

# Multiple Techniques Enable Quantitative Proteomics

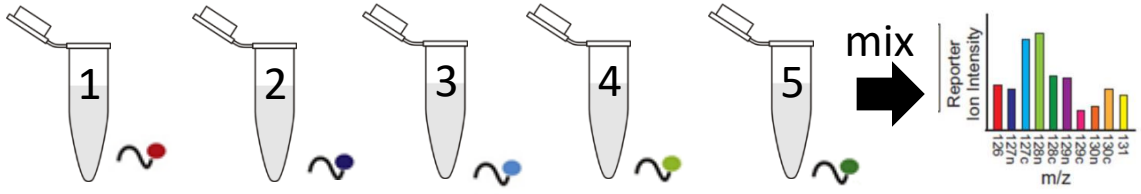
**Label Free**
-many, many replicates
-indirect quant




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

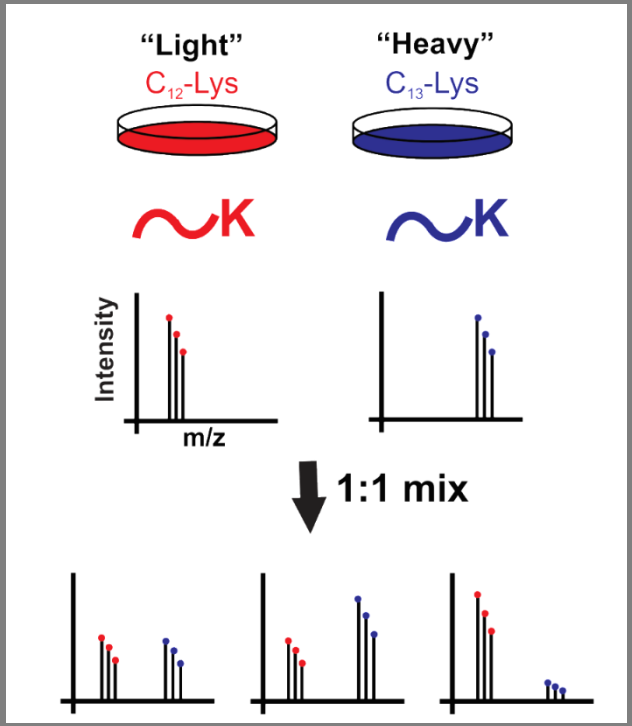



**Barcoding**
-increased multiplex
-direct quant



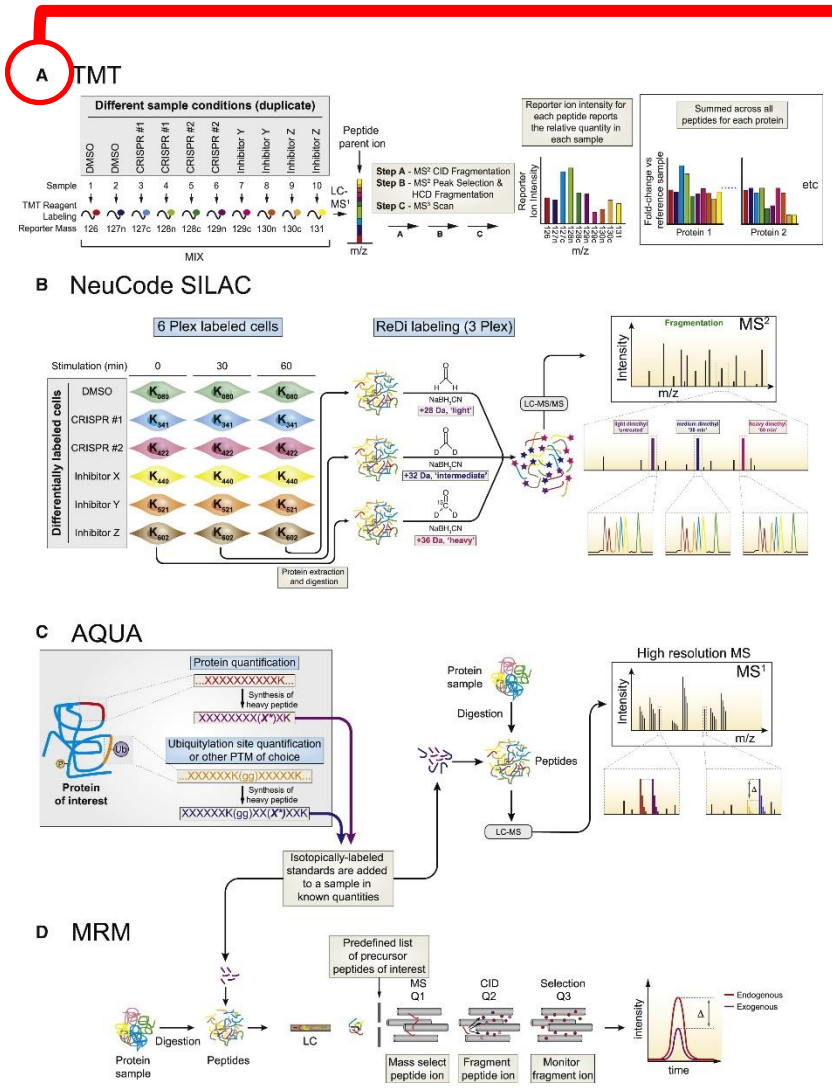
# S.I.L.A.C. - Stable isotope labeling with amino acids in cell culture

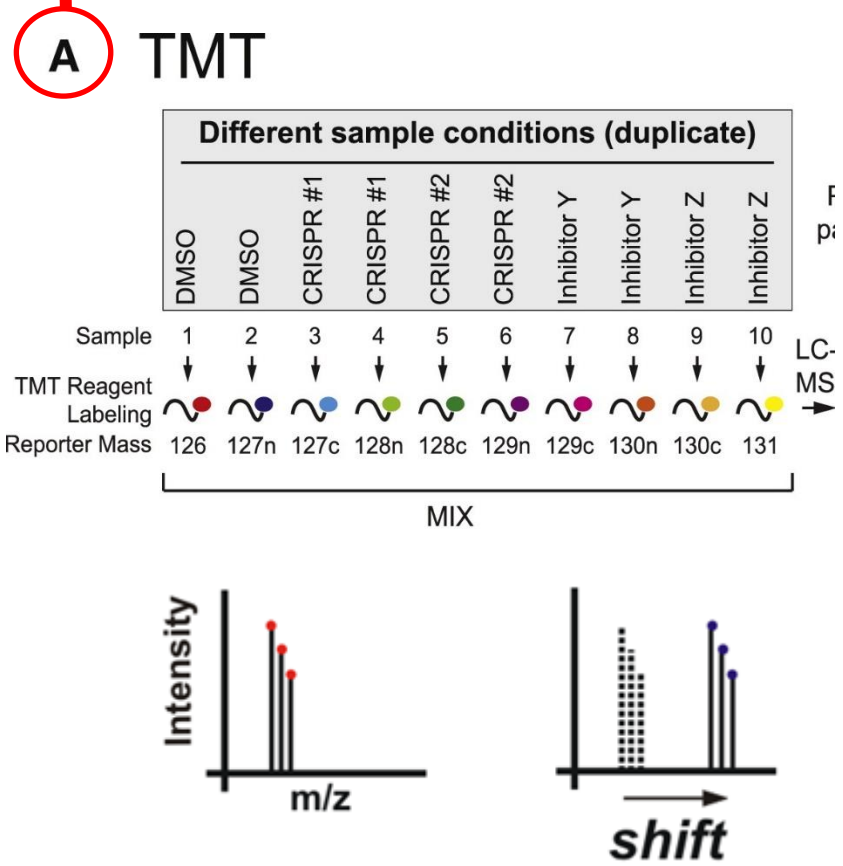
- Stable isotopes, such as carbon-13 ($^{13}C$), introduced into cultured cells
- Normal lysine has 6 ($^{12}C$)
- "Heavy" lysine has 6 ($^{13}C$)
- This yields a 6 Dalton difference in mass that translates to a fixed difference in the m/z and mixtures of two samples yields pairs of spectra




Ong S.E. et al. PMID: 12118079

# Heavy labels can be used for “barcoding” proteomes



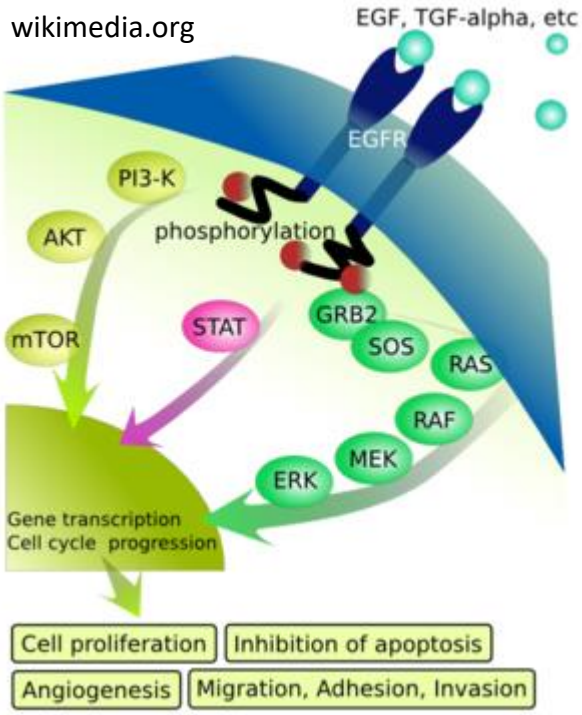




Quantifying ubiquitin signaling; Ordureau A, Münch C, Harper JW. 2015 **PMID: 26000850**

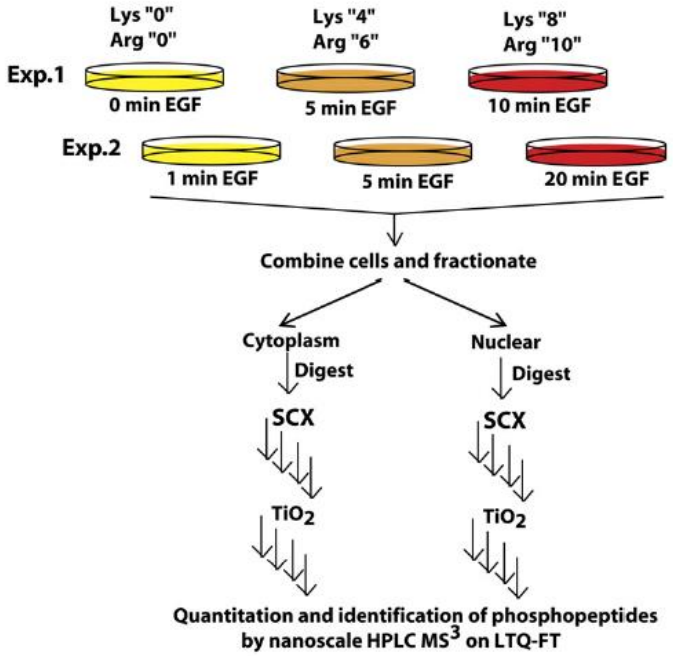
# Quantitative Proteomics Reveals Dynamics in Signaling Networks

Phosphorylation dynamics after EGF stimulation

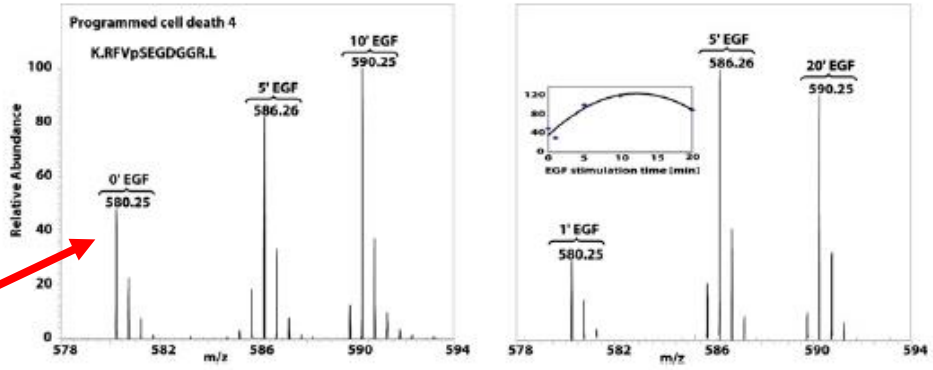
wikimedia.org




SILAC approach enables dynamic analysis




MS spectra triplets

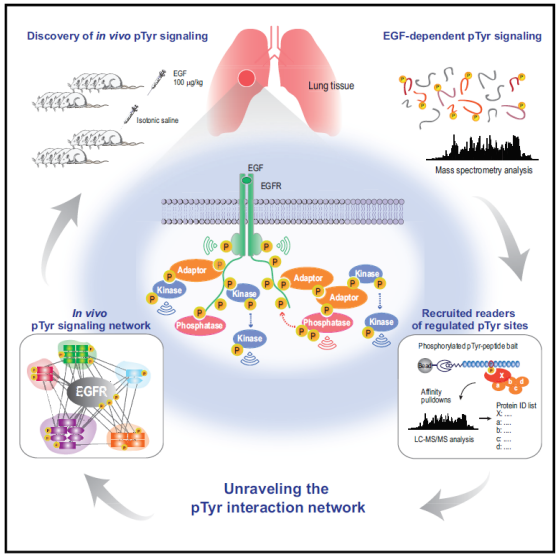



Olsen, et al. *Cell*, 2006

# 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

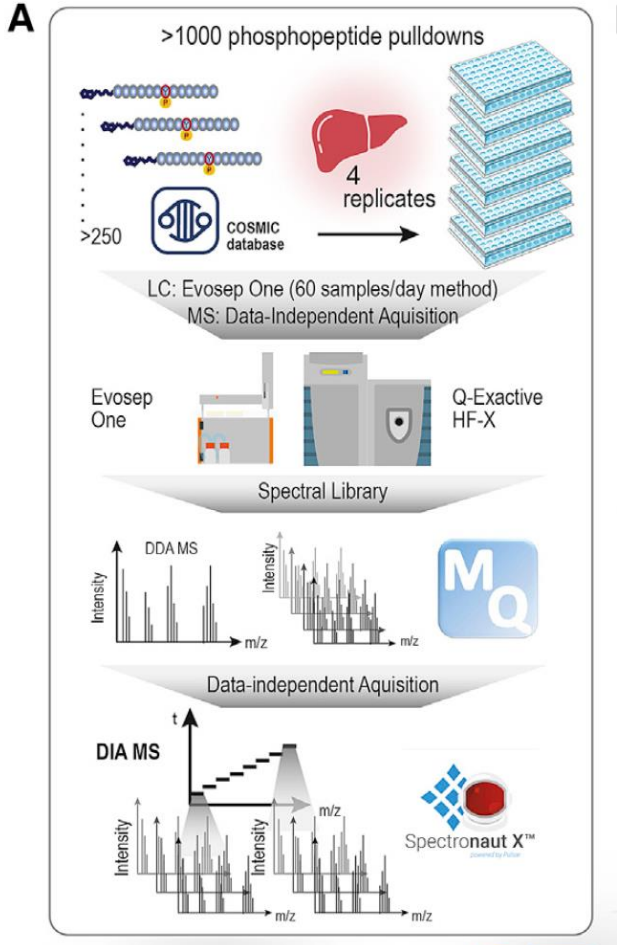
### Correspondence

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.



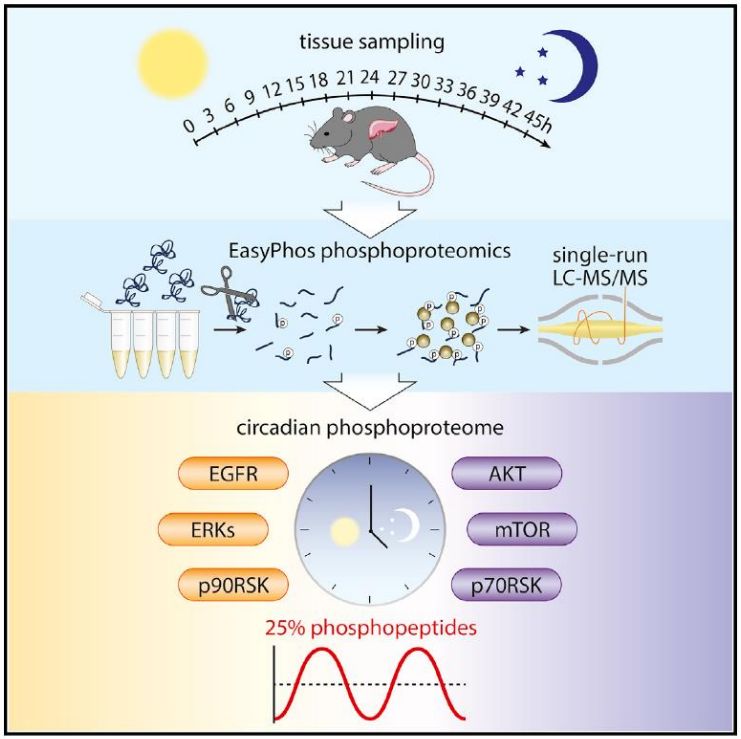
**Lundby et al., & Olsen, Cell 2019** PMID: 31585087

# 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

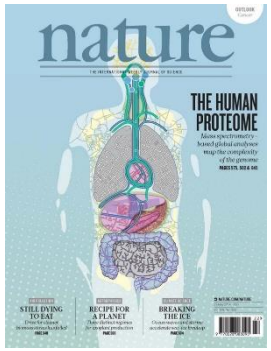
## The Sequence of the Human Genome

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[1], Emanuel Ziegler[2], Lars Butzmann[2], Siegfried Gessulat[2], Harald Marx[1], Toby Mathieson[1], 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[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],

**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

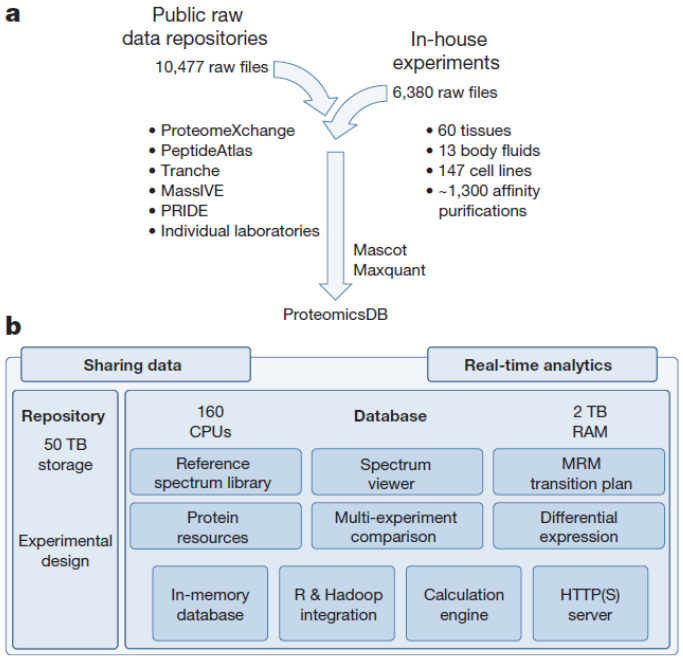
ARTICLE

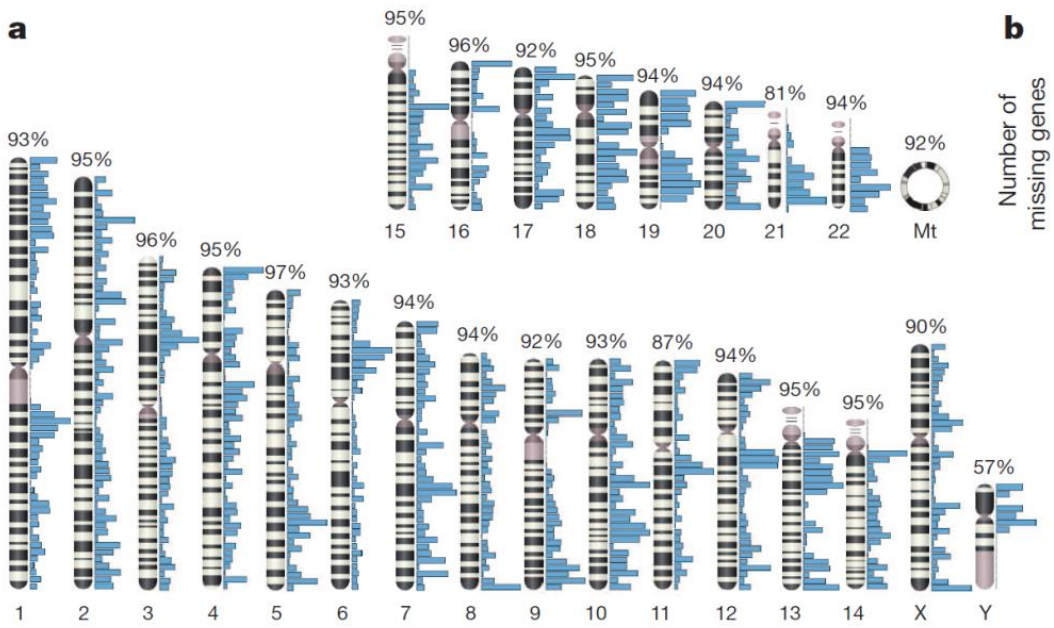
doi:10.1038/nature13319

# 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]

- 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

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]

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


**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**

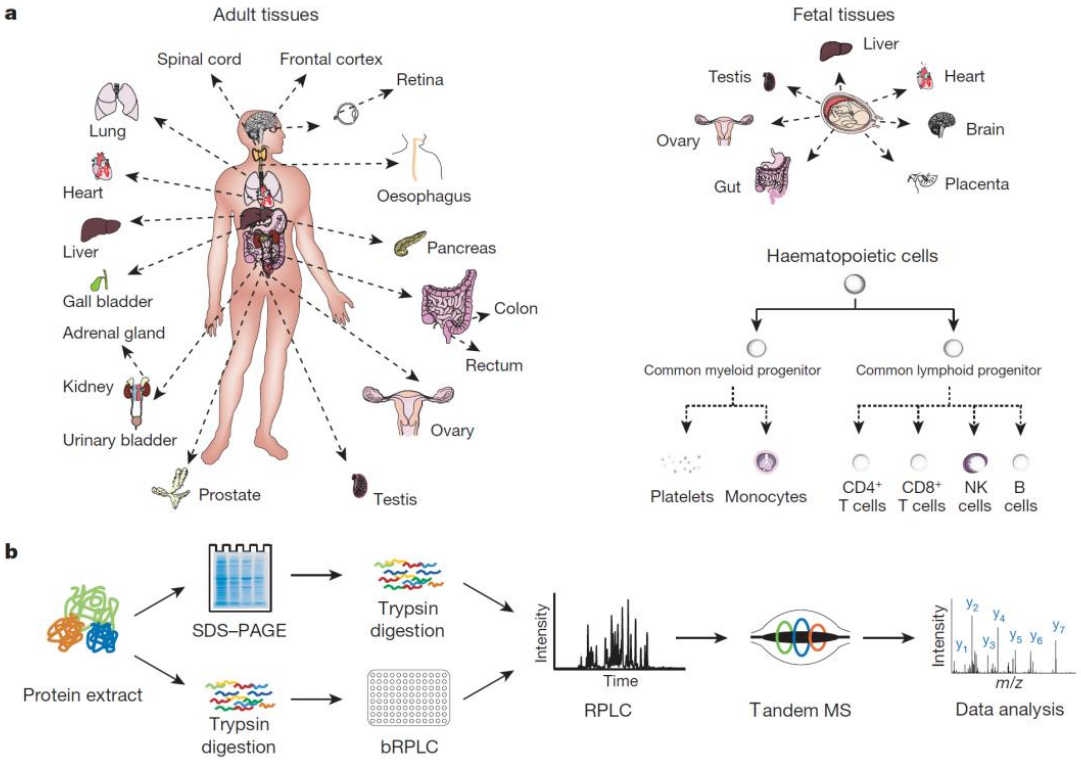
ARTICLE

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[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

http://thegpm.org/GPMDB/index.html


## The Global Proteome Machine

Proteomics data analysis, reuse and validation for biological and biomedical research.

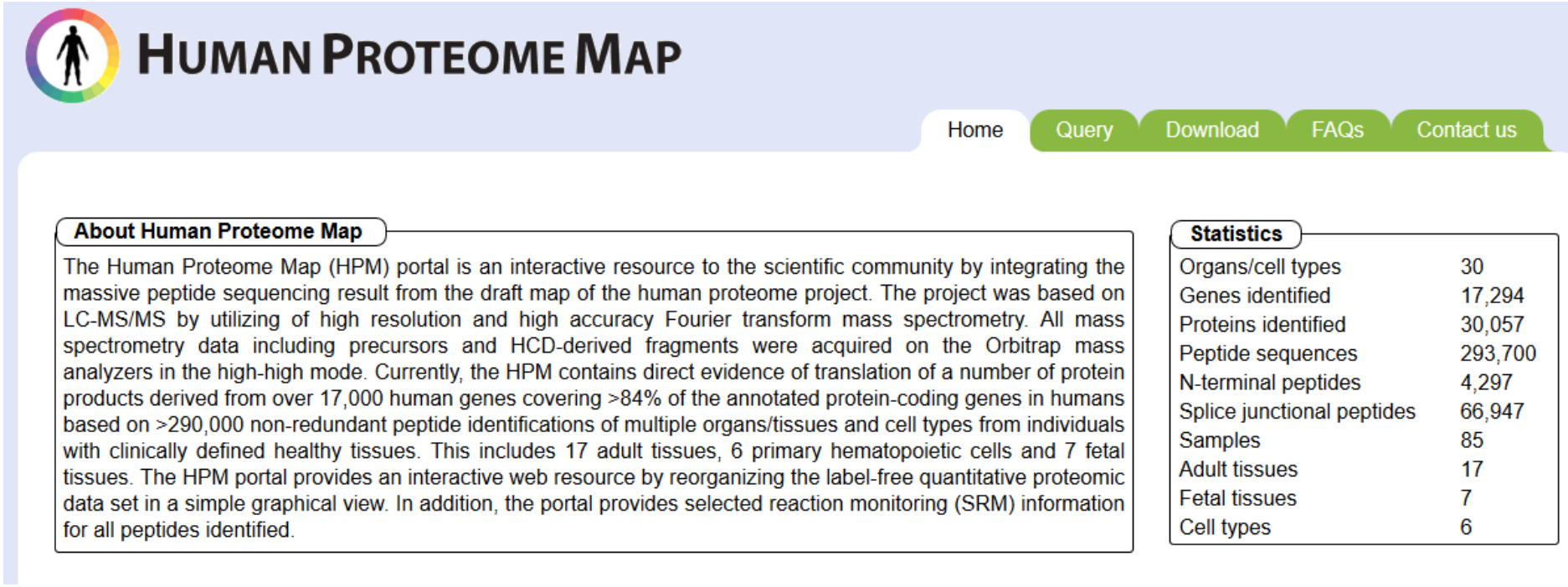
## The GPMDB Project

## gpmDB: Design

gpmDB was designed to be a simplification and extension of the MIAPE scheme proposed by the PSI committee of HUPO. Rather than being a complete record of a proteomics experiment, this database holds the minimum amount of information necessary for certain bioinformatics-related tasks, such as sequence assignment validation. Most of the data is held in a set of XML files: the database serves as an index to those files, allowing for very rapid lookups and reduced database storage requirements. We call this combination of a relational database with XML data XIAPE (Xml Information About a Proteomics Experiment).

**The Minimum Information About a Proteomics Experiment (MIAPE)**
**http://www.psidev.info/node/91**
*Nature Biotechnology* 25, 887 - 893 (2007) PMID: 17687369
*Methods Mol Biol.* 2014;1072:765-80. PMID: 24136562

# Proteomics Databases: Peptide depositories




ARTICLE

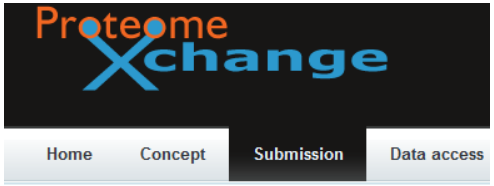
doi:10.1038/nature13302

## A draft map of the human proteome

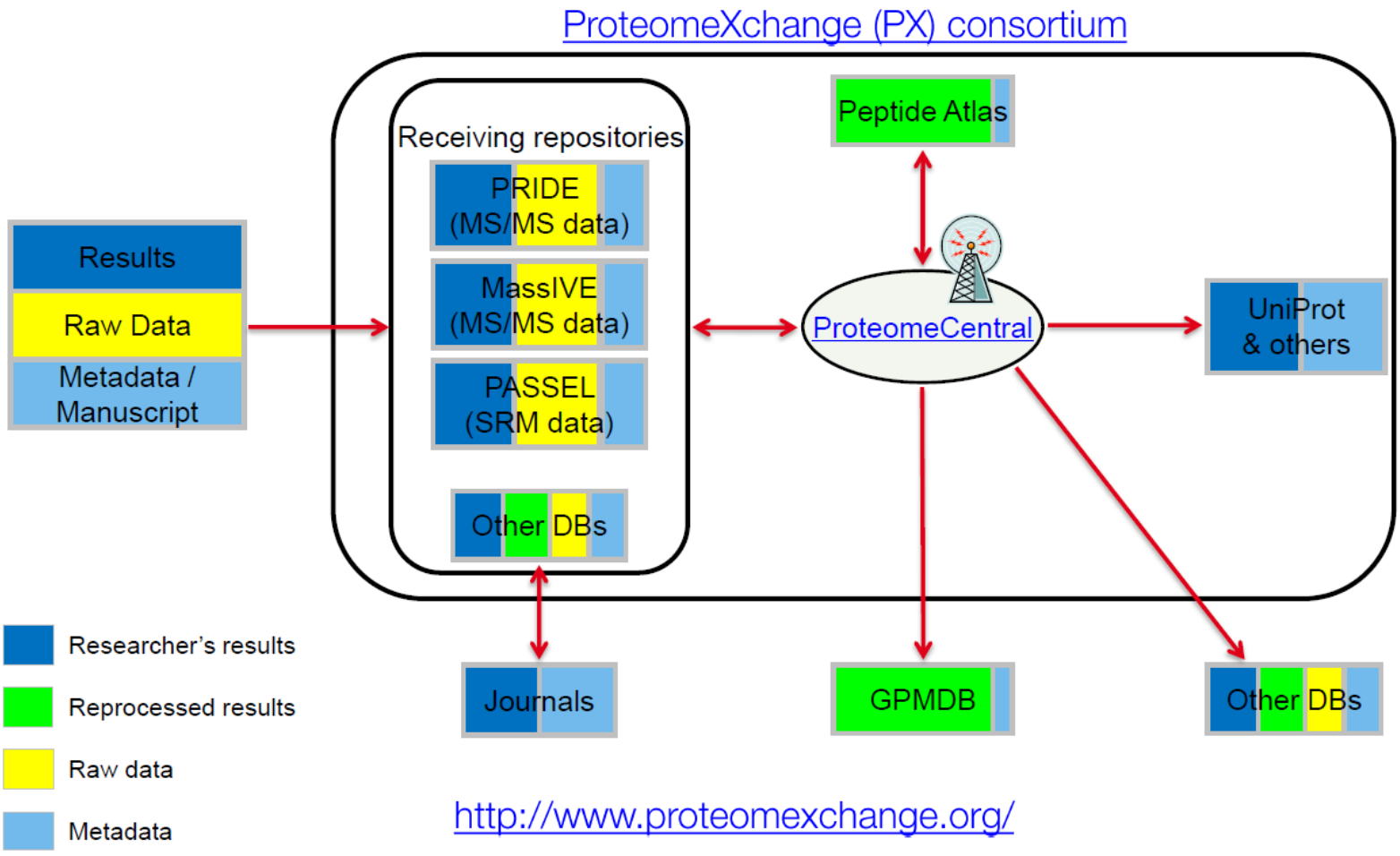
Min-Sik Kim[1,2], Sneha M. Pinto[3], Derese Getnet[1,4], Raja Sekhar Nirujogi[3], Srikanth S. Manda[3], Raghothama Chaerkady[1,2], Anil K. Madugundu[3], Dhanashree S. Kelkar[3], Ruth Isserlin[5], Shobhit Jain[5], Joji K. Thomas[3], Babylakshmi Muthusamy[3], Pamela Leal-Rojas[1,6], Praveen Kumar[3], Nandini 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],

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

# Proteomics Databases: Integrated Resources




http://www.proteomexchange.org/



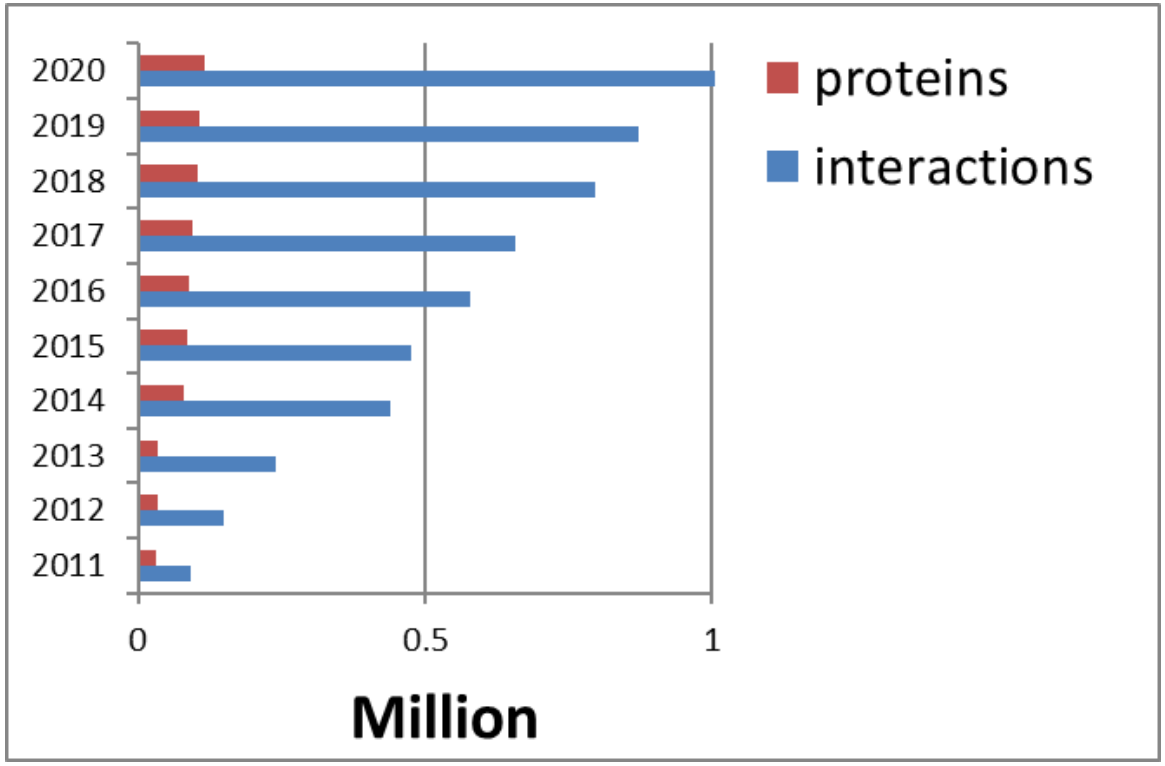

Slide modified from "Computational Mass Spectrometry-Based Proteomics 6th Maxquant Summer School" 21-25 July 2014
Emanuele Alpi, UniProt and PRIDE Development

# Protein-Protein Interaction Databases

EMBL-EBI

IntAct

http://www.ebi.ac.uk/intact/




**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**

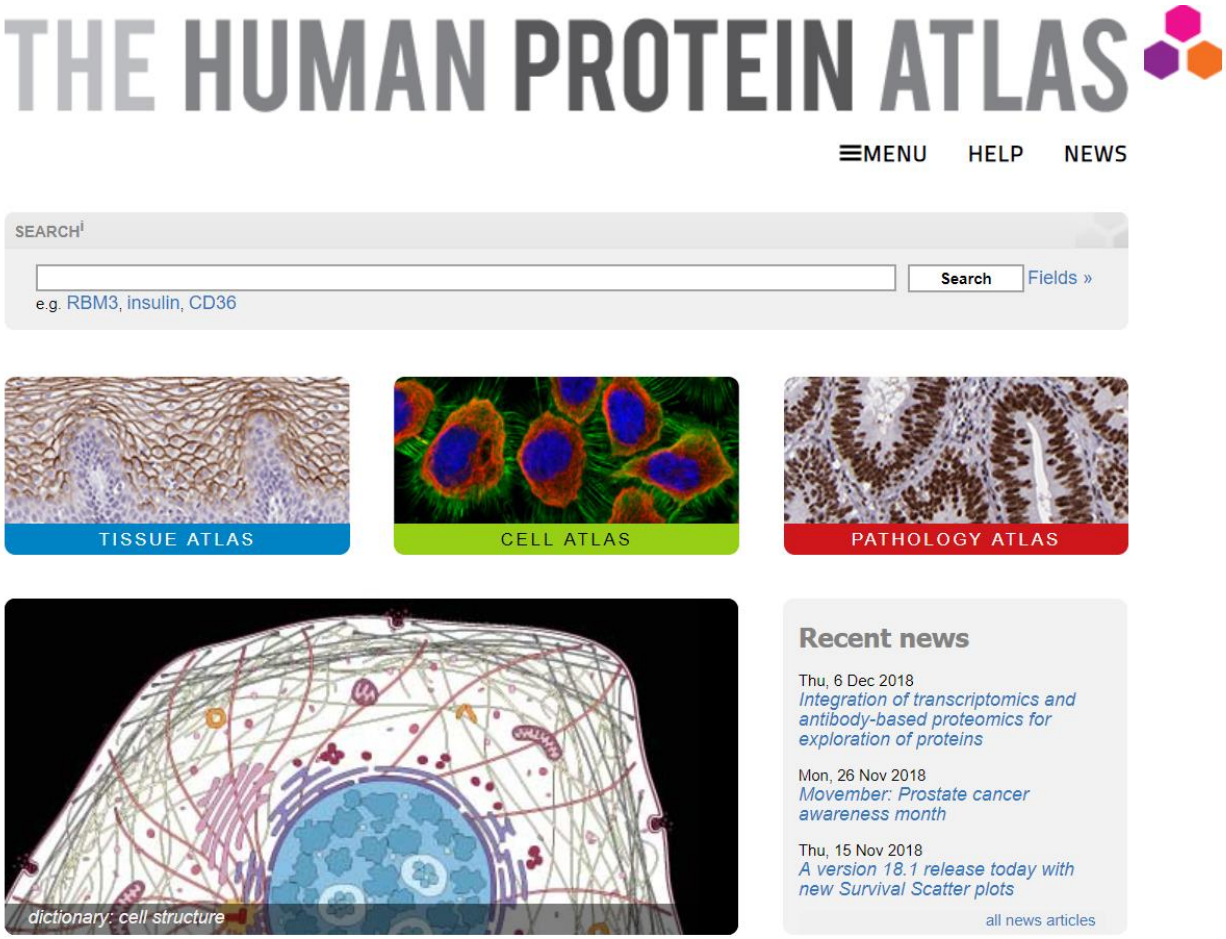
**2019** Data Content

- Publications: **20429**
- Interactions: **872946**
- Interactors: **108492**

**+ 78,024 interactions**
**+ 3,982 proteins**

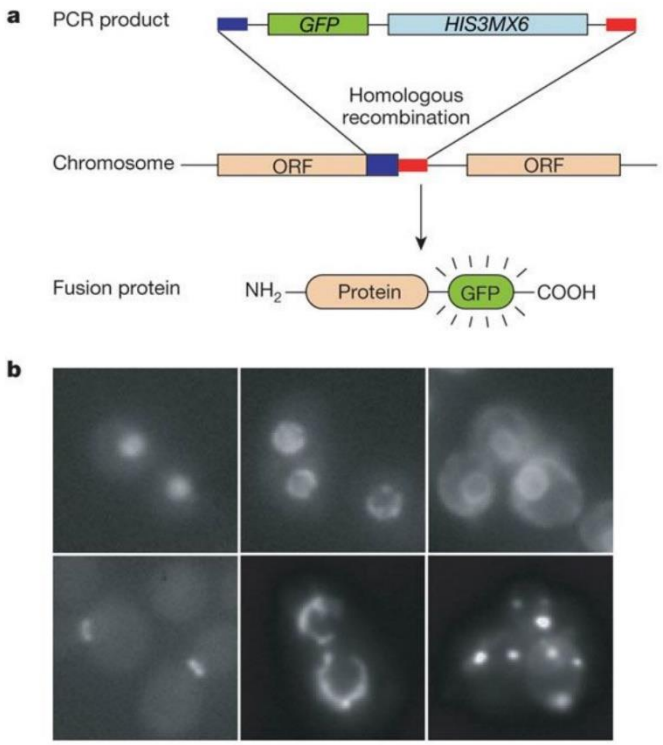
# Proteomics Databases: Integrated Resources Beyond Mass Spectrometry

http://www.proteinatlas.org/



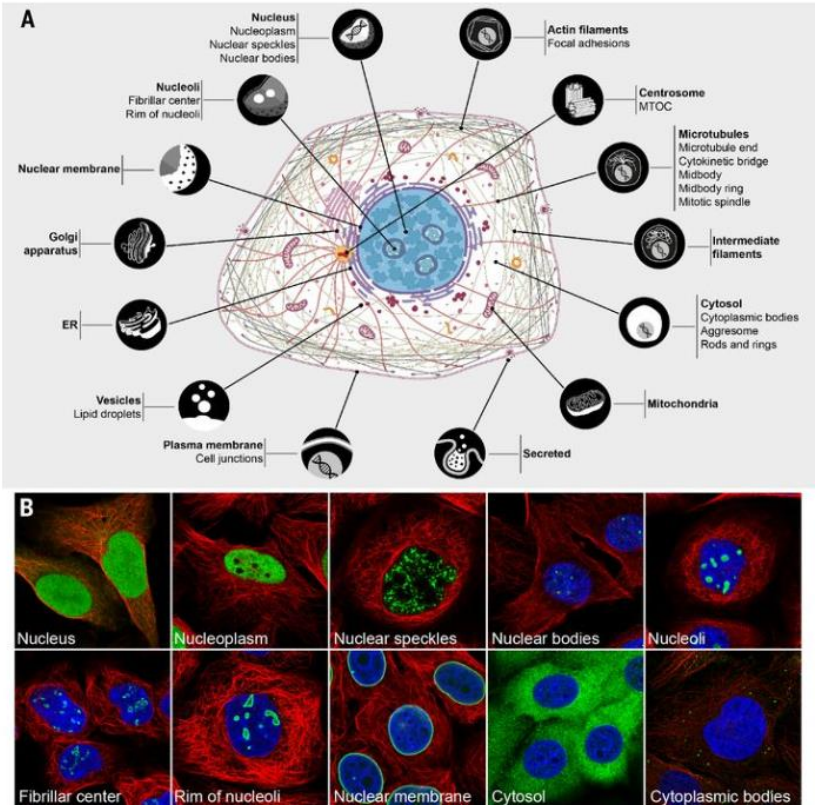
# Proteomics Databases: Integrated Resources Beyond Mass Spectrometry

## >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

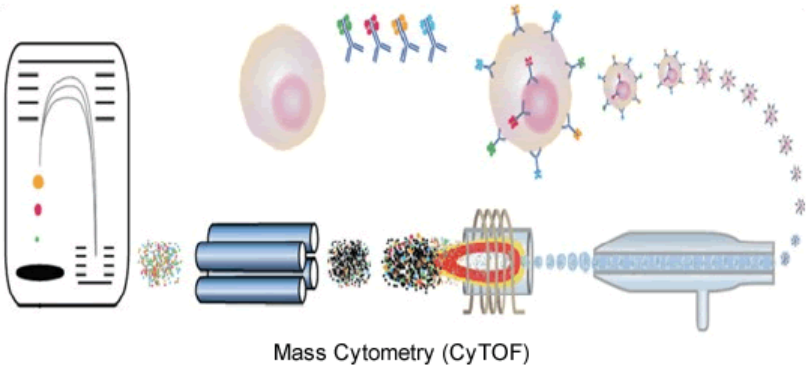
## Single-cell proteomic analysis of *S. cerevisiae* reveals the architecture of biological noise

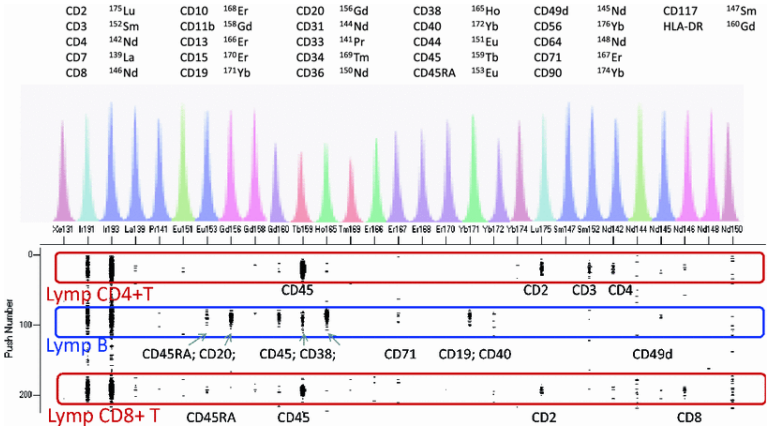
John R. S. Newman[1,2], Sina Ghaemmaghami[1,2]†, Jan Ihmels[1,2], David K. Breslow[1,2], Matthew Noble[1], Joseph L. DeRisi[1,3] & Jonathan S. Weissman[1,2]

**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.**

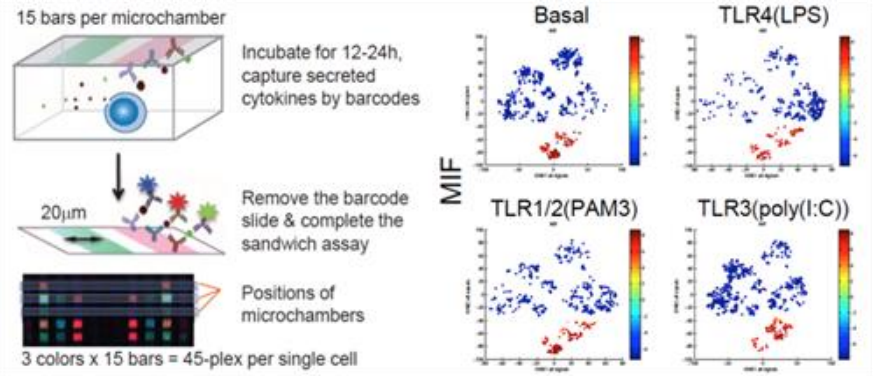
# Proteomics at single cell resolution

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

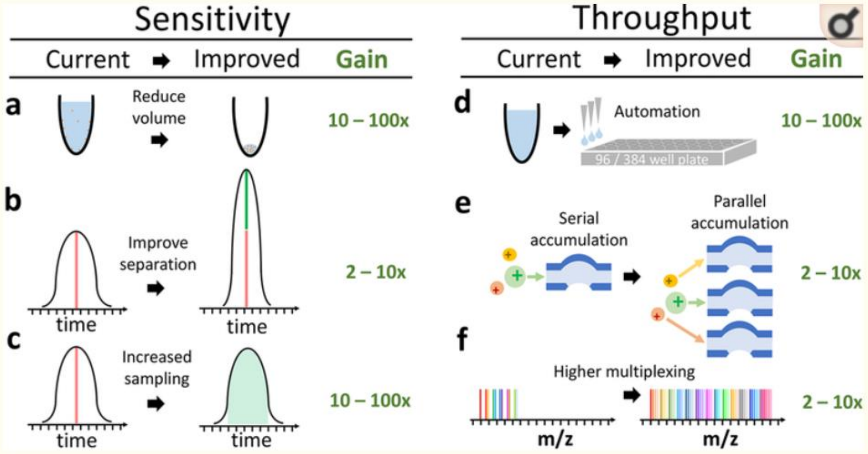



Figure 3.

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/

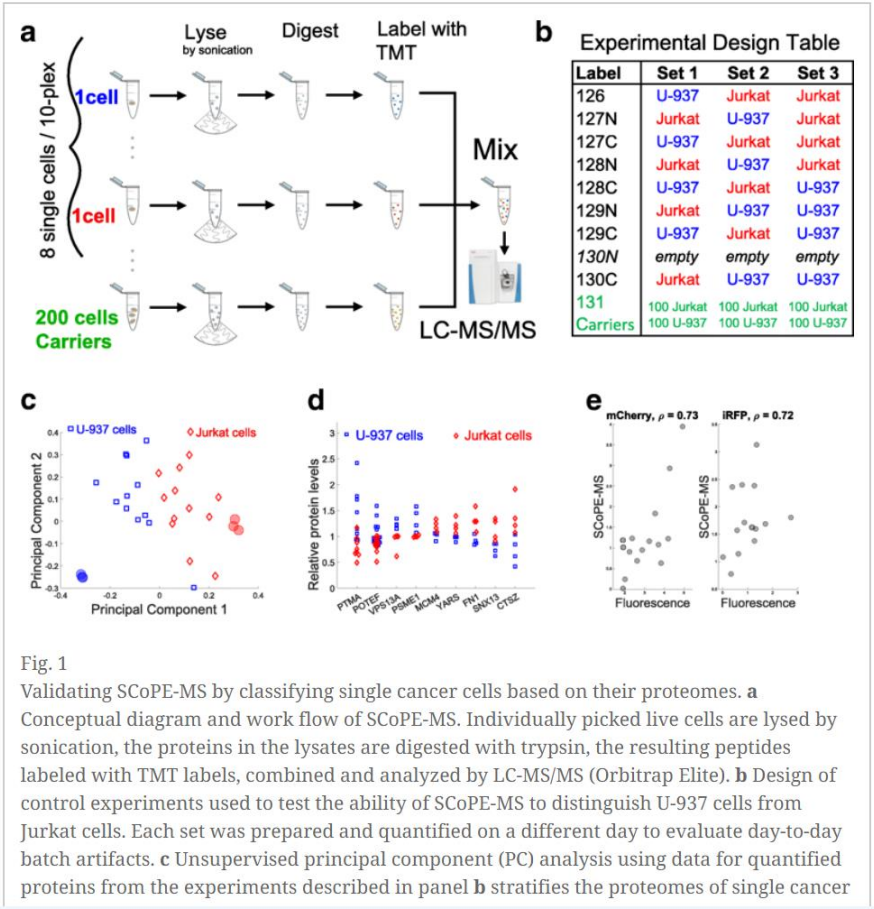
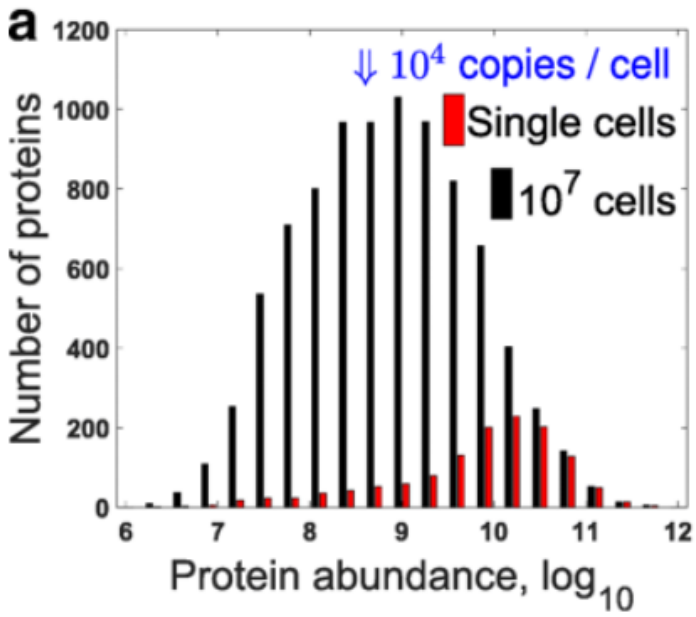



Fig. 1
Validating SCoPE-MS by classifying single cancer cells based on their proteomes. **a** Conceptual diagram and work flow of SCoPE-MS. Individually picked live cells are lysed by sonication, the proteins in the lysates are digested with trypsin, the resulting peptides labeled with TMT labels, combined 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

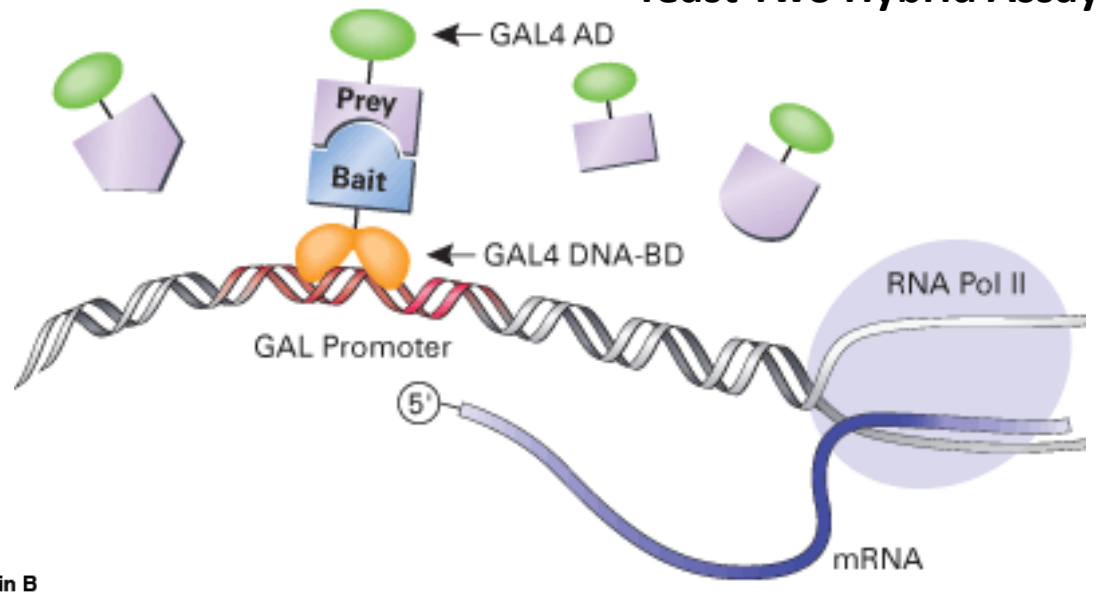
*Genome Biology* 2018 **19**:161
https://doi.org/10.1186/s13059-018-1547-5 
**Received:** 20 February 2018 | **Accepted:** 19 September 2018 | **Published:** 22 October 2018

Supplemental slides

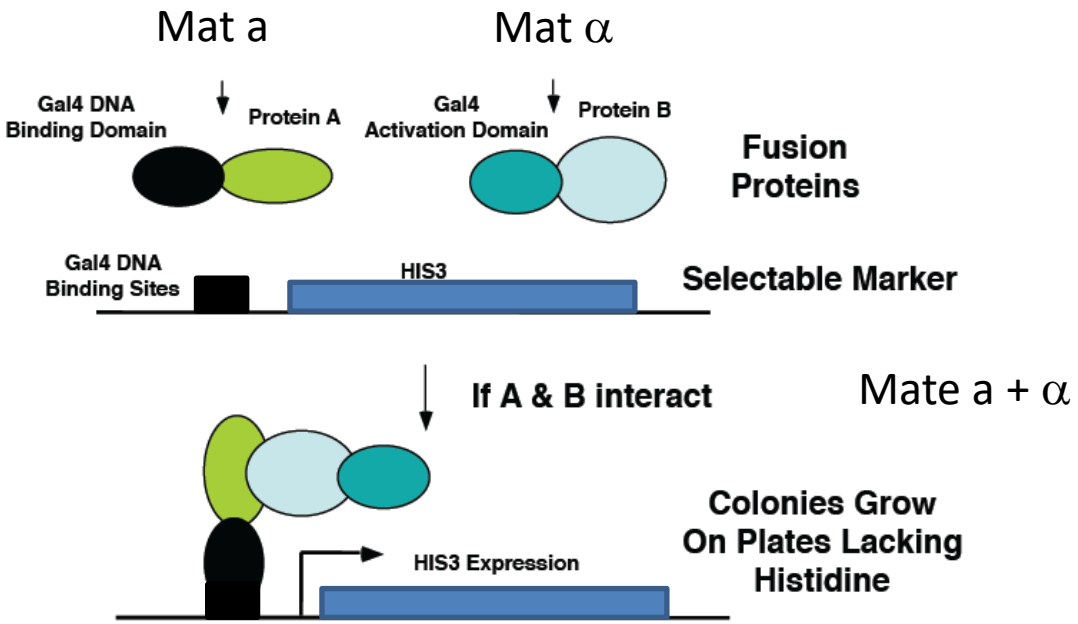
**A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae.**

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

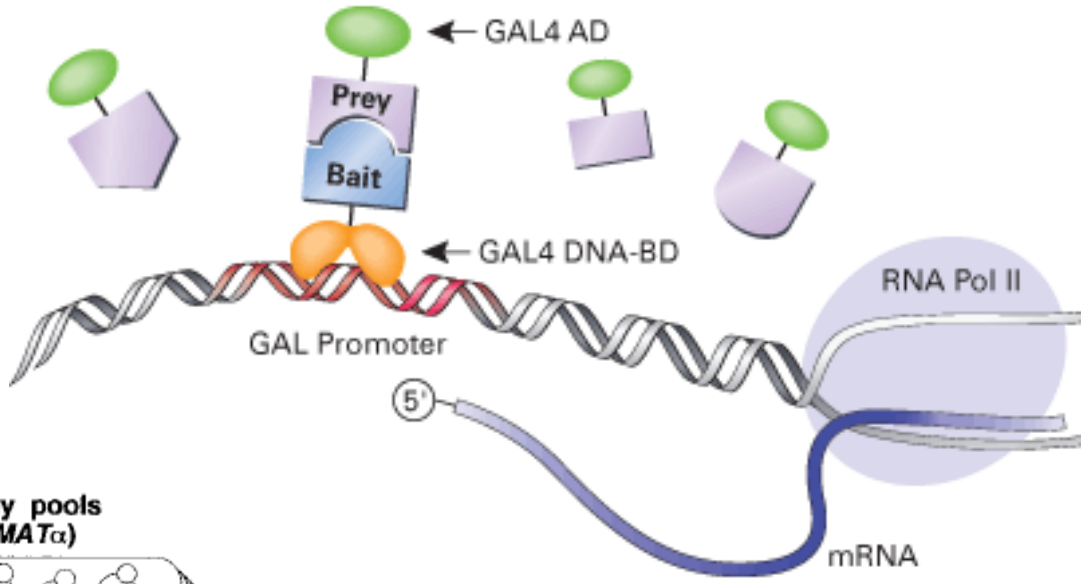
**Yeast Two Hybrid Assay**

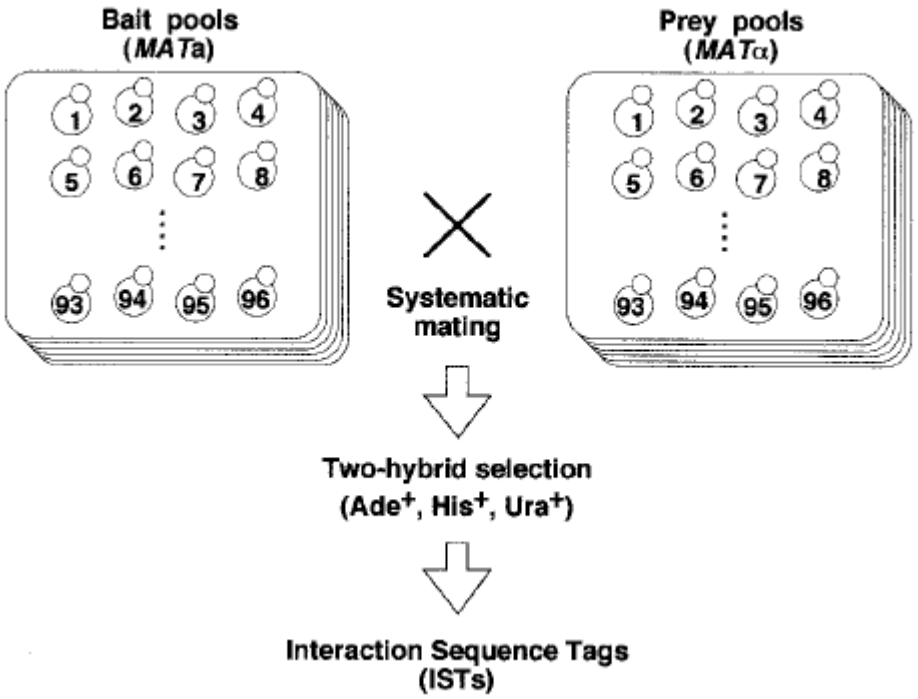



Clone bait and prey constructs and place in separate strains.



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




## Results of Two Studies

1) 4,549 Interactions Among 3,278 Proteins (Ito et al.)

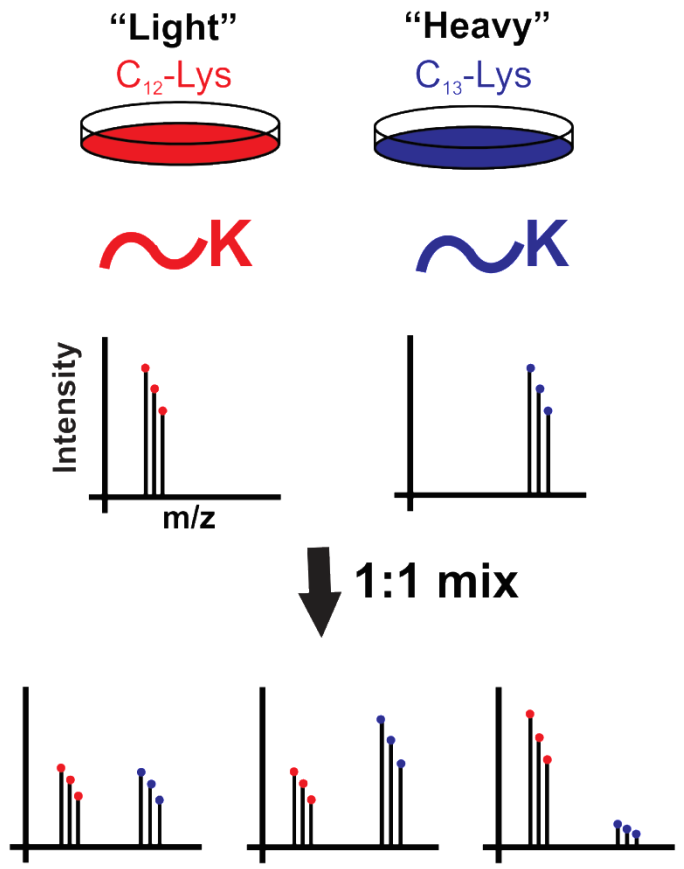
2) 957 Interactions 1004 proteins (Utez et al.)

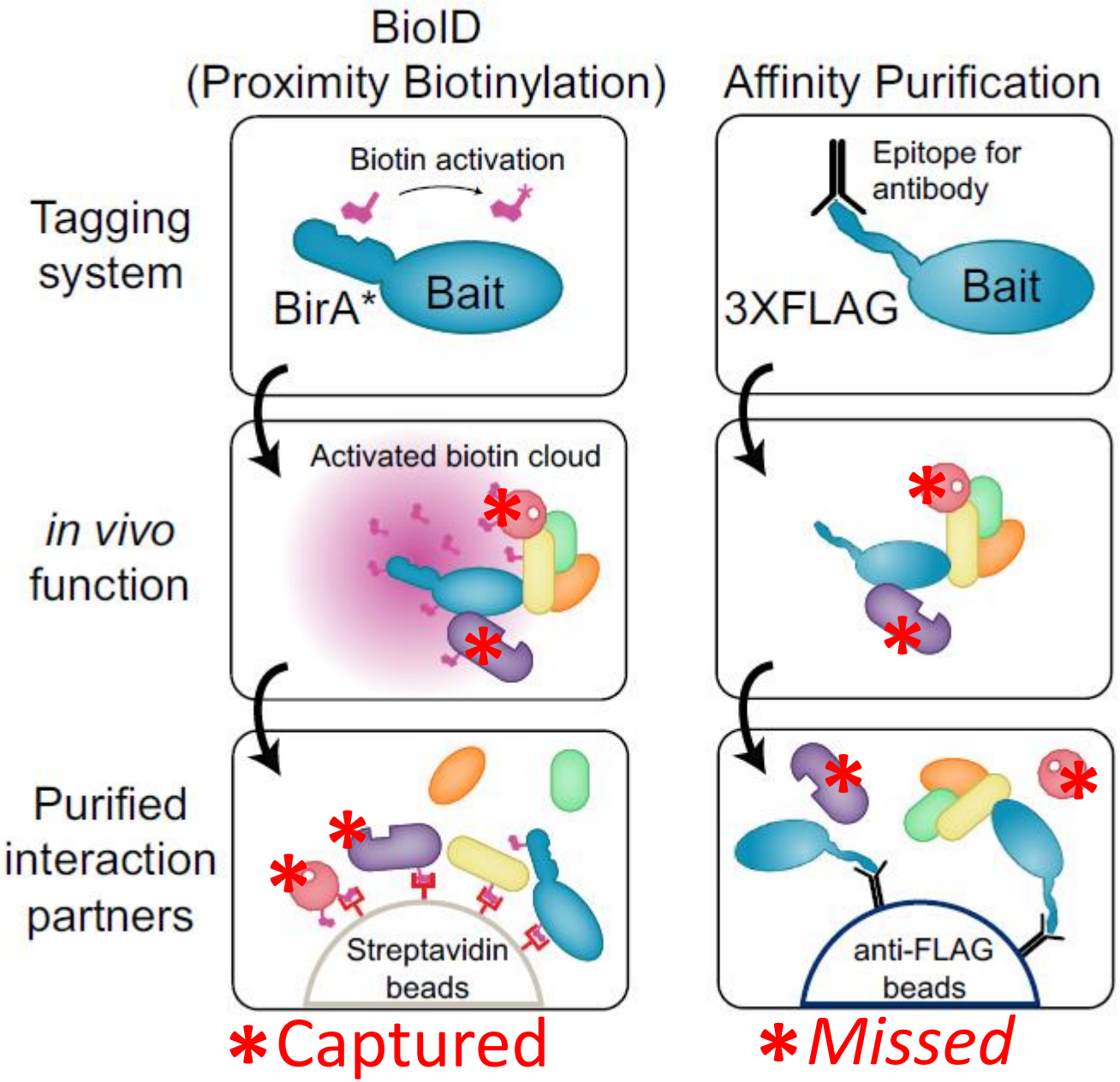
# Quantitative Proteomics

## S.I.L.A.C. - Stable isotope labeling with amino acids in cell culture

-Ong S.E. et al. *Molecular & Cell Proteomics* 2002

- **Stable isotopes are *not radioactive*, and they occur naturally in nature. For example, 99% of all carbon in the world is carbon-12 ($^{12}C$) and 1% is carbon-13 ($^{13}C$).**
- **SILAC reagents have enriched stable isotopes that have been placed into compounds in abundances much greater than their natural abundance.**
- **We can obtain labeled compounds with ~95-99% $^{13}C$.**
- **Because a mass spectrometer separates ions by mass, we use mass spectrometry to distinguish isotopes in compounds by their mass.**
- **Simultaneous comparison in the same MS run is key**






Lambert JP, et al., Gingras AC. J Proteomics. 2015 PMID: 25281560

**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.**

**Table 1 | Yeast ORFs identified by SILAC-based quantitative proteomics**

| | Number of ORFs | TAP | GFP | nanoLC–MS |
|---|---|---|---|---|
| Total yeast ORFs | 6,608 | 4,251 | 4,154 | 4,399 |
| Characterized yeast ORFs | 4,666 | 3,629 | 3,581 | 3,824 |
| Uncharacterized yeast ORFs | 1,128 | 581 | 539 | 572 |
| Dubious yeast ORFs | 814 | 26 (3%) | 23 (3%) | 3 (<1%) |
| Not present in ORF database | | 15 | 11 | 0 |

Comparative sequencing shows that 814 of the 6,608 yeast ORFs are never expressed (dubious ORFs, http://www.yeastgenome.org). Of these only six were identified in this experiment and three were validated by SILAC-assisted *de novo* sequencing of several peptides (Supplementary Table 5 and Supplementary Figs 2–4). Two of the three validated ones were reclassified as genuine yeast genes during writing of this manuscript (YGL041W-A and YPR170W-B). This leaves three potential false-positives (0.37% of 815) and suggests that our estimate of a false-positive identification rate of maximally 1% is conservative.

## 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.**

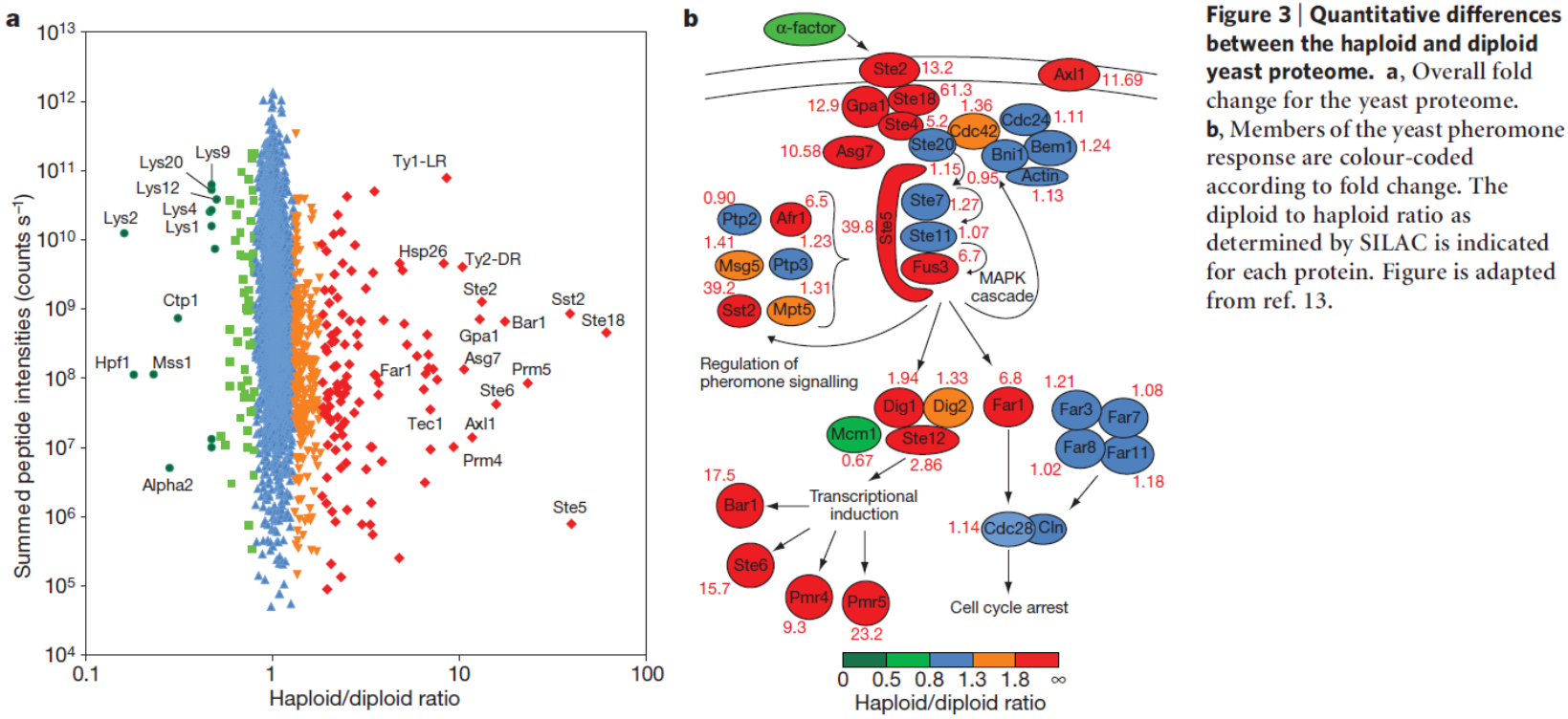


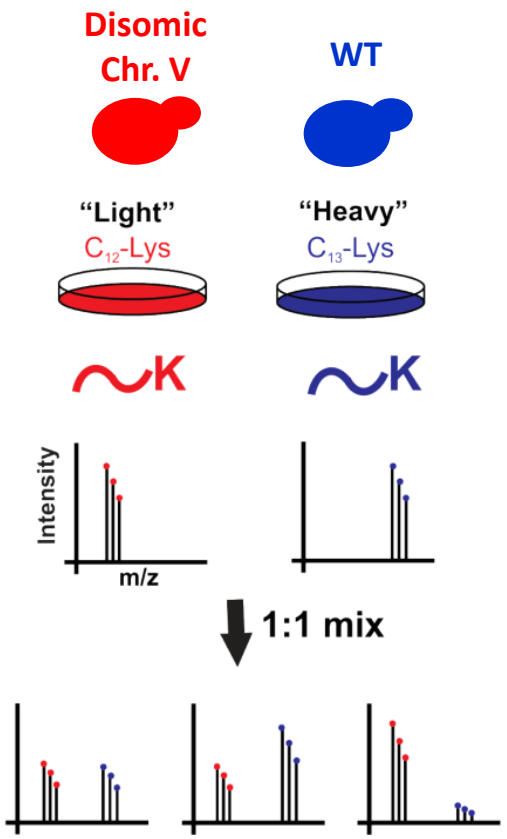

**Figure 3 | Quantitative differences between the haploid and diploid yeast proteome.** **a**, Overall fold change for the yeast proteome. **b**, Members of the yeast pheromone response are colour-coded according to fold change. The diploid to haploid ratio as determined by SILAC is indicated for each protein. Figure is adapted from ref. 13.

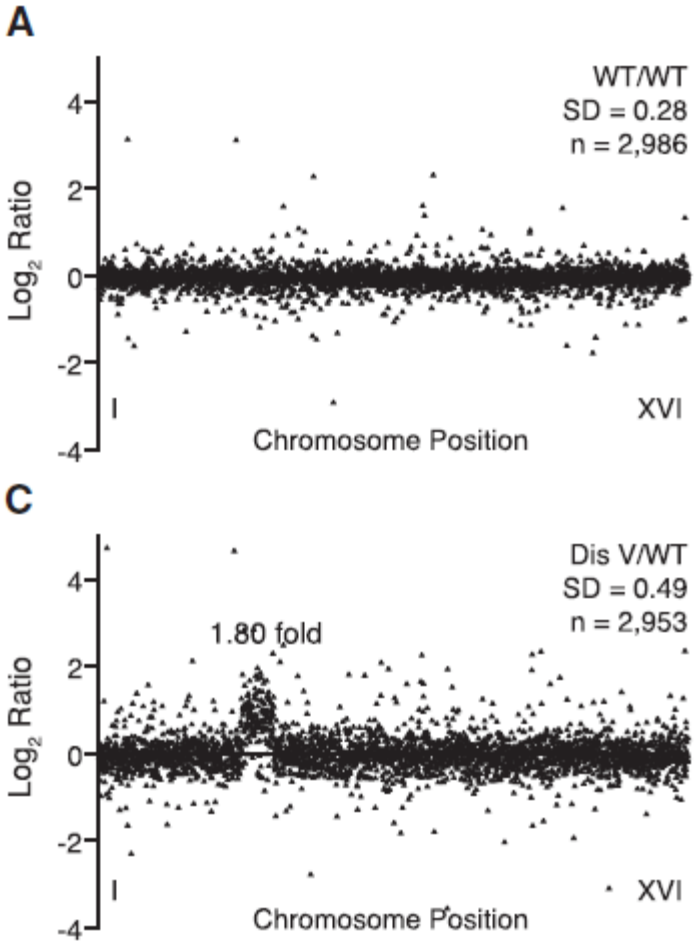
**Pheromone signaling is required for mating of haploid cells and is absent from diploid cells.**

# Identification of Aneuploidy-Tolerating Mutations

Cell *143*, 71–83, October 1, 2010

Eduardo M. Torres,[1,2] Noah Dephoure,[3] Amudha Panneerselvam,[1] Cheryl M. Tucker,[4] Charles A. Whittaker,[1] Steven P. Gygi,[3] Maitreya J. Dunham,[5] and Angelika Amon[1,2,*]



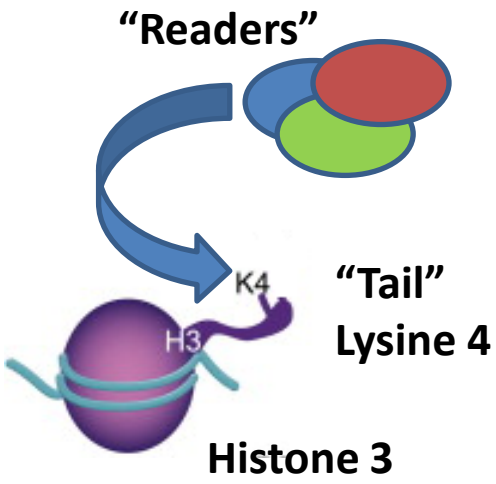



Resource

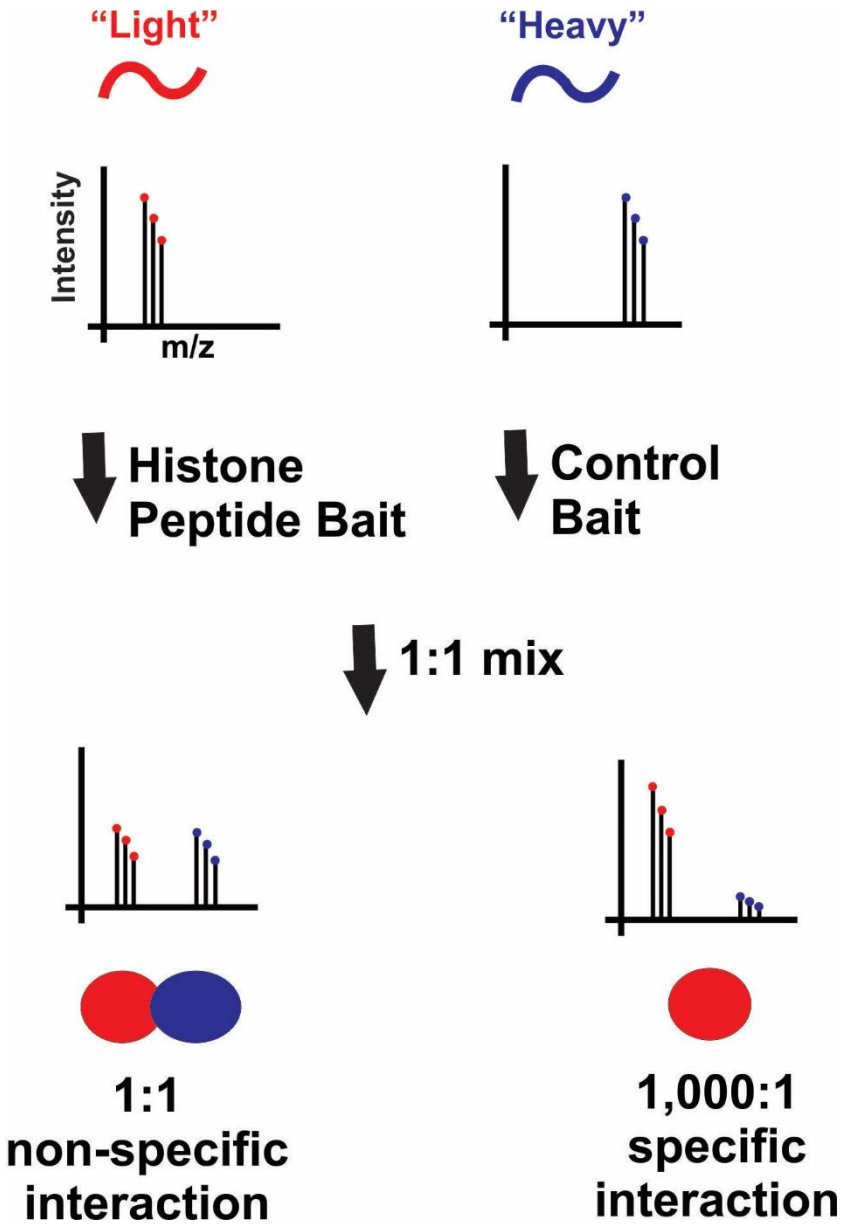
Cell

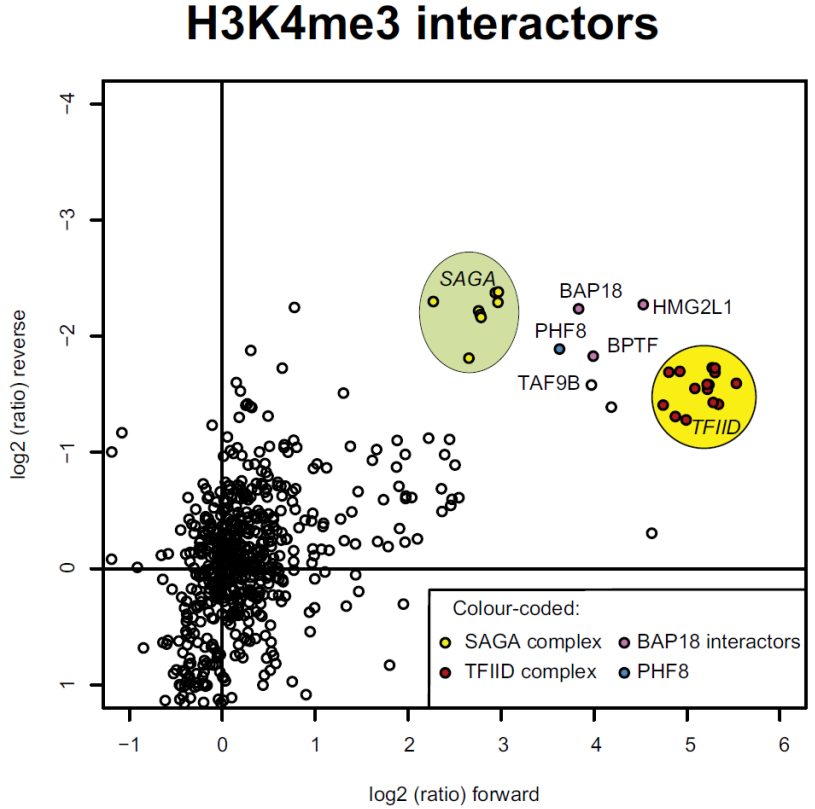
# Quantitative Interaction Proteomics and Genome-wide Profiling of Epigenetic Histone Marks and Their Readers

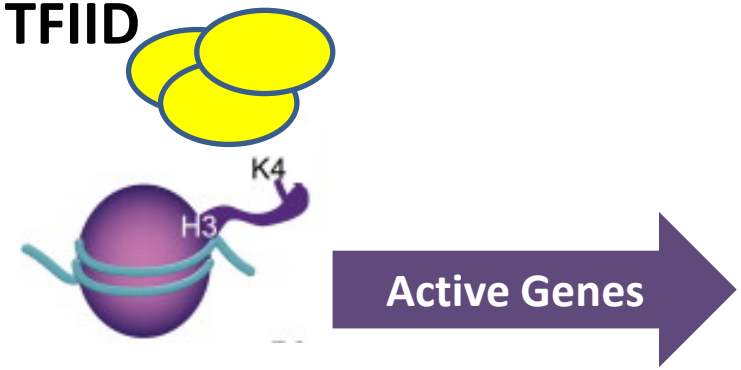
Michiel Vermeulen,[1,6,7,*] H. Christian Eberl,[1,6] Filomena Matarese,[2,6] Hendrik Marks,[2] Sergei Denissov,[2] Falk Butter,[1] Kenneth K. Lee,[3] Jesper V. Olsen,[1,5] Anthony A. Hyman,[4] Henk G. Stunnenberg,[2,*] and Matthias Mann[1,*]




Vermeulen et al., **Cell** 2010

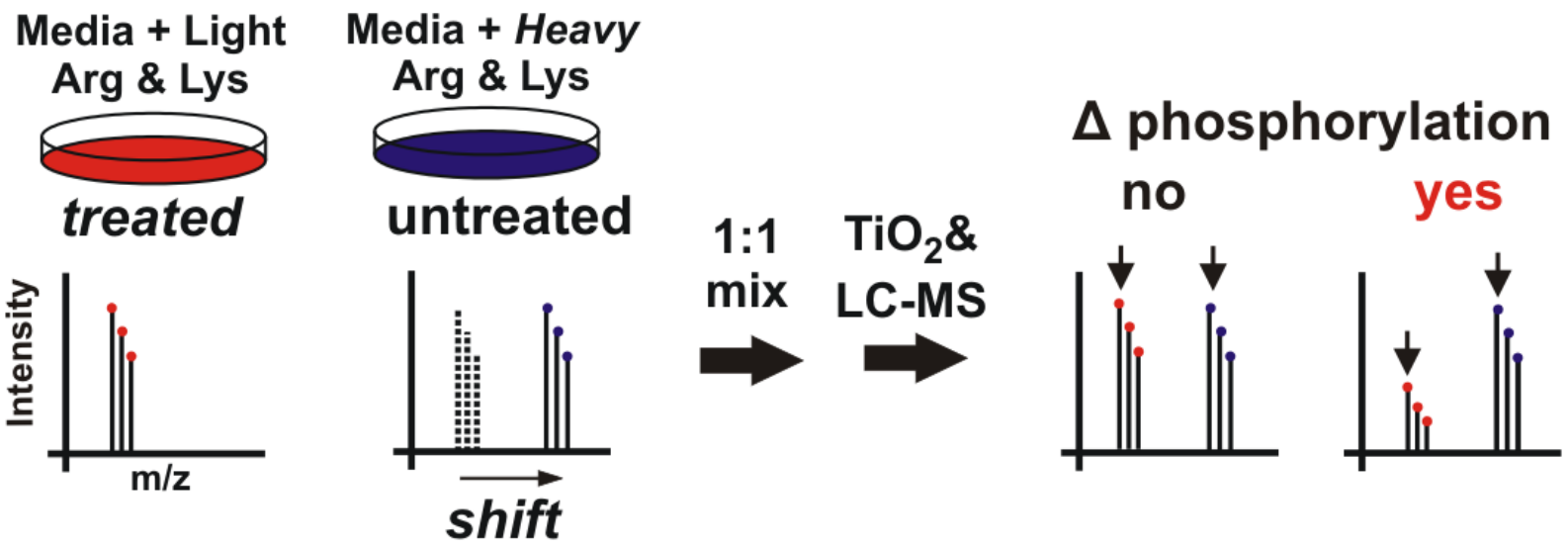


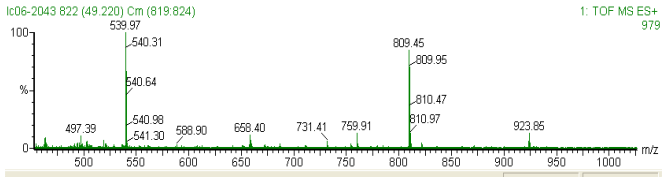

Vermeulen et al., **Cell** 2010

# A SILAC approach to study protein phosphorylation dynamics



# *Phosphopeptide signatures in MS

**MS**



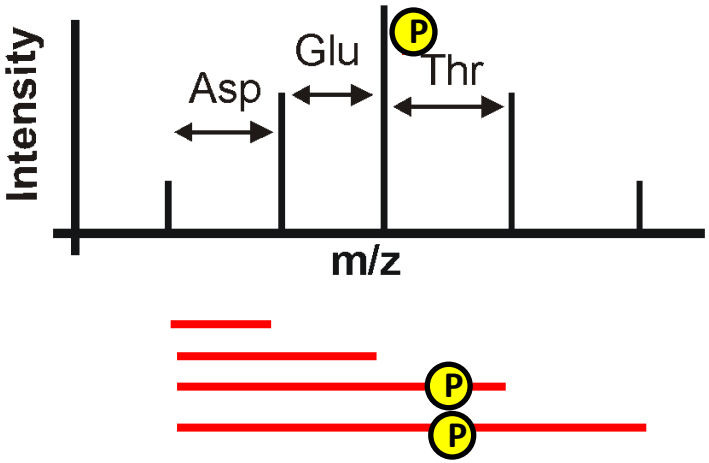

peptide

P peptide

**isolate & fragment**

P peptide

**MS/MS**



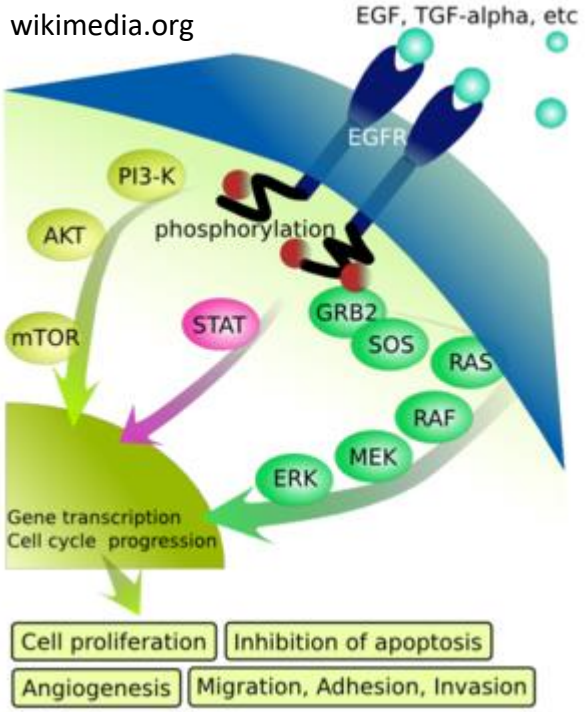

**peptide fragments**

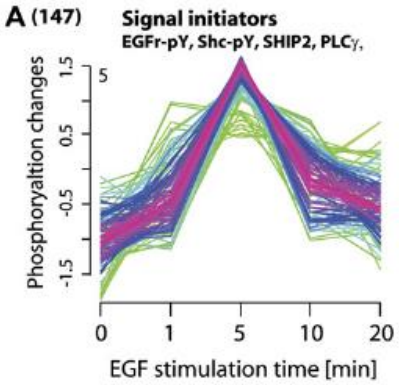
P +80 Da in precursor

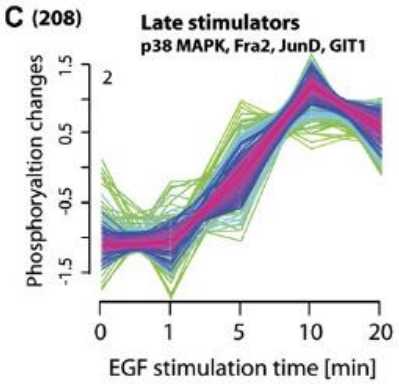
-98 Da loss of phosphoric acid $H_3PO_4$ during fragmentation

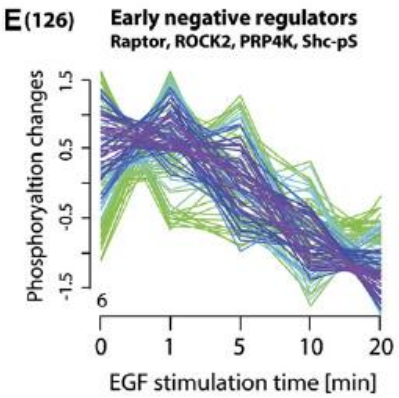
# Phosphorylation dynamics after EGF stimulation

wikimedia.org




**Olsen, et al. *Cell*, 2006**