Proteomics & Protein-Protein Interactions

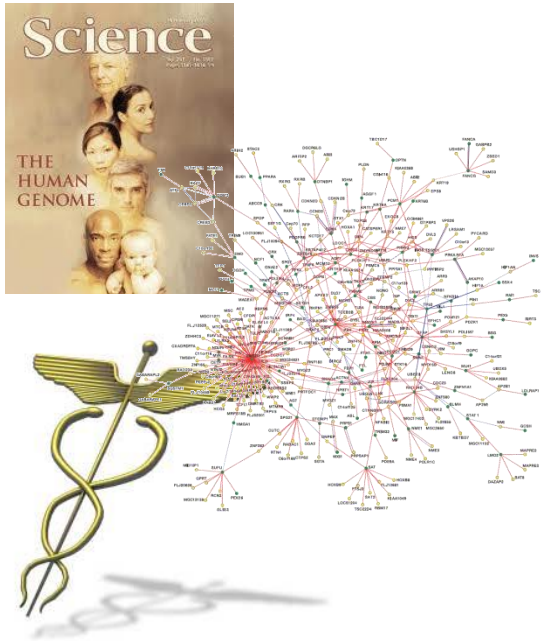
Jesse Rinehart, PhD

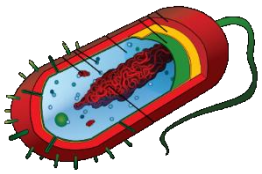
Biomedical Data Science: Mining & Modeling

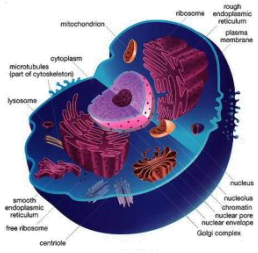
CBB 752, Spring 2020

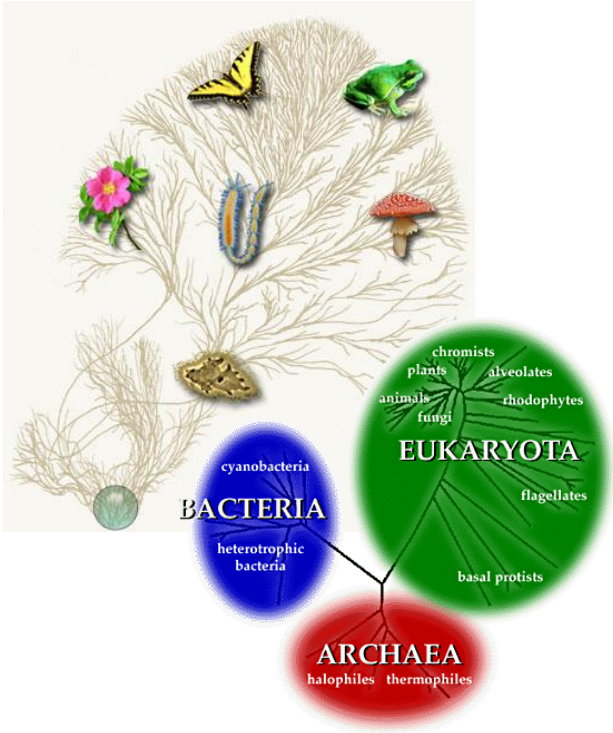
Cellular & Molecular Physiology
Yale University School of Medicine

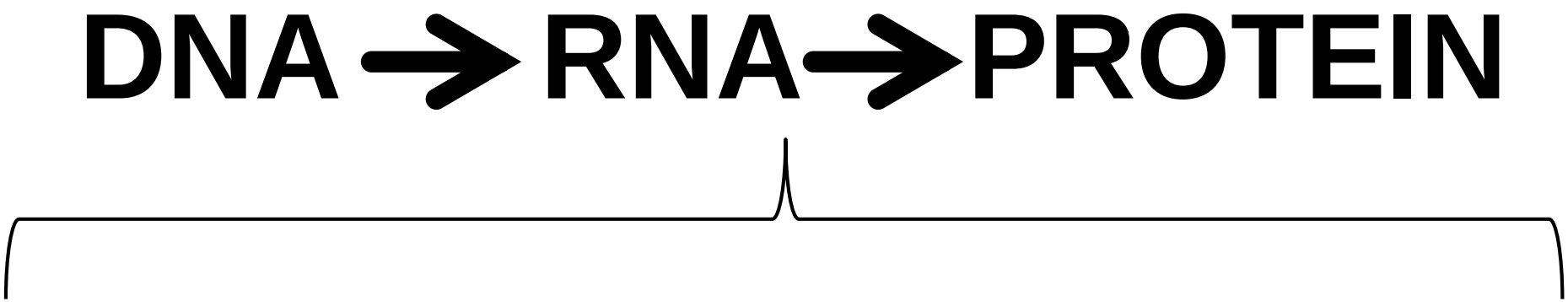
DNA → RNA → PROTEIN










DNA → RNA → PROTEIN
SYNTHETIC BIOLOGY
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†]

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

By Karen Pallarito (HealthDay News)

Uploaded on December 21, 2015

Dec 2015

ARTICLE

Aug. 2017

doi:10.1038/nature23305

Correction of a pathogenic gene mutation in human embryos

Hong Ma[1*], Nuria Marti-Gutierrez[1*], Sang-Wook Park[2*], Jun Wu[3*], Yeonmi Lee[1], Keiichiro Suzuki[3], Amy Koski[1], Dongmei Ji[1], Tomonari Hayama[1], Riffat Ahmed[1], Hayley Darby[1], Crystal Van Dyken[1], Ying Li[1], Eunju Kang[1], A.-Reum Park[2], Daesik Kim[4], Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Ying Gu[5,6,7], Xun Xu[5,6,7], David Battaglia[1,9], Sacha A. Krieg[9], David M. Lee[9], Diana H. Wu[9], Don P. Wolf[1], Stephen B. Heitner[10], Juan Carlos Izpisua Belmonte[3]§, Paula Amato[1,9]§, Jin-Soo Kim[2,4]§, Sanjiv Kaul[10]§ & Shoukhrat Mitalipov[1,10]§

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

The New York Times

By Gina Kolata, Sui-Lee Wee and Pam Belluck

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

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

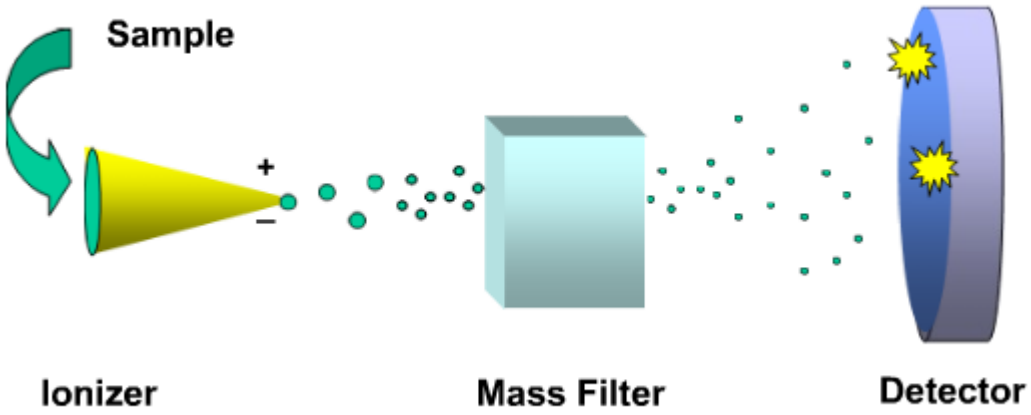
Overview

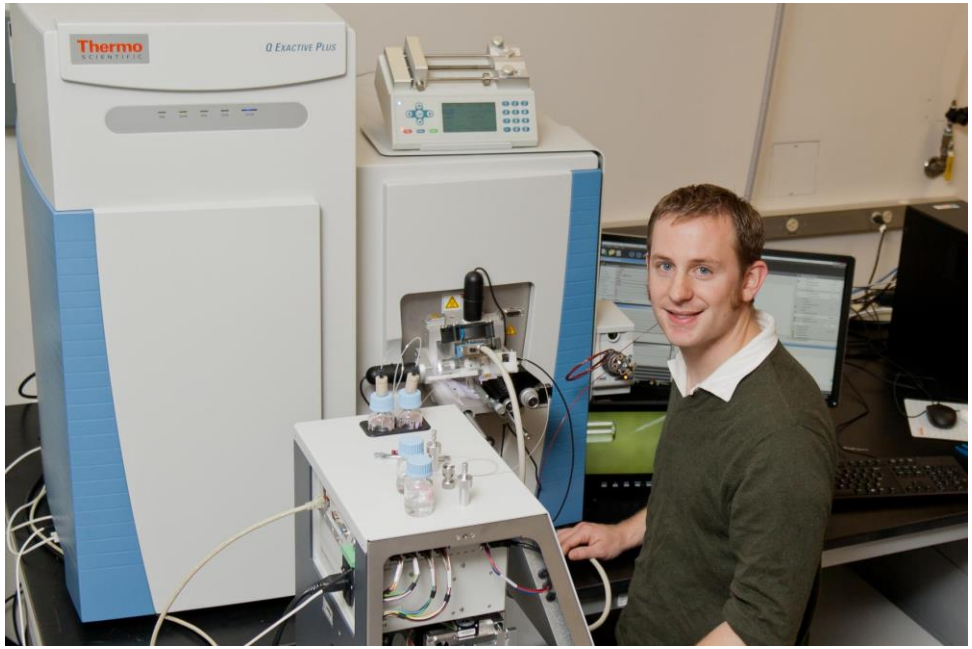
- **Techniques & Technologies**
  - Mass Spectrometry
  - Protein-Protein Interactions
  - Quantitative Proteomics
- **Applications**
  - Representative Studies
- **Putting it all together….**
  - Databases & Pathways

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

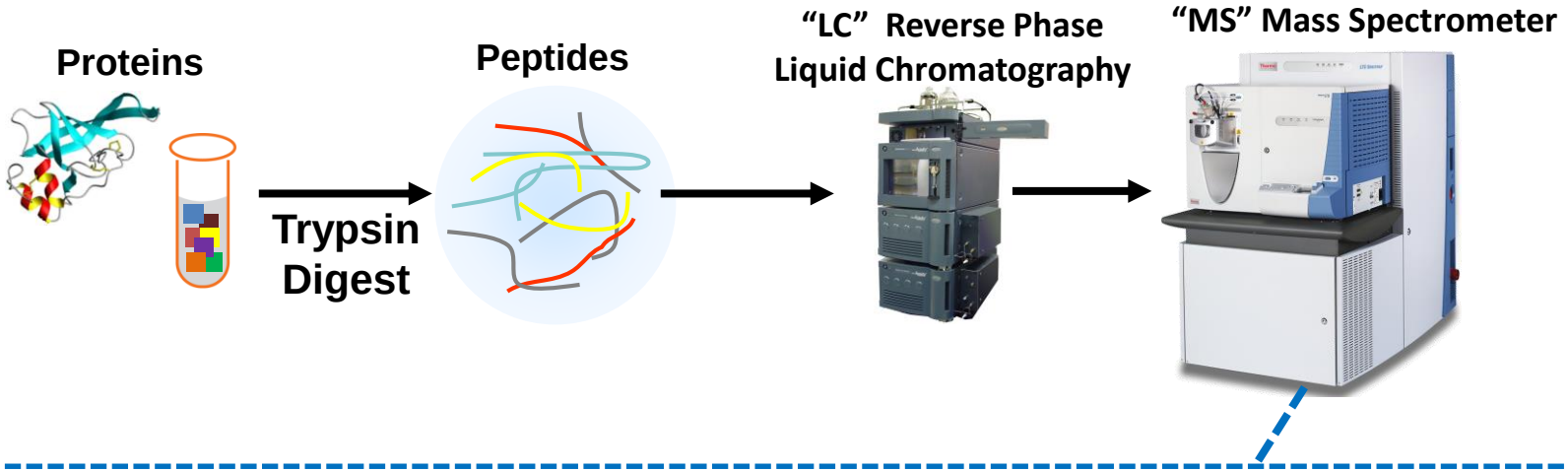
- Matrix Assisted Laser Desorption Ionization (MALDI)
  Tanaka K, Waki H, Ido Y, et al. *Rapid Commun Mass Spectrom* 1988

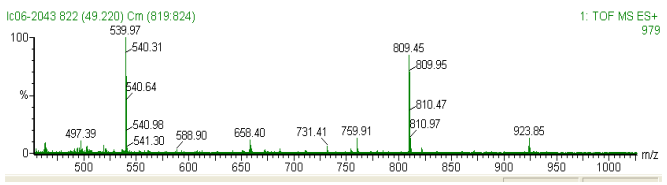
- 2002 Nobel Prize in Chemistry awarded to
  John B. Fenn & Koichi Tanaka

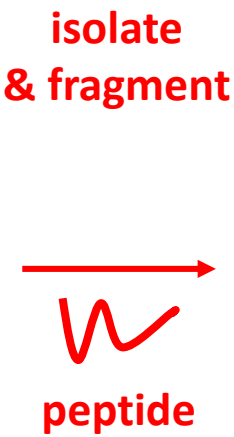
- **Enabled direct measurement and "sequencing" of intact peptides & MS based Proteomics is born**

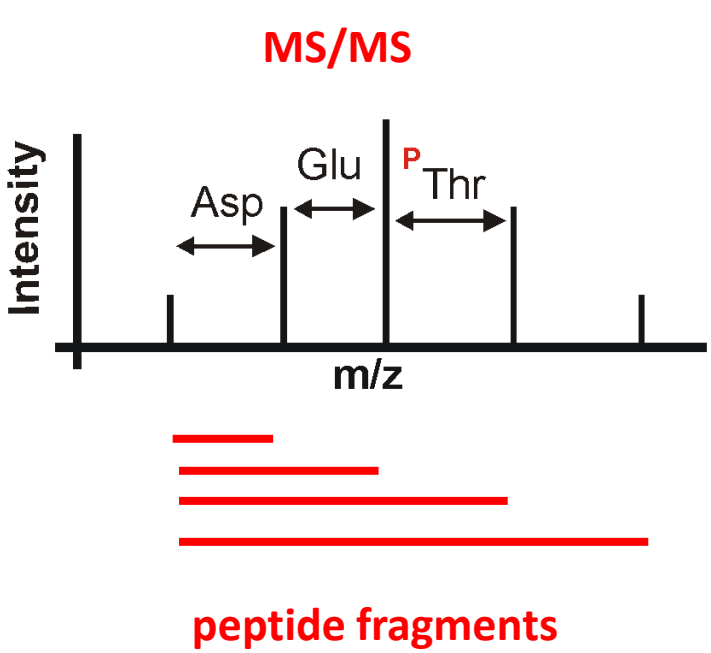
*Matthias Mann (Yale University; Ph.D.; 1988; Chemical Engineering) trained with John Fenn during some of the breakthrough work at Yale

Typical work flow for LC-MS "shotgun proteomics"

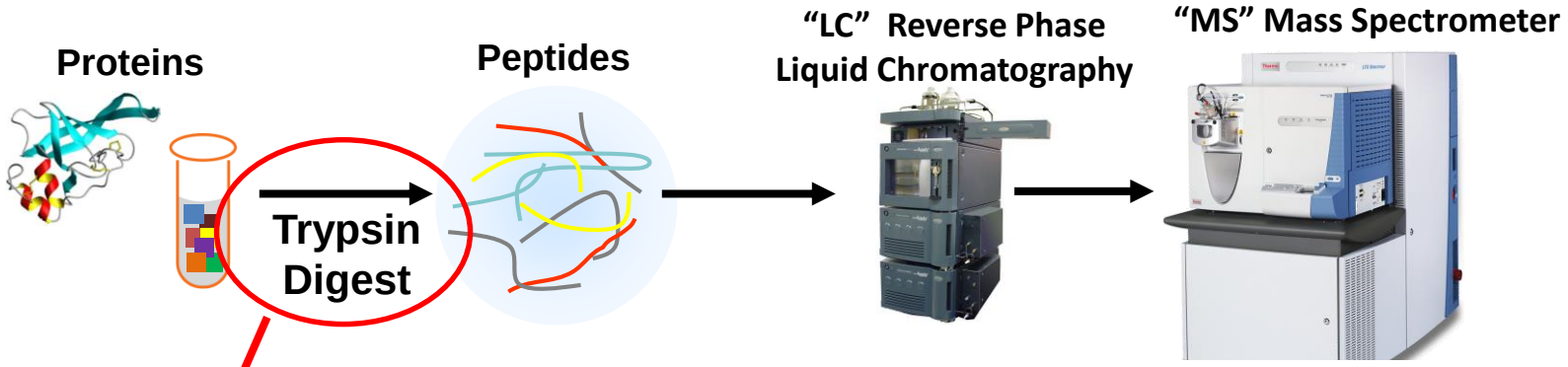


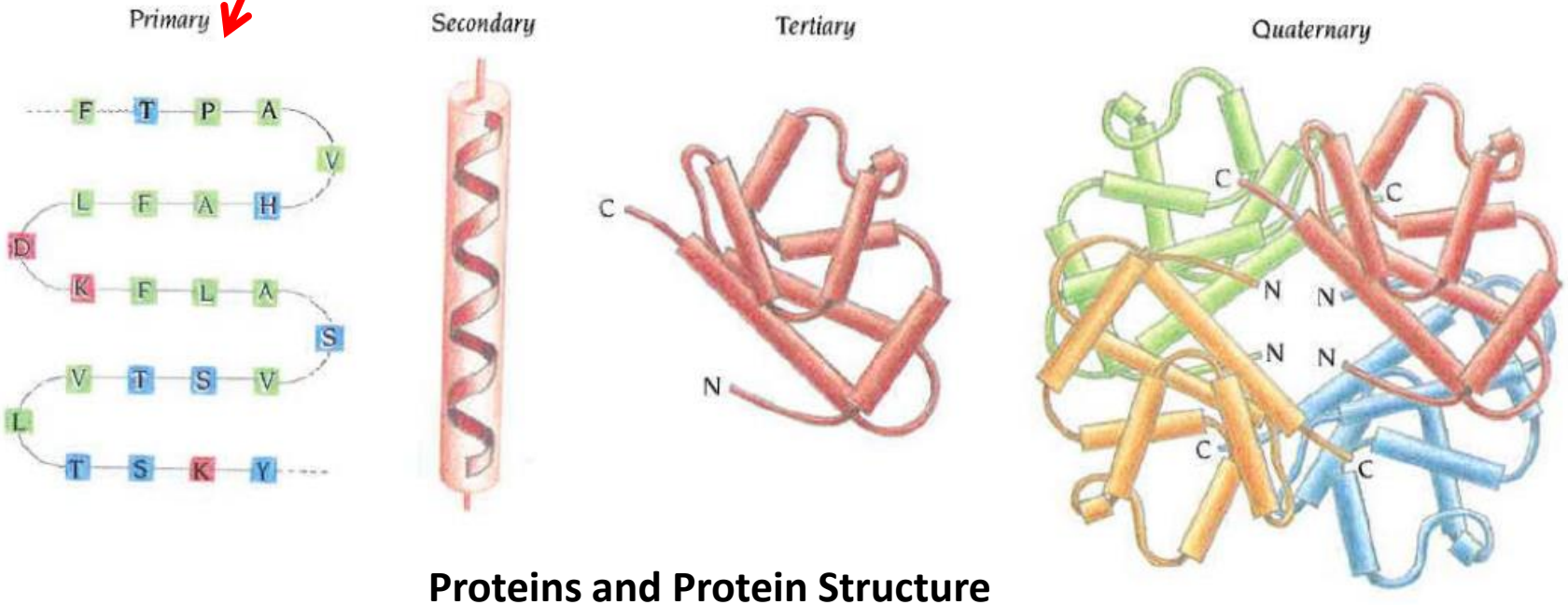






Typical work flow for LC-MS "shotgun proteomics"

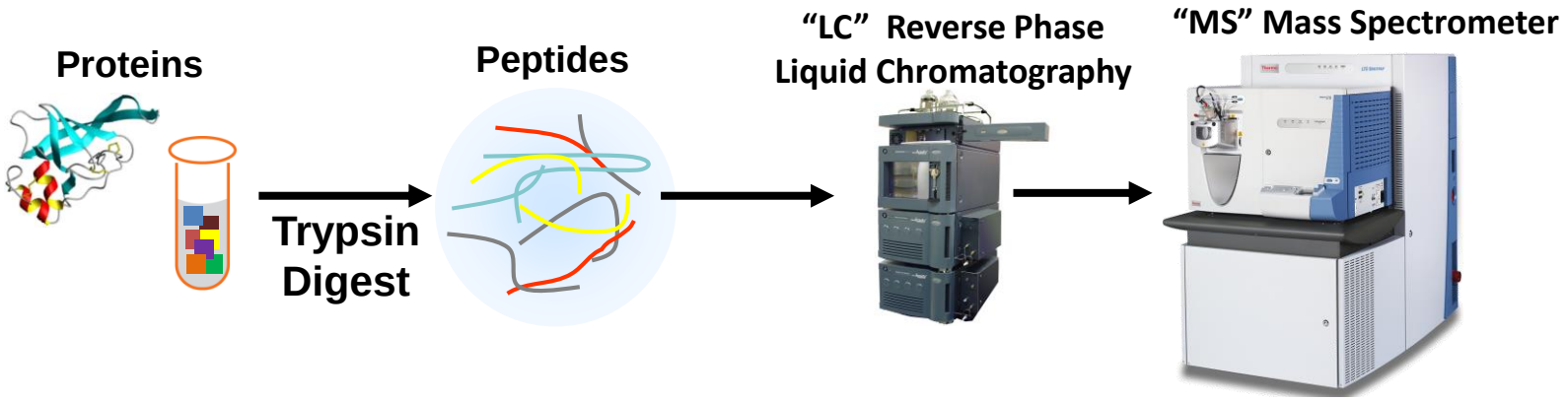


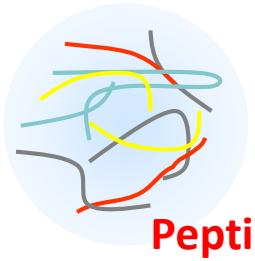


Proteins and Protein Structure

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

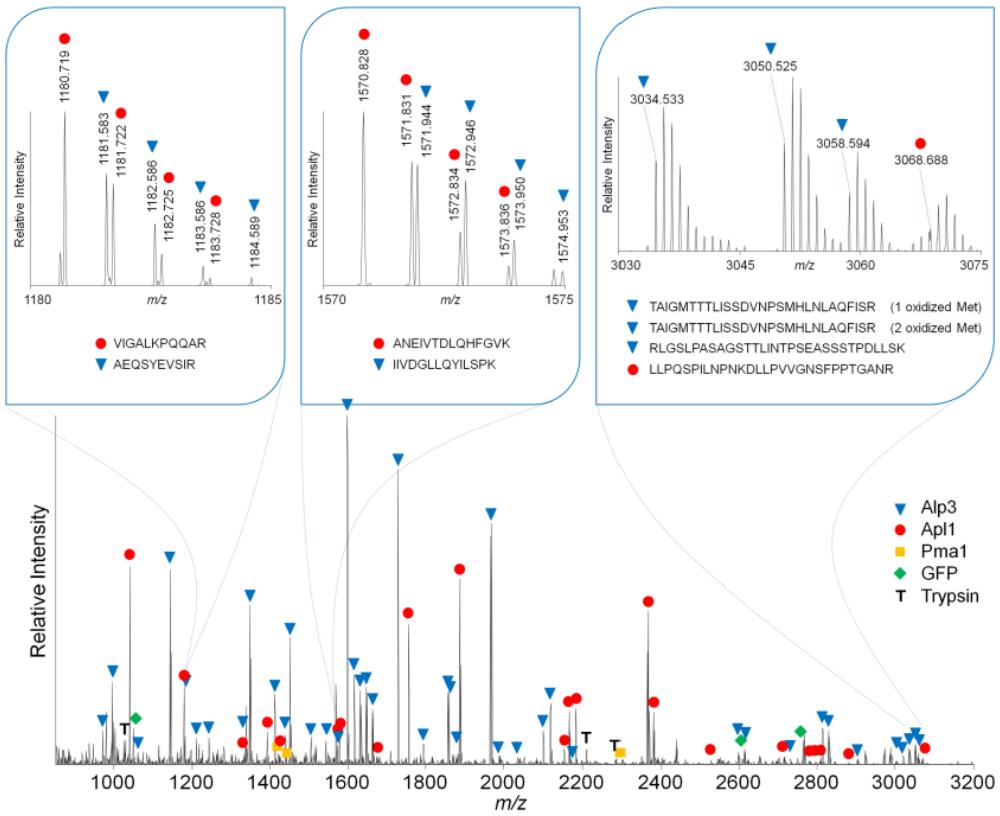
The mass spectra of peptide mixtures are complex



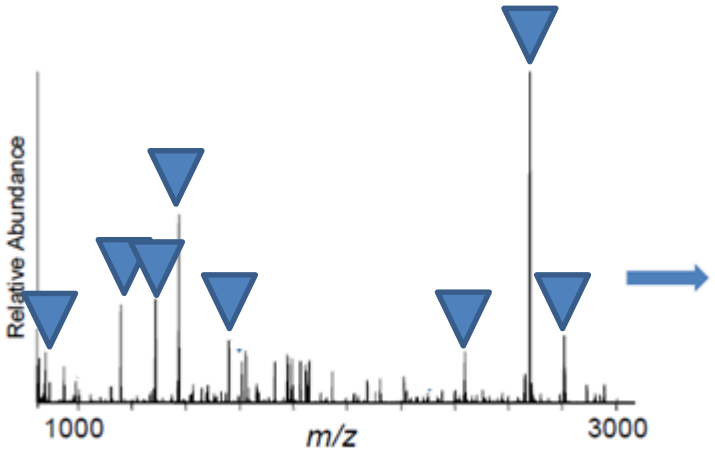


Peptide ions have a mass (m) and a charge (z).

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

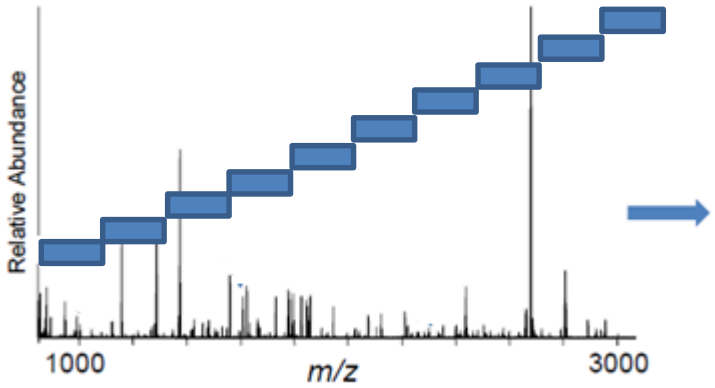


DIA (Data-independent Acquisition) **vs. DDA** (Data-dependent Acquisition)



DDA (Data-dependent Acquisition)

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

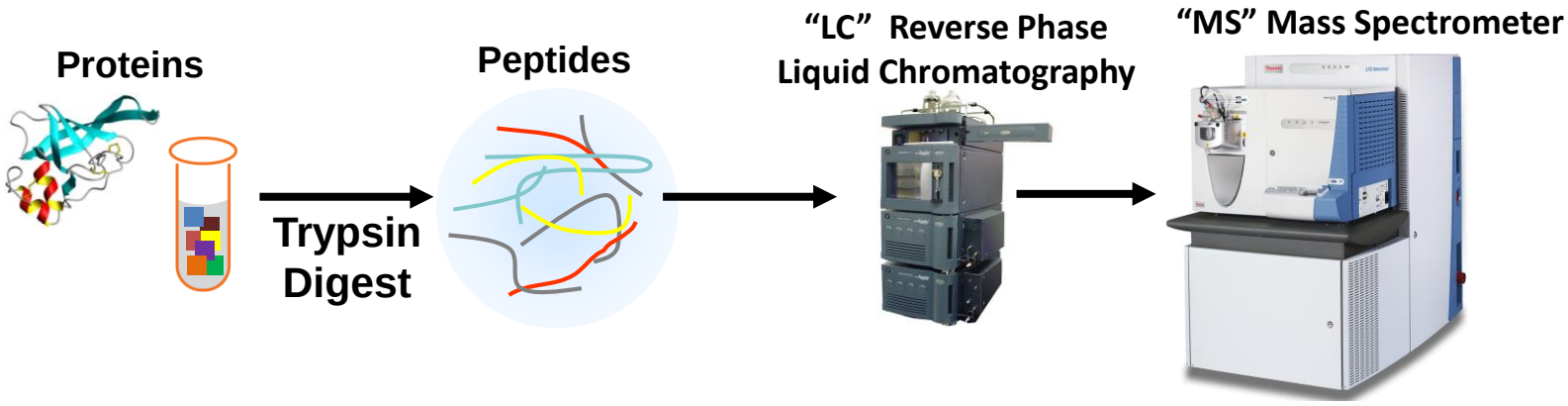


DIA (Data-independent Acquisition)

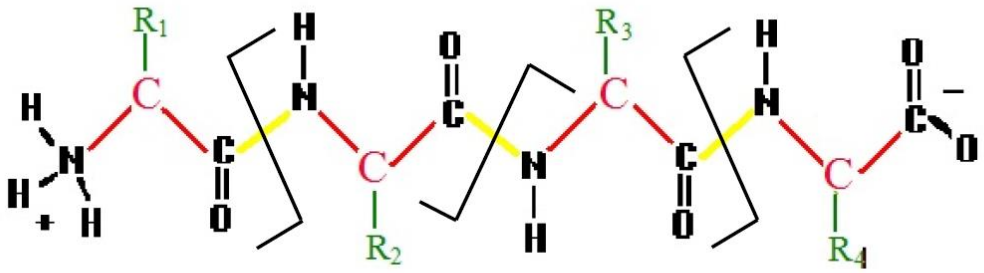
All ions in small M/Z windows are selected for MS/MS sequencing

Further Reading: PMID27092249; PMID30104418

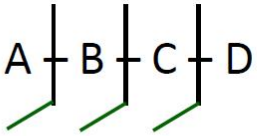
Peptide ions are isolated, fragmented, and “sequenced”



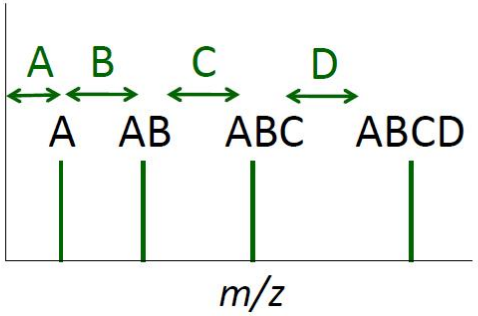
Peptide sequencing



Simplified concept of peptide fragmentation



Peptide “ABCD”



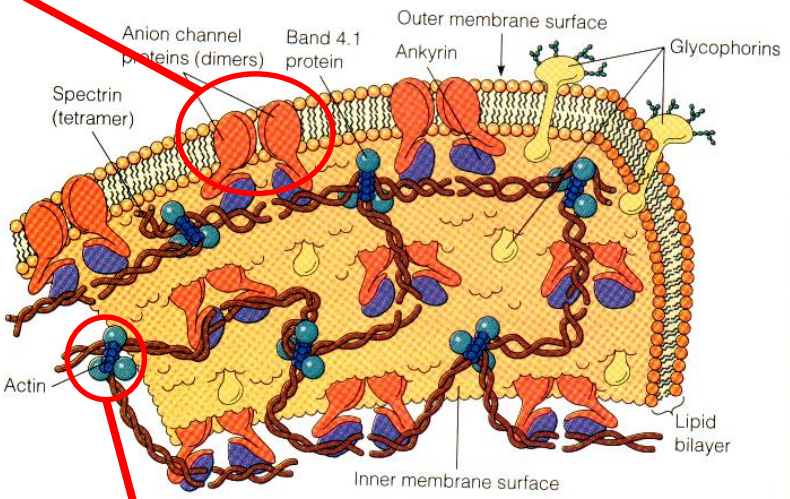
Fragment Spectra of Peptide “ABCD”

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
```



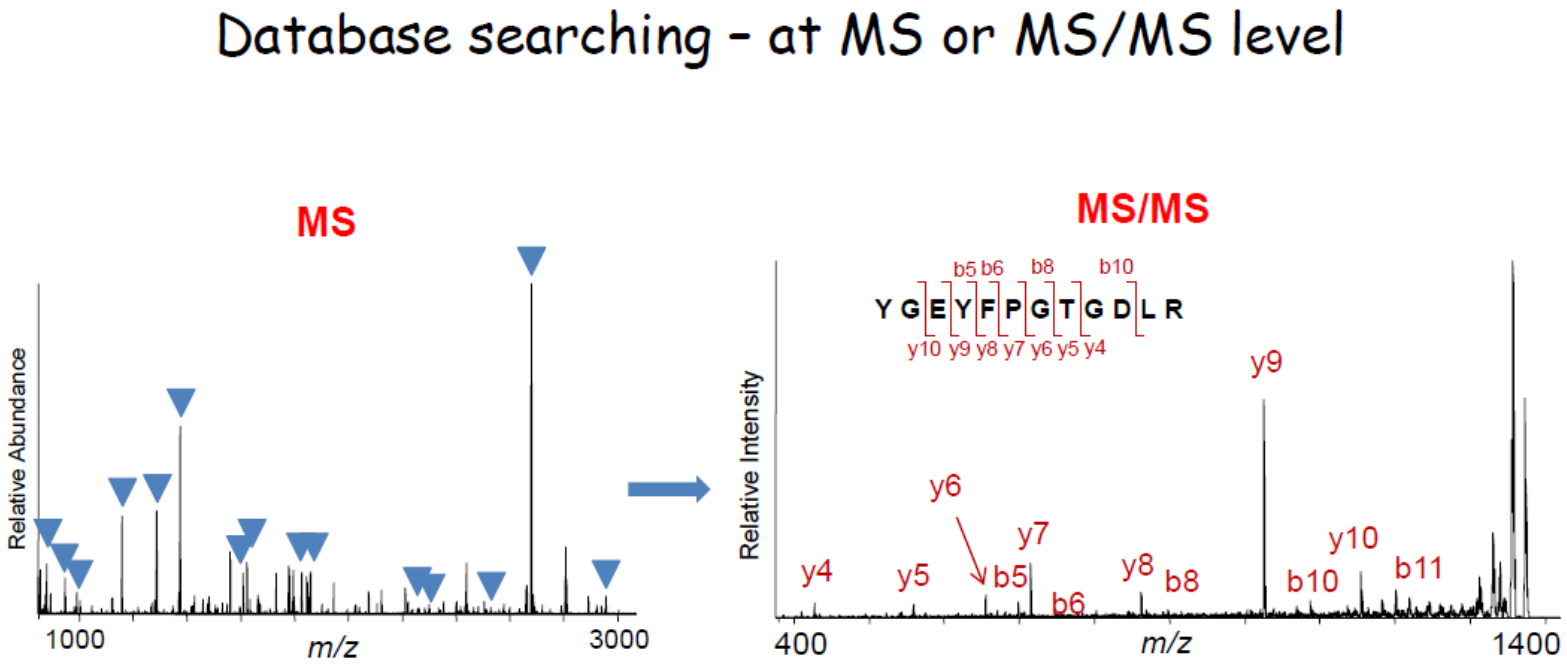
β-actin

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 interpertation
- Genome databases define proteome
- Proteome database used to “match” peptide sequence data



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**

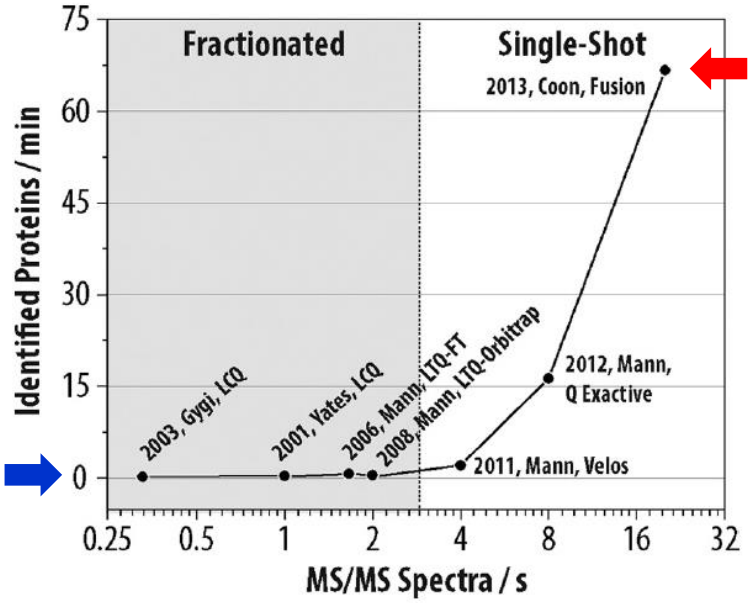


FIG. 5. **Rate of protein identifications as a function of mass spectrometer scan rate for selected large-scale yeast proteome analyses over the past decade.** Each data point is annotated with the year, corresponding author, type of MS system used, and reference number.

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

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

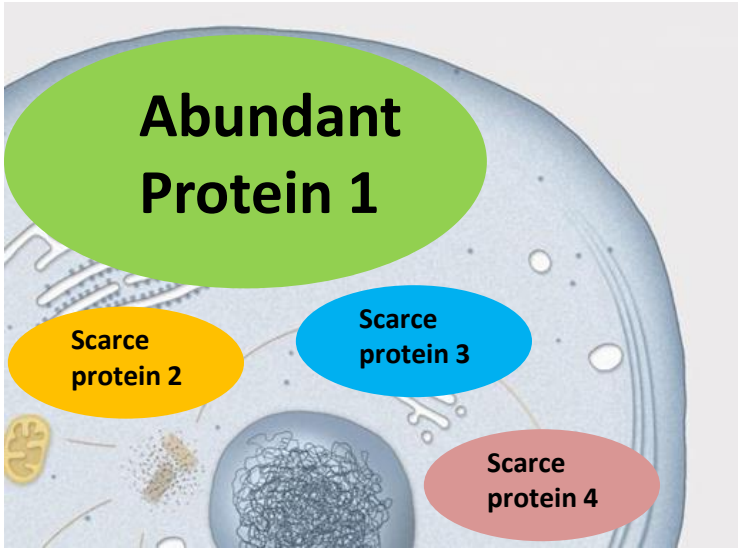
The one hour yeast proteome. Hebert AS, et a, Coon JJ.
Mol Cell Proteomics. 2014 PMID: 24143002 & *Nat Protoc.* 2015. PMID: 25855955

Major challenges prevent complete proteome analysis

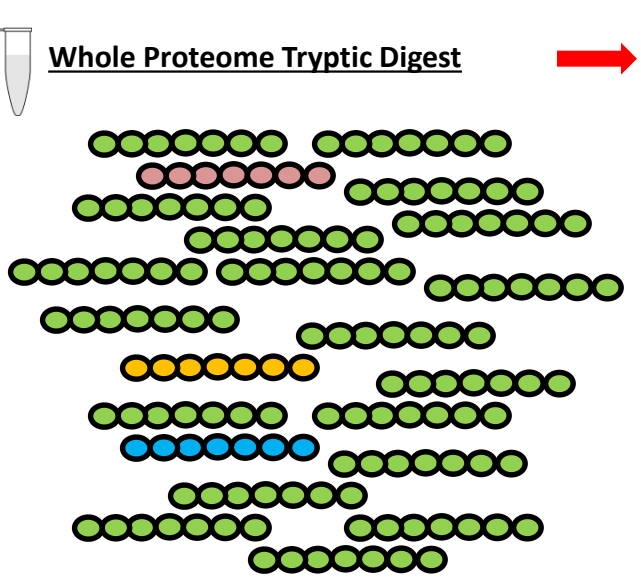
- **Proteomics is sample limited**
  - Recombinant DNA polymerases revolutionized genome sequencing by allowing for amplification of DNA samples
  - Proteomics has no “polymerase” or amplification method and must contend with natural abundancies
- **Mass spectrometry has limitations**
  - No mass spectrometer,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

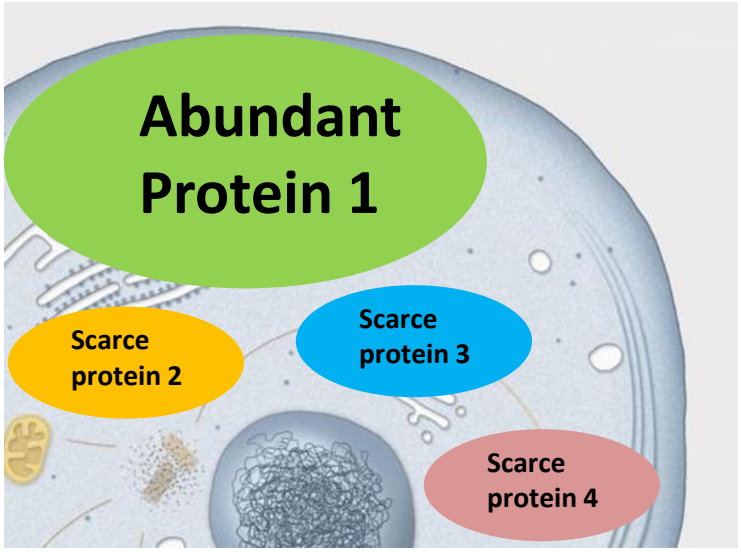
(Hypothetical MS that can only identify one peptide)

Protein 1 Identified

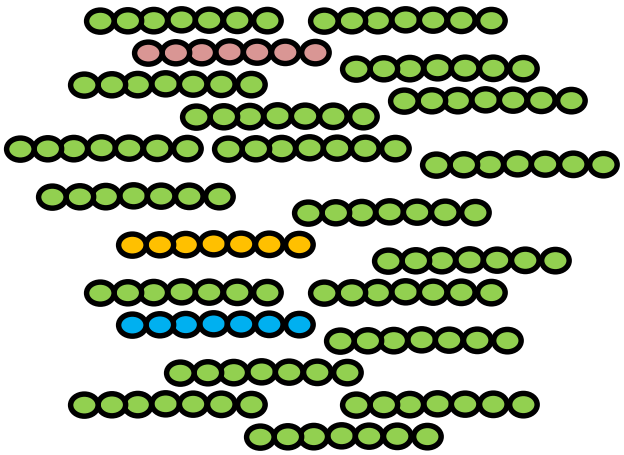
Challenge Question:

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

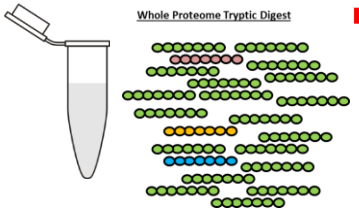


One LC-MS run

(Hypothetical MS that can only identify one peptide)

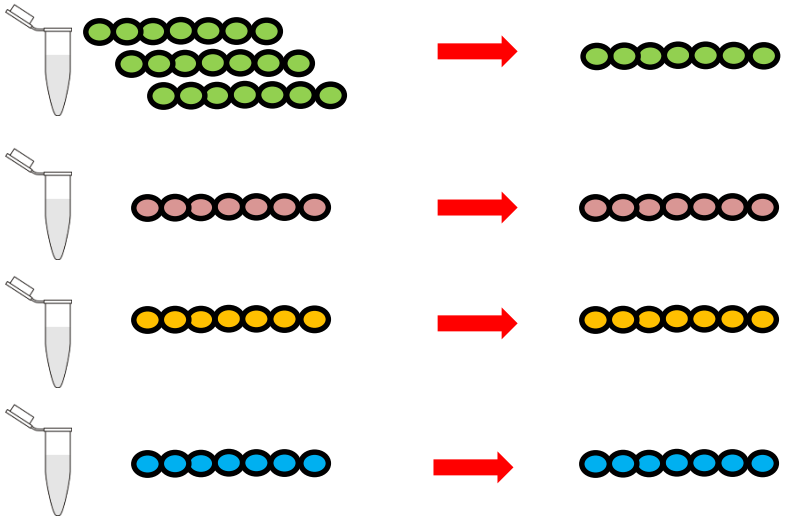
Protein 1 Identified

Option #1: Peptide Fractionation



Chromatography + fractionation

4 separate LC-MS runs



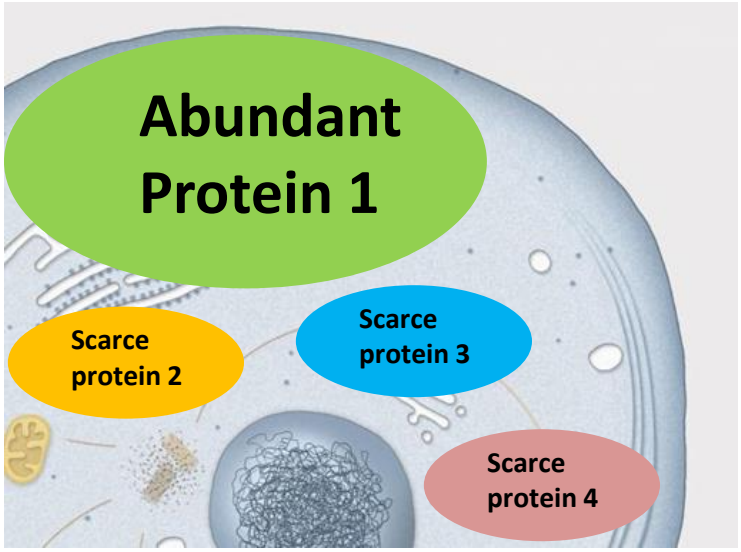
Protein 1 Identified

Protein 2 Identified

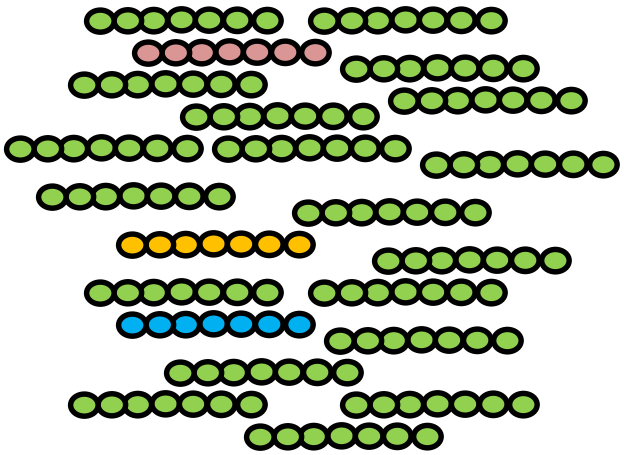
Protein 3 Identified

Protein 4 Identified

Cell with a 4 protein proteome



Whole Proteome Tryptic Digest

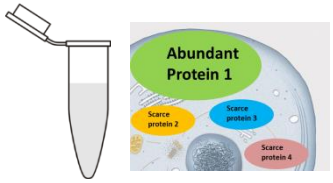


One LC-MS run

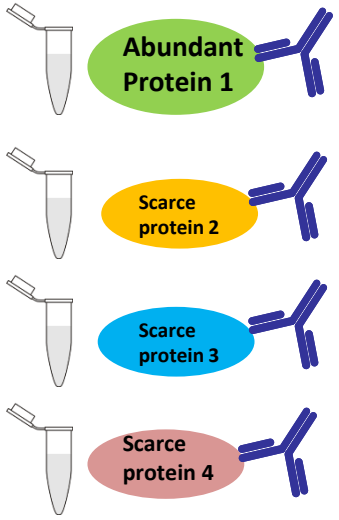
(Hypothetical MS that can only identify one peptide)

Protein 1 Identified

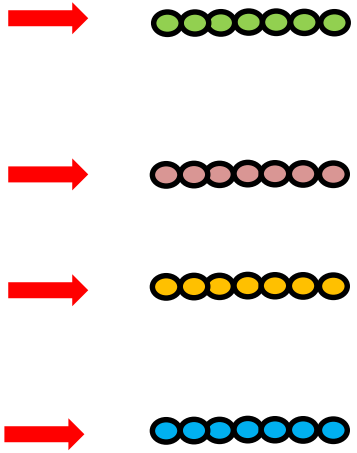
Option #2: Proteome Fractionation (e.g. Immunoprecipitation)



Separate IP Tryptic Digest



4 separate LC-MS runs



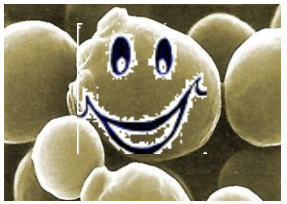
Protein 1 Identified

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

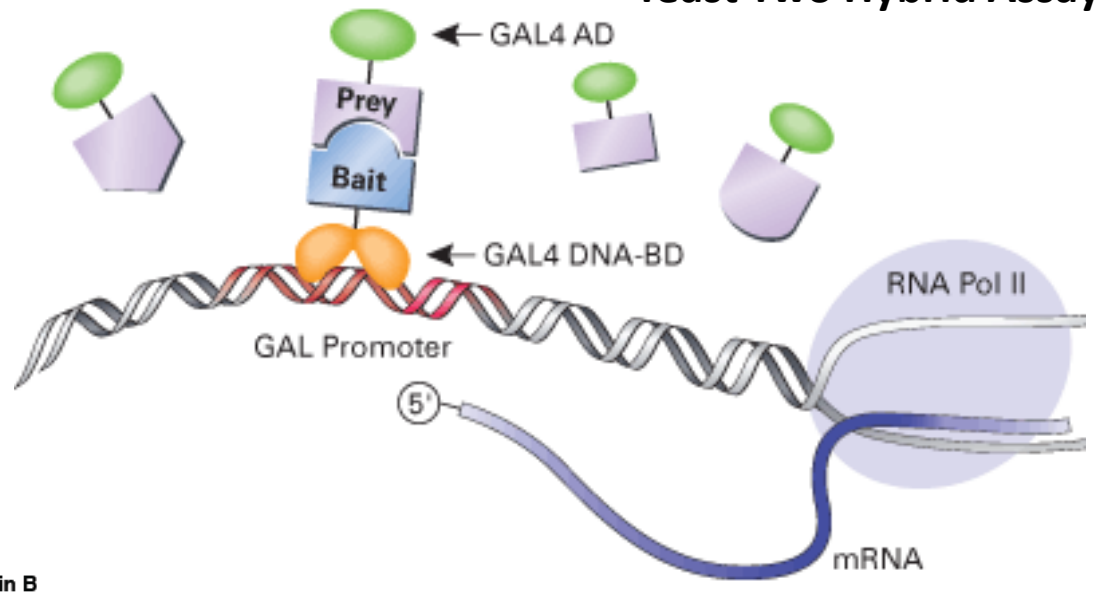
Picotti P, et al. Full dynamic range proteome analysis of S. cerevisiae by targeted proteomics. *Cell.*

➲ **Towards proteome wide targeted proteomics.**

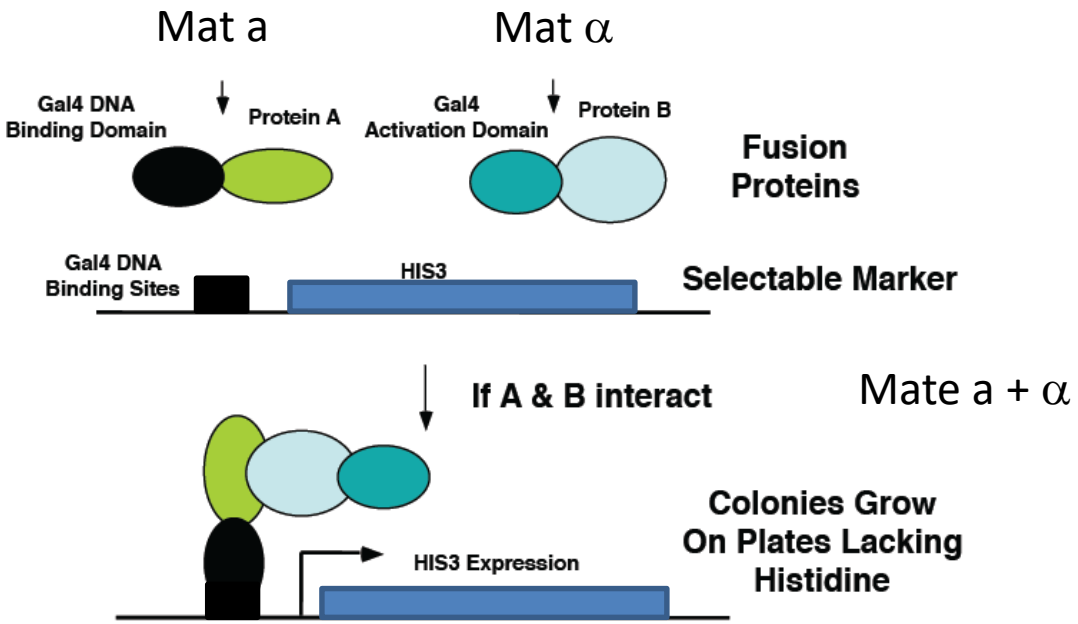
A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae.

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

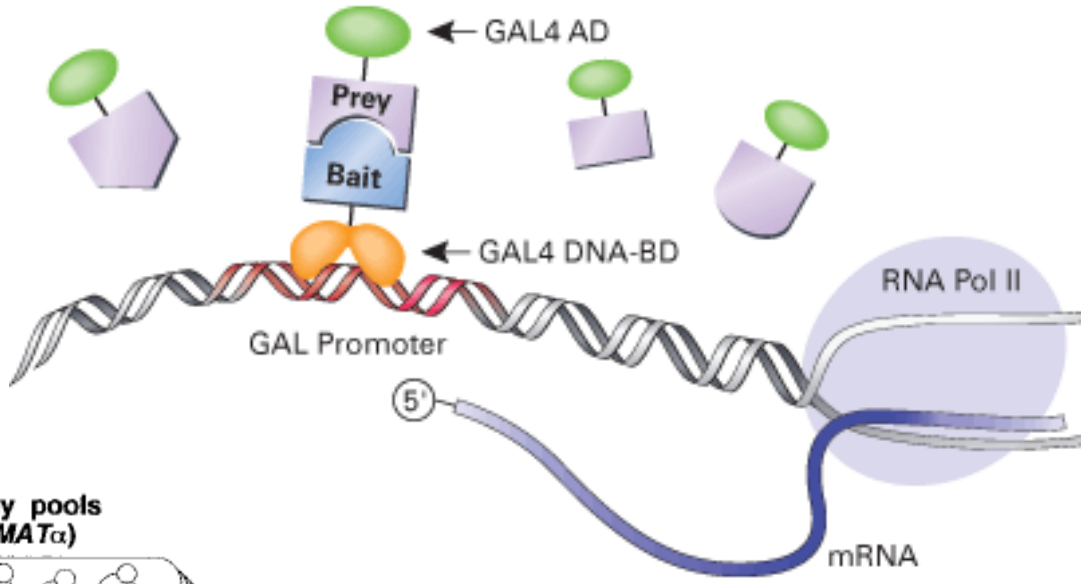
Yeast Two Hybrid Assay

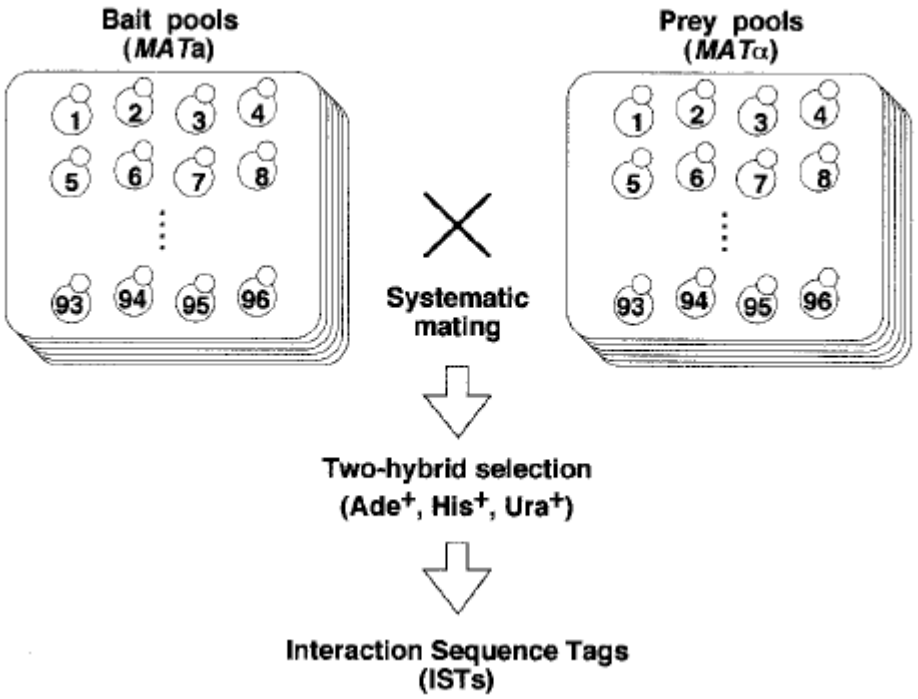


Clone bait and prey constructs and place in separate strains.



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





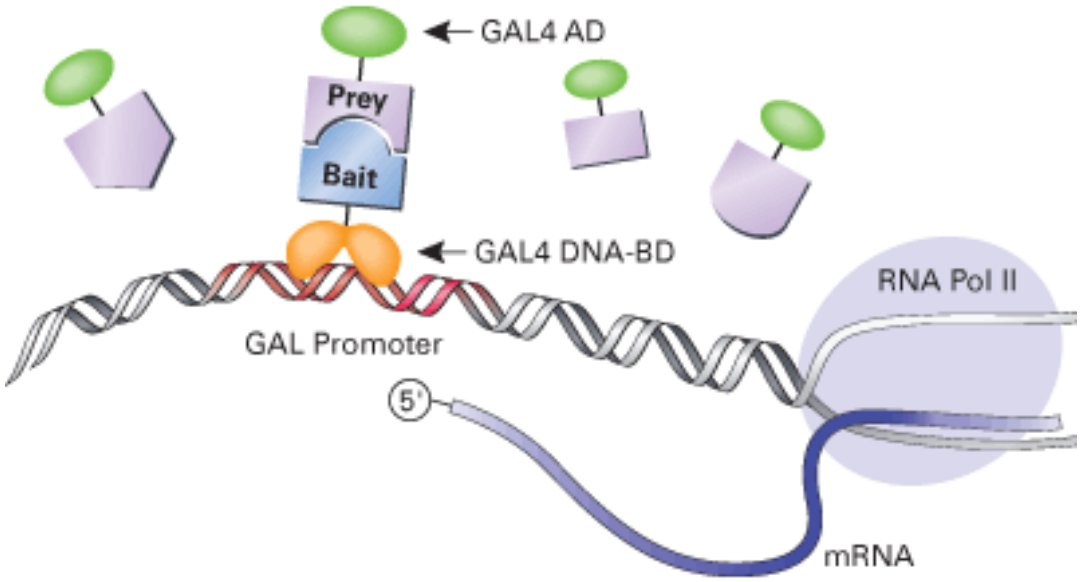
Results of Two Studies

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

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

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**

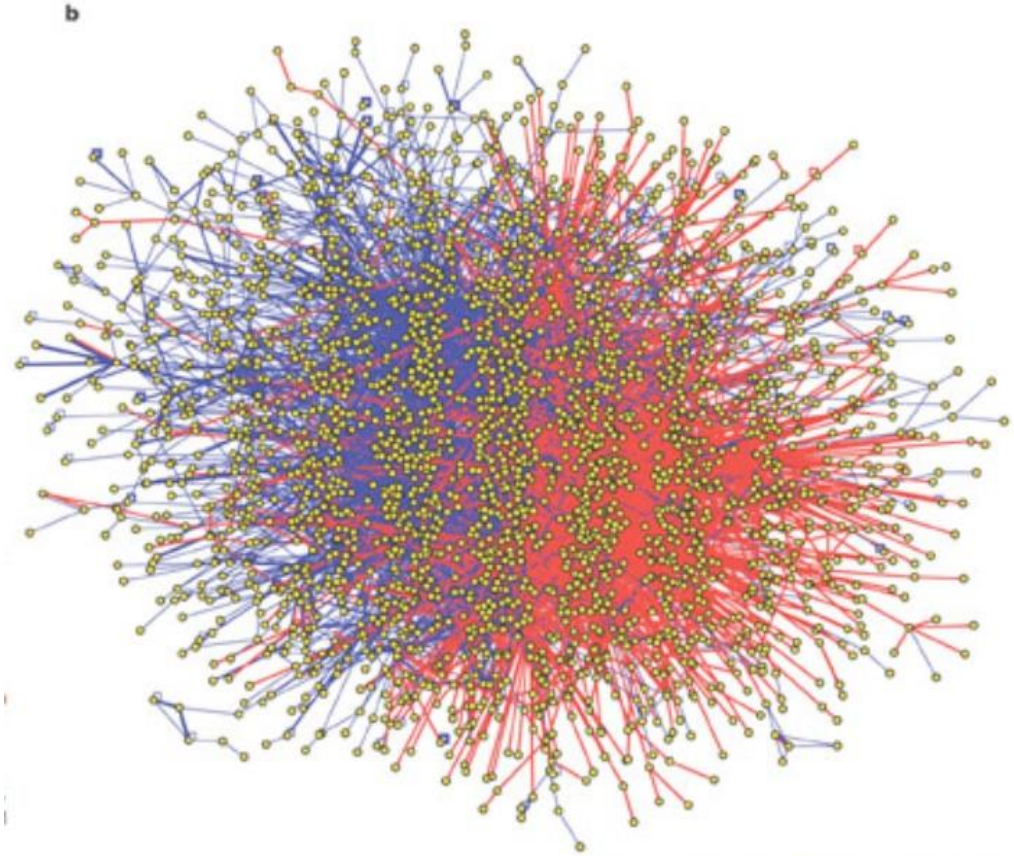
Some Disadvantages

- **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)

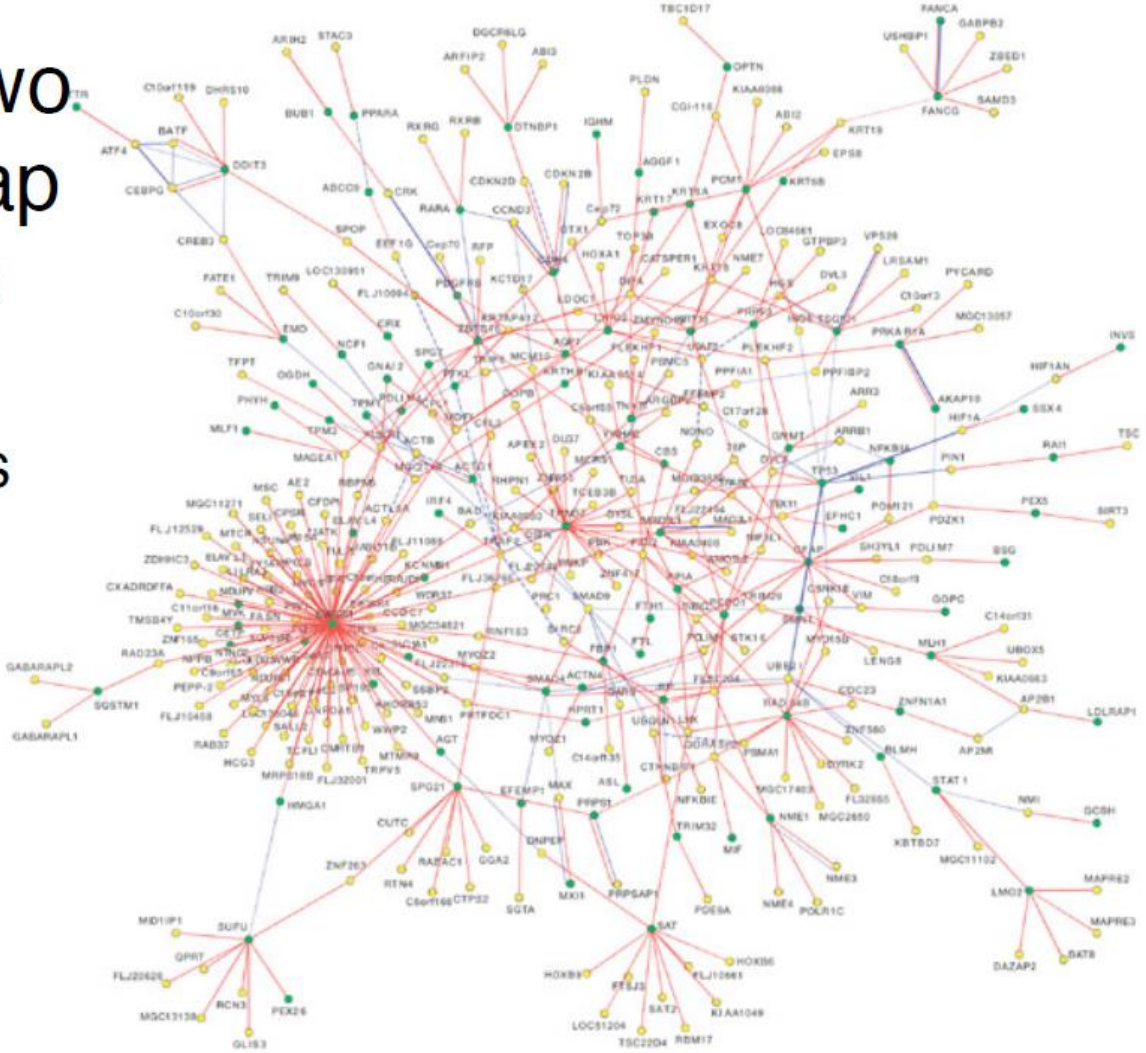
10,597 interactions



Rual et al. Nature 2005

Human Two Hybrid Map Disease Genes

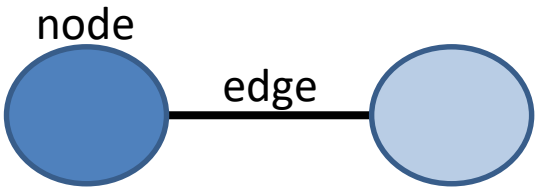
(121 genes (green))

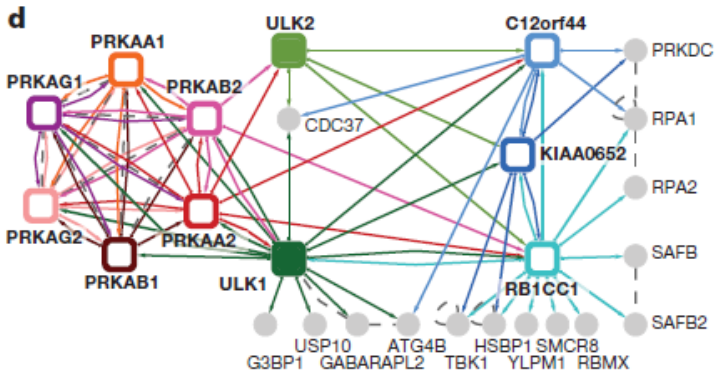


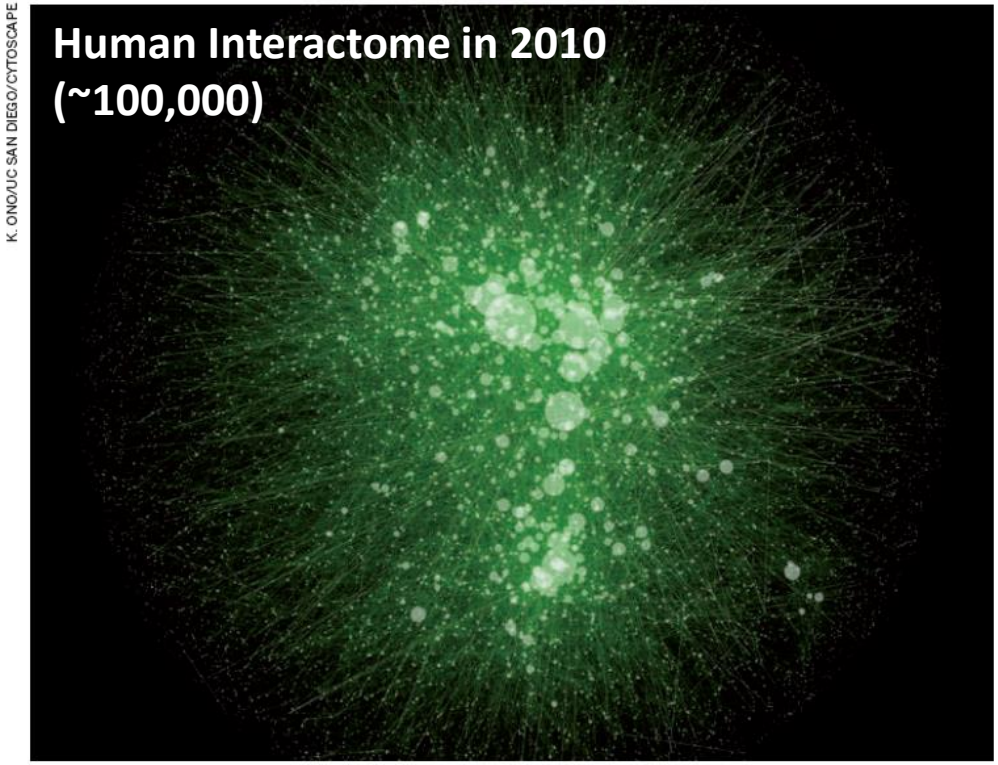
Rual et al. Nature 2005 Vol 437

Protein-Protein interaction maps:

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

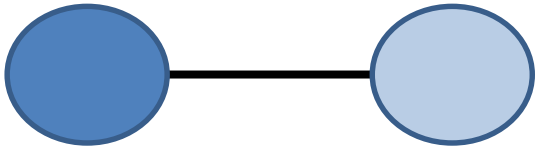






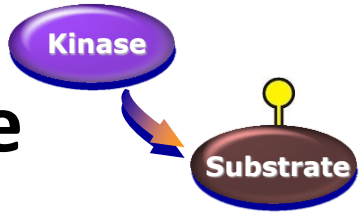
Bonetta, *Nature* 2010

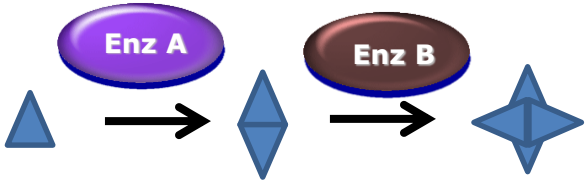
Protein-Protein interactions:



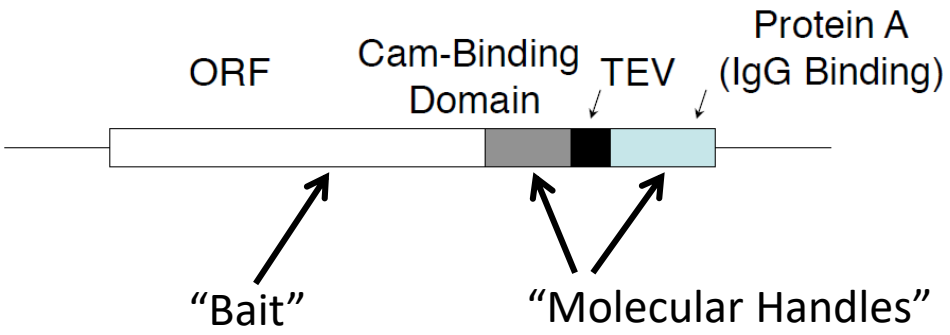
Some examples:

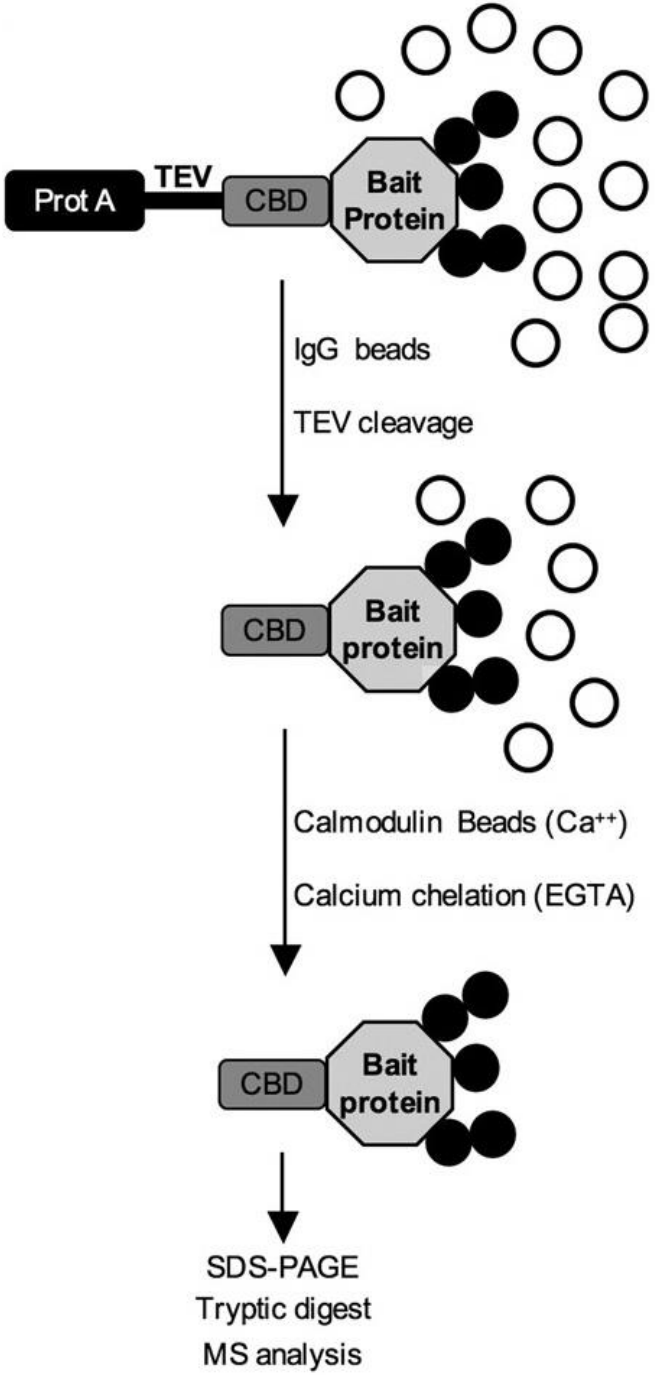
- Physical and direct
- Physical and indirect
  - Multi-protein complexes
  - Scaffolds
- Transient
  - Kinase & substrate
- Metabolic





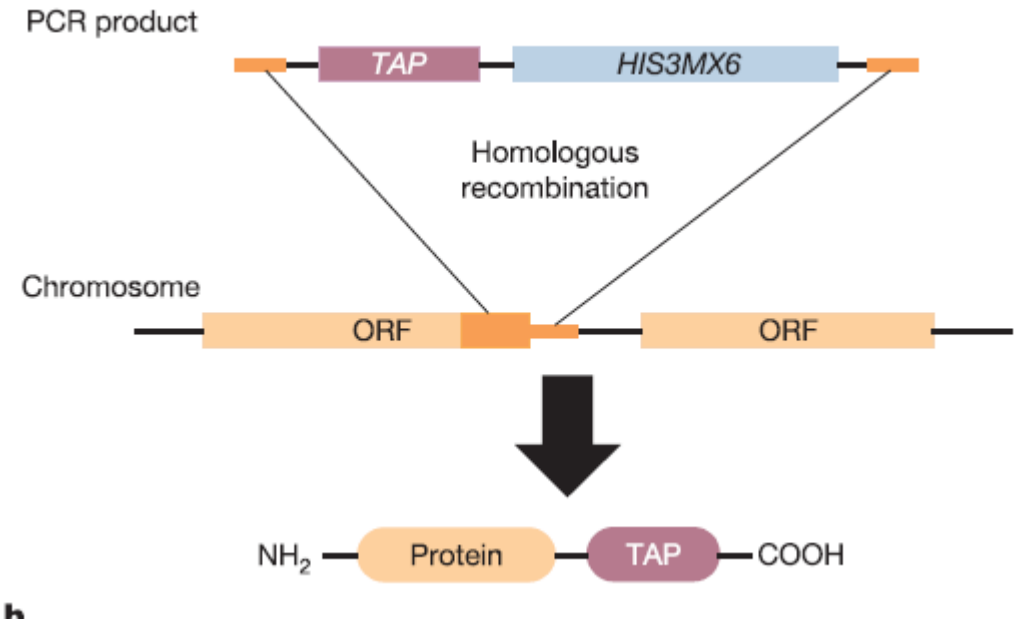
Tandem Affinity Purification (TAP) Tagging





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

Global TAP Tagging in yeast



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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*.

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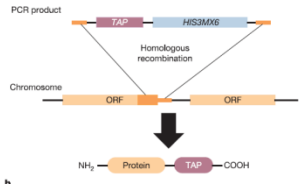
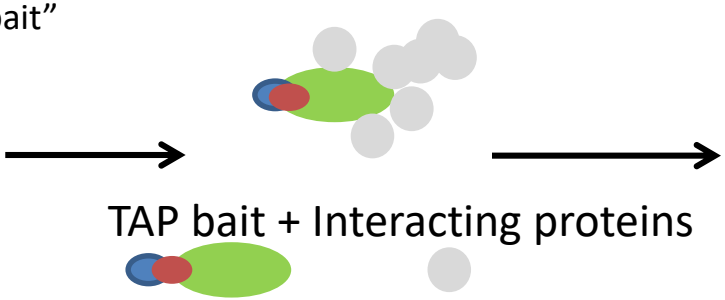
2006

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

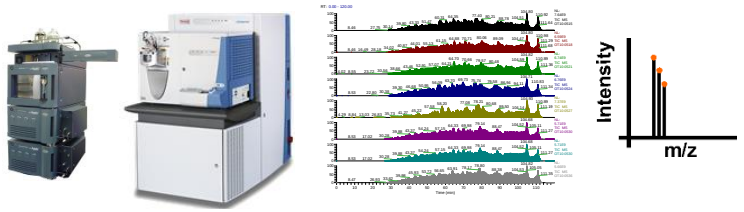
➲ TAP-Tag and Protein-Protein Interaction

Collection of tagged "bait" expression strains





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



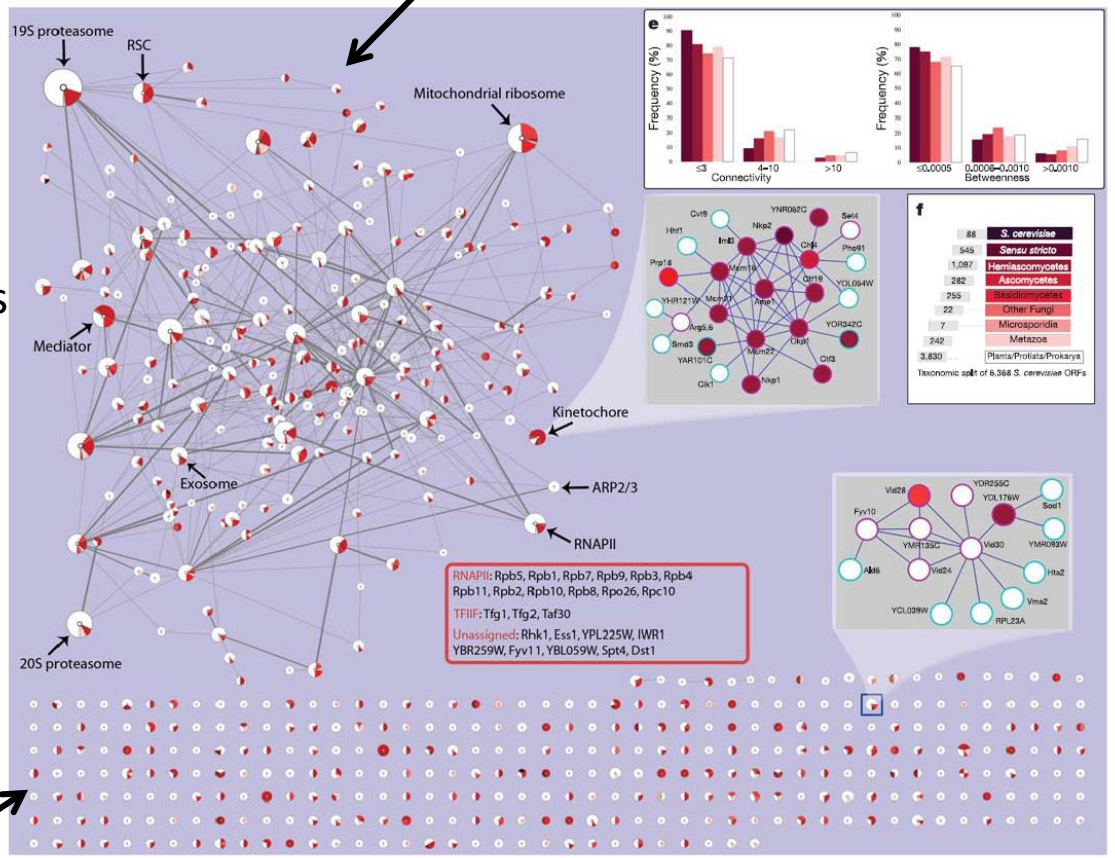
Krogan et al. observed 7,123 protein–protein interactions:

<u>Important aspects:</u>

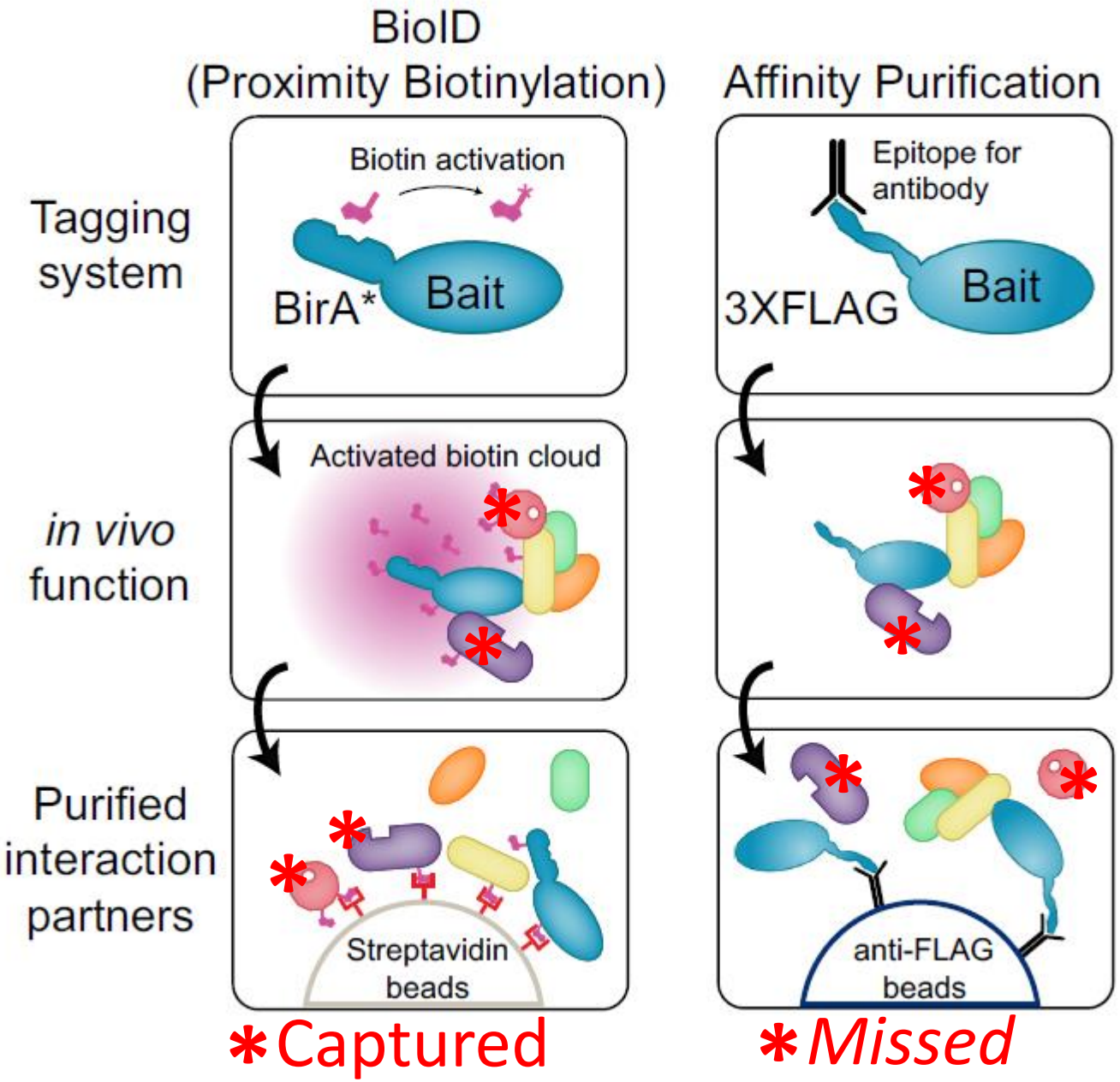
- **Tagged the native genes and did not overexpress the fusion proteins**
- **Could immediately validate partners (reciprocal purification in data set)**
- **Complementary MS techniques, deeper coverage of complexes**
- **Authors state, "…rigorous computational procedures to assign confidence values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

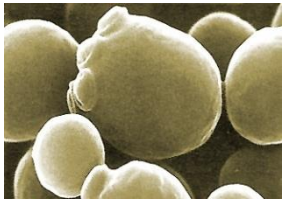


Krogan NJ, et al. *Nature.* 2006



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

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

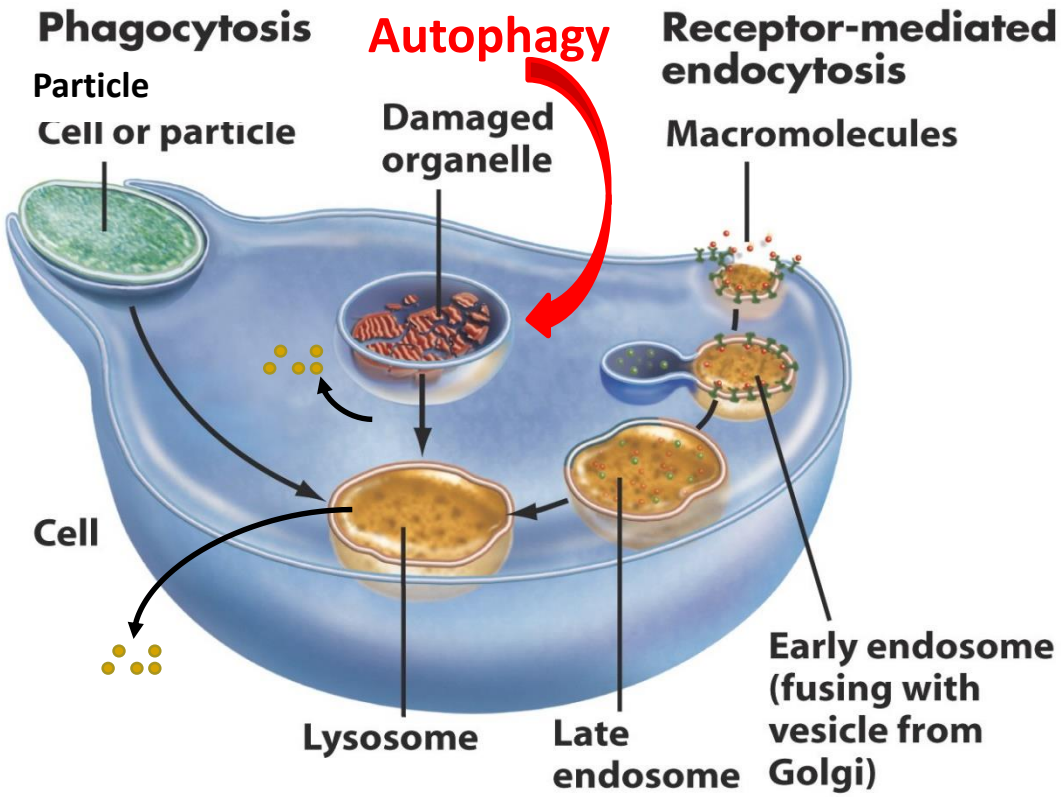


nature Vol 466|1 July 2010|doi:10.1038/nature09204

ARTICLES

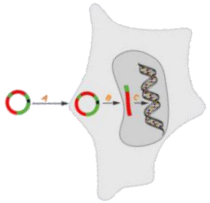
Network organization of the human autophagy system

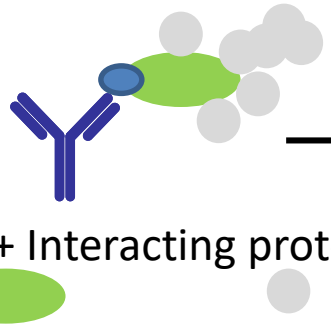
Christian Behrends[1], Mathew E. Sowa[1], Steven P. Gygi[2] & J. Wade Harper[1]



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

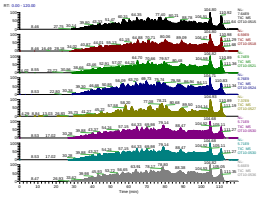
Transfect tagged "bait"





IP Bait + Interacting proteins

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



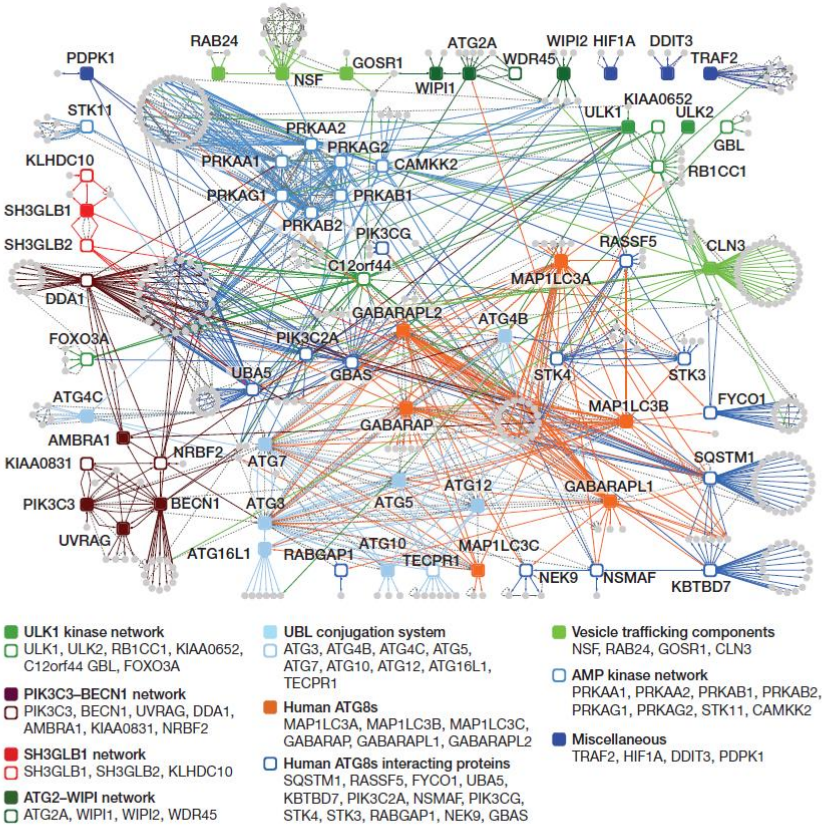
~65 bait proteins
LC-MS/MS identifies
2553 proteins

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]†

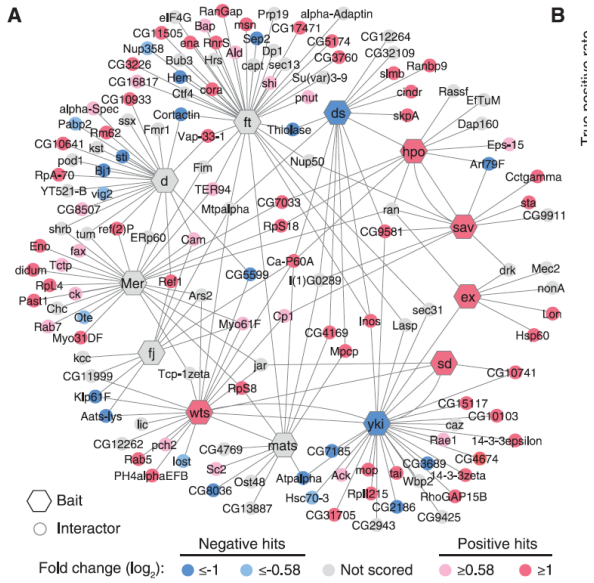
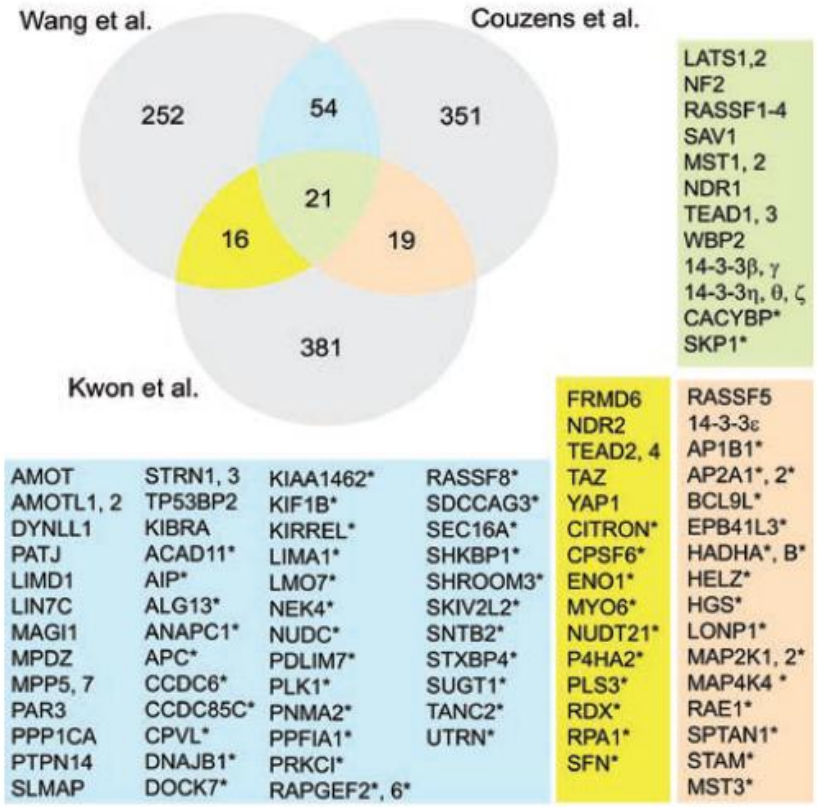


Fig. 2. Validation of Hippo-PPIN with functional RNAi screen and co-IP. (**A**) Distribution of Yki-reporter values for individual double-stranded RNAs (dsRNAs) in our focused RNAi screen. About 70% of genes are covered by two dsRNAs. (**B**) Recovery of Hippo pathway components from RNAi screen [fold-change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

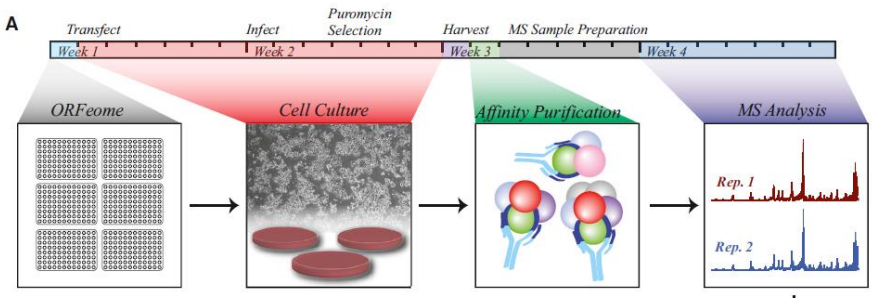
Kwon Y, *et al*. *Science* 2013; Couzens AL, *et al*. *Sci Signal* 2013; Wang W, *et al*. *Mol Cell Proteomics* 2014

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



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]

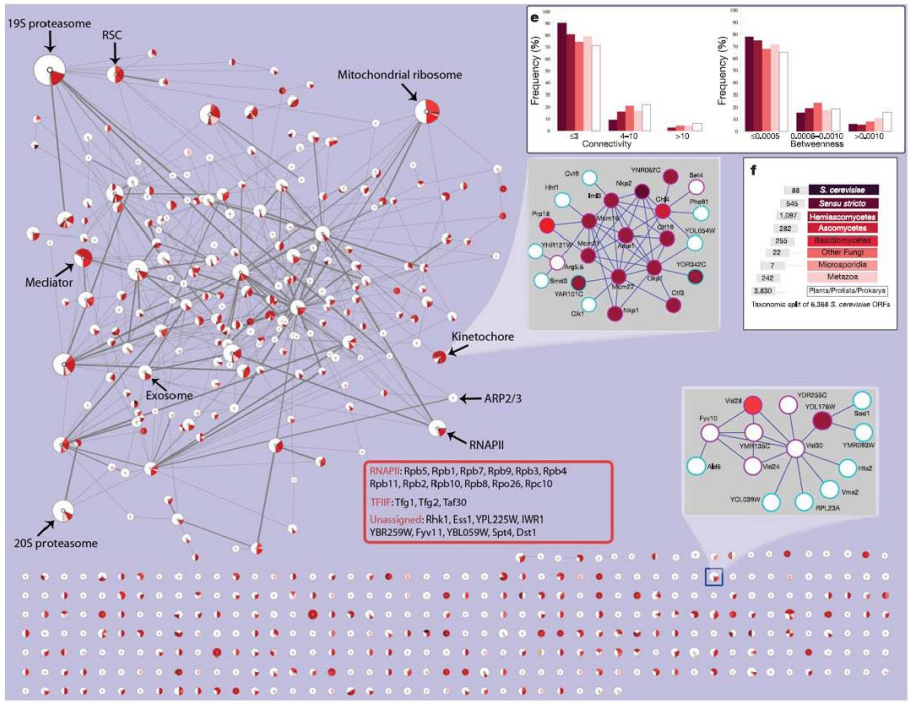
BioPlex 2.0 Huttlin et al, *Nature*. 2017 PMID: 28514442

BioPlex 3.0 *submitted*

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

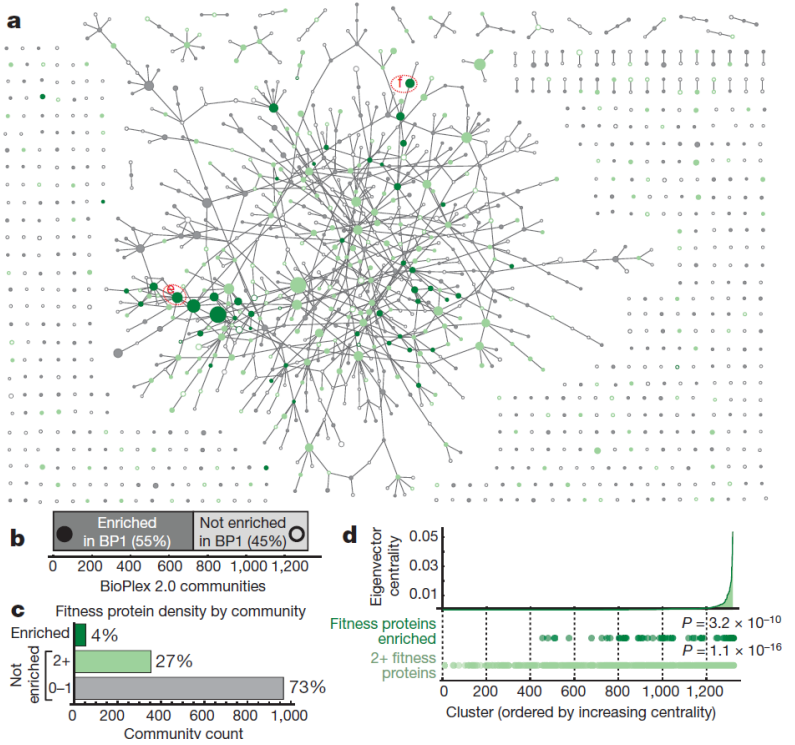
Cellular proteins are organized into complexes and this proteome organization is conserved

Yeast: Interaction Network of Complexes



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

Human: Protein Complex "Communities"



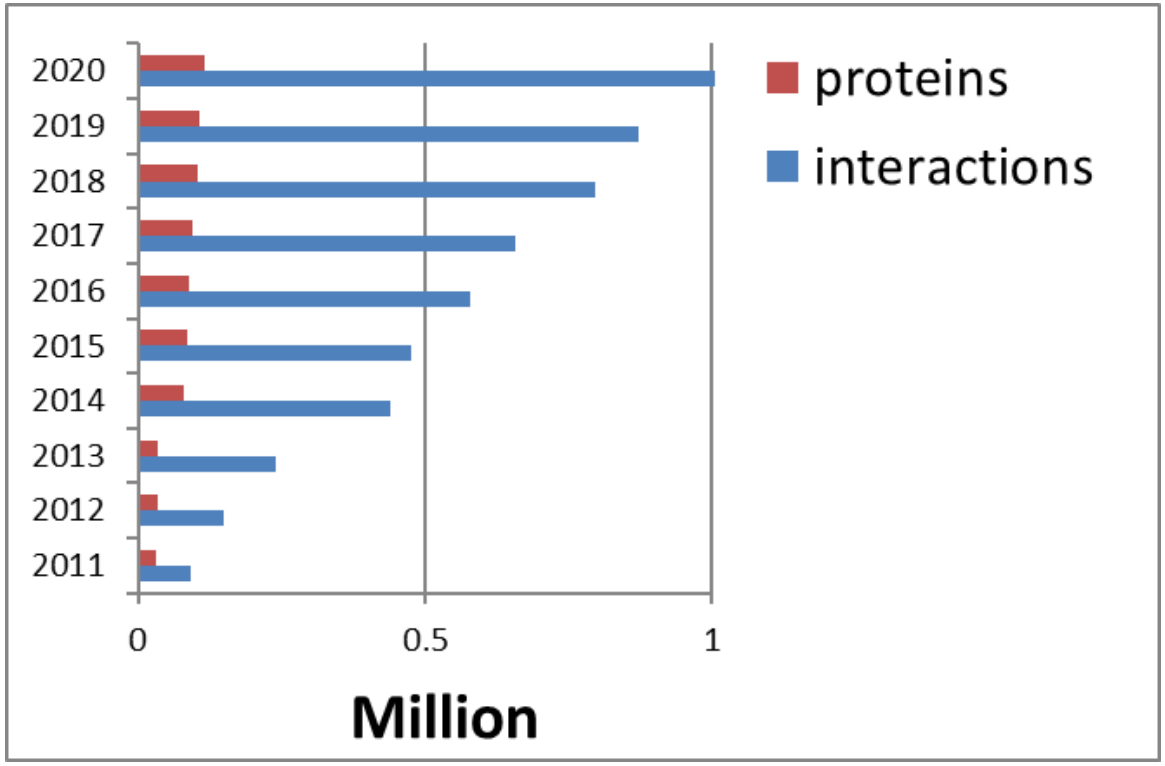
Huttlin et al, *Nature*. 2017 PMID: 28514442

Protein-Protein Interaction Databases

EMBL-EBI

IntAct

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



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

2018

Data Content

- Publications: **20047**
- Interactions: **794922**
- Interactors: **104510**

Protein-Protein Interaction Databases

EMBL-EBI

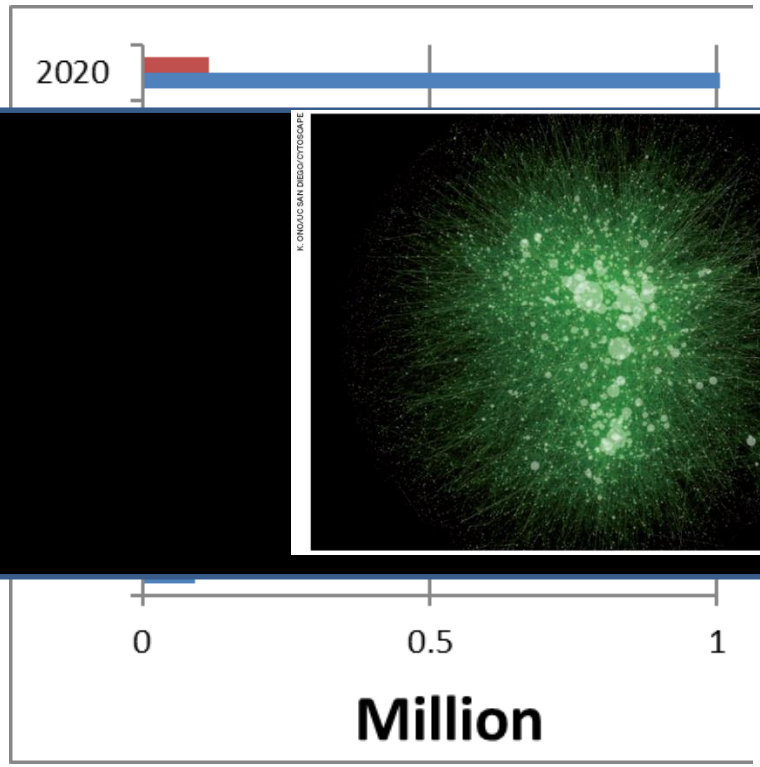
IntAct

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

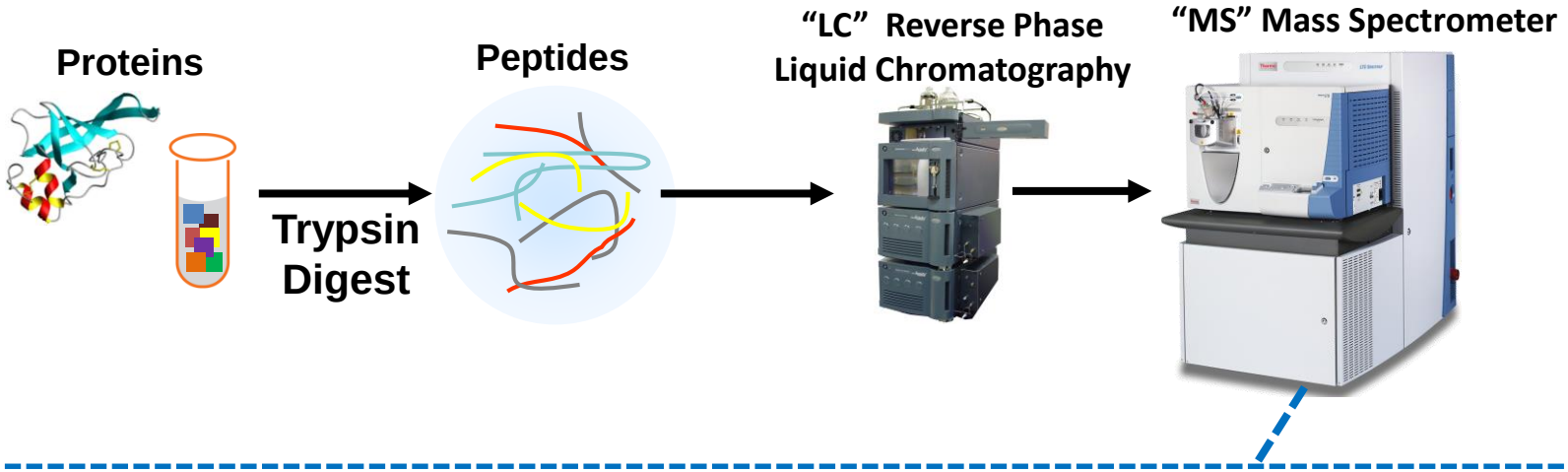
However:

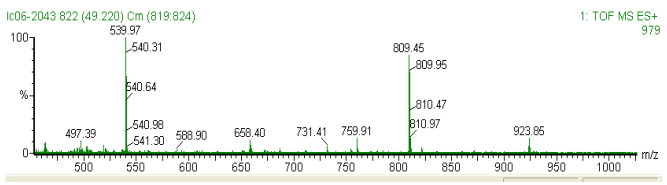
- Interaction data is largely static

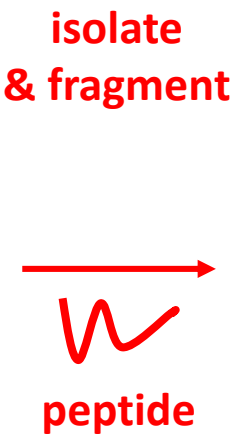
Next Step:

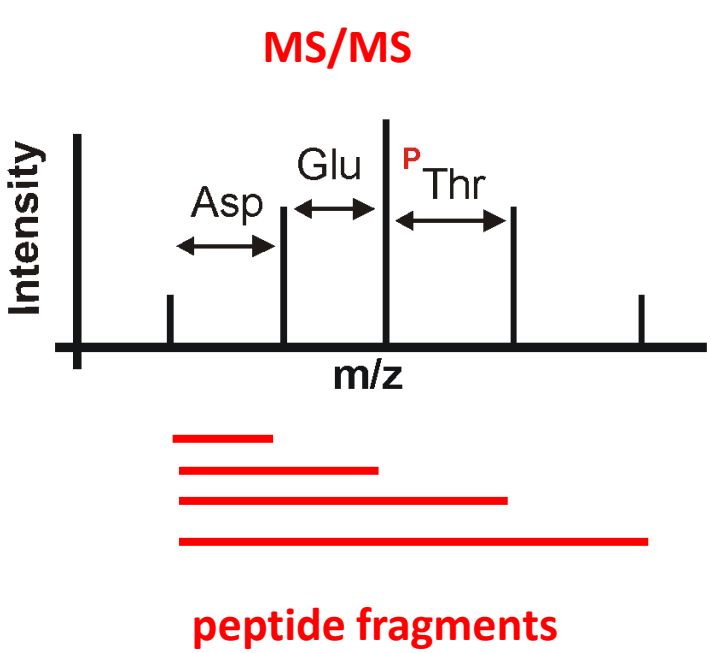
- How do protein interaction networks change over time?

Typical work flow for LC-MS "shotgun proteomics"





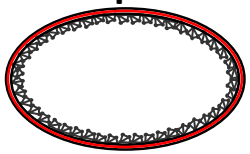


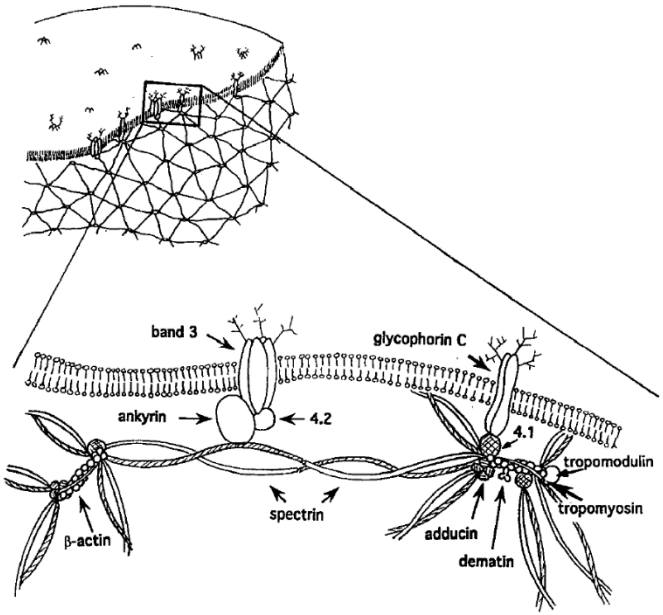


MS Data is not inherently quantitative, *but …*

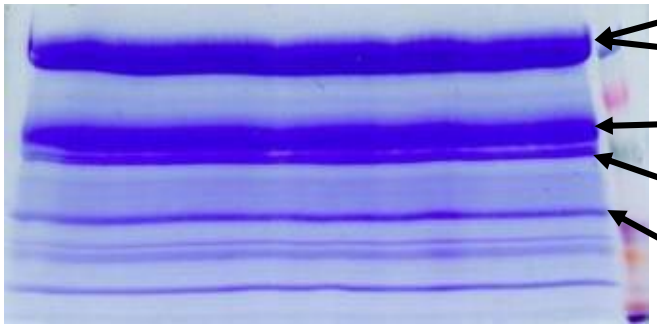
Red Blood Cell

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

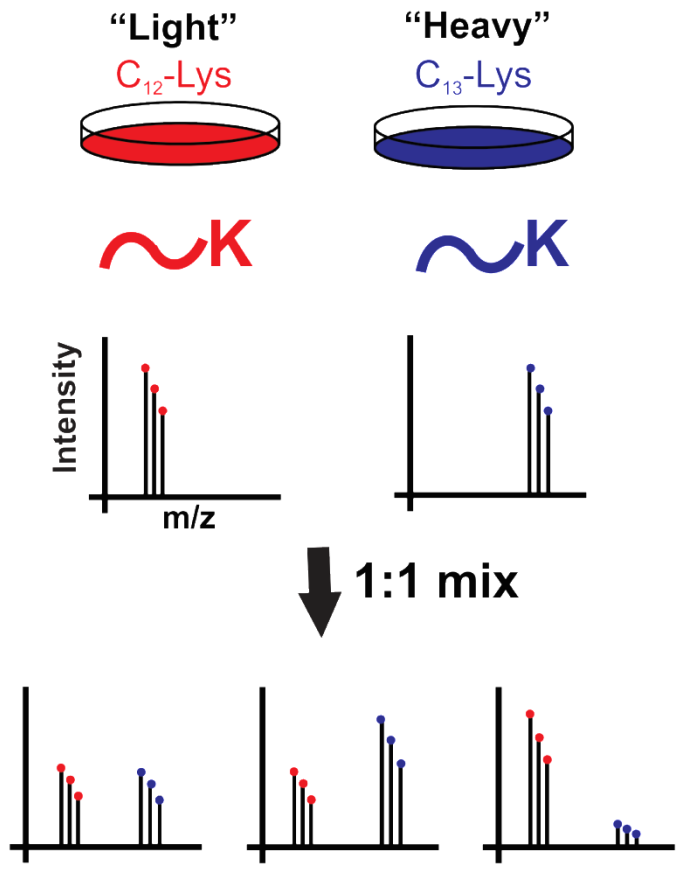
Rinehart et al., *unpublished*

Quantitative Proteomics

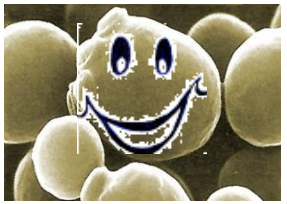
S.I.L.A.C. - Stable isotope labeling with amino acids in cell culture

-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**



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.**

2008

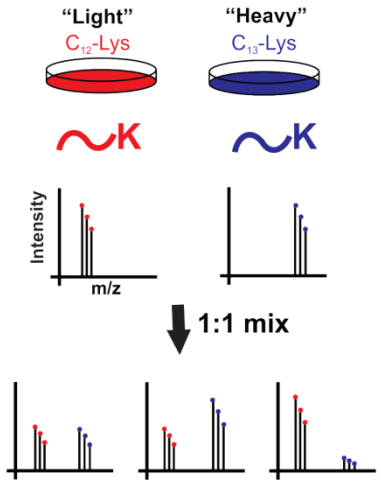
de Godoy LM, et al. Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast. *Nature*.

➲ **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.**

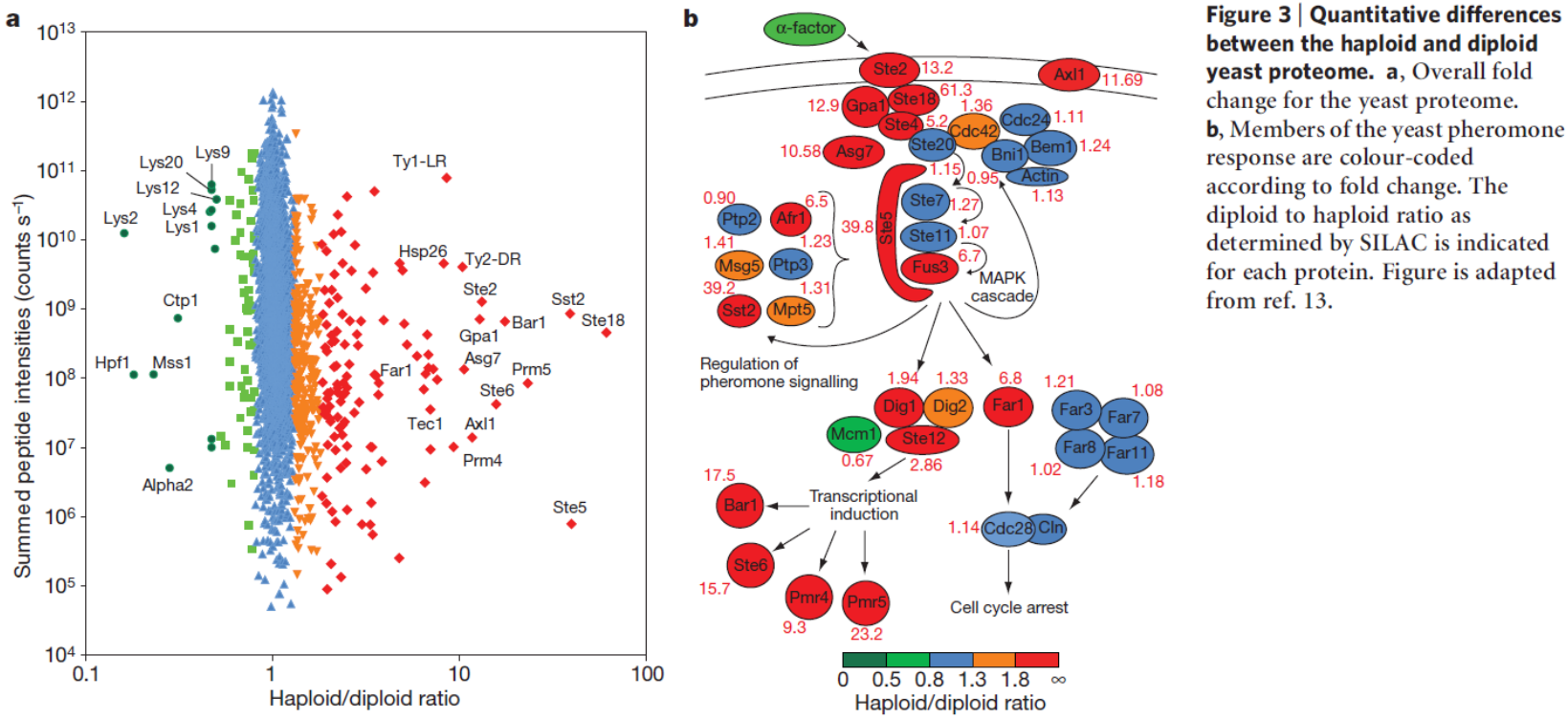


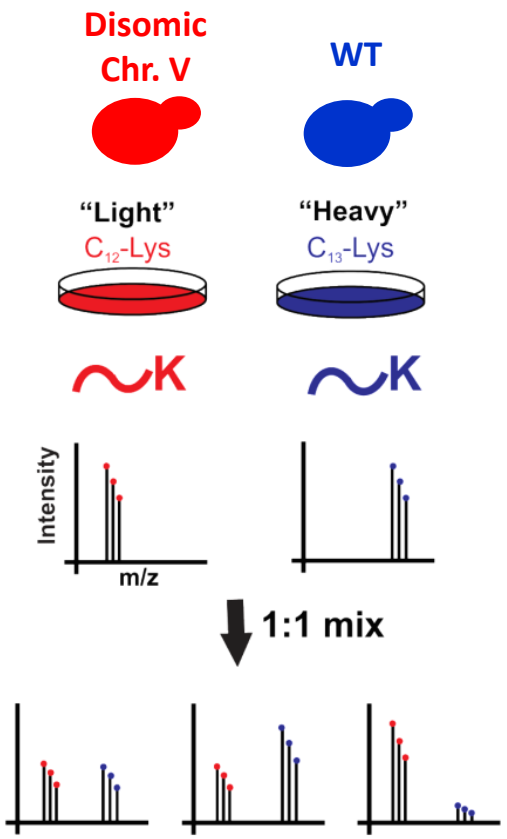
Figure 3 | Quantitative differences between the haploid and diploid yeast proteome. **a**, Overall fold change for the yeast proteome. **b**, Members of the yeast pheromone response are colour-coded according to fold change. The diploid to haploid ratio as determined by SILAC is indicated for each protein.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

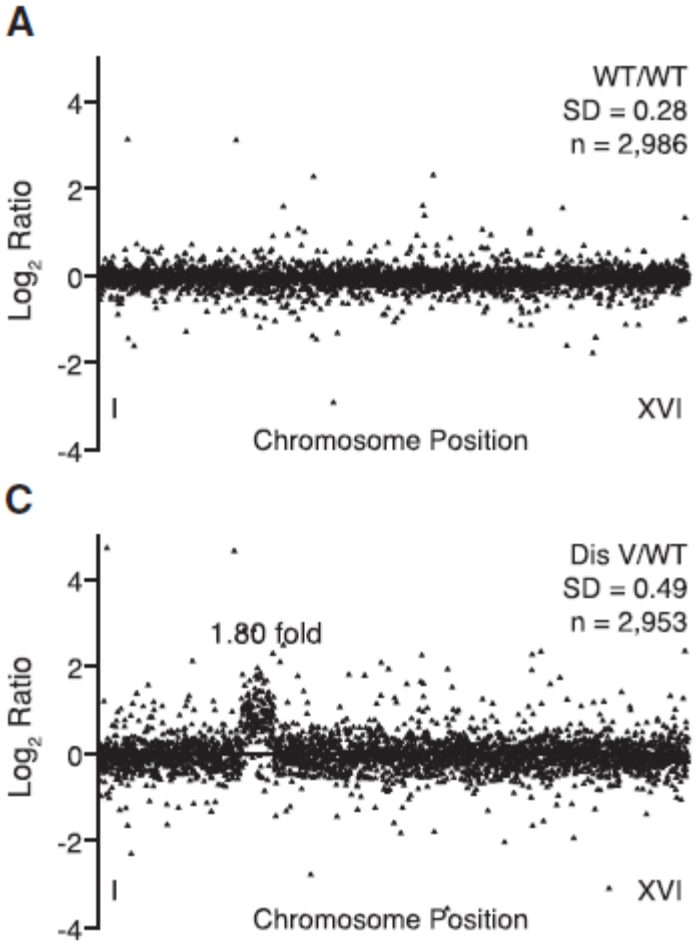
Pheromone signaling is required for mating of haploid cells and is absent from diploid cells.

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,*]



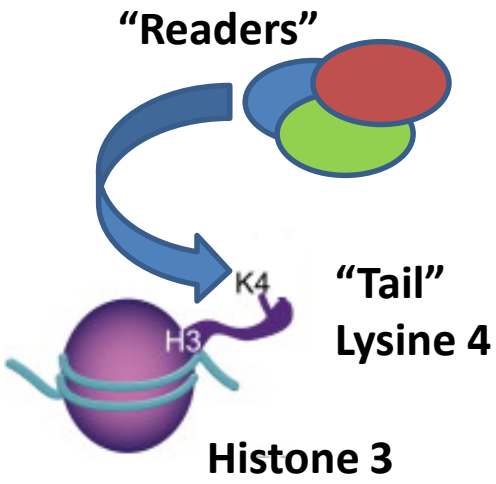


Resource

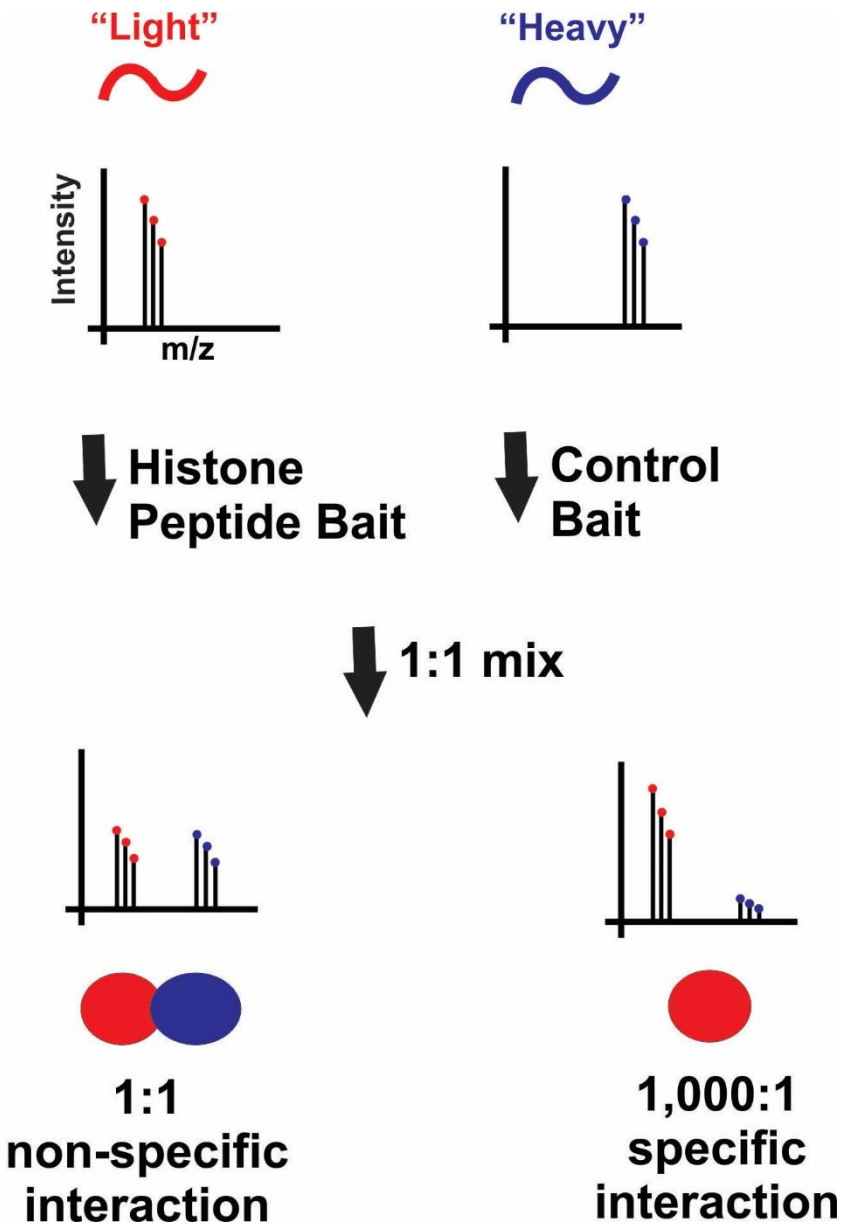
Cell

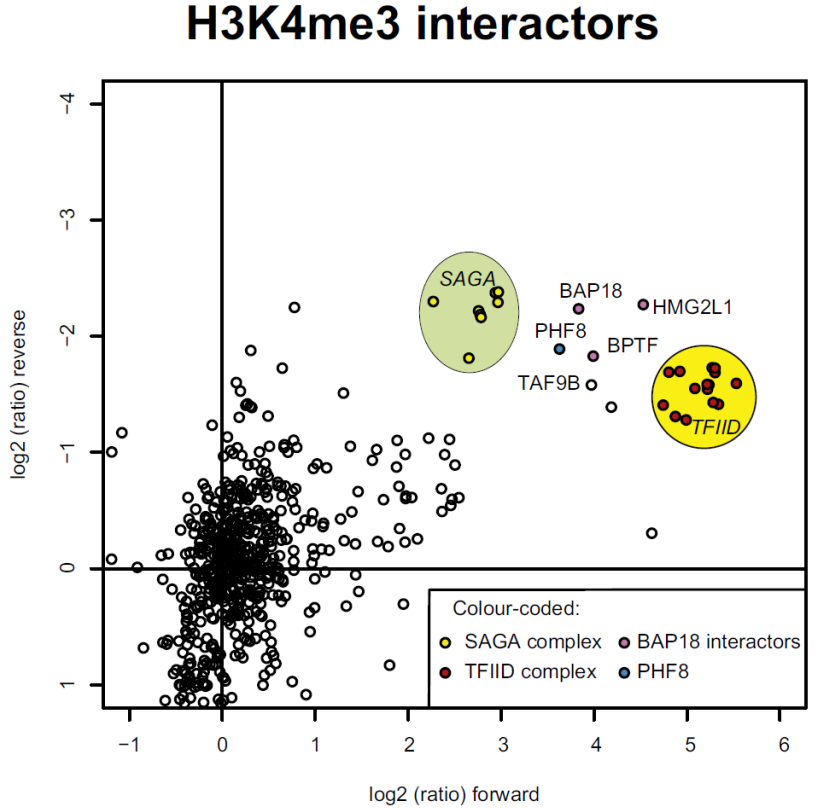
Quantitative Interaction Proteomics and Genome-wide Profiling of Epigenetic Histone Marks and Their Readers

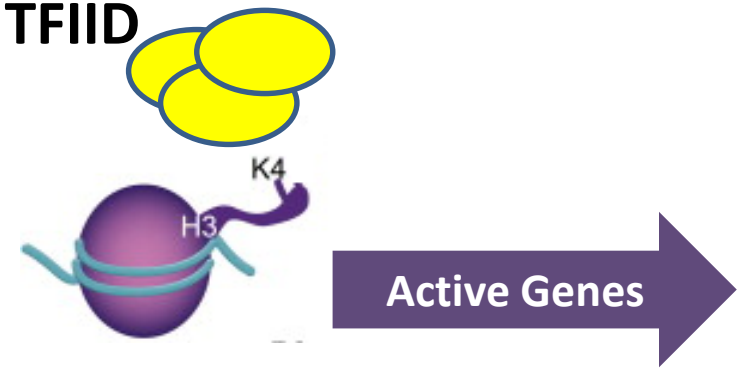
Michiel Vermeulen,[1,6,7,*] H. Christian Eberl,[1,6] Filomena Matarese,[2,6] Hendrik Marks,[2] Sergei Denissov,[2] Falk Butter,[1] Kenneth K. Lee,[3] Jesper V. Olsen,[1,5] Anthony A. Hyman,[4] Henk G. Stunnenberg,[2,*] and Matthias Mann[1,*]



Vermeulen et al., **Cell** 2010

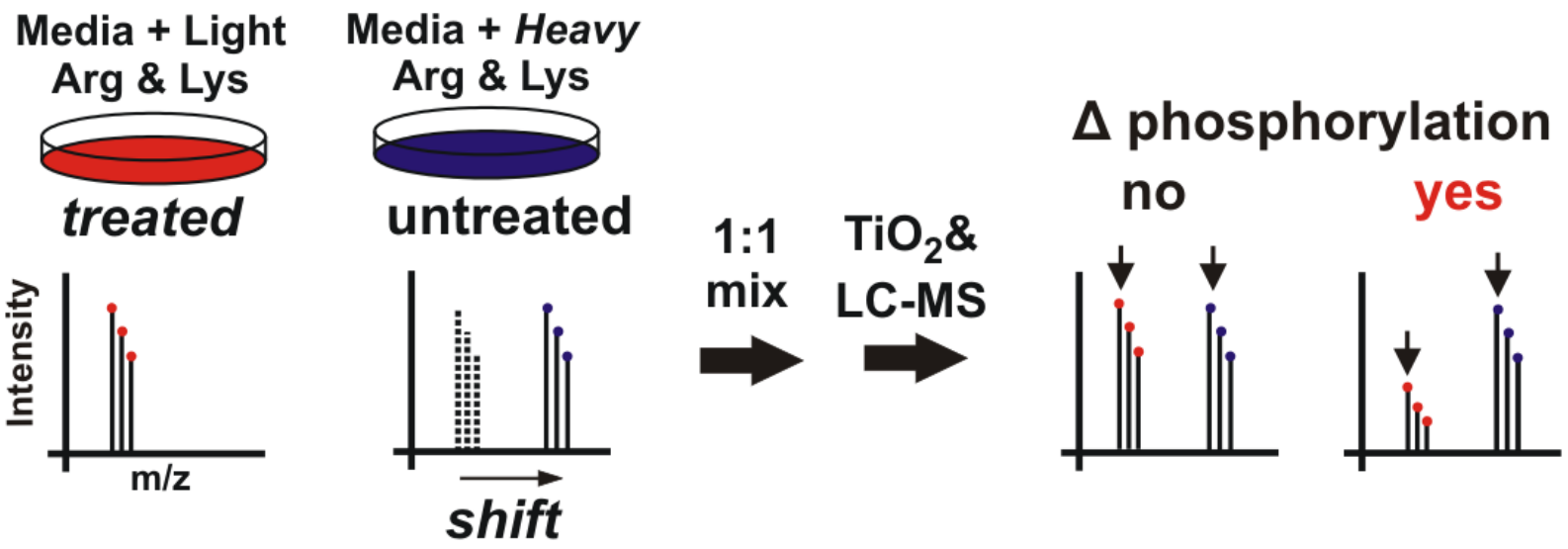






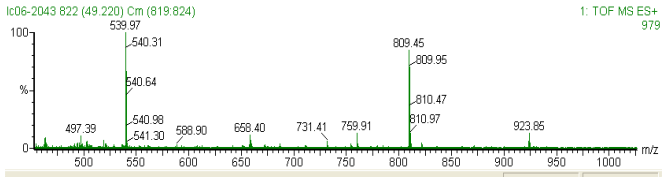
Vermeulen et al., **Cell** 2010

A SILAC approach to study protein phosphorylation dynamics



*Phosphopeptide signatures in MS

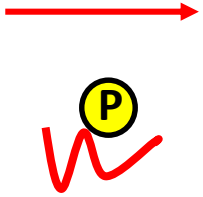
MS



peptide

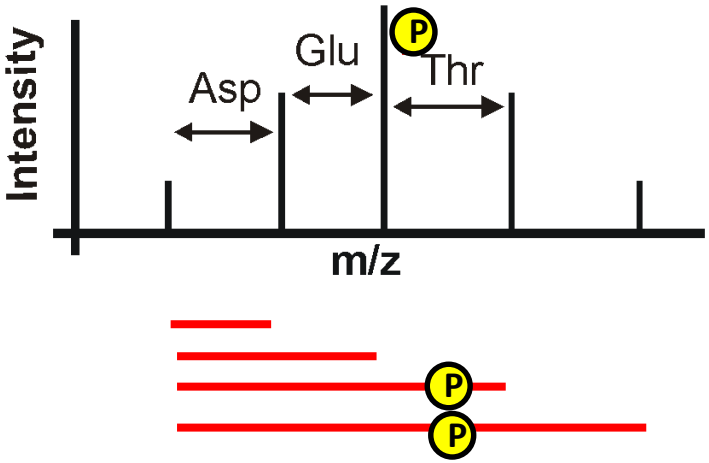
peptide

isolate & fragment



peptide

MS/MS



peptide fragments

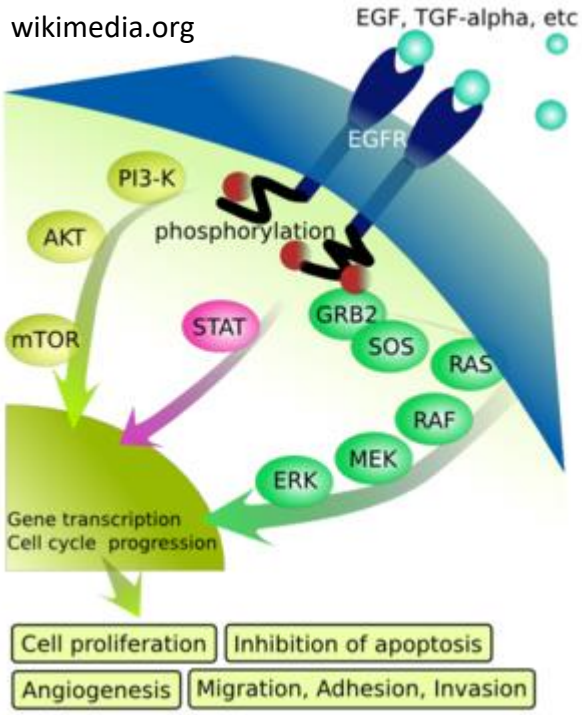
P +80 Da
in precursor

-98 Da loss of phosphoric acid H_3PO_4
during fragmentation

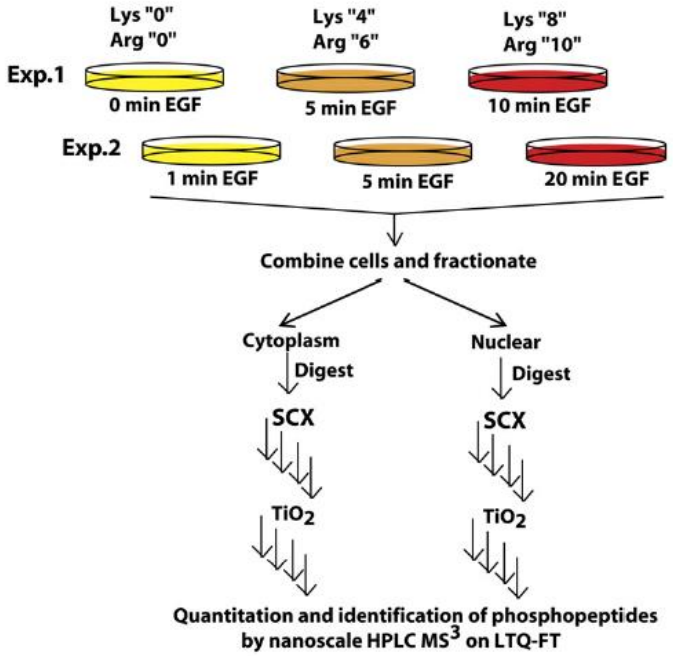
Quantitative Proteomics Reveals Dynamics in Signaling Networks

Phosphorylation dynamics after EGF stimulation

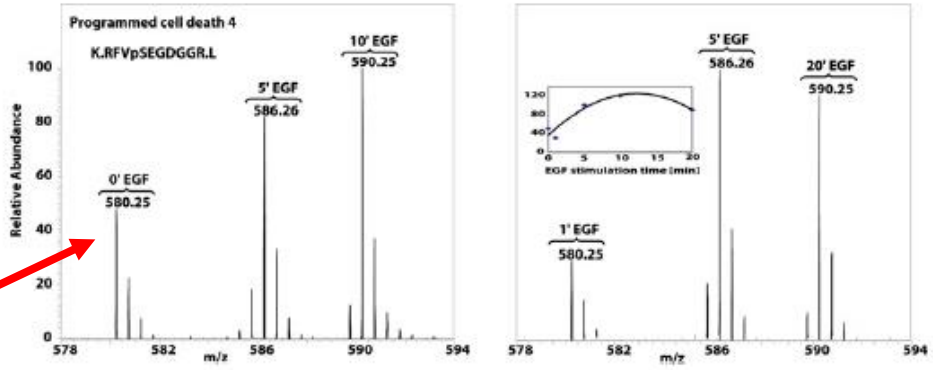
wikimedia.org



SILAC approach enables dynamic analysis



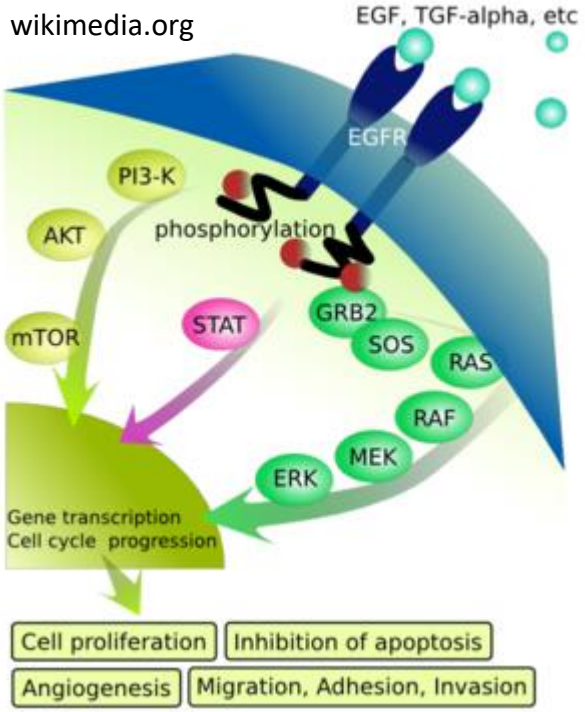
MS spectra triplets

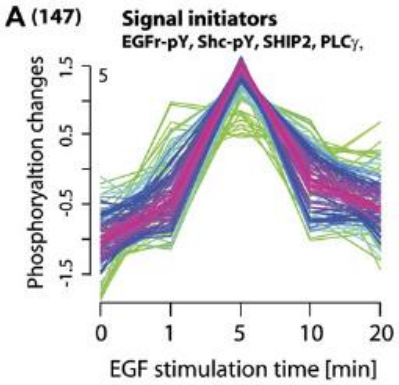


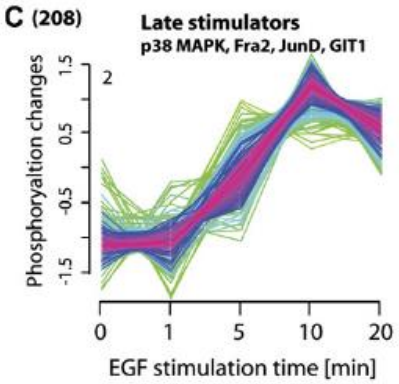
Olsen, et al. *Cell*, 2006

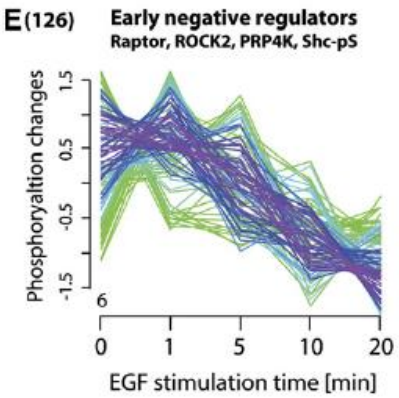
Phosphorylation dynamics after EGF stimulation

wikimedia.org



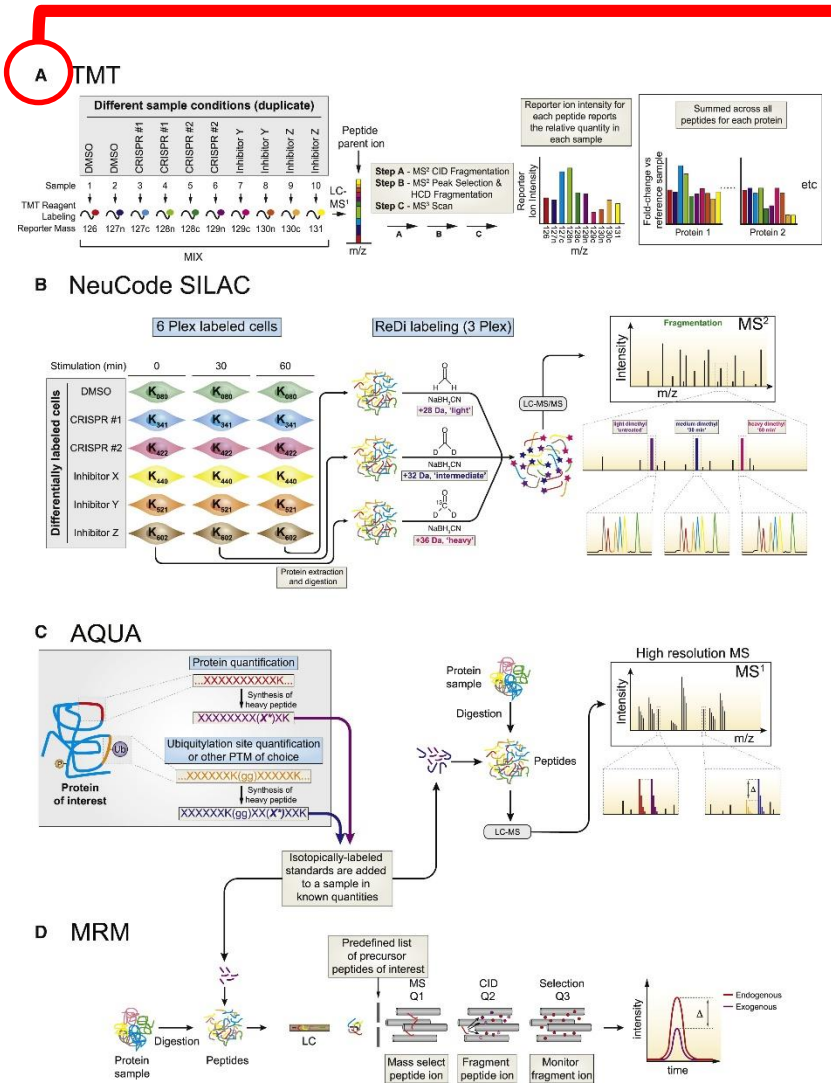


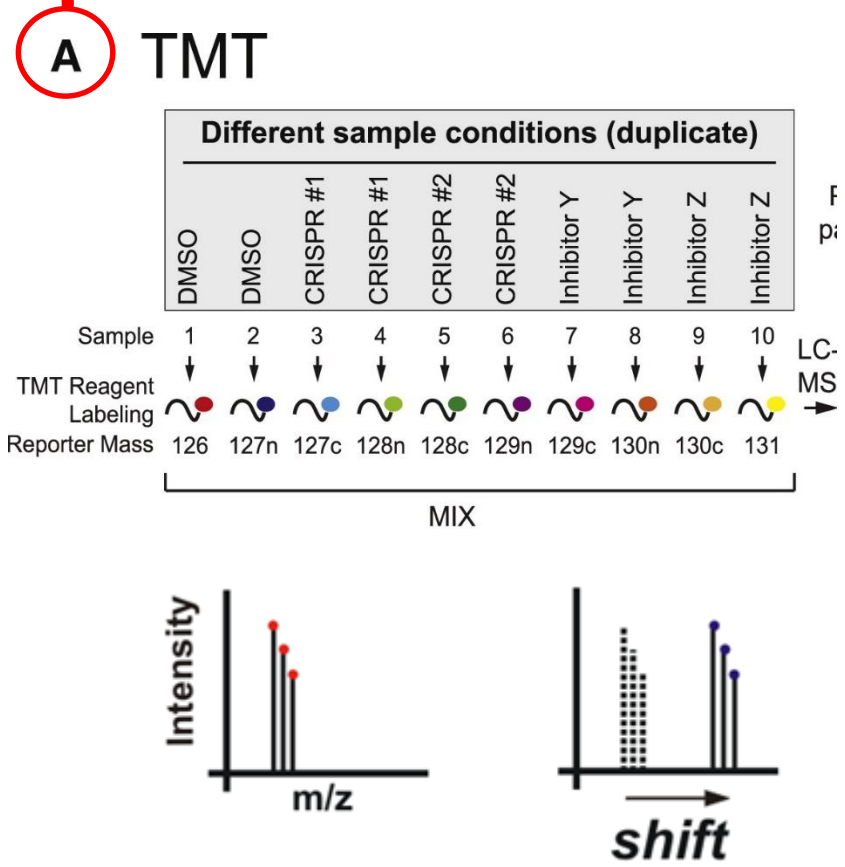




Olsen, et al. *Cell*, 2006

Heavy labels can be used for “barcoding” proteomes





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

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

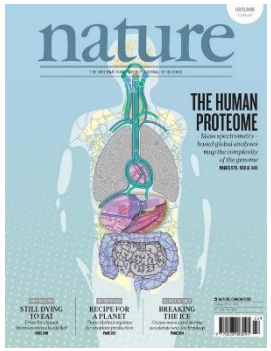
The Sequence of the Human Genome

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[4],

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

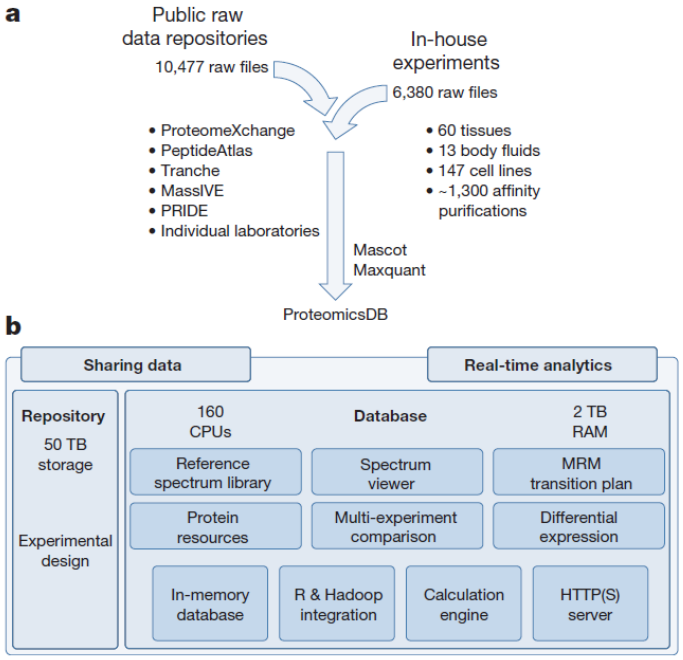
ARTICLE

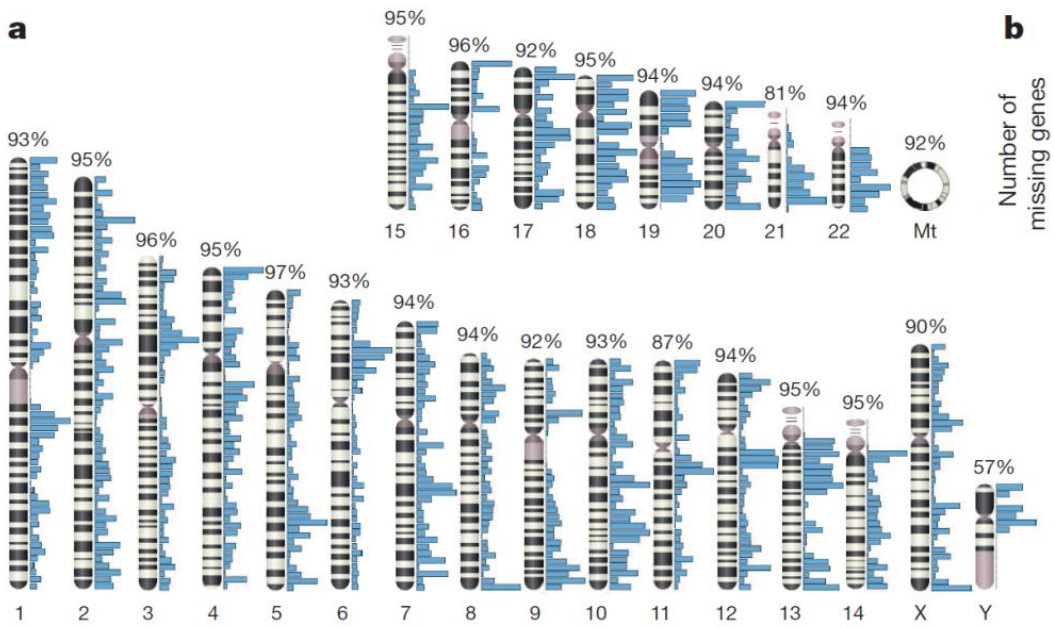
doi:10.1038/nature13319

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**

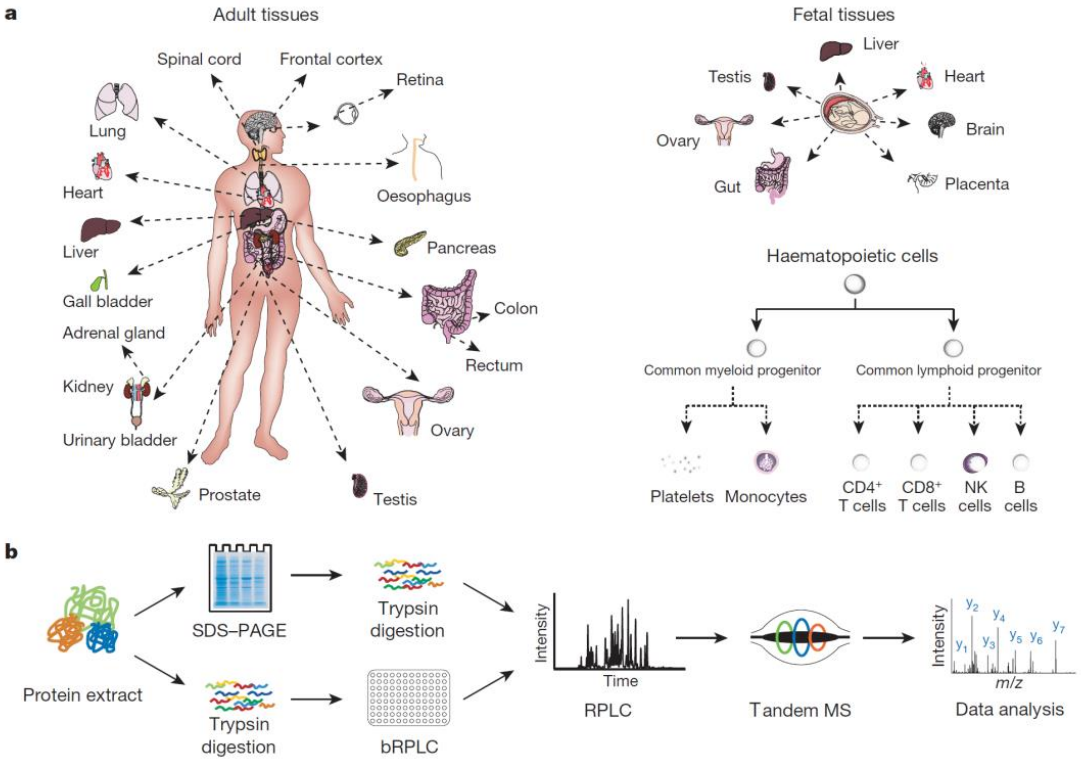
ARTICLE

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.

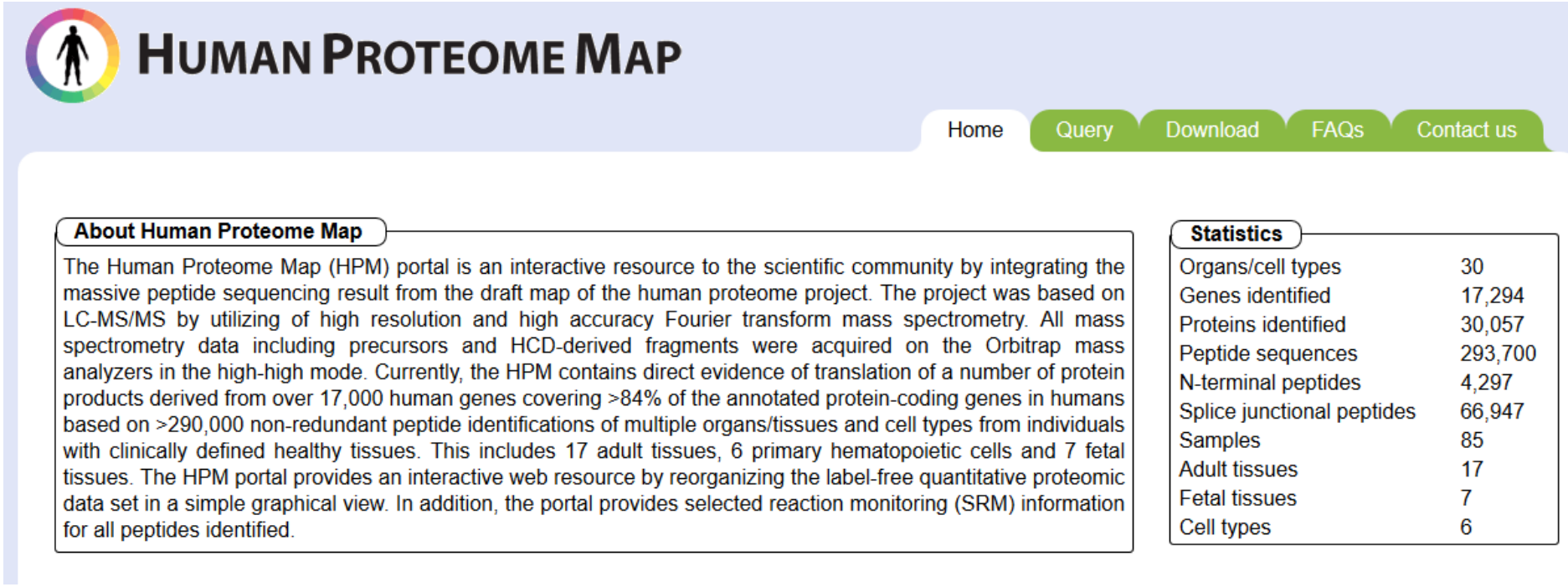
The GPMDB Project

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

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A draft map of the human proteome

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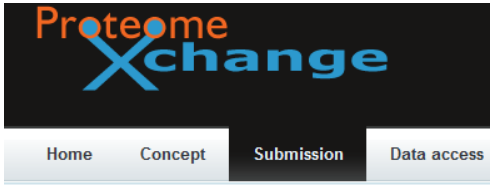
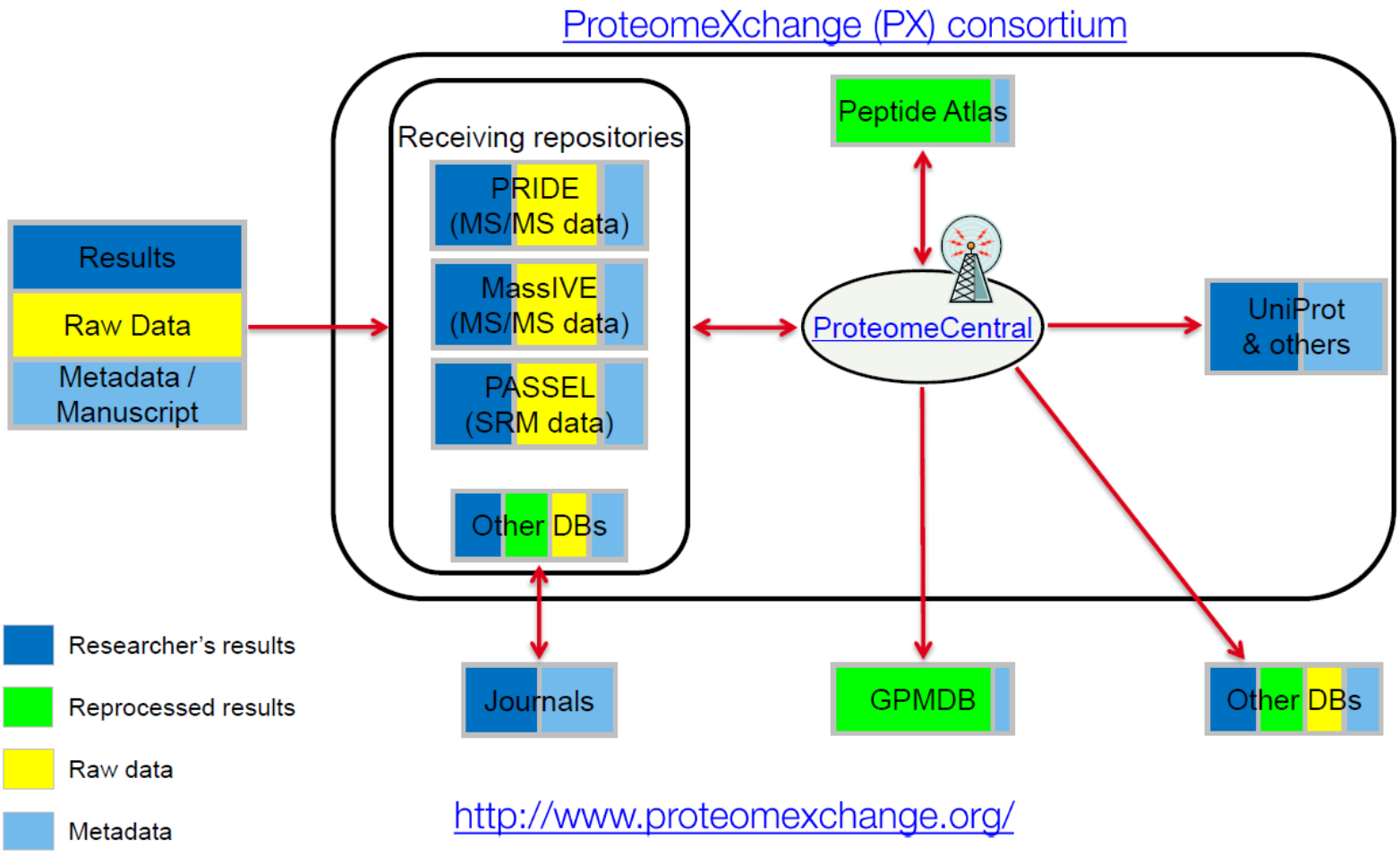
Kim & Akhilesh Pandey et al., ***Nature*** , 2014. PMID: 24870542

Proteomics Databases: Integrated Resources



http://www.proteomexchange.org/



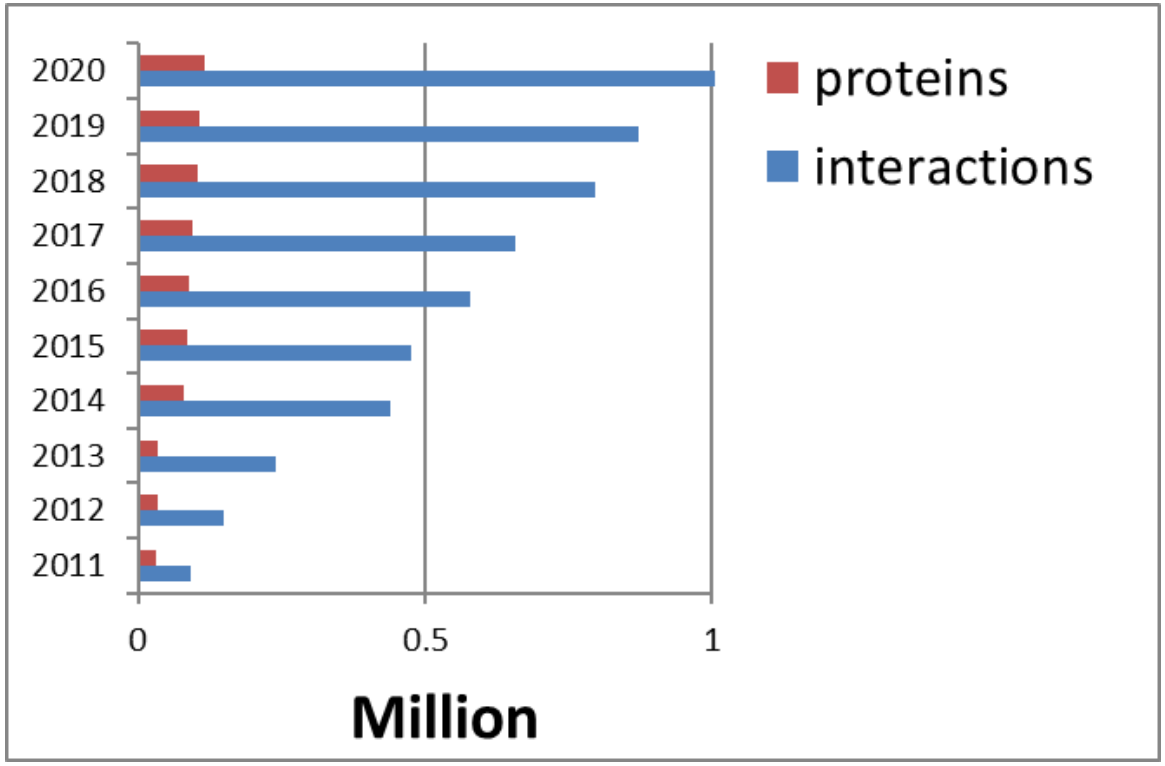
Slide modified from "Computational Mass Spectrometry-Based Proteomics 6th Maxquant Summer School" 21-25 July 2014
Emanuele Alpi, UniProt and PRIDE Development

Protein-Protein Interaction Databases

EMBL-EBI

IntAct

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



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

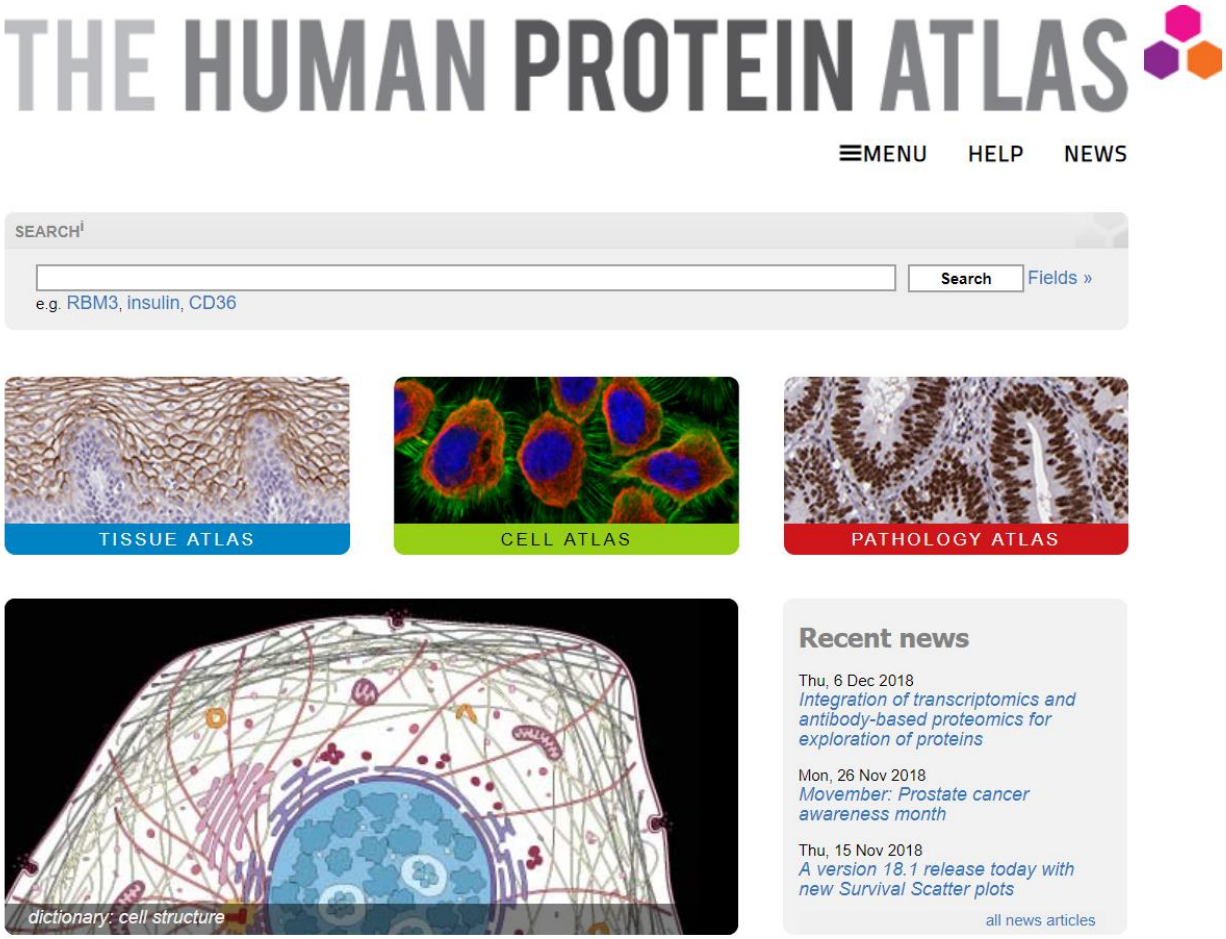
2018

Data Content

- Publications: **20047**
- Interactions: **794922**
- Interactors: **104510**

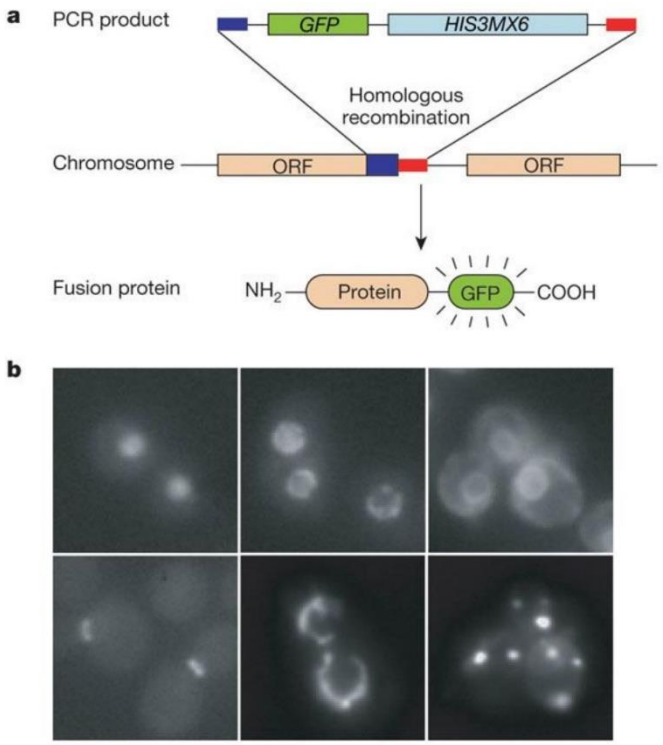
Proteomics Databases: Integrated Resources Beyond Mass Spectrometry

http://www.proteinatlas.org/



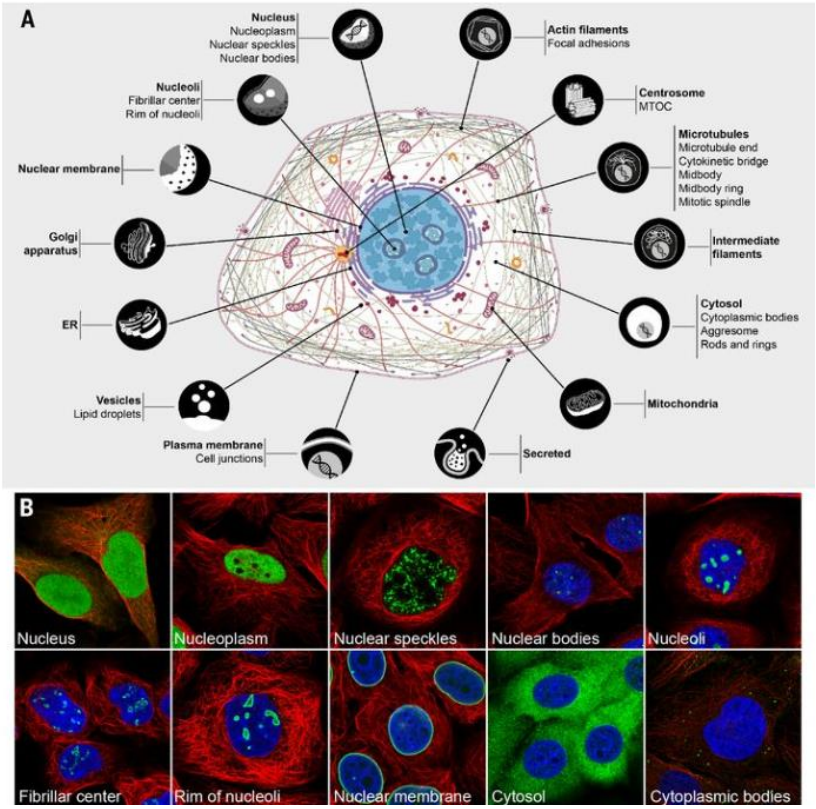
Proteomics Databases: Integrated Resources Beyond Mass Spectrometry

>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

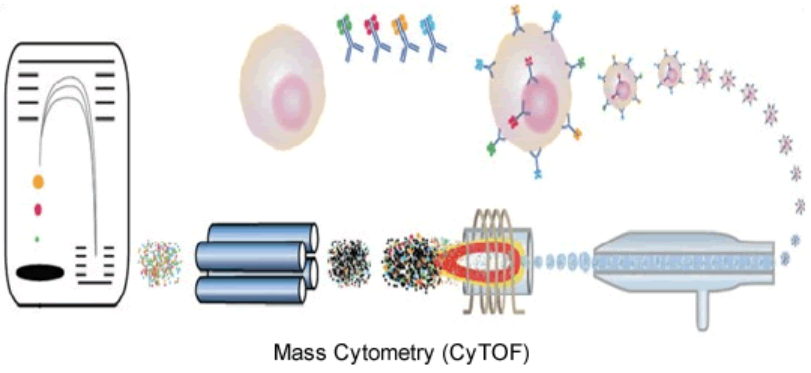
Single-cell proteomic analysis of *S. cerevisiae* reveals the architecture of biological noise

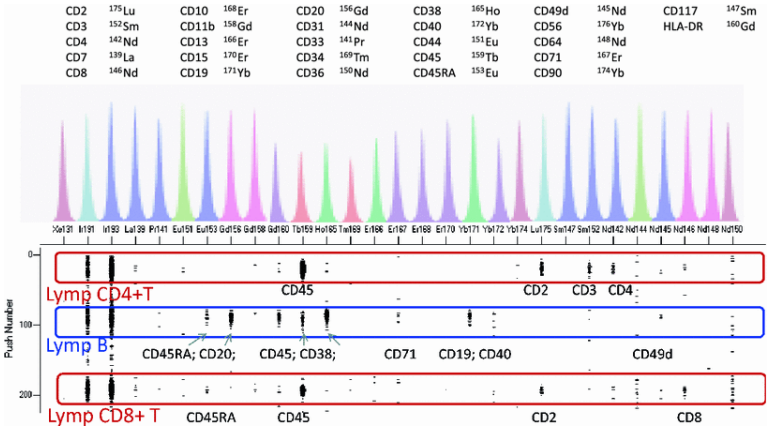
John R. S. Newman[1,2], Sina Ghaemmaghami[1,2]†, Jan Ihmels[1,2], David K. Breslow[1,2], Matthew Noble[1], Joseph L. DeRisi[1,3] & Jonathan S. Weissman[1,2]

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.

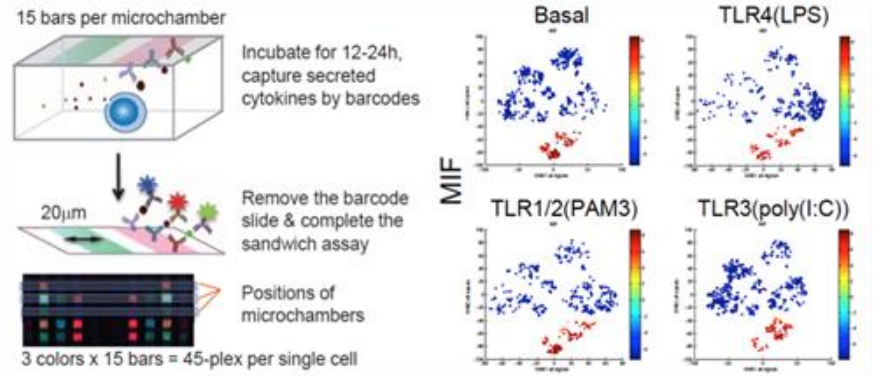
Proteomics at single cell resolution

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

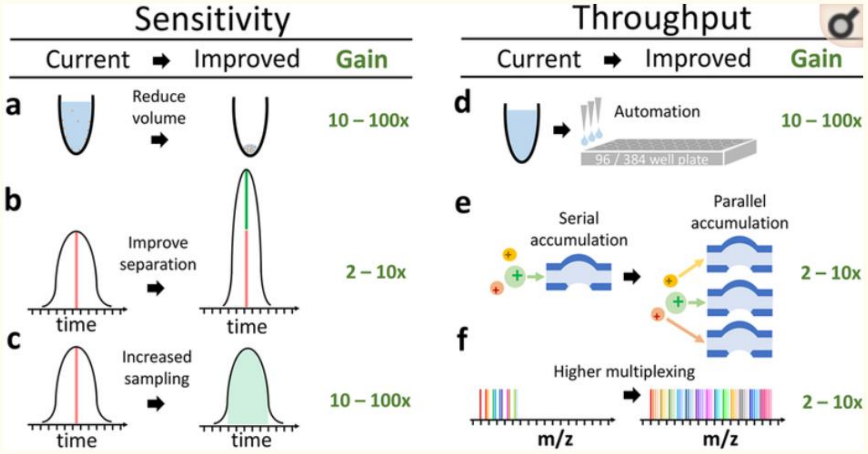


Figure 3.

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/

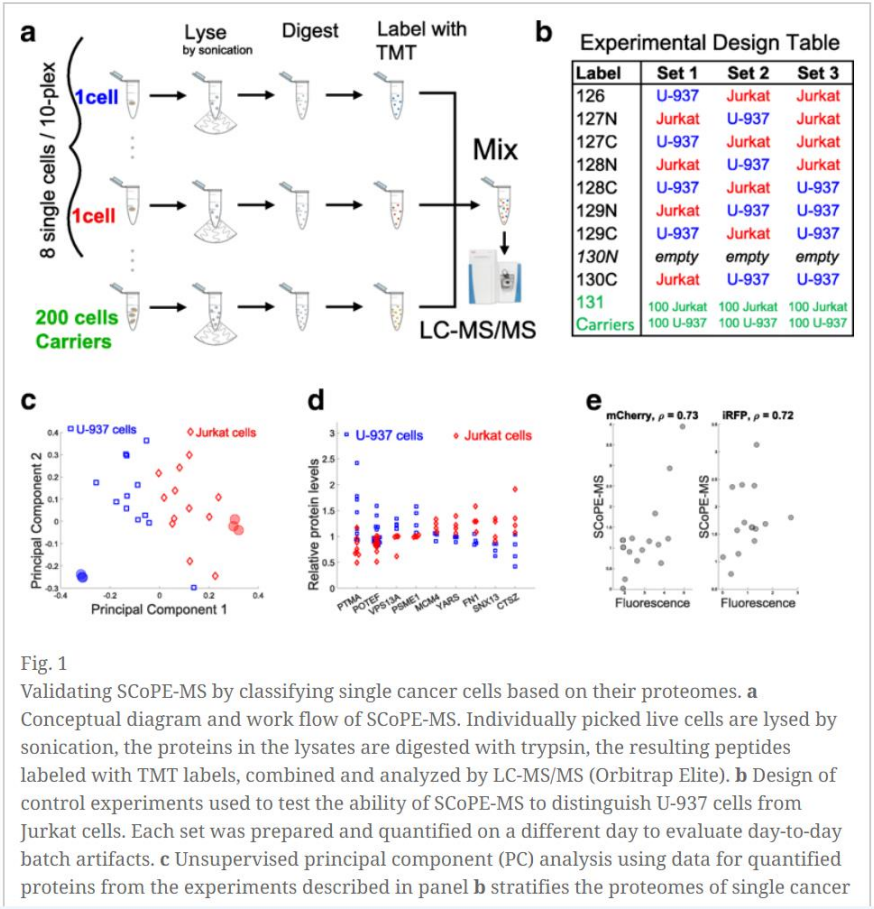
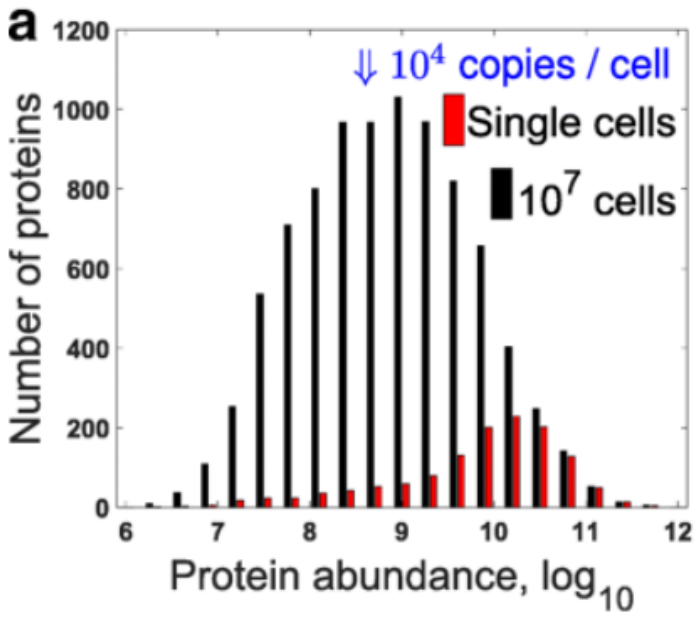


Fig. 1
Validating SCoPE-MS by classifying single cancer cells based on their proteomes. **a** Conceptual diagram and work flow of SCoPE-MS. Individually picked live cells are lysed by sonication, the proteins in the lysates are digested with trypsin, the resulting peptides labeled with TMT labels, combined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