

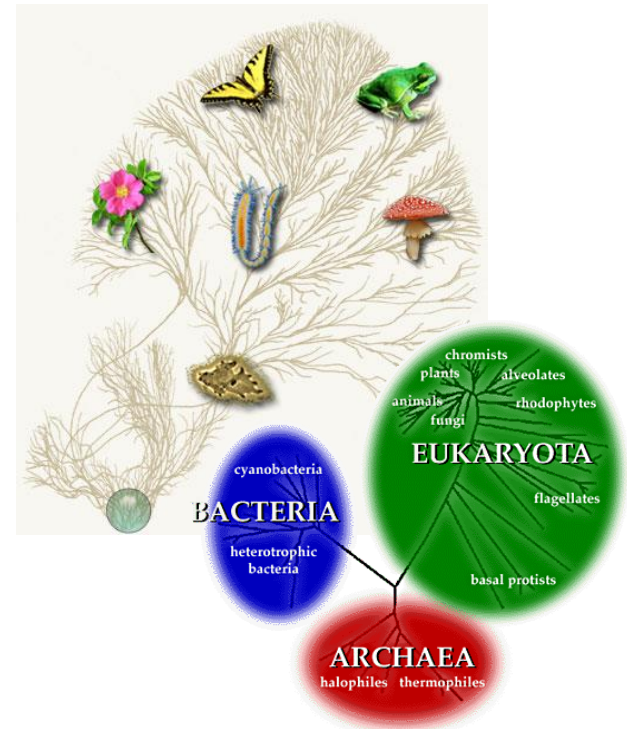
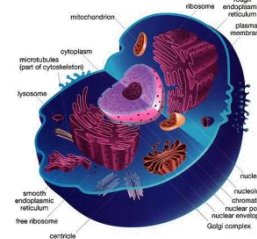
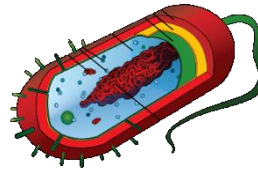
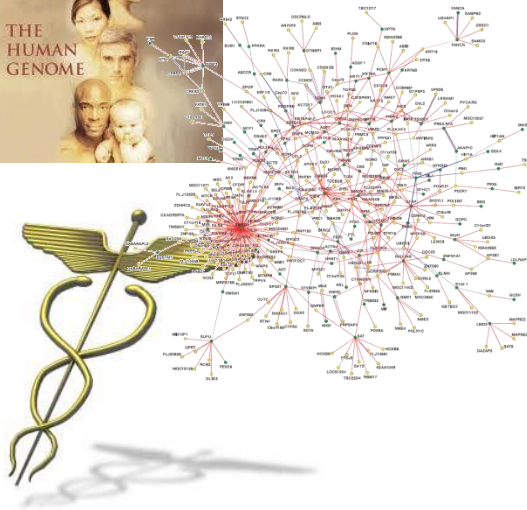
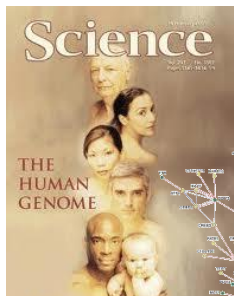


Proteins: Proteomics & Protein-Protein Interactions Part I

Jesse Rinehart, PhD

Department of Cellular & Molecular Physiology
Systems Biology Institute

DNA → RNA → PROTEIN



DNA → RNA → PROTEIN

Cell

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,¹ Koji Tanabe,¹ Mari Ohnuki,¹ Megumi Narita,^{1,2} Tomoko Ichisaka,^{1,2} Kiichiro Tomoda,³ and Shinya Yamanaka^{1,2,3,4,*}

¹Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

²CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

³Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA

⁴Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan

*Correspondence: yamanaka@frontier.kyoto-u.ac.jp

DOI 10.1016/j.cell.2007.11.019

The New York Times

Bird Flu Paper Is Published After Debate

Science

AAAS

Science, 2012 Jun 22;336(6088):1534-41.

Airborne transmission of influenza A/H5N1 virus between ferrets.

Herfst S, Schrauwen EJ, Linster M, Chutinimitkul S, de Wit E, Munster VJ, Sorrell EM, Bestebroer TM, Burke Fouchier RA.

Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands.

Proteins: Proteomics & Protein-Protein Interactions

Overview

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

Proteins: Proteomics & Protein-Protein Interactions

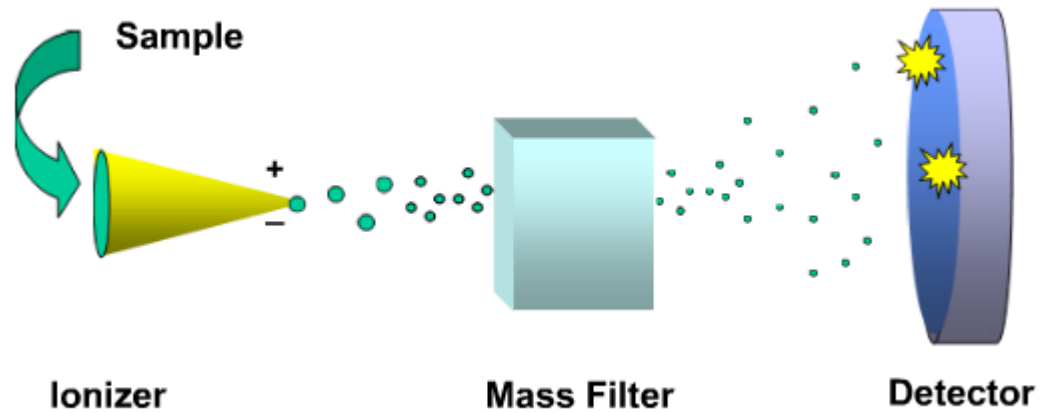
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Principles of Mass Spectrometry (MS)

- 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, J.B. et al, Science, 1989, 246, 64.

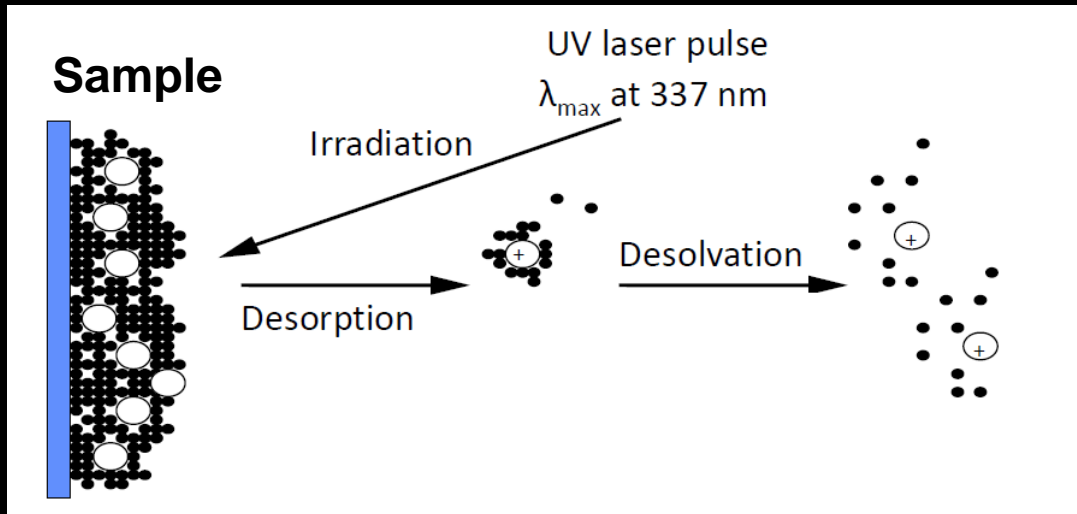
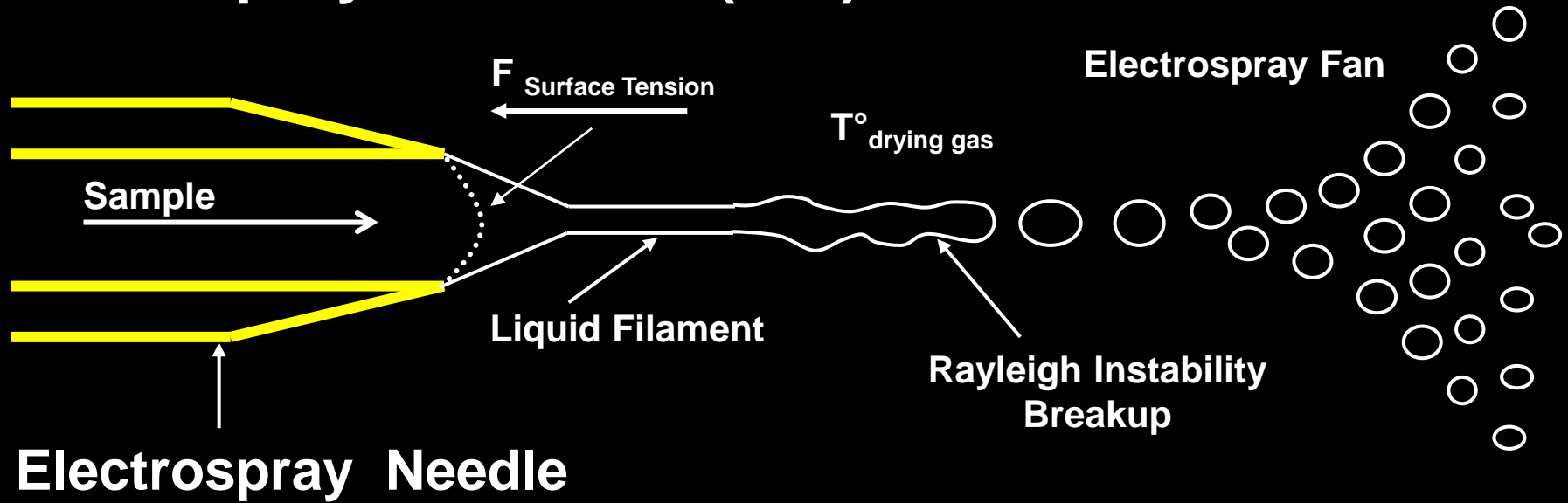
- **Matrix Assisted Laser Desorption Ionization (MALDI)**

Karas, M.; Hillenkamp, F., Anal. Chem., 1995, 60, 2299

MS based Proteomics is born:

- MS to measure weight of large intact proteins
- Non-covalently bonded protein complexes can also be measured (ESI only)
- **Intact peptides measured and “sequenced”**

Electrospray Ionization (ESI)



Matrix Assisted Laser Desorption Ionization (MALDI)

Mass Spectrometry takes the 2002 Nobel Prize in Chemistry

Awarded to John B. Fenn & Koichi Tanaka

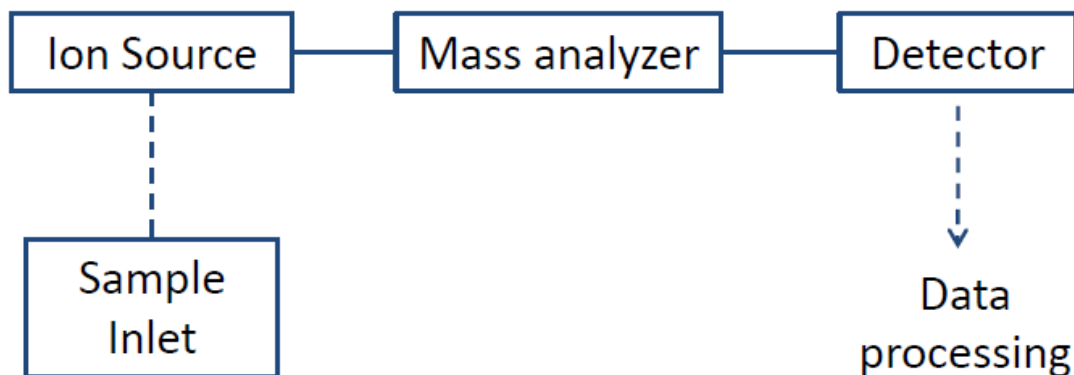


***Fenn** - Discovered Electrospray Ionization (ESI)

***Pioneering work at Yale University in the Department of Chemical Engineering**

Tanaka - Discovered Matrix Assisted Laser Desorption Ionization (MALDI)

Basic Mass Spectrometer

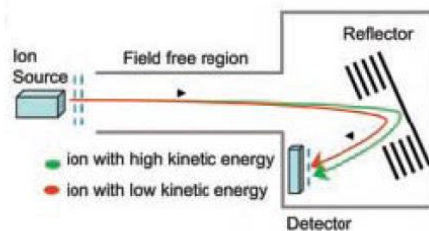


Typical LC-MS Setup

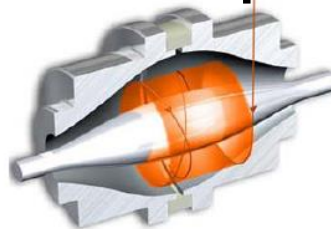


Mass Analyzers

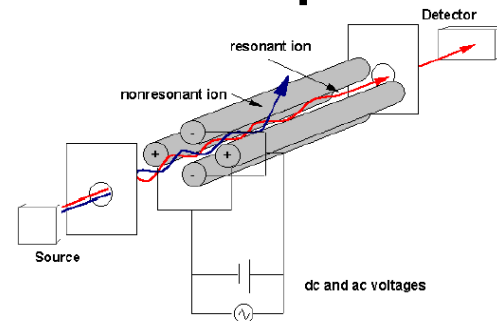
Time of Flight (TOF)



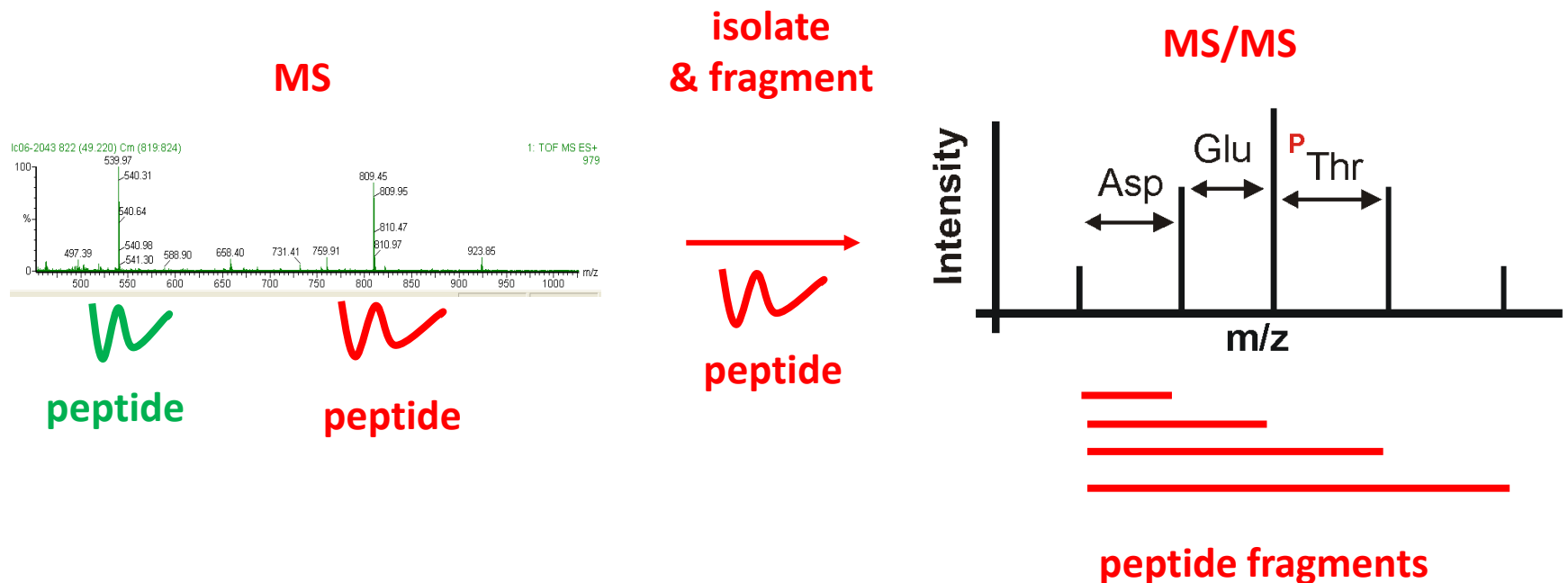
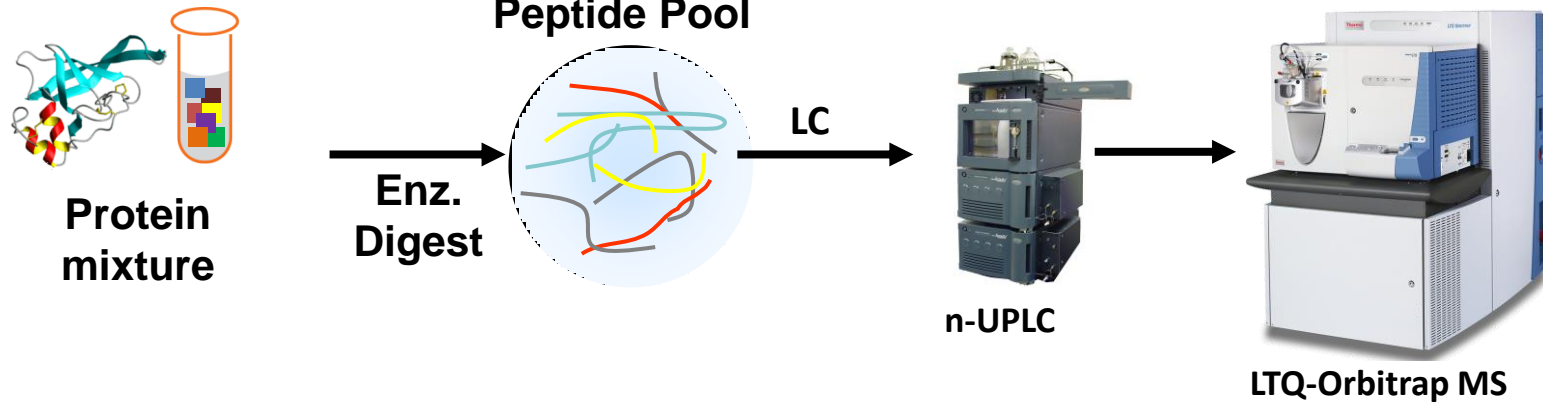
Orbitrap



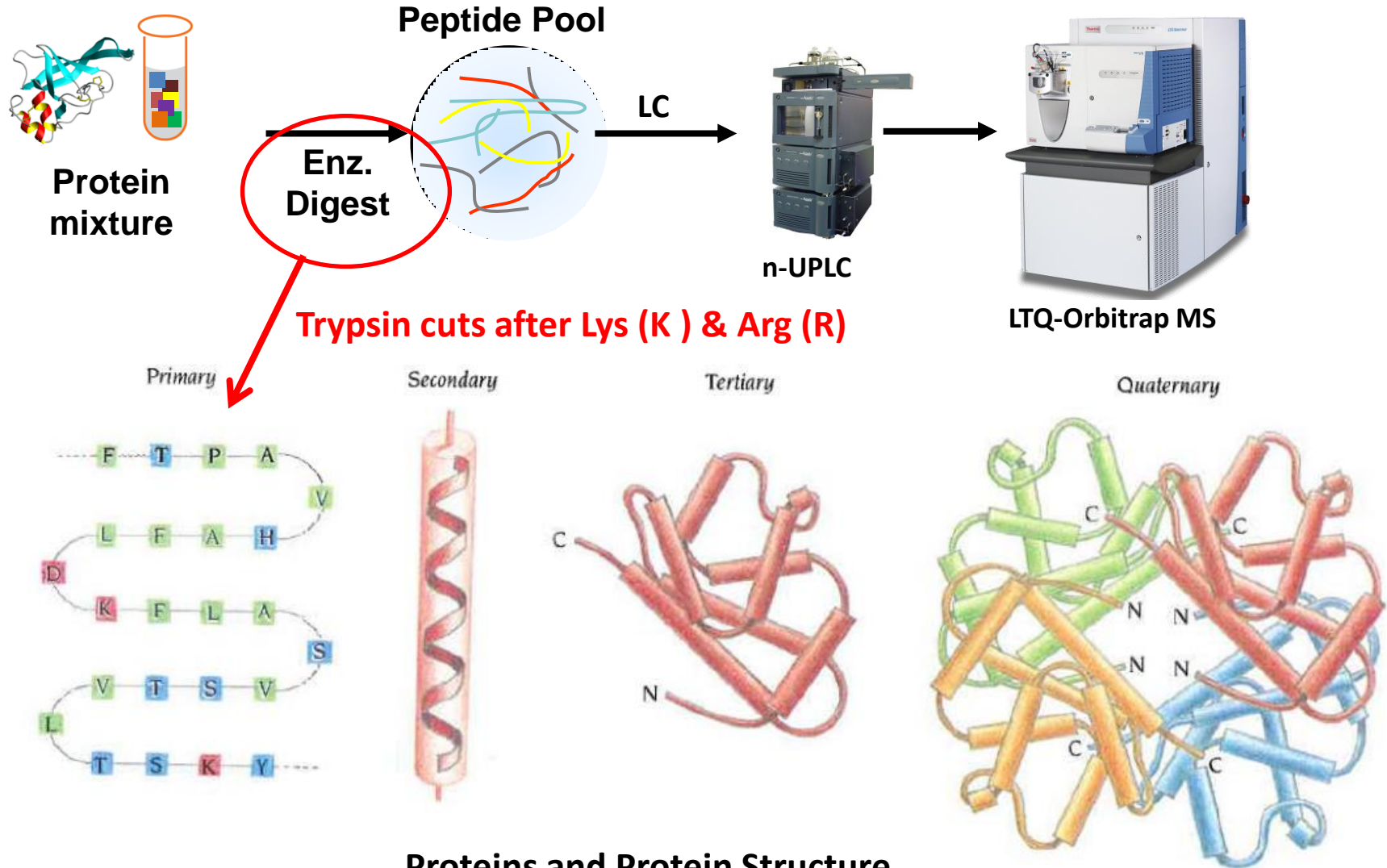
Quadrupole



Typical work flow for LC-MS “shotgun proteomics”



Typical work flow for LC-MS “shotgun proteomics”



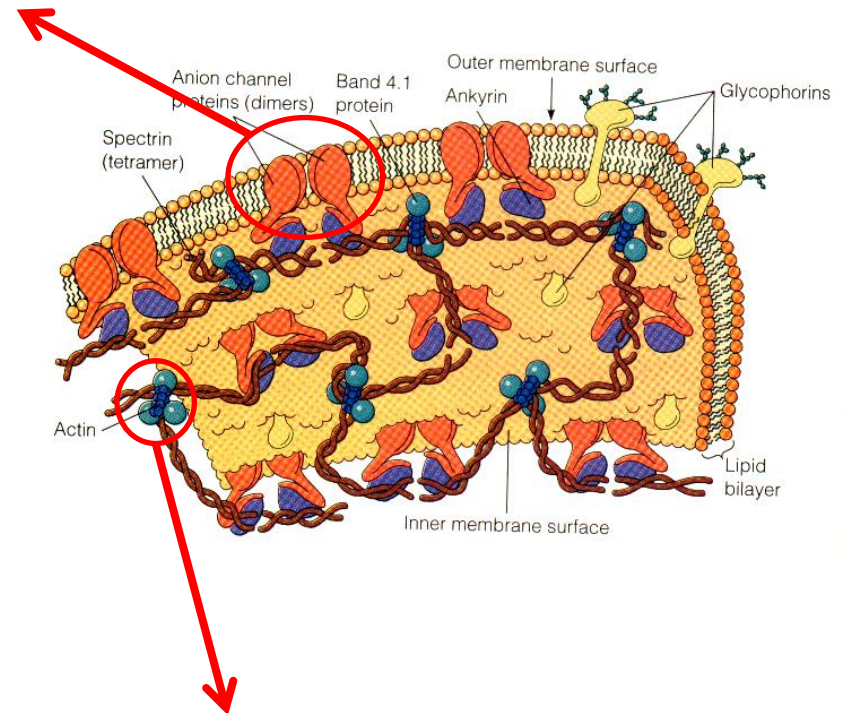
(Branden, C. and Tooze, J. *Introduction to Protein Structure*)

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

Matched peptides shown in **Bold Red**

Band 3 Anion Transporter

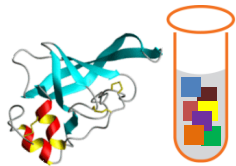
```
1 MEELQDDYED MMEENLEQEE YEDPDIPESQ MEEPAAHDE ATATDYHTTS
51 HPGTHKVVVE LQELVMDEKN QELRWMEAAAR WVQLEENLGE NGAWGRPHLS
101 HLTFWSLLEL RRVFTKGTVL LDLQETSLAG VANQLLDRFI FEDQIRPQDR
151 EELLRALLLK HSHAGELEAL GGVKPAVLTR SGDPSQPLLP QHSSLETQLF
201 CEQGDGGTEG HSPSGILEKI PPDSEATLVL VGRADFLEQP VLGFVRLQEA
251 AELEAVELPV 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 VVLVVAFEVS FLVRFISRYT QEIFSFLISL IFIYETFSKL IKIFQDHPLQ
551 KTYNYNVLMV PKPQGPLPNT ALLSLVLMAG TFFFAMMLRK FKNSSYFP GK
601 LRRVIGDFGV PISILIMVLV DFFIQDITYTQ KLSVPDGFKV SNSSARGWVI
651 HPLGLRSEFP IWMMFASALP ALLVFILIFL ESQITTLIVS KPERKMKVGS
701 GFHLDLLLVV GMGGVAALFG MPWLSATTVR SVTHANALTV MGKASTPGAA
751 AQIQEVKEQR ISGLLVAVLV GLSILMEPIL SRIPLAVLFG IFLYMGVTSL
801 SGIQLFDRIL LLFKPPKYHP DVPYVKRVKT WRMHFLTGIQ IICLAVLWVV
851 KSTPASLALP FVLILTVP LR VLLPLIFRN VELQCILDADD AKATFDEEEG
901 RDEYDEVAMP V
```



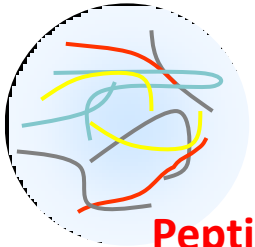
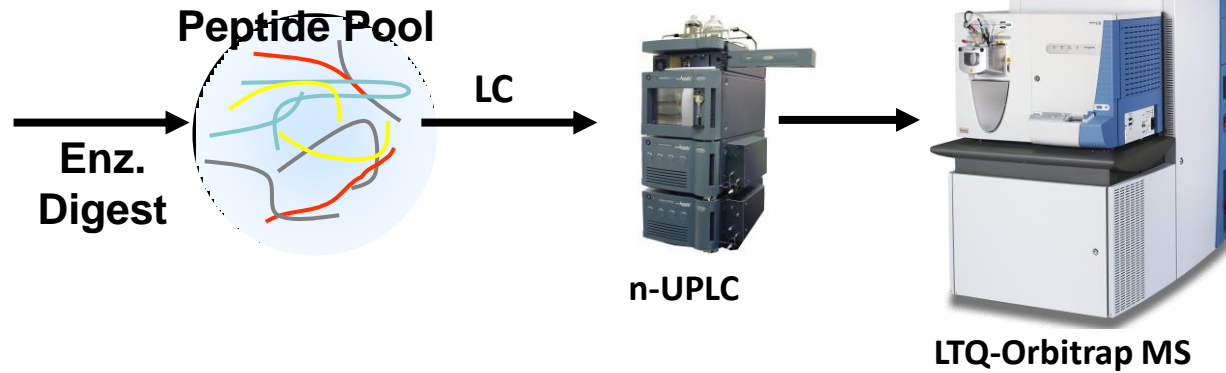
Matched peptides shown in **Bold Red**

β -actin

```
1 MDDDIAALVV DNGSGMCKAG FAGDDAPRAV FPSIVGRPRH QGVVMGMGQK
51 DSYVGDEAQS KRGILTLYP IEHGIVTNWD DMEKIWHHTF YNELRVAPEE
101 HPVLLTEAPL NPKANREKMT QIMFETFNTP AMYVAIQAVL SLYASGRTTG
151 IVMDSGDGVV HTVPIYEGYA LPHAILRLDL AGRDLTDYLM KILTERGYSF
201 TTTAEREIVR DIKEKLCYVA LDPEQEMATA ASSSSLEKSY ELPDGQVITI
251 GNERFRCP EA LFQPSFLGME SCGIHETTFN SIMKCDVDIR KDLYANTVLS
301 GGTTMYPGIA DRMQKEITAL APSTMKIKII APPERKYSVW IGGASILASLS
351 TFQQMWISKQ EYDESGPSIV HRKCF
```



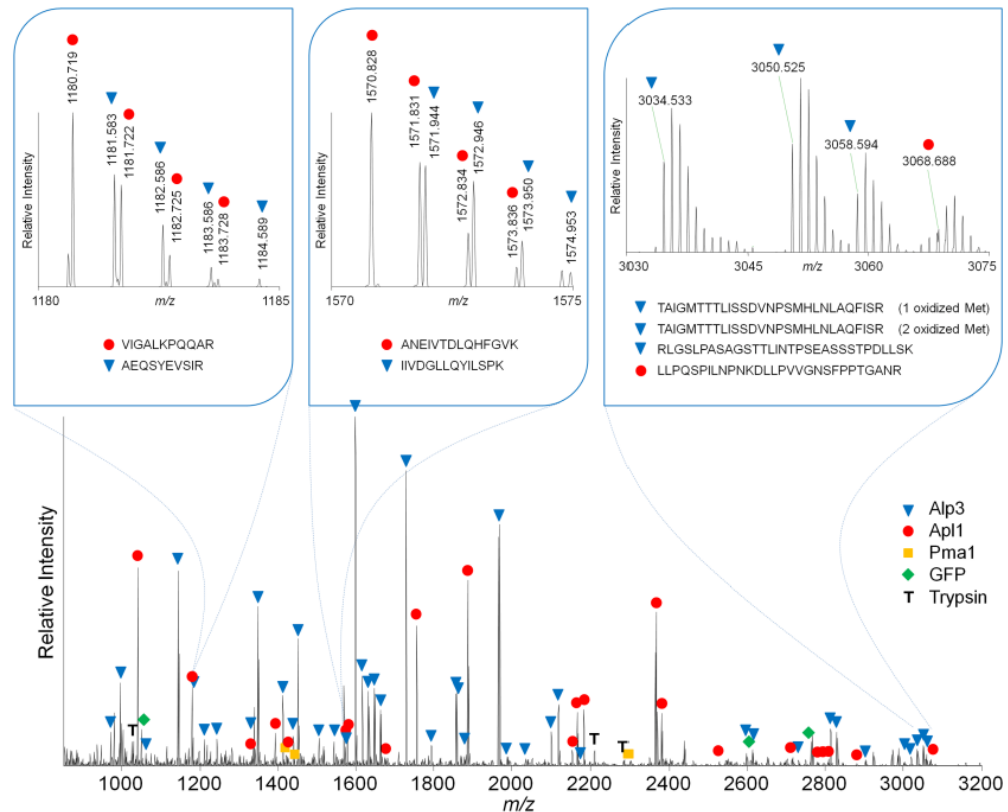
Protein mixture

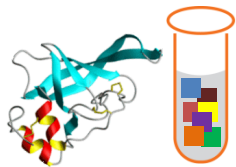


Peptide ions have a mass (m) and a charge (z).

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

Mass Spectrum





Protein mixture

Enz. Digest

Peptide Pool

LC

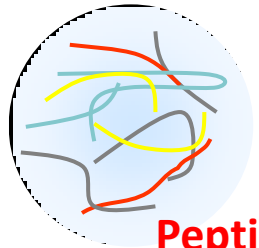


n-UPLC

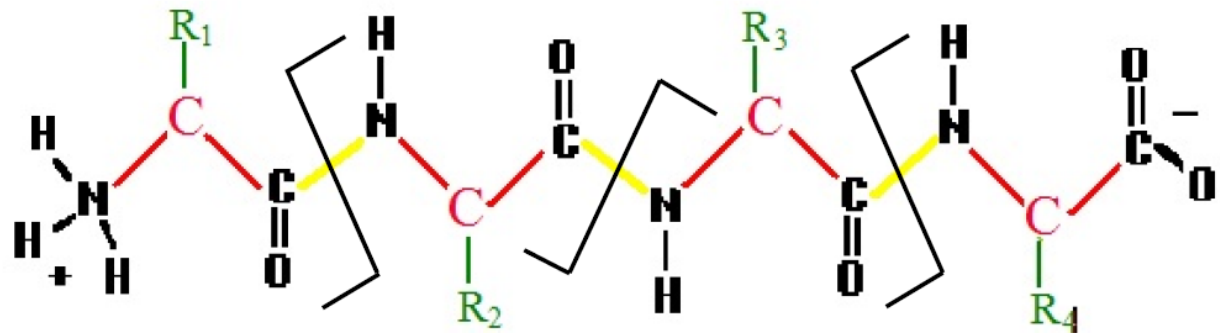


LTQ-Orbitrap MS

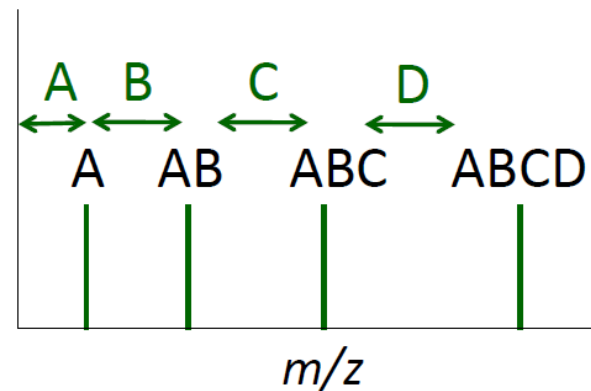
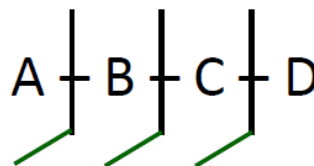
Peptide sequencing

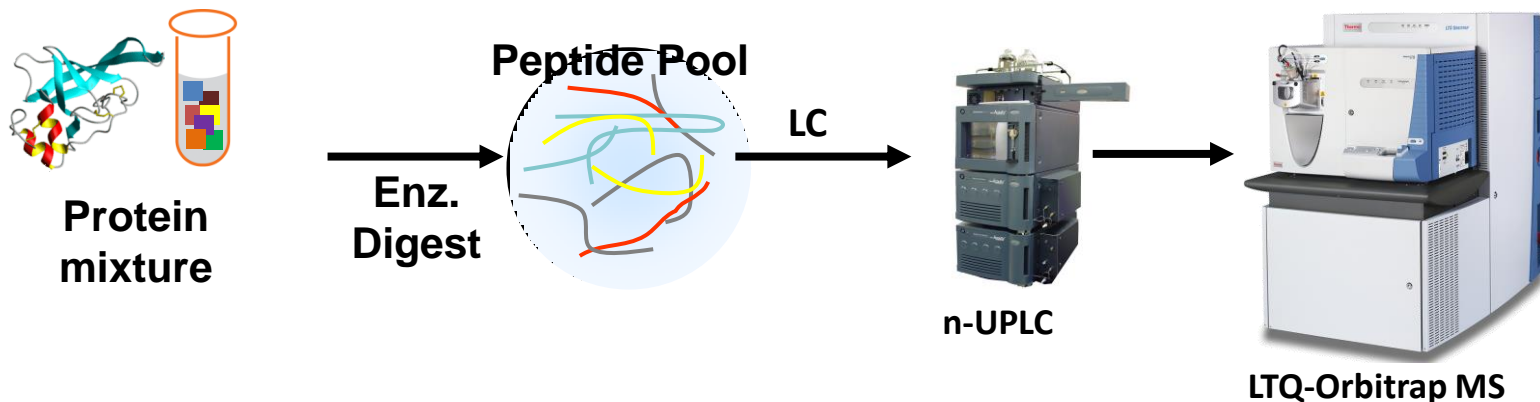


Peptide ions are isolated and "sequenced"

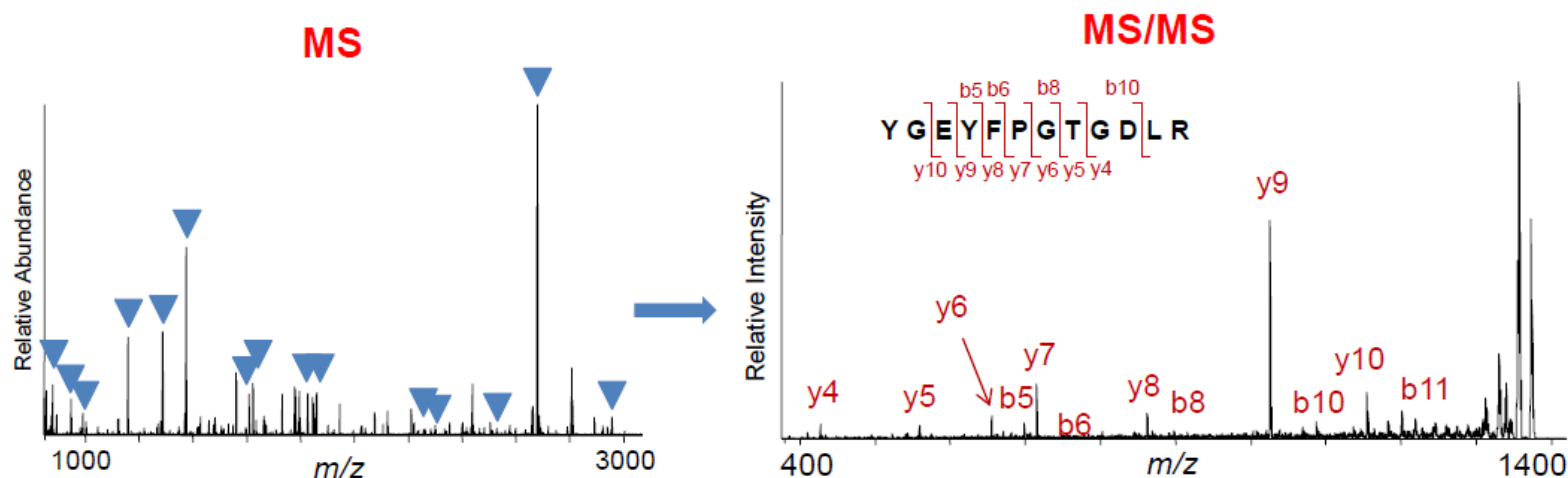


Simplified concept of peptide fragmentation





Database searching - at MS or MS/MS level



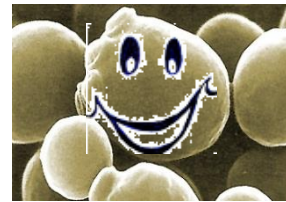
Computational Steps: massive amounts of MS data are read & interpreted. Databases searched to match peptide sequences.

Proteomics

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

[Study of post-translational modifications (protein phosphorylation, acetylation, glycosylation ...) via MS has grown in recent years to dramatically expand the field of Proteomics]

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.**

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➡ **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.**

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

*orders of magnitude behind genomics and transcriptomics

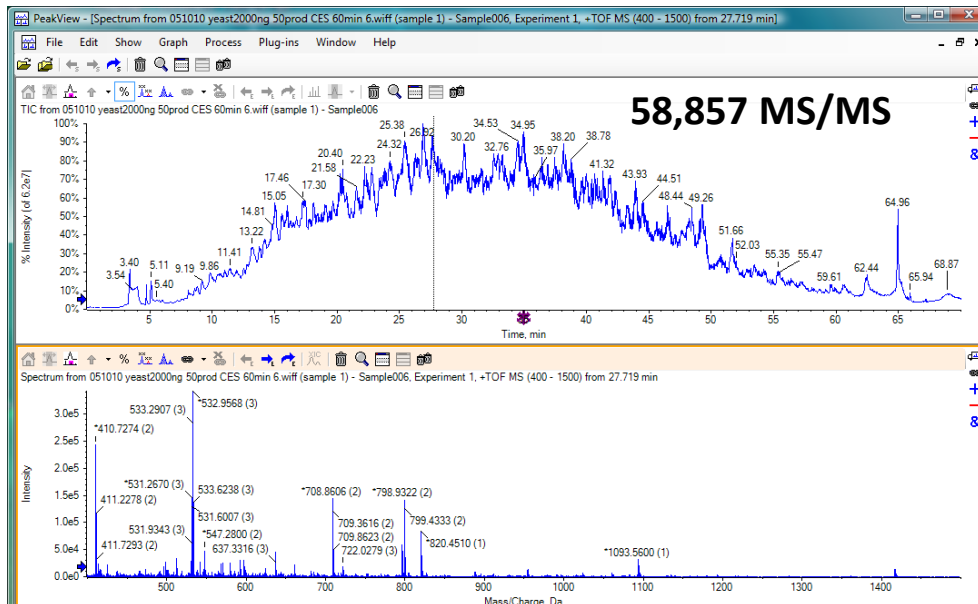
Yeast proteome reported in **Washburn et al. *Nature Biotech* 2001:**

~82 hours* = 1,484 proteins

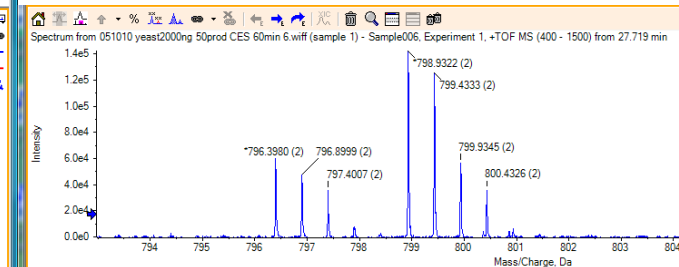
*estimates from paper: 3 fractions @ 15 X 110 minute “runs” for each fraction

Yeast proteome by **Hunter, Colangelo, Rinehart, et al *unpublished* 2010:**

One 60 minute run = 1,286 proteins



AB SCIEX TripleTOF 5600



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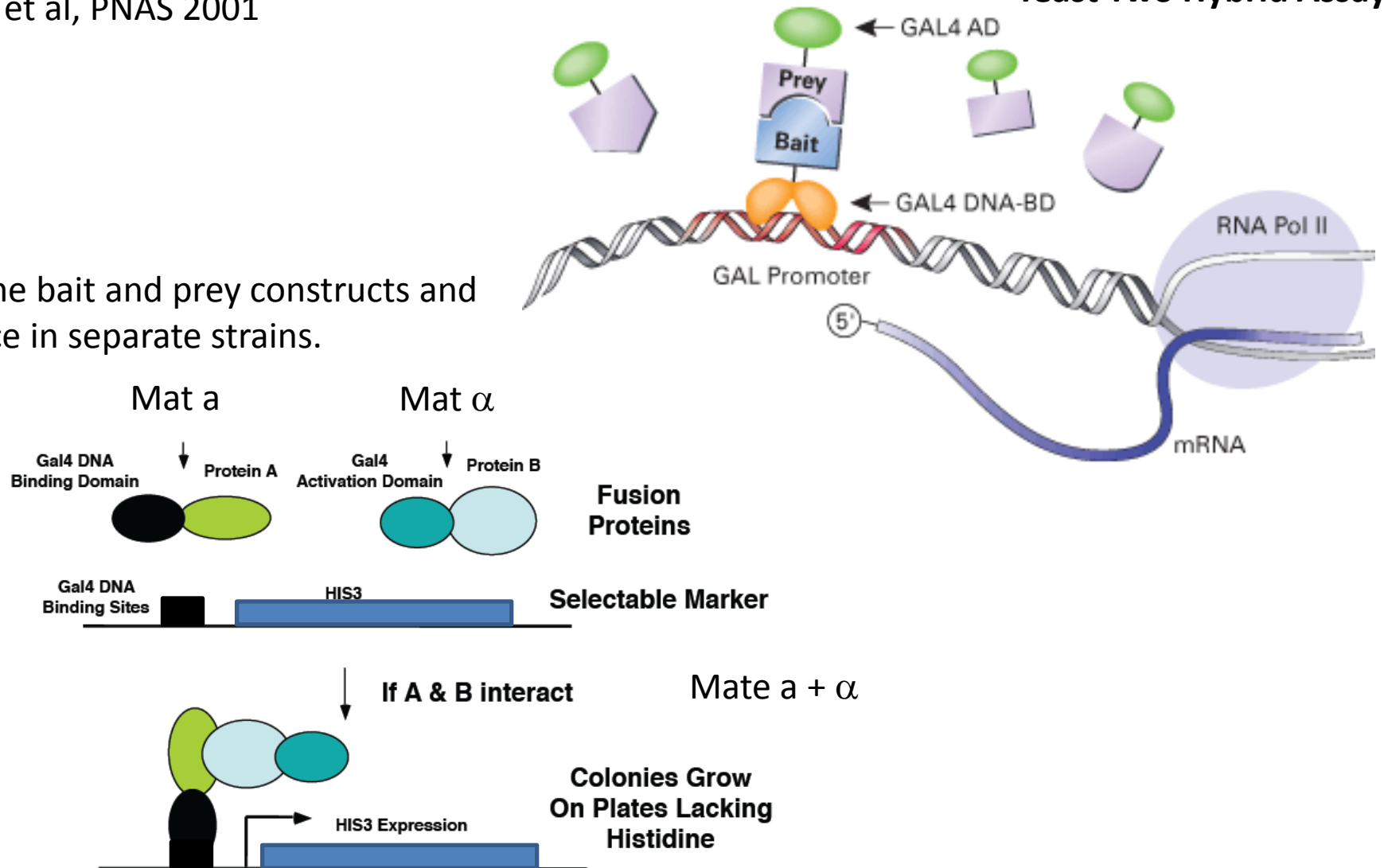
A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*.

Uetz et al, Nature 2000

Ito et al, PNAS 2001

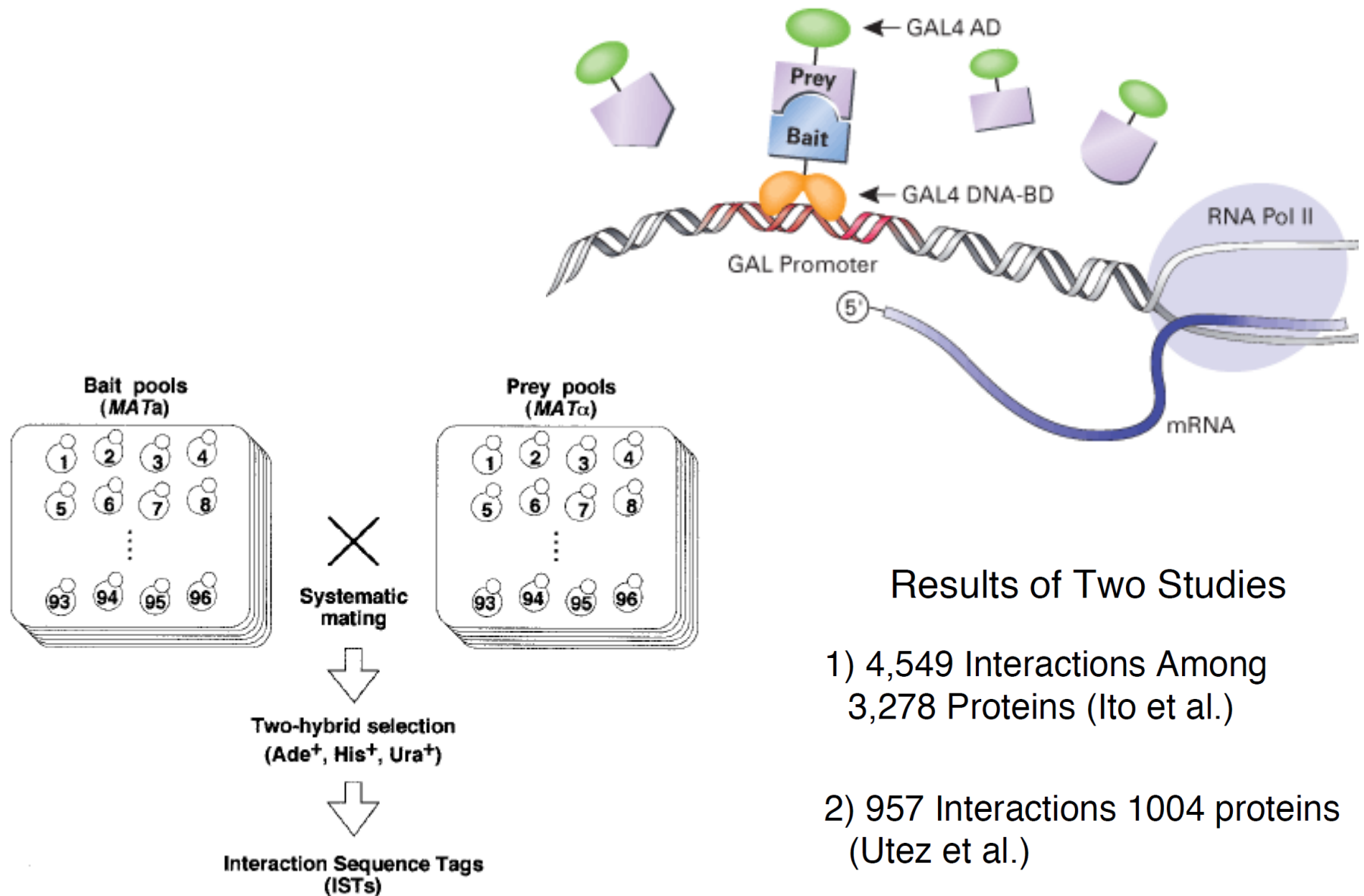
Yeast Two Hybrid Assay

Clone bait and prey constructs and place in separate strains.



Uetz et al, Nature 2000

Ito et al, PNAS 2001



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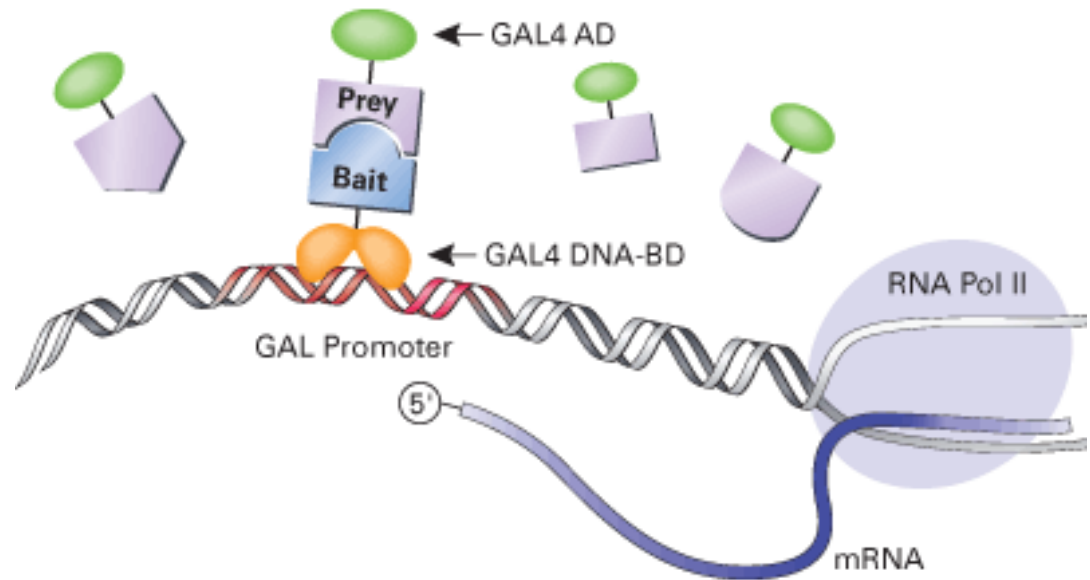
Yeast Two Hybrid Assay

Advantages:

- *In vivo* assay
- Simple

Some Disadvantages

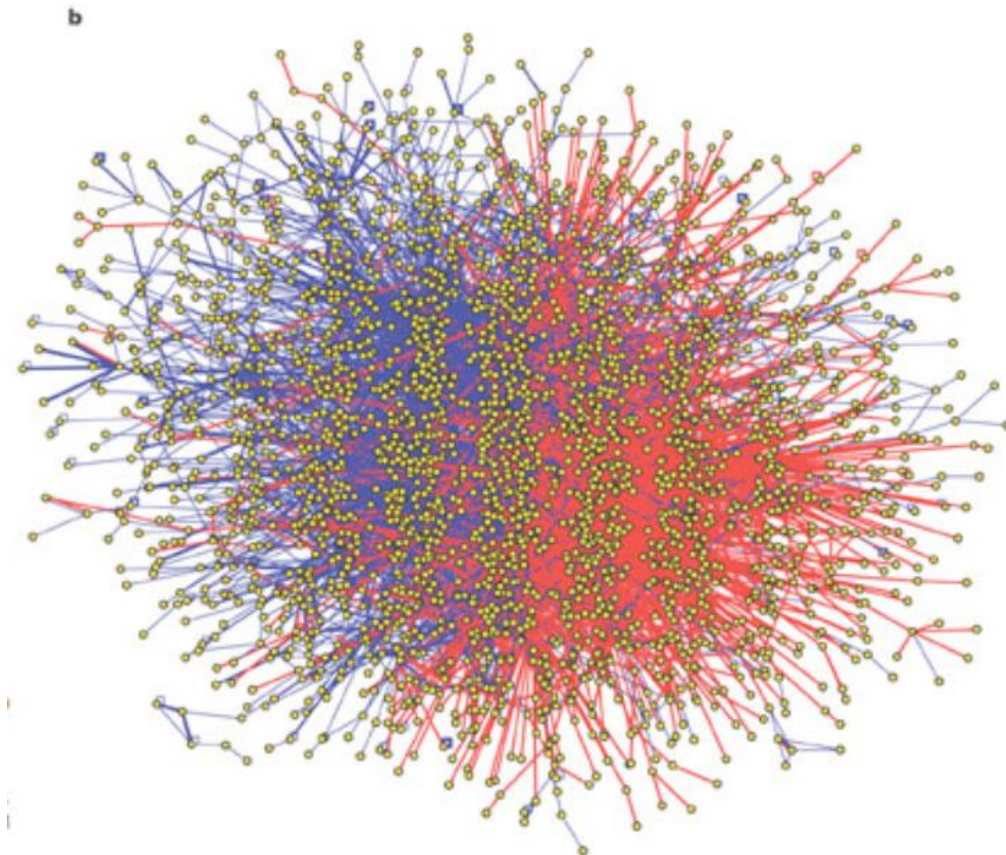
- Hard to execute on large scale
- False positives: a real interaction or “possible” interaction
- Interaction in nucleus (required for GAL system)
- Clones are fusion proteins and sometimes “partial” proteins
- Multiple protein complexes not “captured”



Human Two Hybrid Map

8,100 ORFs (~7,200 genes)

10,597 interactions

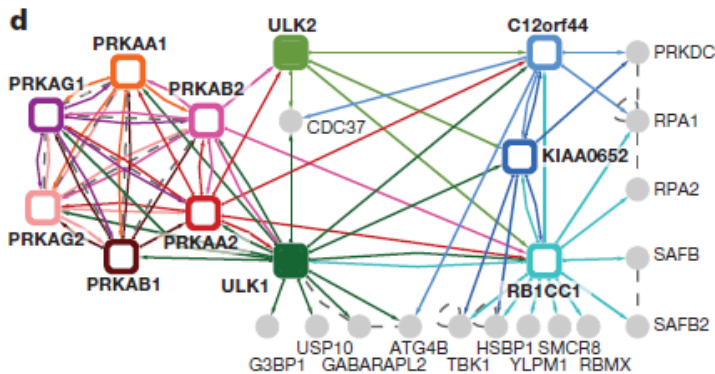
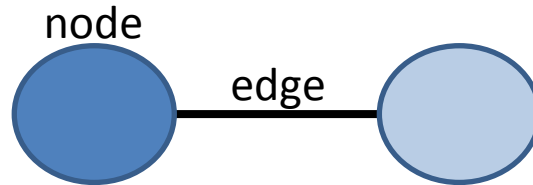


Rual et al. Nature 2005

Rual et al. Nature 2005 Vol 437

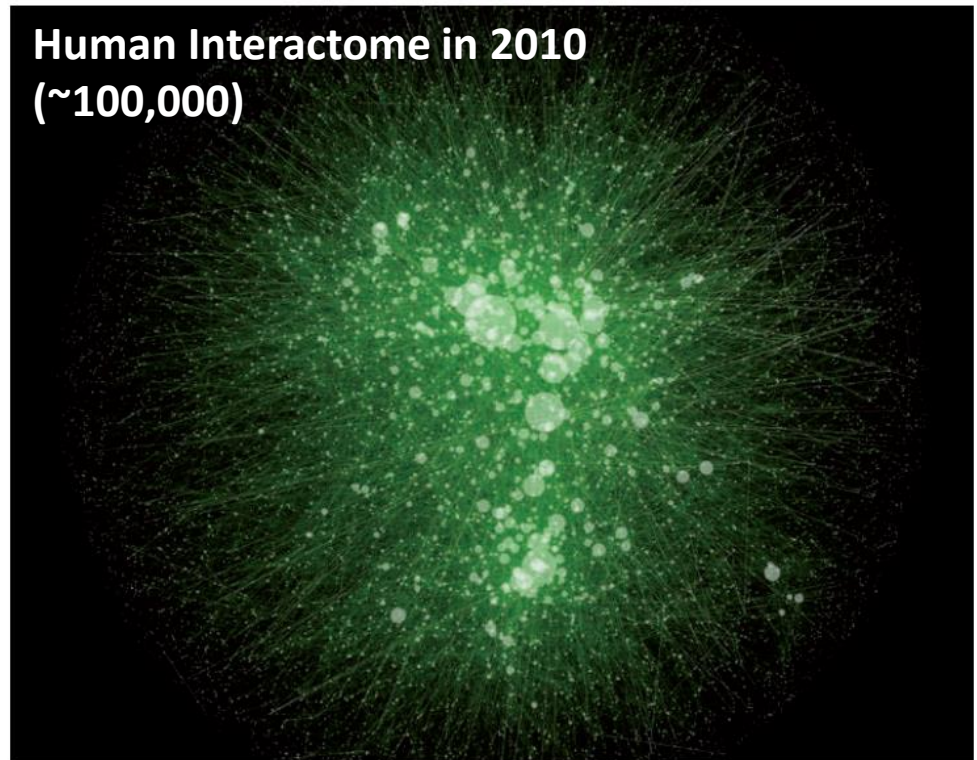
Protein-Protein interaction maps:

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



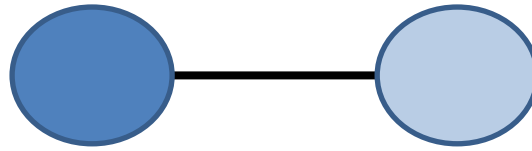
K. ONO/AUC SAN DIEGO/CYTOSCAPE

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



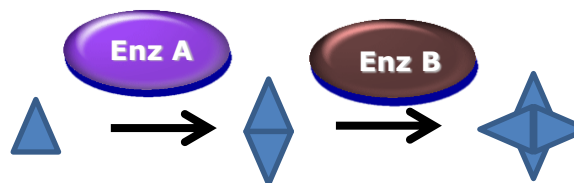
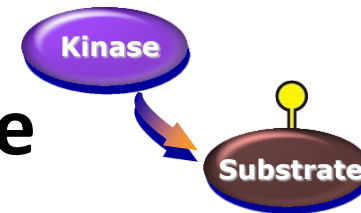
Bonetta, *Nature* 2010

Protein-Protein interactions:

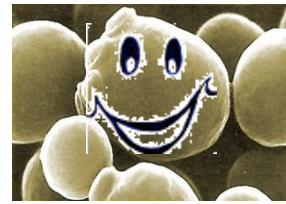


Some examples:

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



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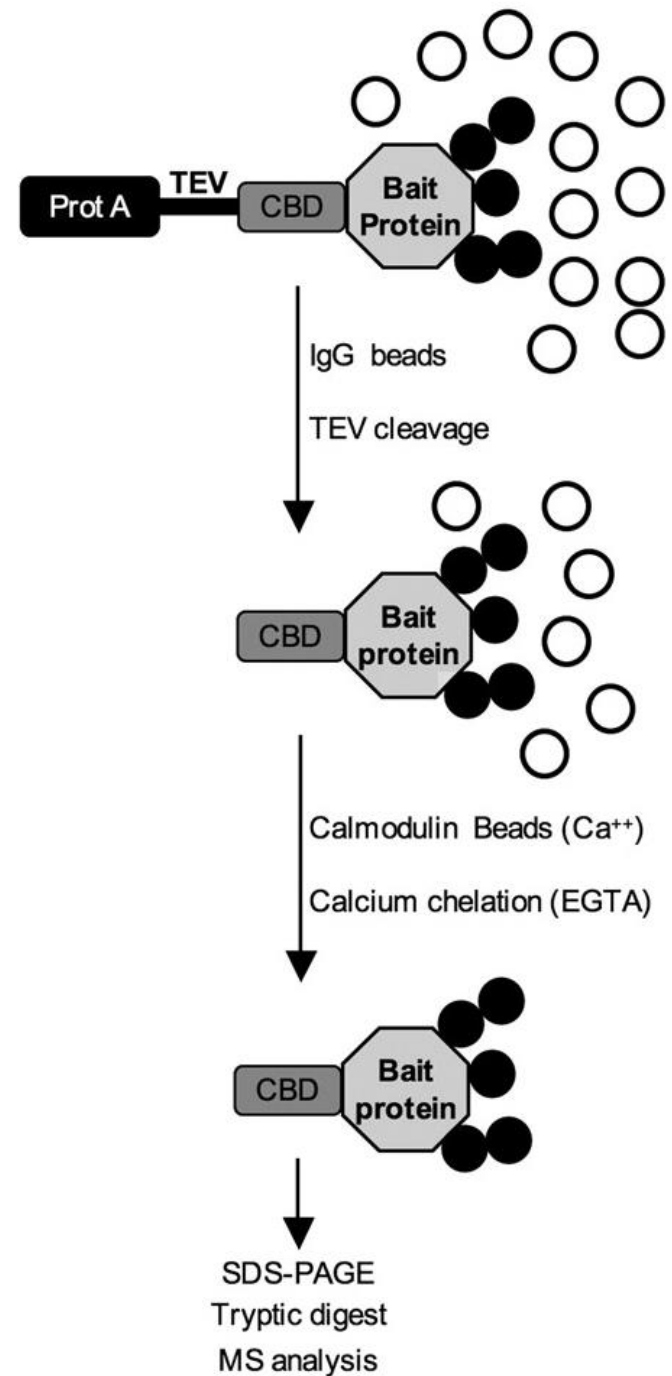
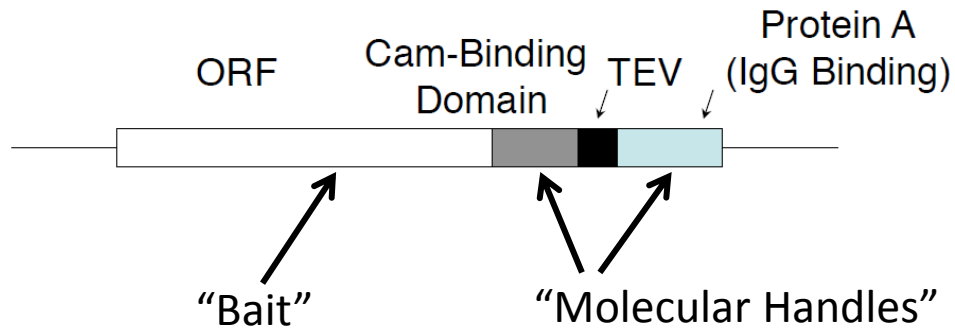
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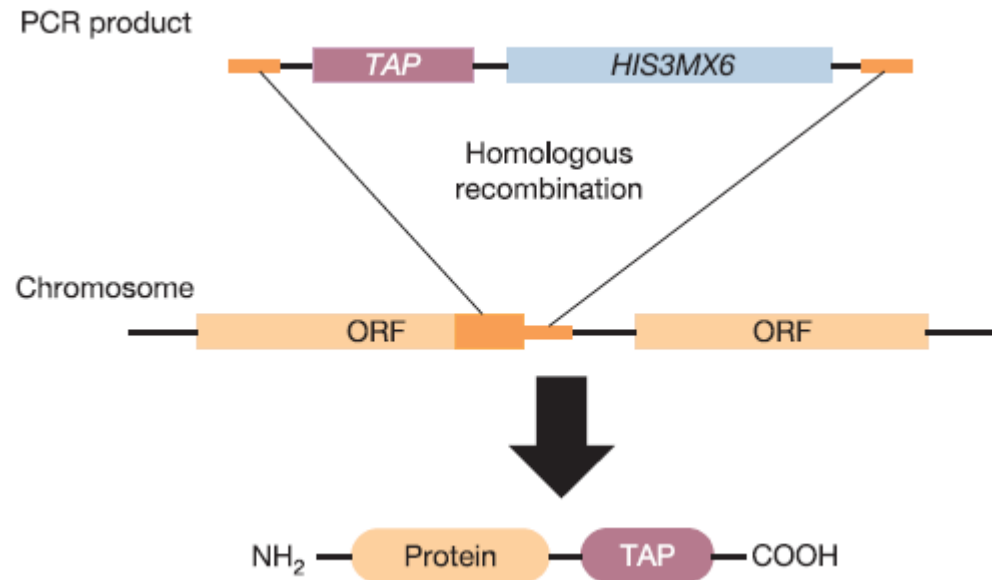
Picotti P, et al. Full dynamic range proteome analysis of *S. cerevisiae* by targeted proteomics. *Cell*.

➡ **Towards proteome wide targeted proteomics.**

Tandem Affinity Purification (TAP) Tagging



Global TAP Tagging in yeast



h

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*.

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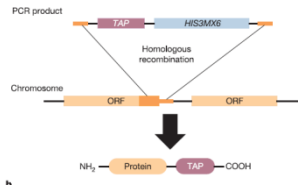
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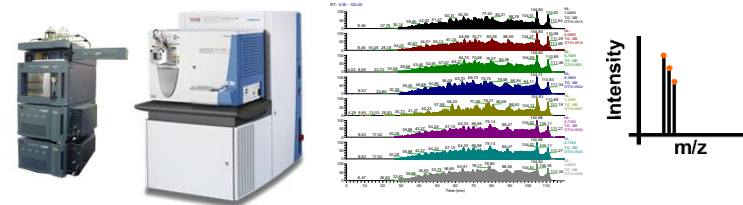
➔ TAP-Tag and Protein-Protein Interaction

Collection of **tagged** “bait”
expression strains



TAP bait + Interacting proteins

Multiple runs of “shotgun” MS
& SDS-PAGE with MS on individual proteins

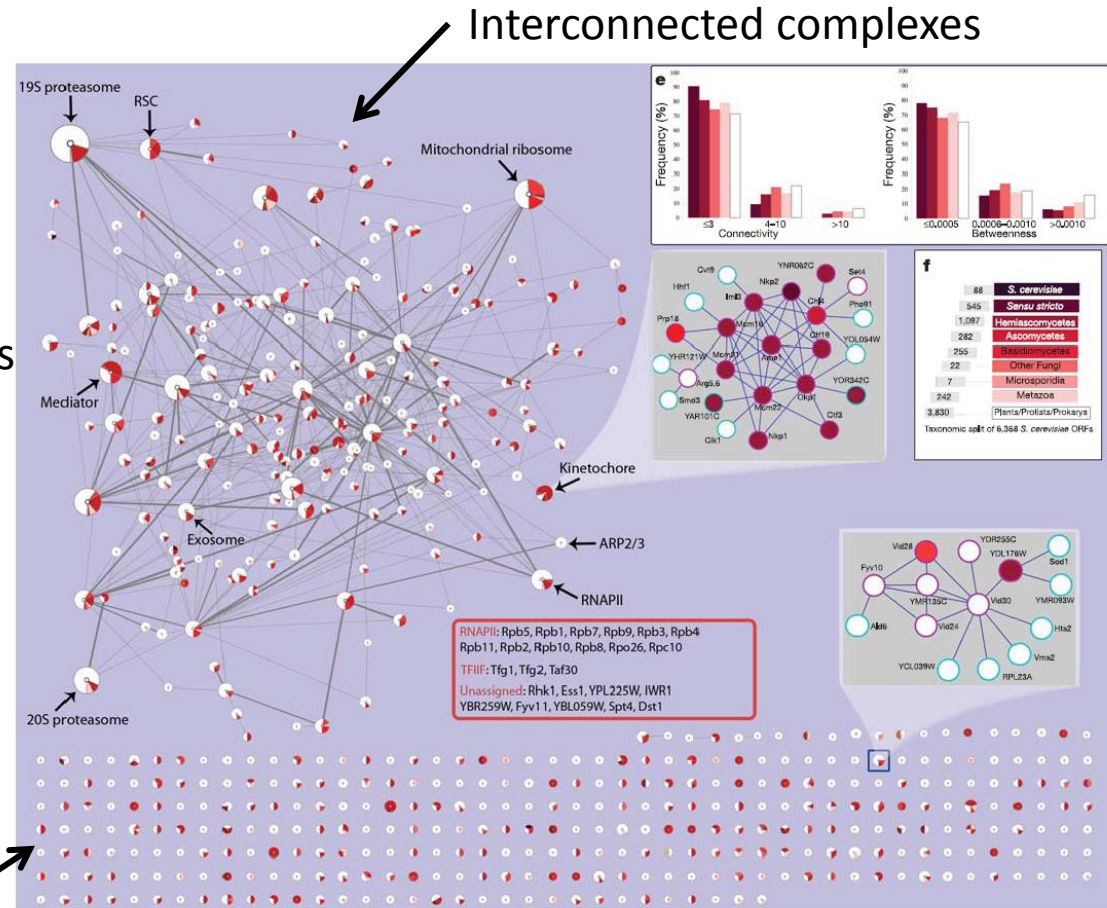


Krogan *et al.* observed 7,123 protein–protein interactions:

Important aspects:

- 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...”

- 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



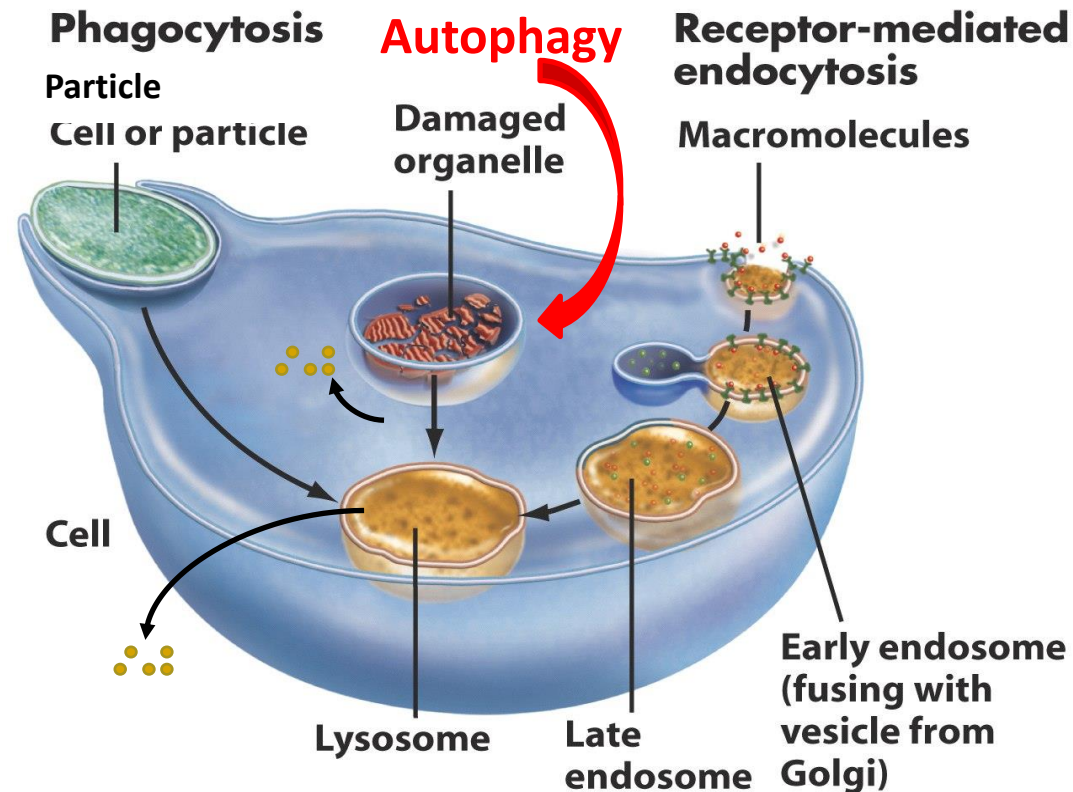
Complexes with little or no interconnectivity

Krogan NJ, et al. *Nature*. 2006

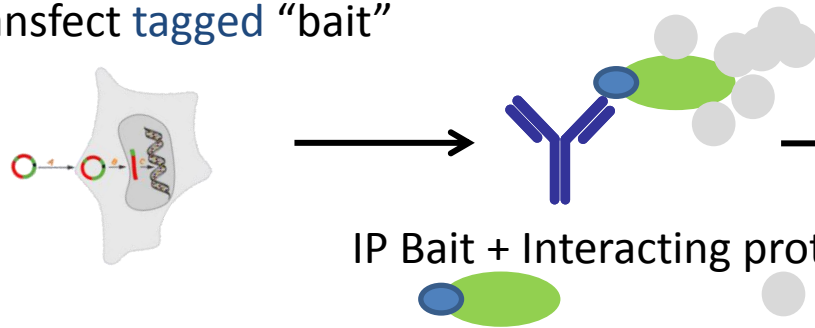
ARTICLES

Network organization of the human autophagy system

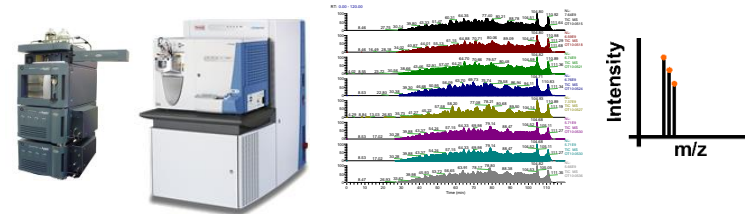
Christian Behrends¹, Mathew E. Sowa¹, Steven P. Gygi² & J. Wade Harper¹



Transfect **tagged** “bait”



Multiple runs of “shotgun” LC-MS/MS



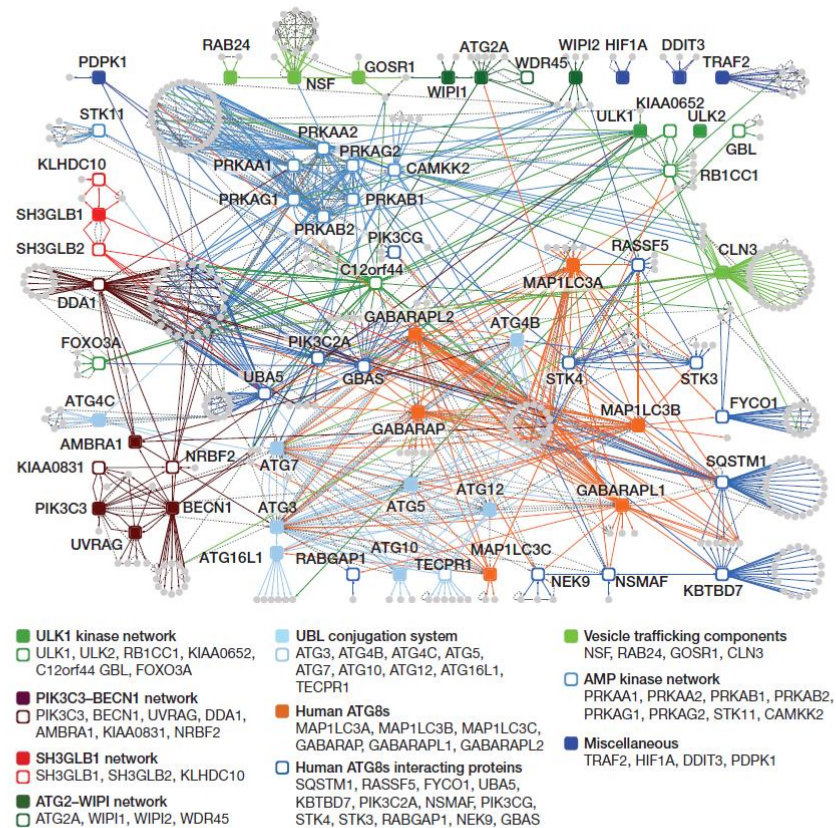
~65 bait proteins
LC-MS/MS identifies
2553 proteins

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

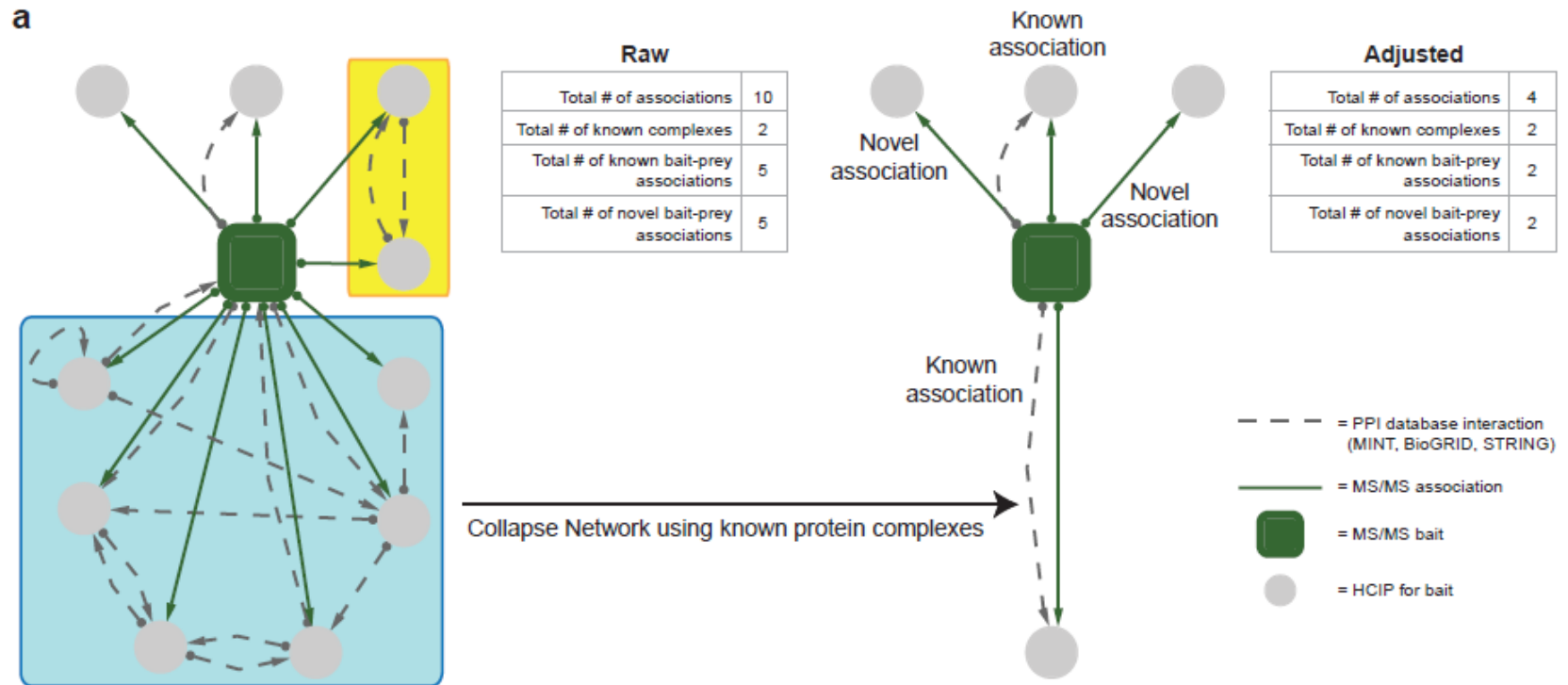
Autophagy Interaction Network



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.

Concatenating Data: Supplementing observed protein complexes with the Protein-Protein Interaction Databases: MINT, BioGRID, STRING



HCIPs	751	} Raw Network
Known Protein Complexes (KPC)	84	
Known Bait-KPC Interactions	40	
<hr/>		
Total Associations	497	} Collapsed Network
Known Bait-HCIP/KPC Associations	68	
Novel Bait-HCIP Associations	429	

A Functional Organization of the Network

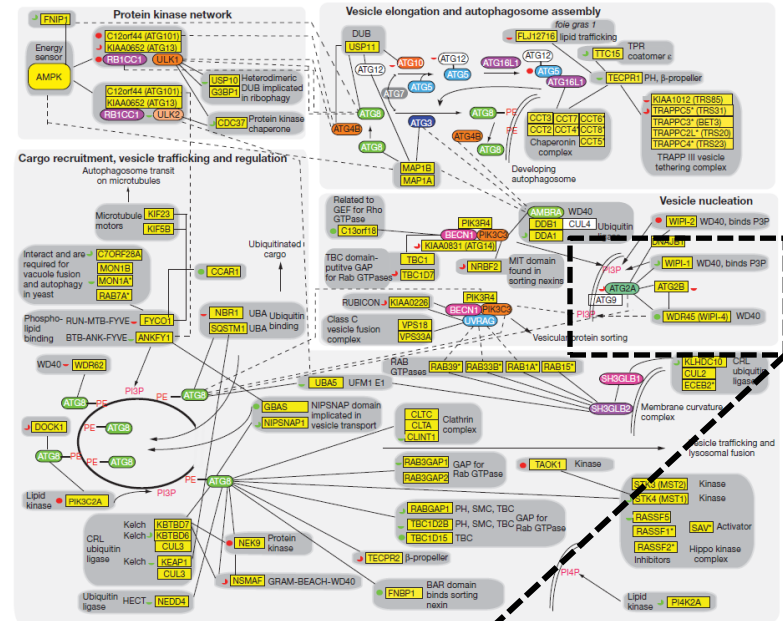
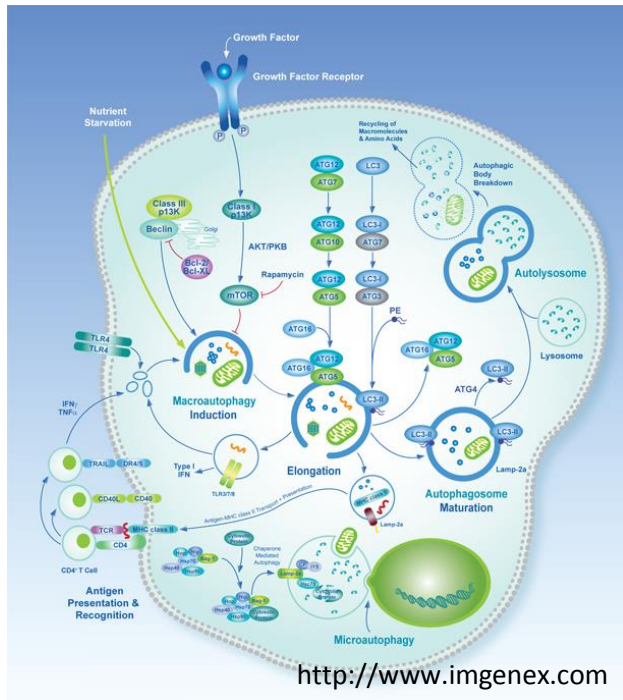
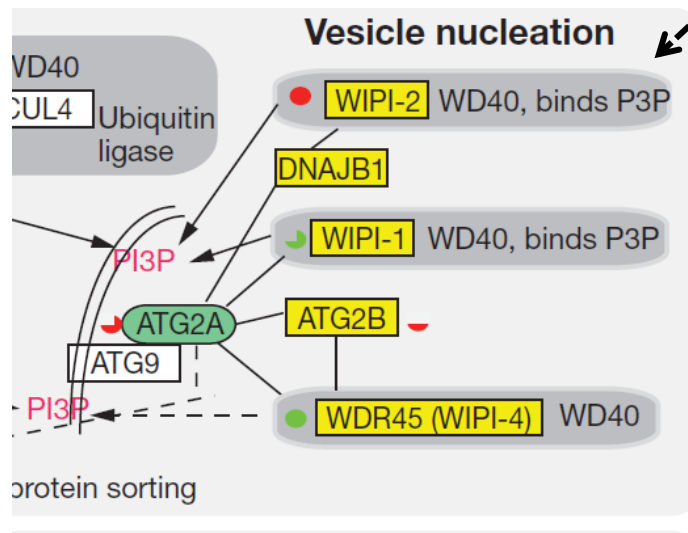


Figure 6 | Functional integration of the autophagy interaction network. Proteins in yellow boxes are HCIPs and proteins labelled with an asterisk were subthreshold (WD^N-score <1.0) for HCIP identification. Dotted lines, cross-module interaction; solid lines, this study; dotted arrows, potential functional interactions. Red or green full circle, three-quarter circle and

half-circle represent a reduction or increase in autophagosomes with 4, 3 and 2 siRNAs, respectively, in GFP-MAP1LC3B-expressing U2OS cells without rapamycin. Proteins in white boxes were not found by proteomics. The six ATG8 family members are represented by ATG8.

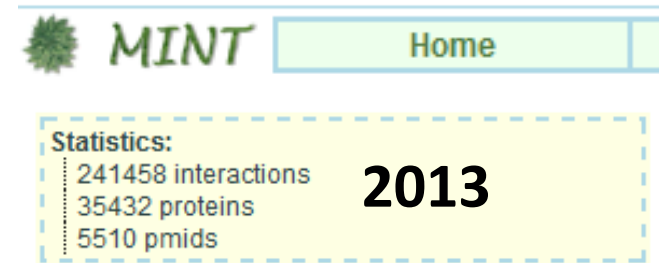
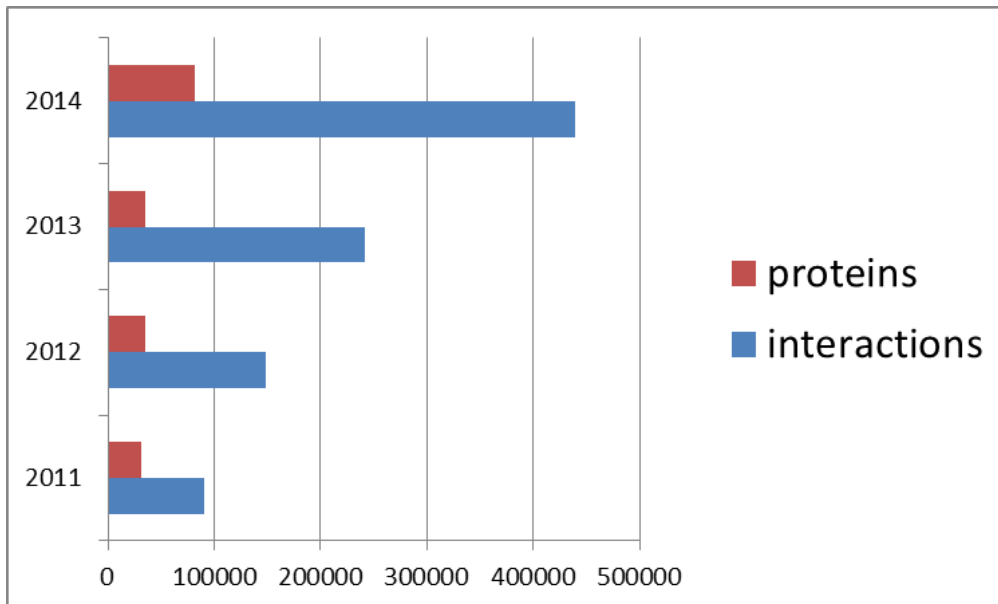
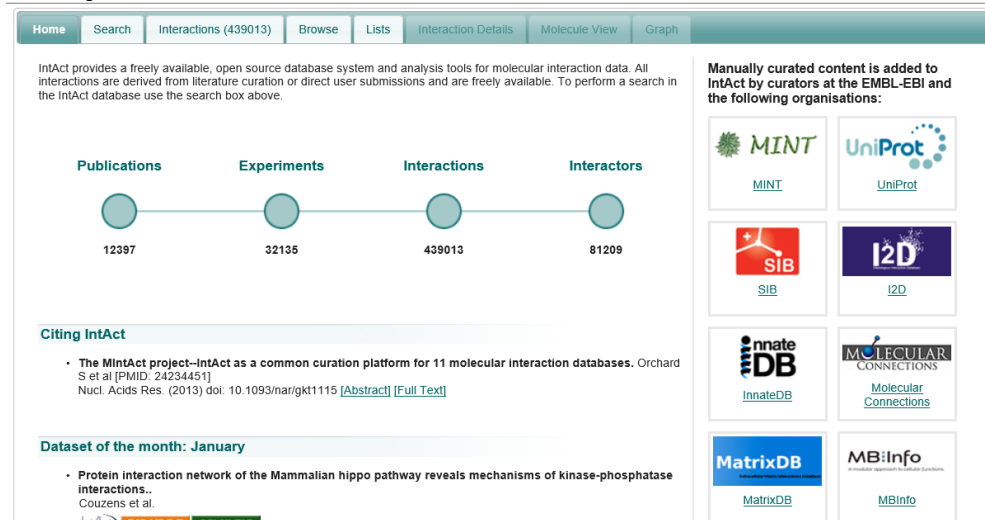


Phenotype after siRNA

- Less autophagosomes
- More autophagosomes

Protein-Protein Interaction Databases

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



2014 + 197,555 interactions
+ 45,777 proteins

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:

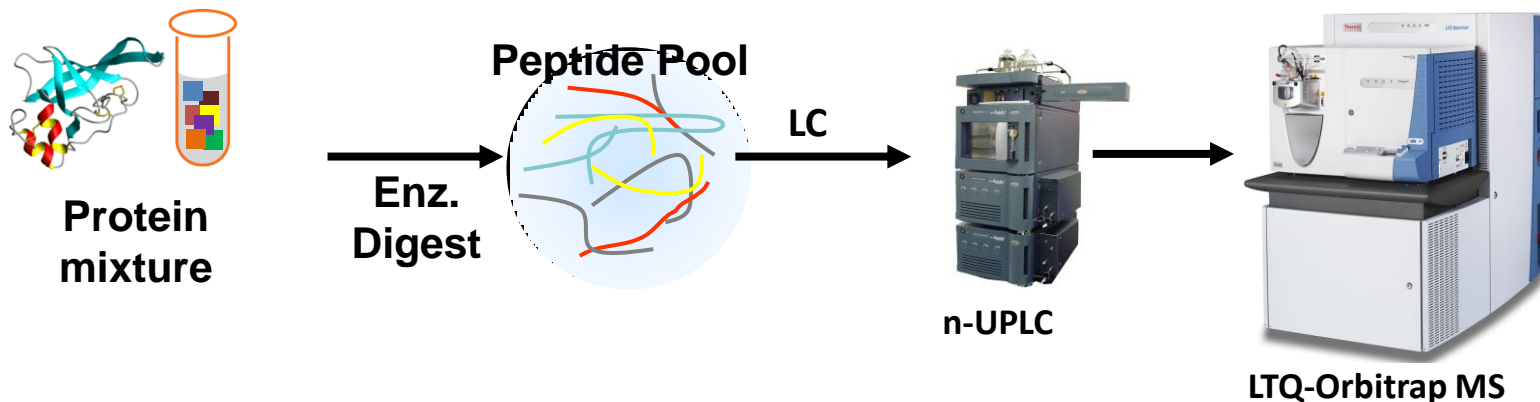
- How do protein interaction networks change over time?**



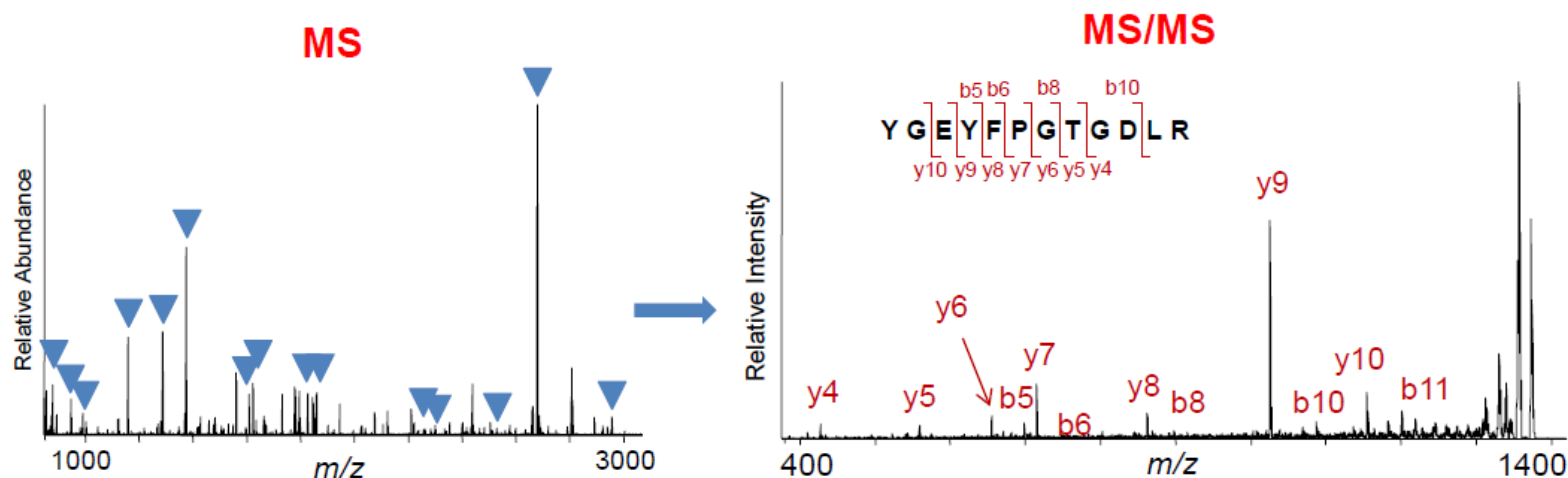
Proteins: Proteomics & Protein-Protein Interactions

Overview

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

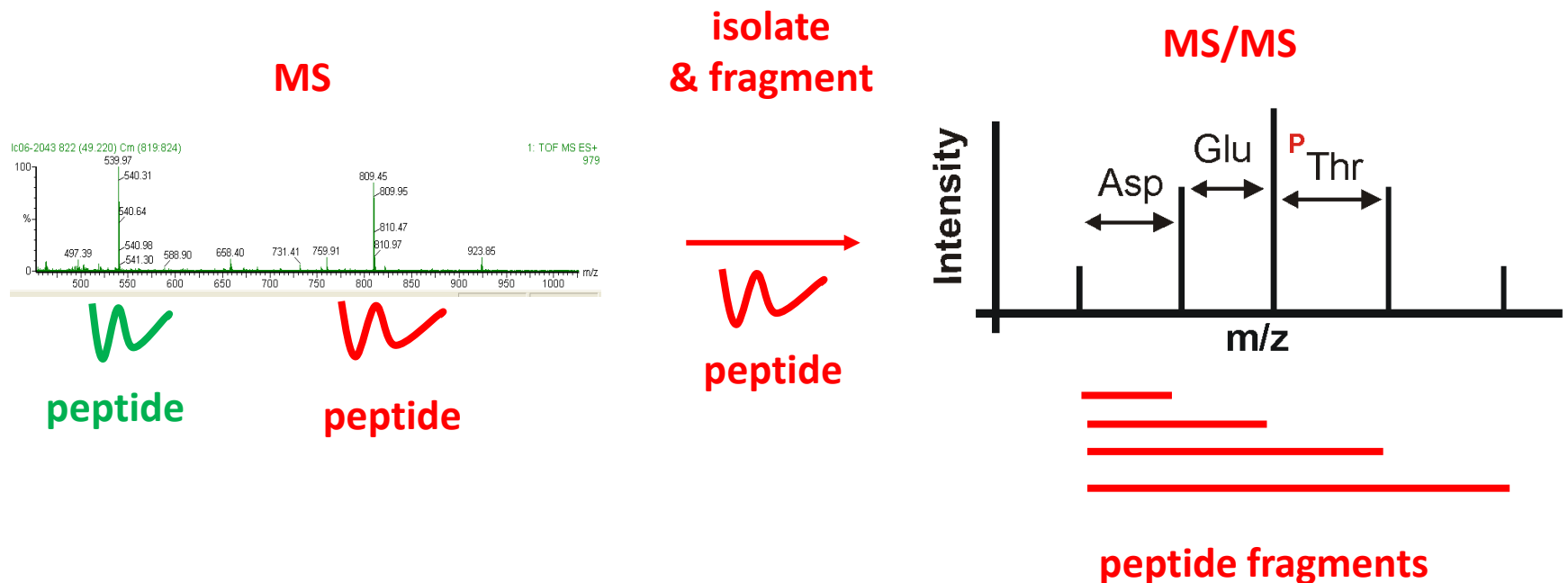
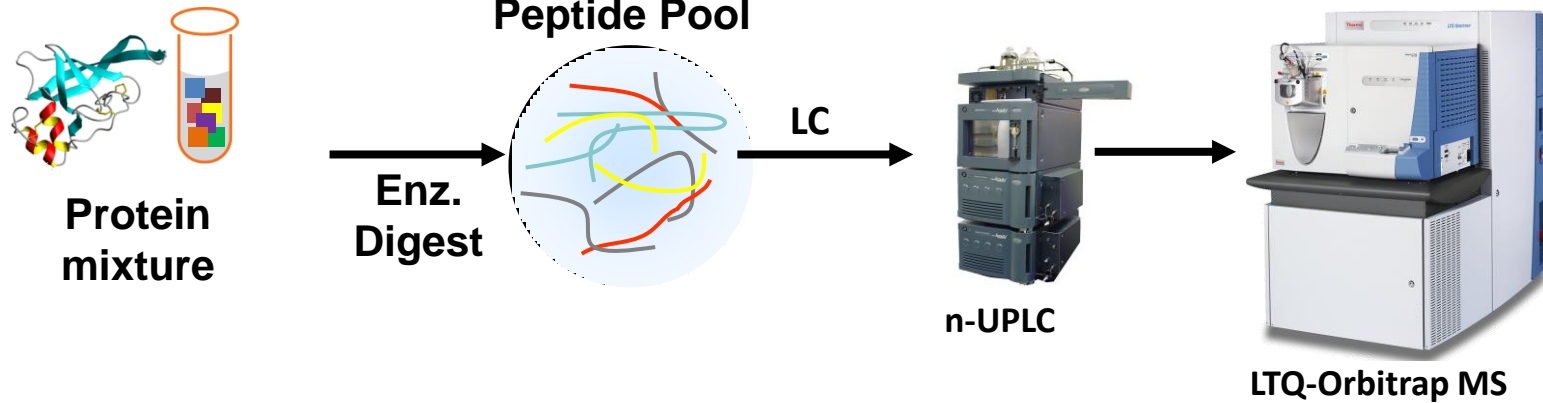


Database searching - at MS or MS/MS level



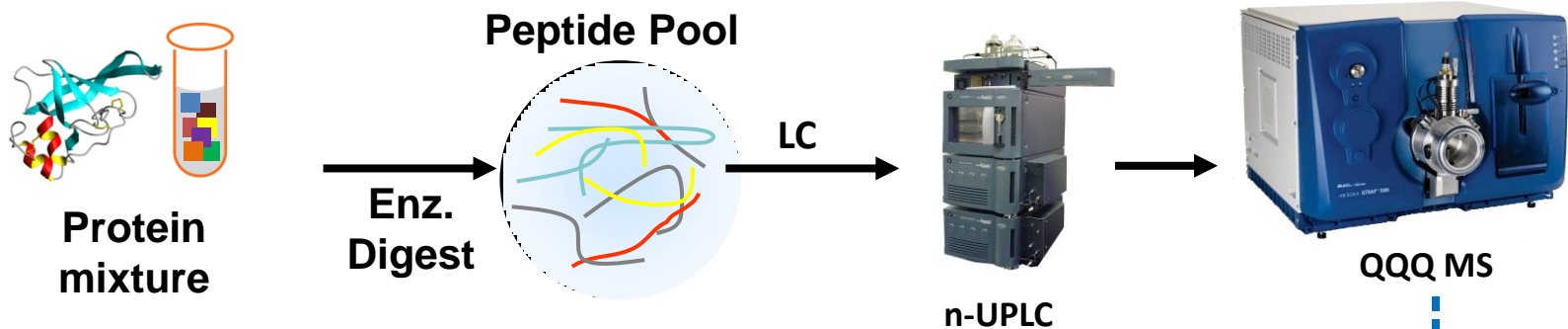
Computational Steps: massive amounts of MS data are read & interpreted. Databases searched to match peptide sequences.

Typical work flow for LC-MS “shotgun proteomics”

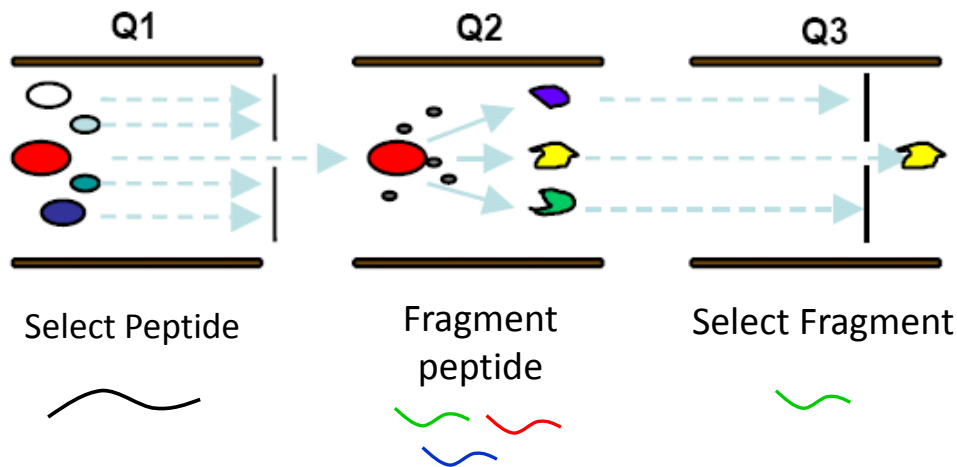


Typical work flow for LC-MRM

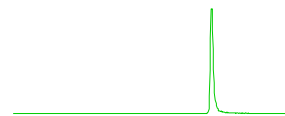
“targeted proteomics”



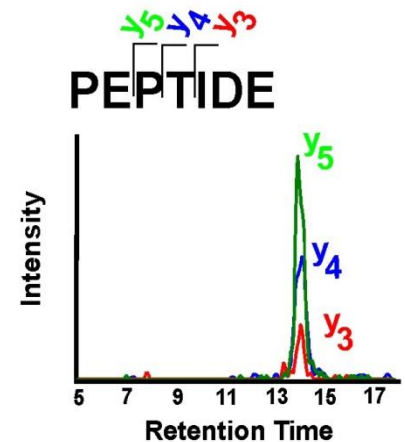
Multiple Reaction Monitoring (MRM)



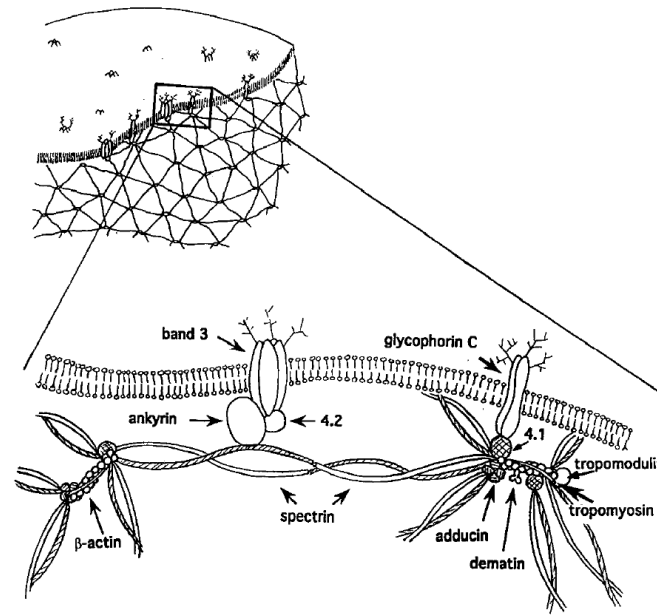
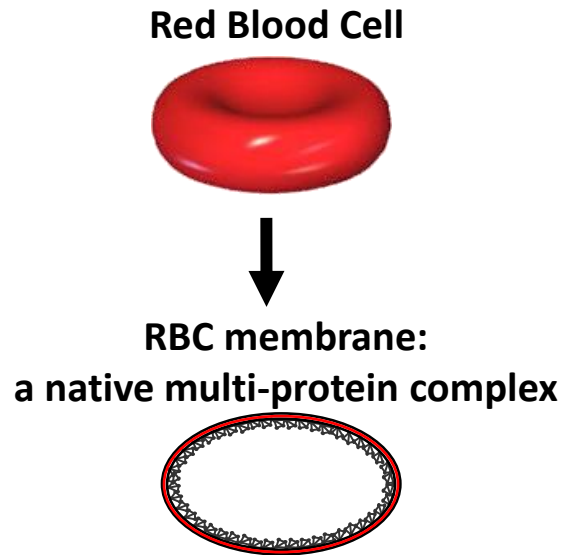
Detect Fragment



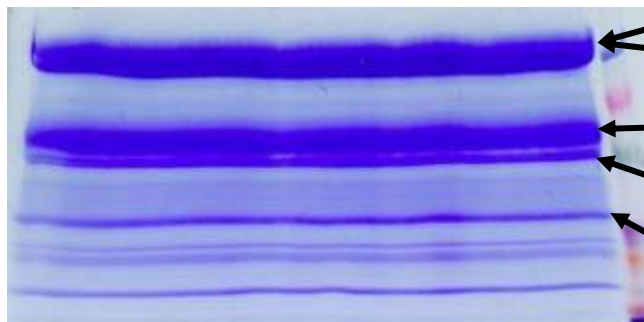
MRM Spectra



MS Data is not inherently quantitative, *but ...*



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



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

peptides (unique)

Spectrin α	352 (291)	Spectrin alpha chain, erythrocyte OS=Homo sapiens GN=SPTA1 PE=1 SV=5
Spectrin β	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
Band 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
Band 4.1	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
β -actin	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

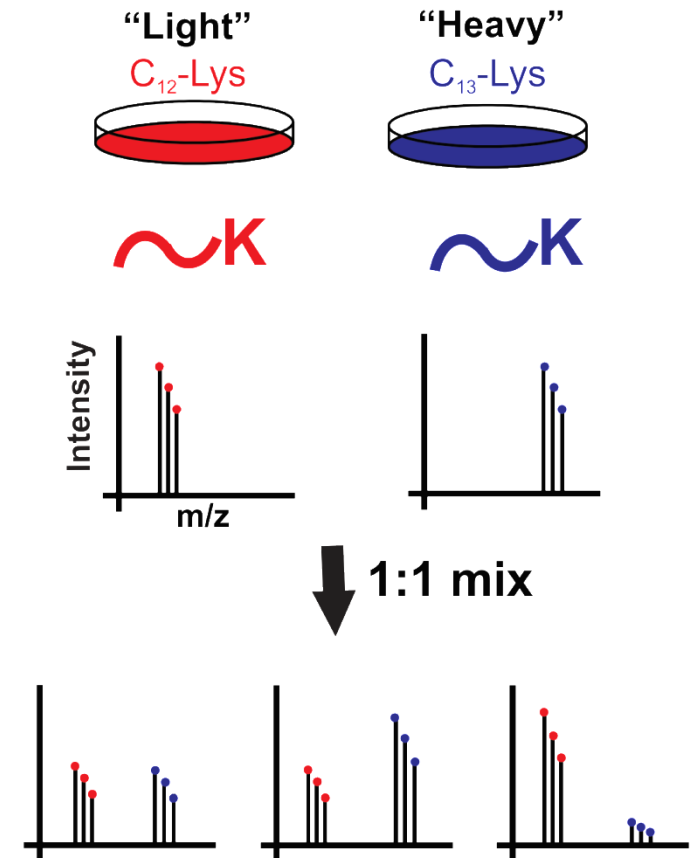
Rinehart et al., *unpublished*

Quantitative Proteomics

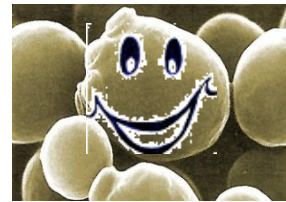
S.I.L.A.C. - Stable isotope labeling with amino acids in cell culture

-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

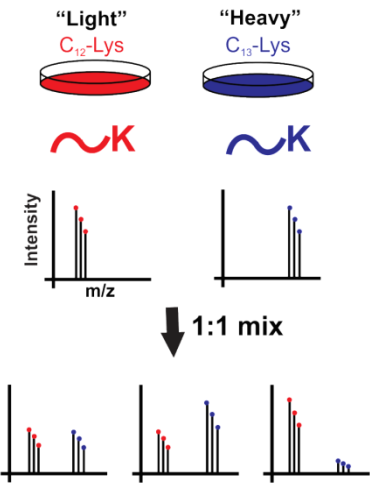
Picotti P, et al. Full dynamic range proteome analysis of *S. cerevisiae* by targeted proteomics. *Cell*.

➡ **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.



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

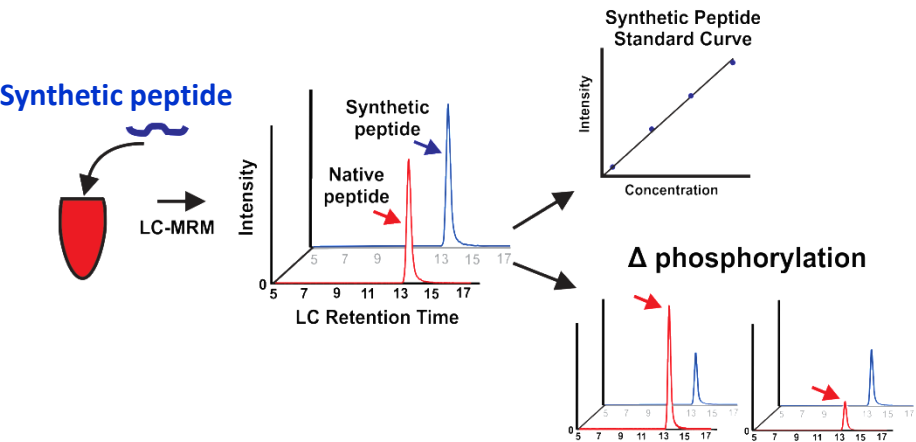
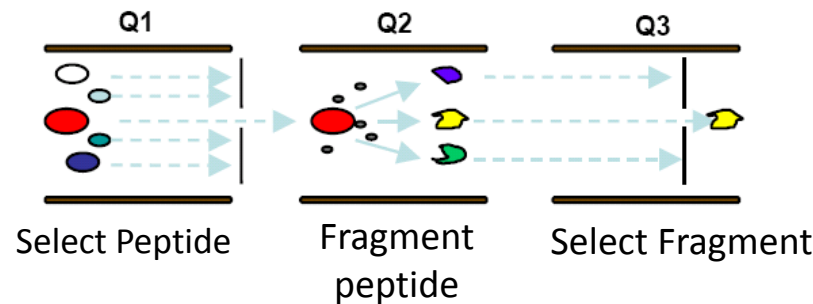
-Ong SE et al. *Molecular & Cell Proteomics* 2002.

2009

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

➡ Towards proteome wide targeted proteomics.

Multiple Reaction Monitoring (MRM)



2008

de Godoy LM, et al. Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast. *Nature*. 30;455(7217):1251-4.

➡ SILAC based quantitation of an entire proteome.

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

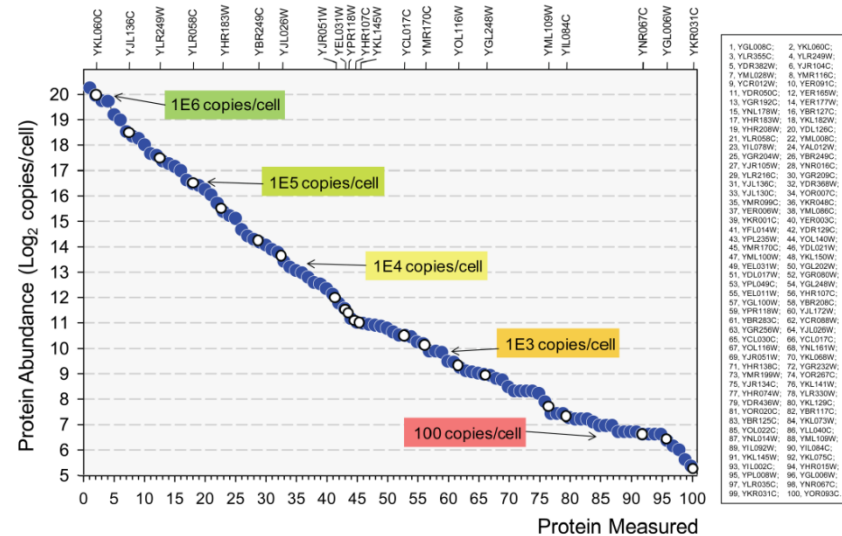
	Number of ORFs	TAP	GFP	nanoLC-MS
Total yeast ORFs	6,608	4,251	4,154	4,399
Characterized yeast ORFs	4,666	3,629	3,581	3,824
Uncharacterized yeast ORFs	1,128	581	539	572
Dubious yeast ORFs	814	26 (3%)	23 (3%)	3 (<1%)
Not present in ORF database		15	11	0

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.

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.

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

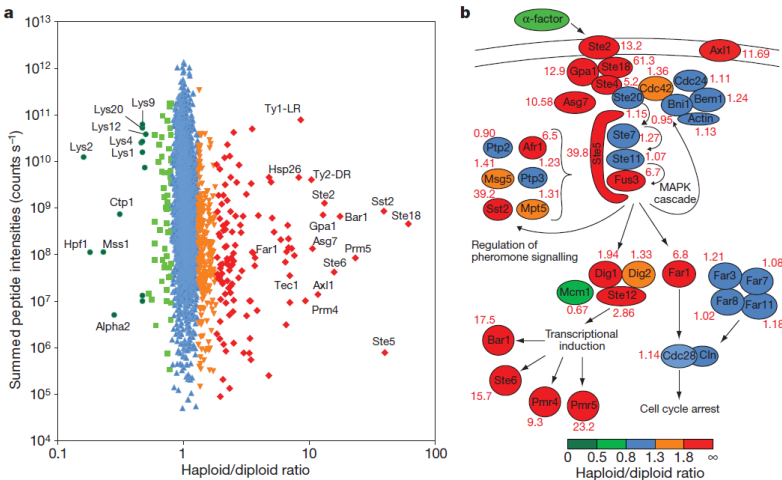


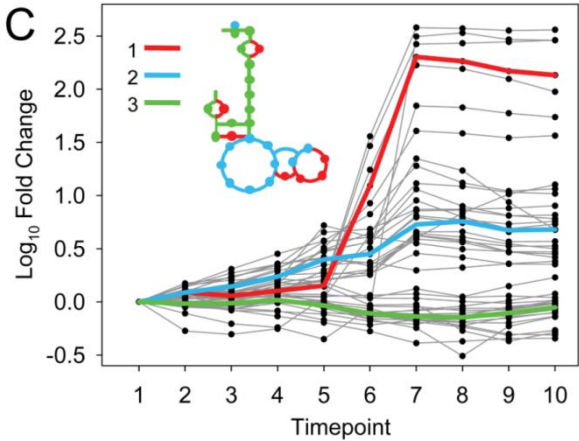
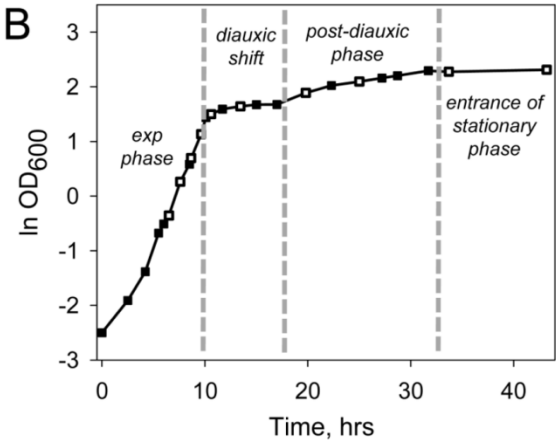
Figure 3 | Quantitative differences between the haploid and diploid yeast proteome. a, Overall fold change for the yeast proteome. b, Members of the yeast pheromone response are colour-coded according to fold change. The diploid to haploid ratio as determined by SILAC is indicated for each protein. Figure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

2009

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

➡ Towards proteome wide targeted proteomics.

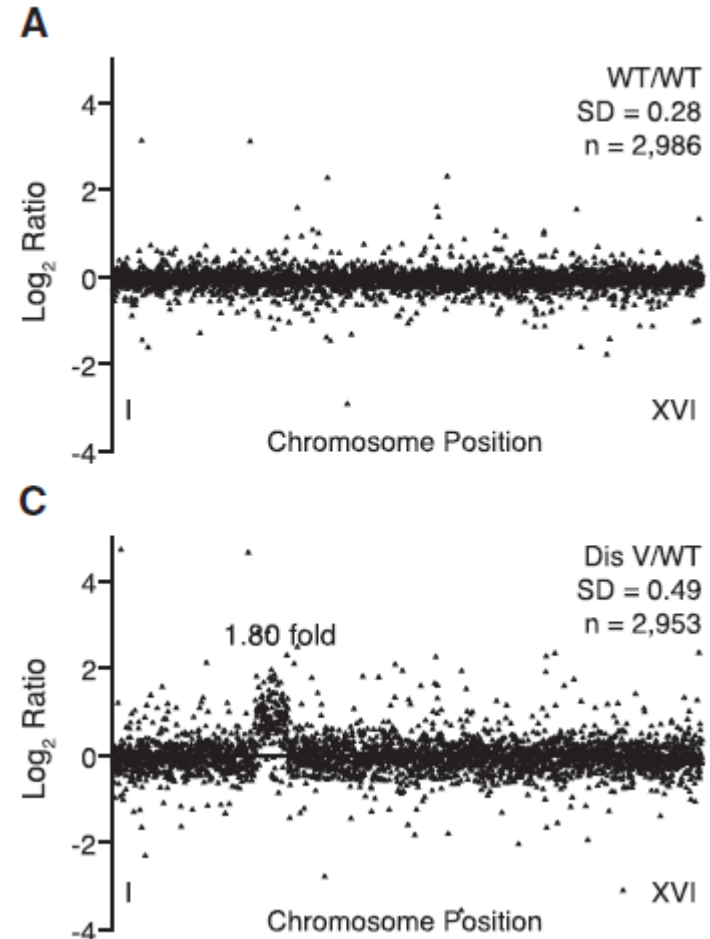
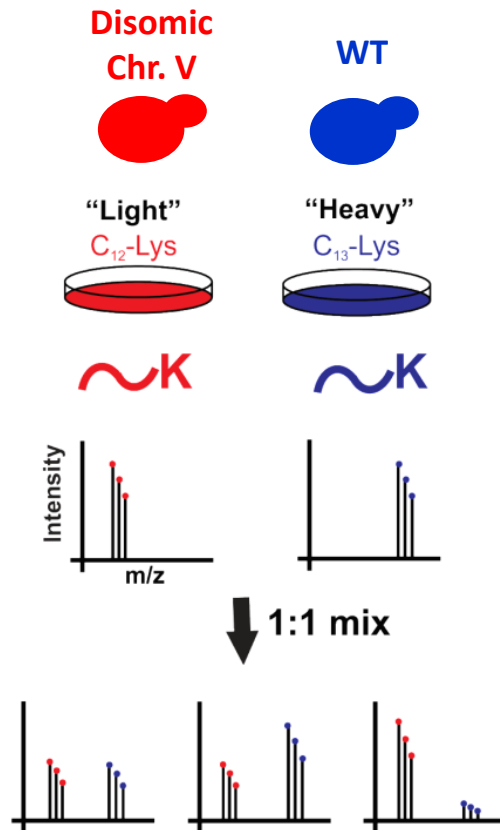
Network expression dynamics



Identification of Aneuploidy-Tolerating Mutations

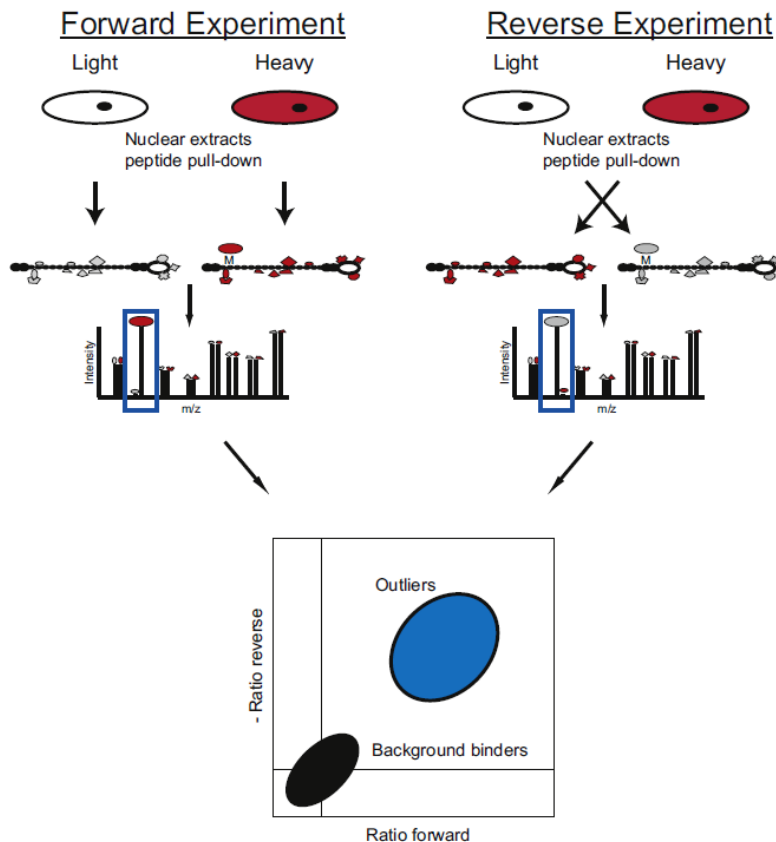
Cell 143, 71–83, October 1, 2010

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,*}

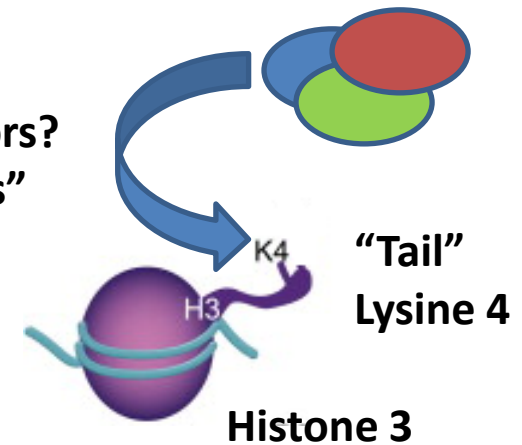


Quantitative Interaction Proteomics and Genome-wide Profiling of Epigenetic Histone Marks and Their Readers

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,*}

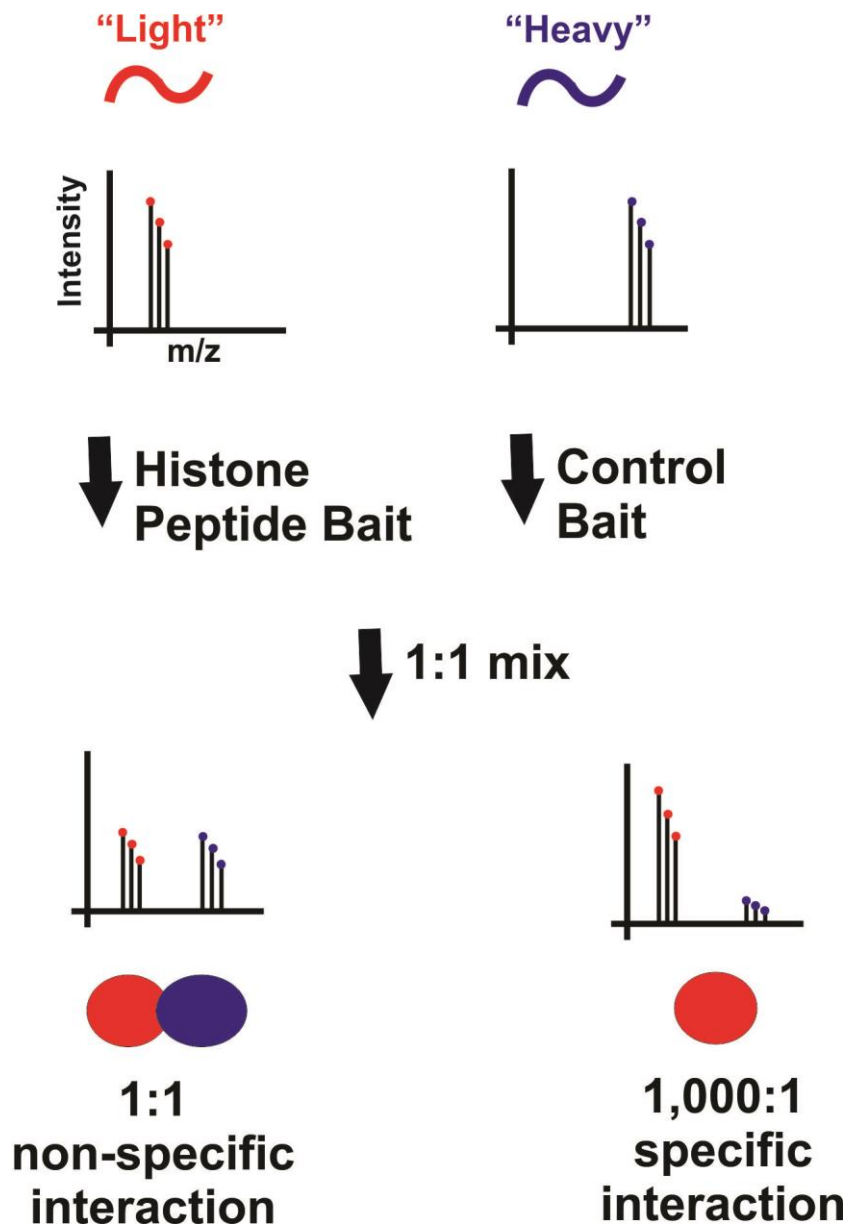


Protein
Regulators?
“Readers”

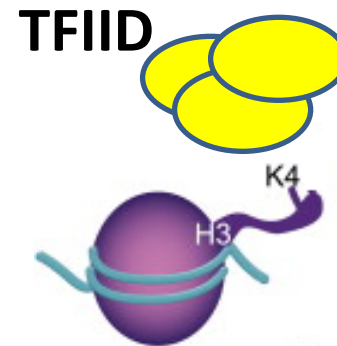
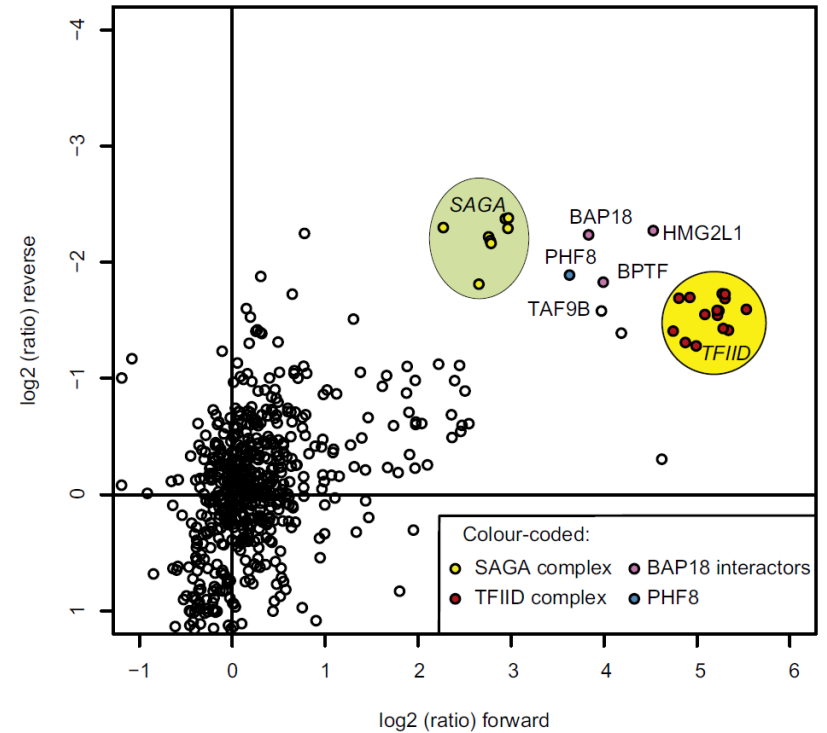


Vermeulen et al., **Cell** 2010

The major lysine methylation sites on the N terminus of histone H3 and histone H4 with a clearly defined biological function are H3K4me3, H3K9me3, H3K27me3, H3K36me3, and H4K20me3, which are associated with different functional states of chromatin. **H3K4me3** is almost exclusively found on promoter regions of **actively transcribed genes** while **H3K36me3** is linked to **transcription elongation**. **H3K9me3**, **H3K27me3**, and **H4K20me3** are generally found on **silent heterochromatic regions** of the genome. Part of the functional distinction between these methylation sites relates to the proteins interacting with them. A number of these “chromatin readers” for various histone methyl lysine sites have already been identified and characterized (Kouzarides, 2007; Shilatifard, 2006; Taverna et al., 2007),

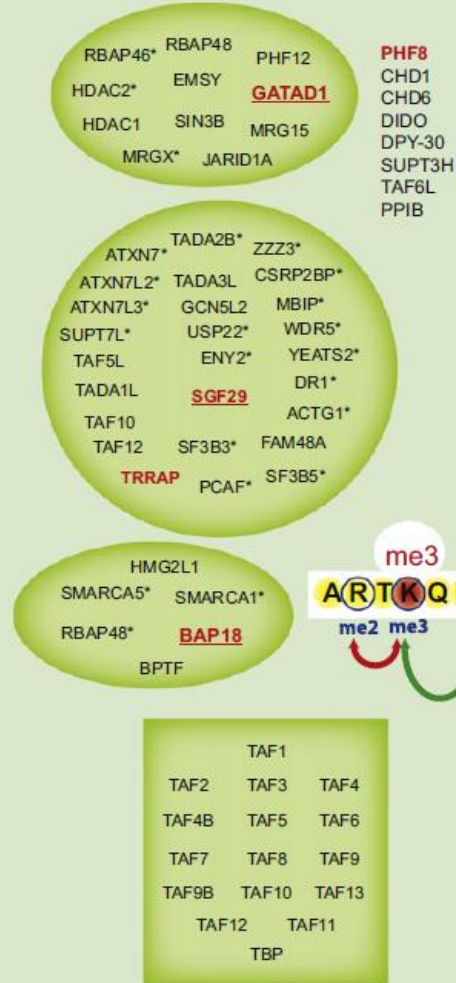


H3K4me3 interactors

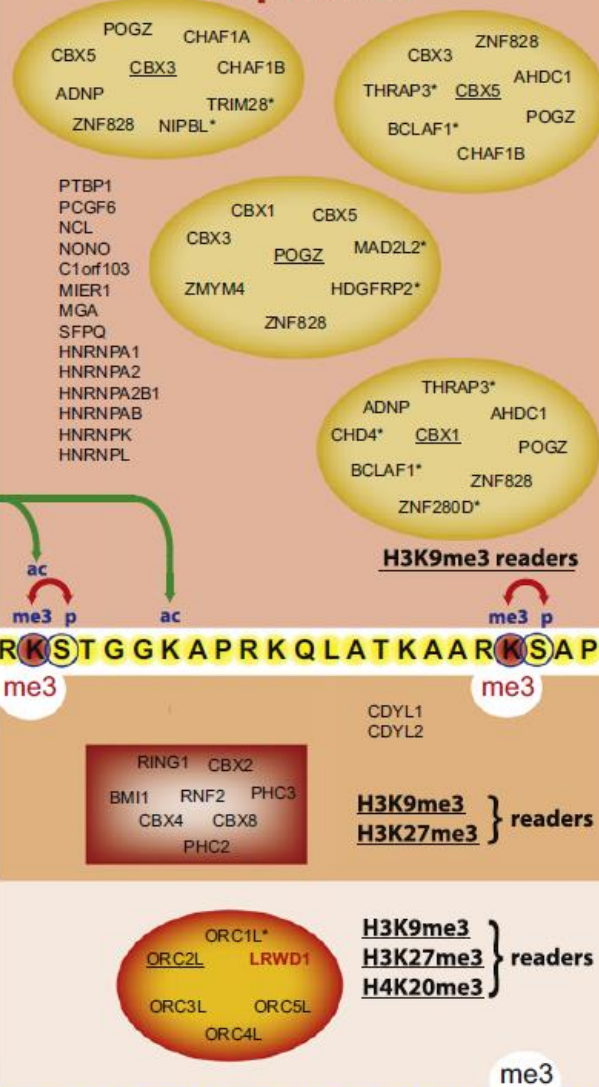


Active Genes

Activation



Repression



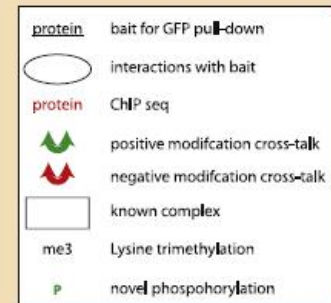
Elongation

PWWP domain:
N-PAC
NSD2

(NSD1)
(NSD3)
(MSH6)

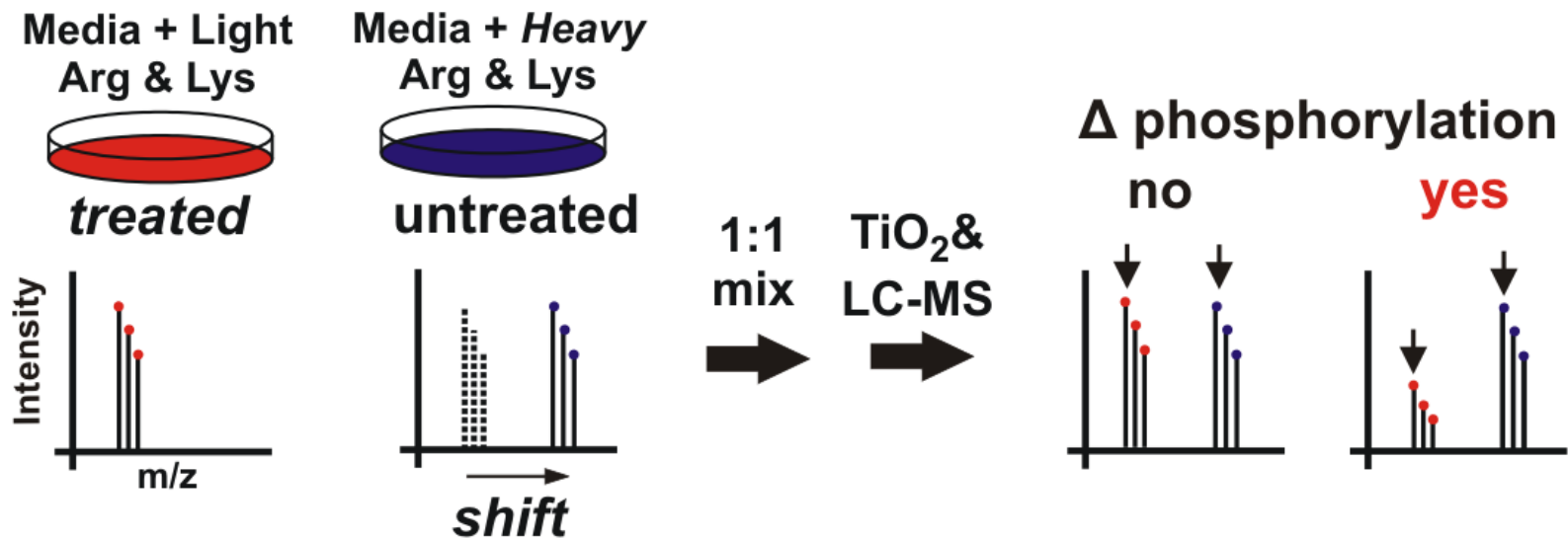
H3K36me3 readers

Histone H3

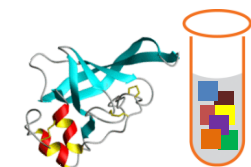


Histone H4

A SILAC approach to study protein phosphorylation dynamics



Major technological advances in mass spectrometers and phosphopeptide enrichment



Protein mixture



Digest



Peptides

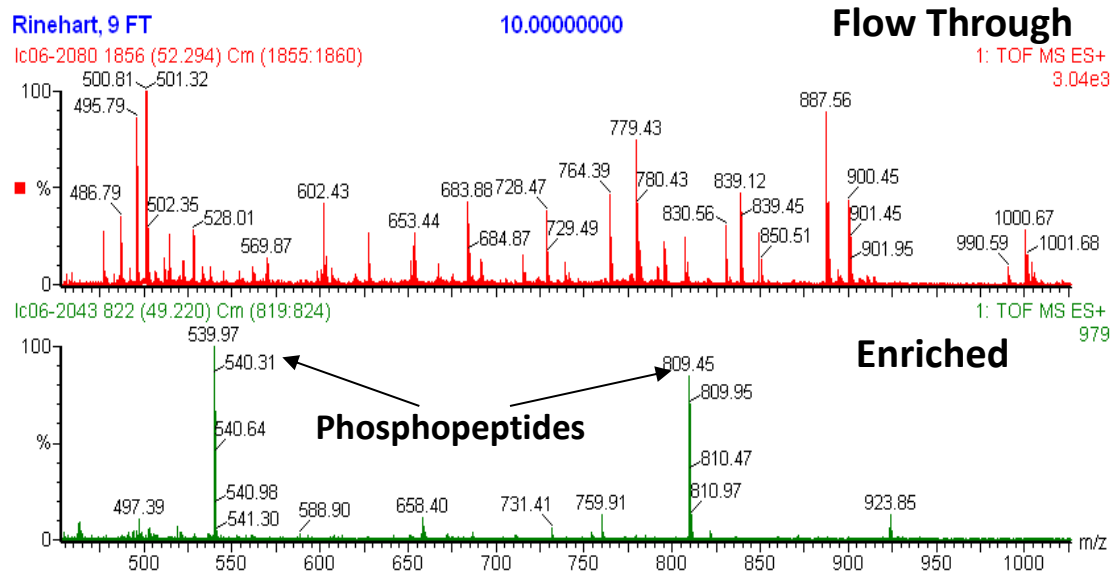
TiO₂ Enrich



Phosphopeptides

MS

TiO₂ Enrichment

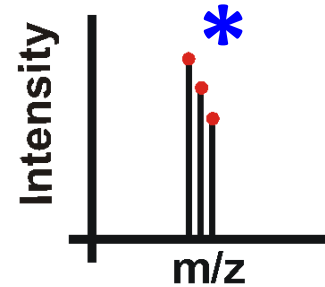


*Phosphopeptide signatures in MS

Phosphopeptide

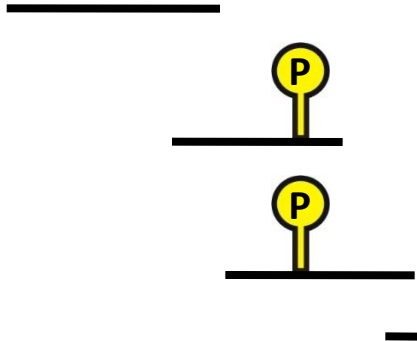


MS

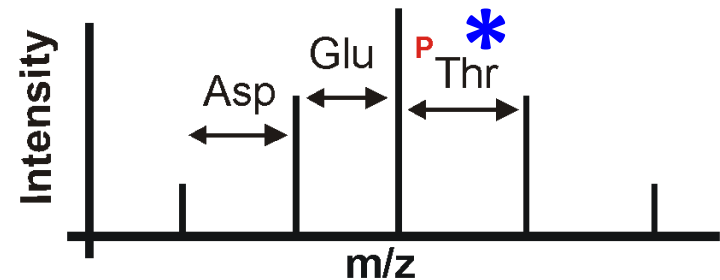


+80 Da
in precursor

isolate
& fragment



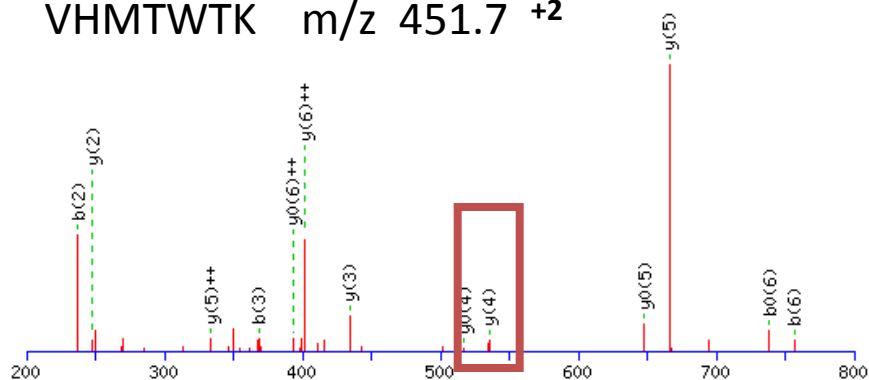
MS/MS



-98 Da loss of phosphoric acid H_3PO_4
during fragmentation

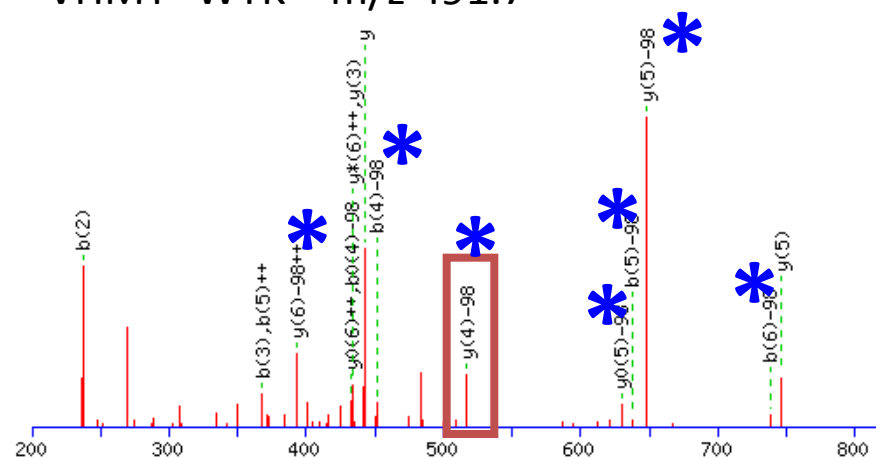
V
H 803.3869
M 666.3280
T 535.2875
W 434.2398
T 248.1605
K 147.1128

VHMTWTK m/z 451.7 ⁺²



V
H 785.3763
M 648.3174
-18 T 517.2769
W 434.2398
T 248.1605
K 147.1128

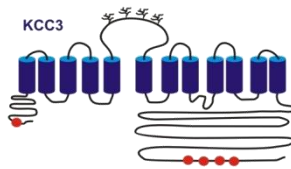
VHMT^PWTK m/z 491.7 ⁺²



P +80 Da
in precursor

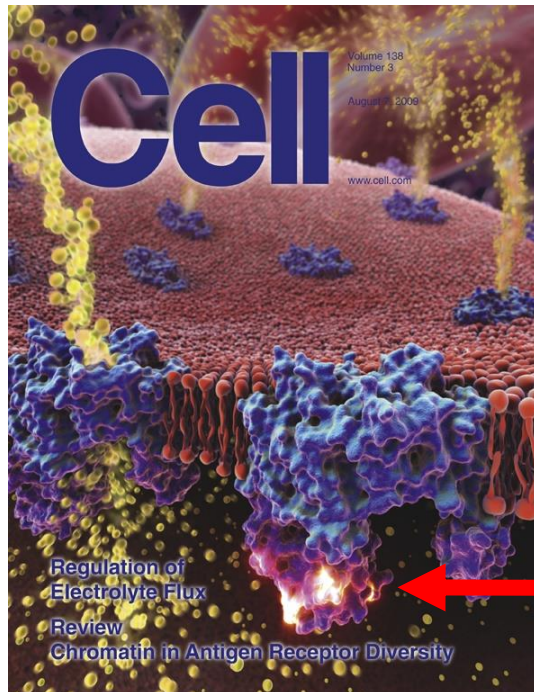
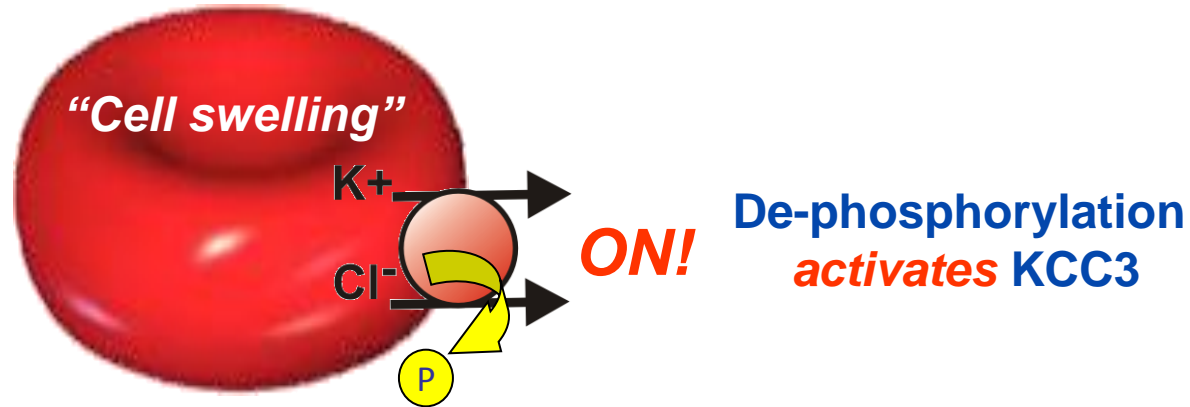
***** - H₃PO₄, 98 Da

(Threonine changes to 2-aminodehydrobutyric acid, -18 Da)

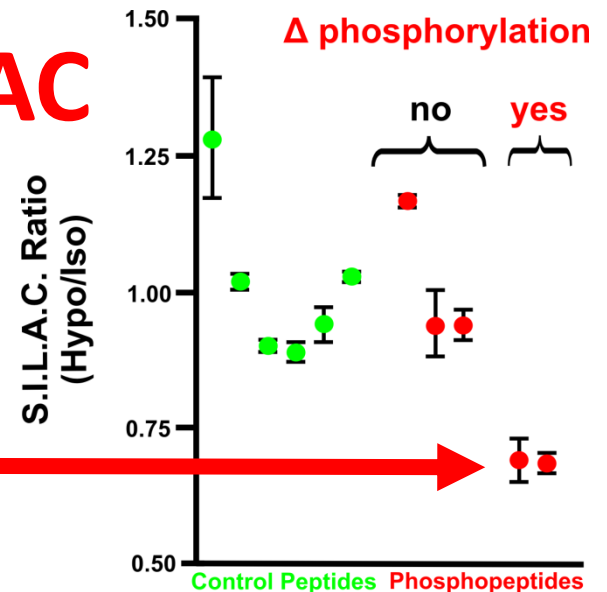


Sites of Regulated Phosphorylation that Control K-Cl Cotransporter Activity

Jesse Rinehart,^{1,5} Yelena D. Maksimova,² Jessica E. Tanis,³ Kathryn L. Stone,^{5,6} Caleb A. Hodson,¹ Junhui Zhang,¹ Mary Risinger,⁷ Weijun Pan,⁴ Dianqing Wu,⁴ Christopher M. Colangelo,^{5,6} Biff Forbush,³ Clinton H. Joiner,⁷ Erol E. Gulcicek,^{5,6} Patrick G. Gallagher,² and Richard P. Lifton^{1,5,*}



SILAC

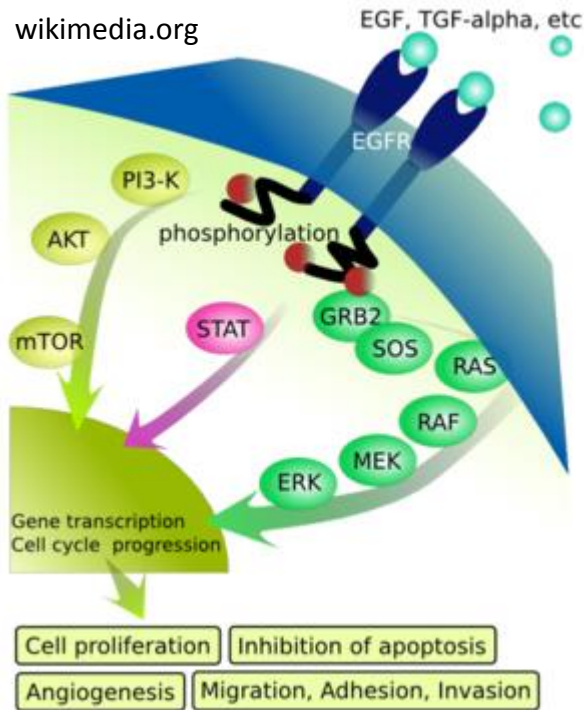


Adapted from Rinehart, et al. *Cell*, 2009

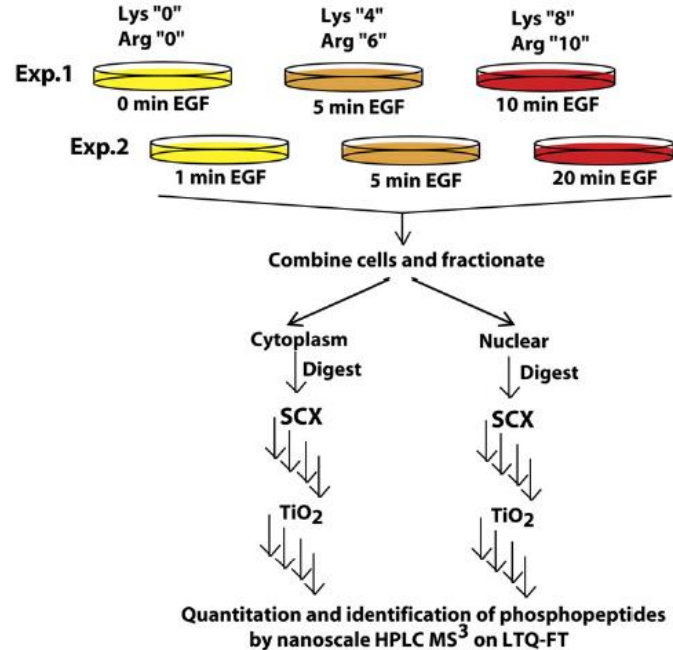
Quantitative Proteomics Reveals Dynamics in Signaling Networks

Phosphorylation dynamics after EGF stimulation

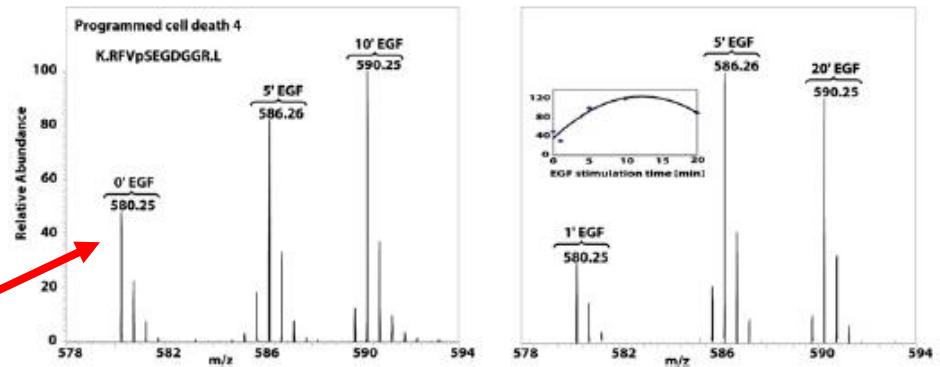
wikimedia.org



SILAC approach enables dynamic analysis

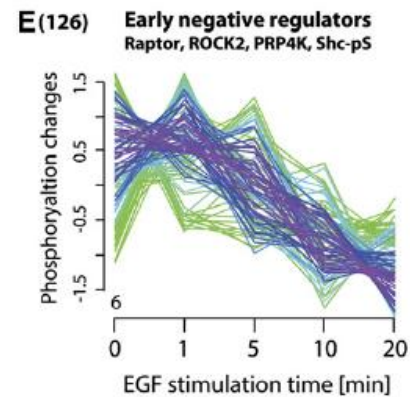
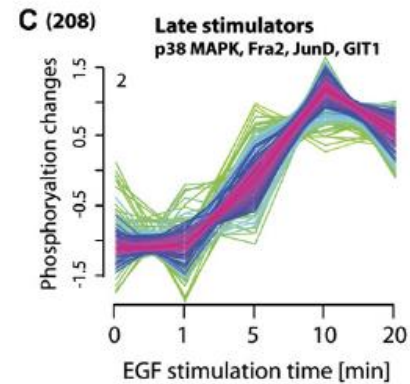
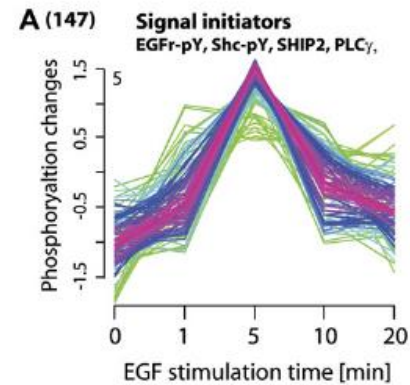
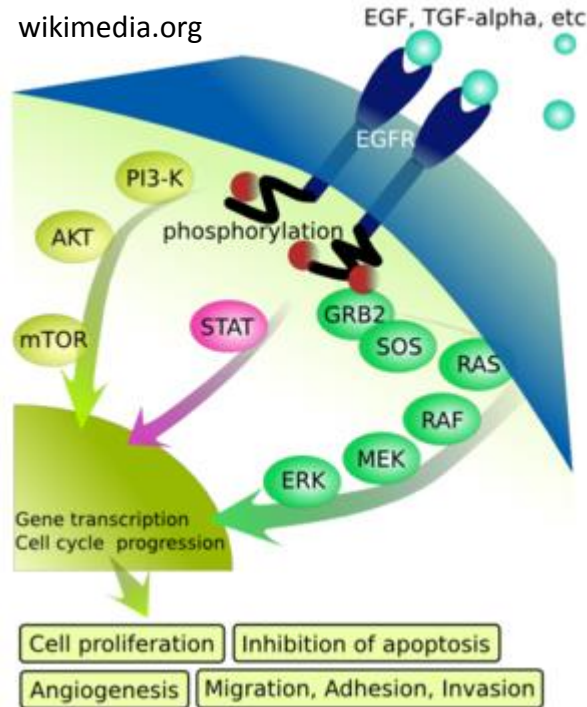


MS spectra triplets



Phosphorylation dynamics after EGF stimulation

wikimedia.org



Proteins: Proteomics & Protein-Protein Interactions

Overview

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