Introduction to Proteomics

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CCBB Short Course
April 1, 2020

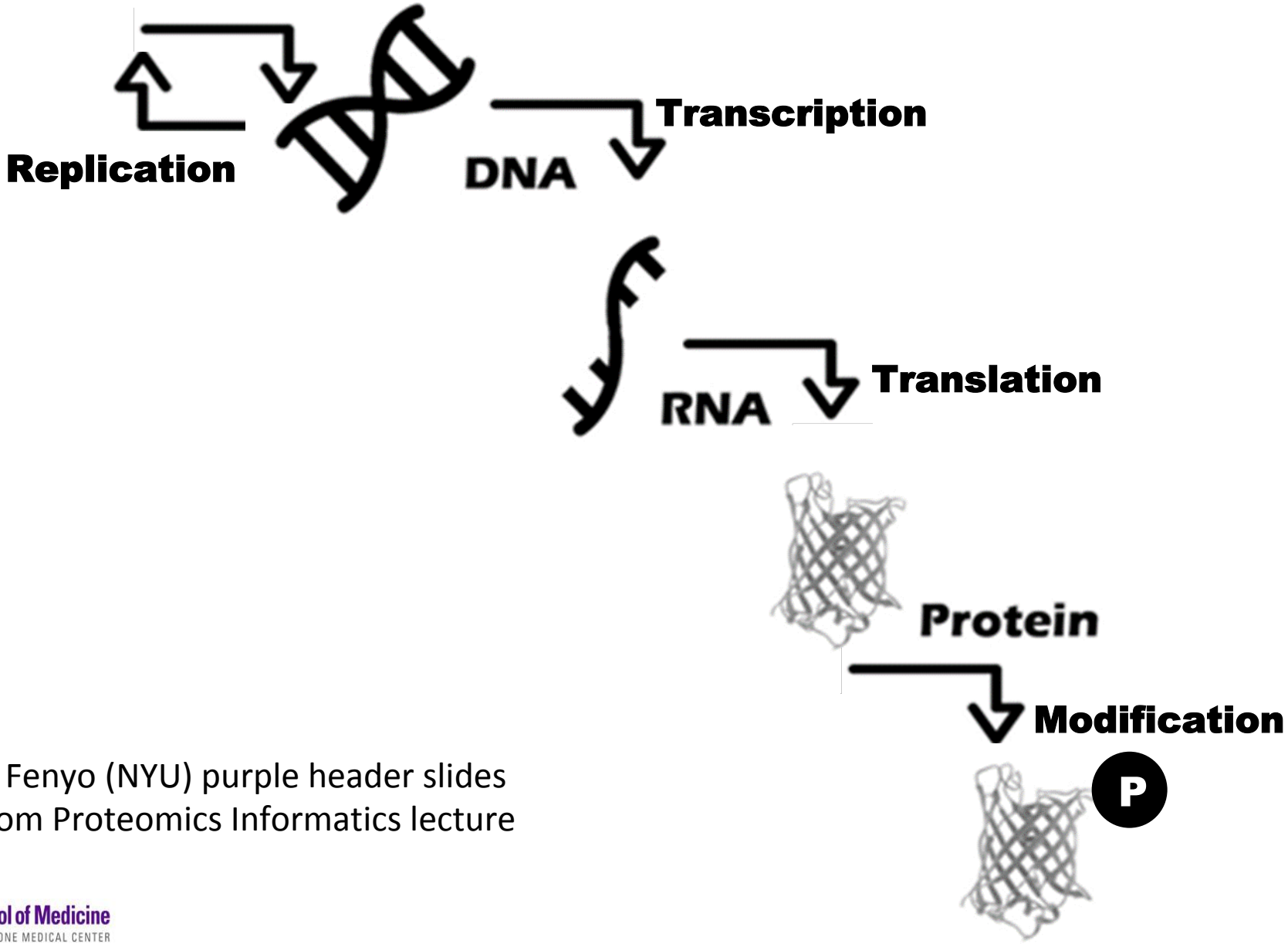
Intro to Proteomics Short Course

- 9 am: Lecture on proteomics
- 10 am: Break out groups design proteomics experiment and present to class
- 11 am: Protein identification tutorial using Scaffold Q/Q+S. Download free viewer at http://www.proteomesoftware.com/products/free-viewer/

Outline

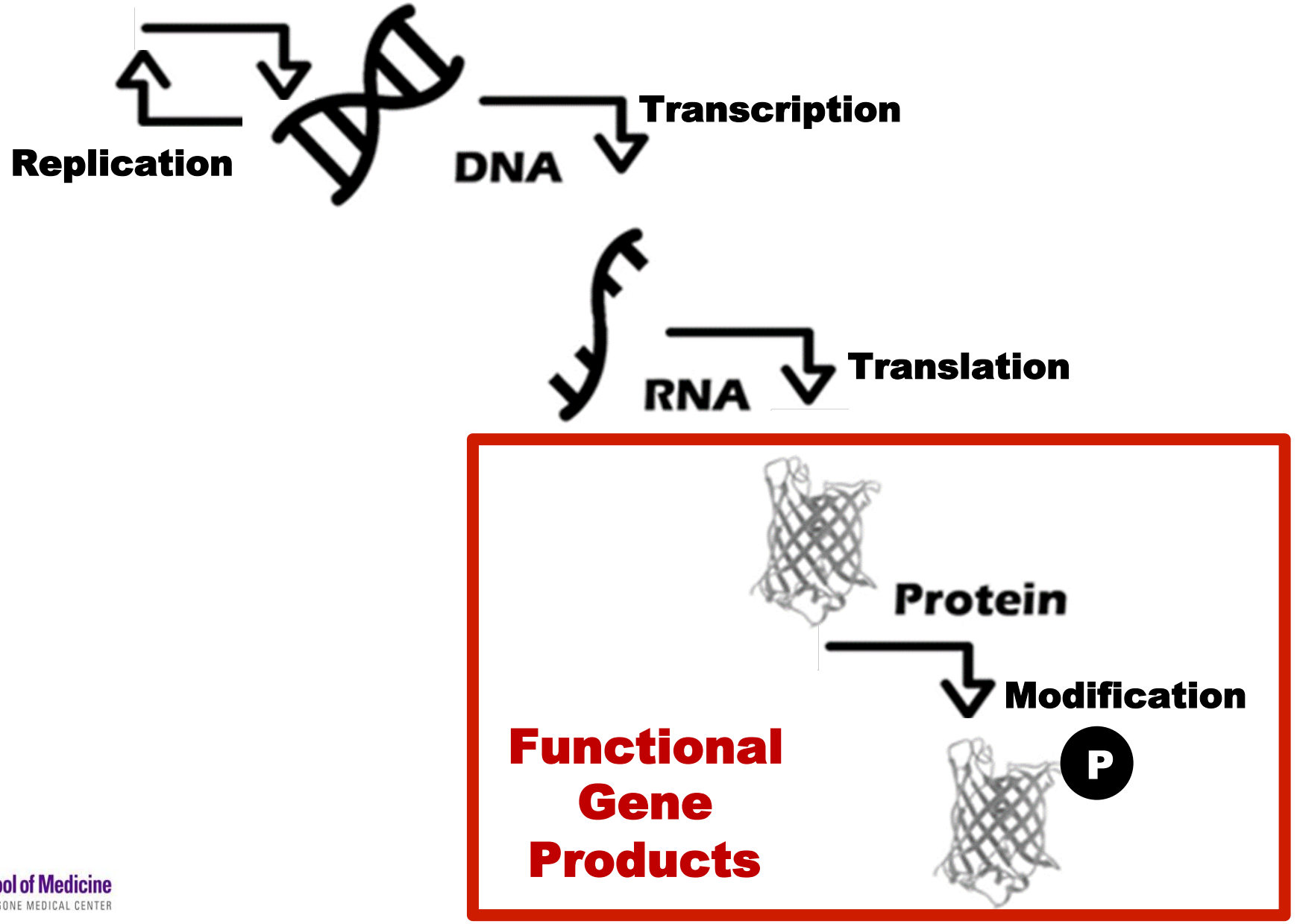
- DNA, RNA, and Protein Correlation
- Protein Identification
- Biological Mass Spectrometry Facility
- Molecular Weight Determination
- Protein-Protein Interactions
- Quantitative Proteomics
- Protein Arrays
- Post-translational Modifications
- Structural Proteomics
- Imaging Mass Spectrometry
- Cellular Location

Central Dogma of Molecular Biology

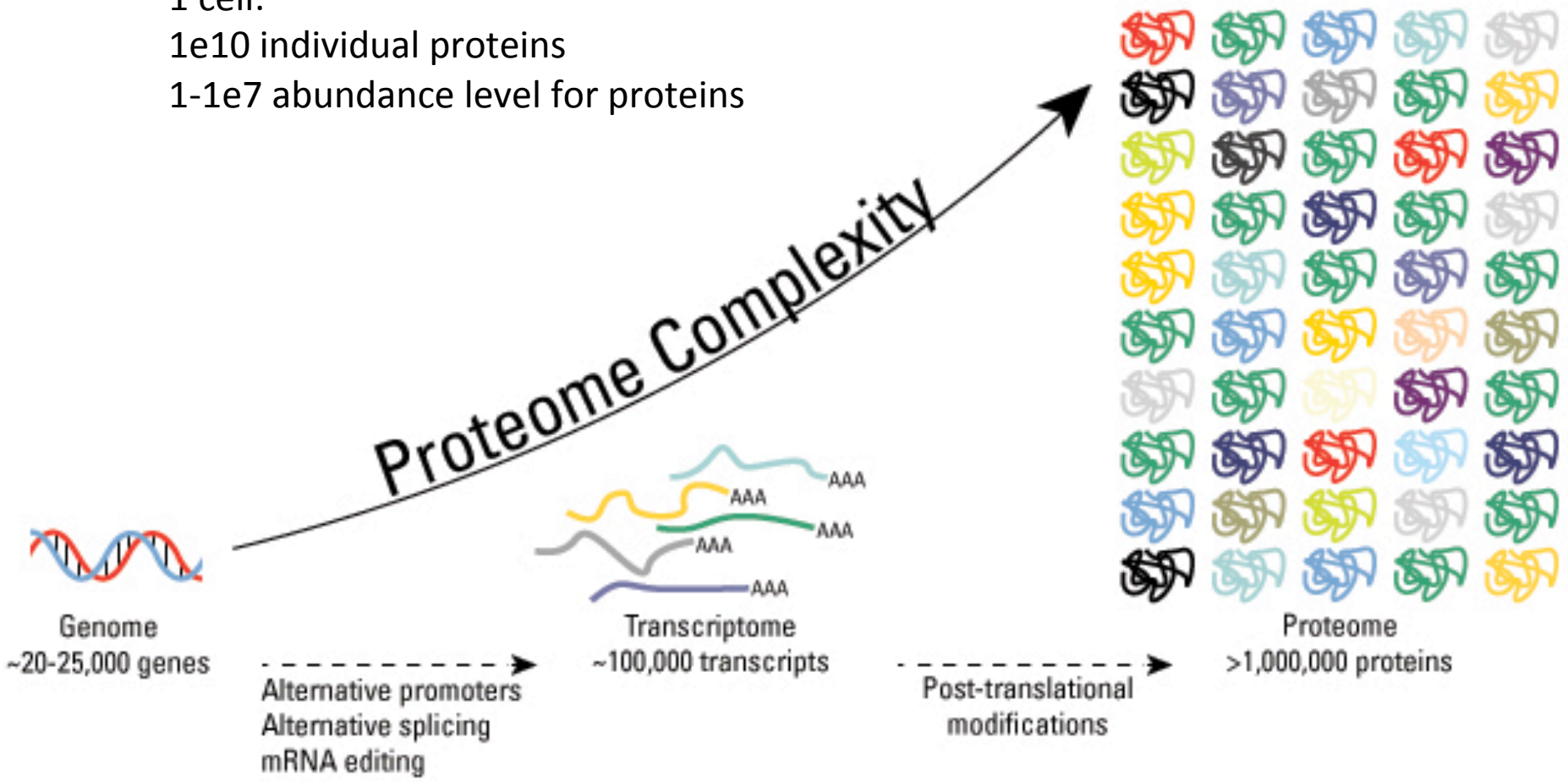


D. Fenyo (NYU) purple header slides
From Proteomics Informatics lecture

Central Dogma of Molecular Biology

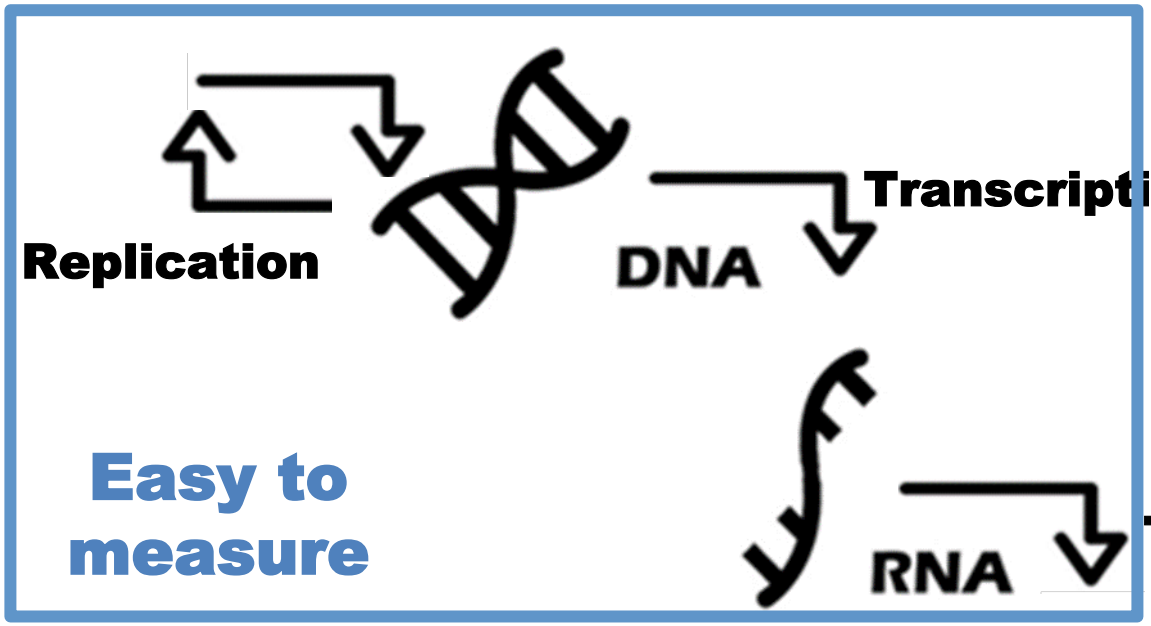


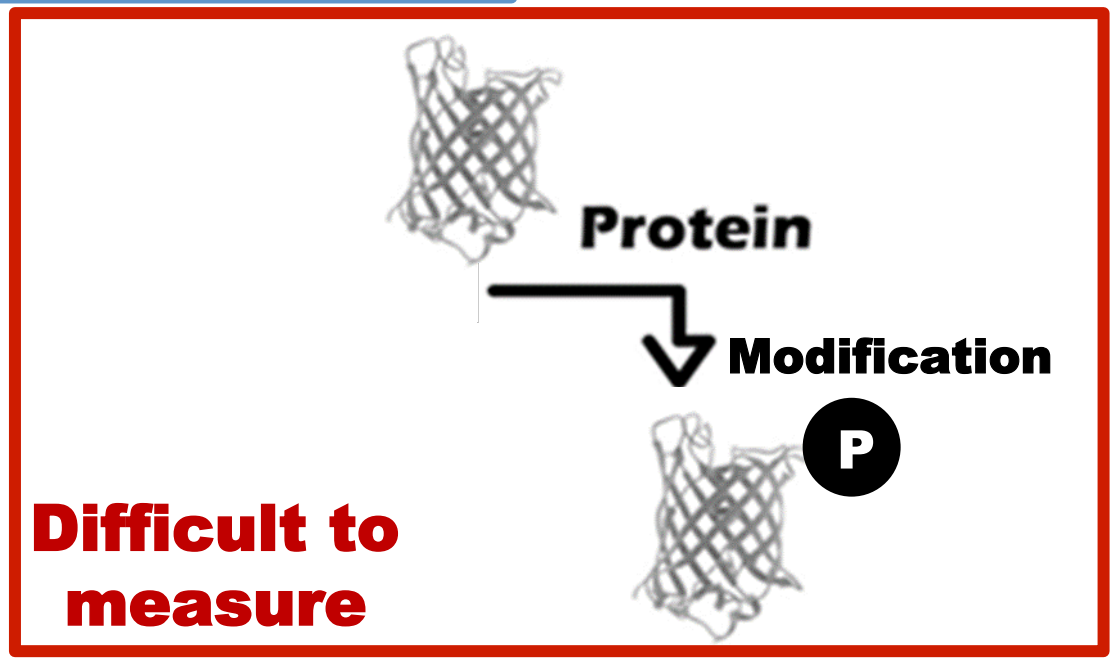
1 cell:
1e10 individual proteins
1-1e7 abundance level for proteins



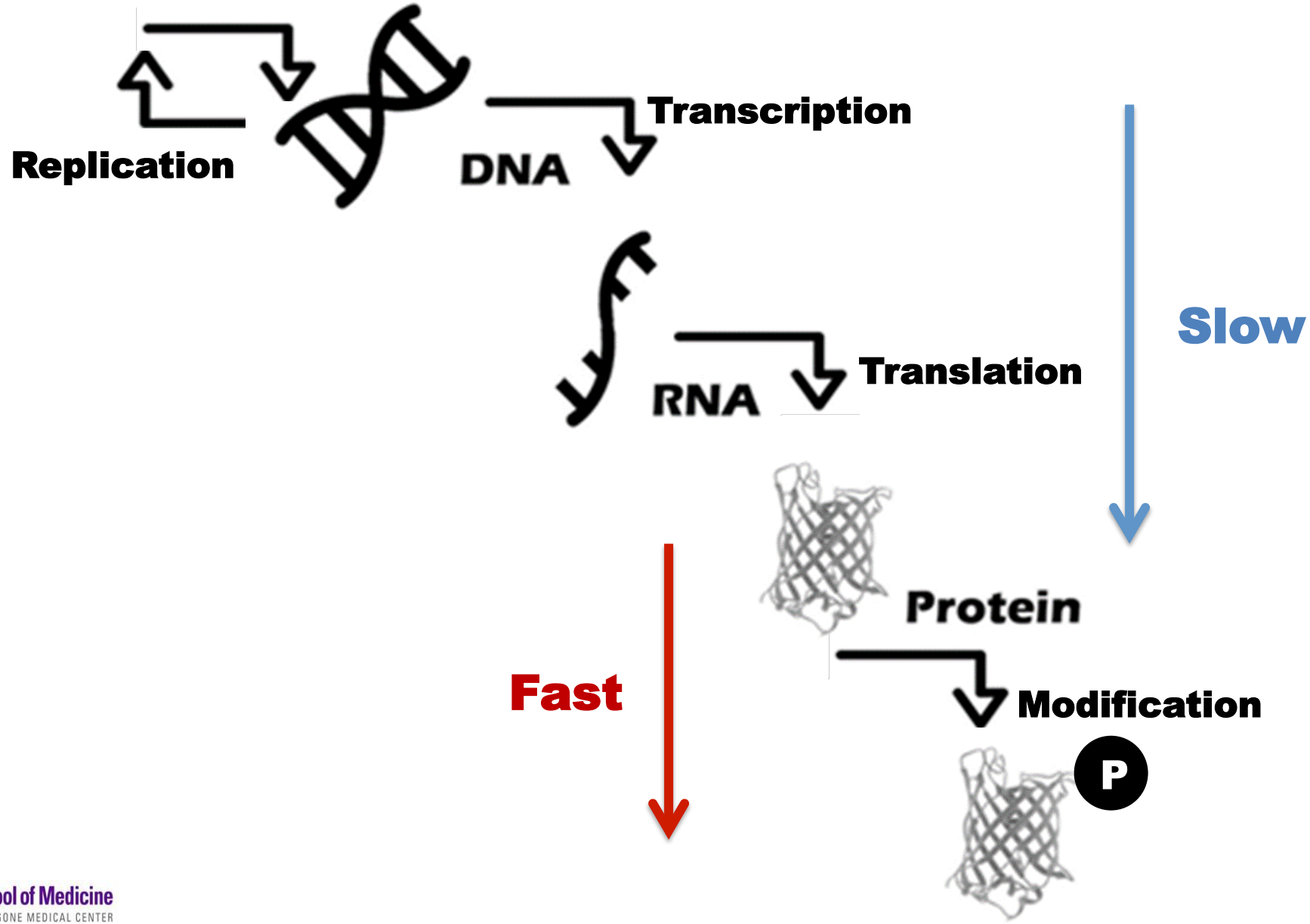
http://www.piercenet.com/browse.cfm?fldID=7CE3FCF5-0DA0-4378-A513-2E35E5E3B49B

Central Dogma of Molecular Biology

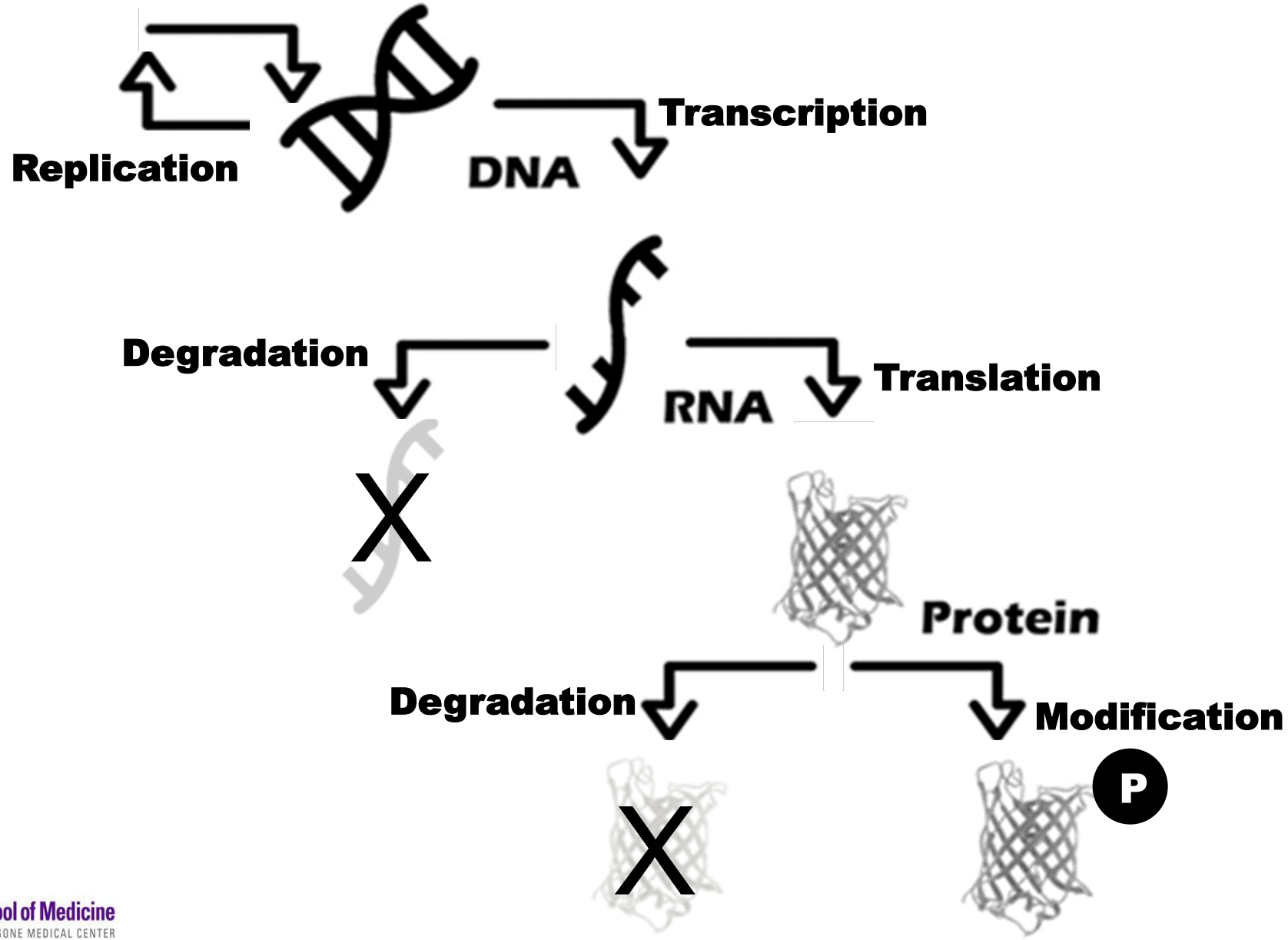




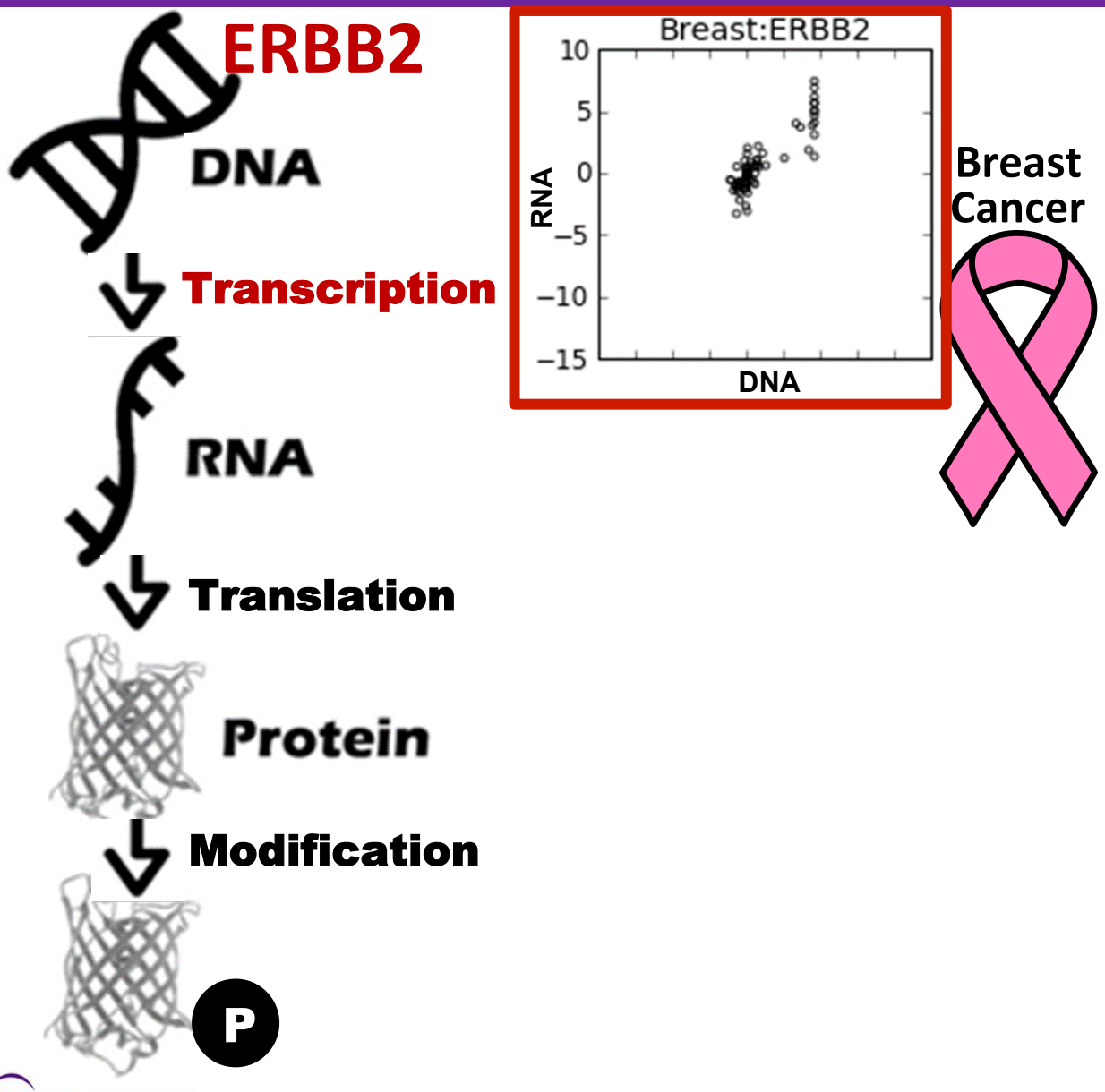
Central Dogma of Molecular Biology



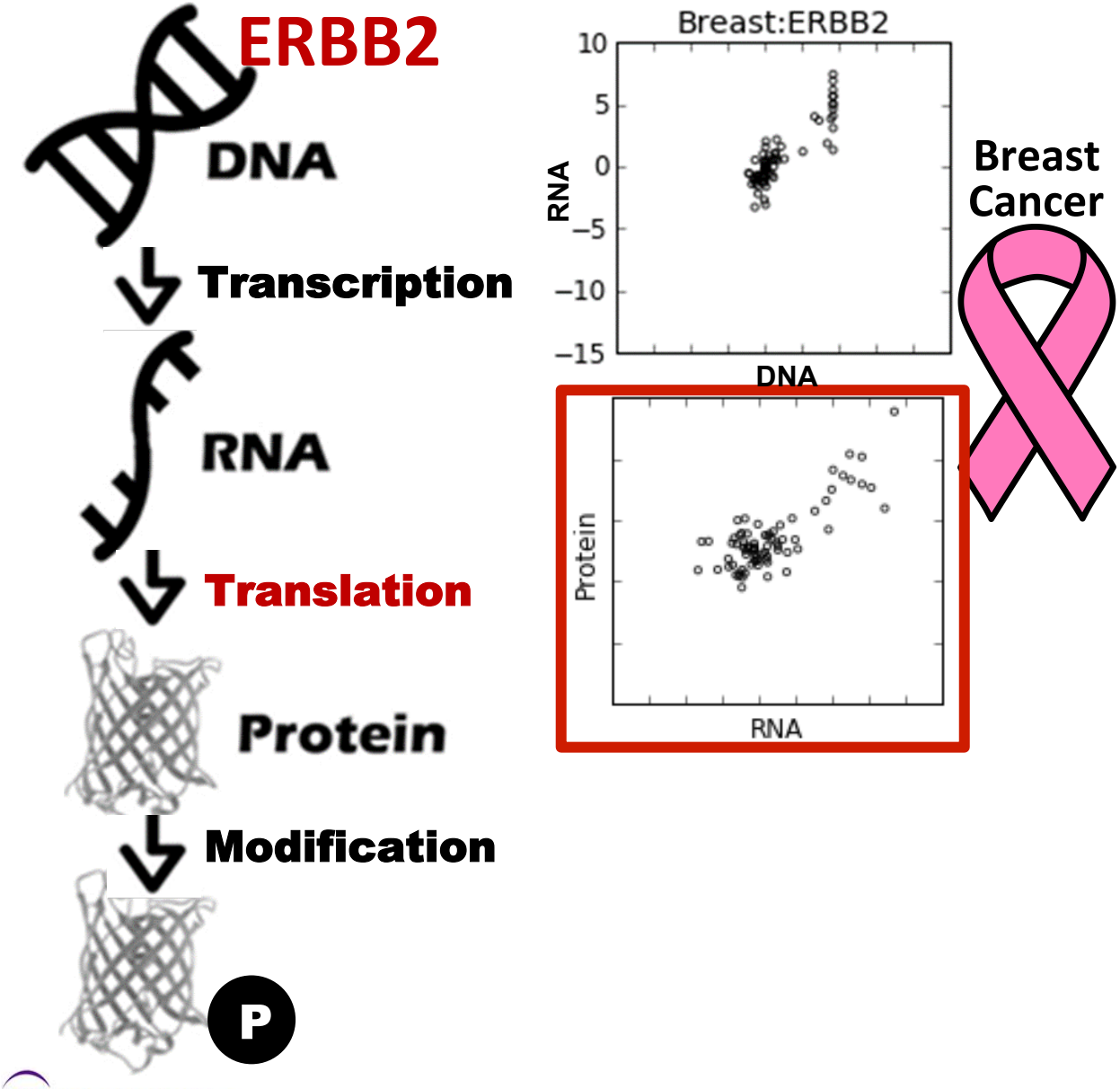
Central Dogma of Molecular Biology



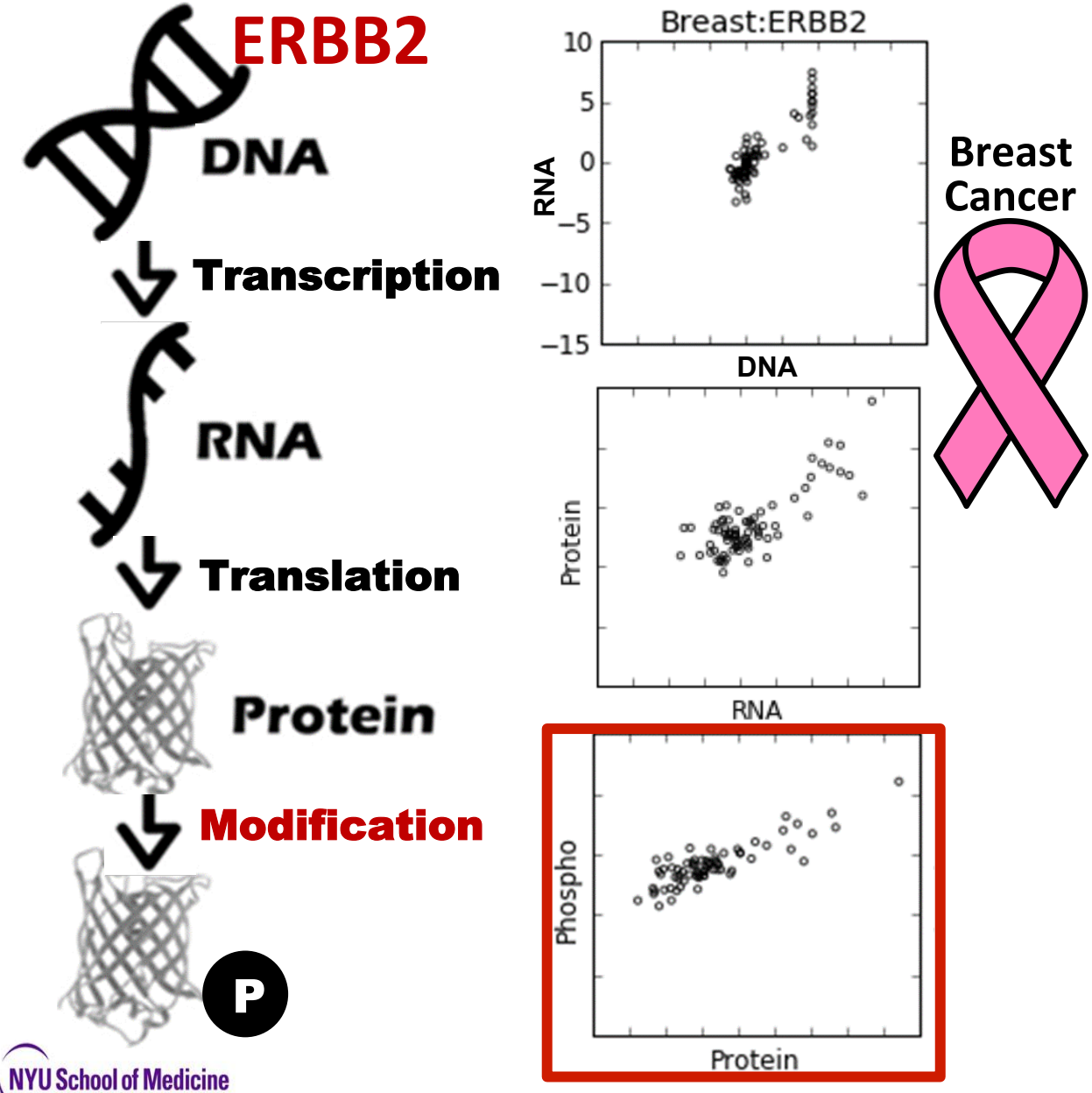
Central Dogma of Molecular Biology



Central Dogma of Molecular Biology

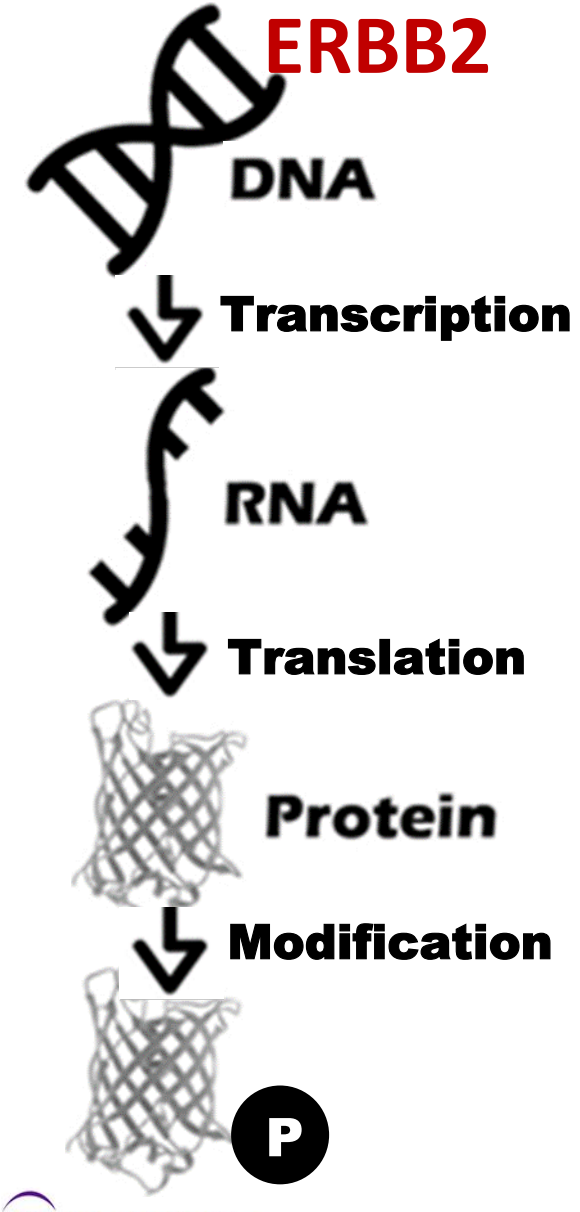


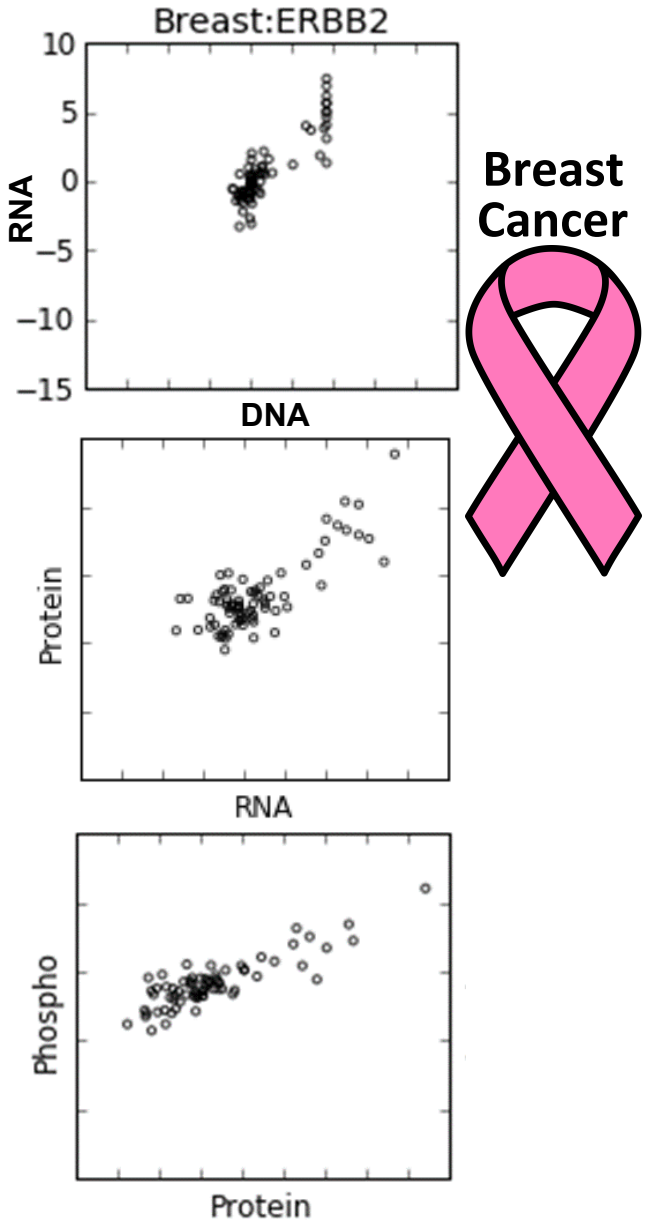
Central Dogma of Molecular Biology

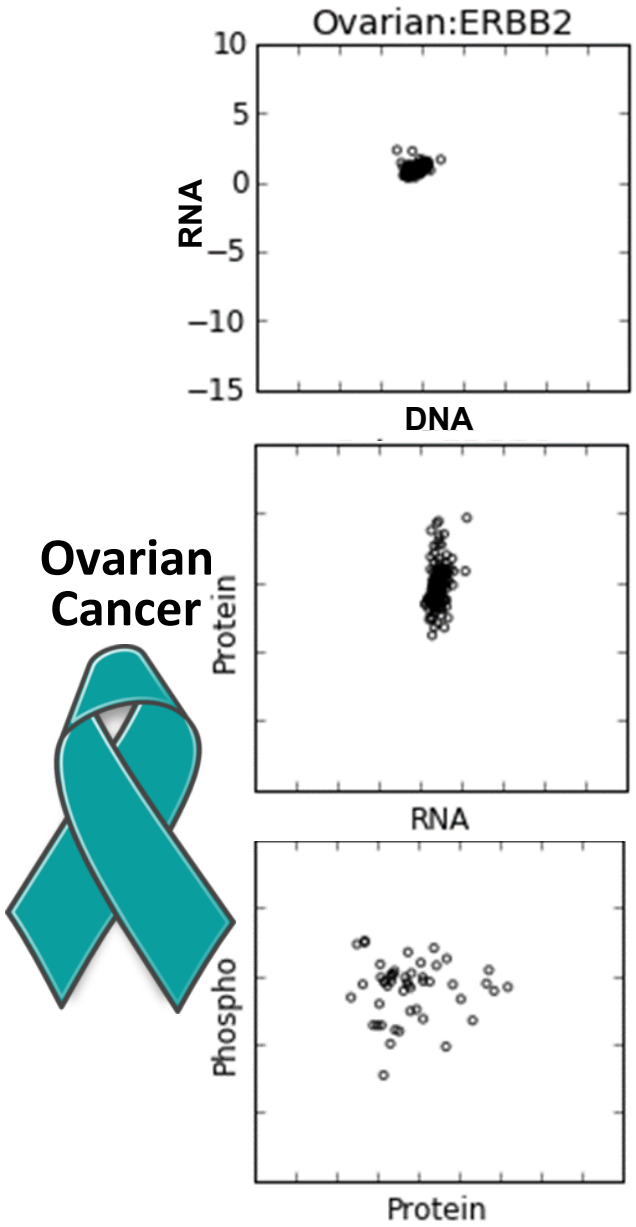


NYU School of Medicine
NYU LANGONE MEDICAL CENTER

Central Dogma of Molecular Biology







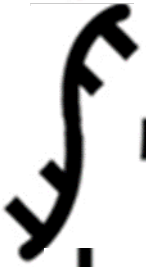
Correlation coefficient intuition

Central Dogma of Molecular Biology

KRT5

DNA

Transcription

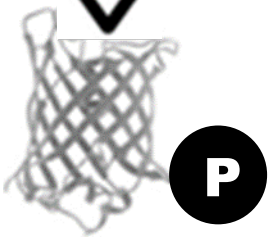


RNA

Translation

Protein

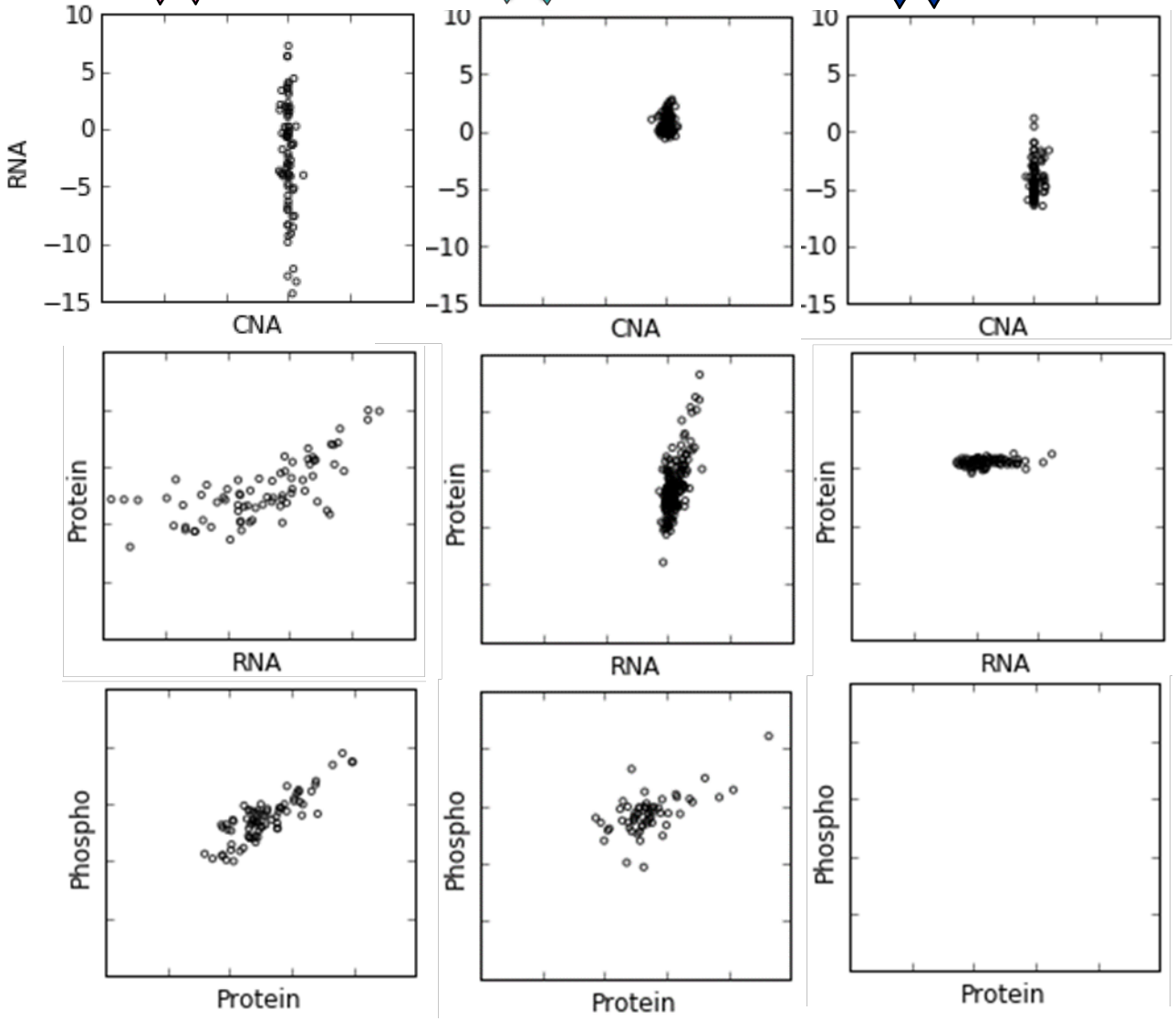
Modification



Breast Cancer

Ovarian Cancer

Colon Cancer



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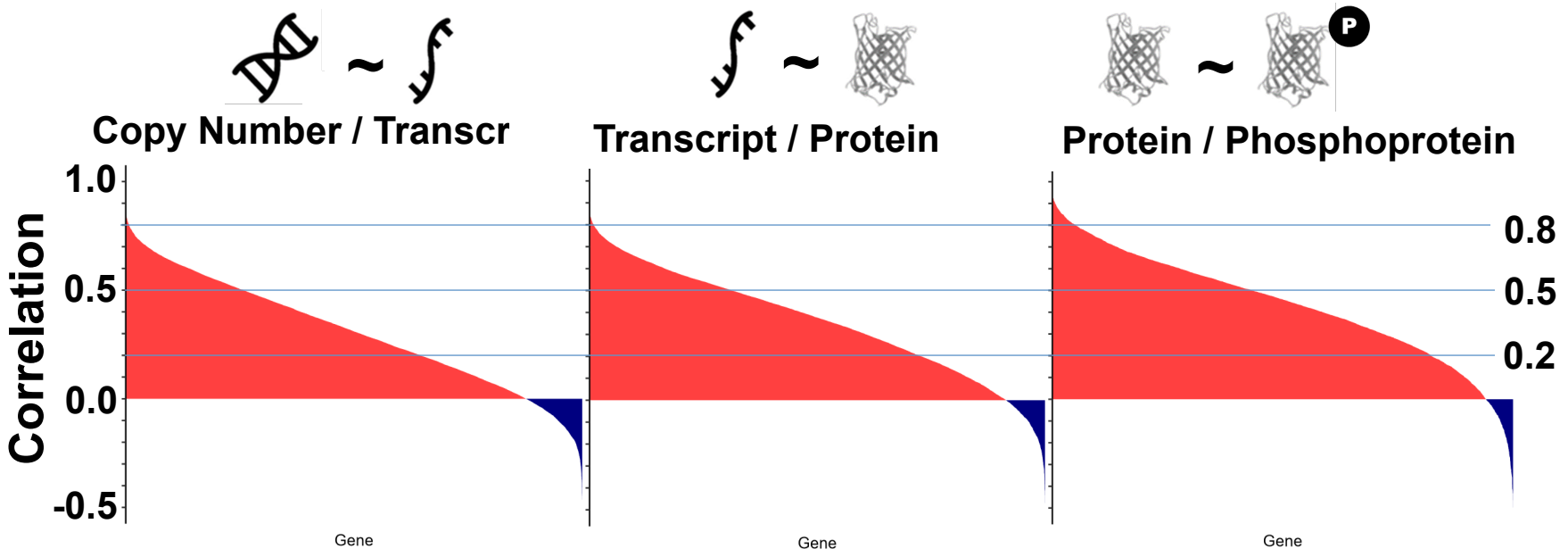
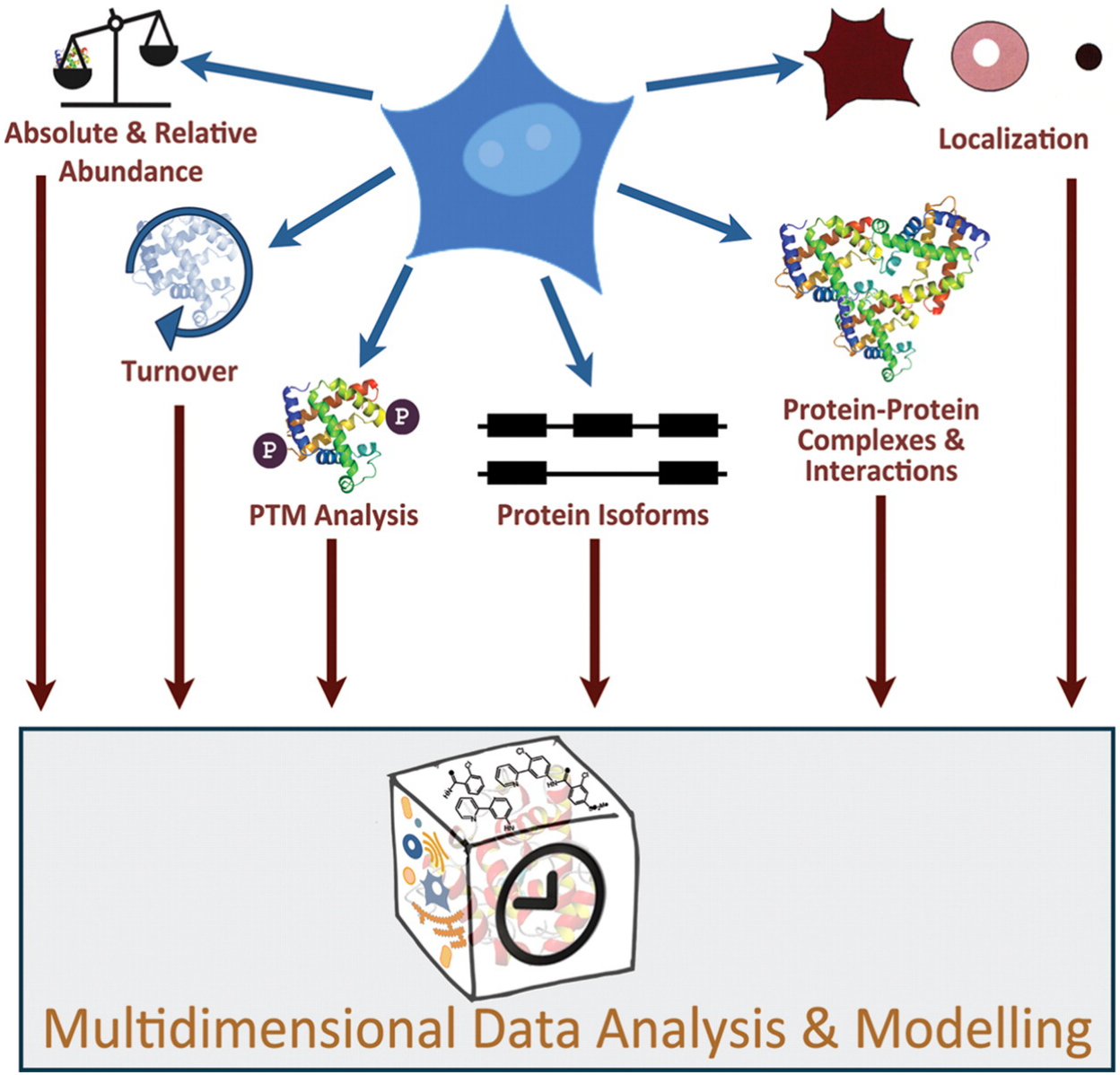
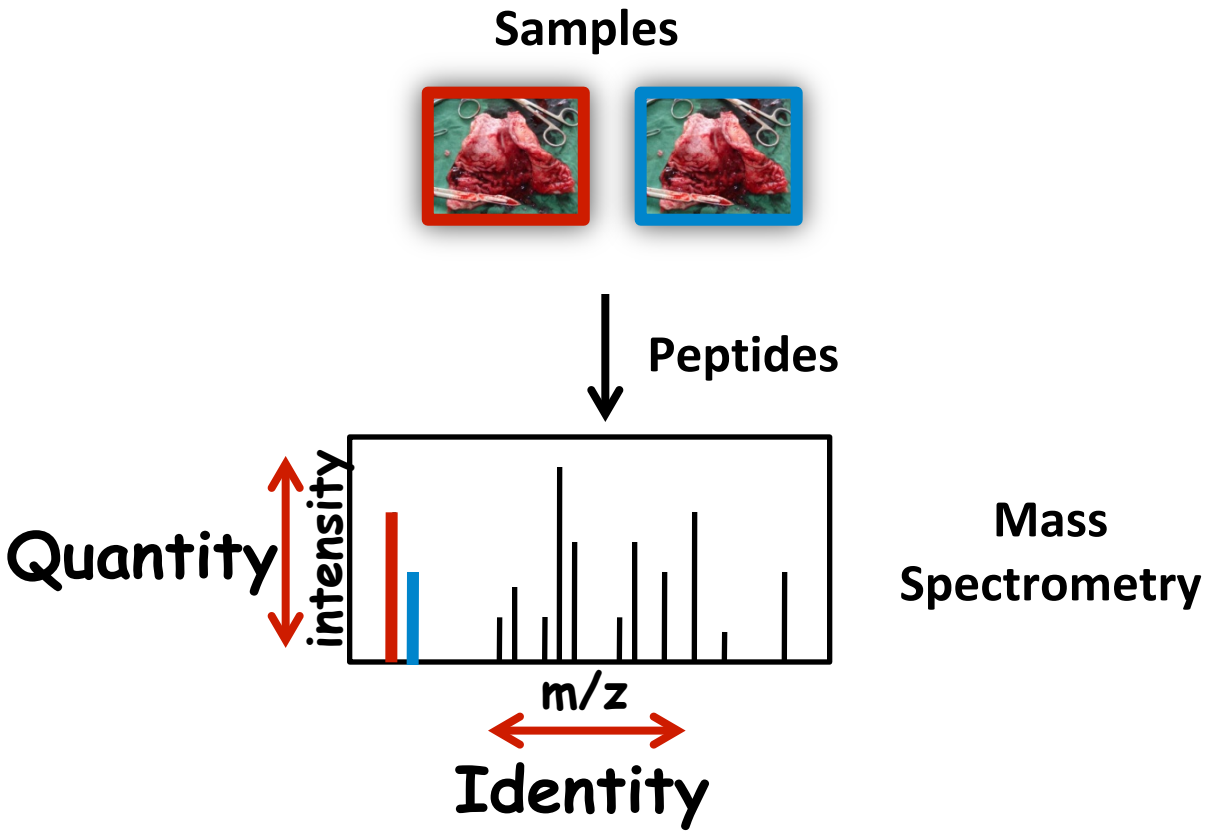
Correlations between copy number, transcript, protein and phosphoprotein quantities



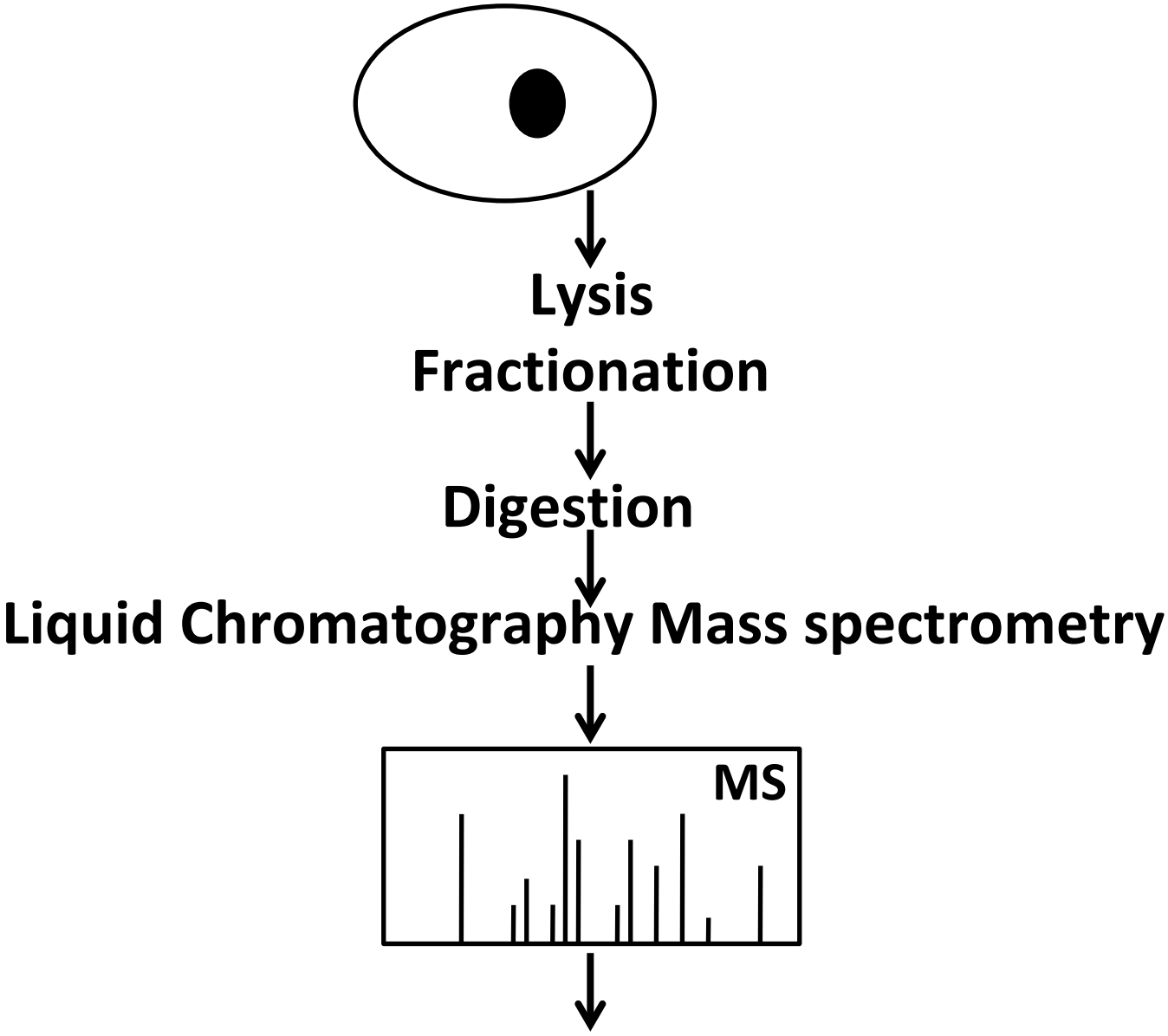


Lamond A I et al. Mol Cell Proteomics 2012;11:O112.017731

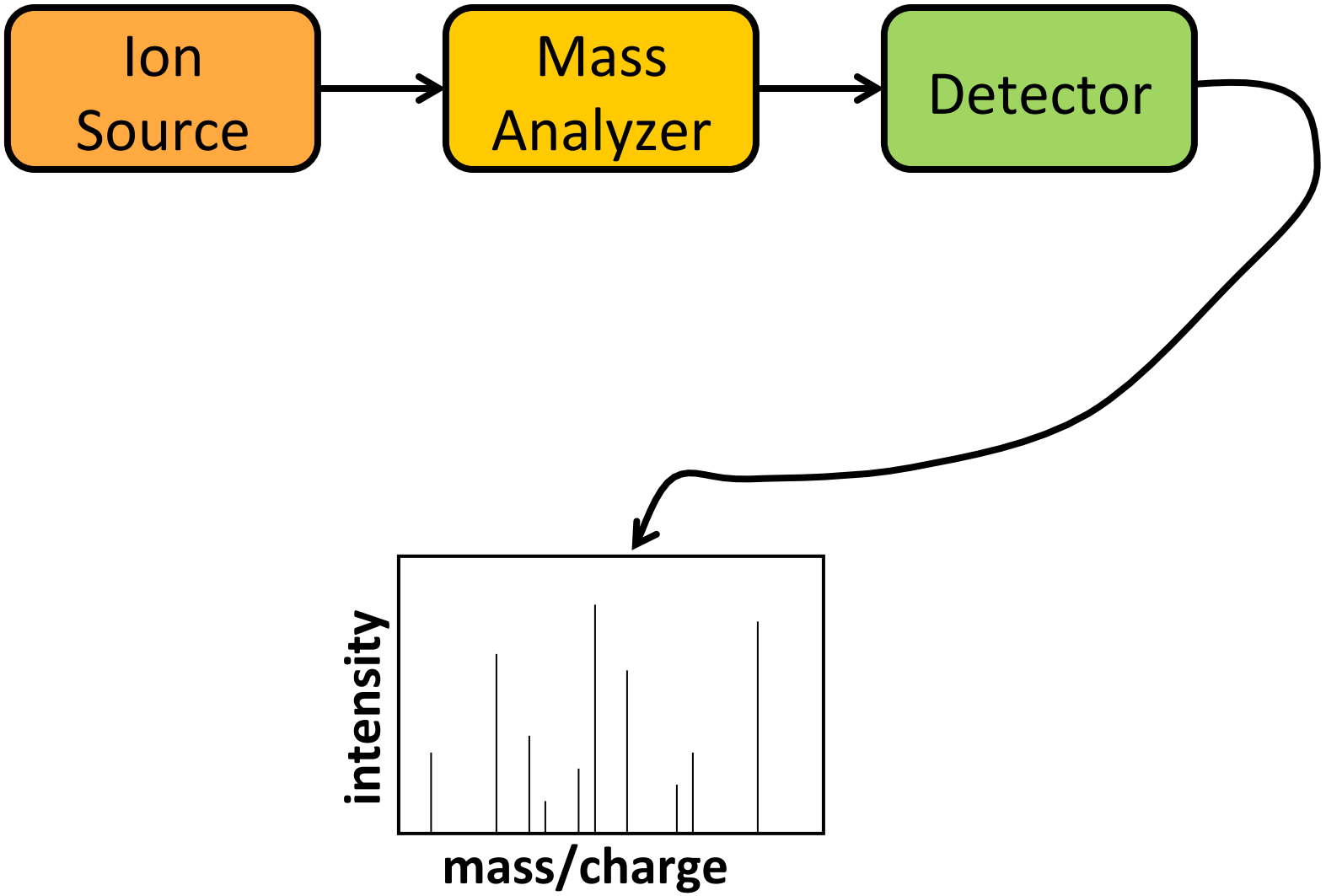
Protein Identification and Quantitation



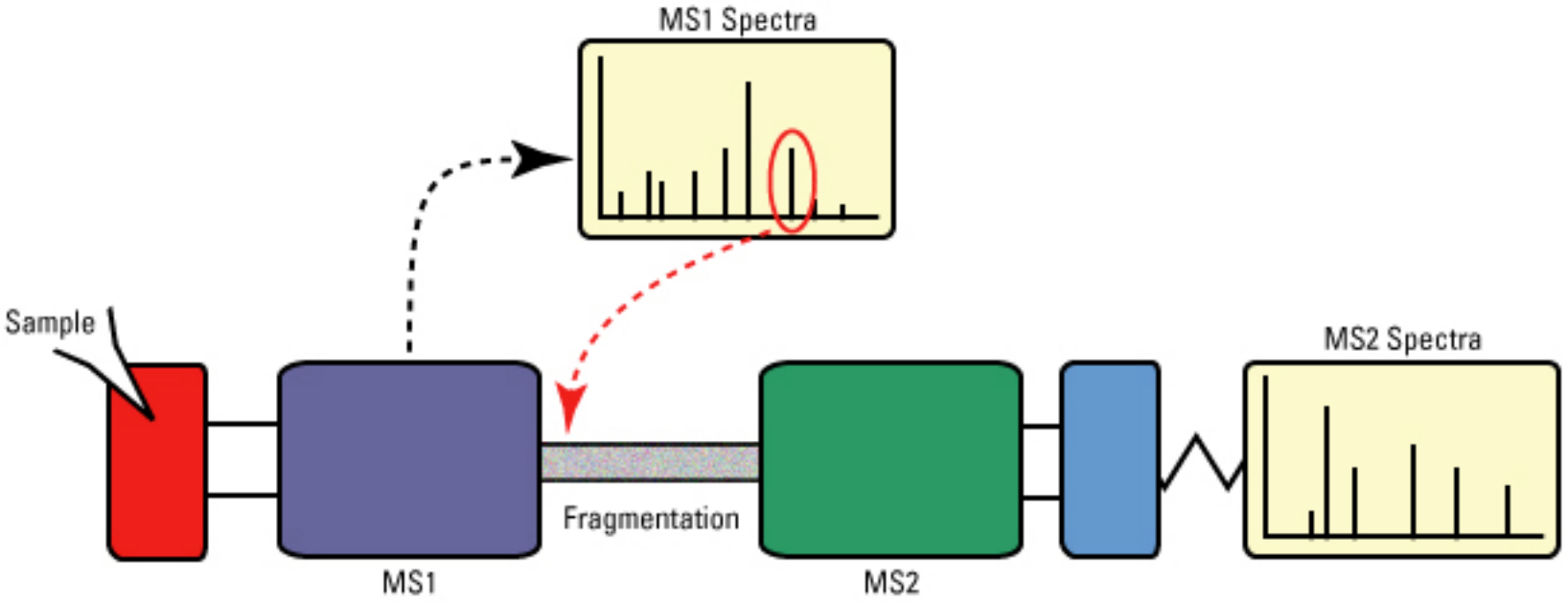
Mass Spectrometry Based Proteomics



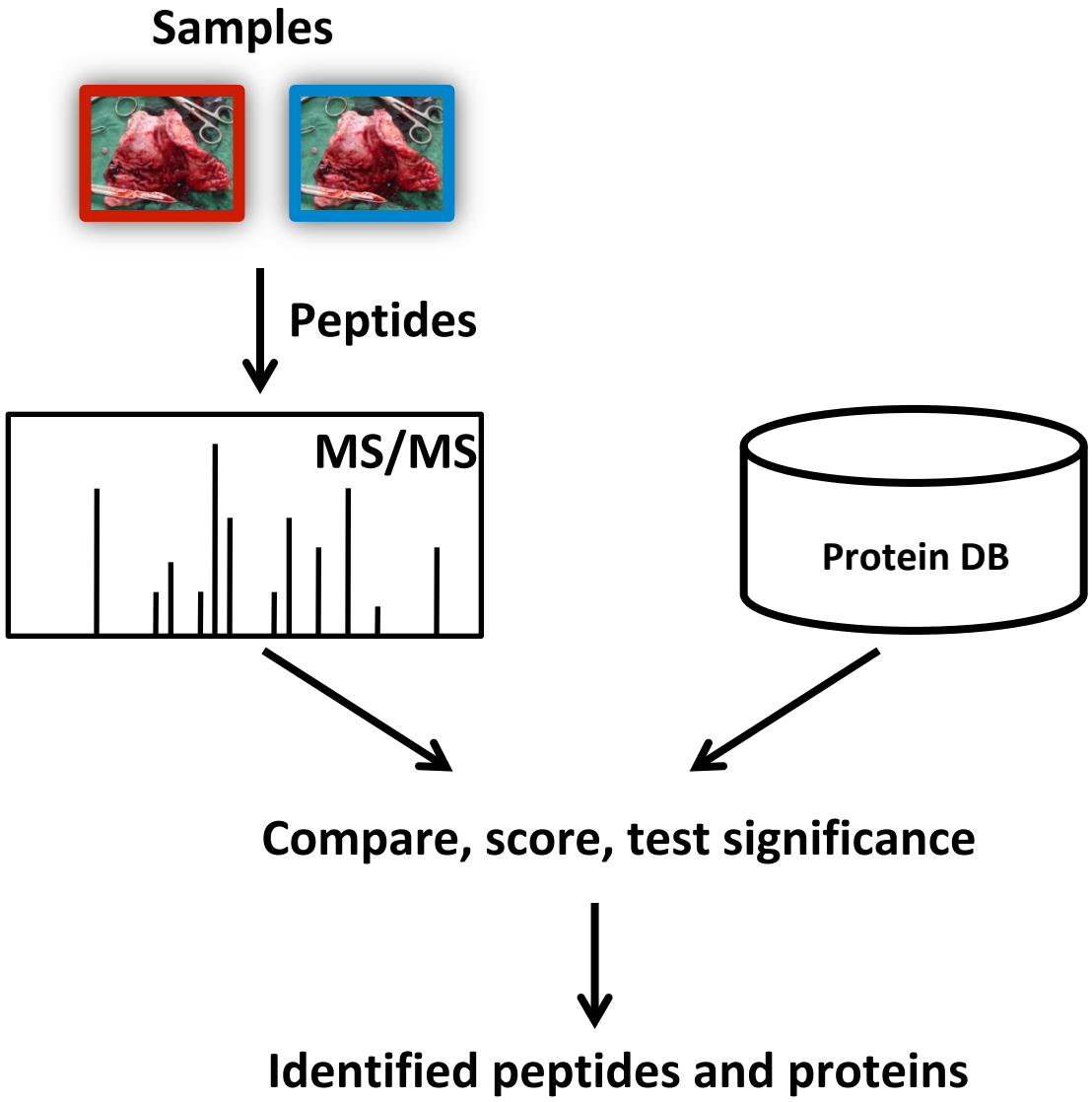
Mass Spectrometry



Tandem Mass Spectrometry



Protein Identification by Mass Spectrometry



Proteomics Facility

Staff:

- Maria Person, Ph.D.
- Michelle Gadush, M.S.
- Hamssika Chandrasekaran, M.S.

Instrumentation

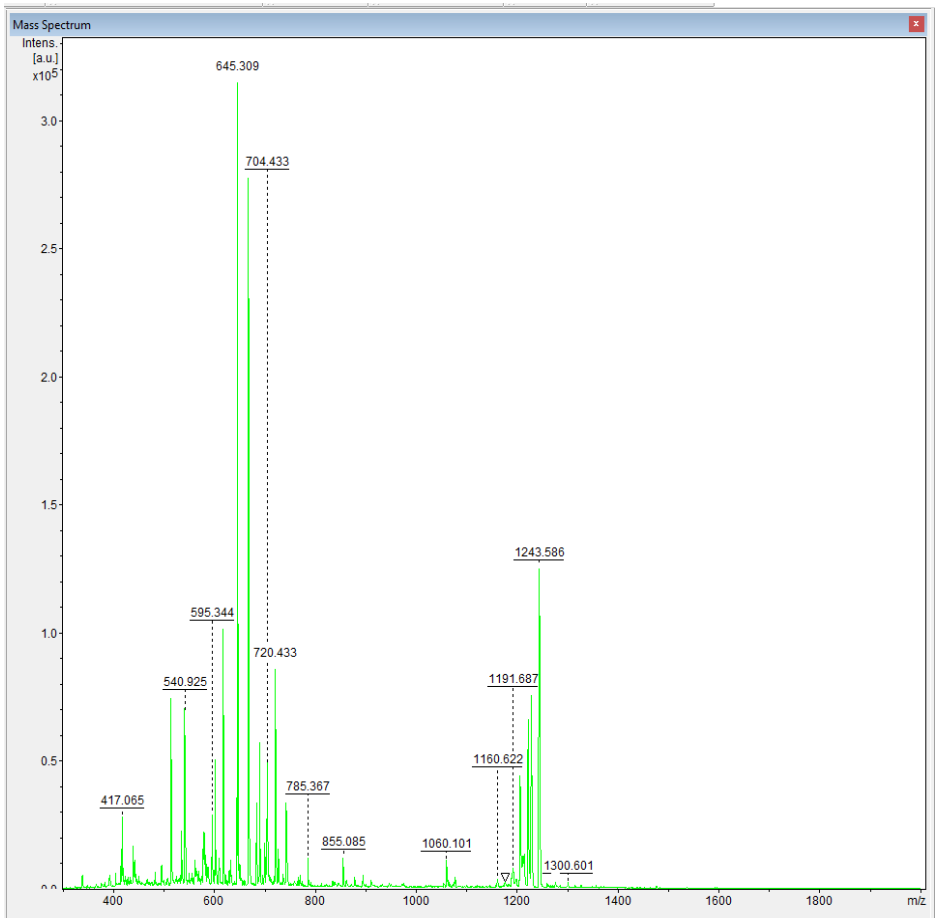
- Two Thermo nanoUPLC-Orbitrap Fusion Tribrid mass spectrometers for proteomics
- Thermo UPLC-QExactive for metabolomics
- Bruker Autoflex MALDI-TOF/TOF for chemicals, polymers, biomolecules, imaging
- Intavis DigestPro for protein digest and desalting

Facility Services

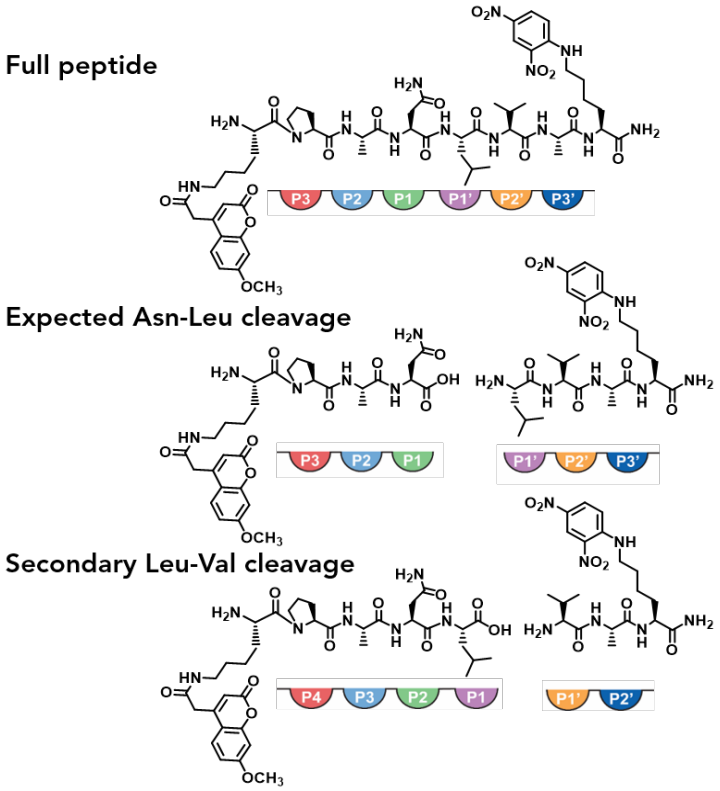
- LC-MS/MS based service and collaborative work: proteolytic digest and desalting, protein fractionation, protein identification, protein modifications, protein quantitation, protein/ peptide molecular weight determination, untargeted metabolomic profiling
- Self-service: chemical, polymer and biological molecular weight determination by MALDI, protein quantitation by FTIR

Protein, Peptide, Polymer, Metabolite, Chemical Molecular Weight Determination

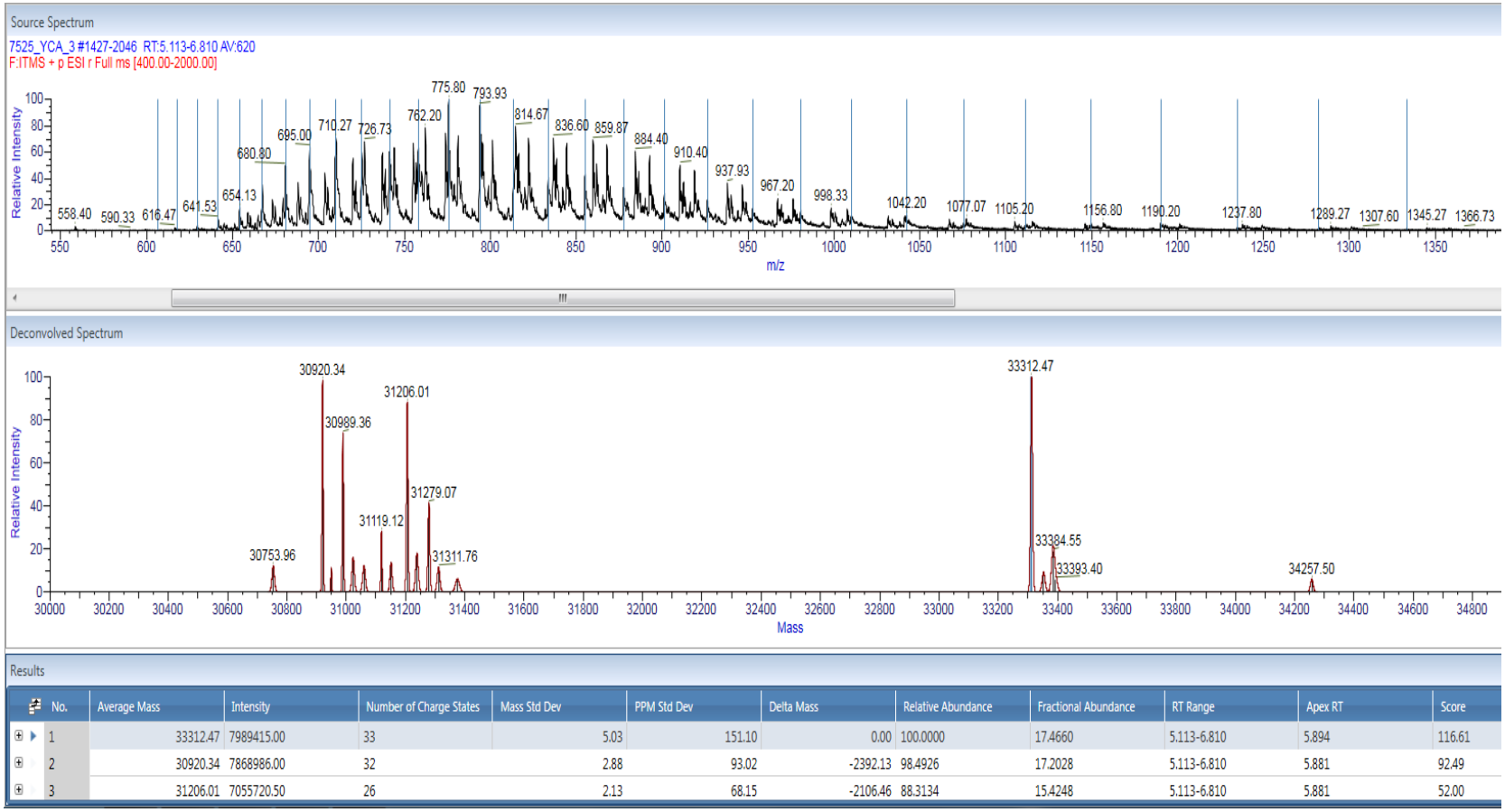
MALDI MS checks peptide synthesis and enzyme reactivity for Rosales lab



Mariah Austin



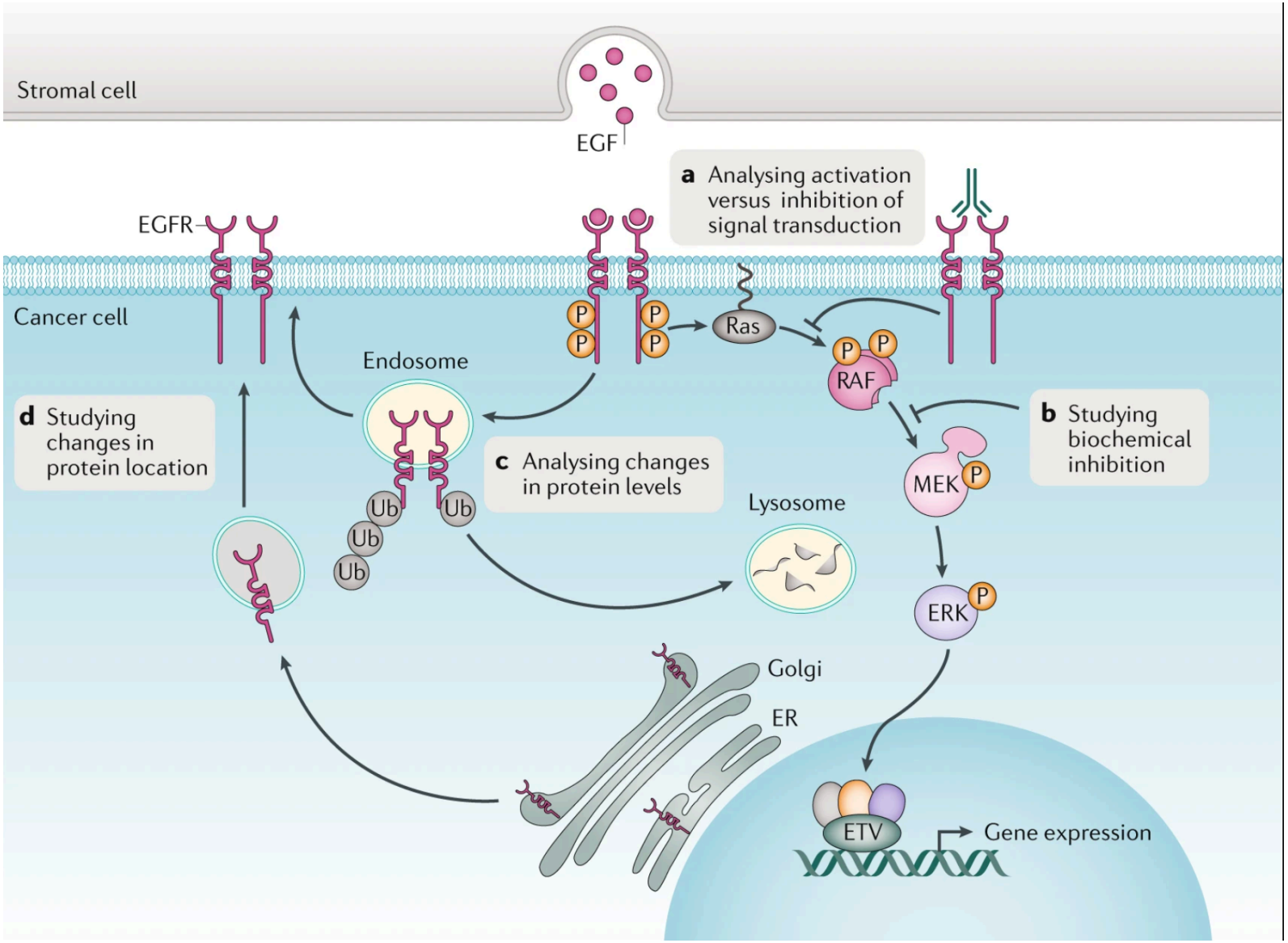
Protein molecular weight by ESI-MS to observe protein covalent modification for Fast lab



Alex Ahn

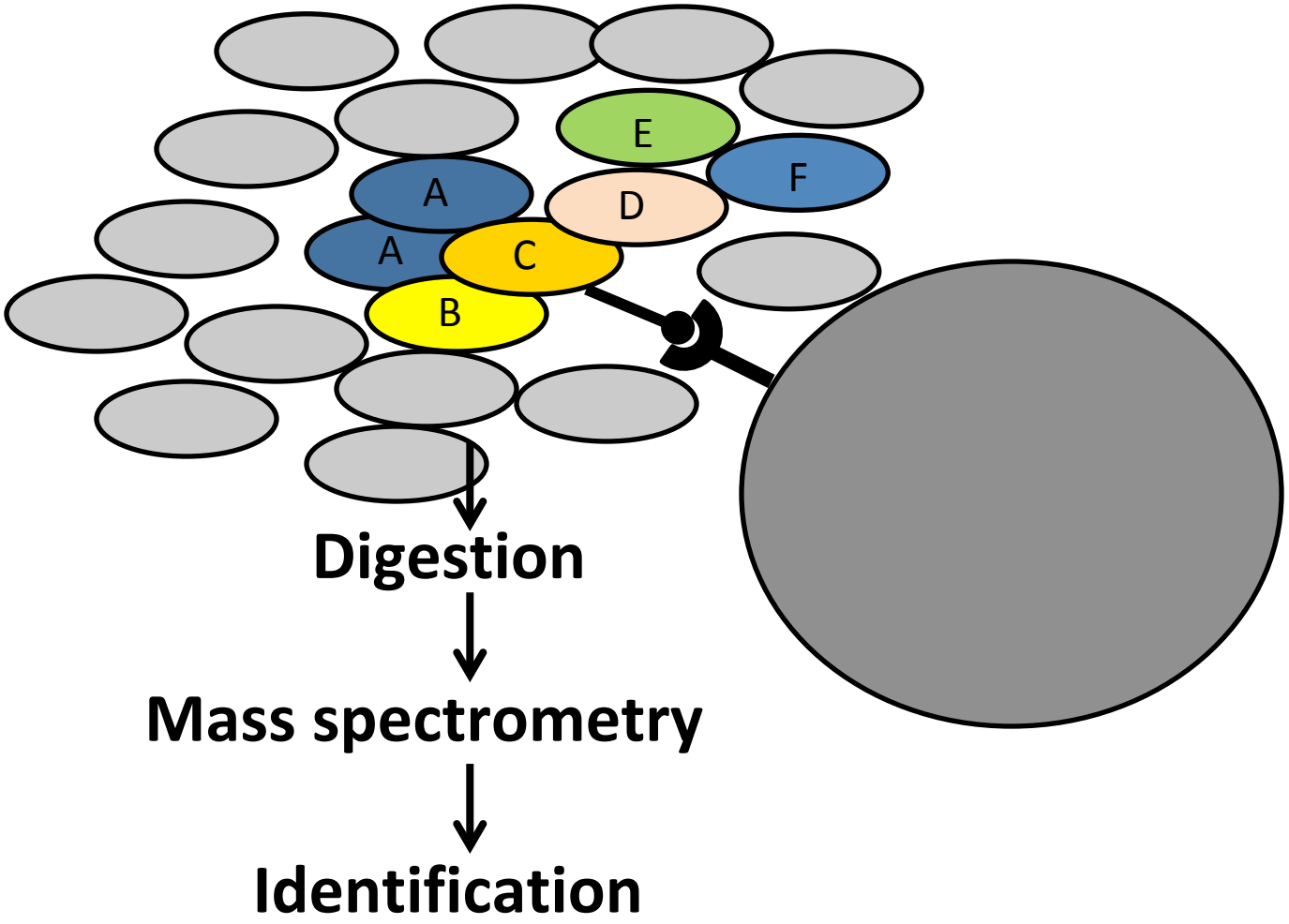
Functional Proteomics: Protein-Protein Interactions

Protein Communities

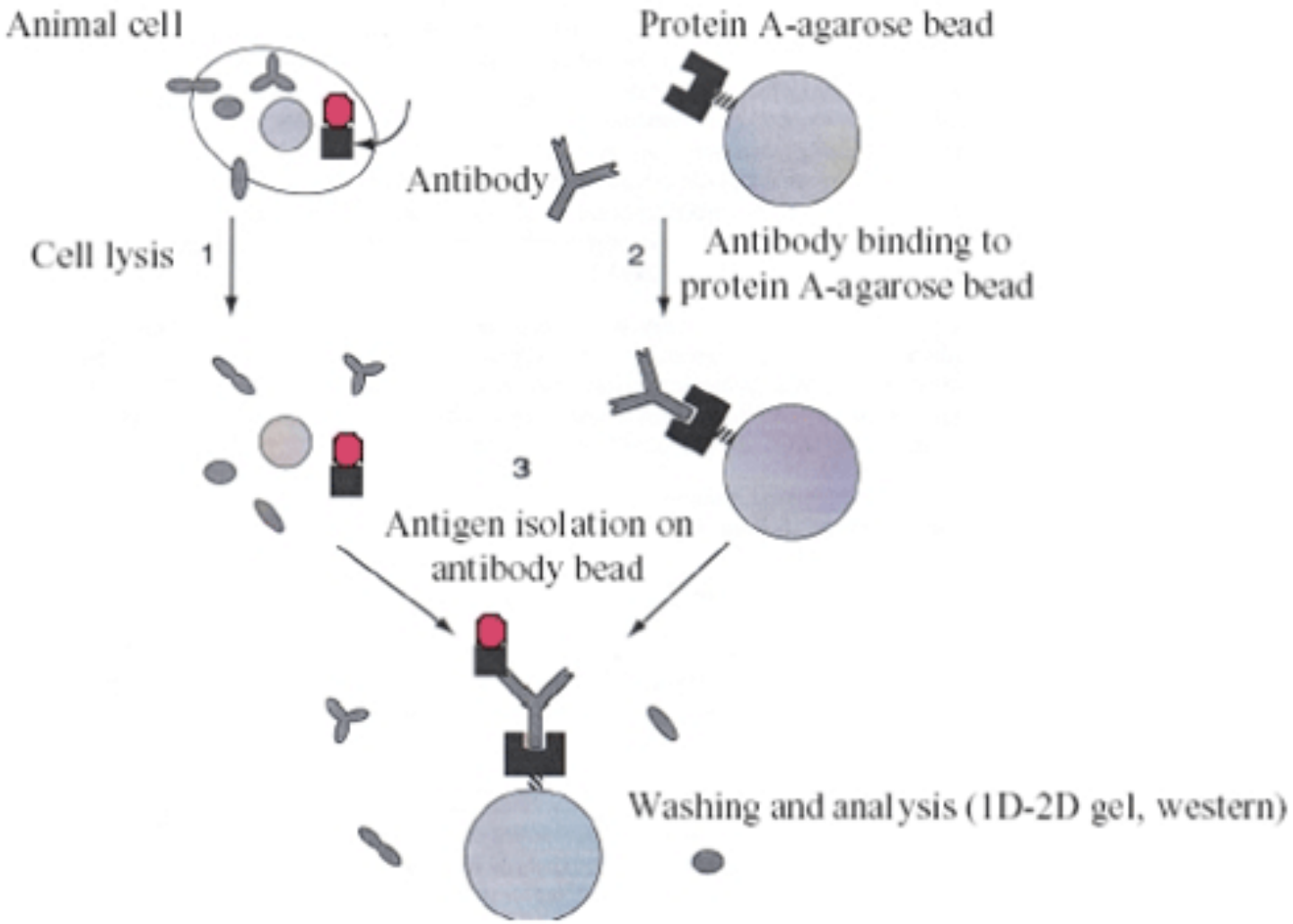


Budayeva, H.G., Kirkpatrick, D.S. Monitoring protein communities and their responses to therapeutics. *Nat Rev Drug Discov* (2020). https://doi-org.ezproxy.lib.utexas.edu/10.1038/s41573-020-0063-y

Affinity Purification Mass Spectrometry



Co-Immunoprecipitation: Protein specific antibodies



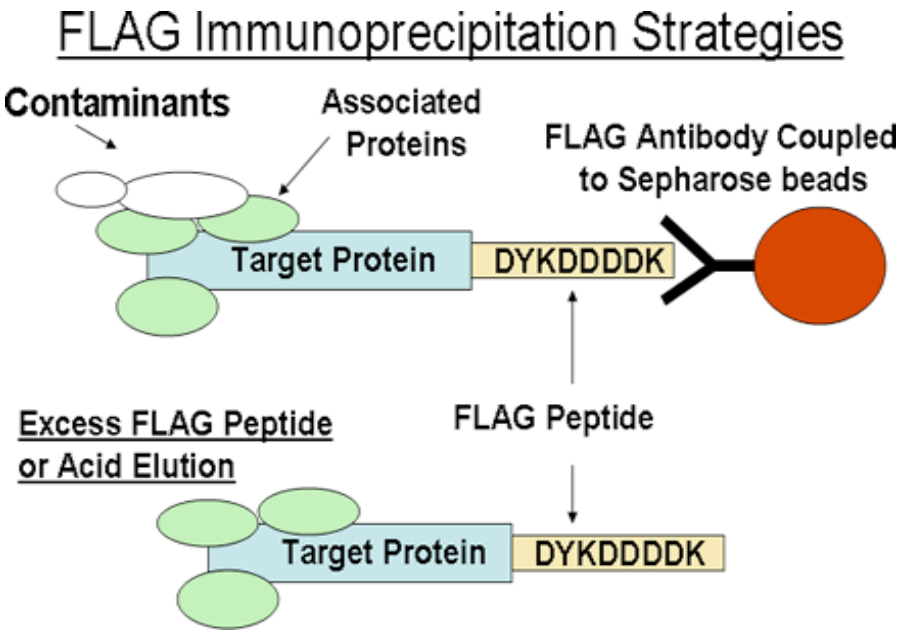
Most common epitope tags are:

His-tag

Flag-tag

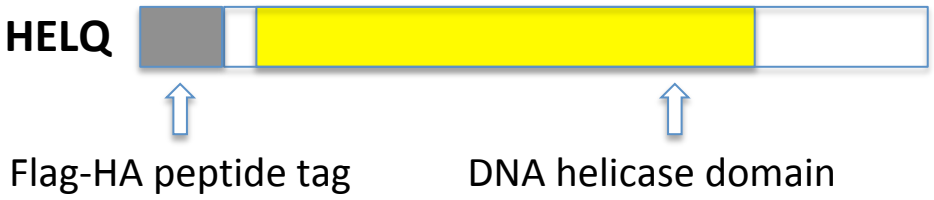
Myc-tag

HA-tag



Problems – antibody cross-reactivity.

Wood lab does affinity capture for HELQ interacting proteions



Use retrovirus to obtain HeLa cells expressing epitope-tagged protein as “bait”

⇓

Grow 9 L cell cultures and prepare cell-free protein extracts

⇓

Sequentially immunoprecipitate extract with anti-FLAG and anti-HA antibody

⇓

Identify associated proteins with LC-MSMS

⇓

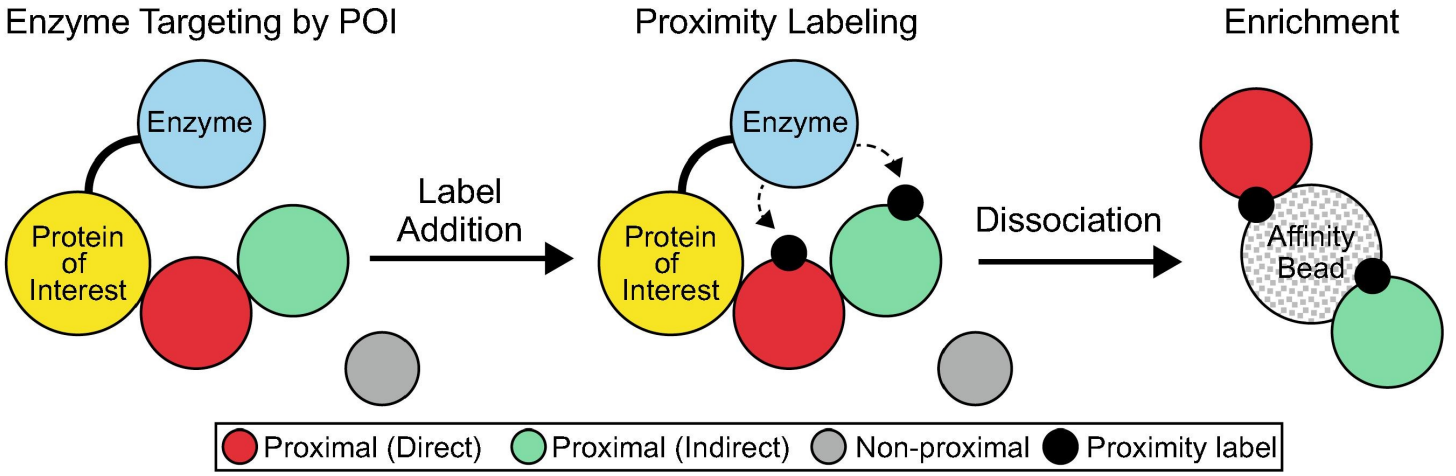
Filter results to eliminate background proteins from recurrent hits obtained with unrelated bait proteins in the same system

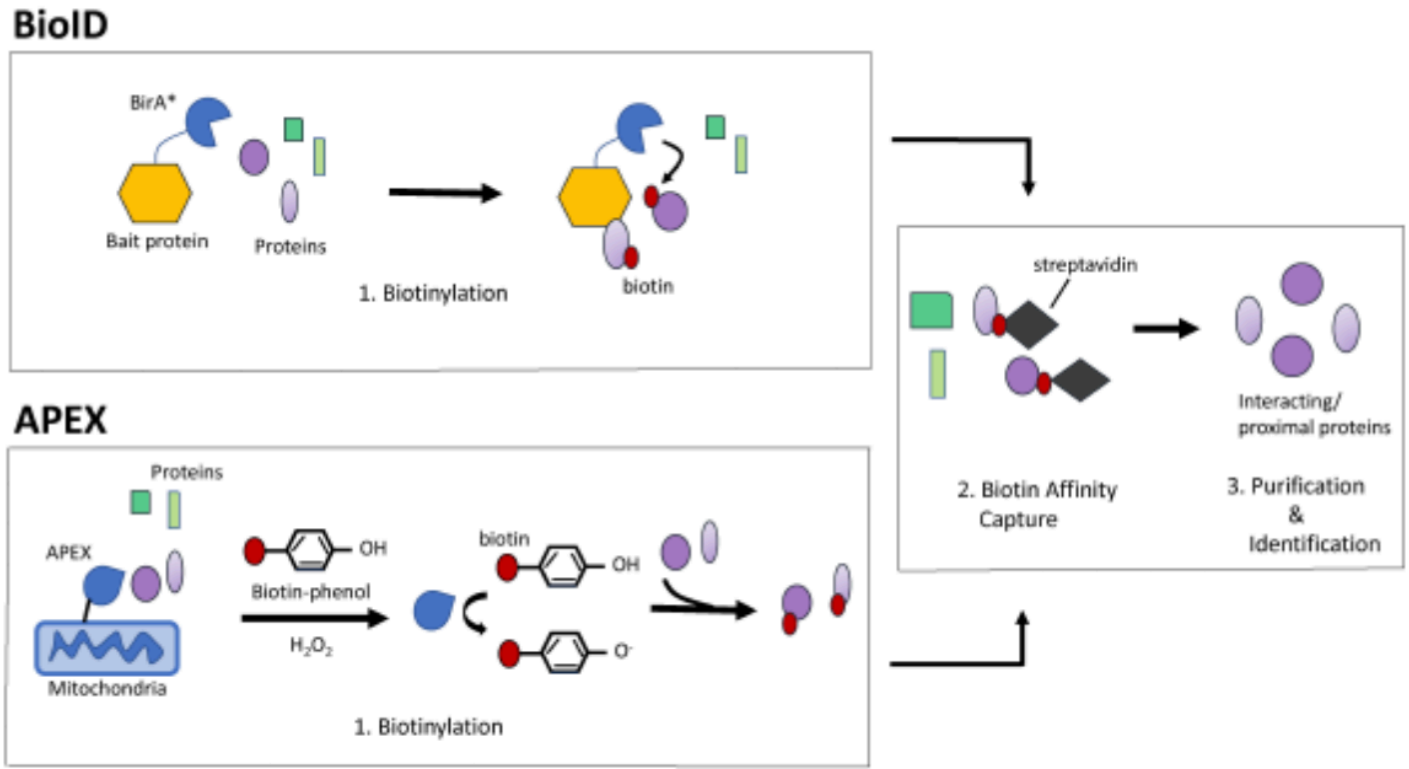
⇓

Results: Interaction with RAD51 paralogs and ATR suggest that HELQ operates in a pathway of DNA recombinational repair and as part of a “checkpoint” response to damaged DNA

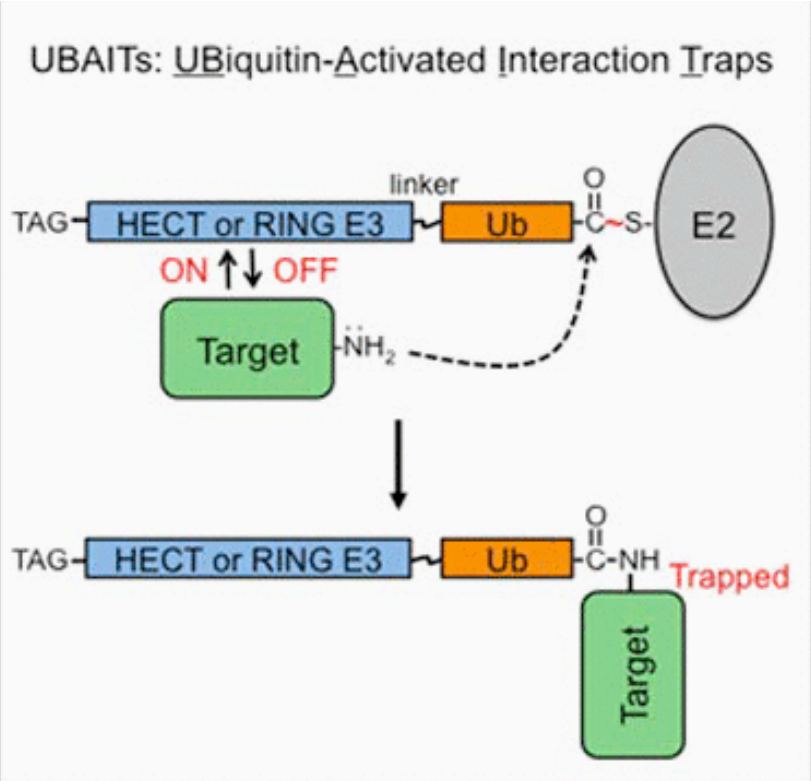
Kei-ichi Takata, Junya Tomida, Rick Wood (MDACC-Smithville)

Proximity labeling tags





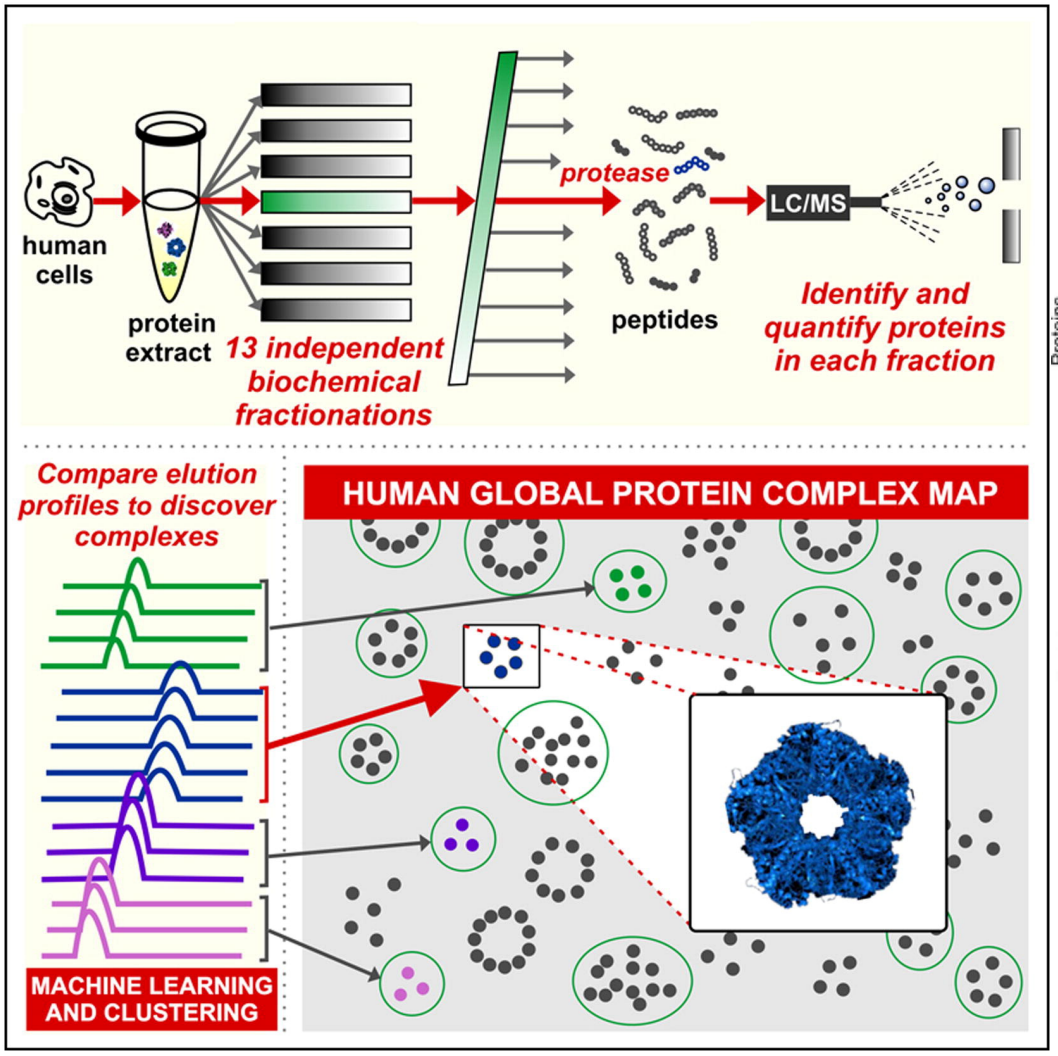
Huibregtse lab: Develop covalent trapping for functional proteomics of E3 ligases

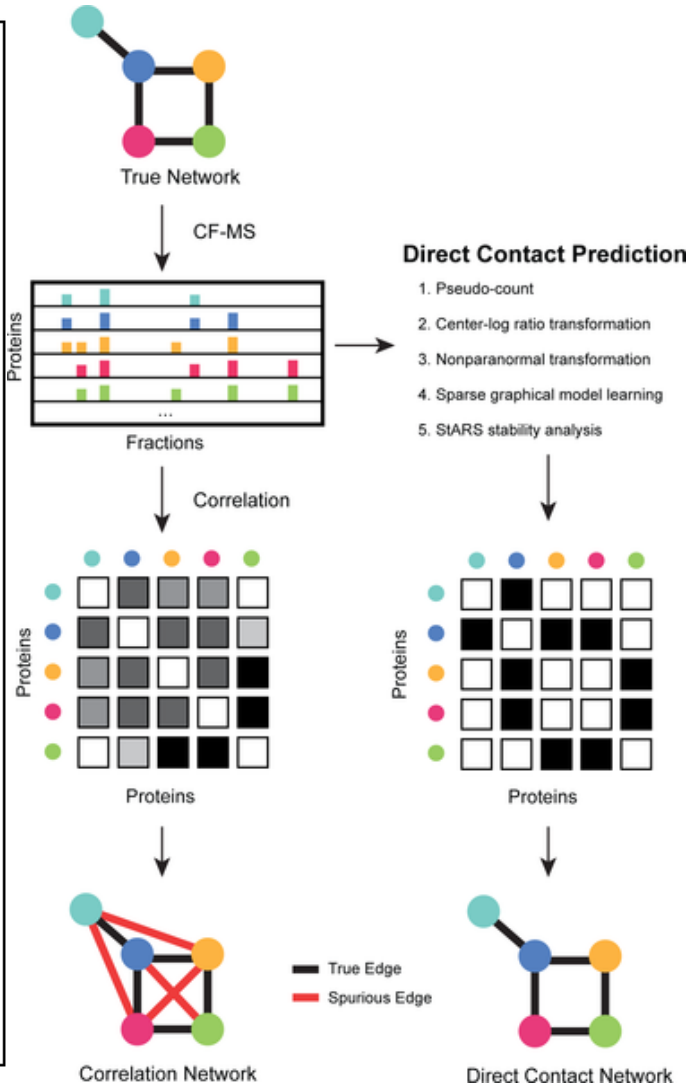


- Identification of ubiquitin E3 ligase-interacting proteins through covalent trapping, enabling co-purification
- H2A.Z is identified as a new RNF168 substrate

- Hazel O'Connor, Nancy Lyon, et al. EMBO Rep. 16(12):1699-712.

CoFractionation-Mass Spec





Havugimana PC et al. A census of human soluble protein complexes. Cell. 2012 Aug 31;150(5):1068-81.
Drew K et al. PLoS Comput Biol. 2017 Oct 12;13(10):e1005625.

Protein Complexes

- hu.MAP

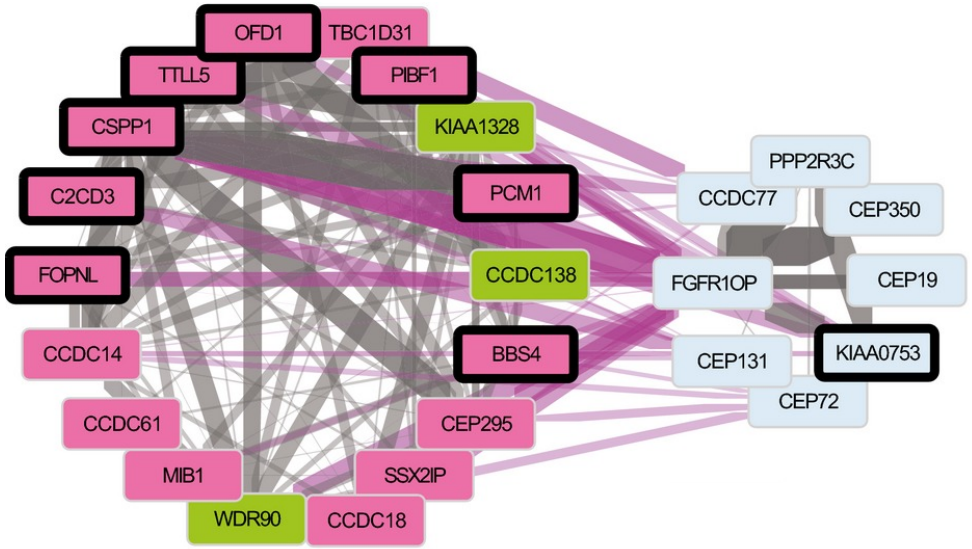
Marcotte lab

Human Protein

complex map

Based on results of

9,000 MS experiment

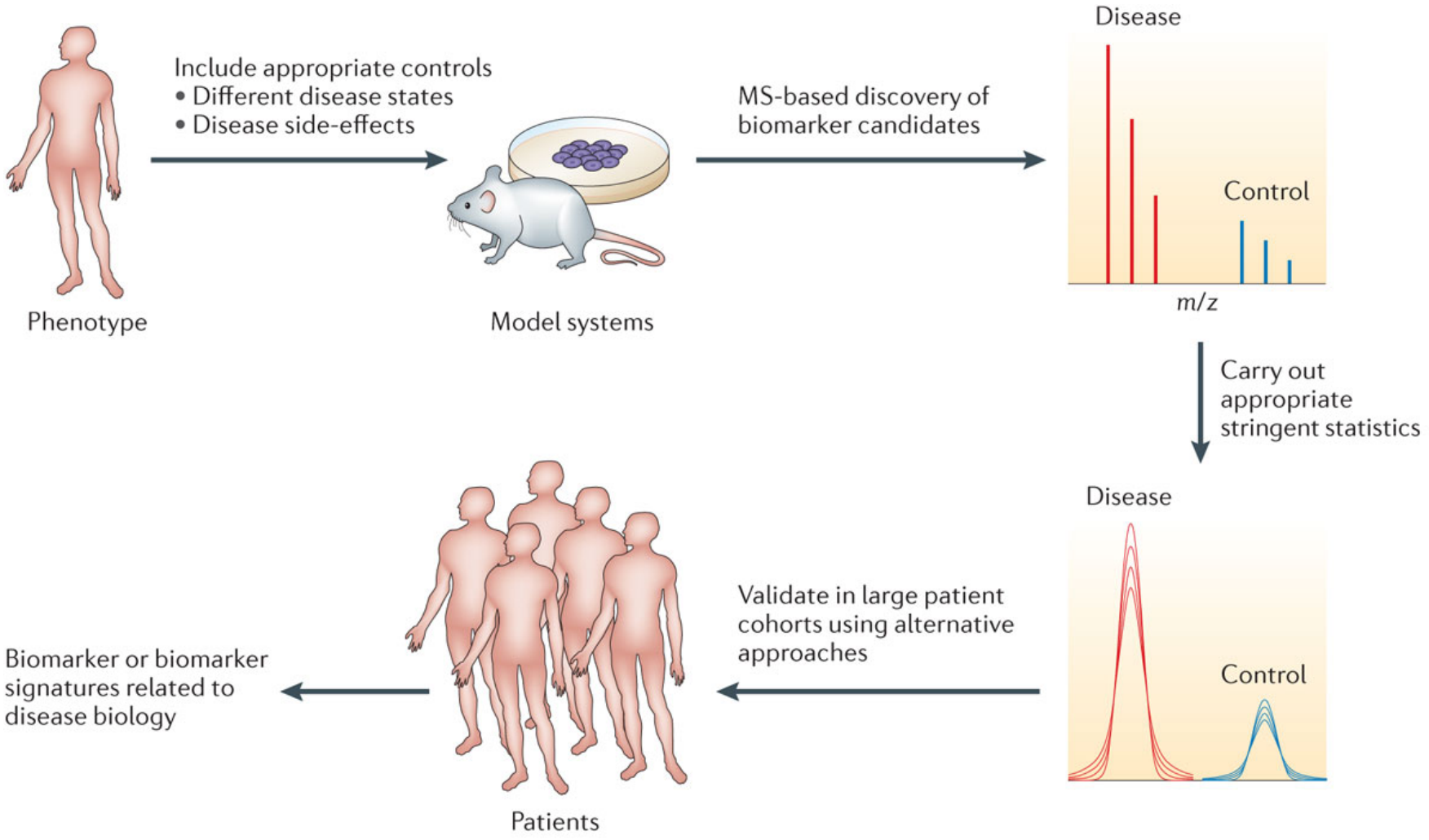


Mol Syst Biol, Volume: 13, Issue: 6, First published: 09 June 2017, DOI: (10.15252/msb.20167490)

- CORUM-resource of manually annotated protein complexes from mammalian organisms.

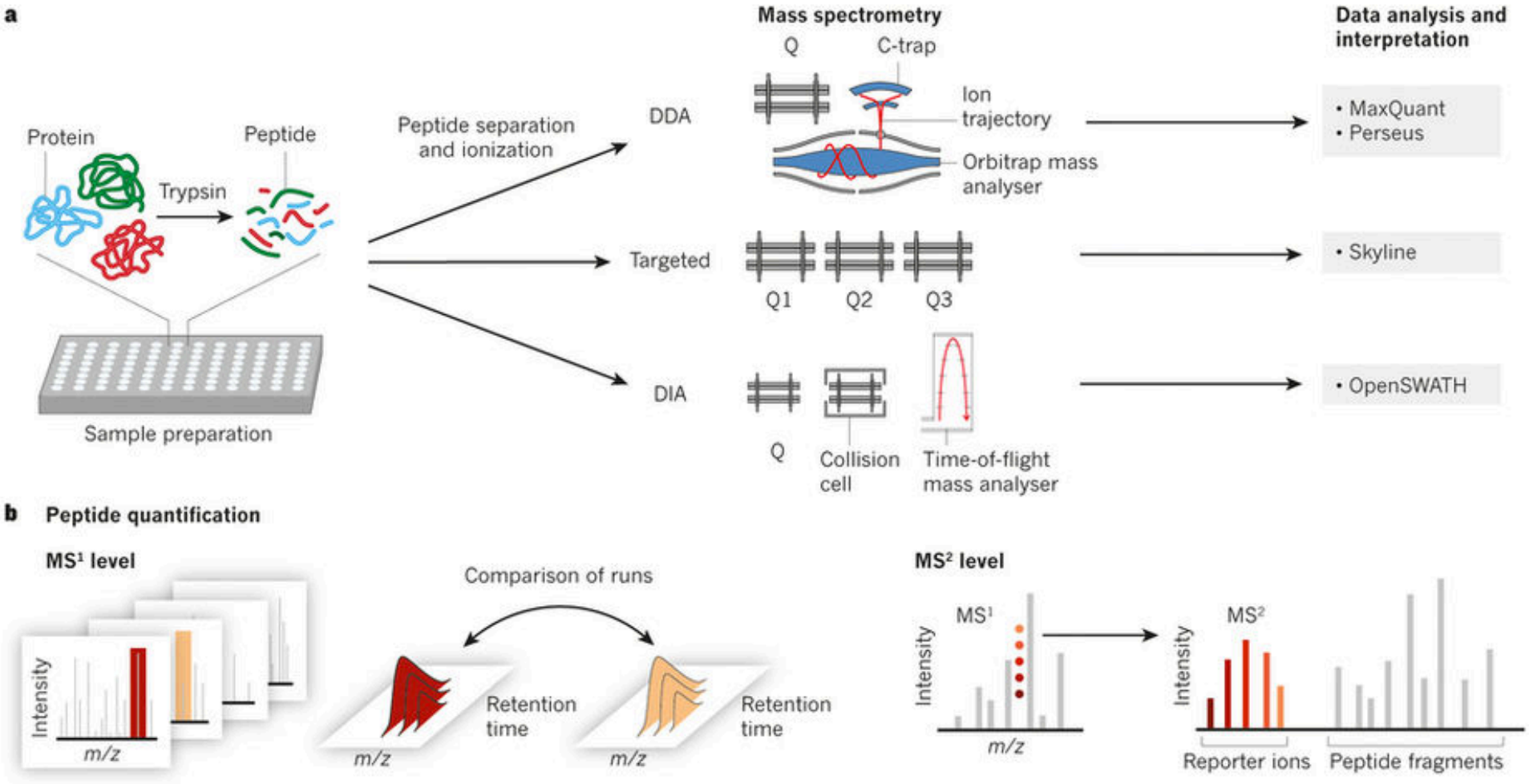
Quantitative Proteomics

Biomarker discovery workflow



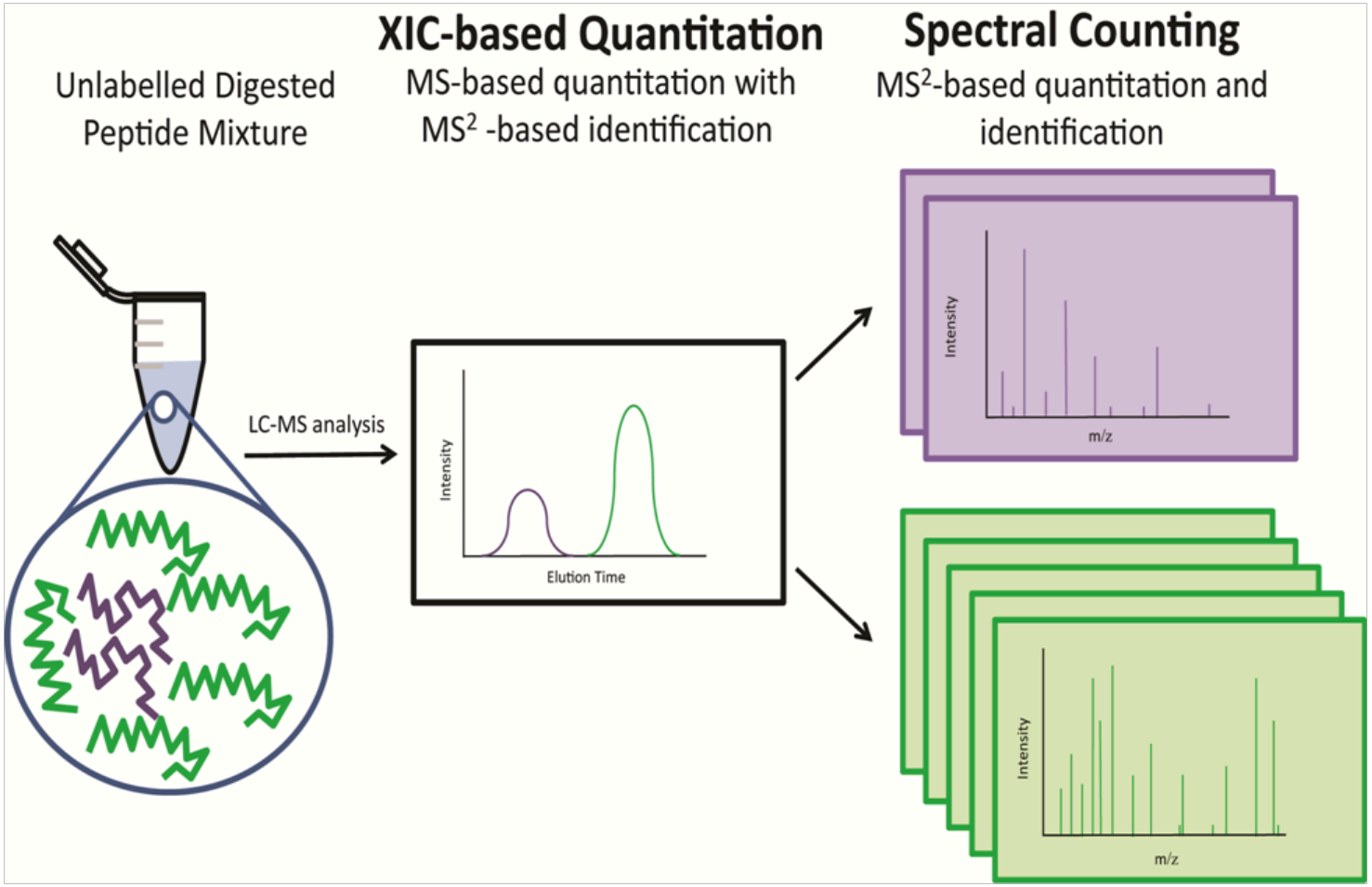
Altelaar et al. Nat Rev Genet. 2013 Jan;14(1):35-48. doi: 10.1038/nrg3356.

Quantitative Proteomics



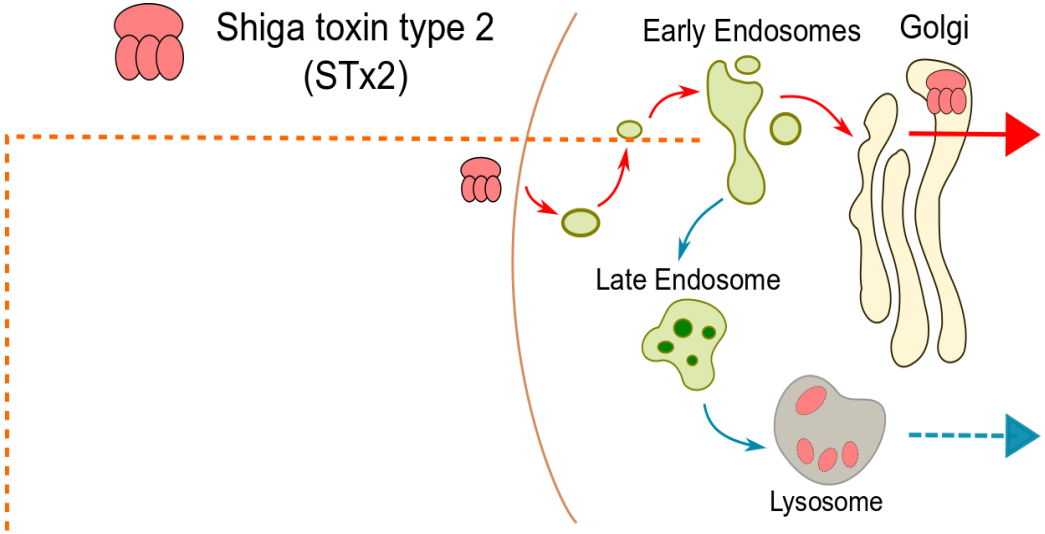
Nature **537**, 347–355 (15 September 2016) doi:10.1038/nature19949

Quantitation uses peptide peak intensity or counts number of MS/MS



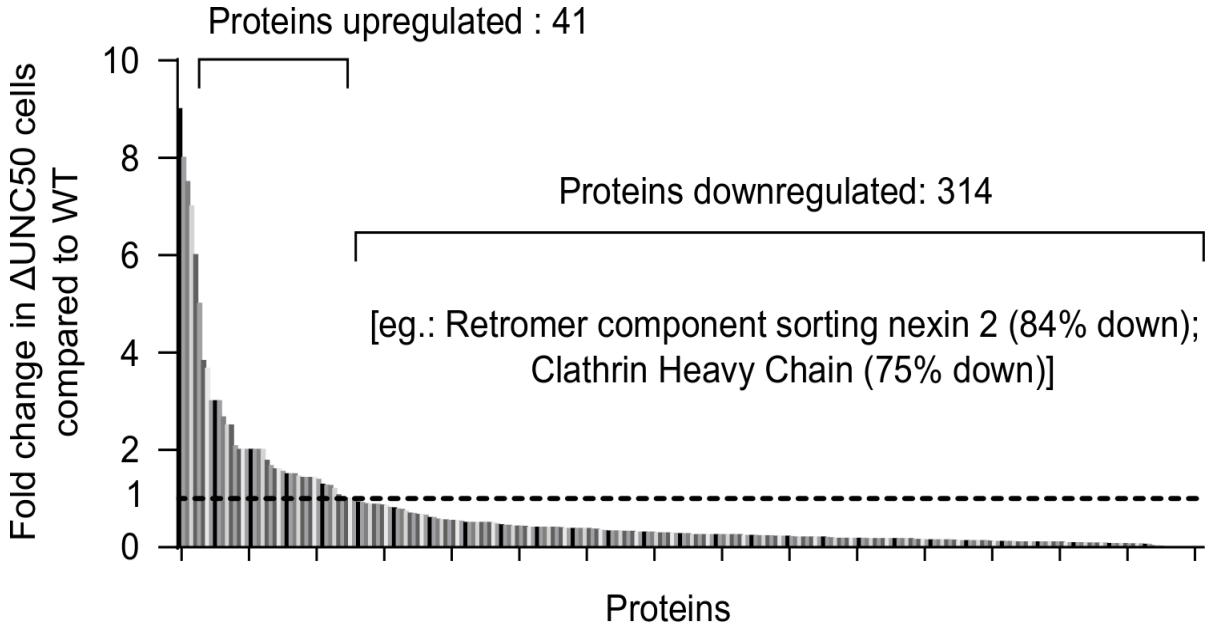
https://www.bioanalysis-zone.com/2016/02/08/chapter-5-modern-techniques-in-quantitative-proteomics/ Smith and Macklin from the series Advanced LC-MS applications for proteomics

Som Mukhopadhyay lab: Shiga toxin transport and routing studied through quantitative proteomics



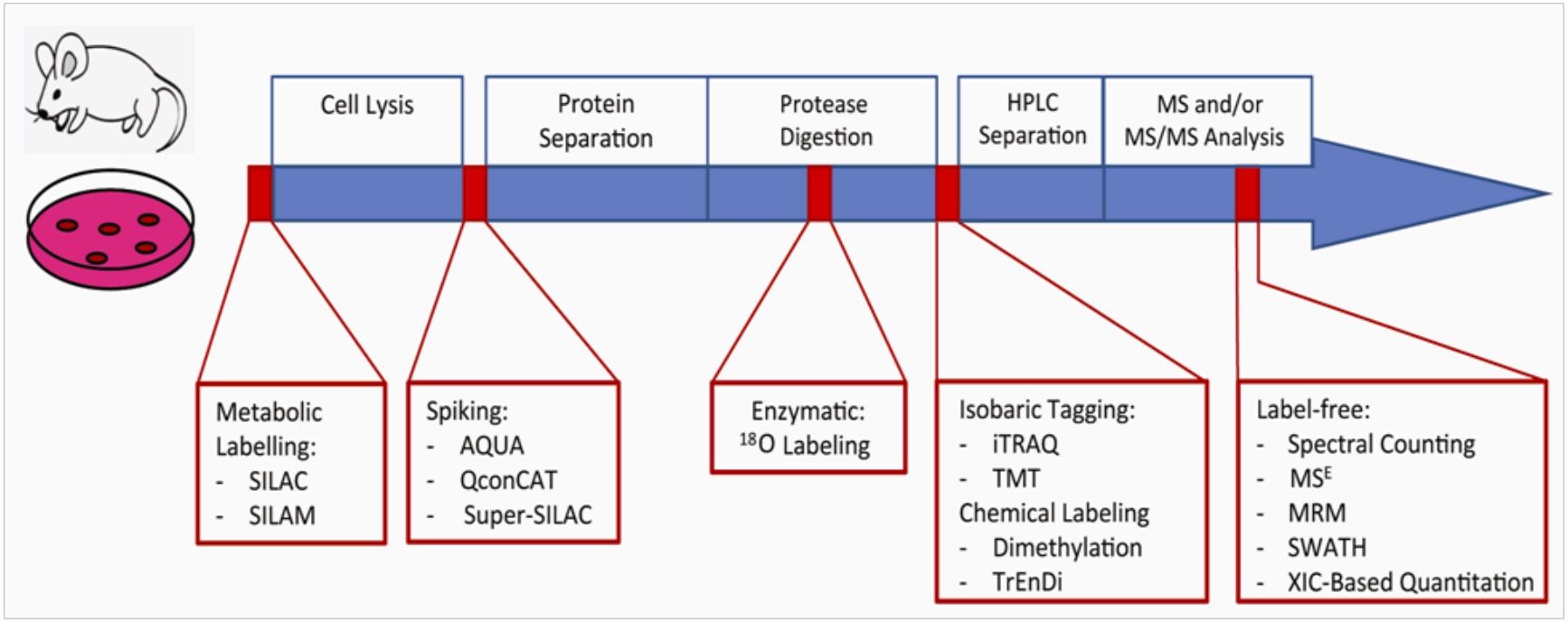
WT cells:
Protein synthesis arrest
Cell death

ΔUNC50 cells:
STx2 Fails to reach Golgi
Routed to lysosomes
No cell death



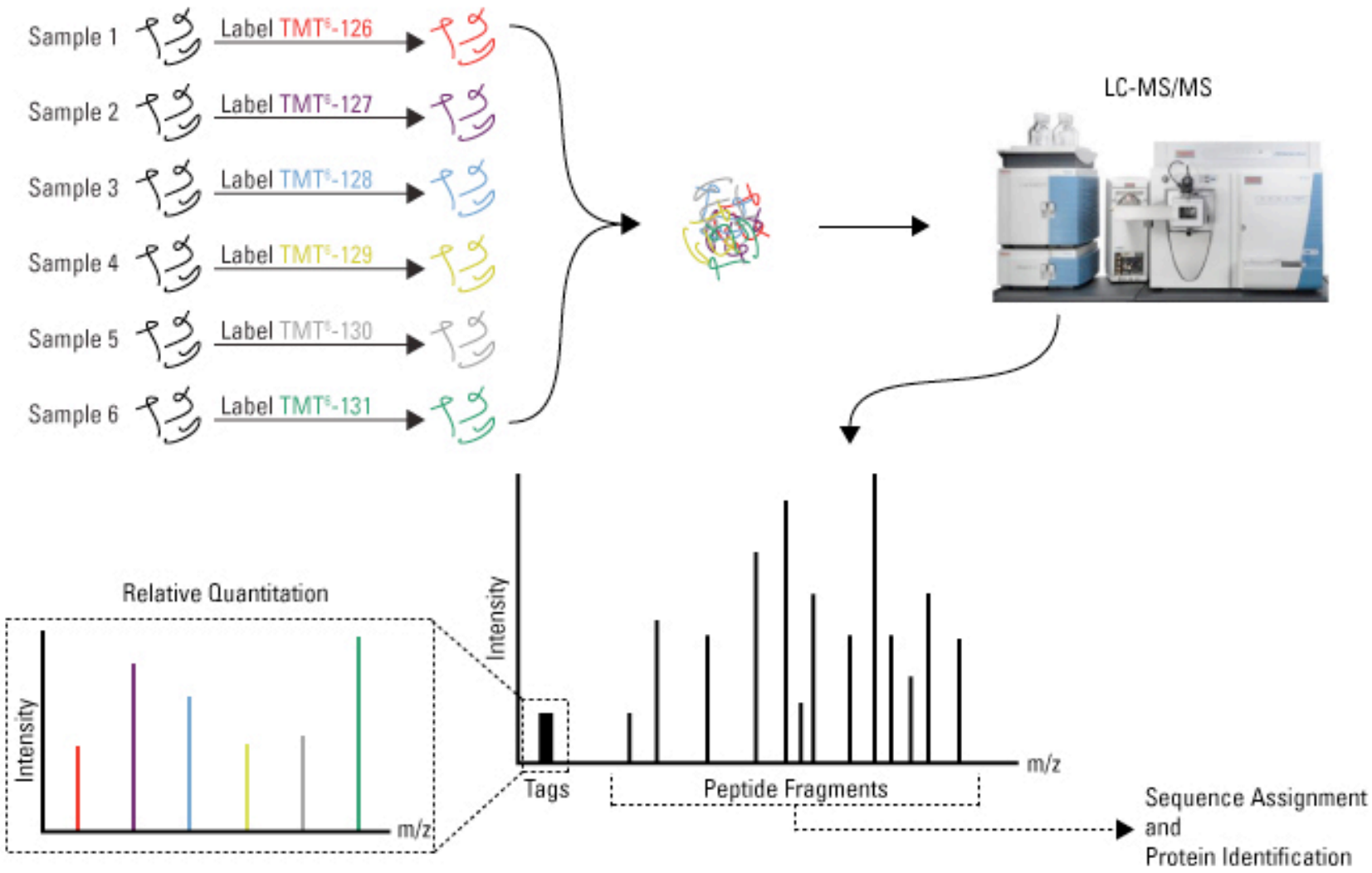
Andrey Selyunin

Experimental stages for initiating quantitation protocol



Smith and Macklin, https://www.bioanalysis-zone.com/2016/02/08/chapter-5-modern-techniques-in-quantitative-proteomics/

Isobaric Tagging: iTRAQ/TMT

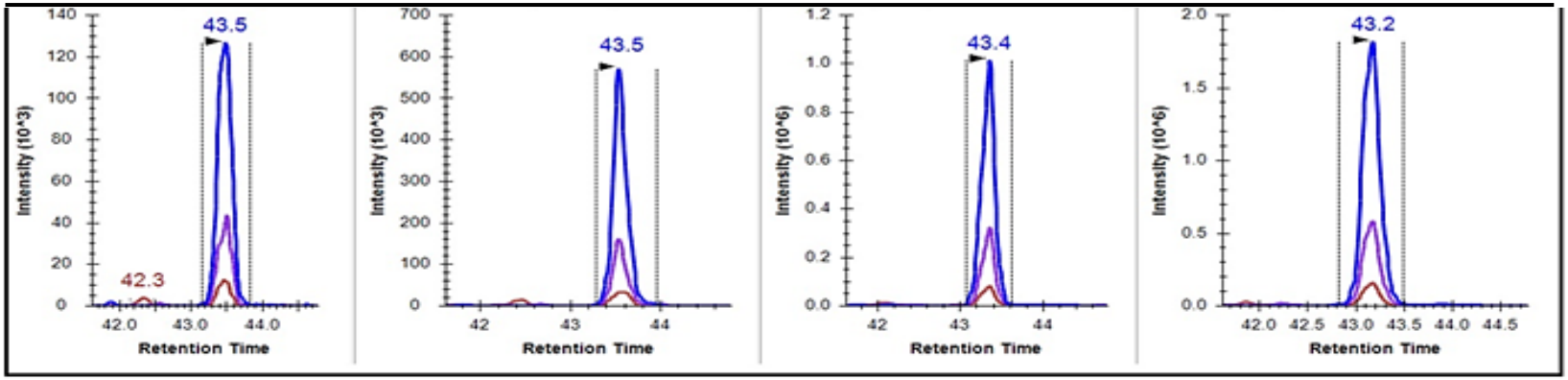


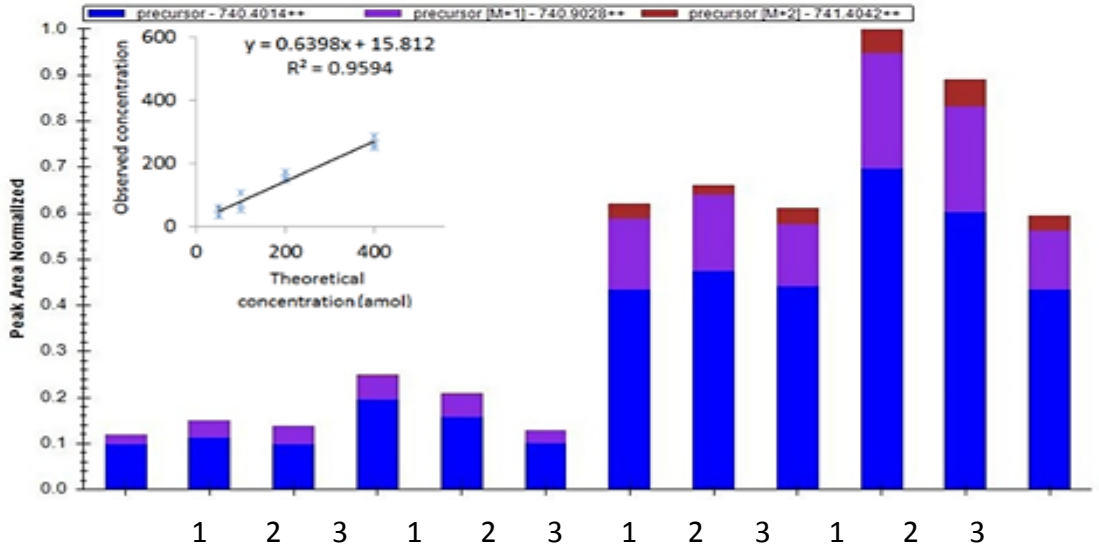
http://www.piercenet.com/method/quantitative-proteomics

Absolute Quantitation with a Standard

BSA

50 amol | 100 amol | 200 amol | 400 amol

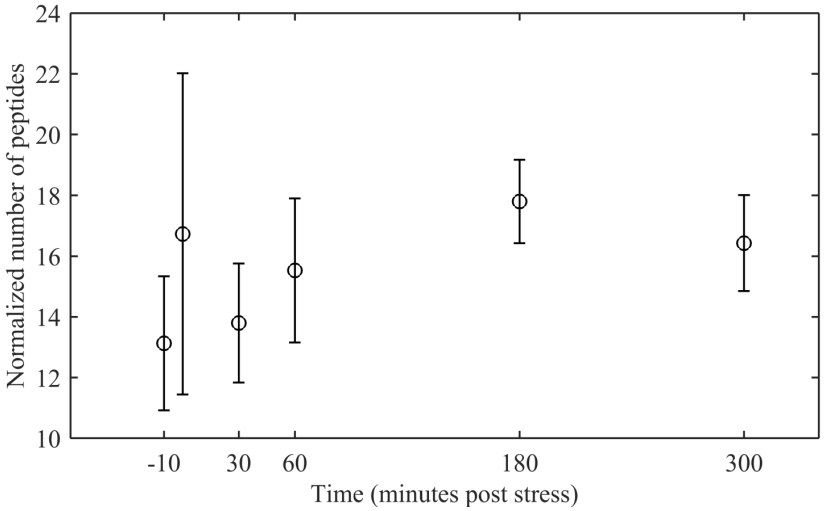




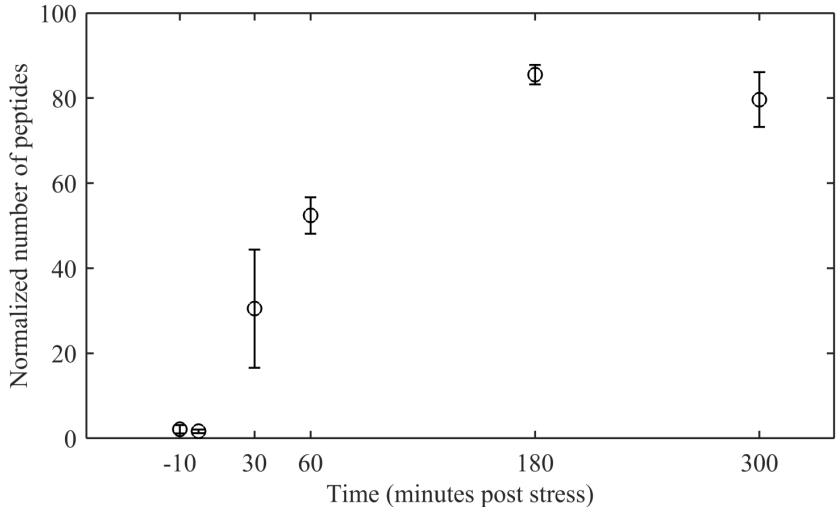
Replicate peak areas

Contreras lab measures time dependent protein expression

Protein regulator



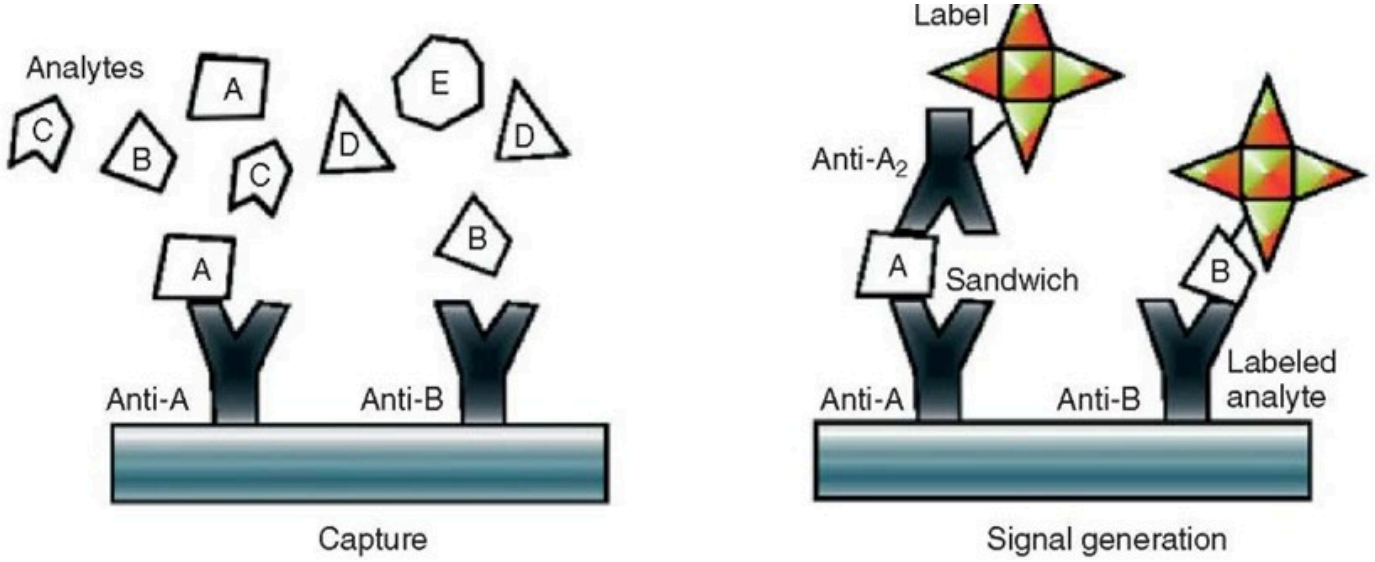
Putative ABC transporter periplasmic-binding protein YdcS



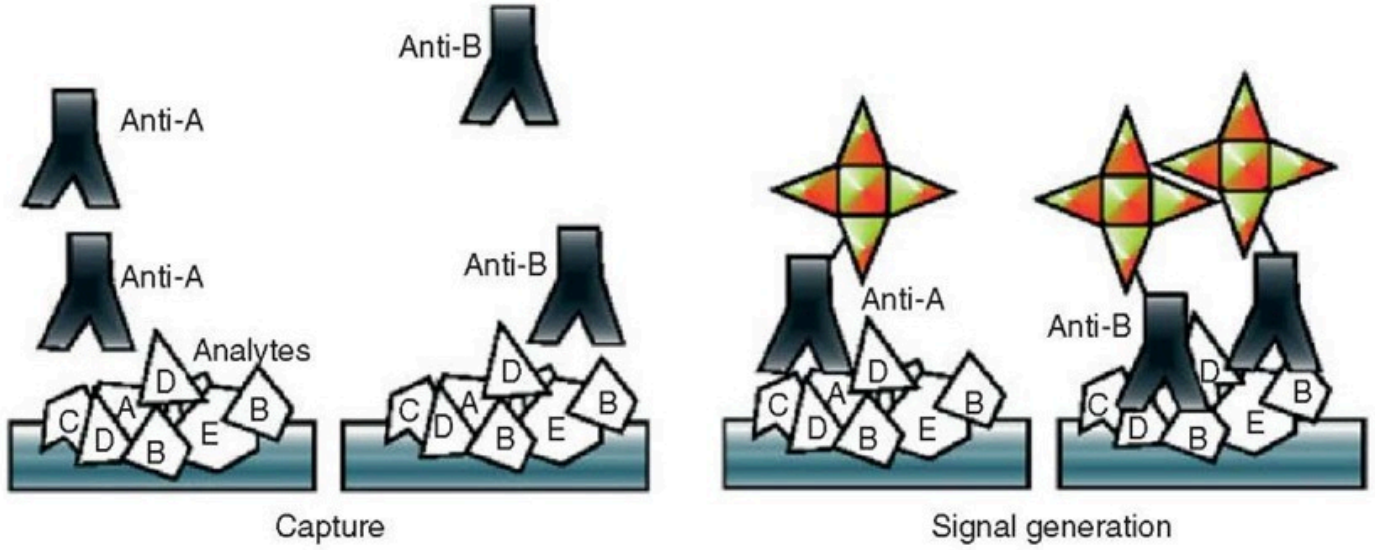
Sowa et al. Nucleic Acids Res. 2017 Feb 28;45(4):1673-1686. doi: 10.1093/nar/gkx048.

Protein Arrays

Forward Phase Protein Microarray Spots Antibody and Probes with Multiple Samples



Reversed Phase Protein Microarray Spots Many Lysates and Probes with an Antibody



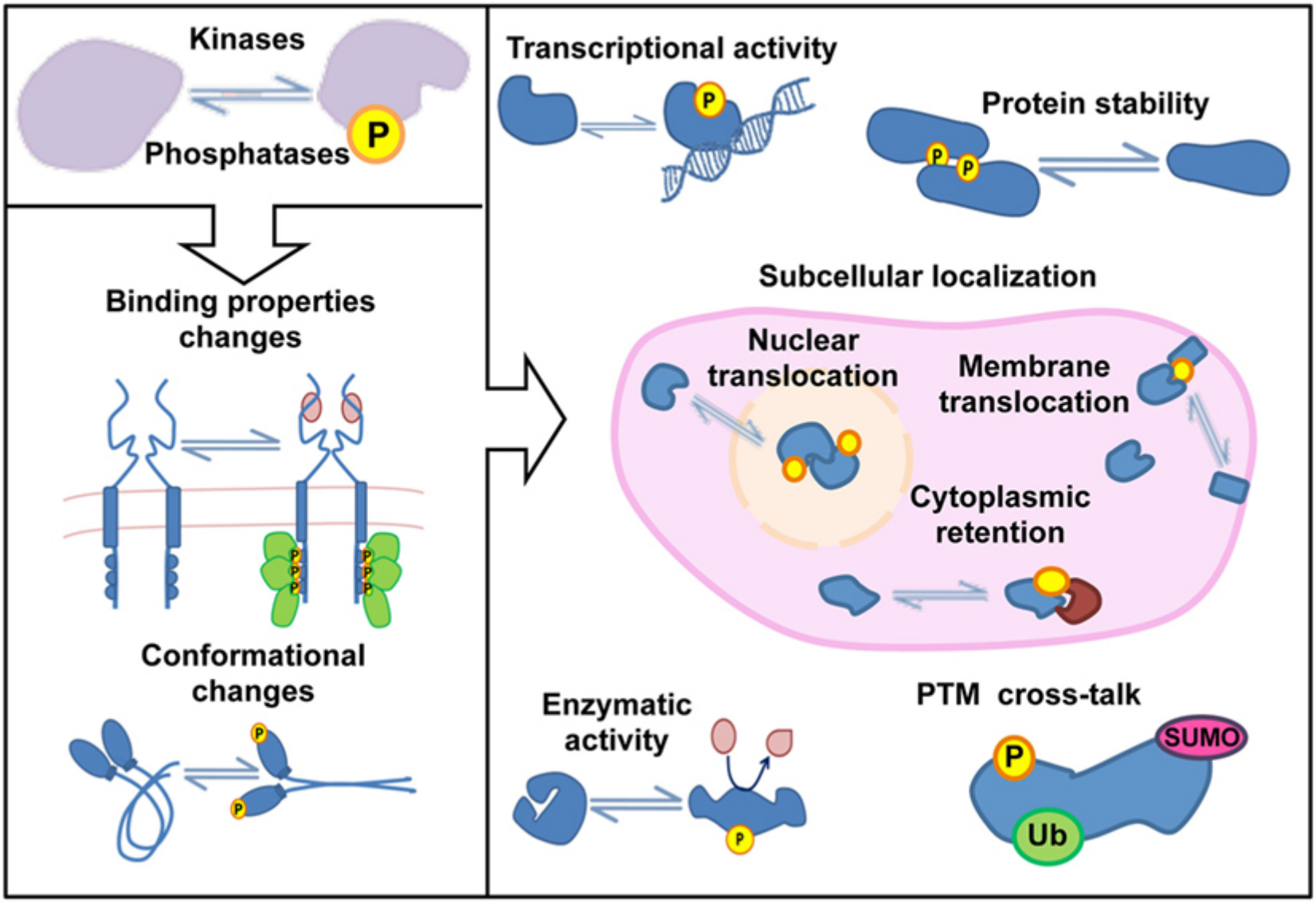
http://what-when-how.com/proteomics/basic-techniques-for-the-use-of-reverse-phase-protein-microarrays-for-signal-pathway-profiling-proteomics/

The Cancer Proteome Atlas

https://tcpaportal.org/tcpa/

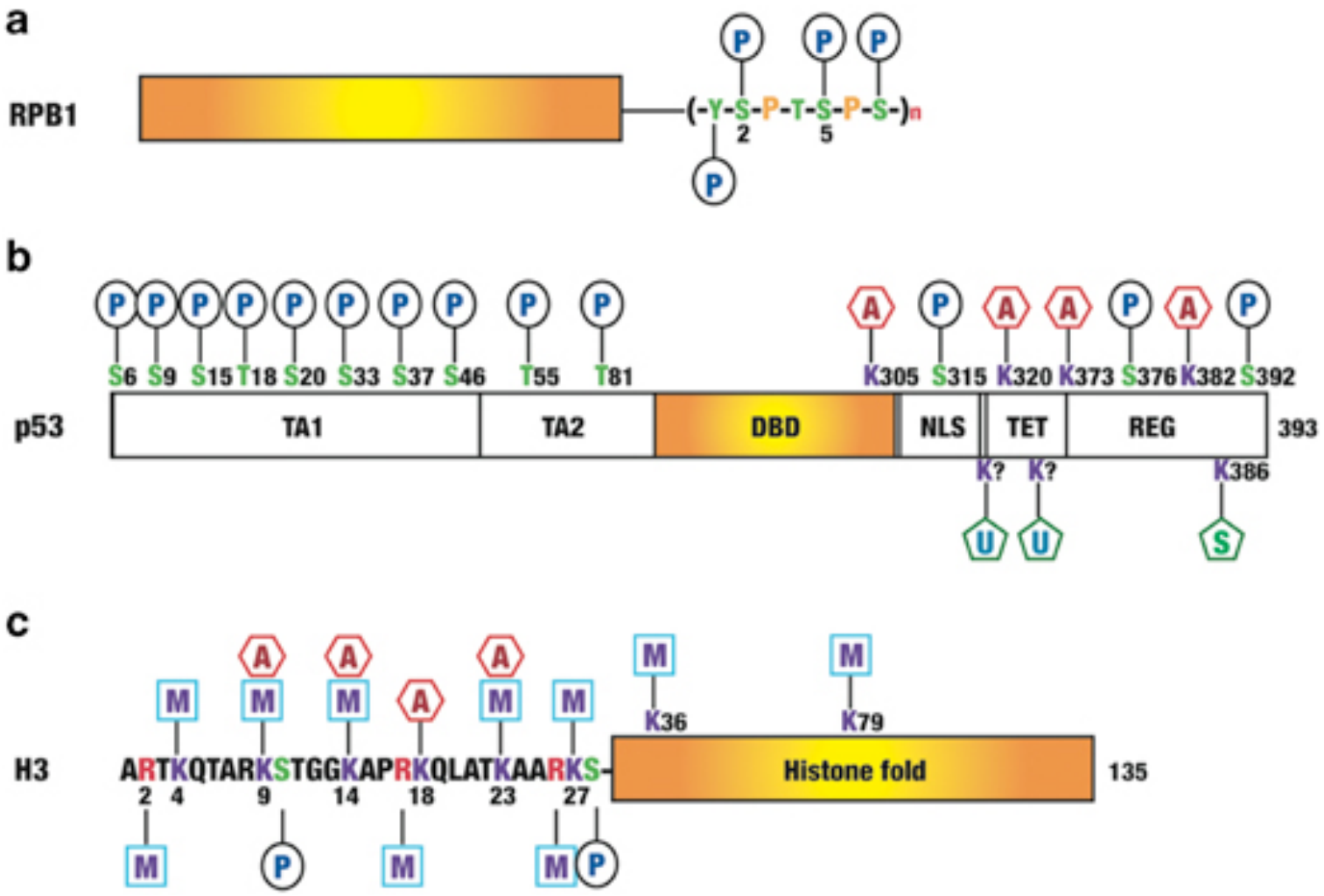
Post-translational Modifications

Functional effects of phosphorylation



Front. Immunol., 09 August 2017 | https://doi.org/10.3389/fimmu.2017.00938

Many kinds of modifications and combinations



P in oval, phosphorylation; A in hexagon, acetylation; U in pentagon, ubiquitination; S in pentagon, sumoylation; M in square, methylation

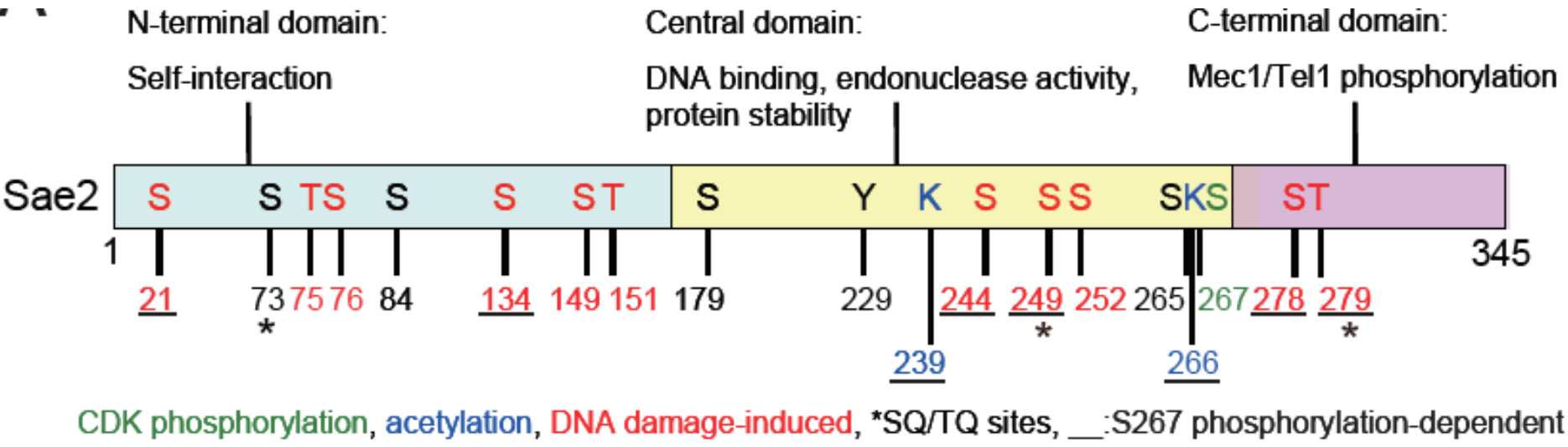
Xiang-Jiao Yang, **Multisite protein modification and intramolecular signaling** Oncogene (2005) 24, 1653–62

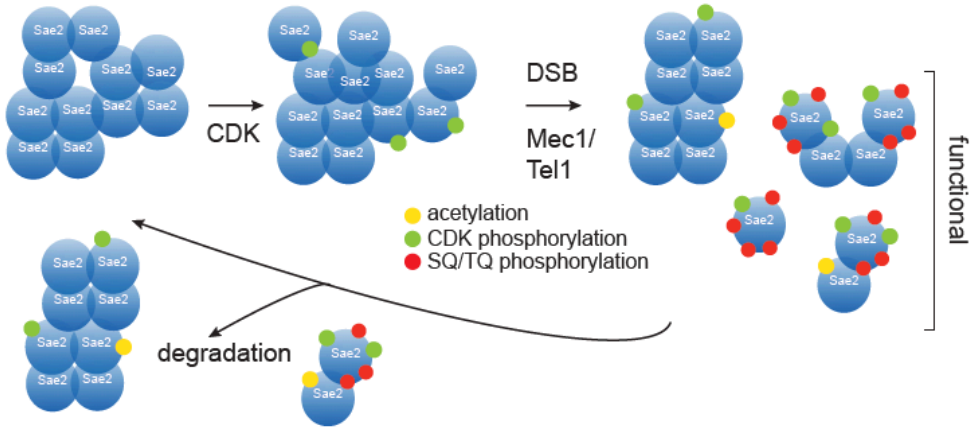
Detecting Modifications by MS

- Start with microgram levels of single protein or mg of complex sample
- Use modification enrichment: affinity chromatography, antibody pulldown, biotinylation, click chemistry
- Purify protein/protein complex
- Use multiple proteases to increase coverage
- Try targeted MS/MS on modified peptide
- Use Ascore to asses site localization
- Validate with synthetic modified peptide standard or antibody

Single Protein: PTM controls activation of Sae2

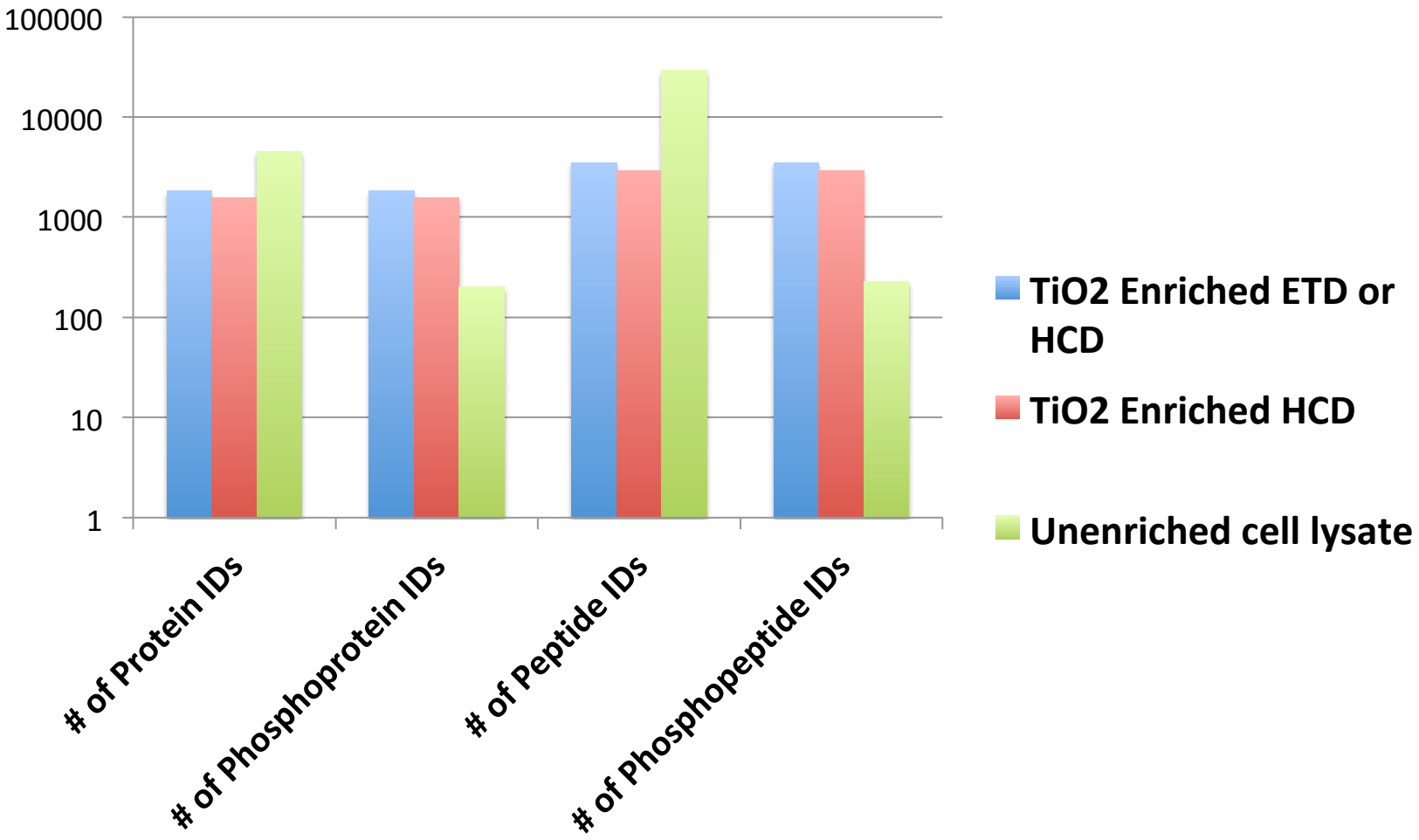
LC-MS/MS using multiple proteases map modifications of Sae2



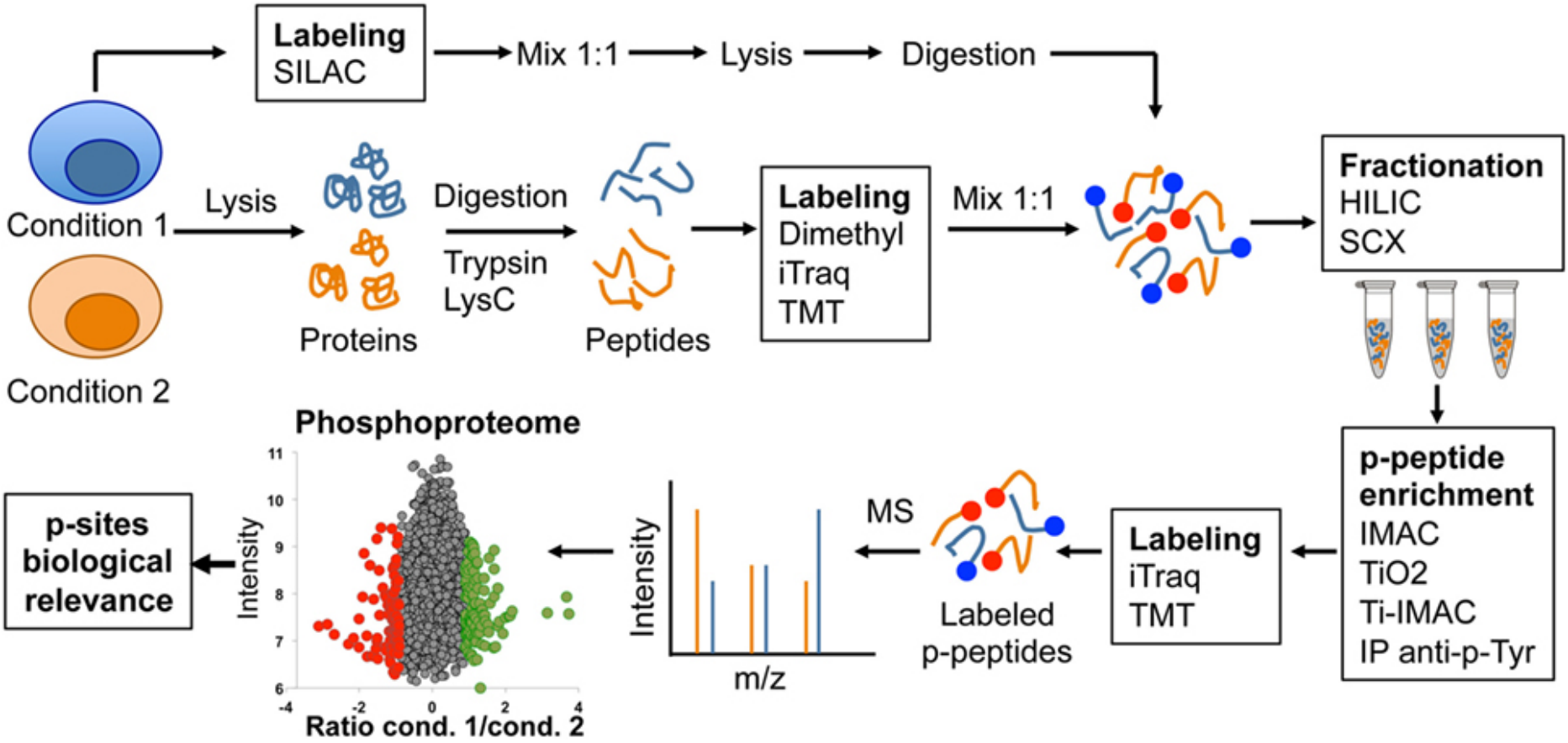


Fu et al. Mol Cell Biol. 2014 Mar;34(5):778-93. doi: 10.1128/MCB.00963-13.

Proteome wide: phosphopeptide enrichment



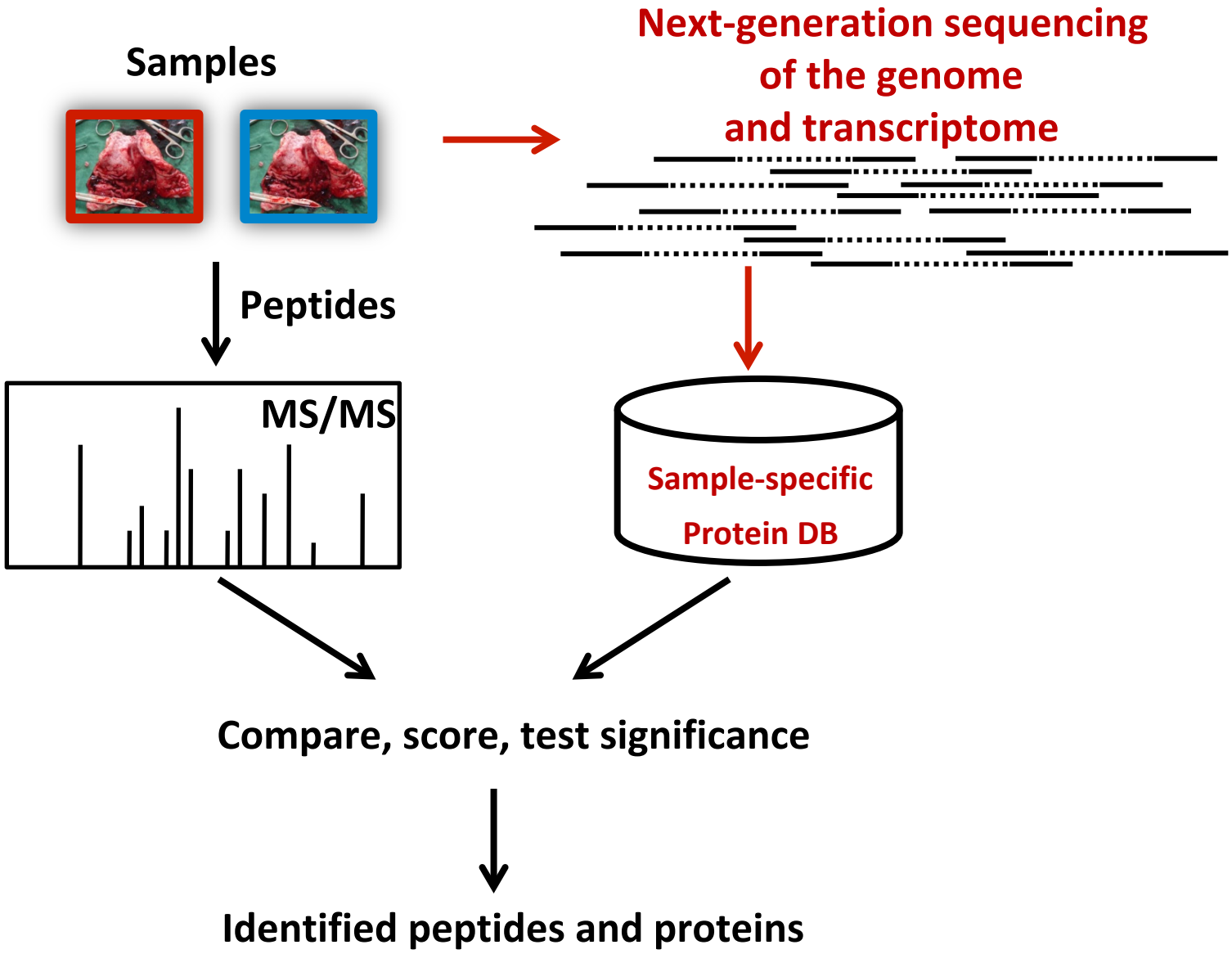
Phosphoproteomics workflow



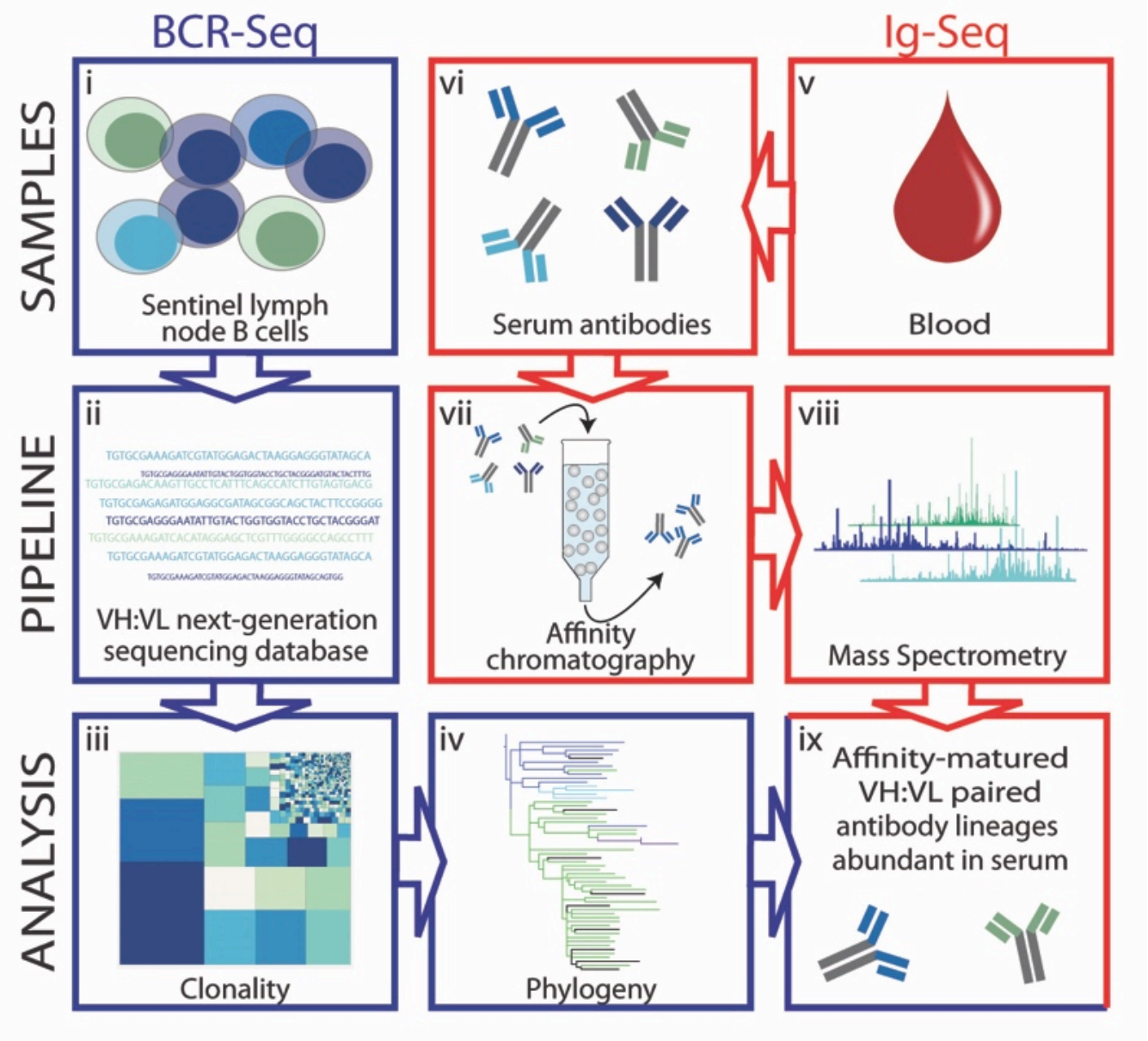
Front. Immunol., 09 August 2017 | https://doi.org/10.3389/fimmu.2017.0093

Proteogenomics

Tumor Specific Databases

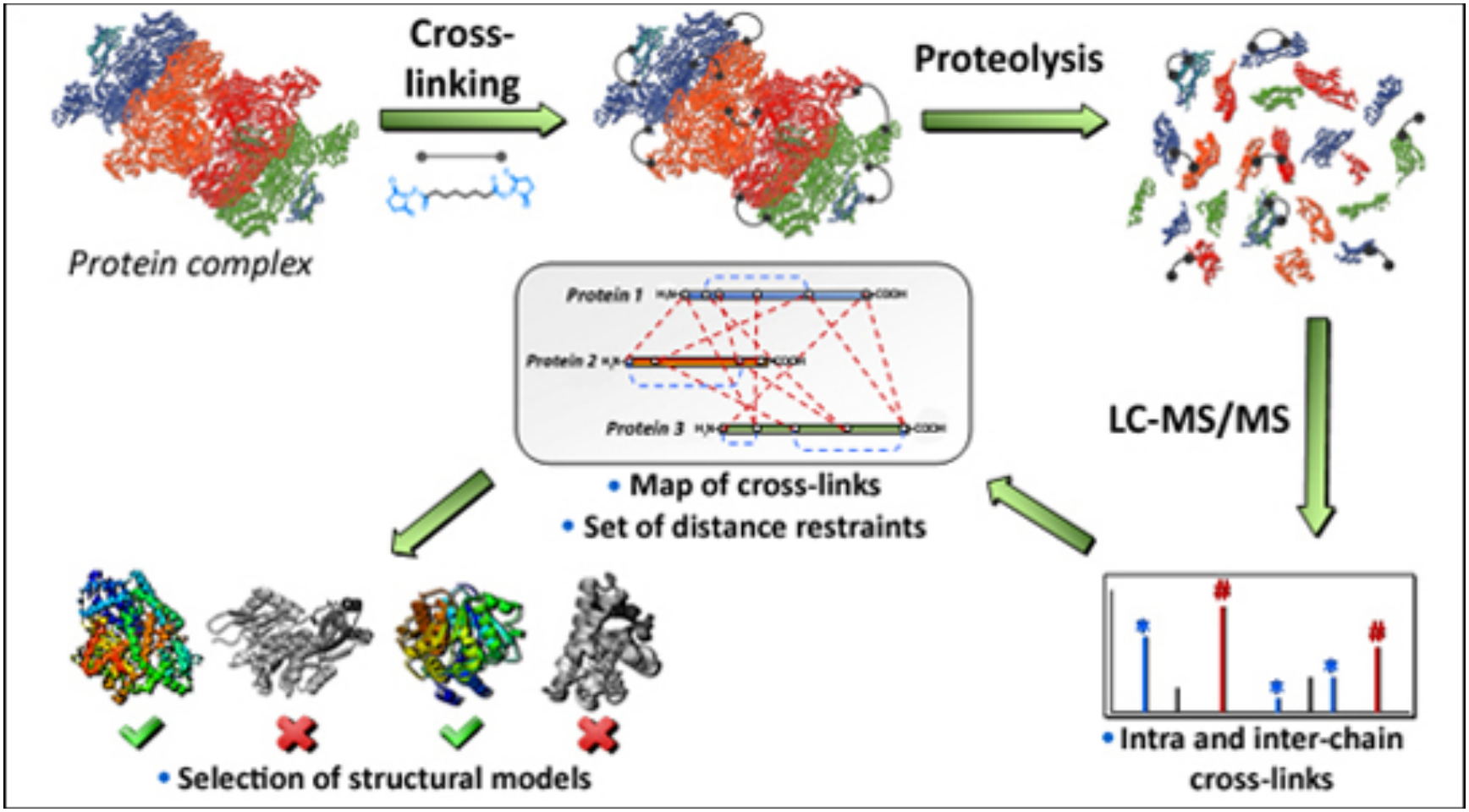


Georgiou lab: B-cell sequencing and quantitative immunoproteomics in breast cancer patients



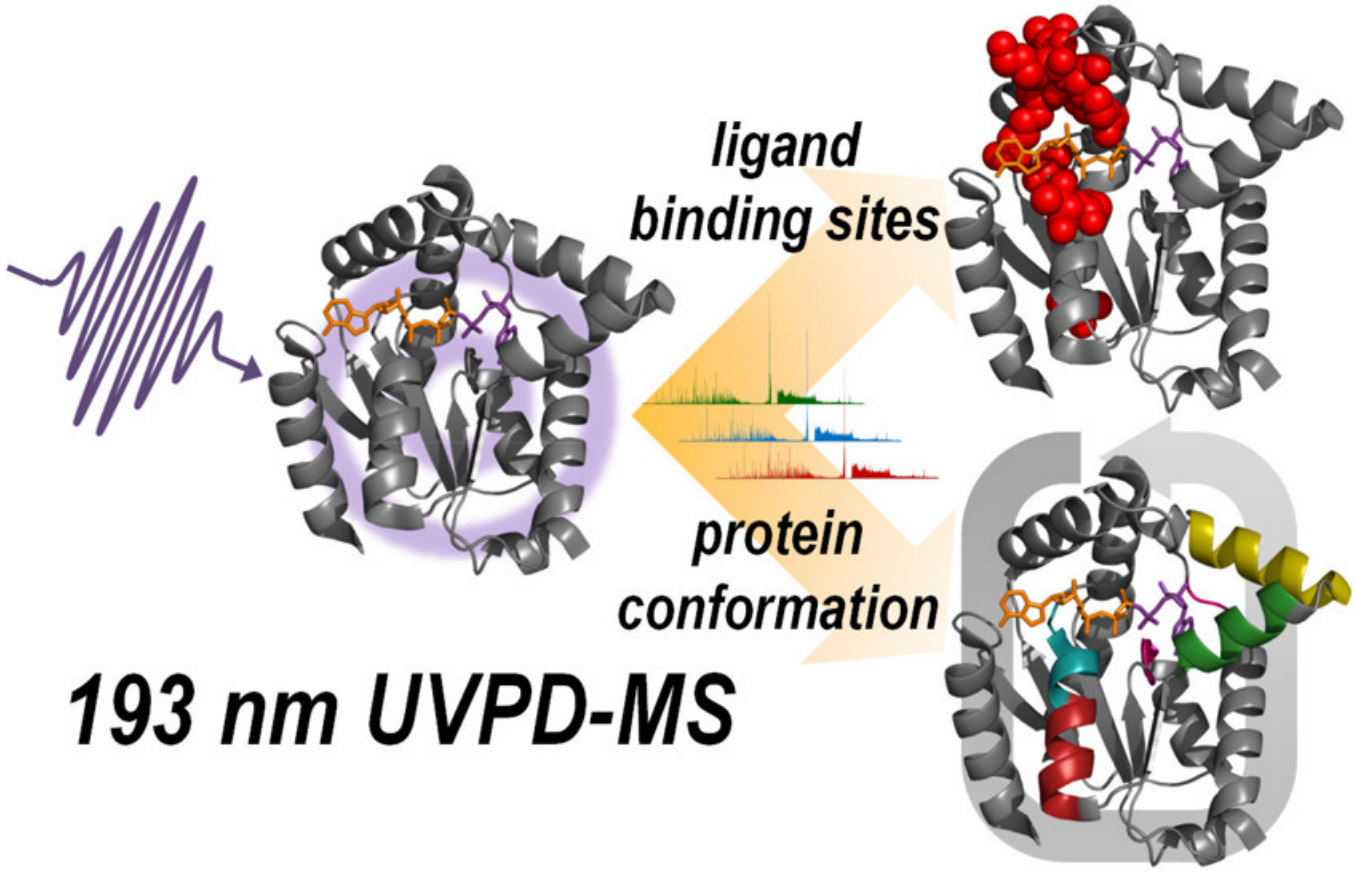
Structural Proteomics

Crosslinking MS



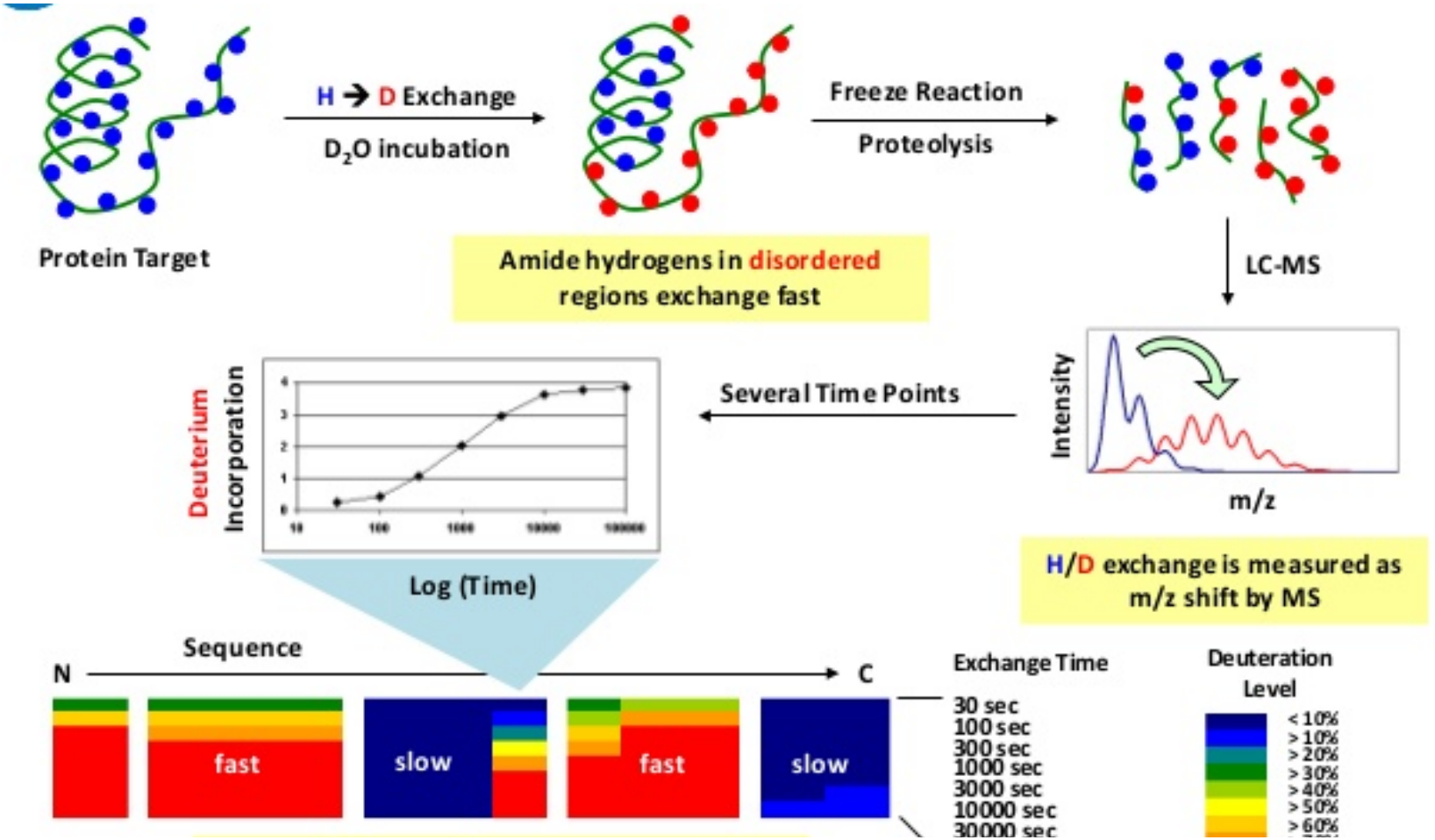
http://daltonlab.iqm.unicamp.br/research.html Fabio Gozo Dalton Mass Spectrometry Lab

Brodbelt lab uses top-down UVPD to measure protein conformation changes and ligand binding sites



Published in: M. Rachel Mehaffey; Michael B. Cammarata; Jennifer S. Brodbelt; *Anal. Chem.* **2018,** 90, 839-846.
DOI: 10.1021/acs.analchem.7b03591

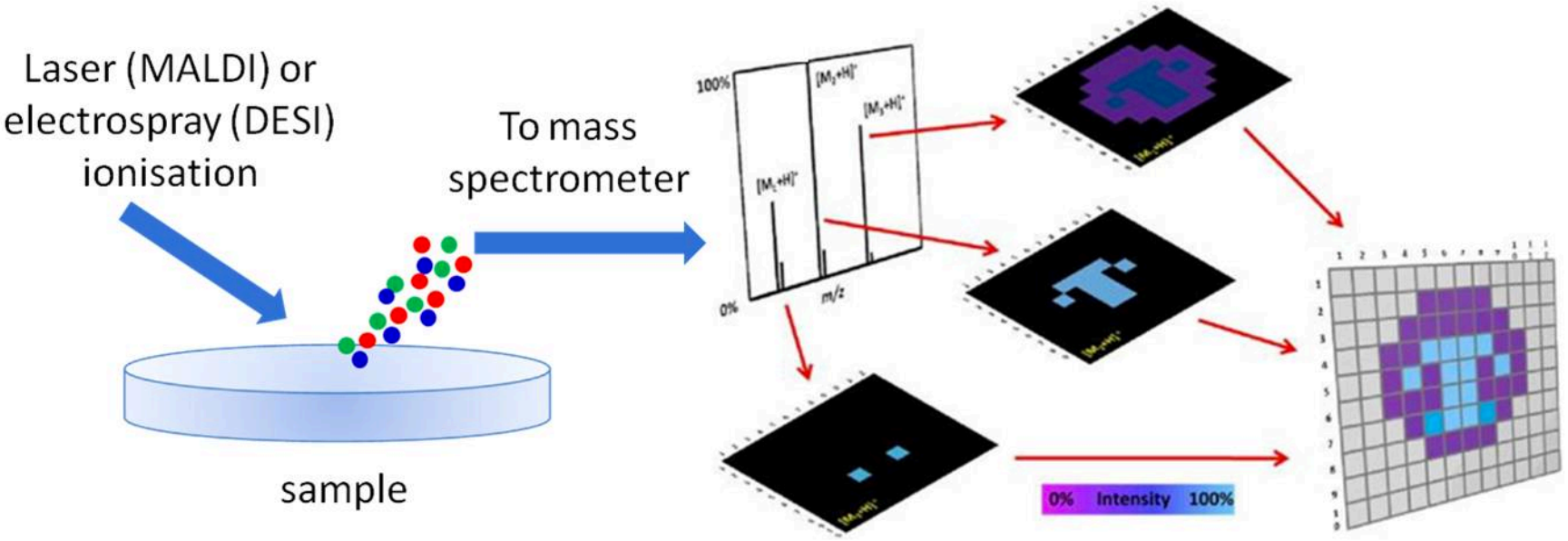
Hydrogen Deuterium Exchange



Hamuro http://www.slideshare.net/Chrom_Solutions/biopharmfocusonhdxappswebinar

Imaging Mass Spectrometry

Imaging Mass Spectrometry (IMS)



- MS from tissue sections generate multiple images based on m/z from selected biomolecules

http://blog.waters.com/do-you-see-what-we-see-mass-spectrometry-imaging-is-revealing-insights-in-biomedical-research

Imaging MS talk tomorrow

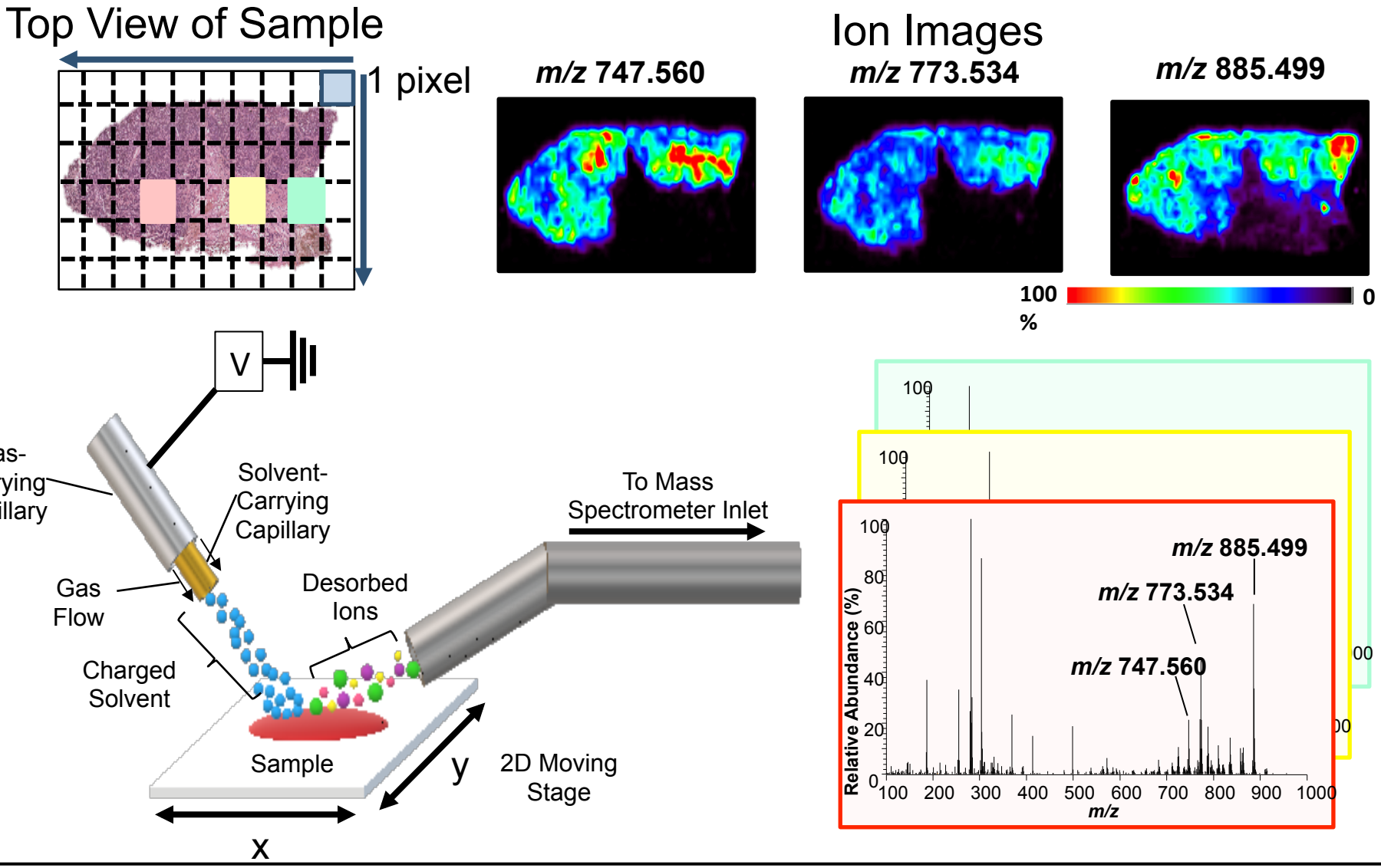
Dr. Erin Seeley, UT Austin Mass Spectrometry Imaging Facility Director

"Mass Spectrometry Imaging for Molecular Histology and Clinical Biomarker Discovery"

April 2, at 3 pm via Zoom, contact mperson@austin.utexas.edu for link

Review paper available on Proteomics Facility Wiki page

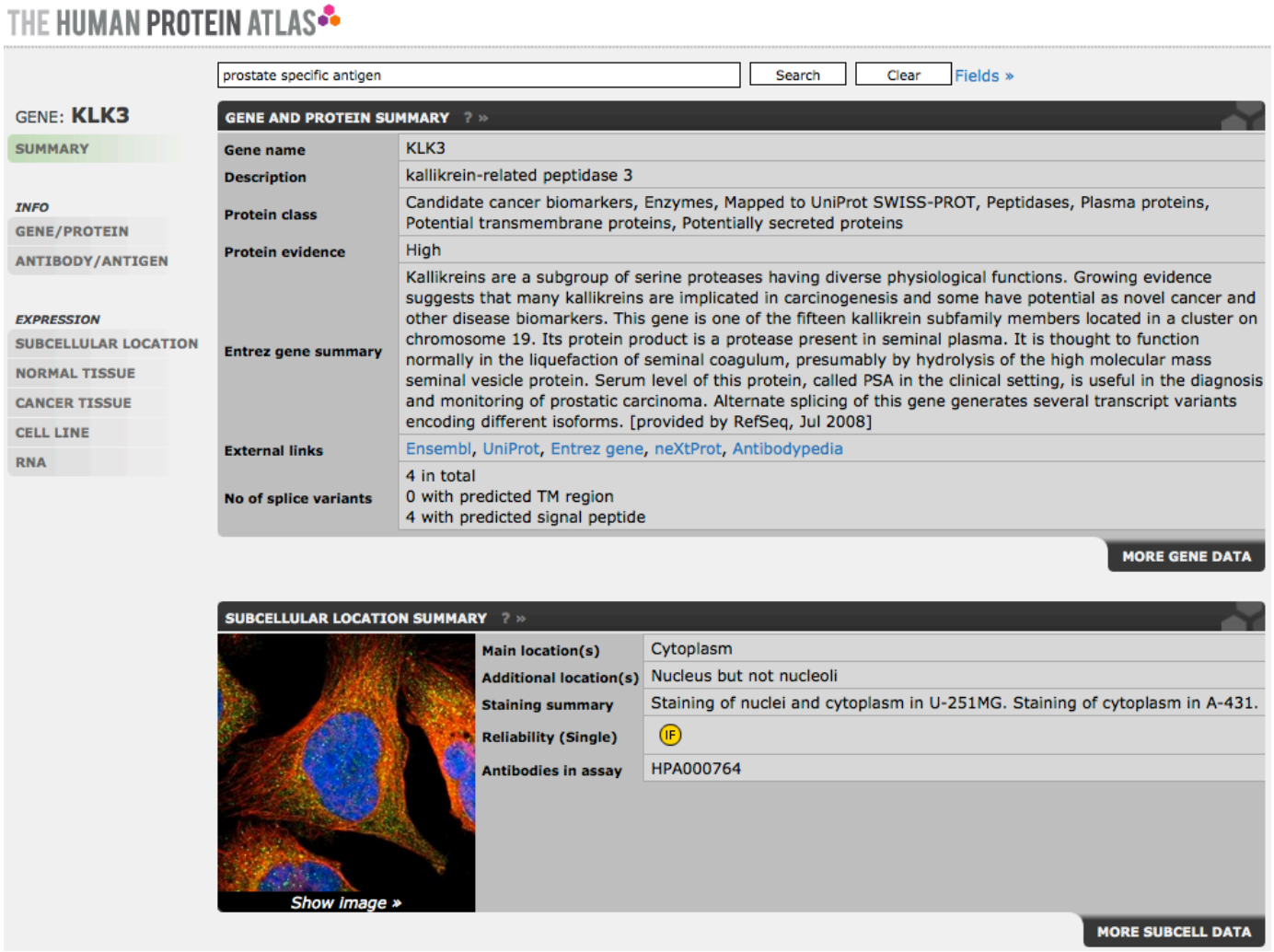
Livia Eberlin lab: Desorption Electrospray Ionization-Mass Spectrometry (DESI-MS) Tissue Imaging



Cellular Localization

Cellular Maps using Tissue Array and Omics Human Protein Atlases: Tissue, Cell and Pathology

v16 with more than 25,000 antibodies, targeting proteins from 17,000 human genes

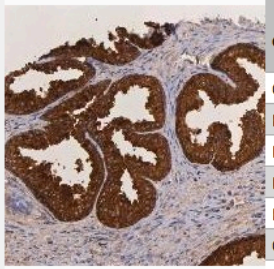


http://www.proteinatlas.org/

PSA localized in prostate tissue and expressed in prostate cancer

NORMAL TISSUE & ORGAN SUMMARY

Expression summary	Cytoplasmic expression exclusively in prostate. Caution: Based on antibodies targeting proteins from multiple genes.
Tissue specificity	Expressed in 1 out of 82 cell types
Reliability (APE)	High
Antibodies in assay	CAB000070, HPA000764



Show image »

Organ	No of cell types	Protein expression
CNS (brain)	11	
Hematopoietic (blood)	8	
Liver and pancreas	5	
Digestive (GI-tract)	13	
Respiratory (lung)	4	
Cardiovascular	1	
Female tissues	13	
Placenta	2	
Male tissues	5	
Urinary tract (kidney)	3	
Skin and soft tissues	14	
Endocrine tissues	3	

Level of annotated protein expression: High, Medium, Low, None

MORE TISSUE DATA

CANCER TISSUE SUMMARY

Staining summary	Antibody staining in 5% of the cancers
Antibodies in assay	CAB000070, HPA000764

Tissue	Cancer staining	Protein expression of normal tissue	Tissue	Cancer staining	Protein expression of normal tissue
Breast cancer			Melanoma		
Carcinoid			Ovarian cancer		
Cervical cancer			Pancreatic cancer		
Colorectal cancer			Prostate cancer		
Endometrial cancer			Renal cancer		
Glioma			Skin cancer		
Head and neck cancer			Stomach cancer		
Liver cancer			Testis cancer		
Lung cancer			Thyroid cancer		
Lymphoma			Urothelial cancer		

Level of antibody staining: Strong, Moderate, Weak, Negative