A *very short* introduction to proteomics

BP205 – 2020

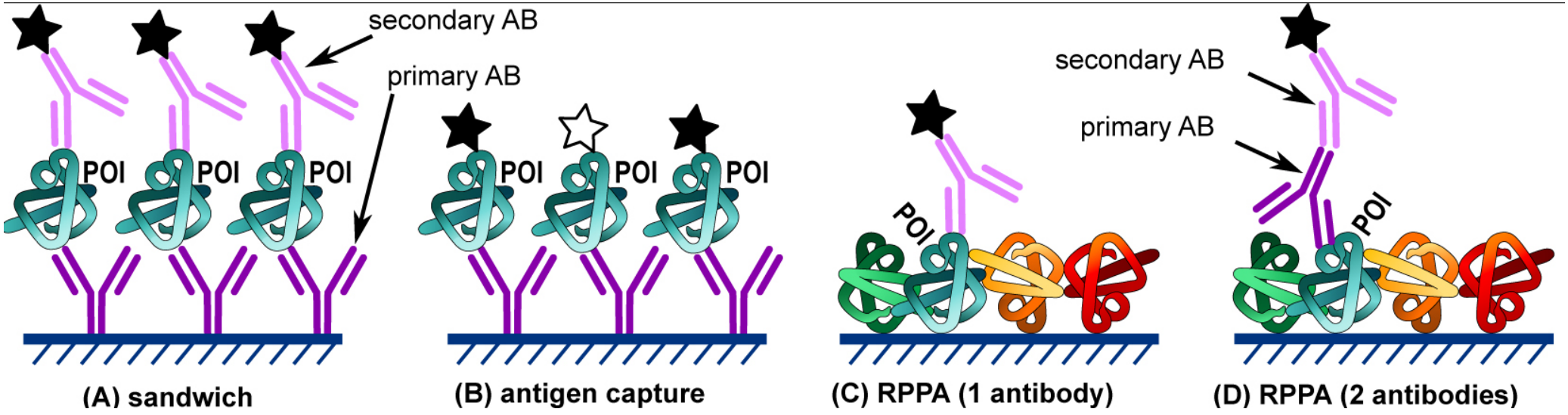
3/25/2020

HARVARD
MEDICAL SCHOOL

Proteomics overview

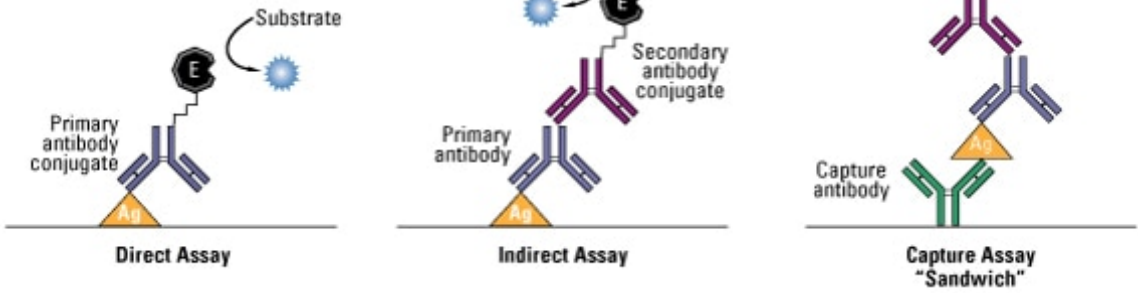
- **A diverse set of assays focused on measuring the identities, abundances and modification states of proteins**
- **Discovery proteomics is typically focused on determining the components of specific macromolecular complexes or compartments**
- **Obtain data on functions and mechanisms of action**
    - Who are the players and what do their interact with?
    - Where and when are they expressed or active?
    - Where are they found in the cell?
    - How does abundance or state vary across perturbation, time or tissue

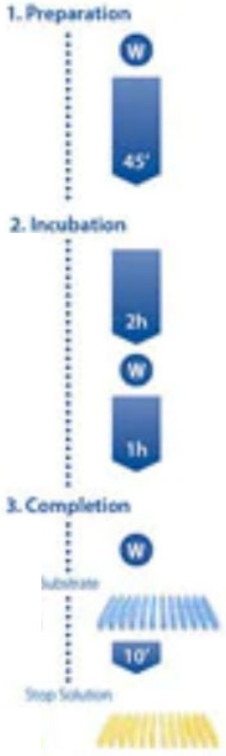
Protein Arrays



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

The classic protein array: ELISA Assays

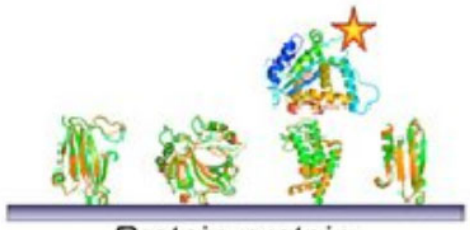




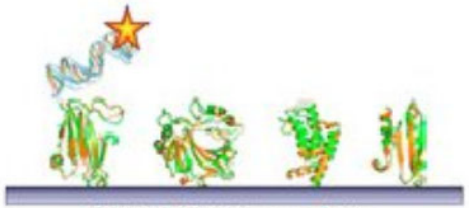
https://www.thermofisher.com/us/en/home/life-science/protein-biology

Other ways to use protein arrays

Protein interactions



Protein-protein

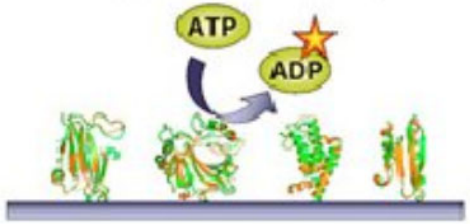


DNA/RNA-protein

Small molecule/Drug-protein

Post-translational modifications

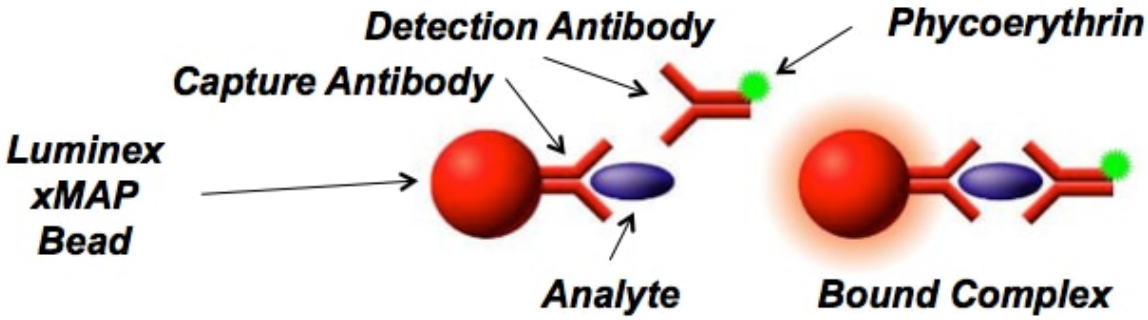
Enzyme activity

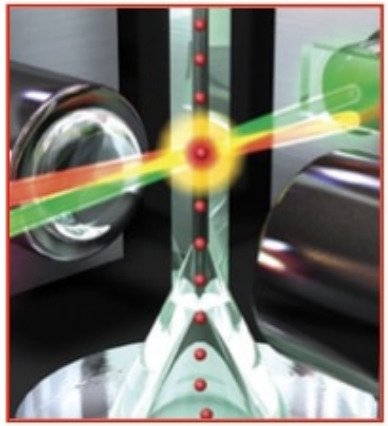


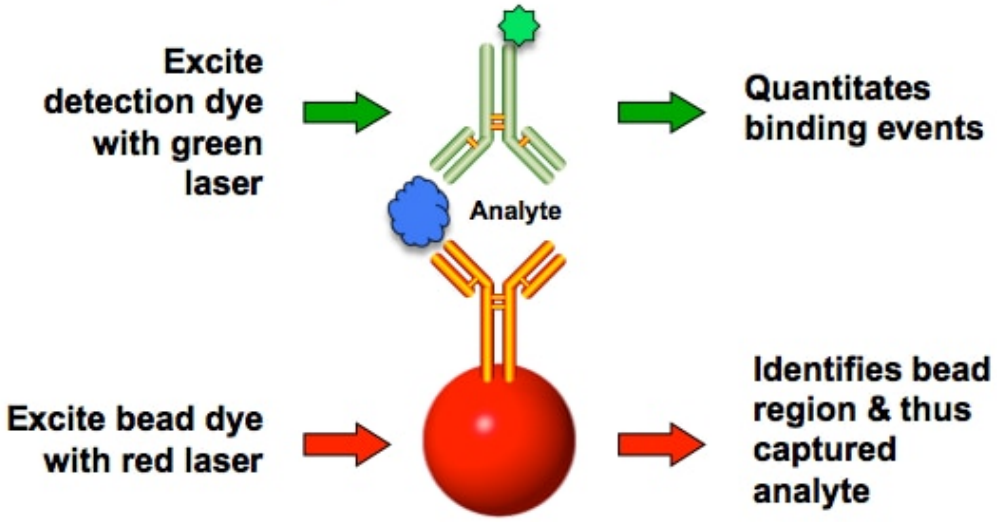
Protein binding
Pathway building
Drug discovery
PTM analysis

•DOI: 10.2174/1389450097884884O4

Sandwich immunoassay on beads ("Luminex Assay")





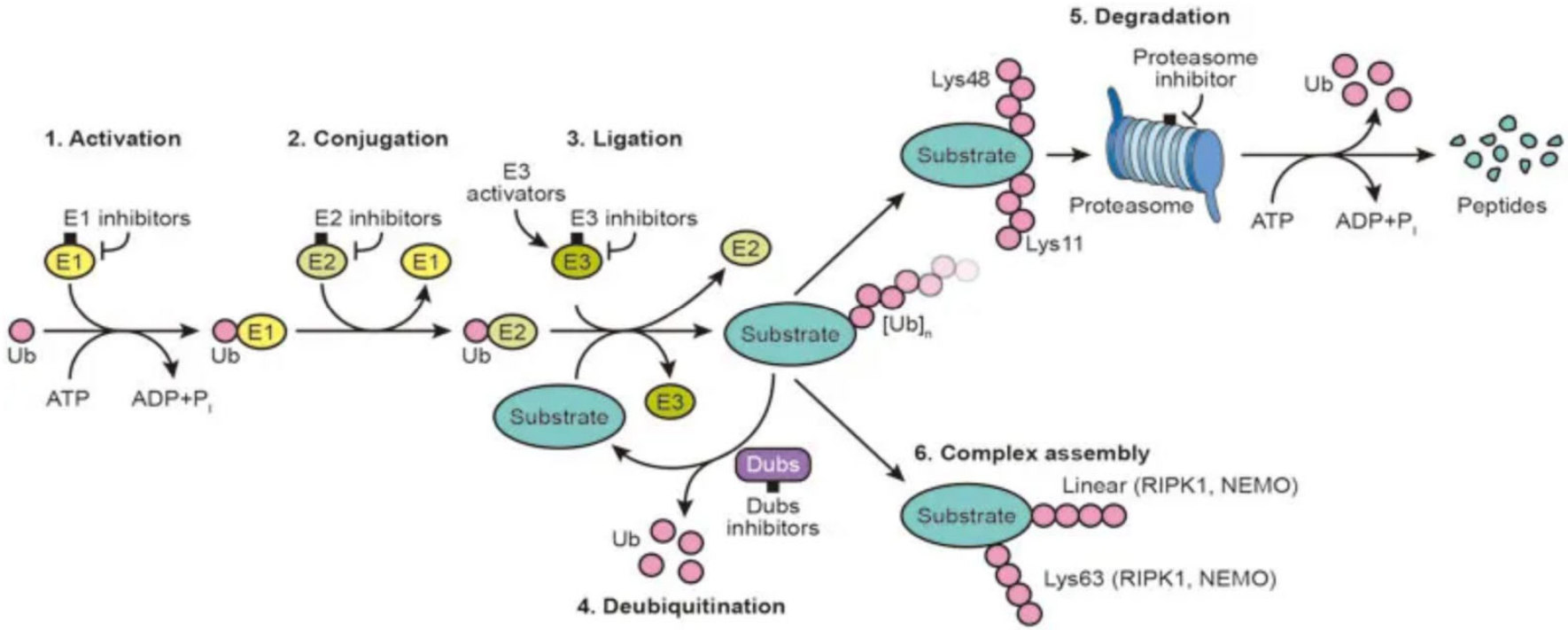


https://www.thermofisher.com/us/en/home/life-science/protein-biology

Post-translational protein modifications

Phosphorylation Ser, Thr, and Tyr
Ubiquitination Lys
Proteolytic Cleavage N-term Met of all proteins removed by aminopeptidases
N-terminal Acylation formyl, acetyl, myristyl, etc. by acyltransferases
Glycosylation Asn, Ser, and Thr
Sulfation Tyr
Carboxyl Terminal Amidation
Hydroxylation Pro, Lys, Asp
N-Methylation Lys, Arg, His, Gln
Carboxylation Glu, Asp
Modifications introduced experimentally: Met [O], Cys-acrylamide

Many modifications are elaborately regulated: ubiquitin modification

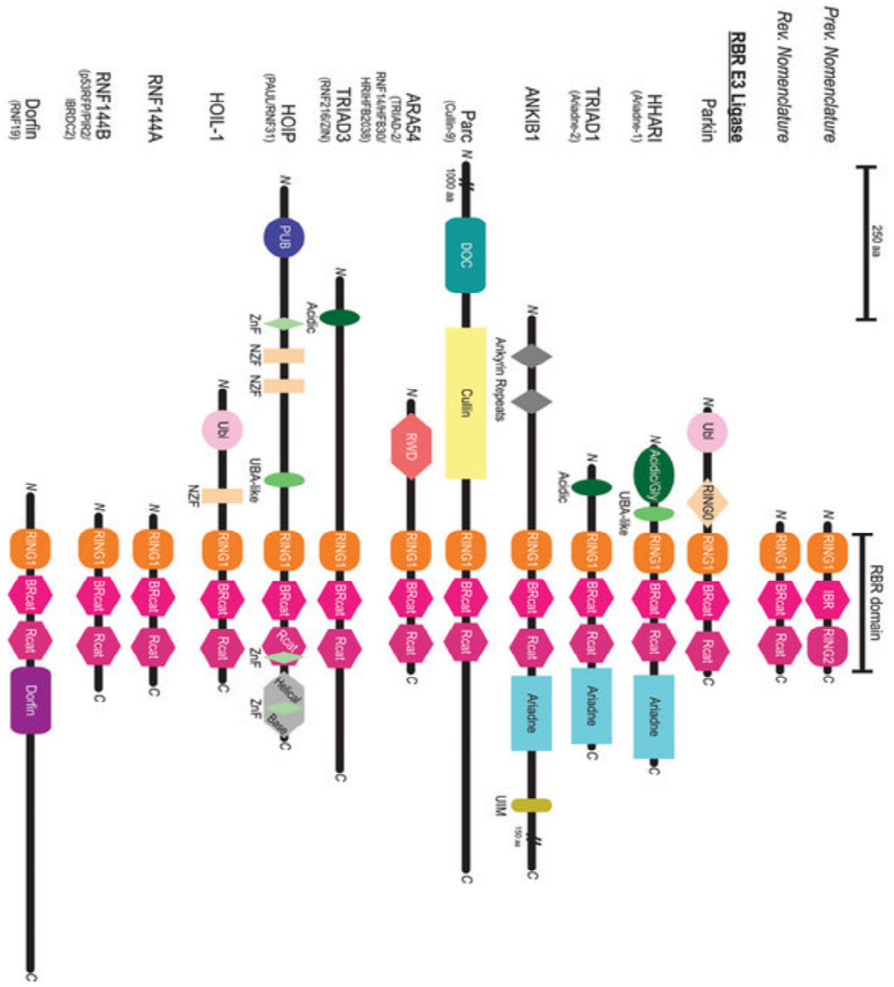


https://www.nature.com/articles/cr201631/figures/1

600–700 E3 ligase genes representing ~5% of the human genome.

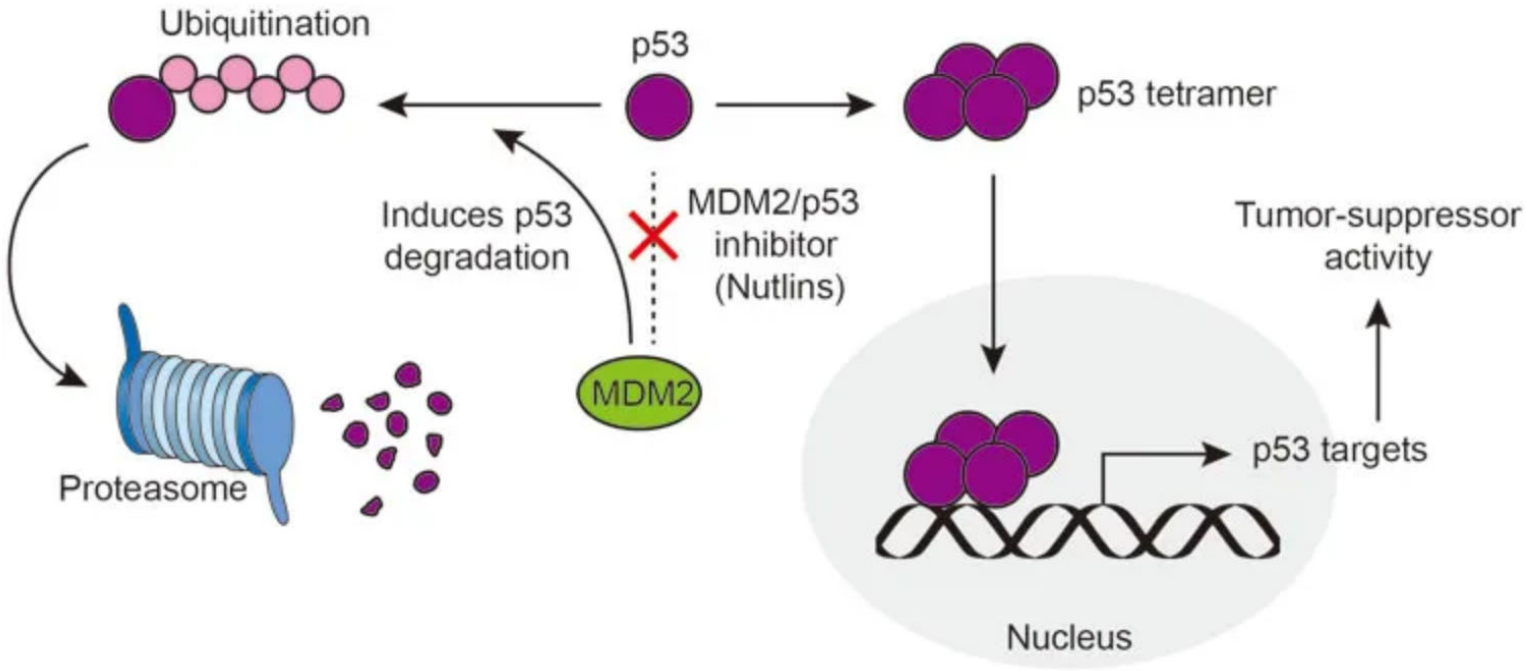
RBR E3 Ligase Family

Repeat domain structure



•DOI: 10.1042/BJ20140006

Drugging the undruggable: MDM2 as a potential anti-cancer target.



https://www.nature.com/articles/cr201631/figures/3

Protein mass spectrometry: dominant proteomic technology

Orbitrap

Q-Exactive

Triple Quadrupole

Discovery/Global Experiments

Targeted MS

Mass-spectrometric exploration of proteome structure and function

Ruedi Aebersold[1,2] & Matthias Mann[3,4]

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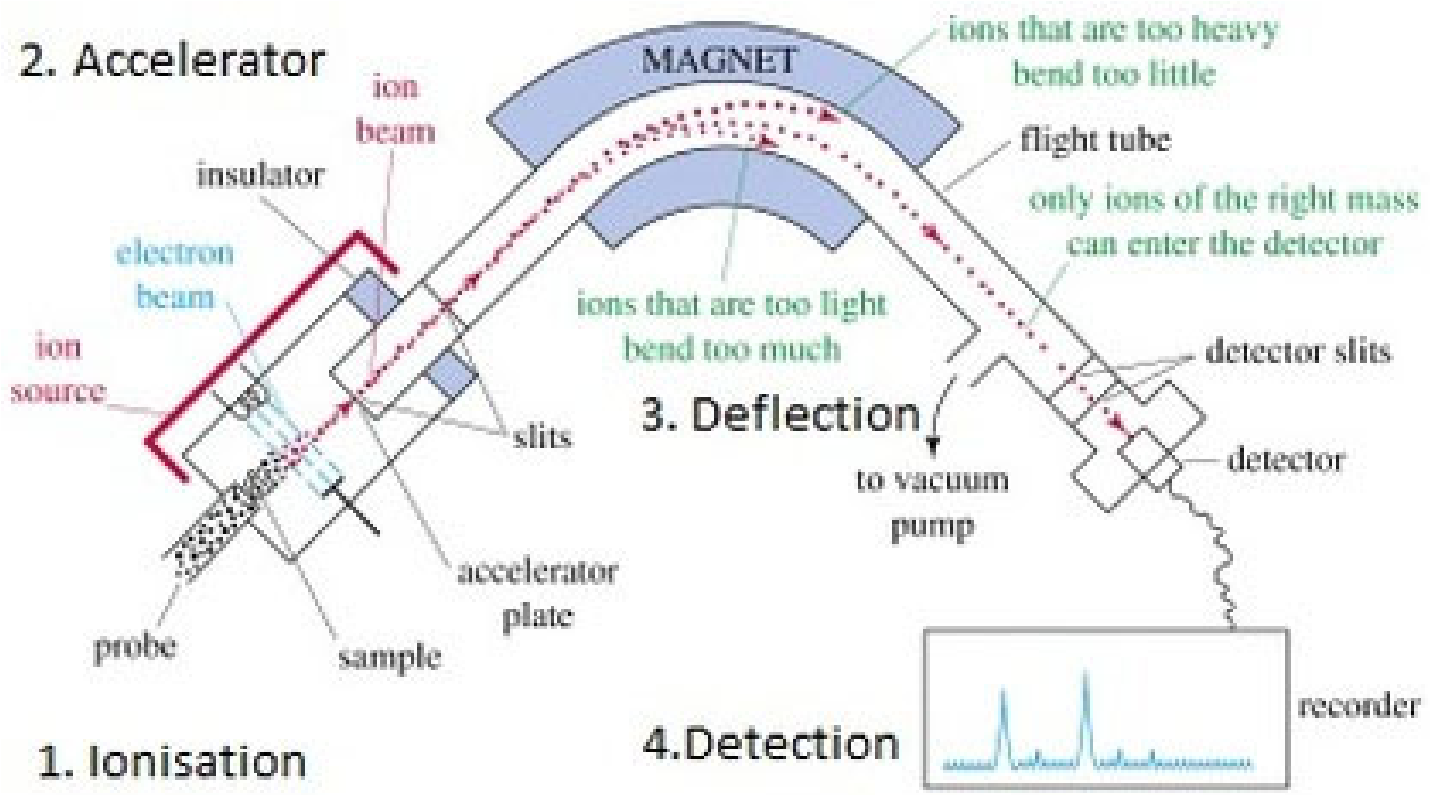
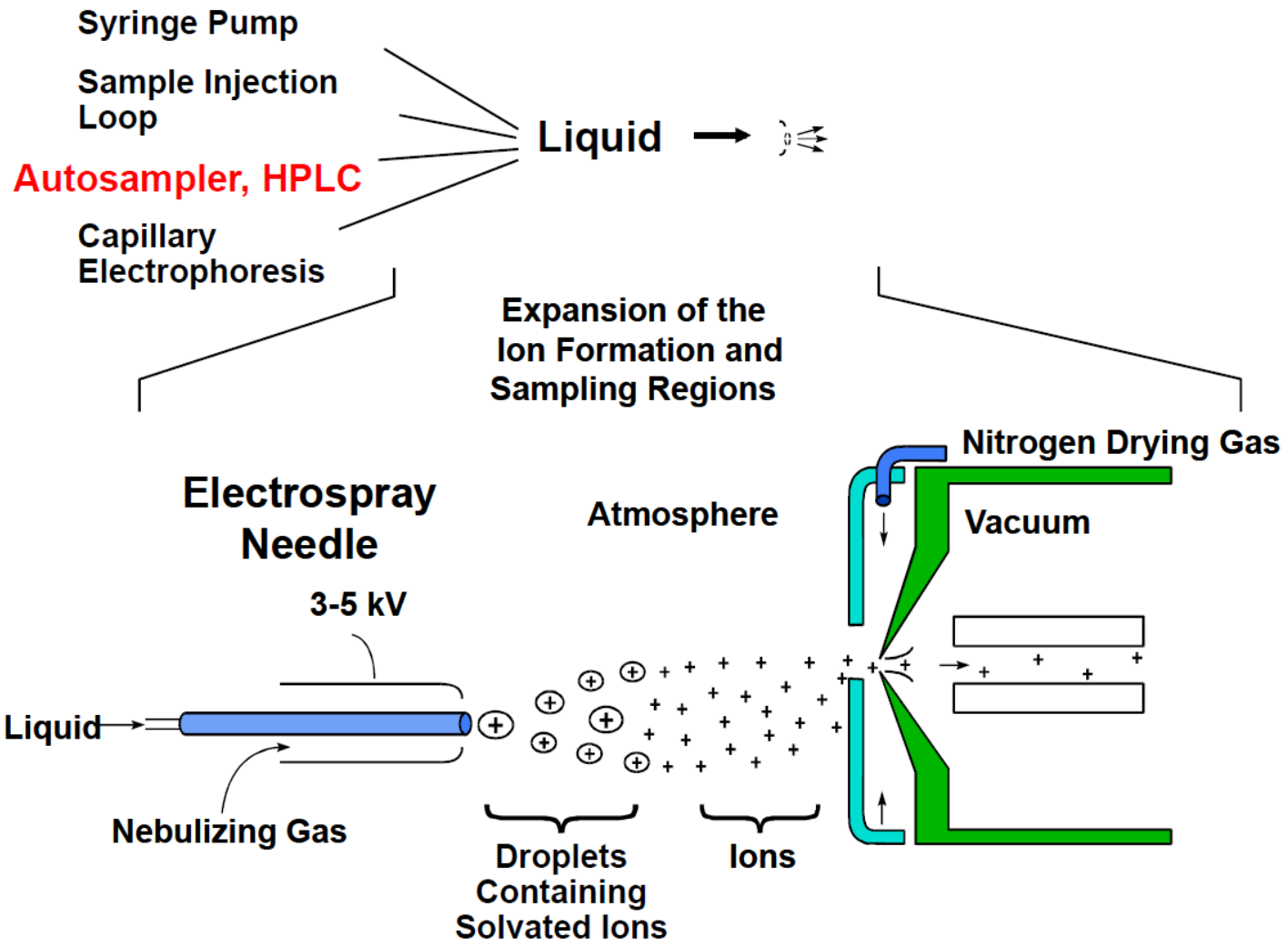
Four steps fundamental to mass spectrometry



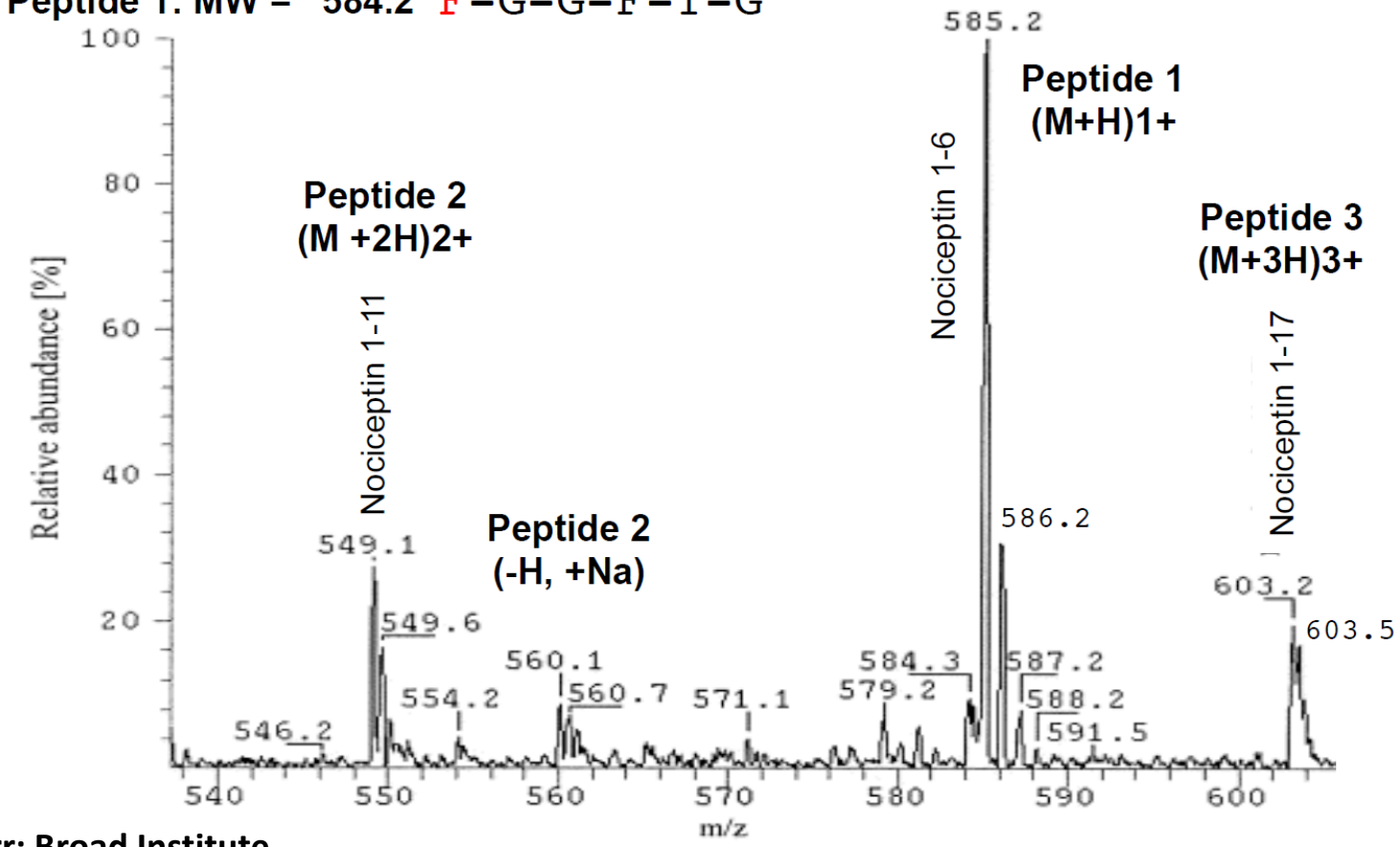
http://chemicalinstrumentation.weebly.com/mass-spectrometry.html

Getting the sample into the instrument: electrospray



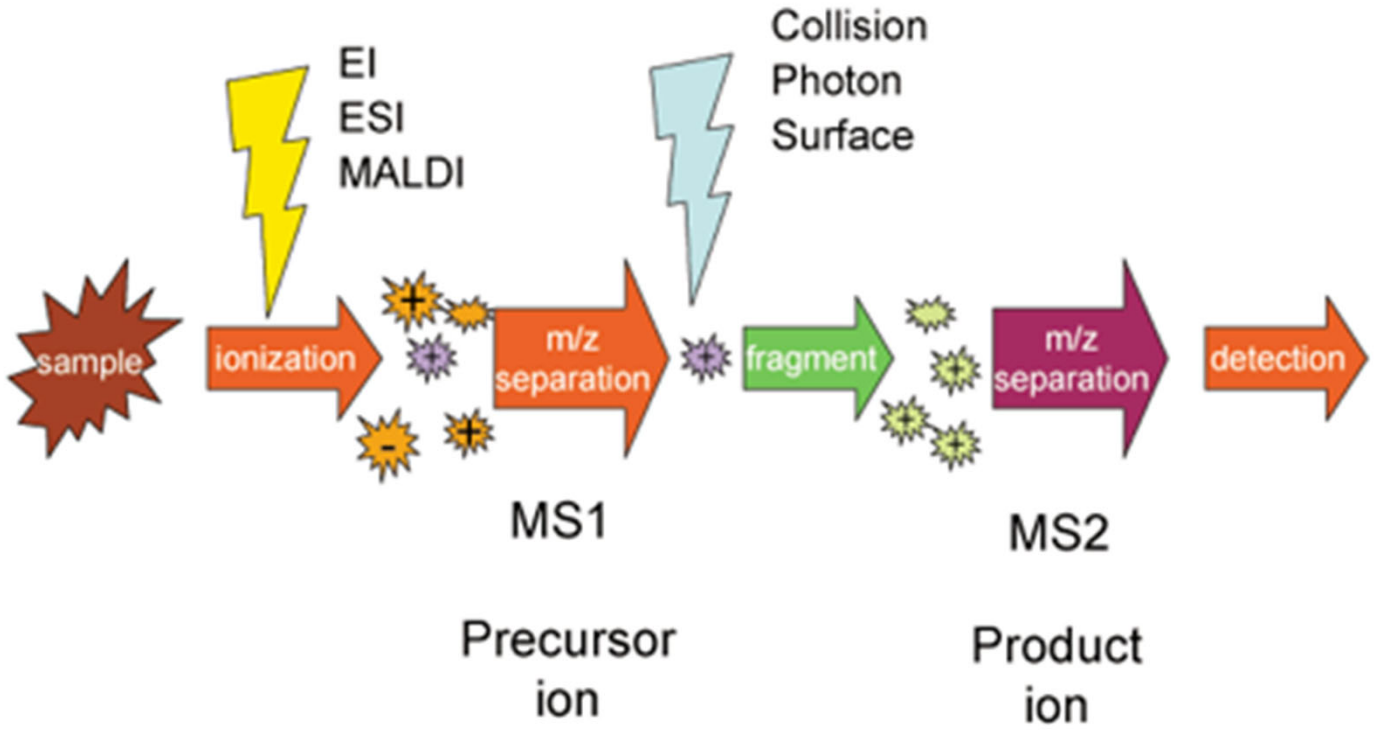
Electrospray MS Spectrum

Peptide 3: MW = 1806.6 F–G–G–F–T–G–A–R–K–S–A–R–K–L–A–N–Q
Peptide 2: MW = 1096.2 F–G–G–F–T–G–A–R–K–S–A
Peptide 1: MW = 584.2 F–G–G–F–T–G



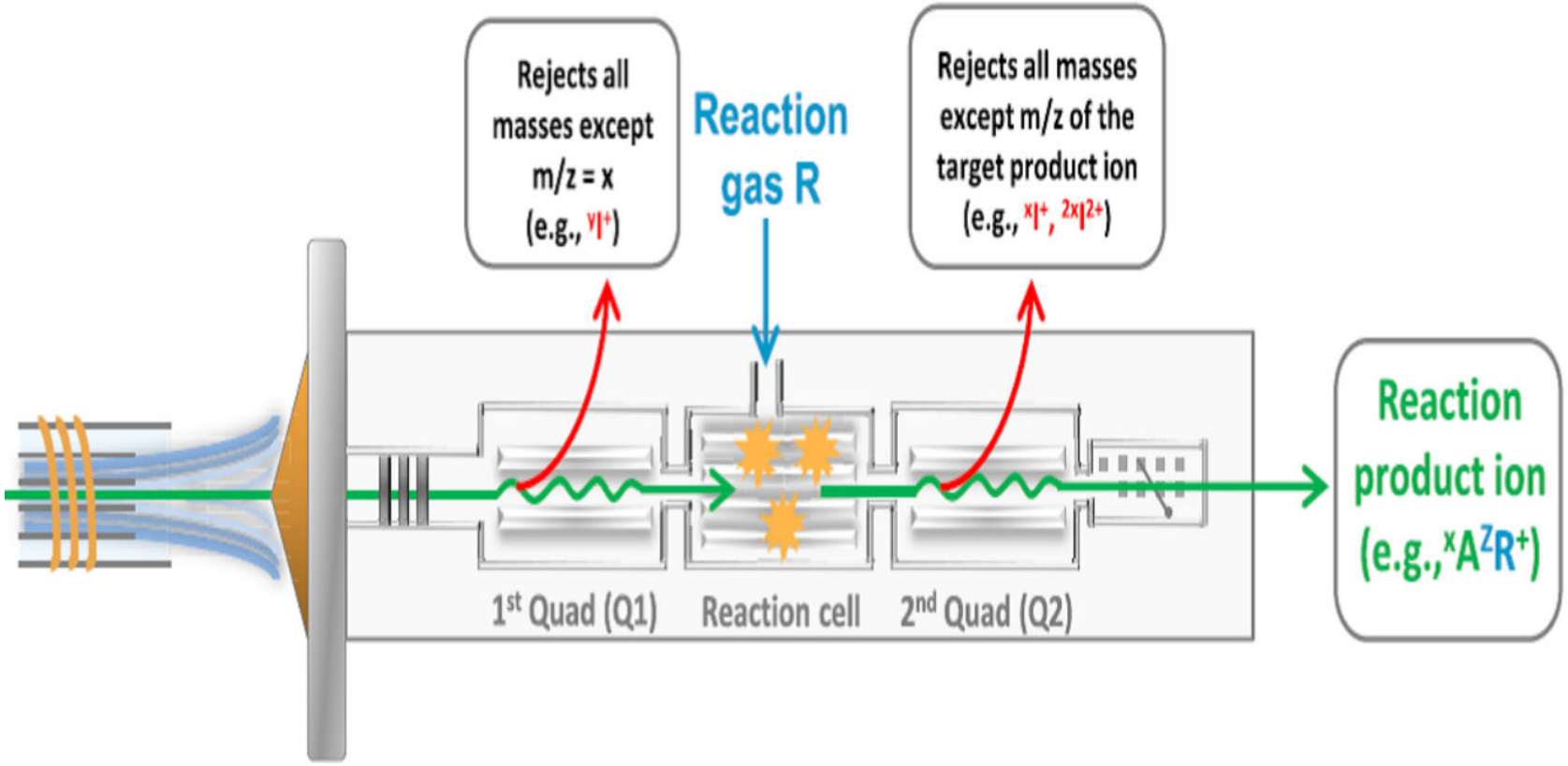
c/o Steve Carr; Broad Institute

MS/MS fragments a peptide to enable identification



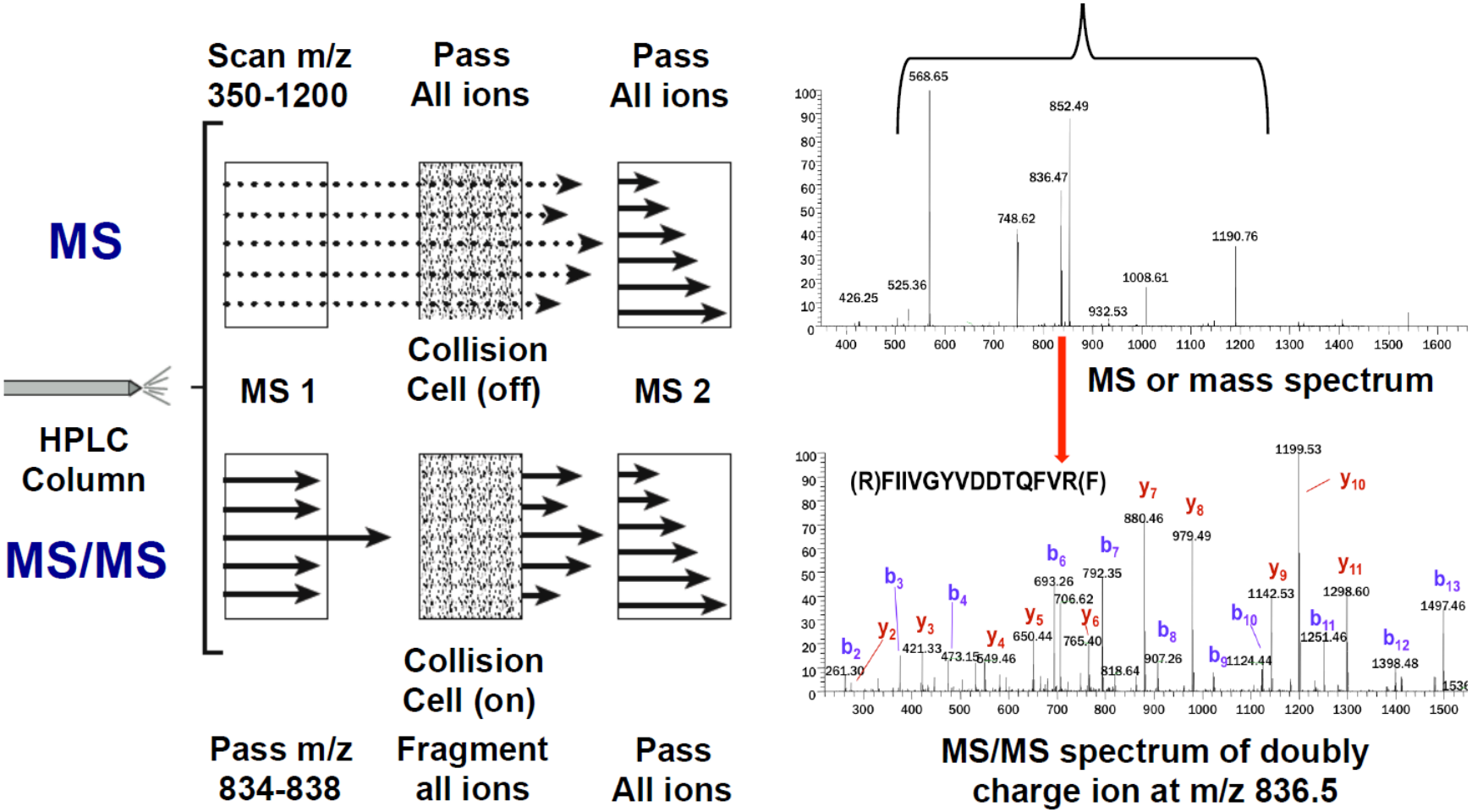
https://nationalmaglab.org/user-facilities/icr/techniques/tandem-ms

MS/MS is accomplished using two (or more) quadrupole magnets



http://chemicalinstrumentation.weebly.com/mass-spectrometry.html

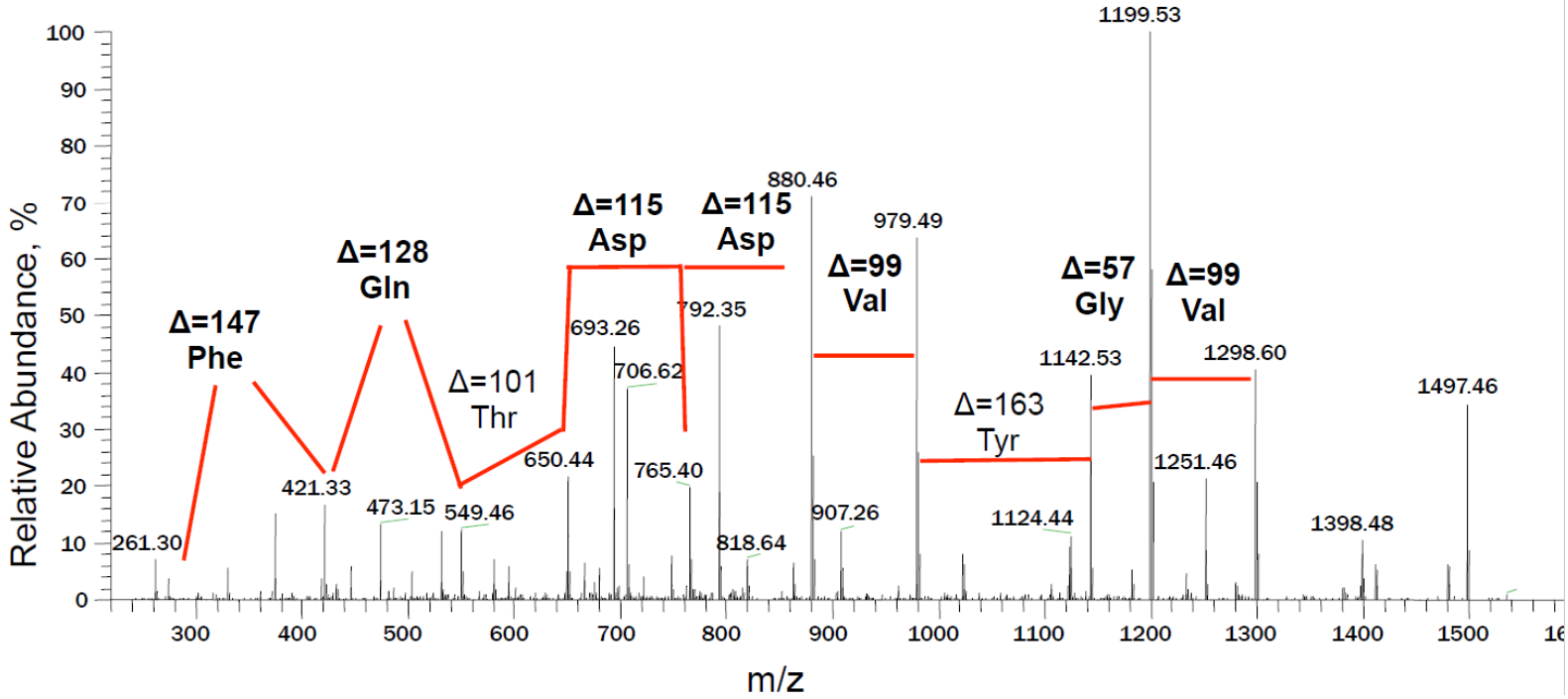
Sequencing proteins by MS/MS



c/o Steve Carr; Broad Institute

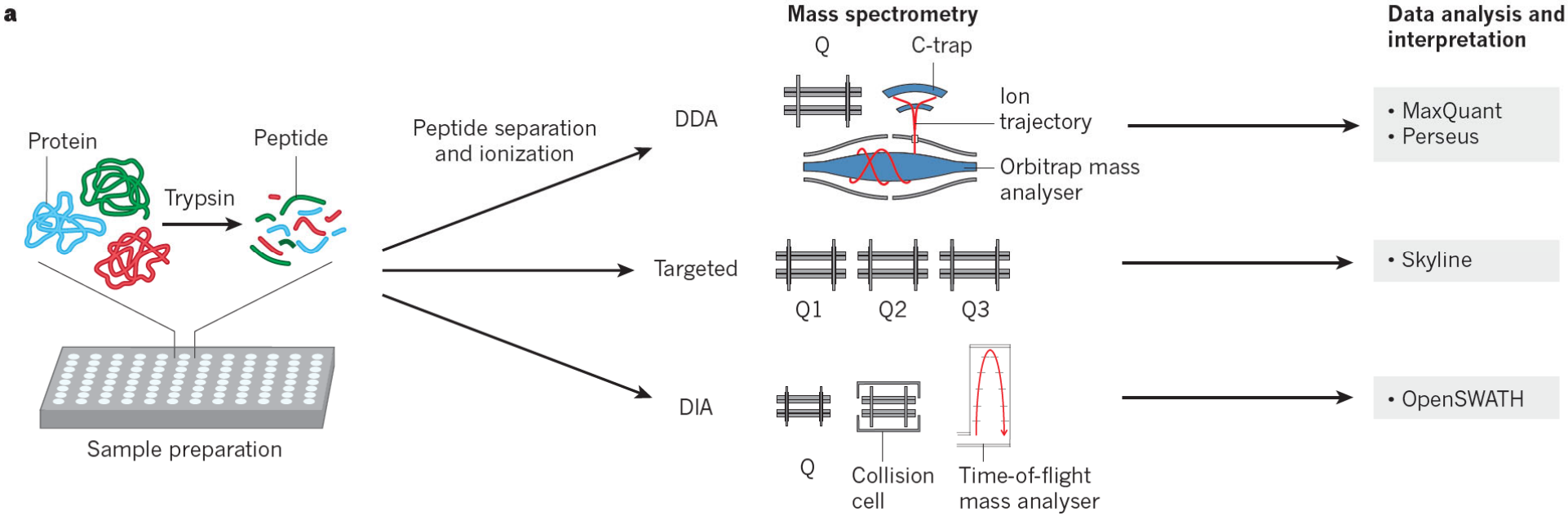
Sequencing proteins by MS/MS

F-I-I-V-G-Y-V-D-D-T-Q-F-V-R



c/o Steve Carr; Broad Institute

LC-MS/MS-based quantitative proteomics



DDA: Data dependent acquisition: top 10–20 peptides per cycle, are sequentially selected from a full mass MS1 scan for fragmentation and acquisition in the MS/MS mode
DIA: Data-independent acquisition identification and quantitation of fragment ions that generated from multiple peptides contained in the same m/z selection window

Mass-spectrometric exploration of proteome structure and function

Ruedi Aebersold[1,2] & Matthias Mann[3,4]

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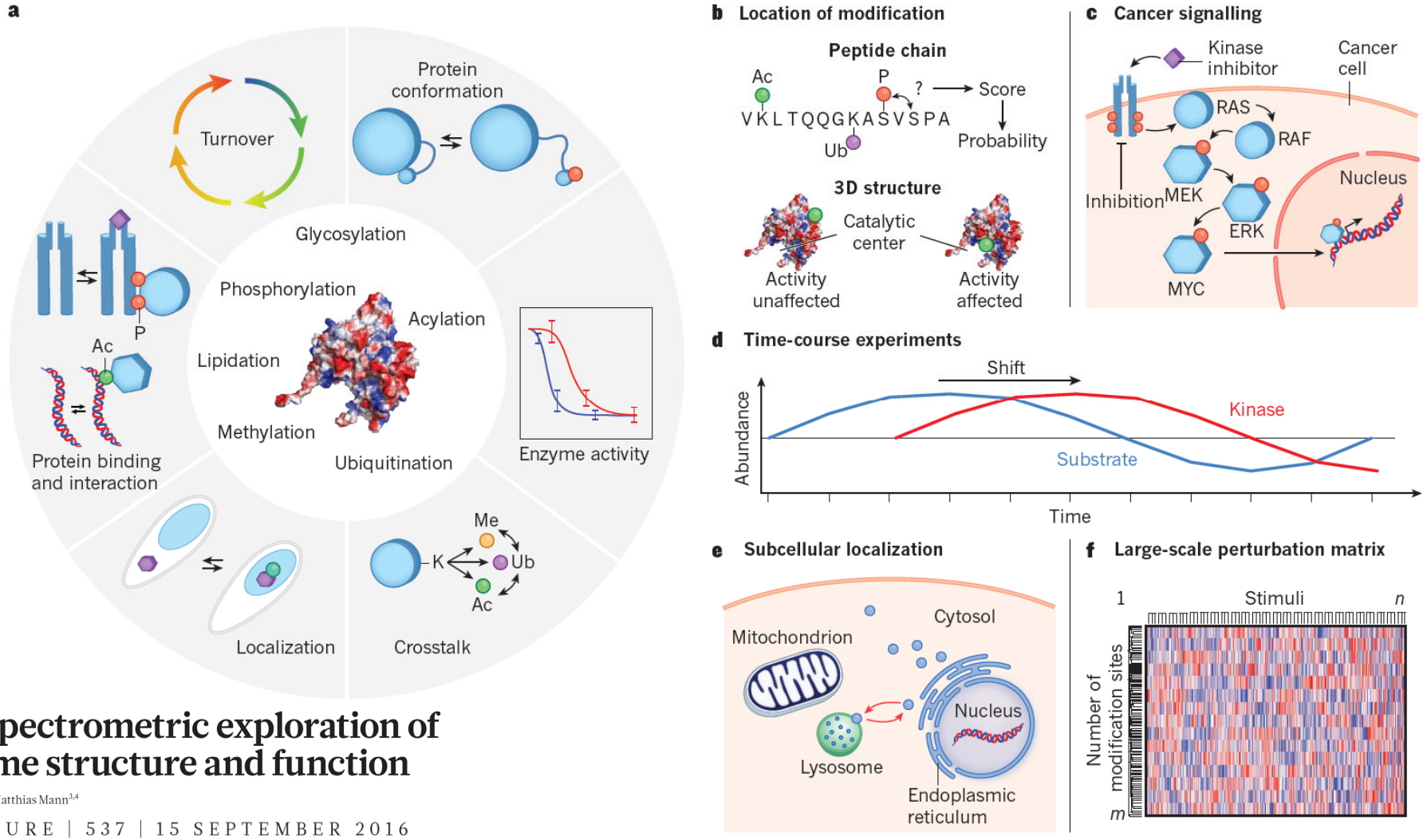
Analysis of post-translational modifications



Mass-spectrometric exploration of proteome structure and function

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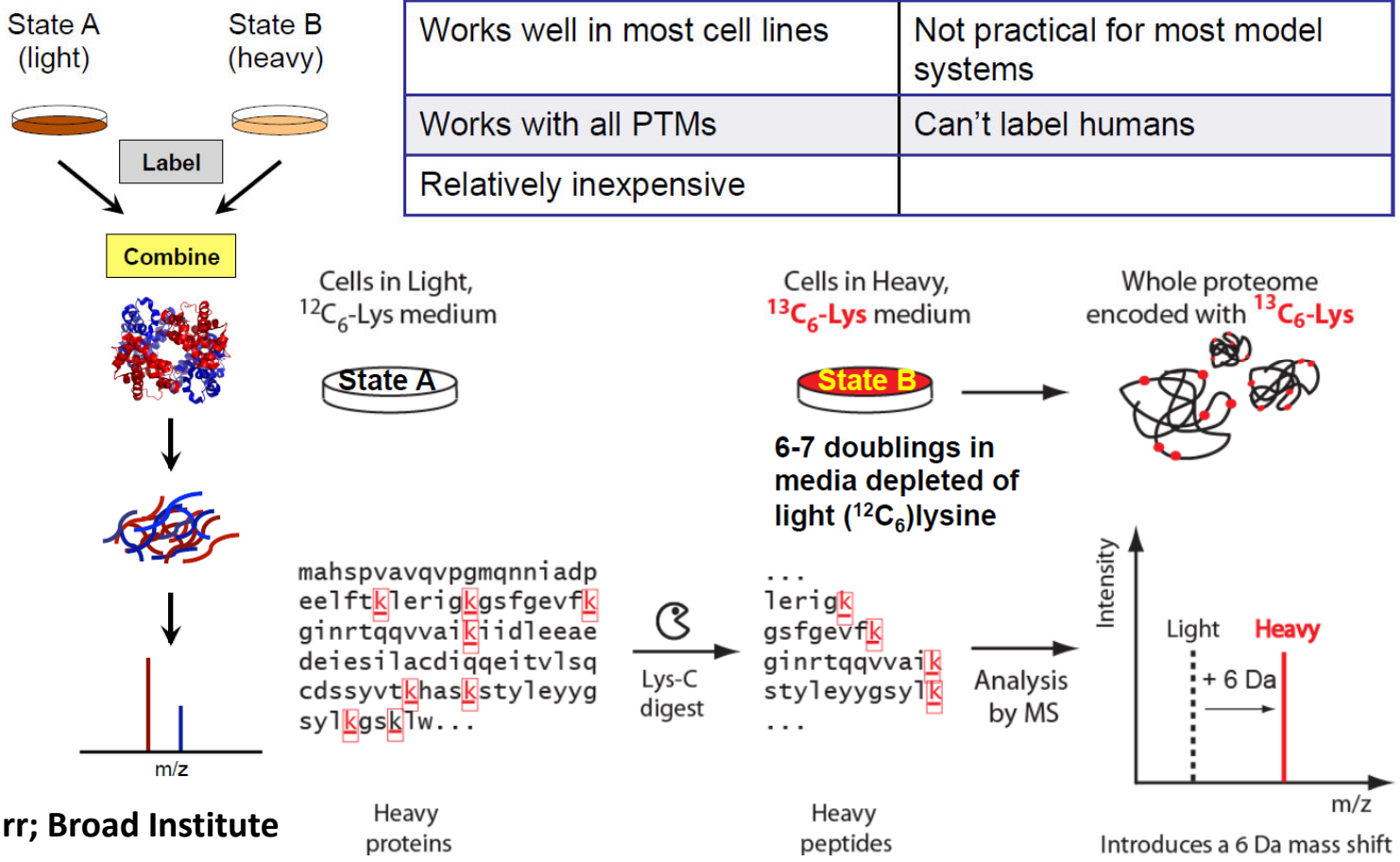
Comparing samples using SILAC

Metabolic labeling (SILAC)
(up to 3 samples at a time)

Pros	Cons
Deep, highly precise quant.	Limited plex level (3 max)
Works well in most cell lines	Not practical for most model systems
Works with all PTMs	Can't label humans
Relatively inexpensive	



c/o Steve Carr; Broad Institute

Latest and greatest: Oribtrap Fusion Lumos

Orbitrap Fusion Lumos MS

The Orbitrap Fusion Lumos is a mass spectrometer that contains three mass analyzers and includes an external syringe pump, a divert/inject valve, and the EASY-Max NG API source. The instrument requires two forepumps.

Ion O

Schematic of the Orbitrap Fusion Lumos Tribrid MS ion transmission path

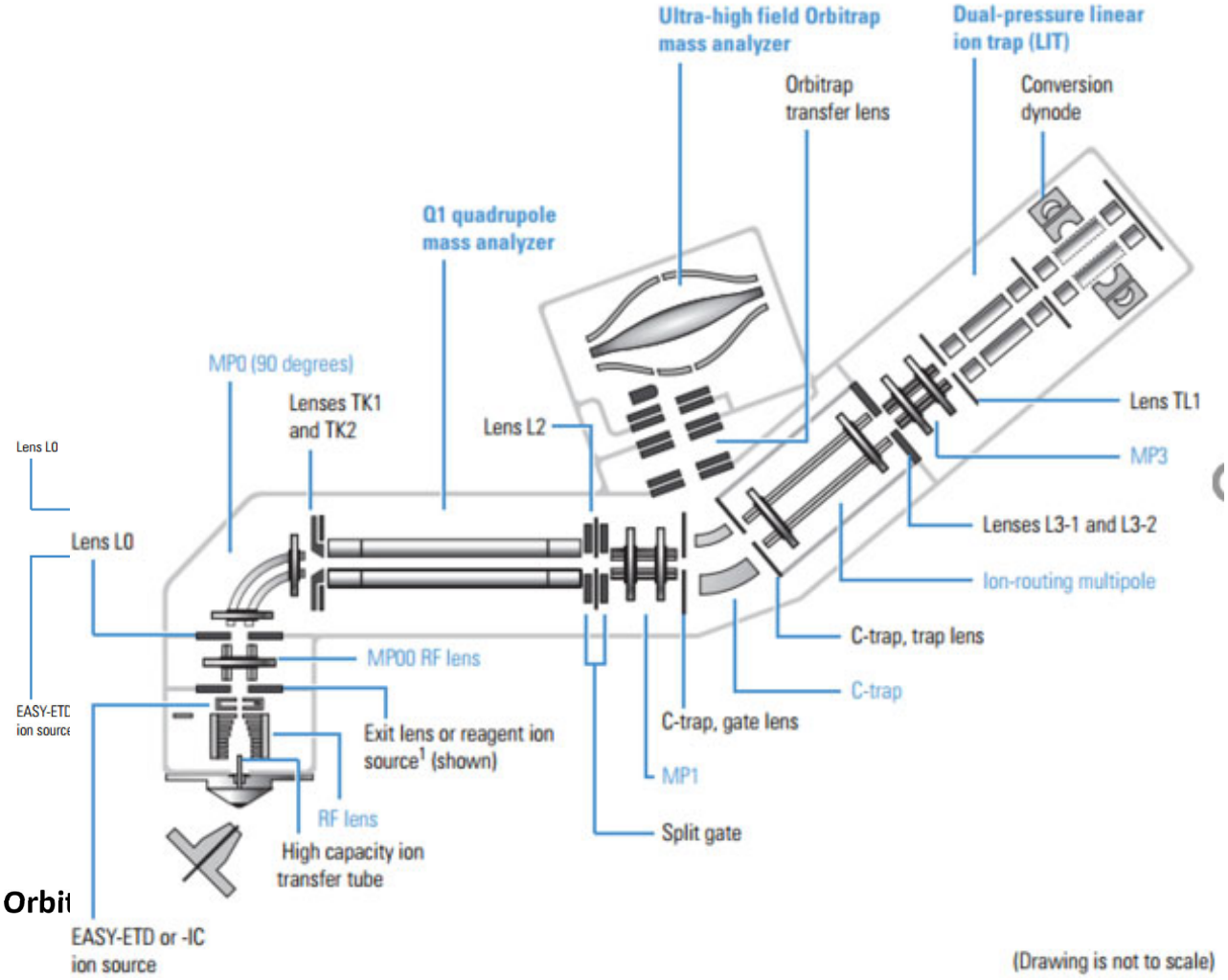
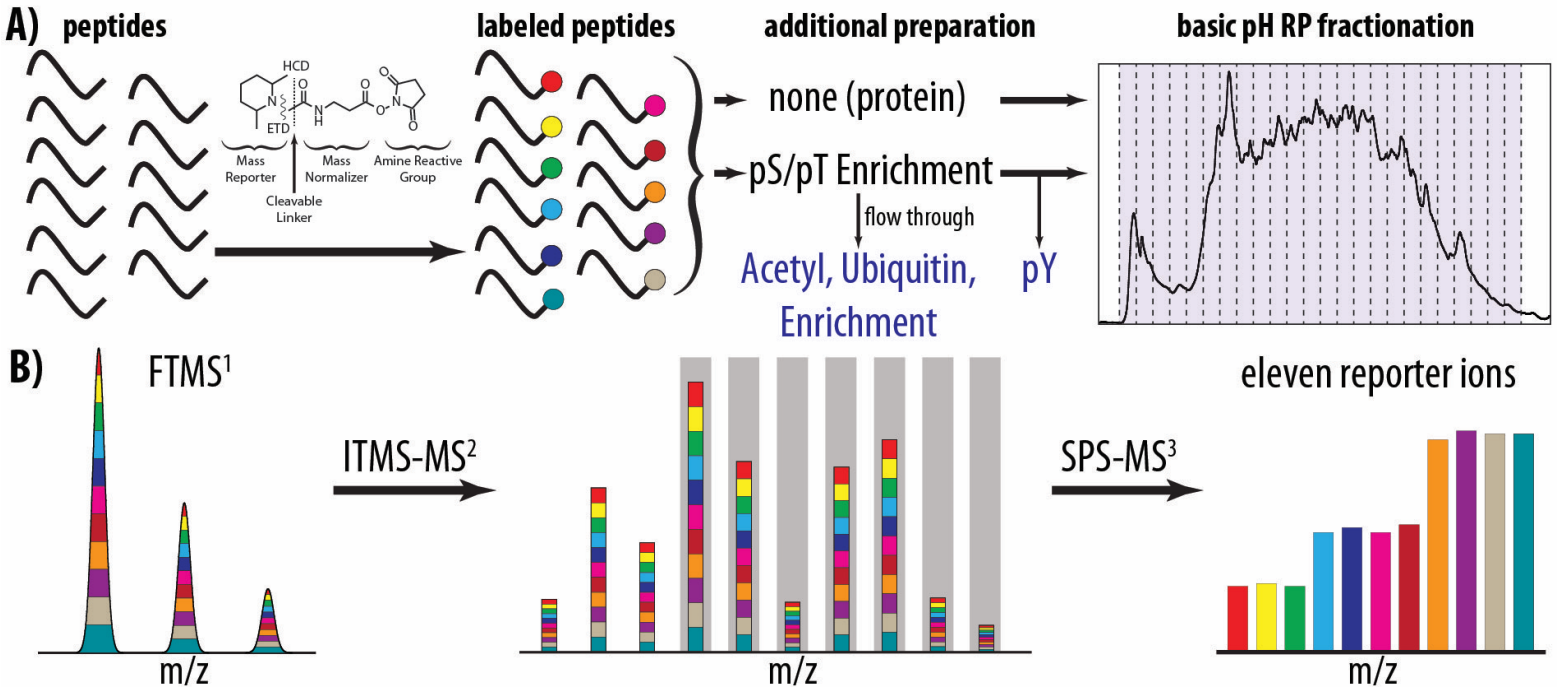


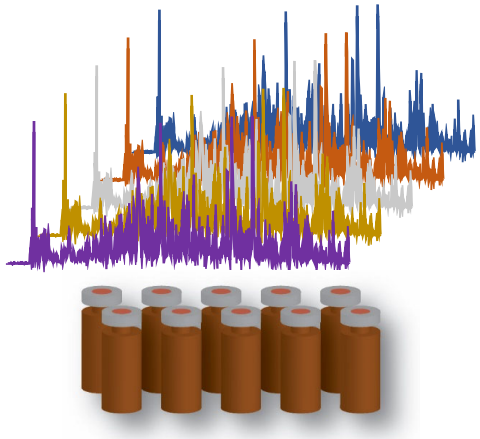
Figure 1: Proteomics Platform technologies of an Orbi coupled to an EASY-nLC 1200 System.

Isobaric Labeling for Quantitative, Multiplexed Proteomics

11-plex

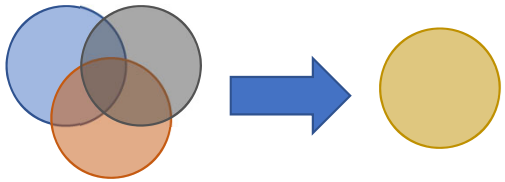


Why Sample Multiplexing?



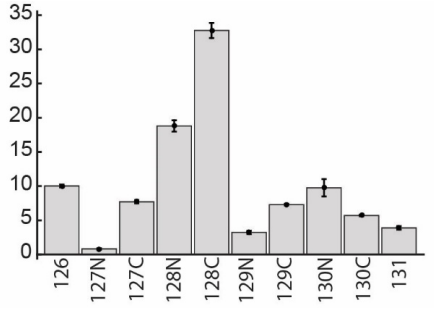
Sample Throughput:

- Increasing experiment scale means lots of samples
- Cancer studies can involve hundreds of samples
- Clinical studies even bigger



Consistency of Detection:

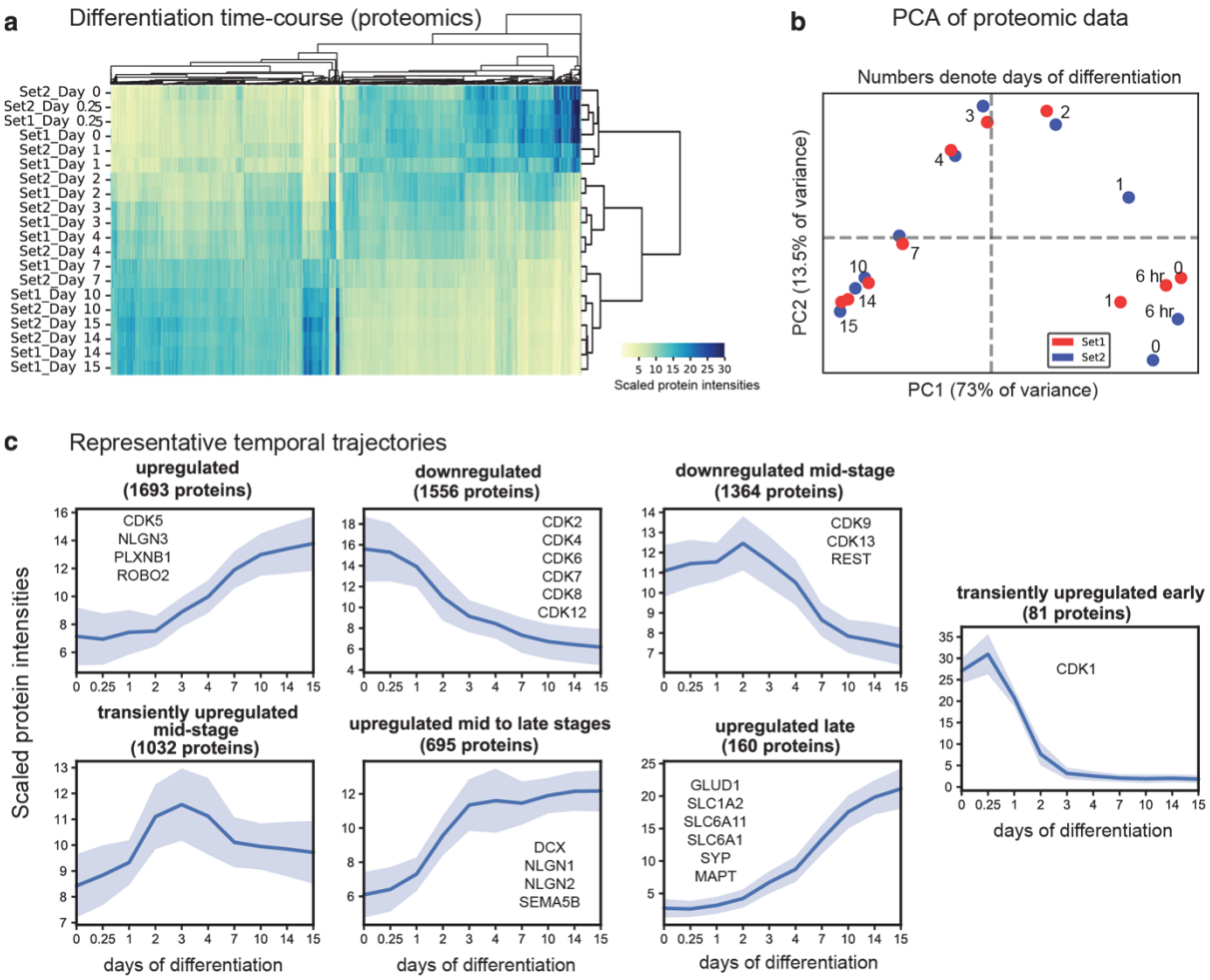
- Proteins can only provide useful biological insight when they are consistently detected across most or all samples.
- This is especially important in studies that involve very large sample sizes.



Precision Quantitation:

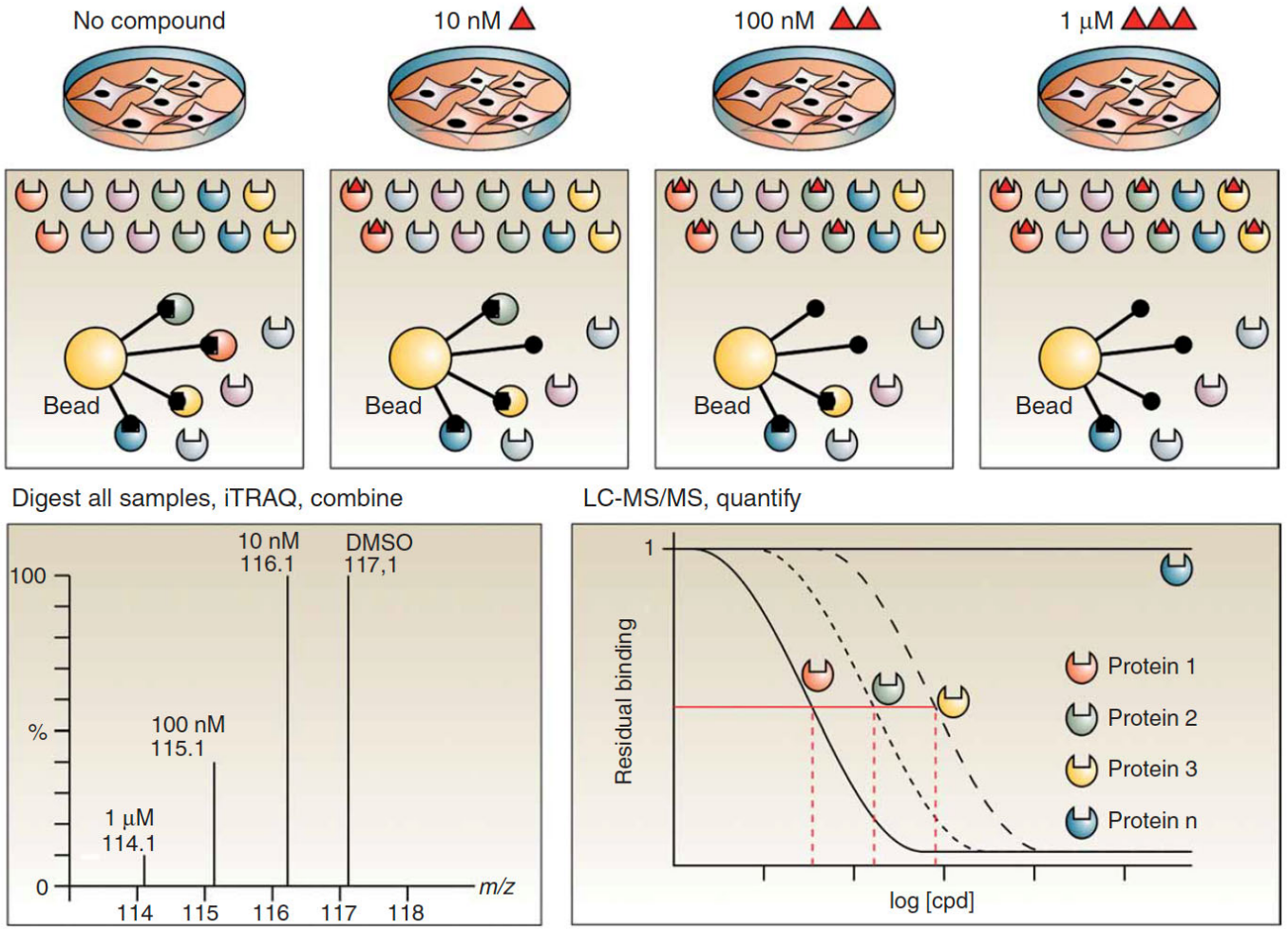
- Precise quantitation is required to maximize power and sensitivity of quantitative proteomics experiments.

Example: proteomics of a differentiating cell culture



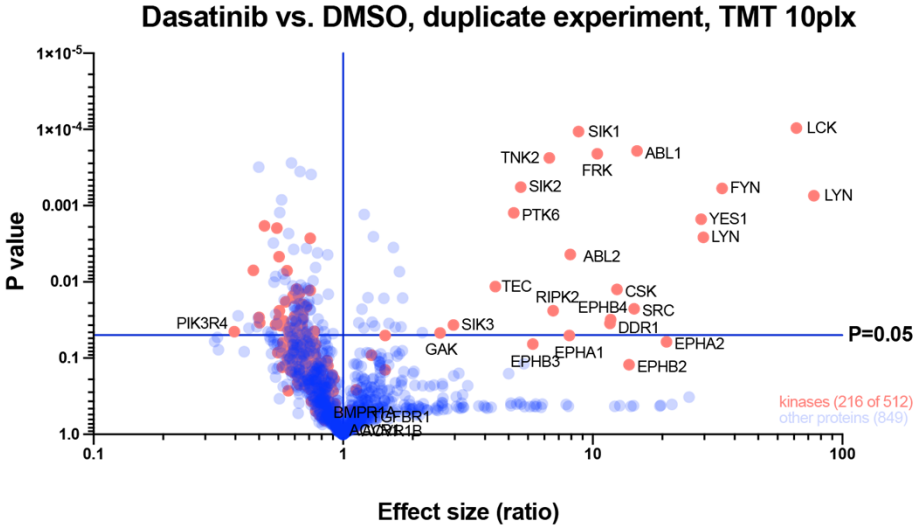
Song, Subramanian, Berberich *et al.*, 2018

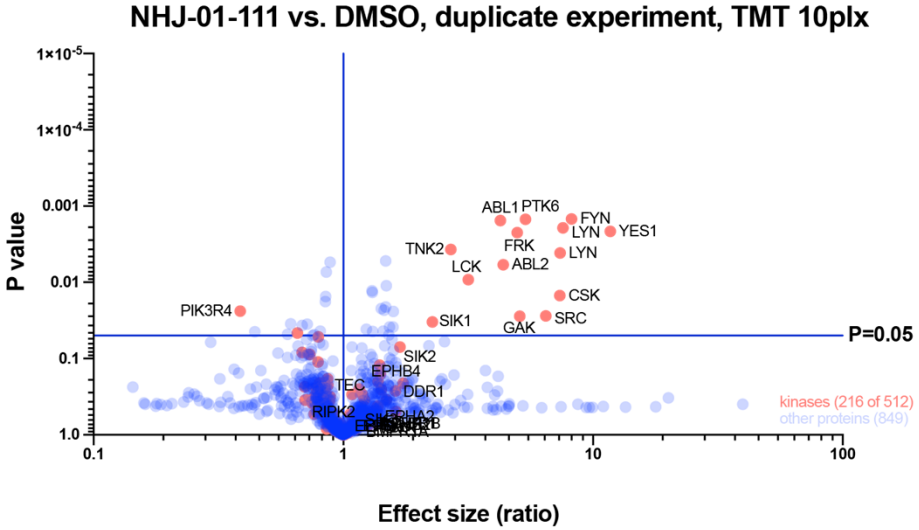
Combining Methods: Kinobeads competition binding assay for mass spec profiling

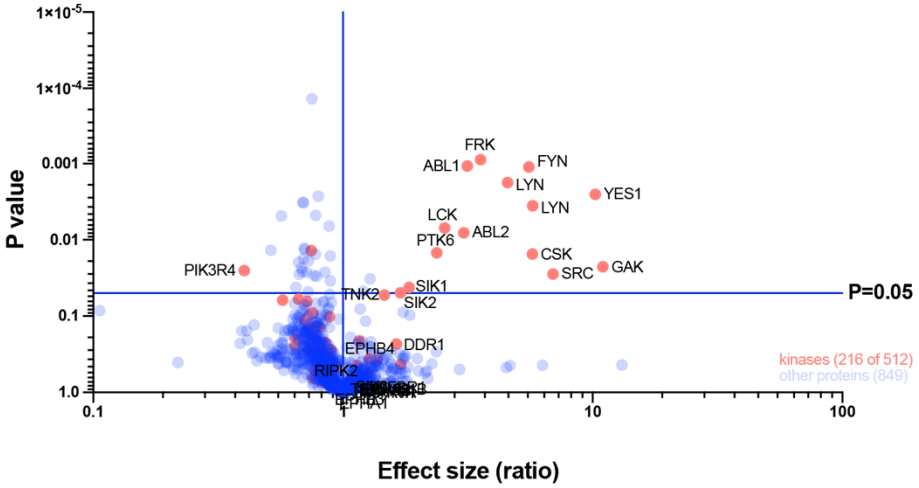


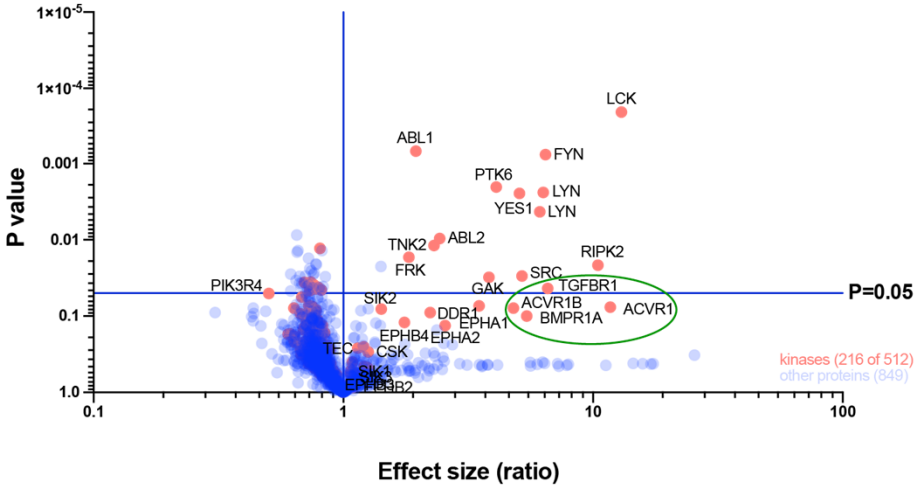
Bantscheff *et al.*, 2007

Kinobeads competition binding assay for mass spec profiling

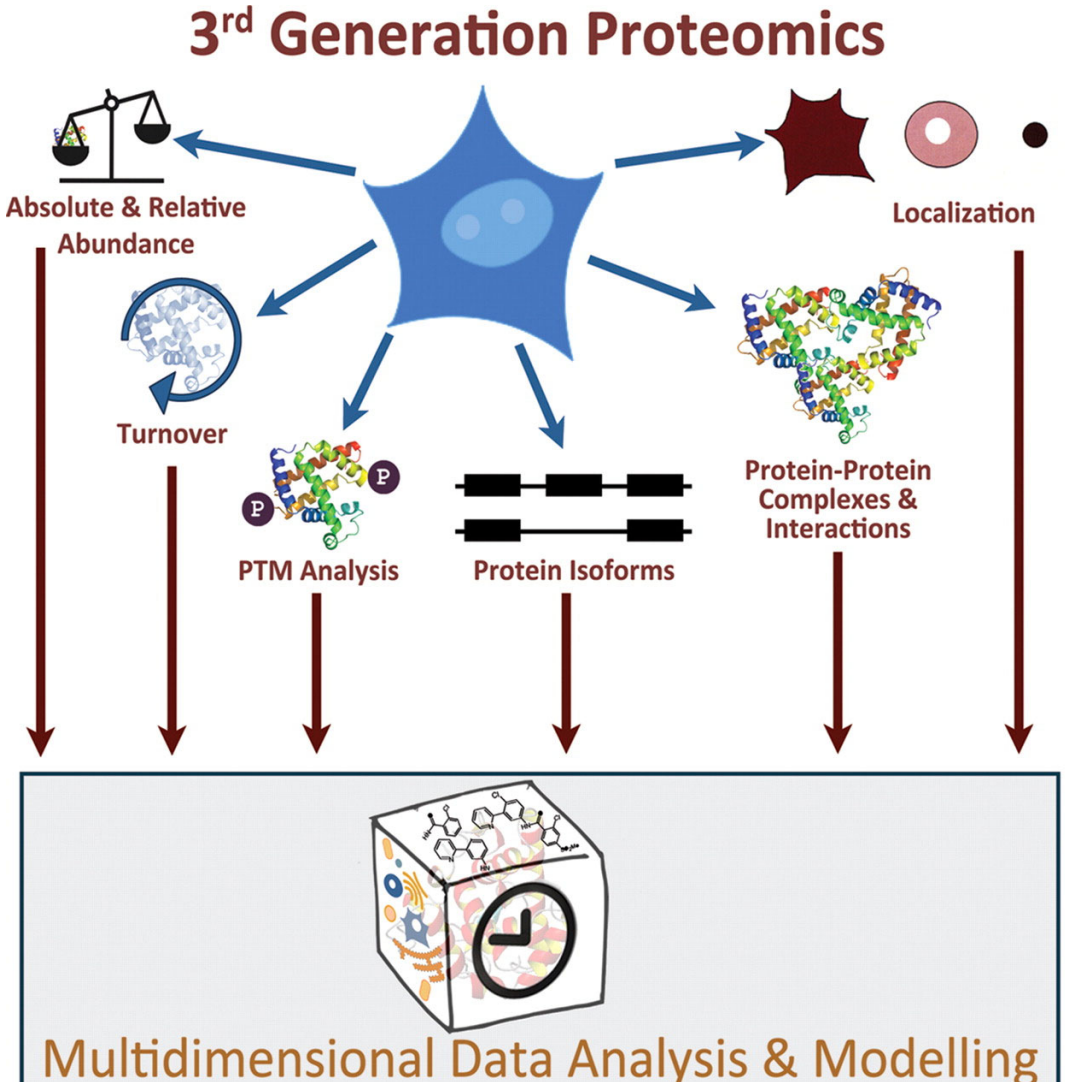








The future



https://www.mcponline.org/content/11/3/O112.017731