Phosphoproteomics

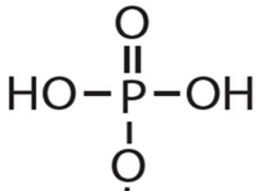
PhD Henri Blomster

Human Genome Project

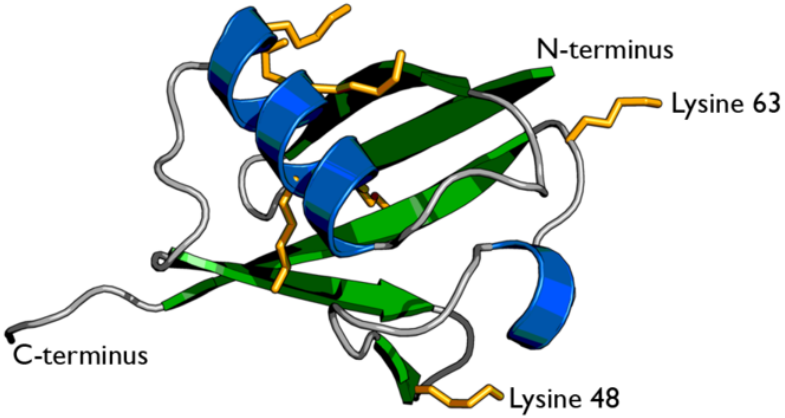
Budding yeast 6,000 genes

Nematode 15,000 genes

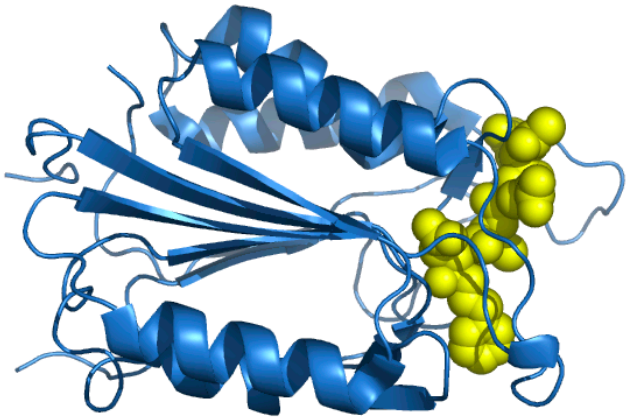
Human 25,000 genes



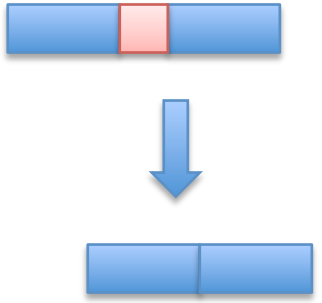
Attachment of chemical groups



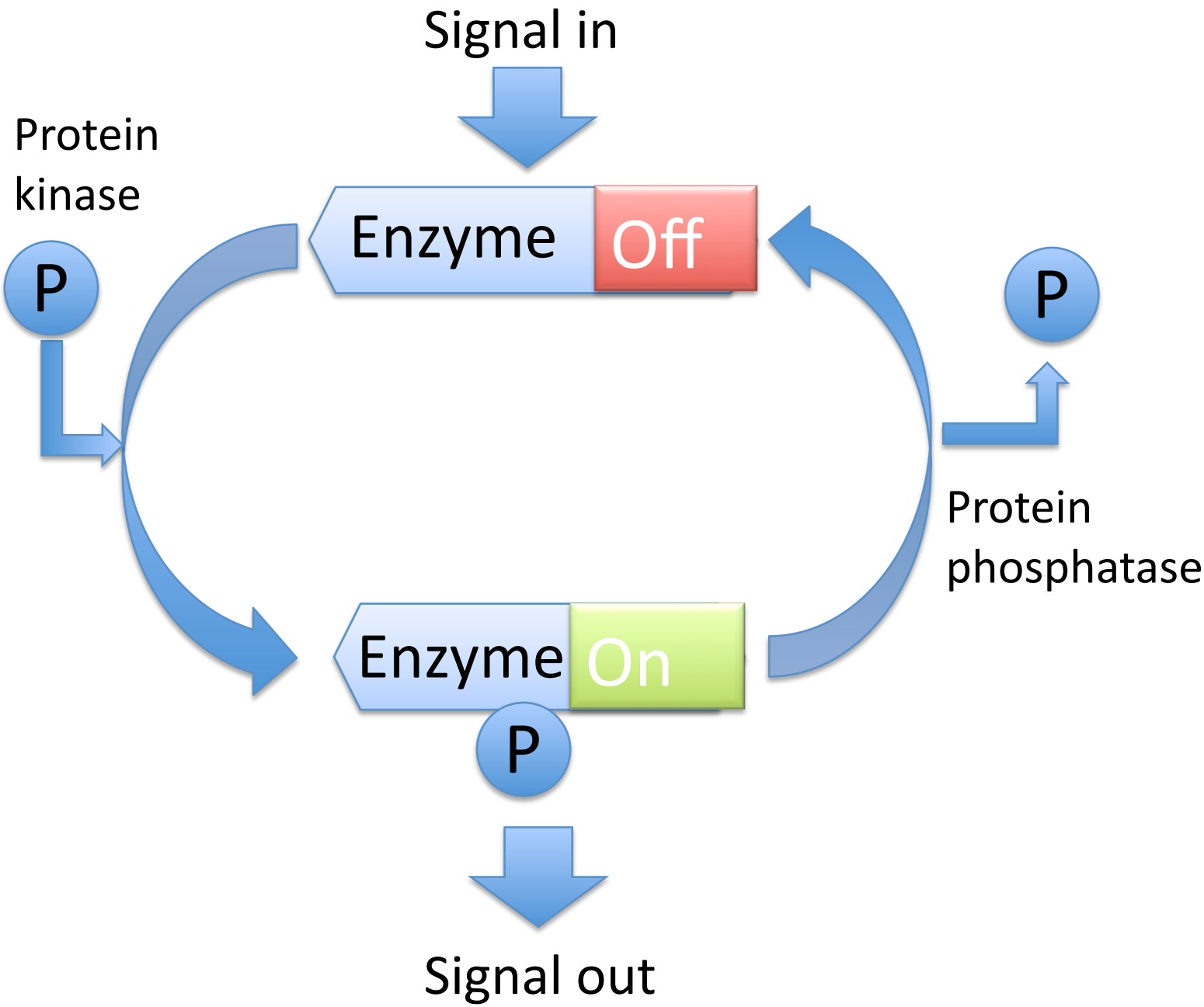
Covalent attachment of polypeptides



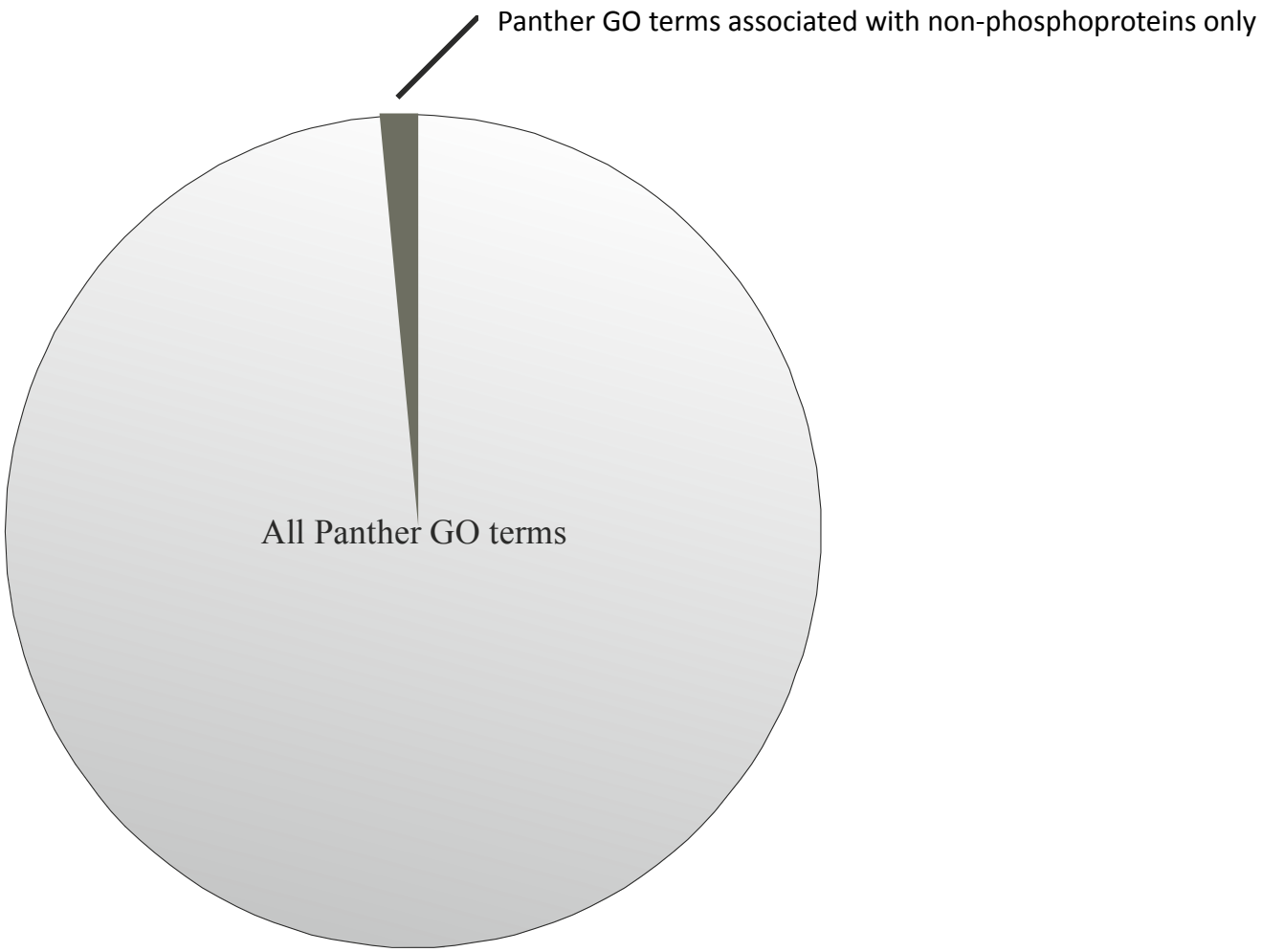
Protein cleavage

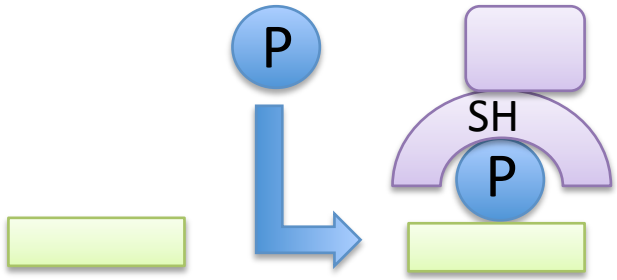


Protein splicing

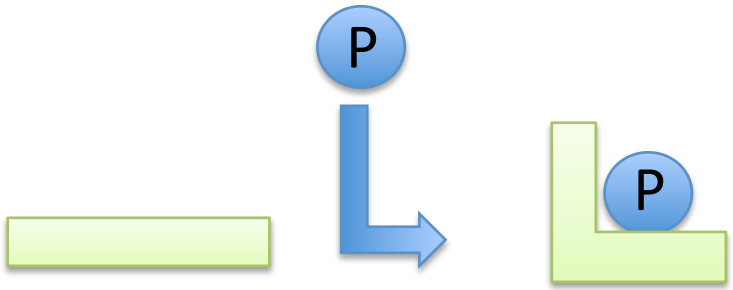

Signal in
Protein kinase
Enzyme
Off
P
P
Protein phosphatase
Enzyme
On
P
Signal out

Why to map PTM sites: a systems biology view

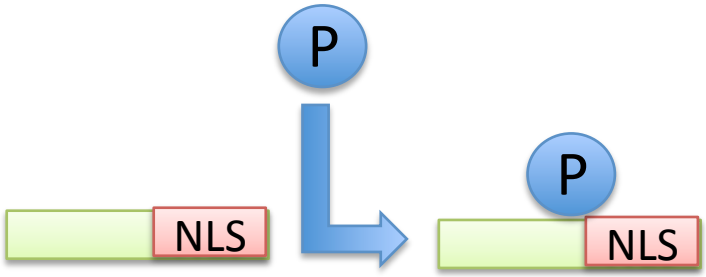




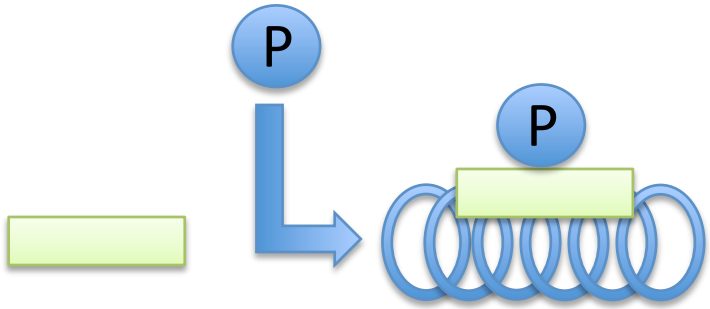
Recruitment of effector proteins



Conformational change

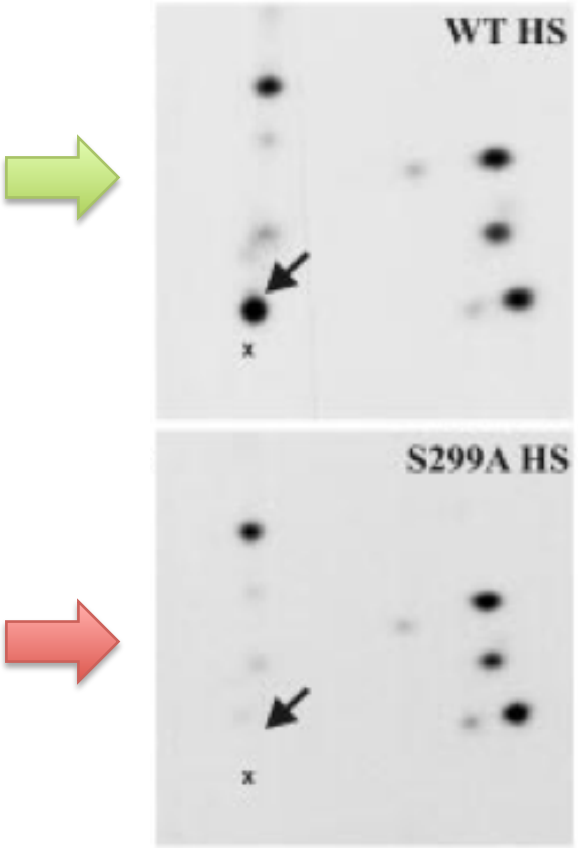


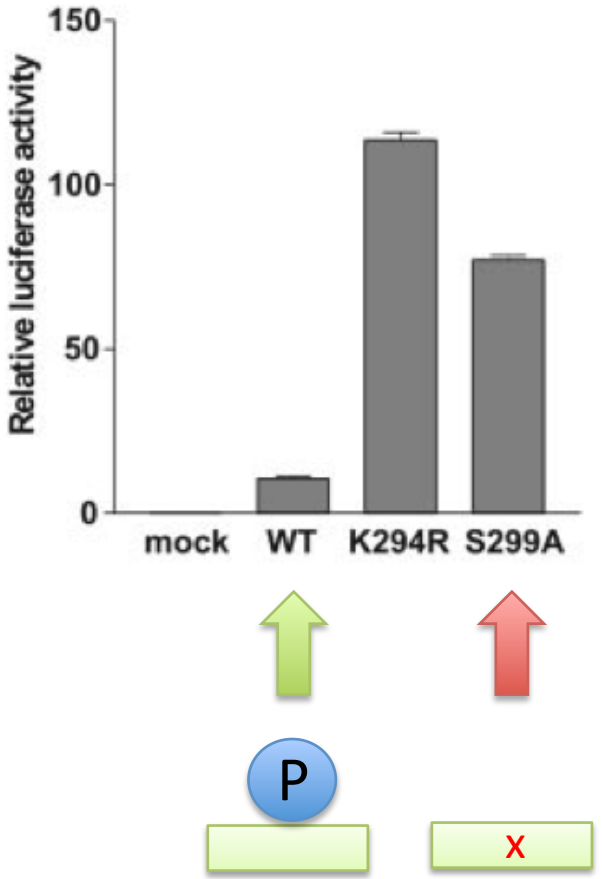
Cellular localisation



DNA binding

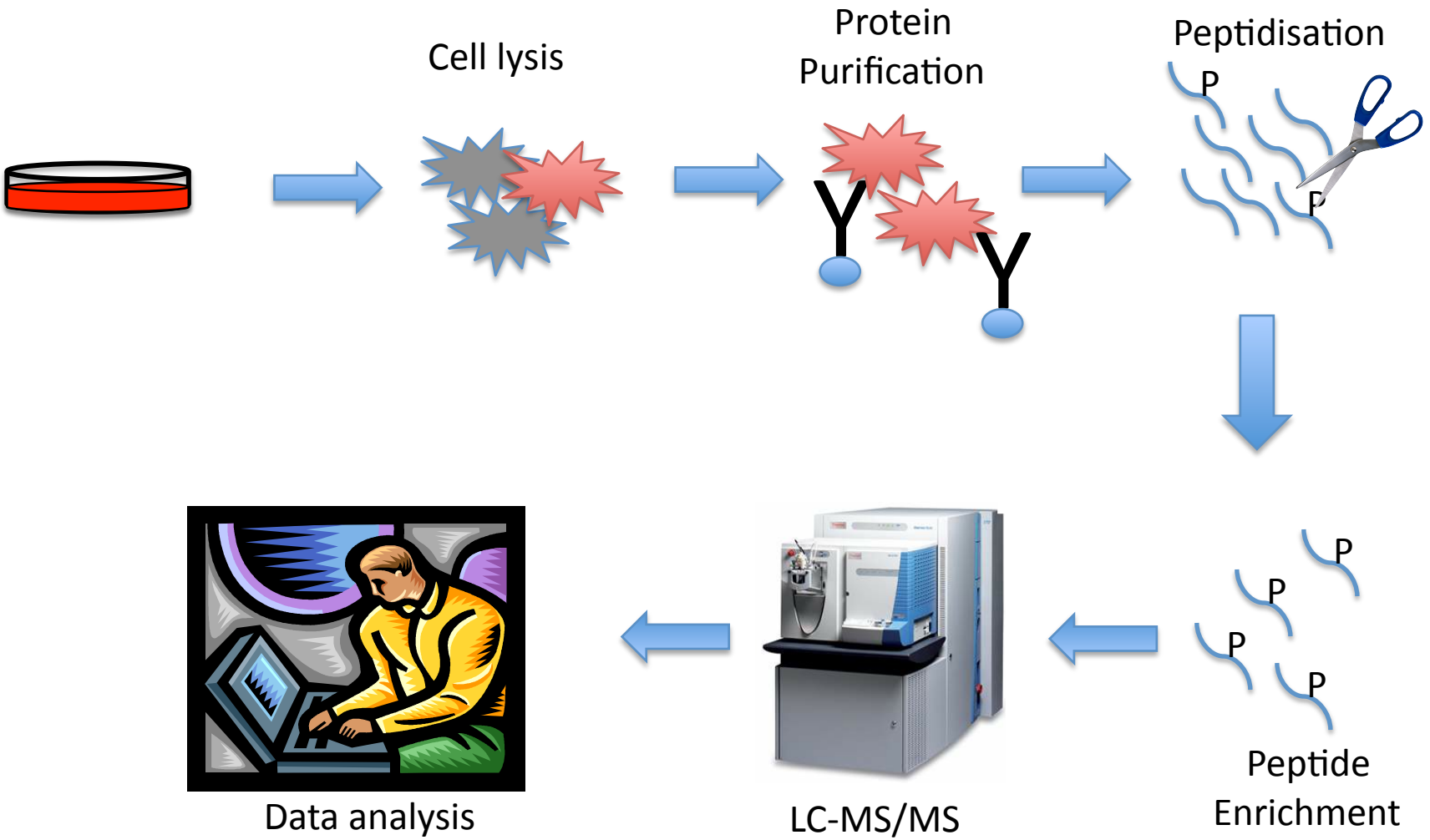
Why to map PTM sites: a single proteins view, HSF4b





Hietakangas et al. *PNAS*, 2006

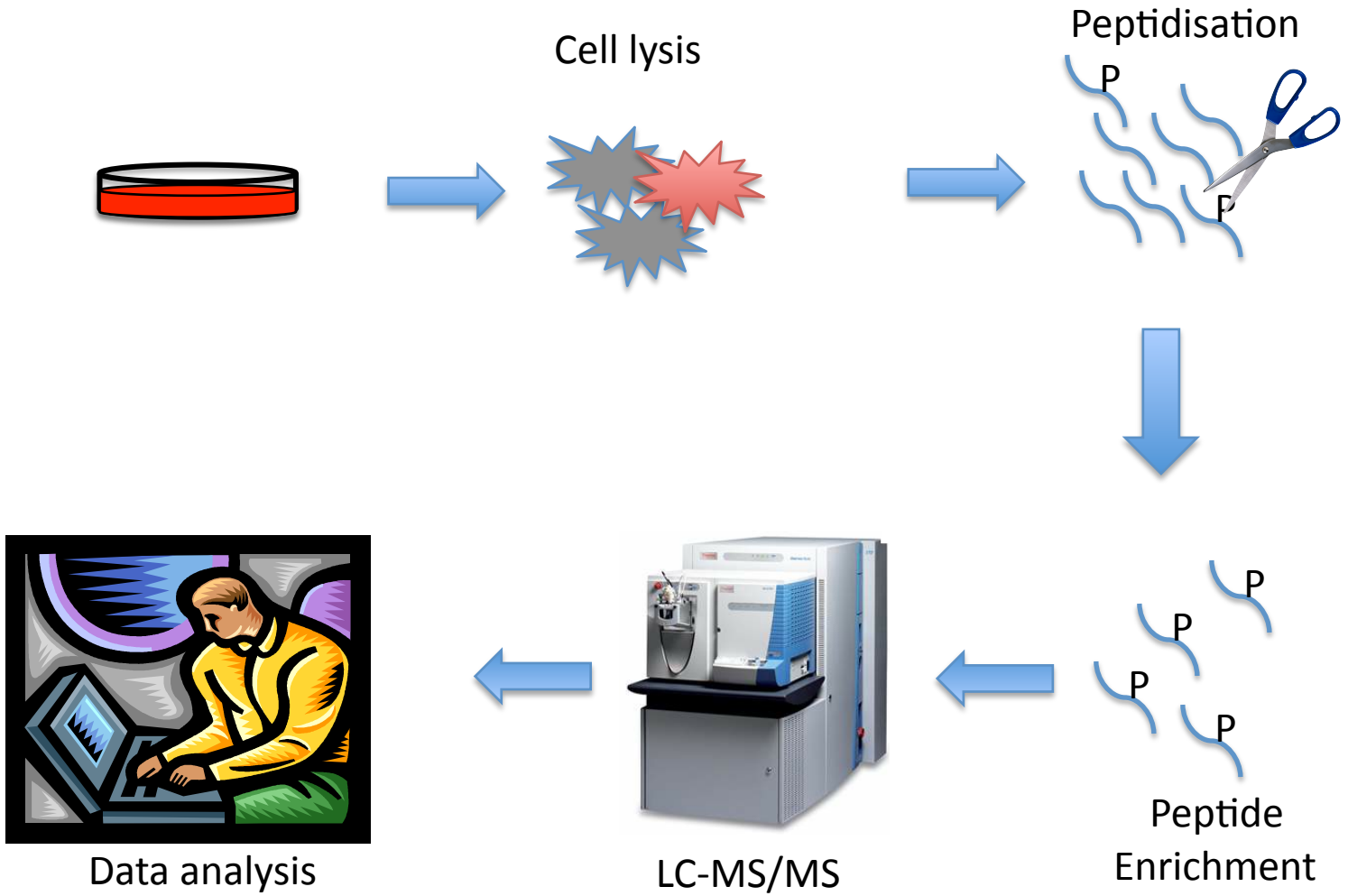
A targeted phosphoproteomics approach



A targeted phosphoproteomics approach

- Advantages
  - Sensitive approach especially for proteins that are expressed at low level like transcription factors
- Disadvantages
  - Low throughput
  - Detect only things that are looked at

Global phosphoproteomics approach

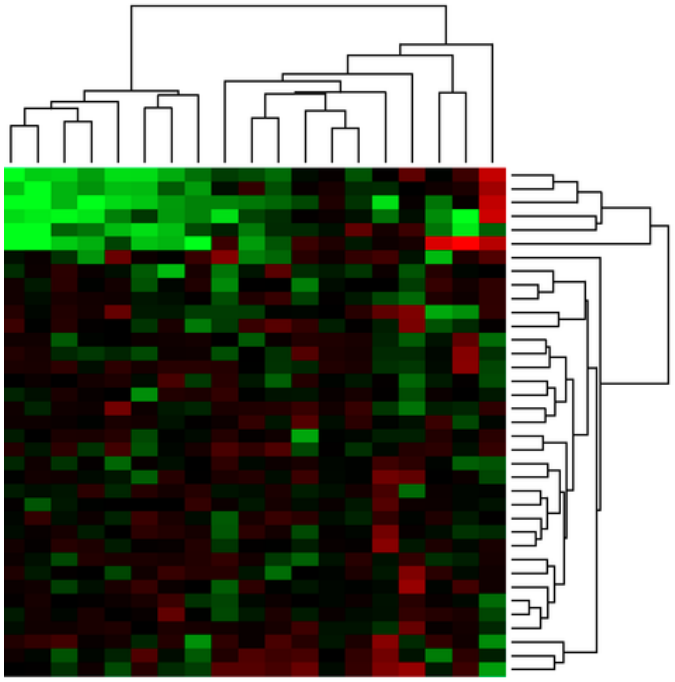


A global approach

- Advantages
  - high number of phosphorylation sites identified

- Disadvantages
  - low-abundant proteins not detected

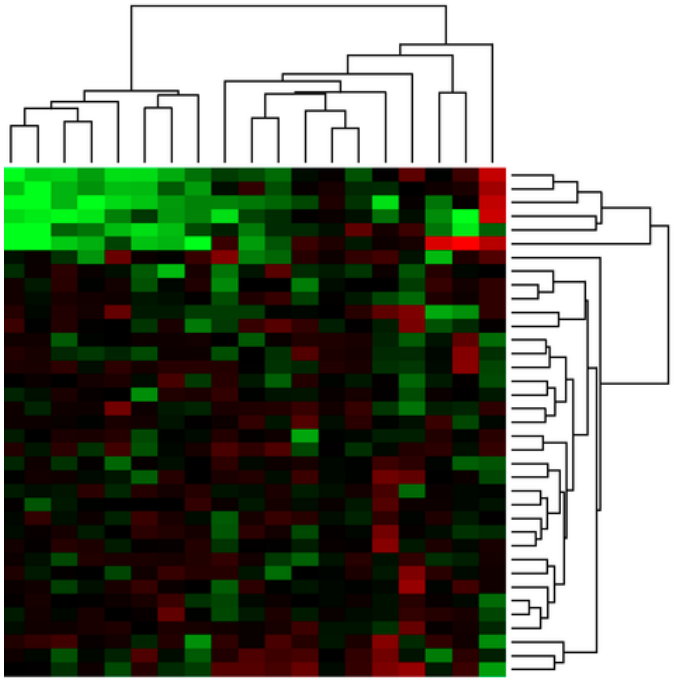
The phosphoproteome is dynamic



State 1 ⟷ State 2

Physiological change

The phosphoproteome is dynamic



State 1

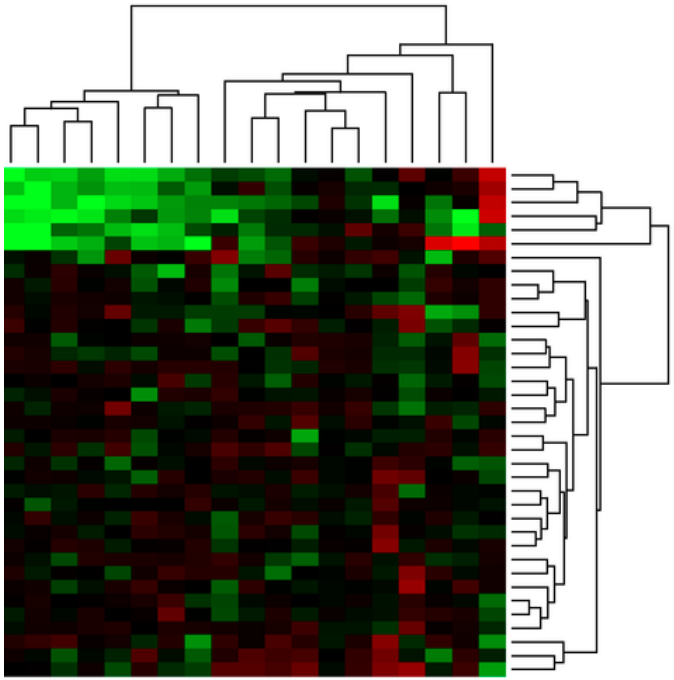
M phase

Physiological change

State 2

S phase

The phosphoproteome is dynamic



State 1

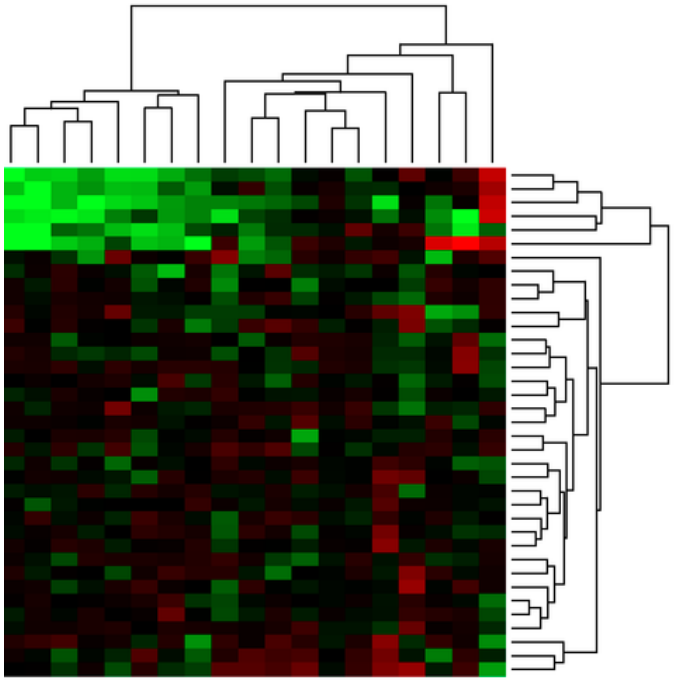
Healthy cell

Physiological change

State 2

Cancer cell

The phosphoproteome is dynamic



a) Understand
b) Design therapies

State 1

Healthy cell

Physiological change

State 2

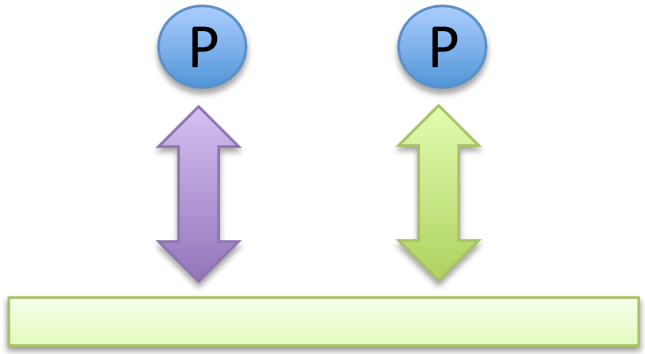
Cancer cell

Why to quantitate, a single proteins view

- Biological function is difficult to find
  - Many phosphorylation sites are background
  - Many biological functions
- Quantitative proteomics is a good starting point

Why to quantitate, a global view

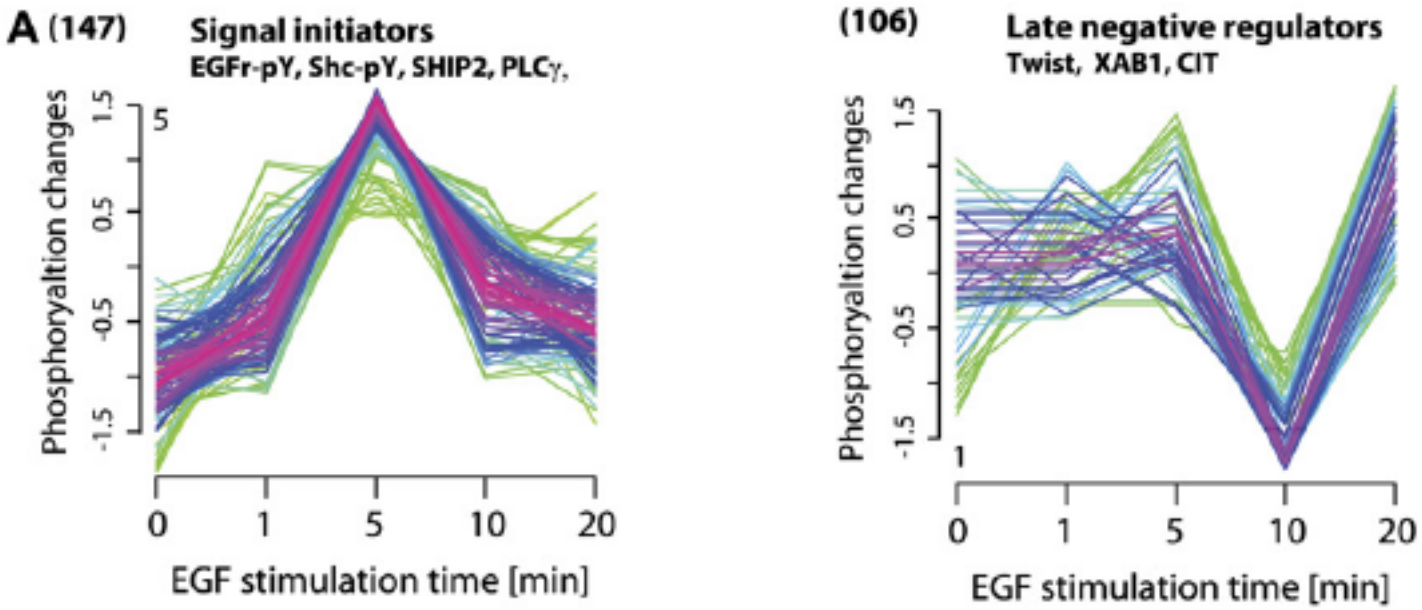
Global quantitation of phosphorylation sites after growth factor stimulation



Olsen et al. *Cell* 2006

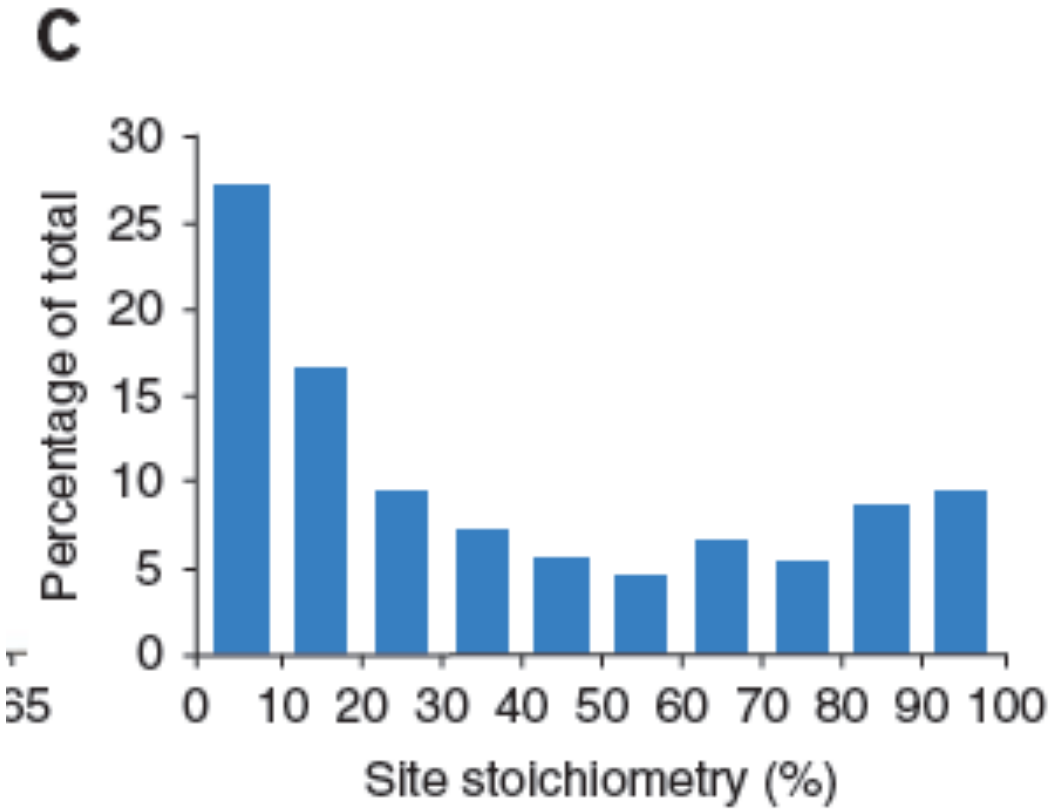
Why to quantitate, a global view

Global quantitation of phosphorylation sites after growth factor stimulation



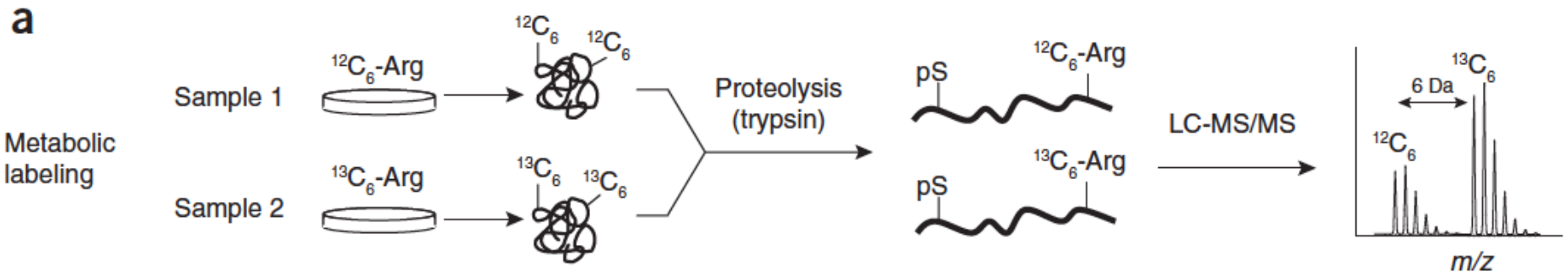
Classification by temporal profiles

Olsen et al. *Cell* 2006

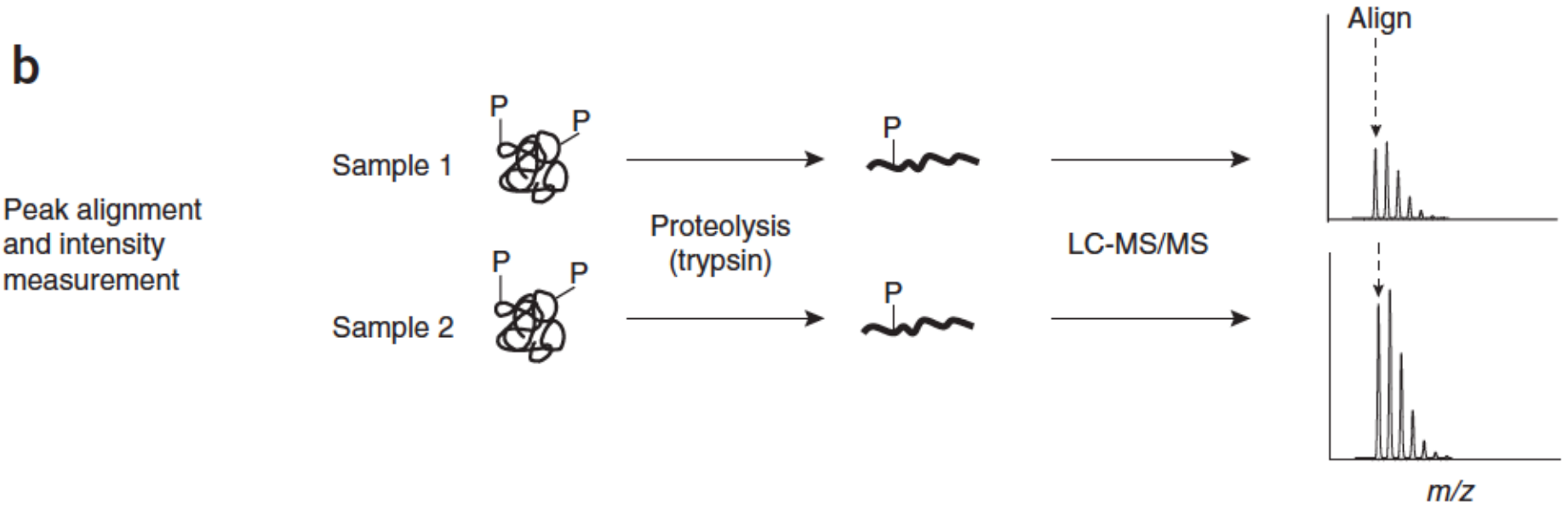


Site stoichiometry distribution for 5,033 events from wild-type yeast undergoing exponential growth

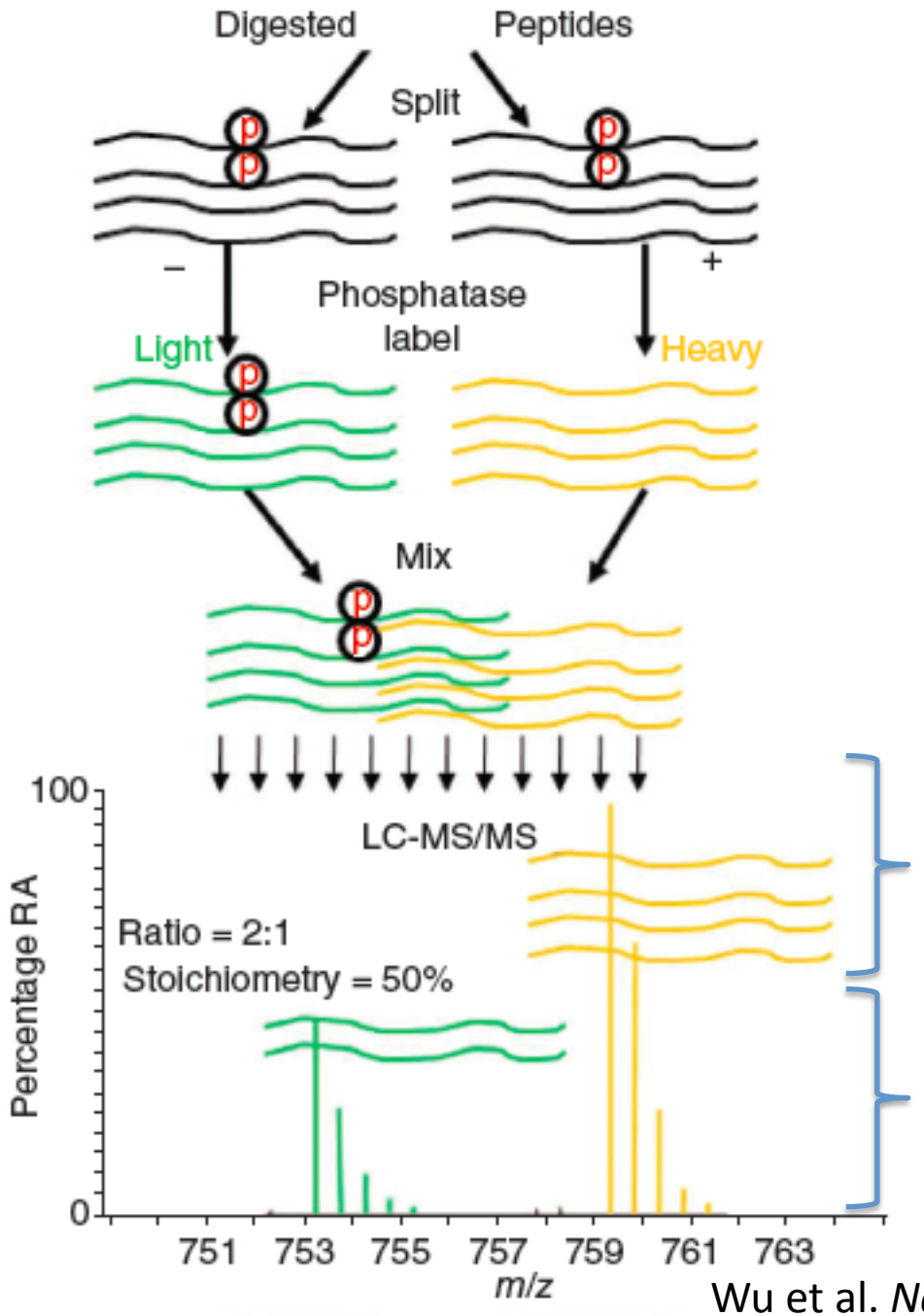
Wu et al. *Nature Methods*, 2011



Witze et al. *Nature Methods* 2007



Witze et al. *Nature Methods* 2007



Amount of phosphorylated

Amount of non-phosphorylated

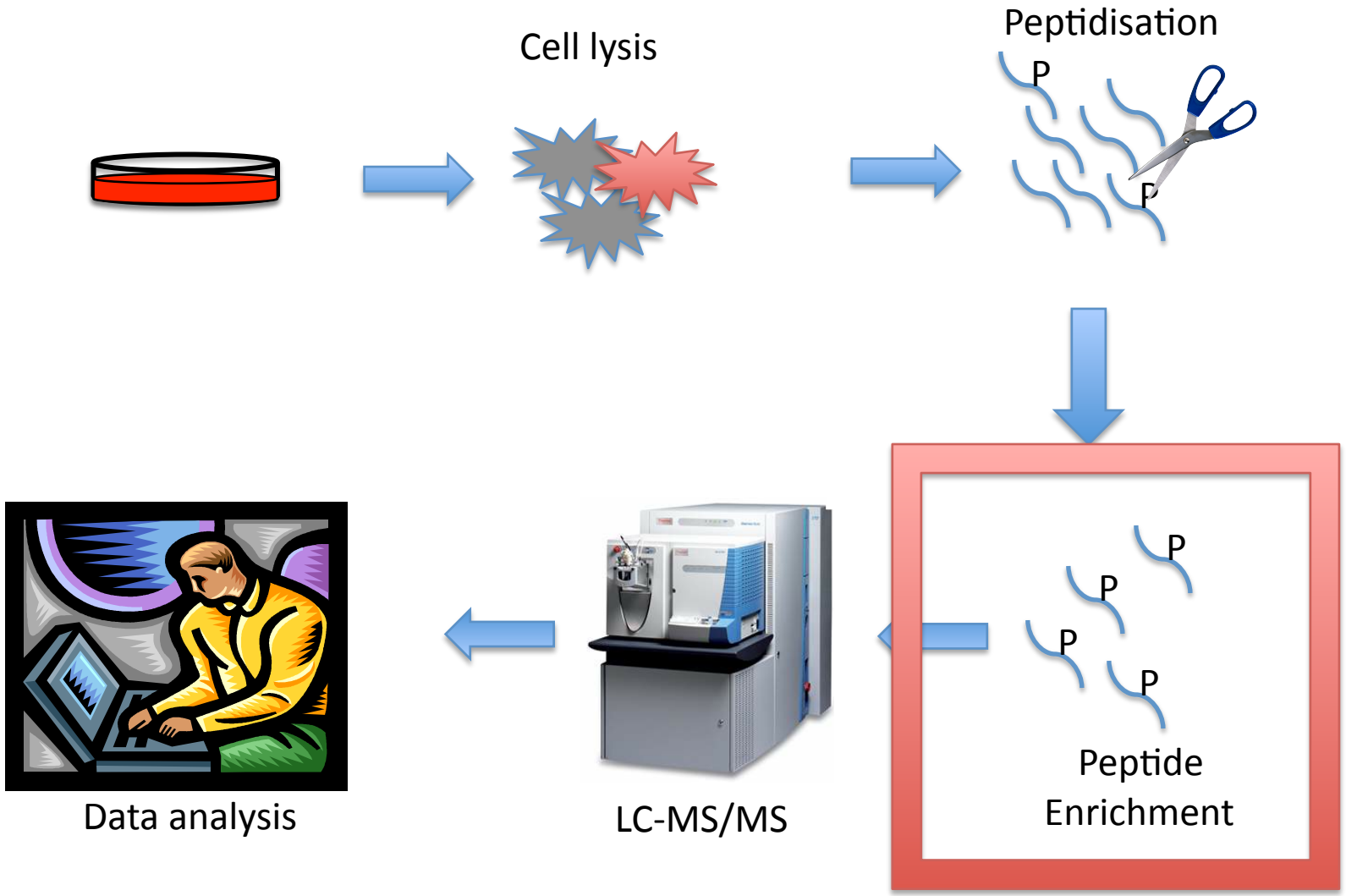
Wu et al. *Nature Methods*, 2011

Summary of Part I

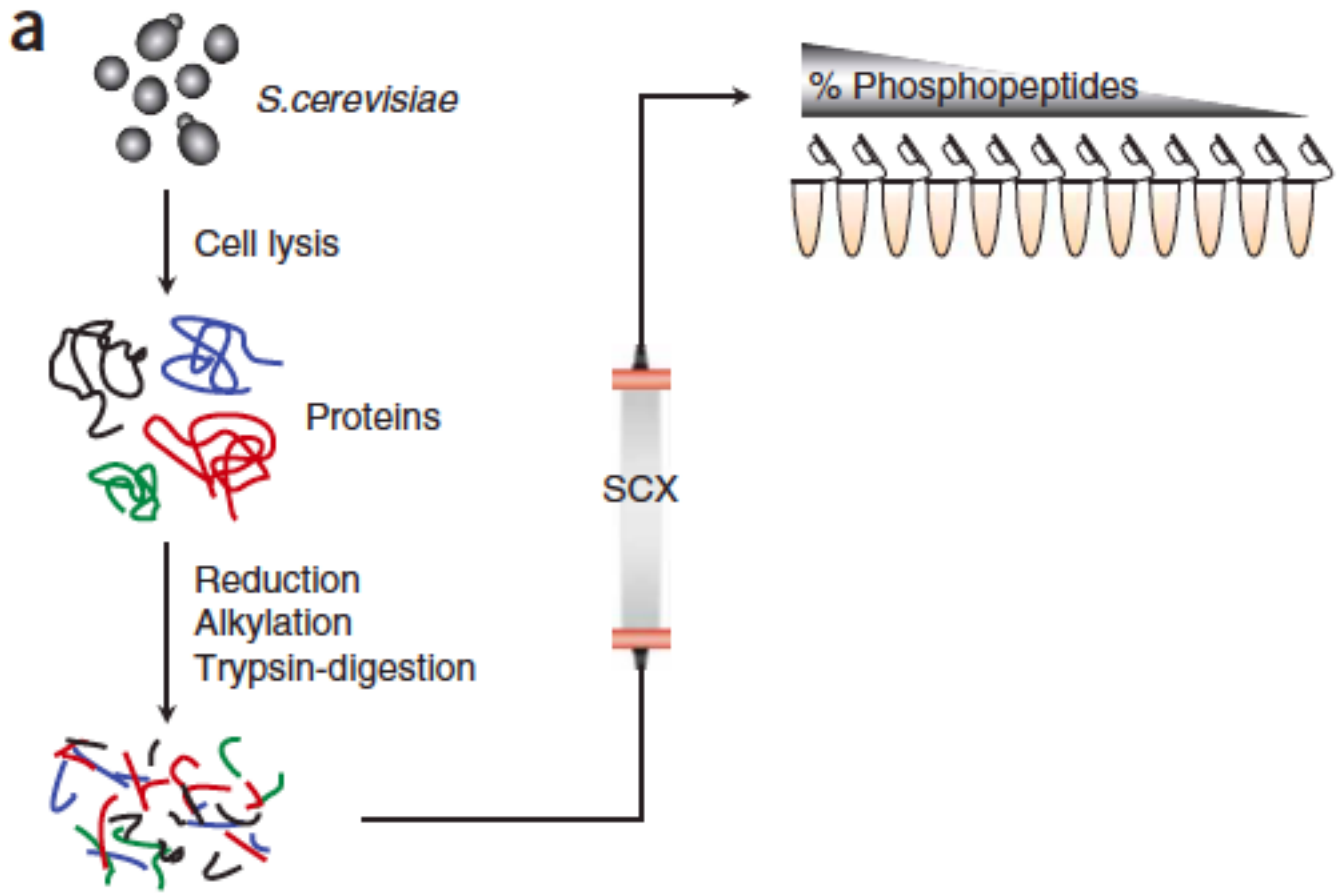
- Importance of PTM's
- Why to map PTM sites
  - targeted approach
  - global approach
- How to map PTM sites
  - targeted approach
  - global approach

How to map phosphorylation sites with mass spectrometry

Global phosphoproteomics approach



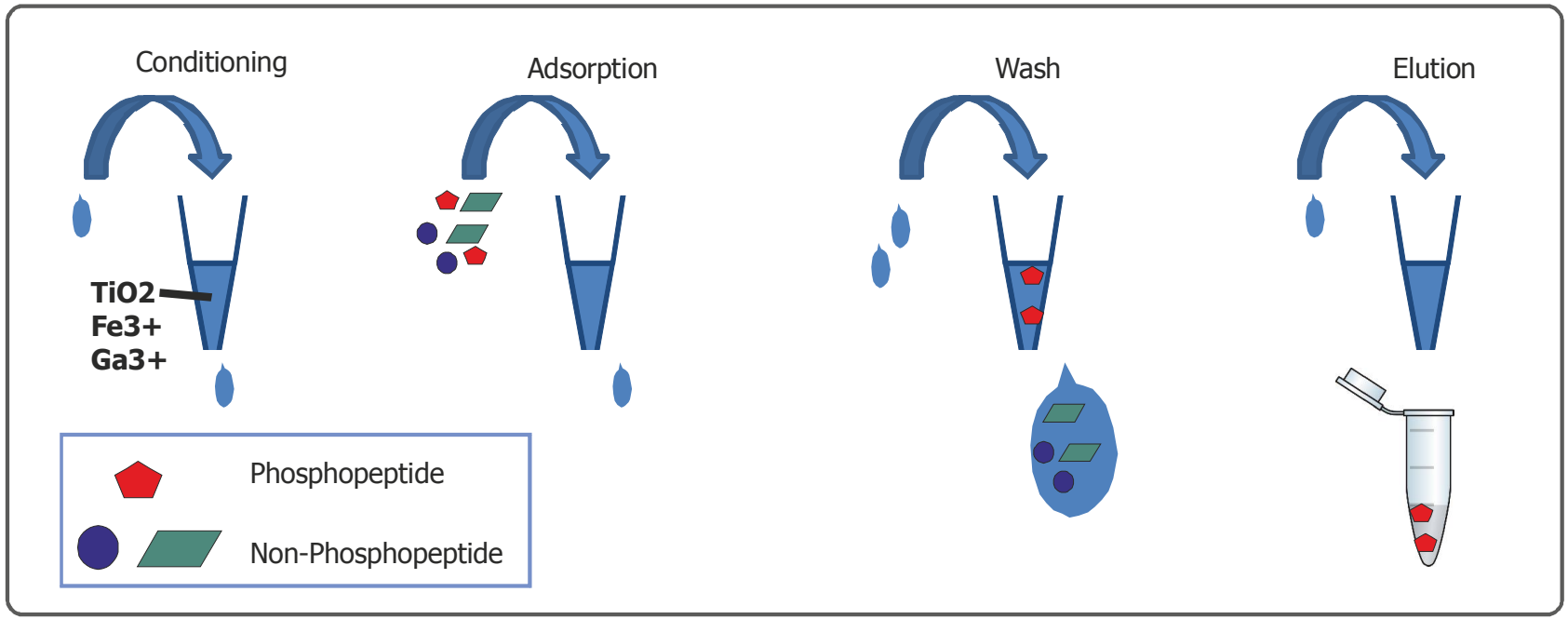
Strong cation exchange, SCX separates peptides by charge. Phosphate groups add negative charge and phosphopeptides are expected to elute earlier

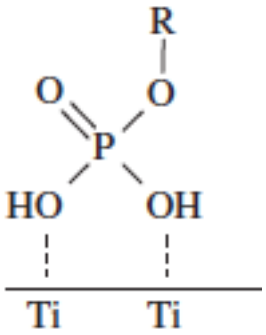


Villen and Gygi, *Nature Protocols*, 2008

IMAC and TiO2 enrichment

Immobilised metal ion chromatography (IMAC) makes use of
matrix-bound metals to affinity purify phosphopeptides. (positive metal, negative phosphate)





Titanium dioxide affinity purification makes use of
matrix-bound TiO2 to affinity purify phosphopeptides.

Engholm-Keller et al. *Journal of Proteomics*, 2011

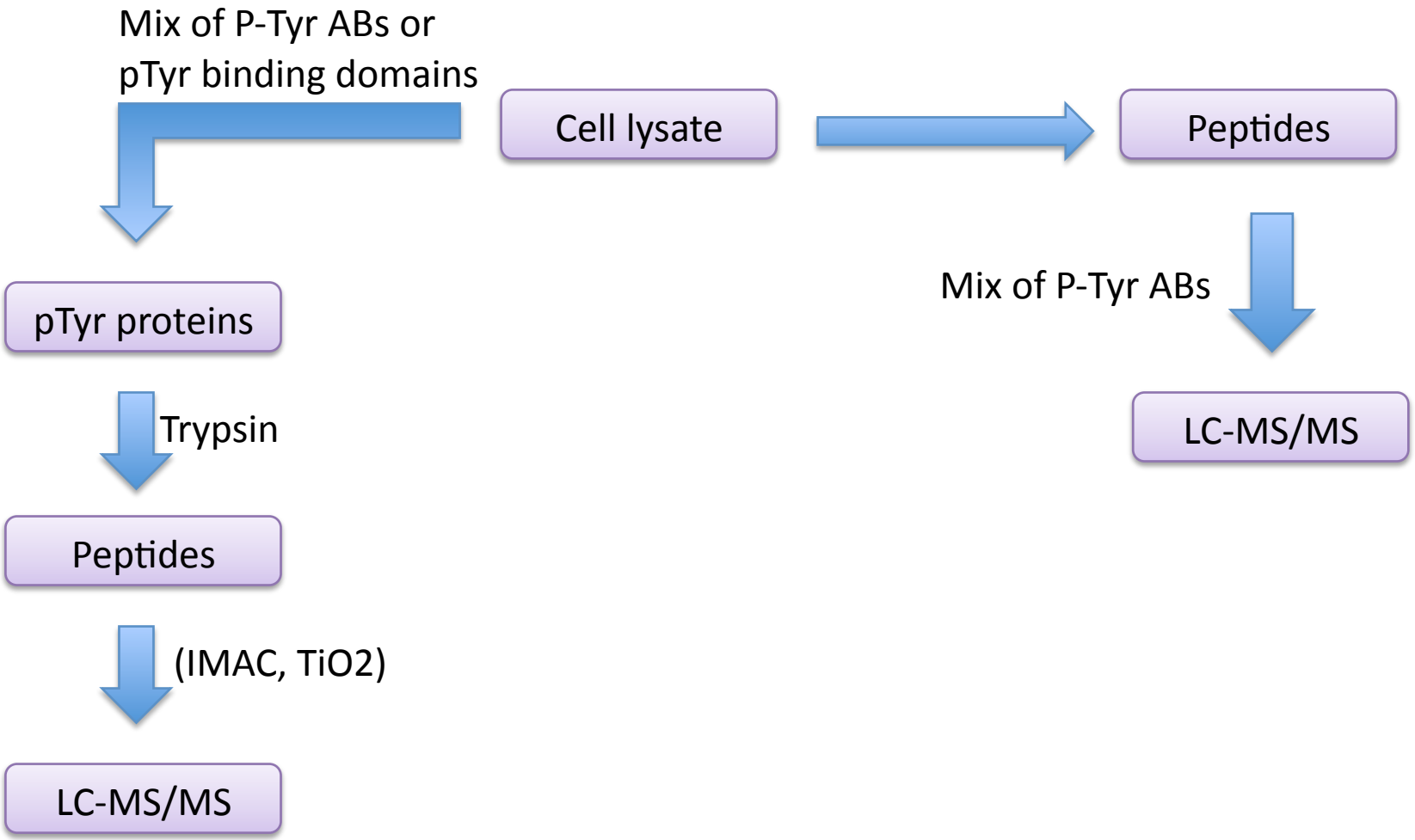
Phosphotyrosine signalling

Distribution of phosphorylation sites by amino acid

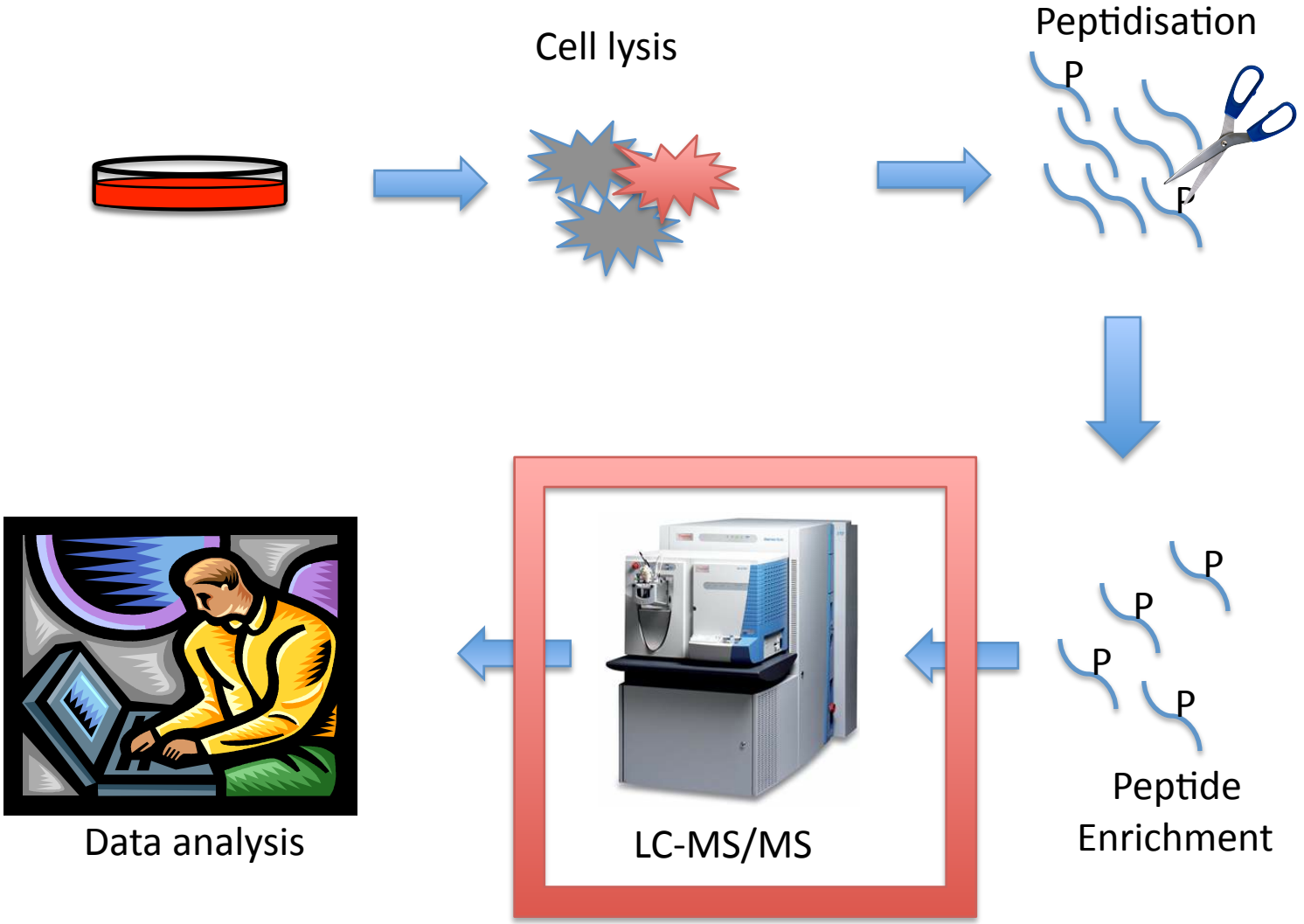
Site	Class I	Percent	EGF-regulated	Percent
pSer	4901	86.4%	724	82.0%
pThr	670	11.8%	106	12.0%
pTyr	103	1.8%	53	6.0%

Olsen et al. *Cell* 2006

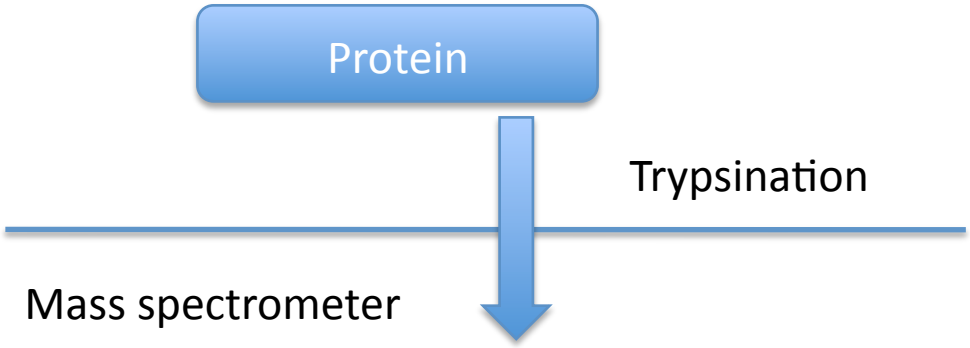
Phosphotyrosine signalling



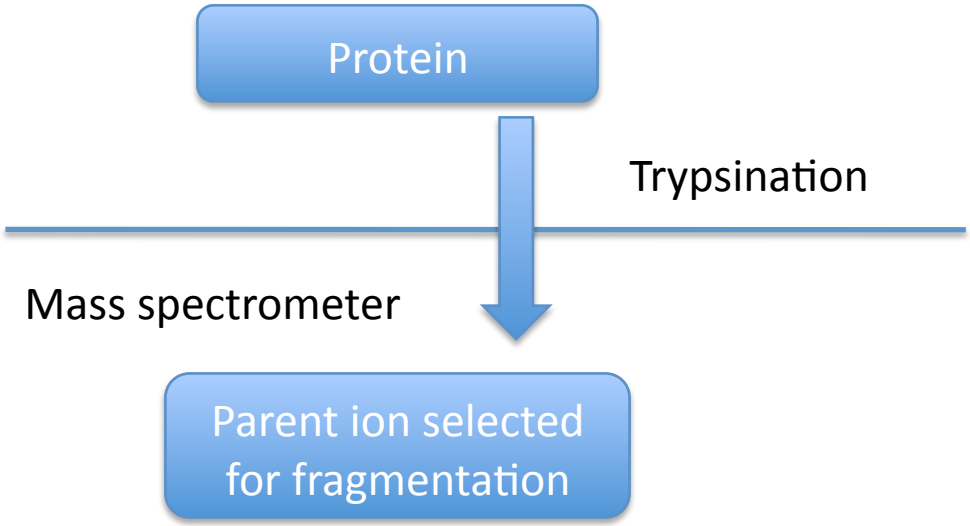
Global phosphoproteomics approach



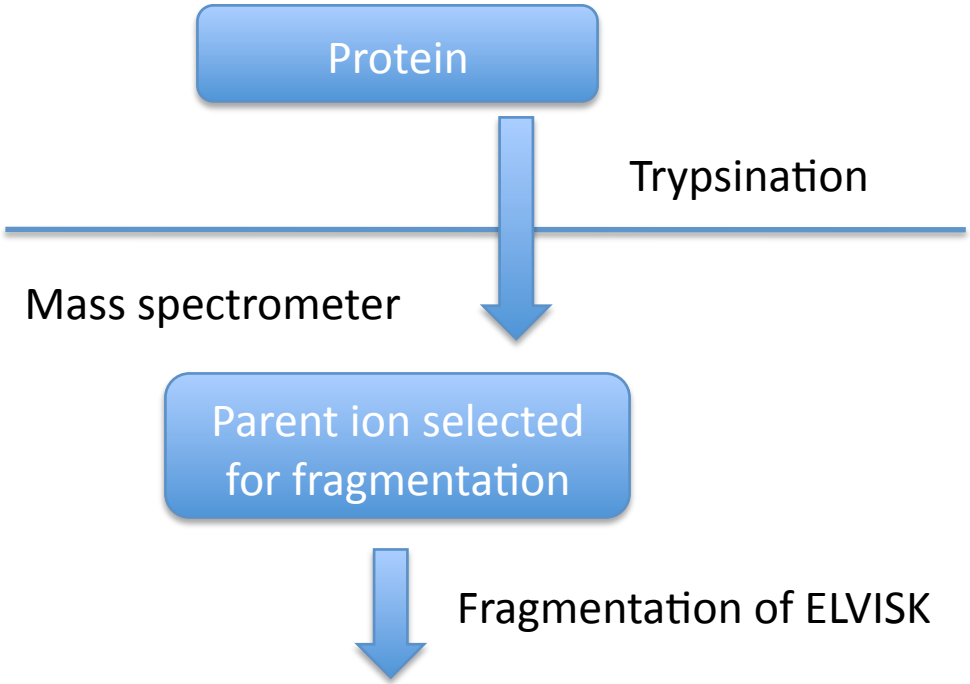
Wet lab



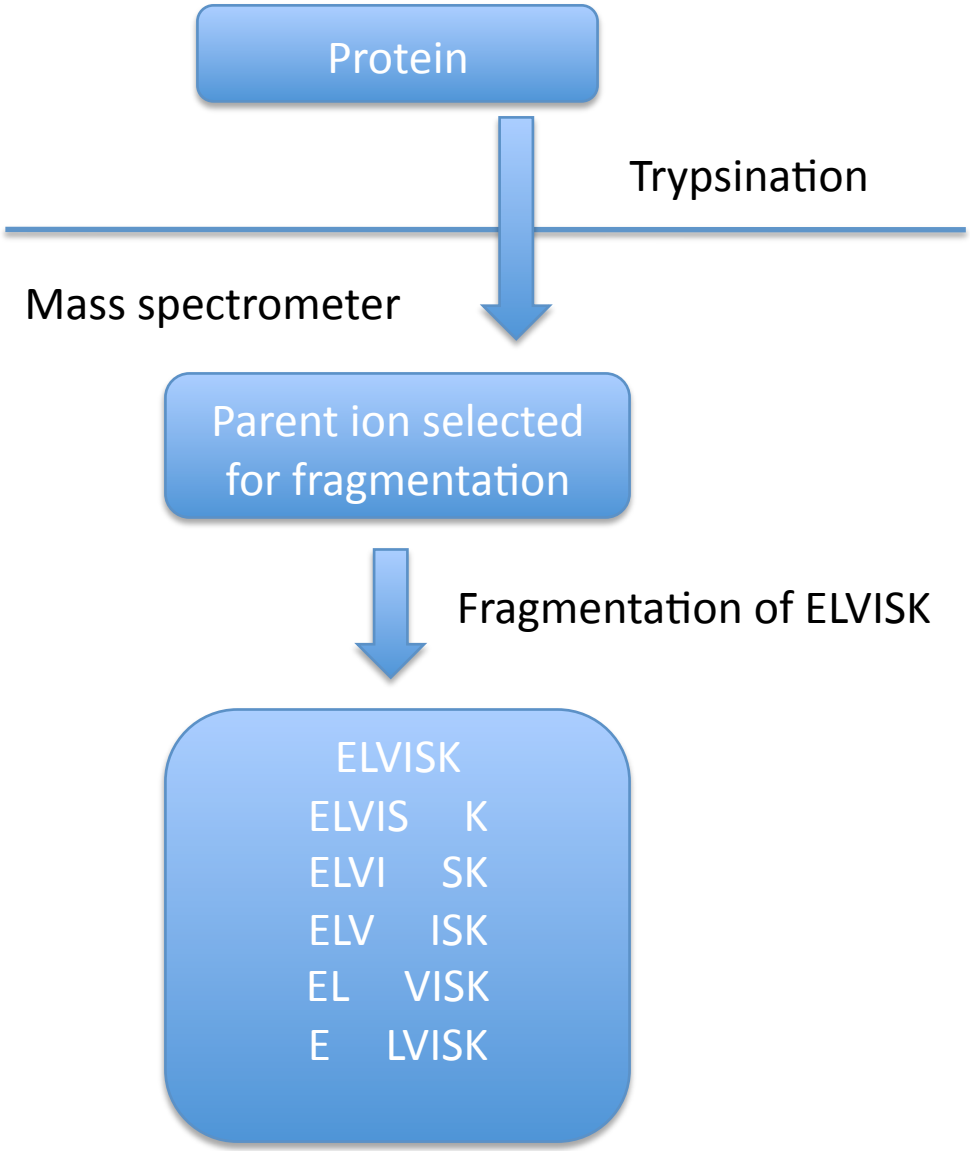
Wet lab


Protein
Trypsination
Mass spectrometer
Parent ion selected for fragmentation

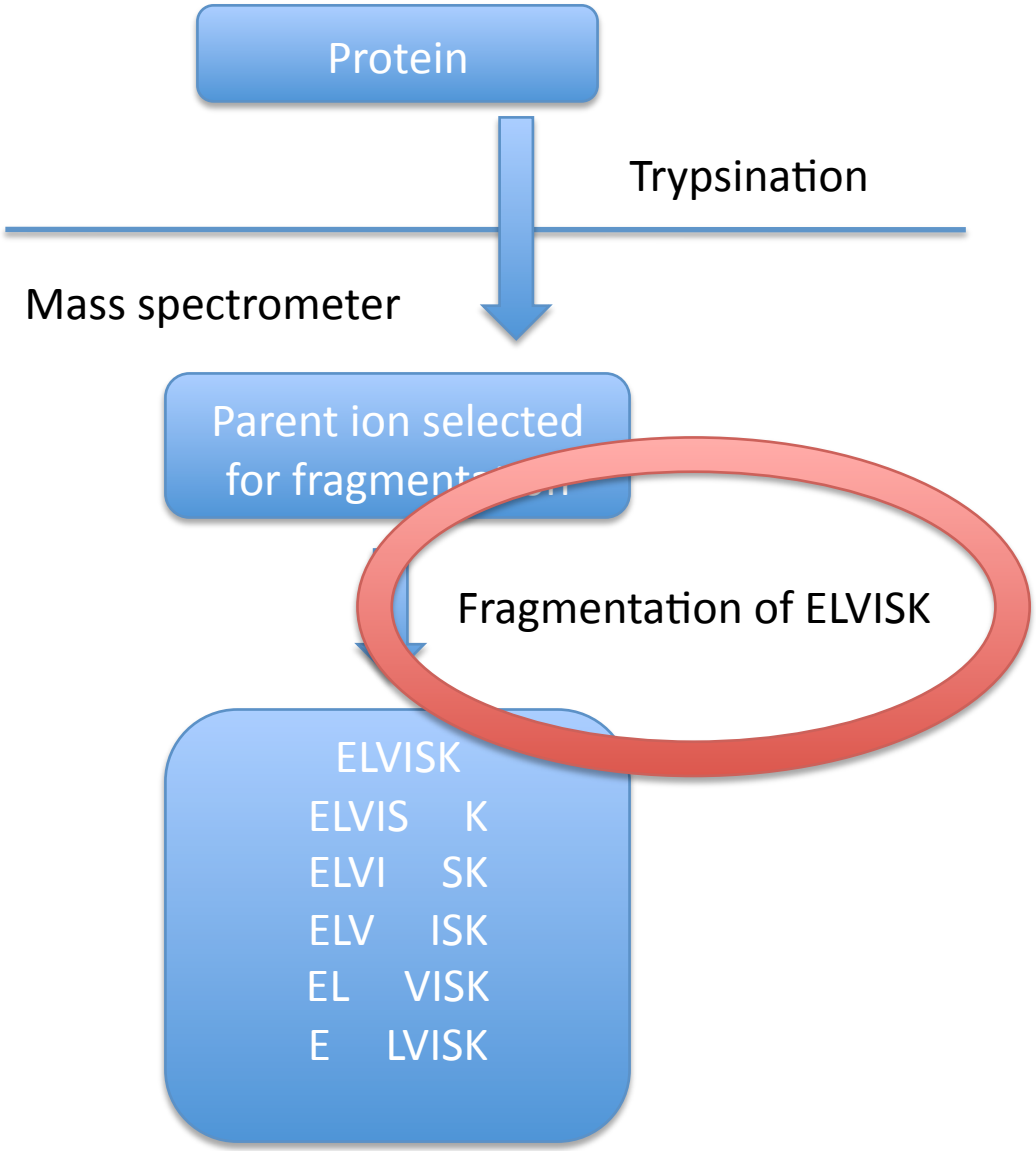
Wet lab


Protein
Trypsination
Mass spectrometer
Parent ion selected for fragmentation
Fragmentation of ELVISK

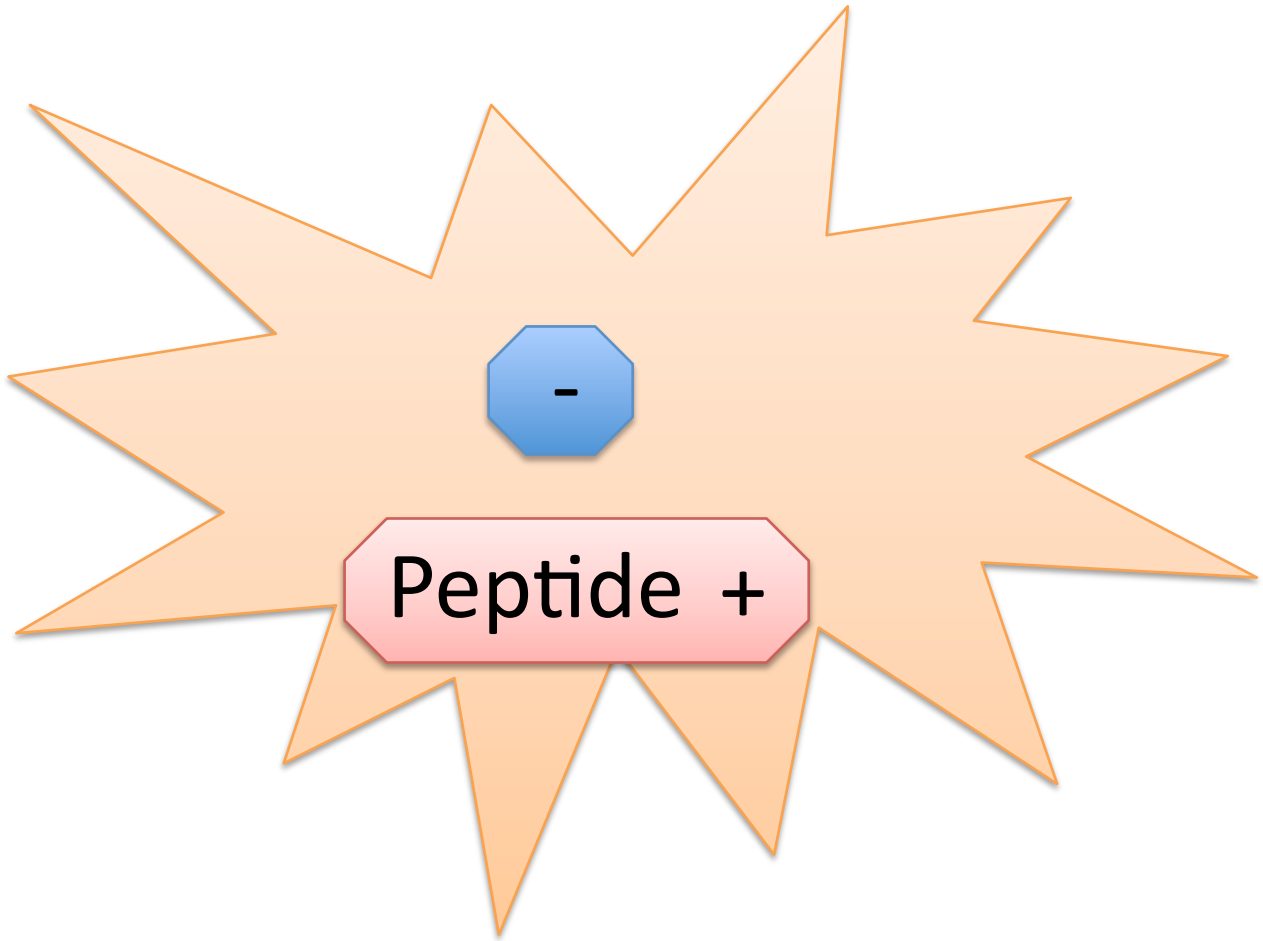
Wet lab


Protein
Trypsination
Mass spectrometer
Parent ion selected for fragmentation
Fragmentation of ELVISK
ELVISK
ELVIS K
ELVI SK
ELV ISK
EL VISK
E LVISK

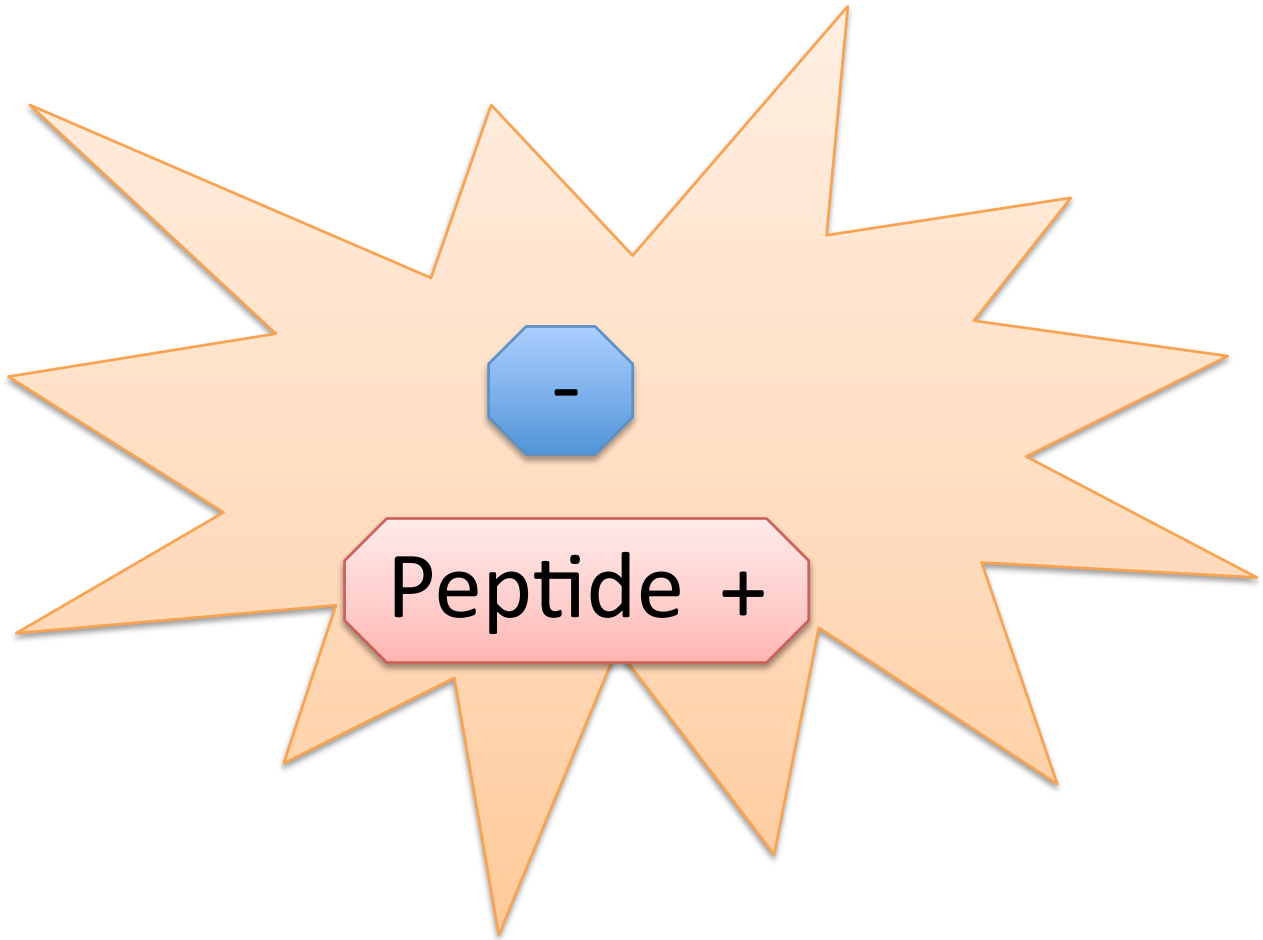
Wet lab


Protein
Trypsination
Mass spectrometer
Parent ion selected
Fragmentation of ELVISK
ELVISK
ELVIS K
ELVI SK
ELV ISK
EL VISK
E LVISK

Electron transfer dissociation ETD



Electron transfer dissociation ETD



ETD, no neutral loss of H3PO4

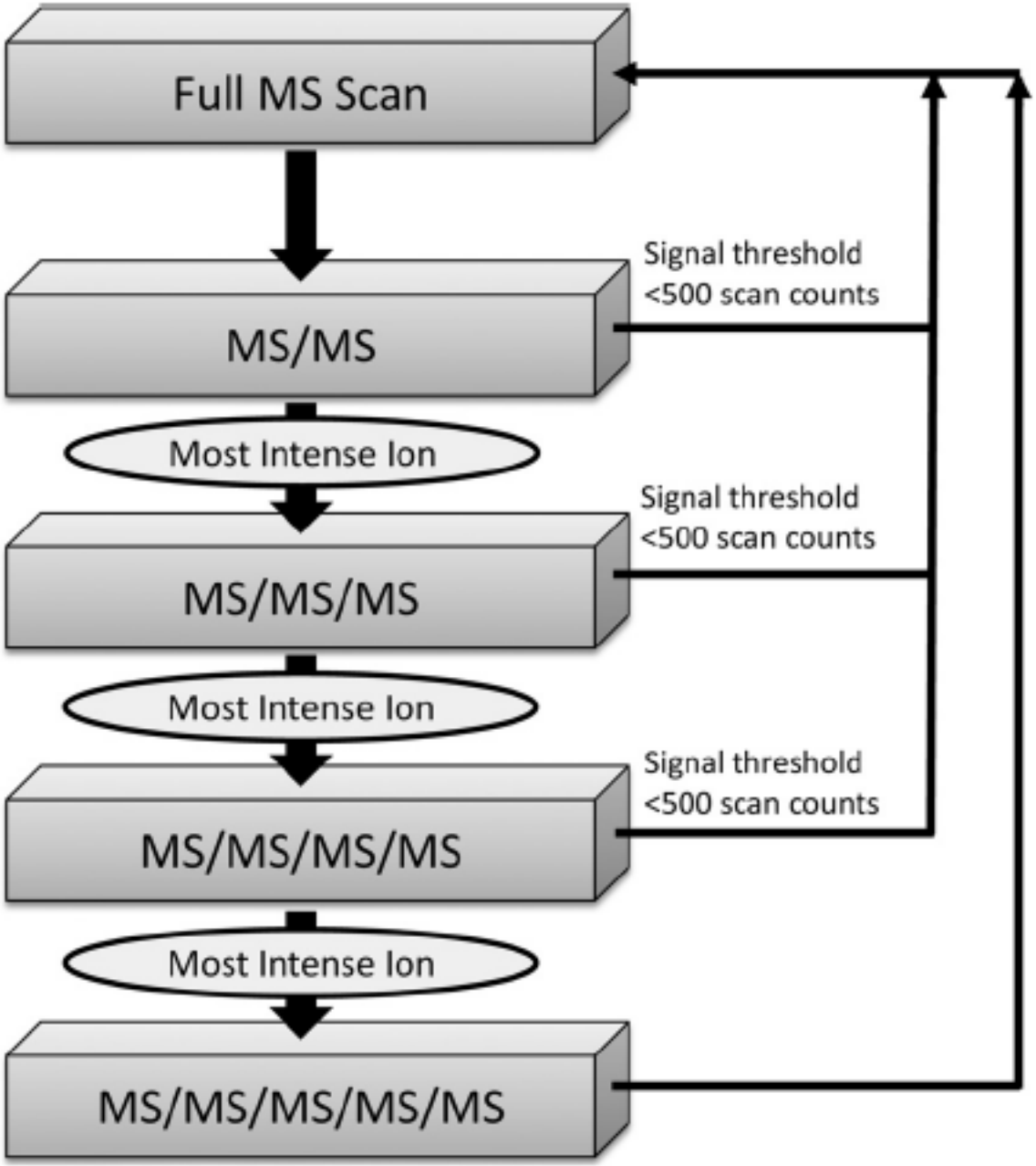
Collision induced dissociation CID

Peptide

Collision induced dissociation CID

Peptide

MS/MS using CID usually generates the dominant neutral loss of H3PO4 (-97.98Da) from Serine and threonine, absorbs energy



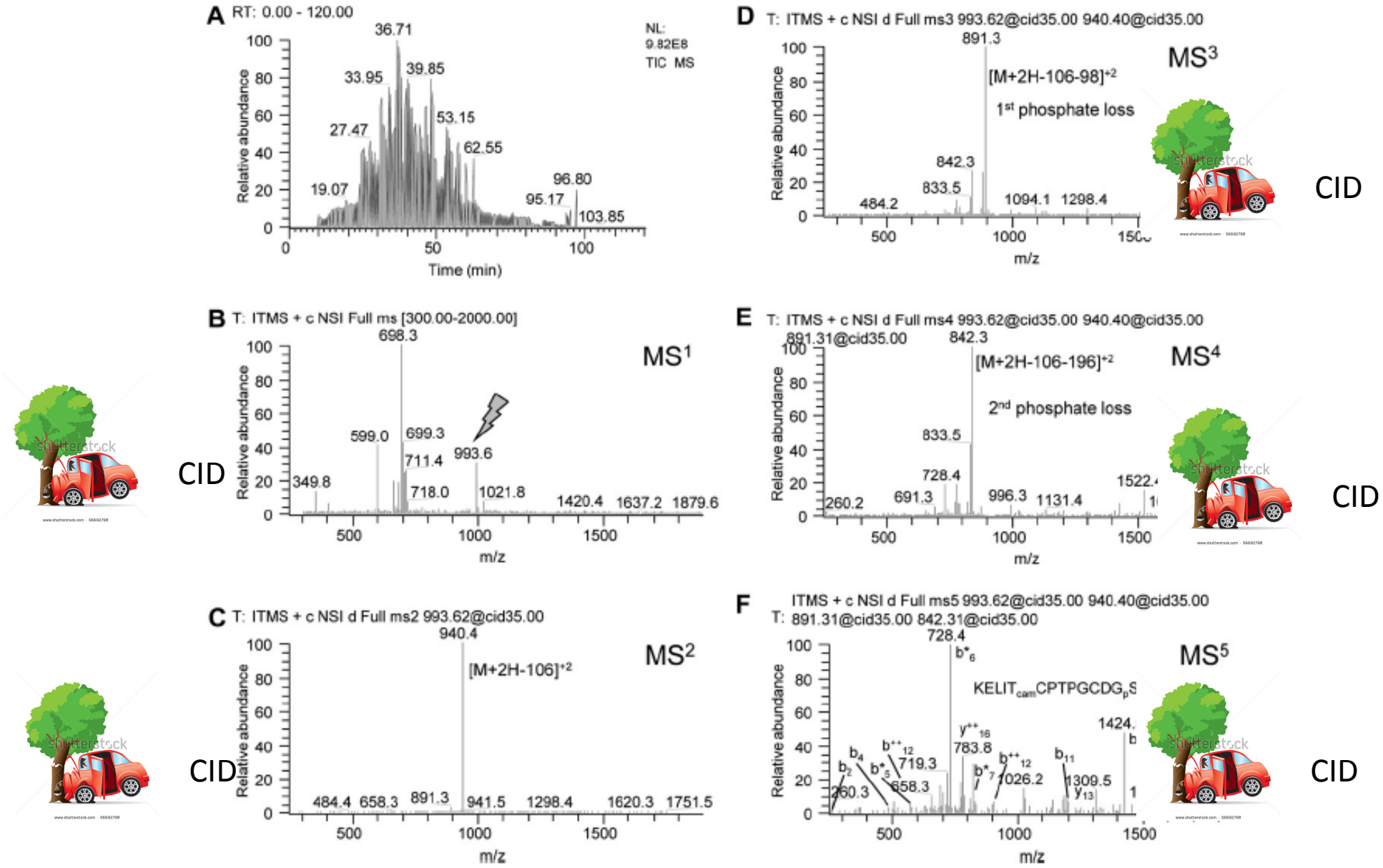
CID

CID

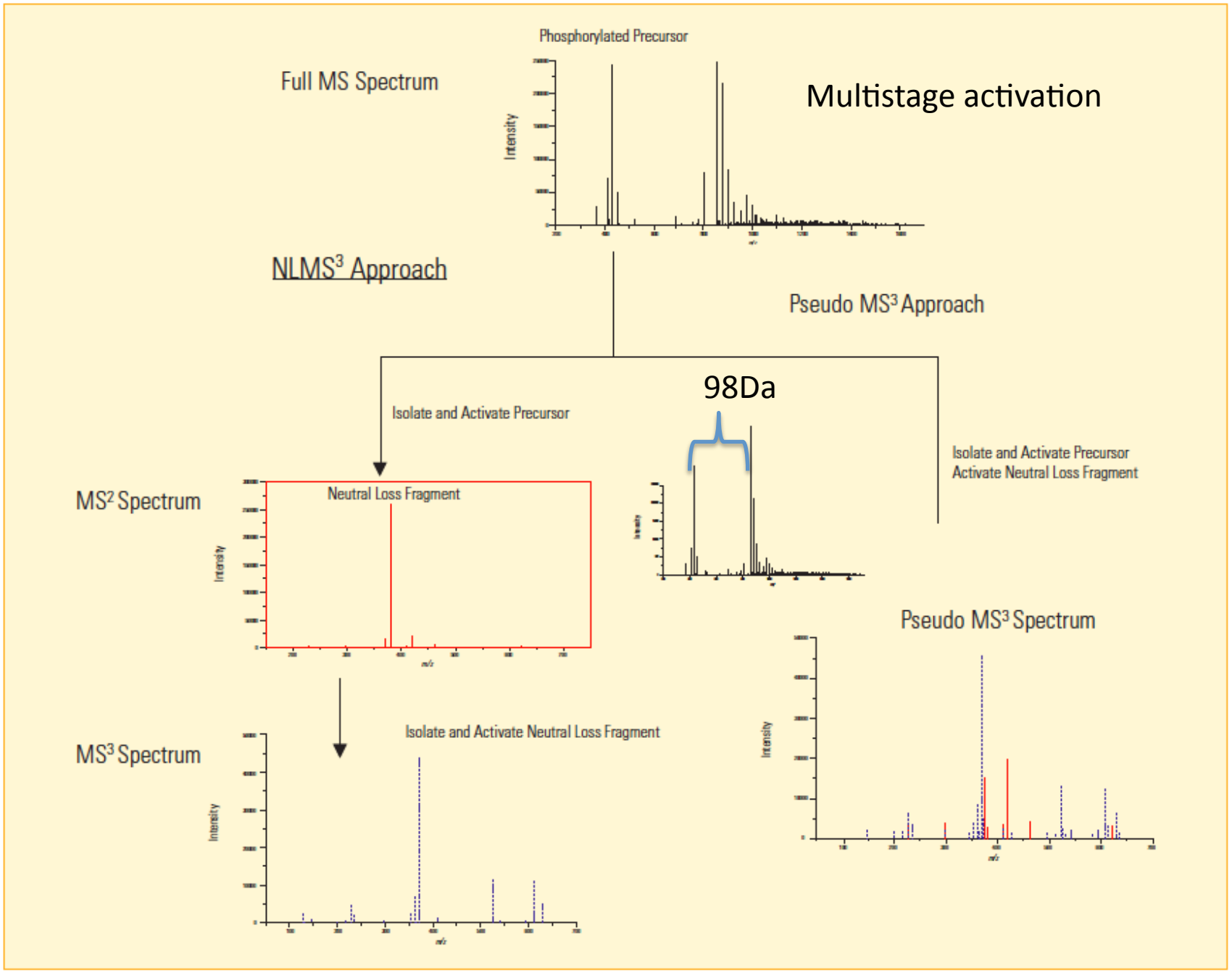
CID

CID

Chesnik et al. *Analytical Biochemistry*, 2011

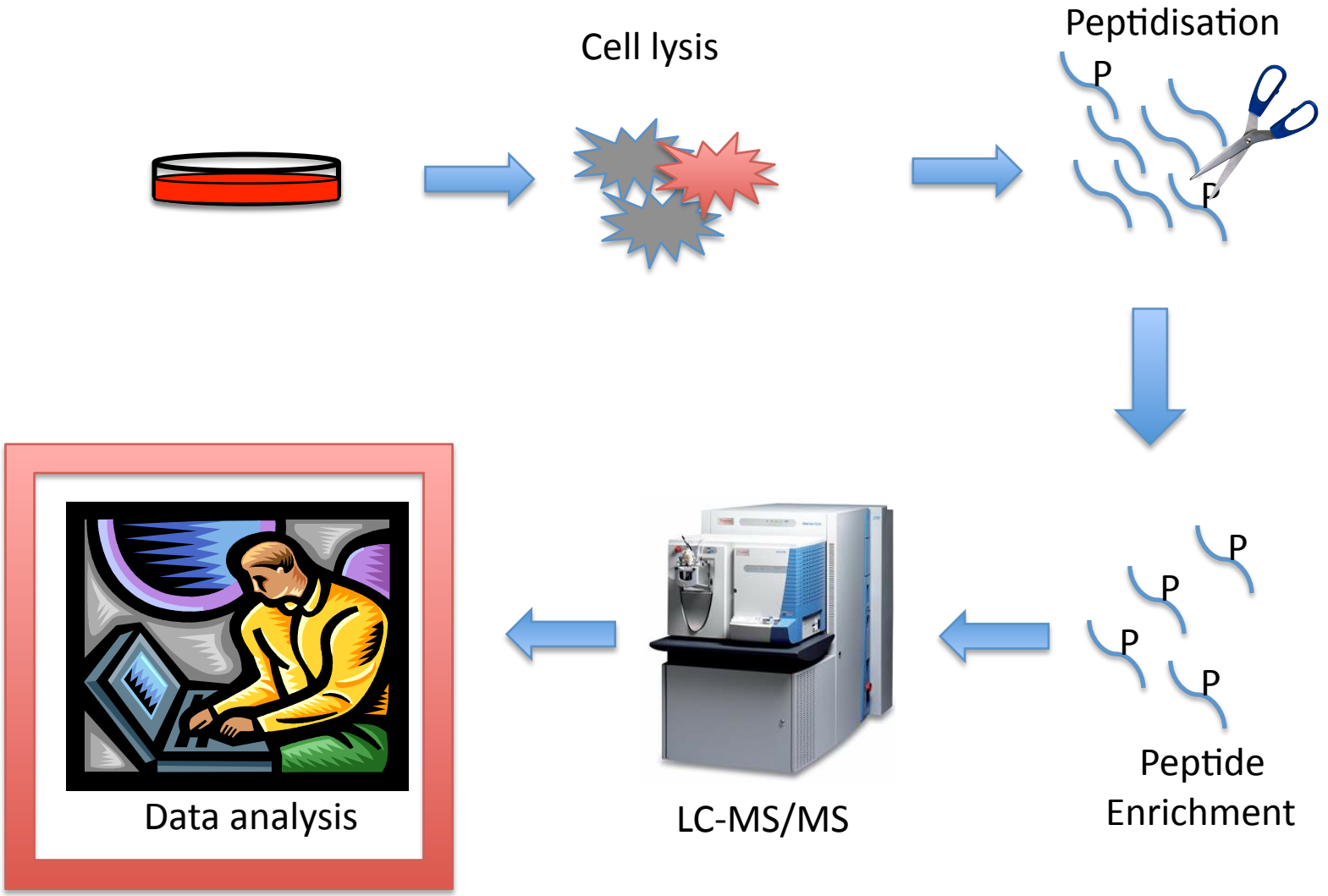


Chesnik et al. *Analytical Biochemistry*, 2011

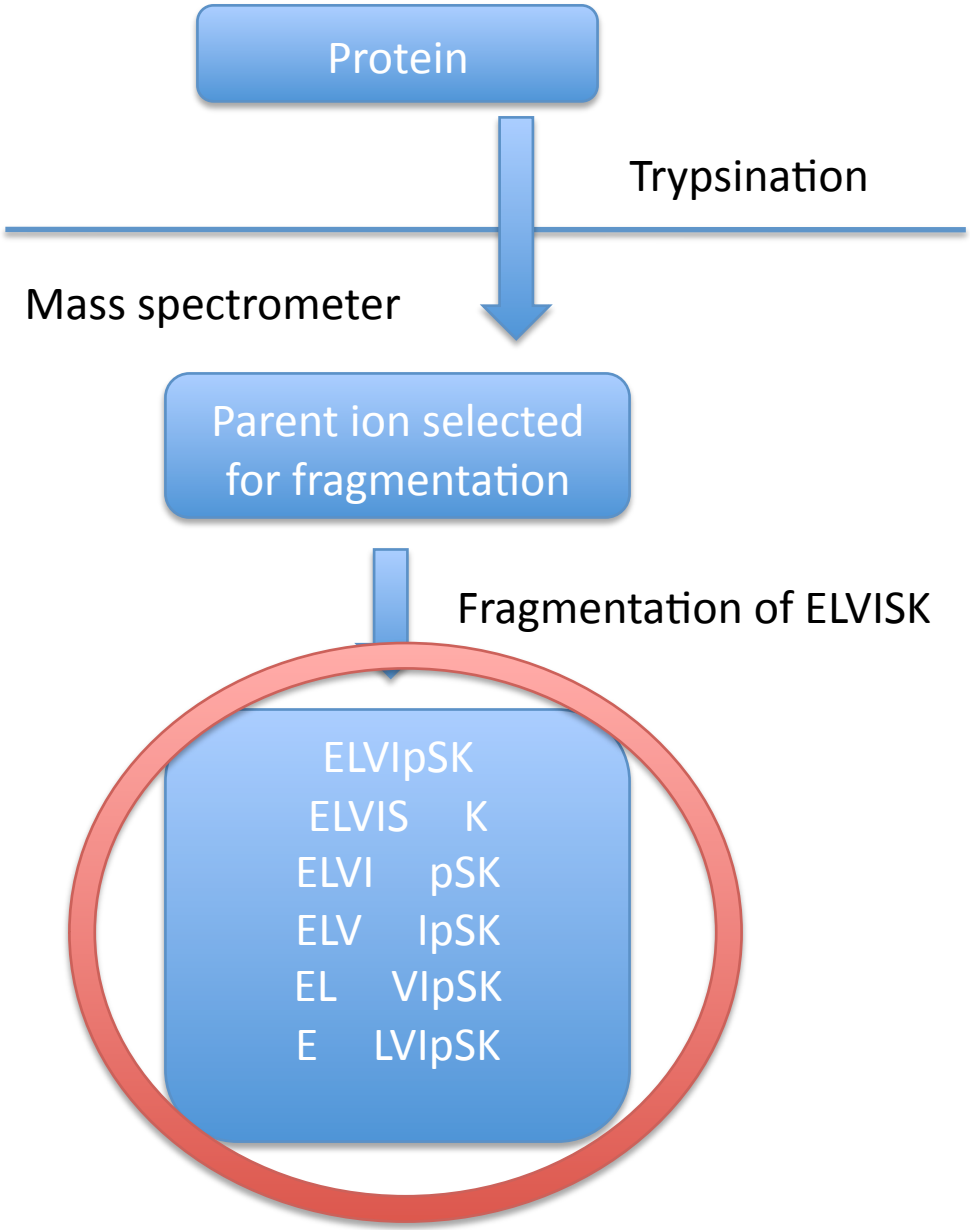


Thermo Scientific

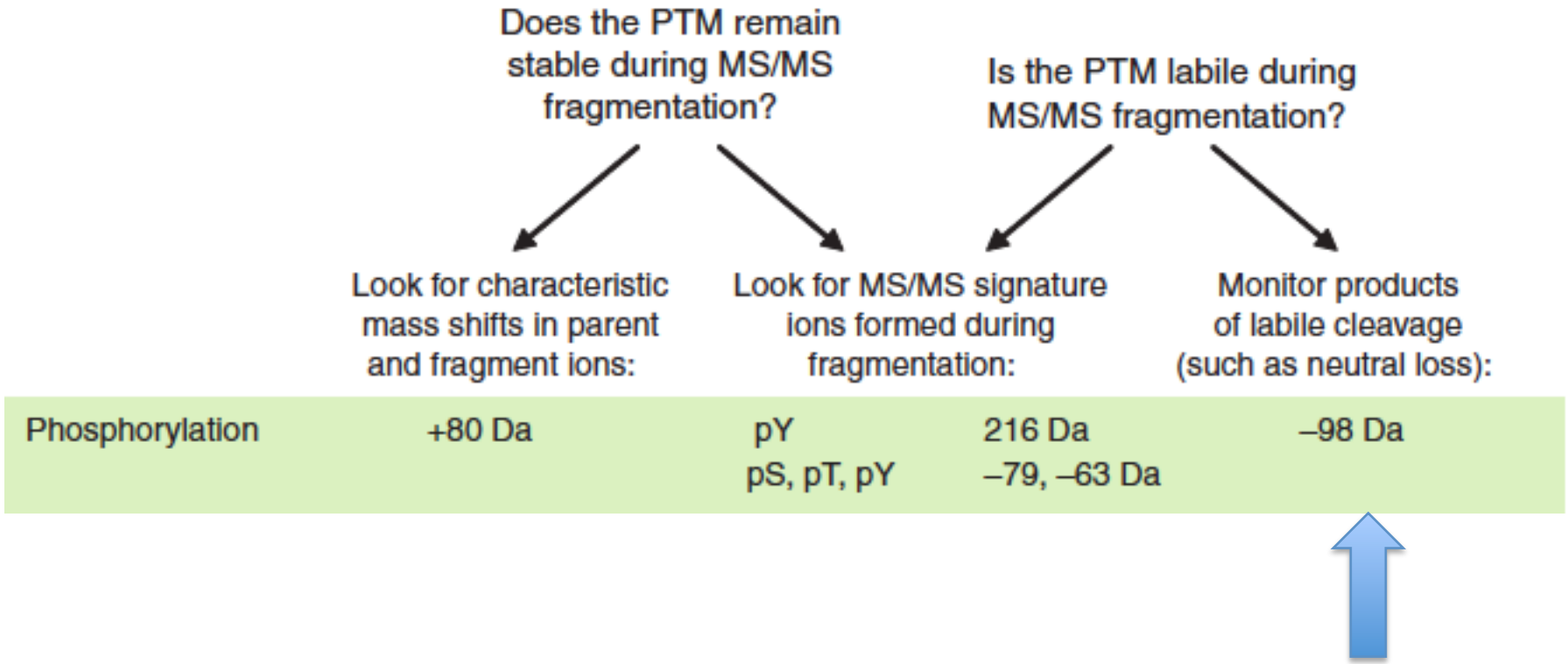
Global phosphoproteomics approach



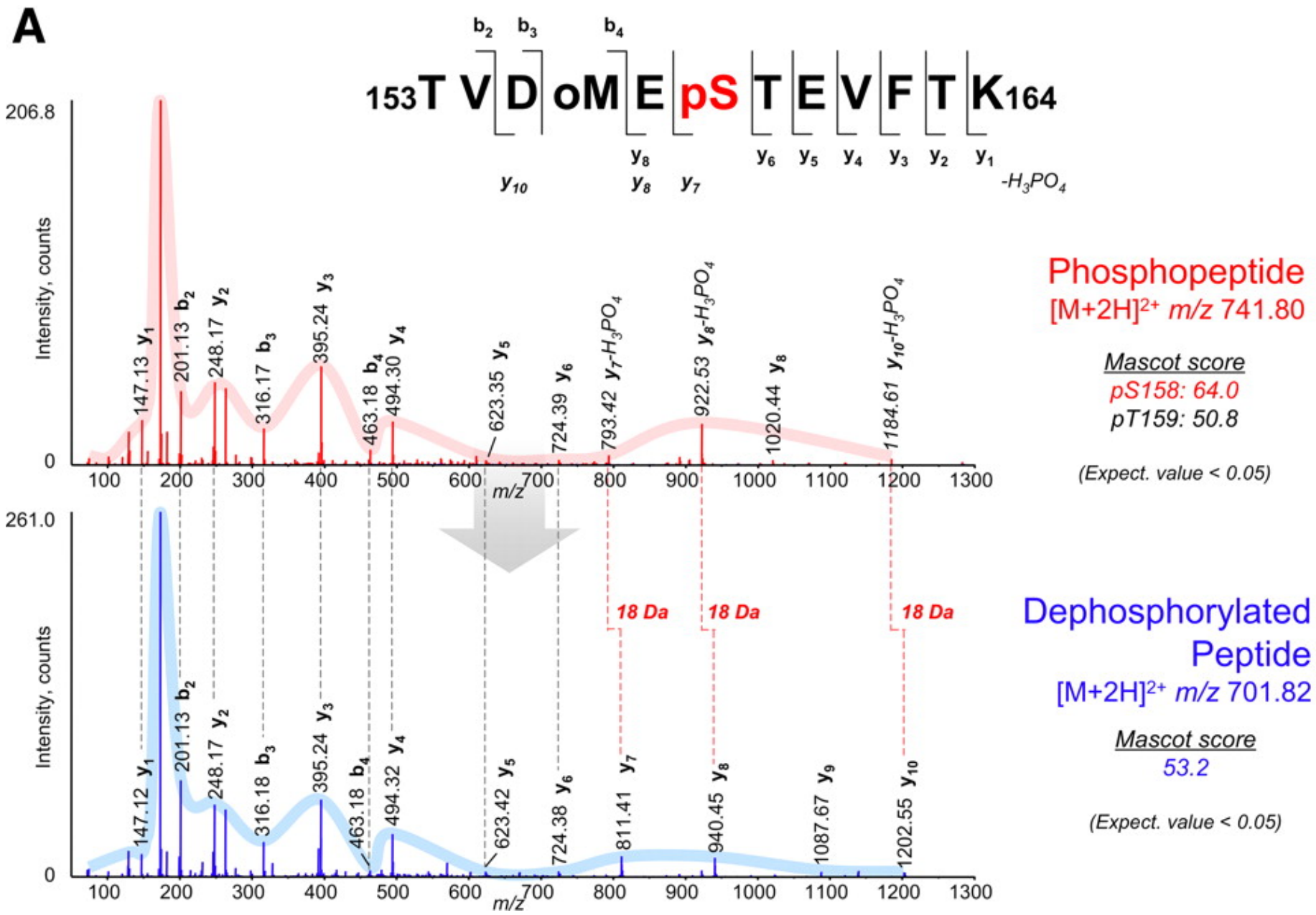
Wet lab


Protein
Trypsination
Mass spectrometer
Parent ion selected for fragmentation
Fragmentation of ELVISK
ELVIpSK
ELVIS K
ELVI pSK
ELV IpSK
EL VIpSK
E LVIpSK

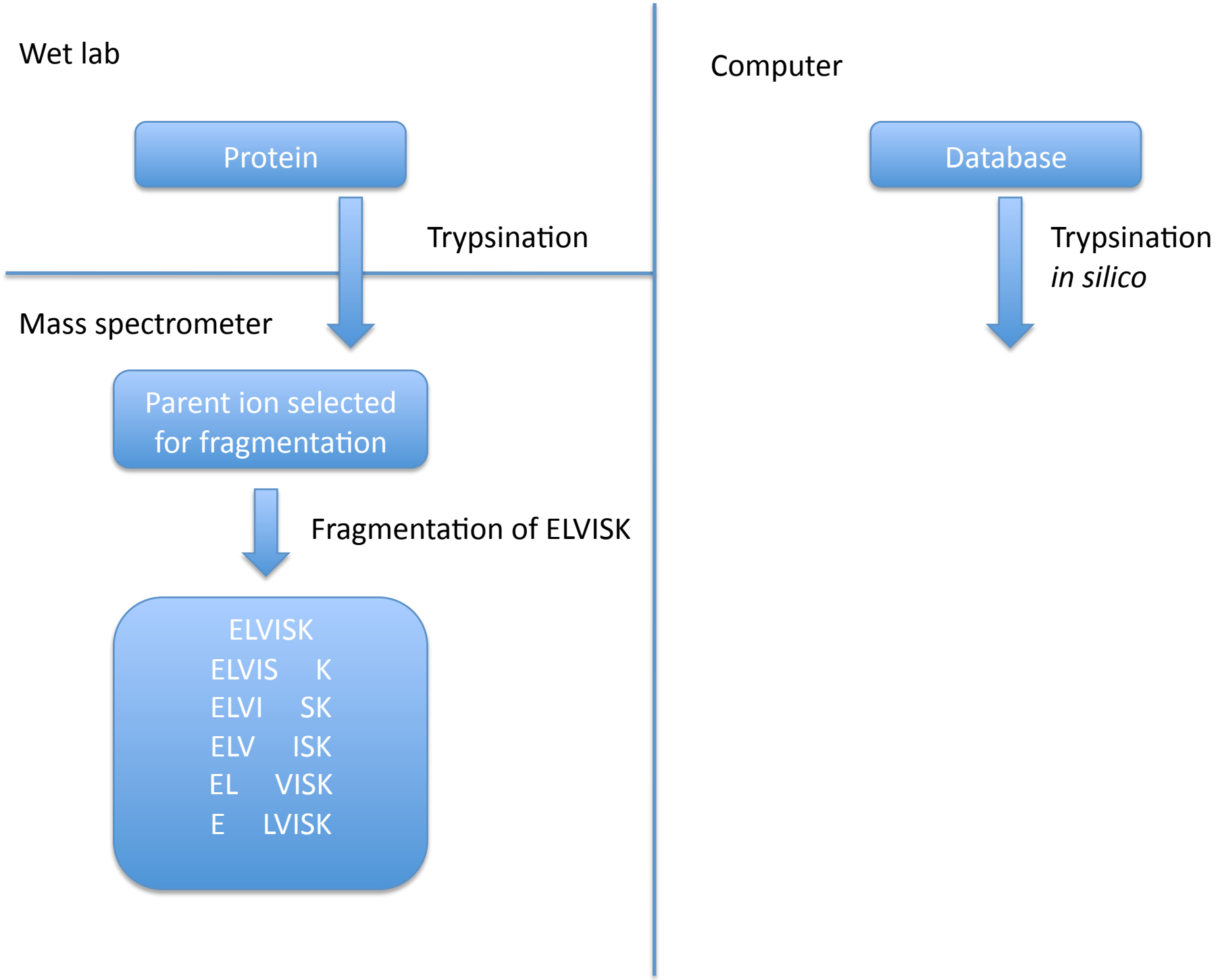
Mapping of PTMs with MS



Witze et al. *Nature Methods* 2007



Imanishi S Y et al. *Mol Cell Proteomics* 2007;6:1380-1391


Wet lab
Protein
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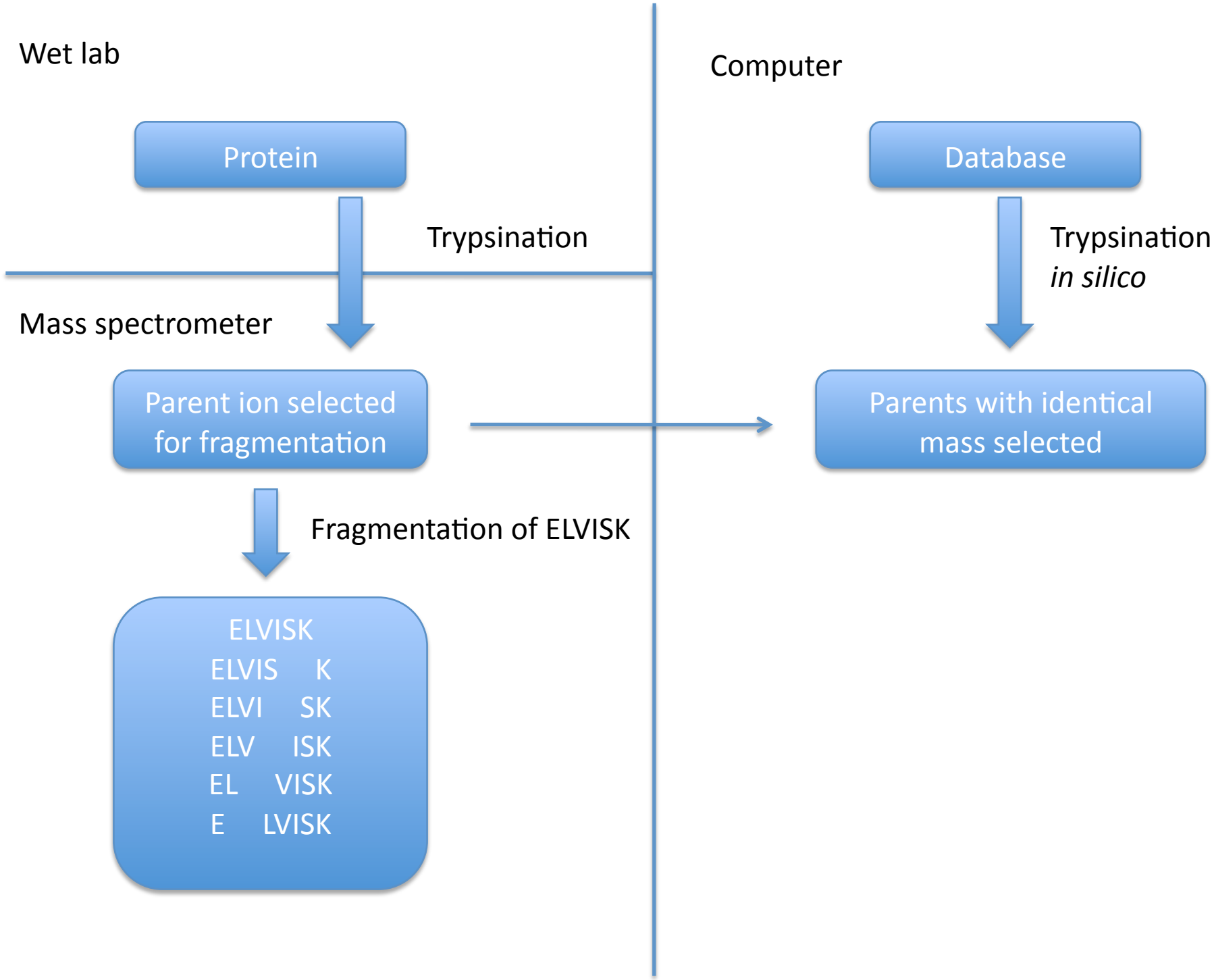
Fragmentation of ELVISK
ELVISK
ELVIS K
ELVI SK
ELV ISK
EL VISK
E LVISK
Computer
Database
Trypsination
in silico


Wet lab
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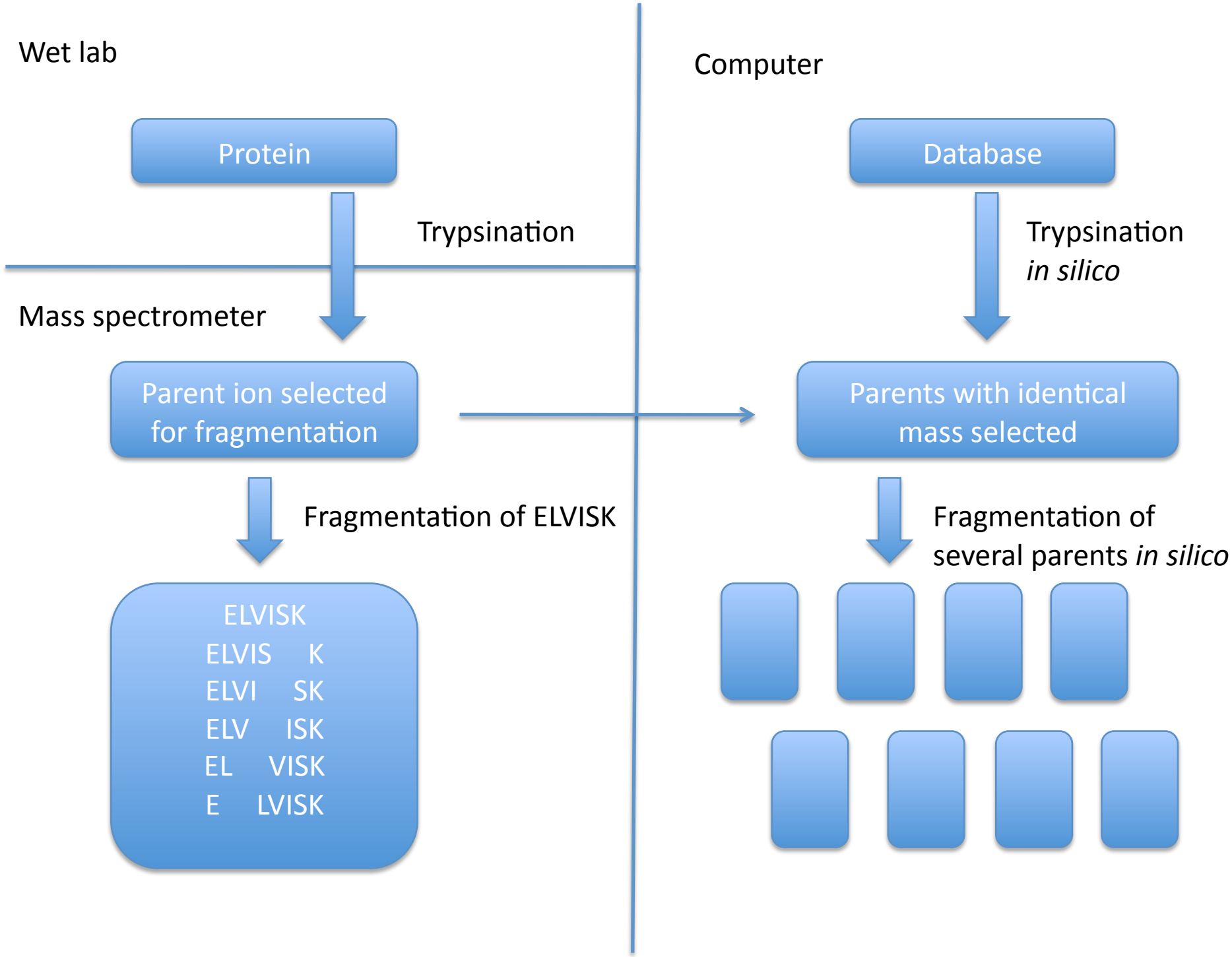
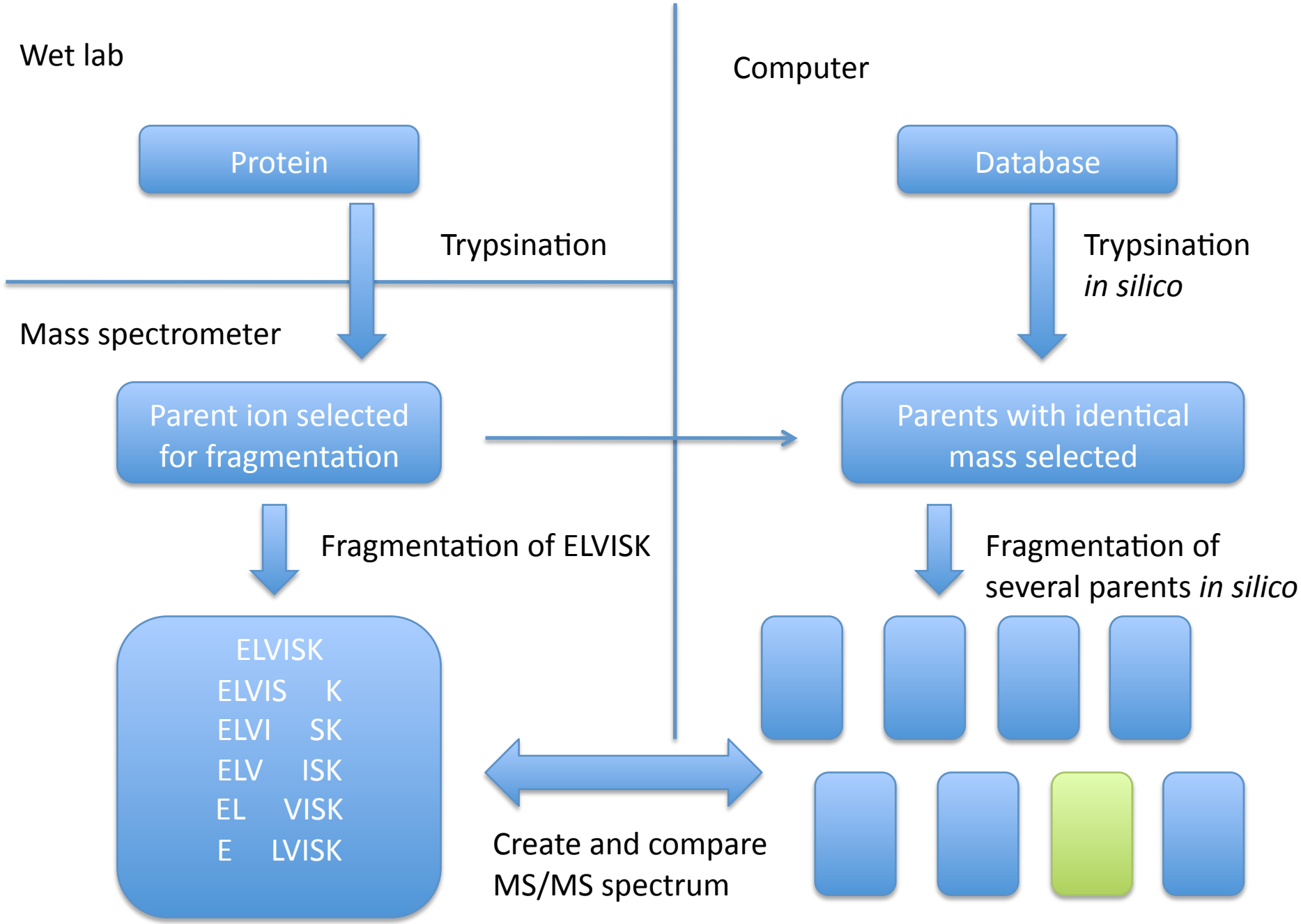
in silico
Mass spectrometer
Parent ion selected for fragmentation
Parents with identical mass selected
Fragmentation of ELVISK
ELVISK
ELVIS K
ELVI SK
ELV ISK
EL VISK
E LVISK


Wet lab
Computer
Protein
Trypsination
Mass spectrometer
Parent ion selected for fragmentation
Fragmentation of ELVISK
ELVISK
ELVIS K
ELVI SK
ELV ISK
EL VISK
E LVISK
Database
Trypsination *in silico*
Parents with identical mass selected
Fragmentation of several parents *in silico*


Wet lab
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Trypsination
in silico
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Parent ion selected
for fragmentation
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mass selected
Fragmentation of ELVISK
Fragmentation of
several parents in silico
ELVISK
ELVIS K
ELVI SK
ELV ISK
EL VISK
E LVISK
Create and compare
MS/MS spectrum

Why to map phosporylation sites
How to map phosphorylation sites