Phosphoproteomics

PhD Henri Blomster
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
Protein kinase

Protein phosphatase

Signal in

Signal out
Why to map PTM sites: a systems biology view

Figure 1. Panther Gene Ontology terms of Biological Processes and Molecular Functions. Out of 412 Panther GO terms, 406 are associated with phosphoproteins. Panther GO terms associated with non-phosphoproteins only.
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

Data analysis

Cell lysis

Protein Purification

Peptidisation

Peptide Enrichment

LC-MS/MS
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

Cell lysis → Peptidisation

Data analysis → LC-MS/MS → Peptide Enrichment
A global approach

• Advantages
  – high number of phosphorylation sites identified

• Disadvantages
  – low-abundant proteins not detected
The phosphoproteome is dynamic
The phosphoproteome is dynamic

1. State 1
2. State 2
- M phase
- Physiological change
- S phase
The phosphoproteome is dynamic
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


**b**

Peak alignment and intensity measurement

Sample 1 → Proteolysis (trypsin) → LC-MS/MS

Sample 2 → Proteolysis (trypsin) → LC-MS/MS

Align

$m/z$
Amount of non-phosphorylated

Amount of phosphorylated

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

Cell lysis

Peptidisation

Peptide Enrichment

Data analysis

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

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.

Phosphotyrosine signalling

Olsen et al. Cell 2006
Phosphotyrosine signalling

Mix of P-Tyr ABs or pTyr binding domains

Cell lysate

Peptides

Mix of P-Tyr ABs

LC-MS/MS

pTyr proteins

Trypsin

Peptides

(IMAC, TiO2)

LC-MS/MS
Global phosphoproteomics approach

Cell lysis

Data analysis

LC-MS/MS

Peptidisation

Peptide Enrichment
Protein

Trypsination

Mass spectrometer
Wet lab

Protein

Trypsination

Mass spectrometer

Parent ion selected for fragmentation
Protein

Parent ion selected for fragmentation

Fragmentation of ELVISK

Wet lab

Mass spectrometer

Trypsination
Wet lab

Protein

Trypsination

Mass spectrometer

Parent ion selected for fragmentation

Fragmentation of ELVISK

ELVISK
ELVIS K
ELVI SK
ELVIS K
EL VISK
EL VISK
E LVISK
Wet lab

Protein

Trypsination

Mass spectrometer

Parent ion selected for fragmentation

Fragmentation of ELVISK

ELVISK
ELVISK
ELVI SK
ELVIS K
ELVISK
ELVISK
ELVISK
Electron transfer dissociation ETD

- Peptide +
Electron transfer dissociation ETD

ETD, no neutral loss of H3PO4
Collision induced dissociation CID

He2

Peptide
Collision induced dissociation CID

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

Parent ion selected for fragmentation

Fragmentation of ELVISK

ELVIpSK
ELVIS K
ELVI pSK
ELV IpSK
EL VIpSK
EL VIpSK
Mapping of PTMs with MS

Does the PTM remain stable during MS/MS fragmentation?
- Look for characteristic mass shifts in parent and fragment ions:
  - Phosphorylation: +80 Da
  - pY
  - pS, pT, pY

Is the PTM labile during MS/MS fragmentation?
- Look for MS/MS signature ions formed during fragmentation:
  - Phosphorylation: 216 Da
  - pY
  - pS, pT, pY
  - 79, -63 Da

Monitor products of labile cleavage (such as neutral loss):
- Monophosphorylation: -98 Da

Phosphopeptide

[M+2H]^2+ m/z 741.80

Mascot score
pS158: 64.0
pT159: 50.8
(Expect. value < 0.05)

Dephosphorylated Peptide

[M+2H]^2+ m/z 701.82

Mascot score
53.2
(Expect. value < 0.05)

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

Parent ion selected for fragmentation

Mass spectrometer

Fragmentation of ELVISK

Database

Trypsination in silico

Wet lab

Trypsination
Wet lab

Protein

Parent ion selected for fragmentation

Mass spectrometer

Trypsination

Database

Trypsination *in silico*

Parents with identical mass selected

Fragmentation of ELVISK

- ELVISK
- ELVIS K
- ELVI SK
- ELV ISK
- EL VISK
- E LVISK
Protein

Parent ion selected for fragmentation

Parent ion selected for fragmentation

Fragmentation of ELVISK

Fragmentation of ELVISK

Trypsination

Database

Trypsination

in silico

Wet lab

Computer

Mass spectrometer

Parents with identical mass selected

ELVISK
ELVIS K
ELVI SK
ELV ISK
EL VISK
E LVISK

Fragmentation of several parents in silico
Wet lab

Protein

Parent ion selected for fragmentation

Fragmentation of ELVISK

ELVISK
ELVIS K
ELVI SK
ELV ISK
EL VISK
E LVISK

Trypsination

Mass spectrometer

Computer

Database

Trypsination in silico

Parents with identical mass selected

Fragmentation of several parents in silico

Create and compare MS/MS spectrum
Why to map phosphorylation sites
How to map phosphorylation sites

Data analysis

LC-MS/MS

Peptidisation

Peptide Enrichment