Quantitatively Probing Cellular Membrane Proteome Dynamics Using Membrane-Impermeable Chemical Probes and Proteomics Analysis


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Chemical proteomics: catch the fish you want

**Affinity group**

- **Biotin-Avidin, Click Chemistry, FLAG Tag...**

**Linker**

- **Length**
- **Isotopic labeling**
- **Hydrophobicity**
- **Chemical-cleavable**
- **Photo-cleavable**
- **Mass spectrometry cleavable**

**Reactive group**

- **Amino acids: lysine, cysteine**
- **Enzyme activity**
- **PTM: phosphorylation, glycosylation**

...
Cell membrane impermeable chemical probe

Affinity Group

Link

Reactive group
Schematic strategy for membrane protein enrichment

1. Labeling
2. Cell lysis
3. Protein enrichment
4. Enzymatic digest
5. (Peptide enrichment)
6. Elution

LC-MS/MS
Membrane protein enrichment specificity for bacterial cells

**Shewanella oneidensis MR-1**

Envelope membrane of gram-negative bacteria

Protein identifications of whole cell lysate

- Cytoplasm: 28%
- Envelope Membrane: 72%

Protein identifications by chemical probe enrichment

- Cytoplasm: 21%
- Envelope Membrane: 79%

From: PNNL
Hematite
Membrane protein enrichment specificity for human cells (HMEC)

Whole cell lysate
- Plasma membrane associated: 375
- Intracellular: 91
- Unknown: 17

Amine-reactive chemical probe (Sulfo-NHS-Biotin)
- Plasma membrane associated: 167
- Intracellular: 40
- Unknown: 27

N-linked glyco-protein (Aebersold, 2009) (Hydrazide-Biocytin)
- Plasma membrane associated: 121
- Intracellular: 9
- Unknown: 9

Intersection:
- Plasma membrane associated: 153
- Intracellular: 81
- Unknown: 62
Multiple labeling sites on extracellular region of cell surface protein

Chemical probe labeled peptides of epidermal growth factor receptor (EGFR)

Cell membrane protein dynamics

Membrane impermeable chemical probe
+
Quantitative proteomics
Application I: Membrane protein transportation

Proposed models depicting electron transfer pathways for *S. oneidensis* MR-1.

Experimental strategy

Membrane protein abundance changes observed via chemical-probe enrichment

Expression Ratio = \frac{\text{wild-type}}{\text{mutant}}

Log_2^{16/O/18/O) = -4 \text{ to } +4

Enriched protein by chemical probe

Whole cell lysate

Application II: Cell signal transduction

HMEC, 80% confluence

EGF starving, 18 hrs

EGF treatment, 7.5 min

Control

Chemical probe

Cell lysis

Peptide enrichment

Membrane protein redistribution induced by EGF signaling

Label-free quantitative proteomics
Cell surface receptor internalization induced by EGF

EGF Receptor peptide abundance profile

Control

EGF treatment


Integrin β1 peptide abundance profile

Control

EGF treatment

Translocation of Annexin A1 from cytoplasm to cell surface

Solito, E. et al., Post-translational modification plays an essential role in the translocation of annexin A1 from the cytoplasm to the cell surface, *FASEB*, 2006, 20, 1498-1500
Selected cell surface protein dynamics induced by EGF

- Unique aspects of surface proteome dynamics
- Direct results of cell signaling
- Complementary to other approaches for studying cell signaling (i.e., phosphorylation
- May reveal unique novel targets for functional studies or new biomarkers
Summary

• Membrane impermeable chemical probe demonstrated high specificity of membrane protein enrichment.

• Quantification strategy of cell surface membrane protein dynamics has been developed using membrane impermeable chemical probe in combination with quantitative proteomics analysis.

• Two biological application including membrane protein transport of bacterial cells and cell signal transduction of human cells were demonstrated using this quantification strategy.

• Other applications: environmental media stimuli, cell surface biomarker, membrane phospho-proteomics (pTyr)…
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