

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

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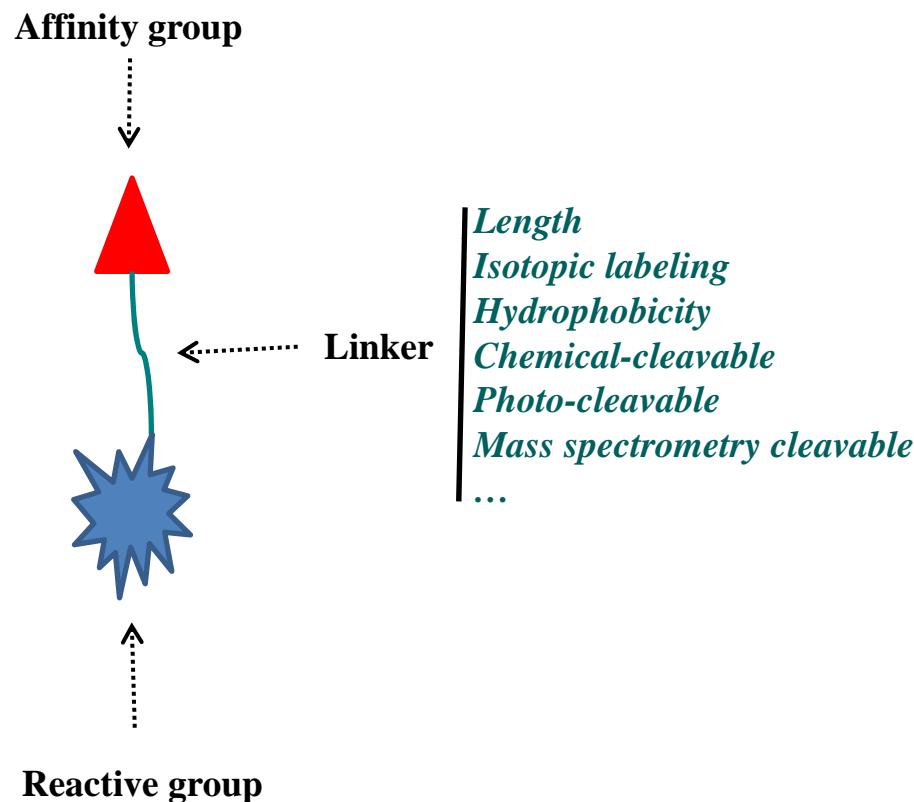
*Biological Sciences Division and Environmental Molecular Sciences Laboratory,
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Chemical proteomics: catch the fish you want

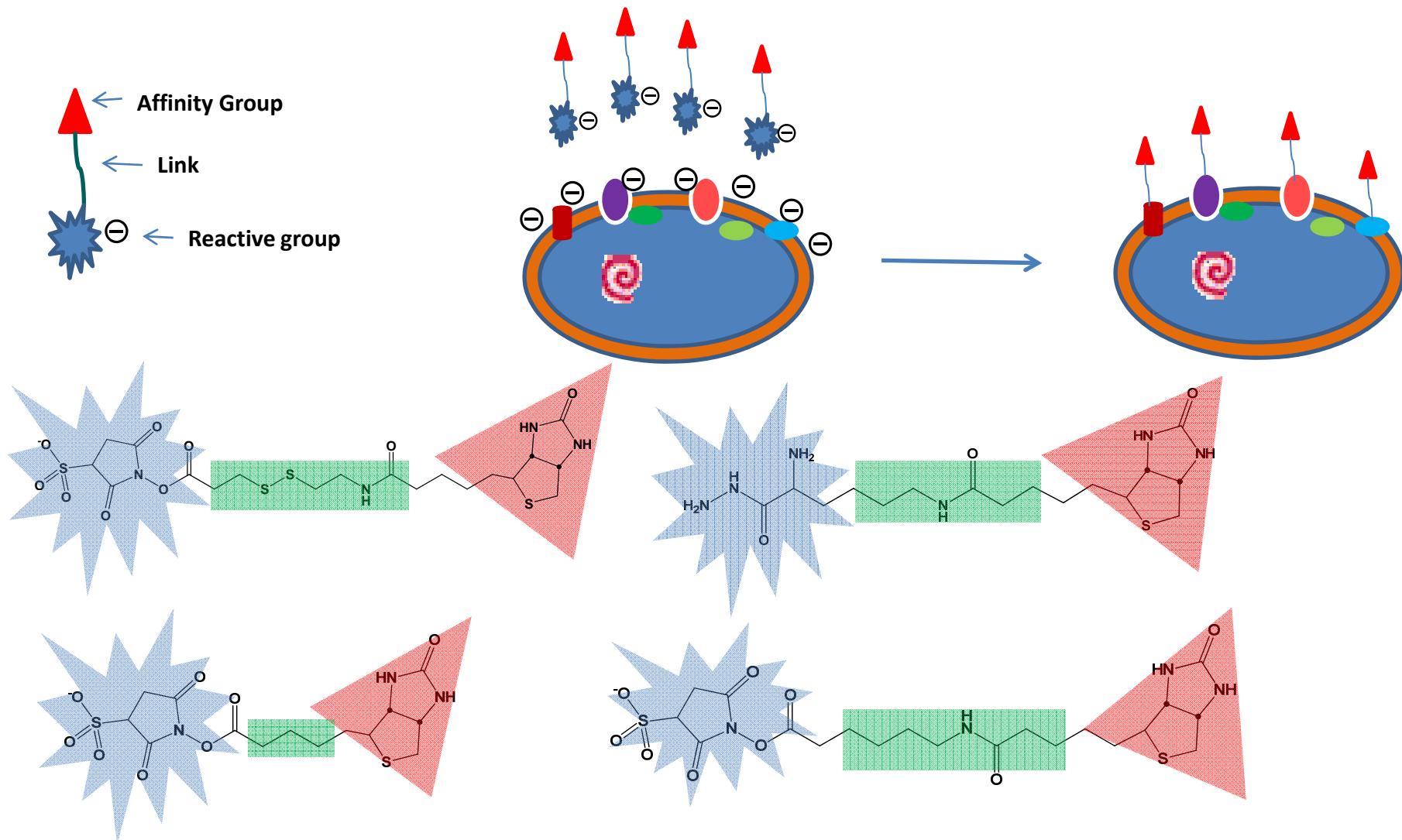
Biotin-Avidin, Click Chemistry, FLAG Tag...



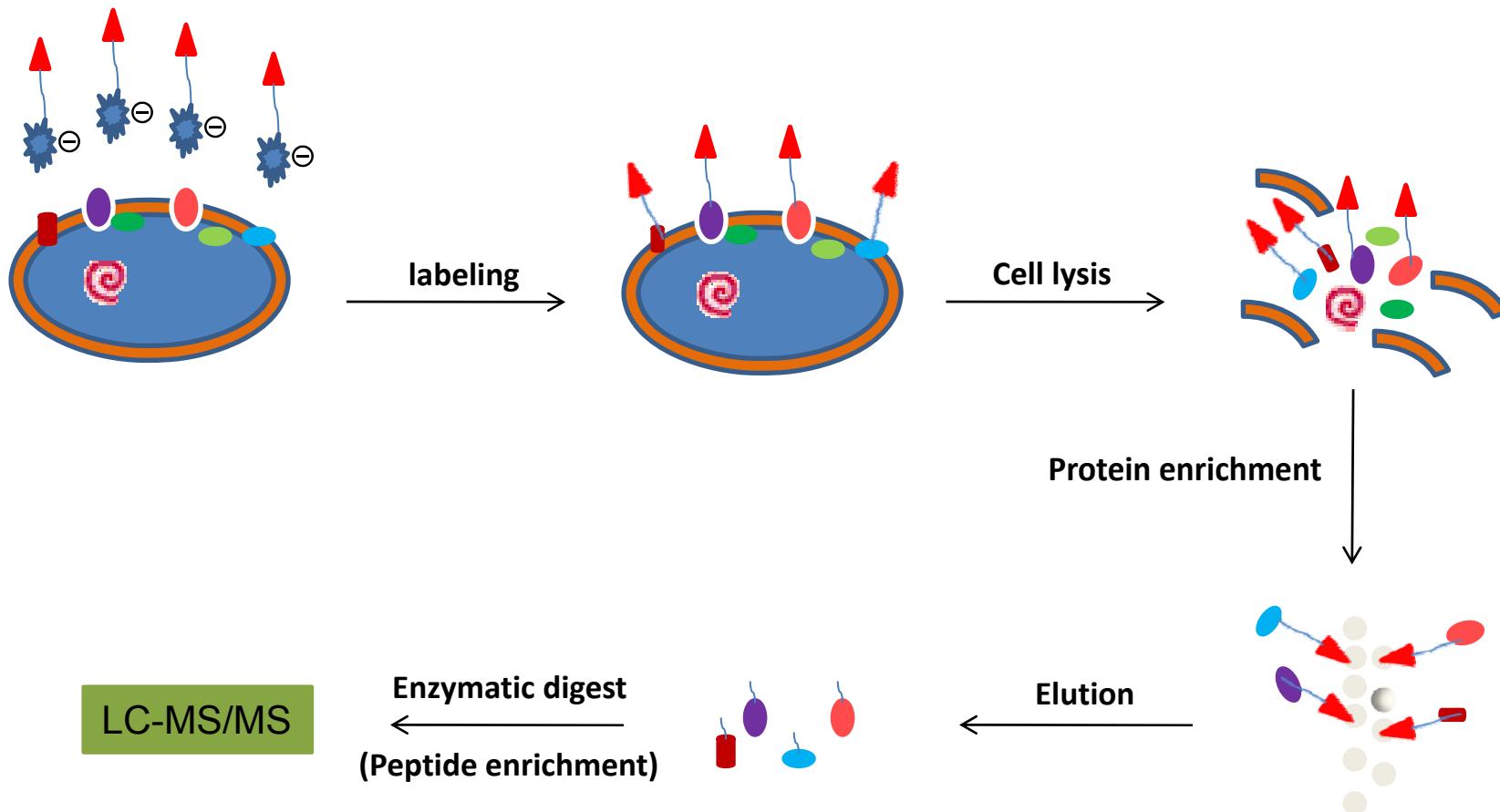
Amino acids: lysine, cysteine
Enzyme activity
PTM: phosphorylation, glycosylation
...



Cell membrane impermeable chemical probe



Schematic strategy for membrane protein enrichment

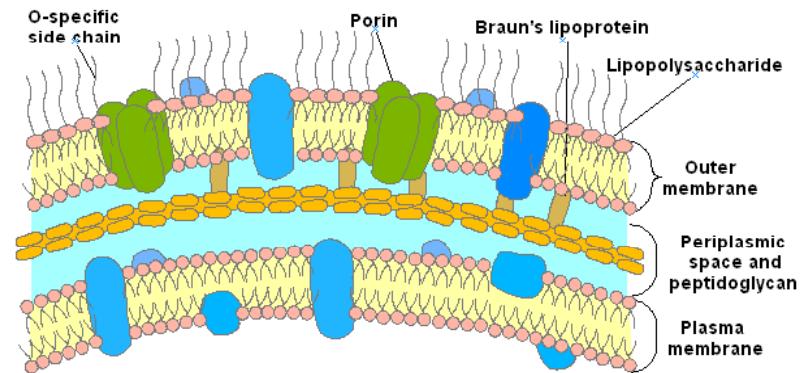


Membrane protein enrichment specificity for bacterial cells

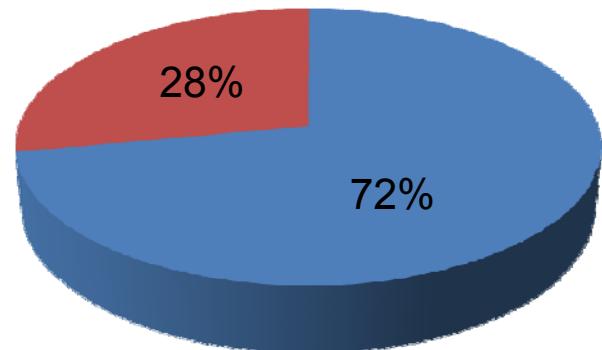
Shewanella oneidensis MR-1



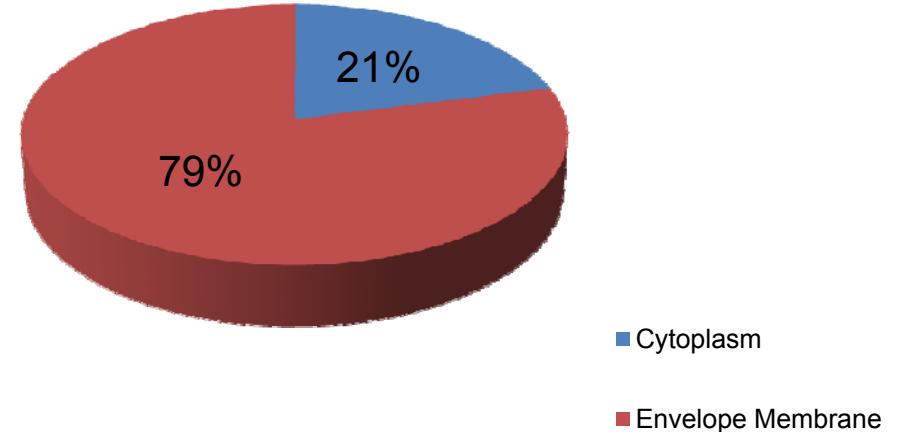
Envelope membrane of gram-negative bacteria



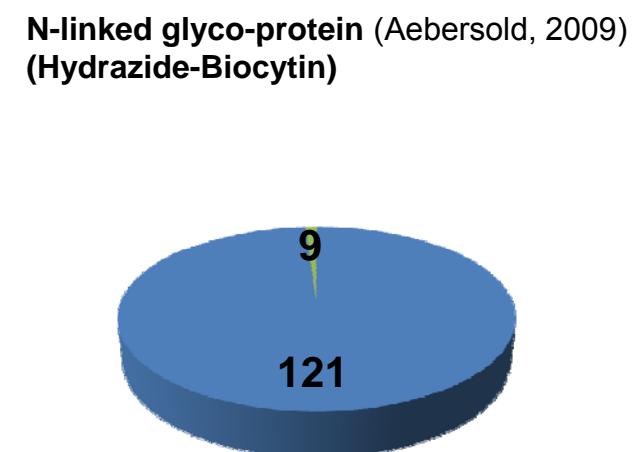
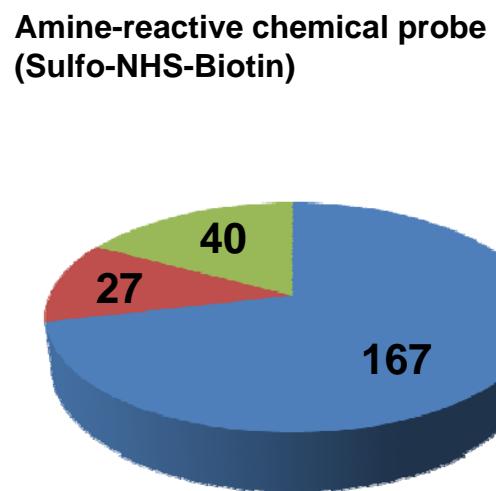
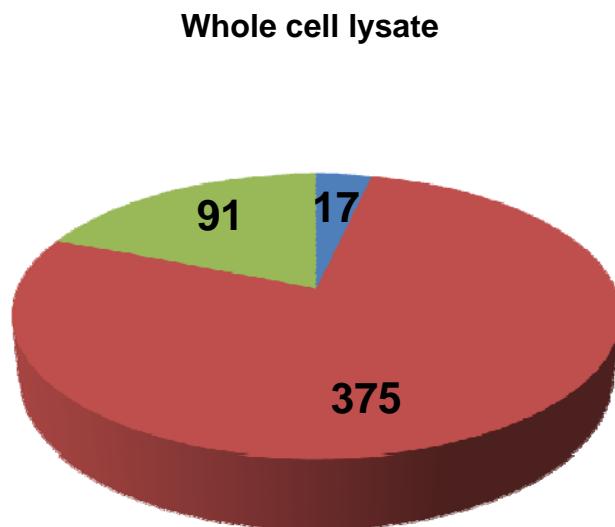
Protein identifications of whole cell lysate



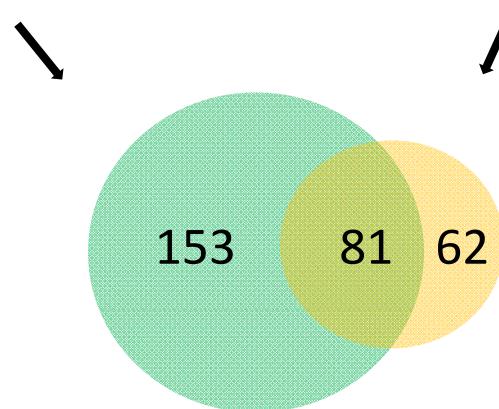
Protein identifications by chemical probe enrichment



Membrane protein enrichment specificity for human cells (HMEC)

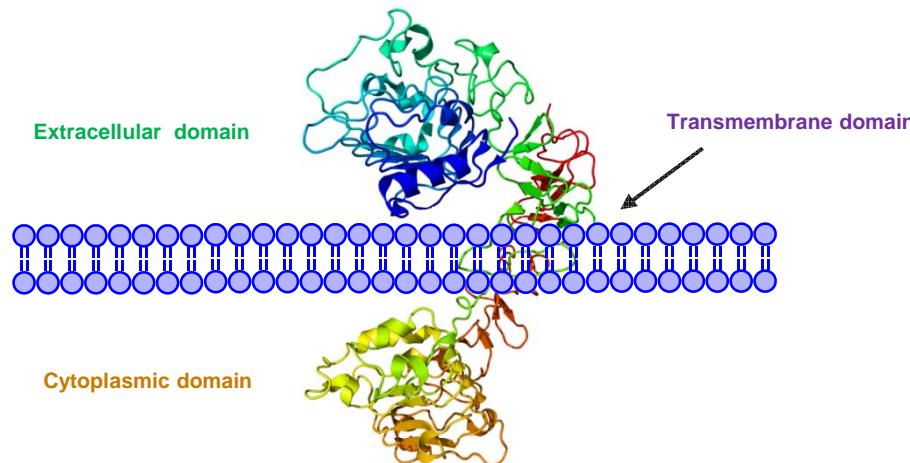


- Plasma membrane associated
- Intracellular
- Unknown



Multiple labeling sites on extracellular region of cell surface protein

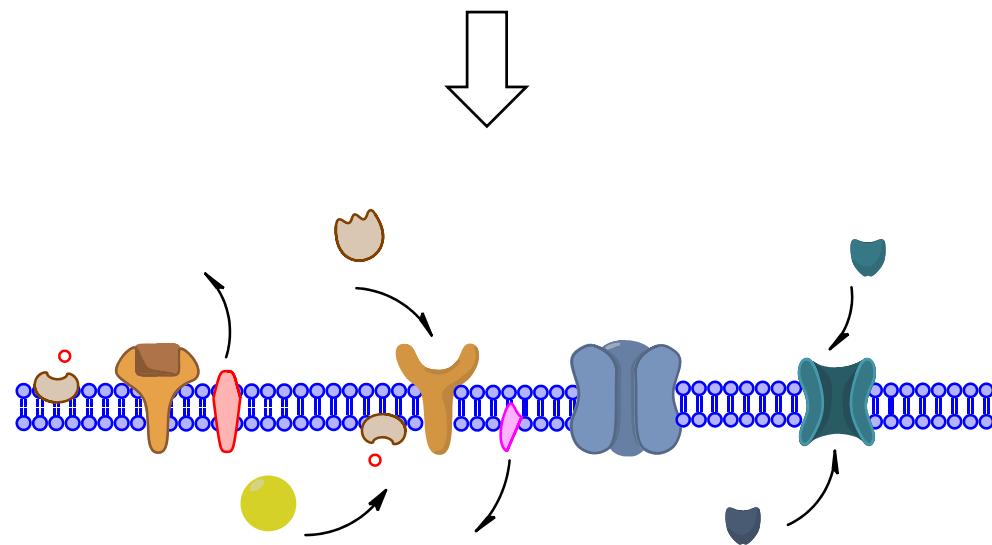
Chemical probe labeled peptides of epidermal growth factor receptor (EGFR)



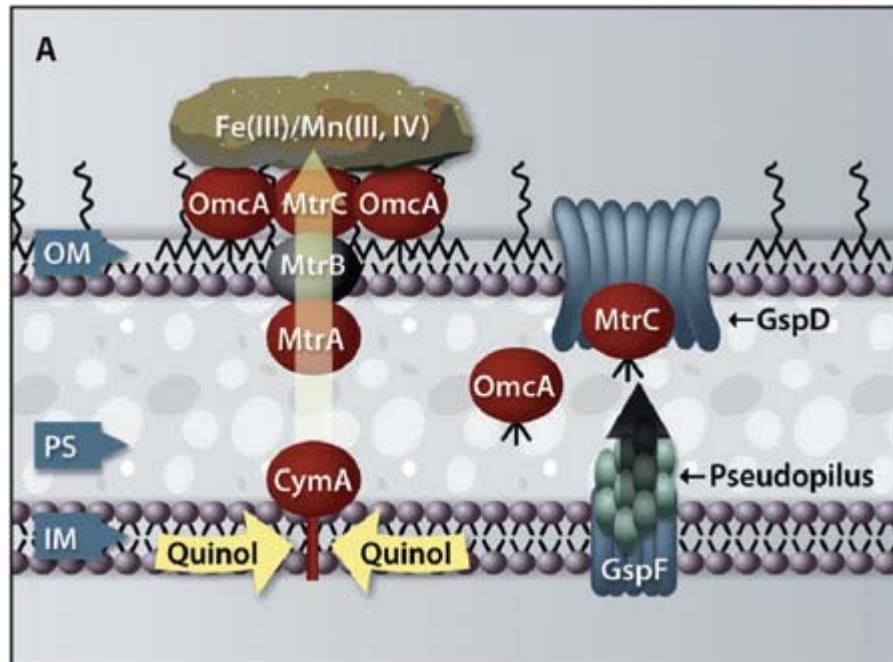
Downward J, Parker P, Waterfield MD (1984). "Autophosphorylation sites on the epidermal growth factor receptor". *Nature* **311** (5985): 483–5

Cell membrane protein dynamics

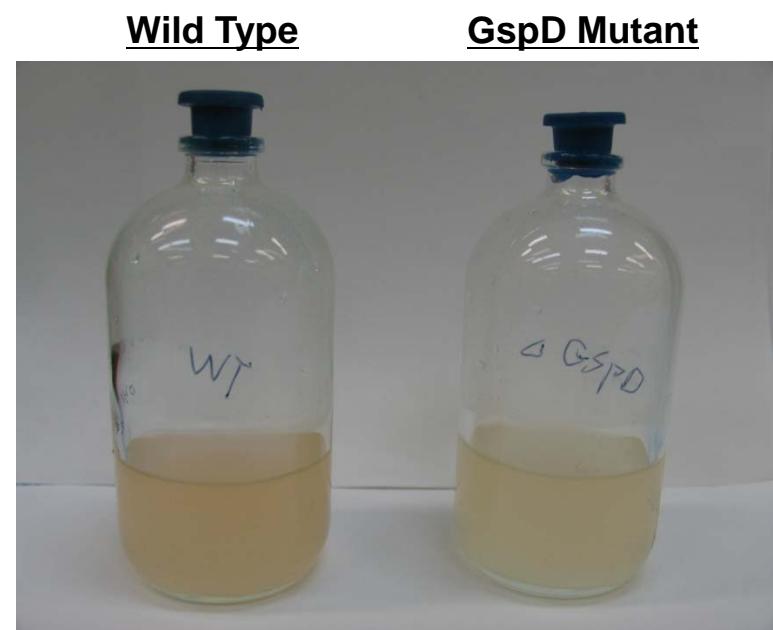
Membrane impermeable chemical probe
+
Quantitative proteomics



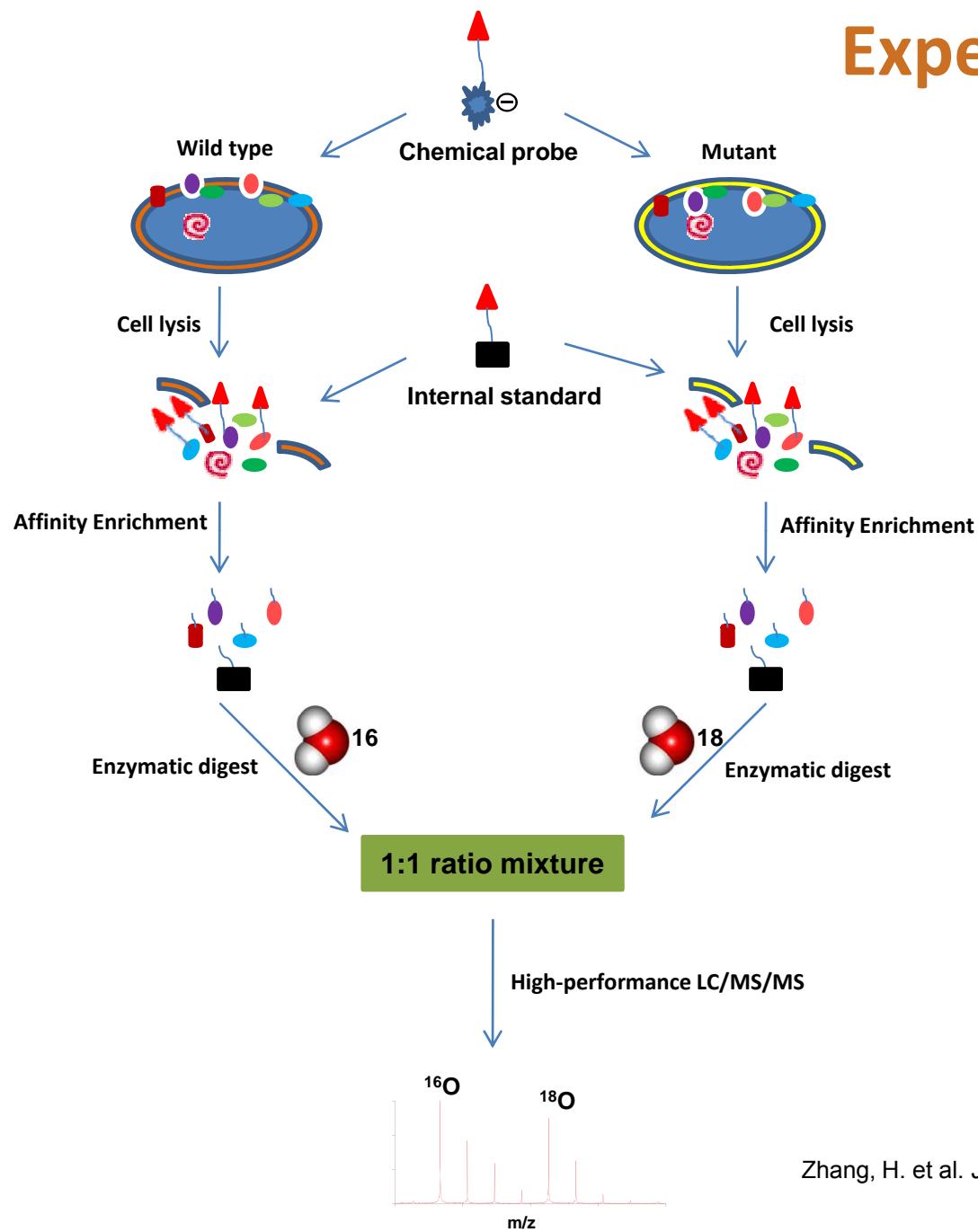
Application I: Membrane protein transportation



L. Shi, T. C. Squier, J. M. Zachara and J. K. Fredrickson.
Molecular Microbiology (2007) 65(1), 12–20

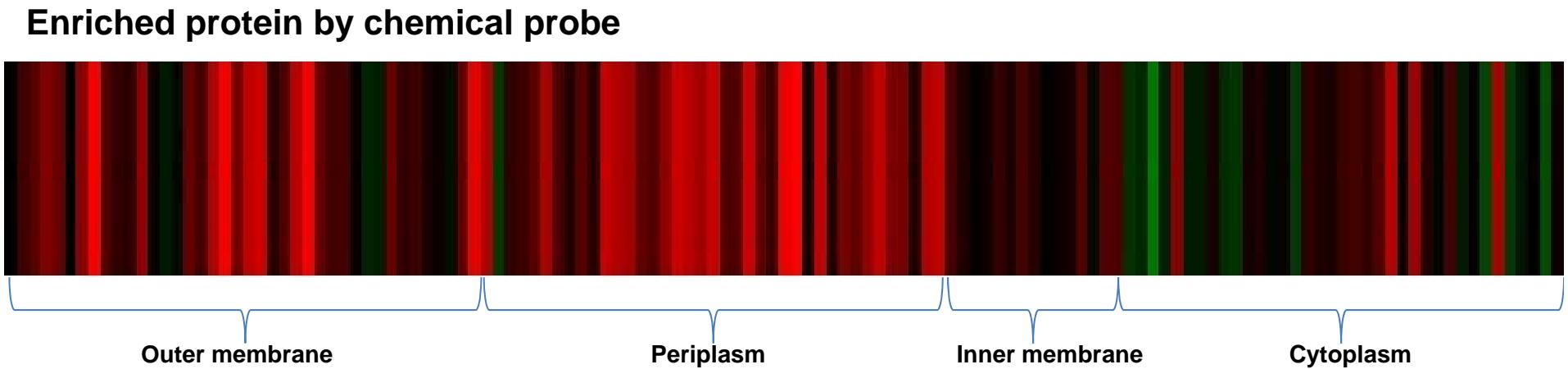


Experimental strategy

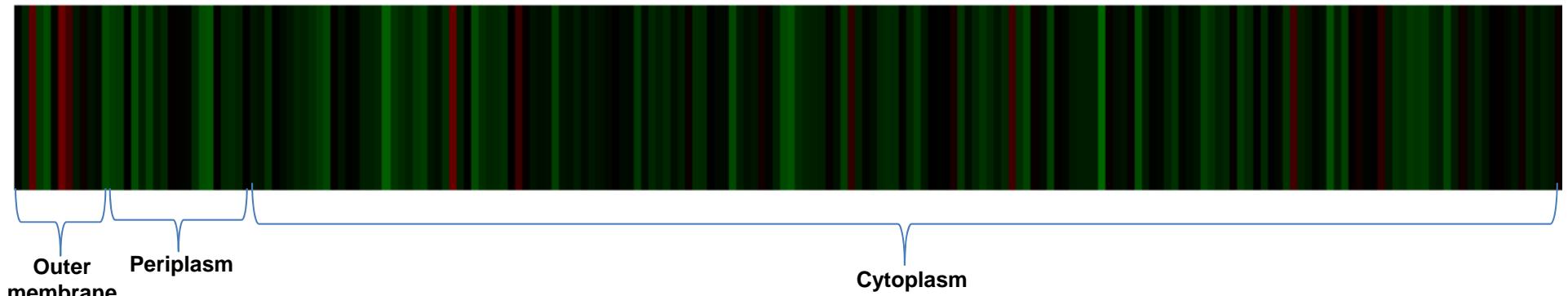


Zhang, H. et al. *Journal of Proteome Research*, 2010, 9, 2160-2169.

Membrane protein abundance changes observed via chemical-probe enrichment

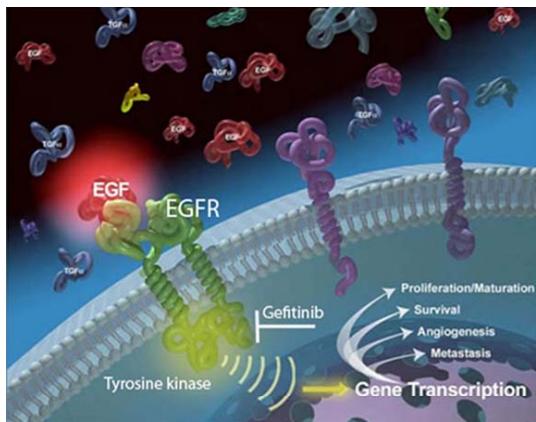


Whole cell lysate

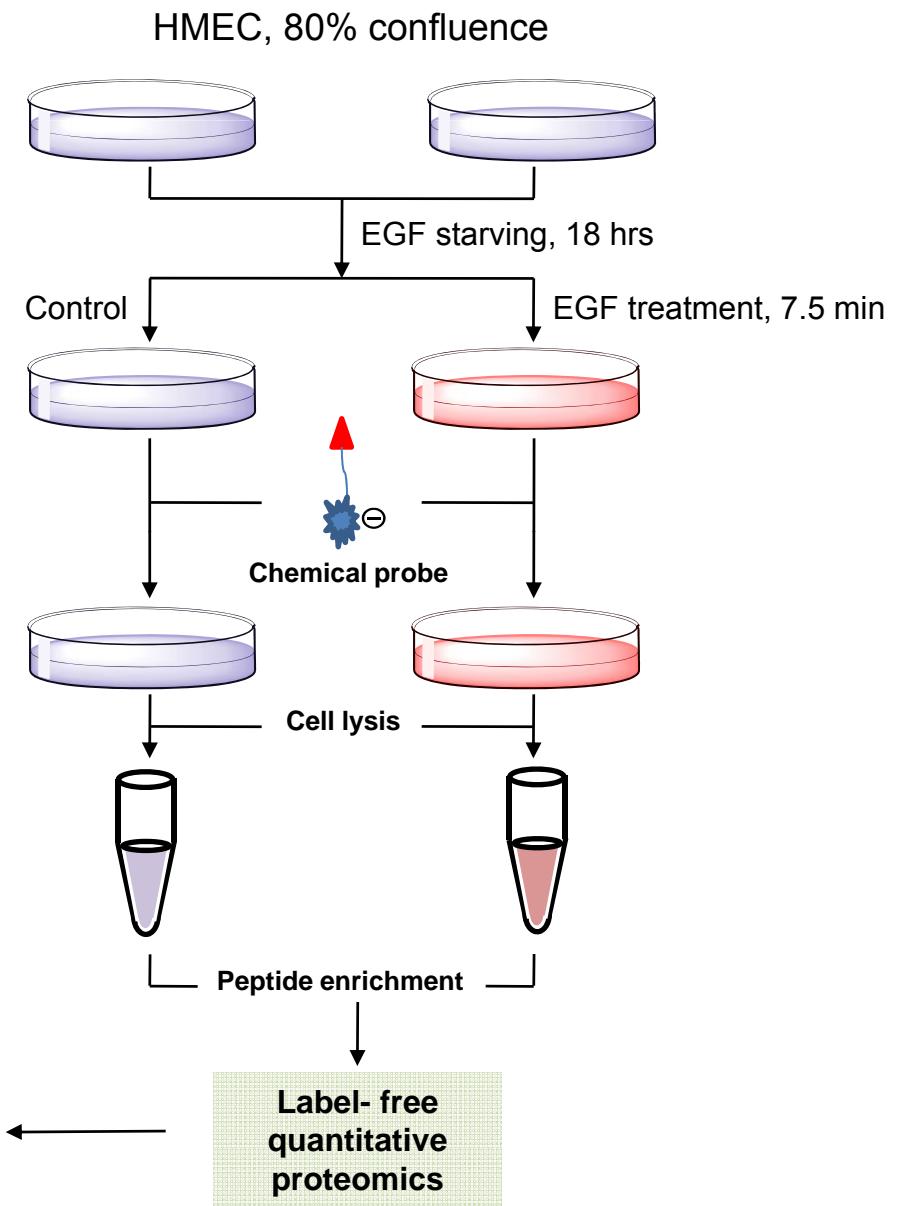


Zhang, H. et al. *Journal of Proteome Research*, 2010, 9, 2160-2169.

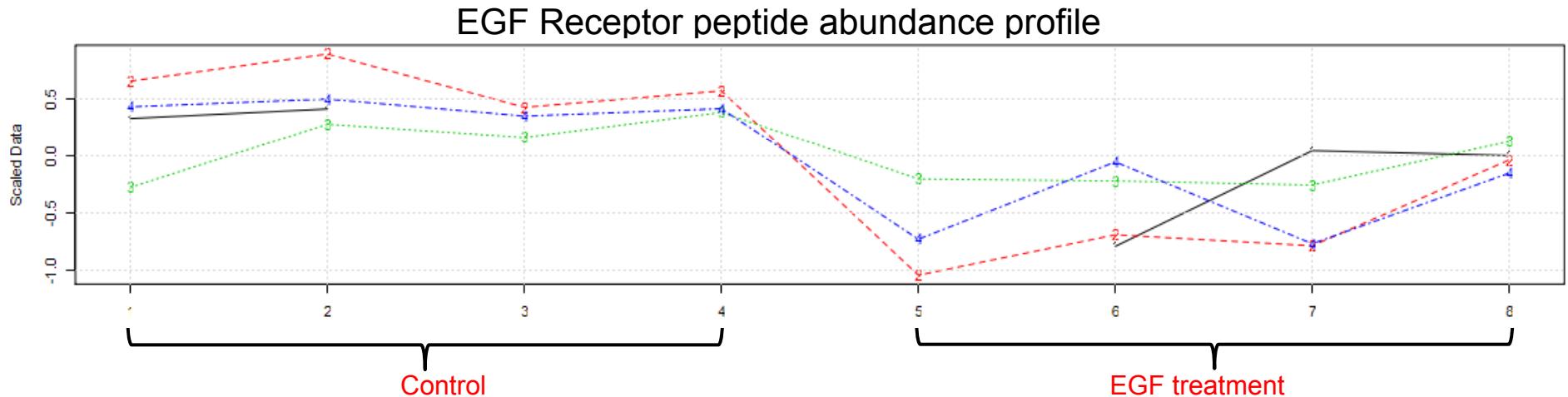
Application II: Cell signal transduction



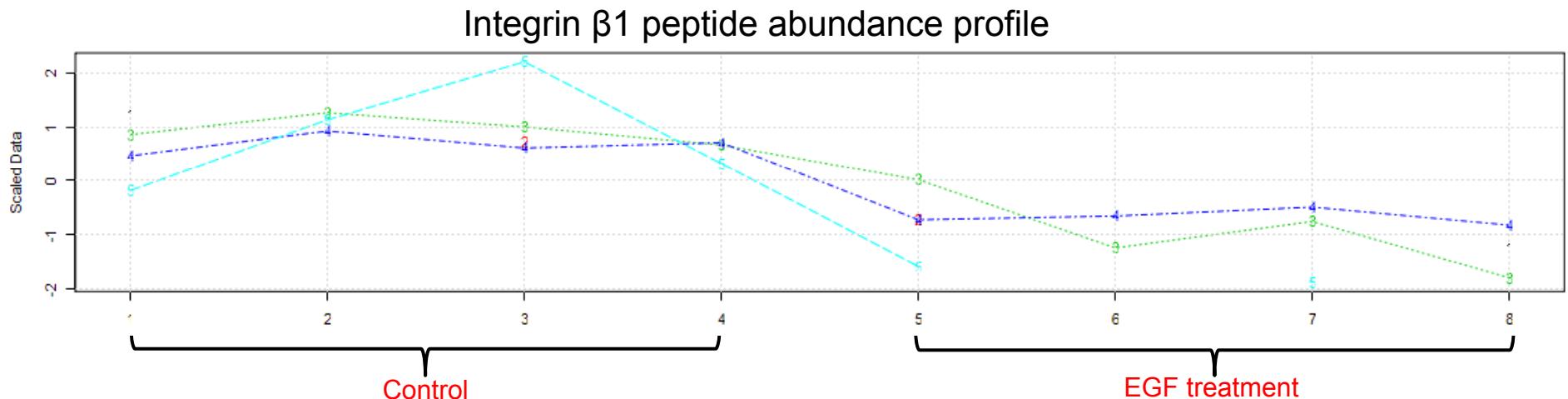
www.cancercell.org



Cell surface receptor internalization induced by EGF

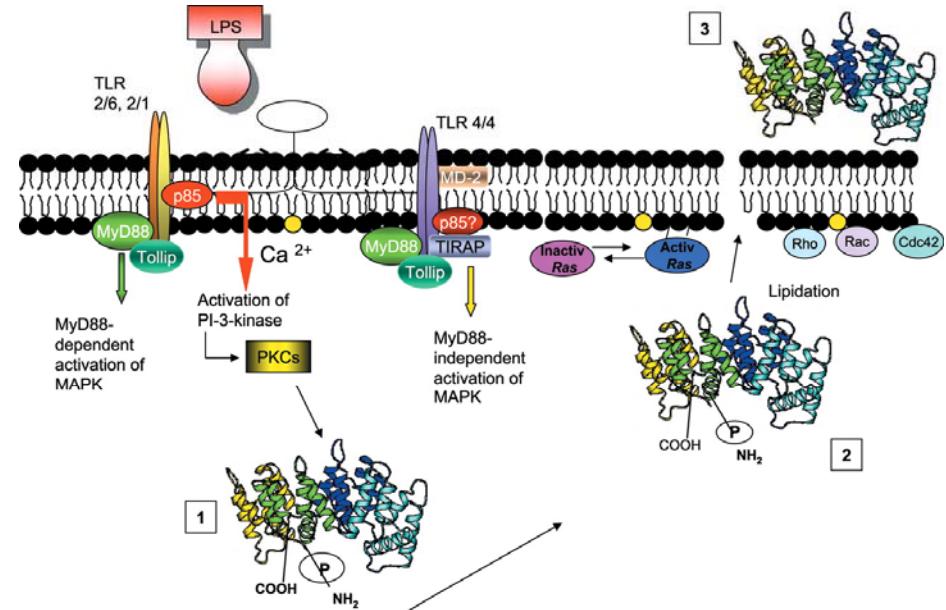


Decker SJ., et al. Epidermal Growth Factor (EGF)-stimulated Tyrosine Phosphorylation and EGF Receptor Degradation in Cells Expressing EGF Receptors Truncated at Residue 973. *J. Bio. Chem.*, 1992, 267, 1104-1110.

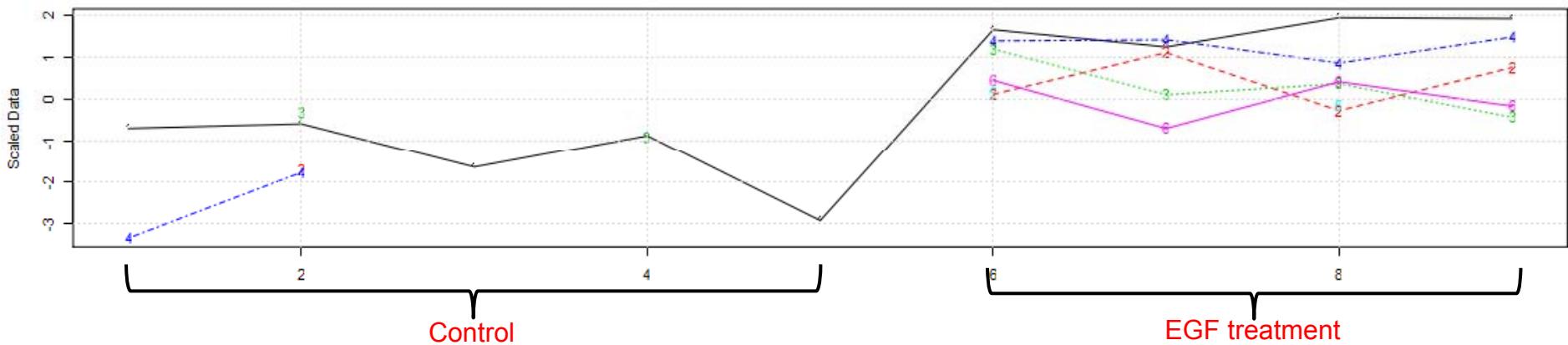


Wang, F., et al. Reciprocal interactions between $\beta 1$ -integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: A different perspective in epithelial biology. *PNAS*, 1998, 95, 14821-14826.

Translocation of Annexin A1 from cytoplasm to cell surface

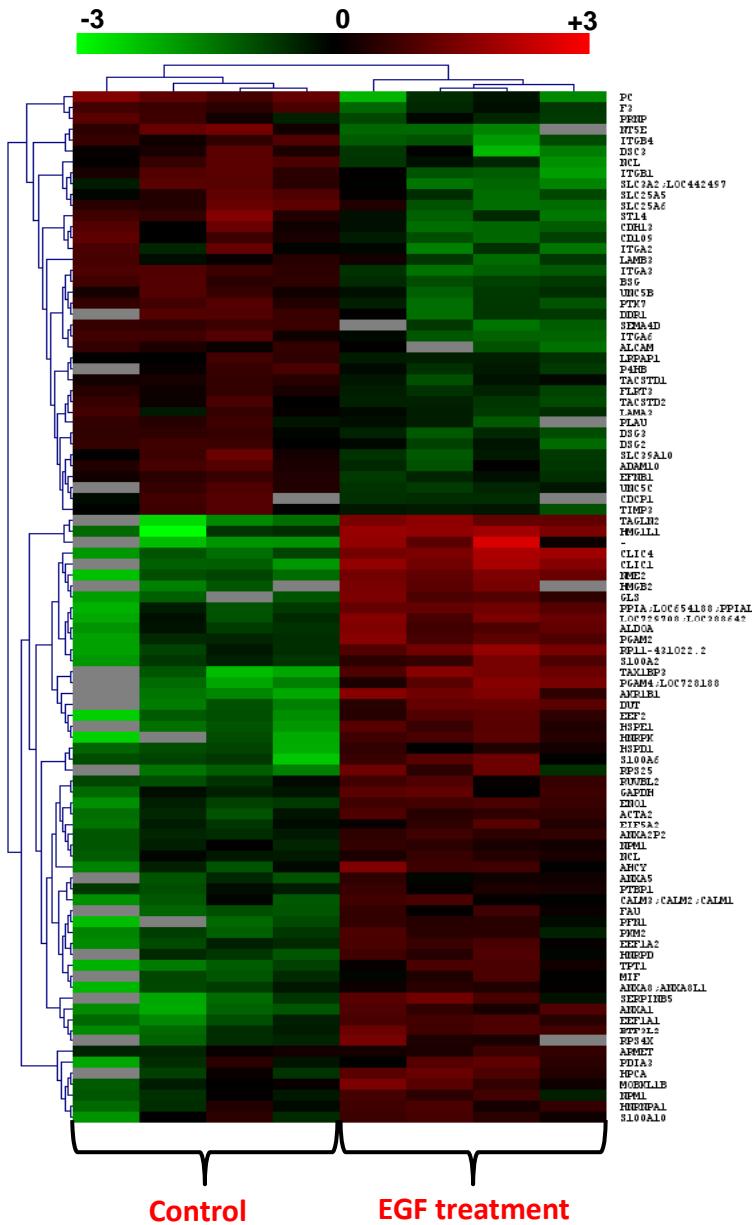


Annexin A1 peptide abundance profile



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)...

Acknowledgements

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