

Targeted quantification of protein phosphorylation in the epidermal growth factor receptor signaling pathway



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Lian Yi¹, Tujin Shi¹, Thomas L. Fillmore², Marina A Gritsenko¹, Adam C. Swensen¹, Therese RW Clauss¹, Yuqian Gao¹, Tao Liu¹, Richard D. Smith^{1,2}, H. Steven Wiley², Wei-Jun Qian¹

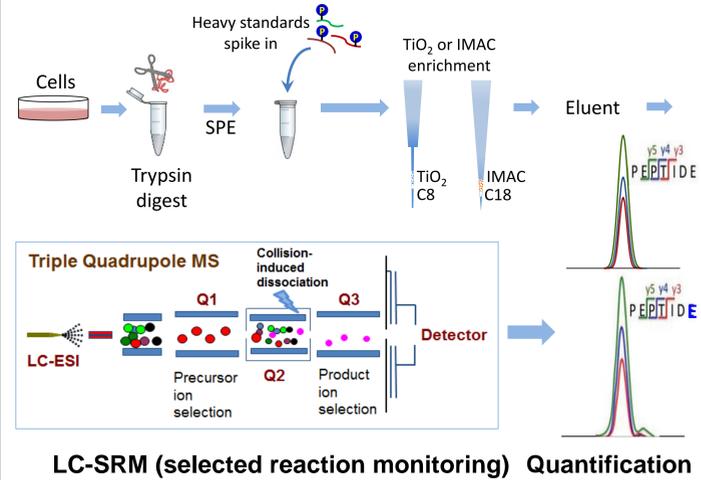
¹Integrative Omics, Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA

²Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA

Introduction

- MS-based targeted quantification is a promising technology for accurately measuring protein phosphorylation events for specific pathways or networks.
- Direct targeted quantification of phosphopeptides in biological samples often suffers from insufficient sensitivity due to the low levels of phosphorylation within highly complex samples.
- Herein, we assess the performance of MS-based targeted quantification coupled with affinity chromatography (Fe-IMAC or TiO₂) in terms of recovery, reproducibility, and sensitivity.¹⁻³
- Next, IMAC-SRM was applied to quantify the phosphorylation dynamics in EGFR network using a human breast cancer cell line Hs 578T.

Targeted phosphoproteomics



Recovery of affinity enrichment

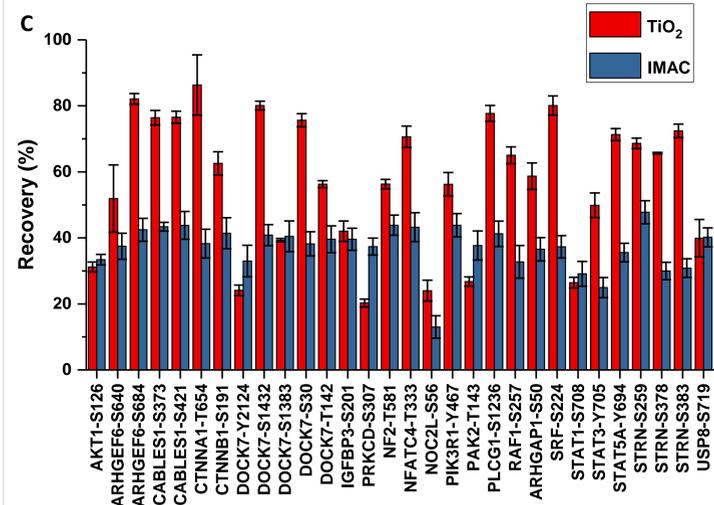
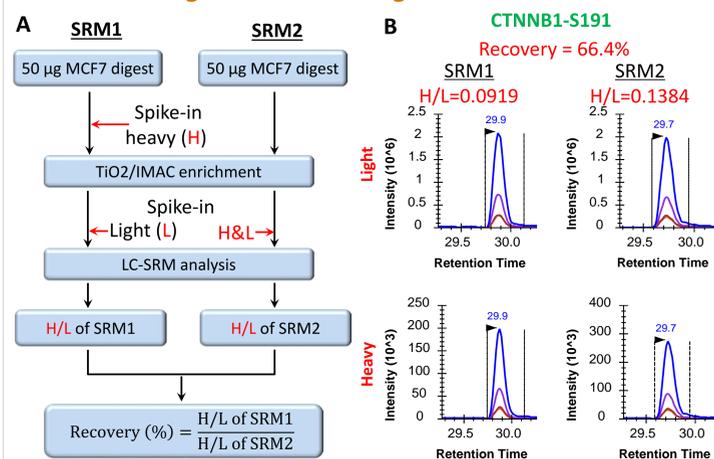
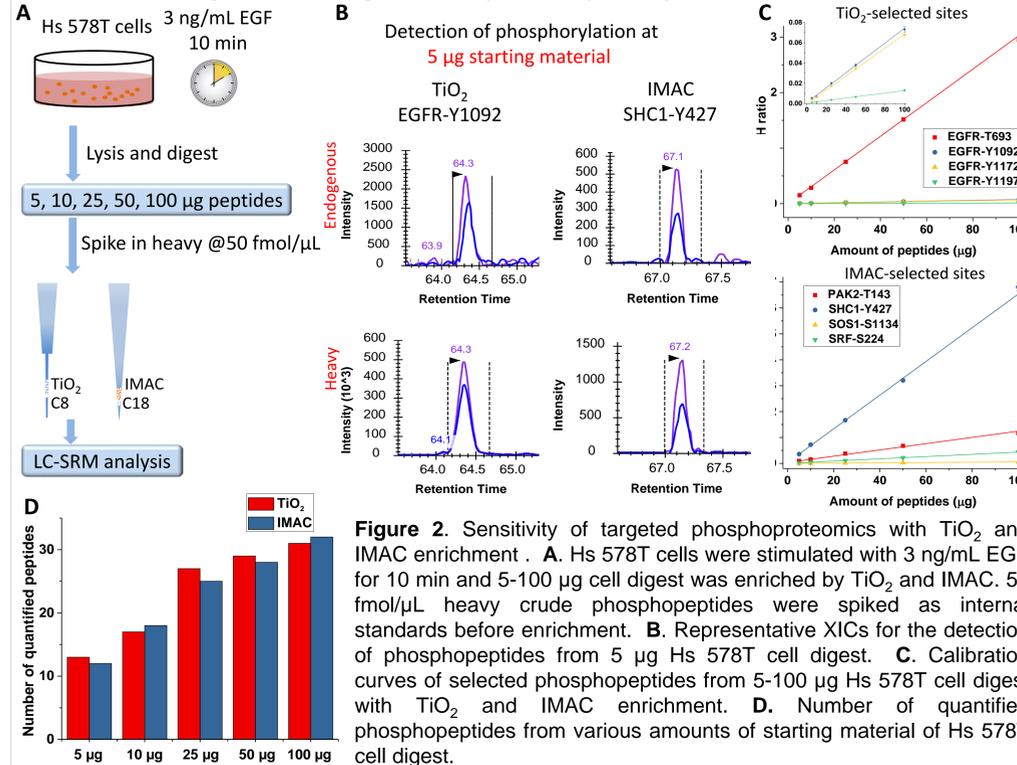


Figure 1. Recovery assessment of TiO₂ and IMAC enrichment by LC-SRM. **A.** Pure light synthetic phosphopeptides (n=30) were spiked at 10 fmol/µL after the enrichment as internal standards. Corresponding heavy crude phosphopeptides were spiked at 10 fmol/µL before (SRM1) and after (SRM2) TiO₂ or IMAC chromatography enrichment. **B.** Representative XICs for CTNNB1-S191 peptide and its recovery evaluation for TiO₂ enrichment. **C.** Recoveries of phosphopeptides for TiO₂ and Fe-IMAC enrichment. Error bars indicated standard deviation within 3 technical replicates.

Sensitivity of targeted phosphoproteomics



Phosphorylation dynamics of the EGFR network

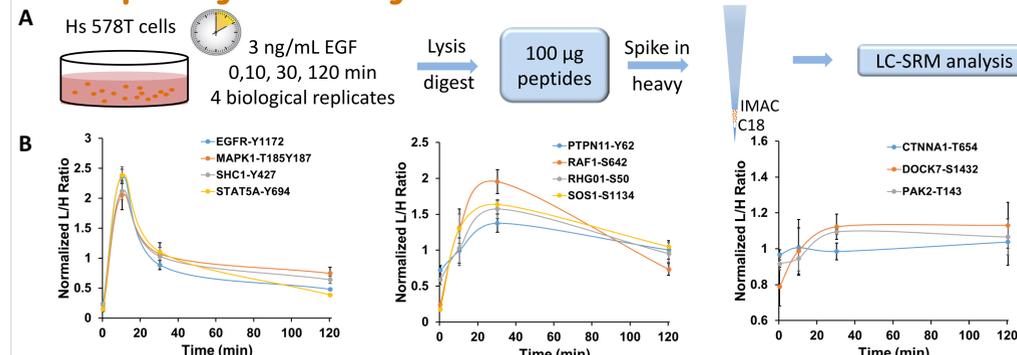


Figure 3. Phosphorylation dynamics in the EGFR pathway. **A.** General workflow. **B.** Phosphoprotein dynamics for different phosphosites. Error bars represent standard errors of 4 biological replicates.

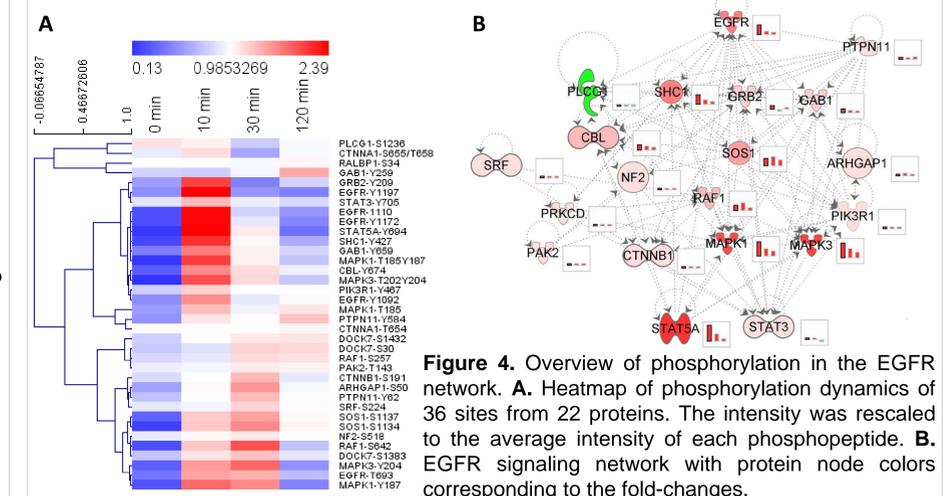


Figure 4. Overview of phosphorylation in the EGFR network. **A.** Heatmap of phosphorylation dynamics of 36 sites from 22 proteins. The intensity was rescaled to the average intensity of each phosphopeptide. **B.** EGFR signaling network with protein node colors corresponding to the fold-changes.

Conclusions

- TiO₂ and IMAC enrichment indicated comparable recovery, reproducibility, and sensitivity.
- The combination of affinity enrichment with LC-SRM enables quantification of phosphorylation from starting materials as low as 5 µg peptides, equal to ~50,000 cells.
- IMAC enrichment with more consistent recoveries was selected for the study of phosphorylation dynamics in the EGFR signaling pathway. 22 phosphorylation sites of 13 key proteins in EGFR pathway were upregulated significantly, including kinase, phosphatase, adaptor proteins, and transcription factors.

Acknowledgements

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Career Opportunities: For potential openings in Integrative Omics Group at PNNL please visit <http://omics.pnl.gov/careers> or <http://jobs.pnnl.gov/>

CONTACT: Lian Yi, Ph.D.
Biological Sciences Division
Pacific Northwest National Laboratory
E-mail: lian.yi@pnnl.gov

www.omics.pnl.gov

