

What do we aim for?

- Investigation, optimization and validation of the electrostatic repulsion chromatography (ERLIC) principle to magnetic beads.
- Validation of literature results for phosphopeptide enrichment using magnetic beads in a high-throughput format for automated handling with robotic system.

What is this project about?

Phosphorylation of proteins as post-translational modification (PTM) plays an important role in the regulation of biological processes across all species. Hence, analytical methods that result in comprehensive understanding of the phosphoproteome are of utmost importance. Until now, this analysis remains challenging, since the applied sample preparation strategies are often not robust and suffer from certain bias [1]. Usually, these methods require a high amount of sample material, which in clinical setting is not often available.

In this project, we aim to improve our current Phosphoproteomics workflow and validate previously published methods for high-throughput phosphoproteomics [2,3]. In addition, we intend to investigate the possibilities of electrostatic repulsion chromatography (ERLIC) as novel retention mechanism on magnetic beads [4]. This allows the simultaneous enrichment and fractionation of phosphopeptides in high-throughput format, which would be an enormous improvement to currently applied methods.

Selection of methods you will use

- Cell lysis from different organs (mouse organs / fungi) with different buffers and techniques

- Tryptic digestion (In-solution / FASP / SP3)
- Proteome analysis using Waters nanoUPLC and Synapt G2S qTOF in DDA and DIA mode
- Database search with PEAKS / PLGS
- Phosphopeptide enrichment (IMAC / MOAC / ERLIC)
- Data evaluation, visualization and statistical analysis
- (Design of Experiments / R / Graphpad Prism / Volcano Plot / Venn Diagram)
- Gene ontology / enrichment analysis

What do you need?

- Under-graduate degree (BSc. or similar) in biology, chemistry or related life sciences

Literature

[1] Beltran, L., & Cutillas, P. R. (2012). Advances in phosphopeptide enrichment techniques for phosphor-proteomics. Amino Acids, 43(3), 1009–1024.

[2] Humphrey, S. J., Karayel, O., James, D. E., & Mann, M. (2018). High-throughput and high-sensitivity phosphoproteomics with the EasyPhos platform. Nature Protocols, 13(9), 1897–1916.

[3] Tape, C. J., Worboys, J. D., Sinclair, J., Gourlay, R., Vogt, J., McMahon, K. M., Jørgensen, C. (2014). Reproducible Automated Phosphopeptide Enrichment Using Magnetic TiO₂ and Ti-IMAC. Analytical Chemistry, 86(20), 10296–10302.

[4] Alpert, A. J. (2018). Effect of salts on retention in hydrophilic interaction chromatography. Journal of Chromatography A, 1538, 45–53.

Exited to push the borders of what is possible?

Please send your CV and some words about your motivation until 28.02.2020 by email to:

Thomas Michna

Universitätsmedizin der Johannes Gutenberg-Universität thmichna@uni-mainz.de