Excellent sensitivity through excellent recovery; ERLIC outperforms TiO$_2$-MOAC in quantitative phosphoproteomics with low sample amounts

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1. Introduction

• Sensitive phosphospecies requires phosphopeptide enrichment strategies with excellent quantitative recovery
• Here we present a systematic comparison of TiO$_2$-MOAC [1] and ERLIC [2, 3] regarding their applicability in phosphoproteomics, especially for experiments with low sample amounts

2. Phosphopeptide intensities

Phosphopeptide-enriched fractions from TiO$_2$-MOAC (SILAC-light, 400 µg, HeLa) and ERLIC (SILAC-heavy) were mixed and analyzed by 2D-LC-MS to determine relative signal intensities (2 fractions, high-pH-RP).

- 80% of all phosphopeptides had higher intensities using ERLIC
- In TiO$_2$-MOAC recovery of p-peptides correlates with hydrophilicity and share of acidic amino acids
- ERLIC shows unbiased recovery

3. Quantitative losses

Phosphopeptide-enriched fractions (from 50 µg SILAC-light, HeLa) were mixed with a corresponding amount of complex digest (50 µg SILAC-heavy) and subjected to another round of enrichment to determine the quantitative losses.

- 62% quantitative recovery with ERLIC vs 37% with TiO$_2$
- In TiO$_2$-MOAC recovery correlates with phosphopeptide length and share of acidic amino acids
- ERLIC shows unbiased recovery

4. Quantification with minute amounts

Analysis of a 2-plex SILAC sample of 2 x 25 µg (cytomegalovirus-infected fibroblasts vs mock) in triplicates. TiO$_2$-MOAC 1 fraction with 200 min total acquisition time, ERLIC: 10 fractions, each 30 min acquisition time.

5. References