Zirconium(IV) IMAC-based enrichment for mass spectrometry driven phosphoproteomics

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Introduction

Phosphopeptide enrichment is an essential step in large-scale, quantitative phosphoproteomics studies by mass spectrometry .

Several phosphopeptide affinity enrichment techniques exist, such as Immobilized Metal ion Affinity Chromatography (IMAC) and Metal Oxide Affinity Chromatography (MOAC).

We optimized sample loading conditions and compared Zirconium (IV) IMAC (Zr-IMAC) magnetic microparticles to more commonly used Titanium (IV) IMAC (Ti-IMAC) magnetic microparticles and Iron (III) IMAC (Fe-IMAC LC) for phosphopeptide enrichment from complex protein samples prior LC-MS/MS.

Aims

- To improve phosphopeptide enrichment efficiency of zirconium and titatium based magnetic microparticles.
- Use hydroxy acids to improve phosphopeptide binding to Zr-IMAC, Ti-IMAC and TiO2 magnetic microparticles.
- Assess Zr-IMAC performance in large scale phosphoproteomics experiments.
- Compare Zr-IMAC with well-known enrichment methods for phosphopeptide enrichment.

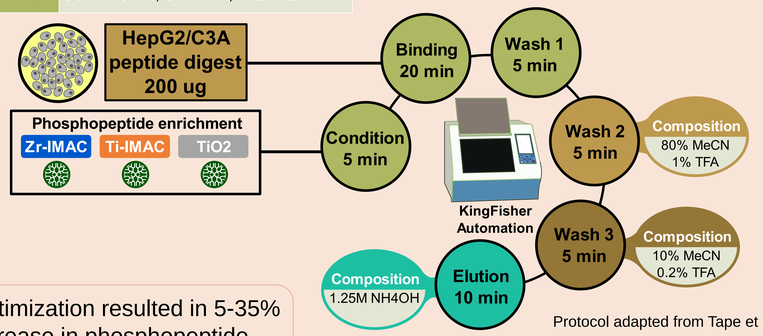
Conclusions

- Zr-IMAC magnetic microparticles selectively and efficiently captures phosphopeptides.
- Optimized Zr-IMAC outperforms more popular methods like Ti-IMAC and Fe-IMAC.
- Optimized Zr-IMAC showed largest complementarity with Fe-IMAC LC.

Hydroxy acid concentration affects phosphopeptide binding to Zr-IMAC/Ti-IMAC/TiO2 magnetic microparticles but not their selectivity

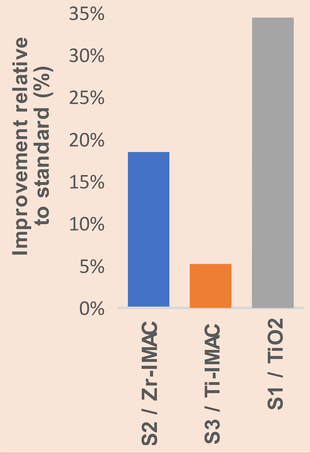
Std	80% MeCN, 5% TFA, 1M GA
S1	50% MeCN, 0.1% Acetic, 0.1M GA
S2	80% MeCN, 5% TFA, 0.1M GA
S3	80% MeCN, 5% TFA, 0.1M LA

Glycolic acid (GA, 1M) is typically used for lowering non-specific binding in IMAC and MOAC. Reduced concentrations of GA and lactic acid (LA) (S1, S2, S3) were tested for Zr-IMAC, Ti-IMAC and TiO2 magnetic microparticles.

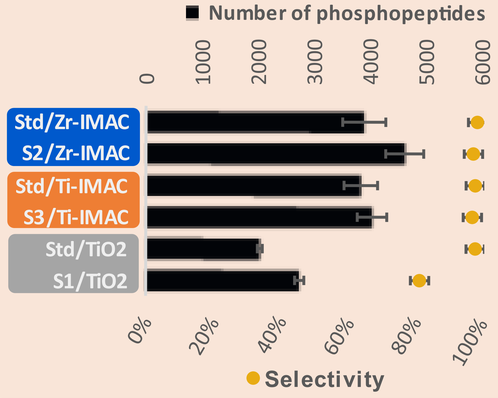


Protocol adapted from Tape et al. 2014

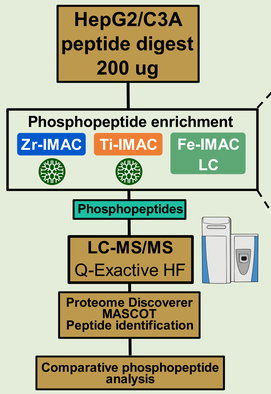
Optimization resulted in 5-35% increase in phosphopeptide identifications.



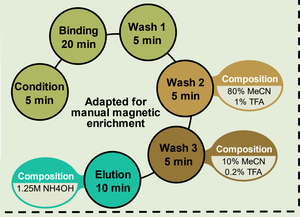
S2/Zr-IMAC outperformed standard conditions, as well as Ti-IMAC and TiO2.

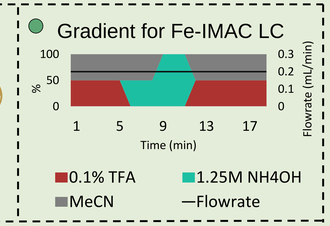


Optimized Zr-IMAC outperforms Ti-IMAC magnetic microparticles and Fe-IMAC LC



Solvent	Composition
Std	80% MeCN, 5% TFA, 1M GA
S2	80% MeCN, 5% TFA, 0.1M GA

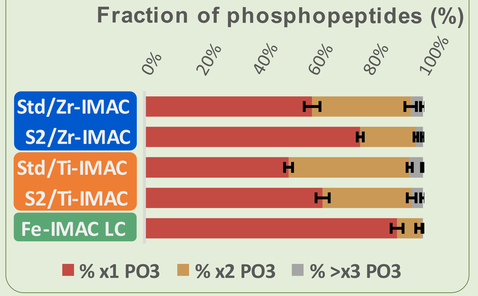




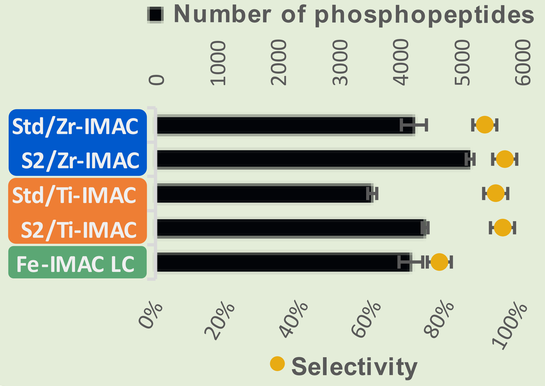
We hypothesized S2 solvent would improve both Zr-IMAC and Ti-IMAC and we tested this by using standard and S2 solvents.

We then compared performance of optimized Zr-IMAC and Ti-IMAC magnetic microparticles to LC-based Fe-IMAC.

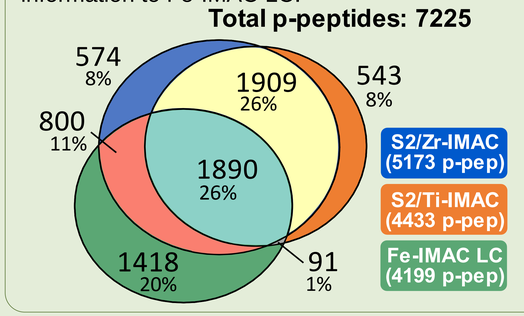
Solvent S2 affects the ratio of mono- and multi-phosphorylated peptides.



S2/Zr-IMAC surpassed both Ti-IMAC and Fe-IMAC LC with excellent selectivity (>95%).



S2/Zr-IMAC recovered more phosphopeptides than S2/Ti-IMAC and provides complementary information to Fe-IMAC LC.



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