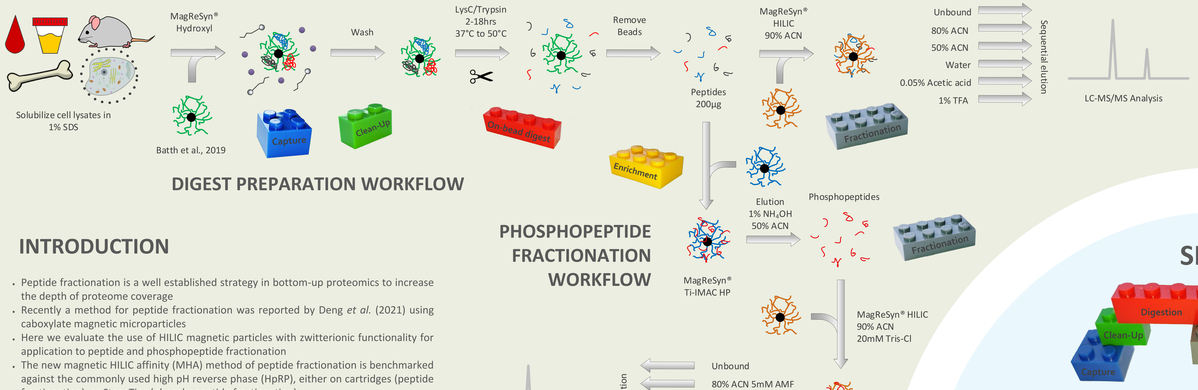
RAPID & SCALABLE OFF-LINE PEPTIDE FRACTIONATION ON ZWITTERIONIC MAGNETIC MICROPARTICLES

Previn Naicker[a,b], Claire Koenig[c], Ireshyn Govender[a,b], Ana Martinez del Val[c], Isak Gerber[a,b], Sipho Mamputha[a], Justin Jordaan[b,d], Stoyan Stoychev[a,b], Jesper V Olsen[c]

[a] *Next Gen Health, CSIR, Pretoria, South Africa,* [b] *ReSyn Biosciences, Pretoria, South Africa,*
[c] *Faculty of Health and Medical Sciences, Novo Nordisk Foundation Center for Protein Research, Copenhagen, Denmark*
[d] *Rhodes University, Biotechnology Innovation Centre, Grahamstown, South Africa, E-mail: sstoychev@resynbio.com*

PEPTIDE FRACTIONATION WORKFLOW



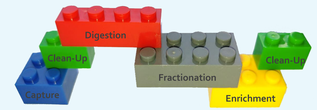
DIGEST PREPARATION WORKFLOW

PHOSPHOPEPTIDE FRACTIONATION WORKFLOW

INTRODUCTION

- Peptide fractionation is a well established strategy in bottom-up proteomics to increase the depth of proteome coverage
- Recently a method for peptide fractionation was reported by Deng *et al.* (2021) using caboxylate magnetic microparticles
- Here we evaluate the use of HILIC magnetic particles with zwitterionic functionality for application to peptide and phosphopeptide fractionation
- The new magnetic HILIC affinity (MHA) method of peptide fractionation is benchmarked against the commonly used high pH reverse phase (HpRP), either on cartridges (peptide fractionation), or StageTips (phosphopeptide fractionation)
- We outline the benefits of the method, including speed of generating fractions for analysis, simple automation (including up front on-bead protein digestion), high technical reproducibility in manual and automated formats, and scalability for both proteome and phosphoproteome applications.

SIMPLER



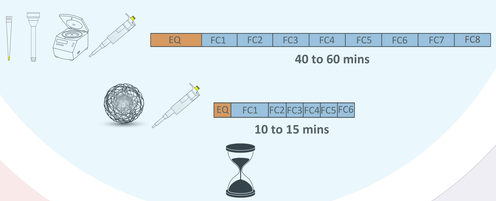
Seamless coupling to up-front protein capture, clean-up and digestion as well as the ability to automate without requiring auxiliary equipment

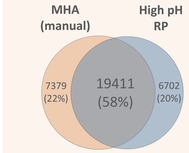
BETTER

High technical reproducibility in both manual and automated formats with ability to scale for both proteome and phosphoproteome applications

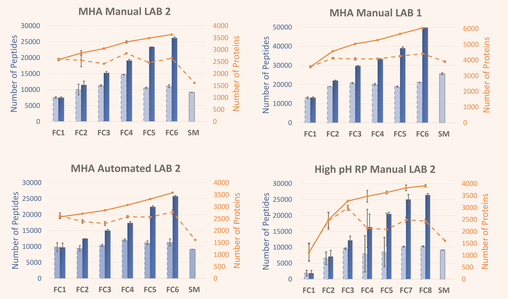
FASTER

Rapid magnetic separation without the need for time consuming centrifugation

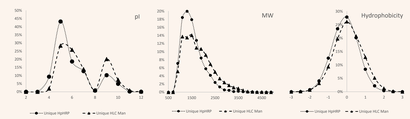




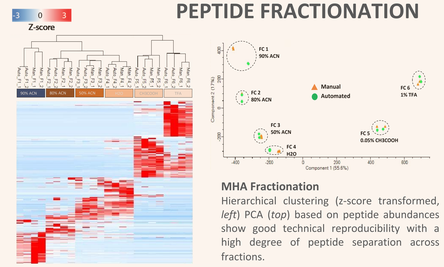
Peptide overlaps showed over 20% uniqueness, indicating the two methods are complementary.



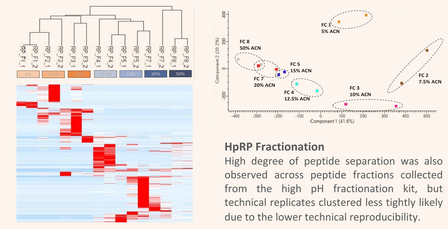
Above: MHA peptide fractionation resulted in a **gain of 2-3 fold in protein and peptide ID's** in comparison to the original starting material (SM). This was consistent for manual and automated formats, and across laboratory sites. Similar gains were observed with high pH RP fractionation, but with a higher number of fractions, and lower reproducibility. *Below:* possible complementarity is confirmed by analyzing the peptide properties, unique MHA peptides were generally more basic, higher MW and more hydrophobic as compared to HpHRP



PEPTIDE FRACTIONATION



MHA Fractionation
Hierarchical clustering (z-score transformed, *left*) PCA (*top*) based on peptide abundances show good technical reproducibility with a high degree of peptide separation across fractions.



HpRP Fractionation
High degree of peptide separation was also observed across peptide fractions collected from the high pH fractionation kit, but technical replicates clustered less tightly likely due to the lower technical reproducibility.

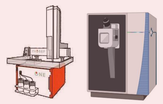
METHODS

MANUAL

AUTOMATED

LAB 1

OLSEN GROUP
The Novo Nordisk Foundation Center for Protein Research



LAB 2

CSIR Touching lives through innovation

Manual peptide fractionation
- Evosep 1, 30 SPD, 40 min gradient
- Exploris 480, DIA, 45k, 2s

Phosphopeptide fractionation
- KingFisher™ Flex (auto)
- *Non-fractionated*: Evosep1, 30 SPD, 40 min gradient, Exploris 480, DIA, 15k, 2s
- *Fractionated:* Evosep1, 60 SPD, 21 min gradient, Exploris 480, DIA, 45k, 2s

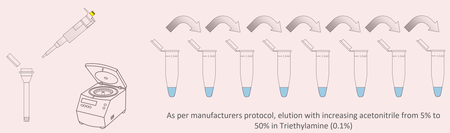
Peptide fractionation
- Manual and KingFisher™ Duo (auto)
- Dionex nanoRSLC, 30min gradient, Sciex 6600, SWATH 60VW

DirectDIA

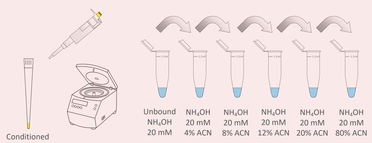
Total Proteome
- The MHA method for peptide fractionation was benchmarked against a HpRP peptide fractionation kit (Pierce)
- The manual MHA workflow took 10 to 15 min to complete, while the HpRP kit required 40 to 60 min due to the centrifugation steps
- The MHA method required a lower elution volume per fraction (⅓ of the volume), and potentially lower for coupling directly to LCMS analysis

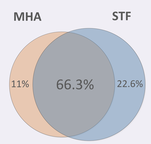
Phosphoproteome
- Similarly, the manual MHA workflow took 10 to 15 minutes to complete, while the 3M™ Empore™ C18 StageTip fractionation (STF) required 40 to 60 minutes (centrifugation, and excluding StageTip assembly of ~60 minutes)
- Phosphopeptides were eluted in 200µl for the automated MHA workflow, and StageTips were eluted with 50µl. A reduced elution volume of 50µl can be achieved by using low volume plates with the MHA method

HIGH PH RP CARTRIDGE: PEPTIDE FRACTIONATION

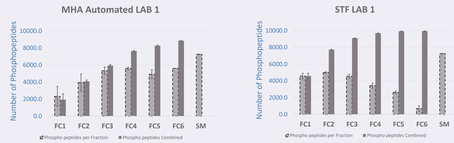


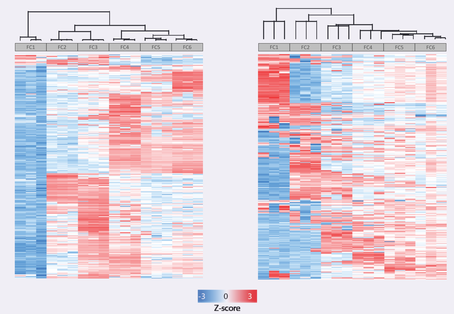
STAGE TIP: PHOSPHOPEPTIDE FRACTIONATION





More than 65% of identified phosphopeptides overlapped between the MHA and STF methods





PHOSPHOPEPTIDE FRACTIONATION

The binding and elution conditions of the MHA method were adapted for phoshopeptide fractionation, after phosphopeptide enrichment using MagReSyn® Ti-IMAC HP. The workflow is currently under development, but under these conditions performance is on par with STF in terms of the number of identified phosphopeptides, and gain in comparison to starting material. MHA is however significantly faster to complete, and can be automated on a KingFisher™ magnetic bead handling station.

Technical replicates of each elution fraction clustered closely for both MHA and STF methods. The orthogonality of the MHA method can be further improved, in particular for FC4 to FC6. This has the potential to increase the overall depth of phosphoproteome coverage.

REFERENCES

- Batth ST *et al.*, 2019. *Protein aggregation capture on microparticles enables multi-purpose proteomics sample preparation.* Mol. Cell Proteomics. DOI:10.1074/mcp.TIR118.001270
- Bekker-Jensen DB *et al.*, 2020. *A Compact Quadrupole-Orbitrap Mass Spectrometer with FAIMS Interface Improves Proteome Coverage in Short LC Gradients.* DOI: 10.1074/mcp.TIR119.001906
- Bruderer *et al.*, 2015. Extending the limits of quantitative proteome profiling with data-independent acquisition and application to acetaminophen-treated three-dimensional liver microtissues. MCP, DOI: 10.1074/mcp.M114.044305
- Deng W *et al.*, 2021. *Carboxylate-Modified Magnetic Bead (CMMB)-Based Isopropanol Gradient Peptide Fractionation (CIF) Enables Rapid and Robust Off-Line Peptide Mixture Fractionation in Bottom-Up Proteomics.* DOI: 10.1074/mcp.RA120.002411
- Tyanova S *et al.*, 2016. *The Perseus computational platform for comprehensive analysis of (prote)omics data.* DOI: 10.1038/nmeth.3901

ReSyn Biosciences