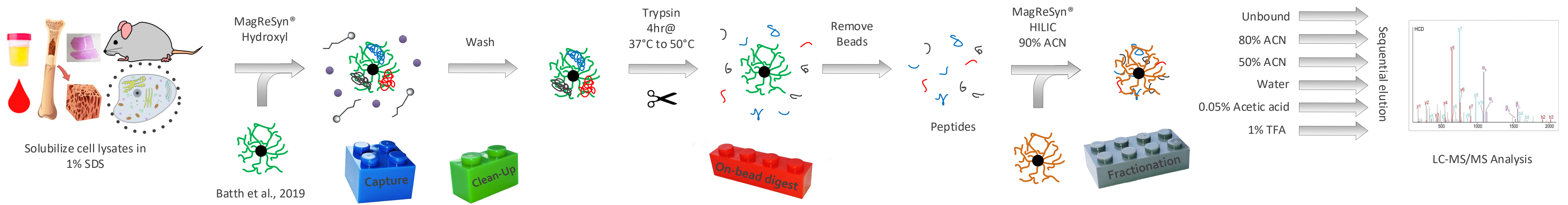
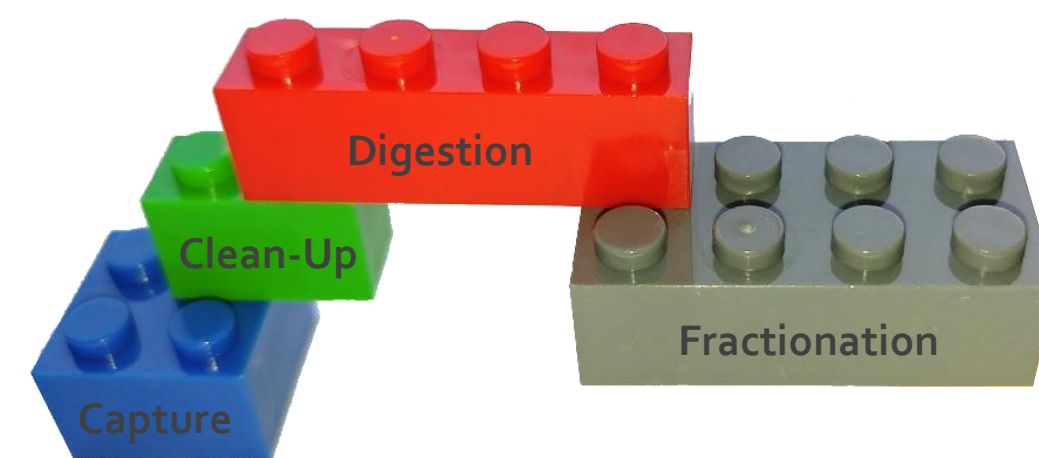


GRAPHICAL ABSTRACT



INTRODUCTION

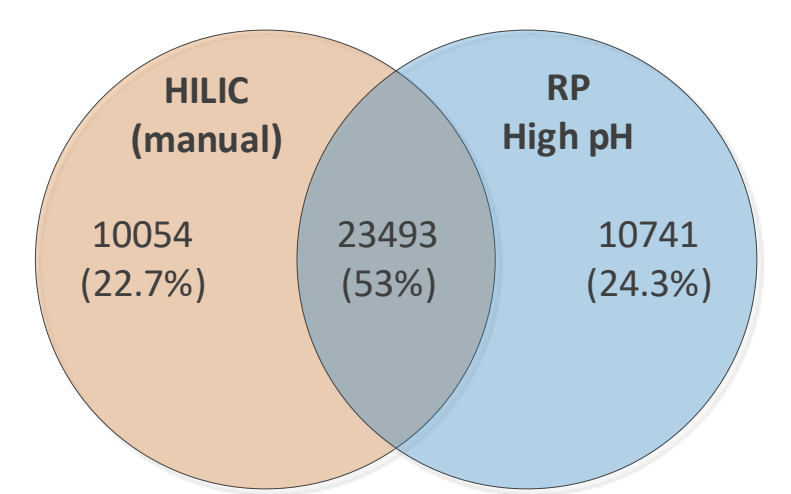
- Peptide fractionation is a well established strategy in bottom-up proteomics to increase the depth of proteome coverage
- Recently a method for peptide fractionation using carboxylate magnetic microparticles was reported by Deng et al.
- Here we evaluate the use of HILIC magnetic particles with zwitterionic functionality for this application
- The new method is benchmarked against the most common method for fractionation, namely high pH reverse phase (RP)
- We outline the benefits of the method, including speed of generating fractions for analysis, simple automation (including up front on-bead protein digestion), and high technical reproducibility in manual and automated formats.



RESULTS

Inter-laboratory Comparison and Automation

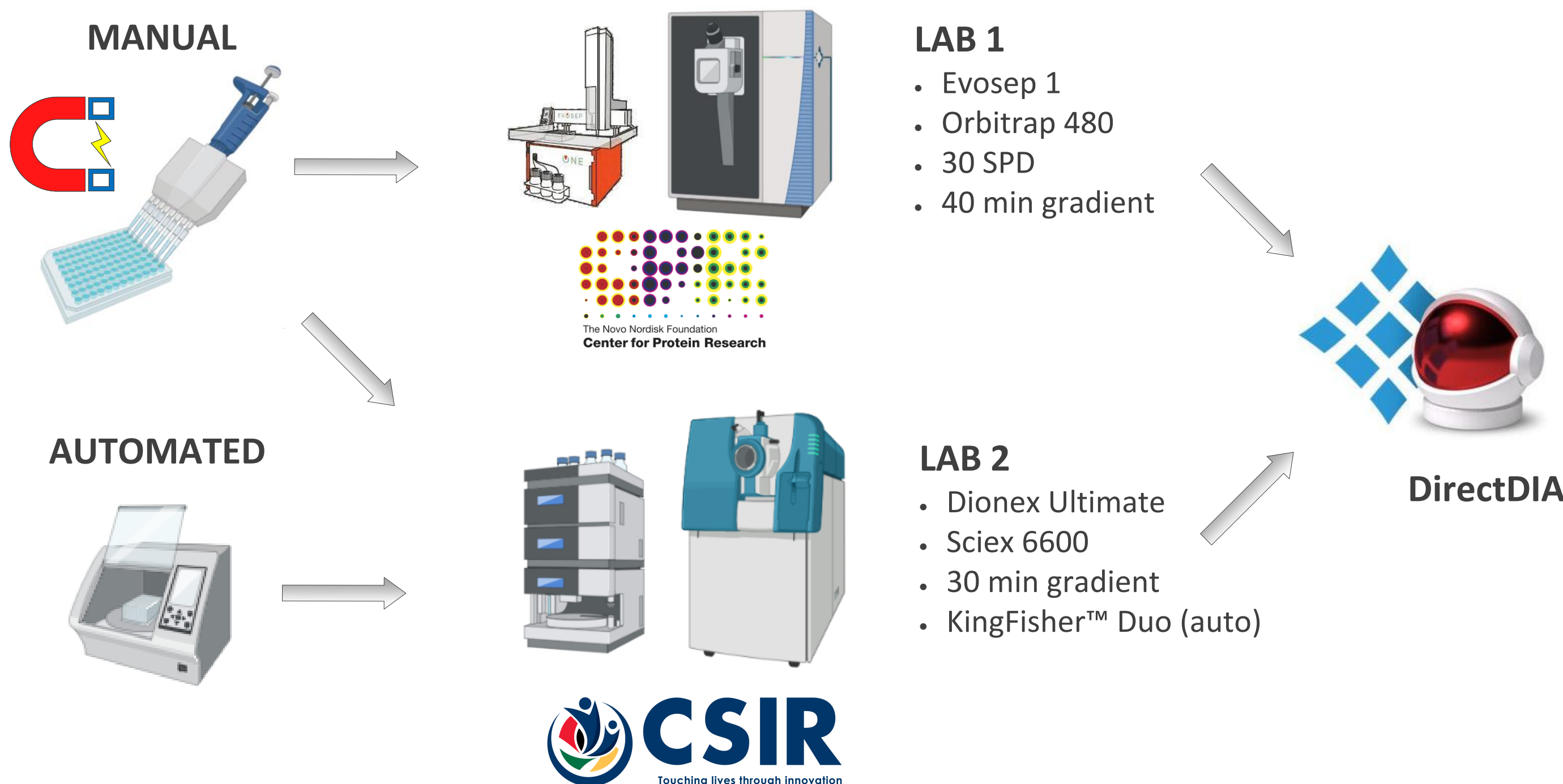
Magnetic hydrophilic affinity (MHA) peptide fractionation resulted in a **gain of 2-3 fold in protein and peptide identifications** in comparison to the non-fractionated starting material (SM). This was consistent for both manual and automated formats, and across laboratory sites (LAB1 and LAB2). Similar gains in ID's were observed with high pH RP fractionation, but with a higher fraction number being analysed, and lower technical reproducibility. Peptide overlaps (right) showed over 20% uniqueness, illustrating that the 2 approaches are complementary.



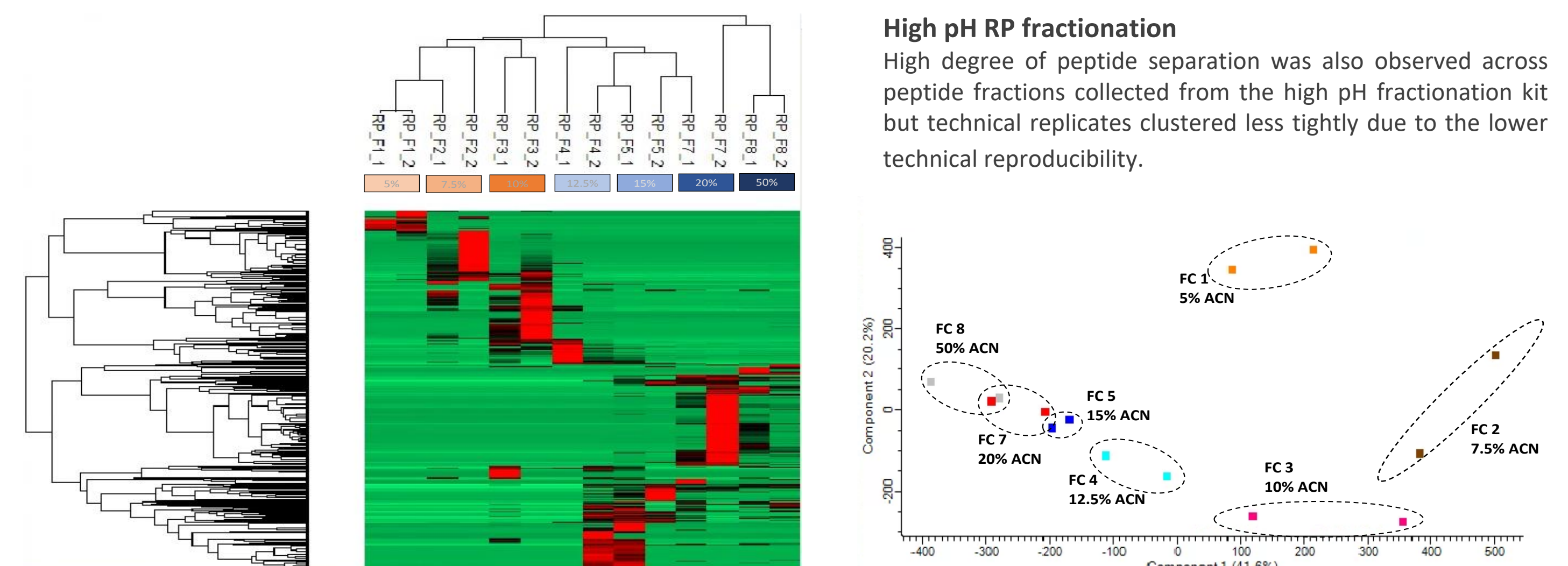
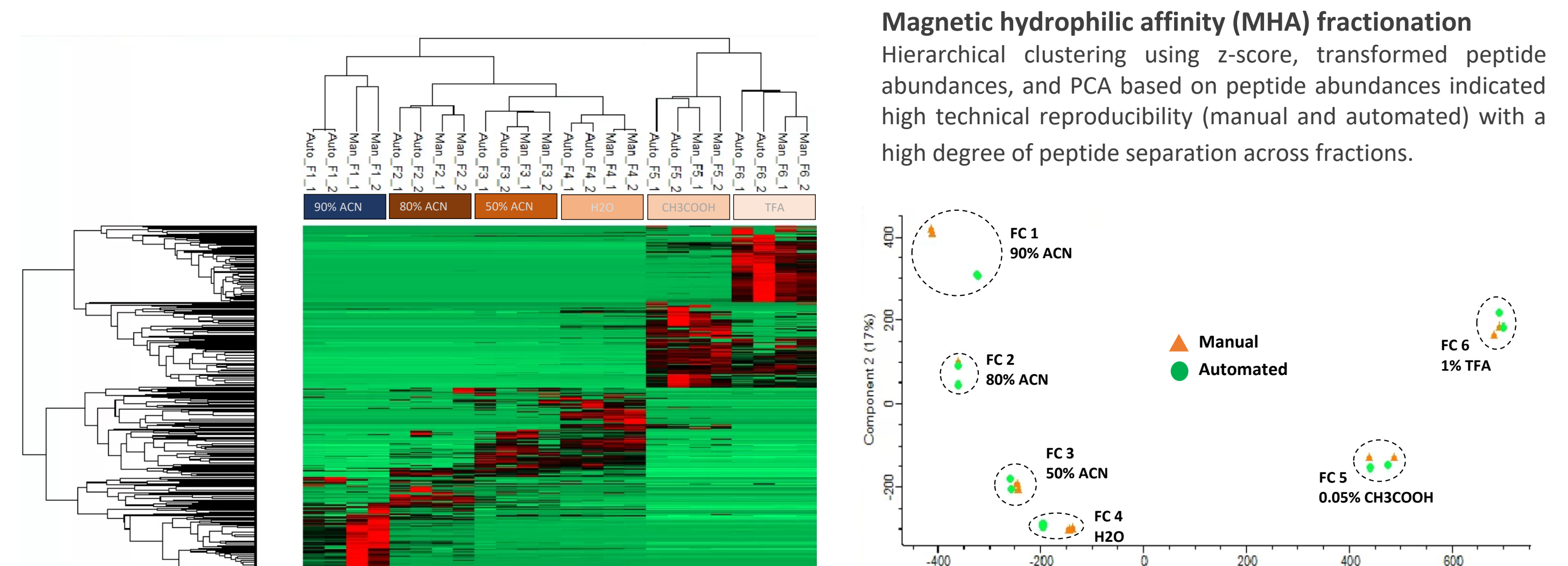
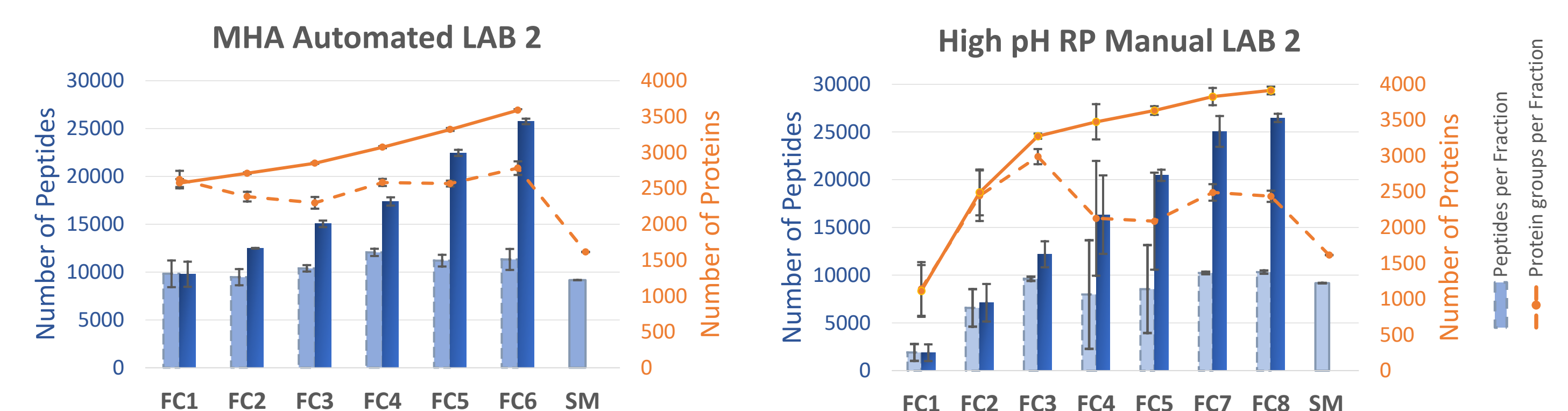
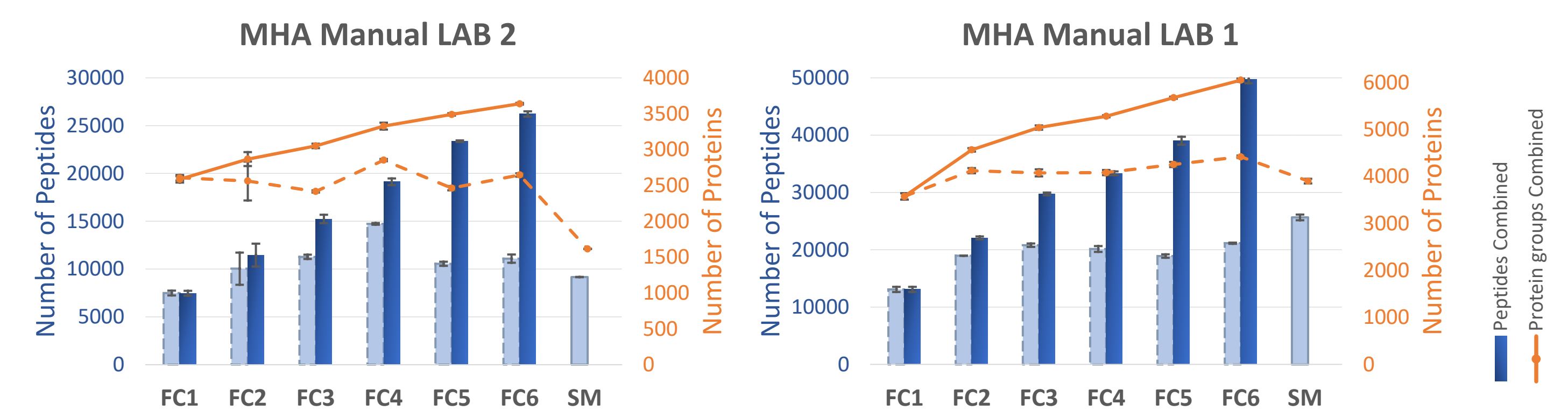
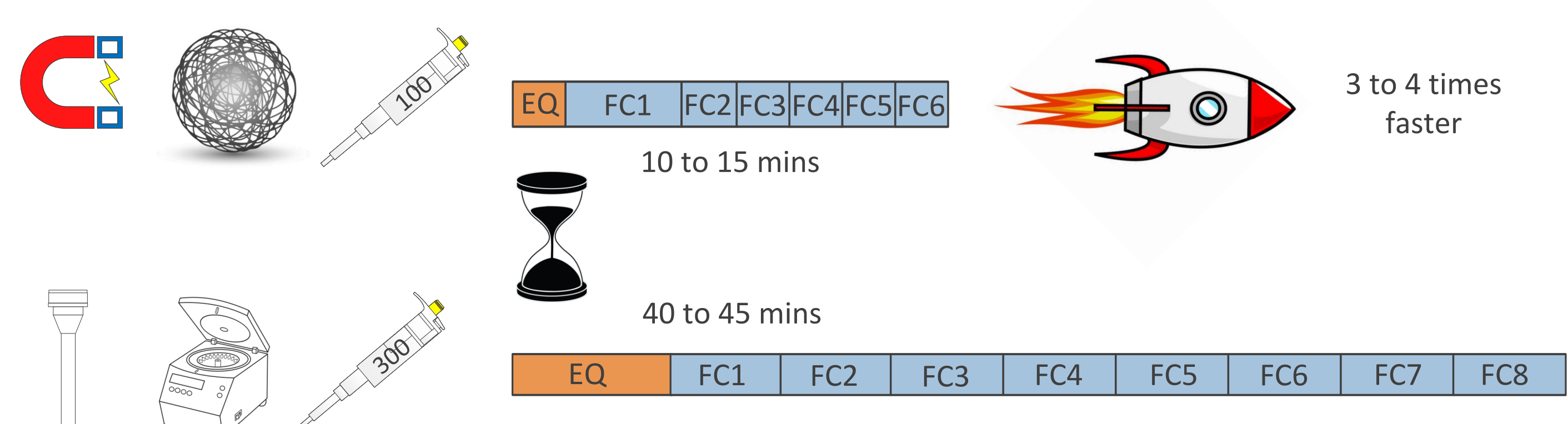
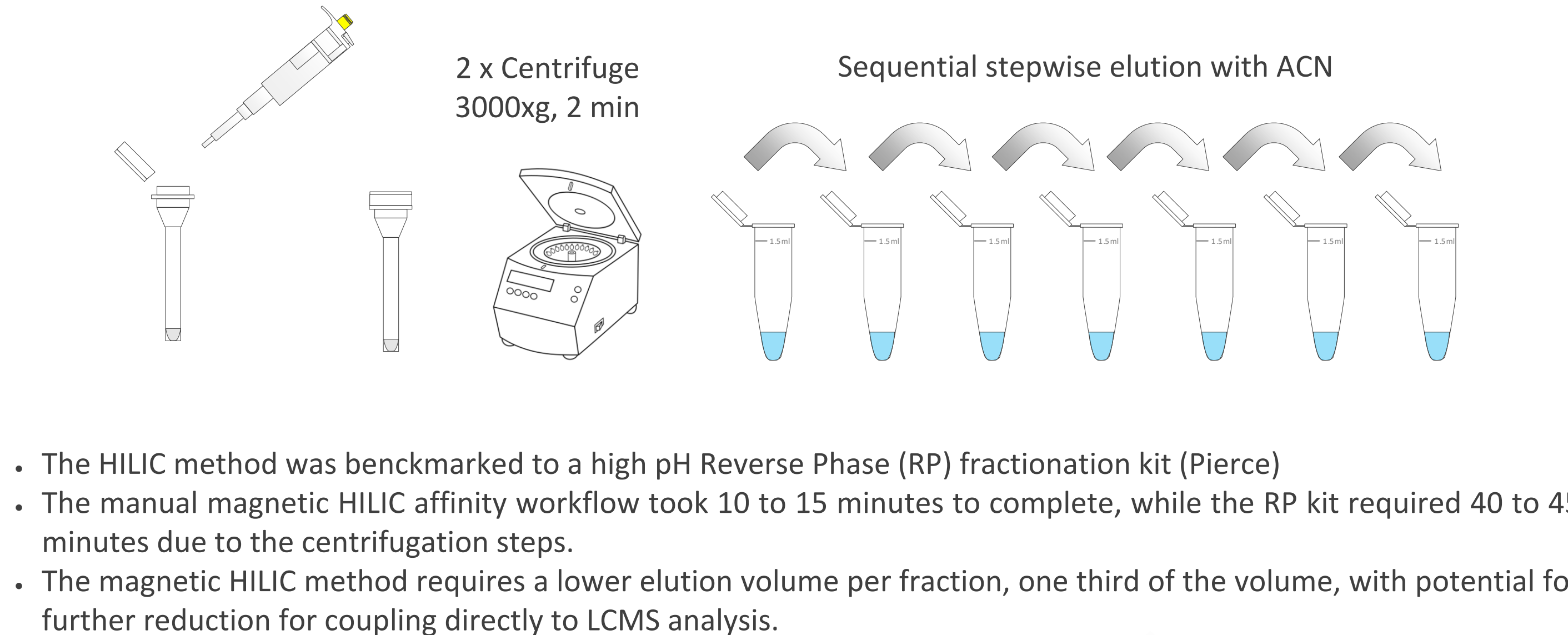
METHODS

- Cell lysates were solubilised in 1% SDS and proteins precipitated on MagReSyn[®] Hydroxyl microparticles.
- Contaminants were removed by washing with high organic solvents, followed by addition of digestion buffer, and on-bead Trypsin digestion.
- Hydroxyl microparticles were removed, and MagReSyn[®] HILIC was added to the digest
- Peptides were bound with 90% ACN, with sequential elution using two organic buffers, water and 2 acidic solutions.
- The reproducibility of the methods was illustrated by performing the manual workflow across two sites, CPR in Copenhagen Denmark, and CSIR in Pretoria South Africa.
- The method was automated at CSIR using a KingFisher[™] Duo magnetic bead handling station.

MAGNETIC HILIC FRACTIONATION



REVERSE PHASE FRACTIONATION



The benefits of this HILIC based fractionation workflow are considered to be:

- The protocol is rapid and can be performed without auxiliary equipment
- Compatible with low elution volumes for coupling directly to LCMS
- High technical reproducibility in both manual and automated formats
- High orthogonality across fractions
- Seamless coupling to up-front protein capture, clean-up and digestion for full automation of the sample preparation workflow

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
- 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