Express protein digestion by automated, enzyme microReaction cartridges

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Overview

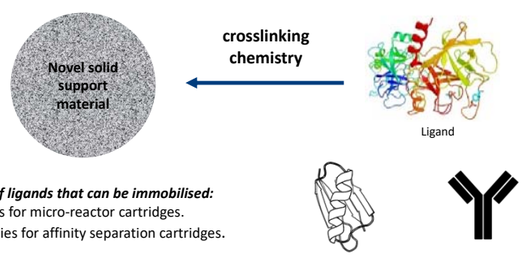
- Enzymes (pepsin and trypsin) were covalently immobilised onto ePrep µSPEed-Cxyl cartridges through crosslinking chemistry in ~6 minutes.
- Efficient digestion of bovine serum albumin (BSA) and cytochrome c by immobilised enzyme cartridges was achieved in 4 minutes.
- Proteins and peptides analysed by liquid chromatography time of flight mass spectrometry (LC-QTOF) revealed efficient digestion of proteins (e.g. 80% sequence coverage of BSA in Figure 4).

Introduction

The rapid identification and quantification of biomarkers are of increasing significance in clinical settings, and this is achievable on small sample sizes by integrating the ePrep customisable micro solid phase extraction devices (µSPEed) with mass spectrometry. The µSPEed cartridges are comprised of a packed sorbent bed compartment, a unidirectional valve and a simple press fit connector for use with a digital syringe drive or an automated sample preparation workstation. The use of positive displacement digital syringes ensures precise and reproducible flow kinetics, providing the opportunity to perform time-dependent assays, such as speeding up protein digests.

Figure 1: *ePrep Automated Sample Preparation Workstation and µSPEed cartridge with a one-way check valve for unidirectional flow.*

Ligand Customisable Cxyl-µSPEed Sorbent



Example of ligands that can be immobilised:
- Enzymes for micro-reactor cartridges.
- Antibodies for affinity separation cartridges.

Method Workflow

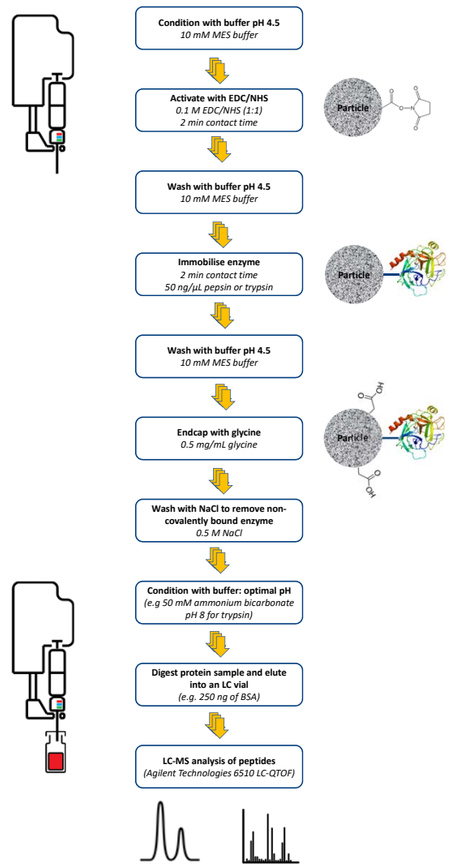


Figure 2: *Workflow for enzyme immobilisation onto µSPEed-Cxyl cartridges for protein digestion.*

Trypsin Digest of Cytochrome C

- ~83 pmol of cytochrome c (100 µL) was digested at a flow rate of 25 µL/min at ambient temperature.
- No undigested cytochrome c remaining (see Figure 3).
- A peptide mass list was automatically generated by the Agilent Data Acquisition software and searched against the Mascot database [1].

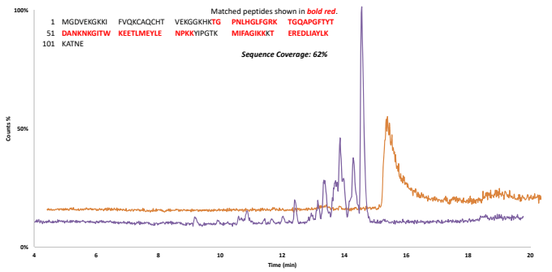


Figure 3: *Mass chromatograms of undigested cytochrome c (orange), digested cytochrome c peptides (blue), and sequence coverage from the Mascot database search. Injected: 16 pmol of protein. Column: Thermo Fisher Accucore C18+ 2.1 x 100 mm (with optimisations required). Mobile Phase: A% 0.1% formic acid, B% ACN + 0.1% formic acid. Gradient: 5% B to 60% B over 25 minutes.*

Trypsin Digest of Bovine Serum Albumin (BSA)

- ~4 pmol of BSA (100 µL) was digested at a flow rate of 25 µL/min at ambient temperature.

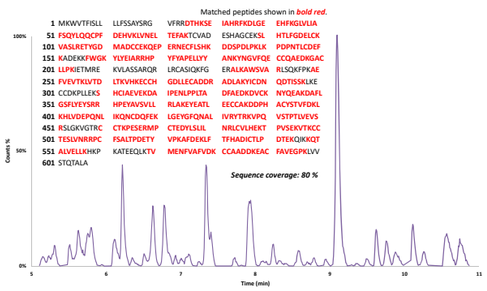


Figure 4: *Extracted mass chromatogram of digested BSA peptides using trypsin and sequence coverage from the Mascot database search. Injected: 0.75 pmol of protein.*

Pepsin Digest of Bovine Serum Albumin (BSA)

- ~4 pmol of BSA (100 µL) was digested in 4 minutes at a flow rate of 25 µL/min at ambient temperature.

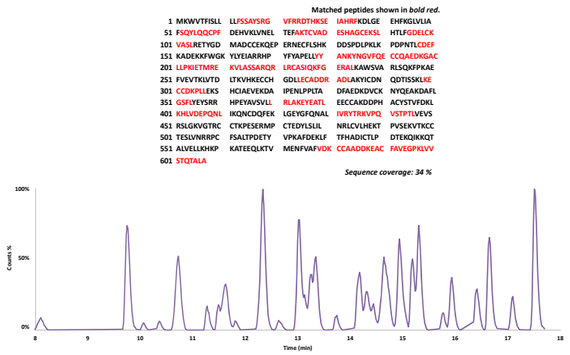


Figure 5: *Extracted mass chromatogram of digested BSA peptides using pepsin and sequence coverage from the Mascot database search. Injected: 0.75 pmol of protein.*

Conclusion

- A workflow was developed for the immobilisation of ligands onto µSPEed cartridges.
- Pepsin and trypsin were covalently immobilised using EDC/NHS crosslinking chemistry.
- Bovine serum albumin and cytochrome C were digested in 4 minutes (100 µL at 25 µL/min) at ambient temperature using various enzymes.
- There was no undigested protein remaining showing efficient digestion.
- The % sequence coverage is based on automated peptide mass list which is not optimised to extract all peptides.
- Future work will improve on chromatography columns to enhance LC-MS peptide recovery.
- The method workflow (Figure 2) is being further optimised for faster digests and including other proteases.

Acknowledgements

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References

1. Matrix Science, *Mascot Peptide Mass Fingerprint*. *http://www.matrixscience.com*.