Integrating a Chromatographic Separation for Removal of Lipids from Membrane Protein Samples into an Automated HDX MS Workflow

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INTRODUCTION

The study of membrane proteins in their native state ideally requires them to be situated in their native lipid membrane environment, as it has been established that protein-lipid interactions may be important in modulating protein structure and function (Laganowsky 2017). Strategies have been described that permit HDX-MS interrogation of membrane proteins within lipid nanodiscs, including within so-called 'native nanodiscs' which contain membrane proteins within a lipid bilayer of native composition (Reading 2017). In the context of studying membrane proteins in such native environments by continuous labeling / bottom-up HDX MS, this presents the problem of lipids potentially interfering with effective protein digestion, and / or efficient chromatographic separation prior to infusion into the mass spectrometer.

Researchers have previously demonstrated selective removal of lipid from membrane protein samples, post labeling and quenching, by manual addition of zirconia coated silica particles followed by filtration (Hebling 2010). Here, we describe a system designed to utilize a column packed with similar zirconia coated silica beads to perform lipid removal. The column is configured in a fluidic pathway such that it can be washed and re-equilibrated during MS data acquisition. This configuration was integrated into a Waters automated HDX MS system using existing components, allowing researchers ready access to evaluate and optimize this approach. Initial data acquired using an example membrane protein (E. coli AcrB) prepared in native nanodiscs provide preliminary evidence of a fully automated workflow that could be developed successfully.

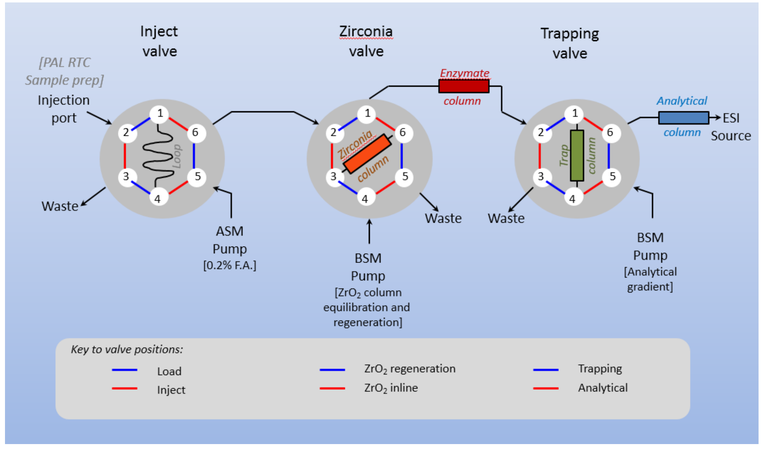

Figure 1. Schematic diagram of the modified fluidic flowpath.

METHODS

Zirconia column, additional UPLC pump and valve

A column (2.0 x 20 mm) was packed with zirconia beads (Supelco) by Eamonn Reading. This was integrated into the fluidic path of a Waters HDX Manager using an external 6-port valve. An additional binary solvent manager M-Class pump was used to pre-equilibrate the zirconia column (in water, 0.2% formic acid), and for its regeneration (50% acetonitrile, 2.5% ammonia). The additional six port valve (referred to as the zirconia valve in figure 1) allowed the zirconia column to be regenerated and re-equilibrated during analytical chromatography / MS data acquisition. Valve switching was controlled by the UPLC method in MassLynx software, such that the full workflow was automated.

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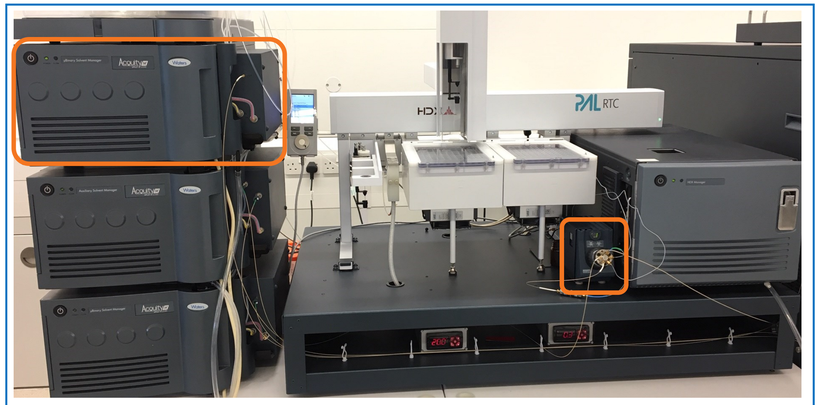

Figure 2. Illustration of the modified system. The additional Binary Solvent Manager (BSM) and electronically-controlled valve are highlighted with boxed orange outlines.

Chromatographic details

ZrO_2 separation / Pepsin digestion / Trapping:

ZrO_2 column: packed by ER, 2.0 x 20 mm, ~64 µL volume
Waters Enzymate™ BEH Pepsin column
2.1 x 30 mm part no. 186007233
Trap column: Acquity BEH C18 VanGuard
2.1 x 5.0 mm part no. 186003975

Eluents:
A: Water, 0.2% formic acid pH 2.5
B: 50% acetonitrile, 2.5% NH_4OH (ZrO_2 regeneration only)
105.0 µL/min, 7.0 minutes duration trapping phase
Approx. pressure during ZrO2 inline / digestion / trapping ≥9000 psi

ZrO_2 regeneration:

Time	Flow rate (µL/min)	% B
Initial	80	0.0
0.5	80	0.0
5.0	80	100.0
6.5	80	100.0
6.6	80	0.0
17.0	80	0.0

Analytical chromatography:

Time	Flow rate (µL/min)	% B
Initial	40	5.0
6.0	40	35.0
7.0	40	40.0
8.0	40	95.0
10.0	40	95.0
10.2	40	5.0
10.8	40	5.0
12.0	40	95.0
12.2	40	95.0
13.0	40	5.0
13.3	40	5.0
15.0	40	95.0
15.1	40	5.0
17.0	40	5.0

Analytical column: BEH C18 1.0 x 100 mm, part no. 186002346
A: Water, 0.2% formic acid pH 2.5
B: Acetonitrile, 0.2% formic acid

METHODS (contd.)

Sample

E. Coli membrane protein AcrB (~114 kDa) was cloned, expressed, and purified by Eamonn Reading as a native nanodisc sample using styrene maleic acid lipid particles (SMALP).

Method Parameters

The PAL RTC automated system was used for 5-fold dilution of AcrB (20 uM) into equilibration buffer (50 mM Tris, 150 mM NaCl, 0.03% DDM pH7.4), quenching (equal volume of 3.0 M Gdn HCl, 0.1% DDM, 0.8% formic acid), and injection into the HDX Manager. The fluidic pathway inside the HDX Manager had been modified as illustrated in figure 1. Samples first passed through the zirconia column to remove lipid prior to pepsin digestion. Eluted peptic peptides were detected via an MS^E method using a Synapt G2-Si HDMS mass spectrometer. Data processing was performed using ProteinLynx Global Server 3.0.3 and DynamX 3.0. The remaining parameters used in this experiment were typical Waters HDX workflow conditions as described previously (van Hateren, 2017).

RESULTS

Data from four replicate injections were processed in ProteinLynx Global Server 3.0.3 using low energy / high energy thresholds of 135 and 30 respectively. Processed data were searched against a fasta database containing the sequences of bovine pepsin and E. coli AcrB. The resulting .csv files were imported and filtered in DynamX 3.0. using the parameters shown in the figure below, resulting in sequence coverage of 41.9%. Although low by conventional standards, this level of coverage for AcrB prepared in native lipid nanodiscs is favourable and suggests future potential for this approach.

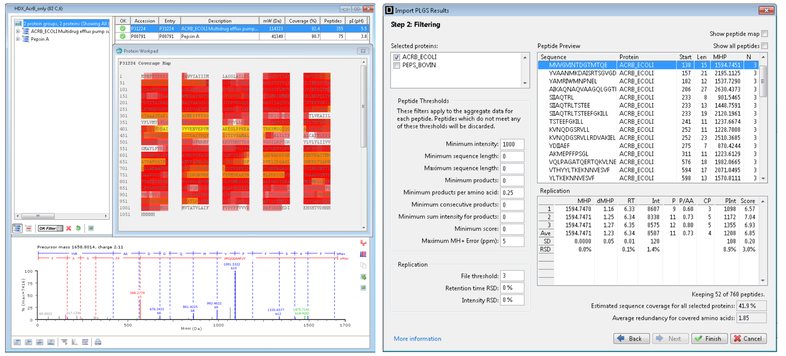

Figure 3. Example outputs from data processing in ProteinLynx Global Server 3.0.3 (left) and DynamX 3.0 (right).

FUTURE WORK

The workflow described here is at the early stage of development. The information presented is intended to demonstrate the potential of the approach with respect to lipid -containing membrane protein samples, as well as the possibility of enabling other forms of chromatographic separation to be integrated into the Waters HDX MS workflow. In the future, we intend to modify hardware to control the temperature of the additional valve, and to optimise parameters for the most effective lipid removal / sequence coverage of the target protein.

REFERENCES

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