Electronic Supplementary Material (ESI) for Analyst.

Characterization and usage of the EASY-spray technology as part of an on-line 2D SCX-RP ultra-high pressure system

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ABSTRACT:

Ultra high pressure liquid chromatography (UHPLC) systems combined with state of the art mass spectrometers have pushed the limit of deep proteome sequencing to new heights making it possible to identify thousands of proteins in a single LC/MS experiment within a few hours. However, the relationship between gradient length and the number of proteins identified is not linear and as gradient time increases to several hours, performance gain diminishes. Proteome coverage can be extended using 2-dimensional chromatography, but traditionally this comes at the expense of sample losses and much longer analysis times. Here, we asked the question whether a fast and sensitive online 2D SCX-RP UHPLC-MS/MS workflow, could compete with the current 1D long gradient analyses, using total analysis time- versus proteome coverage and sample used as benchmark parameters. Our new automated 2D-LC/MS system is robust and easy to use, consisting of a homemade SCX column, a trap column and a 50 cm analytical EASY-Spray

column. We benchmark the system using small amounts of a human cell lysate digest (~10 µg). The 2D SCX-RP UHPLC-MS/MS workflow allowed us to identify almost 37000 unique peptides and 5958 proteins in a total analysis time of about 7 hours. On the same system a 1D RP UHPLC-MS/MS workflow gave 'only' 20000 peptides and 4400 unique proteins in approx. 8 hours of analysis time. We report, here, that with this fast online SCX-RP UHPLC-MS/MS workflow proteome coverage can be substantially extended without significantly compromising analysis time and sample usage.

Figure S-1:

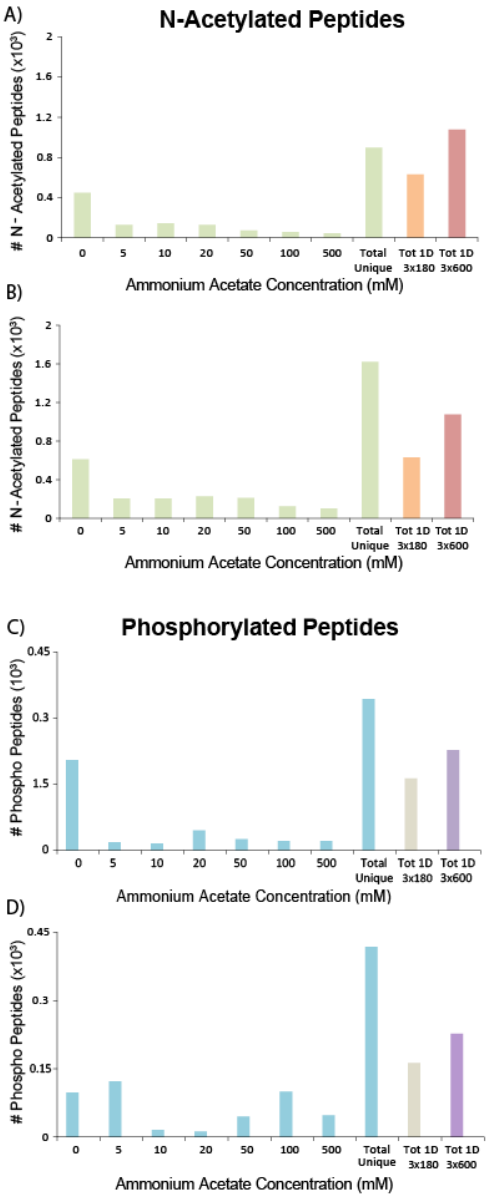

Figure S-1:

The chart shows in light green N-terminus acetylated peptides identified in each salt step for the 2D short (A) and long experiment (B) and the total unique ones identified. In orange and light red the total unique N-terminus acetylated peptides for the combined triplicates of the 1D 180 and 600 min experiments.

In light blue are represented the phosphopeptides identified in each salt step for the 2D short (C) and long experiment (D) and the total unique ones identified. While respectively in gray and

violet the total unique phosphopeptides are shown covered by the combined triplicates of the 1D 180 and 600 min experiments.

Figure S-2:

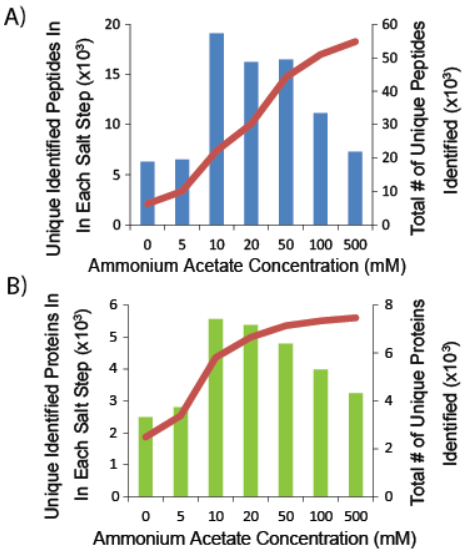

Figure S-2:

Total and per fraction number of unique peptides and proteins identified in the 2D optimized experiment

A) The plot shows for the 2D short experiment the number of unique peptides identified for each salt plug injected (0 mM to 500 mM) and with the red line the cumulative number of unique peptides identified.

B) Number of unique proteins for each salt plug and the cumulative number of unique proteins identified.

Figure S-3:

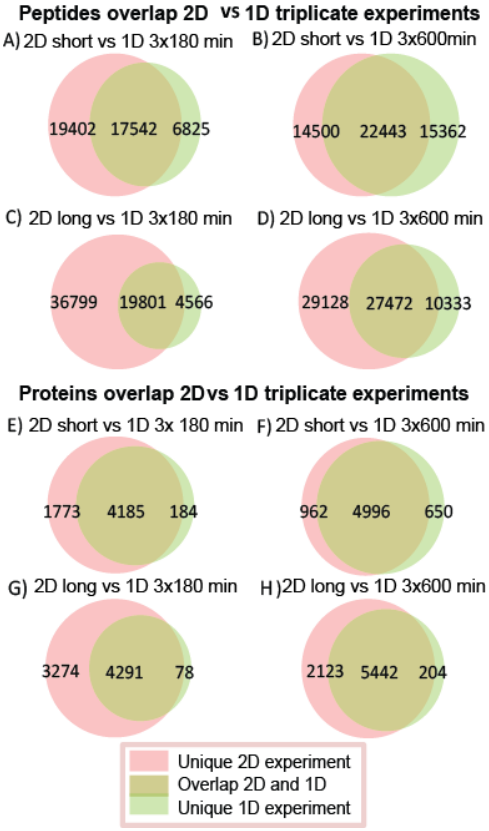

Figure S-3:

Protein and peptide overlap between 2D and 1D combined triplicates experiments.

A-B) The 2D short experiment is respectively compared for the number of peptides covered to the 1D 180 and 600 min combined triplicates. While the same comparison in E-F is made for the proteins.

C-D) The 2D long experiment is respectively compared for the number of peptides covered to the 1D 180 and 600 min combined triplicates. The same comparison in G-H is made at protein level.

Table S-1:

Lists of unique peptides and proteins identified using the 1D setup applying different analysis times. Each experiment was performed in triplicate.

Table S-2:

List of all the unique peptides, proteins, unique phospho- and N-terminal Acetylated peptides identified for combined triplicate of the 180 and 600 min 1D analysis.

Table S-3:

List of all the unique peptides, proteins, unique phospho- and N-terminal Acetylated peptides identified with the 2D short and long experiments.

Table S-4:

Overview of all results in the context of experiment type, number of dimensions, number of salt steps used for the 2D experiments, sample amount injected, analysis time, total number of unique peptides and proteins. Note that for the 1D experiments the total number of unique peptides and proteins is reported as average of three replicates.