

## **Supporting information**

### **S-Trap eliminates cell culture media polymeric surfactants for effective proteomic analysis of mammalian cell bioreactor supernatants**

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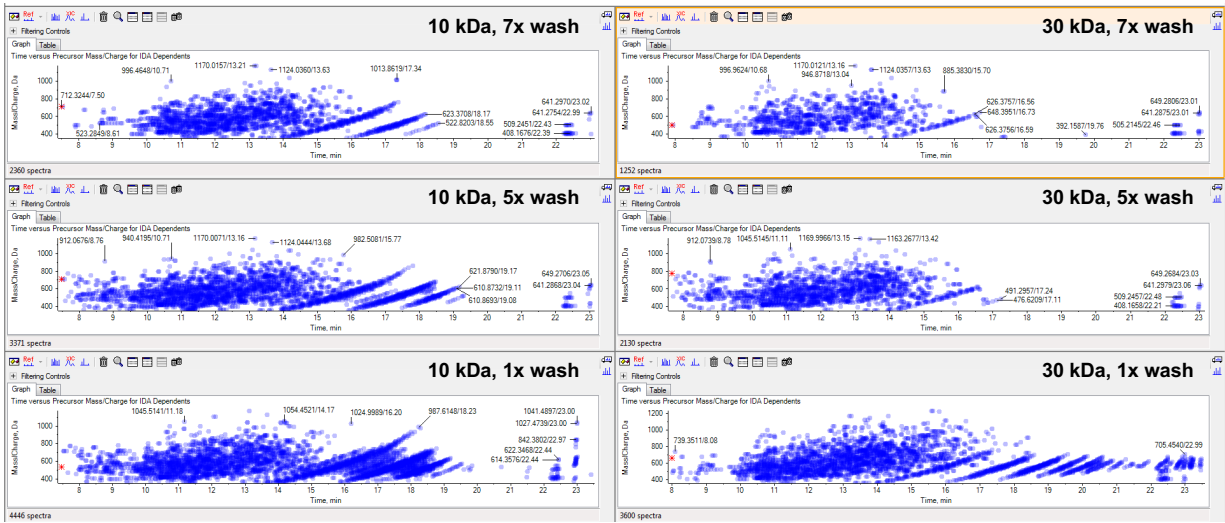
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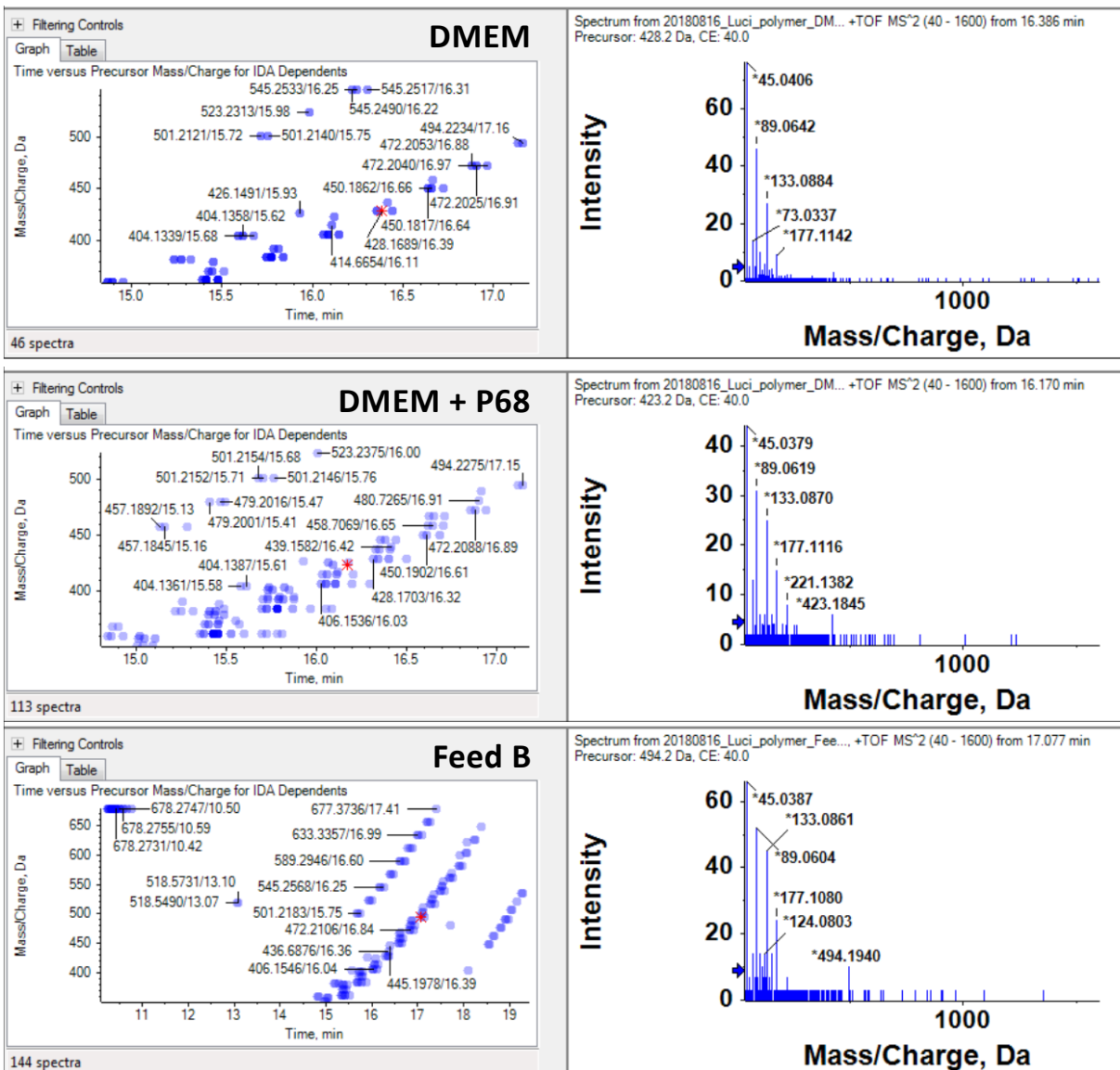
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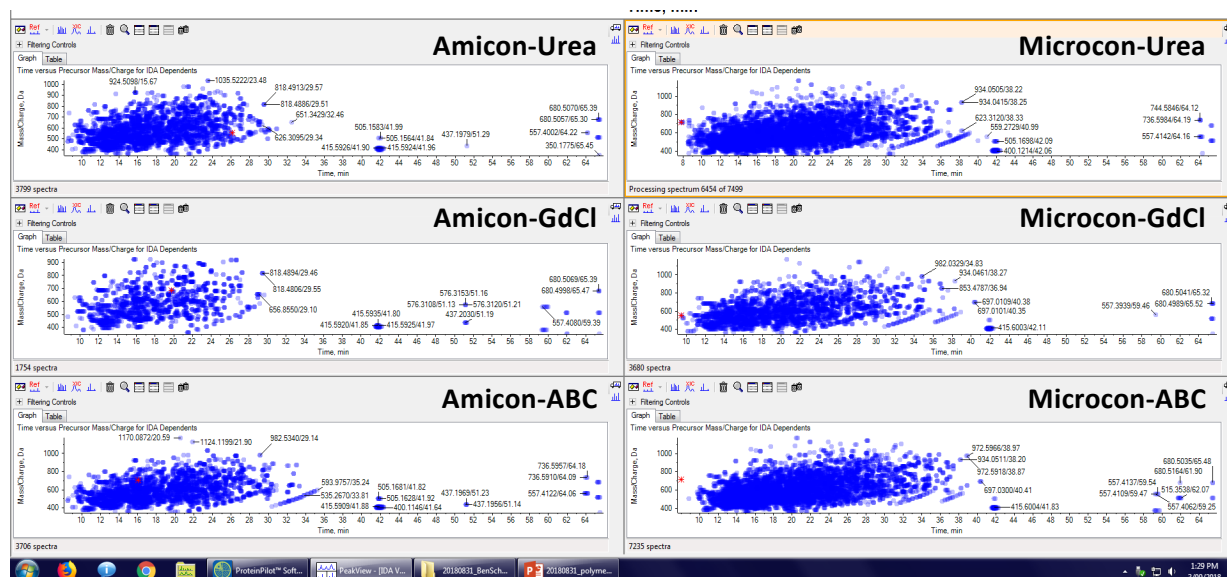


Sample	Nr of Proteins (>0.05, 10%)	Nr of peptides
10kDa, 1x wash	130	538
10kDa, 5x wash	128	585
10kDa, 7x wash	112	474
30kDa, 1x wash	119	701
30kDa, 5x wash	114	509
30kDa, 7x wash	90	334

**Supplementary Figure S1. Increasing the number of washes of Amicon columns with 50 mM ammonium bicarbonate during FASP does not effectively remove polymeric surfactants but does reduce the number of proteins and peptides identified.** 25  $\mu$ L of cell-free spent CD CHO medium supplemented with anti-clumping agent and Glutamax was processed using the FASP protocol as described in the Experimental Section, with one, five, or seven washes with 500  $\mu$ L of 50 mM ammonium bicarbonate. Representation of the fragmented precursors across time ( $m/z$  vs min). The table below shows the number of proteins and distinct peptides identified with ProteinPilot in each sample.



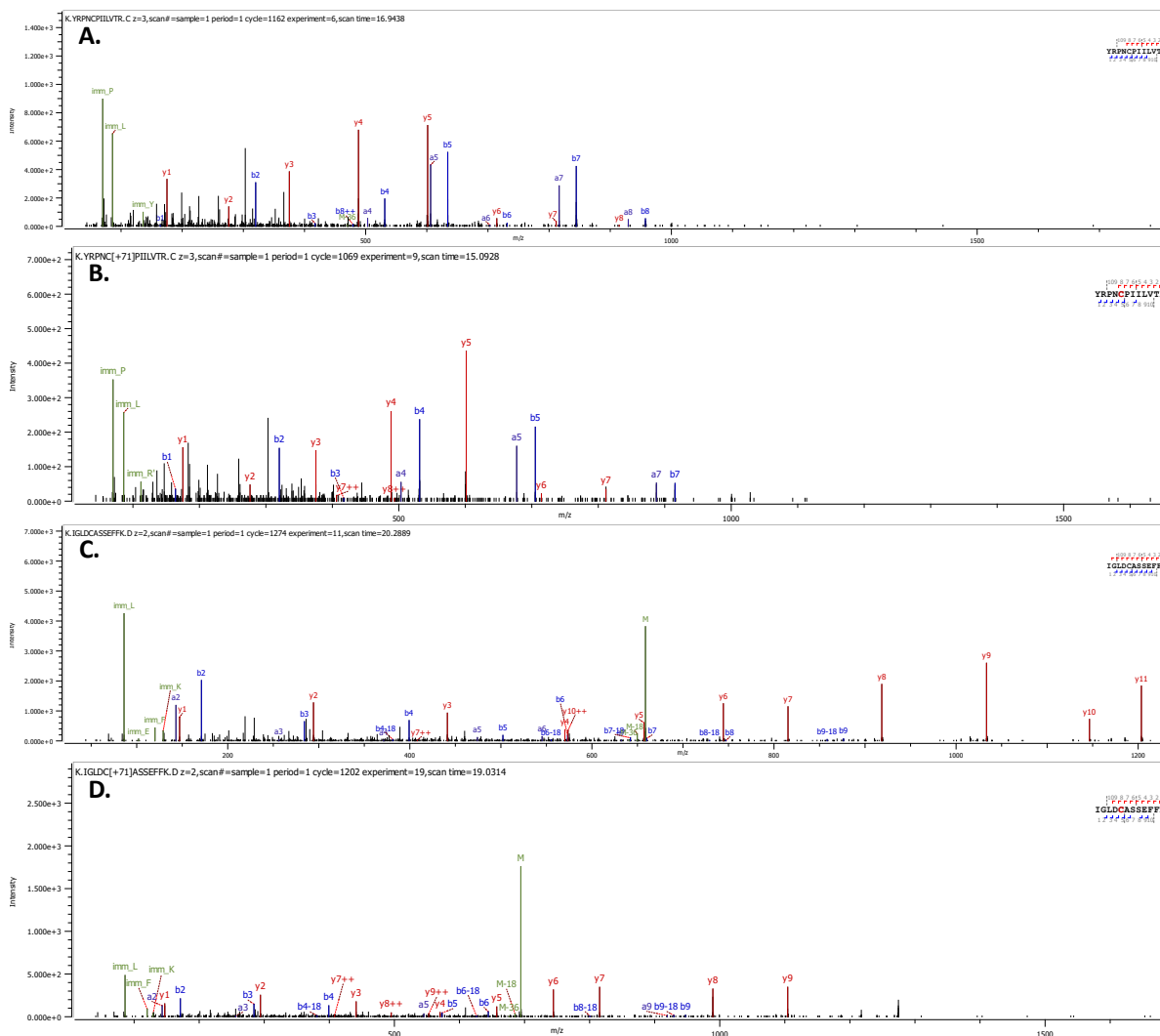
**Supplementary Figure S2. Presence of a polymer with similar MS/MS spectra in DMEM, DMEM supplemented with Pluronic F68, and EfficientFeed B.** DMEM, DMEM supplemented with 1 g/L Pluronic F68 (DMEM + P68), and EfficientFeed B (Feed B) were prepared using the FASP method. Left, representations of the fragmented precursors across time ( $m/z$  vs min); right, representative MS/MS spectra of the polymeric contaminants.



Sample	Proteins	Peptides
11.Amicon_2xABC	73	549
12.Amicon_2xGdCl	49	253
13.Amicon_2xUrea	74	510
14.Microcon_2xABC	94	765
15.Microcon_2xGdCl	39	316
16.Microcon_2xUrea	91	793

**Supplementary Figure S3. Washing denatured protein samples with strong denaturing buffers does not remove polymer in 10 kDa cut-off Amicon or Ultracel PL-30 Microcon columns.** 25  $\mu$ L of cell-free spent CD CHO medium supplemented with anti-clumping agent and Glutamax were processed using the FASP protocol as described in the Experimental Section, with the columns washed 2 times with 500  $\mu$ L of 6 M guanidinium chloride in 50 mM Tris pH 8 (GdCl), 8 M Urea in 50 mM Tris pH 8 (urea), or 50 mM ammonium bicarbonate (ABC) followed by 500  $\mu$ L ABC before trypsinization. The graphs show the fragmented precursors across time ( $m/z$  vs min). The table shows the number of proteins and peptides identified in each sample.





**Supplementary Figure S5. MS/MS spectra of peptide Y<sub>414</sub>RPNCPILVTR<sub>425</sub> from protein yeast Pyruvate Kinase 1 (P00549, UniProtKB) and peptide I<sub>244</sub>GLDCASSEFFK<sub>255</sub> from yeast protein Enolase 2 (P00925, UniProtKB).** Yeast whole cell extracts were prepared with S-Trap or SP3 methods and the samples analysed with a TripleTof 5600 instrument in DDA mode. Shown are the MS/MS spectra of the non-alkylated and alkylated (+71) versions of the peptides identified in the SP3 (A,C) or S-Trap (B,D) samples, respectively. The peptides shown are: **(A)** YRPNCPIILVTR: 482.2746 *m/z*, *z*=3, **(B)** YRPNC(+71)PIILVTR: 505.9537 *m/z*, *z*=3, **(C)** IGLDCASSEFFK: 658.8132 *m/z*, *z*=2, and **(D)** IGLDC(+71)ASSEFFK: 694.3318 *m/z*, *z*=2.

## **Supplementary Tables (.xls)**

**Supplementary Table S1.** ProteinPilot output of DDA data analysis (Protein and distinct peptides) from bioreactor supernatant samples (working day 12, CHO cells grown in CD CHO medium supplemented with CHO CD EfficientFeed B) processed with FASP, S-Trap, SP3, or precipitation.

**Supplementary Table S2.** ProteinPilot output of DDA data analysis (Protein and distinct peptides) from yeast whole cell extract samples (yeast grown overnight in YPD medium) processed with S-Trap, SP3, or precipitation. SP3 samples were prepared using a denaturation step of 95 °C for 10 min.

**Supplementary Table S3.** Re-analysis of published data using SP3 sample preparation <sup>24</sup> using Preview. Three samples from the published dataset PXD008698 were downloaded and analyzed in Preview and Byonic.

**Supplementary Table S4.** Summary of the different proteomic workflows tested in Figure 1, 2, and 3 in this work.