Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Optimization and comparison of bottom-up proteomic sample preparation for early-stage *Xenopus laevis* embryos

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216_2016_9564_MOESM2_ESM.xlsx

Content

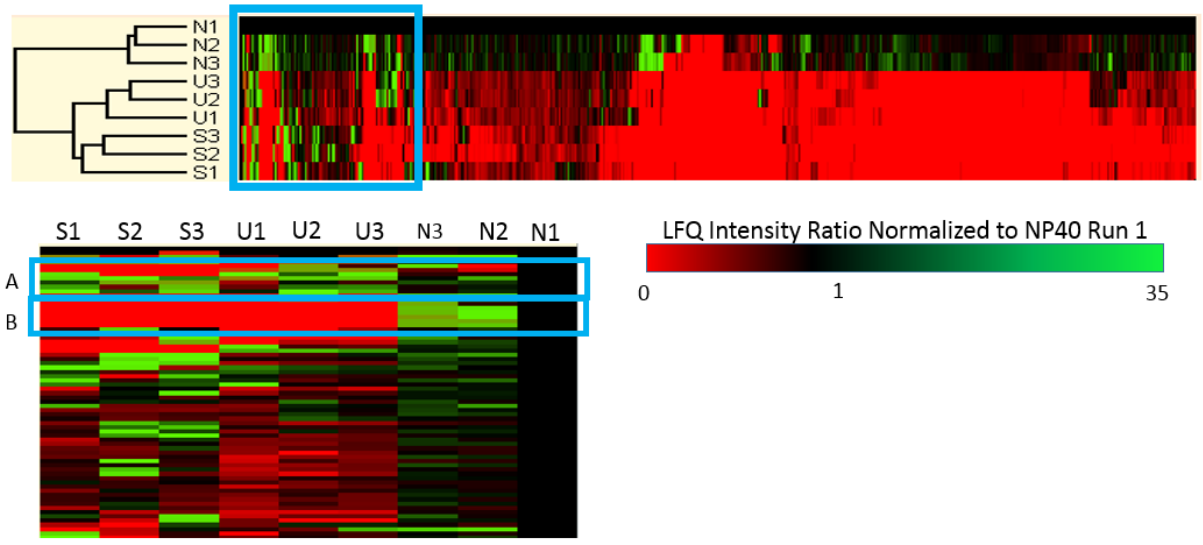


Fig. S1 Heat map and clustering of the data for each of the three lysing methods. Samples were normalized to NP40 run 1 label free quantification intensity. Designation of each run was labeled with the first letter of the lysing method (N: NP40, U: 8M Urea, S: SDS with Freon treatment) followed by the run number. S1A demonstrates increased expression of proteins using SDS with the Freon treatment or 8M urea as a lysing method. S1B demonstrates increased expression of proteins with NP40. Any sample that was not able to identify in all the samples was excluded from the heat map and clustering analysis

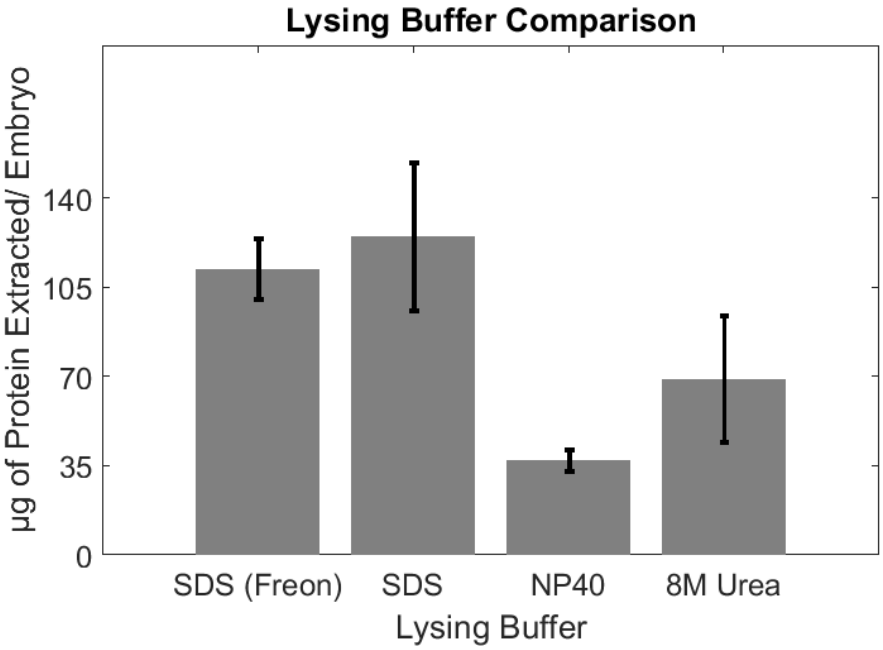


Fig. S2 Comparison of lysing buffer extraction methods. Corresponds with Table 1

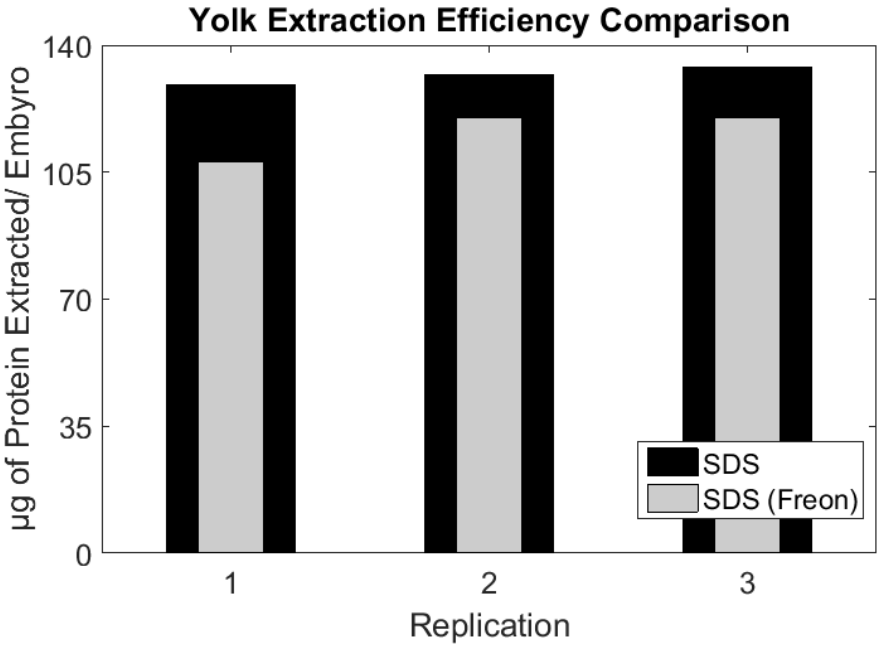


Fig. S3 Ability of Freon to remove hydrophobic proteins which are primarily yolk proteins (shown in Figure 1)

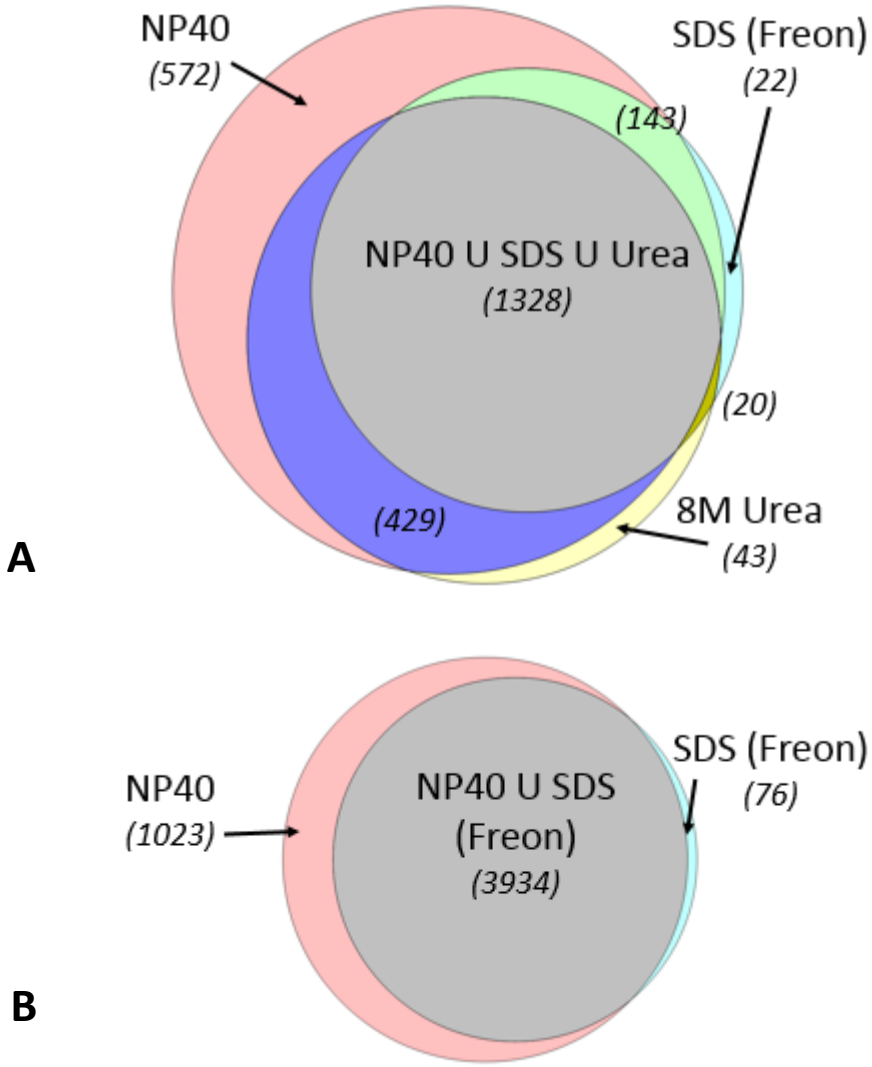


Fig. S4 Venn diagrams showing the ability of each lysing method to identify proteins with a specific extraction method. S4A Corresponds with the proteins identified in table 2. S4B corresponds with results heading: Large-scale proteome analysis of *Xenopus* stage 2 embryo lysate when NP40 and SDS were fractionated before analysis

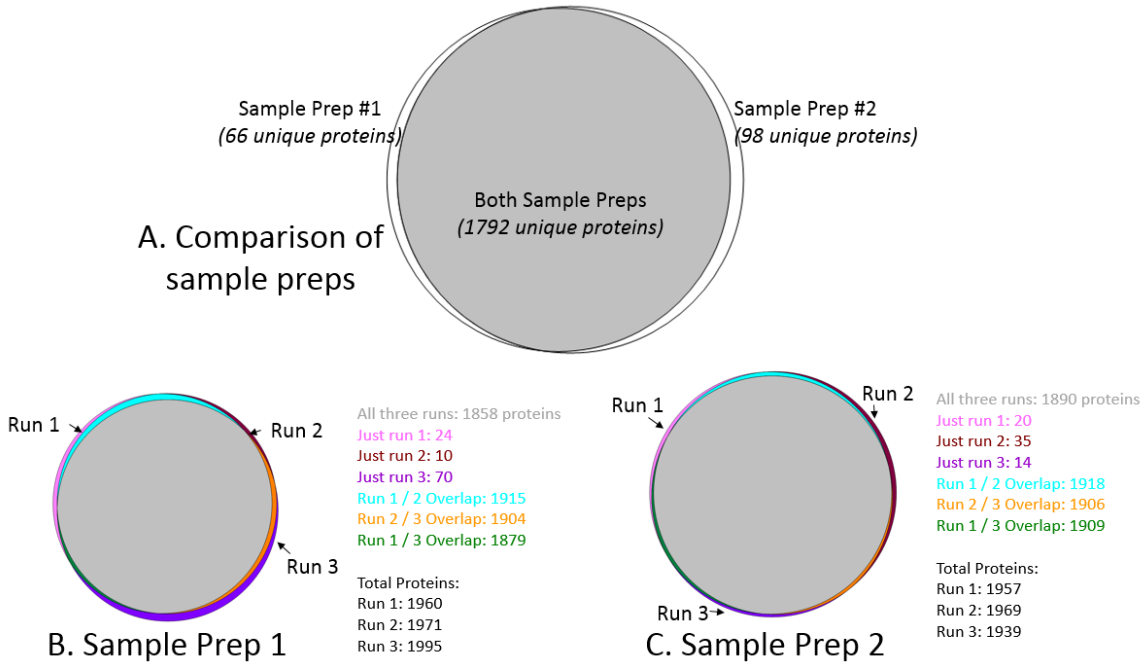


Fig. S5 Venn diagrams showing the reproducibility of protein extraction between two individual sample preparation methods and their overlap in proteins identified. 5A shows the comparison of the two individual sample preparations. To count a protein as identified – it was required to be identified in all three runs for this venn diagram. 5B and C show individual runs in triplicate for each of the sample preparation methods

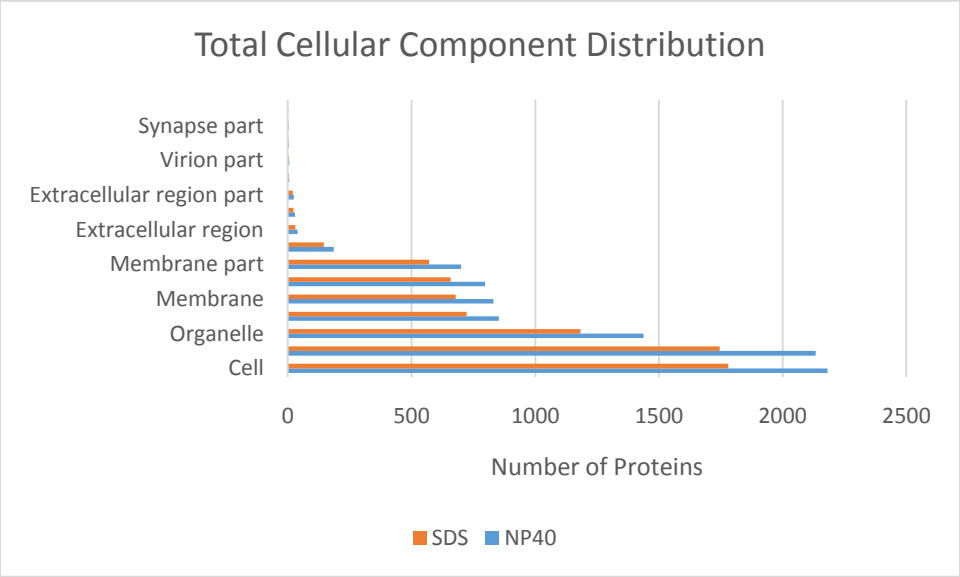


Fig. S6A Bar graph highlighting the distribution of cellular extraction. Overall, NP40 is able to extract additional proteins for all groups. All data for figures S6 was obtained from Uniprots gene ontology, cellular ontology analysis

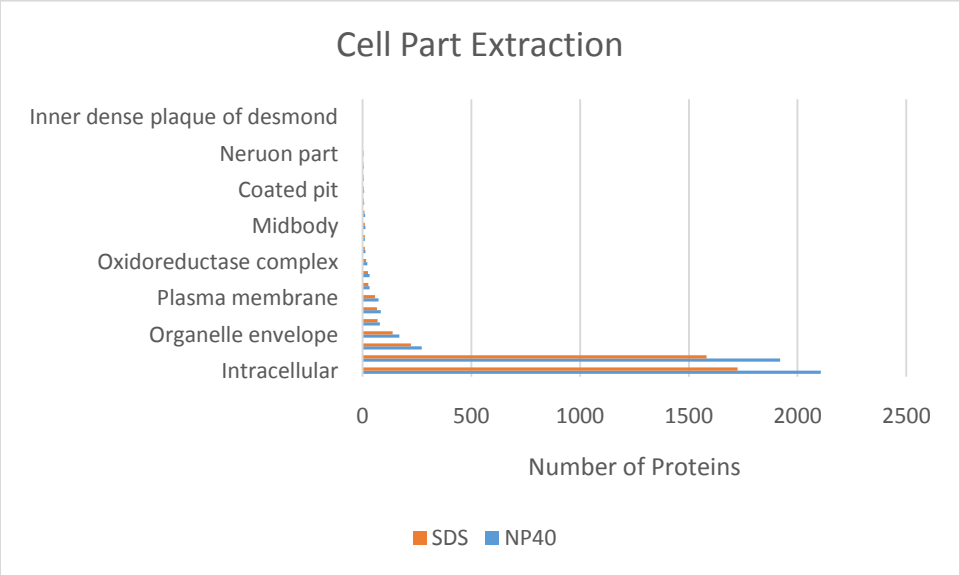


Fig. S6B From Fig. S6A, the extraction of cell parts within the sample

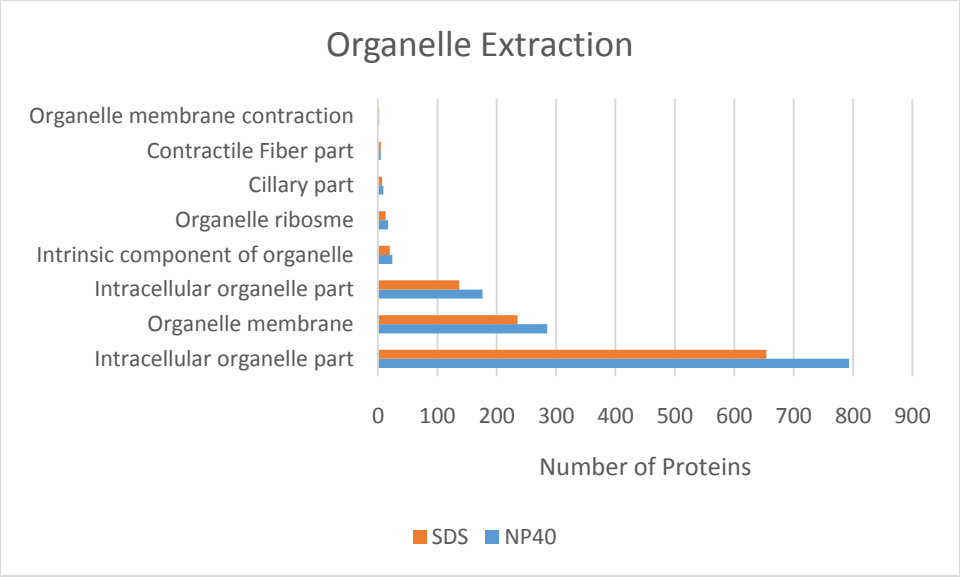
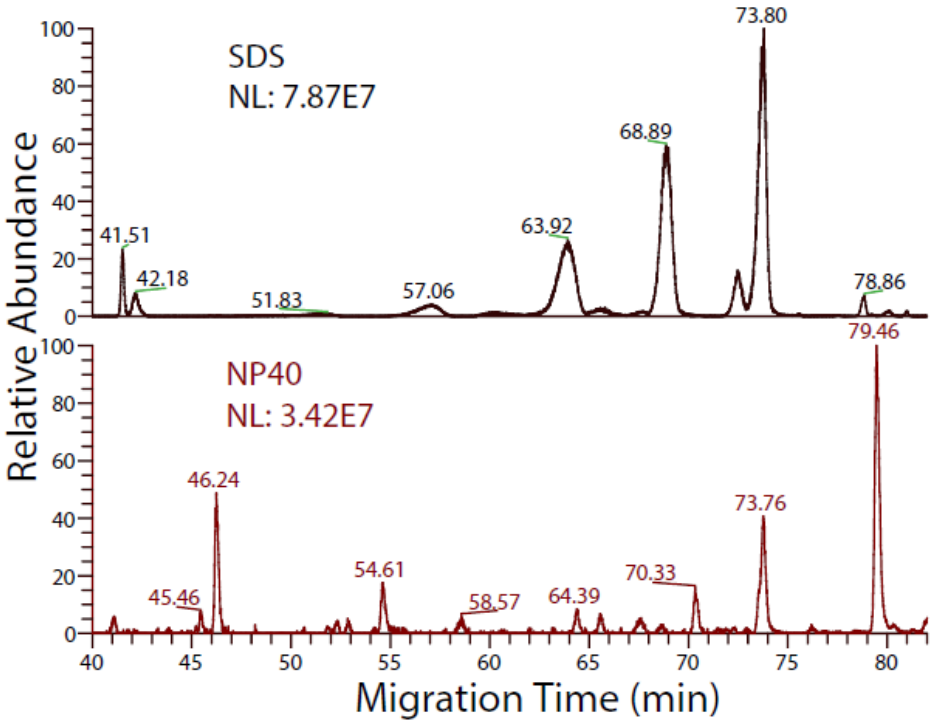


Fig. S6C From Fig. S6A, the extraction of organelle parts within the sample

S7A



S7B

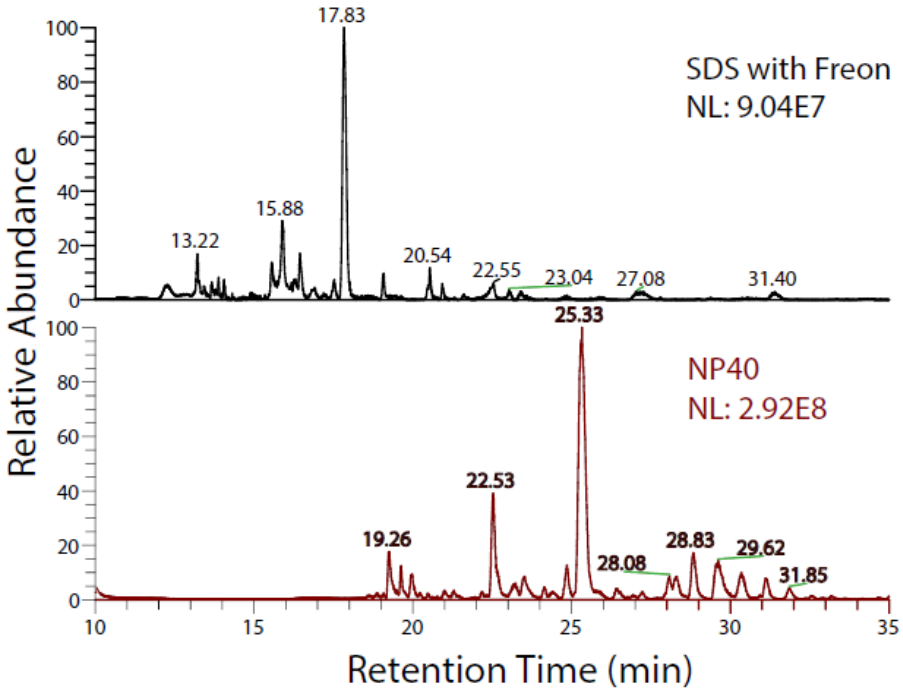


Fig. S7 Extracted chromatograms showing the shift in retention time for SDS, because of the high abundance of yolk protein in the sample initially. (A) is extracted at 458.02 m/z and (B) is extracted at 1023 m/z

In-House Recipes:

Marc's Modified Ringer's (MMR)

0.1 M NaCl

2.0 mM KCl

1 mM MgSO4

2 mM CaCl2

5 mM HEPES (pH 7.8)

Adjust pH to 7.4

MBS (Modified Barth's Saline)

Prepare two solutions: 0.1M CaCl2 and 10X MPS salts (Nacl/KCl/MgSO4/HEPES/NaHCO3)

0.1M Cacl2

11.1 g/liter

autoclave and store aliquots at -20 deg C or 4 deg C

10X MBS salts

880 mM NaCl

10 mM KCl

50 mM HEPES (pH 7.8)

25 mM NaHCO3

Adjust final pH to 7.8 with NaOH; autoclave.

Prepare the final MBS solution by missing 100 ml of 10X salt solution with 7 ml of 0.1M CaCl2; adjust the volume up to 1 liter with distilled water. The following are the final concentrations:

88 mM NaCl

1 mM CaCl2

1 mP MgSO4

5 mM HEPES (pH 7.8)

2.5 mM NaHCO3

*All reagents for MMR and MBS were from Fisher Scientific (Waltham, MA)