
Article:
Zougman, A orcid.org/0000-0002-7168-1339, Wilson, JP, Roberts, LD orcid.org/0000-0002-1455-5248 et al. (1 more author) (2020) Detergent-Free Simultaneous Sample Preparation Method for Proteomics and Metabolomics. Journal of Proteome Research, 19 (7). pp. 2838-2844. ISSN 1535-3893

https://doi.org/10.1021/acs.jproteome.9b00662

Supporting information

Detergent-free simultaneous sample preparation method for proteomics and metabolomics

Authors:

Alexandre Zougman[1*], John P. Wilson[2], Lee D. Roberts[3], Rosamonde E. Banks[1]

Author information:

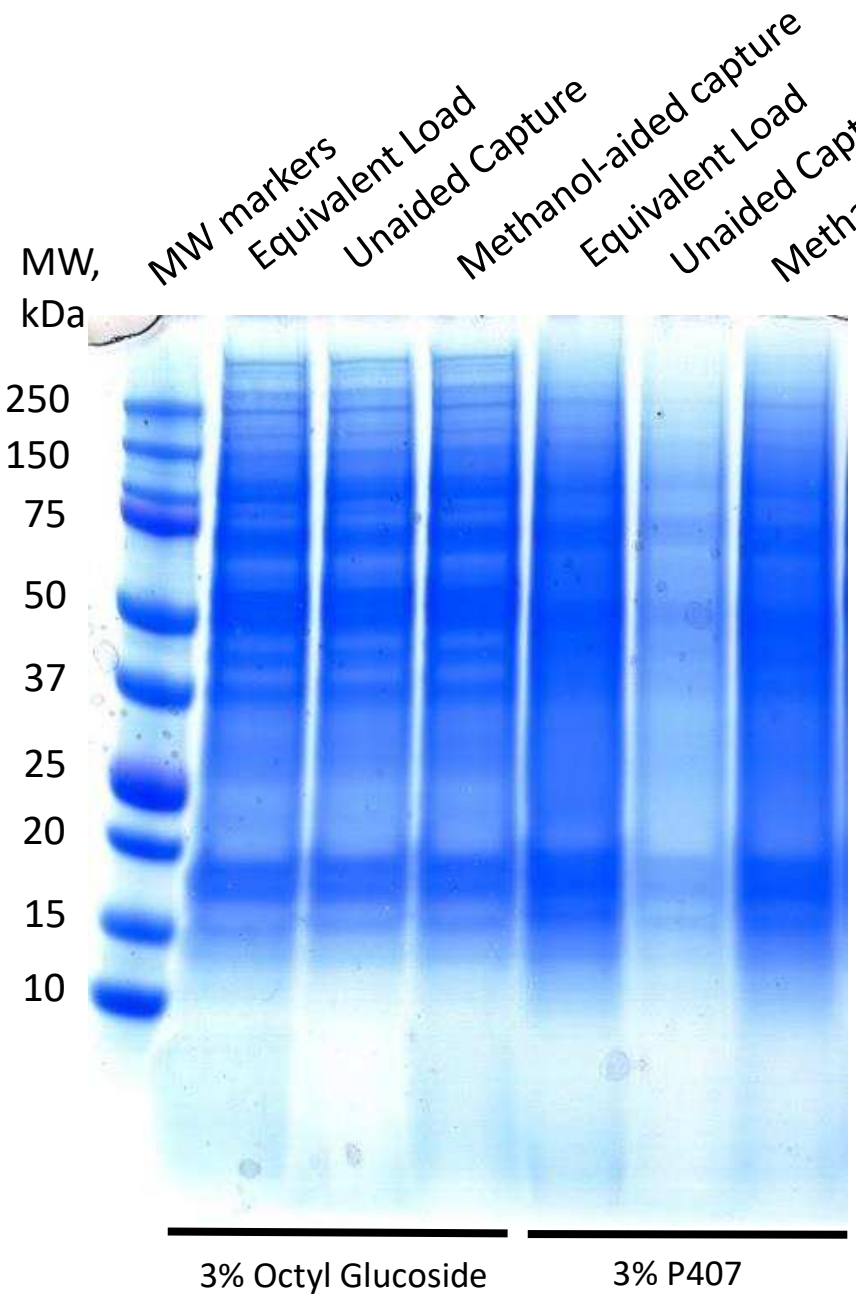
[1]Clinical and Biomedical Proteomics Group, Leeds Institute of Medical Research, St James's University Hospital, Leeds LS9 7TF, UK

[2]Protifi LLC, PO Box 2317, Huntington NY 11743, USA

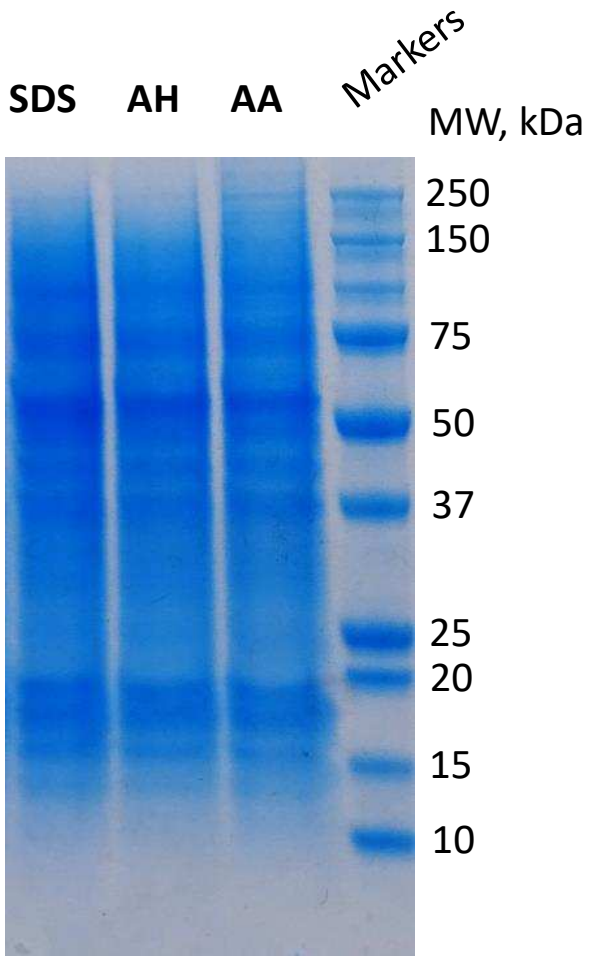
[3]Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds LS2 9JT, UK

[*]To whom correspondence should be addressed:

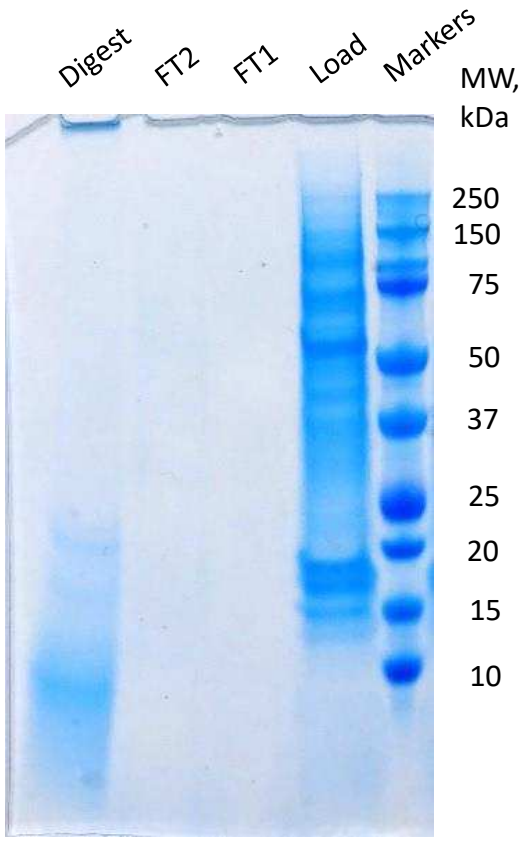
Alexandre Zougman, Leeds Institute of Medical Research, St James's University Hospital, Beckett Street, Leeds LS9 7TF, UK. Email: a.zougman@leeds.ac.uk



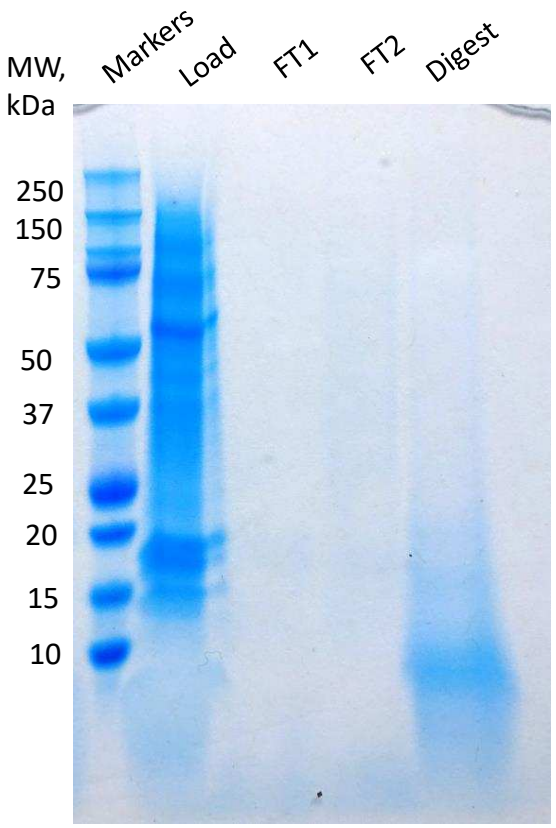
Supplementary Figure S1. Protein capture in cellulose depth filter tips from non-ionic detergent lysates. 3% Octyl Glucoside (OG) and 3% Poloxamer 407 (P407) lysates were prepared in 30 mM ammonium acetate from MDA-MB-231 cells by sonication on ice. The lysates were loaded into the tips immediately or diluted with equal volumes of methanol in 30 mM ammonium acetate (final methanol concentration was 50%). The captured proteins were eluted with 2X Laemmli buffer and run on NuPAGE 4-12% Bis-Tris Protein Gels.



Supplementary Figure S2. Protein capture in cellulose depth filter tips from SDS and detergent-free lysates. MDA-MB-231 cells were lysed by probe sonication on ice using 30 mM ammonium acetate (AA), 1.8% ammonium hydroxide (AH) or 3% SDS in 30 mM ammonium acetate (SDS). The lysates were centrifuged at 11,000 x g for 2 min to remove debris. For AA and SDS lysates 4 volumes of methanol in 30 mM Ammonium acetate were added to the samples; for AH lysates equal volume of 1 M acetic acid was added to the sample followed by addition of 2 volumes of methanol. The proteins were then captured in cellulose depth filters and consequently eluted with 2X Laemmli buffer and run on NuPAGE 4-12% Bis-Tris Protein Gels.



A



B

Supplementary Figure S3. Digestion of a cellular lysate by SiTrap using cellulose tips. MDA-MB-231 cells were lysed by probe sonication on ice either with 30 mM ammonium acetate (AA) **(A)** or 1.8 % ammonium hydroxide (AH) **(B)**. The 30 µg of lysate was loaded into SiTrap tip according to the described protocol and the flow-through (FT1) was collected. Trapped proteins were reduced and alkylated in-situ for 30 min with 10 mM TCEP and 25 mM chloroacetamide in 60 mM TEAB at 80 °C (FT2), digested with trypsin at 47 °C for 45 min and eluted with 2X Laemmli buffer. Samples were run on NuPAGE 4-12% Bis-Tris Protein Gels.

Supplementary Table S1. Multiple Reaction Monitoring Parameters for acylcarnitine species. Acylcarnitines are designated by acyl chain length in carbons and degree of unsaturated double bonds. Internal standard (IS).

Acylcarnitine	Parent Ion (*m/z*)	Fragment Ion (*m/z*)	Cone Voltage (v)	Collision Energy (ev)
C18:2	424.3	85	50	28
C18:1	426.4	85	50	28
C18:0	428.4	85	50	28
C16:1	398.3	85	50	26
C16:0	400.3	85	50	26
C14:2	368.3	85	46	26
C14:1	370.3	85	46	26
C14:0	372.3	85	46	26
C12:1	343.3	85	46	24
C12:0	344.3	85	46	24
C10:1	314.2	85	42	24
C10:0	316.2	85	42	24
C8:1	286.2	85	42	22
C8	288.2	85	42	22

C6	260.2	85	54	20
C5:1	244.2	85	38	22
C5	246.1	85	38	22
C4	232.1	85	34	20
C3	218.1	85	32	18
C2	204.1	85	32	18
C16:0-d3 IS	403.4	341.26	8	18

Supplementary Table S2. Multiple Reaction Monitoring Parameters for free fatty acid species. Free fatty acids are designated by acyl chain length in carbons and degree of unsaturated double bonds. Internal standard (IS).

Free Fatty Acid	**Parent Ion (*m/z*)**	**Fragment Ion (*m/z*)**	**Cone Voltage (v)**	**Collision Energy (ev)**
C22:6	327.25	327.25	45	7
C22:5	329.25	329.25	45	7
C22:4	331.25	331.25	45	7
C22:1	337.25	337.25	45	7
C22:0	339.25	339.25	45	7

C20:5	301.25	301.25	45	7
C20:4	303.25	303.25	45	7
C20:3	305.25	305.25	45	7
C20:2	307.25	307.25	45	7
C20:1	309.25	309.25	45	7
C20:0	311.25	311.25	45	7
C18:3	277.25	277.25	45	7
C18:2	279.25	279.25	45	7
C18:1	281.25	281.25	45	7
C18:0	283.25	283.25	45	7
C17:1	267.25	267.25	45	7
C17:0	269.25	269.25	45	7
C16:2	251.25	251.25	45	7
C16:1	253.25	253.25	45	7
C16:0	255.25	255.25	45	7
C15:1	239.25	239.25	45	7
C15:0	241.25	241.25	45	7
1	225.25	225.25	45	7
C14:0	227.25	227.25	45	7

C12:1	197.25	197.25	45	7
C12:0	199.25	199.25	45	7
C16:0-d31 IS	286.62	286.62	45	7

Supplementary Table S3. Multiple Reaction Monitoring Parameters for bile acid species. Internal standard (IS).

Bile Acid	**Parent Ion (*m/z*)**	**Fragment Ion (*m/z*)**	**Cone Voltage (v)**	**Collision Energy (ev)**
Glycoursodeoxycholic acid	448.25	74	60	35
Tauroursodeoxycholic acid	498.25	80	60	60
Taurohyodeoxycholic acid	498.25	80	60	60
Taurocholic acid	514.25	80	60	64
Glycocholic acid	464.25	74	60	34
Taurochenodeoxycholic acid	498.25	80	60	60
Taruodeoxycholic acid	498.25	80	60	60
Ursodeoxycholic acid	391.25	391.25	60	16
Cholic acid	407.25	343.25	60	34
Glycochenodeoxycholic acid	448.25	74	60	35

Glycodeoxycholic acid	448.25	74	60	35
Taurolithocholic acid	482.25	80	60	60
Chenodeoxycholic acid	391.25	391.25	60	16
Glycolithocholic acid	432.25	74	60	35
Deoxycholic acid	391.25	391.25	60	16
Lithocholic acid	375.2	373.25	60	32
Deoxycholic acid-d6 IS	397.23	331.32	80	36