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Technical Note

Detergent-free simultaneous sample preparation method for proteomics and metabolomics

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Abstract

The integration of omics techniques has seen a step change in our understanding of biological systems. However, multiomics has been impaired by mutually exclusive omic separation methods and the destructive nature of the techniques when sample is limited. We describe Simultaneous Trapping (SiTrap), a simple and effective detergent-free method which facilitates direct measurement of the proteome and metabolome in the same sample extract. This 'single pot' multiomics processing is particularly beneficial in cases when sample amounts are limited and/or are heterogeneous, e.g. tissue biopsies. We demonstrate the value of the SiTrap methodology as an essential multiomics tool in a proof-of-principle integrated study of renal cancer tissue biopsy samples. We believe SiTrap has the potential to become an indispensable tool in translational medical research.

Keywords: multiomics sample preparation, detergent-free sample preparation, SiTrap

Introduction

The combination of omic technology and techniques is gaining in popularity and advancing our understanding of biological systems and human pathologies. However, integrating analyses across omics platforms has introduced new technical challenges. Sample preparation techniques for omics are often platform dependent and mutually exclusive. Our ability to conduct multiomic studies is limited by the destructive techniques, as samples are degraded to extract the DNA, RNA, proteins, metabolites or lipids. This is especially disadvantageous when working with limited or heterogeneous samples where multiple sampling is either not practical or generates contradictory results.

We recently reported the STrap proteomics sample preparation method [1]. As with most of the current bottom-up proteomics methods, STrap is protein-centered and detergent-dependent. STrap was designed as an exclusively proteomics tool for digestion of detergent-solubilized proteins, as an alternative to Filter-Aided Sample Preparation (FASP)[2]. Many labs have found the worth of this robust methodology [3 4 5]. The original STrap concept is simple - instant creation of a fine protein particulate suspension from sodium dodecyl sulfate (SDS)-solubilized denatured and alkylated proteins, particulate entrapment by a depth filter (SDS and contaminants are removed in the flow-through), and digestion. This path however poses a problem if downstream 'omics' profiling, such as metabolomics, of the flow-through fraction is required. The presence of detergents and contaminants (such as reduction/alkylation reagents) can interfere with the downstream metabolomic analysis. Parallel sample handling, where portions of the same sample are processed for different molecular classes, e.g. proteins and metabolites, is one solution. However, when the sample amounts are limited, as is often the case with clinical material, or heterogeneity exists, for example across different tissue sections, using a simultaneous extraction methodology for several molecular classes is essential [6 7]. To date only a handful of simultaneous extraction methods has been reported [8 9]. The methods, based on phase separation, e.g. chloroform-methanol extraction, are limited by their complexity, time-consuming and not practical for either implementation with small sample amounts or high-throughput analyses. Therefore we developed the method described here that would match the protein processing power and simplicity of STrap but with the use of a detergent-free lysis and post-capture *in situ*

reduction/alkylation of the trapped proteins, providing a contaminant-free flow-through fraction for complementary 'omics' analysis.

Experimental Section

Sample processing

SiTrap tips

SiTrap tips were constructed by inserting either quartz depth filter (MK 360, Munktell, 537-4519, VWR or QM-A, Whatman, 516-2611, VWR) or cellulose depth filter (available from Protifi, LLC upon request) plugs, 1.6 mm in diameter, into 200-µl pipette tips similarly to the previously described [1]. SiTrap cellulose tips were used for cellular and tissue analyses. For the sample processing steps involving centrifugation (load, wash and elution), the tips were placed in 2.0 or 1.5-ml sample tubes with the aid of tube adapters [1].

Cell pellets

MDA-MB-231 cell pellets (1,000,000 cells per pellet) were lysed by probe sonication on ice in 250 µl of lysis solutions (30 mM ammonium acetate and 1.8% ammonium hydroxide (prepared by dilution of the stock 28% ammonium hydroxide solution (Sigma)) for SiTrap, and in 3% SDS in 50 mM Tris-HCl, pH 7.6 for STrap. The extracts were clarified by centrifugation at 11,000xg for 2 min at 18°C. Protein concentration was measured by Pierce BCA Protein Assay Kit (Thermo). 30 µg of protein was processed in six replicates in each case. For ammonium acetate (AA) extraction SiTrap processing, four volumes of methanol in 30 mM AA were added to the lysate (the methanol in 30 mM AA reagent is prepared by mixing 1 M ammonium acetate solution with anhydrous methanol). For ammonium hydroxide (AH) extraction SiTrap, an equal volume of 1 M acetic acid was added to the lysate followed by the addition of two volumes of methanol. The samples were loaded into SiTrap cellulose tips. The tips were inserted into 2.0-ml sample tubes and were centrifuged at 2000xg to capture the proteins. Captured proteins were washed by adding 80 µl of 50% methanol in 30 mM AA to the tips followed by centrifugation at 2500xg for 30 sec. The tips were removed and placed into 1.5-ml sample tubes. The captured proteins were further denatured, reduced and alkylated *in situ* by adding 60 mM triethylammonium bicarbonate (TEAB), 10 mM tris(2-carboxyethyl)phosphine (TCEP), 25 mM chloroacetamide (CAA) solution to the tips followed by heating at 80°C for 30 min (please note that the

reduction/alkylation solution should be prepared prior to the start of experiment and thoroughly vortexed right before use). After a wash with 80 µl of 20 mM TEAB at 2500xg for 30 sec, the tips were removed and placed into new 1.5-ml sample tubes. 20 µl of Sequencing Grade trypsin (Promega) in 100 mM ammonium bicarbonate at a concentration of 0.07 µg/µl was added to the tips. The trypsin solution was pushed down using a syringe with a customized tip adapter [1] till the solution meniscus was positioned ~ 3 mm above the top of the cellulose plug. Tryptic digestion was done by incubation at 47°C for 1 hour. Post-digest elution was performed consecutively with 70 µl 300 mM ammonium bicarbonate and 70 µl of 3% formic acid. The peptides were concentrated using C_8 Stage tips for the downstream analysis by mass spectrometry. For STrap processing: 30 µg of protein was processed using only quartz (OQ) tips and standard STrap sample introduction as described previously [10]. The digestion, peptide elution and concentration were the same as for SiTrap.

Renal tissues

Frozen renal tissue from three matched clear cell renal carcinoma (G2 pT3a, G2 pT1b, G1 pT2) /adjacent normal sample pairs were obtained from The Leeds Multidisciplinary Research Tissue Bank. Approximately 1 cm^2 sections with a thickness of 10 µm were cut for each sample and placed into 1.5 ml sample tube. 80 µl of 1.8% ammonium hydroxide was added to the tube and the tissue was lysed by probe sonication. The tube was centrifuged at 11,000xg for 2 min at 18°C to remove the debris. The supernatant was removed for further processing. The SiTrap load was normalized by protein concentration. 50 µg of protein was loaded into the SiTrap cellulose tips as described above for ammonium hydroxide lysates. The collected flow-through fraction, devoid of proteins, was dried down using a Speed-Vac for targeted metabolomics analysis. The captured protein fraction, in turn, was digested as described above, the resulting peptides were concentrated for proteomics analysis.

Proteomics

Peptides were separated online by reversed-phase capillary liquid chromatography using an EASY-nLC 1000 system (Proxeon) connected to a custom-made 30-cm capillary emitter column (inner diameter 75 µm, packed with 3 µm Reprosil-Pur 120 C_{18} media, Dr. Maisch). The chromatography system was hyphenated with a linear quadrupole ion trap - orbitrap

(LTQ-Orbitrap) Velos mass spectrometer (Thermo). The total acquisition time was 100 min for cellular and 140 min for tissue analyses; the major part of the chromatographic gradient was 3% - 22% acetonitrile in 0.1% formic acid. Survey MS scans (scan range of 305–1350 amu) were acquired in the orbitrap with the resolution set to 60,000. Up to 20 most intense ions per scan were fragmented and analyzed in the linear trap. Data were processed against a Uniprot human protein sequence database (October, 2018) with MaxQuant 1.5.2.8 software package (www.maxquant.org) [11]. Carbamidomethylation of cysteine was set as a fixed modification, with protein N-terminal acetylation and oxidation of methionine as variable modifications. Up to three missed cleavages and at least one unique peptide for valid protein identification were chosen. The following default values for mass tolerance were used: Orbitrap (MS) - first search 20 ppm, main search 4.5 ppm; Ion trap (MS/MS) – 0.5 Da. The maximum protein and peptide false discovery rates were set to 0.01. Analysis of Gene Ontology (GO) features was undertaken with Panther 14.0 (www.pantherdb.org) [12]. Perseus software package 1.6.2.3 (https://maxquant.net/perseus/) [13] was used for volcano plot significance analysis – the mean LFQ intensities of proteins were log2-transformed and their differences plotted against the corresponding p values from t-test, the significance cut-offs were set to 0.05 for FDR and 0.01 for S0. For data comparison only proteins identified with at least two peptides and one unique peptide were used.

Metabolomics

Targeted metabolomic LC-MS analysis of acylcarnitines, free fatty acids and bile acids

A solution of 10 µM palmitoyl-L-carnitine-(N-methyl-d3) (Sigma), 10 µM palmitic acid-d31 (Sigma) and 10 µM deoxycholic acid-d6 (Sigma) in LC-MS grade methanol was prepared as internal standard spiking solution (ISSS). Samples were reconstituted in 100 µl LC-MS grade water and 100 µl ISSS, vortex mixed and sonicated for 30 min before being transferred to LC vials. Chromatography was performed using an ACQUITY UPLC system (Waters) equipped with a CORTECS T3 2.7 µm (2.1 X 30 mm) column, which was kept at 60°C. The ACQUITY UPLC system was coupled to a Xevo TQ-XS mass spectrometer (Waters Corporation). The binary solvent system used was solvent A comprising LC-MS grade water, 0.2 mM ammonium formate and 0.01% formic acid, and solvent B comprising analytical grade acetonitrile /isopropanol 1:1, 0.2 mM ammonium formate, and 0.01% formic acid. For all

analyses a 10 µl injection was used and mobile phase was set at a flow rate of 1.3 ml/min. For acylcarnitine analysis, the column mobile phase was held at 2% solvent B for 0.1 min followed by an increase from 2% to 98% solvent B over 1.2 min. The mobile phase was then held at 98% solvent B for 0.9 min. The mobile phase was then returned to 2% solvent B held for 0.1 mins to re-equilibrate the column. For free fatty acid analysis, the column mobile phase was increased from 50% to 98% solvent B over 0.7 min. The mobile phase was then held at 98% solvent B for 0.5 min. The mobile phase was then returned to 50% solvent B held for 0.1 min to re-equilibrate the column. For bile acid analysis, the column mobile phase was held at 20% solvent B for 0.1 min followed by an increase from 20% to 55% solvent B over 0.7 min. The mobile phase was increased to 98% solvent B and held for 0.9 min. The mobile phase was then returned to 20% solvent B held for 0.1 mins to re-equilibrate the column. Analyses were performed using multiple reaction monitoring (MRM). Transitions and ionization conditions are given in Supplementary Tables 1, 2 and 3. For acylcarnitine analyses the Xevo TQ-XS was operated in positive electro-spray ionization (ESI) mode. For free fatty acid and bile acid analyses the Xevo TQ-XS was operated in negative ESI mode. A cone gas flow rate of 50 ml/hr and desolvation temperature of 650°C was used.

Metabolomics data analysis

Data were processed and peak integration performed using the Waters Targetlynx application (Waters Corporation). Integrated acyl-carnitine, free fatty acid, and bile acid peak areas were normalized to the palmitoyl-L-carnitine-(N-methyl-d3), palmitic acid-d31 or deoxycholic acid-d6 internal standard respectively. Multivariate data analysis was performed using MetaboAnalyst version 4.0 [14]. Data sets were mean-centered and analyzed using principal components analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). Metabolite changes responsible for clustering or regression trends within the pattern recognition models were identified by interrogating the corresponding loadings plot. Metabolites identified in the variable importance in projections/coefficients plots were deemed to have changed globally if they contributed to separation in the models with a confidence limit of 95%. These were verified using univariate volcano plots with a fold change cut off of 1.2 and P-value cut off of 0.05.

Data Availability

Raw data are available at ProteomeXchange [15] with identifiers PXD015677 and PXD015678.

Results and Discussion

While working with cell lysates and non-ionic detergents such as octyl glucoside and Poloxamer 407 we noticed that proteins in their native state could be simply captured by cellulose or quartz depth filters at near-neutral pH **(Suppl. Figure 1)**. The capture allowed further denaturation, reduction, alkylation and wash steps *in situ* with the following digest routine performed in a manner similar to STrap. We embarked on finding the optimal means of lysing cells without detergents, capturing and digesting extracted proteins *in situ* with the same efficiency as STrap. We found that sonication of a cell pellet either at near-neutral or basic pH efficiently releases proteins into solution with a similar extraction efficiency to SDS **(Suppl. Figure 2)**; the proteins then could be captured by either cellulose or quartz depth filter trap. This is achieved through a combination of two capture mechanisms - precipitating protein particles are physically trapped in the filter pores and the rest of the protein material is adsorbed on the filter due to non-covalent interactions with the filter surface. Notably, the flow-through after this capture will contain extracted physiological small molecules and does not include contaminants save for the volatile buffer components. This provides a suitable medium for profiling of metabolites. Importantly, the captured proteins could still be reduced and alkylated while in the trap, consequently facilitating downstream *in situ* protein digestion and proteomics analysis.

To outline SiTrap cellular processing, firstly a cell pellet is sonicated in excess of either 30 mM ammonium acetate (AA) or 1.8 % ammonium hydroxide (AH) with subsequent centrifugation to remove debris. Using AH for lysis, and analyzing UV absorbance at 280 nm in a microvolume spectrophotometer, may provide a coarse direct estimation of protein concentration in cell lysates [16]. If AA extraction is used then four volumes of methanol in 30 mM AA are added to the lysate. The sample is loaded into a SiTrap tip containing a depth filter compartment where proteins are instantly trapped. If AH extraction is used then an equal volume of 1M acetic acid is added to the lysate which brings pH close to neutral, two volumes of methanol are then added before loading into the SiTrap tip. The resulting flow-through is collected for additional 'omics' processing. The captured proteins are denatured, reduced and alkylated *in situ* by heating at 80 °C in 60 mM triethylammonium bicarbonate

(TEAB), 10 mM tris(2-carboxyethyl)phosphine (TCEP), 25 mM chloroacetamide (CAA) solution. After a wash, trypsin is added and the sample is incubated at 47°C for one hour to provide digestion of the proteins. The peptides are eluted and then concentrated using reverse phase Stage tips for analysis by mass spectrometry (MS) **(Figure 1A)**.

To test the proteomics performance of the new SiTrap methodology we compared it with STrap. MDA-MB-231 cells were extracted using either cell lysis and probe sonication on ice with AA or AH followed by SiTrap tryptic processing in cellulose SiTrap tips **(Suppl. Figure 3)** or cell lysis and probe sonication with SDS followed by STrap tryptic processing in quartz (OQ) STrap tips [10]. 30 µg of protein was processed in six replicates in each case. Tryptic digestion was performed at 47°C for one hour both for SiTrap and STrap samples. We identified 1293 (±12 SD) and 1278 (±44 SD) proteins on average with at least two peptides using AA or AH SiTrap lysis, respectively. This was comparable with the 1230 (± 27 SD) average number of proteins identified for SDS lysis with STrap **(Figure 1B)**. The average percentage of identified MS/MS spectra for AA and AH SiTrap lysis (54.6 (±0.5 SD) and 52.5 (±2.9 SD), respectively) was comparable with that of STrap (51.8 (±0.9 SD)). The protein distributions in the main GO cellular component categories were very similar in all cases **(Figure 1C)** and the majority of proteins were identified by all three approaches indicating the absence of bias **(Figure 1D)**.

The ability of the SiTrap method to provide a simultaneous multiomics analysis platform was probed using a comparative proof-of-principle proteomics/metabolomics profiling study of clear cell renal carcinoma and corresponding adjacent noncancerous tissue sections. The tissue sections (three normal/tumor pairs) were lysed by sonication with AH, the lysates were loaded into the SiTrap cellulose tips, the flow-through fractions were collected for targeted metabolomics profiling whereas the captured proteins were digested for proteomics analysis. Proteomics analysis resulted in a proteome dataset of 2655 proteins. The targeted metabolomics screen included 62 species across three metabolite classes – 26 free fatty acids, 20 acyl carnitines, and 16 bile acids. Of these 59 metabolites were observed and quantified – 25 free fatty acids, 19 acylcarnitines, and 15 bile acids. The metabolomics analysis indicated a decrease both in short-chain acylcarnitines (C5, C5:1 and C3) and in polyunsaturated free fatty acids (C20:5, C20:4, C22:6) in the tumor samples **(Figure 2A, Figure 3A)**. Carnitine O-acetyltransferase (CRAT), Carnitine O-

palmitoyltransferase 2 (CPT2) and Carnitine O-palmitoyltransferase 1 (CPT1A), the enzymes with crucial roles in acylcarnitine metabolism, were identified, quantified and found to be significantly decreased in the tumor samples, in concordance with the metabolomics results **(Figure 2B, Figure 3B)**. The carnitine system has been recently suggested to play a pivotal role in cancer metabolic plasticity [17]. A recent study of hepatocellular carcinoma tissues discovered a decrease in short- and medium-chain acylcarnitines, inhibited carnitine shuttle system and downregulation of CPT2 [18] which is consistent with our findings. We were unable to identify desaturases, an important group of enzymes in polyunsaturated fatty acid metabolism, Acyl-CoA (8-3)-desaturase (FADS1) and Acyl-CoA 6-desaturase (FADS2)[19], reduced expression of FADS1 is known to be linked with worse prognosis in non-small-cell lung cancer [20]. However, we were able to detect a significant decrease in the tumor samples of other enzymes relevant to the polyunsaturated fatty acid metabolism, in concordance with the metabolomics results: Acyl-CoA Thioesterase 1 (ACOT1) which releases C20:4, C20:5 and C22:6 from their CoA equivalents, and long chain Fatty acid-CoA ligase (ACSL1) which activates long-chain fatty acids to form acyl-CoAs **(Figure 2B)**.

Outlook

This work outlines the concept of the Simultaneous Trapping technology, SiTrap, for detergent-free proteomics and metabolomics sample processing. Because we have demonstrated the method's utility only for a handful of metabolite classes – free fatty acids, bile acids and acylcarnitines, further work will have to be done to test the method's broader or universal applicability in metabolomics field which may require tuning of the SiTrap sample processing steps to facilitate the yield of certain metabolite classes. Nevertheless, in our view, the described methodology provides the opportunity for simple and robust multiomics profiling performed on the same sample which has significant impact for comparative biological inference in omics data, high-throughput omics analysis, and is of key importance when working with limited sample amounts. We believe SiTrap has the potential to become an indispensable tool in translational medical research.

Supporting information

The following supporting information is available free of charge at ACS website http://pubs.acs.org

Figure S1. Protein capture from non-ionic detergent lysates.

Figure S2. Protein capture from SDS and detergent-free lysates.

Figure S3. Digestion of cellular lysates by SiTrap.

Table S1. Multiple Reaction Monitoring Parameters for acylcarnitine species.

Table S2. Multiple Reaction Monitoring Parameters for free fatty acid species.

Table S3. Multiple Reaction Monitoring Parameters for bile acid species.

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Contributions

A.Z. envisioned and developed the methodology. J.W. contributed to the method's development. A.Z., L.R. and R.B. designed the experiments. A.Z. and L.R. performed the experiments. A.Z., J.W., L.R. and R.B. analyzed the data. A.Z., J.W., L.R. and R.B. wrote the manuscript.

Competing interests

A patent application related to this work is filed by Protifi, LLC.

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Abbreviations

AA ammonium acetate

AC acylcarnitine

ACOT1 acyl-CoA Thioesterase 1

ACSL1 fatty acid-CoA ligase

AH ammonium hydroxide

CAA chloroacetamide

CPT1A carnitine O-palmitoyltransferase 1

CPT2 carnitine O-palmitoyltransferase 2

CRAT carnitine O-acetyltransferase

ESI electro-spray ionization

FADS1 acyl-CoA (8-3)-desaturase

FADS2 acyl-CoA 6-desaturase

FDR false discovery rate

GO gene ontology

IS internal standard

ISSS internal standard spiking solution

LC-MS liquid chromatography-mass spectrometry

LFQ label-free quantitation

MRM multiple reaction monitoring

MS mass spectrometry

OQ only quartz

PCA principal components analysis

PLS-DA partial least squares-discriminant analysis

ppm parts per million

SD standard deviation

SDS sodium dodecyl sulfate

SiTrap simultaneous trapping method

STrap suspension trapping method

TCEP tris(2-carboxyethyl)phosphine

TEAB triethylammonium bicarbonate

UPLC ultra performance liquid chromatography

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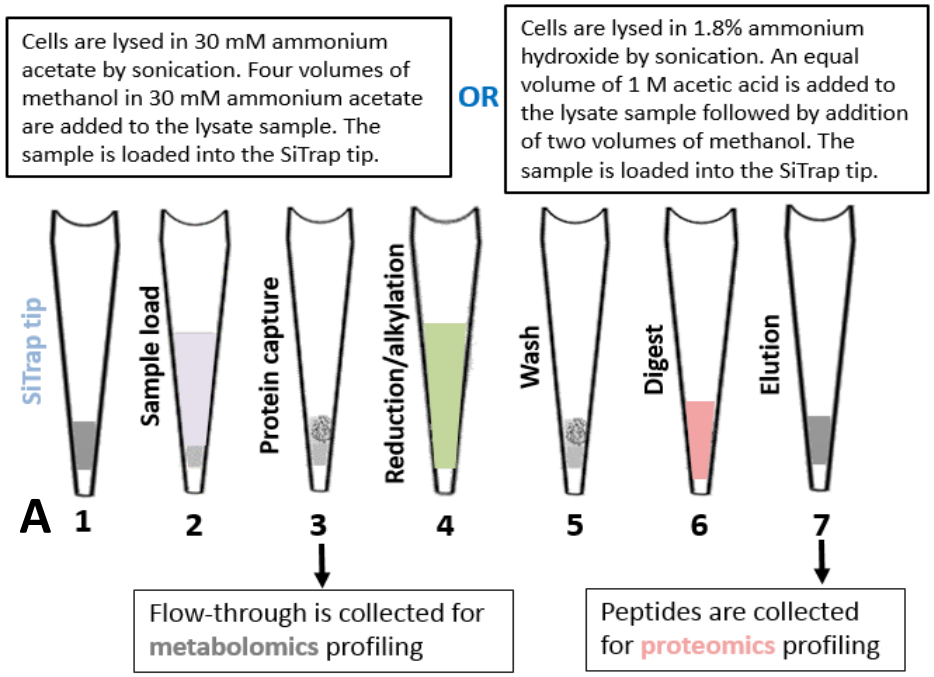
Figure legends

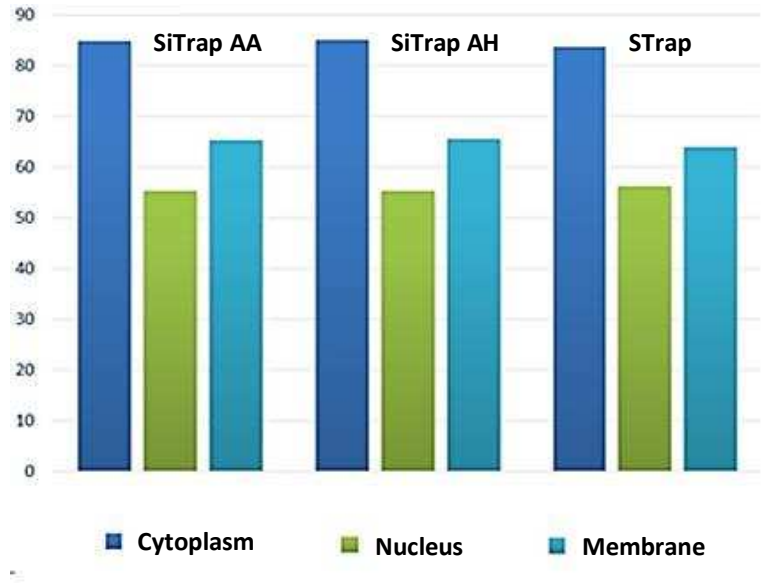
Figure 1. SiTrap processing of cellular material. **(A)** Basic scheme. A cell pellet is sonicated in excess of either 30 mM ammonium acetate (AA) or 1.8 % ammonium hydroxide (AH). For AA extraction four volumes of methanol in 30 mM AA are added to the lysate. For AH extraction an equal volume of 1M acetic acid is added to the lysate followed by two volumes of methanol. The resultant mix is loaded into the SiTrap unit (1), the proteins are captured in the depth filter trap and the flow-through is collected (2, 3). Following a wash with 50% methanol, the proteins are denatured, reduced and alkylated *in situ* by heating at 80 °C in 60 mM triethylammonium bicarbonate (TEAB), 10 mM tris(2-carboxyethyl)phosphine (TCEP), 25 mM chloroacetamide (CAA) solution (4). After the wash (5) an enzyme is introduced to the trapped proteins (6). After the digestion, the peptides are eluted from the SiTrap tips (7). The peptides are concentrated by Stage tips for downstream analysis by mass spectrometry. **(B-D)** Proteomics comparison of SiTrap ammonium hydroxide (AH), SiTrap ammonium acetate (AA) and standard SDS-based STrap digests of MDA-MB-231 cells. **(B)** Box-plot diagram of identified protein numbers (at least two peptides were required for protein identification). **(C)** Protein distributions in the main GO cellular component categories. **(D)** Venn diagram showing distributions of the number of proteins identified with at least two peptides for each of the three sample preparation methods.

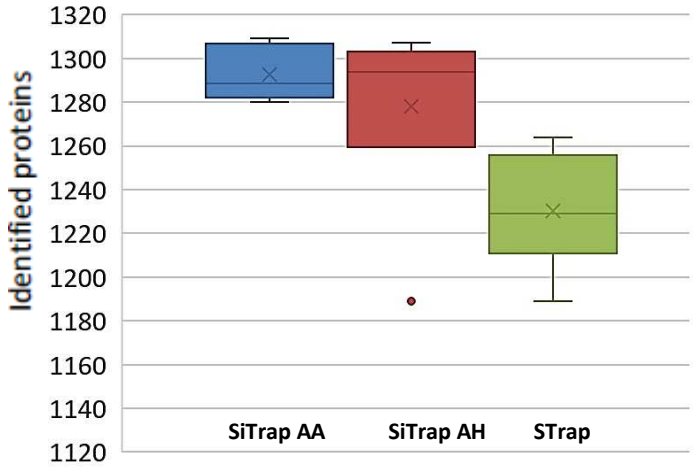
Figure 2. Volcano plot significance analysis of the metabolomics and proteomics profiling data for normal vs tumor renal sections. The significance cut-offs were set to 0.05 for false

discovery rates (FDR). **(A)** The results of the metabolomics analysis indicate a decrease in both short chain acylcarnitines (C5, C5:1 and C3) and in polyunsaturated free fatty acids (C20:5, C20:4, C22:6) in the tumor samples. **(B)** The results of the proteomics analysis indicate downregulation of enzymes in the carnitine pathway, Carnitine O-acetyltransferase (CRAT), Carnitine O-palmitoyltransferase 2 (CPT2) and Carnitine O-palmitoyltransferase 1 (CPT1A) in the tumor samples. Downregulation of enzymes in the polyunsaturated fatty acid pathway, Acyl-CoA Thioesterase 1 (ACOT1) and long chain Fatty acid-CoA ligase (ACSL1), is also observed in the tumor samples.

Figure 3. SiTrap proteomic and metabolomic analysis of renal tumors identifies dysfunctional acylcarnitine (AC) metabolism. **(A)** Metabolomics analysis identifies decreased short chain acylcarnitines (C5, C5:1 and C3) in the tumor samples. The Y axes represent mean-centered relative concentrations. **(B)** Proteomics analysis indicates downregulation of Carnitine O-acetyltransferase (CRAT), Carnitine O-palmitoyltransferase 2 (CPT2) and Carnitine O-palmitoyltransferase 1 (CPT1A) in the tumor samples. The Y axes represent label-free quantitation (LFQ) intensity values.


Cells are lysed in 30 mM ammonium acetate by sonication. Four volumes of methanol in 30 mM ammonium acetate are added to the lysate sample. The sample is loaded into the SiTrap tip.
OR
Cells are lysed in 1.8% ammonium hydroxide by sonication. An equal volume of 1 M acetic acid is added to the lysate sample followed by addition of two volumes of methanol. The sample is loaded into the SiTrap tip.
SiTrap tip
Sample load
Protein capture
Reduction/alkylation
Wash
Digest
Elution
A
1
2
3
4
5
6
7
Flow-through is collected for metabolomics profiling
Peptides are collected for proteomics profiling


C
SiTrap AA
SiTrap AH
STrap
90
80
70
60
50
40
30
20
10
0
Cytoplasm
Nucleus
Membrane


Identified proteins
1320
1300
1280
1260
1240
1220
1200
1180
1160
1140
1120
SiTrap AA
SiTrap AH
STrap
B

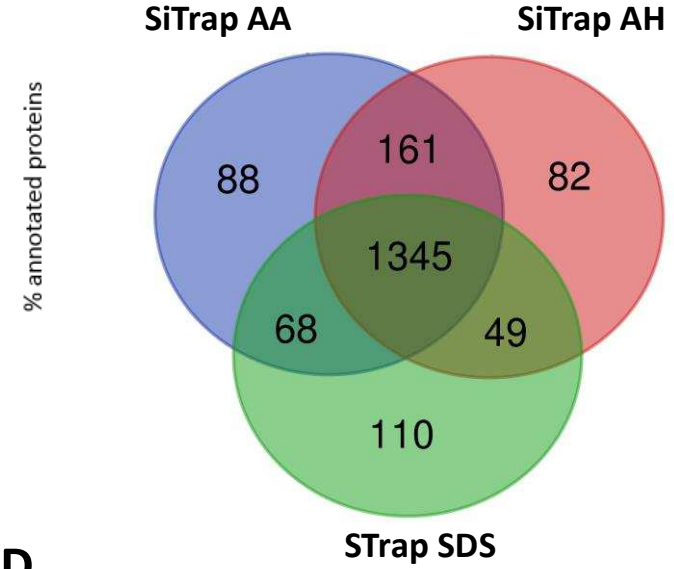
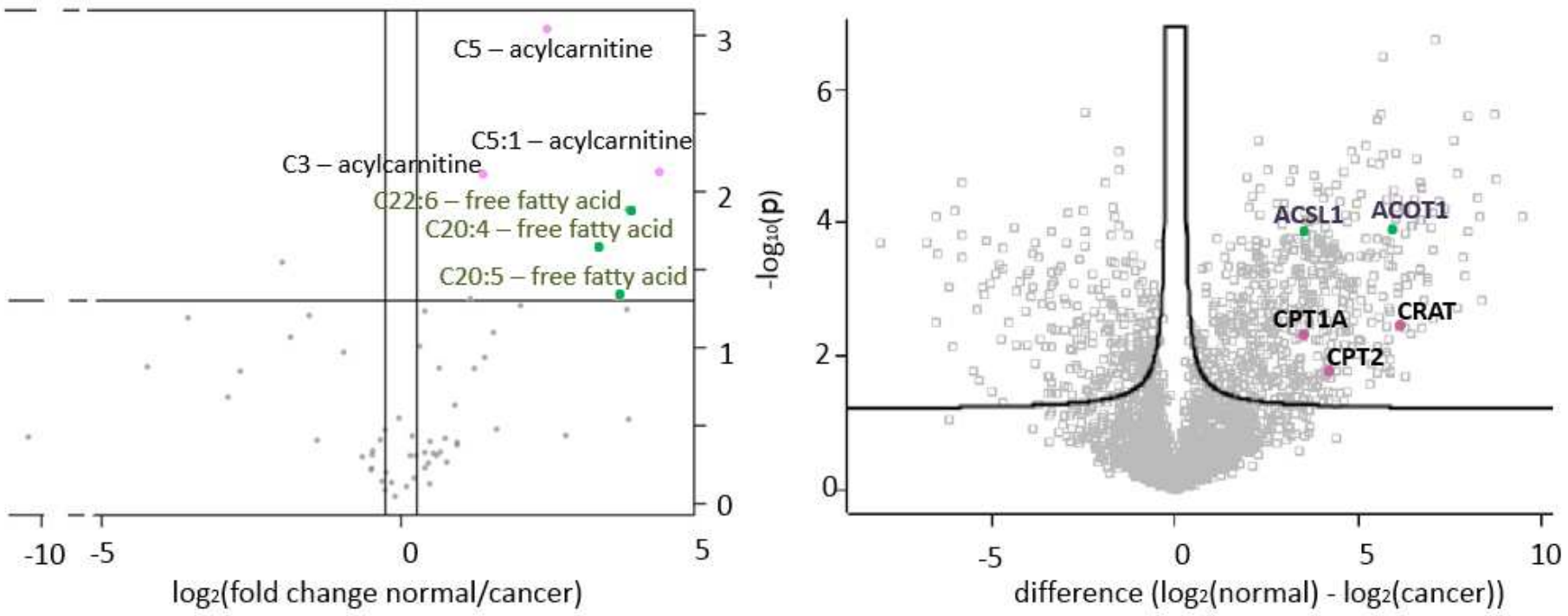

SiTrap AA
SiTrap AH
% annotated proteins
88
161
82
1345
68
49
110
STrap SDS
D

Figure 1


C5 – acylcarnitine
C5:1 – acylcarnitine
C3 – acylcarnitine
C22:6 – free fatty acid
C20:4 – free fatty acid
C20:5 – free fatty acid
-log10(p)
log2(fold change normal/cancer)
ACSL1
ACOT1
CPT1A
CRAT
CPT2
difference (log2(normal) - log2(cancer))

A **B**

Figure 2

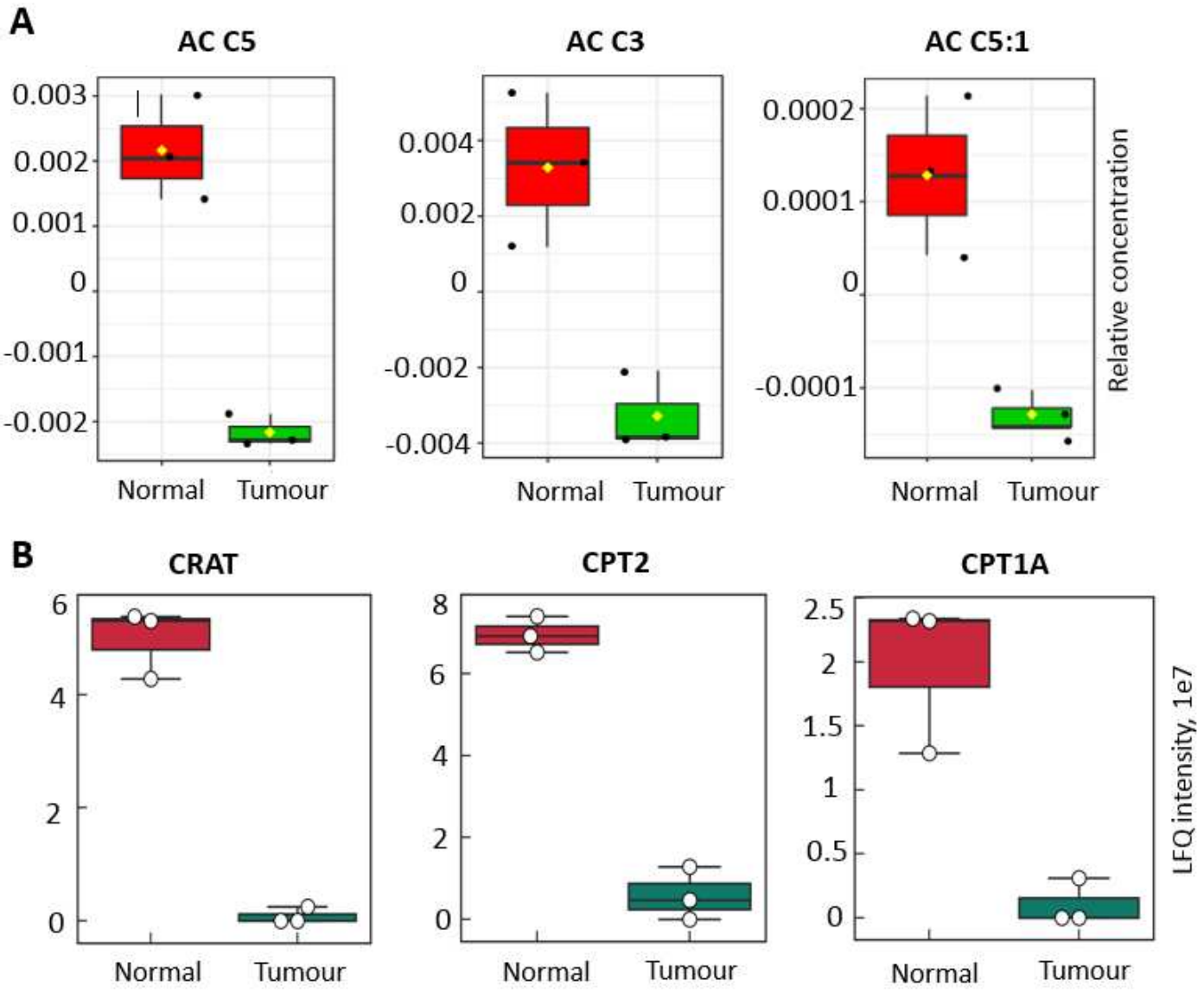
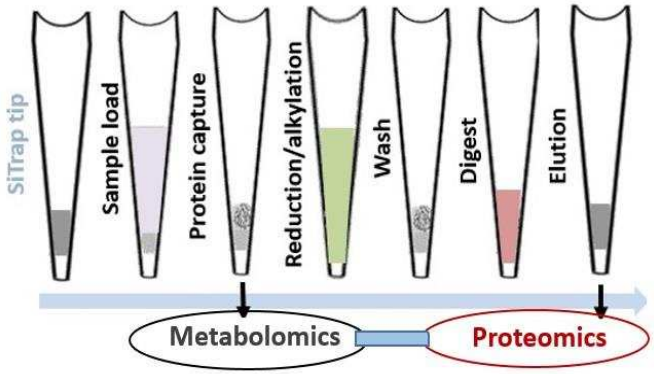

A
AC C5
AC C3
AC C5:1
0.003
0.002
0.001
0
-0.001
-0.002
0.004
0.002
0
-0.002
-0.004
0.0002
0.0001
0
-0.0001
Relative concentration
Normal
Tumour
B
CRAT
CPT2
CPT1A
6
4
2
0
8
6
4
2
0
2.5
2
1.5
1
0.5
0
LFQ intensity, 1e7
Normal
Tumour

Figure 3

FOR TOC ONLY


SiTrap tip
Sample load
Protein capture
Reduction/alkylation
Wash
Digest
Elution
Metabolomics
Proteomics