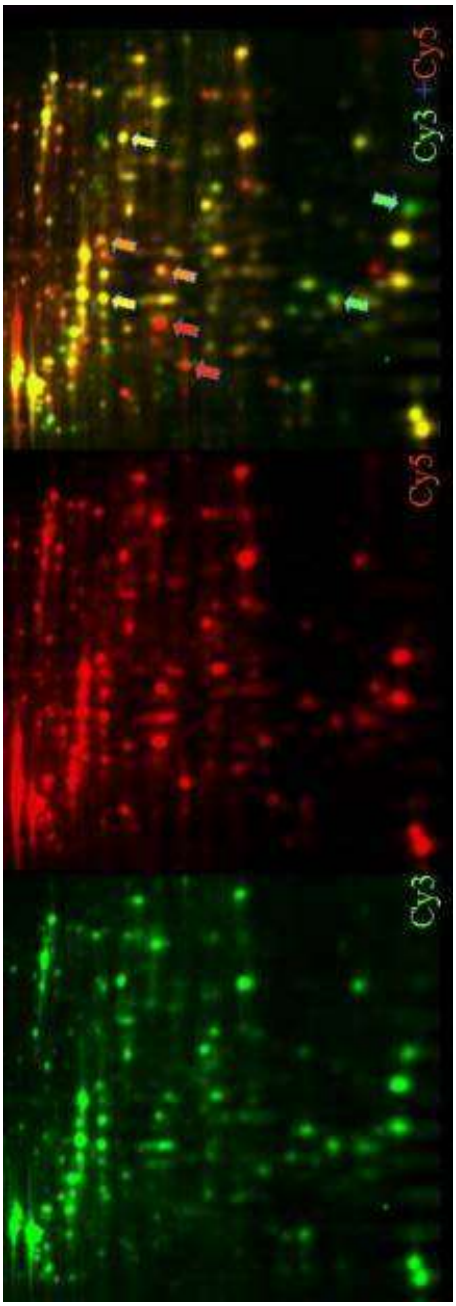

UNIVERSITY OF CAMBRIDGE

Cambridge Centre for Proteomics

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ccpcore@bioc.cam.ac.uk

Gel and mass spectrometry-based proteomic services

The Cambridge Centre for Proteomics, established in 2000, is a world leading facility tackling proteomic-based problems and actively engages in method development. We provide a considerable amount of expertise in proteomics and mass spectrometry based techniques to both academic and industrial researchers.

Experiments are performed using state-of–the-art instrumentation, which includes a range of nano-LC systems coupled with Orbitrap/Q-TOF mass spectrometers. We currently offer full quantitative analysis on virtually any sample of any complexity.

All our proteomics applications are supported by robust informatics pipeline and data analysis.

Protein Identification by Mass Spectrometry

CCP has expanded its range of LC systems and mass spectrometers in recent years which has meant that the throughput of samples has increased considerably, and therefore, turn-around times are continually being reduced.

We currently have state-of-the-art instrumentation (Orbitrap family mass spectrometers and associated nano-LCs) for the identification of proteins from gel bands or solution.

Each experiment is tailored to suit each sample, ensuring that maximum sequence coverage is obtained. Full sample preparation from gel-based samples or proteins in solution is performed including reduction, alkylation and enzymatic digestion.

Peptides are separated by high-resolution nanoscale chromatography and high mass accuracy MS/MS spectra are automatically acquired. Finally, data are searched against standard or custom databases using the Mascot search algorithm and search results are then emailed with a secure dropbox connection.

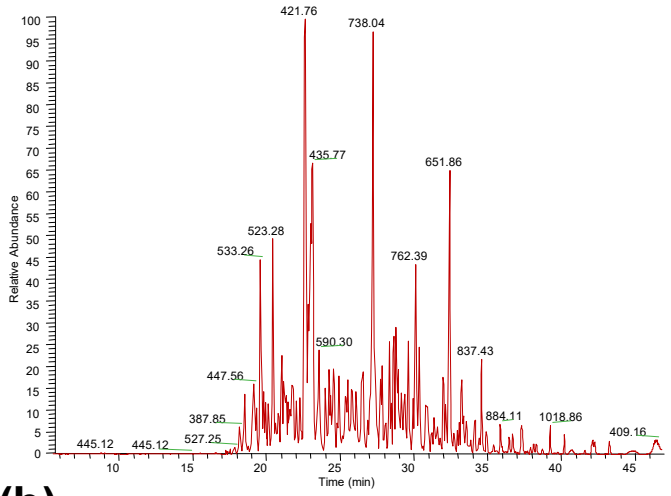
CCP has a range of software to analyse and quantify proteomic data including MASCOT, Proteome Discoverer, Scaffold and MaxQuant.

Support is always available for data interpretation or any data-related queries.

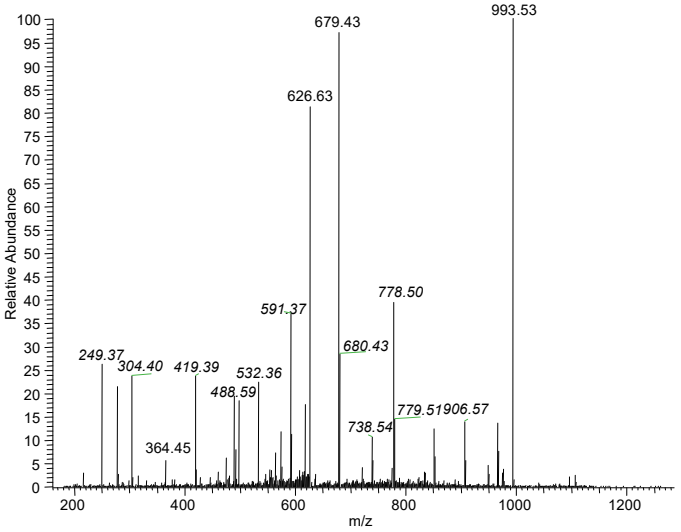
Services available:

- Full sample processing, including reduction, alkylation and enzymatic digestion of proteins within the gel bands or solutions.
- High resolution reverse-phase chromatography followed by high mass accuracy MS/MS.
- Mascot database searching and interpretation of data using standard or custom databases.

(a)



(b)



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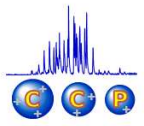


Cambridge Centre for Proteomics
Mascot Search Results

```
User           : CCP Core
Email          :
Search title   : CCP Core Brochure File Human Database 2019 (\\prot-filesvr1\data\CORE\PARAMETERS\Mascot_search_parame
MS data file   : \\prot-filesvr1\data\CORE\CCP Brochure 2019\CCP Brochure test file.mgf
Database 1     : cRAP 20181217 (120 sequences; 39582 residues)
Database 2     : CCP_UniProt_homo_sapiens UniProt_homo_sapiens_proteome_20180409 (93609 sequences; 37041084 residues)
Timestamp      : 14 Jan 2019 at 13:39:22 GMT
Protein hits   : 2::Q15149-4          Isoform 4 of Plectin OS=Homo sapiens OX=9606 GN=PLEC
                 2::Q15149            Plectin OS=Homo sapiens OX=9606 GN=PLEC PE=1 SV=3
                 2::Q15149-2          Isoform 2 of Plectin OS=Homo sapiens OX=9606 GN=PLEC
                 2::Q15149-3          Isoform 3 of Plectin OS=Homo sapiens OX=9606 GN=PLEC
                 2::Q15149-8          Isoform 8 of Plectin OS=Homo sapiens OX=9606 GN=PLEC
                 2::Q09666            Neuroblast differentiation-associated protein AHNAK OS=Homo sapiens
                 2::P21333            Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4
                 2::Q60FE5            Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=1
                 2::P35579            Myosin-9 OS=Homo sapiens OX=9606 GN=MYH9 PE=1 SV=4
                 2::O75369-8          Isoform 8 of Filamin-B OS=Homo sapiens OX=9606 GN=FLNB
                 2::O75369            Filamin-B OS=Homo sapiens OX=9606 GN=FLNB PE=1 SV=2
                 2::Q14315            Filamin-C OS=Homo sapiens OX=9606 GN=FLNC PE=1 SV=3
```

Figure 1. Data dependent acquisitions was performed using the Q Exactive Orbitrap instrument to automatically select and fragment peptides formed from tryptic digests. (a) LC-MS/MS traces are generated which give a readout of intensity versus time (b) MS/MS spectra are generated which give specific information relating to the sequence of the peptides (c) the MS/MS data is converted and searched against specific databases using the Mascot search algorithm. The output is a search result file which lists all identified proteins.

Post-translational modification (PTM) mapping and synthetic modifications mapping of non-complex samples

This experiment involves the identification and location of modifications by LC-MS/MS. It usually involves the purification by immunoprecipitation or pull-down followed by separation of the enriched proteins on a SDS-PAGE, excision of the protein of interest, digestion and analysis by LC-MS/MS.

LC-MS/MS data are analysed using Mascot database searches and annotated spectra are then manually verified by analysing specific sequence ions where PTM sites may be identified.

In principle there is no limit to the type or number of modifications that can be searched. Modifications which can be routinely searched include phosphorylation, methylation, acetylation, ubiquitination, oxidation and sulfation.

Furthermore, we can also routinely map custom synthetic modifications to specific amino acids.

Services Available

- Full sample preparation.
- LC-MS/MS experiments.
- Full data analysis including assignment of PTM sites, where possible.

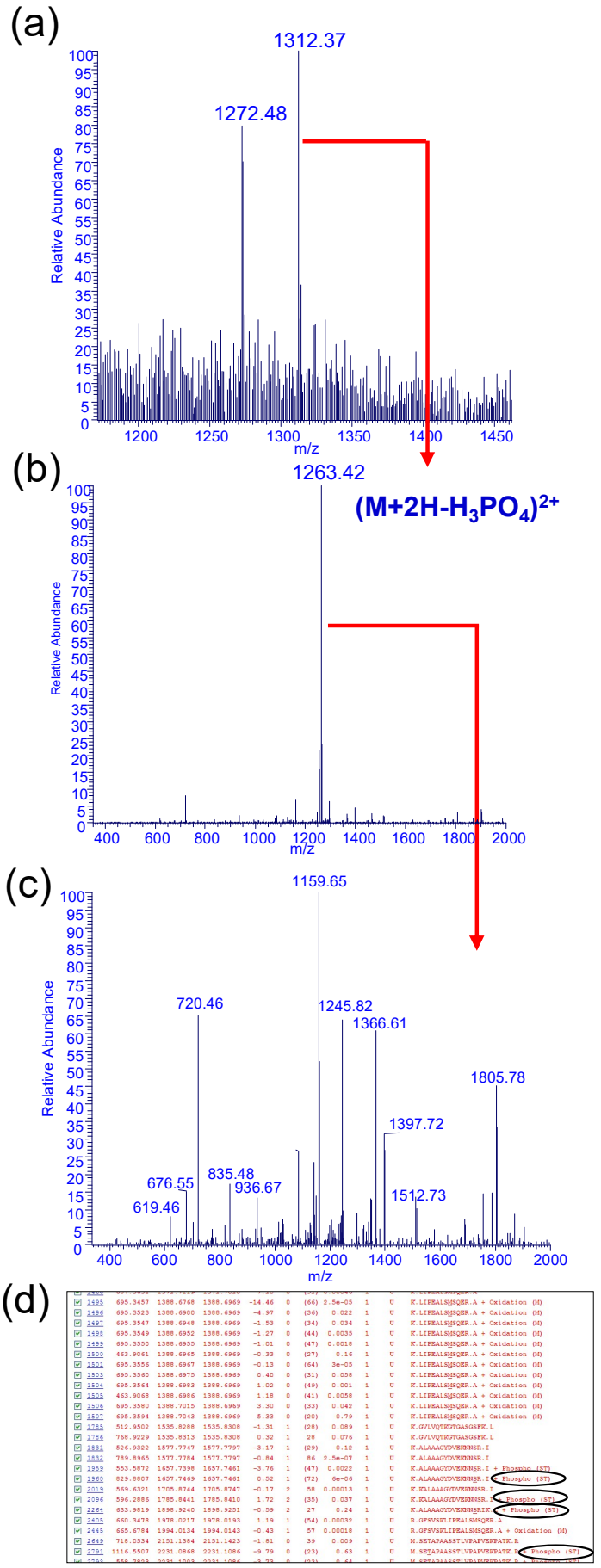


Figure 2. (a) Mass spectrum of a phosphopeptide which is selected for MS/MS to give (b) a prominent fragment ion which has lost phosphoric acid. Few backbone cleavage ions are produced, and so the fragment ion is selected for a second stage of MS/MS (i.e. MS^3) (c) which yields abundant sequence ions. (d) The data are searched using Mascot and the output reveals potential phosphopeptides which are then interrogated further.

High-Throughput Protein Quantitation using chemically tagged peptides

One of the of the major strengths of CCP is the high-throughput quantitative analysis of proteins by mass spectrometry-based techniques. In recent years, CCP has carried out extensive method development utilising isobaric tags (Tandem Mass Tags™, Thermo) for the quantitation of multiple protein samples.

Individual protein samples which are to be compared are reduced, alkylated and digested. Each digest is then labelled with isobaric amine-reactive tags (TMT, up to 18 tags). The samples are then pooled and fractionated according to hydrophobicity by high pH reverse-phase chromatography. Fractions are collected, lyophilised and desalted before being individually analysed by LC-MS/MS. TMT-labelled samples are analysed by the SPS-MS3 method using an Orbitrap Lumos mass spectrometer for high quantitative accuracy.

The resulting MS/MS spectra are then analysed using Proteome Discoverer platform (PD, Thermo) which outputs protein identifications, quantitation and FDR estimation.

As well as quantitation of isobarically tagged peptides, we can also quantify light and heavy peptides which have been produced by Stable Isotope Labelling of Amino Acids in Cell Culture (SILAC). SILAC can be used for the relative quantitation of proteins for two or more samples.
It involves in vivo incorporation of a heavy amino acid tag into proteins, followed by relative quantitation at the MS level.

Services Available

• Full sample preparation including protein estimation, reduction, alkylation, digestion and TMT (from 6-plex to 18-plex) labelling.

• We don't provide SILAC labelling but can process SILAC-labelled samples.

• Reverse-phase fractionation and collection of peptides.

• Sample desalting.

• LC-MS/MS of fractions.

• Data processing including database search and quantitation using our in-house R scripts. Limma package is utilised for statistical analysis.

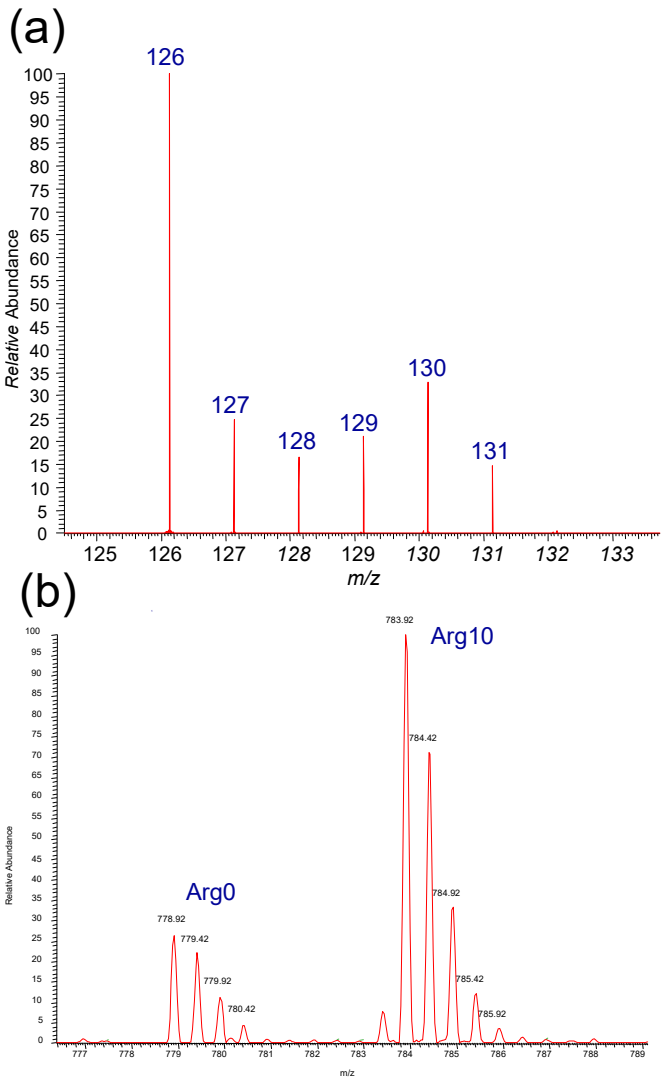


Figure 3. (a) Detail showing the MS/MS spectrum of a TMT reporter ions from six samples. The intensity of each reporter ion in the MS/MS spectrum is measured and compared with each of the others for relative quantitation. (b) Detail showing light and heavy SILAC labelled peptide peaks from two different samples. Again, the areas under both the light and heavy peaks are calculated and compared , this time at the MS level.

Data Independent Acquisition using Orbitrap Astral or timsTOF instrumentation

Traditionally, peptide data using LC-MS/MS experiments using Data Dependent Acquisitions (DDA) in which precursor ions are sequentially isolated and fragmented. This generally results in the selection and fragmentation of the most abundant peptide ions at the expense of the lower abundance peptide ions. Data Independent Acquisitions select all peptide ions across the *m/z* range followed by fragmentation which results in fragment ion information from both the abundant and much less abundant peptide ions. This results in superior sensitivity and quantitation, particularly for complex samples (several 1000s of proteins).

We can currently perform DIA experiments using the Bruker timsTOF and Thermo Astral mass spectrometers. Both mass spectrometers have ion mobility separation capabilities, which are able to remove singly charged contaminant ions, which can result in ion suppression of the peptide ions. This is particularly important in the analysis of very low amounts of proteins (picogram levels). The fast scan rates of these mass spectrometers allows for very short LC-MS/MS run times which increases throughput.

The data is processed using DIA-NN, which can generate in-silco libraries which contains deep learning peptide characteristics, fragment ion profiles and protein annotations. DIA-NN is now used worldwide and was developed in collaboration with CCP (Demichev *et al*, *Nat. Meth.*, 17(1):41-44 (2020).

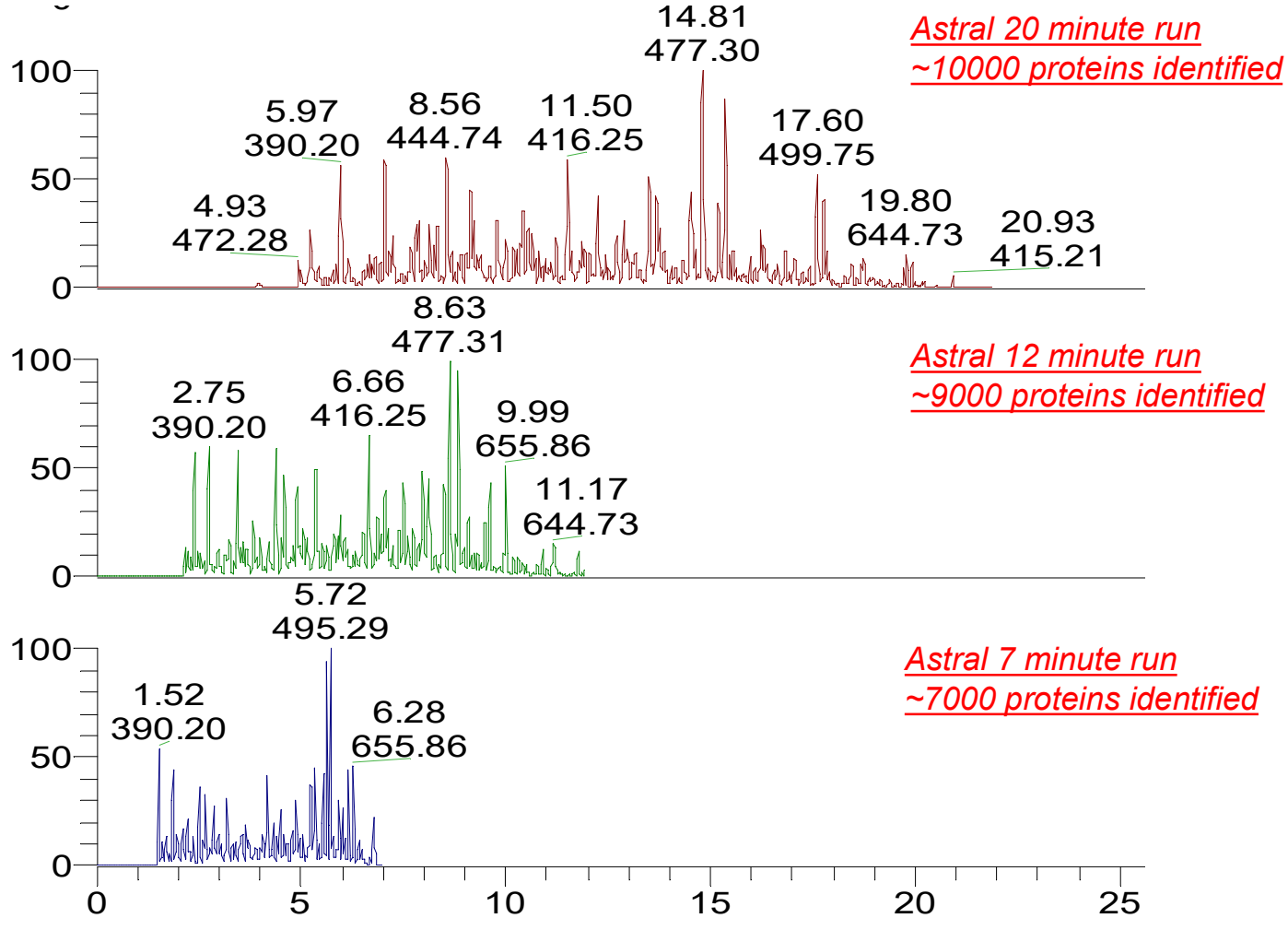
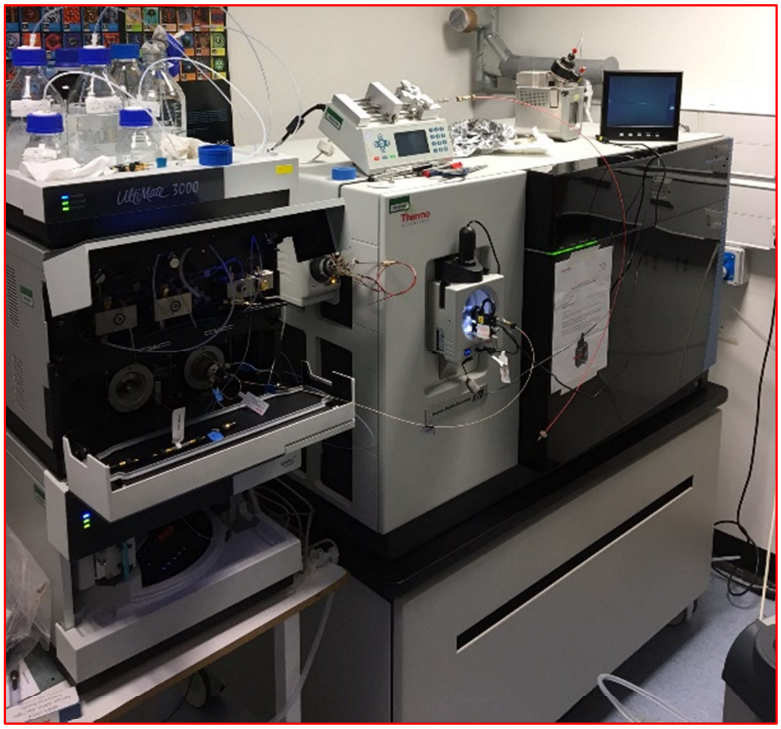


Figure 4. LC-MS/MS chromatograms obtained from the Astral MS acquired in DIA mode, showing rapid run times and corresponding number of protein identifications/

Mass Spectrometers

- Thermo Scientific Lumos and Dionex 3000 RSLCnano LC **(1)**
- Thermo Scientific Orbitrap Astral and Vanquish Neo LC **(2)**
- Bruker timsTOF HT and nanoElute 2 LC **(3)**

(1)



(2)

(3)

CCP Charges

MASS SPECTROMETRY METHODS

LC-MS/MS

Pricing guidance notes

1. Purified single proteins from gels or solution generally only require a 60 minute LC-MS/MS run.

2. More complex samples (e.g pulldown/IP experiment or total lysates which have been run on a gel or pull-downs containing proteins in solution) generally require a longer 120 min to reduce the complexity of the sample and generate a greater number of protein identifications.

3. Please note that VAT is payable if users work in the UK

	Academic (£)	Industry (£)
Sequencing of proteins from 1D gel bands (includes sample preparation and database searching, analysed on Lumos instrument).		
< 5 bands/spots (prices per spot/band)		
60 min LC-MS/MS run	220	330
120 min LC run	250	400
5 or more bands or spots (prices per spot/band)		
60 min LC-MS/MS run	180	270
120 min LC-MS/MS run	250	300
Sequencing of proteins from solutions (solutions must be free of salts and detergents. If desalting is Required, a £65 charge will be applied).		
60 min LC run	220	330
120 min LC run	250	370

MASS SPECTROMETRY METHODS (Continued)

TMT™ quantitation

	Academic (£)	Industry (£)
Sample preparation including protein precipitation, reduction/ alkylation/digestion, TMT™ Labelling (6-Plex), 1st dimension LC and collection of up to 30 fractions:		
6-plex	1320	1650
10-plex	1700	1900
16-plex	2300	2600
18-plex	3000	3300
LC-MS/MS per run (long gradients, including Mascot searching, performed on the Lumos)	275	440
Quantitation analysis from above data (custom statistical analysis is not included, typically 4 hours of analysis time is required)	90 per hour	120 per hour

Label-free quantitation

	Academic (£)	Industry (£)
Sequencing of and quantitation using either the Orbitrab Astral or timsTOF mass spectrometers		
LC-MS//MS (DIA) runs	300	400
Quantitation analysis from above data (custom statistical analysis is not included, typically 2 hours of analysis Is required).	90 per hour	120 per hour

Additional enzyme requirements

For the majority of experiments, trypsin or chymotrypsin are usually the enzymes of choice to cleave most proteins. However, there are occasions when other enzymes may offer greater protein coverage. For any other enzymes requested, the user will be required to pay the cost incurred by CCP for purchasing the enzyme.

Protein precipitation and protein/peptide estimation

For some experiments, samples which require analysis directly from solutions may have been prepared in buffers, which are incompatible with LC-MS/MS analysis, particularly detergents. In these cases, the protein will need to be extracted from the buffer by precipitation methods. For accurate label-free quantitation, the protein or peptide concentration will need to be measured.

TCA/acetone precipitation

	Academic (£)	**Industry (£)**
Up to 5 samples:	130	165
For each additional 5 samples	65	80

Protein estimation:

Up to 10 samples:	85	120
For each additional 10 samples:	60	90

Peptide estimation:

Pierce fluorometric assay

Up to 10 samples:	140	170
For each additional 10 samples:	100	135

Desalting of samples

For samples in solution which contain salts, it is necessary to desalt before they are analysed.

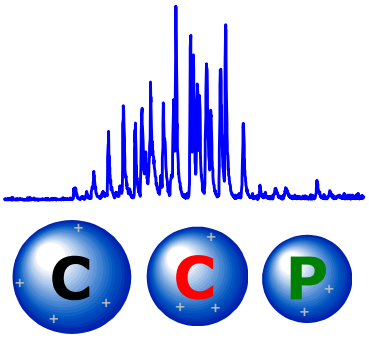
Desalting using Pierce desalting columns

1 sample:	65	100
Each additional sample:	35	50

1D gels

Running gels, including sample prep. and staining with Coomassie/silver and scanning

1D minigel	95	170



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