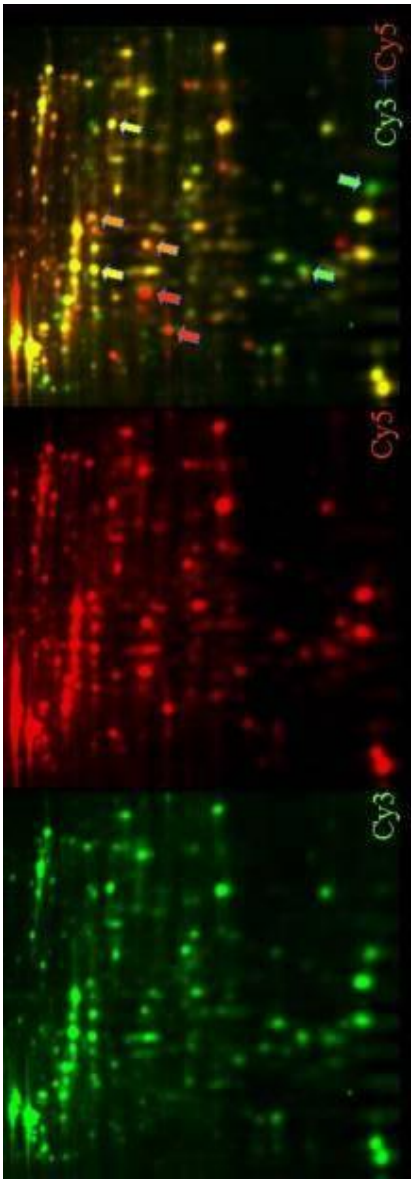


UNIVERSITY OF CAMBRIDGE

Cambridge Centre for Proteomics

http://proteomics.bio.cam.ac.uk

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 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.

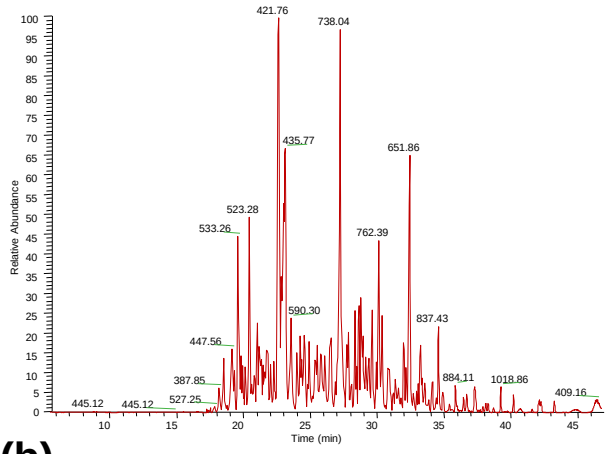
CCP has a range of softwares to analyse and quantify proteomic data including MASCOT, Proteome Discoverer, Scaffold and MaxQuant.

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)



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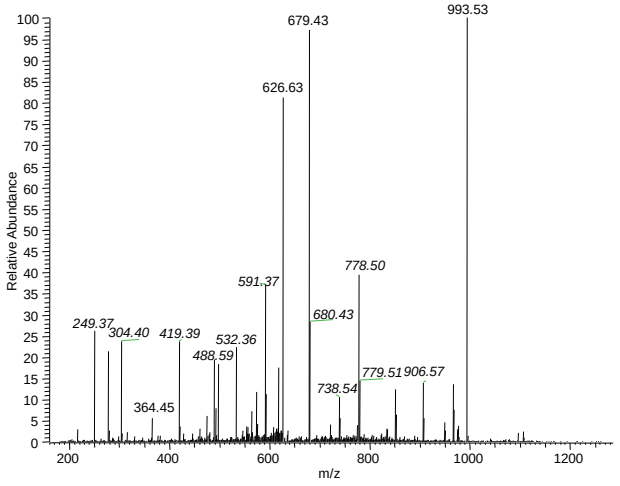

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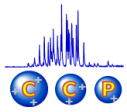


Cambridge Centre for Proteomics
Mascot Search Results

```
User           : CCP Core
Email          :
Search title   : CCP Core Brochure File Human Database 2019 (\\prot-filesvr1\data\CORE\PARAMETERS\Mascot_search_paramet
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.

High-Throughput Protein Quantitation by Mass Spectrometry

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). 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 (6-plex or 10-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.

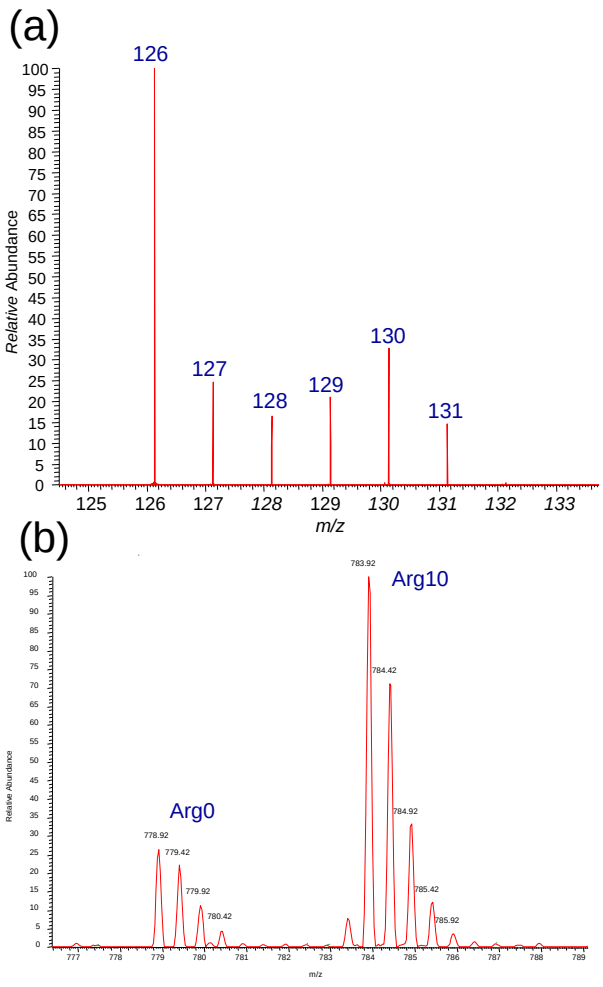


Figure 2. (a) Detail showing the MS/MS spectrum of a TMT reporter ions from six samples. The intensity of each reporter ion in the MS/MS spectrum is measured and compared with each of the others for relative quantitation. (b) Detail showing light and heavy SILAC labelled peptide peaks from two different samples. Again, the areas under both the light and heavy peaks are calculated and compared , this time at the MS level.

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.

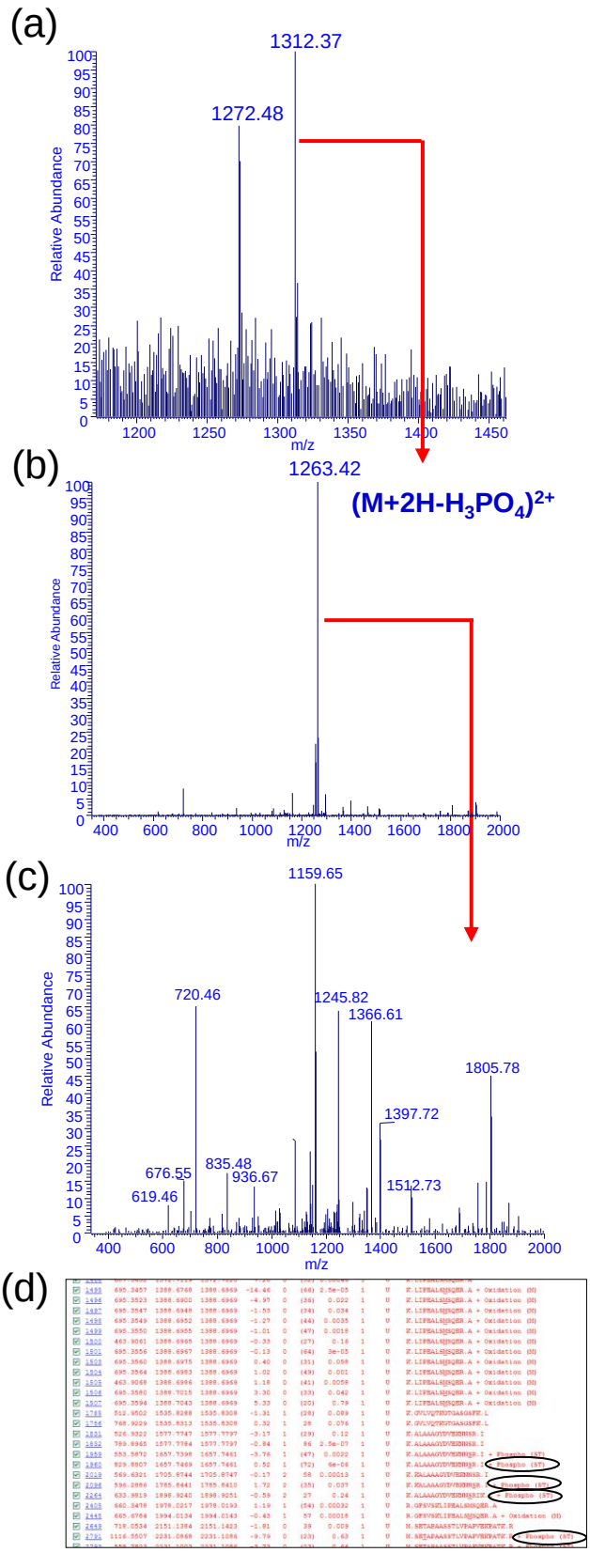
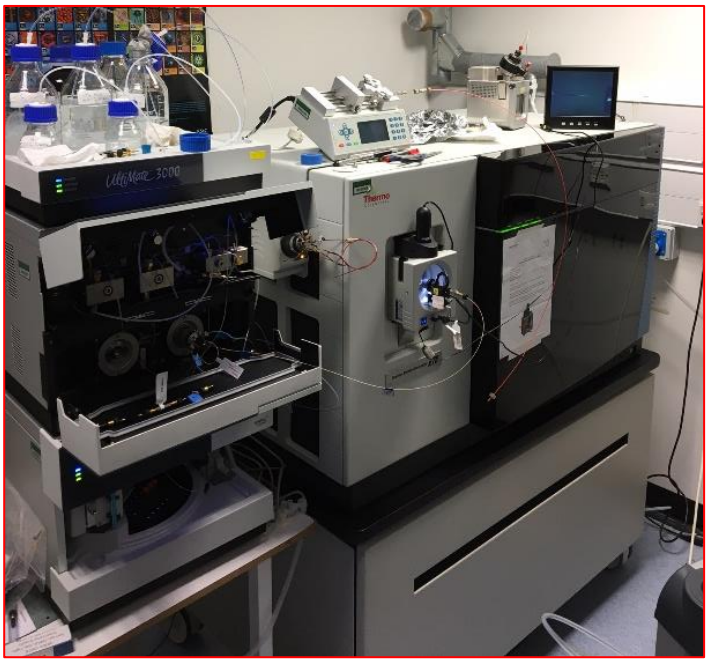


Figure 3. (a) Mass spectrum of a phosphopeptide which is selected for MS/MS to give (b) a prominent fragment ion which has lost phosphoric acid. Few backbone cleavage ions are produced, and so the fragment ion is selected for a second stage of MS/MS (i.e. MS^3) (c) which yields abundant sequence ions.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

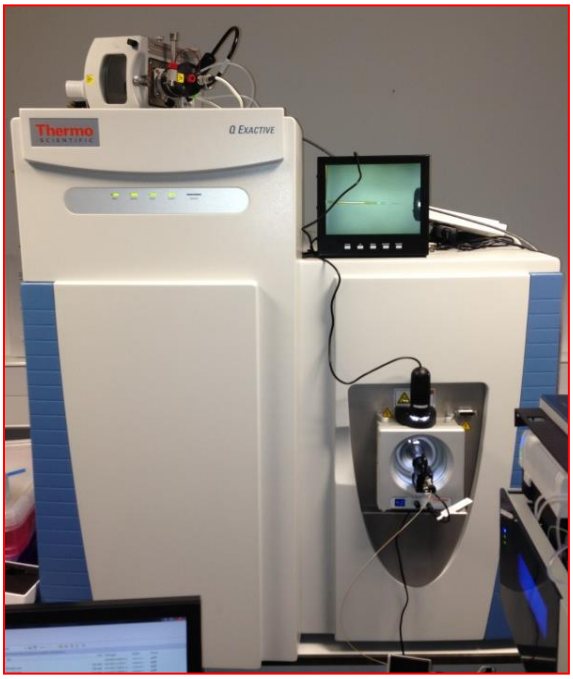
Mass Spectrometers

- Thermo Scientific Lumos and Dionex 3000 RSLCnano **(1)**
- Thermo Scientific Q Exactive and Dionex 3000 RSLCnano **(2)**
- Thermo Scientific LTQ Orbitrap Velos and Waters NanoAcquity UPLC **(3)**

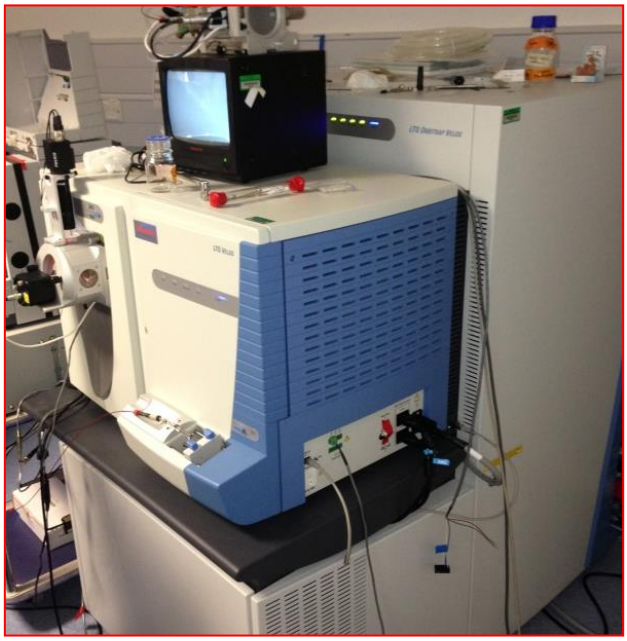
(1)



(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.

	Academic (£)	Industry (£)
Sequencing of proteins from 1D gel bands (includes sample preparation and database searching)		
< 5 bands/spots (prices per spot/band)		
60 min LC-MS/MS run	185	270
120 min LC run	210	310
5 or more bands or spots (prices per spot/band)		
60 min LC-MS/MS run	150	220
120 min LC-MS/MS run	185	250
Sequencing of proteins from solutions (solutions must be free of salts and detergents. If desalting is Required, a £10 charge will be applied).		
60 min LC run	185	270
120 min LC run	210	310

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. Pricing: 6-plex (*10-plex*) (16-plex)	1100 (*1400*) (1900)	1400 (*1600*) (2200)
LC-MS/MS per run (long gradients, including Mascot searching)	225	370
Quantitation analysis from above data (custom statistical analysis is not included)	70 per hour	100 per hour

GEL-BASED METHODS

Protein/peptide quantitation (BCA assay):

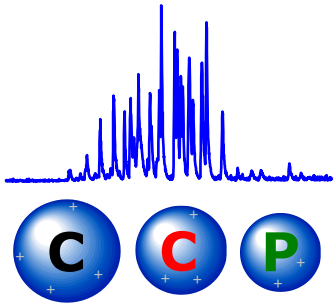
	Academic (£)	Industry (£)
	70	125

Running gels (including sample prep., per gel and staining with coomassie or silver and scanning)

	Academic (£)	Industry (£)
1D minigel	75	140

Additional enzyme requirements

For the majority of experiments, trypsin is usually the enzyme of choice to cleave most proteins. However, there are occasions when other enzymes may offer greater protein coverage. The most common alternative enzyme is chymotrypsin and because of the additional cost of this enzyme, we charge an additional £35 to the cost of all experiments. For any other enzymes requested, the user will be required to pay the cost incurred by CCP for purchasing the enzyme.



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http://proteomics.bio.cam.ac.uk

CCP Group email:
ccpcore@bioc.cam.ac.uk

UNIVERSITY OF CAMBRIDGE

Mailing address:

Cambridge Centre for Proteomics
c/o MRC Toxicology Unit
Gleeson Building
University of Cambridge
Tennis Court Road
Cambridge
CB2 1QR

Contact details

To discuss using the service, either for the standard experiments described or custom experiments, please contact:

Dr. Mike Deery

Proteomics Facility Manager

Tel: 01223 760253

Email: md289@cam.ac.uk

Prof Kathryn Lilley

Proteomics Facility Group Leader

Tel: 01223 760255

Email: ksl23@cam.ac.uk