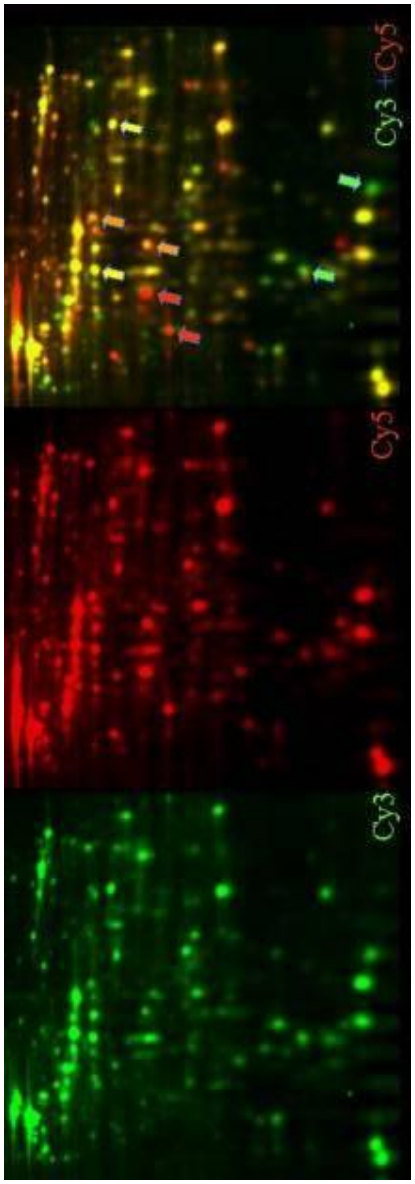


UNIVERSITY OF CAMBRIDGE

Cambridge Centre for Proteomics

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

ccpcore@hermes.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.

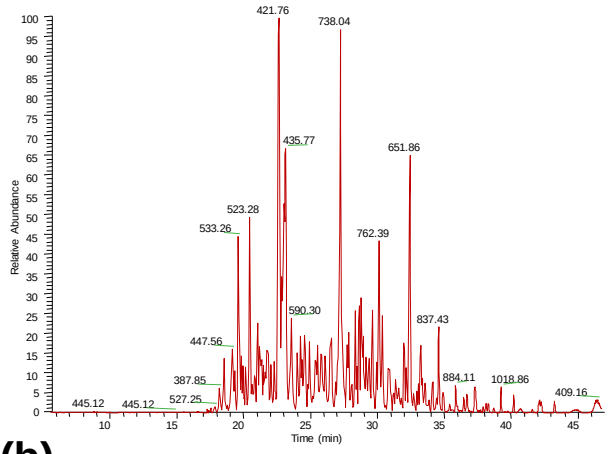
CCP has a range of softwares to analyse and quantify proteomic data including MASCOT, Proteome Discoverer, Scaffold and MaxQuant.

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

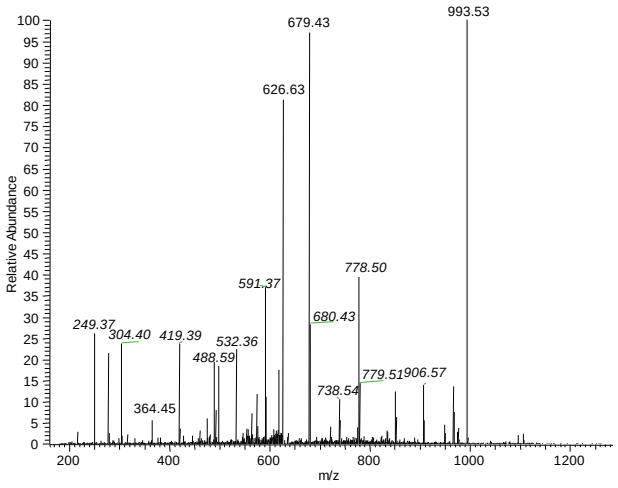
Services available:

- 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)



(b)



(c)

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_param
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.

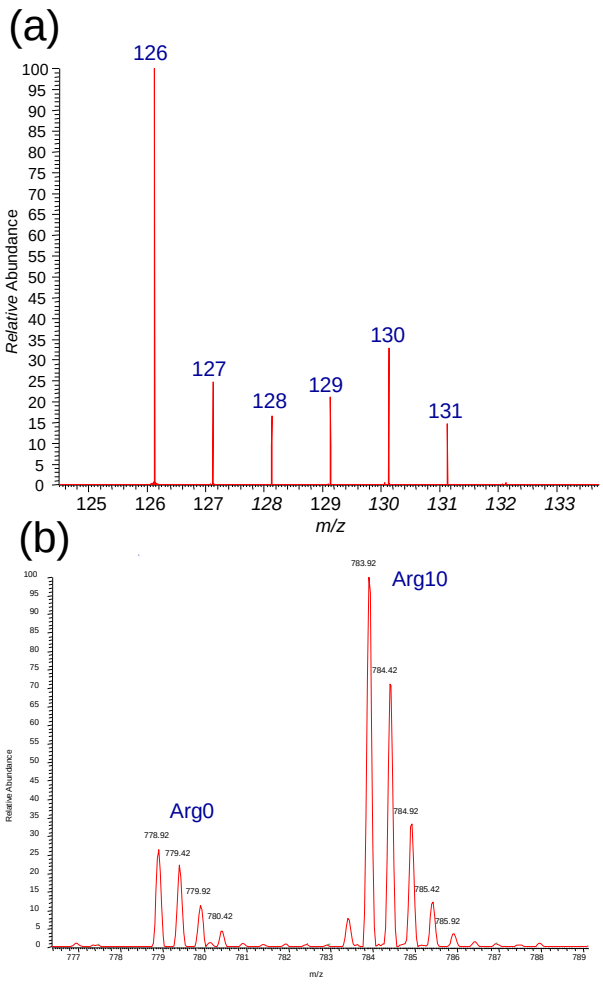


Figure 2. (a) Detail showing the MS/MS spectrum of a TMT reporter ions from six samples. The intensity of each reporter ion in the MS/MS spectrum is measured and compared with each of the others for relative quantitation. (b) Detail showing light and heavy SILAC labelled peptide peaks from two different 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 modificatios to specific amino acids.

Services Available

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

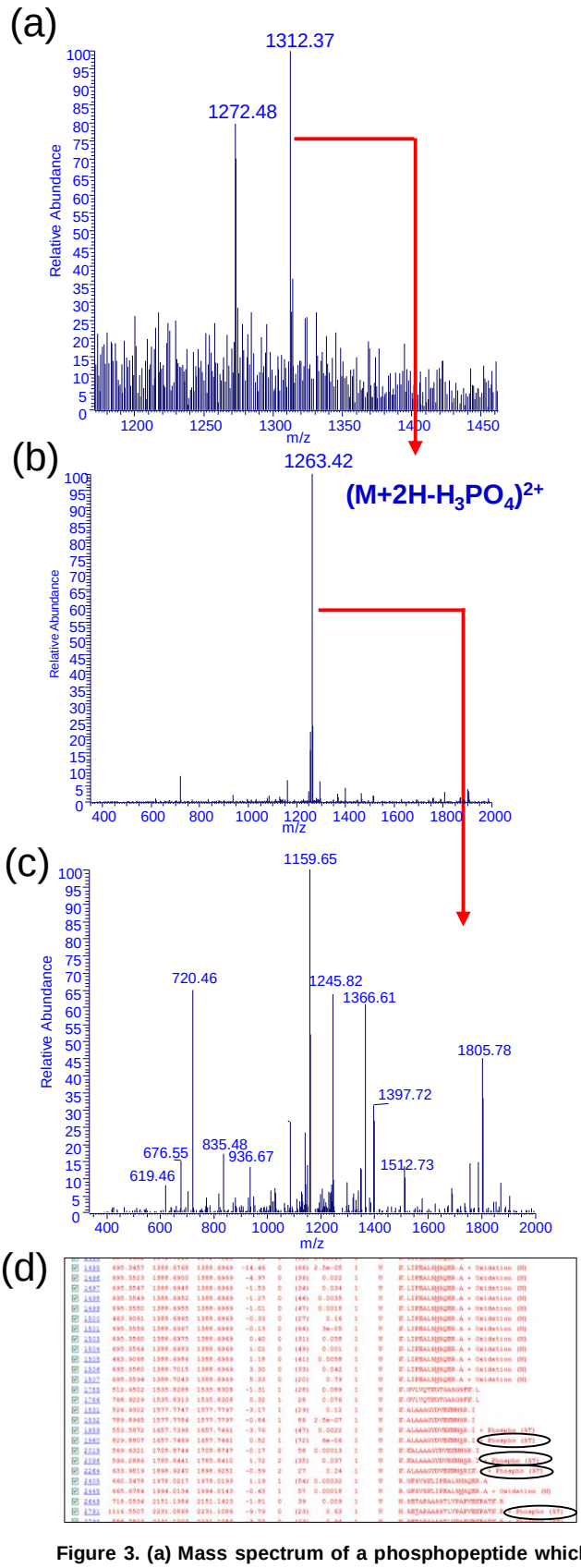
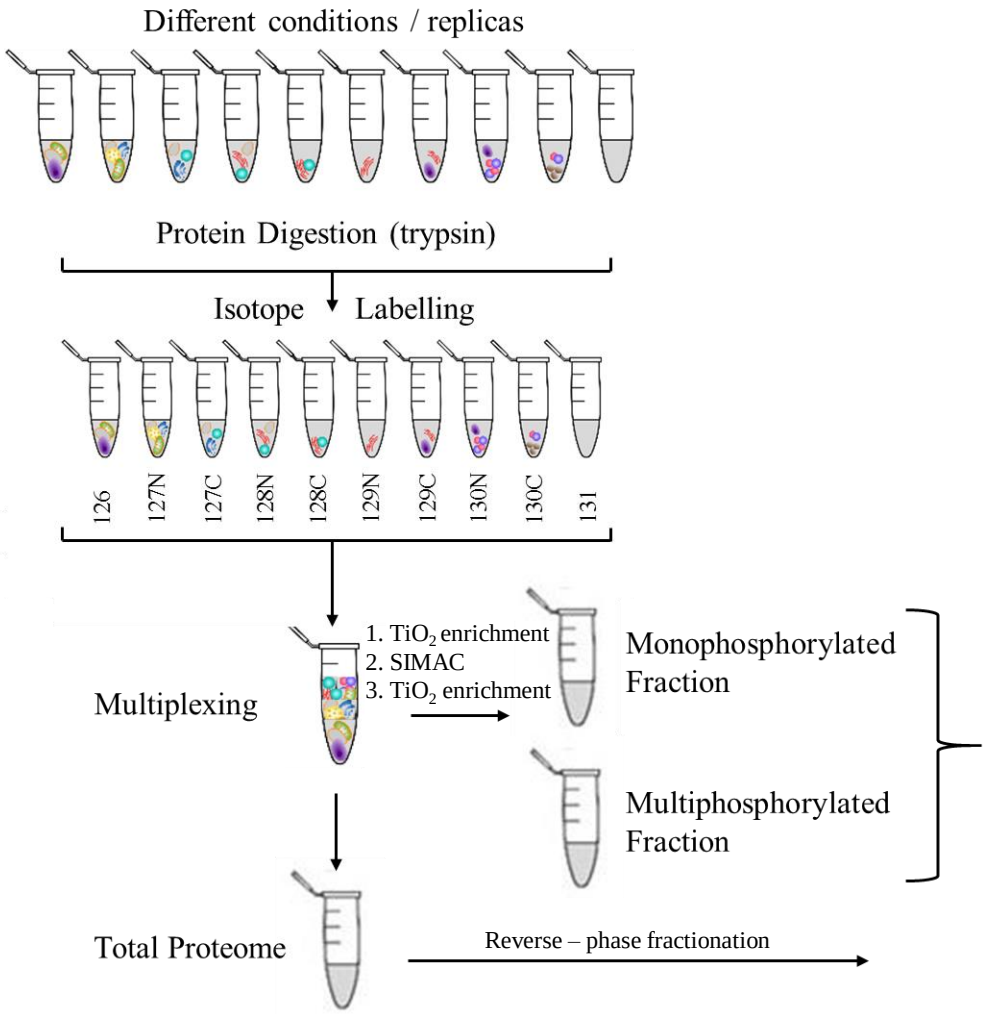
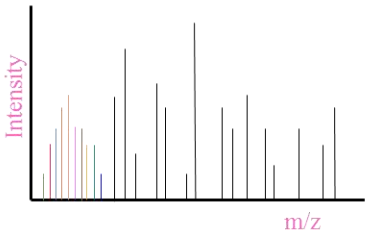


Figure 3. (a) Mass spectrum of a phosphopeptide which is selected for MS/MS to give (b) a prominent fragment ion which has lost phosphoric acid. Few backbone cleavage ions are produced, and so the fragment ion is selected for a second stage of MS/MS (i.e. MS^3) (c) which yields abundant sequence ions.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

Quantitative Phosphoproteomics

Phosphoproteomics studies require phosphopeptide enrichment prior to mass spectrometry analysis, especially when performed on a large scale, due to low detection owing to the large number and abundance of non-phosphopeptides.





Thermo Orbitrap Fusion Lumos

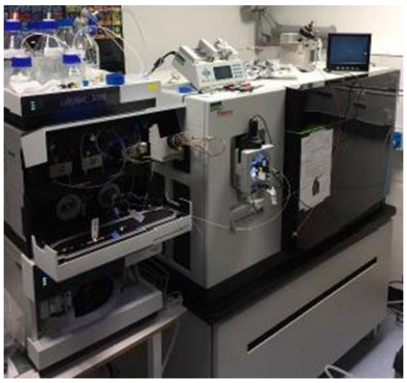


Figure 4. Quantitative Phosphoproteome Analysis workflow.
Cell lysates are resolubilised, digested and quantified prior to TMT labelling. Labelled samples are then combined in equal proportions and enriched. An aliquot of the combined sample is taken for quantitative total proteome analysis. Phosphorylated peptides are identified from the enriched fraction and compared to the protein abundances in the total proteome samples to asses reliable regulation in phosphorylation sites.

CCP enriches TMT (or SILAC) labelled samples through TiO_2 affinity chromatography followed by a Sequential Elution from Immobilised Metal Affinity Chromatography (SIMAC) methodology providing mono- and multi- phosphorylated enriched fractions. The monophosphorylated fraction is then further enriched with TiO_2 and alkaline reverse-phase HPLC fractionation is employed on the monophosphorylated peptide fraction to further increase the yield and coverage of the phosphoproteome. Analysis of the total proteome together with the phosphopeptides provides reliable identification of regulated proteins and phosphorylated sites.

CCP Core Method is an adaptation of the following methods:
1. TE Thingholm et al., Nat. Protoc., 2006, 1(4), 1929 – 35 2. TE Thingholm et al., MCP., 2007, 7(4), 661 - 671

2D-SDS PAGE

2D-SDS PAGE is an important tool in proteomics due to the fact that thousands of proteins can be resolved, resulting in a global view of the state of the proteome. Proteins are firstly separated according to charge in the first dimension (isoelectric focussing (IEF)) and by mass in the second dimension (SDS-PAGE).

At CCP, we offer a full range of 2D-gel electrophoresis services, performed by highly skilled personnel, for the separation of proteins in complex samples. A wide range of pH ranges in the first dimension can be accommodated, ranging from pH 3-11 for the widest separation to a pH range of 1 (e.g. pH 5-6) for the narrowest separation. This ensures maximum resolution for the separation of proteins at any pH range of interest. Furthermore, we offer different sizes of 1st dimension separations ranging from 7cm strips (minimal resolution) up to 13cm strips (maximum resolution). CCP has an extensive array of gel equipment which means that multiple protein samples can be performed simultaneously, to maximise throughput.

Once the gels have been run, the protein spots can be visualised using any stain available. We typically work with visible dyes such as colloidal coomassie and silver and fluorescent dyes such as SYPRO ruby and Deep Purple.

Services available:

• Sample preparation for most samples (please contact CCP discuss this, as not all samples can be handled/processed in-house) including tissue/cell lysis and protein concentration measurements.

• Suitable for separation of soluble proteins (non-membrane) which have pH values between 3-11.

• Separation of proteins in the 1st dimension using 7cm or 13cm strips

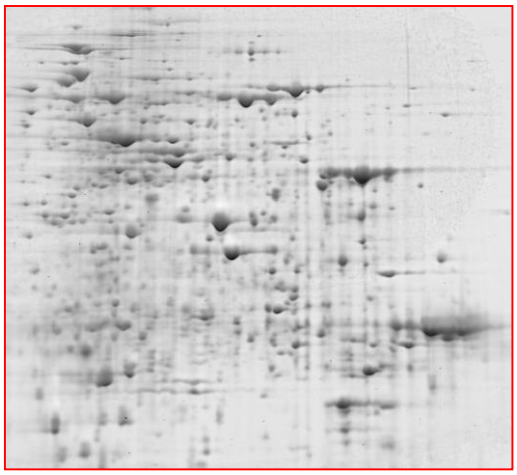
• Separation of proteins in the second dimension, again using multiple size formats of SDS-PAGE gels.

• Spot picking prior to MS analysis

• 1D gels and subsequent staining.

• Western blots and visualisation, providing suitable primary and secondary antibodies are supplied.

(a)



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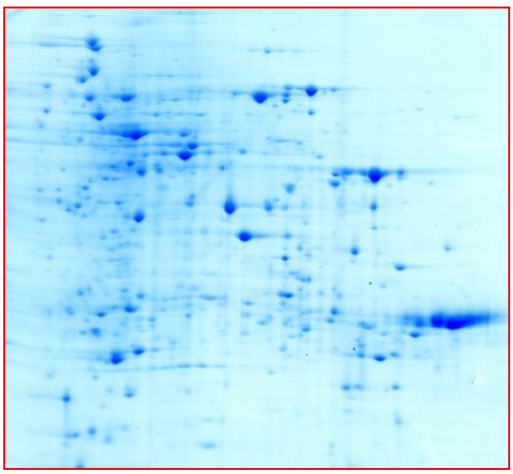
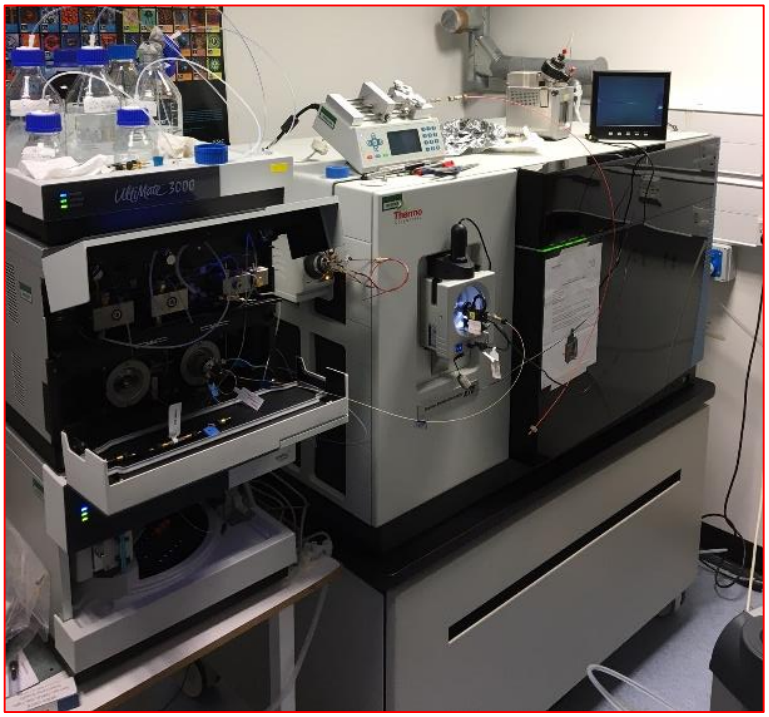


Figure 6. (a) Silver stained and (b) coomassie stained 2D gels of a mouse brain extract.

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)



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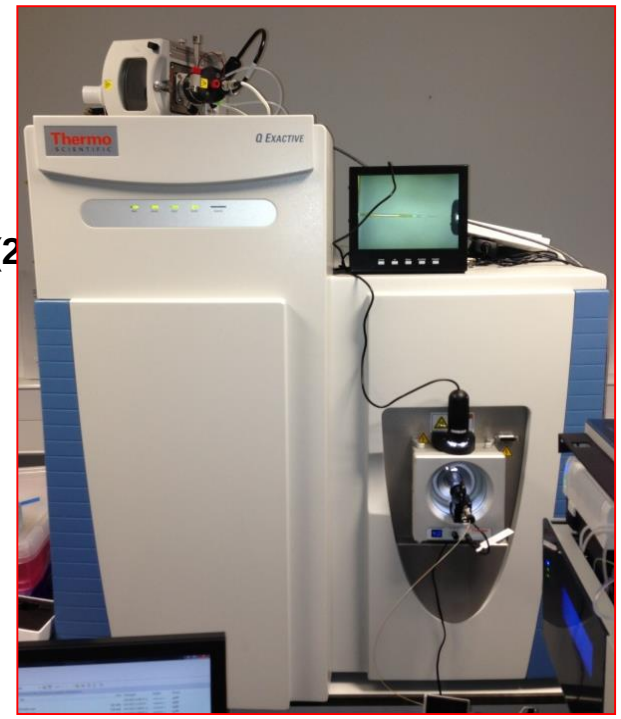

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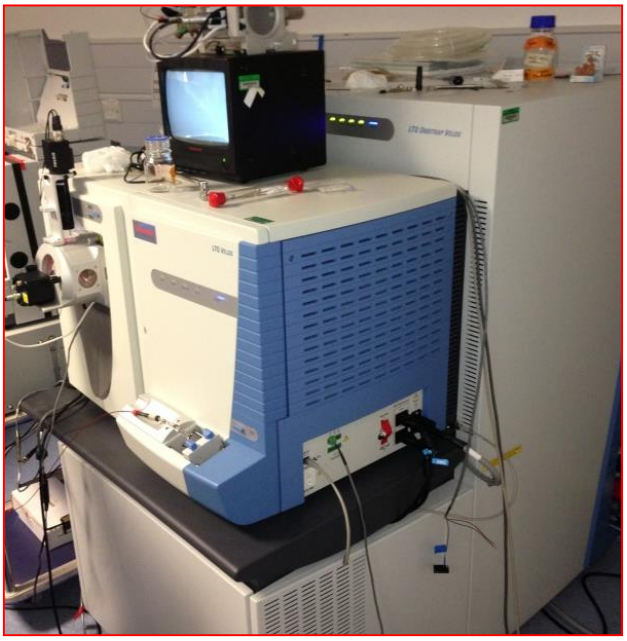


CCP Charges

MASS SPECTROMETRY METHODS

LC-MS/MS

PLEASE NOTE: From 1st April 2019, the charges associated with the proteomics facility will increase. The new increases (shown below) reflect the rising costs associated with consumables, service contracts and personnel costs.

Pricing guidance notes

1. Purified single proteins from gels or solution generally only require a 60 minute LC-MS/MS run. Similarly, 2D gels spots will only require the 60 minute 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	160	235
120 min LC run	185	270
5 or more bands or spots (prices per spot/band)		
60 min LC-MS/MS run	130	190
120 min LC-MS/MS run	160	220
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	160	235
120 min LC run	185	270

MASS SPECTROMETRY METHODS (Continued)

TMT™ quantitation

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*)	**Academic (£)** 850 (*1050*)	**Industry (£)** 1075 (*1275*)
LC-MS/MS per run (long gradients, including Mascot searching)	195	320
Quantitation analysis from above data.	60 per hour	80 per hour

Quantitative Phosphoproteomics

Sample preparation including protein precipitation, reduction/ alkylation/digestion, TMT™ Labelling (6-Plex), LC fractionation of up to 30 Fractions. Pricing: 6-plex (*10-plex*)	**Academic (£)** 850 (*1050*)	**Industry (£)** 1075 (*1275*)
Phosphoproteome Enrichment	1100	1700
LC-MS/MS per run (long gradients, including PD searching)	195 (2 hours) 300 (4 hours)	320 (2 hours) 425 (4 hours)

The enrichment of the phosphoproteome will result in 5 fractions: 1 multiphosphorylated peptide fraction and 4 Monophosphorylated peptide fractions. The multiphosphorylated peptide fraction requires a 4 hour LC-MS/MS run.

Quantitation analysis from above data.*	60 per hour	80 per hour

* Standard CCP TMT analysis workflow includes database searching, quantitation and statistical analysis.

GEL-BASED METHODS

Protein quantitation (BCA assay):

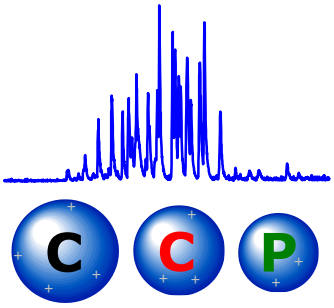
	Academic (£)	Industry (£)
	60	110

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

	Academic (£)	Industry (£)
1D minigel	65	120
2D minigel	120	230
2D (13cm)	150	290

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 £25 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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Contact details

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

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Email: md289@cam.ac.uk

Prof Kathryn Lilley

Proteomics Facility Group Leader

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