

**Terms and conditions for working with cMaLL.**

The core Metabolomics and Lipidomics (cMaLL)provide ready to use and bespoke analytical methods for small molecule measurement by LCMS. cMaLL has a very wide experience in metabolomics and lipidomcis and all work will be collaborative and expected to be on cost recovery basis.

We provide different levels of support for small molecule measurements. The breadth and depth of the analysis determines both the cost and turn-around time for analysis and needs to be decided with cMaLL team.

The LCMS methods within cMaLL are based on high resolution mass spectrometry. This allows us to combine targeted and untargeted methods. Our aim is to provide (semi)-quantitative measurements of known molecules, but untargeted data processing is possible, although we expect that for publication identifications are confirmed against standards. We can provide support in experimental design, sample extraction, LCMS, data processing and data analysis. Before the start of the experiment, we need to be clear on what level of support is expected for the project to allow for realistic times.

All requests for analytical work done by cMaLL have to be done through completion of the attached application form. These will be shared with the cMaLL’s SAB (Currently: Prof Sue Ozanne, Prof Steve O’Rahilly, Prof Mike Murphy & Prof David Savage). The SAB can make decisions on suitability or priority.

**Lipid(omics) analysis.**

An individual project is expected to consist of multiples of 80 samples. Projects that consist out of smaller sample set need to be combined with other smaller experiments and are therefore difficult to prioritise.

Full lipidomics The LCMS method can determine more than 1000 different lipid species covering over 25 different lipid classes. For a full lipidomic profile covering all lipids of all classes we expect a turnaround time of at 3 to 4 months based on an experiment with 1 to 4 multiples of 80 samples. This will cost £32.50/£50/£75 per sample (for IMS MRL & BRC NOME/ CIMR, MBU & MedSchl / Academic Outside).

Focussed lipidomics It is also possible to only process the data for the major lipids (ca 200 covering of ca 22 classes depending on sample type). Based on experience we provide a list of the lipids that are most likely to be present in most or all of the samples. The data processing of the shortlist is faster and more efficient reducing the turnaround time and per sample costs. For this shortlist analysis we expect a turnaround time of about 6 to 8 weeks based on an experiment with 1 to 4 multiples of 80 samples. This will cost ££20/£30/£45 per sample (for IMS MRL & BRC NOME/ CIMR & MedSchl / Academic Outside).

Lipid class analysis: We can also process only 1 or 2 specific lipid classes. Again, this will reduce data processing time and cost. It is important to realise that after data processing further lipid classes are asked to be processed that this requires further scheduling of work and cost. For this class specific analysis we expect a turnaround time of at 4 to 6 weeks based on an experiment with 1 to 4 multiples of 80 samples. This will cost £17.50/£23/£35 per sample (for IMS MRL & BRC NOME/ CIMR & MedSchl / Outside).

All of these approaches will result in a spreadsheet with molar concentration (per mg tissue, or per 100k cells, or per ml fluid) that can be used in further data analysis. This data will be suitable for publication and can uploaded to data repositories like [Metabolights](https://www.ebi.ac.uk/metabolights/) or [Metabolomics Workbench](https://www.metabolomicsworkbench.org/). We can help with further data analysis and interpretation. This will be very dependent on the underlying question on what the timeline is for this work and dependent on the information provided with the samples.

Untargeted data processing. It is possible to use the raw data for untargeted data processing. This is using software for peak picking followed by a data driven approach to find differentiating signals. It is expected that further work will be done to fully identify the lipids of interest.

**Metabolomics and other small molecule analysis.**

For metabolomics we will set-up two different methods. The first will be based on HILIC (Hydrophilic interaction chromatography) for very polar metabolites like sugar phosphates and a reversed phase based approach to analyse organic acid, bile acids and carnitines. Further developments will be implemented based on the directions and interests of collaborators.

The data processing will be based on quantitation of known metabolites using standards and untargeted analysis will be done using fragmentation (MS2) with post hoc identification against standards.

It is possible to do one class or one compound specific analysis (such as bile acids, carnitines or metformin). The development of new methods for specific metabolites will be possible as well, but timelines will be dependent on the type of molecule and expected concentration.

Full metabolomics: Comprehensive metabolic profiling covering all non-lipid classes, where sample will be analysed using two methods to maximise comprehensiveness. The work will provide quantitation on ca 120 metabolites (but list will build up further). This can be extended with untargeted data process for all metabolic features. We expect a turnaround time of at 2 to 3 months based on an experiment with 1 to 4 multiples of 80 samples. This will cost £32.50/£50/£75 per sample (for IMS MRL & BRC NOME/ CIMR & MedSchl / Academic Outside).

Focussed metabolomics: This will focus on using one method and cover known metabolites, the coverage will be discussed with the collaborator. For this shortlist analysis we expect a turnaround time of at 4 to 6 weeks based on an experiment with 1 to 4 multiples of 80 samples. This will cost ££20/£30/£45 per sample (for IMS MRL & BRC NOME/ CIMR & MedSchl / Academic Outside).

Metabolite & Metabolite class analysis: The analysis of one specific class of metabolites only or one specific metabolite. Expected turnaround time of at 4 to 6 weeks based on an experiment with 1 to 4 multiples of 80 samples. will cost £17.50/£23/£35 per sample (for IMS MRL & BRC NOME/ CIMR & MedSchl / Academic Outside).

All of these approaches will result in a spreadsheet with molar concentration (per mg tissue, or per 100k cells, or per ml fluid) that can be used in further data analysis. This data will be suitable for publication and can uploaded to data repositories like [Metabolights](https://www.ebi.ac.uk/metabolights/) or [Metabolomics Workbench](https://www.metabolomicsworkbench.org/). We can help with further data analysis and interpretation. This will be very dependent on the underlying question on what the timeline is for this work and dependent on the information provided with the samples.

Untartgeted data processing. It is possible to use the raw data for untargeted data processing. This is using software for peak picking followed by a data driven approach to find differentiating signals. It is expected that further work will be done to fully identify the metabolites of interest.

Stable isotope analysis (isotope ratio and tracer analysis/fluxomics).

All methods described above can be adjusted to include specific isotopologues (metabolites labelled with known (and sufficient) amount of stable isotopes like 13C, 18O, 15N or deuterium. The sample prep and analysis is the same but we will require more time for the data analysis. Costs are therefore dependent on the type of labelling and level of incorporation. Please contact cMaLL for the expected costs.

It is also possible to do isotope ratio measurements of specific metabolites or lipids, but this will require bespoke methods.

Training

cMaLL will provide bespoke training for ECRs and students, where full training in the methodology will be provided for the specific methods of interest based on samples relevant to the project. At the end of the training, you will have publication ready data. The full cost of the training is £1000 based on sample set of 80 samples for team members in of the IMS MRL or BRC NOME themes. £1500 for team members in of CIMR or the Medical School and £2000 for academic scientists from outside. We can also provide training for non-academic and commercial partners and the costs for this are on request.

A full training will enable the ECR to prepare their own samples and process their own data. This will reduce the turnaround time for analysis and reduce the per sample costs

List of metabolites that we will aim to quantify in an initial method and build out further either in collaboration with other teams or from our own observation.

**Nucleosides, nucleotides, and analogues**

1-methyladenosine,

Adenosine

Uridine

Xanthosine

S-adenosyl-L-homocysteine

Organic acids and derivatives

2-aminoadipic\_acid

Methionine

Threonine

**Amino acids**

Alanine

Arginine

Asparagine

Glutamic acid

Glutamine

Histidine

Isoleucine

Leucine

Lysine

Serine

Tyrosine

Valine

Glycine

Ornithine

Proline

Phenylalanine

Tryptophan

Aspartic acid

Cystine

**Amino acids derivatives and organic a**cids

1-methyl-L-histidine

2-aminobutyric acid

2-aminooctanoic acid

3-methyl-L-histidine

Citrulline

gamma-glutamylvaline

Homocitrulline

Hydroxyproline

Hypotaurine

N-acetylglutamic acid

N-acetylglycine

N-acetyl-L-glutamine

N-acetyl-L-histidine

N-acetyl-L-ornithine

Phenylacetylglutamine

N-acetyl-3-methylhistidine

N-acetylalanine

N-acetylaspartic acid

N-acetyl-L-citrulline

N-acetylserine

Nalpha-acetyl-L-arginine

Nalpha-acetyllysine

Nepsilon-acetyllysine

SDMA

Sarcosine

Betaine

Carnosine

Creatine

Creatinine

Glutaric acid

Guanidinoacetic acid

Homoarginine

Methionine sulfoxide

Taurine

Urea

4-oxo-L-proline\_Pipecolic acid

**Organic nitrogen compounds**

Agmatine

Carnitine

Ethanolamine

Choline

Histamine

N-acetylputrescine

Trimethylamine\_N-oxide

**Organoheterocyclic compounds**

Kynurenine

Adenine

1-methylnicotinamide

Cytosine

Hydroxytryptophan

Hypoxanthine

Kynurenic acid

Pyridoxamine

Riboflavin

Thiamine

**Acyl carnitines**

arachidoylcarnitine

decatrienoylcarnitine

eicoseneoylcarnitine

hydroxydecanoylcarnitine

hydroxydodecenoylcarnitine

hydroxyoctenoylcarnitine

Butyrylcarnitine

Hydroxybutyrylcarnitine

Hydroxyisovalerylcarnitine

Isovalerylcarnitine

Propionylcarnitine

Succinylcarnitine

Acetylcarnitine

decadienoylcarnitine

Decanoylcarnitine

Decenoylcarnitine

Dodecanoylcarnitine

dodecenoylcarnitine

Glutarylcarnitine

Hexadecadienoylcarnitine

Hexanoylcarnitine

Hexenoylcarnitine

Hydroxypalmitoylcarnitine

hydroxytetradecenoylcarnitine

linoleoylcarnitine

Myristoylcarnitine

N,N-dimethylglycine

Octadecanoylcarnitine

octanoylcarnitine\_ hexenedioylcarnitine

octenoylcarnitine

Oleoyl L-carnitine

palmitoleoylcarnitine

Palmityol-L-carnitine

tetradecadienoylcarnitine

tetradecenoylcarnitine

Targeted analysis of specific metabolites, lipids or compounds

We can develop bespoke targeted assays for specific metabolites or compounds of interest. Timelines for the development of a fully validated method are difficult to give. The method development will involve optimal extraction method and determine extraction efficiency, LCMS method, selection optimal internal standard, linearity, precision, accuracy, background check and identification of interfering ions.