

Operation Model

December 2013

1. Structure of the ITQB/iBET Mass Spectrometry Unit (UniMS)

Mission

The goal of UniMS is to guarantee the continuing increase of Mass Spectrometry know-how and infrastructures at ITQB and iBET in order to provide the appropriate support of MS services to the scientific community and to the industry.

Organization

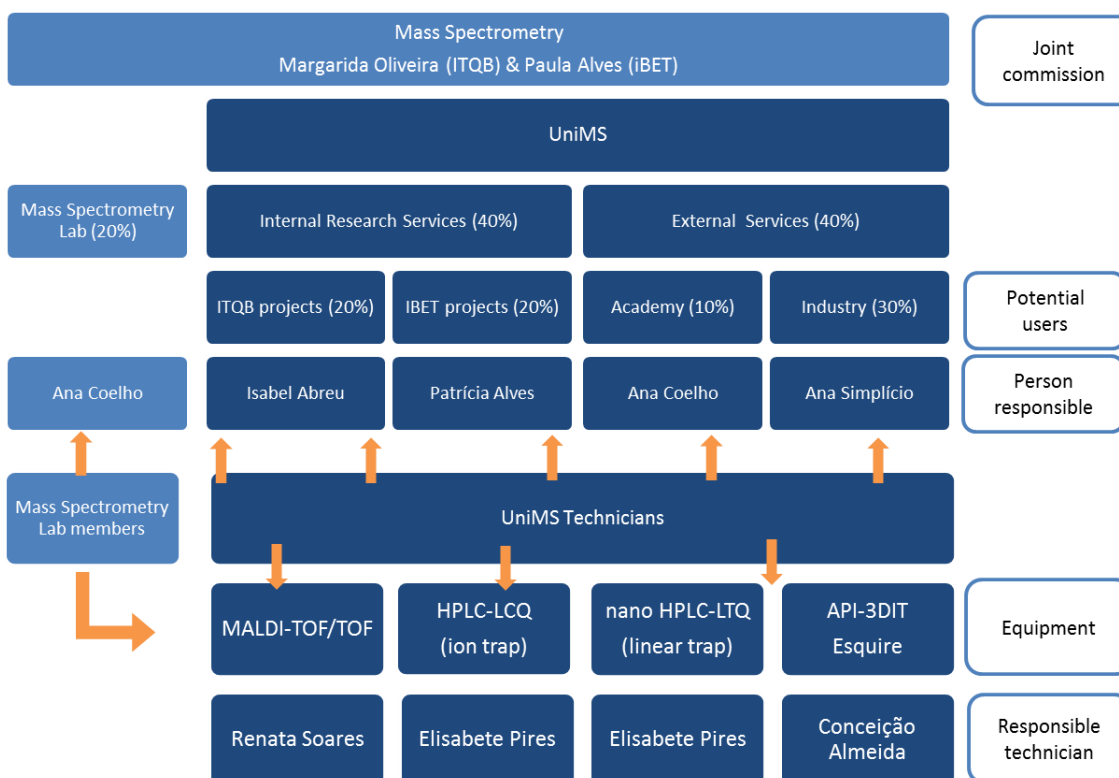
The UniMS has been formally separated from the mass spectrometry research laboratory of ITQB (currently under the management of Ana Coelho).

Coordination of activities within UniMS is now assured by the Users Direction (UD) that reports to the directions of ITQB and iBET through the Joint Commission (JC) constituted by Margarida Oliveira and Paula Alves.

The UD is presently constituted by Ana Luísa Simplício, Isabel Abreu, Patrícia Alves and Susana Araújo which are responsible for the management and the sustainability of UniMS, ensuring UniMS access to the researchers from Laboratório Associado de Oeiras (LAO), predominantly ITQB and iBET researchers, and also to external users. The UD is therefore responsible for the design and implementation of operation models and procedures, for suggesting pricing, for managing equipment and human resources as well as establishing the link between technicians and researchers that need support in experimental design.

Decisions by the UD shall be presented to the JC for approval. The UD shall also advise the JC and/or the Institutes' Directions about issues impacting the normal functioning of UniMS, namely those related to equipment acquisitions, refurbishments or maintenance.

Distribution of the operation time between the Mass Spectrometry research laboratory and the potential users of UniMS were defined by the JC and UD and are depicted in the following scheme:



Human Resources

Technicians presently dedicated to UniMS are responsible for maintenance of equipment, assay development and execution, maintenance of the Quality System, and daily management of the service (sample reception, acquisitions, billing, ...). The technicians are the contact point (unims.technicians@itqb.unl.pt/unims.technicians@ibet.pt) for users requesting any of the services available and listed in the next section of this document.

- Conceição Almeida (x1744) is responsible for the API-Ion Trap Bruker (Esquire).
- Elisabete Pires (x1418, x1364, x1407) is responsible for the two API – Ion Trap Thermo mass spectrometers (LCQ and LTQ), and for the HPLC (microAS and Surveyor) and the nanoLC.
- Renata Soares (x1418, x1364, x1407) is responsible for the MALDI-TOF/TOF equipment and Spotter.

The UD is the contact point for researchers and industry clients requesting assays which are not listed in the pricing table or development of new methodologies (unims.direction@itqb.unl.pt/unims.direction@ibet.pt).

- Ana Luísa Simplício (x1741) is responsible for contacts with industry clients.

- Isabel Abreu (x1660) is responsible for contacts with researchers requesting services through ITQB or IGC.
- Patrícia Alves (x1434) is responsible for contacts with researchers requesting services through iBET or CEDOC.

Ana Coelho (which is responsible for the Mass Spectrometry research laboratory of ITQB, x1451) serves UniMS as interface for external researchers to the Laboratório Associado de Oeiras.

In case of unavailability of the corresponding UD member or Ana Coelho, other UD members are responsible for replying to any request. In order to assure this, all requests should be submitted through one of the following e-mails: unims.direction@itqb.unl.pt/
unims.direction@ibet.pt.

2. UniMS Services

The services currently available within the UniMS, according with the existing equipment (for more information on equipment please consult the UniMS site: <http://www.itqb.unl.pt/facilities/UniMS/>), are the following:

- MALDI-TOF/TOF

MALDI-TOF/TOF (ABI 4800 Plus MALDI TOF/TOF™ Analyzer) is a high-throughput tandem time-of-flight (TOF) MS/MS system with soft ionization (MALDI - matrix-assisted laser desorption/ionization). For analysis in the MALDI-TOF/TOF, the samples are previously co-crystallized with an organic matrix and then ionized by the action of a laser. After ionization the charged species of the analytes, fly through the TOF and their mass-to-charge ratios are determined.

Sample analysis can be performed using linear mode for analytes with molecular weights above 4000 Da or in reflector MS mode for analytes below the 4000 Da. Samples previously analyzed in reflector MS mode can be further analyzed in MS/MS mode to obtain a fragmentation pattern.

The MALDI-TOF/TOF is mainly used for protein identification, intact mass measurements and purity profile determination. This MS system can also be coupled (off-line) to a nanoLC-Spotter system for previous separation of complex samples.

The system is easily operated and therefore the UniMS may provide training for independent users if the workload justifies.

Currently the services that can be provided under this equipment are the following:

- Protein identification
- Determination of the molecular mass up to 70 kDa
- Determination of protein purity
- Evaluation of protein heterogeneity

- ESQUIRE

This is a direct infusion ion-trap that can be coupled to soft (electrospray ionization - ESI or nanoESI) or chemical (APCI, Atmospheric Pressure Chemical Ionization) ionization sources. This equipment is used mostly for characterization of biomolecules and small molecules synthesised by ITQB/iBET research groups.

Currently the services that can be provided under this equipment are the following:

- Molecular mass determination and fragmentation profiles of peptides, ionic liquids, organic and organometallic compounds.
- LCQ and LC-MS

This ion trap mass analyzer can be used for sample direct infusion or coupled (on-line) to an HPLC system (SURVEYOR, THERMOFINNIGAN) for previous separation of complex samples. The LCQ can use soft- (ESI), or chemical ionization (APCI). This MS system is mainly used for molecular mass and fragmentation pattern determination of proteins, peptides, organic and organometallic compounds.

Experiments and possible applications:

- Separation and/or identification of small molecules;
e.g.: macrocyclic compounds; polymers; ionic liquids; organic and organometallic compounds; dye metabolites.
- Metabolite and degradation product identification;
e.g.: stability studies of drugs and chemicals in biological fluids and chemical media.
- Enzyme reactions;
e.g.: enzymatic bioremediation in the textile industry and pharmacokinetic studies.
- Characterization of the composition of natural products and their fractions ;
e.g.: Characterization/identification of the phenolic and antioxidant composition in plant extracts.

- LTQ and LC-MS/MS (nano, micro and conventional)

LTQ is a linear trap mass analyzer that can be used for sample direct infusion, but is mainly used when coupled to an HPLC (microAS, ThermoFinnigan) or to a nano-flow LC system (Easy nanoLC, ThermoFinnigan) for previous complex sample separation. This MS has soft ionization sources for conventional and nano-flow rates (ESI and nanoESI). The LTQ is capable of MSⁿ analysis, which can fragment molecules (MS/MS) numerous times, allowing the identification and characterization of for example post-translational modifications of proteins/peptides.

This MS equipment is used in assays of several types of molecules, namely mixtures of proteins, peptides, oligosaccharides, lipids, and organic molecules. It is also used in assays of relative quantification.

Experiments and possible applications:

- Intact protein;
- Proteome and sub-proteome analysis;
- Identification of synthesis or natural compounds;

e.g.: polymers; saponins; phytochelatins; phenolic compounds.

3. Method development and implementation (D&I) at UniMS

UniMS can provide D&I of methods in order to:

- i) Accommodate specific needs of ITQB/iBET research groups;
- ii) Enlarge the portfolio of services available to offer to the community and therefore increase the sustainability of UniMS;
- iii) Motivate as well as maintain and increase the experience and know-how of the UniMS team through active contribution to the scientific production of LAO and industry.

Therefore several methodologies are under development and/or implementation and will soon be made available as services:

- Identification and quantification of phospholipids;
- Oligosaccharide quantification;
- Identification and characterization of post translational modifications of proteins: N-glycosylation and phosphorylation;
- 2D-Electrophoresis e 2D-DIGE separations.

Model of access to this service:

- a. UniMS may unilaterally decide and finance the implementation of new techniques considered of potential interest to several clients.
- b. D&I services may also be requested by internal groups but must be financially supported by the requesting group namely in terms of consumables and equipment time.
 - In such cases, UniMS may decide to support development by allocation of human resources, free of charge, as long as that does not interfere with ongoing strategic services. As repayment, the research group will allow UniMS to offer the developed method as a UniMS service in the future.
 - The technicians may be co-authors in publications involving the implementation.

In order to have access to this option, the research group should present a one-page formal proposal justifying the study, identifying its potential, describing what financial support will be provided and a prediction of the necessary equipment and human resources time. The proposal will be assessed by the UD (unims.direction@itqb.unl.pt / unims.direction@ibet.pt) and presented to the JC for approval.

4. Access to UniMS services

All researchers of ITQB, iBET and the rest of LAO will have access to the services of UniMS. Work performed for these researchers will be considered as internal and the services will be paid according to the prices indicated in the table below.

To request for UniMS services, a request form is available on-line (<http://www.itqb.unl.pt/facilities/UniMS/services>) and should be filled by the researchers and sent by e-mail or it can be delivered with the samples.

The table lists the prices for both independent users and services provided.

Independent users are people with specialized training in MS that have been trained specifically for the UniMS machines and methods by the UniMS technicians. This users are granted direct access to the equipment with minimal supervision by the technicians and will pay only the hourly cost of use of equipment, not the human resources cost (specific reagents, chromatographic columns, etc..., will be acquired by the research group using the equipment).

In case of damage to the equipment, independent users will be financially accounted, shall it be proved that damages occurred due to incorrect use or negligence and permission for independent use may be withdrawn.

Researchers external to LAO will also have access to services. Ana Coelho will manage requests by those users; this request should also be directed to the UD e-mail to assure prompt reply.

Prices for these services are based on the table presented bellow depending if the request is from an external research group or if it is from a member of RNEM, but in some cases a quotation may be necessary.

Requests by industrial clients are managed by Ana Simplício. Prices are subjected to individual quotation, upon request.

Prices for services not considered in the price table below will be subjected to an individual assessment and quotation.

Training

Any internal user may request independent use of the equipment if the volume of work justifies this type of access. However, the technician responsible for the equipment has to train the user to be independent and determine when the researcher is qualified for such use.

Training will be provided individually or in group depending on specific requests, needs and availability of technicians and equipment time.

The updated list of qualified users is with the technicians.

The use of equipment by independent users is subjected to time availability, specific rules for each equipment and instructions from the technicians.

Opening hours

Except under particular circumstances, a technician will be available during the normal working hours (9h-18h, Monday to Friday).

Booking

People authorized as independent user may book the equipment (or request booking by the technicians) using the calendars available on-line in <http://unims.itqb.unl.pt>.

Booking is effective only after confirmation by the technician.

5. Obligations of users and potential users

In project submission:

All researchers that want to include MS services in project applications must confirm previously with UniMS the feasibility of the work proposed and request a quotation. The value accorded must be included in the project under “services and acquisitions” with the justification “acquisition of maintenance services and replacement parts for mass spectrometers” and “consumables for mass spectrometry”.

In independent equipment utilization:

All independent users must abide by the pre-established rules and fill the log books for each equipment (specific equipment rules are printed and available close to each equipment).

When publishing:

When the results of services provided by the UniMS are subjected to publication, an acknowledgement/reference must be added in the adequate section. An example of the text that should be added is: “MS data provided/obtained by the UniMS – Mass Spectrometry Unit, ITQB/iBET, Oeiras, Portugal”.

Acknowledgement to RNEM should also be added when applicable (for work developed in LTQ equipment).

6. Prices and modes of payment

The table below presents the actual prices by type of assay/equipment and/or the hourly rate.

Payment may be accomplished in the following modes:

When predicted in projects as described in the section above: making the agreed budget available for use by the technicians.

In a service not under the scope of projects

ITQB: direct payment to the UniMS ITQB line from lines not allocated to projects.

iBET: to project 10.

Assay	# samples or time	LAO internal price (€)	RNEM Price (€)	External price (€)	Independent users (€)
Atmospheric pressure ionization					
ESI small molecule*		15 ⁼	20 ⁼	30 ⁼	-
Additional fragmentation		10 ⁼	15 ⁼	20 ⁼	-
ESI Proteins		20 ⁼	40 ⁼	55 ⁼	-
LC-ESI-MS					
€/hour	1 sample	90 ⁼	120 ⁼	175 ⁼	28 ¹
	>1 samples	40 ⁼	50 ⁼	70 ⁼	
nanoLC-ESI-MS					
€/hour	1 hour	150 ²	150 ²	150 ²	-
€/hour	>8 hours	100 ²	100 ²	100 ²	-
MALDI-TOF/TOF					
Intact mass measurement		20 ⁼	30 ⁼	50 ⁼	-
Protein identification	1 sample	40 ⁼	45 ⁼	50 ⁼	-
	>1 sample	20 ⁼	25 ⁼	30 ⁼	-
	>50 samples	10 ⁼	10 ⁼	10 ⁼	-
nanoLC-spotter MALDI MS (€/hour)		70 ²	70 ²	110 ²	50 ³
nanoLC-spotter MALDI MS/MS (€/hour)		100 ²	100 ²	150 ²	
€/hour		28 ⁴	-	-	28 ⁴
Other					
Protein digestion	1 sample	20 ⁼	35 ⁼	50 ⁼	-
	>1 sample	10 ⁼	15 ⁼	20 ⁼	-
Cleaning procedure		5 ⁼	10 ⁼	15 ⁼	-

IVA (VAT) tax not included.

⁼ Prices as before.

¹ Prices established for small molecules, peptides and proteins. Include maintenance and depreciation costs. Do not include method development, HPLC columns, consumables and human resources costs.

² Prices established for small molecules, peptides and proteins. Prices were based on the prices of other RNEM nodes and include consumables, HPLC columns, human resources, maintenance and depreciation costs. Do not include method development.

³ Prices established for small molecules, peptides and proteins. Prices were based on the prices of other RNEM nodes and include consumables, maintenance and depreciation costs. Do not include method development, HPLC columns and human resources costs.

⁴ Prices include maintenance and depreciation costs. custos de manutenção de de amortização do equipamento. Do not include consumables or human resources costs.

7. Equipment maintenance and calibration

Equipment maintenance and calibration is under the responsibility of the UniMS technicians. Costs will be under the responsibility of UniMS whenever possible. Costs exceeding the budget will be supported by ITQB and iBET in the proportion of utilization from the previous year.