



EuPA-EC

**Education Committee (EC)
2006-2008 working plan**

EuPa

EUROPEAN PROTEOMICS ASSOCIATION

Proposal of Members for the EuPa Education Committee



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3. Concha Gil, conchagil@farm.ucm.es (Spain)
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6. Ole Jensen jenseno@bmb.sdu.dk (Denmark)
7. Patricia Palagi Patricia.Palagi@isb-sib.ch (Switzerland)
8. Deborah Penque Deborah.Penque@insa.min-saude.pt (Portugal)

Experience in educational related fields such as coordinators of international projects, proteomics platforms, well known proteomics courses, educational activities, etc. Representation of different European countries.



Juan Pablo Albar - jpalbar@cnb.uam.es (Spain)

Head of Proteomics Facility at the National Center for Biotechnology-CSIC. Coordinator of ProteoRed, the Spanish Network of Proteomics Facilities. Member of the Spanish Proteomics Society Board.



Garry Corthals - garry.corthals@btk.fi (Finland)

Group Leader of the Protein Research Laboratory at the Turku Centre for Biotechnology, and Director of the CBT VTT Proteomics Facility. Board member of the Finnish Proteomics Society - FinnProt.



Concha Gil - conchagil@farm.ucm.es (Spain)

Professor of Microbiology at Complutense University of Madrid. Group Leader in the Microbiology Department. and Director of Proteomic Facility of UCM-Parque científico de Madrid. Vice-President of the Spanish Proteomics Society.



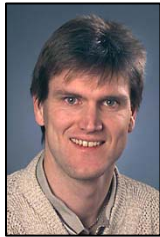
Hubert Hondermarck - hubert.hondermarck@univ-lille1.fr (France)

Professor of molecular and cellular biology at the University of Lille. Head of an INSERM unit entitled "Growth factor signaling in breast cancer - functional proteomics". President of the French Proteomics Society (SFEAP).



Peter James - peter.james@elmat.lth.se (Sweden)

Professor of Protein Technology at Lund University. Director of the Molecular Anatomy of the Cancer Cell program and the Swegene proteomics platform.



Ole Jensen - jenseno@bmb.sdu.dk (Denmark)

Professor of Protein Mass Spectrometry at the University of Southern Denmark at Odense. Group leader, Protein Research Group. Vice-dean Biotechnology. Member of the HUPO Council. Interim Chair of the Danish Proteomics Society.



Patricia Palagi - Patricia.Palagi@isb-sib.ch (Switzerland)

Senior Scientist at the Proteome Informatics Group of the Swiss Institute of Bioinformatics. Coordinator of the Master's in Proteomics and Bioinformatics of the Geneva University. Vice-President and General-Secretary of the Swiss Proteomics Society.



Deborah Penque - deborah.penque@insa.min-saude.pt (Portugal)

Group Leader of the Laboratory of Proteomics at the Human Genetics Centre, National Institute of Health Dr Ricardo Jorge, Lisbon; Coordinator of Portuguese Proteomics Network-ProCura.

Proteomic training is needed



- **Proteomics is a new area of research**
- **Proteomics technologies are being developed very fast and researchers need to be up date**
- **Proteomics can be applied at different knowledge areas**
- **Proteomics is still not being taught at almost all universities**

Objectives of the Education Committee



- *The main missions of of EuPA Education Committee are to promote and enhance the quality of proteomics knowledge creating educational programs, and to challenge European-wide scientific exchange programs for young researchers.*
- 1. Coordination of **workshops** and **courses** to promote and enhance the quality of proteomics knowledge through the life sciences community, general public and governmental bodies.
- 2. Coordinate and promote **European-wide scientific exchange** programs for young researchers to enhance their scientific career and promote cultural exchange and acceptance throughout Europe.
- 3. **Promote collaborative research projects between academic research institutions and the life sciences industries. Any ideas?**
- 4. **Knowledge dissemination of validated and quality assured methodologies and procedures to the bioscience community (via world-wide accessible information resources. Any ideas?**

Could these objectives be shared with the other sub-committees?

Educational Programs of proteomics technologies and applications



- **Coordination of Courses and Workshops, G. Corthals, P. James, P. Palagi and C. Gil.**
- **Coordination of Courses at EuPa meetings, D. Pengue and H. Hondermark.**
- **Coordination of Laboratory networking, O. Jensen and J.P. Albar**
- **Other Education activities: Educational material at web page and in proteomics journals P. James, G. Corthals and C. Gil**

Educational Programs of proteomics technologies and applications



- **Courses**
- **Workshops**
- **Laboratory networking**
- **Other Education activities: educational material at web page and in proteomics journals**

Courses



1. Coordination of courses to enhance the quality of proteomics knowledge:
 1. life sciences community
 2. general public
- **Courses held by research centre and facilities**
- Courses held at proteomics meetings (EuPA meetings)
- Assemble database of courses and contents, We are going to make a list of the courses that we have already planned and try to support them as EuPA courses.
- To hold identical courses in different countries throughout Europe
 - In different locations throughout the yearly
 - Scotland, Spain, Finland, Sweden, other countries (AU, CZ, HU, DE, CH, NL, ...)

Database of courses and contents that have already planned and could be supported by EuPA



- Proteomics and Bioinformatics (ProteoRed-INB, Spain). Possible dates: 6-8 February, 27 February-1 March or 6-8 March. Madrid.
- 2D gel image analysis. (ProteoRed-INB, Spain). Barcelona. 28-29 September
- Mass spectrometry : spectra analysis (ProteoRed-INB, Spain). 13-14 June, Madrid.
- Bioinformatics used in post-ID of proteins (ProteoRed-INB, Spain) 10-11 of July, Barcelona.
- "EMBO Practical Course on Bioinformatics for Mass Spectrometry in Proteomics". 18-22 September 2006, SIB Geneva.
- Introductory course in bioinformatics for proteomics. (Patricia Palagi proposal, This course could be spread out in Europe)

We might also consider on-line tutorials/WebEx sessions

GENERAL COUNCIL MEMBERS. Please send to the Education Committee the list of courses that have already planned in your country and you want to be supported by EuPA.

Eupa New Courses for 2006



- **Eupa Basic Training Course**

To hold identical courses in different countries throughout Europe

- In different locations throughout the yearly
- Finland, Spain, Scotland, Sweden, **other countries (AU, CZ, HU, DE, CH, NL, ...)**

- Similar programme and Material.
- Some conferences can be included in the web page.

- **Introductory course in bioinformatics for proteomics(P. Palagi)**

Basic training courses (protein separation and identification by mass spectrometry)

- *2D-PAGE PRACTICAL PROTEOME COURSE (P.James)*
- *2D-course (G. Corthals)*
- *Protein identification course outline. This course was run with the help of people from Matrix Science (Mascot) and Finnigan (Sequest, de novo).(Peter James).*
- *MS-course (G. Corthals)*
- *2D and protein identification course (C. Gil).*
- *Mass spectrometry for proteomics (BMPRG and PAF)*

The course could be run with the help of people from companies

many others: we need your input

EuPA course in different countries throughout Europe

Courses Funding

- **National Proteomics Societies or government of the different European Countries (Example: in Spain we have some money to organize courses. It means that some courses could be free for students but they have to pay traveling and accommodation. We are organizing two bioinformatic courses that we can propose as EuPA courses).**
- **Marie Curie Conference and Training Courses. Deadline 17th of May 2006.**
- **Companies**



EuPA Course Proposals

- Title:
- Description
- Location:
- Number of participants (5-10/10-20)
- Duration (1-5 days)
- Proposed dates
- Organizing laboratory
- Budget
- Contact person (address, phone and E-mail)
- Detailed program

Courses Organization



REQUIRED RESOURCES

- ***Human resources***
 - Organizer
 - Expert researchers
 - Technical personnel
- ***Material resources***
 - Class room
 - Computer room
 - Proteomics Laboratory

Educational Programs of proteomics technologies and applications



- **Courses**
- ***Workshops***
- **Laboratory networking**
- **Other Education activities: educational material at web page and in proteomics journals**

Workshops



1. Coordination of workshops to enhance the quality of proteomics knowledge to the life sciences community, general public and governmental bodies.
 1. life sciences community
 2. general public

- Workshops held at proteomics and other meetings
- Workshops held by research centres and facilities

- **Assemble database of workshops and topics**

- Identical workshops can be held throughout Europe
 - Numerous times annually

Workshops

GENERAL COUNCIL MEMBERS. Please send to the Education Committee the list of workshops that have already planned in your country and you want to be supported by EuPA.

Eupa Workshops



- 2006 *Phosphoproteomics*
- 2007 **Proposals of the National Proteomics Societies**
- 2008 **Proposals of the National Proteomics Societies**

Phosphoproteomics workshop

As phosphoproteomics is increasingly becoming a highly demanded discipline, we are planning to organize a workshop to address the state-of-the-art developments on this branch of science.

The different aspects on phosphoproteomics to cover on this workshop should range from techniques for enrichment on phosphopeptides to interpretation of mass spectra of phosphopeptides.



	Introduction to phosphoproteomics: <i>Where are we?</i>
First Session	<i>Protein extraction and cellular fractionation</i>
	Protein extraction methods for phosphoproteins: Subcellular fractionation and large scale phosphoproteomics: <ul style="list-style-type: none">▪ Plasma membrane▪ Nuclear fraction▪ Organelles▪ Soluble
Second Session	<i>Phosphopeptide enrichment</i>
	SCX-HPLC principles and its applications to phosphopeptide enrichment
	IMAC using different resins and tips for improvement
	Modified IMAC and Rotofor for protein pre-fractionation
Third Session	<i>Quantitative phosphoproteomics</i>
	Different Mass analysers for Phosphoprotein analysis
	SILAC phosphoproteomics
	Application of I-TRAQ to quantitative phosphoproteomics



Education activities in the EuPa meetings



- Collaborate with local organizing committee about courses in proteomics technologies or applications.
- Promote and collaborate in the organization of small meetings for young people (similar to “club-jeunes SFEAP), before EuPa meetings.

Other courses

- Basic courses for Master students.
- Courses for clinicians

Educational Programs of proteomics technologies and applications



- **Courses**
- **Workshops**
- ***Laboratory networking***
- **Other Education activities: educational material at web page and in proteomics journals**

Laboratory networking

Youth scientific exchange



- Promote and assist in European-wide scientific exchange programs for young researchers to enhance their scientific career and promote cultural exchange and acceptance throughout Europe.
- Make a list of Proteomics laboratories that would like to be involved in training (3-6 months) and specify their key research interests and the number of students that they can take.

There are Marie Curie Fellowships for transfer of knowledge (deadline 25th January 2006).

Educational Programs of proteomics technologies and applications



- **Courses**
- **Workshops**
- **Laboratory networking**
- ***Other Education activities: educational material at website and in proteomics journals***

Other education activities



- **Educational material**
 - Slides on website
 - Links with educational web pages
- **Tutorials (web page and journals).**
 - Invite scientist to write tutorials to be published on the EuPA website and in journals e.g. Proteomics.
- **Videos and any material from companies (Peter James).**

Committee meetings



**WE ARE GOING TO HAVE ONE DAY MEETING IN
JANUARY/FEBRUARY**

WE NEED THE PROPOSALS OF THE DIFFERENT SOCIETIES

Video Conferences using skype,

<http://www.skype.com/intl/es/download/>

Committee meetings: Once a month.

Meetings of working groups: once a week or every two weeks

- Juan Pablo Albar
- Garry Corthals *Garry Corthals*
- Concha Gil *conchagil*
- Hubert Hondermarck
- Peter James *peterhyweljames*
- Ole Jensen
- Patricia Palagi *patpal1234*
- Deborah Pengue



EuPA-EC

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EuPa

EUROPEAN PROBOIONICS ASSOCIATION

Currently available slides from tutorials



- Who has slides and work from previous courses or lectures:
- List:
 - General proteomics [GC, CG]
 - MS [GC, CG]
 - Sample preparation [CG]
 - Gels and IEF [GC, CG]
 - Quantitation [GC]
 - Protein-proteins interaction [GC]
 - Modifications
 - Phosphorylation [GC]
 - Glycosylation
 - Ubiquitination
 - Lipidation
 - Sumoylation
 - ...
 - Applications
 - Biomedical applications [CG]

WEB SITES OF INTEREST

http://www.proteomesoftware.com/Proteome_software_link_tutorials.html

http://www.forumsci.co.il/HPLC/lcms_page.html

<http://www.ionsource.com/>

http://ncrr.pnl.gov/training/tutorials/mass_spec_tutorial.stm#info
: <http://www.opdac.com/>,

<http://www.bio.davidson.edu/courses/genomics/Front/surfingenomics.html>

Collaborative research projects



1. Promote collaborative research projects between academic research institutions and the life sciences industries:
 - Knowledge transfer through collaborative projects between for example academic institutions and biotechnology, pharmaceutical and agricultural industries.

Quality assured technologies (protocols)



1. Knowledge transfer of validated and quality assured technologies and procedures to the life science community via world-wide public information resources.
 - Publish ‘validated’ protocols and procedures
 - Publish lectures and manuals for promotion of best practices and ‘validated’ procedures

NOTE: Validated procedures are procedures that have been standardised

Example of Tutorial or detailed protocol
Isoelectrofocusing using Rotofor

ISOELECTROFOCUSING WITH ROTOFOR

MATERIALS

Buffers and solutions

1. *Protein extraction buffer*: 100 mM HEPES-KOH, pH 7.5, 5% glycerol, 50 mM sodium pyrophosphate, 1 mM sodium molybdenate, 25 mM sodium fluoride, 15 mM EGTA, 5 mM EDTA, 0.5% polyvinylpyrrolidone, 1% triton (3 mM DTT added on day of use).
2. 100 mM PMSF in isopropanol (store 4°C)
3. 10 mM Leupeptin (store -20°C)
4. 10 µM Calyculin A (store -20°C)
5. Phenol
6. *Back extraction buffer*: 100 mM Tris-HCl, pH 8.4, 20 mM KCl, 10 mM EDTA, 0.4% β-mercaptoethanol
7. 100 mM ammonium acetate in methanol
8. 80% acetone, 50 mM Tris-HCl, pH 8.0
9. Sonicating water bath
10. *Low stringency buffer*: 9 M urea, 1% Triton X-100, 5% ampholytes, 0.5% DTT
11. *High stringency buffer*: 7 M urea, 2 M thiourea, 4% Chaps, 5% ampholytes, 2% DTT
12. 50% glycerol

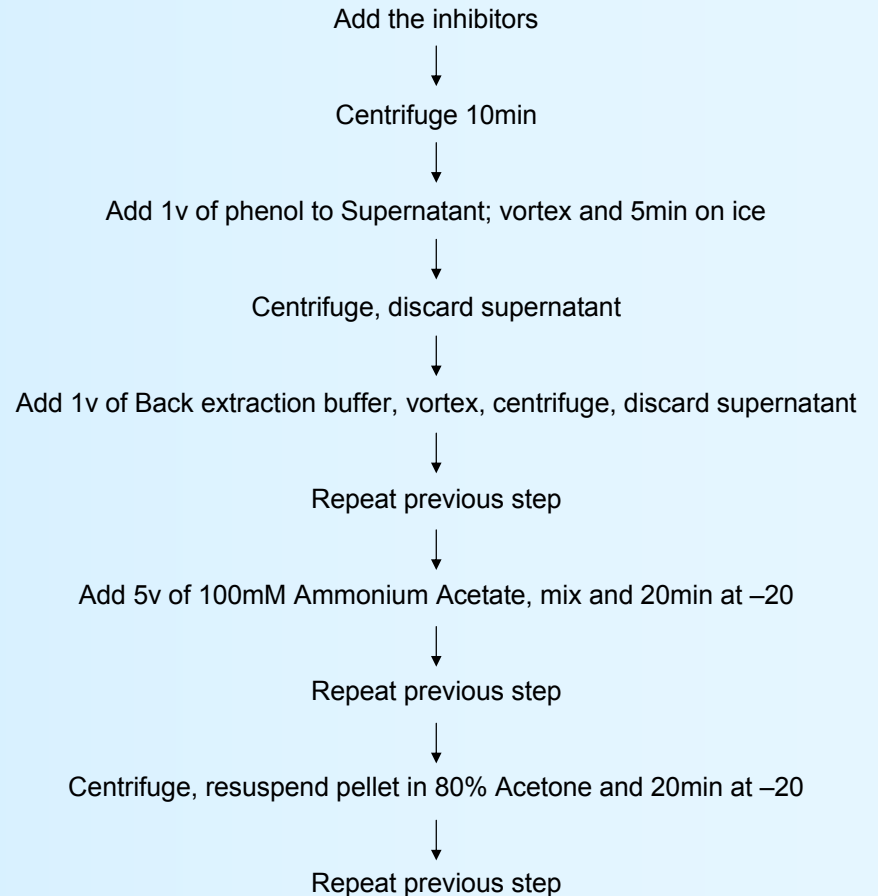
Reagents

Ammonium Acetate
Ampholytes
Calyculin
DTT
EDTA
EGTA
Glycerol
HEPES-KOH
Leupeptin
Methanol
Phenol buffered pH 8
PMSF
PVP
Sodium Fluoride
Sodium Molybdate
Sodium Pyrophosphate
β-Mercaptoethanol
Tris-Base
Triton
Urea

PROTOCOL

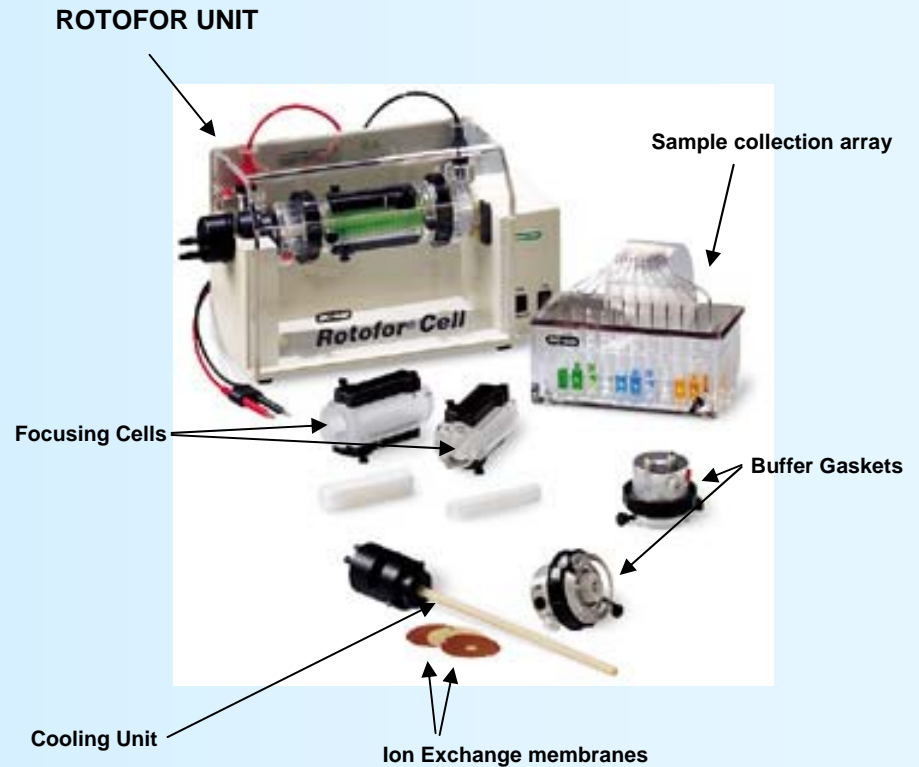
1. Previously described by Peck et al (2001) yields proteins of good quality for IEF separation.
2. Add inhibitors to protein extraction buffer [final concentrations of 1 mM PMSF, 10 μ M leupeptin, and 10 nM Calyculin A .
3. Centrifuge (10,000g; 10 min) to clear cell debris.
4. Transfer the supernatant to a tube containing one volume of phenol, vortex, and keep on ice for 5 min.
5. Centrifuge (10,000g; 10 min) and discard the aqueous phase. (protein will be in the phenol phase and interface).
6. Add one volume of back extraction buffer, vortex, and centrifuge (10,000g; 10 min). Discard the aqueous phase, and repeat the back extraction. Steps 4 to 6 will help remove both nucleic acids and sugars.
7. Add five volumes of 100 mM ammonium acetate in methanol, vortex, and place at -20°C for 20 min to precipitate proteins from the phenol.
8. Centrifuge (10,000g; 10 min) to pellet protein.
9. Wash the pellet with 100 mM ammonium acetate in methanol, using a sonicating water bath to break up the pellet. Centrifuge (10,000g; 10 min) and repeat wash.
10. Wash the protein pellet from previous step with 80% acetone buffered. Centrifuge (10,000g; 10 min) and repeat wash. Protein can be stored as an acetone suspension indefinitely at -20° C.

FLOW CHART



PREPARATIVE IEF

1. Use an amount equivalent to 20 – 40 mgs of protein. Centrifuge (10,000g; 10 min), discard supernatant, and allow pellet to air dry.
2. Re-suspend pellet in denaturing IEF buffer. Determine the volume capacity for the IEF unit (see manufacturer's instructions). Prepare the proper amount of chemicals for half this volume to be prepared with double distilled water and the other half to be prepared with 50% glycerol. The glycerol is necessary to prevent protein precipitation during focussing, but the proteins will dissolve more easily in the glycerol free buffer.
3. Re-suspend the pellet in the buffer without glycerol for 1 h with continuous shaking. Centrifuge (10,000g; 10 min) to remove unsolubilised material.
4. Mix the supernatant with the glycerol-containing buffer. The proteins are ready to be loaded in the IEF cell.
5. Follow manufacturer's instructions for assembling the IEF unit, loading the sample, and focussing conditions. (Specifics may vary depending on IEF unit.)
6. After focussing, you will have many (~ 20) fractions representing a range of pIs
7. Fractions need to be back-extracted to remove ampholytes. Perform steps 4-10 from Protein extraction.



NOTES

1. The extraction buffer given is for isolation of phosphoproteins, it can be omitted for other proteins. PMSF and Calyculin A are unstable in solution; so they should be added only immediately before protein isolation. The polyvinylpyrrolidone (PVP) is present to bind and remove polyphenolics that might otherwise damage proteins. It may be necessary to increase its concentration or to include the insoluble polyvinylpolypyrrolidone (PVPP) to remove all polyphenolics. A simple indication is that if the protein extract is turning purple, more PVP or PVPP should be used.
2. After the first methanol precipitation, the protein tends to be spread along the wall of the tube, use a pipette to remove it from the wall. Failure to do so will result in significant losses of protein. This step is generally not necessary after subsequent centrifugations.
3. Protein concentrations of ~ 0.5 mg/ml are common. Higher protein concentrations are possible but could result in protein precipitation because of the desalting effect of focusing or the increase of local protein concentration. To overcome these problems increase ampholyte concentrations (we use 5%, but they can be raised up to 40%) or add more glycerol to the buffer (we use 12.5 %, but it may be possible to increase the concentration to 20%).
4. We sometimes find it advantageous to decrease the complexity of the proteome using differential protein extraction from the acetone pellet. The low stringency buffer resolubilizes a subset of proteins with a general bias towards smaller (< 60 kDa) proteins. The remaining pellet can then be resuspended with the high stringency buffer. In both cases, the amount of urea and/or thiourea is substantial and affects the volume of the solution. Therefore, only add about 2.9 mL water for each 5 mL total volume of low stringency buffer or 2.5 mL water for each 5 mL of the high stringency buffer. Once the chemicals are fully resuspended, adjust the final volume. For the solutions containing glycerol, perform the same procedure but use 50% glycerol instead of water to make the buffers.



Training in ProteoRed- INB (Spain)

<http://www.proteored.org/pages/index.html>

Aim of the training courses

- General training courses in bioinformatics for proteome analysis
- Specialized seminars

Bioinformatics and proteomics

- **Unit 1: 2D electrophoresis**
- 1.1.- Introduction
- 1.1.1.- Required experiments to obtain a 2D gel
- 1.1.2.- Important aspects to consider in the analysis of a 2D gel image
- 1.1.3.- Software package
- 1.1.4.- Accuracy of current systems
- 1.2.- Ge Analysis
- 1.2.1.- Image correction
- 1.2.1.1.- Space correction
- 1.2.1.2.- Intensity correction
- 1.2.2.- Spot detection
- 1.2.3.- Spot analysis (quantification)
- 1.3.- Differential image analysis
- 1.3.1.- Image recording
- 1.3.1.1.- *Image warping*
- 1.3.1.2.- Spot matching
- 1.3.2.- Analysis of differential expression
- 1.3.2.1.- Detection of mutations
- 1.3.2.2.- Trend analysis
- 1.4.- Large scale analysis
- 1.5.- 2D-E protein databases

Unit 2: MALDI-TOF mass spectrometry. Fingerprinting

- 2.1.- Introduction
- 2.1.1.- Peptide fingerprint
- 2.1.2.- Interpreting data of mass spectra.
- 2.1.3.- Mass list calibration
- 2.2.- IDing your protein on Databases
- 2.2.1.- Public databases
- 2.2.2.- Database engines.
- 2.3.- Identifying PTMs.

Unit 3: CID mass spectrometry.

- 3.1.- Introduction
- 3.1.1.- Obtaining the fragmentation spectra
- 3.1.2.- Processing the mass spectra.
- 3.2.- Using databases for protein ID
- 3.2.1.- Public databases
- 3.2.2.- Database engines.
- 3.3.- De Novo sequencing
- 3.3.1.- Classical approach
- 3.3.2.- Software package
- 3.3.3.- New approaches

Unit 4: Data integration and post-ID

- 4.1.- Introduction
- 4.1.1.- Getting to know LIMS
- 4.1.2.- Software package
- 4.1.3.- Data integration and standards
- 4.1.3.1.- pEDRO
- 4.1.3.2.- HUPO-PSI
- 4.2.- Public information systems
- 4.3.- Knowledge extraction systems

Specialized seminars

- **2D gel image analysis:**

- Melanie,
- Decyder
- Virtual gels (Presenting data)
- Integration of experimental data

- **Mass spectrometry : spectra analysis**

- Filtering and Processing MS and MS/MS spectra: *main tools*.
- Treatment of MS/MS spectra to improve the De Novo sequencing
- Phenix

- **Bioinformatics used in post-ID of proteins**

- Similarity search: analysing the aminoacidic sequence
- Protein-Protein interaction search
- Specific tools: PIKE, Garban, String, GO, etc.

Course Proposals

- Title:
- Description
- Location:
- Number of participants (5-10/10-20)
- Duration (1-5 days)
- Proposed dates
- Organising laboratory
- Budget
- Contact person (address, phone and E-mail)

Courses Organization



REQUIRED RESOURCES

- Human resources
 - Organizer
 - Expert researchers
 - Technical personnel
- Material resources
 - Class room
 - Computer room
 - Proteomics Laboratory



A. Sample preparation for mass spectrometry analysis

- 1.- Proteomes, Subproteomes (membranes, nuclei..., etc.)

B. 2D electrophoresis

- 2.- DIGE
 3.- Rotofor (preparative IEF)
 4.- Cell surface labelling with fluorocromes
 5.- Blue Native

C. Peptide fractionation by LC

- 6.- Multidimensional Chromatography

D. Analysis of differential protein accumulation by MS-quantification

- 7.- ITRAQ
 8.- Other labellings:

E. Phosphoproteomics

- 9.- Phosphopeptide enrichment and MS analysis

F. Bioinformatics

- 10.- Analysis and interpretation of Mass spectrometric results (MS y MS-MS)
 11.- Bioinformatic tools for protein analysis
 12.- Image analysis of 2D gels
 13.- Data integration and post-ID.
 14.- Software management for the prediction of protein-protein interactions.

G. Interpreting Spectra

- 15.- De novo sequencing

H. Practical courses on Mass Spectrometry

- 16.- MALDI-TOF and TOF/TOF
 17.- Applications of TRAPs and Q-TOFs to protein sequencing and PTM analysis
 18.- SELDI-TOF in the search of biomarkers

I. Other training courses of special interest for the technician of proteored