Program Overview

- Session 1. Course Presentation: Mass spectrometry-based proteomics for molecular and cellular biologists
- Session 2. Principles of Mass Spectrometry
- Session 3. Mass spectrometry based proteomics I: Main workflow in mass spectrometry based proteomics
- Session 4. Sample and methods preparation I: Digestion and peptide separation
- Session 5. Bioinformatics applied to proteomics analyses I: Software for MS/MS data interpretation
- Session 6. Bioinformatics applied to proteomics analyses II: Software for MS/MS data interpretation
- Session 7. Sample and methods preparation II: Peptide enrichment
- Session 8. Mass spectrometry based proteomics II: Quantitative Proteomics
- Session 9. Mass spectrometry based proteomics III: Introduction to targeted proteomics
- Session 10. Data analysis I: Protein quantification and down-stream analysis
- Session 11. Sample and methods preparation III: Selected reaction monitoring
- Session 12. Current trends in Proteomics
- Session 13. Data analysis II: Targeted proteomics data analysis and interpretation
- Session 14. Student presentations and discussions
- Session 15. Final session: feedback

Course Program

Monday, July 1st, 2013

Session 1. Course Presentation: Mass spectrometry-based proteomics for molecular and cellular biologists

When: 9:30 to11am
What: Lecture
Where: Charles Darwin Room (Ground Floor)

Contents

- Welcome and presentation of the lecturers
- Setting the frame: time, topics, targets, roles, and rules
- Getting introduced to each other
- Expectations from the students about the course
- Goals of the course
- Assignment for Friday

- COFFEE BREAK (11 – 11.30am) – Inner Square –

Session 2. Principles of Mass Spectrometry

When: 11:30am to 1pm
What: Lecture
Where: Charles Darwin Room (Ground Floor)

Contents

- Definition of mass spectrometry and its applications to molecular and cellular biology (broad vision)
- Definition of a mass spectrum
- Definition of mass, isotopes, monoisotopic mass, and isotopic pattern of multiply charged species
- Parts of a mass spectrometer

- LUNCH BREAK (1 – 2.30pm) – PRBB Canteen -

Session 3. Mass spectrometry based proteomics I: Main workflow in mass spectrometry based proteomics

When: 2.30 to 5 pm
What: Lecture
Where: Charles Darwin Room (Ground Floor)

Contents

- Bottom up versus top down proteomics approaches
- Protein identification from MS/MS data (MS/MS definition: combining mass spectrometric analyzers)
- MS/MS precursor selection algorithms: DDA (vs DIA), dynamic exclusion, etc.
- Fragmentation: Types, patterns of fragmentation, definition of fragment types and examples (CID, ETD, HCD)
- Manual interpretation of MS/MS data
- Automated database search and assessment of false discovery rate (decoy databases)
- Peptide and protein identification
- Identification Post-translational Modifications (Phosphorylation, Ubiquitinylation, Methylation, Acetylation, etc.)

Tuesday, July 2nd, 2013

Session 4. Sample preparation I: Digestion and peptide separation

When: 9am to 1.15pm
What: Lecture combined with Laboratory Practicals
Where: Teaching laboratory (6th floor)

Practical

- Preparation of several tryptic digestions: reduction, alkylation and tryptic digestion.

Contents

- Overview and explanation of the sample digestion protocol (reduction, alkylation and digestion)
- Main proteases used in protein digestion and types of peptides generated (charge distribution, end amino acid, etc.)
- Chromatography theory and chemical principles of peptide separation
- Types of chromatographic techniques for peptide separation (C18, C8, ERLIC, Strong Cation Exchange, Strong Anion Exchange)

- **LUNCH BREAK (1.15 – 2.15pm) – PRBB Canteen -**

Session 5. Bioinformatics applied to proteomics analyses I: Software for MS/MS data interpretation

When: 2.15 to 4.15pm
What: Lecture combined with computational practical
Where: Room 468 (4th floor)

Practical

- Manual interpretation of MS/MS data (exercises)

Contents

- Introduction to automated database search: types of automated interpretation of MS/MS data (de novo, tag-based, spectrum match)

- COFFEE BREAK (4.15 – 4.30pm) – 5th floor terrace -

Session 6. Bioinformatics applied to proteomics analyses II: Software for MS/MS data interpretation

When: 4.30 to 6.30pm
What: Lecture combined with computational practicals
Where: Room 468 (4th floor)

Contents

- Search engine algorithms heuristics
- Search space and database size
- Available protein databases (Uniprot, NCBInr, Spectral Libraries, and organism specific databases)
- Assessment of false discovery rate (decoy databases, Gygi's Nat Methods Paper)

Wednesday, July 3rd, 2013

Session 7. Sample preparation II: Peptide enrichment

When: 9am to 1.15pm
What: Lecture combined with laboratory practicals
Where: Teaching laboratory (6th floor)

Practicals

- C18 clean-up of the tryptic digests + speedvac
- phospho-enrichment of the samples + speedvac
- MS method review and queue setting (runs overnight)

Contents

- Enrichment strategies: phospho-enrichment (TiO2 and variants, IMAC, pTyr antibody), Ubiquitin enrichment (GlyGly antibody), Glyco-enrichment (chemical oxidation, lectins and TiO2)

- **LUNCH BREAK (1.15 – 2.30 PM) – PRBB Canteen -**

Session 8. Mass spectrometry based proteomics II: Quantitative Proteomics

When: 2.30 to 4pm
What: Lecture
Where: Room 473 (4th floor)

Contents

- Importance of obtaining quantitative results, and introduction to different quantitative approaches (label free versus isotopic labelling)
- Label free quantitation: Methods based on number of peptides, spectral counting, and area under the curve (top3, ibaq, direct comparison)
- Isotopic label quantitation: Methods based on metabolic labelling (SILAC), chemical labelling (iTRAQ, Dimethyl), and isotopically labeled standards (AQUA)

- COFFEE BREAK (4 – 4.30am) – 5th floor terrace -

Session 9. Mass spectrometry based proteomics III: Introduction to targeted proteomics

When: 4.30 to 6pm
What: Lecture
Where: Room 473 (4th floor)

Contents

- Introduction to hypothesis-driven proteomics

- Usage of the triple-quadrupoles as double filter to monitor the peptides of interest
- Selected-reaction monitoring workflow
- Method development: protein selection, peptide selection and making a method with Skyline

Thursday, July 4th, 2013

Session 10. Data analysis I: Protein quantification and down-stream analysis

When: 9am to 1.15pm
What: Computer practicals
Where: Room 468 (4th floor)

Practicals

- Data base search and analysis of the acquired raw files
- Comparison of different label-free quantification approaches e. g. spectral count, top3 and direct comparison
- Down-stream analysis (GO-enrichment, Pathway-enrichment, Clustering, PCA, etc.)

- **LUNCH BREAK (1.15 – 2.15 PM) – PRBB Canteen –**

Session 11. Sample and methods preparation III: Selection reaction monitoring

When: 2.15 to 4.15pm
What: Practical Session
Where: Teaching laboratory (6th floor) & Room 468 (4th floor)

Practical

- SRM method review and queue setting (runs overnight)

Contents

- Revision of the concepts introduced in Session 9 regarding the selected reaction monitoring pipeline

- COFFEE BREAK (4.15 – 4.30pm) – 5th floor terrace -

Session 12. Current trends in proteomics

When: 4.30 to 6pm
What: Lecture
Where: Marie Curie Room (Ground Floor)

Contents

- Biomarker discovery: Pipeline of biomarker discovery and SISCAPA
- Protein-protein interactions using AP-MS
- High-throughput targeted proteomics: Data independent acquisition (SWATH & others)
- Measurements of complete mammalian proteomes with reasonable throughput
- Increase in sensitivity: "Single type" cell proteomics (CyTOF)

Friday, July 5th, 2013

Session 13. Data analysis II: Targeted proteomics data analysis and interpretation

When: 9:00-11:00h
What: Practical Session
Where: Room 468 (4th floor)

Practical

- Analysis of the SRM acquisition with Skyline and calculation of absolute quantification values for the selected proteins.

- **LUNCH BREAK (12 – 2 PM) – PRBB Canteen –**

Session 14. Student presentations, discussions and feedback

When: 2 to 6pm
What: Presentations
Where: Marie Curie Room (Ground Floor)

Contents

- Student presentations on their current projects and how to apply proteomics experiments in them (10 min each)
- Group discussion about the proposed approach of each couple and suggestions to improve them (5 min discussion)
- Retrieve feedback from the students on the course and listen to suggestions to improve it

- **FAREWELL DRINK & END OF THE COURSE – Inner Square**