



UCD Conway Institute of Biomolecular & Biomedical Research

Graduate Education Modules 2010/2011

APPLIED PROTEOMICS - MODULE SCHEDULE & OUTLINE 2011

Course Organiser: Dr. Giuliano Elia

Module Co-ordinator: Dr Giuliano Elia

Credits: 5

Venue: UCD Conway Institute (AM: seminar rooms 1 or 2; PM: undergraduate lab 1 or core facility)

Date & Time	Session & Topic	Coordinator
9th May L1 10.00 – 11.15 L2 11.30 – 13.00 Seminar Rm 2	1. Protein biochemistry refresher Course introduction; Fundamentals on proteins and peptides; Planning a proteomic study: Top-down and Bottom-up approach – <i>G. Elia</i> Sample preparation for Proteomics; Protein digestion and peptide purification – <i>G. Elia</i>	G. Elia
Mass Spec Lab 1 14.00 (G047)	Practical: Protein digestion and peptide purification – <i>K. Wynne and G. Manning</i>	
10th May L1 10.00 – 11.15 L2 11.30 – 13.00 Seminar Rm 1	2. Gel-based and gel-free separations I Separation of proteins and separation of peptides – <i>G. Elia</i> Gel-based protein separation techniques: 1D SDS-PAGE, 2D-PAGE, 2D-DIGE – <i>G. Elia</i>	G. Elia
Undergrad Lab 1 14:00	Practical: Sample preparation & IPG strip rehydration – <i>C. Scaife</i>	
11th May Undergrad Lab 1 09:00-17.00	3. Gel-based and gel-free separations II Practical: Isoelectric Focusing – <i>C. Scaife</i>	G. Elia
L1 10.00 – 11.15 L2 11.30 – 13.00	Chromatographic techniques for protein or peptide separation – <i>J. English</i> MudPIT, RP-RP, Offgel fractionation-RP – <i>J. English</i>	
12th May L1 10.00 – 11.15 L2 11.30 – 13.00 Seminar Rm 1	4. Mass spectrometry (MS) techniques I Fundamentals of mass spectrometry; Ionization methods and instrumentation for protein and peptide analysis by MS – <i>M. Wilm</i> Protein identification by MS techniques: Peptide Mass Fingerprinting, MS/MS search, Sequence Mass Tags; De novo protein sequencing – <i>M. Wilm</i>	M. Wilm
Undergrad Lab 1 14:00	Practical: DIGE gel imaging – <i>C. Scaife</i>	
13th May L1 10.00 – 11.15 L2 11.30 – 13.00 Seminar Rm 2	5. Mass spectrometry (MS) techniques II Analysis of intact proteins – <i>M. Wilm</i> Quantitative proteomics: stable isotope labeling techniques and label-free quantitation – <i>M. Wilm</i>	M. Wilm
Mass Spec Lab 1 14.00 (G047)	Practical: MALDI mass spectrometry of peptides and proteins – <i>K. Wynne and G. Manning</i>	



17th May L1 10.00 – 11.15 L2 11.30 – 13.00 Seminar Rm 1	6. Clinical Applications of MS-based proteomics. Proteomic applications in biology and medicine – <i>S. Pennington</i> Clinical proteomics – <i>S. Pennington</i>	S. Pennington
Lecture Theatre 14.00	Practical: Mid-course assessment	
23th May L1 10.00 – 11.15 L2 11.30 – 13.00 Seminar Rm 2	7. Applications of MS-based proteomics Study of protein post-translational modifications (PTMs) – <i>G. Cagney</i> Study of protein-protein interactions; Chemical proteomics – <i>G. Cagney</i>	G. Cagney
Mass Spec Lab 1 14.00 (G047)	Practical: LC-MS/MS mass spectrometry of peptides and proteins – <i>K. Wynne and G. Manning</i>	
25th May L1 10.00 – 11.15 L2 11.30 – 13.00 Seminar Rm 1	8. Protein arrays Types of protein arrays; Protein sources; Formats and surfaces – <i>D. Cahill</i> Immobilisation considerations; Detection systems; Specificity and cross-reactivity; Related formats; Applications of protein arrays – <i>D. Cahill</i>	D. Cahill
Expression Factory 14.00 (G084)	Practical: Protein expression – <i>D. O'Connell</i>	
27th May L1 10.00 – 11.15 L2 11.30 – 13.00 Seminar Rm 2	9. Proteomics bioinformatics Languages and formats; Algorithms for raw data file management – P. O'Gaora Algorithms for statistical validation of data; Software tools for proteomic data interpretation – P. O'Gaora	P. O'Gaora
Interactive Hub 14.00 (G026)	Practical: Proteomics bioinformatics – <i>P. O'Gaora</i>	
31st May L1 10.00 – 11.15 L2 11.30 – 13.00 Seminar Rm 2	10. Validation strategies Antibody-based techniques: Western-blot, ELISA, Imaging techniques - <i>G. Elia</i> Antibody-phage display; MS-based techniques – <i>G. Elia/R. Inzitari</i>	G. Elia
Lecture Theatre 14.00	Practical: Final Assessment	



APPLIED PROTEOMICS COURSE OUTLINE

This course focuses on the description of techniques for Applied Proteomics, i.e., the genome-wide, high-throughput identification, characterization and quantification of proteins and peptides in a variety of biological systems. It provides an overview of current and developing concepts in Proteomics and of its applications to biology and medicine. It aims to familiarise participants with:

- Techniques for protein/peptide separation, their mass spectrometry- or protein array-based identification and quantitation, validation with complementary techniques
- Bioinformatic aspects of proteomic science and its biological and clinical application

The course comprises 10 x 3hr seminar-style sessions designed to encourage interaction and discussion between lecturers and participants

An outline of the content and schedule for each of the seminars is indicated below.

Session 1: Protein biochemistry refresher

10.00 – 11.15 Introduction & Overview – *Giuliano Elia*

To cover:

- *Introduction to the course*
- *Fundamentals on proteins and peptides*
- *Planning a proteomic study*
- *Top-down and Bottom-up approach*
- *Questions and Discussion*

11.15 – 11.30 Break

11.30 – 13.00 Sample preparation – *Giuliano Elia*

To cover:

- *Sample preparation for Proteomics*
 - *Protein digestion and peptide purification*
 - *Questions and Discussion*
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Session 2: Gel-based and gel-free separations I

10.00 – 11.15 Gel-based techniques for protein separation – *Giuliano Elia*

To cover

- *Separation of proteins and separation of peptides*
- *Gel-based protein separation techniques*
- *1D SDS-PAGE*
- *Questions and Discussion*

11.15 - 11.30 Break

11.30 – 13.00 Gel-based techniques for protein separation – *Cont'd- Giuliano Elia*

To cover



- *Questions and Discussion*

Session 3: Gel-based and gel-free separations II

10.00 – 11.15: Gel-free techniques for separation of proteins and peptides – *Jane English*

To cover

- *Chromatographic techniques for protein and peptide separation*
- *Strong cationic exchange*
- *Strong anionic exchange*
- *Questions and Discussion*

11.15 – 11.30 Break

11.30 – 13.00: Gel-free techniques for separation of proteins and peptides – *Cont'd– Jane English*

To cover

- *MudPIT*
 - *RP-RP*
 - *Offgel fractionation-RP*
 - *Questions and Discussion*
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Session 4: Mass spectrometry techniques I

10.00 – 11.15 Introduction to mass spectrometry – *Matthias Wilm*

To cover:

- *Fundamentals of mass spectrometry (MS)*
- *Ionization methods*
- *MS instrumentation for protein and peptide analysis*
- *Questions and Discussion*

11.15 – 11.30 Break

11.30 – 13.00: Mass spectrometry-based protein identification – *Matthias Wilm*

To cover:

- *Protein identification by MS techniques*
 - *Peptide Mass Fingerprinting*
 - *De novo protein sequencing*
 - *MS/MS search*
 - *Sequence Mass Tags*
 - *Questions and Discussion*
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Session 5: Mass spectrometry techniques II

10.00 – 11.15 MS Analysis of intact proteins – *Matthias Wilm*

To cover:

- *Use of MALDI mass spectrometry*



11.15 – 11.30 Break

11.30 – 13.00: Protein quantification – **Matthias Wilm**

To cover:

- *Label-based and label-free quantification*
 - *Stable isotope labeling techniques: ICAT, iTRAQ, ¹⁸O exchange*
 - *Label-free quantitation: Spectral counts, Analysis of spectral features*
 - *Questions and Discussion*
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Session 6: Clinical applications of MS-based proteomics

10.00 – 11.15 Proteomic applications in biology and medicine – **Stephen R. Pennington**

To cover:

- *Biomarkers discovery*
- *Target discovery*
- *Signalling pathway analysis*
- *Questions and Discussion*

11.15 – 11.30 Break

11.30 – 13.00: Clinical proteomics – **Stephen R. Pennington**

To cover:

- *Translational medicine*
 - *Neuroproteomics*
 - *Cardioproteomics*
 - *Questions and Discussion*
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Session 7: Applications of MS-based proteomics

10.00 – 11.15 Analysis of protein post-translational modifications (PTMs) – **Gerard Cagney**

To cover:

- *Most common protein PTMs*
- *Use of mass spectrometry applied to PTM studies*
- *Phosphoproteomics*
- *Questions and Discussion*

11.15 – 11.30 Break

11.30 – 13.00: Protein-protein interactions – **Gerard Cagney**

To cover:

- *Study of protein-protein interactions*
 - *Immunoprecipitation, TAP-tagging, use of stable isotope labelling*
 - *Chemical proteomics*
 - *Questions and Discussion*
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10.00 – 11.15 Protein arrays technique – **Dolores J. Cahill**

To cover:

- Types of protein arrays; Antibody arrays
- Protein sources, formats and surfaces
- Immobilisation considerations, detection systems
- Specificity and cross-reactivity
- Questions and Discussion

11.15 – 11.30 Break

11.30 – 13.00: Clinical applications of protein arrays – **Dolores J. Cahill**

To cover:

- Antibody profiling
- The Protein Expression Factory™
- Questions and Discussion

Session 9: Proteomics bioinformatics

10.00 – 11.15 Introduction to proteomic bioinformatics – **Peadar O’Gaora**

To cover:

- Languages and formats
- Algorithms for raw data file management
- Databases and database search
- Questions and Discussion

11.15 – 11.30 Break

11.30 – 13.00: Statistics and validation – **Peadar O’Gaora**

To cover:

- Algorithms for statistical validation of data
- Software tools for proteomic data interpretation
- Questions and Discussion

Session 10: Validation strategies

10.00 – 11.15 Antibody-based techniques – **Giuliano Elia**

To cover:

- Sources of antibodies
- Antibody-phage display libraries
- Western-blot, ELISA, imaging techniques
- Questions and Discussion

11.15 – 11.30 Break

11.30 – 13.00: MS-based validation techniques – **Rosanna Inzitari**

To cover:

- Triple quadrupole mass spectrometry
- Selected reaction monitoring, multiple reaction monitoring



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Questions and Discussion
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