INDEPTH Virtual Training School on Protein-Protein Interactions PPI studies: objectives and technologies (February 8th- 10th 2021)

February 8th Day 1 (5hr plus breaks)

OPENING OF THE PPI WORKSHOP
(20 minutes)
Christophe Tatout[1] and Marta Koblowska[2]
[1]. Laboratoire de Génétique Reproduction et Développement, Université Clermont Auvergne, Faculté de Medecine, France

[2]. Laboratory of Systems Biology, Faculty of Biology, University of Warsaw, Poland

Presentation of the participants (1 h)
21 students from 11 countries

Belgium	2
Czech Republic	2
Denmark	1
France	1
Germany	4
Portugal	1
Spain	1
Sweden	1
Switzerland	1
Turkey	1
United Kingdom	6

List of attendees:

ADAMUSOVA	Katerina	University of Copenhagen	Frederiksberg	Denmark	mus@plen.ku.dk
CURRAN-FRENCH	Seamus	John Innes Centre	Norwich	United Kingdom	Seamus.Curran-French@jic.ac.uk
GU	Yangtao	Swedish University of Agricultural Sciences	Uppsala	Sweden	yangtao.gu@slu.se
GUMUS	Berivan Ozlem	Ege University	Izmir	Turkey	b.ozlemgumus@gmail.com
HU	Bingyan	University of Hamburg	Hamburg	Germany	bingyan.hu@uni-hamburg.de
JAMIE	Orr	The James Hutton Institute	Invergowrie	United kingdom	jamie.orr@hutton.ac.uk
JIMENEZ JIMENEZ	Alvaro Luis		Malaga	Spain	jimenezjimenezalvaroluis@gmail.com
LOURENCO	Tiago Filipe	ITQB NOVA	Oeiras	Portugal	tsantos@itqb.unl.pt
MUEHLENBECK	Henning	Institute for Plant and Microbial Biology, University of Zurich	Wetzikon ZH	Switzerland	henning.muehlenbeck@botinst.uzh.ch
NESPOR DADEJOVA	Martina	Mendel Centre for Plant Genomics and Proteomics CEITEC, Masaryk University	Brno	Czech Republic	nespor.dadejova@centrum.cz
OBOMIGHIE	Osi	University of Essex	Colchester	United Kingdom	icobom@essex.ac.uk
PERTUISOT	Brice	Université Clermont Auvergne	Clermont-Ferrand	France	brice.pertuisot@etu.uca.fr
PONNU	Jathish	University of Cologne, Germany	Cologne	Germany	jponnu@uni-koeln.de
RATNIKAVA	Maryia	Leibniz Institute of Plant Genetics and	Gatersleben	Germany	maryiaratnikava21@gmail.com

		Crop Plant Research (IPK)			
REHNEKE	Laura	molekulare Botanik Universität Ulm	Ulm	Germany	laura.rehneke@uni-ulm.de
ROBERTS	Jemma	University of Warwick	Coventry	United Kingdom	J.Roberts.6@warwick.ac.uk
SKIRTACH	Andre	UGent	Ghent	Belgium	Andre.Skirtach@UGent.be
STOJILKOVIC	Boris	Faculty of Bioscience Engineering - UGent	Gent	Belgium	boris.stojilkovic@ugent.be
TOWNSEND	Felix	Aberystwyth University	Aberystwyth	United kingdom	fet7@aber.ac.uk
VELLUTINI	Elisa	University of Glasgow	glasgow	United Kingdom	e.vellutini.1@research.gla.ac.uk
VLADEJIC	Jovanka	Centre of Plant Structural and Functional Genomics, Institute of Experimental Botany of the Czech Academy of Sciences, Centre of the Region Haná for Biotechnological and Agricultural Research	Olomouc	Czech Republic	vladejic@ueb.cas.cz

MASS SPECTROMETRY METHODS TO INVESTIGATE PROTEIN-PROTEIN INTERACTIONS

Vicente Rubio, Centro Nacional de Biotecnología, Madrid (3 h)
Introduction to proteomics
AP-MS approaches, and data analysis. Hands-on training for the MS data filtering and interactomics analyses. Introduction to methods used to confirm the detected interactions: Y2H, in vitro and semi in vivo pull downs, and BiFC.

Rafal Archacki, Laboratory of Systems Biology, Faculty of Biology, University of Warsaw, Poland (30 min)

Bromodomain-containing proteins are subunits of Arabidopsis SWI/SNF chromatin remodeling complexes - case study
SWI/SNF chromatin remodeling complexes have been studied in different organisms since early 1990s, however their exact composition and structure have been uncovered only recently, in yeast and mammals. In plants, the composition of SWI/SNF complexes and the identity of several subunits still remain unknown. During the presentation I will show our recent studies, in which by combining IP/MS approach with Y2H, Y3H, and BiFC analyses, we could identify three bromodomain-containing proteins as new subunits of Arabidopsis SWI/SNF complexes.

BIOPHYSICAL METHODS TO INVESTIGATE PROTEIN-PROTEIN INTERACTIONS

Marcin Nowotny
Laboratory of Protein Structure, International Institute of Molecular and Cell Biology in Warsaw, Poland (1 h)

Overview of the methods used to study protein-protein interactions and introduction to cryo-electron microscopy - lecture**.**

This lecture will present an overview of the available methods that can be used to detect and characterize protein-protein interactions. They range from simple biochemical experiments such a pull-downs (affinity chromatography) and gel filtration to biophysical methods such as, among others, fluorescence measurements, analytical ultracentrifugation, surface plasmon resonance or, more recently, thermophoresis. The second part of the lecture will cover the basic principles of structural biology will allows visualization of protein-protein interfaces at atomic resolution. In particular, I will introduce cryo-electron microcopy which is ideally suited for the structural studies of large protein assemblies and recently underwent a technological revolution greatly expanding its applicability.

Mariusz Czarnocki-Cieciura
Laboratory of Protein Structure, International Institute of Molecular and Cell Biology in Warsaw, Poland (2x45 minutes)

Size-Exclusion Chromatography coupled to Multi-Angle Light Scattering (SEC-MALS) – a powerful tool for characterization of macromolecular complexes - seminar and data analysis

Light scattering techniques allow for rapid characterization of macromolecules in solution. They can be divided into two main categories, Static Light Scattering (SLS) and Dynamic Light Scattering (DLS). Multi-Angle Light Scattering (MALS) is a special type of SLS technique that can be used to measure absolute molecular weight based on the intensity of light scattered by the sample. For complex, heterogeneous samples the result is a weight-averaged molecular weight of the entire mixture. This can be overcome by coupling MALS detector with separation technique, such as Size-Exclusion Chromatography (SEC). With such setup, it is possible to characterize independently each component separated by the SEC column. During my lecture, I will explain briefly the principles and applications of SLS and DLS methods and show, how SEC-MALS technique can be used for the determination of the composition and stoichiometry of macromolecular complexes.

Wojciech Bal
Department of Biophysics, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland (2x45 minutes)

Introduction to Microscale Thermophoresis - seminar and data analysis

Microscale Thermophoresis is one of the newest general purpose methods of detecting intermolecular interactions and determining binding constants. It is based on the physical principle discovered in the 19th century, but implemented practically only very recently. The phenomenon of thermophoresis is based on different responses of molecules to the force of the solvent flow driven by a temperature gradient. The effect is mediated by the solvation sphere of the molecule, hence it is sensitive to the change of the molecule shape, e.g. due to the molecular complex formation. The microscale implementation has been empowered by using an infrared laser for controlled heating of the sample in a capillary, and detecting the generated microscale movement by fluorescence excited by another laser. Systems that can be investigated range from small molecules to proteins and nucleic acids to supramolecular structures, liposomes, viruses and organelles, and the

values of binding constants range from millimolar down to nanomolar and subnanomolar, depending on the specific application.

February 10th Day 3 (7hours plus breaks)

PPI PREDICTION and MODELING

Piotr Zielenkiewicz, Department of Bioinformatics, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland (1 h)

Computer modeling of the protein structure and interactions – lecture

Basics of protein-protein recognition will be described, both from thermodynamic and kinetic perspective. Methods of protein quaternary structure prediction from known tertiary structures of subunits will be critically evaluated. Finally, methods of successful design of protein-protein interaction inhibitors and their pharmaceutical as well as biotechnological applications will be covered.

Alessandra Carbone, Department of Computational and Quantitative Biology, Sorbonne Université, CNRS, France (1.5 h)

Ab initio PPI predictions from sequences and structures - lecture

PPI can be predicted at large scale based on structures, with coarse-grained docking, and based on sequences, with deep learning approaches. These reconstructions are independent of lab experiments and can provide important information on not yet identified interactions.

PPI prediction from sequences and structuresfrom Alessandra Carbone's lab – hands-on training (2 h)

- **Yasser Mohseni Behbahani (PhD):** Prediction of the Effect of mutations on PPI + presentation of Levelnet to draw PPI and predict PPI
    - **GitLab project:** http://gitlab.lcqb.upmc.fr/mohseni/Warsaw-PPI

- **Laurent David (PhD) :** Sequenced-based PPI prediction using RCNN
    - **GitLab project:** https://github.com/LaurentDavid711/PPI_Workshop/tree/main/PIPR_Network

Elodie Laine, Department of Computational and Quantitative Biology, Sorbonne Université, CNRS, France (1.5 h)

Mutational scanning predictions – seminar and hands-on training

The huge amount of sequence data available today can be leveraged to identify functional sites in protein, e.g., involved in interactions with cellular partners, and to predict the effect of mutations at these sites. I will present **GEMME**, a very efficient method to predict mutational outcome at large scale by reconstructing the evolutionary history of natural sequences and by accounting for the whole sequence context.

GEMME Webservice: http://www.lcqb.upmc.fr/GEMME/Home.html

Christophe Tatout

Laboratoire de Génétique Reproduction et Développement, Université Clermont Auvergne, Faculté de Medecine, France

Round-table discussion about

- Feed-backs from the training school: attendees were satisfied. Comment about hands on training that were a little too fast (cf computational training needed specific environment to run the examples)

- Do we need to organize a new workshop? Suggestion: Post-translational marks (PMTs) in chromatin (ChIP)
- Discussion about limitation in plant science regarding PPI: cryoEM is risky and is difficult to fund in plant research, plant datasets are missing in database