CSSB Spring School 2015

From Molecules to Organisms:

Approaches for Structural Systems Biology

13. – 17. 4. 2015

<table>
<tr><th>Mo</th><th>Tu</th><th>We</th><th>Th</th><th>Fr</th></tr>
<tr><td colspan="5">Breakfast 7:00-7:45</td></tr>
<tr><td>Opening (Labahn) 8:00-8:15</td><td colspan="4" rowspan="2">Lectures 8:00-9:00

(Grünewald,Marlovits,Meents,Preller)</td></tr>
<tr><td>Keynote (Wilmanns) 8:15-9:15</td></tr>
<tr><td>Lectures

9:15- 10:45</td><td>Transport
9:00-9:45</td><td rowspan="2">EM-Single Particle

(Marlovits)

9:15-11:45</td><td rowspan="2">Xtal-Beamline & Data

Meents & Schneider

9:15-11:45</td><td rowspan="2">Modelling

(Preller)

9:15-11:45</td></tr>
<tr><td>Protein stability

(Hallberg)
11:00 -12:45</td><td>Microscopy

(Grünewald)

9:45-12:15</td></tr>
<tr><td>Lunch
12:45-13:45</td><td>Lunch
12:15-13:15</td><td colspan="3">Lunch
12:00-13:00</td></tr>
<tr><td rowspan="3">Protein-interaktion
(Löw)

Protein-crystallization
(Labahn)

13:45-17:00</td><td rowspan="2">Microscopy

13:15-18: 15</td><td rowspan="3">EM-Single Particle

13:00-18:45</td><td rowspan="3">Xtal - Modelling & Structure

(Schneider)

13:00-18:45</td><td>Modelling

(Preller)

13:00 – 16:00</td></tr>
<tr><td>Discussion
16:00 – 17:00</td></tr>
<tr><td>Transport
18:15-19:00</td><td rowspan="4">End</td></tr>
<tr><td rowspan="2">Dinner

18:30-19:15</td><td colspan="3">Superresolution LM (Reimer)
Xray-Microscopy (Meents)
XFEL (Chapman)
19:00 -20:00</td></tr>
<tr><td colspan="3">Dinner

20:15-21:00</td></tr>
<tr><td>Socializing</td><td colspan="3">Socializing</td></tr>
</table>

Day 1: Sample preparation

Opening Lecture:

The Center for Structural Systems Biology – an Introduction

M. Wilmanns (EMBL/CSSB)

Optimization of protein sample stability

M. Hällberg (KI/CSSB)

High sample quality – meaning that the sample needs to be pure, homogenous and as stable as possible – is a prerequisite for different structural biology techniques. The quality of the sample is strongly influenced by the buffer system it is in. The Thermofluor assay is an easy-applicable, high-throughput method, which helps to identify optimized buffer compositions and/or small molecule stabilizing agents beneficial for diverse sample preparation steps. During the practical, a Thermofluor experiment will be performed and data analysis and interpretation will be discussed.

Monitoring the interaction of the *Mycobacterium tuberculosis* type VII secretion system chaperone $EspG_5$ with protein effectors PE25-PPE41

C. Löw, (EMBL/CSSB)

The growth of *Mycobacterium tuberculosis* bacilli depend on their type VII secretion system. This system exports effector proteins (PE and PPE proteins), associated with virulence and persistence in the host, across membranes to the bacterial surface. These proteins require system specific chaperones for secretion. In the practical course the interaction of *Mycobacterium tuberculosis* type VII secretion system chaperone $EspG_5$ with the protein effectors PE25-PPE41 will be characterized using isothermal titration calorimetry (ITC). This method allows the quantitative determination of thermodynamic binding parameters such as binding affinity K_a, binding enthalpy ΔH and the binding stoichiometry of the reaction.

Meso-Phase Crystallisation of Membrane Proteins

J. Labahn (FZJ/CSSB)

Meso-phase crystallization means the crystallization of membrane proteins in a quasi-natural environment and allowed for the recent progress in determining structures of important target proteins like GPCRs at atomic resolution.

Membrane crystallization will be achieved by Controlled in meso-phase crystallization (CIMP) which combines the advantages of crystallization in cubic lipidic phase with the well-established vapor-diffusion technique.

Day 2: Advanced Microscopy

Integrative cryo microscopies: bridging native information from dynamics to high resolution

K. Grünewald (HPI/CSSB)

The presentation will introduce the methods and current state of the art of electron cryo microscopy (cryo-EM) and tomography (cryo-ET) and their combination with complementary techniques, like advanced super-resolution fluorescence (cryo) microscopy and soft X-ray cryo microscopy/tomography. A wide range of dedicated sample preparation techniques enabling optimal biological (sub) systems for analyses of different levels of complexity will be introduced. CryoEM/ET are currently undergoing a 'resolution revolution' due to the introduction of direct electron detectors. The power of an integrated structural biology approach to cell biology at the macromolecular level will be exemplified along our group's analyses of crucial steps in the molecular interactions between viruses and their host cells with a main emphasis being on understanding mechanisms of membrane modulation.

Evening Lecture:

Superresolution light microscopy,

R.Reimer (HPI)

Day 3. Single particle Analysis

Molecular Machines: Single Particle Analysis and Modelling

W.Lugmayr (UKE)

The course will provide fundamental understanding of molecular mechanisms of molecular machines and will be structured in a morning talk and a training session. The talk will present the theoretical knowledge on how an electron microscope and the corresponding data analysis works. It will be followed by a practical analysis of electron microscope data starting from raw data to a 3D model by using an empirical Bayesian approach performed by a state-of-the-art software suite.

Evening Lecture:

X-ray microscopy of biological samples

A. Meents (Desy)

Day 4: Crystallographic Analysis

Macromolecular X-ray crystallography at synchrotrons

A. Meents (Desy), T. Schneider (EMBL)

X-ray crystallography at synchrotron sources is a powerful method for the structure determination of biological macromolecules at atomic resolution. After a short introduction to the PETRA III synchrotron and the macromolecular crystallography beamlines the students will have the opportunity to perform synchrotron data collections from their previously grown protein crystal and to analyze the data. A second lecture will address structure solution and refinement procedures followed by a practical exercise on protein structure refinement based on the data sets collected in the morning.

Evening lecture:

Structure determination with free electron lasers,

H. Chapman (CFEL)

Day 5: Bioinformatics and Modelling

Strukturelle Modellierung und computergestützte Analyse molekularer Interaktionen in der Infektionsbiologie

M.Preller (MHH/CSSB)

Topics are computer-assisted methods in Structural Systems-biology and Infection-biology for structural modelling , investigation and prediction of molecular interactions, a well as simulation of dynamic processes. The course will provide an overview of the multitude of currently available techniques in the fields of Molecular Modeling and Structural Bioinformatics. The course specifically targets the topic Molecular Interaction of Proteins with low molecular weight compounds.