15th International School of Biophysics 2020
Split-Primošten, Croatia
27/8-5/9 2020

Hands-on training modules:

theoretical/calculational/computer or pen and paper based modules:

1 TH - Molecular modeling (C. Oostenbrink, Vienna) - 2 DAY: This workshop will help you gain first experiences in performing molecular dynamics simulations. After a short theoretical introduction, you will find out about the basic ingredients for a molecular simulation. You will setup your own simulation of a small peptide and run some short simulations of it. Together with your colleagues, you will explore the effect of different simulation conditions on the molecular behavior. You will analyse the resulting trajectories and find out what computational methods can offer in addition to experimental approaches. Held by Prof. Oostenbrink. Work on Linux PCs at the Faculty of Science, or you bring your own Linux laptop.

2 TH - Simulating large scale conformational changes in biomolecular systems (L. Kamerlin, Uppsala) - 1 DAY: This workshop will provide attendees with the capability to perform and analyze a powerful enhanced sampling molecular dynamics simulation technique known as Hamiltonian replica exchange (HREX). The method enables for the efficient exploration of conformational space orders of magnitude faster than conventional molecular dynamics simulations, alongside the ability to obtain information about the underlying free energy landscape. Following a theory session with a mind to possible applications of HREX, attendees will setup, run and analyse a HREX simulation on the enzyme DHFR, exploring how its loop dynamics regulate its catalytic activity. Simulations will be run using GROMACS simulation software. Held by Prof. Lynn Kamerlin. Work on Linux PCs at the Faculty of Science, or you bring your own Linux laptop.

3 TH - CryoEM image analysis (H. Stark, Goettingen) - 1 DAY:

4 TH - Nuclear Magnetic Resonance (NMR) spectroscopy (E. Cabrita, Lisbon) - 1 DAY: We will introduce fundamental concepts concerning the application of NMR in a drug discovery context to characterize protein-ligand interactions.

Task I: One of the most powerful ligand based NMR techniques, namely the Saturation transfer difference NMR technique (STD NMR). Students will learn how to design the experiments, prepare the sample and interpret the results. Students will use STD NMR raw data obtained for different ligands in the presence of a protein, to determine the thermodynamic dissociation constant and characterize the interaction by identifying the ligand epitope (those parts of the ligand in close proximity to the protein).

Task II: The use of protein detected NMR techniques (namely protein 15N-HSQC NMR) to study protein-ligand interactions from the protein viewpoint. Students will interpret and analyse experimental spectra to characterize the interaction by identifying the residues of the protein that are more likely interacting with the ligand and determine an apparent dissociation constant.

5 TH - Macromolecular biophysics - 2 DAY: **(A. Šiber, Zagreb)**: The course will cover fundamental aspects of physical descriptions of biological systems. These include the interactions in biological context and how they reduce and proceed from elementary physical forces, but also some important applications of equilibrium thermodynamics. The relevant physics will be applied to molecular structure of proteins and nucleic acids and interactions between them.

experimental modules:

6 EXP - *Bio AFM – applications of AFM in studies of macromolecules, supramolecular complexes and cells* (N. Santos, Lisbon & W. Roos, Groningen), supported by Bruker/JPK - 1 DAY: AFM is a flexible tool to image and probe samples at the nanoscale. The workshop addresses Ph.D students and post-docs, as well as scientists, core facility technicians and engineers that are interested in the application of AFM to biology. A major emphasis will be on the preparation of biological samples for AFM imaging and spectroscopy and experimental activity will be the core of the workshop

7 EXP - Electron Paramagnetic Resonance (EPR) spectroscopy – (F. MacMillan, UK), supported by Bruker BioSpin - 1 DAY: Molecules containing an unpaired electron (a free radical) are important in both chemical and biological systems and may be studied by EPR. Students will develop the skills needed to characterise stable paramagnetic molecules as well as learn the methods to chemically reduce and study unstable molecules to assess the impact of oxidation and reduction events on magnetic spectra. Students will use Bruker Biospin's *State-of-the-Art* benchtop instrument to record their own EPR spectra. A modern computational package (Matlab©) will be introduced and used to interpret and analyse their experimental spectra. Both simulation and fitting routines will be taught in order to extract parameters necessary to describe the local environment and the strength and multiplicity of couplings to nearby magnetic nuclei. Using this knowledge and their EPR spectra, students will identify the chemical structure of an unknown molecule.

8 EXP - *Protein-ligand interactions, kinetics, assoc/dissoc/equilibrium constants*, supported by NanoTemper - 1 DAY: The workshop will cover principles of biomolecular interaction analytics using Microscale Thermophoresis (MST) as well as protein stability and aggregation assessment with use of nanoDSF technology. We strongly encourage to bring your samples for analysis. Experts, application sepecialists from Nanotemper will train the students in experimental setup, instrument operation and data analysis.

9 EXP - Introduction to ion channel electrophysiology and electrophysiological investigation of pore-forming bacterial toxins on a single molecule level, **supported by Nanion - 1 DAY**: Nanion will showcase two instruments, Port-a-Patch mini and Orbit mini. Application scientist will give an introduction into ion channel and bilayer electrophysiology on these instruments, respectively. The Orbit mini device will be used for live experiments on artificial lipid bilayers and the alpha-Hemolysin and Gramicidin protein channels. Worksteps include: introduction to ion channel electrophysiology, analysis of ion currents in cells, generating lipid bilayers, preparing asymmetric buffer conditions for the bilayer and reconstitution of proteins into bilayers. Orbit mini device will be then used to characterize the pores formed.

10 EXP - Protein Identification by Mass Spectrometry - (M. Cindrić, Zagreb) - 2 DAY: Mass spectrometry is routinely used to identify pathogens and malignant cell strains by directly fingerprinting their proteome. Prof. Mario Cindric has developed this method to the next level where it can be used for de-novo sequencing of peptides. The method is based on a proprietary chemical derivatization agent for preparation of the protein samples for MS analysis and heavily involves bioinformatics tools to analyze the MS spectra.