Preparation and characterization of macromolecular complexes

November 19th to 23rd 2018

IGBMC, Strasbourg-Illkirch, France

INSTRUCT-FRISBI course

Preliminary program

Monday, Nov. 19th

Morning: Lectures

9h00-9h30	Course introduction
9h30-10h00	Participants will present their projects and aims
10h30-12h30	Methods for isolation of macromolecular complexes: ▪ Engineering cells by genome editing to facilitate purification of complexes ▪ Baculovirus production of recombinant multiprotein complexes: General principles

Afternoon: Practicals

13h30-15h30	Engineering cells by genome editing: design of gene tagging experiments
16h00-18h00	Baculovirus production of recombinant multiprotein complexes: manipulation of insect cells and infection with baculovirus expressing exogenous complexes

Tuesday, Nov. 20th

Morning: Practicals

9h00-10h30	Biophysical characterization of complexes**:** NanoDSF for stability optimization of protein samples: presentation of the method and data acquisition
11h00-12h30	EM Grid preparation and analysis of sample homogeneity

Afternoon: Practicals

13h30-15h30	Engineering cells by genome editing: CRISPR/Cas9 reagents and gene tagging
16h00-18h00	Baculovirus production of recombinant multiprotein complexes: transfection of constructs for virus production

Wednesday, Nov. 21st

Morning: Practicals

9h00-10h00	Baculovirus production of recombinant multiprotein complexes: construct design
10h00-11h00	Biophysical characterization of complexes**:** NanoDSF data analysis
11h00-12h00	Biophysical characterization of complexes**:** SEC MALLS (Multiple Angle Laser Light Scattering coupled to Size Exclusion Chromatography) for protein sample quality control: presentation of the method and data acquisition

Afternoon: Mini-Symposium

"State-of-art strategies technologies for preparation and biophysical characterization of macromolecular complexes in view of biochemical and structural studies"

13h30–14h00	Identification & reconstitution of multiprotein complexes: the Exon Junction Complex as model system – **H. le Hir**
14h15–14h45	New opportunities offered by genome editing strategies - **J.P. Concordet**
15h00–15h30	Strategies for determining the 3D structure of macromolecular complexes at atomic or pseudo-atomic resolution - **B. Klaholz**
15h45–16h15	X-ray Free Electron Laser: Opportunities for drug discovery – **M. Hennig**
16h15–16h45	*Coffee Break*
16h45–17h15	Nanobodies as tools for structural proteomics and beyond - **J. Steyaert**

Thursday, Nov. 22nd

Morning: Practicals

9h00-10h00	Biophysical characterization of complexes**:** SV-AUC (Sedimentation Velocity Analytical Ultracentrifugation) for protein sample quality control: presentation of the method and sample preparation
10h00-11h00	Biophysical characterization of complexes**:** SEC MALLS (Multiple Angle Laser Light Scattering coupled to Size Exclusion Chromatography)
11h00-12h00	Lecture on co-expression in *E. coli* for the reconstitution of multiprotein complexes (Christophe Romier)

Afternoon: Practicals

13h30-15h30	Engineering cells by genome editing: Testing integration with a luciferase assay
16h00-18h00	Baculovirus production of recombinant multiprotein complexes: collect and analyze culture for expression of exogenous complexes

Friday, Nov. 23rd

Morning: Practicals and lecture/discussion

9h00-10h00	Biophysical characterization of complexes**:** SV-AUC data analysis
10h00-11h00	Biophysical characterization of complexes**:** SEC MALLS and NanoDSF data analysis
11h00-12h00	Lecture on the vaccinia virus expression system for the reconstitution of multiprotein complexes (Marc Ruff & Robert Drillien)

Afternoon: Wrap-up session

13h30-14h00	Summary of experiments
14h00-15h30	General discussion

End of the workshop