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

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**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) and NanoDSF data analysis

11h00-12h00 Lecture on co-expression in *E. coli* for the reconstitution of multiprotein complexes (Christophe Romier)

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**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

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**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)

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**Afternoon: Wrap-up session**

13h30-14h00 Summary of experiments

14h00-15h30 General discussion

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End of the workshop