Structural Biology and Genomics Platform in Strasbourg: strategies for the crystallization of macromolecular complexes

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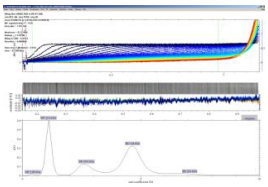
The Integrative Structural Biology Department is a centre of expertise for the determination of the structures of proteins (and of their complexes with DNA and RNA) mainly related to human health - notably those involved in the regulation of gene expression (transcription, translation). As a part of this department, the Structural Biology and Genomics Platform has been implemented, and consists of different interconnected modules, from protein production to X-ray crystallography. We have developed flexible methods, using automation when appropriate, in order to adapt each step to project requirements. A major effort is put on the characterization of samples; at the crystallization level, we especially focused on developing techniques for screening and optimization of crystal growth. The platform is open to academic and industrial users.

Activities : Protein production and purification - Biophysical characterization of macromolecules - Crystallization and 3D structure determination.

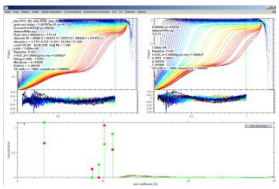
Biophysical characterization of macromolecular complexes in solution

Sedimentation velocity experiments (Analytical Ultracentrifuge XL-I)

This allows for example to assess the homogeneity of a hetero-complex or reveal an interaction with DNA.



No homogeneity

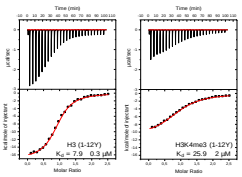


Complex formation with DNA

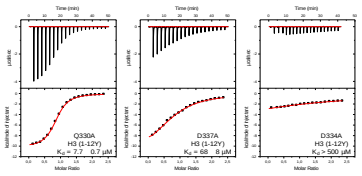
Global analysis of two protein:DNA ratios — 1:3 — 1:5

Isothermal Titration Calorimetry experiment (Microcal ITC200)

Among the applications of ITC are the quantification of binding affinity or the determination of binding specificity and stoichiometry.



Affinity of a histone tail reader for H3 peptides.

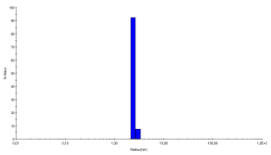


Critical residues for interaction.

Dynamic Light Scattering experiment (Dynapro Nanostar)

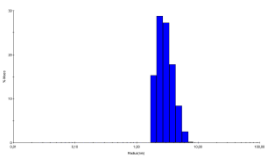
DLS is a technique for measuring the size of particles. We use it to characterise newly purified complexes for size and homogeneity.

DNA Binding Protein + DNA 1



Rh = 2,45 nm
Polydispersity = 7,0%

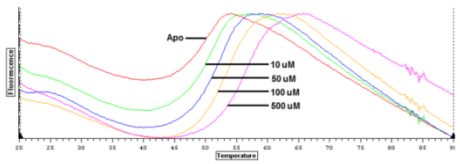
DNA Binding Protein + DNA 2



Rh = 3,01 nm
Polydispersity = 32,6%

Thermal Shift Assay (MiniOpticon)

TSA is a rapid and sensitive tool for monitoring protein thermostability. It allows for the identification of optimal conditions or conformations that favour protein stability, including the investigation of protein-ligand interactions.
Here we observe a significant increase of the protein's stability when it binds specific ligands.



Crystallization and X-ray structure determination

Crystallization

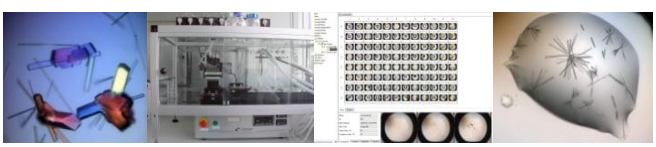
Screening is carried out in microplates, using both the techniques of vapour diffusion and micro-batch.

Sitting drops (from 100nl to 500nl) are dispensed by a Cartesian Honeybee 8+1 robot, and reservoir filling is performed by a Tecan 96-channel head.

1400 conditions are available from either commercial or home-made screens (prepared by a Tecan Miniprep).

Optimization of the crystal growth is carried out using the robots, by screen refinement, additive screening, varation of volumes and ratios for the drops and automated microseeding.

Plates are stored and automatically imaged at 20°C in a Formulatrix Rock Imager system. Images are accessible to users and can be scored via a web-interface. Plates can also be stored at 4°, 17°, 24°C and 37°C and inspected through a Leica stereo-microscope.



Data Collection and Structure Solution

The department is equipped with a MicroMax 007 HF rotating anode source with VariMax HF optics and a Saturn 944 CCD camera from Rigaku for the collection of data.

High resolution and anomalous data are regularly collected at synchrotron sources including the ESRF (Grenoble), Soleil (Paris), SLS (Zurich) and DESY (Hambourg).

Data can also be collected under humidity controlled conditions using a Free Mounting System from Proteros and a MAR345 image plate coupled to the second port of the MicroMax 007 HF generator.

Data can also be collected directly from the drop using Crystalquick™ X plates mounted on the FIP-BM30A beamline at the ESRF.