1. Holzmann et al. RNase P without RNA: identification and functional reconstitution of the human mitochondrial tRNA processing enzyme. Cell (2008), 135, 462-74.
2. Gobert et al. A single Arabidopsis organellar protein has RNase P activity. NSMB (2010), 17, 740-4.
3. Gobert et al. Structural insights into protein-only RNase P complexed with tRNA. Nat Commun (2013), 4, 1353.

L16

CHARACTERIZATION OF DNA-BINDING PROTEINS

Christian Biertümpfel

Max Planck Institute of Biochemistry, Am Klopferspitz 18, 821 52 Martinsried, Germany

All organisms use a plethora of proteins interacting with DNA. They fulfill essential roles during cell homeostasis and proliferation. The functions of DNA-binding proteins are divers and reach from modifying, labeling, checking, processing, regulating, opening, locking, shielding, degrading, editing, transcribing to structuring DNA. Studying DNA-binding proteins is often a challenging task as their function and abundance is tightly controlled, and usually restricted to certain conditions, functional states or cell cycle stages. However, remarkable findings about their cellular roles can be discovered when taking the properties and functional requirements of DNA-binding proteins into account. The interaction with DNA facilitates additional strategies for the purification of DNA-binding proteins and it offers many methods for their functional characterization. The presentation will focus on special features of DNA-binding proteins and techniques for their functional characterization. In addition, various ways of forming, stabilizing and analyzing protein-DNA complexes will be discussed. Understanding the mode of action of proteins interacting with DNA is crucial not only to comprehend complex biological processes but also to shed light onto their defects and roles in diseases like cancer.

Wednesday, June 28, Session VI

L17

FROM PROTEIN CHEMICAL MODIFICATION TO CROSS-LINKING AND BEYOND

Petr Novák

Institute of Microbiology, v.v.i., BIOCEV, Prumyslova 595, Vestec, Czech Republic

Even the first protein structural models were built using X-ray crystallography and NMR spectroscopy sixty and thirty years ago, respectively. There are still many protein sequences and protein complexes with unknown 3-D structure. The tremendous progress in mass spectrometry in last decades opened the space for studying protein folding, protein/ligand interactions and protein dynamics in solution. The potential of ion mobility, chemical cross-linking and hydrogen/deuterium exchange for structural biology will be discussed.

This work has been supported by grants from the Ministry of Education, Youth and Sports of the Czech Republic (LH15010, LD15089), the Czech Science Foundation (16-24309S) and European Regional Development Funds (CZ.1.05/1.1.00/02.0109).

L18

NMR TOOLBOX FOR PROTEIN STUDIES

Pavel Srb

Institute of organic chemistry and biochemistry, Czech Academy of Sciences, Flemingovo nam. 2, 166 10 Praha 6

We are witnessing a silent revolution in application of the NMR spectroscopy to biological questions. NMR together and X-ray crystallography complement each other in structural studies of proteins and their complexes. I will briefly cover basic principles of NMR relevant for biomolecular studies, quickly moving to examples of handiness of NMR for projects involving proteins and protein interactions with either small or bigger molecules. NMR spectroscopy can not compete with crystallography in speed and straightness but, unlike it, often, using very simple experiments, can answer specific questions about protein interactions with proteins, nucleic acids or drugs. Especially, when it comes to more specific questions and the structure is not the ultimate goal.

L19

CURRENT CHALLENGES OF OBTAINING CRYSTALS FOR X-RAY AND NEUTRON MACROMOLECULAR CRYSTALLOGRAPHY

M. Budayova-Spano, N. Junius, C. Berzin, Y. Sallaz-Damaz, J.L. Ferrer

Institut de Biologie Structurale, UMR5075 CEA-CNRS-UGA, Grenoble, FRANCE
monika.spano@ibs.fr

Knowledge of the phase diagram has key importance when designing and controlling a crystallization process for a substance. In the case of proteins, accurate phase diagram data is limited due to the complexity of their structure caused by the diversity of the amino acid residue groups that form proteins, with this process being easily influenced by environmental conditions. The solubility of a protein depends strongly on the protein-protein interactions as well as on the protein-solvent interactions. Any slight modification of the composition can influence the solubility dramatically, or even alter the nature of these macromolecules. Independently of the complexity of protein behaviour, the phase transformation is still governed by both the thermodynamics and the kinetics of the system. It is still possible to describe all this information in phase diagrams. In the case that crystallization conditions or nucleation points are identified the information can be plotted in phase diagrams, represented in a simplified form in Figure 1, and in this case the information that is provided relates to both thermodynamics and kinetics. Thermodynamic data are the solubility curves, the presence of metastable phases, polymorphs, liquid-liquid separation… They depend on multiple parameters such as temperature, pH, solvent, impurities, etc. In addition, kinetic trajectories in the phase diagram are relevant to control most of the final properties of the synthesized crystals. The path followed in the diagram controls the nucleation and growth of the crystals, and thus their number, size, and morphology.

Two new and emerging uses result in specific challenges for crystallization of proteins. In both, precise control of crystal size is essential. New approaches to serial (time-resolved) crystallography, where crystals in the 1-20 m size are used to solve structures including those/structures of short-lived intermediates with reactions initiated by light or rapid mixing. Serial crystallographic methods are being increasingly used at synchrotron sources (serial synchrotron crystallography) due to advances in micro- and nano-focus beamlines, as well as at rapidly developing ultra-bright free-electron laser sources (serial femtosecond crystallography [1]), enabling structure determination of previously intractable proteins. At the other extreme are the requirements of the next-generation flagship neutron sources, such as the ESS (European Spallation Source, Lund). Because neutrons interact very weakly with matter, much larger, and ideally cubic crystals are needed with volumes of > 0.01 mm^3 (i.e. 200 m on a side) for neutron crystallography [2]. This is, however, the only way to visualise all of the protons in a protein structure, important information for drug design.

The detailed knowledge of the phase diagram is at the basis of the devices [3-6] we have developed especially with the focus on X-ray and Neutron Macromolecular Crystallography. The 1st generation instrument combines the use of temperature control and seeding and allows for grow of large crystals in crystallization batch [3]. A crystallization batch in the metastable zone is seeded with small protein crystals. The seeds are maintained inside this region of the phase diagram for as long as possible by doing a temperature step each time the crystal solution equilibrium is achieved. The temperature steps are repeated until crystals of suitable size for diffraction measurement are obtained.

The 2nd generation instrument, represented in Figure 2, adds new functionality to the first instrument thanks to a fluidic cell enabling to perform a temperature controlled dialysis crystallization experiment [4,5]. The new crystal growth apparatus combines accurate temperature control with control of the chemical composition of the crystalliza-

tion solution and therefore it allows very sophisticated experiments to be performed. Systematic phase diagrams in multi-dimensional space can be investigated using far less protein material than previously. We have demonstrated that it can be beneficial to provide sufficient scattering volumes for neutron studies that require large-volume well-ordered single crystals. Based on this macro-scale instrument we have also conceived a miniaturizing apparatus that allows precise control of the experiment parameters using microfluidics [6]. The functional microfluidic chips integrating microdialysis with the volume less than 1 µL have been already successfully tested with model proteins. The microchips have multiple designs in order to achieve single or multiple crystallization experiments at the same time. They are transparent to X-ray radiation and allow performing *in situ* X-ray crystallography experiments at room temperature. The recently developed fluidic devices, once adapted, are expected to be useful in monitoring and controlling the crystallization processes of challenging biological macromolecules, such as membrane proteins.

1. H.N. Chapman, *Synchrotron Radiation News*, **28**, (2015), 20.
2. M.P. Blakeley, S.S. Hasnain, S.V. Antonyuk*, IUCrJ,* **2***,* (2015), 464.
3. M. Budayova-Spano, F. Dauvergne, M. Audiffren, T. Bactivelane, S. Cusack, *Acta Cryst. D.*, **D63**, (2007), 339.
4. M. Budayova-Spano, *Patent FR10/57354, UJF*, (extension : EP117730945, US13821053, JP2013528746), (2010).
5. N. Junius, E. Oksanen, M. Terrien, C. Berzin, J.L. Ferrer, M. Budayova-Spano, *J. Applied. Cryst.*, **49**, (2016), 806.
6. M. Budayova-Spano, N. Junius, J.B. Salmon, *Patent FR2016/053185,* (2016).

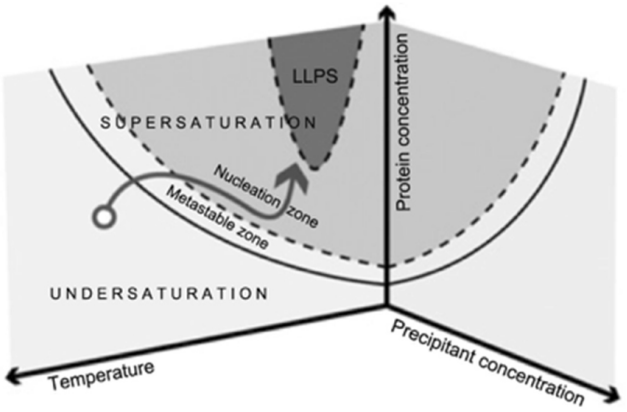

Figure 1. Schematic view of a multidimensional phase diagram. The arrow illustrates a specific pathway taken during crystallization.

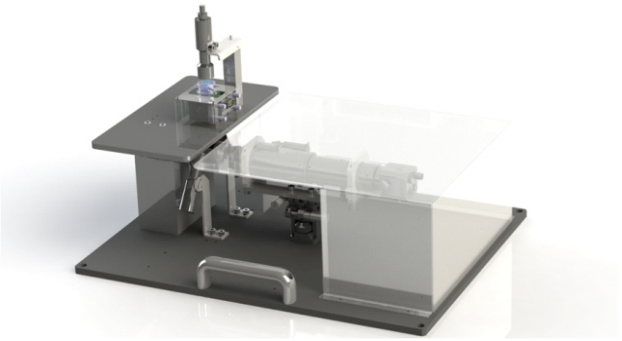

Figure 2. Simplified view of a crystallization apparatus for temperature controlled flow cell dialysis with real time visualization.

L20

CURRENT TRENDS IN IMMUNOLOCALISATION ON ULTRASTRUCTURAL LEVEL

Jana Nebesářová

Faculty od Science, University of South Bohemia, České Budějovice, Czech Republic
Biology Centre of CAS, v.v.i. – Institute of Parasitology, České Budějovice, Czech Republic
nebe@paru.cas.cz

The immune-electron microscopic localization of molecules of interest by monoclonal and polyclonal antibodies in cells and tissues has become an indispensable routine technique in numerous fields of biological and biomedical research. It is based on a specific bond between antigen and specific antibodies conjugated with a high-density gold nanoparticle tag, which can be visualized usually by transmission electron microscope (TEM). The aim of my contribution is to give an overview of new methods of immunolocalization aimed at simultaneous multiple detection of molecules of interest, which are based on using low voltage electron microscopy and correlative light and electron microscopy.