P8-4

CRYSTALLOGENESIS STUDIES OF BACTERIAL AMINOACYL-tRNA SYNTHETASES AS POTENTIAL TARGETS FOR DRUG DESIGN

R. de Wijn, B. Lorber, C. Sauter

Architecture et Réactivité de l'ARN, Institut de Biologie Moléculaire et Cellulaire (IBMC-CNRS), Université de Strasbourg, 15 rue René Descartes, F-67084 Strasbourg, France
r.dewijn@ibmc-cnrs.unistra.fr

Aminoacyl-tRNA synthetases (aaRSs) are ubiquitous enzymes that catalyze the first step of protein biosynthesis or translation. In the presence of ATP, they activate the amino acids as adenylates and subsequently bind the amino acid moity onto the 3' CCA end of transfer RNAs [1]. The resulting aminoacyl-tRNAs are then carried by the elongation factors to the ribosome to be incorporated into nascent polypeptide chains. AaRSs are a target of choice for drug design because they are essential enzymes having a high specificity for their substrates.

Our study is focused on bacterial aspartyl-tRNA synthetases (AspRSs) that bind specifically L-aspartate. We have initiated the structural characterization of the binding mode of two families of inhibitors. One is a natural antibiotic produced by *E. coli* strains that targets the catalytic site of AspRSs (collaboration with Prof. S. Rebuffat, Museum National d'Histoire Naturelle, Paris) and the other a series of chemically synthesized peptides that were selected against an AspRS from the opportunistic human pathogen *Pseudomonas aeruginosa* (collaboration with Prof. Hiroaki Suga, University of Tokyo).

We apply various crystallogenesis approaches to prepare crystals that are suitable for the X-ray diffraction analysis. They involve the optimization of crystal production either by cocrystallization or by soaking of native crystals with ligands. In the case where the enzyme of one bacterial species does not yield exploitable crystals, the protein is either chemically methylated to change its surface properties and crystallizability, or a close structural homolog with a conserved active site is substituted to take advantage of genetic diversity. In the final step crystallization systematically takes place in an agarose gel with a low gelling temperature to improve crystal quality, stability during the soaking with inhibitors, and handling [2]. The rationale of our crystallogenesis strategy will be presented and illustrated with examples.

1. M. Ibba, C. Francklyn and S. Cusack. *Aminoacyl-tRNA Synthetases*, Goergetown, TX: Landes Biosciences. 2005.
2. B. Lorber, C. Sauter, A. Théobald-Dietrich, A. Moreno, P. Schellenberger, M.-C. Robert, B. Capelle, S. Sanglier, N. Potier, R. Giegé. *Crystal growth of proteins, nucleic acids, and viruses in gels. Progr. Biophys. Mol. Biol.*, **101** (2009), 13-25.

Posters - Teaching Crystallography

P10-1

CRISTALES: A WORLD TO DISCOVER. AN EXHIBITION FOR SCHOOLS AND UNIVERSITIES

Juan Manuel García-Ruiz, Fermín Otálora, Alfonso García-Caballero, Luis A. González-Ramírez, Cristóbal Verdugo-Escamilla

Laboratorio de Estudios Cristalográficos, Instituto Andaluz de Ciencias de la Tierra (CSIC-UGR). Avenida de las Palmeras 4, Armilla, Granada. 18100, Spain.
juanmanuel.garcia@csic.es

The exhibition *CRISTALES: a world to discover* is a teaching/outreach activity whose main goals are to increase awareness of the importance of crystallography and its role in everyday life in modern society, motivate young people, and promote education and research in crystallography.

CRISTALES is designed to inspire the audience with a careful design and a view of crystallography that places the emphasis not only on the most important contributions of crystallography to society´s welfare, including new materials and biomedical research, but also on those aspects of crystallography related to art and the mind.

This work describes the simplest version of the exhibition, composed of 14 posters that have been created specifically for schools and universities. Each poster displays an image that is both aesthetically powerful and scientifically intriguing, so as to provoke the curiosity of the students. The posters also contain a brief text explaining the image and its relation to crystallography and a QR code that links the poster to a web page containing further information [1].

1. J. M. García-Ruiz, F. Otálora, A. García-Caballero, L. A. González-Ramírez, C. Verdugo-Escamilla, *J. Appl. Cryst.*, **48**, (2015), 1264.

P10-2

THE MICROGRAVITY PROGRAM'S BIOPHYSICS INITIATIVE

S. Gorti, George C. Marshall

Space Flight Center, National Aeronautics and Space Administration, Huntsville, AL 35812

Biophysical microgravity research on the International Space Station using biological materials has been ongoing for several decades. The well-documented substantive effects of long duration microgravity include the facilitation of the assembly of biological macromolecules into large structures, e.g., formation of large protein crystals under μ-gravity. NASA is invested not only in understanding the possible physical mechanisms of crystal growth, but also promoting two flight investigations to determine the influence of μ-gravity on protein crystal quality. In addition to crystal growth, flight investigations to determine the effects of shear on nucleation and subsequent formation of complex structures (e.g., crystals, fibrils, etc.) are also supported. It is now considered that long duration microgravity research aboard the ISS could also make possible the formation of large complex biological and biomimetic materials. Investigations of various materials undergoing complex structure formation in microgravity will not only strengthen NASA science programs, but may also provide invaluable insight towards the construction of large complex tissues, organs, or biomimetic materials on Earth.

Posters - Neutron Diffraction

P11-1

X-RAY COMPATIBLE MICROFLUIDIC DEVICE FOR PROTEIN CRYSTALLIZATION AND MAPPING PHASE DIAGRAMS

N. Junius[3,1,2], Y. Sallaz-Damaz[2,1,3], F. Borel[3,1,2], J-L. Ferrer[3,1,2], J-B. Salmon[4], M. Budayova-Spano[1,2,3*]

[1]Univ. Grenoble Alpes, IBS, F-38044 Grenoble, France
[2]CNRS, IBS, F-38044 Grenoble, France
[3]CEA, IBS, F-38044 Grenoble, France
[4]CNRS, Solvay, LOF, UMR 5258, Univ. Bordeaux, F-33600 Pessac, France
**monika.spano@ibs.fr*

We have developed the prototype of an integrated apparatus for the rational optimization of crystal growth by mapping and manipulating temperature-precipitant concentration phase diagrams [1]. This so-called crystallization bench comprises a flow cell dialysis set-up to exchange crystallization conditions and control temperature during experiment.

Based on this macro-scale instrument we have focused on a miniaturizing apparatus that allows precise control of the experiment parameters using microfluidics. The first functional microfluidic chips integrating microdialysis with the volume less than 1μL already exist [2]. These microchips have multiple designs in order to perform single or multiple crystallization experiments at the same time. As a proof of principle, the experiments, notably using dyes, have been performed to demonstrate the high resistance of the dialysis membrane, its proper integration in the chip as well as lack of any leakages during the crystallization experiments.

The success of these preliminary studies allows to drive crystallization experiments with model proteins like lysozyme and a plant kinase. The chemical composition in the chip can be systematically exchanged during crystallization experiment using a pressure-driven pump. It enables to investigate a multidimensional phase diagrams.

The materials that compose the chips have been chosen carefully and tested at the ESRF on synchrotron beamline FIP-BM30A in order to limit significant scattering background. These results were compared to traditional crystallization plates used for in-plate crystallization. We demonstrate that these chips are X-ray compatible allowing to collect *in-situ* diffraction data at room temperature of a large number of protein crystals grown on the chip.

1. Budayova-Spano, M. (2010). Patent FR10/57354, UJF, (extension: EP117730945, US13821053, JP2013528746).
2. Budayova-Spano, M., Junius, N., Salmon, J-B. (2015) Patent FR1561715, UJF.