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THE MICROGRAVITY PROGRAM'S BIOPHYSICS INITIATIVE

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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

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

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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.

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"GROWING LYSOZYME CRYSTALS TO THE SIZE OF MM^3 FOR NEUTRON DIFFRACTION"

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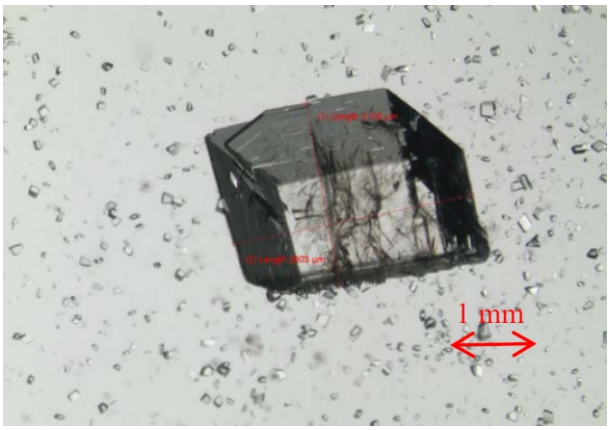
Crystallization remains a bottleneck for protein structure determination, and it can be a challenge to get crystals that are of a size that enables structure determination by use of X-ray crystallography. Crystals of a size around 10^5 μm^3 are sufficient for data collections at most synchrotrons, but with the use of microfocus beamlines crystals smaller than 10^3 μm^3 are used for data collection and structure determination. The X-ray synchrotron beamlines have been automated and improved so data collections can be performed in minutes. Compared to this there are major challenges associated with obtaining crystals of a size that will enable structural studies at high resolution of proteins by neutron diffraction. Here mm^3 size protein crystals are needed and it requires days of data collection.

To compare high resolution data (better than 1.2Å resolution) collected on crystals of Hen Egg White Lysozyme (HEWL) from both an X-ray source and a neutron source, triclinic crystals of HEWL are required that should be larger than 5.0 mm^3 in size.

Triclinic crystals of HEWL are difficult to obtain, but following the procedures by Heijna *et al* and Legrand *et al* a reproducible path for making triclinic HEWL was achieved. The procedure involves a precipitation step at 4°C, before crystal growth at room temperature.

Optimizing batch crystallization conditions starting with 0.2 M $NaNO_3$, 0.05 M Na-acetate pH 4.5 and a protein concentration in the drop of 10 mg/ml, using repeated serial macro seeding and months of growing we have obtained more than 10 crystals bigger than 5 mm^3 for planned neutron diffraction experiments at ILL, Grenoble.



1. M.C.R Heijna, P.B.P van den Dungen, W.J.P van Enckevort, E. Vlieg, Crystal Growth and Design, **6**, (2006), 1206.
2. L. Legrand, M. Ries-Kautt, M. Robert, Acta Crystallogr. Sect. D, **58**, (2002), 1564.