

## Use of Microgravity for the Preparation of the Large Volume Crystals Required in Neutron Diffraction Studies.

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Neutron crystallography provides direct positional and occupancy information of hydrogens (deuterons) in macromolecules. The signal from hydrogens in X-ray diffraction is very weak and their positions only appear at very ultra-high resolution, 1 Å and beyond. Neutron scattering from deuterons is comparable to carbon and nitrogen. Using joint refinement, the combination of X-ray and neutron diffraction data, deuterons will appear as spherical peaks in the difference maps. Joint refinement is accomplished using modules available in both Phenix and nCNS. Combining data can be challenging where data completeness and accuracy are magnified in the Fourier transform. The flux of the reactors and spallation sources generating neutrons are many orders of magnitude less than current X-ray sources. To overcome the issues of low flux, very large volume crystals, typically > 1 mm<sup>3</sup>, are necessary. Even with extremely large crystals, the difference in resolution is quite pronounced. Our current studies of aspartate amino transferase utilize X-ray data that extends to 1.2 Å while our neutron data, acquired at ILL Grenoble, extends to 2.1 Å<sup>1</sup>. To improve crystal size and quality, we are utilizing the microgravity environment provided by the International Space Station. Our initial experiments flew on SpaceX CRS4 in 2014 in which we utilized the High-Density Protein Crystal Growth (HDPCG) apparatus. Most recently, we tested a new apparatus, which we have nicknamed the “Toledo Crystallization Box (TCBs)”, that utilizes capillary dialysis. Hydrogenated proteins were flown on SpaceX CRS15 in June, 2018 and improvement in both size and quality are noted. The duration of flight was five weeks and the setups were retrieved early August. Initial inspection showed that nucleation had occurred. Growth was limited but negligible defects appeared. Crystal quality was measured at APS IMCA 17-ID with improvements in both resolution and mosaicity noted. A next generation of TCBs are included in the payload manifest for SpaceX CRS18 July, 2019 and will carry both hydrogenated and perdeuterated proteins. These experiments involve minimal astronaut intervention where the samples are moved from the Dragon capsule to a storage locker and back. A new program referred to as “Real Time Protein Crystal Growth”, proposed by a consortium of investigators, is a series of experiments in which the astronauts will complete crystals setups on the ISS.

<sup>1</sup>Dajnowicz, S., Johnston, R.C., Parks, J.M., Blakeley, M.P., Keen, D.A., Weiss, K., Gerlits, O., Kovalevsky, A., Mueser, T.C., (2017) Nature Communications 8 (1), 955.

See also, Neutron Diffraction Studies of PLP-Dependent Enzymes, Victoria N. Drago, this meeting.