Crystal Growth in Microgravity for Neutron Diffraction Studies

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Single crystal diffraction methods provide high-resolution views of macromolecules. The X-ray diffraction signal is correlated to the number of electrons associated with an atom and hydrogen is essentially unobservable. In neutron diffraction, the signal is correlated to the atomic scattering length and deuterium is nearly equivalent to carbon, nitrogen, and oxygen. The joint refinement of X-ray and neutron diffraction data from crystals of perdeuterated protein provides a complete atomic model. The importance of complete models for our computational studies justifies the effort involved in completing a neutron diffraction structure. The flux of neutrons at spallation (SNS, ORNL) and reactor (HFIR, ORNL and ILL, Grenoble) sources are several orders of magnitude less than X-rays available at synchrotrons. To obtain a neutron data set, the crystals need to be very large, on the order of 1 mm^3 or greater. Grown in unit gravity, crystals of this size are typically highly mosaic and full of defects which decrease coherency and limit resolution. In microgravity aboard the ISS, crystals grow larger and more uniform. For our first attempt (Space X-CRS4, 2014), we used the High-Density Protein Crystal Growth (HDPCG) apparatus, a vapor diffusion setup manually activated on the ISS. While our crystals improved in quality, the size remained similar to unit gravity. Another apparatus approved for microgravity experiments (but no longer available), the Grenada Crystallization Box (GCB), utilized capillary liquid diffusion and reports indicate improvement in both size and quality. We have created a new liquid diffusion setup comparable to the GCBs nicknamed the Toledo Crystallization Box (TCBs). The TCBs are simple, inexpensive, and very small in comparison to the other available apparatus. The new setup works with all precipitating agents and uses the neutron mounting capillaries to eliminate crystal transfer. The ability to adjust the timing of equilibration to match the duration of flight has been evaluated. Several hydrogenated samples are planned for the next flight (Space X-CRS15, 2018) followed by perdeuterated samples (Space X-CRS16, 2018).