Microgravity crystallization for improving uniformity and homogeneity of crystals for time-resolved diffusive mixing XFEL experiments

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Riboswitches control gene expression through ligand-driven conformational changes that switch transcription or translation ON/OFF in response to intracellular ligand concentrations. In 2016, we demonstrated, for the first time, the capability of serial crystallography using an X-ray free electron laser (XFEL) using very small (1-5 μm) crystals for time-resolved crystallographic (TRX) measurements to study molecular conformational changes of the adenine riboswitch aptamer (riboA) upon ligand binding. We also demonstrated that the crystal phase transition that accompanies conformational switching involves at least three unique crystal lattices, driven by the ligand-induced conformational switch and molecular reorganization. We determined the structures of riboA in each of these lattice states. However, further derivation of transitional structures from the diffraction data is complicated by factors such as crystal non-uniformity and heterogeneity. In collaboration with the Center for the Advancement of Science in Space (CASIS), we aim to investigate the effects of crystallization under microgravity on lattice order and the uniformity of the ligand-induced phase transition. By mitigating detrimental effects of gravity and convection on nucleation and crystal growth, our goal is to obtain a crystalline sample of higher quality and homogeneity that is most suitable for riboA TRX experiments. Using an XFEL, the study will compare the diffraction of crystals generated under 1) atmospheric conditions (reference sample), or 2) microgravity aboard the International Space Station, before (no ligand) and after (premixed with ligand) the phase transition. In collaboration with TechShot, Inc. we have successfully tested and verified large-volume batch crystallization of riboA crystals using a syringe-based mixing device that will be utilized by astronauts aboard the ISS.