Preparation and Characterization of Samples for Imaging Using X-Rays from Free Electron Lasers

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Structure determination of biological particles is ever evolving and has come a long way. Many techniques were discovered to overcome the shortcomings of the previously used methods. In this ever changing field of structure determination, we are now looking into using x-rays from free electron lasers (X-FEL) as the next stage of evolution.

X-FEL uses a bunch of fast moving electrons from the electron source for the production of the coherent x-rays. These free electrons are used as the active media for the production of an x-ray laser and thus the name 'Free Electron Laser'. The high resolving and penetrating power of x-rays can be used to image the surface characteristics and unique internal arrangements of the viral particle.

The sample selection for the experiment was based on criteria that they have consistent size and shape with a particle size that fits in the XFEL beam focus and ease with which they can be produced in lab conditions. With these basic criteria in mind we selected MS2, PR772 and Gold Nanoparticles. MS2 is a 26nm icosahedral coliphage belonging to Leviviridae with +ssRNA genome and infects F+ E.coli only. PR772 is a 53nm sized lipid containing bacteriophage belonging to the Tectiviridae family. It is icosahedral and consists of +dsDNA. Gold Nanoparticles are octahedra which can be tailored to required size by Polyol process. These samples were prepared for the structure analysis at LCLS, SLAC Stanford, USA in the month of May, 2013.

The viral samples were prepared by growing the phage particle on the host cells. They were purified by using tangential flow filtration followed by size exclusion chromatography. We could produce $10^{10} – 10^{11}$ PFU/mL particles. The Gold octahedra were prepared using Polyol process by reducing gold salt to metallic gold. The size of the tailored particles was about 100nm.

A preliminary study of viability with MS2 at different stages of sample preparation and delivery showed that there is a decrease in viability of the sample at every stage. This emphasises the need of high PFU/mL of viral particles and also to refine the preparation and delivery process to reduce the stress on the sample particles.

In future, it would be beneficial to understand how different biological particles behave in different volatile buffers and the effect of beam line conditions on various other particles. A study on the types of forces that the particles are subjects to during the sample injection and delivery process could also help in reducing the damages by these forces on the particles.

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