Calibration of Beamline 17 ID-BM Using Bovine Insulin

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Abstract

Beamline 17 ID-BM is operated by IMCA, a collection of pharmaceutical companies, to analyze samples for drug discovery. Since all beamlines are unique many who use it spend an unnecessary amount of time shooting their samples due to the unknown calibration settings of the beamline. The student researchers propose to provide other beamline users with statistical data of preset calibration settings that will help them understand the parameters of beamline 17 ID-BM. This will potentially reduce the time that is needed to shoot their samples; therefore, beamline users' time would be more cost-effective.

The student researchers have chosen to use insulin as their experimental molecule in order to refine the beamline settings needed to find the optimal image resolution. Insulin was chosen because it is easily accessible and potentially has similar characteristics to other samples that are tested on beamline 17 ID-BM. Although other beamline users may not use insulin, the statistical data collected by the student researchers on the beamline may be used as a starting point for the testing of their sample. The students manipulated x-ray exposure time and beamline attenuation while keeping the x-ray dose constant.

Procedure

1. All chemicals were obtained from Sigma Aldrich and Flinn.
   From Sigma Aldrich: insulin from bovine pancreas (I5500) and ethylenediaminetetraacetate acid trisodium salt hydride (ED3SS).
   From Flinn: disodium phosphate (s0100), glycerol (G007), sodium Phosphate (s0101), and sodium acetate (S0037).
2. A protein solution was created containing 20 mg ml^{-1} insulin in 20 mM Na_2HPO_4 and 10 mM Na_3EDTA pH 10.10. A reservoir solution was created containing 175 or 256 mM Na_2HPO_4/Na_3PO_4 (pH 10.12) and 10 mM Na3EDTA.
3. Bovine insulin crystals were prepared using hanging drop method by mixing 4uL of protein solution with 4uL of reservoir solution and equilibrating the drop against the reservoir.
4. Crystals appeared overnight and were allowed to grow undisturbed for 1-2 weeks.
5. A cryo-protectant solution was created containing 175 mM Na_2HPO_4/Na_3PO_4 pH 10.10, 10 mM Na_3EDTA and 30%(v/v) glycerol.
6. Crystals were placed on loops, immersed in cryo-protectant, and kept under liquid nitrogen conditions.
7. The x-ray dose was kept constant by manipulating exposure time and beam attenuation. Samples were examined using APS beamline 17 ID-BM and the resulting data was analyzed using autoPROC 1.1.7.

Results/Conclusion

The purpose of this experiment was to determine if a shorter or longer exposure time would yield clearer images of crystals if the x-ray dosage was kept constant. Our data shows that an increase in exposure time resulted in a higher resolution and thus a clearer image of crystals. This is likely due to the fact that we kept our overall radiation dose constant, so as our exposure time increased, the radiation intensity was decreased. Lower intensities are not as likely to damage the crystals during the imaging process even over longer exposure times.

The data analysis gave us multiple R factors of which we used R-merge on all I+ and I-. R factors are a measure of how different our actual data is compared to theoretical, or calculated, data. An R-factor of less than 10% is generally considered to indicate a good data set. Our higher exposure times yielded inner shell R-merge values as well as overall R-merge values close to or under 5%.

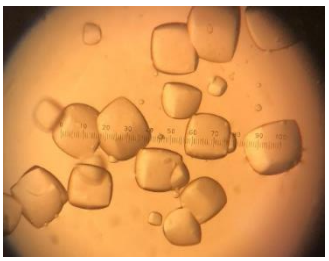

Fig. 1: Bovine Insulin Crystals

Fig. 2: Harvesting of insulin crystals

Fig. 3: The research team at Beamline 17 ID-BM

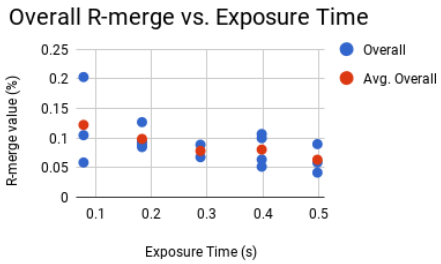

Fig. 4: Overall R-merge vs Exposure Time

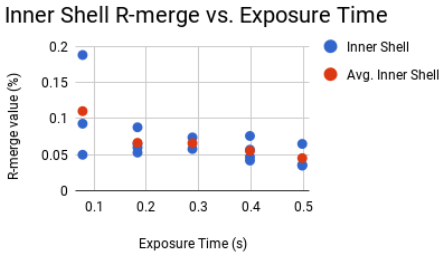

Fig. 5: Inner Shell R-merge vs Exposure Time

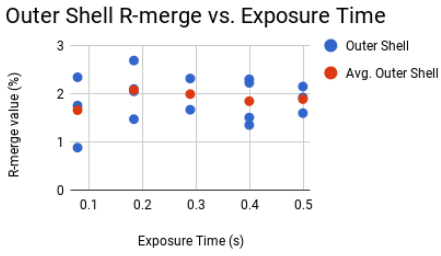

Fig. 6: Outer Shell R-merge vs Exposure Time

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