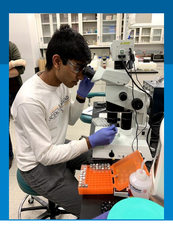
Sulfur Single-Wavelength Anomalous Diffraction

Sahaja Danthurthy, Pravallika Padyala, Hariharan Kannan, Metea Valley High School



Abstract

The purpose of this experiment was to learn the mechanics of x-ray crystallography in conjunction with modern technology in order to discern the atomic and molecular structure of bovine insulin. Bovine insulin is a two-chain polypeptide hormone found in the pancreatic cells of cows, and it was chosen as the subject protein due to easy accessibility and an easily comprehensible structure. Initially, the bovine insulin was crystallized with the hanging drop method within a well of reservoir solution made up of Na3PO4, H2O, and EDTA, yielding cuboidal crystals of medium-large size. At the Advanced Photon Source at Argonne National Laboratories, the S-SAD process was used, a method in which a particle accelerator shoots electrons at extremely high velocities to find the structure of the molecule. The crystallized samples were placed on nylon loops and shock-cooled before being placed into the beamline. These crystals were hit by high-powered x-ray photons generated from the advanced photon source. While on the beamline the sample will be turned 180 degrees. This will create an electron configuration chart from the diffractions of the photons based upon the position of the electrons in the samples. The data will then be analyzed then used to map the structure of the protein bovine insulin.

Motivation

Understanding the structure of bovine insulin is valuable to medical researchers and pharmaceutical companies, as with the structure they can determine what it does and thus treat disease relating to it easier. Insulin is a crucial player in diabetes mellitus, and bovine insulin can be used as a therapeutic for patients suffering from this condition. Furthermore, the S-SAD process can be utilized to determine the structures of other proteins that are important to medicine and the human body, allowing researchers to further their knowledge of proteomics and future pharmaceuticals. This has a great potential application to medicine and can allow researchers to figure out new ways to kill proteins in cancerous cells or discover novel therapeutics. The research we performed was to test the efficiency and accuracy of the S-SAD data process while determining the structure of bovine insulin.

Procedure

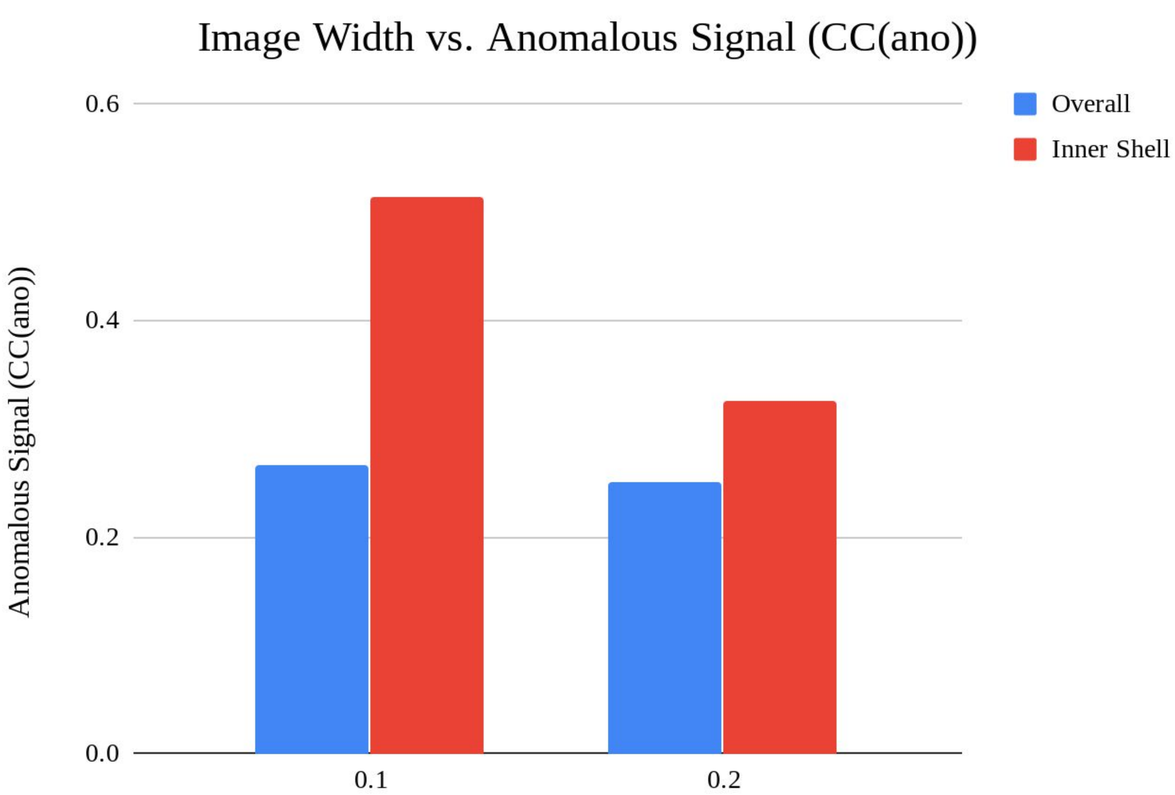
Hang Drop Bovine Insulin Crystallization Procedure:

1. Make 6 different 1M stock solutions of the Na2HPO4 protein solution at each pH (9.8, 10.0, 10.2, 10.4, 10.6, and 10.8) using 1M NaOH to reach target pH.
2. Microcentrifuge bovine insulin solution to settle out undissolved insulin
3. Add 10 µl of 1M Na3 EDTA into each reservoir
4. Add an appropriate amount of 1M Na2HPO4 to wells to reach target concentrations.
5. Fill each well to the 1 ml level with distilled water.
6. Pipette protein and reservoir solution (with the same concentration as in the well) amounts into a drop on each lid on each well (24). One drop:
   a. 4 µl of protein solution
   b. 4 µl of reservoir solution (what's in the well)
7. Label reservoir plate "Bovine insulin" and write down the conditions of each well
8. Let sit for a few days for crystals to form.
9. After a few days use a microscope to determine which wells have usable crystals

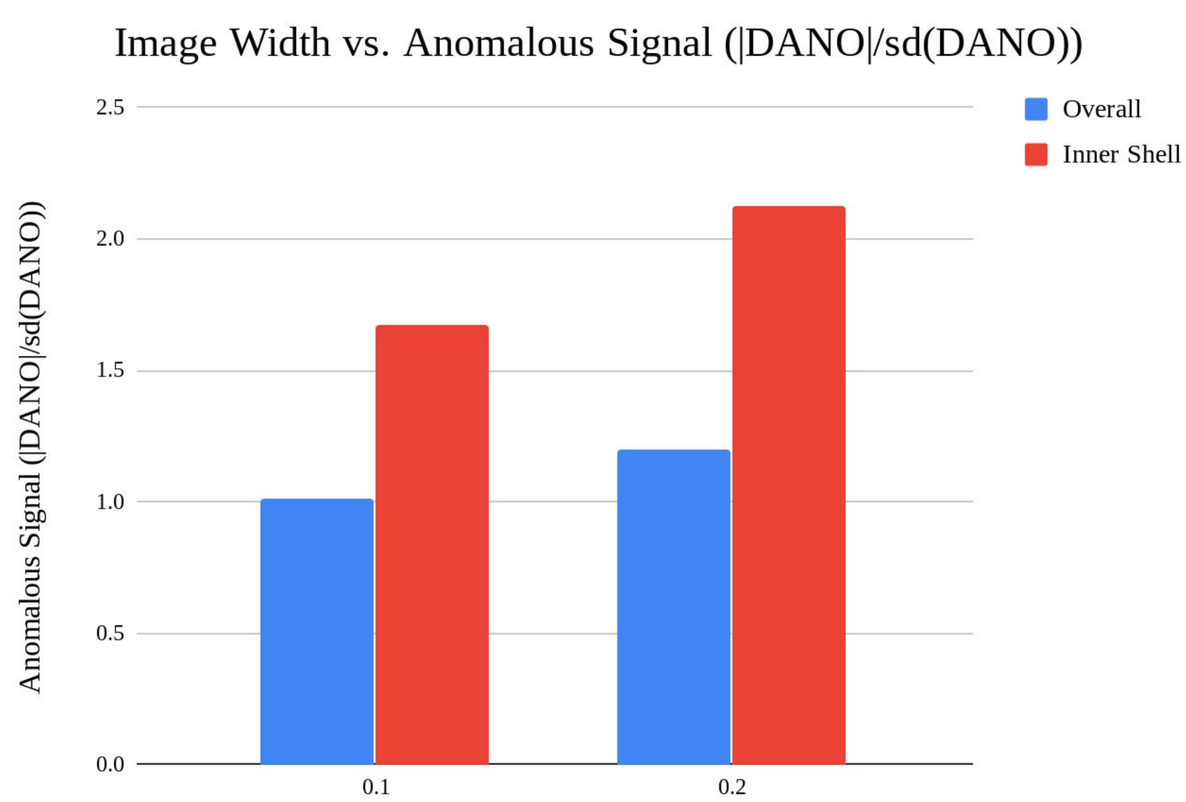
Upon travel to Argonne National Laboratories...

10. Take a puck and completely submerge it in a bowl of liquid nitrogen. Fill bowl as needed as the nitrogen boils off through step 17
11. Prepare a petri dish by placing dam paper towel around the edge and wiping the surface off
12. Using tweezers, pop coverslip slides off well and place on a petri dish.
13. Add a separate drop, 2 µl, of the cryogenic solution adjacent to the crystal.
14. Using a microscope and cryogenic loop, scoop up a crystal and place it into the cryogenic solution
15. Wait roughly 10 seconds
16. Scoop the crystal out of the cryoprotectant solution and place the entire sample pin in the puck submerged in liquid nitrogen
17. Repeat steps 10-16 until there is a large sample of crystals
18. Place the puck in a dewar full of liquid nitrogen face down and use the cap screw to remove the top so only the lid with the samples are in the dewar
19. Set desired image width, number of images, transmission, and exposure time.
20. Using computer program and robot, center the crystal on all axis and specify zones for x-ray beams to hit, be sure to cover the whole crystal as it is often hard to see
21. Based on the resulting data, determine the region of crystal that best results and center camera on that region.
22. Once centered on all axis, draw a narrow, vertical zone for x-ray beams to hit.
23. Repeat step 21.
24. Change file name in association to crystal number and click "collect" on computer program.

Graph 1



Graph 2



Conclusion

When analyzing the data with CC(ano) measure, the greatest anomalous signal can be seen in the inner shell with an image width of 0.1 degrees. The inner shell, on average, yielded higher anomalous signals when compared to both the inner and outer shell. Within the inner shell, the image width of 0.1 degrees possessed the strongest signals overall. Based on the data that we have, we cannot determine whether or not fine slicing helps or harms data. With the lack of variety in the data, it is difficult to come to a conclusion on what the advantages and disadvantages of fine slicing are.

References

Faust, A., Panjikar, S., Mueller, U., Parthasarathy, V., Schmidt, A., Lamzin, V. S., & Weiss, M. S. (n.d.). A tutorial for learning and teaching macromolecular crystallography [PDF]. *Journal of Applied Crystallography*.

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Next Steps

In order to truly determine the reliability and efficiency of the S-SAD data collection method, it is required to conduct further trials on Bovine Insulin to receive and analyze more data. Having more data will allow to understand the advantages and shortcomings of the S-SAD method. In the future, depending on the reliability of the S-SAD method, putting it into use in a real world setting can further knowledge of proteomics and pharmaceuticals. Conducting trials that include fine slicing would impact whether or not it helps or harms the amount of anomalous signal yielded.

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