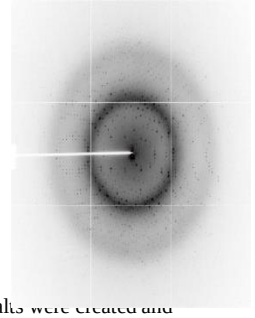
Identifying Protein Crystals Using Known Structures



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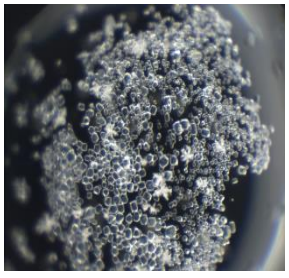
Abstract

Bovine insulin was purchased from a commercial supplier. Using commercially available chemicals, well solutions containing polyethylene glycols, a buffer system, and a variety of salts were created and mixed with the protein solution. Crystallization trails were initiated using the hanging drop method in 24 well plates specifically designed for such experiments. After the protein crystals were grown, they were harvested and frozen in liquid nitrogen using a cryogenic solution to prevent ice formation within the crystals. The crystals were then analyzed using the x-rays at NE-CAT to create data sets. These data sets were then processed using a variety of software suites to produce the three dimensional structure of the protein.

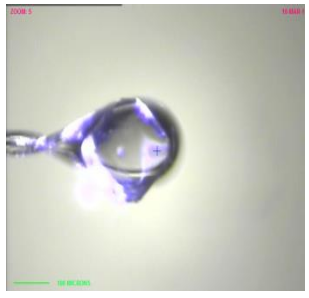
Introduction

Argonne performs a variety of experiments everyday. A selection of Amundsen Students performed a crystallization experiment at Argonne laboratory. During this process, students learned about stock solutions, protein solutions, and the crystallization process. Students made their own solutions, crystallized known protein and observed the results with the help of scientists at Argonne lab.



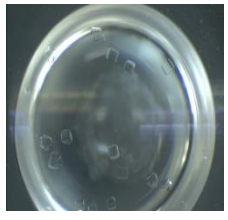
Procedure

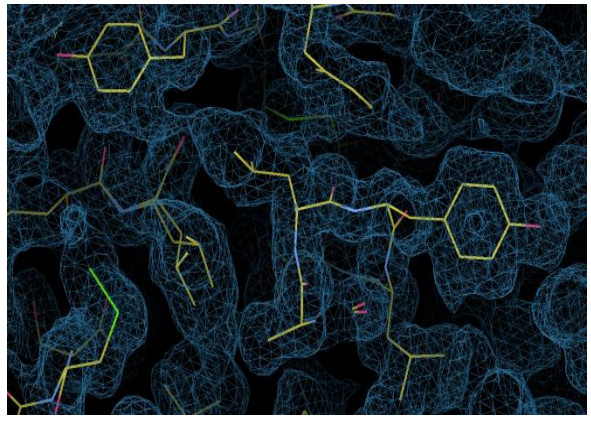
1. Set up 24 well crystallization tray
   Pipette solutions into each well
   Mix solutions in each well
2. Make crystallization drop on silicon cover slip by mixing
   2µlprotein solution with 2µl well solutions. Do not make any bubbles
3. Invert cover slip over well and seal.
4. Label lid with experimenter, experiment, and date.
5. Place in 21˚C incubator.
6. Crystal were beamed in LS-CAT Sector 21 of the APS at 25 keV, 101.9 mA, and .49591 Å for 5 minutes per sample using the optical layout.

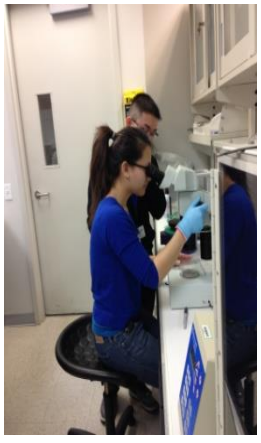


Results and Discussion

Results were viewed via beam line monitor.
After the x-ray of each protein solutions, the results were diffracted. The results of x-raying the protein is various small dots that have to be complied and re-arranged to reveal the structure of the protein. This process can vary in difficulty since different proteins are harder to crystallize. The experiment of Amundsen students to crystallize insulin was successful.







Conclusion

The students were able to complete the process of crystallizing bovine insulin coming up with viable samples that were successfully frozen, ,free from ice crystals and then obtained data from the Photon beam line trials which was processed with software to compare the three dimensional structure of the sample protein to known crystal protein structures.

Acknowledgements

This research was made possible through the Exemplary Student Research Program, supported by Argonne National Laboratory's Educational Programs (CEPA), and conducted at the Advanced Photon Source on the Northeastern Collaborative Access Team beamlines, which are supported by grants from the National Center for Research Resources (5P41RR015301-10) and the National Institute of General Medical Sciences (8 P41 GM103403-10) from the National Institutes of Health. Use of the Advanced Photon Source, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science by Argonne National Laboratory, was supported by the U.S. DOE under Contract No. DE-AC02-06CH11357.Argonne National Laboratory is managed by UChicago Argonne, LLC . Major assistance from Amundsen High School teachers E. Sarhan and C. Hawist.