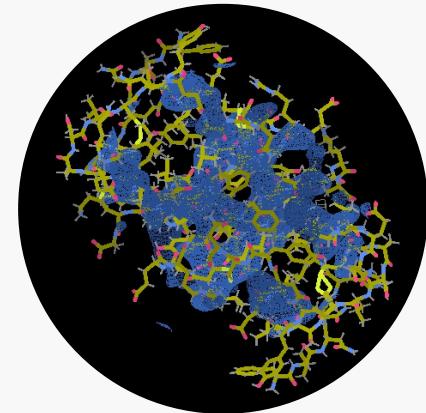


2022

Students on the Beamline

Radiation Exposure on Protein Stability



Meadowridge School

Rayan Ramadan, Tsz Wun Wong, Jason Lee, Steven Wei, Hugh Gao, Grace Yu, Peter Yin, Akira Yoshiyama, Jerome Wong, Isabel Kong, Phoebe Xu

Maple Ridge, Greater Vancouver, British Columbia





Meadowridge School



- Location: Maple Ridge, British Columbia
- Founded: 1985
- International Baccalaureate
- Junior Kindergarten - Grade 12

“Learning to live well, with others and for others, in a just community”



Land Acknowledgement

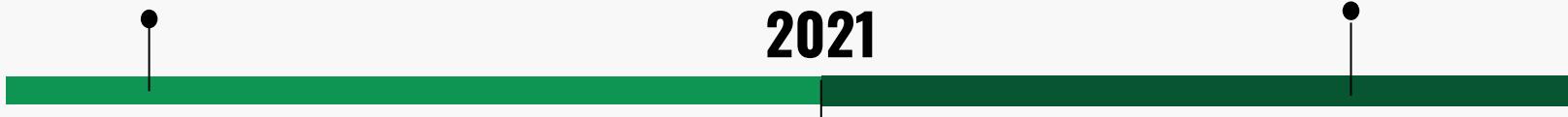
Meadowridge School is located on the ancestral, unceded territory of the Katzie, the Kwantlen, and Stó:lō First Nations.

The CLS, in Saskatoon, SK, is located on Treaty Six land in the traditional territories of the Nêhiyawak (Cree), Anishinabek, Lakota, Dakota and Nakota Nations, and the homeland of the Métis.



Our Journey

- Prepared for the Beamline for Schools competition
- Targeting negatively charged cancer cells using positrons



**Mar.
2021**

- Started working on proposal for beamtime through SotB

**Aug.
2021**

- Inhibiting zinc dendrites in batteries
- Exploring the Fibonacci sequence in nature

**Nov.
2021**



Our Journey

- Began investigating in proteins
- Later, narrowed topic to insulin
- Investigation of data analysis methods



- In-person experiment at the CLS



Research Question

How does the X-ray exposure time of insulin impact its structural stability at cryogenic and room temperatures during crystallography experiments?



Importance

“X-ray crystallography has made the largest contribution to our understanding of protein structure” (Aminpour et al., 2019).

- Protein structure is crucial to drug development (Hillisch, 2008).
- Pharmaceutical companies use X-rays at cryogenic temperatures to determine protein structures (Aminpour et al., 2019).

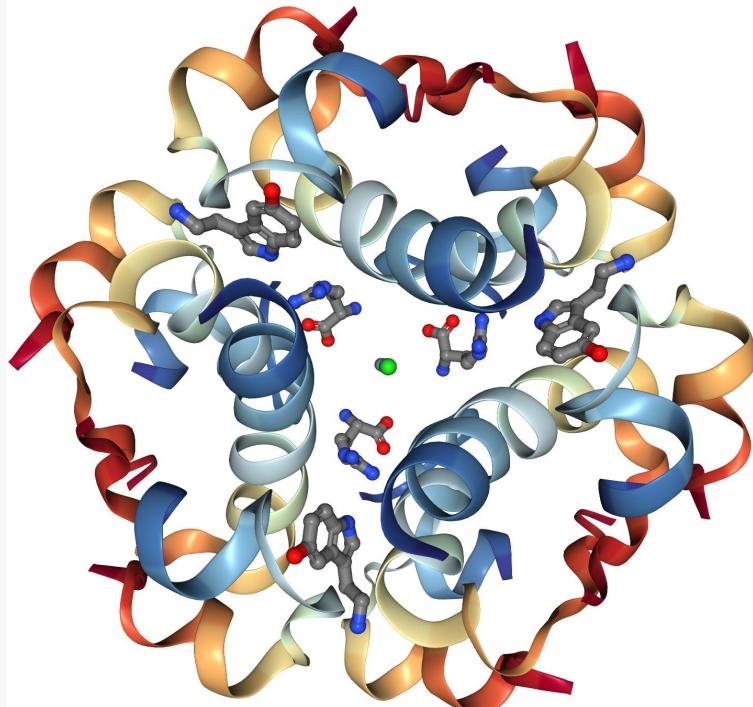


Proteins

Macromolecules composed of long chains of amino acids called polypeptides.

Four protein structures make up the overall structure.

Insulin is a hormone produced in the pancreas that regulates blood glucose levels.



(Zhang et al., 2018)

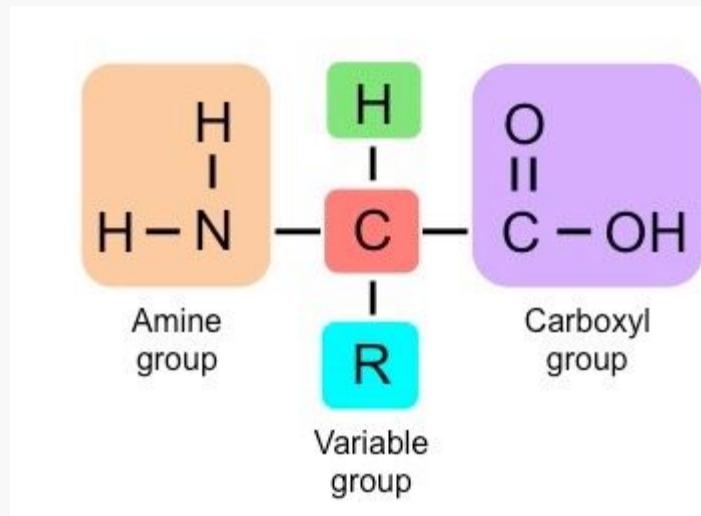


Amino Acid

Amino acids all share a common basic structure.

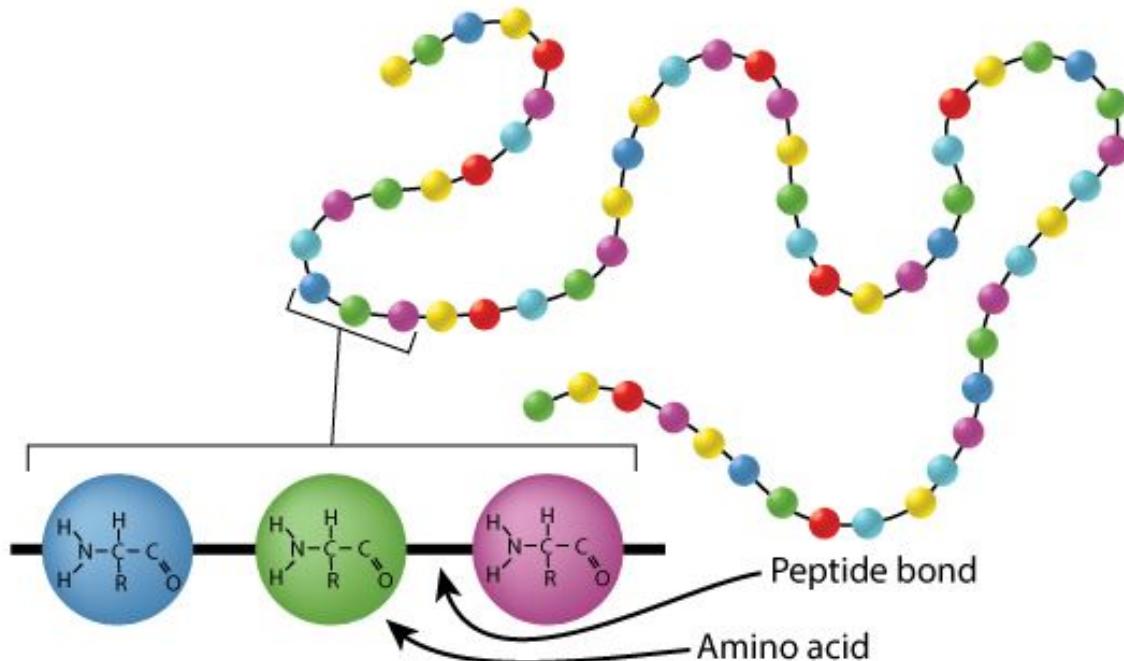
Distinct chemical properties cause the protein to fold and function uniquely.

Joined together by peptide bonds.





Protein Structure



Primary structure:
A sequence of
amino acids
linked together to
form a
polypeptide chain

- Peptide bonds

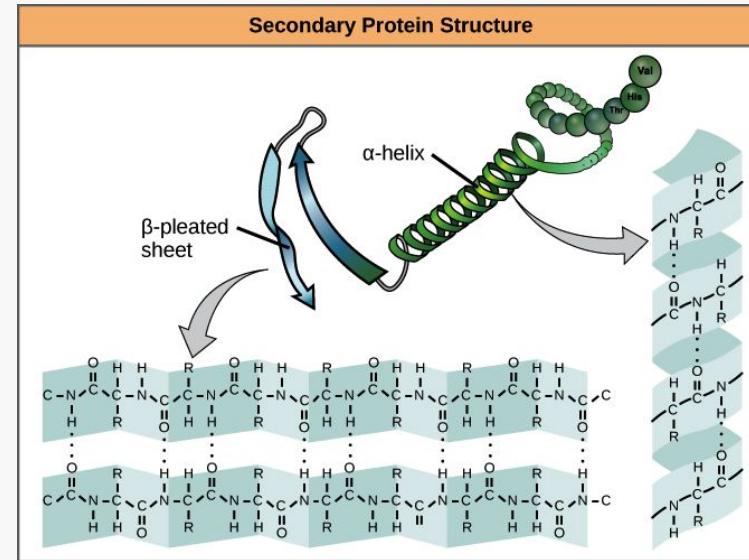
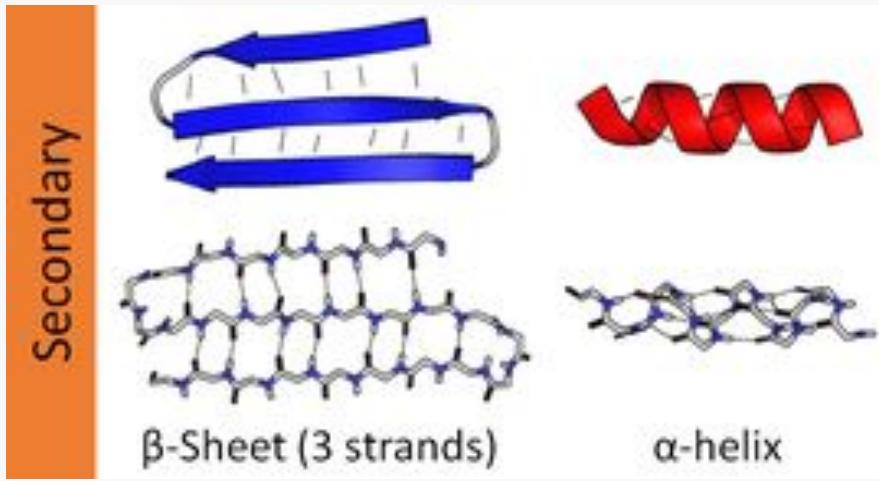


Protein Structure

Secondary structure:

Alpha helices and beta-pleated sheets

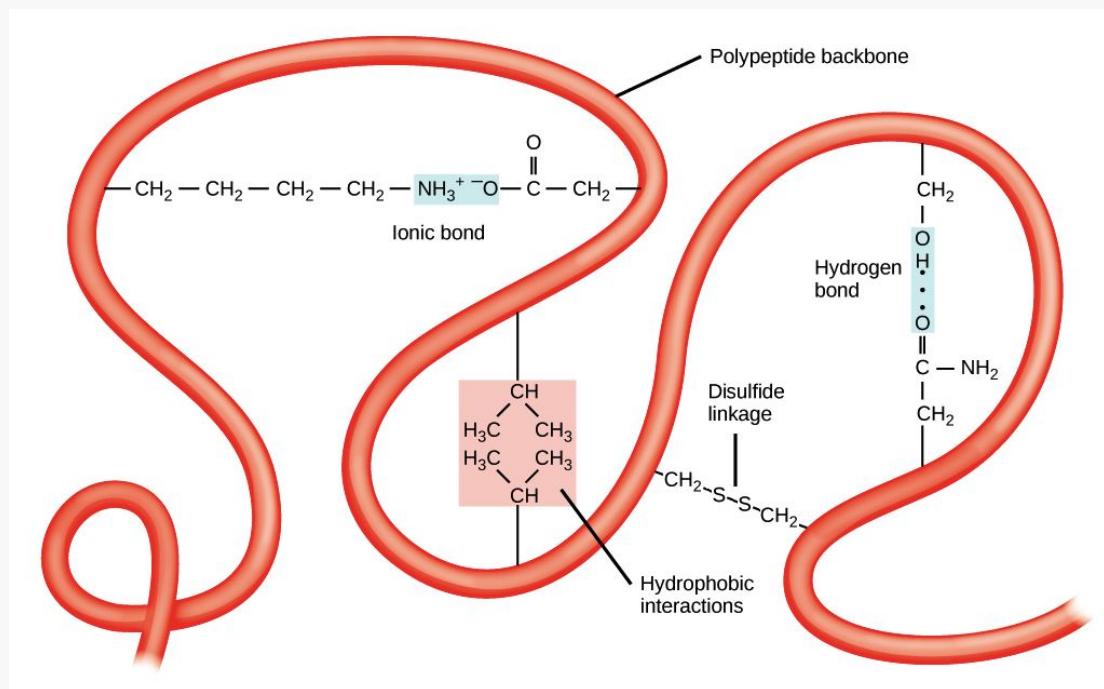
- Hydrogen bonds





Protein Structure

Tertiary structure:
Overall folding three-dimensional arrangement of its polypeptide chain in space

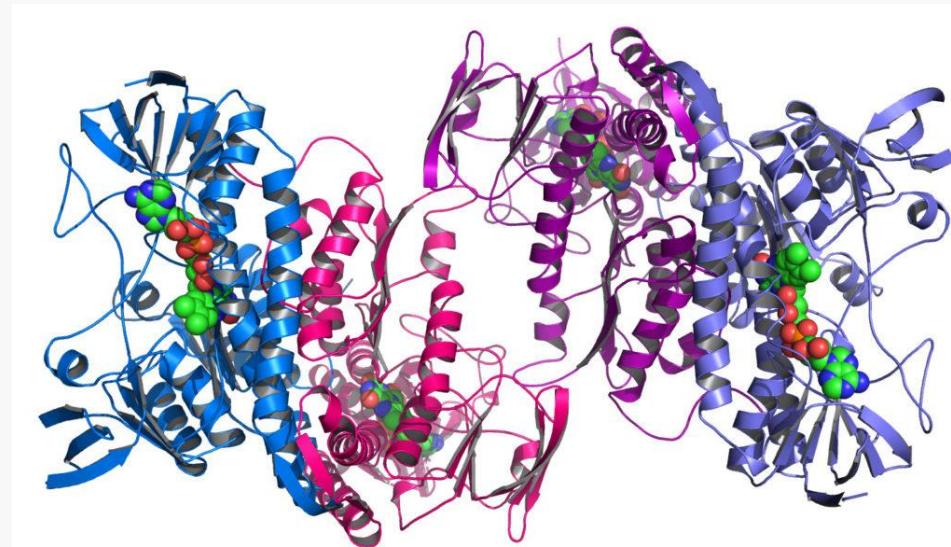
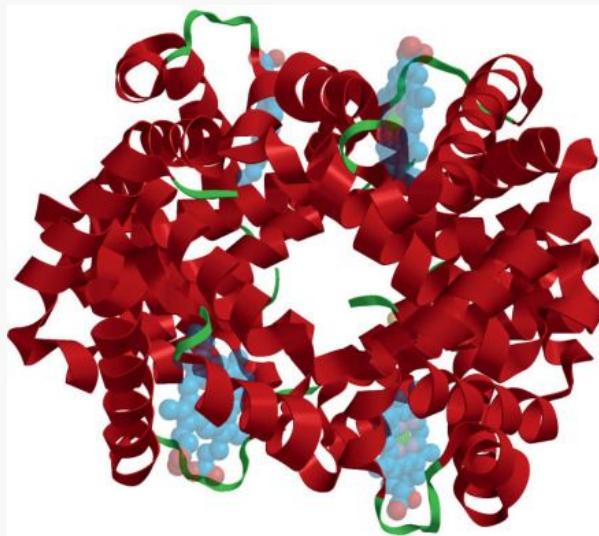




Protein Structure

Quaternary structure:

Protein subunits interact to form a larger protein complex.

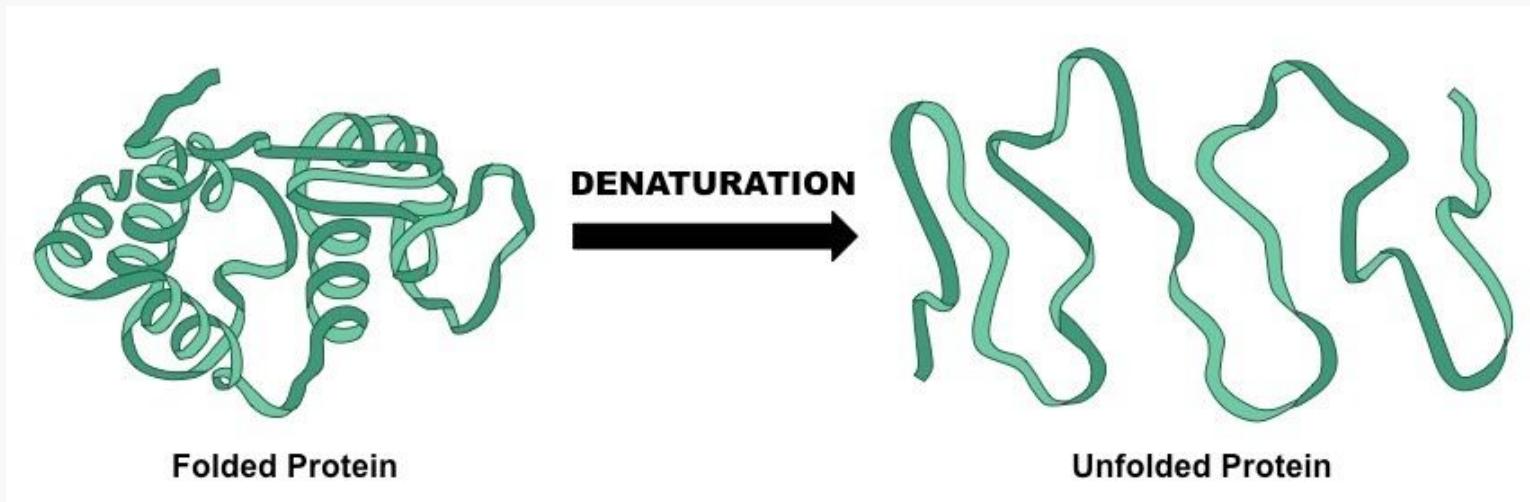




Protein Denaturation

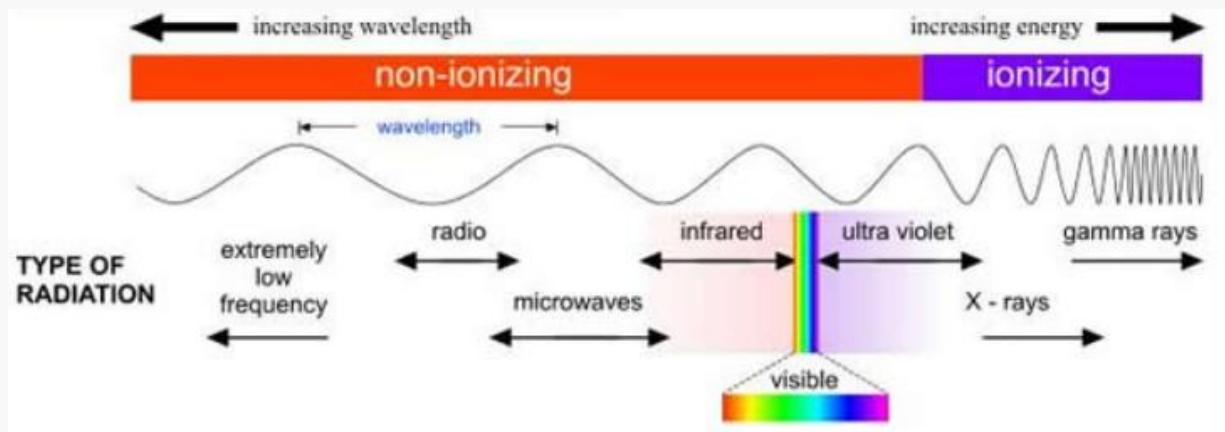
The **molecular structure** of a protein is irreversibly broken down through radiation

- Overcoming the weak intermolecular forces
- Ex. egg





Ionizing Radiation



(ARPANSA)

- **Ionizing radiation** is a form of energy that acts by removing electrons from atoms and molecules.
- X-ray radiation knocks off electrons and destabilizes proteins.



Research Question

How does the X-ray exposure time of insulin impact its structural stability at cryogenic and room temperatures during crystallography experiments?

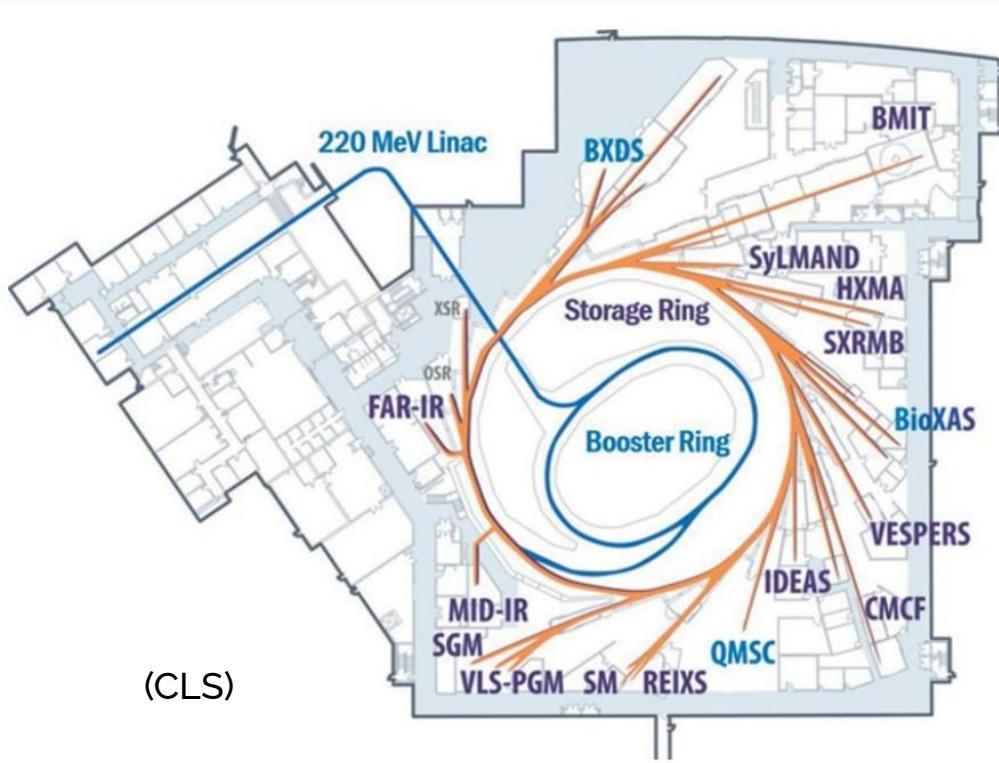


Hypothesis

As the X-ray exposure time and temperature increases, the stability of the insulin decreases because ionizing radiation overcomes the intermolecular forces.



The Synchrotron



- Electrons are accelerated to 99.99% of the speed of light.
- Magnets bend the path of the electrons, releasing light into the beamlines.



CMCF-BM Beamline

Canadian Macromolecular Crystallography Facility - Bending Magnet



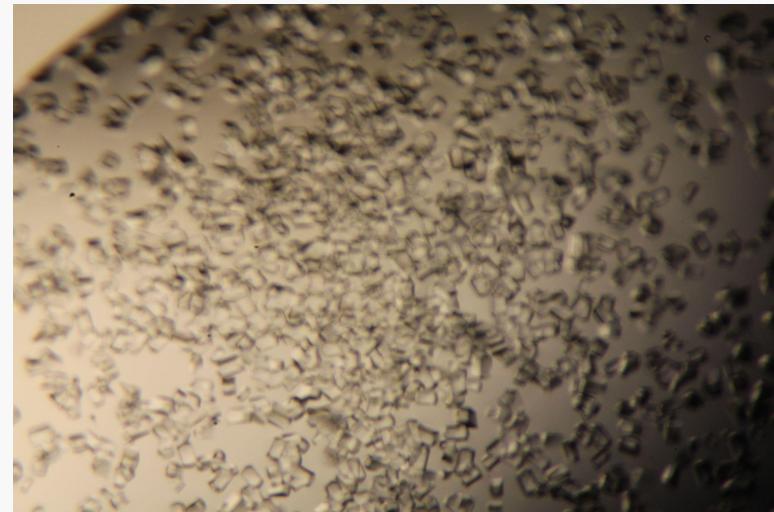
- Single-crystal X-ray diffraction.
- Great for studying the structures of proteins.
- Crystallized samples are mounted on sample pins.



Protein Crystallisation



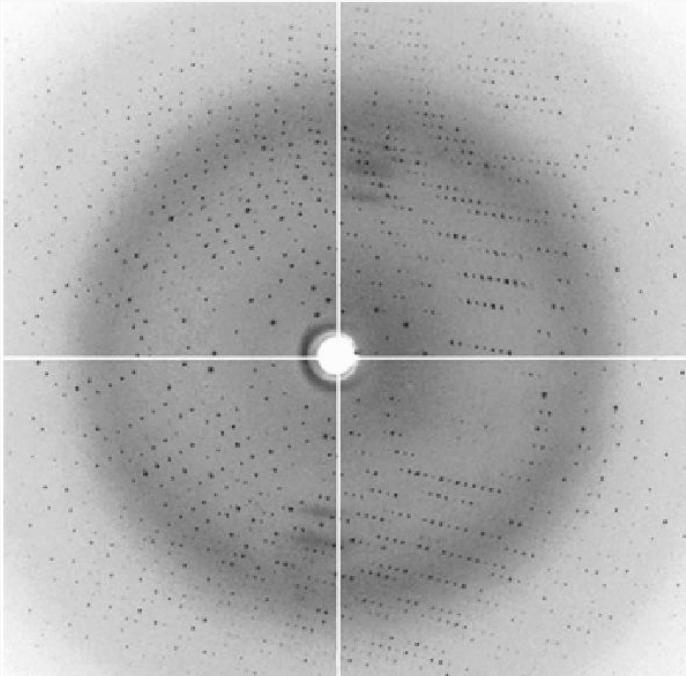
- Process in which protein is organised into a 3D lattice structure.



- Proteins arranged such that the X-rays hitting the crystal create a pattern.



X-ray Crystallography

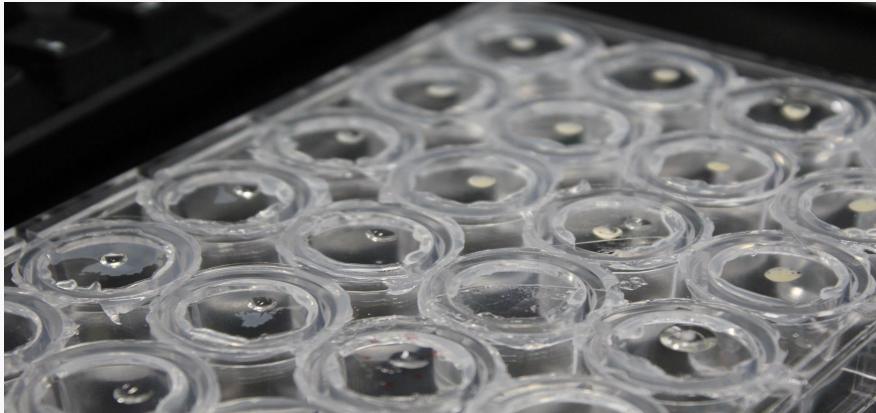


(Chen et al, 2001)

- Diffracted X-rays are projected onto a screen and recorded.
- Protein structure is deduced from the pattern.
- Requires a large amount of computational power.



Creating the Crystals

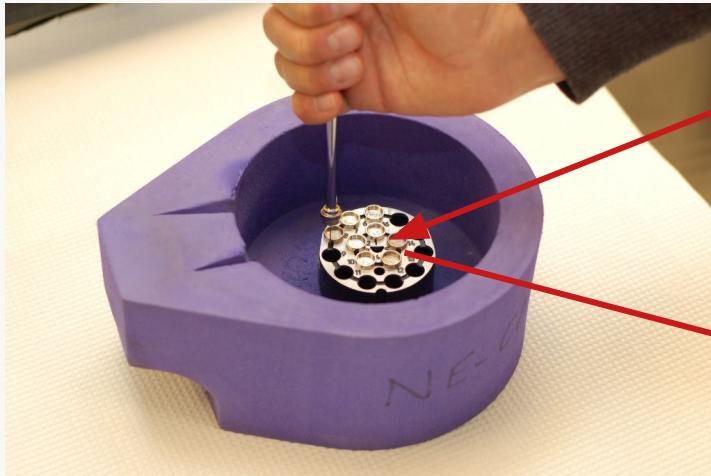


- Crystallisation by hanging drop method.
- Fished out from droplets.
- Placed in sample puck to transfer to beamline.





Loading the Crystals



(NE-CAT, 2016)

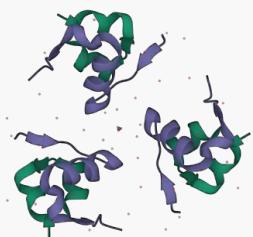
Cryogenic puck

Transferred to
beamline into the
tank



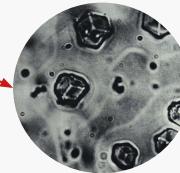
- Very cold condition: need to store in liquid nitrogen.
- Temperature of liquid nitrogen: 77 Kelvin / -195.8°C.
- Robotic arm loads crystals into experimental space.

🔬 Sample Distribution



6 Bovine insulin
crystals

3 room-
temperature crystals

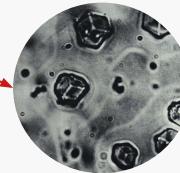


0.25 seconds

0.5 seconds

1 second

3 cold crystals



0.25 seconds

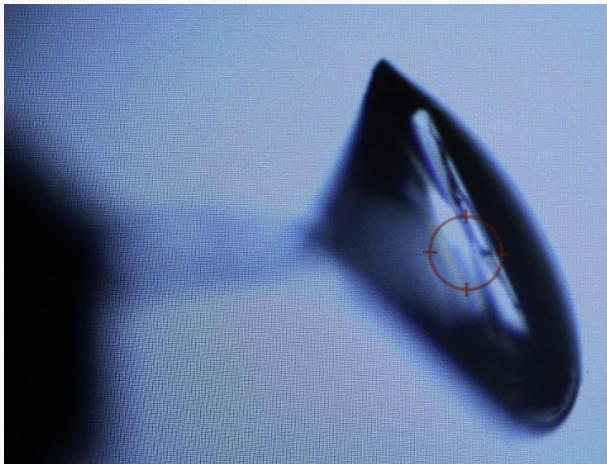
0.5 seconds

1 second

Screening for Crystals

100 micron beam

Used when we are able to see the crystal clearly



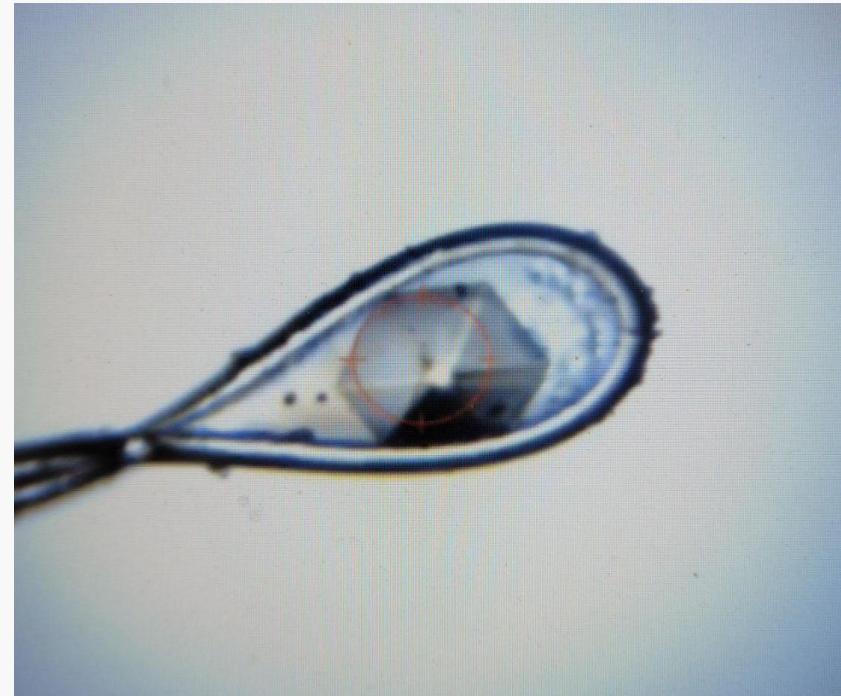
200 micron beam

Used to find the crystal when we are not able to see the crystal clearly



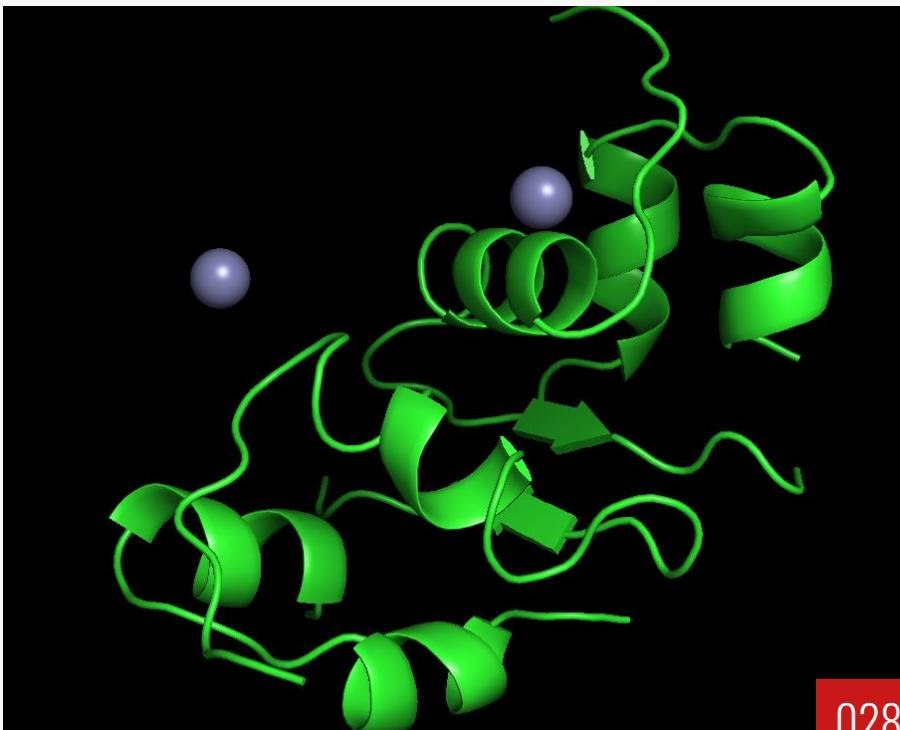
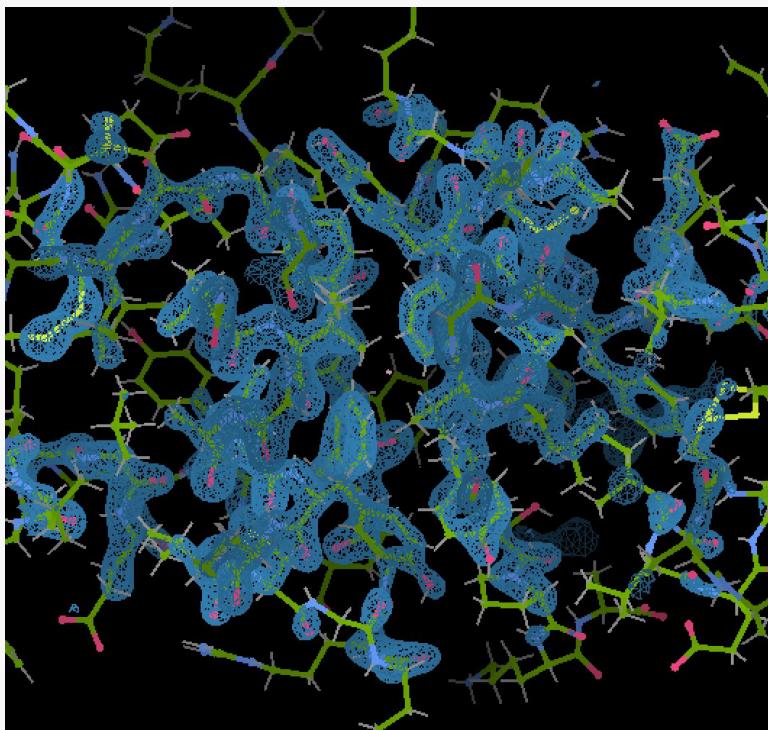


Qualitative Observations





Solved Protein Structure





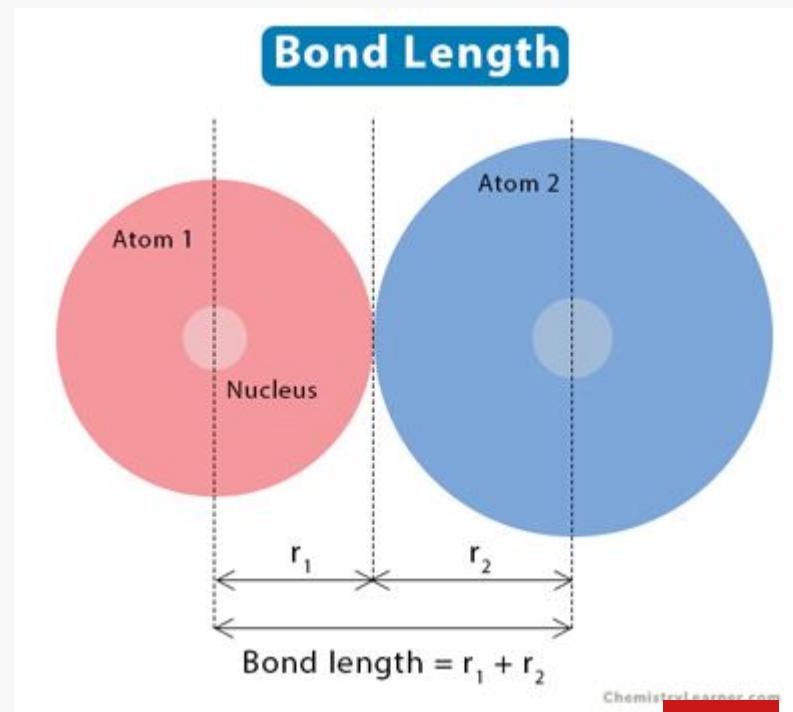
Average Bond Length

Bond Length

The direct distance between the nuclei of two atoms.

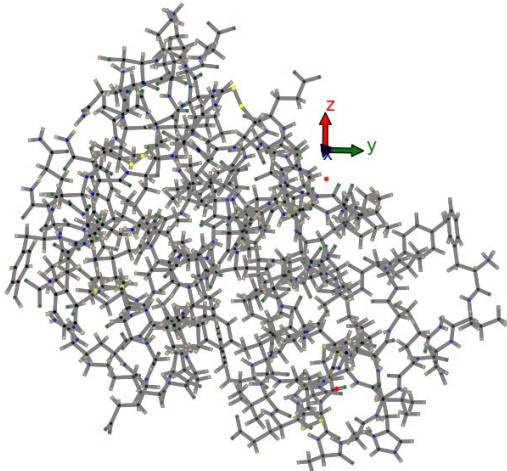
Average Bond Length

All the bond lengths are added together and divided by the total number of bonds.





Average Bond Length Result



Across all four increments, there are no significant difference between their average bond lengths.



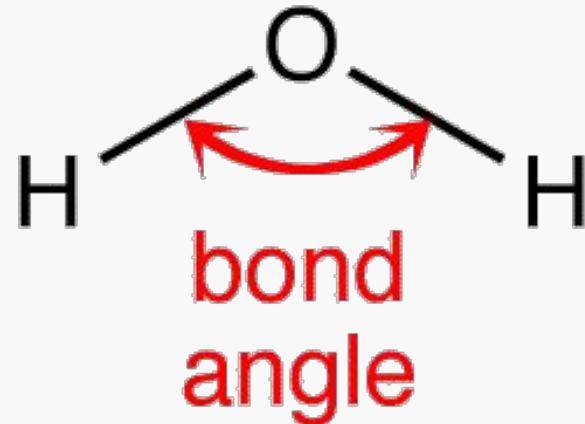
Average Bond Angle

Bond Angle

- In a 3-D plane, there are x, y, z coordinates of each atom.
- We use bond angles to determine the location of each atom.

Average Bond Angle

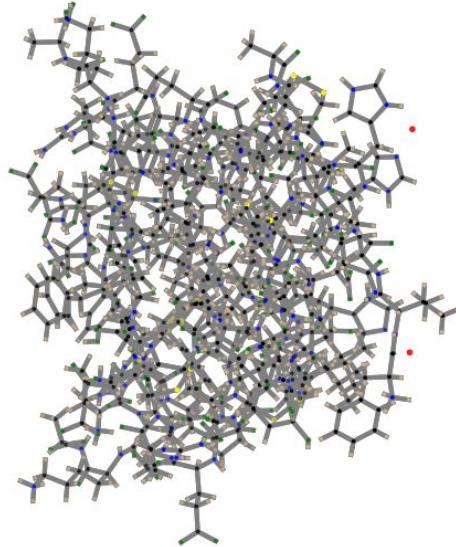
- By adding all the bond angles in the protein, and divided by the number of bonds, we find the average bond angles.



Chemistry LibreText n.d



Average Bond Angle Result

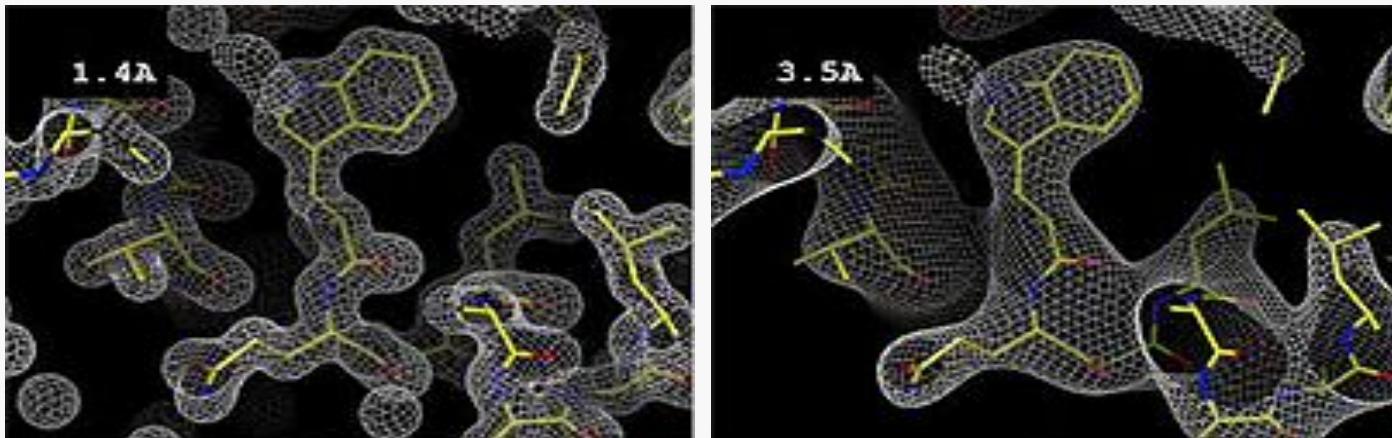


Across all four increments, there are no significant difference between their average bond angles.



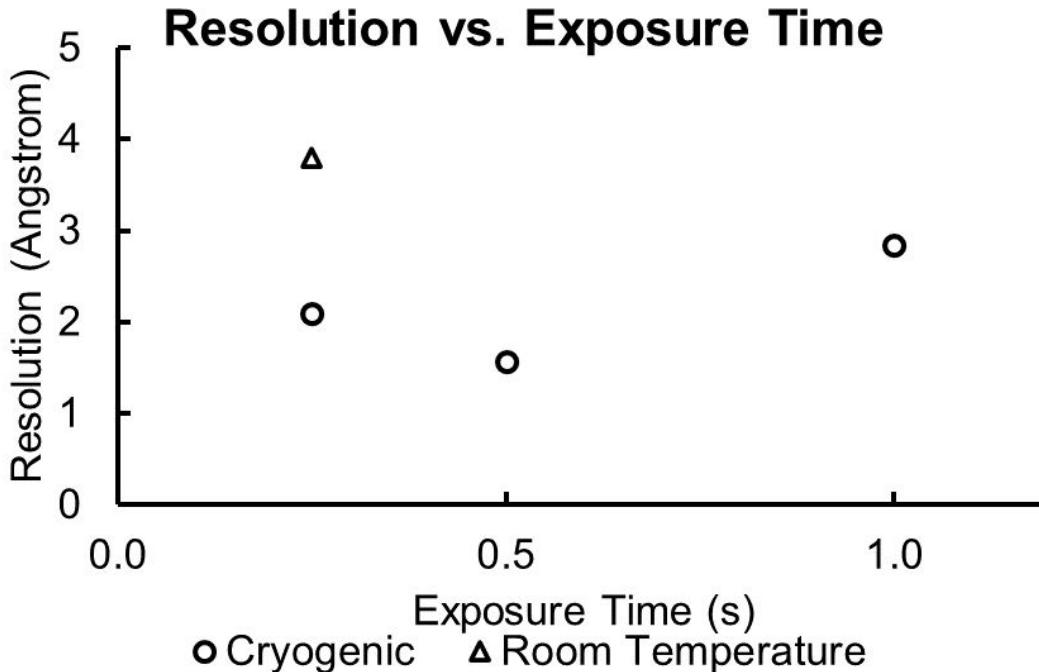
Resolution

- Proteins can be seen as stacked planes.
- Resolution is the smallest distance between the planes (like a pixel).
- The smaller the number of Angstroms (\AA), the higher the resolution.
 - An Angstrom is a measure of length ($1 \text{ \AA} = 10^{-10} \text{ m}$).





Resolution



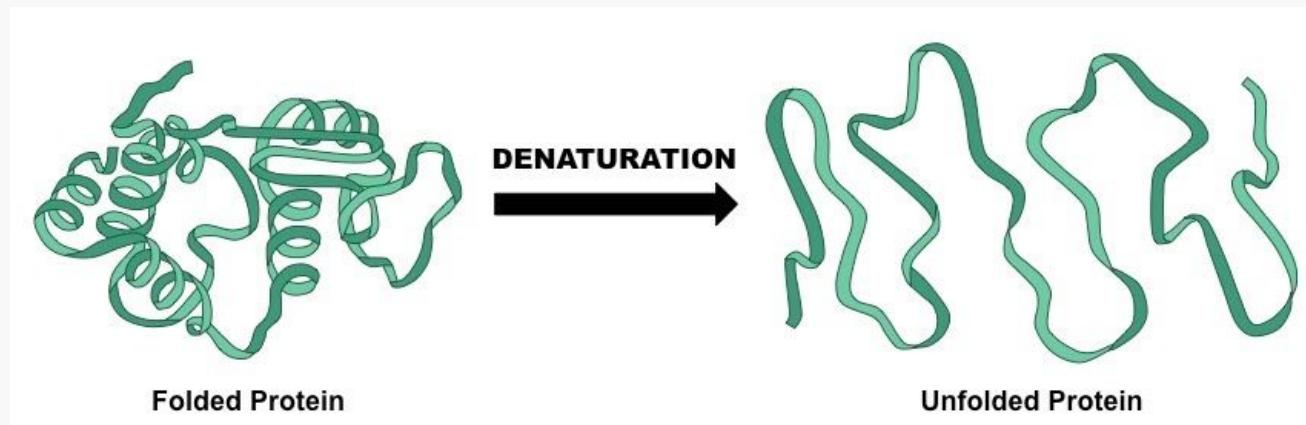
- There are no clear trends between exposure time and resolution.

*the other two crystals from room temperature are damaged beyond solvable for the program



Radiation Damage

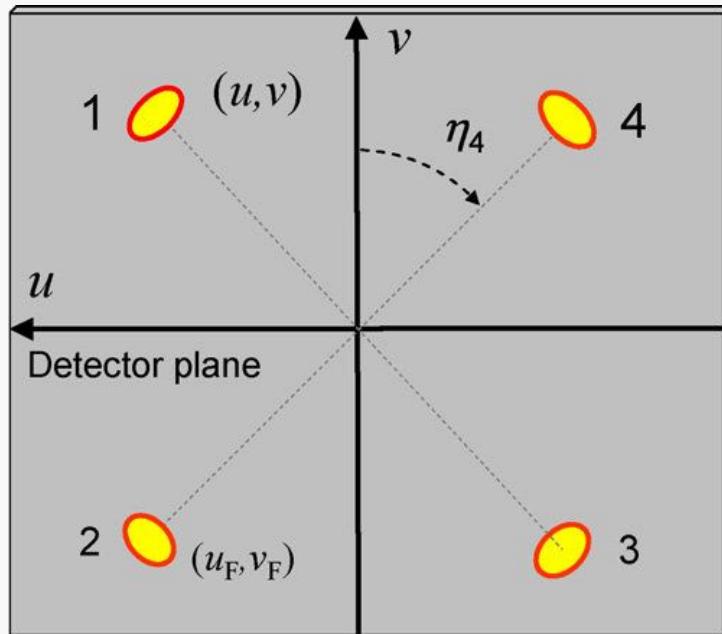
- Refers to any destruction caused by exposure to radiation.
- Radiation causes disruption in the lattice, preventing the crystal containing the protein to diffract.
- Also causing fragmentation of the protein.



(BioNinja, n.d)



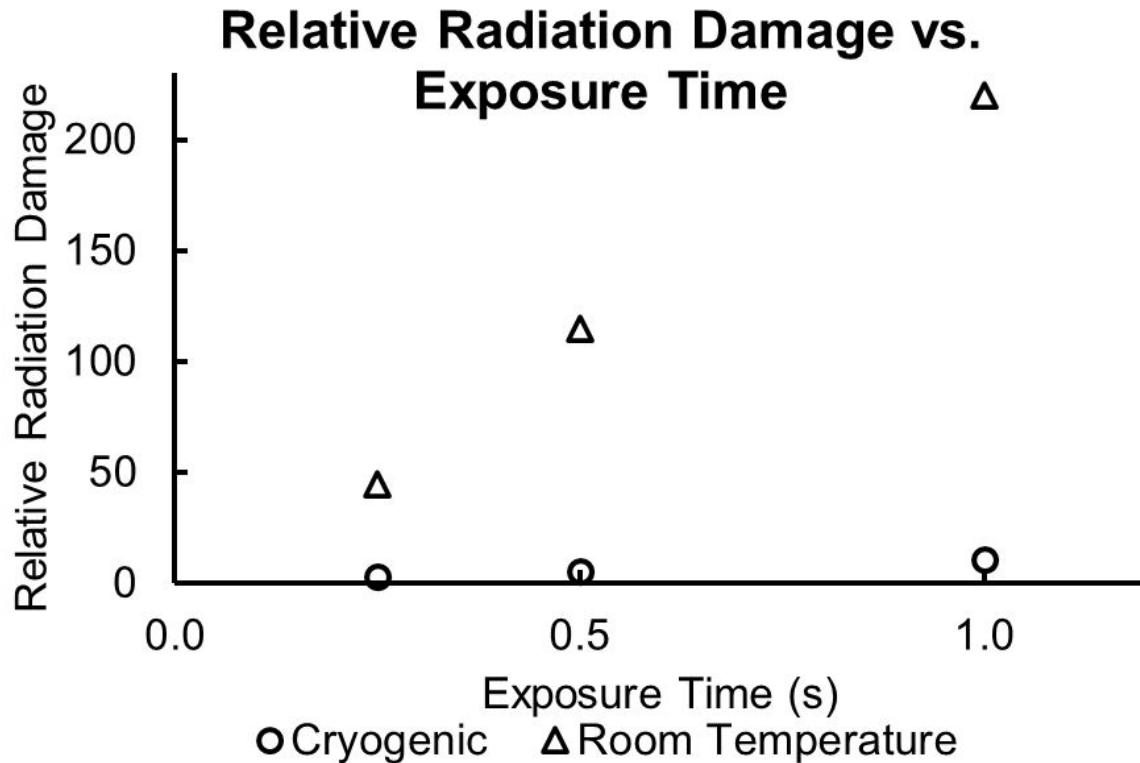
Relative Radiation Damage



- Relative radiation damage is calculated using a different in x-ray image symmetry between **Friedel pairs**.
- Samples at Room Temperature at 0.5 s and 1.0 s exposure had too high a difference between Friedel pairs that no structure was generated.



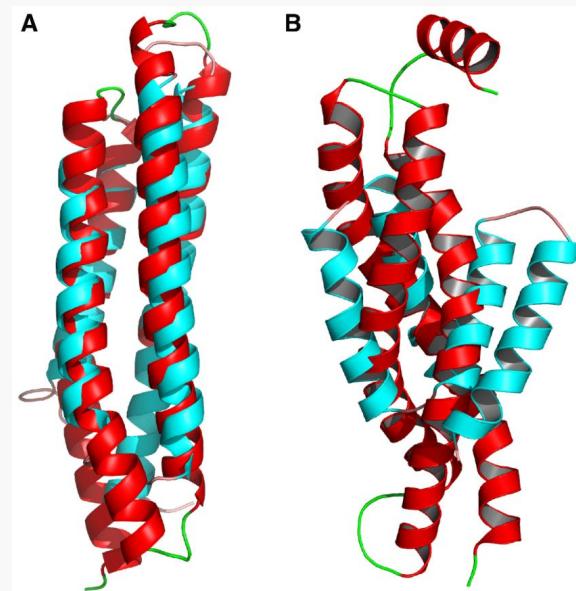
Relative Radiation Damage



- Relative radiation damage is produced from a difference in symmetrical Friedel pairs.

🔬 RMSD (Root Mean Square Deviation)

RMSD is used to measure the average displacement between identical locations on a reference object and the targeted object.



(Sabban, 2020)



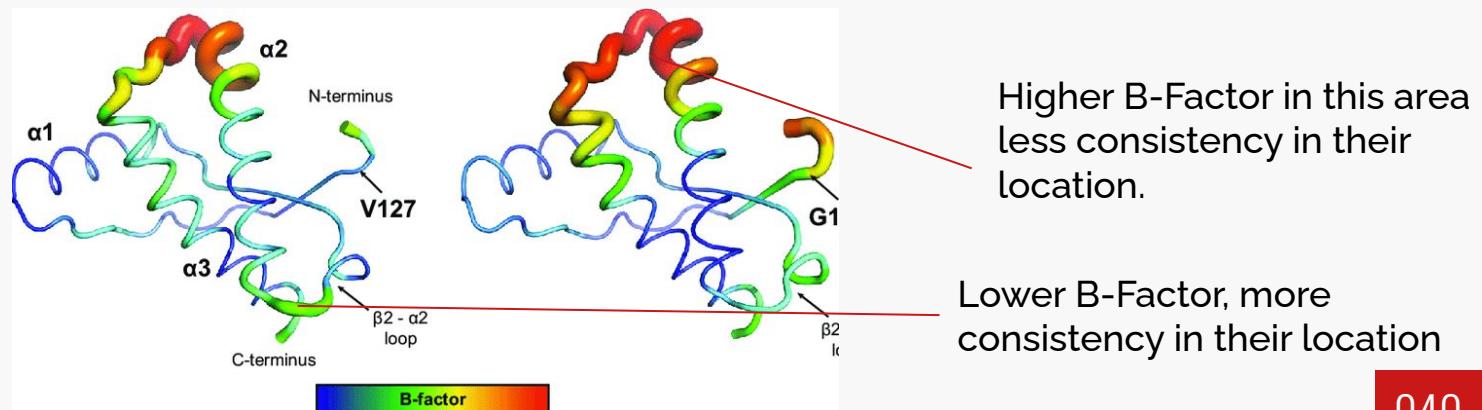
RMSD Result

- Across all six increments, there are no significant difference between the RMSD values.



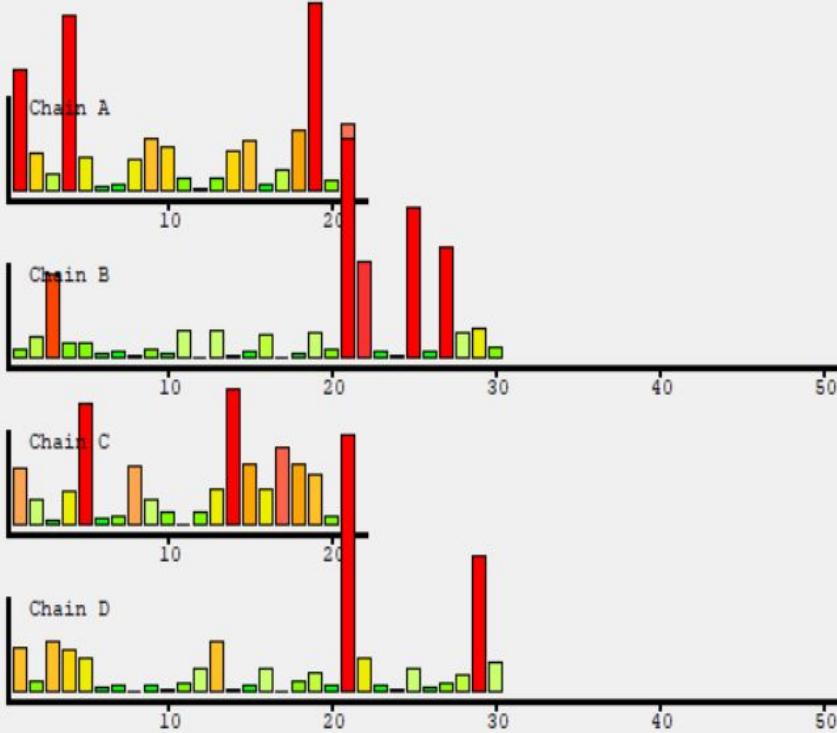
B-Factor

- Also known as Debye-Waller factor/temperature factor/atomic displacement parameter.
- The higher thermal motion results in higher B-Factor.
- Therefore higher B-factor indicates less consistent of location of atoms.





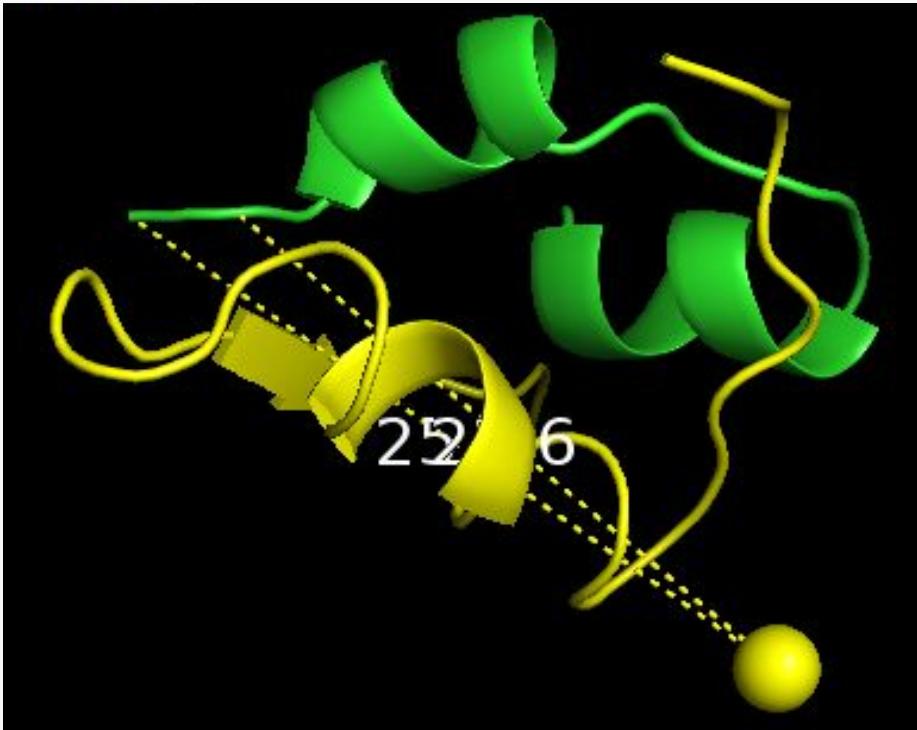
B-Factor Results



- The B-Factor did not change, across all 3 increments of 0.25s, 0.5s, and 1.0s.



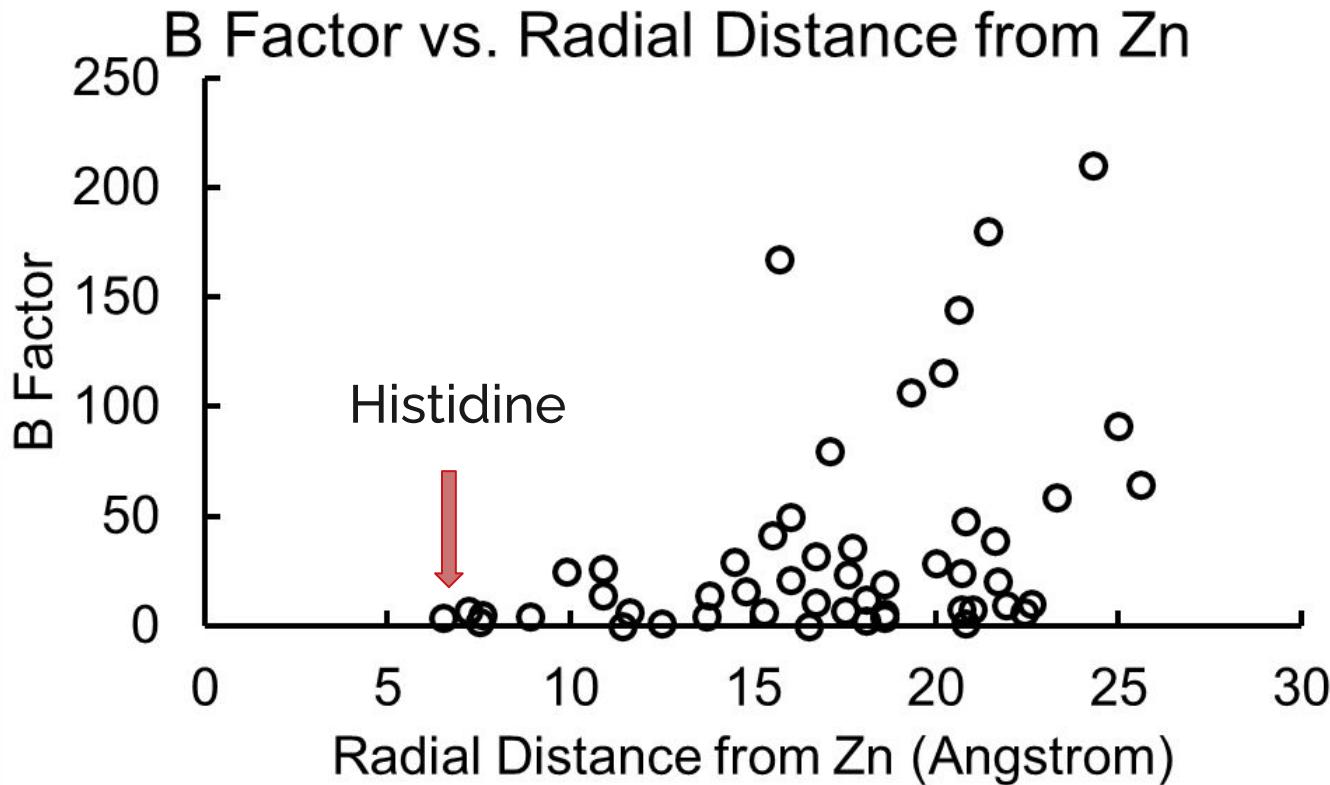
B-Factor Results



Distances between amino acids and the zinc ions were manually measured using software.



B-Factor Results



The B-Factor of amino acids increased the farther away it from the zinc ion.

Significance of the B-Factor and Zinc

Mapping B-Factors

B-Factors represent how much the amino acids “move”.



There are more amino acids that have high B-factors when farther away from zinc.



We want to know if zinc may play a stabilizing role in insulin.



Summary of Results

- No significant change in **RMSD, B-factor, bond length, and bond angle.**
- Cryo temperatures significantly reduced radiation damage.
- Exposures beyond 0.25 seconds in room temperature destroyed crystals.



Summary of Results

- Radiation damage increased with exposure.
- Zinc maintained a **B-factor of 0**.
- **Resolution** did not increase at the **1s** increment but radiation damage did.
- **B-Factor** increased the further away the amino acid was from zinc.



Impact on Society

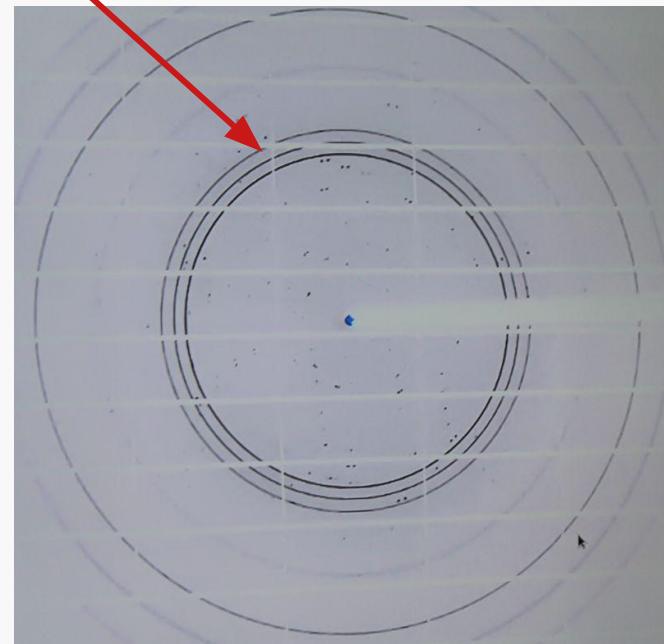
- **Drug development:** measuring the effect of x-ray radiation on symmetry/accuracy of data used in drug synthesis.
- **Disease specific research,** ie. the role of metal ion binding to proteins in diseases such as Parkinson's, Alzheimer's, etc.
- Increased research in this area can help evaluate and/or improve the accuracy of data in the Protein Data Bank (PDB).



Caveats

- 14 crystal were exposed in the CMCF-BM beamline, but so far only 6 structures have been solved for.
- Ice in crystals and on diffraction pattern caused by decentred liquid nitrogen stream.
- Diffraction patterns due to salt.

Ice Ring





Future Investigations

Exposure times

Investigate for the most optimal exposure time for higher resolutions

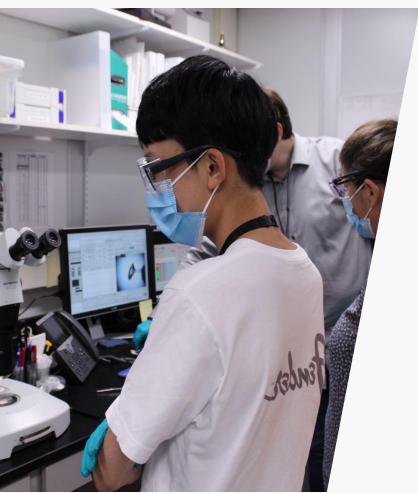
Computational Modelling

Attempt to reduce radiation damage through serial crystallography

Role of Metal Ions

Explore the role of different ions in different proteins.

Our Experience





Acknowledgements



Canadian
Light
Source

Centre canadien
de rayonnement
synchrotron



MEADOWRIDGE
SCHOOL





Acknowledgements

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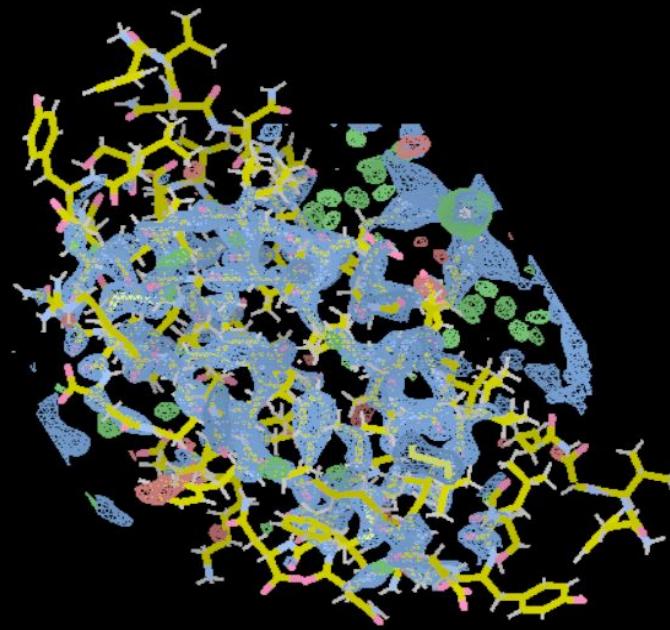
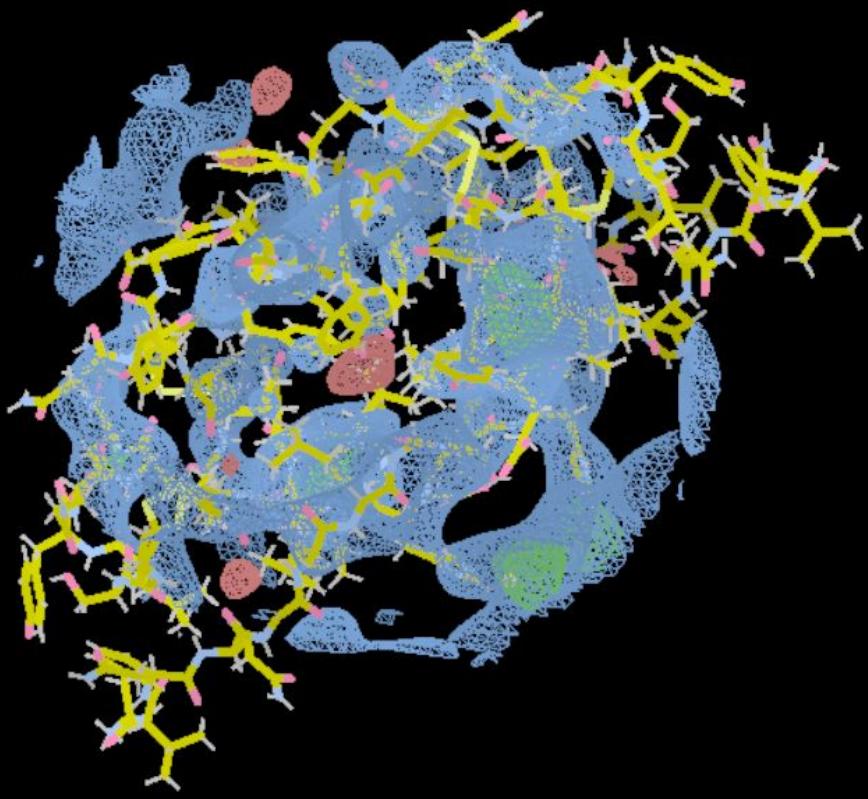


Amanda Pfeiffer
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Meadowridge School

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