SUMMARY OF MY SUMMER RESEARCH

STRUCTURAL BIOLOGY RESEARCH CENTER, KEK

Maria Papamanousaki

University of Crete, Greece

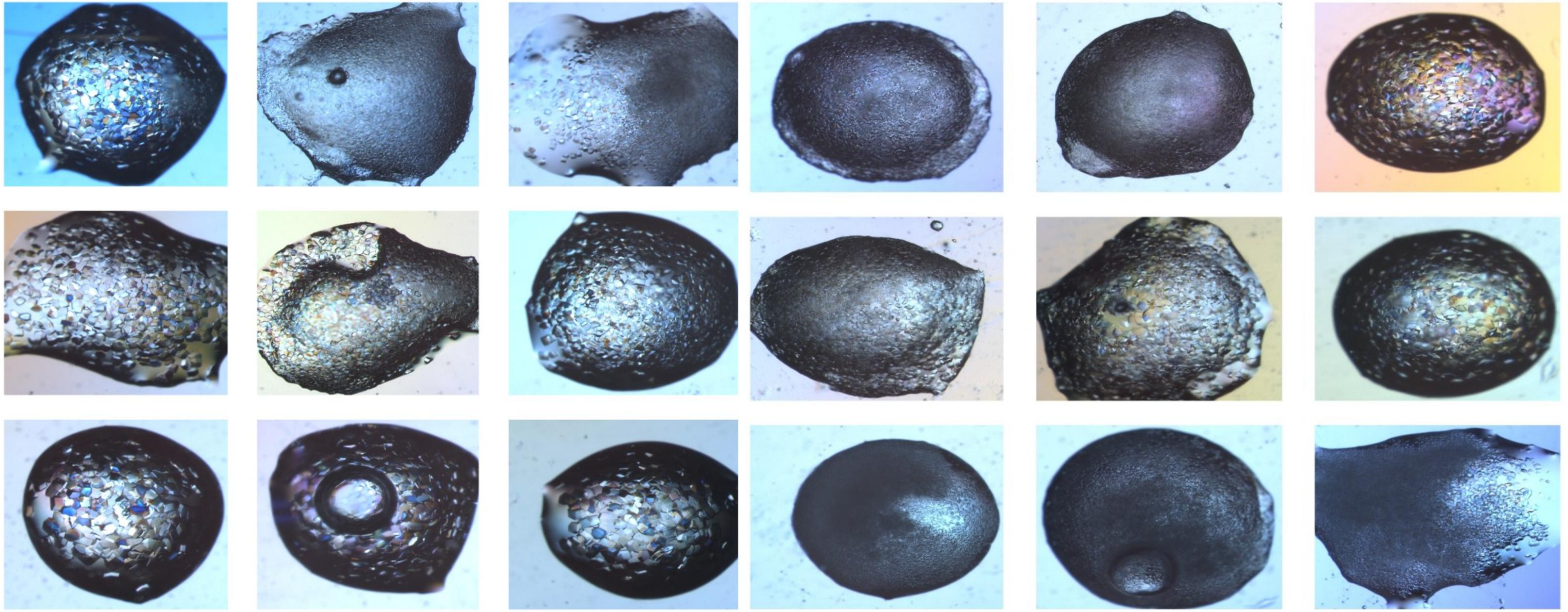
22 June 2026 – 30 July 2026

Project Supervisor: Miki Senda

RESEARCH PROJECTS

Project	My contribution
Lysozyme	Protein crystallization under various conditions and presentation of the results
STX	Practice of crystallographic refinement
DSD	Practice of crystallographic refinement
Glycose Isomerase	Observation of robotic crystallization screening and discussion of optimization strategies
BphA4	Observation of robotic crystallization screening, optimization of crystallization conditions, and experimental crystallization based on the optimized conditions

LYSOZYME CRYSTALLIZATION



LYSOZYME CRYSTALLIZATION

Objective

- Learn the fundamentals of protein crystallization.
- Compare various crystallization conditions.

My work

- Prepared crystallization solutions.
- Set up hanging-drop vapor diffusion experiments.
- Tested three different crystallization conditions.
- Evaluated crystal morphology and quality.

Outcome

- Successfully obtained protein crystals.
- Presented the project during the Summer Student Event.

STX: INTRODUCTION TO MODEL BUILDING WITH COOT

Objective

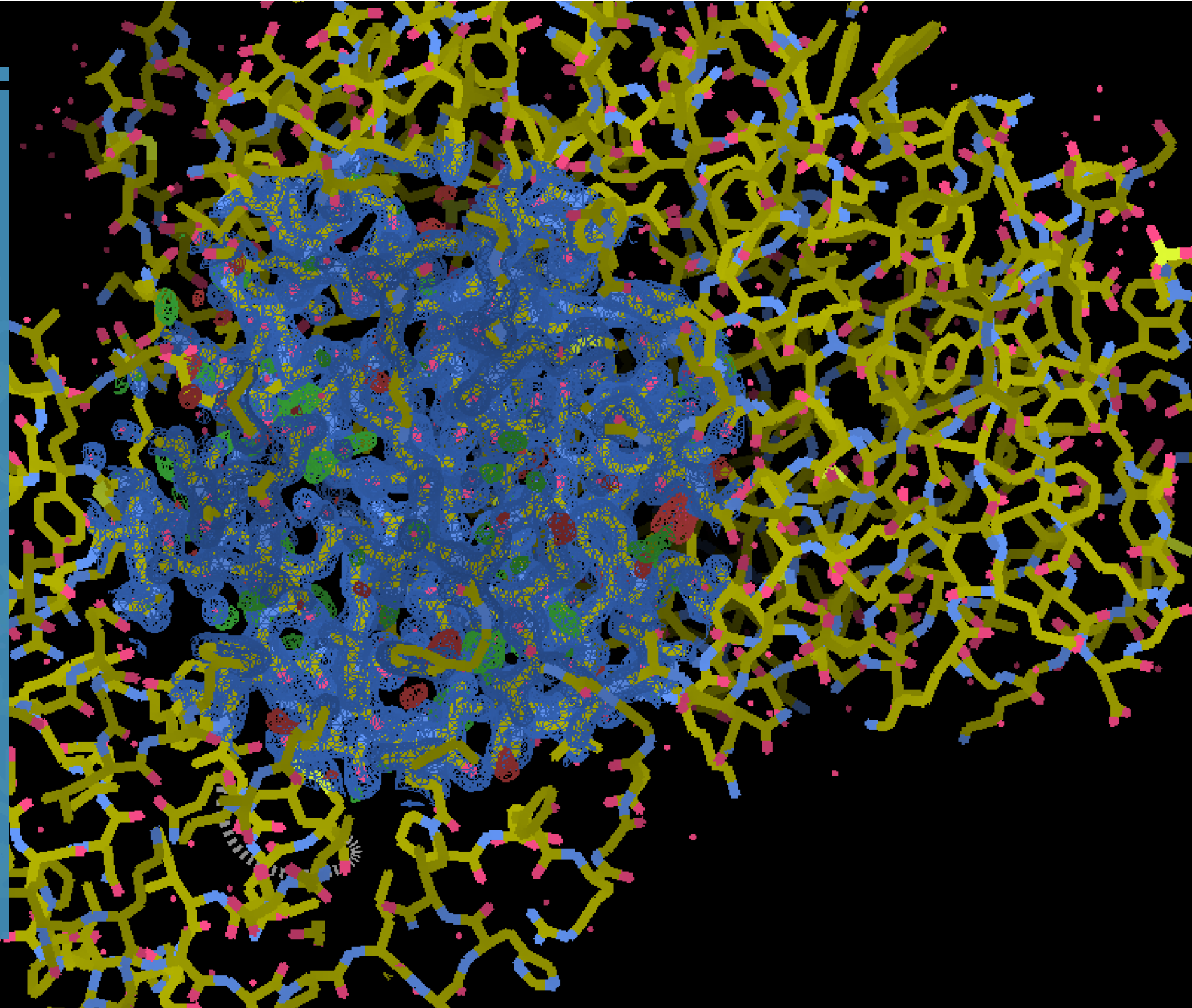
- Become familiar with model visualization and manual model building in Coot.

My work

- Explored protein structures and electron density maps.
- Practiced manual model corrections.
- Learned the basic tools for model building.

Outcome

- Acquired the fundamental skills required for later structure refinement projects.



DSD PROTEIN: DATA PROCESSING AND STRUCTURE REFINEMENT

Objective

- Learn the workflow from diffraction data to a refined protein structure.

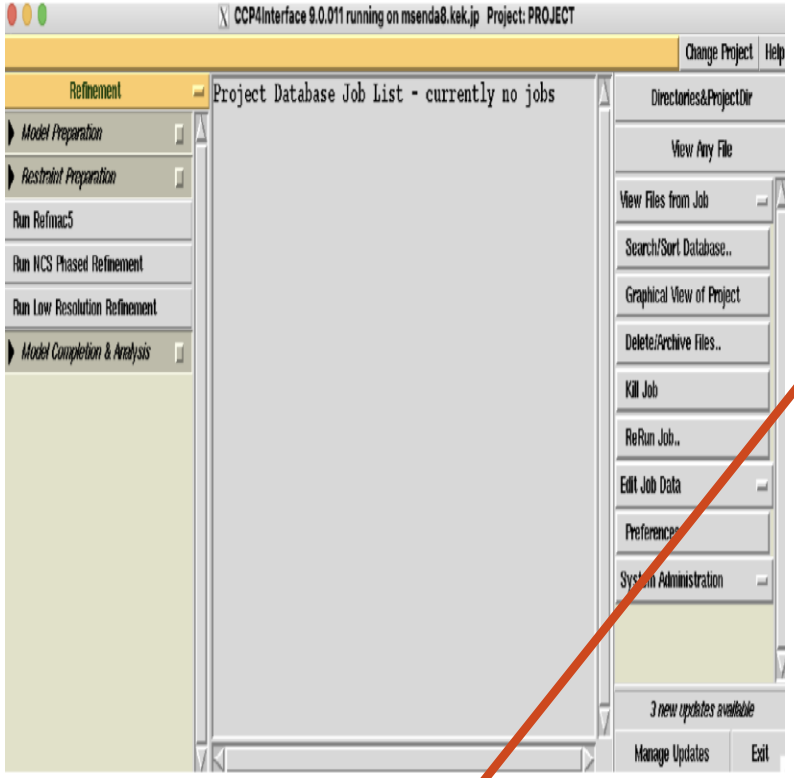
My work

- Scaling using **Aimless**.
- Performed model building and corrections in **Coot**.
- Carried out structure refinement using **Phenix**.
- Evaluated model quality throughout the refinement process.

Outcome

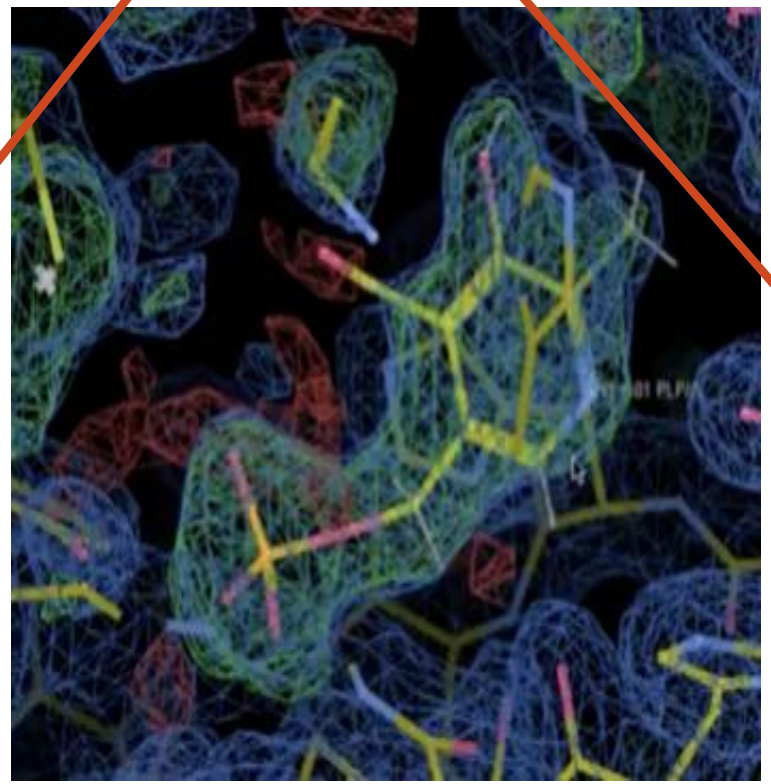
- Gained practical experience with the complete structure determination workflow.

DSD PROTEIN: DATA PROCESSING AND STRUCTURE REFINEMENT



1) Aimless

2) Coot



3) Phenix

Figure 1	Starting	Final
R-work	0.2450	0.2454
R-free	0.2776	0.2786
Bonds	0.007	0.007
Angles	0.901	0.862

WARNING: R-free increased during refinement; the selected strategy may not be appropriate.

Unsuccessful refinement

If **R-work** and **R-free** increase after refinement, the refinement strategy or the model may need to be revised.

In this case:

- Review the model for incorrect residues, ligands, or water molecules.
- Verify the refinement settings.
- Correct the model in Coot before running another refinement cycle.

(Figure 1: R-work and R-free increased after refinement.)

Figure 2	Starting	Final
R-work	0.2408	0.2390
R-free	0.2758	0.2756
Bonds	0.006	0.006
Angles	0.864	0.842

Successful refinement

A successful refinement generally results in lower **R-work** and **R-free** values while maintaining good model geometry.

In this example:

- **R-work** decreased from **0.2408** to **0.2390**.
- **R-free** decreased from **0.2758** to **0.2756**.
- The bond and angle RMS values remained acceptable.

(Figure 2: Refinement statistics improved after refinement.)

GLUCOSE ISOMERASE: CRYSTALLIZATION SCREENING AND OPTIMIZATION

Objective

- Gain experience with automated crystallization screening.
- Evaluate crystallization outcomes and identify potential optimization strategies.

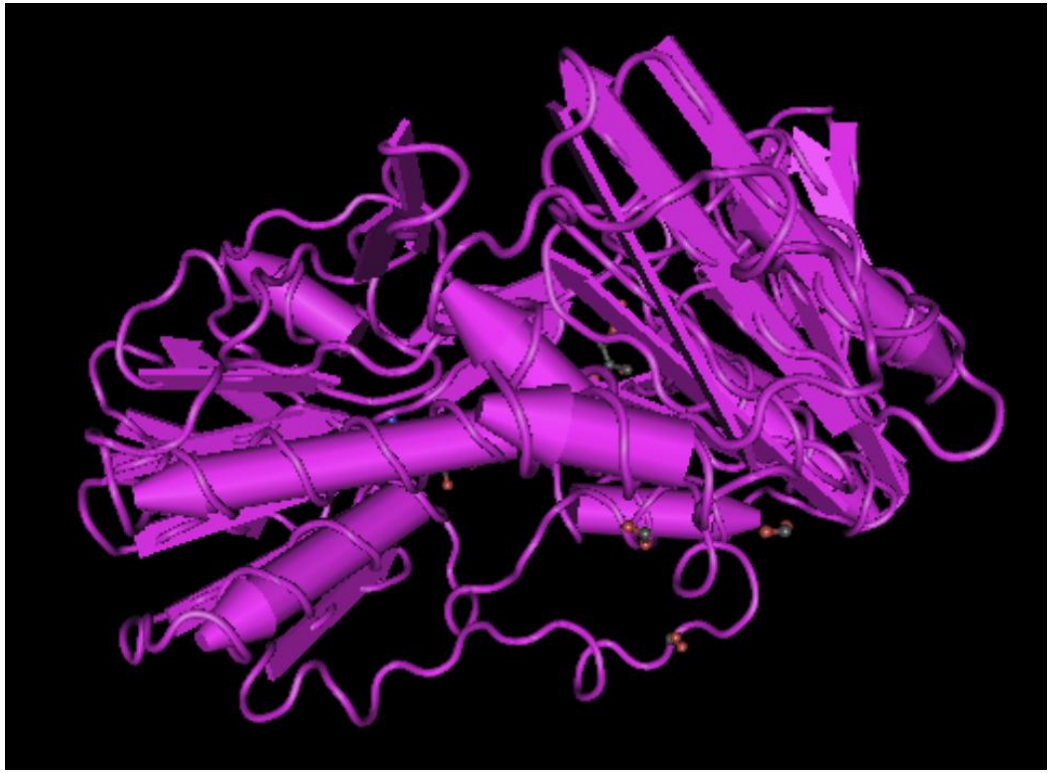
My work

- Observed crystallization experiments performed using a robotic system.
- Analyzed the obtained crystallization plates and crystal formation.
- Discussed possible optimization strategies based on the screening results.

Outcome

- Learned how automated platforms are used to efficiently screen a large number of crystallization conditions.
- Understood the process of selecting promising conditions for further optimization

BPHA4: INITIAL CRYSTALLIZATION SCREENING FOR MONOMER BPHA4



Objective

- Evaluate the initial robotic crystallization screening.
- Select promising conditions for optimization.

My work

- Observed the robotic crystallization screening results.
- Evaluated crystal formation across different conditions.
- Selected one promising condition for further optimization.

Outcome

- Identified an initial condition for subsequent optimization experiments.

BPHA4 WILD-TYPE : STRUCTURE DETERMINATION

Objective

Analyze the selected crystal dataset and determine the protein structure.

My work

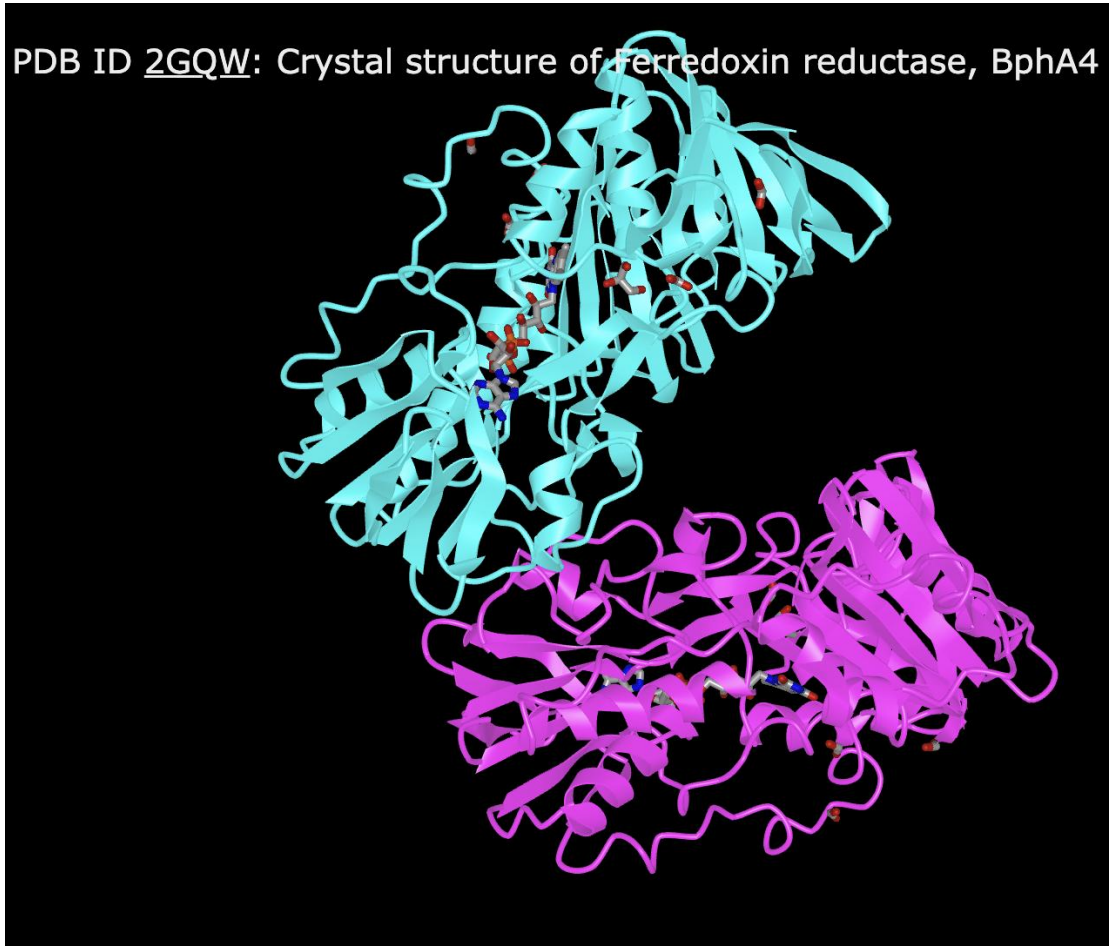
Processed diffraction data using **Aimless**.

Built and inspected the model in **Coot**.

Refined the structure using **Phenix**.

Outcome

Gained experience in the complete structure determination workflow.



BPHA4 MONOMER: CRYSTALLIZATION OPTIMIZATION

The original condition

Protein solution:
14 mg/mL BphA4

Reservoir solution:
2 M sodium formate
100 mM acetate buffer (pH 4.6)
final pH=unknown

Droplet solution:
7mg/mL BphA4
1 M sodium formate
50 mM acetate buffer (pH 4.6)

	plate		BphA4 WT	Lot. 260403 16.95mg/ml				
	aerobic	seed(-)						
	1	2	3	4	5	6	sitting/hanging	droplet size
1-260727	A1	A1	A1	B1	B1	B1	hanging	1.2μL+1.2μL=2.4μL
2-260727	A2	A2	A2	B2	B2	B2	hanging	1.2μL+1.2μL=2.4μL
3-260727	A3	A3	A3	B3	B3	B3	hanging	1.2μL+1.2μL=2.4μL
4-260727	A4	A4	A4	B4	B4	B4	hanging	1.2μL+1.2μL=2.4μL

A = Sodium formate optimization

A1 = 1.8 M, pH 4.0
A2 = 1.9 M, pH 4.0
A3 = 2.0 M, pH 4.0
A4 = 2.2 M, pH 4.0

B = pH optimization

B1 = 2.0 M, pH 3.8
B2 = 2.0 M, pH 4.0
B3 = 2.0 M, pH 4.4
B4 = 2.0 M, pH 4.8

Objective

- Improve the initial BphA4 crystallization condition identified from robotic screening.

My work

- Evaluated the initial crystallization condition obtained from the robot.
- Proposed two optimization strategies:
  i. Sodium formate optimization
  ii. pH optimization
- Applied the optimized conditions in laboratory crystallization experiments.

Outcome

- Gained experience in designing follow-up experiments based on screening results.
- Learned how crystallization conditions can be systematically optimized.

BPHA4 MONOMER: EXPERIMENTAL CRYSTALLIZATION

CRYSTALLIZATION EXPERIMENT

- Preparation of optimized crystallization solutions
- Setup of crystallization experiment
- Application of the proposed optimization strategy

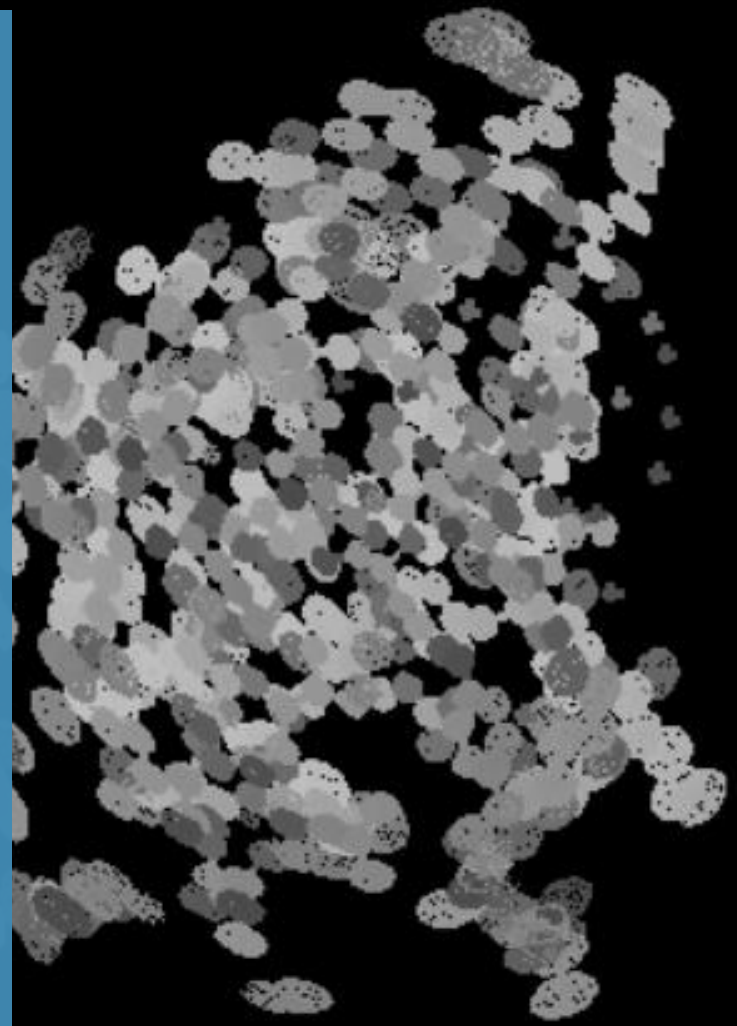
FROM TECHNIQUES TO RESEARCH EXPERIENCE

Scientific & Technical Skills

- Protein crystallization and optimization strategies
- Automated and manual crystallization workflows
- Evaluation of crystallization conditions
- Diffraction data processing
- Model building and visualization
- Structure refinement and validation
- Understanding of the complete structural biology workflow

Beyond the Techniques

- Learned how to approach scientific problems and design follow-up experiments
- Improved my ability to analyze results and discuss research strategies
- Experienced the importance of collaboration and communication within a research group
- Gained confidence working in an international scientific environment



Special Thanks

🌱 Dr. Miki Senda

For your guidance and support

🔬 Structural Biology Center members

For welcoming me into the group

🌏 KEK & SOKENDAI

For this incredible opportunity