EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

	Experiment title: Studies of changes in the structure of xylose/glucose isomerase induced by high pressure	**Experiment number**: MX-2517
Beamline: ID30B	**Date of experiment**: from: 05.05.2023 to: 06.05.2023	**Date of report**:
Shifts: 3	**Local contact(s)**: Christoph Mueller-Dieckmann	*Received at ESRF:*

Names and affiliations of applicants:

Agnieszka Klonecka [1, 2, 3], Joanna Sławek[1], Maciej Kozak[1, 4]

[1] SOLARIS Polish National Synchrotron Radiation Research Centre, Jagiellonian University, Kraków, Poland

[2] Jagiellonian University, Doctoral School of Exact and Natural Science, Jagiellonian University, Kraków, Poland

[3] Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University, Kraków, Poland

[4] Faculty of Physics, Adam Mickiewicz University, Poznań, Poland.

Report:

This report presents the experimental results and analysis conducted as part of the research project on studying the influnce of high-pressure on the crystal structure of glucose/xylose isomerase (XI) from *Streptomyces rubiginosus*. The aim of this study was to investigate the behavior of the protein under high-pressure conditions using X-ray diffraction, specifically focusing on the unfolding or denaturation of the tetrameric structure.

The protein crystals of glucose/xylose isomerase were grown before the experimnt in the EMBL laboratory. Subsequently, the crystals were subjected to freezing under high-pressure using a pressure range of 60-200 MPa. All crystals crystallized in space group I222.

The frozen protein crystals were then measured using X-ray single-crystal diffraction. The measurements were carried out at the ID30B (MASSIF-3) beamline at the ESRF.

During data collection we were able to made 121 diffraction data sets. This resulted in receiving different data, listed below:

The analysis focused on understanding the impact of high-pressure on the structure of glucose/xylose isomerase. The obtained diffraction data revealed changes in the crystal structure as the pressure increased. The resulting data sets for glucose/xylose isomerase without substrate showed the following resolutions:

- Ambient pressure: 1.48 Å
- 60 MPa: 1.20 Å
- 100 MPa: 1.08 Å
- 200 MPa: 1.26 Å

The study also examined the effect of different substrates (glucose and fructose) on the protein structure under high-pressure conditions. For glucose/xylose isomerase with glucose as a substrate, the following resolutions were obtained:

For glucose:
- Ambient pressure: 1.99 Å
- 100 MPa: 1.22 Å
- 200 MPa: 1.25 Å

For fructose:
- Ambient pressure: 1.68 Å
- 100 MPa: 1.19 Å
- 150 MPa: 1.23 Å
- 200 MPa: 1.27 Å

All measurements were conducted in series to monitor radiation damage.

To date, we have successfully refined the structure of glucose/xylose isomerase at 200 MPa and compared it to the structure obtained at ambient pressure (Figure 1). By superimposing the two structures, we calculated the RMSD to be 0.342 for 365 Cα atoms, indicating the presence of noticeable differences between the two structures. Additionally, we observed variations in the elementary cell parameters, as shown in table 1.

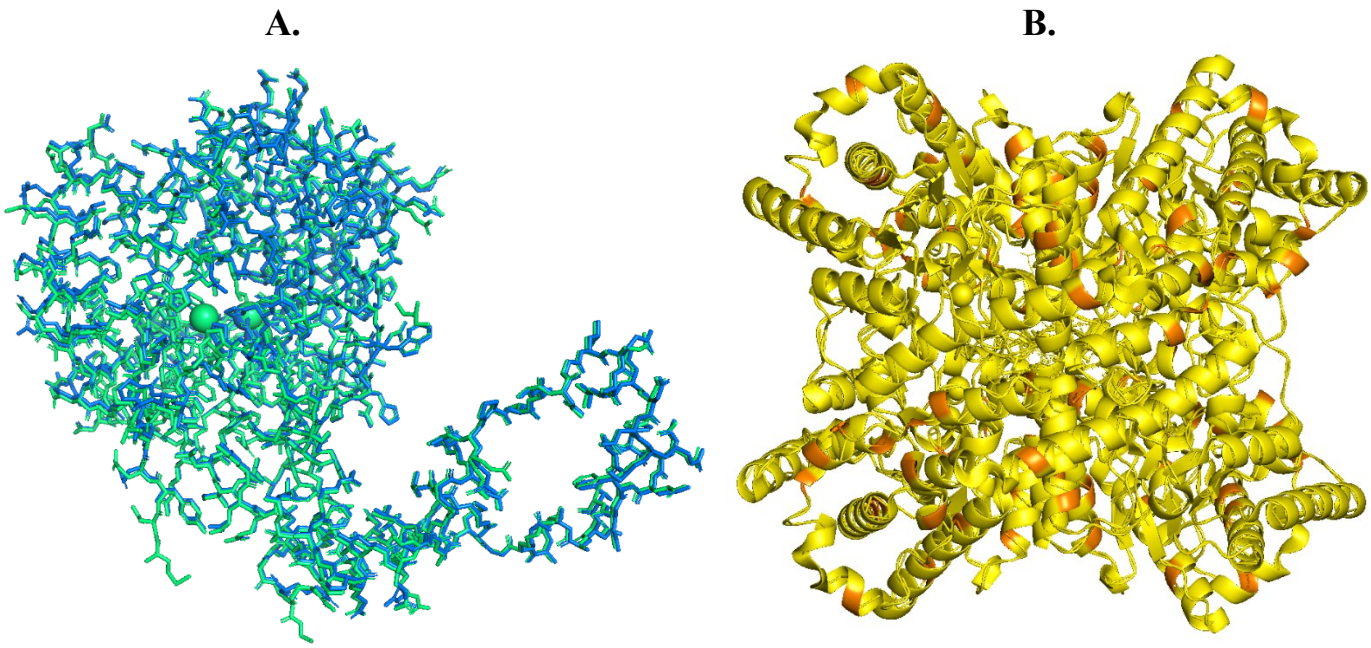

Figure 1. (A) Superimposed structures of glucose/xylose isomerase at 200 MPa and ambient pressure, revealing differences in their conformation. (B) Amino acids most affected by pressure, estimated after packing analysis.

Table 1. Comparison of elementary cell parameters between glucose/xylose isomerase structures at 200 MPa and ambient pressure.

Parameter	Ambient pressure	200 MPa
a [Å]	94.49	92.68
b [Å]	99.92	98.36
c [Å]	100.19	102.24
Volume of cavities [Å^3]	54313	60223

Currently, we are in the data processing phase. We have successfully elucidated significant structural details through the measurements conducted with the addition of sugar. These observations shed light on the transition state and the binding of the substrate occurring in a different location than previously known.

To provide visual representation of our findings, Figure 2 displays an elemental map obtained during the measurement at 200 MPa. Notably, the map reveals the presence of a glucose molecule, indicating its binding to the protein structure.

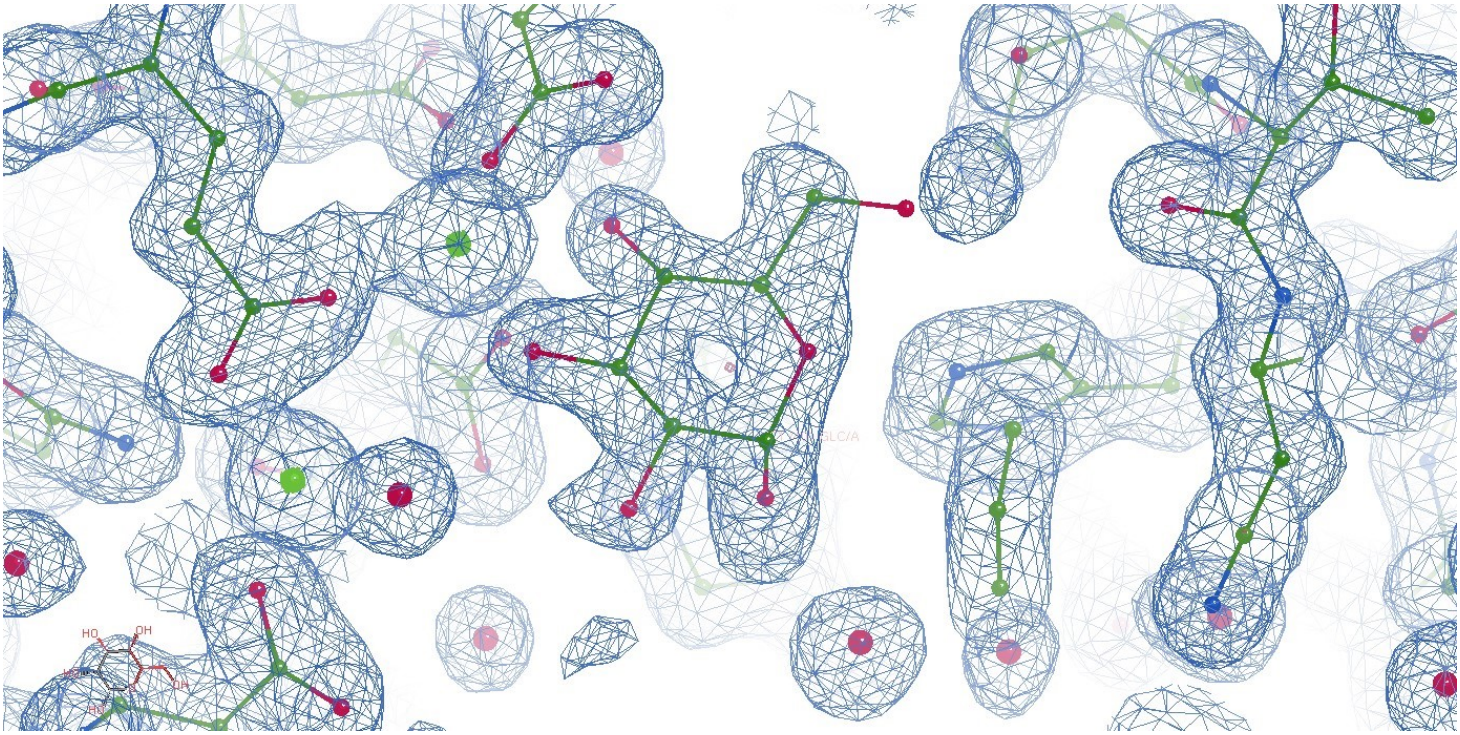

Figure 2. Electron density map of XI structure under 200 MPa with glucose addition.

The results obtained will be usd in Agnieszka Klonecka's PhD thesis and will be published in the future, and presented at the IUCr 2023 meeting.