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Sample preparation and characterization for time-resolved serial crystallography with MurA

The basic concept of serial femtosecond crystallography (SFX), diffraction-before-destruction, made it possible to collect radiation-free data with nano- and micro-crystals at room temperature. As the crystals are usually only shot once by a single short X-ray pulse and destroyed, sample preparation requires production of large numbers of crystals with homogeneous size distribution and high density. While more research groups are interested in conducting SFX experiments, the know-how on sample preparation and characterization has not been widely shared. In addition, the equipment for the microcrystal characterization is not available in all institutes. Thus, the winter school would provide the student the opportunity to learn and practice the entire process of protein crystal sample preparation and characterization for serial crystallography. [1]

The target protein for this project will be MurA (UDP-N-acetylglucosamine 1-carboxyvinyltransferase), a key enzyme in the biosynthesis of peptidoglycan, which is a major component of the bacterial cell wall. The pathway of peptidogycan biosynthesis has been an important target for antibacterial agents, such as the beta-lactams. MurA is of particular interest because it is inhibited by the naturally occurring antibiotic fosfomycin. The enzyme catalyzes the enolpyruvyl transfer from phosphoenolpyruvate (PEP) to a second substrate (UNAG), however, the detailed molecular mechanism is controversial due to lack of direct evidence. [2, 3] To design novel drugs, it is important to know the precise enzymatic mechanism as it provides the reaction intermediate structure to mimic.

The ultimate objective of this project is to visualize the intermediate structure and identify the actual mechanism. We would begin with MurA+UNAG crystals and plan to observe the structural changes caused by addition of PEP using mixing injection for time-resolved serial crystallography. During the winter school, we will work on protein preparation and micro-crystallization. As MurA has a well-established preparation protocol and macro-crystallization conditions, the student should be able to complete the entire process of sample preparation, from cell culture to micro-crystallization and injection test, for serial crystallography within five weeks. In addition, if there is a possibility (i.e. if any SFX experiment is scheduled during winter school period), the student can also join in our sample delivery experts to learn how the actual SFX experiment is performed at the XFEL beamline. Overall, the effort to optimize the crystallization conditions for the production of micro-crystals and to gain experience with characterization and injection tests would be beneficial for student to be involved in future SFX experiment.

References

1. Han H et al. (2021), J Appl Cryst, 54, 7-21.
2. Eschenburg S et al. (2003), J Biol Chem, 278, 49215-49222.
3. Zhu JY et al. (2012), J Biol Chem, 287, 12657-12667.

Field

A2: Molecular sciences (application oriented)

DESY Place

Hamburg

DESY Division

other

DESY Group

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Bio chemistry lab experience

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