Optimized approach for serial synchrotron crystallography using chips

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Abstract

Serial crystallography is a rapidly developing field that makes it possible to determine the structure of biomolecules at room temperature with atomic resolution. Numerous advances in detectors, data analysis pipelines, sample delivery methods, and crystallization protocols expand the scope of structural biology to understand the fundamental processes that take place in living cells. At the same time, all stages of experiments should be maximally optimized to avoid loss of beamtime. Thus, this paper proposes a strategy for optimizing beamtime utilization while using a fixed target sample delivery method such as chips. The strategy consists of two steps: first, a fast raster scan of the chip is performed to determine the positions of the crystals, and then small rotational series are measured at predetermined positions. Such an approach skips empty positions during data acquisition, saving valuable beam time and, as an additional consequence, reducing the volume of measured data.

***Keywords*:** serial crystallography, fixed-target sample delivery, hit-rate optimization, chip-scanning

Introduction

Proteins play an important role in our lives: they support most of the important molecular processes that occur in all living cells, and they have been used in the development of biotechnology for decades. In addition to many applications in industry, protein structures play a central role in the computer-aided development of drugs [1–5]. The function of a protein derives from its 3D structure, which can be resolved with atomic resolution using X-ray sources such as synchrotrons and Free Electron Lasers (FELs) [6]. Usually to get a 3D structure a rotational series of diffraction snapshots from a crystallized protein is acquired. One bottleneck of this technique is the damage to the sample by the radiation. This radiation damage generally results from ionization caused by X-ray exposure of the crystal, which can lead to conformational changes in the macromolecule or crystal lattice [7,8].
To overcome the problem of radiation damage, a single-shot technique, named serial crystallography (SX) was developed [9–12]. In this method, crystals are illuminated by the beam one by one in different orientations so that a full 3D structure can be obtained. Serial crystallography can help to understand the dynamics of structural fluctuations and the mechanisms of macromolecules by resolving protein structures at different time points after a uniform induction [13,14]. First SX experiments were demonstrated with short-pulses X-ray of FELs [9] and therefore is usually referred to as Serial Femtosecond Crystallography (SFX). Later similar approach was implemented at synchrotrons [11,12] and is often called Serial Synchrotron Crystallography (SSX). In conventional rotational crystallography each diffraction pattern captures some wedge of angles in reciprocal space, therefore the integrated

intensity of each reflection (Bragg peak) can be determined quite precisely. In SX each pattern is a section through a reciprocal space (its thickness depends on the beam bandwidth and divergence), so each reflection is measured only partially. Therefore, SX usually requires many snapshots for proper structure determination. The advantages of serial crystallography, however, are the ability to collect data close to physiological conditions such as room temperature and measuring small crystals, which gives access to faster dynamics, and yet SX has a lower susceptibility to radiation damage, due to the principle of "diffract before destroy" [15].

In the last decades, X-ray crystallography has developed rapidly: there are new generations of X-ray sources with brighter beams, more advanced detectors with better properties (wider dynamic range, single-photon sensitivity, fast frame rate), improvements in focusing optics, and the latest crystal delivery systems have paved the way for the study of a wider range of possible protein samples. In addition, innovations in data acquisition and analysis have also emerged, expanding the scope of structural biology research. It also allows us to optimize the sample delivery methods for more efficient use of the proteins and the beamtime.

In serial crystallographic experiments, different systems are used to deliver crystal samples of micro- to nanometer scale into the X-ray. For example injector methods with liquid or extrusion jets, fixed-target methods, and hybrid methods. These techniques for XFEL sources and instruments are discussed in detail in the literature [16]. The choice of sample delivery method depends on the experimental design and the parameters of the crystals (like size or quantity). Furthermore, physical parameters (room temperature or cryogenic conditions, ambient or high pressure, vacuum, dynamical or static measurements) limit the experimental design to various delivery methods. If more than one sample delivery method can be used in an experiment, their advantages and disadvantages should be considered for each step of data acquisition, availability at the beamline, and impact on further data processing steps. In the following, we will give a brief overview of existing sample feeding methods and their main features.

The idea behind crystal-injector methods is to obtain a fine stream of crystals by ejecting a suspension of crystals through a small nozzle that usually flows orthogonally to the beam direction. The X-ray beam interrogates the crystal stream and a diffraction pattern is produced each time an X-ray pulse hits a jet with crystal or without. Injectors techniques could be divided into the following types:

- injectors with liquid jet (or high-flow injector techniques like dynamic virtual nozzles, GDVN [17], double-flow focusing nozzle, DFFN [18]): the main advantage of such injectors is relatively low background, can be used in air and vacuum, is capable of delivering the samples at a very high rate (up to MHz) and can be used for different time-resolved measurements (light-activated of mixing). The main disadvantage of this method is high sample consumption due to the high injection speed.
- Injectors with extrusion jet (or low-flow injector technique such as Lipidic cubic phase injector (LCP-jet) [19]): in comparison with using liquid jet such sample-

delivery method is slower and gives higher sample consumption efficiency, but the jet is thicker, so it produces higher background.

- microfluidic electrokinetic sample holder (MESH) [20,21]: this method is characterized as a low-flow method compatible with both liquid, and moderately viscous carrier media, but can affect the observed structure of the protein due to the electric potential. In comparison to a thin liquid jet, MESH has a higher background.
- other methods of sample delivery, based on jets, that are widely used in synchrotrons and FELs such as a droplet-based injection method [22,23], sample extractor method [24], laser ablation of crystal-containing solutions [25,26] and so on.

Alternative to jets, different fixed-target sample delivery systems were developed. The main concept of such a sample-delivery method is to fix the crystal on a solid support which is manipulated to translate and, sometimes, rotate the crystal in the X-ray beam. The fixed-target approach allows efficient usage of the protein since each crystal can be measured, therefore, it reduces sample consumption dramatically. Thus, it can be considered an appropriate method for delivering protein samples that are in short supply due to their difficulties with the expression, purification, and crystallization steps. This method also allows on-the-chip crystallization [27], thus it is more suitable for brittle crystals to prevent damage. This is a huge advantage over the injection methods, which can create risks in the filtering steps, transfer, and loading stages, as well as pressures and forces associated with the injection process itself. By creating a bigger support allowing to hold a large number of crystals, preferably in well-defined positions, and moving the support with respect to the X-ray beam in such a way that each X-ray pulse hits an individual crystal, it is possible to reach 100% sample efficiency and high hit rate. It is also possible to collect data at room temperature and controlled humidity or cryogenic temperatures. Some beamlines have a robot for mounting holders on the goniometer that eliminates the need to enter the experimental hutch during the experiment.

Several different fixed-target designs for serial crystallography have been developed in recent years [28–33]. The crystals are usually placed on a thin membrane chip made of silicon or silicon nitride periodically patterned with microscopic wells or pores. If the pores are of an appropriate size, the crystals get trapped when the excess mother liquor is removed by blotting [33], resulting in a very low background [32,34,35]. It is necessary to avoid dehydration of the crystals, which is usually achieved by sealing the chip between two membranes or by keeping it under a humid environment such as a stream of humidified gas. The first approach is suitable for vacuum measurements, while the second one has the advantage of giving a lower background as it does not introduce any extra material into the beam. Various types of chips are being used as a fixed-target for protein crystallography, such as a nylon mesh and an enclosed film (NAM)-based sample holder [36] and robust polymeric microfluidic chips [37]. New fixed-target delivery approaches such as a combined inject-and-transfer system (BITS) are also presented [38].

Delivery methods using jets, acoustic droplet injection, or the crystal extractor are hard to optimize to reduce the time of data acquisition: the decision to stop data collection will be made by the research group based on the desired or optimum data quantity and/or quality. Usually, in these methods, during or after the experiment, hit-finding is launched [39] and only useful patterns are selected. With a fixed target there are two standard modes for data collection: either “fly” scans (data acquired during the movement) at different angles, or doing mini-rotations at each position of a step-scan. To optimize the scanning of the fixed-target chips one can try to locate the crystals and do the measurements at the found positions. The task of finding the crystals can be done in different ways: using second-harmonic imaging of protein crystals (SONICC) or UV tryptophan fluorescence imaging (UV imaging) or even manually selecting crystals using an in-line microscope. Unfortunately, all these methods have their drawbacks: SONICC is difficult to integrate at the beamline, optical auto-search of crystal positions often fails since the crystals have very different shapes and sizes and manual centering requires a lot of concentration and user intervention. Another approach for automating the data acquisition of hundreds to thousands of microcrystals with fixed targets is to use micro-meshes or microchips [30] with known geometry of pores. The idea is to load such chips with protein crystals and then blot them further to get crystals rearranged according to the position of the pores. Then, the data collection is performed only for the predefined positions of pores, assuming the crystals are actually in the pores. Nevertheless, the main problem of this technique is that crystals, especially the big ones, do not always get into the pores and could be found anywhere on the chip including the edge of the chip. Another limitation of this method is that the crystals, smaller than the pore size, will be sucked in together with the liquid during the blotting process.

To improve the data collection with fixed target sample delivery we propose to use two-stage scanning of the chip: first a short fly scan, to find the positions with diffracting crystals, followed by the mini-rotation series only for those positions. In practice it is implemented in the following way: the fly-scan is performed with low-intensity of incident X-rays to prevent damage to the crystals and at the maximum scanning speed. Then the collected data is analyzed to determine the positions of the scan at which the crystal diffraction was observed. The data analysis is performed for each scan line individually and in parallel, thus, the results are available shortly after the scan is finished. Then at each scan position, where the typical crystal diffraction pattern (with sharp Bragg peaks) was detected, small rotation series are collected. Such an approach lets us speed up the data collection and reduce the total volume of generated data by preliminary applying a non-hits rejection approach. Here we are going to present the proof of principle of the smart X-ray chip scanning by introducing the intermediate step of crystal localization into the CrystalControl software developed at P11, Petra III, Hamburg, DESY.

Materials and Methods

Detailed information about the status of the crystallography beamlines at Petra III for 2016 can be found in [40]. The control of the experiment at the P11 beamline, PETRA III, is carried out using a custom-made Python-based Graphical User Interface (GUI) named

CrystalControl (CC). In addition to standard MX data acquisition, the GUI provides several features for micro-crystallography, such as the ability to scan a grid. Two different modes for the grid scans are implemented: a fly scan and a step scan. In the fly scan mode, the measurements are performed at a fixed angular position of the chip during the horizontal translation at a constant velocity. This mode allows fast scanning, which is limited by the detector speed (133 Hz in the case of the Eiger 2X 16M) and has a limited positioning accuracy. The main disadvantage of this method is the fact that measuring is performed at a constant angle of the chip to the beam, which can lead to low completeness and anisotropy of measured reflections if the crystals have preferential orientation at the chip. Taking into account that crystals usually survive a single measurement with a short acquisition time, there is a chance to measure the same chip several times at different angles. The drawback of such an approach is the proportional increase in measurement time and the chance that the crystals might get dehydrated during the long measurement. The step scan is performed differently: the chip is shifted to a predefined position and then the data collection starts. In this mode, a rotation series can be acquired at each position, which generates more usable data per crystal. The drawback of this method is that it is much slower than the fly scan since positioning at each point of the predefined grid usually requires more time. The common issue of both scans is that the entire chip is scanned regardless of the presence of crystals. This is especially noticeable if the chip contains a small number of crystals. In this study, we propose an alternative approach for chip scanning.

We used 4 x 10 mm silicon Chips, manufactured by Suna precision [41] (see Fig. 2), covered by a suspension of lysozyme crystals. The excess solution was removed by blotting and subsequently protected by a sealed cover sleeve. Then the chip was manually installed at the goniometer using the standard magnetic mount and the approach of smart chip screening was performed.

First, a fast fly scan with low exposure, with the deposited dose of several kGys per position to minimize the damage of the crystals [Schulz 2022], is performed. This dataset is run through a hit-finder (for example [39] or [42]) to determine the frames containing useful diffraction. Then a step scan with preset parameters (angular range and the acquisition time) is started only for those positions, where the useful diffraction was detected. This allowed us to greatly speed up the serial measurements using chips by collecting the data only at the positions of the located crystals. One more advantage of the proposed scanning mode is the reduction in the volume of the collected data: instead of measuring every position at the grid, regardless if it contains a crystal or not, only the useful data is stored. Only the first fly scan has every position at the grid, but this scan can be just deleted because it does not contain more useful information than the following rotation micro-series collected at the position of the determined crystals.

To speed up the process of the hit finding we have used the following strategy. Each line in a fly scan is saved as a separate HDF5 file, so as soon as the saving of the file is finished a job is submitted to the DESY Maxwell HPC Cluster (https://confluence.desy.de/display/IS/Maxwell) to perform the hit-finding. Since the

Maxwell cluster contains a lot of quite powerful nodes, this strategy does the hit-finding almost on-the-fly. Alternative hit-finding was performed using the Dozor program [42] that was also run for each line separately at the dedicated P11 cluster. The two approaches were used simultaneously and compared - see Fig 4. The result of the hit-finding step for each scanned line was a text file with recorded coordinates of crystal positions along that line. All individually processed files were merged to make a two-dimensional map of the crystal positions at the chip. This map was automatically loaded by the step-scan for data collection and the step-scan with predefined positions was launched.

The latest version of CC installed at P11 was modified by including some intermediate steps to demonstrate the proof of principle of our scanning approach. The main workflow involves drawing a grid, which we further scan at low intensity to find hits. As a result of the previous step, we get positions with detected crystals, and only certain positions will remain on the drawn grid. At the final stage, we start scanning the modified grid with user-specified parameters (angular step, number of exposures per position, and transmission). The core idea of the algorithms can be seen in Fig. 1. Such peak finding algorithms as Dozor [42] and peakfinder8 [39] were tested with Dozor and peakfinder8, and then visually compared (see Fig. 4).

To test the developed scanning mode three experiments were performed at the P11 beamline. The experiments were carried out at the photon energy 18 keV using the detector Eiger 16M placed 155 mm behind the sample, the beam was focused on the spot of 9x5 um. The beamline transmission during the fly-scan was set to the value of 1% and the exposure time to 40 ms (1.3kGy), while during the measurements of rotation mini-series, the transmission was set to 10% (approximately 5e11 ph/s in the focus of 9x5 um2) and the exposure to 100 ms. Rotation mini-series were performed with 10 frames, 0.50 degrees/frame at each position. Such measurement deposits the dose of 325kGy at each position, that can be considered as acceptable [de la Mora 2020].

Results

To check if the positions of the determined crystals were enough for the further data processing pipeline we have performed the following test: we indexed using CrystFEL [43] the whole measured during the fly-scan dataset (containing both useful diffraction and non-hits) and compared the results to the indexing of only found hits. Table 1 demonstrates 6 different scans confirming that in both cases the number of indexed patterns was the same, so all positions of the grid with useful crystal diffraction were determined at the hit-finding stage.

Table 1

Dataset	Number of indexed patterns while indexing all frames	Number of indexed patterns, while indexing only frames determined as

		hits
Lyso1_grid1	27	27
Lyso1_grid2	94	94
Lyso2_grid1	232	232
Lyso3_grid1	545	545
Lyso4_grid1	511	511
Lyso5_grid1	155	155

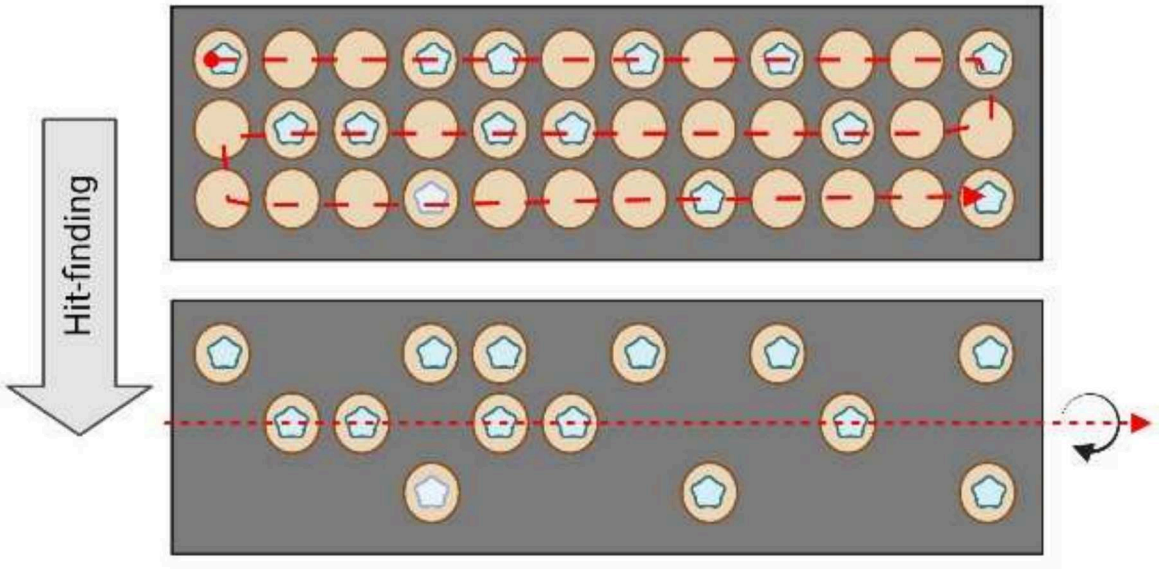


Figure 1. The principle of the smart chip-scanning approach. First, an on-the-fly scan is performed to locate well-diffracting protein crystals and then mini-rotation series were measured at each detected crystal position.

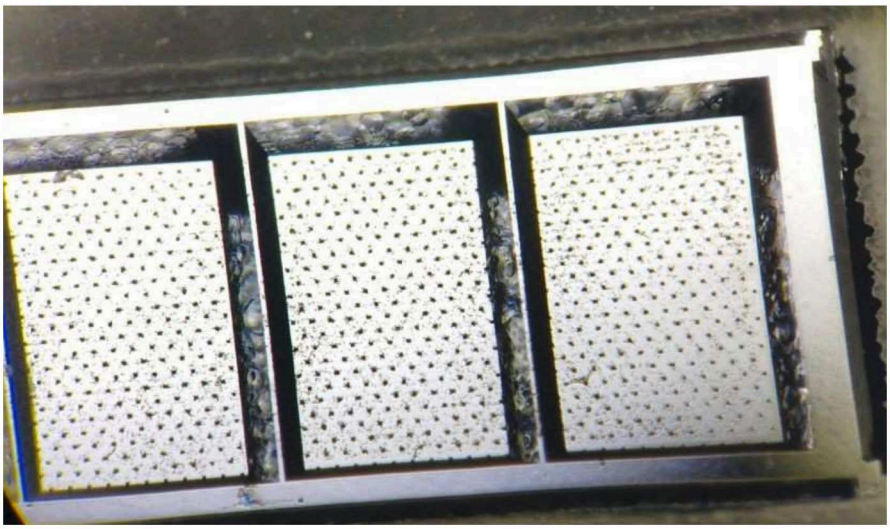


Figure 2. The photo of used 4 x 10 mm silicon Chips, manufactured by Suna precision [https://www.suna-precision.com/products/serial-crystallography/micro-patternd-silicon-chips/]

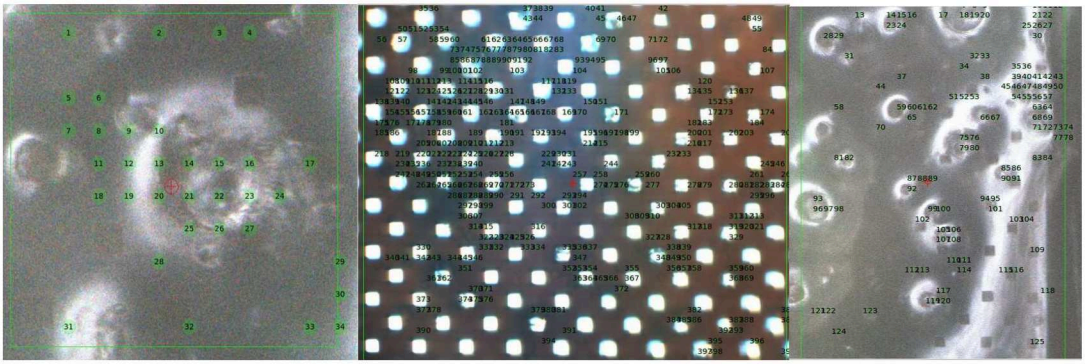
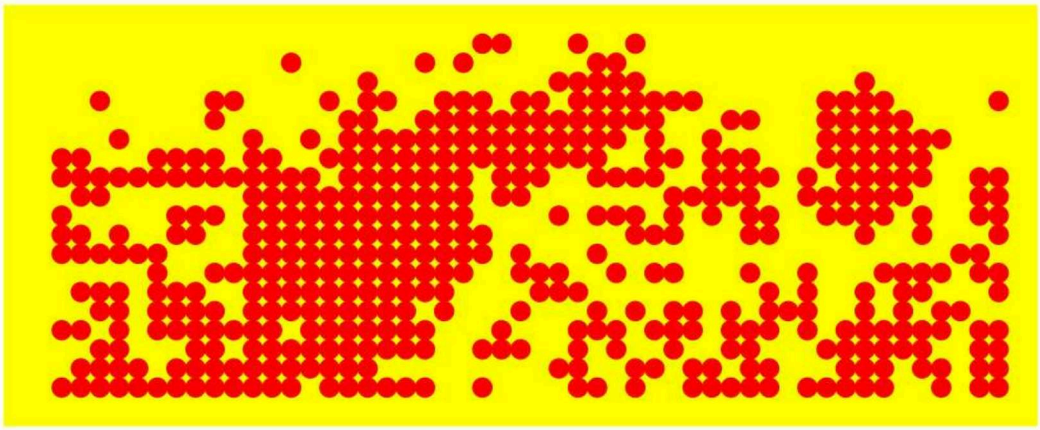


Figure 3. Examples of the grids with detected protein crystal positions as obtained automatically as a result of the developed approach.

peakfinder8



Dozor

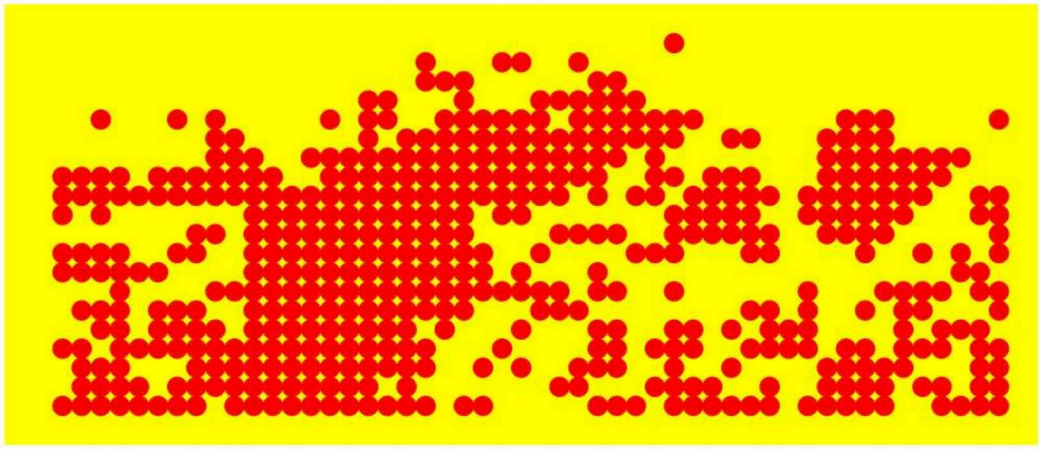


Figure 4. The positions of detected protein crystals by peakdinder8 and Dozor.

Lysozyme sample preparation

The following protocol for sample crystallization was used [27]: hen egg white lysozyme was purchased from Sigma-Aldrich and dissolved in 50 mM sodium acetate pH 3.5 (140 mg/mL) and filtered through a 0.2 µm filter. A cold solution (4°C) of 60 mg/mL lysozyme was mixed 1:1.5 with a prechilled (4°C) precipitation solution (50 mM sodium acetate pH 3.5, 0.75 M sodium chloride, 30% ethylene glycol, 11.25% polyethylene glycol 400), adapted from Lieske et al. 2019. The mixture was incubated at 4 °C for 16 hours and mixed at intervals using an Eppendorf Thermomixer C (1600 rpm for 30 seconds, every 5 minutes).

Setup information

A micro-patterned silicon chip (Suna precision) with a size of 4 x 10 mm was used as a fixed target sample holder [41]. The silicon chip was perforated with 25 µm holes through which excess liquid can be sucked. The silicon chip holder has a cavity that serves as a mother liquor reservoir and provides an equilibrated humid environment for the sample. Batch crystals with a size range of 25-40 µm were deposited on the chip and the excess reservoir solution was sucked through the chip holes with a tissue. A thin meiler foil sleeve was used as humidity protection against drying out.

Discussion

Structural biology helps us to understand the connection between the protein structure and the function it provides within living cells. Due to the high interest in structural biology applications, more methods for structure determination are being developed. One of the most thrilling methods is X-ray serial protein crystallography. Because of rapid developments in such areas as detectors, sample preparation protocols, sample delivery systems, and optics, new strategies for data collection have appeared. Of the two main types of sample delivery systems, fixed-target methods offer several advantages over jet injectors, including lower sample consumption, clog-free delivery, often lower background, and the ability to control crystal-on-chip density for optimal hit rates. This work is focused on the optimization of the

measurement of crystals deposited at fixed-target support (chips). This is achieved by using a two-step scanning mode: first, the chip is scanned with high speed at a low-dose mode and then the positions at which the crystals were detected are measured as mini rotation series. The determination of the crystals' positions is done during the first fly-scan and a grid of interesting positions is formed automatically. Such optimization of the scans helps not only to achieve a higher speed of data collection but also results in reducing data volume due to avoiding collecting empty frames from positions lacking crystals, which prevents the storage of unusable data and thus saves a lot of resources. The developed “smart” chip-scanning approach was implemented in a separate branch of the CrystalControl software at the P11 beamline and its integration into the controlling software at P09 (protein crystals screening station) is ongoing.

Conflict of interest statement

Nothing declared.

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References

1. Blundell T.L. Protein crystallography and drug discovery: recollections of knowledge exchange between academia and industry // IUCrJ. 2017. Vol. 4, № 4. P. 308–321.
2. Pomés A. et al. 100 Years later: Celebrating the contributions of x-ray crystallography to allergy and clinical immunology // Journal of Allergy and Clinical Immunology. 2015. Vol. 136, № 1. P. 29-37.e10.
3. Srinivasan V. et al. Antiviral activity of natural phenolic compounds in complex at an allosteric site of SARS-CoV-2 papain-like protease // Commun Biol. 2022. Vol. 5, № 1. P. 805.
4. Ennifar E. X-ray Crystallography as a Tool for Mechanism-of-Action Studies and Drug Discovery // CPB. 2013. Vol. 14, № 5. P. 537–550.
5. Wu S. et al. Biocatalysis: Enzymatic Synthesis for Industrial Applications // Angew. Chem. Int. Ed. 2021. Vol. 60, № 1. P. 88–119.
6. Parker M.W. Protein Structure from X-Ray Diffraction // Journal of Biological Physics. 2003. Vol. 29, № 4. P. 341–362.
7. Nass K. Radiation damage in protein crystallography at X-ray free-electron lasers // Acta Crystallogr D Struct Biol. 2019. Vol. 75, № 2. P. 211–218.
8. Garman E.F. Radiation damage in macromolecular crystallography: what is it and why should we care? // Acta Crystallogr D Biol Crystallogr. 2010. Vol. 66, № 4. P. 339–351.
9. Chapman H.N. et al. Femtosecond X-ray protein nanocrystallography // Nature. 2011. Vol. 470, № 7332. P. 73–77.
10. Boutet S. et al. High-Resolution Protein Structure Determination by Serial Femtosecond Crystallography // Science. 2012. Vol. 337, № 6092. P. 362–364.
11. Stellato F. et al. Room-temperature macromolecular serial crystallography using

synchrotron radiation // IUCrJ. 2014. Vol. 1, № 4. P. 204–212.
12. Gati C. et al. Serial crystallography on *in vivo* grown microcrystals using synchrotron radiation // IUCrJ. 2014. Vol. 1, № 2. P. 87–94.
13. Schmidt M. Time-Resolved Macromolecular Crystallography at Pulsed X-ray Sources // IJMS. 2019. Vol. 20, № 6. P. 1401.
14. Schmidt M. Reaction Initiation in Enzyme Crystals by Diffusion of Substrate // Crystals. 2020. Vol. 10, № 2. P. 116.
15. Neutze R. et al. Potential for biomolecular imaging with femtosecond X-ray pulses // Nature. 2000. Vol. 406, № 6797. P. 752–757.
16. Martiel I., Müller-Werkmeister H.M., Cohen A.E. Strategies for sample delivery for femtosecond crystallography // Acta Crystallogr D Struct Biol. 2019. Vol. 75, № 2. P. 160–177.
17. DePonte D.P. et al. Gas dynamic virtual nozzle for generation of microscopic droplet streams // J. Phys. D: Appl. Phys. 2008. Vol. 41, № 19. P. 195505.
18. Oberthuer D. et al. Double-flow focused liquid injector for efficient serial femtosecond crystallography // Sci Rep. 2017. Vol. 7, № 1. P. 44628.
19. Weierstall U. et al. Lipidic cubic phase injector facilitates membrane protein serial femtosecond crystallography // Nat Commun. 2014. Vol. 5, № 1. P. 3309.
20. Kern J. et al. Room temperature femtosecond X-ray diffraction of photosystem II microcrystals // Proc. Natl. Acad. Sci. U.S.A. 2012. Vol. 109, № 25. P. 9721–9726.
21. Sierra R.G. et al. Concentric-flow electrokinetic injector enables serial crystallography of ribosome and photosystem II // Nat Methods. 2016. Vol. 13, № 1. P. 59–62.
22. Roessler C.G. et al. Acoustic methods for high-throughput protein crystal mounting at next-generation macromolecular crystallographic beamlines // J Synchrotron Rad. 2013. Vol. 20, № 5. P. 805–808.
23. Roessler C.G. et al. Acoustic Injectors for Drop-On-Demand Serial Femtosecond Crystallography // Structure. 2016. Vol. 24, № 4. P. 631–640.
24. Mathews I.I. et al. The Conformational Flexibility of the Acyltransferase from the Disorazole Polyketide Synthase Is Revealed by an X-ray Free-Electron Laser Using a Room-Temperature Sample Delivery Method for Serial Crystallography // Biochemistry. 2017. Vol. 56, № 36. P. 4751–4756.
25. Franjic K., Miller R.J.D. Vibrationally excited ultrafast thermodynamic phase transitions at the water/air interface // Phys. Chem. Chem. Phys. 2010. Vol. 12, № 20. P. 5225.
26. Schulz E.C. et al. Protein crystals IR laser ablated from aqueous solution at high speed retain their diffractive properties: applications in high-speed serial crystallography // J Appl Crystallogr. 2017. Vol. 50, № 6. P. 1773–1781.
27. Lieske J. et al. On-chip crystallization for serial crystallography experiments and on-chip ligand-binding studies // IUCrJ. 2019. Vol. 6, № 4. P. 714–728.
28. Hunter M.S. et al. Fixed-target protein serial microcrystallography with an x-ray free electron laser // Sci Rep. 2014. Vol. 4, № 1. P. 6026.
29. Mueller C. et al. Fixed target matrix for femtosecond time-resolved and in situ serial micro-crystallography // Struct. Dyn. 2015. Vol. 2, № 5. P. 054302.
30. Roedig P. et al. A micro-patterned silicon chip as sample holder for macromolecular crystallography experiments with minimal background scattering // Sci Rep. 2015. Vol. 5, № 1. P. 10451.
31. Oghbaey S. et al. Fixed target combined with spectral mapping: approaching 100% hit rates for serial crystallography // Acta Crystallogr D Struct Biol. 2016. Vol. 72, № 8. P. 944–955.
32. Owen R.L. et al. Low-dose fixed-target serial synchrotron crystallography // Acta Crystallogr D Struct Biol. 2017. Vol. 73, № 4. P. 373–378.
33. Kisselman G. et al. X-CHIP: an integrated platform for high-throughput protein crystallization and on-the-chip X-ray diffraction data collection // Acta Crystallogr D Biol Crystallogr. 2011. Vol. 67, № 6. P. 533–539.
34. Roedig P. et al. High-speed fixed-target serial virus crystallography // Nat Methods. 2017. Vol. 14, № 8. P. 805–810.

35. Meents A. et al. Pink-beam serial crystallography // Nat Commun. 2017. Vol. 8, № 1. P. 1281.
36. Park S.-Y. et al. Fixed-Target Serial Synchrotron Crystallography Using Nylon Mesh and Enclosed Film-Based Sample Holder // Crystals. 2020. Vol. 10, № 9. P. 803.
37. Gilbile D. et al. Plug-and-play polymer microfluidic chips for hydrated, room temperature, fixed-target serial crystallography // Lab Chip. 2021. Vol. 21, № 24. P. 4831–4845.
38. Lee K. et al. Combination of an inject-and-transfer system for serial femtosecond crystallography // J Appl Crystallogr. 2022. Vol. 55, № 4. P. 813–822.
39. Barty A. et al. *Cheetah*: software for high-throughput reduction and analysis of serial femtosecond X-ray diffraction data // J Appl Crystallogr. 2014. Vol. 47, № 3. P. 1118–1131.
40. Burkhardt A. et al. Status of the crystallography beamlines at PETRA III // Eur. Phys. J. Plus. 2016. Vol. 131, № 3. P. 56.
41. Roedig P. et al. A micro-patterned silicon chip as sample holder for macromolecular crystallography experiments with minimal background scattering // Sci Rep. 2015. Vol. 5, № 1. P. 10451.
42. Zander U. et al. *MeshAndCollect*: an automated multi-crystal data-collection workflow for synchrotron macromolecular crystallography beamlines // Acta Crystallogr D Biol Crystallogr. 2015. Vol. 71, № 11. P. 2328–2343.
43. White T.A. et al. *CrystFEL*: a software suite for snapshot serial crystallography // J Appl Crystallogr. 2012. Vol. 45, № 2. P. 335–341.