Abstract: Radiation damage and cryogenic sample environment are an experimental limitation observed in the traditional X-ray crystallography technique. However, the serial crystallography (SX) technique not only helps to determine structures at room temperature with minimal radiation damage, but it is also a useful tool for profound understanding of macromolecules. Moreover, it is a new tool for time-resolved studies. Over the past 10 years, various sample delivery techniques and data collection strategies have been developed in the SX field. It also has a wide range of applications in instruments ranging from the X-ray free electron laser (XFEL) facility to synchrotrons. The importance of the various approaches in terms of the experimental techniques and a brief review of the research carried out in the field of SX has been highlighted in this editorial.

Keywords: serial crystallography (SX); serial femtosecond crystallography (SFX); serial millisecond crystallography (SMX); serial synchrotron crystallography (SSX); sample delivery
Moreover, the crystal structure determined at cryogenic temperatures is less flexible than that at room temperature [20,21]. Therefore, the process followed in traditional X-ray crystallographic techniques can sometimes determine structures that are less biologically relevant [22,23].

Serial crystallography is a technique that can overcome the experimental limitations observed in the traditional X-ray technique [6,24,25]. SX uses X-ray free electron laser (XFEL) or synchrotron X-ray, wherein the crystals are exposed to the X-rays only once, thus minimizing radiation damage compared to the traditional X-ray crystallography technique. Moreover, these experiment approaches allow data collection at room temperature [26].

Serial femtosecond crystallography (SFX) using XFEL provides an extremely high peak brilliance and an ultrashort pulse width at a femtosecond jitter, thereby providing an opportunity to implement not only SX data collection using small crystal samples but also to enable a markedly short time-scaled pump-probe study [6,27]. Thus, SX using XFEL is beneficial for time-resolved studies using micron or sub-micron crystals. However, owing to the structure of the XFEL facility for XFEL production, the supply of beam time cannot meet the demand. As an alternative to this technique, serial millisecond crystallography (SMX) or serial synchrotron crystallography (SSX) based on synchrotron X-rays has been recently used [28–32]. In order to perform the SX using synchrotron X-rays, it is important not only to provide a high photon flux at the sample position but also to enable fast readout by a detector [33]. Currently, many macromolecule crystallography beamlines at synchrotrons have achieved X-ray focusing using optics (e.g., K-B mirror) by up to a few microns and high-speed readout detectors are commercially available. Therefore, it is expected that several SX-related studies will utilize more synchrotrons in the near future.

The process of collecting diffraction data by exposing the crystal to X-rays in SX is the same as that observed in the traditional X-ray crystallography technique. However, because X-rays are only exposed to the crystal volume in the SX experiment, determination of the three-dimensional structure will require a large number of crystals to collect diffraction data [26]. Moreover, a sample delivery method is required to continuously and reliably deliver a large number of crystal samples to the X-ray interaction point for diffraction data collection [34,35]. Various sample delivery methods such as the use of injectors [36,37], syringes [38,39], injection with carrier matrix [35,37–44], fixed-target scanning [33,45–48], microfluidics [49–51], and conveyor belt approaches [52,53] are developed. These techniques are used to carry out the SX experiment and crystal structure can be determined successfully.

The sample delivery method is selected based on the characteristics of the crystal, crystallization solution, amount of crystal, and the purpose of the study. Moreover, the method is selected according to the specifications of the sample delivery device and the facility in which the SX is carried out. Currently, there are five X-ray XFEL facilities that carry out SFX, namely, Linac Coherent Light Source (LCLS) [54], SPring-8 Angstrom Compact Free Electron Laser Facility (SACLA) [55], Pohang Accelerator Laboratory X-ray Free Electron Laser (PAL-XFEL) [56], European XFEL [57], and SwissFEL [58]. In SFX, beamline is performed in these XFEL facilities, and the sample environment (vacuum, He, or ambient air), beam characteristics (repetition rate, photon flux, beam size, pulse width, and so on), and other devices (detector, optical laser, and so on) are different. Moreover, various experimental devices (injector, translation stage, and crystal support for fixed-target scanning) are applied to deliver samples. In SX or SMX using synchrotron X-rays, sample delivery devices, beam characteristics (photon flux and beam size), detector (specification and readout speed), and sample environment (temperature and air path) vary in each beamline. As a result, research approaches vary according to the specifications and characteristics of the experimental hutch that performs SX and the devices that deliver the sample.

In the early stages of SX research, sample delivery and data collection strategies based on injectors were common; however, now diverse sample delivery methods and data collection strategies have been reported [26,33–35,59,60]. Although the SX research approach used by each facility cannot be applied in the same manner to all other facilities, reporting and sharing of SX approaches followed in each research institute and facility will provide new insights to the SX community in the future.
and provide useful information for development of a universal system that can be easily accessed by general researchers.

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