ejection (ADE) methods to accurately and gently transfer small protein crystals (roughly 10 μm on each side) within microdroplets of mother liquor from the crystallization well, through a short air column (1-10 cm), to a standard X-ray diffraction mounting mesh. The acoustic droplet ejection instrumentation uses sound energy to transfer nanoliter to picoliter volumes from the surface of liquids. The successful use of ADE to transfer living cells and isolated DNA without inducing strand breaks suggests that it might be gentle enough for protein crystals.

Here, we report that ADE methods are well-suited for transferring 2.5 nanoliter droplets of microcrystal slurries of insulin or lysozyme from a 384-well plate to standard MiTeGen™ (Ithaca, NY) micromesh mounting pins. After cryocooling, the micron-sized crystals are located on the mesh with the X-ray beam via a rasterscan strategy by the presence or absence of diffraction. Once microcrystals are located, partial datasets are collected and crystal structures solved to better than 2 Å resolution from merged datasets. Importantly, high resolution structures can be solved from slurries of microcrystals that traditionally would have been discarded as unsuitable for X-ray diffraction studies.

Keywords: microdiffraction, method, synchrotron

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The use of kinoform lenses as an option for microbeams in macromolecular crystallography

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Most new generation macromolecular beam lines offer small beams in the tenth of micron range. Significant reductions in beam size imply in significant beam flux losses. The inclusion of kinoform lenses in the existing beam line optics may be a simple and cheap alternative to obtain beams in the 1 micron range.

A kinoform lens designed to focus in the one-to-one configuration was inserted in the beam path of the bending magnet beam line X6A at the National Synchrotron Light Source. The optical design for the X6A beam line is very simple, with a channel cut Si (111) monochromator and a toroidal focusing mirror, the focused beam size on the sample is of the order of several hundred microns. Using the image of a precision adjustable slit 1m upstream of the sample position the kinoform lens allowed us to produce a beam size that is adjustable from the smallest measured size of 20 microns up to the size normally produced by the beamline optics. Advantages of this approach are 1) an improved signal to noise, 2) a conveniently adjustable trade-off between spot size and flux on sample, and 3) simple configuration change from small beam mode to the normal, larger beam size mode.

Keywords: kinoform lens, macromolecular crystallography

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Protein micro-crystallography at the micro focus beamline BL32XU at SPring-8

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A newly developed micro-focus beamline, BL32XU at SPring-8, is dedicated to the protein-micro crystallography[1]. Available focused beam size is from 1 to 10 μm square with photon flux density of 10^{10} phs/sec/μm^2. The user operation of this beamline started from May 2010 for the domestic users, and now opens its 20% beam time for public user worldwide. The beamline is operated mainly for the National Project, named "Targeted Proteins Research Program".

Structure determinations of proteins are often hindered when the size of available crystals is small, even though using the synchrotron radiation. However, proteins involved in recent target such as membrane proteins or protein complexes, tend not to grow largely enough for providing good diffraction signals. Thus, demands for achieving protein micro-crystallography are getting larger. Accordingly, the construction of this beamline was started from 2007 at SPring-8.

We had successfully completed the commissioning of the beamline at the end of 2009. The achieved beam size at sample position corresponded to 0.9 x 0.9 μm^2 with 6 x 10^{10} photons. The beam size is easily changeable by users from 1 to 10 μm square with the almost same flux density.

An equipped automatic sample changer, SPACE[2], can mount so-called Hampton-style pins stored in UNIPUCK trays[3]. The robot enabled user to conduct beamline experiments completely from outside of the hutch[4]. This is also important for stabilizing a position of the micro-beam against the temperature change of the hutch inside. By keeping the hutch temperature precisely, the drift can be controlled below 2 μm per a day, which is easily fixed with a few minutes automatic beamline tuning. For reducing background scattering from the air, a helium chamber which sealed sample environment was developed and usable in co-operation with the helium gas cryo-cooler and SPACE which had a compact arm to access to the goniometer.

Through one year user operation of the beamline BL32XU, some experiments, previously considered to be difficult, were achieved by using its micro-beam with high flux density, such as collecting a full diffraction dataset from 3 μm protein crystal, acquiring high quality dataset from a crystal harvested from the initial crystallization condition, probing single-crystal volumes from a heterogeneous protein crystal, and so on.

These results proved that the beamline benefited users by cutting off their time to optimize crystallization conditions especially for smaller and lower quality crystals.

We will also present about the high throughput screening system of protein micro-crystals using the CMOS detector[5].

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The long-wavelength MX beamline I23 at diamond light source

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Experimental phasing exploiting the anomalous signal from protein or RNA/DNA crystals around specific absorption edges has become the method of choice to solve the crystallographic phase problem in macromolecular crystallography (MX) in the absence of molecular replacement models. For metallo-proteins such absorption edges are within the wavelength range from 0.6 to 2.2 Å typically provided by