Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallography on a chip

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Received 14 August 2011
Accepted 22 December 2011

A new chip-based crystal-mounting approach for rapid room-temperature data collection from numerous crystals is described. This work was motivated by the recent development of X-ray free-electron lasers. These novel sources deliver very intense femtosecond X-ray pulses that promise to yield high-resolution diffraction data of nanocrystals before their destruction by radiation damage. Thus, the concept of 'diffraction before destruction' requires rapid replenishment of the sample for each exposure. The chip promotes the self-assembly of an array of protein crystals on a surface. Rough features on the surface cause the crystals to adopt random orientations, allowing efficient sampling of reciprocal space.

1. Introduction

Elucidating the three-dimensional structures of macromolecules by X-ray crystallography is an important step in deducing the chemical mechanisms underlying biological function. Facilitated greatly by synchrotron X-ray sources, the method is limited largely by the quality of the crystals and radiation damage. X-ray free-electron lasers (XFELs) provide extremely brilliant femtosecond X-ray pulses (Emma *et al.*, 2010), allowing room-temperature diffraction of protein nanocrystals before the onset of significant radiation damage (Chapman *et al.*, 2011). This 'diffraction-before-destruction' approach relies on efficiently replacing crystals for each exposure to the FEL beam and creates a need for a platform that allows room-temperature diffraction data to be obtained from a multitude of crystals. The proposed platform must also allow an efficient sampling of reciprocal space.

To meet this challenge, we describe a crystallography chip for the collection of diffraction data at room temperature (Fig. 1). Unlike previous efforts to develop array-like platforms for *in situ* crystallography (Kisselman *et al.*, 2011), in which the array surface offers an environment for both crystal growth and *in situ* diffraction, our chip operates based on principles of the self-localization of liquid (Zarrine-Afsar *et al.*, 2011), and hence crystals suspended therein, onto spatially varying regions that allow stabilization of liquid contacts. Building on previous understanding of the role of surface roughness in enhanced wettability (Quere, 2008), this is achieved here by exploiting both the contrast in hydrophilicity/hydrophobicity as well as in roughness between a mixture of amorphous glass beads of varying sizes (hydrophilic) and a supporting polyimide film (hydrophobic) confined in a silicon mesh (Supplementary Fig. S1[1]). A regular array of crystals self-assembles at prescribed locations with a favourable liquid pinning potential by translating a drop of crystal suspension on the surface of the chip. The loaded chip is kept in a defined atmosphere in a holder allowing room-temperature data collection (Supplementary Fig. S1[1]).

2. Results and discussion

The chip provides a gentle means of crystal-array assembly that is applicable to different mother-liquor compositions and crystal

[1] Supplementary material has been deposited in the IUCr electronic archive (Reference: TZ5001). Services for accessing this material are described at the back of the journal.

morphologies. Using the model systems lysozyme (bar-shaped morphology) and ferritin (cubic shape), we demonstrate that through spatially varying regions of elevated hydrophilicity and roughness on a surface, regular arrays of protein crystals can be self-assembled by pinning the liquid and hence the crystals suspended therein to a surface. To validate the usefulness of the chip, we performed proof-of-principle diffraction experiments on lysozyme and ferritin crystals using synchrotron radiation (Fig. 1, Table 1). Reasonably complete diffraction data sets of acceptable quality can be obtained by merging together the individual data sets collected from multiple crystals in the wells (Table 1). Through using glass beads as a convenient (although strongly absorbing and scattering) test system to vary the surface profile of the chip bottom, we demonstrate that a wide distribution of crystal orientations can be induced for the bar-shaped lysozyme crystals (Fig. 2). This is particularly important in the case of crystal morphologies (such as plates) that would otherwise take up only a few preferred orientations in order to ensure sufficient coverage of reciprocal space for low-symmetry space groups.

Our work, however, also reveals key issues pursuant to further optimization as detailed below. As seen in Table 1, the statistics obtained for the model systems examined are poor compared with values reported through cryomounting of these systems using conventional loop-transfer methods. Further efforts are required in order to minimize X-ray absorption and scattering from the chip structure itself (McPherson, 2000; Glaeser *et al.*, 2000), especially for the study of small crystals (Holton & Frankel, 2010), through the use of alternative materials with favorable absorption properties such as thinner Kapton foils and appropriate hydrophilic particles such as Sepharose- or surface-modified plastic beads and/or surface-modification techniques to engineer roughness. In this work, we chose glass beads for a proof-of-principle demonstration of the utility of surface roughness in inducing random crystal orientations because of their availability and the ease with which they could be introduced to the Kapton surface to provide the roughness needed to orient the crystals.

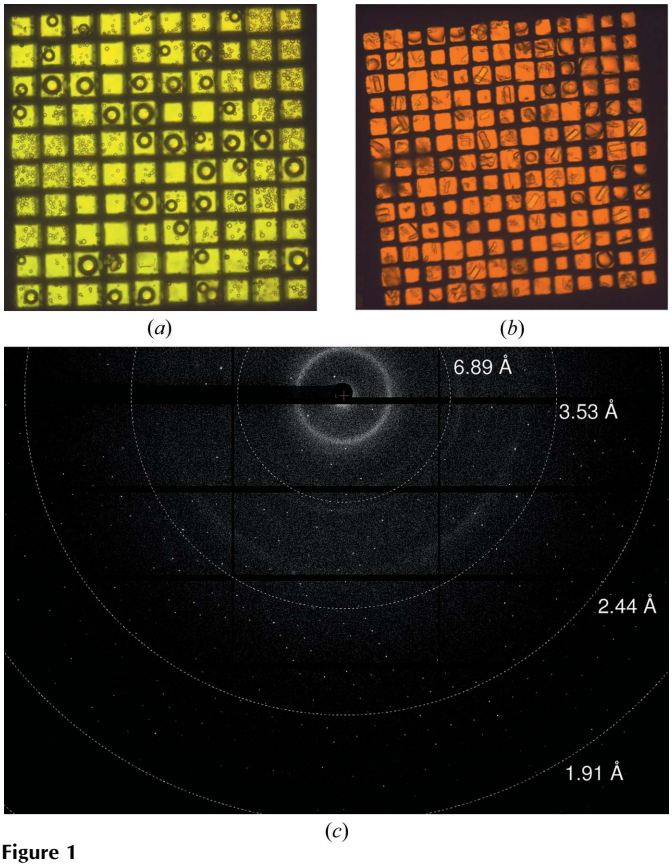

Figure 1
(*a*) The structure of the chip, depicting the silicon mesh (black) with well sizes distributed around 60 µm on a polyimide film (yellow) sprinkled with glass beads of different sizes (a 1:1 mixture of beads with diameters of <106 µm and 4–8 µm). (*b*) Snapshot of a chip with well sizes distributed around 45 µm loaded with tetragonal lysozyme crystals imaged under polarized light, indicating a typical fill factor of ∼90% with close to 30% of the filled wells containing a single crystal. (*c*) A representative diffraction pattern of a lysozyme crystal obtained from the chip. The addition of a 10 µm aperture, followed by a 25 mm Mo tube for collimation and air-scatter reduction close to the crystal (Supplementary Fig. S1), resulted in significantly reduced air scattering, as shown in Supplementary Fig. S2.

Table 1
Data-collection and refinement statistics of chip-mounted crystals.

Values in parentheses are for the highest resolution shell.

	Ferritin	Lysozyme
Data collection		
Space group	*F*432	$P4_32_12$
Unit-cell parameters (Å)	$a = b = c = 182.5$	$a = b = 79.1$, $c = 38.5$
X-ray source	Swiss Light Source, beamline X10SA	Swiss Light Source, beamline X10SA
Wavelength (Å)	1.00	1.00
Resolution (Å)	10–2.5	10–2.3
$R_{\rm merge}$†	0.13 (0.38)	0.19 (0.31)
$\langle I/\sigma(I)\rangle$	8.0 (3.3)	5.0 (2.8)
Completeness (%)	98.6 (98.8)	92.0 (91.1)
Multiplicity	4.2 (4.4)	3.4 (3.1)
Refinement		
Resolution (Å)	41.88–2.5	69–2.3
$R_{\rm work}/R_{\rm free}$	0.19/0.24	0.217/0.256
No. of atoms		
Protein	1377	1001
Ligand/ion	1 [Cd^{2+}]	1 [Cl^-]
Water	44	29
B factors (Å^2)		
Protein	23.9	29.2
Ligand/ion	22.4	29.4
Water	25.3	27.7
R.m.s. deviations		
Bond lengths (Å)	0.01	0.01
Bond angles (°)	1.12	0.94

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

Lastly, since the efficiency of the self-assembly process is largely determined by the pinning potential of the crystal storage solution on a given surface (Zarrine-Afsar *et al.*, 2011), the loading efficiency has to be optimized for each new system. The efficiency of the wetting process that drives crystal capture can be modulated by altering the hydrophilicity contrast between target areas and the chip surface, and tailored to the requirements of the model system(s) under study. Caution must be exercised in extending the applicability of the chip design as presented here to protein nanocrystallography, as the microscopic forces that drive wetting on the submicrometre scale may be substantially different from those in effect on the macroscale. Here, the ability of the chip concept to provide a versatile platform to gauge surface properties tailored to specific requirements of a particular model system and mother-liquor composition must be strongly emphasized. Nevertheless, these efforts may pave the way towards a truly high-throughput platform for room-temperature protein nanocrystallography. The chip developed here constitutes an alternative to *in situ* methods for crystal growth and diffraction (Kisselman *et al.*, 2011; McPherson, 2000). An advantage of our method compared with *in situ* platforms is the ability to assemble large crystalline arrays using concentrated suspensions of crystals.

As illustrated in Supplementary Fig. S2, in the absence of a collimating tube in close proximity to serve as a scatter guard, the air scatter upstream of the chip results in a significant 'checkerboard-

like' shadowing that may overlap with many relevant resolution shells. Although this scatter can be significantly reduced by using a collimating tube with a small aperture in close proximity to the chip (Fig. 1, Supplementary Fig. S1)[2], for FEL applications in which crystals are exposed to very bright coherent X-rays, the experimental setup depicted in Supplementary Fig. 1 can be further modified to include a helium chamber between the collimating tube and the chip, especially in cases where placing a collimating tube in close proximity may be challenging.

X-ray free-electron (XFEL) laser-based serial femtosecond crystallography (Chapman *et al.*, 2011) is an emerging technique for diffraction data collection using tiny crystals in a diffraction-before-destruction approach (Neutze *et al.*, 2000). The chip provides a means of loading large numbers of crystals for data collection. By translating the loaded chip at the repetition rate of an XFEL a 'discrete' sampling of the crystals is achieved, which is expected to be more efficient compared with the currently employed 'continuous' liquid-jet methods (DePonte *et al.*, 2008; Chapman *et al.*, 2011) in which a stream of mother liquor carrying crystals is intercepted by XFEL pulses. In addition to addressing the problem of radiation damage, XFELs offer the prospect of observing protein dynamics on the chemical timescale (Gaffney & Chapman, 2007) in order to reveal motions that couple barrier-crossing to biological function. A major advantage of the chip concept is that it enables collection of reference diffraction data without laser excitation, which is essential to properly normalize the relative changes in the diffracted intensities arising from structural changes (Moffat, 1989). Here, the chip will be a useful addition to recently developed detectors with fast readout times.

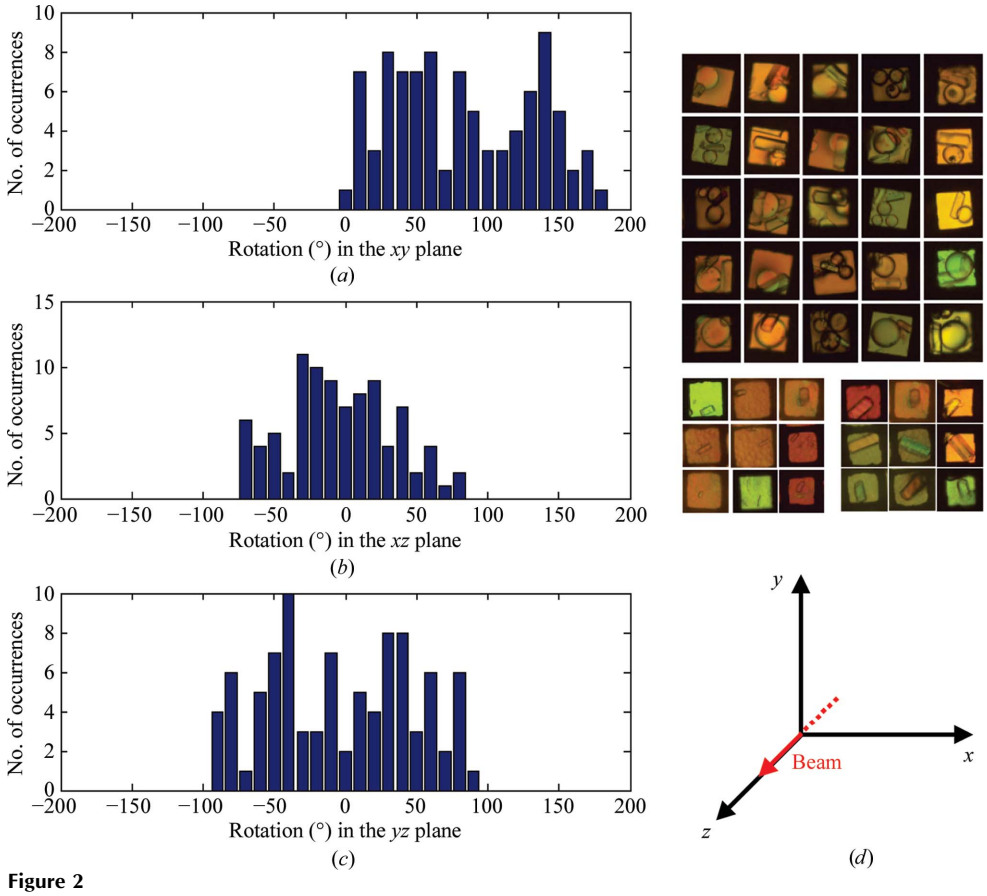

Figure 2
Distribution of orientations of lysozyme crystals mounted in the chip, indicated by the mis-setting angles of the unit-cell axes with respect to the laboratory coordinate system. (*a*) Sampling in the *xy* plane (around the beam). (*b*, *c*) Sampling in the *xz* and *yz* planes. In addition to improving the sample consumption compared with continuous liquid-jet approaches, an important requirement of the chip concept is that it must provide an efficient and complete sampling of the reciprocal space. The brick-shaped lysozyme crystals widely sample the *xy* plane which is perpendicular to the axis of the beam, with close to 170° coverage. In addition, a wide distribution in crystal orientations along the planes *xz* and *yz*, parallel to the beam is seen. (*d*) Selected snapshots of the effect of beads on the intrinsic orientation of crystals, illustrating the importance of matching bead size with crystal size for maximal effectiveness in orientating the crystals. The upper panel depicts crystals with sizes of the order of those of the beads (~30–50 µm). The lower left panel shows smaller ~5–10 µm crystals on a bed of small beads (4–8 µm). The lower right panel shows representative images of larger ~30–50 µm crystals on beads 4–8 µm in size.

We thank Wolfgang Kabsch and Robert Shoeman (MPI Heidelberg), James Rini and Zhijie Li (University of Toronto) and Alexander Marx (Max Planck Group for Structural Dynamics, Hamburg) for stimulating discussions and useful suggestions. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Max Planck Society. Diffraction data were collected on beamline X10SA at the Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland. AZ-A acknowledges support from a postdoctoral fellowship from NSERC and CM is supported by an NSERC Vanier Scholarship.

[2] In this work, all data sets were collected with the collimating tube in place.

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