A Versatile System for High-Throughput In Situ X-ray Screening and Data Collection of Soluble and Membrane-Protein Crystals

Jana Broecker, Viviane Klingel, Wei-Lin Ou, Aidin R. Balo, David J. Kissick, Craig M. Ogata, Anling Kuo, and Oliver P. Ernst

Supporting Information

ABSTRACT: In recent years, in situ data collection has been a major focus of progress in protein crystallography. Here, we introduce the Mylar in situ method using Mylar-based sandwich plates that are inexpensive, easy to make and handle, and show significantly less background scattering than other setups. A variety of cognate holders for patches of Mylar in situ sandwich films corresponding to one or more wells makes the method robust and versatile, allows for storage and shipping of entire wells, and enables automated crystal imaging, screening, and goniometer-based X-ray diffraction data-collection at room temperature and under cryogenic conditions for soluble and membrane-protein crystals grown in or transferred to these plates. We validated the Mylar in situ method using crystals of the water-soluble proteins hen egg-white lysozyme and sperm whale myoglobin as well as the 7-transmembrane protein bacteriorhodopsin from Halobacterium halobium. In conjunction with current developments at synchrotrons, this approach promises high-resolution structural studies of membrane proteins to become faster and more routine.

1. INTRODUCTION

Crystallography is a key investigative method in structural biology. It provides three-dimensional structural information on macromolecules such as proteins or nucleic acids, which is of paramount value for understanding biomolecular function on an atomic level and for applications like designing new drugs. The last 15 years have seen tremendous efforts in structural genomics, including the development of new methods for automation and miniaturization to achieve high-throughput crystallography, thereby significantly decreasing the time and effort with which structures of very high quality can be obtained. A typical workflow in protein crystallography comprises various steps, mainly recombinant protein production, protein purification, functional and biophysical characterization of the protein sample, protein crystallization, diffraction data-collection, and subsequent data analysis. In conjunction with newly developed specialized instrumentation and tools, many steps of this workflow have been improved and accelerated. However, despite the use of robotics, obtaining well-diffracting protein crystals remains a time-consuming, iterative, trial-and-error process. Thus, the two steps of crystal optimization and data collection still are major bottlenecks in protein crystallography, bearing high potential for optimization.

In situ data collection, where crystal harvesting is avoided by directly collecting diffraction images from unperturbed crystals residing in their growth environment, has been a focus of development in recent years. While early in situ attempts primarily served the purpose of screening crystals and validating their diffraction quality, recent approaches aim at collecting full data sets suitable for structure determination. Current in situ technologies combine crystallization and data collection in the form of X-ray compatible crystallization plates, microfluidic devices, which also comprise combinations with microcapillaries and nanodroplets, or fixed-target crystallography chips. While microfluidic technologies drastically reduce sample consumption, fixed-target chips are very successful for time-resolved crystallography on proteins where crystallization conditions and crystal positions are known. Both technologies, however, require specialized equipment and thus can be used with ease for automated high-throughput screening. Limitations, however, are their costs, the requirement of specific hardware such as translational stages to align the X-ray beam with crystals in the plate, and typically

Received: June 21, 2016
Revised: September 30, 2016
Published: October 3, 2016

DOI: 10.1021/acs.cgd.6b00950
Cryst. Growth Des. 2016, 16, 6318–6326
their incompatibility with measurements at cryogenic conditions as well as with the collection of complete high-resolution data sets.

Some of the limitations of in situ data collection have been overcome with the advent of thin sandwich plates developed for the in meso crystallization of membrane proteins in lipidic cubic phases (LCPs).\textsuperscript{9,10} LCPs are membrane-mimetic matrices that stabilize membrane proteins while at the same time allowing for crystal contacts in a lipid-bilayer environment.\textsuperscript{24,25} Traditionally, in meso crystallization trials are set up in glass sandwich plates (Figure 1) either manually or with the help of a robot.\textsuperscript{26} While glass sandwich plates bring along several advantages for crystal imaging like optical clarity and non-birefringence, it is not possible to use them directly (i.e., in situ) for screening or diffraction data-collection. Moreover, it is notoriously difficult to harvest small and fragile membrane-protein crystals from the highly viscous LCP.\textsuperscript{27} Recently, modified sandwich plates, so-called IMISX plates (commercially available from MiTeGen),\textsuperscript{9,10} have been described, where instead of glass plates two synthetic cyclic olefin copolymer (COC) films, this is thin, X-ray-transparent plastic sheets, form the mesophase-containing sandwich. The COC film sandwich is layered in between two glass plates to form a double-sandwich for stability, watertight sealing, and imaging purposes (Figure 1). The advantages are that the inner film sandwich is thin enough to allow for data collection and patches of the sandwich plate corresponding to one or more wells with typically several dozens of crystals can be cut out and taken to an X-ray source. Similar in situ double-sandwich plates were recently described for the growth of soluble protein crystals.\textsuperscript{7}

In order to push the limits of in situ crystallography, we designed a set of tools in a parallel development, henceforth referred to as the Mylar in situ method. Our intention was to produce a low-cost, robust, easy to make and handle, and highly versatile solution of sandwich plates, called hereafter Mylar in situ plates. In addition, we designed cognate holders, which allow automated crystal imaging and screening along with goniometer-based X-ray diffraction data-collection at room temperature and under cryogenic conditions for soluble and membrane proteins grown in or transferred to these plates. We demonstrate the potential of the Mylar in situ method for obtaining high-resolution structural information by using water-soluble hen egg-white lysozyme (HEWL) and sperm whale myoglobin (SWMb) as well as the 7-transmembrane protein bacteriorhodopsin from Haloquadratum walsbyi (HwBR).\textsuperscript{28,29}

2. RESULTS AND DISCUSSION

2.1. Mylar vs COC Film. As a liquid crystal, the mesophase scatters intensely at both low and high angles. At low angles, scattering is characterized by a series of well-defined rings, while at a higher angle it is more diffuse and centered at ~4.6 Å resolution, where it overlaps with scatter from the COC film used in the IMISX method.\textsuperscript{9,10} Both regions of scattering cause a decrease of the signal-to-noise ratio of the diffraction pattern. With very small and/or weakly scattering crystals as well as for challenging crystallographic measurements (e.g., in meso crystallography or phasing) the signal-to-noise ratio is decreased even further, necessitating the use of a different film material with lower background scattering.

The X-ray transparent film used in this study is made from Mylar instead of COC. Mylar film was chosen because it is relatively watertight, optically transparent, inexpensive, and commercially available from Premier Lab Supplies (Port St. Lucie, USA) in continuous rolls of varying thickness (e.g., 1.5 μm, 2.5 μm, 3.0 μm, or 3.5 μm). Most importantly, it is chemically inert and absorbs and scatters X-rays only weakly. Furthermore, Mylar in situ plates are of exceptionally slim design that further reduces X-ray scatter and absorption (Figure 1). At a total thickness of only 7 μm, in comparison to 50 μm in the case of COC films, the two Mylar films through which the X-rays pass in the process of in situ measurements show a lower and narrower background scatter profile, with weak, narrow maxima centered at around 4 Å, 5 Å, and 6 Å (Figure 2). It is conceivable that thinner COC film, which we could not source though, would be suited similarly well or even better, since it would promise a lower and more diffuse scatter profile without the biaxially oriented features seen in Mylar.

The strong background scattering from mesophase-loaded Mylar sandwiches (Figure S1c) can be reduced by working with thinner spacers, which in turn require thinner mesophase boluses (Figure S1a,b). Perforated 96-well double-sticky spacer tape of varying thickness (e.g., 28 μm, 58 μm, or 144 μm) is commercially available from Saunders (St. Paul, USA). Note that in our experience the use of thin spacers can unintentionally result in the preferential orientation of crystals along the dimension closest in size to the spacer’s thickness (Figure S2...
and Note S1). Therefore, in this study, most measurements were made with spacers that were 144 μm thick.

2.2. Mylar-Based In Situ Plates. Mylar in situ double-sandwich plates are assembled essentially the same way as IMIX and standard glass plates (Figure 1 and Supplementary Methods). They can be made simply and quickly in the lab with a small amount of commercially available materials (Supplementary Methods). Plates were designed to be handled both manually and by a robot. The work reported here was performed using a Gryphon LCP robot (Art Robbins Instruments; Sunnyvale, USA) with no changes to the existing protocol. As is the case with IMISX plates, Mylar in situ plates are transparent and can be imaged with a microscope under bright-field and polarized light (Figure 4) as well as UV light (Figure S4), facilitating the identification of protein crystals, especially small initial crystal hits or colorless crystals. Crystallization conditions obtained from glass plates translated almost exactly to Mylar in situ plates. Also, similar hit rate and crystal size and frequency were observed with the two plate types.

2.3. Holders for High-Throughput Screening and Routine Data Collection. In order to facilitate and automate screening and data collection, we have developed a variety of holders, which were designed in a 3D program suited for 3D printing (Supplementary Methods). Printer files are available on request, so that users can print their own holders at a 3D printer.
printer of their convenience. These files are ready-to-use, as the designs have been optimized with respect to printing time, material consumption, orientation on the printer’s base level, and the use of supports and rafts. When 3D printed, all holders can easily be preassembled in large quantities (Supplementary Methods), allowing for an integrated, robust, and reproducible approach when mounting wells. For a quick and easy selection of the best holder for a particular project and setup, please refer to Figure 5.

**Holders for Data Collection.** Single-well holders clamp a single well at a time (Figure 6a,b). The room-temperature holder features a full frame for stability, while the cryo-holder has no frame in order to reduce the interference with the cryo stream. Both holders come with supports at the feet for added stability. The base part and lid are connected by screw and nut, with the screw going through both holes. A bulge at the lower end of the lid allows for a clamping effect when the holder is closed (Figure 6e). For a similar effect, the hole is moved down in the case of the cryo holder. The goniometer base is the same for both holders. A CrystalCap SPINE base (SPINE base) from Hampton Research (Aliso Viejo, USA) was used together with a 3D-printed adapter that can hold both holders (Figure 6c). A slit in the adapter is off-centered (Figure 6d), so that the film sandwich, which holds the crystals, sits in the center of the SPINE base once the holder is fully assembled. By default, crystals are then in or close to the plane of focus of the on-axis microscopes found at synchrotrons.

Importantly, both single-well holders are small enough to be used at a synchrotron and at a home source (Figure 6f), where space is usually limited. Moreover, both holder types can withstand liquid nitrogen (Figure 6g), which allows for optimal

**Figure 5.** Holder-selection scheme. Goniometer-based holders can be used for either screening (with limited suitability for data collection; refer to text) or data collection. Some holders can be used for measurements at room temperature (RT) or under cryogenic (cryo) conditions. Adapter-based holders can be used for screening and data collection at RT or under cryo conditions. Translation stages might be necessary for full coverage of a particular holder. Depending on the number of wells that are supposed to be analyzed, holders can hold patches of Mylar film sandwiches containing up to 96 in situ wells at a time. Please refer to the indicated figures in the main text.

**Figure 4.** Comparison of crystal growth in the LCP. Crystals of bacteriorhodopsin HwBR are shown as grown on (a) standard glass plates and (b) Mylar in situ plates. (Top panel) Mesophase boluses under bright-field light. (Middle panel) Crystals shine brightly under cross-polarized light. (Bottom panel) Close-up views show no significant differences in crystal shape and size.
24 or 96 wells (Figure 7b,c) by using a standard glue stick (Supplementary Methods).

All goniometer-based screening holders are ideal for rapidly screening the diffraction quality of crystals in the different wells. While the 4-well holder can be supported on both GM/CA beamlines without an additional translation stage, the 24- and 96-well holders require one in order to move the goniometer vertically and horizontally, thereby covering all of the wells. For the larger 96-well holder, the GM/CA facility can handle approximately 6 rows and the full horizontal translation on one beamline and about 6 rows and 6 columns on the other beamline, where a vacuum tube prevents further horizontal translation. Within limitations, these holders also allow the collection of diffraction data. However, the axis of rotation will not be aligned with the center of the sample, which hampers optimally centering crystals that are far off the area that would hold crystals in a standard loop. Thus, it is highly probable that upon rotation of crystals during data collection particularly small crystals will eventually move out of the plane of focus. In the worst case, a crystal is not hit by the beam anymore, while it is still stable and would produce good diffraction-quality data.

A set of modified screening holders for 1, 4, 24, 48, and 96 wells at a time (Figure 8a–e) makes the screening holders more versatile. Here, the feet are planar and fit into an adapter (Figure 8f) that was developed by the GM/CA team at the APS and thus will henceforth be referred to as GM/CA adapter. For proper use, the GM/CA adapter includes an additional translation stage that must be attached to the goniometer. As it comes with submicron-level motion control, allowing for centering any crystal in the bolus, screening holders with flat
feet can be used for rapid screening as well as for data collection. Note that theoretically any adapter can be used that can hold plates of different thicknesses and that fits over the goniometer’s normal sample holder. If necessary, users of the Mylar in situ method might want to work with their individual beamlines to implement a suitable translation stage. Also, a combination of holders with a robotic arm is conceivable.

2.4. Flexible Usage of the Mylar In Situ Method.
Besides growing crystals in situ, another approach to circumvent crystal harvesting is to transfer crystallization samples en masse to a chip with windows that are X-ray transparent. Similarly, another way to benefit from our technology is to transfer crystals that have been grown in other setups or assemblies, such as hanging drop, sitting drop, or batch crystallization, onto Mylar in situ plates. Here, we used the soluble protein myoglobin as a model protein. Myoglobin is the iron- and oxygen-binding pigment found in abundance in the muscle tissue of mammals, where it is also responsible for the characteristic red color. The prosthetic group heme in myoglobin can reversibly bind molecular oxygen and carbon monoxide, CO. In this study, sperm whale myoglobin (SWMb) crystals were grown in batch mode in glass vials under CO atmosphere (Figure 9a,b). SWMb-CO crystals were transferred onto Mylar in situ plates. Here, we used the water-soluble protein HEWL as a model protein. HEWL is easy to handle, commercially available, and produces crystals that diffract to high resolution within a few hours (Supplementary Methods) under in meso conditions (Table S1). Crystals were set up on Mylar in situ plates and shipped to the GM/CA beamline at the APS synchrotron ahead of data collection. At the beamline, plates were opened, and wells were mounted and immediately flash-frozen for 100 K measurements. A full diffraction data set was collected in oscillation geometry from a single crystal (grown in space group P4_12_2 with 90° of data required). The data collected under cryo-conditions allowed structure determination to 1.7 Å resolution; the resulting model was refined to Rwork and Rfree values of 0.16 and 0.21, respectively (Table S2).

The final structure agrees well with a HEWL structure solved under similar conditions using the IMIXS method (PDB ID: SDSC; ref 10) (Ca root-mean-square deviation [RMSD] of 0.21 Å). The electron density map revealed the presence of one polyethylene glycol (PEG) molecule, one acetate ion, several chloride ions, and a sodium ion octahedrally coordinated by the backbone carbonyl oxygen atoms of Ser60, Cys64, and Arg73, the oxygen Oγ of Ser72, and two water molecules. Most importantly, no effects were noticed from the Mylar film, the spacers’ adhesive, or the presence of nail polish on crystal growth and stability, diffraction quality, or the electron density map, indicating that Mylar in situ plates are well suited for general protein crystallization and structure determination.

Mass Transfer of Myoglobin Crystals. SWMb-CO crystals were transferred onto Mylar in situ plates as described above. Under cryogenic conditions, a full diffraction data set was collected from a single crystal grown in space group P6 (60° of data required) in oscillation geometry. The data collected under cryo-conditions allowed structure determination to 1.7 Å resolution; the resulting model was refined to Rwork and Rfree values of 0.18 and 0.22, respectively (Tables S1–2). The final structure agrees well with another structure obtained under similar conditions (PDB ID: 3ESS, wild-type protein, space group P2_12_1; ref 36). The corresponding electron density map is
of high quality and revealed the presence of the iron-containing prosthetic group heme bound to CO, several sulfate ions, and two chloride ions. Most importantly, we noticed how easily and quickly Mylar in situ sandwich plates can be used as sample delivery system for other crystals, such as crystals of soluble proteins and/or crystals not grown in situ.

Structure of Bacteriorhodopsin from Haloquadratum walsbyi. Bacteriorhodopsin from *Haloquadratum walsbyi* (HwBR) is a light-driven proton pump with 7 transmembrane α-helices. HwBR is a good model membrane protein, as it is colored and can be produced in *E. coli* in sufficient quantities. In meso crystals grow as hexagonal flat plates (Tables S1–2) that are mechanically sensitive and easily break during harvesting, making an in situ approach for this type of crystals particularly useful. Crystals were grown on Mylar in situ plates and shipped to the GM/CA beamline at the APS synchrotron before data collection. At the beamline, plates were opened, wells were mounted, and immediately flash-frozen for 100 K measurements. Full data sets were collected, and the structure at 100 K could be solved to 2.1 Å with high quality parameters and was refined with values of $R_{work}$ and $R_{free}$ of 0.21 and 0.23, respectively (Table S2). HwBR crystals used in this study pack in space group $C2$ ($180^\circ$ of data needed), and the solved structure agrees well with another structure obtained under similar conditions (PDB ID: 5ITE; to be published) ($Ct$ RMSD of 0.23 Å over 689 residues) (Figure 10). The corresponding electron density map is of high quality and revealed the presence of the cofactor retinal bound to Lys224 (Figure 10c), several monoolein lipids, and water molecules.

### 2.6. Data Collection at Room Temperature (293 K).

Room-temperature measurements do not work well with small, weakly diffraction, and/or radiation-sensitive crystals, as was the case here with HwBR crystals. In such circumstances, it is advisable to perform in situ measurements at the standard cryogenic temperature of 100 K (see above). However, on a more general note regarding in meso crystallography, it was easier to spot even very small crystals in the essentially optically transparent mesophase at 293 K in contrast with comparable measurement at 100 K, where the mesophase has a tendency to turn turbid. This allowed rapidly choosing crystals with the on-axis camera at the beamline and eliminated the need to perform diffraction raster scanning to locate crystals, which is time-consuming and generates a multitude of images that need to be analyzed.

In general, the flat profile of in situ wells allows for data collection over a very wide angular range. However, at room temperature, radiation damage severely limits the amount of data that can be collected from a single crystal. Thus, the data-collection strategy at 293 K included collecting small angular wedges of relatively radiation damage-free data from individual crystals. By repeating this process on randomly oriented crystals, a complete data set of high quality can be produced by merging many small data wedges from a multitude of randomly oriented crystals (Supplementary Methods). For example, using HEWL crystals we found that usually only the first 10° of data (in rare cases, such as big crystals, up to 20° of data) are suitable for use in structure determination by molecular replacement and refinement. Accordingly, for each crystal, a total of 10° of data were collected by rotation, and data from 9 crystals (with nonredundant data) were then processed and merged. Crystals diffracted to 2.0 Å resolution, and the structure was refined with $R_{work}$ and $R_{free}$ values of 0.15 and 0.20, respectively (Table S2). For details on the HEWL structure at the two different temperatures, please see Note S5.

Room-temperature structures can help to shed light on mechanistic questions, such as protein activation, ligand binding, or protein-complex formation, making the Mylar in situ method particularly valuable for studying these and other phenomena. Note that for the small HwBR crystals radiation damage was too severe at room temperature to allow useful data sets to be collected using the rotation method.

### 2.7. High-Throughput Screening at Room Temperature.

The success of protein-structure determination by crystallography relies on screening a large number of crystallization conditions. On many occasions, particularly during the early stages of optimizing crystallization, any project would benefit from a screening approach that immediately reports back on the diffraction quality of crystals despite the limited crystal size (assuming crystals are at least big enough to be detectable and assessable with a microbeam). An integrated approach of Mylar in situ plates with an exhaustive set of screening holders developed in this study (see above) allows for rapidly screening up to 96 crystallization conditions at a time in order to verify the proteinaceous nature of crystals and to assess their diffraction quality as a function of precipitant composition. Also, the quality of several crystals per crystallization condition can be assessed easily. This is helpful, given the experience that often not all crystals in one well are of equal quality. For instance, big crystals with an increased diffraction volume often

![Figure 10. Structure alignment based on sequence alignment with subsequent optimizing fit using the Ct positions for HwBR. (a) Structural alignment of HwBR structures solved under cryogenic conditions: 5ITE harvested from glass plates (green, retinal in blue) and in situ structure from this study (magenta, retinal in orange). Residues with a $Ct$ RMSD $> 0.2$ Å are highlighted in cyan. Retinal molecules are shown as sticks. (b) Top view of HwBR structures in gray. Monooleins are shown as sticks with the color code as in (a). (c) Residues of the retinal binding site are shown as sticks. $\sigma F - \tau F$ electron density maps in blue (5ITE) and orange (in situ structure) are contoured at 1σ. (d) Residues involved in the proton translocation pathway of HwBR are shown in stick representation (carbon green and magenta; oxygen red; nitrogen blue).](image-url)
show a better signal-to-noise ratio than small crystals. Thus, it
remains to be studied how the optimization process of
crystallization conditions might be influenced by the possibility
to screen and optimize for diffraction quality using initial crystal
hits consisting of a few microcrystals prior to optimizing crystal
size (with an apparently additional positive effect on the signal-
to-noise ratio) in contrast to the standard reverse approach.
Note that wells of opened in situ sandwiches after removal of
the glass plates (Figure 3) lost less than 10% of water over the
first 6 h under X-ray exposure (Figure S5).

3. OUTLOOK

Synchrotrons make in situ structural studies of proteins easier,
 faster, and more successful. For instance, developments such as
attenuated microbeams,39,40 faster detectors, 41,42 rastering and/or
vectoring data-collection software, 43 or multi-crystal data-
collection software44,45 aim at automating and facilitating
protein crystallography. Nevertheless, the Mylar in situ method,
particularly obliging the use of a suitable light-source for light-sensitive
collection of data sets,20

XFEL measurements

open in situ plates and treat isolated wells at a
derived from substantial movements along all axes; a cryo-stream
developments such as, among others, goniometers that can
attenuated microbeams,39,40 faster detectors,41,42 rastering and/or

3 This work was supported by aNUANCE Center for Structural & Functional
Research Chair program (to O.P.E.). O.P.E. holds the Anne

ACKNOWLEDGMENTS

We are grateful to Frank Sicheri, P. Lynne Howell, Emil F. Pai,
Gilbert G. Privé, and Raymon Julien (all University of Toronto)
for providing access to X-ray home sources. We thank the
MADLab and the Gerstein Library, in particular Erica Lenton
and Michael Spears (University of Toronto), for admission to
the 3D printing facility. We also thank Yang Shen (University of
Toronto) for help with purifying SWMb and growing
Swmb-Co crystals, as well as John S. Olson (Rice University,
USA) for providing plasmid pMb413a. We also thank Allison
Trnka (Saunders, USA) for supplying us with various spacers
and Jeff Sansome from the Machine Shop (University of
Toronto) for advice with clamping. This research used
resources of the Advanced Photon Source, a U.S. Department
of Energy (DOE) Office of Science User Facility operated for
the DOE Office of Science by Argonne National Laboratory
under Contract No. DE-AC02-06CH11357. We particularly
thank the staff at the GM/CA beamline. For fruitful discussions
and carefully reading the manuscript we are grateful to Emil F.
Pai (University of Toronto). This work was supported by a
Research Fellowship from the German Research Foundation
(DFG) to J.B. (BR 5124/1-1) and by the Canada Excellence
Research Chair program (to O.P.E.). O.P.E. holds the Anne
and Max Tanenbaum Chair in Neuroscience at the University of Toronto.

REFERENCES

(1) Giegé, R.; Sauter, C. HFSP J. 2010, 4, 109.
(2) Joachimich, A. Carr. Opin. Struct. Biol. 2009, 19, 573.
(3) Beteva, A.; Cipriani, F.; Cusack, S.; Delageniere, S.; Gabadinho, J.; Gordon, E. J.; Guijarro, M.; Hall, D. R.; Larsen, S.; Launier, L.; Lavault, C. B.; Leonard, G. A.; Mairs, T.; McCarthy, A.; Meyer, J.; Mitchell, E. M.; Monaco, S.; Murizzo, D.; Pernot, P.; Pieritz, R.; Ravei, R. G. B.; Rey, V.; Shepard, W.; Spruce, D.; Stuart, D. I.; Svensson, O.; Theveneau, P.; Thibault, X.; Turkenburg, J.; Walsh, M.; McSweeney, S. M. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2006, 62, 1162.
(4) Garman, E. F. Science 2014, 343, 1102.
(5) Aller, P.; Sanchez-Weatherby, J.; Foadi, J.; Winter, G.; Lobley, C. M.; Axford, D.; Ashton, D.; Bellini, D.; Brandao-Neto, J.; Culurgioni, S.; Dongamath, A.; Duman, R.; Evans, G.; Fisher, S.; Flagg, R.; Hall, D. R.; Lukacik, P.; Maziorana, M.; McAuley, K. E.; Mykhaylyk, V.; Owen, R. L.; Paterson, N. G.; Romano, P.; Sandy, J.; Sorenson, T.; von Delft, F.; Wagner, A.; Warren, A.; Williams, M.; Stuart, D. I.; Walsh, M. A. Methods Mol. Biol. 2015, 1261, 233.
(6) Bingel-Erlenmeyer, R.; Olieric, V.; Grimson, J. P. A.; Gabadinho, J.; Wang, X.; Eber, S. G.; Isenegg, A.; Schneider, R.; Schneider, J.; Glettig, W.; Pradervand, C.; Panepucci, E. H.; Tomizaki, T.; Wang, M.; Schulze-Briese, C. Cryst. Growth Des. 2011, 11, 916.
(7) Axford, D.; Aller, P.; Sanchez-Weatherby, J.; Sandy, J. Acta Crystallogr., Sect. F: Struct. Biol. Commun. 2012, 72, 313.
(8) Cipriani, F.; Rüwer, M.; Landret, C.; Zander, U.; Felisaz, F.; Marquez, J. A. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2013, 68, 1393.
(9) Huang, C.-Y.; Olieric, V.; Ma, P.; Panepucci, E.; Diederichs, K.; Wang, M.; Caffrey, M. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2015, 71, 1238.
(10) Huang, C.-Y.; Olieric, V.; Ma, P.; Howe, N.; Vogele, I.; Liu, X.; Warschmanage, R.; Weinert, T.; Panepucci, E.; Kobilka, B.; Diederichs, K.; Wang, M.; Caffrey, M. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2016, 72, 93.
(11) Pinker, F.; Brun, M.; Morin, P.; Deman, A.-L.; Chateaux, J.-F.; Olieric, V.; Strinimann, C.; Lorber, B.; Terrier, N.; Ferrigno, R.; Sauter, C. Cryst. Growth Des. 2013, 13, 3333.
(12) Heymann, M.; Ophthalme, A.; Wierman, J. L.; Akella, S.; Szebenyi, D. M. F.; Gruner, S. M.; Fraden, S. J. CIF 2014, J., 349.
(13) Perry, S. L.; Guha, S.; Pawate, A. S.; Henning, R.; Kosheleva, I.; Själer, V.; Kensing, P. J. A.; Ren, Z. J. Appl. Crystallogr. 2014, 47, 1975.
(14) Stroock, A. D.; Dertinger, S. K. W.; Ajari, A.; Mezic, I.; Stone, H. A.; Whitesides, G. M. Science 2002, 295, 647.
(15) Li, L.; Fu, Q.; Kors, C.; Stewart, L.; Nollert, P.; Laible, P.; Ismaglov, R. Microfluid. Nanofluid. 2010, 8, 789.
(16) Khvostchenko, D. S.; Schieferstein, J. M.; Pawate, A. S.; Laible, P. D.; Kensing, P. J. Cryst. Growth Des. 2014, 14, 4886.
(17) Yadav, M.; Gerdes, C. J.; Sanishvili, R.; Smith, W. W.; Roach, L. S.; Ismaglov, R. F.; Kuhn, F.; Stevens, R. C. J. Appl. Crystallogr. 2005, 286, 900.
(18) Pineda-Molina, E.; Daddaoua, A.; Krell, T.; Ramos, J. L.; García-Ruíz, J. M.; Gavira, J. A. Acta Crystallogr., Sect. F: Struct. Biol. Commun. 2012, 68, 1307.
(19) Maeki, M.; Yoshizuka, S.; Yamaguchi, H.; Kawamoto, M.; Yamashita, K.; Nakamara, H.; Miyazaki, M.; Maeda, H. Anal. Sci. 2012, 28, 65.
(20) Mueller, C.; Marx, A.; Epp, S. W.; Zhong, Y.; Kuo, A.; Balo, A. R.; Soman, J.; Schottle, F.; Lemke, H. T.; Owen, R. L.; Pai, E. F.; Pearson, A. R.; Olson, J. S.; Anfinrud, P. A.; Ernst, O. P.; Miller, R. J. J. Struct. Dyn. 2015, 2, 054302.
(21) Oglibba, S.; Sarracini, A.; Ginn, H. M.; Pare-Labrosse, O.; Kuo, A.; Marx, A.; Epp, S. W.; Sherrill, D. A.; Eger, B. T.; Zhong, Y.; Loch, R.; Mariani, V.; Alonso-Mori, R.; Nelson, S.; Lemke, H. T.; Owen, R. L.; Pearson, A. R.; Stuart, D. I.; Ernst, O. P.; Müller-Werkmeister, H. M.; Miller, R. J. D. Acta Cryst. D. Biol. Crystallogr. 2016, 72, 944.