Serial femtosecond X-ray diffraction of enveloped virus microcrystals

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Serial femtosecond crystallography (SFX) using X-ray free-electron lasers has produced high-resolution, room temperature, time-resolved protein structures. We report preliminary SFX of Sindbis virus, an enveloped icosahedral RNA virus with \(700\) Å diameter. Microcrystals delivered in viscous agarose medium diffracted to \(40\) Å resolution. Small-angle diffuse X-ray scattering overlaid Bragg peaks and analysis suggests this results from molecular transforms of individual particles. Viral proteins undergo structural changes during entry and infection, which could, in principle, be studied with SFX. This is an important step toward determining room temperature structures from virus microcrystals that may enable time-resolved studies of enveloped viruses.

I. INTRODUCTION

A broadly distinguishing structural feature of all viruses is the presence or absence of a lipid envelope. Non-enveloped viruses are composed of structural proteins arranged in the form of a shell (capsid) that encloses a genome of either DNA or RNA. Enveloped viruses have, in addition, a lipid membrane that is acquired from host cells. The genomes of enveloped viruses are enclosed in a protein capsid or associated with protein as a helical nucleocapsid. Viral
glycoproteins are anchored in the lipid envelope. While some enveloped viruses are pleomorphic, others have a defined structure with symmetry and a fixed number of envelope proteins. Examples of human viruses that are enveloped and also possess structural symmetry include herpes simplex, varicella zoster, Epstein-Barr (Herpesviridae); dengue, West Nile, Yellow Fever (Flaviviridae); and Chikungunya (Togaviridae) viruses (Carstens, 2012).

A. Virus crystallography

Numerous X-ray crystallography structures have been determined from crystals of non-enveloped viruses, with resolution now reaching to 1.4 Å (Zocher et al., 2014). Enveloped virus crystals, on the other hand, have not yet proven capable of producing such high-resolution diffraction. Although they are not typical enveloped viruses, X-ray structures from the lipid membrane-containing PRD1 (Tectiviridae) and PM2 (Corticoviridae) bacteriophage capsids have been reported to 4.2 Å (PDB ID: 1W8X) and 7.0 Å (PDB ID: 2W0C), respectively (Abrescia et al., 2004; 2008). To date, no X-ray structure has been determined for a typical enveloped virus, which has a lipid membrane with envelope proteins that surrounds an inner capsid or nucleocapsid. Many such enveloped viruses are pleomorphic and lack the structural symmetry required for crystallization. Among the enveloped viruses that do possess a symmetrical physical structure, only Sindbis virus (Togaviridae), Semliki Forest virus (Togaviridae), and West Nile virus (Flaviviridae) have been crystallized (Wiley and von Bonsdorff, 1978; Harrison et al., 1992; and Kaufmann et al., 2010). X-ray diffraction was previously reported for Sindbis crystals at 30 Å (Harrison et al., 1992), and West Nile virus crystals at 25 Å (Kaufmann et al., 2010). Although enveloped viruses such as these possess a fixed number of proteins arranged with icosahedral symmetry in the envelope, high-resolution diffraction data remain elusive. It has been suggested that resolution is limited because the envelope lipids confer an inherent flexibility and heterogeneity to the virus particles (Rossmann, 2013). The motivation to crystallize other enveloped viruses has decreased as cryo-electron microscopy (cryo-EM) techniques have advanced significantly in recent years, leading to higher resolution image reconstructions of enveloped viruses (Grigorieff and Harrison, 2011). Cryo-EM maps have been obtained for Sindbis virus at 7 Å and 10.3 Å for West Nile virus (PDB: 3J0F, 3J0B) (Tang et al., 2011 and Zhang et al., 2013b). The highest resolution obtained for an enveloped virus with cryo-EM methods is currently the 3.5 Å map for dengue virus (Flaviviridae) (Zhang et al., 2013a). The current application of cryo-EM for high-resolution structural studies is outstanding.

Virus particles are several orders of magnitude larger in size and mass than proteins. Consequently, virus crystals often have significantly larger unit cells that typically contain only one or two viruses. The reduced number of unit cells per volume, compared to the same volume of smaller unit cells (smaller macromolecules), results in proportionally weaker X-ray diffraction. Diffraction patterns of crystals with large unit cells feature very densely spaced Bragg reflections and therefore, a larger number of reflections at a given resolution shell (Fry et al., 1999; Rossmann, 1999; and Holton and Frankel, 2010). The dense spacing of the Bragg spots demands a large array of detector pixels to provide adequate sampling of the reflection and the areas between spots at high resolution. Thus, increases in the brilliance of X-ray sources and sensitivity of detectors are particularly beneficial for the study of virus crystals.

B. X-ray free-electron laser (XFEL) with viruses

The new method of SFX with XFEL (Chapman et al., 2011 and Weierstall, 2014) represents a powerful advancement in the field of X-ray crystallography. Damage free crystal structures can now be determined from nano- and microcrystals of proteins that are difficult to crystallize and highly important, such as human G-protein coupled receptors (GPCRs) (Liu et al., 2013; Fenalti et al., 2015; and Zhang et al., 2015). The technique is beginning to
have a significant impact in biology and medicine. The unparalleled brilliance of XFEL beams and short femtosecond pulse duration that outruns radiation damage \cite{Barty2011} makes them uniquely well-suited for studying viruses as crystals and as single particles. Thus far, only low resolution XFEL diffraction has been reported from large viruses that were probed as single particles \cite{Song2008, Seibert2011, Ekeberg2015}. Here, we present the first diffraction results from SFX studies of enveloped virus crystals.

XFELs allow the collection of data prior to onset of radiation damage at room temperature, rather than under cryo-conditions \cite{Barty2011, Chapman2014}. This enables for the first time damage-free collection of X-ray diffraction data from biological samples under physiological conditions. Furthermore, the physiological conditions can be dynamically controlled during delivery of sample to the XFEL beam to produce time-resolved diffraction data \cite{Aquila2012, Kupitz2014, Spence2014, Tenboer2014, Wang2014}. Recently, it has been shown that structural changes can be detected by time-resolved SFX at atomic resolution \cite{Tenboer2014}. Many viruses undergo structural changes during their life cycles, including response to changes in pH during entry via endosomes and viral protein interactions with cellular receptors \cite{Perera2008, Connolly2011, Harrison2013}. The application of time-resolved SFX to understand these changes has significant potential for structural virology studies. Recently, it has been shown that detailed time-resolved structural changes can be detected by wide angle X-ray scattering of single proteins in solution using an XFEL when there is a corresponding initial-state structure available at high resolution that can be used as a reference point \cite{Neutze2014}. The high-resolution static virus structures that are now becoming attainable with cryo-EM can potentially be combined with dynamic room-temperature studies of conformational changes studied with time-resolved SFX in a powerful complementary approach toward producing time-resolved virus structures.

C. Sindbis as a structural model for enveloped virus crystals

Sindbis virus is a member of the Togaviridae family, which also includes other medically important viruses such as Chikungunya virus, Ross River virus, Semliki Forest virus, and Rubella virus. All viruses included in the Togaviridae are enveloped, positive-sense, and single-stranded RNA viruses. Sindbis is transmitted from mosquitoes to humans and other vertebrates \cite{LundstromPfeffer2010}. It has been used as a structural model for the study of enveloped viruses in past decades because it grows to high titers and exhibits icosahedral symmetry in both its capsid and envelope \cite{Zhang2002, HernandezBrown2005}. Small angle neutron scattering (SANS) measurements determined the diameter of Sindbis as $676 \pm 25$ Å at pH 7.2 and $720 \pm 28$ Å at pH 6.4 \cite{He2012}. Sindbis is composed of three major structural components, two envelope glycoproteins (E1 and E2) and one capsid protein (C). There are 240 copies of each of these proteins per virus particle. In the envelope, E1 and E2 form heterodimers that further associate as trimers. The T=4 icosahedral symmetry of Sindbis is defined by the 80 resulting E1-E2 trimeric spikes that are anchored in the lipid envelope. Inside the envelope, the 240 capsid proteins are also organized into an icosahedron with T=4 symmetry. The ~11.7 kb RNA genome is packaged inside the capsid. Protein, RNA, and lipids constitute roughly 64%, 9%, and 27%, respectively, of the total viral mass of Sindbis virus particles \cite{Fuller1987}.

The ability to grow a large amount of Sindbis virus, its icosahedral symmetry, and availability of structural details from cryo-EM make crystals of the virus an ideal model for development of SFX methods to study the structure and dynamics of enveloped viruses.

II. MATERIALS AND METHODS

A. Macro-scale cultivation and purification of Sindbis virus

Protocols for growth and purification of Sindbis virus were followed as previously reported \cite{HernandezBrown2005}, with modifications for large-scale production.
Baby hamster kidney cells (BHK) were cultivated by passage in minimal essential medium (MEM) supplemented with 5% fetal bovine serum (FBS), 5% tryptose phosphate broth, 2 mM L-glutamine, and 50 μg/ml gentamicin. Cells were grown to near confluence in 875 cm² multi-level flasks (Falcon) prior to infection with a heat-resistant strain of the Sindbis virus (SVHR) at a multiplicity of infection (MOI) of 0.02 (0.02 virus particles/cell). Cells were refed with Glasgow MEM (GMEM) containing the same supplements plus an additional 2 g/l of NaHCO₃, following infection. Virus particles were harvested from the growth medium at 25 h post-infection.

Virus particles were purified by centrifugation through gradients of potassium tartrate (dibasic hemihydrate) in PN Buffer (50 mM PIPES pH 7.2, 100 mM NaCl). Growth media containing extracellular virus particles were clarified by slow speed centrifugation prior to being loaded onto continuous 15%–37% (w/v) potassium tartrate density gradients. Gradients were run at 100 000× g for 4 h (Fig. 1). Gradients were fractionated. Fractions containing the virus were combined and applied to a step gradient of 37% and 15% potassium tartrate. The virus particles were collected from the fractions at the interface between the 15% top and 37% bottom layer of potassium tartrate. Fractions were subsequently dialyzed against PN buffer to remove the potassium tartrate. The virus particles were then sedimented by ultracentrifugation at 100 000× g for 2 h. The resulting virus pellets were resuspended in PN buffer and the concentration was adjusted to either 1 mg/ml or 4 mg/ml. The concentration was determined by using the Lowry Assay method for the estimation of membrane protein concentrations (Markwell et al., 1978).

Sample purity was determined by denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2, lane 2). The tricine-SDS-PAGE protocol established by Schägger (2006) was used. Gels were silver stained using a commercial kit (Pierce). Purified virus particles were imaged by transmission electron microscopy (TEM) on copper grids after staining with 2% uranyl acetate for 30 s.

B. Micro-crystallization

The production of Sindbis virus macrocrystals in vapor diffusion drops was previously described by Harrison et al. (1992). This method was adapted to produce showers of microcrys-
tals. The precipitant solution was 5.5% w/v PEG 8000, 7.5% w/v glycerol, and 240 mM KCl. A total of 195 hanging drops were prepared by mixing 5 μl of the 1 mg/ml concentration of purified Sindbis virus in PN buffer with 5 μl of the precipitant solution in each drop. An additional 30 hanging drops were likewise prepared using the 4 mg/ml Sindbis sample. Hanging drops were allowed to equilibrate with 500 μl of the precipitant solution by vapor diffusion for at least 4–5 days at 4°C. EasyXtal (Qiagen) 15-well plates equipped with screw-in crystallization supports were used. The density of microcrystals was estimated by counting the number of crystals in a

![FIG. 1. Purification of Sindbis virus by centrifugation through a continuous potassium tartrate gradient, followed by characterization of the sample using transmission electron microscopy (TEM), and characterization of the crystals using UV fluorescence microscopy is illustrated. The TEM image of purified Sindbis virions is at 53 000× magnification.](image-url)
small volume using a hemacytometer and an optical microscope. Crystallization with a 1 mg/ml and 4 mg/ml sample was expected to produce 25 \( \mu \text{m} \) and 50 \( \mu \text{m} \) crystals, respectively.

\[ \text{C. Virus inactivation} \]

To meet biosafety requirements for SFX data collection at the Linac Coherent Light Source (LCLS), infectivity of Sindbis virus microcrystals was abolished by glutaraldehyde treatment. Once microcrystals had formed, glutaraldehyde was added to the precipitant volume in all crystallization plate reservoirs to a final concentration of 0.5\% (v/v) and allowed to equilibrate with the volume in each crystal drop by vapor diffusion for 4–5 days at 4°C. The volume of all crystal drops was then combined.

Inactivation of all microcrystals used for the experiments was confirmed by plaque assays. A sample of the glutaraldehyde treated microcrystals was diluted ten-fold in PN buffer supplemented with 3\% FBS, and warmed at 37°C for 1 h to dissolve the crystals. Ten-fold serial dilutions were prepared and assayed by duplicate plaque titration. Untreated microcrystals were likewise assayed in parallel as a control. After 48 h, cells were stained with 0.05\% neutral red and plaques were counted. The limit of detection with this assay was 25 plaque forming units (pfu) per ml, a measure of infectious virus particles.

Glutaraldehyde is known to react with various amino acid functional groups to form covalent crosslinks within a protein or among proteins (Migneault et al., 2004). Crosslinking of viral
proteins results in the particles being rendered non-infectious. SDS-PAGE of the glutaraldehyde treated crystals was used to confirm crosslinking and provide additional evidence of inactivation (Fig. 2, lanes 3–4).

D. Serial femtosecond X-ray crystallography

Sindbis virus microcrystals were mixed with an agarose-based viscous medium for delivery to the XFEL beam according to methods previously established with protein crystals (Conrad, 2015). The 25 μm Sindbis crystals were first concentrated by centrifugation at 4000 × g for 3 min, followed by resuspension in 20 μl of the mother liquor (41 mM PIPES pH 7.2, 82 mM NaCl, 4.5% PEG 8000, 7.75% glycerol, and 0.2M KCl). The 50 μm crystals were likewise concentrated and resuspended in 13 μl of the mother liquor. The agarose medium was prepared by dissolving 0.14 g of ultra low-melt agarose (Sigma-Aldrich) in 1.4 ml of crystal buffer and 0.6 ml of glycerol and heating to 95 °C for approximately 30 min. For each sample, four parts of the agarose medium were mixed with one part resuspended crystals, such that approximately 10⁸ microcrystals were embedded in the agarose/sample mixtures that were subsequently injected. Optical light microscopy and UV fluorescence microscopy were used to confirm that the crystals withstood the mixing process.

Sindbis crystals in the agarose-based viscous medium were delivered to the XFEL beam at the Coherent X-ray Imaging (CXI) beamline of the SLAC LCLS (Boutet and Williams, 2010). The high viscosity medium injector (Weierstall et al., 2014), coupled with a 50 μm or 75 μm diameter nozzle capillary, was used to deliver the sample to the X-ray beam. The Cornell-SLAC Pixel Array Detector (CSPAD) was positioned at 582 mm from the sample/beam intersection point. The XFEL pulses were ~47 fs in duration at 6 keV (2.066 Å), with approximately 6 × 10¹⁰ photons/pulse (120 Hz). The XFEL beam was focused to a diameter of ~1.3 μm.

III. RESULTS AND DISCUSSION

A. Sample yield, purity, and crystallization

To produce a sufficient number of virus crystals, large-scale preparations of highly purified virus were necessary. Using the Lowry assay, we determined that ~0.3 mg of purified Sindbis virus was produced from the infection of confluent BHK cell monolayers in each 875 cm² multi-level flask. 1 mg of Sindbis virus is roughly equivalent to 3 × 10¹¹ pfu. Following purification by ultracentrifugation through potassium tartrate gradients, highly purified virus was obtained, and characterized by TEM (Fig. 1) and SDS-PAGE (Fig. 2, lane 2).

The density of microcrystals produced from the pooled vapor diffusion drops was ~10⁵ crystals/ml. Crystals prepared from the 1 mg/ml concentration sample were up to 25 μm across a hexagonal face (sample A) (Fig. 3(a)), while at 4 mg/ml, crystals exhibited diameters up to 50 μm (sample B) (Fig. 3(b)). Crystal thickness was about 1/3 of the diameter. Crystals were neither birefringent nor detectable by second-order nonlinear optical imaging of chiral crystals (SONICC) (Kissick et al., 2011) but did manifest strong UV absorbance (Fig. 1). The microcrystals were dissolved and analyzed by SDS-PAGE, where separation of the structural proteins E1 (47.5 kDa) and E2 (46.7 kDa) and the capsid protein (29.4 kDa) confirmed the presence of the expected proteins in the virus crystals (Fig. 2, lane 3).

B. Inactivation of virus infectivity

Treatment of microcrystals with glutaraldehyde was successful at eliminating infectivity of the Sindbis crystals. SDS-PAGE showed the disappearance of bands corresponding to the individual structural proteins in the treated sample, which is indicative of crosslinking (Fig. 2, lane 4). Complete inactivation was confirmed by the observation of no detectable infectious virus in plaque assays of the treated crystals. The parallel control plaque assay for virus infectivity in the untreated Sindbis crystals produced a titer of 2.5 × 10⁸ pfu/ml.
C. XFEL diffraction

A total of 5685 diffraction patterns were identified as crystal hits from 709,196 events (0.8% average hit rate) during 98 min of sample injection. The hit rates from samples A (maximum diameter of 25 \(\mu\)m) and B (maximum diameter of 50 \(\mu\)m) were very similar. Hit finding and detector signal-calibration were carried out using Cheetah (Barty et al., 2014). An event was considered a crystal hit if it contained over 15 isolated peaks with a signal-to-noise ratio of at least 6. Most of the patterns had fewer than 50 peaks (Fig. 4(a)). The majority of the diffraction patterns featured strong diffuse scattering between Bragg spots, which is discussed in Section III D. The resolution, sharpness of spots, and visibility of diffuse scattering varied significantly between patterns (Fig. 5). The diffuse scattering ranged from concentric hexagons (Fig. 5(a)) in single crystal patterns to isotropic concentric rings (Figs. 5(b)–5(d)) as the number of crystal mosaic blocks in the beam increased (indicating internal disorder). These diffuse rings resembled small angle X-ray scattering (SAXS) from a large spherical particle.

The crystal diffraction patterns were processed with the CrystFEL software suite (White et al., 2013) using MOSFLM (Leslie and Powell, 2007) for indexing. A total of 562 patterns (10%) could be indexed with a rhombohedral unit cell, where \(a = 652 \pm 27 \text{ \AA}\), \(b = 664 \pm 30 \text{ \AA}\),
c = 662 ± 30 Å, α = 114.2 ± 3.4°, β = 114.6 ± 3.8°, and γ = 114.0 ± 3.7°, fitting a single Sindbis virus particle per unit cell. The unit cell distribution is shown in Fig. 6. Within this set, only 65 patterns could be indexed with a strictly rhombohedral cell, with a ∼ 670 Å, α ∼ 115°, and 6 patterns were indexed with a ∼ 785 Å and α ∼ 115°. Despite the high accuracy of predicted peak locations, the low resolution of these data does not permit us to determine whether the broad unit cell distribution is inherent to the crystals (potentially exacerbated by dehydration during sample delivery into vacuum), or a limitation of the indexing due to the small angular spread of observed reflections. The virtual powder pattern from the indexed patterns (Fig. 7) shows the broad rings indicative of a range of unit cell sizes.

The previously reported Sindbis crystal unit cell (Harrison et al., 1992), where a = b = 640 Å and c = 1520 Å, was presumed to contain two Sindbis particles per (hexagonal) unit cell. The volume of their equivalent rhombohedral cell, where a = 627 and α = 61.4°, is somewhat smaller than our cell, which may be due to the temperature and crystallization differences.
The resolution range of the indexed partial datasets is 287–43 Å, with no observable difference between resolution limits of samples A and B (Fig. 4(b)), despite the difference in crystal size. The histogram of the resolution limits slightly underestimates the highest resolution peaks in the patterns by 1–2 diffraction orders. This is a result of necessarily using a higher intensity threshold than the weakest diffraction spots during hit finding, in order to minimize the number of false positive hits.

During the extremely brief XFEL pulses (~45 fs in this experiment), crystals do not have time to rotate and, together with the narrow SASE (self-amplified spontaneous emission) bandwidth (0.1%) and low divergence, this leads to the collection of still diffraction patterns. As a result, almost all observed reflections are partials, requiring high multiplicity (number of times a symmetry related reflection is sampled) to accurately determine the structure factors. Crystals with a high degree of disorder, such as the Sindbis virus crystals, require an even higher multiplicity to be able to average out over the heterogeneities. A significantly larger dataset, which extends to higher resolution, will be necessary to confirm the space group.

D. Analysis of XFEL small angle diffuse scattering

To gain some insight into the observed diffuse scattering, we analyzed the data in the context of small angle scattering. Signal to noise ratio was improved by combining the signal from all 5685 hits and calculating the median intensity for each pixel across all patterns. Combining the diffuse scattering intensities from the 5685 randomly orientated diffraction patterns resulted in a single isotropic pattern (Fig. 8). To improve the signal-to-noise ratio, each pixel was assigned a radial distance from the center of the image, and the average of intensity at each radial bin was calculated to yield a final intensity profile as a function of q, while masking out the gaps between modules in the detector. The background contributed by solvent scattering was determined from images not categorized as hits and subsequently subtracted from the signal. The intensity profile generated from images not categorized as hits did not exhibit a SAXS-like pattern (Fig. 9). This indicates that the observed scattering does not arise from SAXS of free virus particles suspended in the delivery medium but is associated with the microcrystals.

The XFEL experimental setup is designed for high to medium resolution X-ray diffraction studies, rather than very low-resolution data typically collected in SAXS experiments. The resolution necessary to reliably determine size and shape of a particle from SAXS is twice the
maximum dimension of the particle. The estimated dimension of Sindbis, based on SANS studies, is 676–720 Å (He et al., 2012). True SAXS analysis requires data with resolution lower than 1360–1440 Å—well below the minimal 363 Å resolution that we were able to collect with the maximal possible detector distance.

To assess whether the diffuse rings may be attributed to SAXS from Sindbis virus particles, we calculated a SAXS profile from the low-resolution electron density map from a cryoEM
model of Sindbis at pH 6.4 (Cao and Zhang, 2013), taking into account the varying densities present in the particle. The electron density map was converted to a volumetric model of 15 Å diameter beads, and the electron density was stored in the B-factor column of the resulting PDB file (Wriggers, 2010). The Debye equation was then used to calculate the SAXS profile, which yielded a radius of gyration ($R_g$) of 247.3 Å and $D_{max}$ of 680 Å. The spacing between the maxima of the concentric rings of this simulated profile, which is directly related to particle size (Glatter and Kratky, 1982), was 0.0130 Å$^{-1}$. This is consistent with what was observed in the experimental profile from Sindbis microcrystals, which was 0.0132 Å$^{-1}$, suggesting that the diffuse scatter seen in the XFEL data arises from the small angle scattering of Sindbis virus particles. However, direct comparison of the simulated profile and the experimental profile does reveal discrepancies in features such as peak height, and slight variations in spacing between individual peaks (Fig. 10). These differences may result from assuming a solution of monodisperse, non-interacting virus particles when calculating the SAXS profile using the Debye equation. However, in this experiment, the diffuse scattering arises not from particles floating freely in solution but from particles packed tightly in a crystal. Significant inter-particle interactions occur as demonstrated by the bright Bragg spots. These inter-particle interactions may manifest as other forms of diffuse scattering, which might also explain the differences between the simulated and experimental SAXS patterns. Nonetheless, the agreement between the average spacing of concentric rings of the diffuse scattering and the simulated SAXS pattern of the virus particle suggests that the non-Bragg scattering is a consequence of underlying molecular transforms of the individual particles.

Diffuse scattering from virus crystals was also observed in 25 Å diffraction patterns from enveloped virus West Nile virus crystals (Kaufmann et al., 2010). Partially disordered crystals of the internal lipid membrane-containing bacteriophage PRD1 also produced diffuse rings that overlayed with Bragg peaks, and the radial ring separation was consistent with solution scattering (Bamford et al., 2002). This indicates that size of the bacteriophage in the crystals is similar to the solution state. As noted earlier, the presence of lipid makes crystals of enveloped viruses particularly prone to disorder, and any departure from translational symmetry will produce diffuse scattering between the Bragg reflections (Rossmann, 2013). However, X-ray diffraction of crystals of the non-enveloped HK97 bacteriophage head also showed similar rings among the lower-resolution Bragg peaks (Tsuruta et al., 1998). This suggests that the background
scattering could also be caused by other factors, such as heterogeneity in the sample due to ordering of a second phase, rather than an average over many defects, which would produce isotropic scattering. Even if the particles are structurally identical in terms of protein arrangement, an important consideration is the organization of RNA or DNA genome in a capsid, which may differ among the particles (Fry et al., 1999). The fact that the diffuse scattering from individual shots is not entirely isotropic suggests that the genomes may occur in a limited set of orientations, rather than a continuous distribution. Simulations of the diffuse scattering based on models may be used to determine the possible registration of the genome relative to the capsid. Single-stranded RNA genomes, like that of Sindbis, are assembled through interactions between the genome and capsid or nucleocapsid proteins in the confines of the capsid or virus particle, and some do organize in line with their symmetry (Speir and Johnson, 2012).

E. Summary and Conclusions

Serial crystallography with a femtosecond pulsed XFEL has been established in recent years as a viable approach for investigating protein crystal structures (Chapman et al., 2011). Here, we report the first attempts to extend the technique to virus crystallography. Our results provide a foundation of experience that future virus XFEL crystallography and solution scattering experiments can build upon. Although this effort did not produce diffraction at a higher resolution than previously reported, in spite of the high flux XFEL beam, the data provide evidence for a rhombohedral space group for enveloped Sindbis virus crystals. It is apparent that resolution is limited by the inherent nature of the sample, rather than the quality of the X-rays.

Delivery of the virus crystals to the beam in the viscous agarose medium proved to be a significant technical improvement. Agarose enables more efficient delivery of sample to the beam, thus reducing the amount of sample needed for measurement (Conrad et al., 2015). The agarose medium produced minimal background scattering, particularly in the low to mid-resolution range where diffraction was measured for the Sindbis crystals.

One of the most interesting results reported here is the simultaneous appearance of diffuse scattering and Bragg peaks produced from crystals. Our analysis indicates that the SAXS-like diffuse scattering originates from the crystals, not from free virus particles in solution. Information from the scatter produces a SAXS-like profile that is similar to a calculated profile from a model of Sindbis that was derived from cryoEM of virus particles. The extent to which factors that were discussed above contribute to the diffuse scattering will require further investigation. An improved understanding of this phenomenon will lead to an appreciation of how useful this data may be in providing additional structural information about the ordering of virus particles and their genomes in a crystal lattice.

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