Supplemental Material: Three-Dimensional Reconstruction of the Giant Mimivirus Particle with an X-Ray Free-Electron Laser

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A. EXPERIMENTAL SETUP

Experiments were performed at the AMO beam line[1] of the LCLS hard x-ray laser[2], using the CAMP instrument[3]. Far-field diffraction patterns were recorded on a pair of pnCCD detectors[3], operating at the 60 Hz repetition rate of the LCLS. The active area of the two detector halves was 76.8 mm x 38.4 mm and contained 1024 x 512 pixels of area 75 x 75 µm^2. The gap between the two detector halves was 2.1 mm and the sample to detector distance was 740 mm. The electron bunch was 70 fs FDHM. The resulting photon bunch is believed to be shorter[4] and contained about 1.2×10^{12} photons/pulse (0.24 mJ) at 1.2 keV photon energy (1 nm wavelength). The photon pulses were focused to a spot of 10 µm FWHM at the interaction point, giving 1.2×10^{10} photons/µm^2 in the centre of the beam, and a peak power density of about 3.4×10^{15} W/cm^2. The entire experiment was performed at low pressure (10^{-6} mbar) to reduce background scattering. The x-ray background was successfully suppressed to a level where it did not exceed the detector read-out noise. Diffraction data were collected for 103 minutes.

Purified mimivirus particles[5] were transferred into a volatile buffer (250 mM ammonium acetate, pH 7.5) and the suspension was aerosolized with helium in a gas dynamic nebuliser[6]. The aerosol of hydrated and adiabatically cooled particles entered a differentilly pumped aerodynamic lens[7]. The viability of injected mimivirus particles was tested on particles captured on an inert gel surface (AD-23T-00-X0, Smart Materials & More, Nijmegen) placed in front of the injector in the vacuum chamber. These particles were re-suspended in 500 µl harvest buffer[8]. Virus titration was then performed by turbidity measurements at 600 nm wavelength. A 25 µl aliquot of the recovered sample (containing 250 mimivirus particles) was used to infect 140,000 Acanthamoeba castellanii cells. After 24 h all cells were infected, indicating that the initial mimivirus titer was around 1 particle for 1000 cells suggesting that most of the captured virions were infectious.

B. DATA PREPROCESSING

Algorithms described in [9] and implemented in the CASS framework[10] were used to identify 2000 hits. These patterns were further filtered based on the particle having a loose icosahedral envelope. From the 307 diffraction patterns that passed this filter we selected 198 diffraction patterns that did not saturate the detector but still had a signal extending to beyond 83 nm^{-1}. A subset of 24 of these patterns is shown in Fig. 1. in the main article.

The centre positions of the diffraction patterns fluctuates over 750 µm, or 10 pixels, horizontally and vertically[11] due to the divergence of the x-ray pulse. We identified the centre for each diffraction pattern by maximizing centrosymmetry.

The diffraction patterns were cropped to a size corre-

sponding to a full-period resolution of 83 nm and then down sampled to a size of 64 by 64 pixels. This decreases the computational time and ensures a sufficient overlap between slices in adjacent orientations in the orientation recovery.

C. ESTIMATION OF THE REQUIRED NUMBER OF DIFFRACTION PATTERNS

The number of diffraction patterns required for a 3D reconstruction is different for the case where the orientation of the sample is set experimentally (e.g. in tomography) and for the case where particles are exposed to the beam in random and unknown orientations. The number of randomly oriented diffraction patterns required for a 3D reconstruction extending to a resolution d from an object with diameter D, can be estimated by considering the sampling of the 3D Fourier space (these diffraction patterns have strong signal so we don't need to take signal-to-noise ratio into account). The probability of filling the Fourier space with N randomly oriented patterns is calculated by considering the probability of covering individual Shannon pixels in the outermost resolution shell. Because of centrosymmetry, we can limit this calculation to the outer half-shell, which contains $K = 2\pi(D/d - 1/2)^2$ Shannon pixels. The high-resolution half-rim of each diffraction pattern is lined by $k = \pi(D/d - 1/2)$ Shannon pixels. Assuming no preferential orientation, we can calculate the probability of each outer-shell Shannon pixel to be covered by a single pattern as k/K. The probability of a Shannon pixel to be covered at least once by a set of N patterns is then $p_{\text{single}} = 1 - (1 - k/K)^N$. Making the simplifying assumption that the process of covering individual Shannon pixels is independent, the probability of covering K pixels can be expressed as $p = (p_{\text{single}})^K = \left(1 - (1 - k/K)^N\right)^K$. The probability to completely cover Fourier space to the edge of the diffraction patterns (83 nm^{-1}) for a mimivirus particle of 450 nm diameter is then 99.999991 % for 198 images.

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