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Self-terminating diffraction gates femtosecond X-ray nanocrystallography measurements

Anton Barty^{1,†}, Carl Caleman^{1,†}, Andrew Aquila¹, Nicusor Timneanu², Lukas Lomb^{3,4}, Thomas A. White¹, Jakob Andreasson², David Arnlund⁵, Saša Bajt⁶, Thomas R. M. Barends^{3,4}, Miriam Barthelmess⁶, Michael J. Bogan⁷, Christoph Bostedt⁸, John D. Bozek⁸, Ryan Coffee⁸, Nicola Coppola^{1,‡}, Jan Davidsson⁹, Daniel P. DePonte¹, R. Bruce Doak¹⁰, Tomas Ekeberg², Veit Elser¹¹, Sascha W. Epp^{4,12}, Benjamin Erk^{4,12}, Holger Fleckenstein¹, Lutz Foucar^{3,4}, Petra Fromme¹³, Heinz Graafsma⁶, Lars Gumprecht¹, Janos Hajdu², Christina Y. Hampton⁷, Robert Hartmann¹⁴, Andreas Hartmann¹⁴, Günter Hauser^{15,16}, Helmut Hirsemann⁶, Peter Holl¹⁴, Mark S. Hunter¹³, Linda Johansson⁵, Stephan Kassemeyer^{3,4}, Nils Kimmel^{15,16}, Richard A. Kirian¹⁰, Mengning Liang¹, Filipe R. N. C. Maia¹⁷, Erik Malmerberg⁵, Stefano Marchesini¹⁷, Andrew V. Martin¹, Karol Nass¹⁸, Richard Neutze⁵, Christian Reich¹⁴, Daniel Rolles^{3,4}, Benedikt Rudek^{4,12}, Artem Rudenko^{4,12}, Howard Scott¹⁹, Ilme Schlichting^{3,4}, Joachim Schulz¹, M. Marvin Seibert^{2,‡}, Robert L. Shoeman^{3,4}, Raymond G. Sierra⁷, Heike Soltau¹⁴, John C. H. Spence¹⁰, Francesco Stellato¹, Stephan Stern¹, Lothar Strüder^{4,15}, Joachim Ullrich^{4,12}, X. Wang¹⁰, Georg Weidenspointner^{15,16}, Uwe Weierstall¹⁰, Cornelia B. Wunderer⁶, and Henry N. Chapman^{1,18},

¹Center for Free-Electron Laser Science, DESY, Notkestrasse 85, 22607 Hamburg, Germany

²Laboratory of Molecular Biophysics, Department of Cell and Molecular Biology, Uppsala University, Husargatan 3 (Box 596), SE-751 24 Uppsala, Sweden

³Max-Planck-Institut für medizinische Forschung, Jahnstr. 29, 69120 Heidelberg, Germany

⁴Max Planck Advanced Study Group, Center for Free Electron Laser Science, Notkestrasse 85, 22607 Hamburg, Germany

⁵Department of Chemistry, Biochemistry and Biophysics, University of Gothenburg, SE-405 30 Gothenburg, Sweden

Correspondence and requests for materials should be addressed to A.B. and H.N.C., anton.barty@desy.de; henry.chapman@desy.de.

[†]These authors contributed equally to this work.

[‡]Present address: European XFEL GmbH, Albert-Einstein-Ring 19, 22761 Hamburg, Germany (N.C.), LCLS, SLAC National Accelerator Laboratory, 2575 Sand Hill Road, Menlo Park, California 94025, USA (M.M.S.).

Author contributions

H.N.C., J.C.H.S., A.B. and P.F. conceived the experiment, which was designed with T.A.W., R.A.K., J.S., D.D.P., U.W., R.B.D., S.Bo., M.J.B., D.S., I.S., S.M. and J.H. The CAMP instrument was the responsibility of S.W.E., R.H., D.R., A.R., L.F., N.K., P.H., B.R., B.E., A.H., Ch.R., G.W., L.S., G.H., H.G., J.U., I.S., H.So., H.H., L.G., H.G. and C.W., who operated the pnCCD detectors. C.B., J.B. and M.M. set up and aligned the beamline. P.F., M.S.H. and I.G. prepared samples. R.B.D., D.D.P., U.W., J.C.H.S., P.F., L.L. and R.L.S. developed and operated the sample delivery system. H.N.C., A.B., A.A., J.S., D.P.P., U.W., R.B.D., S.Ba., M.J.B., L.G., J.H., M.M.S., N.T., J.A., S.St. and J.C.H.S. developed diffraction instrumentation. M.B., M.L., A.B. and K.N. designed and/or fabricated calibration samples. H.N.C., J.C.H.S., P.F., A.B., T.A.W., R.A.K., C.C., A.A., L.L., J.S., D.P.D., U.W., R.B.D., I.S., N.C., R.L.S., M.S.H., M.B., S.W.E., R.H., D.R., A.R., S.K., T.E., M.L., C.B., J.U., L.F., J.D.B., M.M., M.F., C.Y.H., R.G.S., G.J.W., A.R., M.S., O.J., I.A. and J.H. carried out the experiment. A.B., C.C., N.T. and H.N.C. developed the theory, and analysed the data with L.L., T.A.W., I.S. and T.R.M.B. N.T., C.C. and H.S. carried out the Cretin simulations. A.B., C.C. and H.N.C. wrote the manuscript with discussion and improvements from all authors.

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⁶Photon Science, DESY, Notkestrasse 85, 22607 Hamburg, Germany

⁷Stanford PULSE Institute, SLAC National Accelerator Laboratory, 2575 Sand Hill Road, Menlo Park, California 94025, USA

⁸LCLS, SLAC National Accelerator Laboratory, 2575 Sand Hill Road, Menlo Park, California 94025, USA

⁹Department of Photochemistry and Molecular Science, Uppsala University, Box 523, SE-75120 Uppsala, Sweden

¹⁰Department of Physics, Arizona State University, Tempe, Arizona 85287, USA

¹¹Department of Physics, Cornell University, Ithaca, New York 14853, USA

¹²Max-Planck-Institut für Kernphysik, Saupfercheckweg 1, 69117 Heidelberg, Germany

¹³Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-1604, USA

¹⁴PN Sensor GmbH, Otto-Hahn-Ring 6, 81739 München, Germany

¹⁵Max-Planck-Institut Halbleiterlabor, Otto-Hahn-Ring 6, 81739 München, Germany

¹⁶Max-Planck-Institut für extraterrestrische Physik, Giessenbachstrasse, 85741 Garching, Germany

¹⁷Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA

¹⁸University of Hamburg, Luruper Chaussee 149, 22761 Hamburg, Germany

¹⁹Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, California 94550, USA

Abstract

X-ray free-electron lasers have enabled new approaches to the structural determination of protein crystals that are too small or radiation-sensitive for conventional analysis¹. For sufficiently short pulses, diffraction is collected before significant changes occur to the sample, and it has been predicted that pulses as short as 10 fs may be required to acquire atomic-resolution structural information^{1–4}. Here, we describe a mechanism unique to ultrafast, ultra-intense X-ray experiments that allows structural information to be collected from crystalline samples using high radiation doses without the requirement for the pulse to terminate before the onset of sample damage. Instead, the diffracted X-rays are gated by a rapid loss of crystalline periodicity, producing apparent pulse lengths significantly shorter than the duration of the incident pulse. The shortest apparent pulse lengths occur at the highest resolution, and our measurements indicate that current X-ray free-electron laser technology⁵ should enable structural determination from submicrometre protein crystals with atomic resolution.

The Linac Coherent Light Source (LCLS) at SLAC National Accelerator Laboratory produces individual X-ray pulses with energies of up to 3 mJ and durations that can be varied between ~30 and 400 fs, corresponding to $\sim 1 \times 10^{13}$ photons per pulse at a photon energy of 2 keV (wavelength, 0.6 nm)⁵. On exposure to an X-ray pulse, energy is primarily transferred to matter by photo-absorption. For example, for a 40 fs, 2 keV pulse at an irradiance of $1 \times 10^{17} \text{ W cm}^{-2}$, ~10% of carbon atoms in a protein crystal absorb a photon. Energy is initially released through the emission of photoelectrons and Auger electrons, followed by a cascade of lower-energy electrons caused by secondary impact or field ionizations taking place on a 10–100 fs timescale. The Coulomb repulsion of the ions and the rapid rise in electron temperature of the system causes displacement of both atoms and ions during the pulse. This heating leads to a high pressure that drives the explosion of the

sample. During the pulse, all chemical bonds are broken, and the temperature rises to over 500,000 K, completely vaporizing the sample. Simulations by molecular dynamics^{2,6,7} and hydrodynamic codes^{8–11} have predicted that motions of the ions of 0.5 nm can occur in less than 100 fs, and that pulses as short as 10 fs may be required to achieve atomic resolution.

On the other hand, strong diffraction peaks can be observed from single nanocrystals of Photosystem I (PSI) protein complex¹² flowing across the focused LCLS beam¹ using peak X-ray fluences of 4 kJ cm⁻² and pulse durations up to 300 fs. The single-shot diffraction pattern shown in Fig. 1, for example, was recorded to a resolution of 8 Å using a single 250-fs-duration pulse at a photon energy of 2 keV. The maximum dose to each protein crystal is 3 GGy per pulse, 100 times higher than tolerable doses for cryogenically cooled crystals exposed at synchrotron sources^{13–15}. The observation of strong diffraction for pulses with a duration of up to 300 fs appears at first sight to be at odds with conventional radiation damage models.

This remarkable tolerance to radiation dose can be explained by acknowledging that crystalline order is lost on timescales shorter than the pulse duration. As the atomic disorder increases, Bragg diffraction from the crystal decreases and ultimately turns off before the end of the incident pulse, resulting in apparent pulse lengths significantly shorter than the duration of the incident pulse. As the explosion progresses over the duration of the pulse, disordering of the atomic positions proceeds to ever-longer length scales, as depicted in Fig. 2a. As the correlation of structure between individual unit cells (periodic order) is lost on progressively longer length scales, the scattered photon flux accumulating into the corresponding Bragg peaks diminishes and eventually terminates. The pulse integrated counts in Bragg peaks are proportional to their undisturbed diffraction efficiencies, the pulse irradiance and their lifetimes. Simultaneously, diffuse scattering accumulates until the end of the pulse. The strength of the accumulated Bragg signals relative to this background depends proportionally on the number of unit cells in the crystal and inversely on the pulse duration (see Supplementary Information). Although this background is negligible for the crystal sizes, pulse durations and resolution considered here, it could be minimized using the shortest possible pulses.

The pulse-integrated diffraction pattern can be modelled in terms of the distinct processes of ionization and displacement of the atoms in the crystal. Ionization of atoms in the crystal occurs randomly, modifying atomic scattering factors^{16,17} and leading to both a resolution-dependent reduction in Bragg signal and the addition of uniform diffuse scattering¹⁸. For example, single ionization of half the atoms decreases the Bragg signals by ~20%. Our measurements are not sensitive to this uniform change.

We calculated the atomic displacements caused by the X-ray interaction using the plasma modelling code Cretin¹⁹ on a homogeneous protein sample in water (see Methods). As the ion temperature rises during the X-ray pulse, so too does the root mean square (r.m.s.) atomic displacement, shown in Fig. 2b. At any point in time, the displacement of any particular atom in the unit cell is considered to be random and isotropic with zero mean. As with the familiar analysis of a thermally disordered crystal¹⁸, the diffraction pattern is modified from the perfect crystal by an addition of a diffuse scattering term that increases with increasing scattering angle, and a compensating reduction in the Bragg signal by a term $\exp(-4\pi^2 q^2 \langle D^2(t) \rangle)$, where $q = (2/\lambda) \sin \theta$ for wavelength λ and scattering angle 2θ , and $\langle D^2(t) \rangle = \langle D^2(t) \rangle$ where D is the component of the atomic displacement in the direction of the photon momentum transfer of the Bragg peak at time t during the pulse. Cretin simulations show that for the conditions of our experiment, the mean square displacement $\langle D^2(t) \rangle$ increases approximately as t^3 for high irradiance pulses and times longer than 10 fs (see Fig. 2b). For a pulse of irradiance I_0 and duration T , the accumulated Bragg signal is therefore given by

$$I(q;T)=I_0 T r_e^2 P \Delta \Omega |F(q)|^2 g(q;T) \quad (1)$$

with

$$g(q;T)=\frac{1}{T} \int_0^T \exp(-4\pi^2 q^2 \sigma^2(t)) dt \quad (2)$$

for a detector pixel solid angle $\Delta \Omega$, and where r_e is the classical electron radius, P is the polarization factor, and $F(q)$ is the structure factor of the room-temperature nanocrystal (with an implicit Debye–Waller term due to the initial displacements of the atoms from perfect lattice positions). The dimensionless dynamic disorder factor $g(q; T)$ gives the change in signal relative to the undisturbed sample for pulses of irradiance I_0 and duration T .

As can be seen from equations (1) and (2) and Fig. 2b, as time progresses during the pulse, the Bragg diffraction effectively terminates when the r.m.s. displacement exceeds $1/(2 q) = d/(2 \sin \theta)$, where d is the ‘interplanar spacing’ of that Bragg peak. Higher-resolution peaks turn off sooner, leading to lower counts accumulated on the detector (Fig. 3a). For the derived t^3 dependence of $\sigma^2(t)$, the turn-off time of a Bragg peak is estimated as $t_{\text{off}} = (2 q \sigma_T)^{-2/3} T$ where $\sigma_T = \sigma(T)$ is the r.m.s. atomic displacement at the end of the pulse. Indeed, from equation (2) the disorder factor $g(q; T)$ tends towards

$$g(q;T) \approx \Gamma(4/3) \frac{t_{\text{off}}}{T} \approx \frac{\Gamma(4/3)}{(2\pi q \sigma_T)^{2/3}} \quad (3)$$

for pulse durations $T > t_{\text{off}}$ and where Γ is the gamma function. Given high enough pulse irradiance I_0 , scattering will be observed, even for pulses of longer duration than the explosion dynamics. For example, the Cretin calculations predict an r.m.s. atomic displacement of ~ 0.25 nm by the end of a 40 fs pulse at an irradiance of $1 \times 10^{17} \text{ W cm}^{-2}$ for a homogeneous protein object (Fig. 2b). In this case, the accumulated Bragg signal at a resolution of 1 nm (green line in Fig. 3a) will be $\sim 60\%$ of the undisturbed signal (dashed black line) after 40 fs, as contributed by approximately the first 30 fs of the pulse, while at the end of a 150-fs-duration pulse of the same irradiance the peaks at 1 nm resolution will have accumulated only 25% of the possible undisturbed signal.

We measured the effect of Bragg termination over a wide resolution range, and as a function of pulse duration, by forming ‘virtual powder diffraction’ patterns. An example of such a powder pattern is shown in Fig. 4a, obtained by summing 3,792 single-pulse single-crystal patterns acquired with pulses of 300 fs duration. By averaging these patterns over shells of q , we obtain measures of $I(q; T)$ (Fig. 4b). As with conventional powder diffraction, these measurements are averaged over crystal shapes, orientations, wavelength spread and beam divergence (0.1% and 0.5 mrad, respectively, in this case). Various pulse durations between nominal values of 70 fs and 300 fs were achieved by varying the compression of the free-electron laser electron pulses, which keeps the pulse fluence $I_0 T$ approximately constant. Previous work has indicated that pulses below 100 fs may have less than half the nominal duration²⁰.

The effect of overall Bragg termination can be isolated from other contributions in equation (1), such as the structure factors $F(q)$ and the initial room-temperature Debye–Waller factor, by taking the ratio of $I(q; T)$ to the shortest, least damaging duration, $I(q; T=70 \text{ fs})$. These ratios are plotted versus q in Fig. 4c, showing that the relative Bragg diffraction efficiency at higher scattering angles is diminished as pulse duration increases. Dashed lines are from

Cretin calculations matching the experimental conditions, but assuming the shortest pulses have 40 fs duration and all others follow their nominal values (see Supplementary Information). We expect from equation (3) that the ratios of $I(q; T)$ are independent of q when $T > t_{\text{off}}$, which is indeed the case for $q > 0.75 \text{ nm}^{-1}$. In this regime, and for constant pulse fluence $I_0 T$, the ratios are equal to the ratio of relative turn-off times of the pulses, t_{off}/T . At 300 fs, the 1 nm resolution Bragg signal therefore originates from ~ 0.25 of the contribution compared with 40 fs pulses, which themselves contribute $g(q; T) = 0.6$ (for a turn-off time of $\sim 0.25 \times 300 \text{ fs} \times 0.6 = 50 \text{ fs}$ at this lower irradiance). As the pulse irradiance is increased, the turn-off time t_{off} becomes shorter. However, we find from simulations that σ_T^2 depends linearly on I_0 , whereby, from equation (3) the Bragg counts are proportional to $I_0 t_{\text{off}} \propto I_0^{2/3}$. In other words, the highest signals are achieved with the highest irradiance pulses, and structure factors can be corrected for Bragg peak termination using the observed pulse-length dependence. Comparison of the dynamic disorder factor $g(q; T)$ with conventional Debye–Waller scaling shows appreciable differences for long pulse lengths (Fig. 3d).

Future experiments will be carried out at shorter wavelengths to access atomic resolution. The photoabsorption cross-sections of light elements are about 60 times lower at 8 keV photon energy than at 2 keV, giving rise to a corresponding reduction in the dose that drives the explosion dynamics. This is offset somewhat by the requirement for higher X-ray fluence due to the lower diffraction efficiency. Our calculations show that 0.3 nm resolution can be achieved with a 30-fs-duration pulse of $1 \times 10^{18} \text{ W cm}^{-2}$ (for example, a 1.5 mJ pulse energy focused to a $5 \mu\text{m}^2$ focus) as detailed in Fig. 5. We expect an r.m.s. displacement of only 0.1 nm by the end of the pulse, for a deposited dose of 270 MGy. This will yield strong diffraction from crystals of the same size as used here (Supplementary Table S2), possibly beating extensive ionization of the sample²⁰. Our model predicts that pulses of even higher irradiance will give stronger diffracted signals.

Our experiments and models show that, unlike single-particle diffraction², nanocrystallography does not require X-ray pulses to be strictly shorter than the onset of nuclear motion. Instead, the increasing uncorrelated motion of atoms during plasma formation destroys crystalline order. Bragg diffraction ceases before the incident pulse terminates, producing apparent pulse lengths much shorter than the incident pulse duration. The dynamics of plasma formation depend on the overall atomic composition, not on the unit cell parameters, and hence our results should be indicative for most protein crystals. Similar principles can be applied to diffraction from solid-state samples. Measurements and simulations at various pulse irradiances offer a way to characterize the explosion dynamics and hence correct for the effect of Bragg peak termination, thereby correcting data for structure determination.

Methods

Data collection

Femtosecond nanocrystal diffraction patterns were collected at the Atomic Molecular and Optical (AMO) beamline²¹ at LCLS⁵, in the CFEL-ASG Multi-Purpose (CAMP) instrument²². A schematic of the experiment is shown in Fig. 1, and is similar to previously described experiments¹. Diffraction patterns were recorded on a pair of X-ray p–n junction charge-coupled device (pnCCD) modules located 64.7 mm from the liquid jet carrying the nanocrystal suspension. Detector panels were placed asymmetrically with respect to the X-ray beam, the upper panel 3.9 mm above beam centre and the lower detector 29 mm below beam centre, so that the largest scattering angle intercepted by the detector modules was $2 = 49^\circ$, giving a highest resolution of 0.73 nm at a photon energy of 2 keV. The LCLS

operated at a repetition rate of 60 Hz. X-rays were focused into a focal spot of area $10\ \mu\text{m}^2$ by a pair of Kirkpatrick–Baez mirrors in the AMO beamline.

The liquid jet was focused to a diameter of $4\ \mu\text{m}$ by coaxial gas flow²³, and intersected the LCLS beam in the continuous liquid column, upstream of the breakup of the jet into drops due to Rayleigh instability. The suspension of Photosystem I nanocrystals in their unadulterated mother liquor flowed at a rate of $10\ \mu\text{l min}^{-1}$ and with a protein concentration of $\sim 1\ \text{mg ml}^{-1}$ ($1\ \mu\text{M}$). The crystals intersected randomly with the LCLS pulses, which arrived at a repetition rate of 60 Hz. The pnCCD detectors were read out at the same rate and digitized after each LCLS pulse. The probability of a crystal being in the intersecting volume of the X-ray and fluid beams at any point in time was 20%, in accordance with the observed rate of crystal diffraction patterns. Statistics of the measured patterns are given in Supplementary Table S1.

Data reduction

Each recorded pnCCD frame was corrected for detector offset and gain, and then a time-windowed average background was subtracted for each pixel. The corrected frame was searched for Bragg spots using a thresholding and morphological analysis algorithm. This algorithm generated a list of peak locations (in detector coordinates), with the X-ray counts being the sums of pixel values within a contiguous region identified as belonging to the peak. Detector artefacts and scattering from the liquid jet were identified and excluded from the list of Bragg spots, and only patterns with at least three peaks were included. The magnitude of the photon momentum transfer, $q = (2\sin \theta) / \lambda$, for each peak was determined from the peak location and the shot-to-shot wavelength variation of LCLS (as calculated from the electron beam and undulator parameters⁵). Plots of $I(q; T)$ were generated by averaging the peak intensities in the list, for each pulse duration. These one-dimensional plots are also corrected for the solid angles of the detector pixels and scaled to the integrated counts at the lowest scattering angles to account for fluctuations in pulse energy. The serial data, collected one crystal at a time, can be assembled into a three-dimensional set of structure factors by first indexing each pattern and summing counts for each symmetrically unique reflection (Miller index)^{1,24}. Integrated structure factors may be corrected for Bragg diffraction termination by estimating the dynamic disorder factor from ratios of powder diffraction data, as in Fig. 4c, or fitting this factor as part of a structural refinement step. Corrections in structure factors by accounting for pulse length effects are quantified by the $R_1(I)$ factor (Supplementary Equation S11). The dynamic disorder factor corrections, and the corresponding Debye–Waller factor corrections $\exp(-Bq^2/2)$, where $B = 8\ \text{\AA}^2$, are plotted in Fig. 4d. The B factor is often determined as a fit parameter in a final refinement step in structure determination. As seen in Fig. 4d, the Gaussian form does not adequately account for Bragg termination, requiring a change to these structural refinement methods.

Simulations

Simulations were performed using Cretin, a non-local thermodynamic equilibrium radiation transfer plasma modelling code¹⁹, similar to earlier work²⁵ (see Supplementary Information for details). The Cretin code has been validated at this irradiance level in soft X-ray experiments of saturated ablation and ionization of materials²⁶.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Femtosecond X-ray diffraction from Photosystem I nanocrystals

A suspension of nanocrystals flows in a liquid jet across the X-ray beam, and diffraction is recorded using a pair of $512 \times 1,024$ pixel pnCCD detectors with $75 \mu\text{m}$ pixel pitch²². The lower half detector is placed further from the beam centre than the upper half detector to increase the accessible range of scattering angles. The detector module is located 64.7 mm downstream of the interaction region, giving a maximum measurable resolution of 0.76 nm. Thousands of individual diffraction patterns are recorded from single nanocrystals with pulse lengths up to 300 fs to a resolution of $d=0.76$ nm, and summed to produce virtual powder patterns, which are radially integrated to produce one-dimensional powder plots.

Figure 2. Dynamics of exploding crystals

a, Onset of disorder in a crystalline lattice during an ultra-intense X-ray pulse with a duration of 100 fs, which, for the sake of visual clarity, is shown here for the case of a small molecule (lysergic acid diethylamide). Individual atoms are randomly displaced by atomic displacements (calculated using Cretin). For this small molecule, crystalline order is largely destroyed by 100 fs; however, by this time, Bragg diffraction from the initial ordered crystalline structure has already been measured. **b**, Plot of the one-dimensional component of the r.m.s. displacement, $\sigma(t) = \sqrt{\langle D^2(t) \rangle}$, of atoms in Photosystem I calculated using Cretin for constant-irradiance X-ray pulses with a photon energy of 2 keV. Black triangles show the predicted component of r.m.s. displacement at the end of pulses of a constant fluence of 4 kJ cm^{-2} . The turn-off time for our highest-resolution length $d=0.76 \text{ nm}$ occurs when (t) reaches a value of $d/(2) = 0.12 \text{ nm}$ (dashed horizontal line).

Figure 3. Self-terminating Bragg diffraction

a, Plot of relative accumulation of Bragg signal, $tg(q; T)$, using (t) values from Fig. 2b at $I_0 = 1 \times 10^{17} \text{ W cm}^{-2}$ and a pulse duration $T=150$ fs. Bragg peaks initially accumulate signal at the same rate proportional to the irradiance I_0 (relative to the undisturbed case); however, accumulation of counts into higher-resolution peaks ends as crystal disorder grows.

Termination of signal accumulation can occur before the X-ray pulse itself has terminated, leading to apparent pulse lengths shorter than the duration of the incident pulse. The vertical black line at 40 fs indicates the pulse duration experimentally realized at this fluence, which is close to the turn-off time for the highest-resolution peaks at $d=0.76$ nm. **b**, Visualization of the dynamic disorder factor $g(q; T)$ given by equation (2). The black line shows the turn-off time for Bragg peaks of different resolution. The zero-frequency signal, which depends only on the total electron mass of the crystal, is unaffected by the crystal explosion.

Figure 4. Bragg termination observed at approximately constant X-ray pulse fluence I_0T
a, 'Virtual powder pattern' formed by summing 3,792 single-pulse patterns obtained with X-ray pulses with a duration of 300 fs. The spots in the pattern are Bragg peaks, which are visible out to the corners of the detector, corresponding to a resolution of $d=0.76$ nm. Because of the large unit cell size of the crystal, Debye-Scherrer rings overlap and are not resolved at $q > 0.5$ nm⁻¹. **b**, Bragg signal $I(q; T)$ of Photosystem I nanocrystals averaged over q shells of virtual powder patterns for nominal pulse durations T varying between 70 fs and 300 fs. **c**, Bragg signal relative to the shortest pulses, plotted as solid lines. Dashed lines give the computed ratios of $I(q; T)/I(q; T=40$ fs) from the Cretin simulations of Fig. 2. Previous experiments at LCLS indicate that the nominal '70 fs' pulses are shorter than indicated²⁰. We achieve a best fit assuming these pulses have a duration of 40 fs (see Supplementary Information). **d**, Comparison of the calculated dynamic disorder factor $g(q; T)$ (solid lines) compared to a Debye-Waller factor best-fit to the same data (dashed lines).

Figure 5. Dynamic disorder factor at atomic resolution

a, Plot of the one-dimensional component of atomic displacement in a Photosystem I protein sphere for constant-irradiance 8 keV X-ray pulses (wavelength, 0.15 nm). Higher irradiances than 2 keV are required to achieve similar diffraction signals. The turn-off time for 0.3 nm resolution occurs when $\langle t \rangle$ reaches 0.05 nm. **b**, Plot of $g(q; T)$ for 8 keV pulses at 100 kJ cm^{-2} fluence (8×10^{11} photons μm^{-2}), for different pulse durations. At 100 fs duration, the pulse irradiance is 1×10^{18} W cm^{-2} . The highest diffraction efficiency and signal-to-background is reached with the shortest pulses, but longer pulses do not preclude the observation of Bragg peaks.