

Electronic Supplementary Information

High mobility of lattice molecules and defects during the early stage of protein crystallization

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Supplementary Figures S1–S9

Supplementary Movie Legends S1–S5

Supplementary Figures

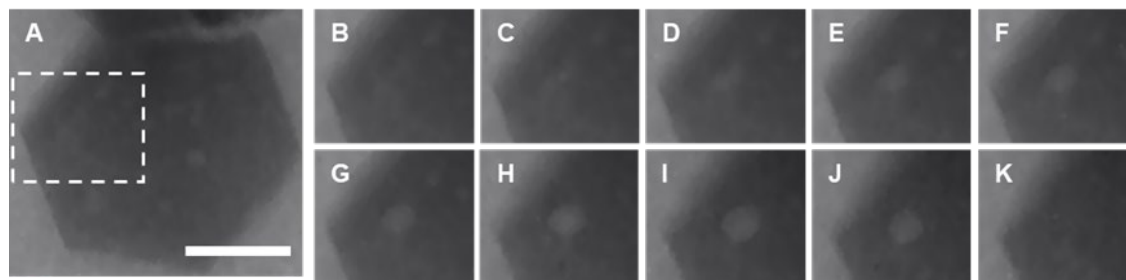


Figure S1. Formation and disappearance of a 60-nm void. (A) Tetragonal lysozyme crystal before the formation of a 60-nm void. (B–K) Time-lapse images of the area enclosed by the broken line in (A). Images B–K were captured 0 (B), 1 (C), 2 (D), 3 (E), 4 (F), 5 (G), 16 (H), 26 (I), 33 (J), and 35 s (K) after Image A (the crystal was started to be irradiated by the electron beam approximately 133 s previously). From (B) to (G), the void rapidly formed and grew to 60 nm in diameter. The void then disappeared from (J) to (K). The void appears to be a facet in (I). The scale bar is 200 nm, and the scale is the same in all images.

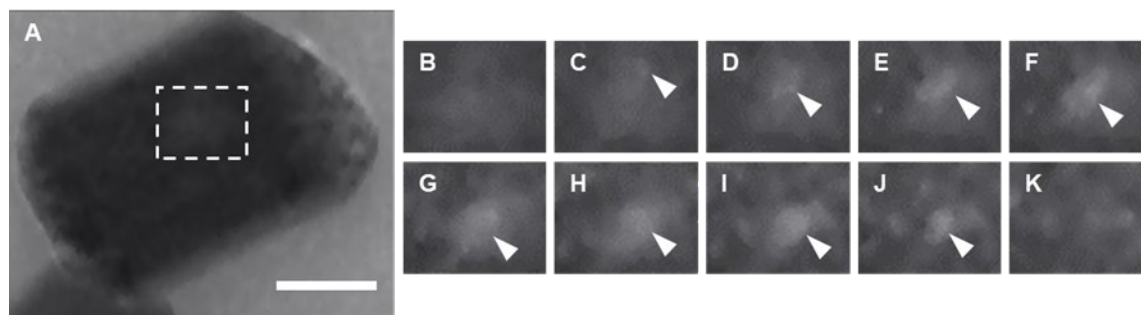


Figure S2. Formation and disappearance of a void in an orthorhombic lysozyme crystal. (A) Orthorhombic lysozyme crystal before the formation of a void. (B–K) Time-lapse images of the area enclosed by the broken line in (A). Images B–K were captured 0 (B), 5 (C), 7 (D), 9 (E), 10 (F), 15 (G), 20 (H), 23 (I), 25 (J), and 30 s (K) after Image A (the crystal was started to be irradiated by the electron beam approximately 362 s previously). The white arrowheads indicate the formed void. From (B) to (F), the void formed and grew; it then disappeared from (I) to (K). The scale bar is 200 nm, and the scale is the same in all images.

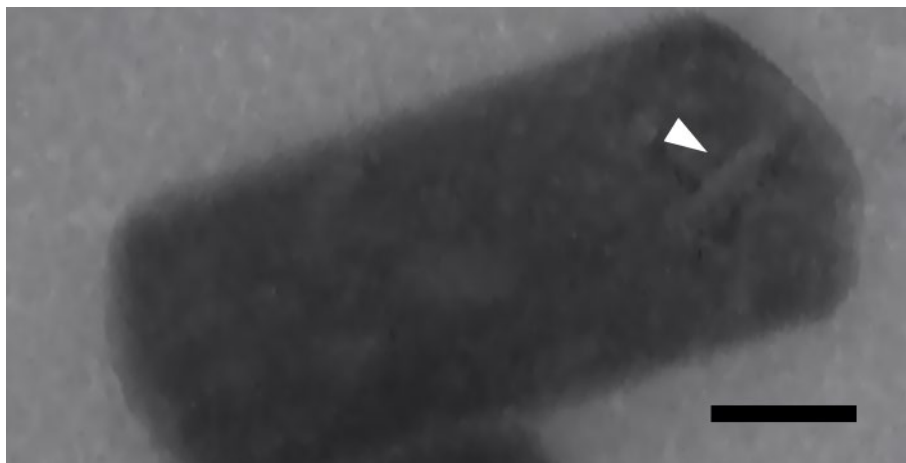


Figure S3. Typical example of an elongated void. The void indicated by the white arrowhead in the orthorhombic lysozyme crystal is elongated and 120 nm long in the direction of elongation. The crystal was started to be irradiated by the electron beam approximately 549 s previously. The scale bar is 200 nm.

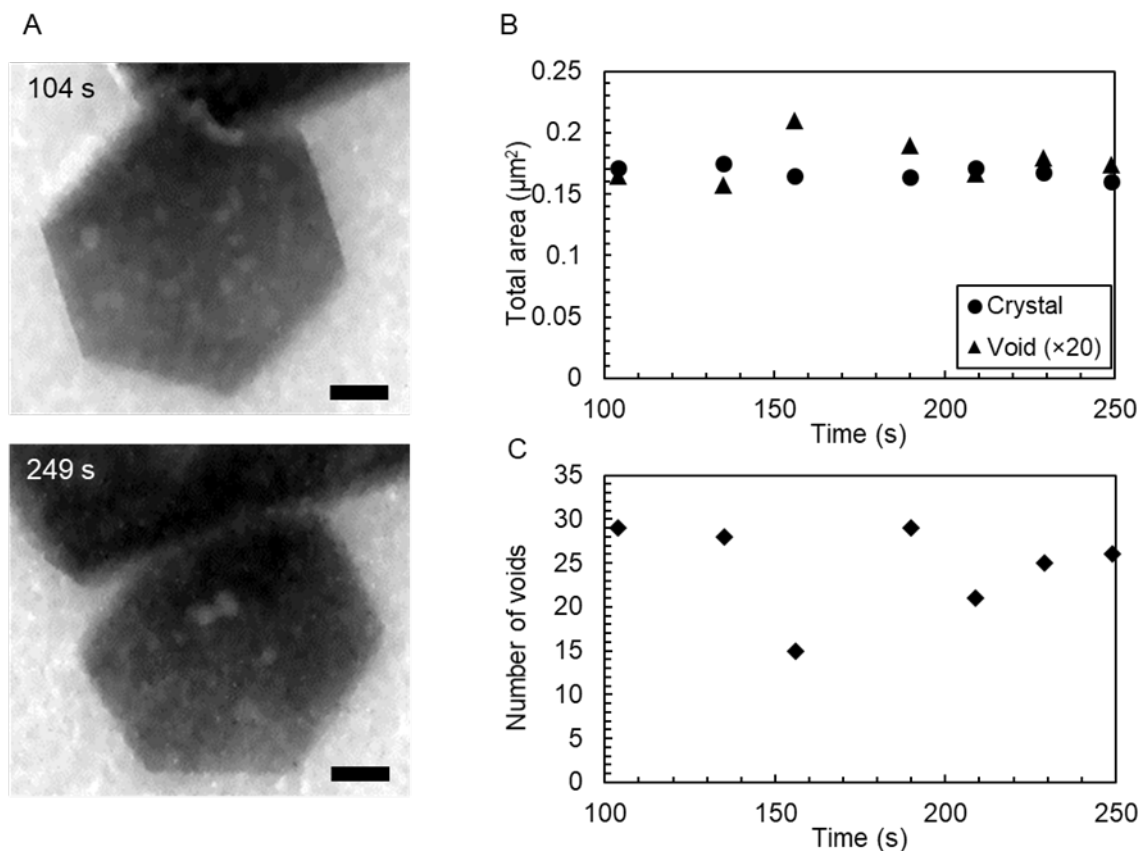


Figure S4. Variation of the total area and number of voids in a tetragonal lysozyme crystal as a function of observation time. (A) The first and last TEM images of the tetragonal lysozyme crystal, where the total area and number of voids were measured. The irradiation time of the crystal by the electron beam is indicated in the images. The accumulated electron dose of the image at 104 and 249 s was between 3.3×10^4 – 3.0×10^5 and 8.0×10^4 – 7.2×10^5 $\text{e} \cdot \text{nm}^{-2}$, respectively. The liquid cell containing the crystal was assembled about 30 min before observation began. The scale bars are 100 nm. (B) The time variation of the total area of voids and the crystal. The area of the voids accounts for 4.4–6.4% of the of crystal. (C) The time variation of the number of voids in the crystal.

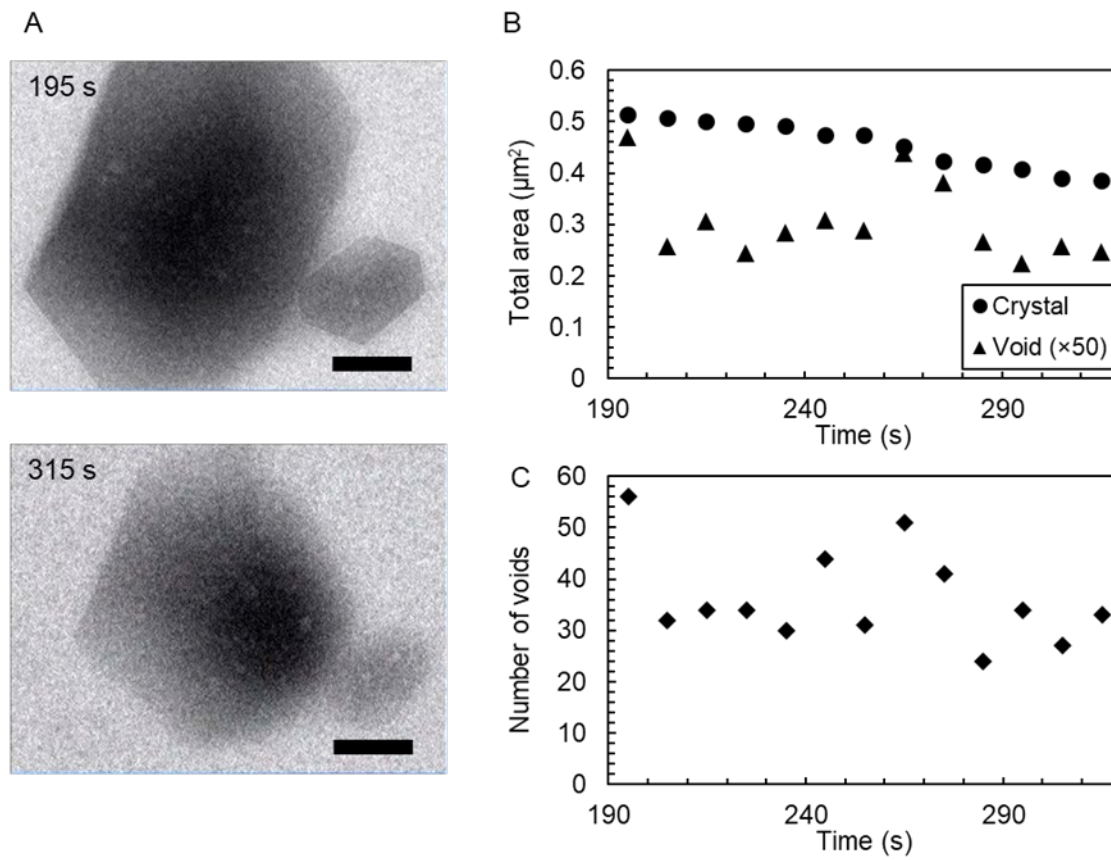


Figure S5. Variation of the total area and number of voids in a dissolving tetragonal lysozyme crystal. (A) The first and last TEM images of the dissolving tetragonal lysozyme crystal, where the total area and number of voids were measured. The irradiation time of the crystal by the electron beam is indicated in the images. The accumulated electron dose of the image at 195 and 315 s was between 6.2×10^4 – 5.7×10^5 and 1.0×10^5 – 9.1×10^5 $\text{e} \cdot \text{nm}^{-2}$, respectively. The liquid cell containing the crystal was assembled about 60 min before observation began. The scale bars are 200 nm. (B) The time variation of the total area of the voids and crystal. The area of the voids accounts for 1–2% of the crystal. (C) The time variation of the number of voids in the crystal.

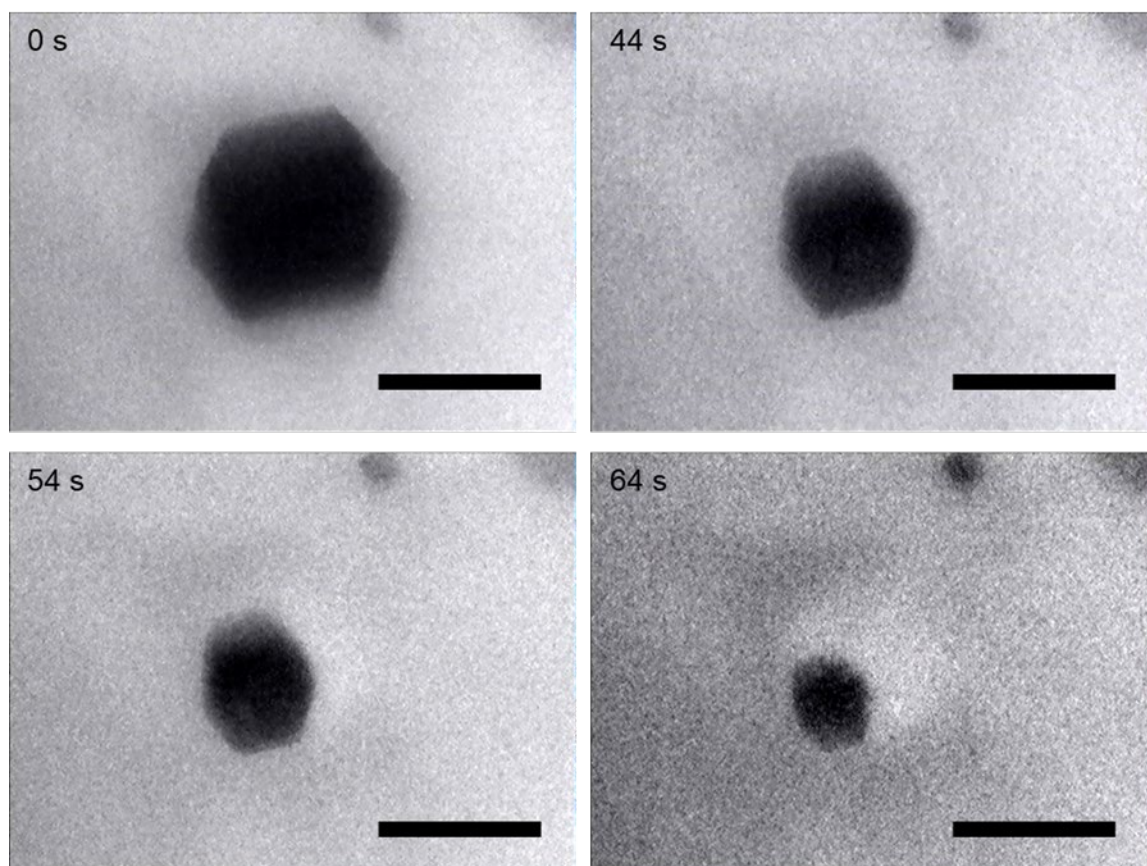


Figure S6. A dissolving tetragonal lysozyme crystal with no voids. A series of TEM images of the dissolving tetragonal lysozyme crystal with no voids. The times indicate the elapsed times after the crystal was irradiated by the electron beam. The accumulated electron dose of the image at 64 s was between 2.0×10^4 – 1.9×10^5 e $\cdot$ nm $^{-2}$. The liquid cell containing the crystal was assembled $\sim$ 24 h before observation. The scale bars are 500 nm.

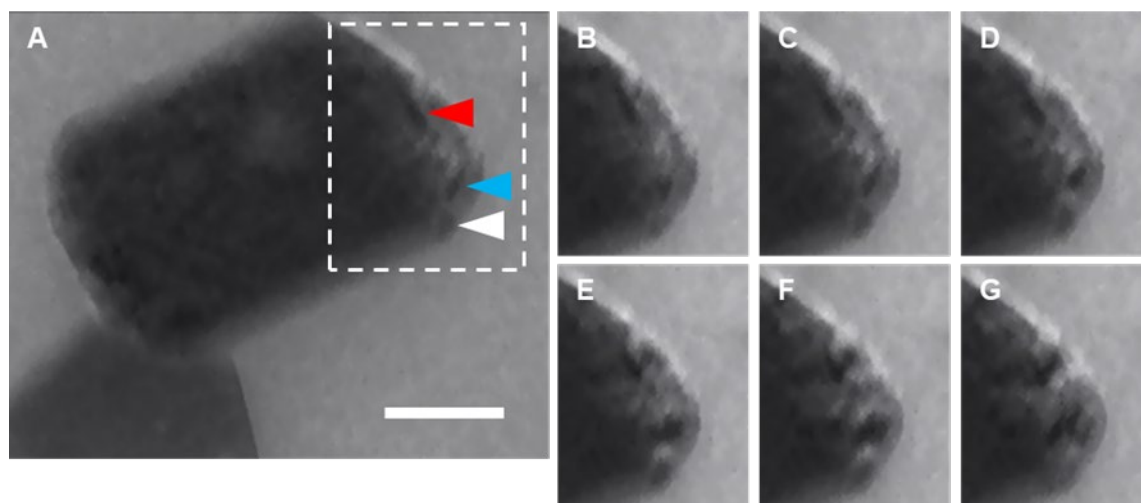


Figure S7. Temporal fluctuations in contrast at the end of an orthorhombic lysozyme crystal. (A) Orthorhombic lysozyme crystal showing paired areas of bright and dark contrast. The scale bar is 200 nm. (B–G) Time-lapse images of the area enclosed by the broken line in (A). Images B–G were captured 1 (B), 2 (C), 3 (D), 4 (E), 5 (F), and 6 s (G) after Image A (the crystal was started to be irradiated by the electron beam approximately 366 s previously). The shape of the area of contrast indicated by the white arrowhead in (A) did not change during (B) to (G), whereas the shapes of the areas indicated by the blue and red arrowheads in (A) changed in each image and between (E) and (F), respectively. The scale in all images is the same as Image A.

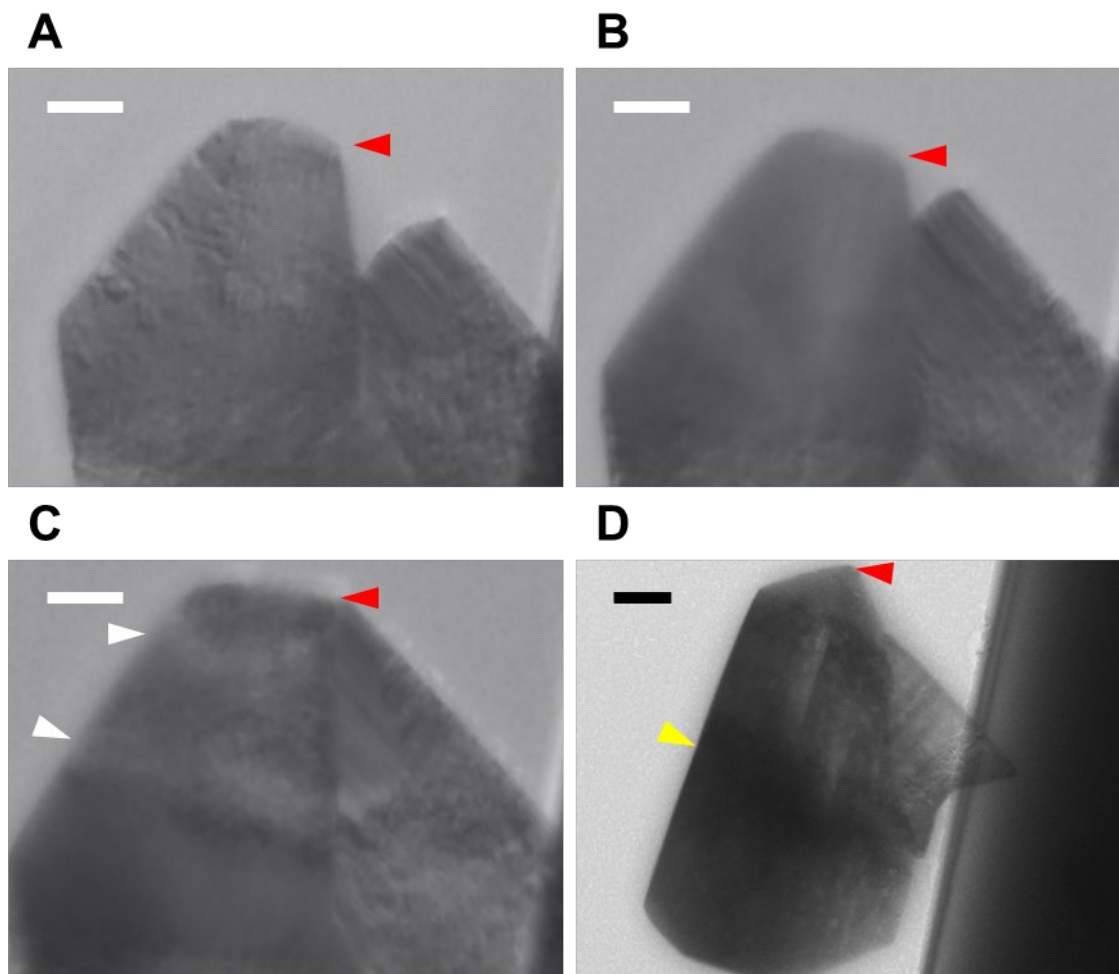


Figure S8. Temporal evolution of paired areas of bright and dark contrast in a growing lysozyme crystal. (A) The paired areas of bright and dark contrast extend across the entire crystal. The red arrowhead indicates one of the points of the crystal. (B–D) The paired areas moved and then disappeared. The red arrowheads indicate the same point in each image. The white and yellow arrowheads indicate bright and dark areas of contrast, respectively. Notably, the shape of the crystal appears to transform from tetragonal (hexagonal-like) to orthorhombic (elongated). Images B–D were captured 59 (B), 140 (C), and 742 s (D) after Image A (the crystals were started to be irradiated by the electron beam approximately 193 s previously). The scale bars are 200 nm (A–C) and 500 nm (D).

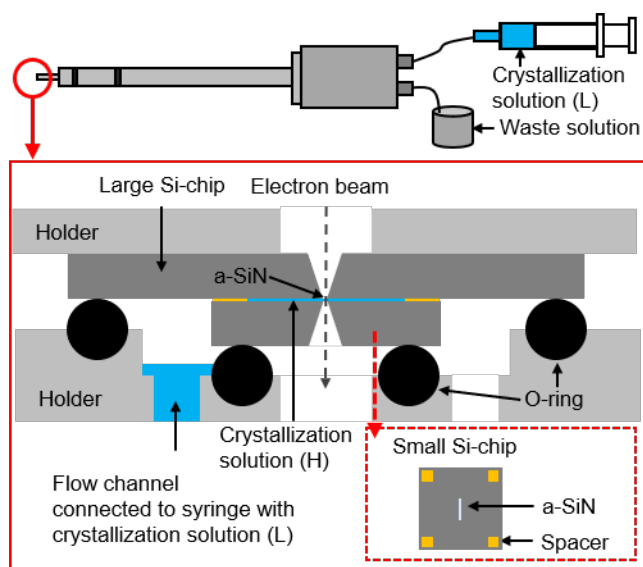


Figure S9. Schematic representations of the TEM holder system and a cross-section of the liquid cell. A syringe containing a crystallization solution with lower lysozyme concentration [Crystallization solution (L)] connects to the TEM holder. An enlarged cross-section of the liquid cell at the tip of the TEM holder is shown in the solid red rectangle. A crystallization solution with higher lysozyme concentration [Crystallization solution (H)] is sandwiched between the large silicon chip (Si-chip) and the small Si-chip, a top view of which is shown in the broken red line. Each Si-chip has an observation window made of amorphous silicon nitride (a-SiN). The small Si-chip is also equipped with 500 nm-thick spacers at its four corners. The schematics are not drawn to scale.

Supplementary Movie Legends

Movie S1. Behavior of defects in a tetragonal lysozyme crystal. The movie is presented at 10× speed. This movie starts from 59 s later when the crystal was started to be irradiated by the electron beam. The scale bar is 200 nm. Screenshots of this movie are presented in Figs. 1, S1, and S4.

Movie S2. Behavior of defects in an orthorhombic lysozyme crystal. The movie is presented at 10× speed. This movie starts from 374 s later when the crystal was started to be irradiated by the electron beam. The scale bar is 200 nm. Screenshots of this movie are presented in Fig. 2, S2, S3, and 7.

Movie S3. Defects highlighted by band contrast in orthorhombic lysozyme crystals. The movie is presented at 10× speed. This movie starts from 234 s later when the crystals were started to be irradiated by the electron beam. The scale bar is 200 nm. A screenshot of this movie is presented in Fig. 3.

Movie S4. Selective dissolution and band contrasts in orthorhombic lysozyme crystals. The movie is presented at 10× speed. This movie starts from 685 s later when the crystals were started to be irradiated by the electron beam. The scale bar is 200 nm. A screenshot of this movie is presented in Fig. 3.

Movie S5. Possible moment of phase transition from tetragonal to orthorhombic lysozyme crystal. The movie is presented at 10× speed. This movie starts from 32 s later when the crystals were started to be irradiated by the electron beam. The scale bar is 200 nm. A screenshot of this movie is presented in Fig. 4.