

# **Atomic structure of granulin determined from native nanocrystalline granulovirus using an X-ray free-electron laser**

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To understand how molecules function in biological systems, new methods are required to obtain atomic resolution structures from biological material under physiological conditions. Intense femtosecond-duration pulses from X-ray free-electron lasers (XFELs) can outrun most damage processes, vastly increasing the tolerable dose before the specimen is destroyed. This in turn allows structure determination from crystals much smaller and more radiation sensitive than previously considered possible, allowing data collection from room temperature structures and avoiding structural changes due to cooling. Regardless, high-resolution structures obtained from XFEL data mostly use crystals far larger than  $1 \mu\text{m}^3$  in volume, while the X-ray beam is often attenuated to protect the detector from damage caused by intense Bragg spots. Here, we describe the 2 Å resolution structure of native nanocrystalline granulovirus occlusion bodies (OBs) that are less than  $0.016 \mu\text{m}^3$  in volume using the full power of the Linac Coherent Light Source (LCLS) and a dose up to 1.3 GGy per crystal. The crystalline shell of

granulovirus OBs consists, on average, of about 9,000 unit cells, representing the smallest protein crystals to yield a high-resolution structure by X-ray crystallography to date. The XFEL structure shows little to no evidence of radiation damage and is more complete than a model determined using synchrotron data from recombinantly produced, much larger, cryo-cooled granulovirus granulin microcrystals. Our measurements suggest that it should be possible, under ideal experimental conditions, to obtain data from protein crystals with only 100 unit cells in volume using currently available XFELs and suggest that single-molecule imaging of individual biomolecules could almost be within reach.