Direct TEM observation of nucleation processes in a solution

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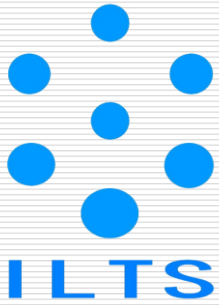
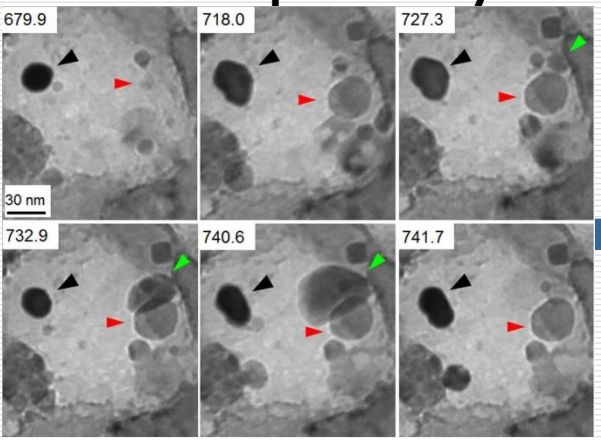


(200) Mathematical Aspects of Surface and Interface Dynamics 14, Tokyo, Oct. 26, 2017.

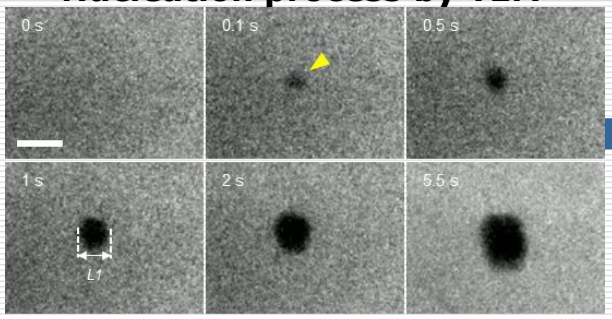
My resent works

Dissolution process by TEM



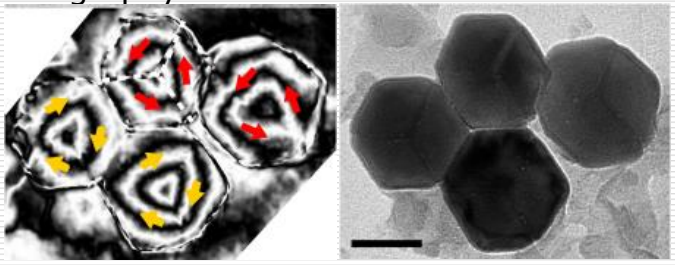
Kimura et al. *JACS* 136 (2014) 1762.

Nucleation process by TEM

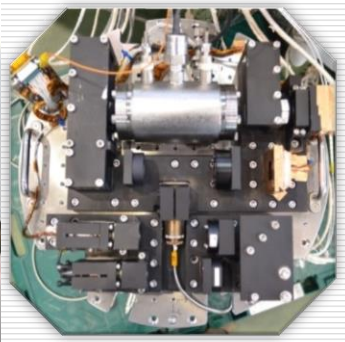


Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

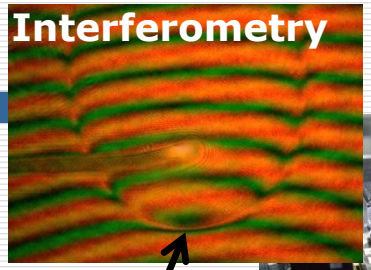
Magnetic remnant by electron holography TEM

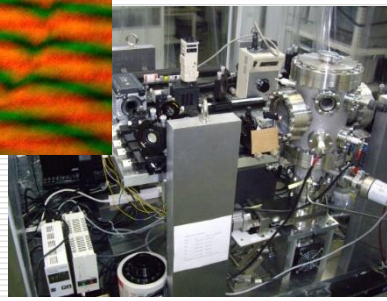


Nucleation is key!



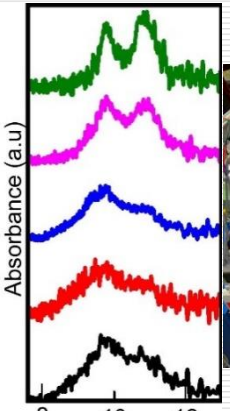
Kimura et al. *Science Adv.*, 3 (2017) e1601992.

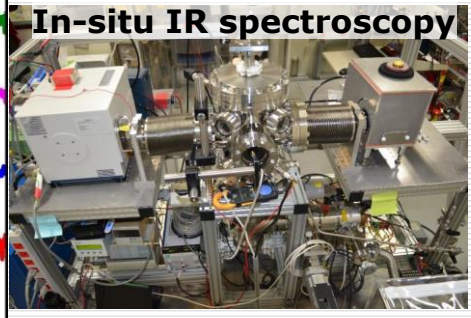




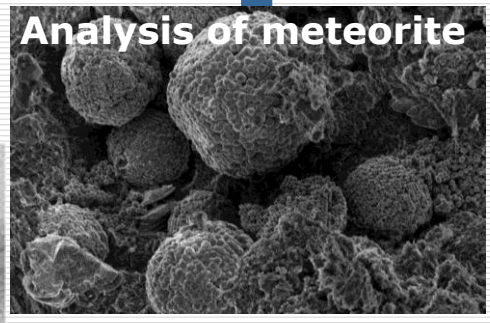
Kimura et al. *Crystal Growth & Design*, 12 (2012) 3278.

Signal of nucleation



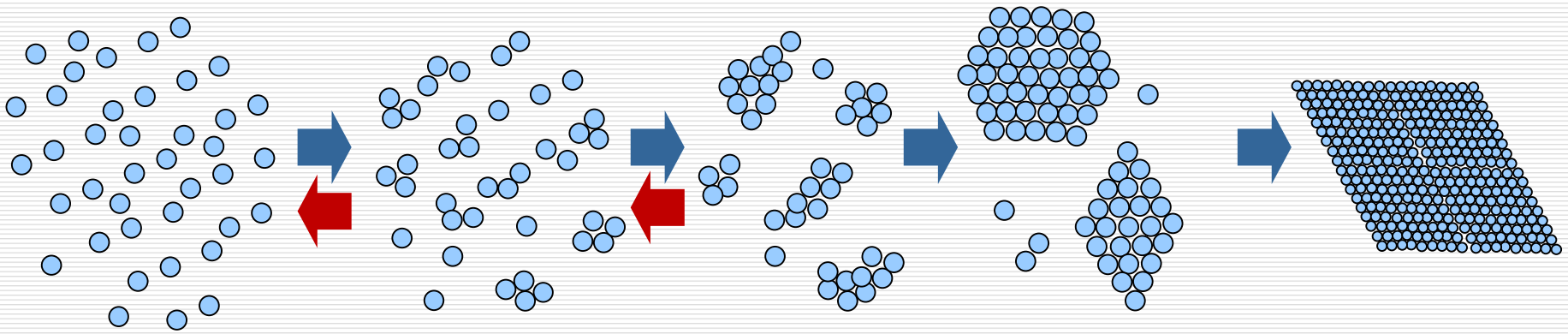


Ishizuka, **Kimura** *et al.*, *Chemistry of Materials*, 28 (2016) 8732.

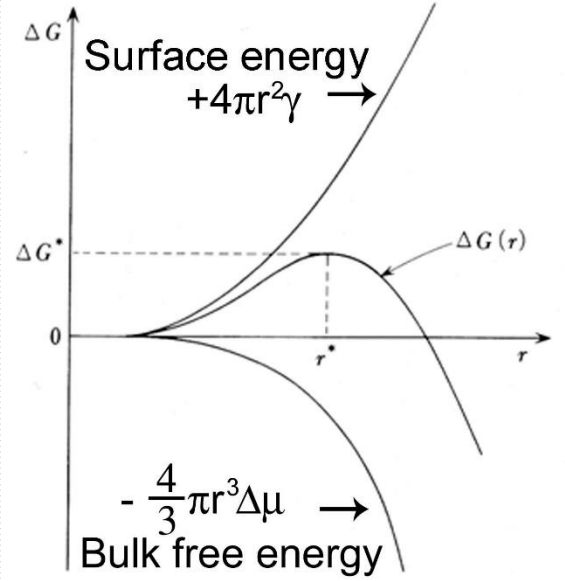


Kimura et al. *Nature Communications*, 4 (2013) 2649.

Steps in Nucleation



Growth unit | Embryo | Nuclei | Nano-particles | Bulk material



Size of critical nuclei "r^*" and changing of Gibbs free energy "ΔG^*" for nucleation

In the classical view, nucleation is a simple process; single growth unit attaches to an *n*-mer to be an (*n*+1)-mer, which process progresses sequentially from a formation of dimer.

Nevertheless, we cannot explain a real process and predict results of crystallization.

Y. Kimura, In: *Nanodust in the Solar System* (2012) 31-46.

Motivation

Nucleation theories give us nucleation rates, J, but having large difference with that by experiments or MD simulations.

$$\frac{J_{theory}}{J_{(experiment\ or\ MD)}} = \text{Several orders } (H_2O, \text{ Methanol}) \text{ to 20 orders } (Ar)$$

Why?

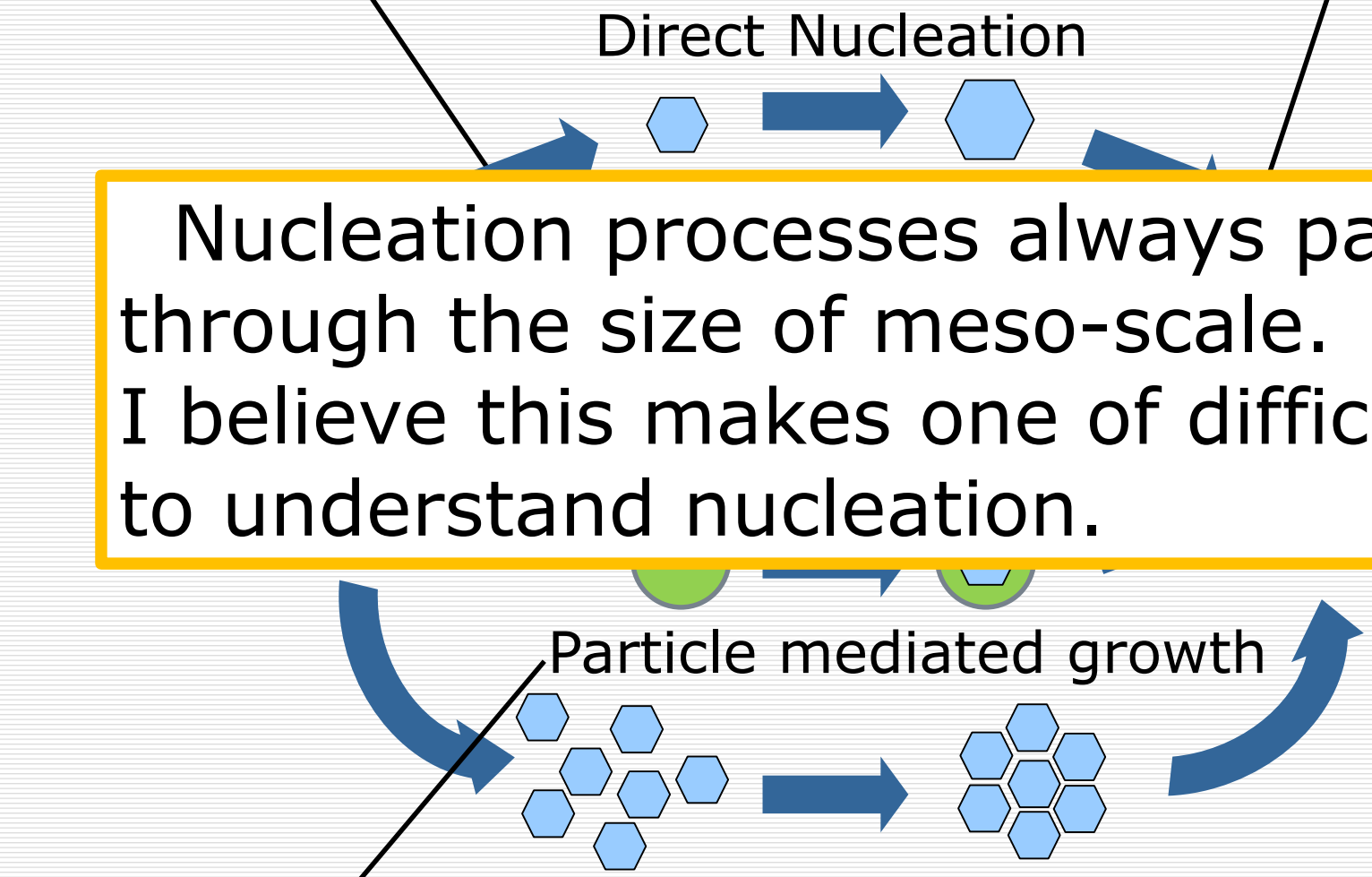
- Limitation of the theories
- Heterogeneous nucleation
- Multistep nucleation

We don't know why nucleation rates are so different.

Steps in early stages of crystallization

by dissolution & precipitation or solid-solid phase transition

from a dense liquid phase or by dehydration of hydrated amorphous particle



Nucleation processes always passes through the size of meso-scale.
I believe this makes one of difficulties to understand nucleation.

Kimura. *Jap. J. Crys. Growth*, 44 (2017) 11.

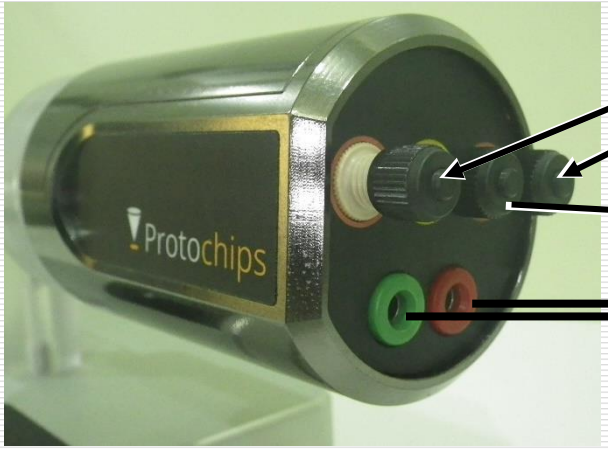
Methods

- Poseidon holder (Protochips Inc.)

EDS Compatible Tip

Liquid Flow

Liquid Heating



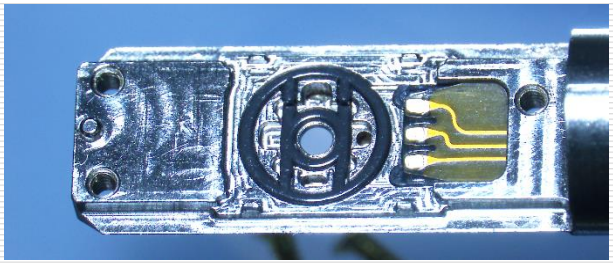
Flow in (PEEK tube、ϕ100 μm)

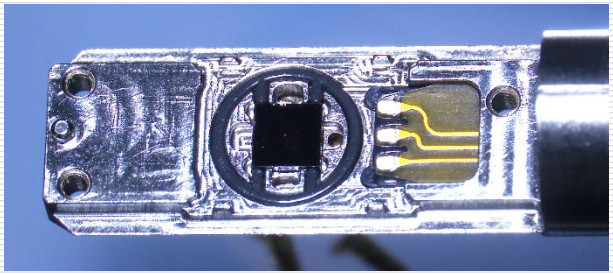
Flow out (PEEK tube、ϕ150 μm)

Electrodes

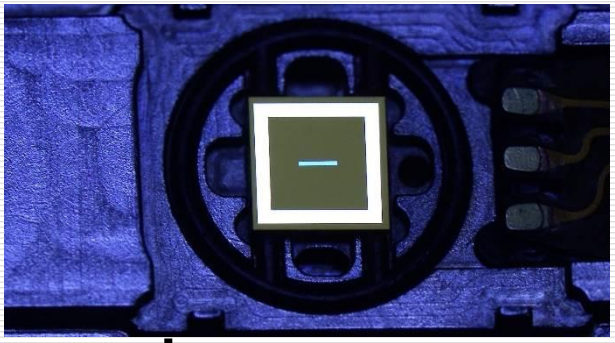
Methods

- Preparation sequences of a liquid cell

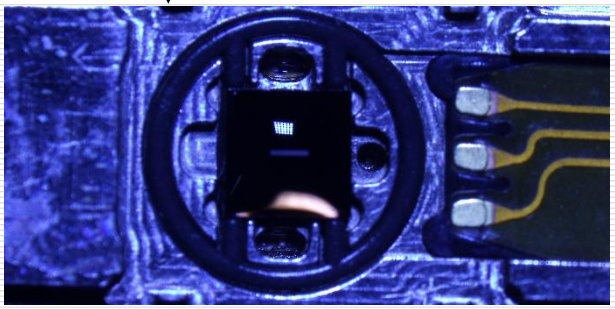


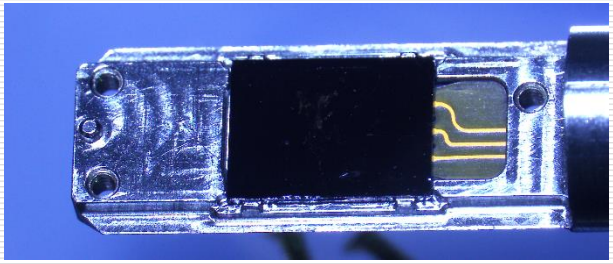


Enlarged, tilted

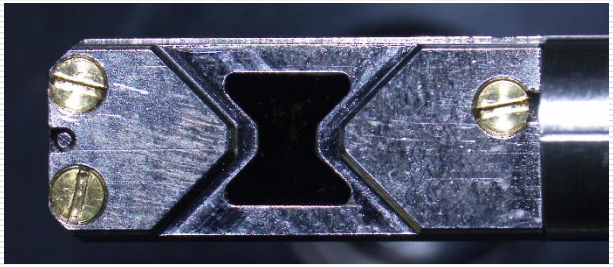


Drop a solution
(~0.5 – 1 μL)

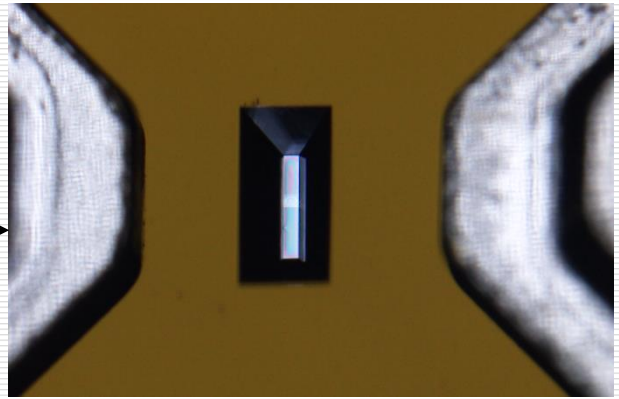




Covered by a
large E-chip

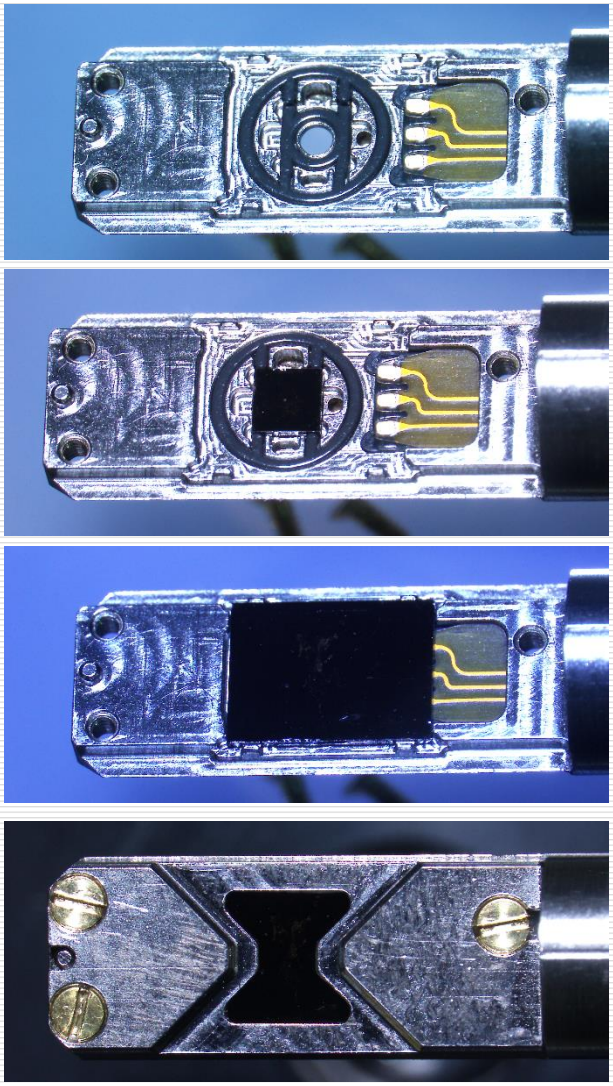


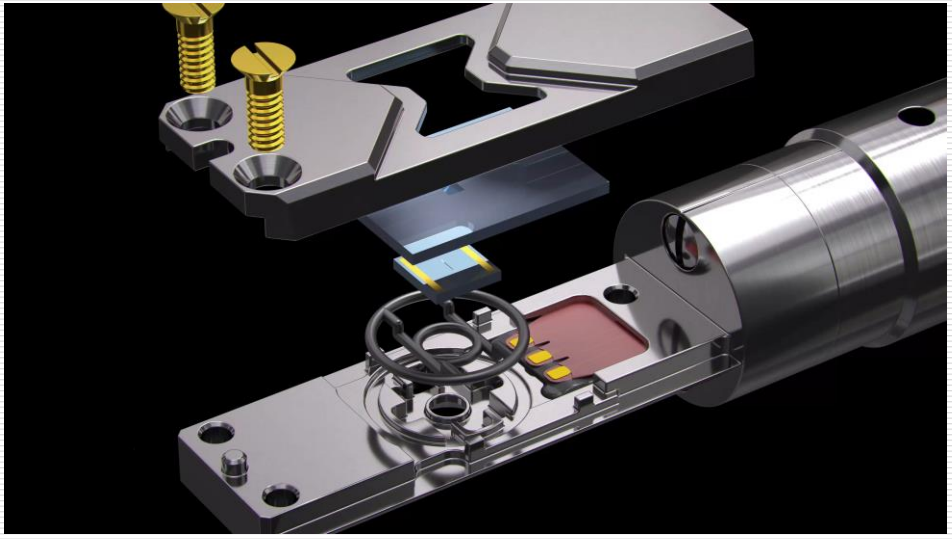
Enlarged, tilted

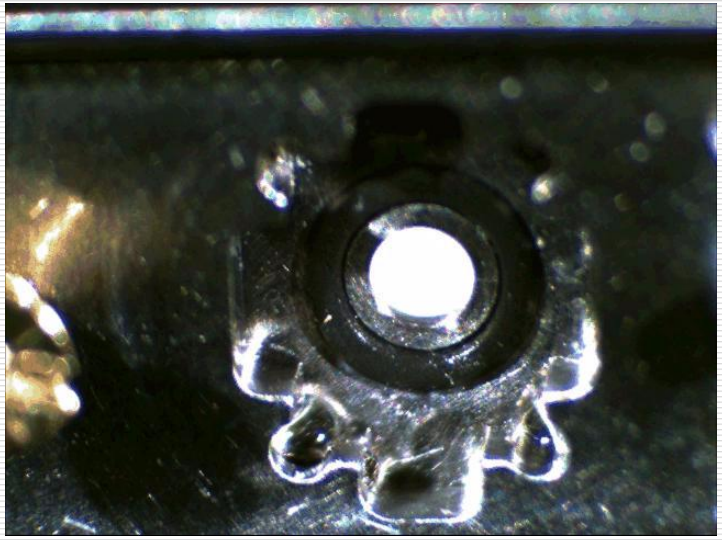


Methods

- Preparation sequences of a liquid cell





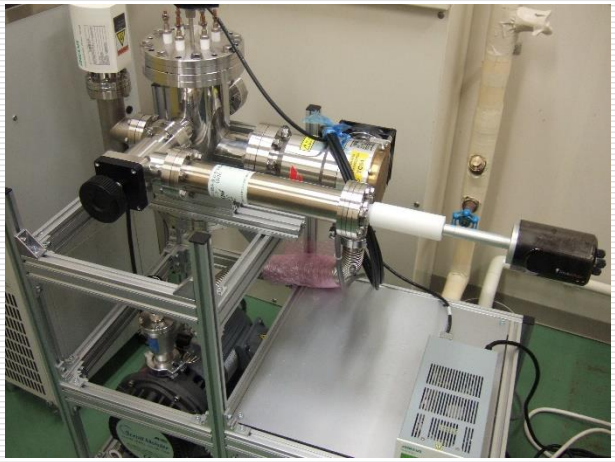


Final amount of a solution in a liquid cell with 500 nm spacer is ~2 nL.

Methods

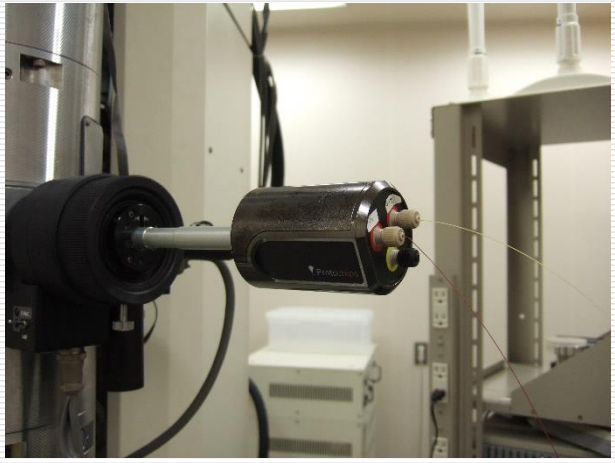
- Preparation sequences of a liquid cell

Check a leakage

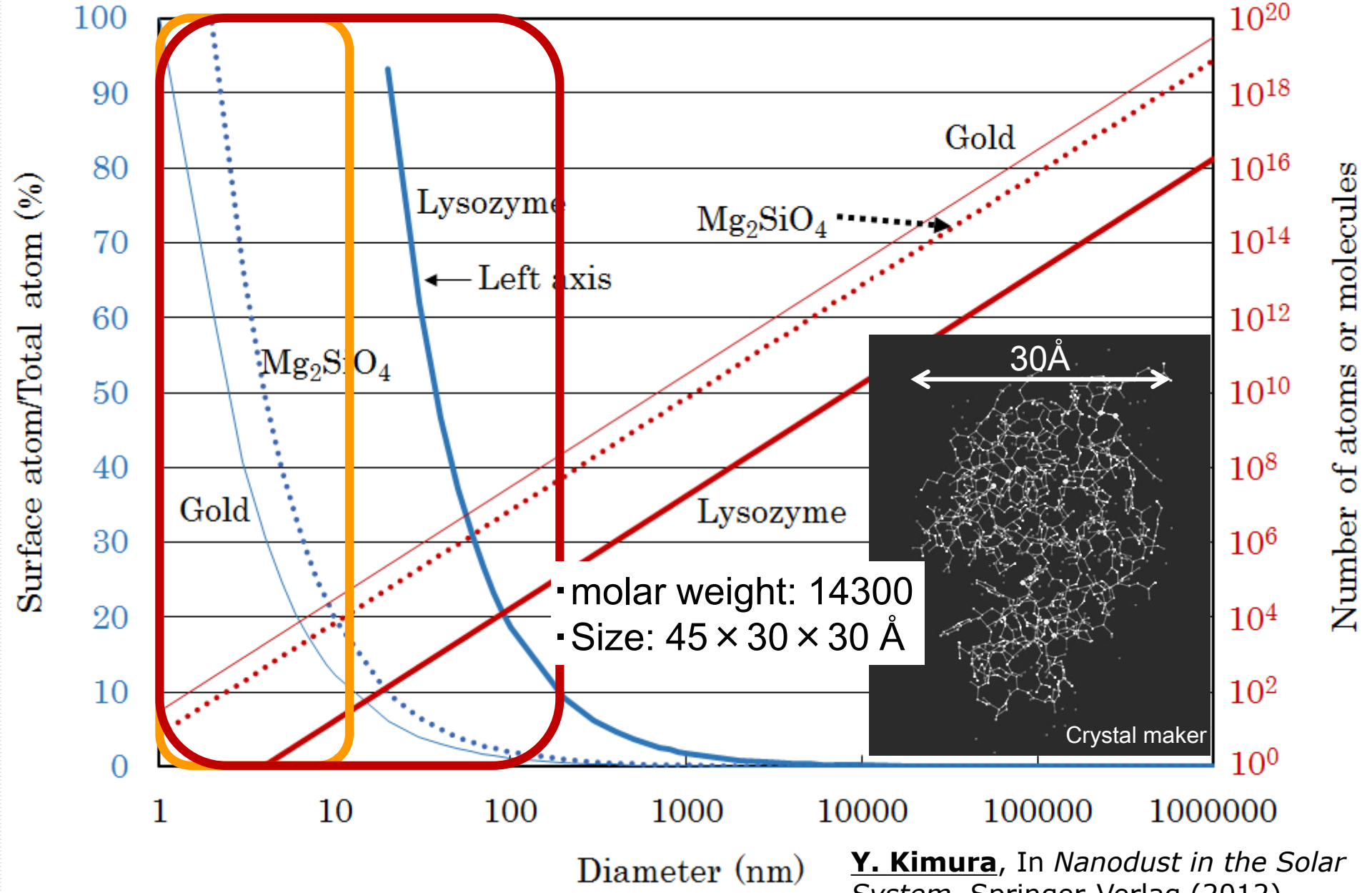


Introduced tinto a TEM

In case of flow, PEEK tube is connectted with a syringe pump



Relation of Size & surface to volume ratio



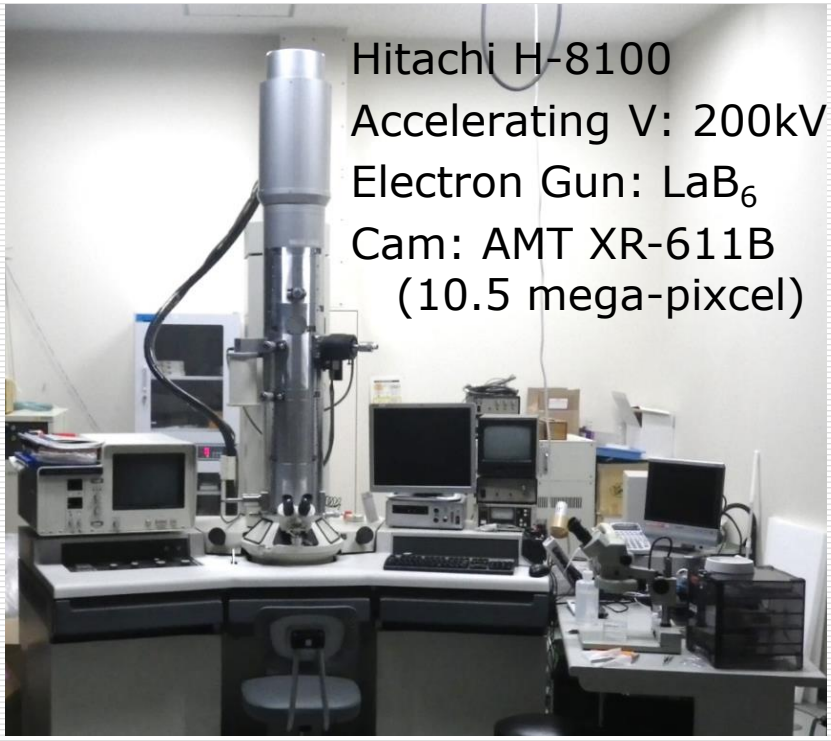
Y. Kimura, In *Nanodust in the Solar System*, Springer-Verlag (2012)

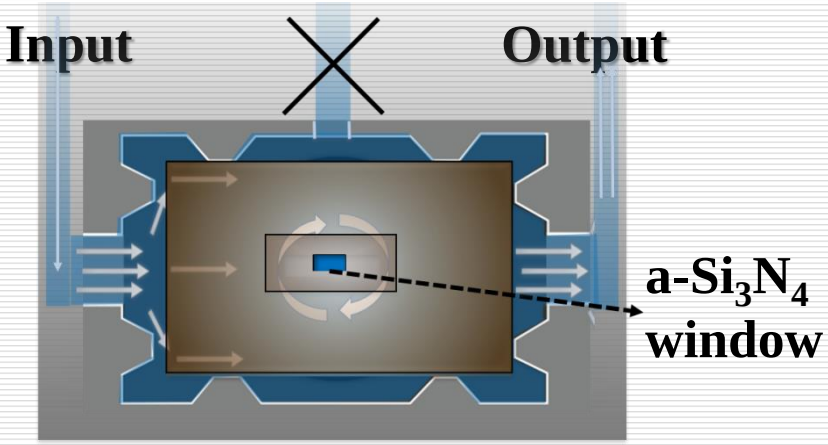
Methods

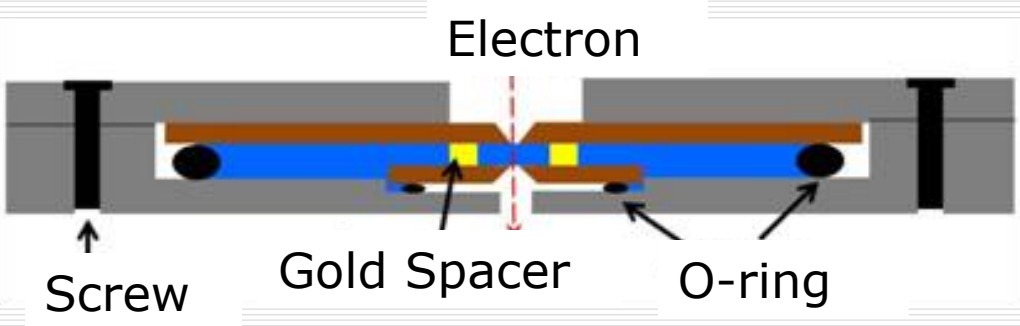
Protein sample: Hen egg white lysozyme
Crystallized using NaCl as a precipitant in sodium acetate buffer solution (pH = 4.5).

Lysozyme 15 [mg ml^{-1}]
NaCl 50 [mg ml^{-1}]

Solution was continuously flowed in the cell.

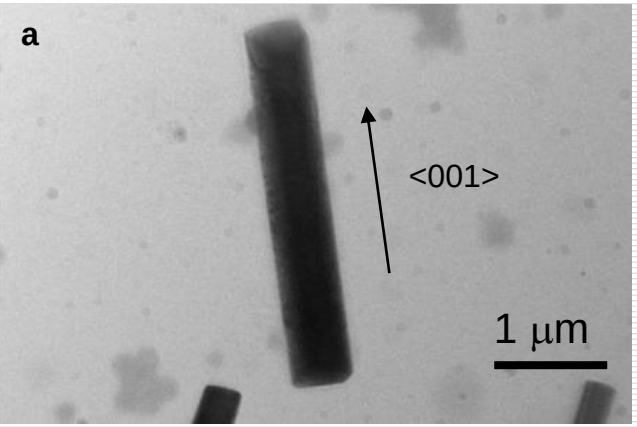


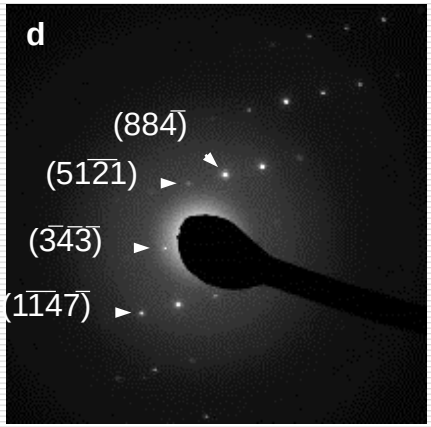




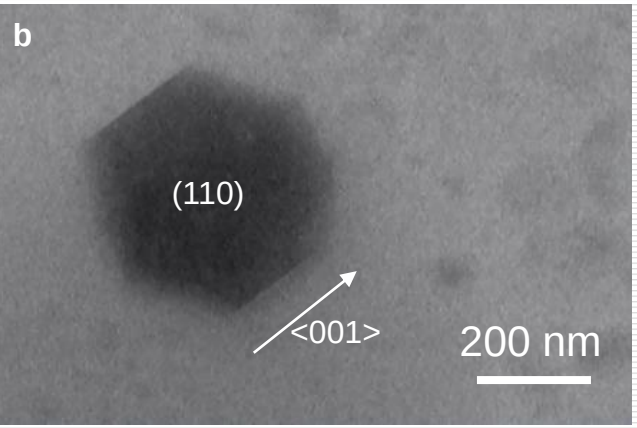
- Thickness of the liquid layer is 150-500 nm.

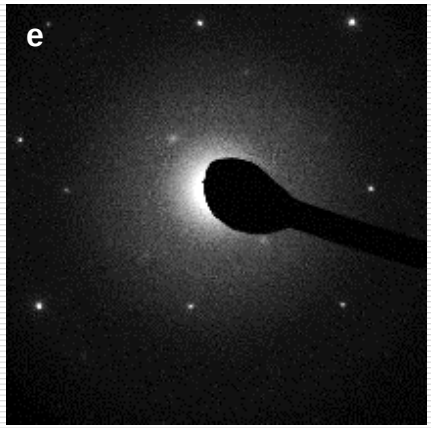
TEM images of protein particles



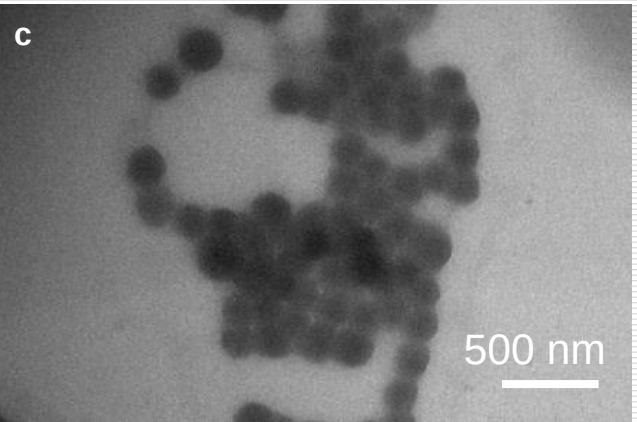


a: An elongated orthorhombic lysozyme crystal. The crystal zone axis is [10 3 –14].





b: A tetragonal lysozyme crystal viewed from its (110) face. The crystal zone axis is [1 1 0].

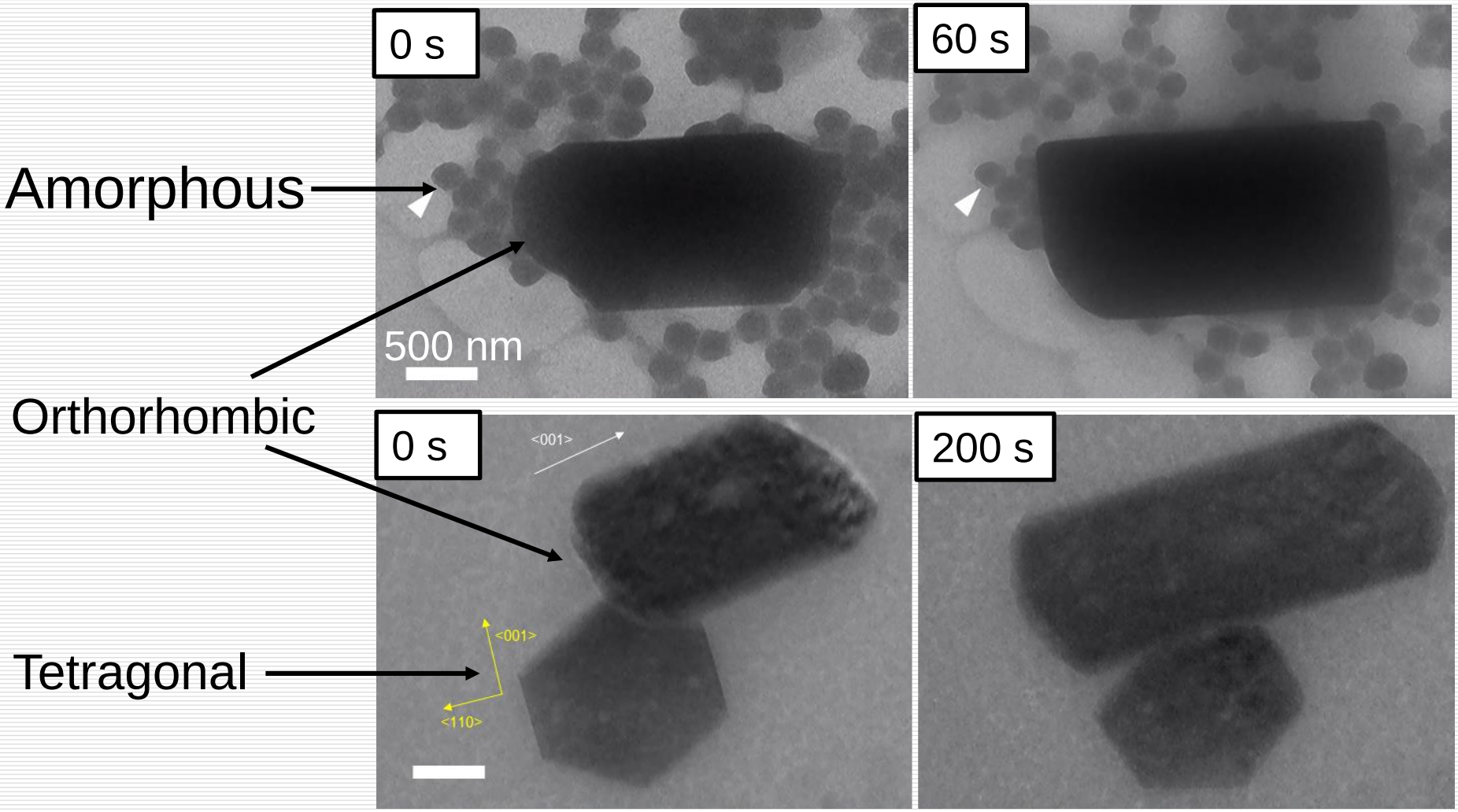


c: Cluster of spherical amorphous particles.

Thickness of E-chips spacer: 500 nm

Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

Growth process of orthorhombic crystal

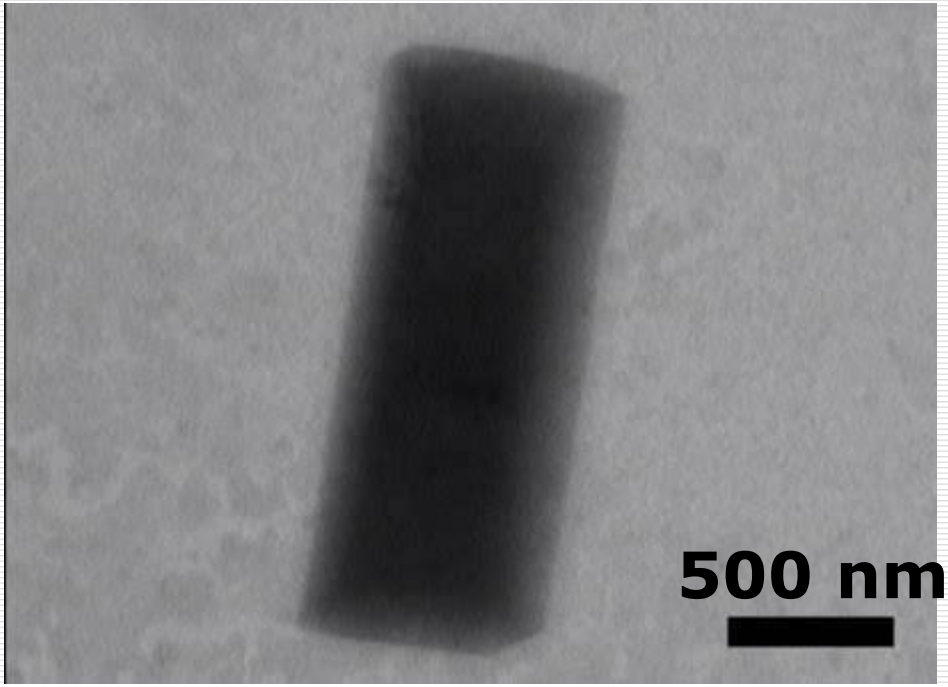


Amorphous and tetragonal particles are incorporated into most stable orthorhombic crystals via dissolution rather than attachment or fusion.

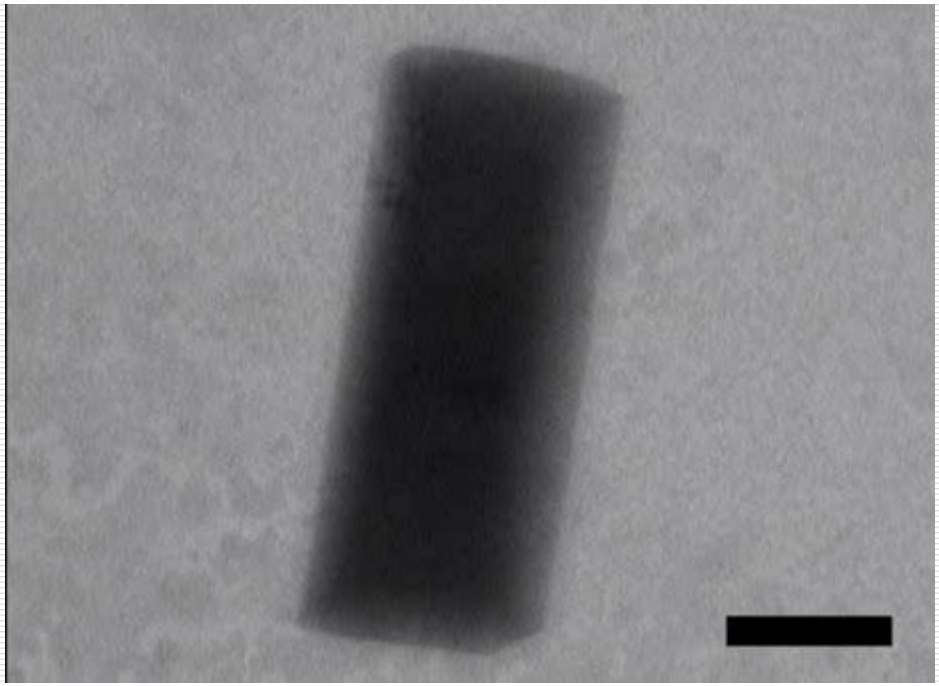
Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

In-situ observation of growing crystal

Initial



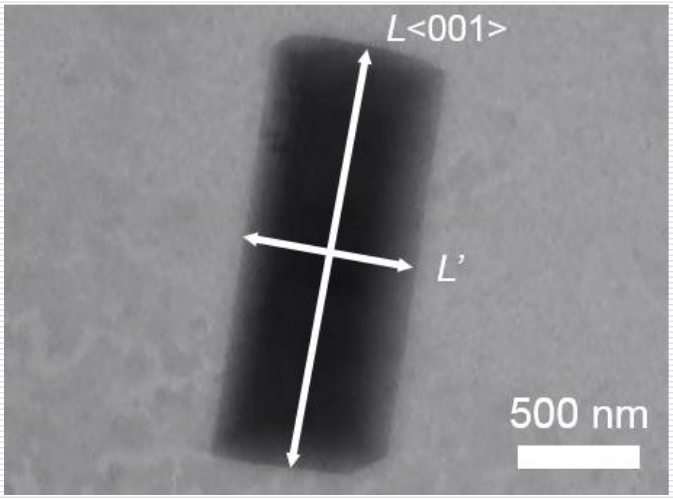
Video

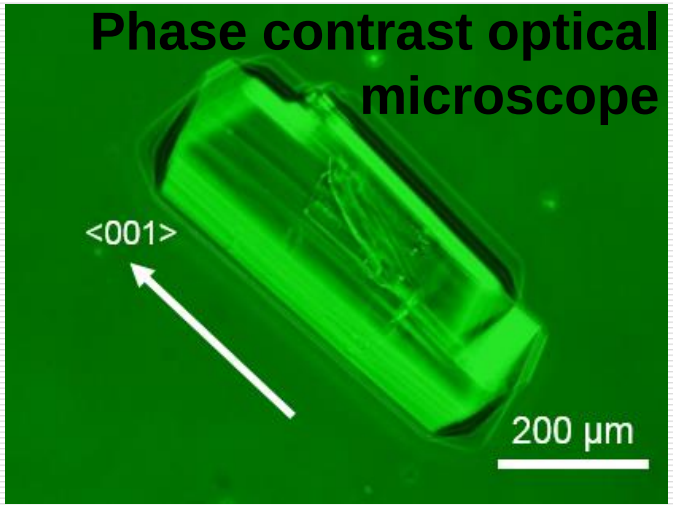


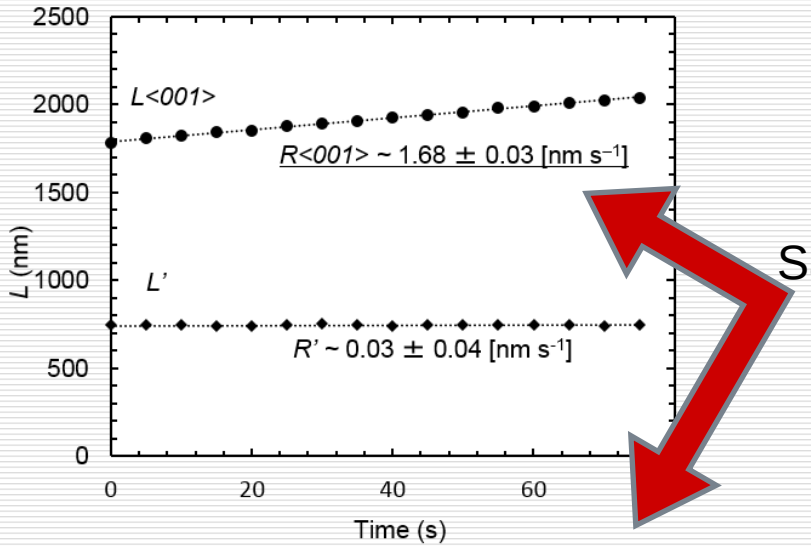
Very small growth rates (~1-2 nm/s) of the growing crystal can be determined in relatively shorter time (<10 s).
In-situ TEM observation of an orthorhombic lysozyme crystal. Play speed of the video is 5 times faster.

Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

Comparison of growth rates at 24°C

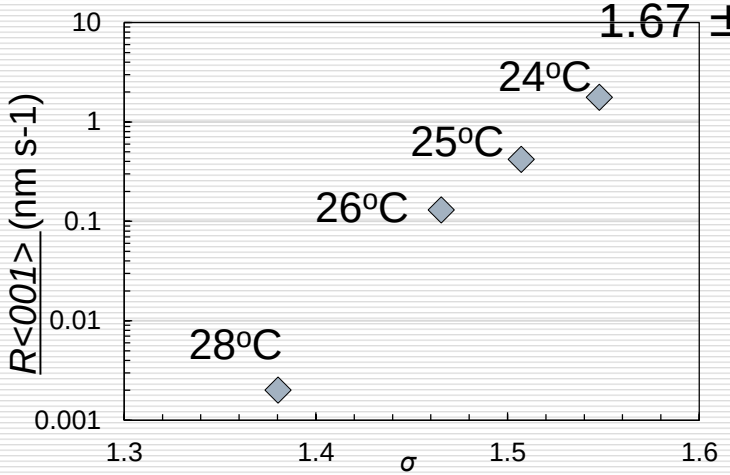






Same value

1.67 ± 0.15 nm s^{-1}

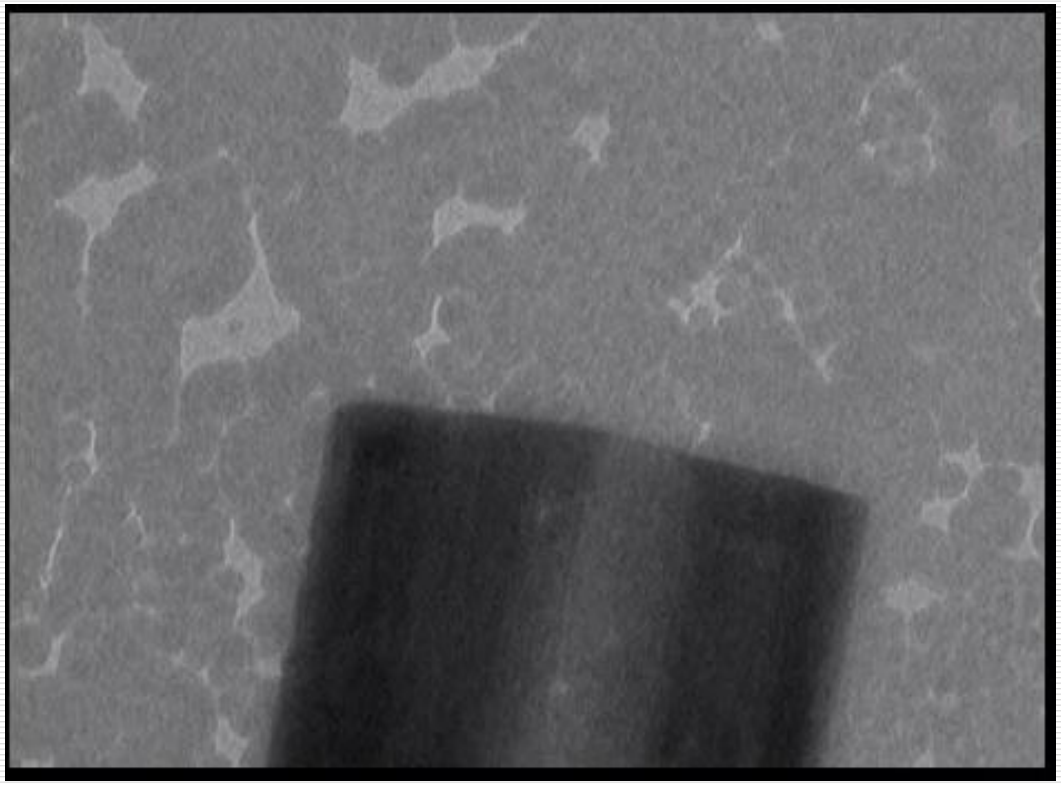


Growth rates of lysozyme crystals as a function of supersaturation $\sigma = \ln(C/Ce)$ (C: lysozyme concentration, Ce: solubility at certain temperature) measured under optical microscopy. There is the threshold of electron dose not to affect the crystallization.

Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

Dissolution of a lysozyme crystal

- Increasing flux of electron



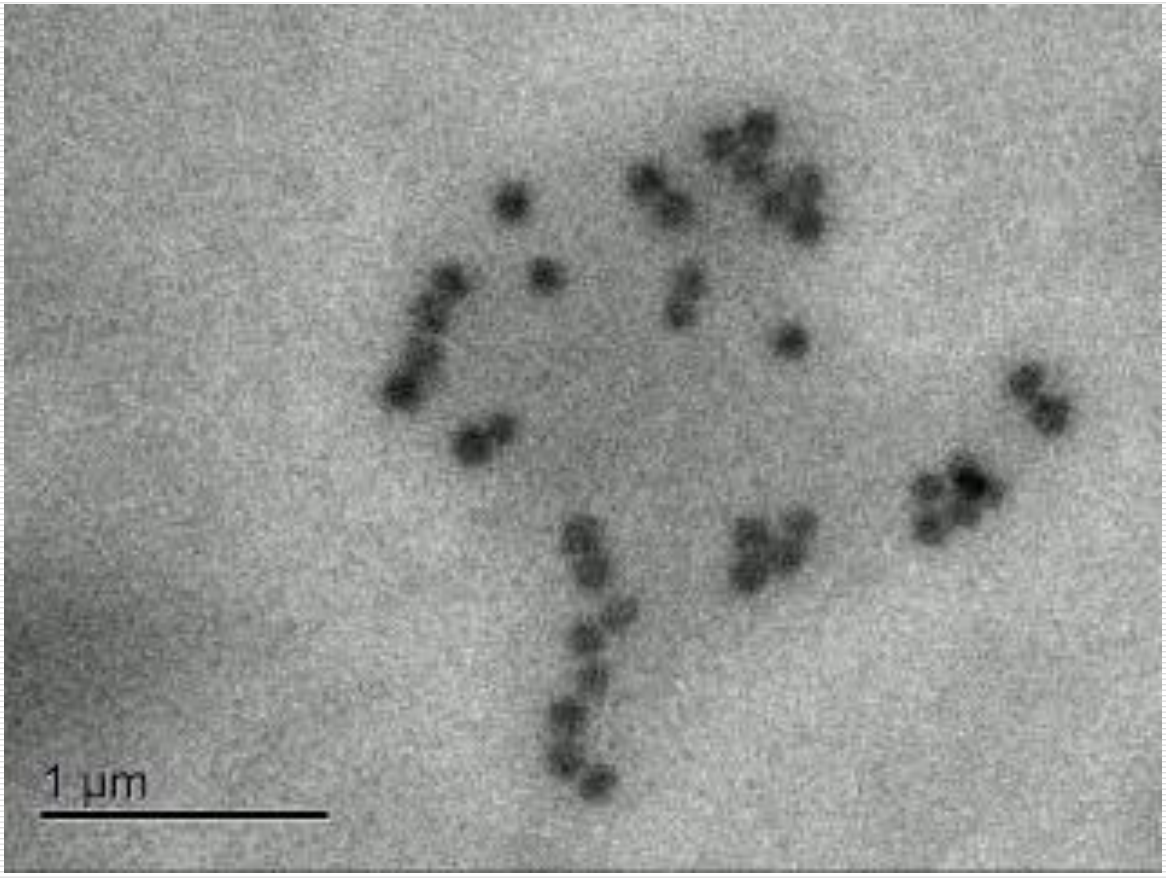
10× faster

Sample : Orthorhombic lysozyme crystal
E-chips : Flow
Spacer : 500 nm
Flow rate : 2 μL/min
TEM : H-8100
Acc. V : 200 kV
E. Gun : LaB_6

There is a threshold of electron flux, which can be neglected to discuss crystallization of lysozyme protein

Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

At the moments of crystallization



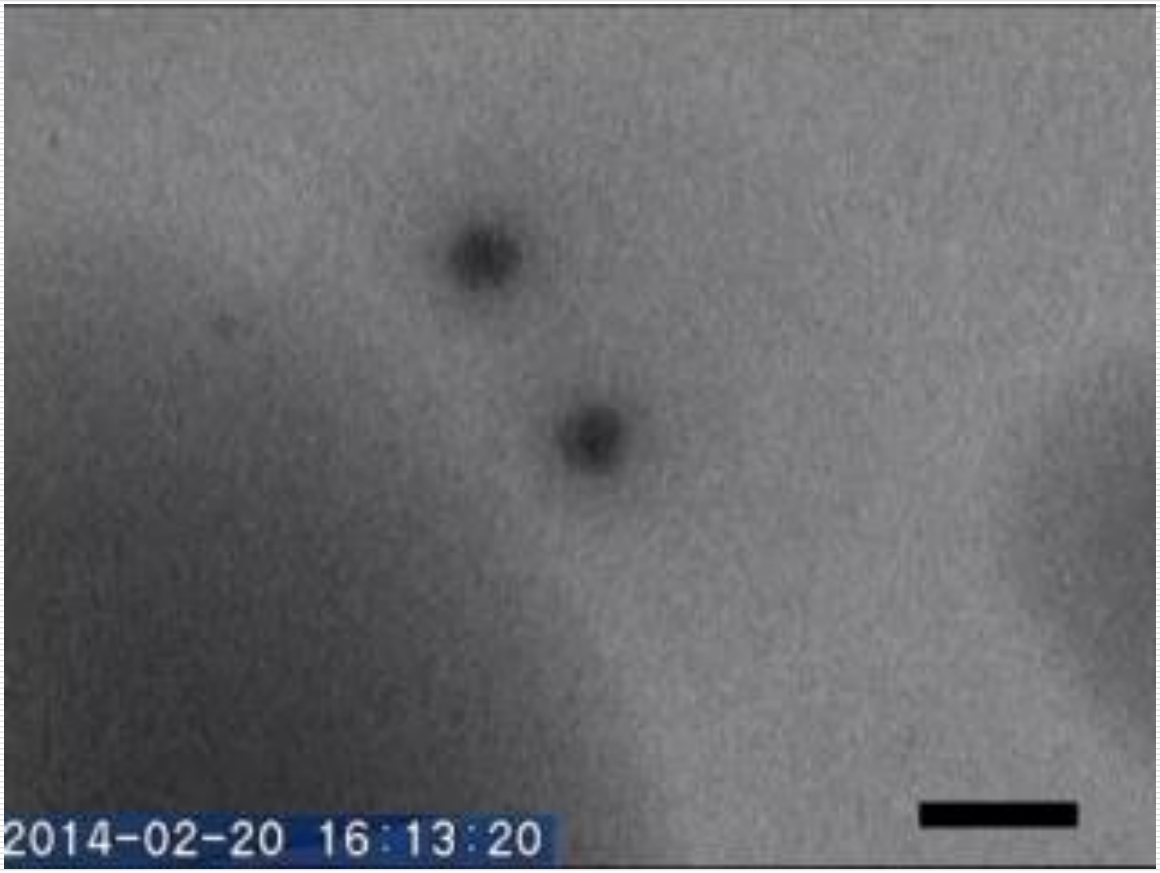
Real time

Sample : Lysozyme crystal
E-chips : Flow
Spacer : 150 nm
Flow rate : 2 μL/min
TEM : HF-3300
Acc. V : 300 kV
E. Gun : Field emission

Orthorhombic lysozyme crystal nucleated separately from the amorphous particles. The amorphous particles did not transferred into a crystal.

Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

At the moments of crystallization



2× faster

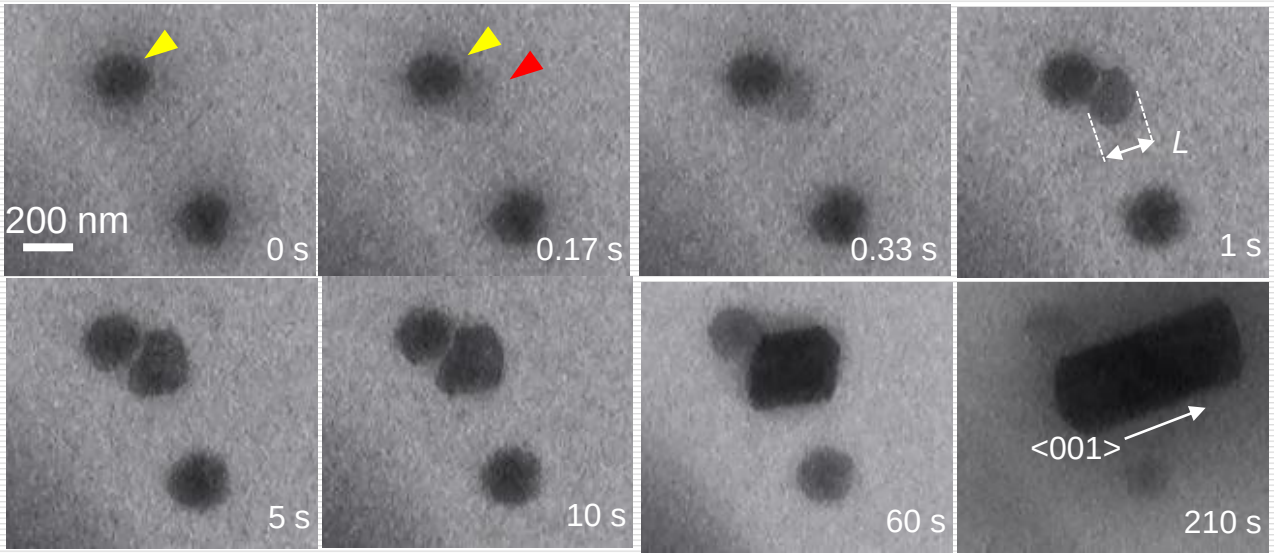
Sample : Lysozyme crystal
E-chips : Flow
Spacer : 500 nm
Flow rate : 2 μL/min
TEM : H-8100
Acc. V : 200 kV
E. Gun : LaB_6

Scale bar: 500 nm

Orthorhombic lysozyme crystal nucleated on the amorphous particle.
The amorphous particles did not incorporated into the crystal.

Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

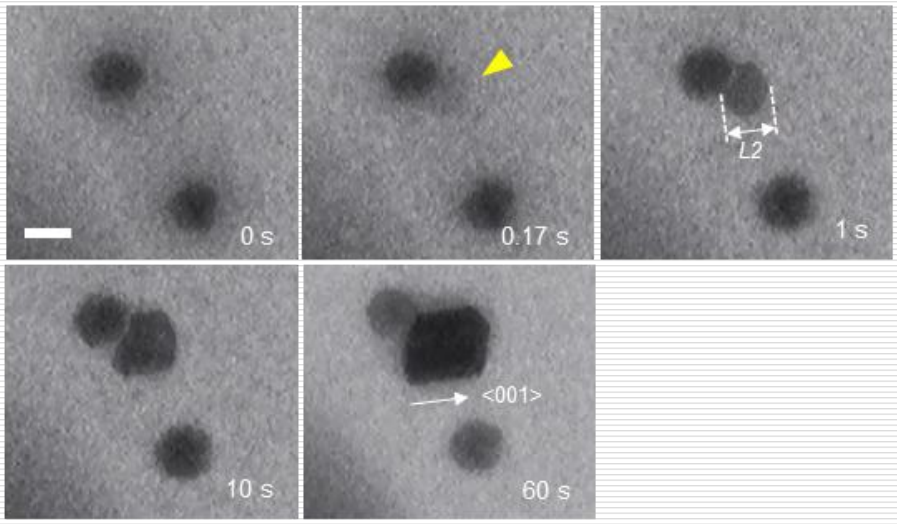
Crystallization of protein (Pattern A)

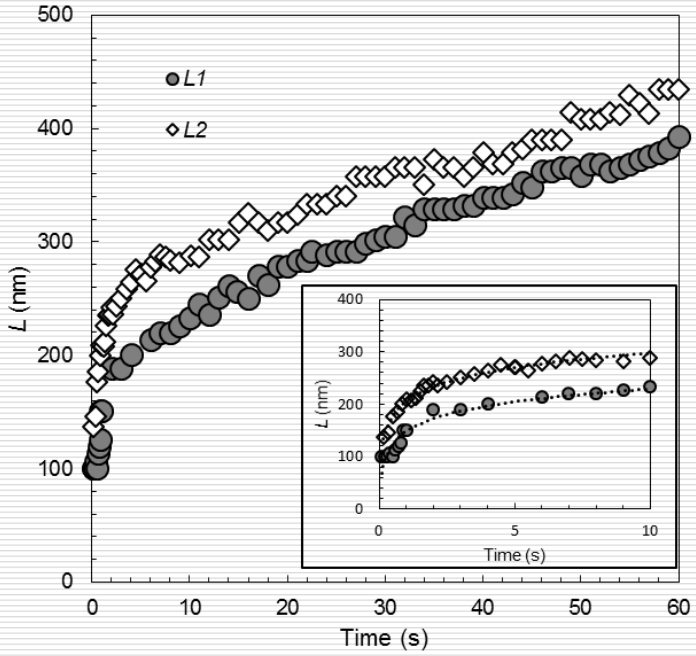


Still micrographs of the nucleation processes as observed by TEM. There are two amorphous particles at 0 s. The yellow triangles indicate a pre-existing amorphous particle. The red triangle shows a spherical particle that nucleated at 0.17 s near the amorphous particle indicated by the yellow triangle. This nucleated particle grew and transformed into an orthorhombic lysozyme crystal.

Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

Nucleation process of lysozyme crystals

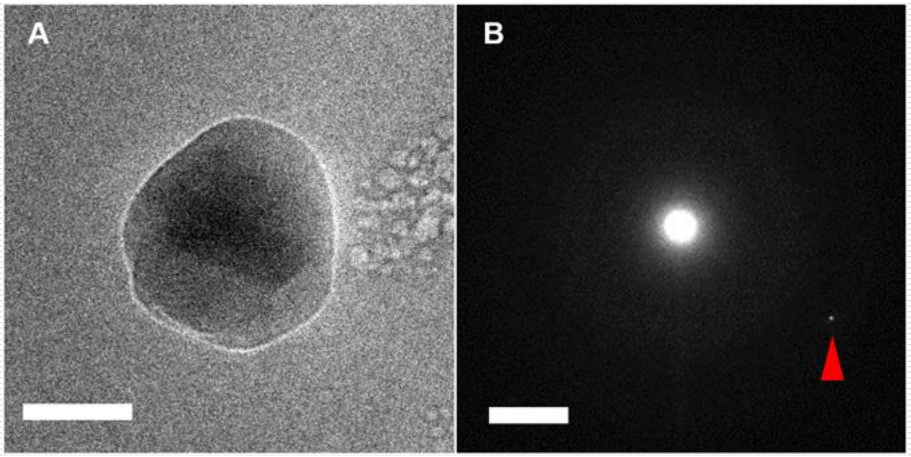




The role of amorphous phases in the nucleation of orthorhombic lysozyme crystals. (**Left**) Time resolved *in situ* TEM images. A spherical cluster, indicated with a yellow arrowhead, forms at 0.17 s near an amorphous solid particle (ASP) and transforms into an orthorhombic crystal. The scale bar is 200 nm.
(**Right**) The size evolutions of particles reveal decreasing, on the average, growth rates. (Inset) Zoom-in of times 0 – 10 s. Dotted lines are logarithmic fits to each data set.

Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

Nucleation process of lysozyme crystals



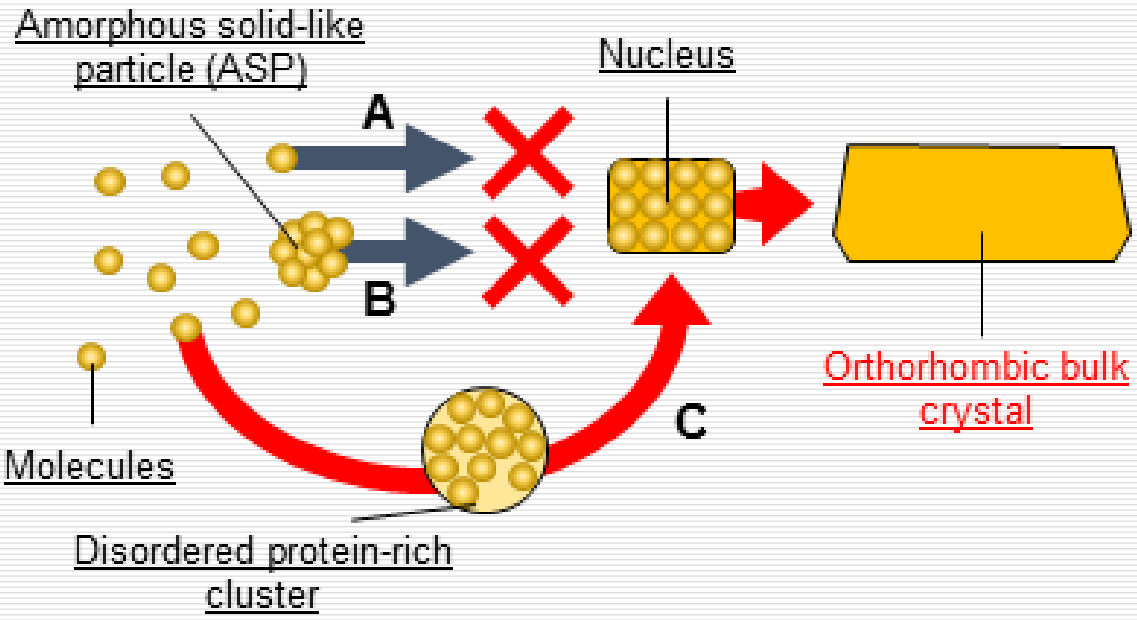
Frozen particle with a crystal in its center observed by cryo-TEM.

(A) The particle has a different contrast than the faceted faces, indicating a crystalline structure. (Scale bar: 50 nm)

(B) The corresponding diffraction pattern of the particle shows a Laue spot (indicated by a triangle). (Scale bar: 2 nm^{-1})

Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

Nucleation process of lysozyme crystals



Schematic of the nucleation pathway of lysozyme crystals. Crystals are not directly nucleated by assembly of molecules from the bulk solution (**A**) or transformation of the ASP (**B**). Instead, orthorhombic crystal nucleate within disordered protein-rich clusters (**C**), which heterogeneously nucleate on the surface of the ASPs or the container walls.

Yamazaki, **Kimura** *et al.*, *PNAS* 114 (2017) 2154.

Summary

- Competition is an actual event before nucleation.
- Amorphous protein particles with 150-200 nm in size is not dense liquid, but solid, and not precursor of lysozyme crystal.
- Lysozyme crystals seem to be nucleated via dense liquid phase.
- Even in protein, physical properties of nanoparticles may play important roles in nucleation processes.

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