Time-resolved Cryo-EM

Jack Fu
Joachim Frank's lab
Columbia University

Questions to address?

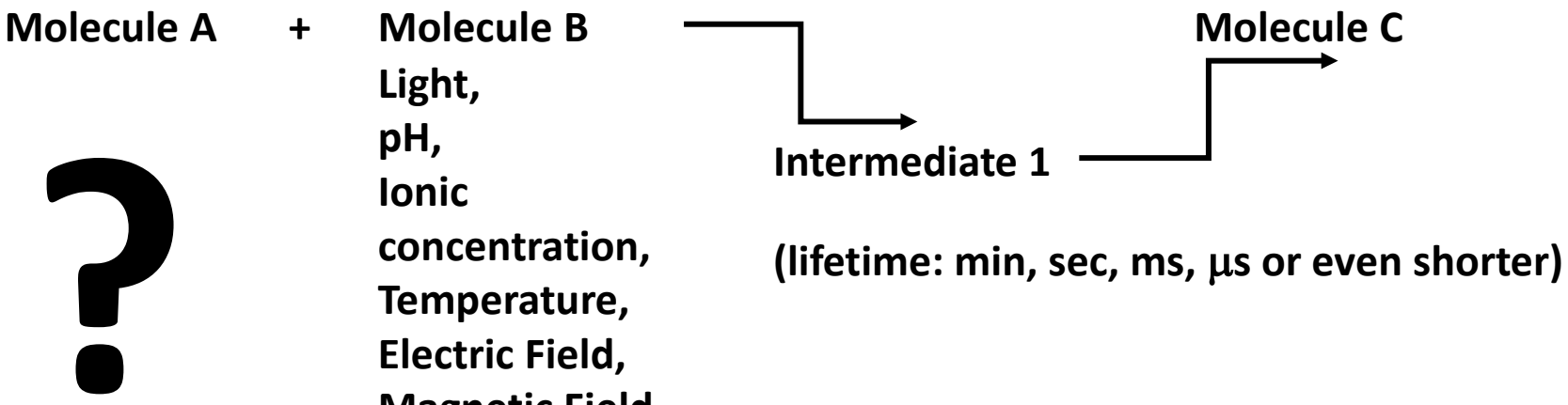
- ~~How can time-resolved cryo-EM help you in your research?~~
- **We need your help.**
- ~~What are the obstacles to success?~~
- **There are a lot of issues in time-resolved Cryo-EM method.**

Time-resolved cryo-electron microscopy

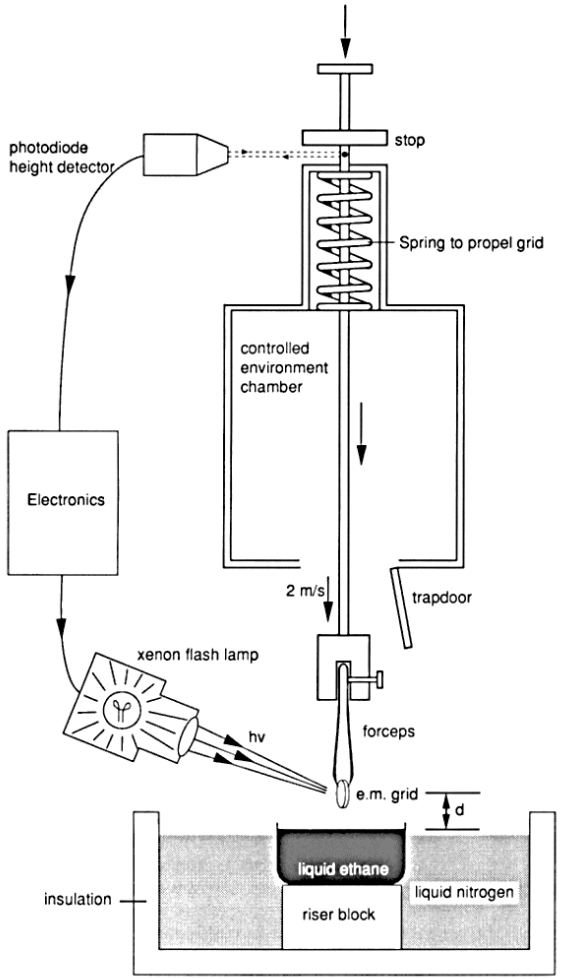
- Time-resolved cryo-electron microscopy (cryo-EM) combines the known advantages of **single-particle cryo-EM in visualizing molecular structure** with the ability **to dissect the time progress of a reaction between molecules in vitro.**

Time-resolved cryo-electron microscopy

- Time-resolved cryo-electron microscopy (cryo-EM) combines the known advantages of **single-particle cryo-EM in visualizing molecular structure** with the ability **to dissect the time progress of a reaction between molecules in vitro.**



What has been tested?



The EMBO Journal vol.12 no.1 pp.1–8, 1993

Electron diffraction analysis of structural changes in the photocycle of bacteriorhodopsin

Sriram Subramaniam[1,2], Mark Gerstein[1], Dieter Oesterhelt[3] and Richard Henderson[1]

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Communicated by R.Henderson

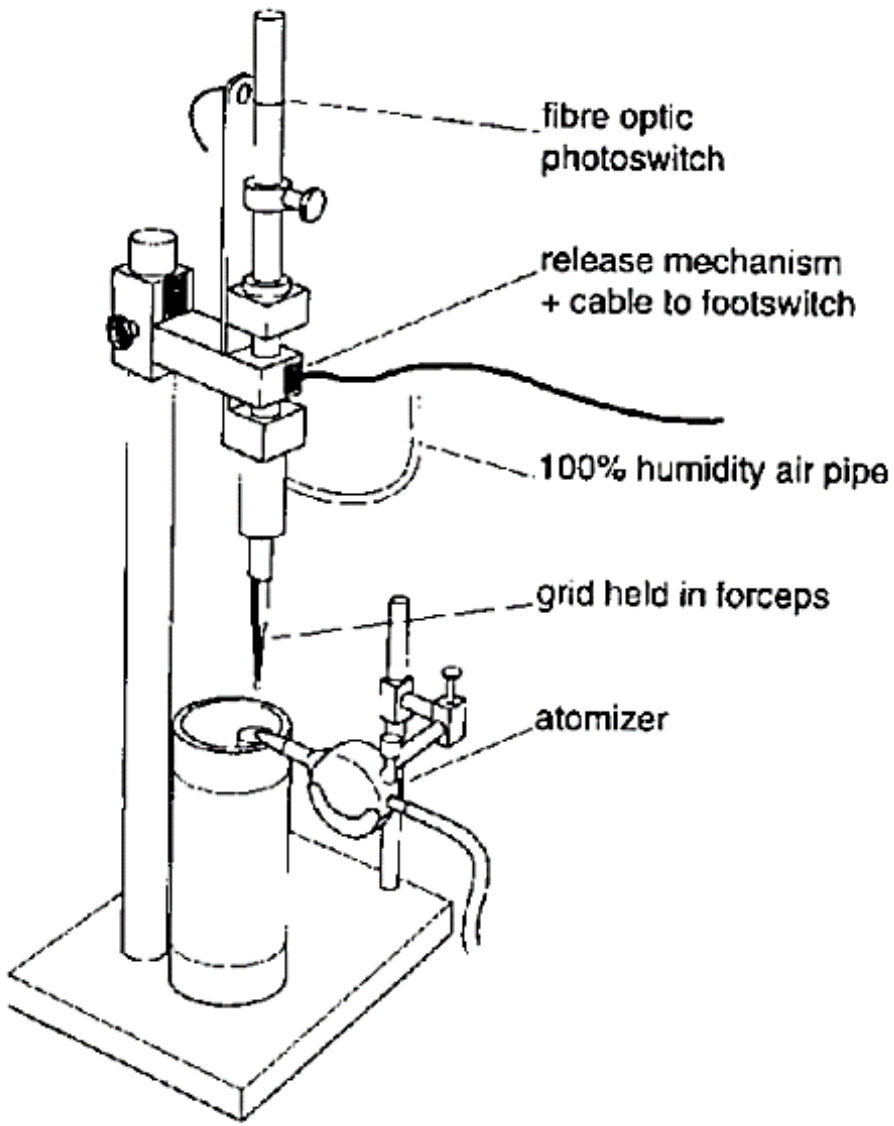
Analysis of transient structures by cryo-microscopy combined with rapid mixing of spray droplets

John Berriman, Nigel Unwin

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK

Received 15 June 1994

a



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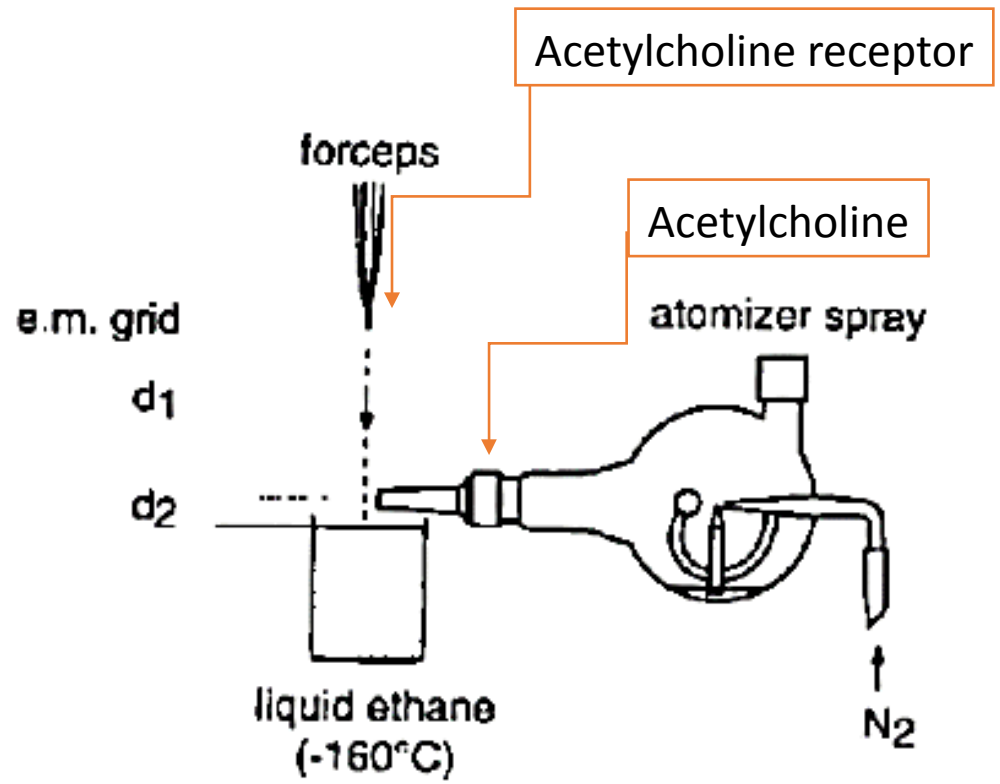

Biophysical Journal Volume 68 April 1995 87s–91s

Millisecond Time Resolution Electron Cryo-microscopy of the M-ATP Transient Kinetic State of the Acto-Myosin ATPase

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JOURNAL OF STRUCTURAL BIOLOGY **121,** 306–313 (1998)
ARTICLE NO. SB983968

A Computer-Controlled Spraying-Freezing Apparatus for Millisecond Time-Resolution Electron Cryomicroscopy

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Received November 18, 1997, and in revised form January 26, 1998

Limitations in the spraying-freezing method

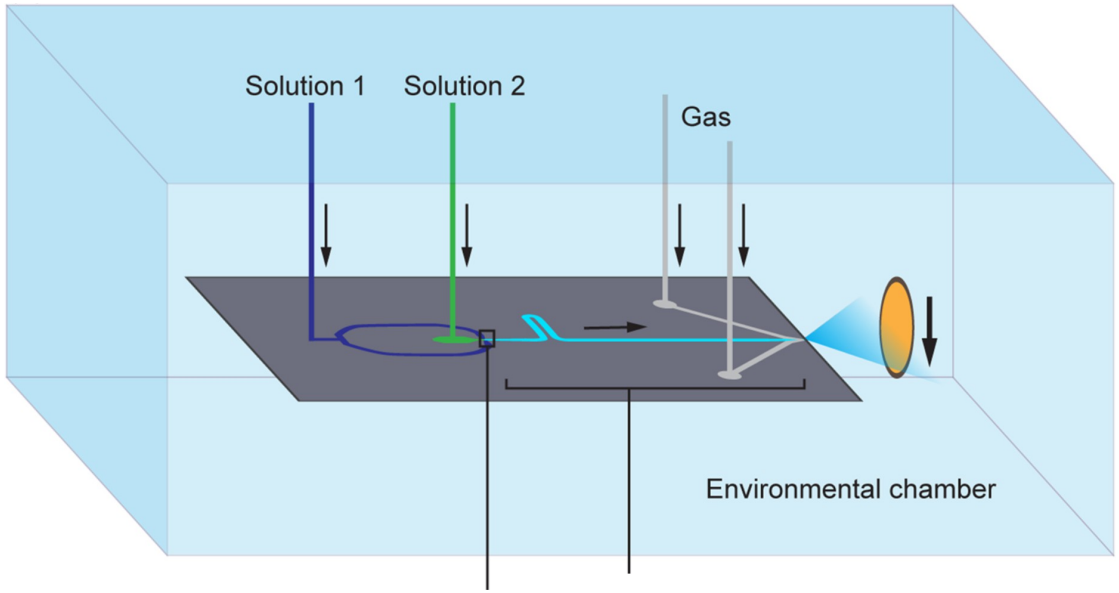
Molecule A	**+**	**Molecule B**
On Cryo-EM grid	**+**	Small molecule Acetylcholine/ ATP Marker to identify the droplets

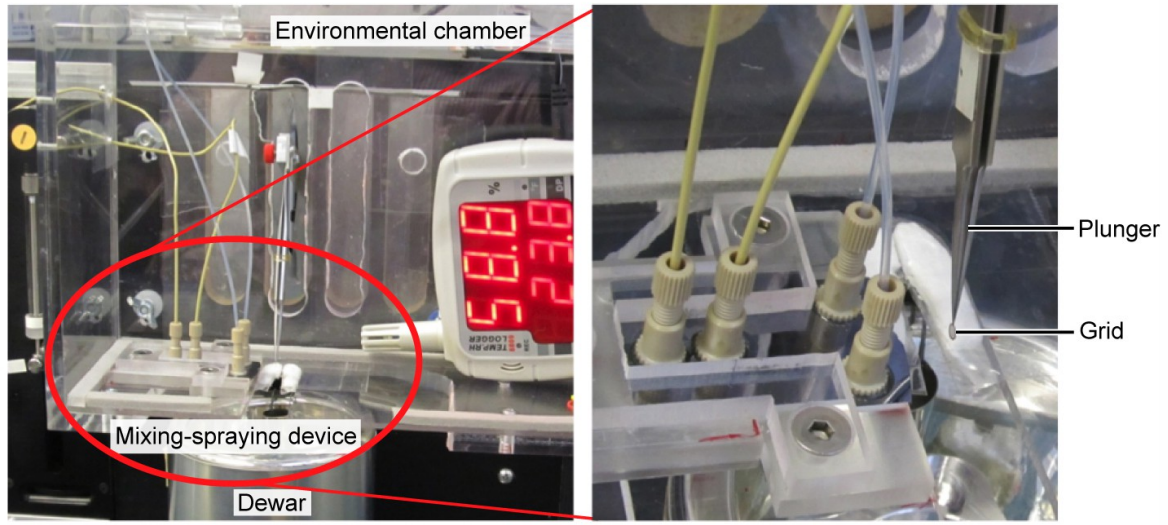
The mixing is dependent on diffusion

1. slow
2. dependence on molecular weight

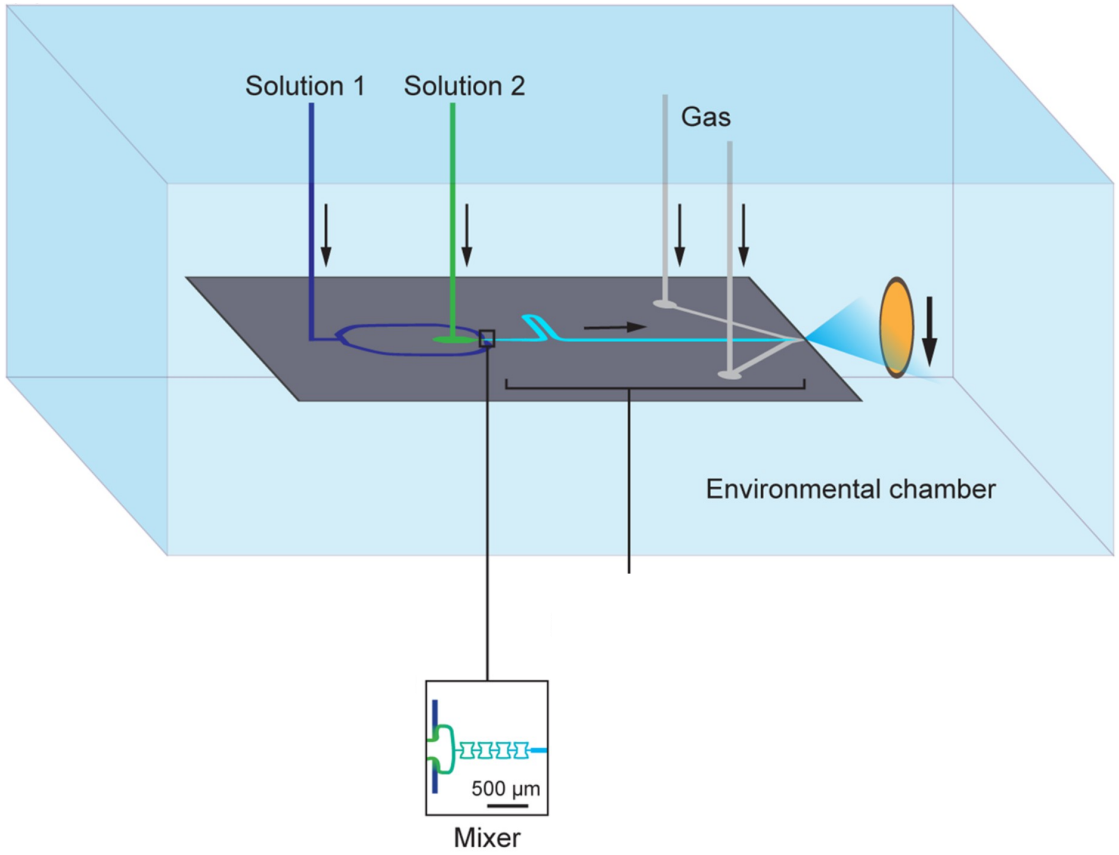
Our solution is the mixing-spraying-freezing method

Experimental setup – Microfluidic chip





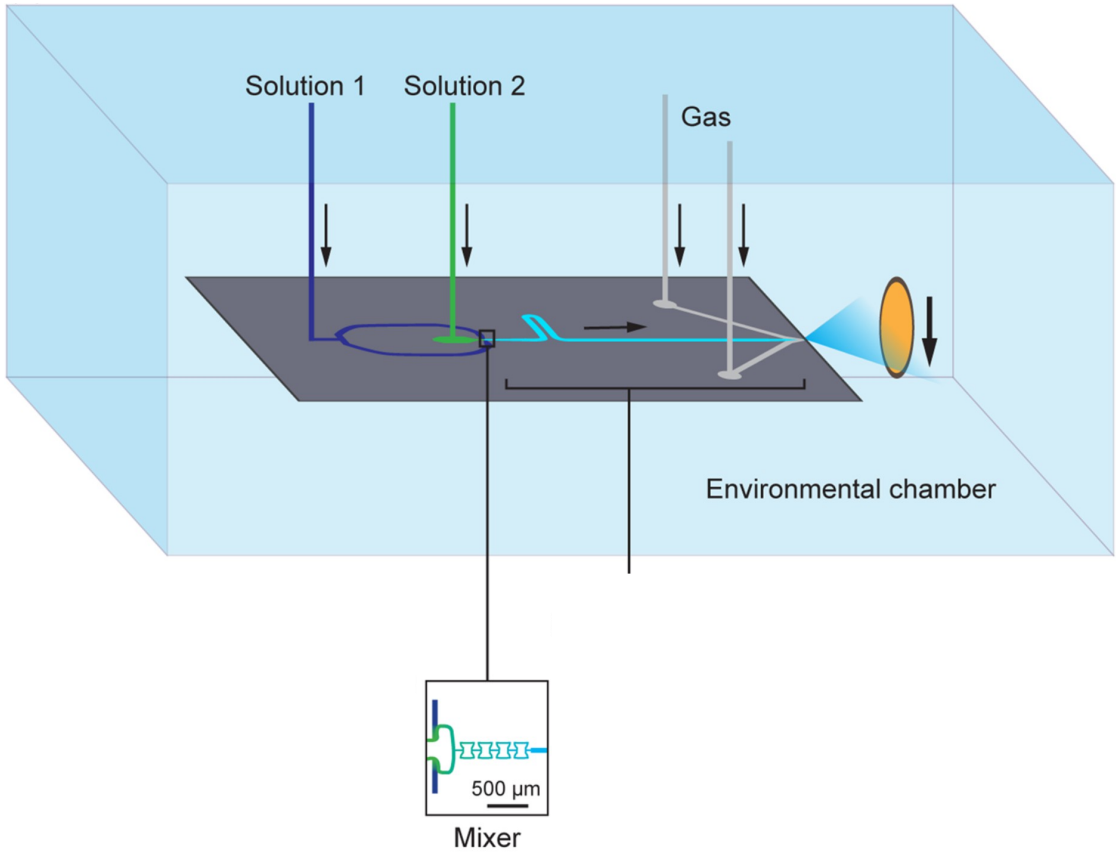
Experimental setup – Mixer performance



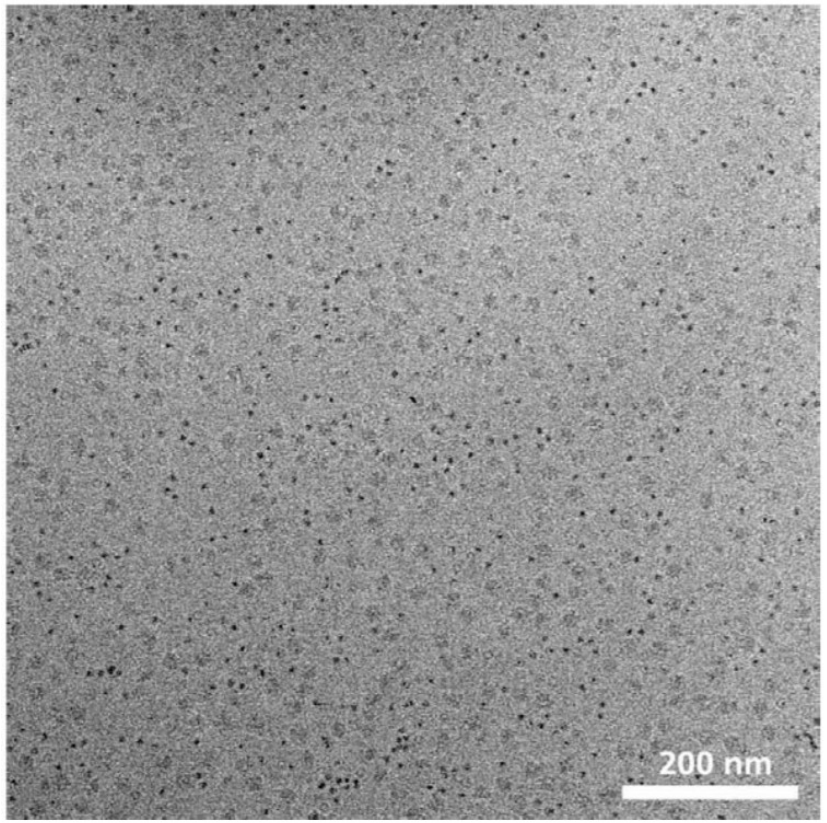
Not mixed well Flow rate: 1µL/s

Very well mixed Flow rate: 6 µL/s

Experimental setup – Mixer performance

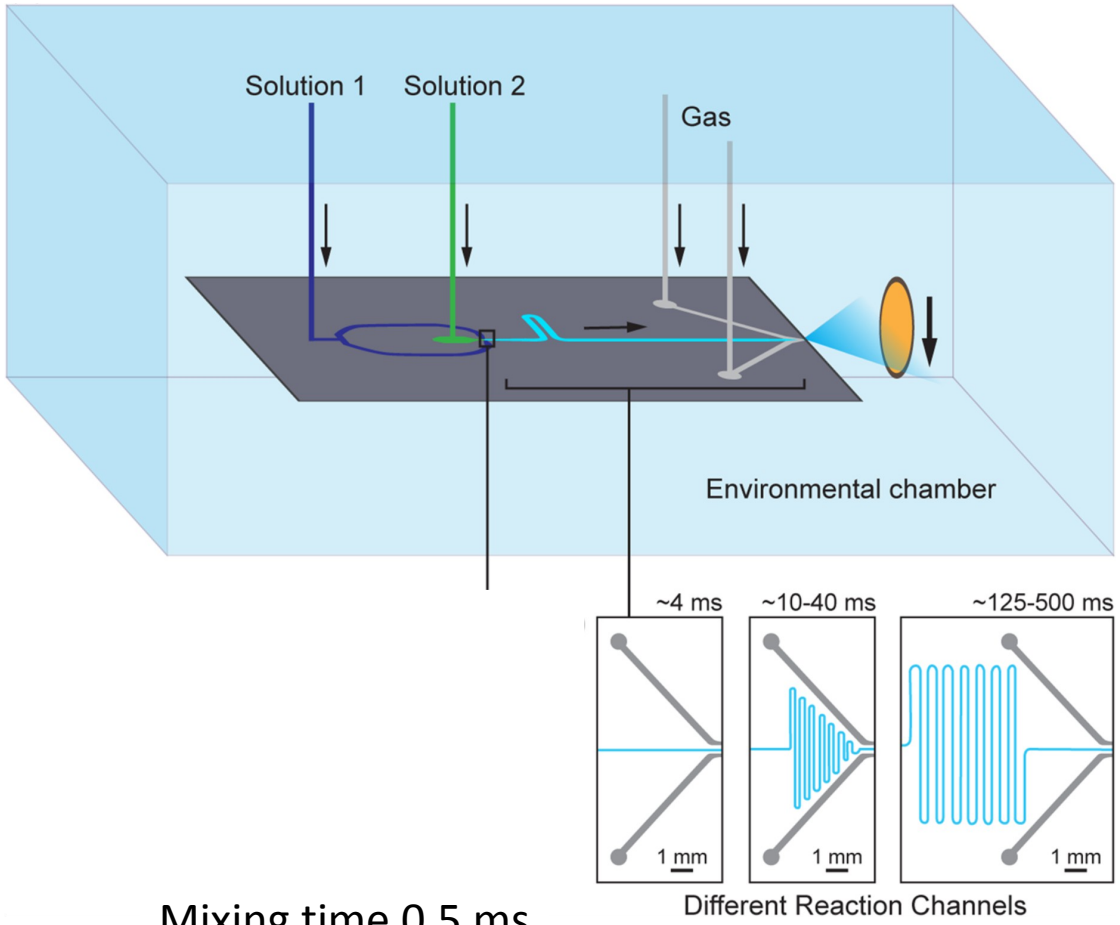


Mixing time 0.5 ms



Mixing 70S ribosomes and Ferritin molecules

Experimental setup – Reaction time

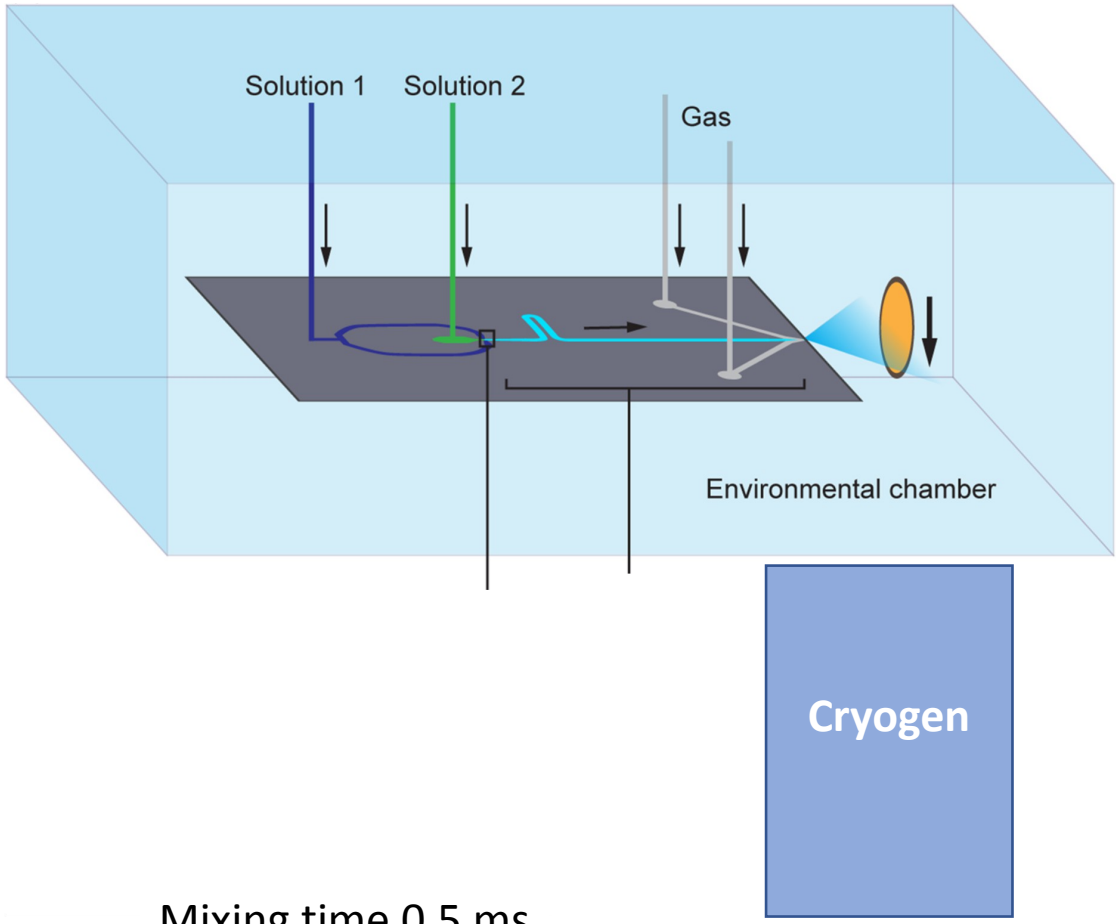


Length of the reaction channels and **the flow rate** determine the reaction time in the microfluidic chips.

Mixing time 0.5 ms

Reaction time 4-500 ms

Experimental setup – Plunging and freezing



Reaction is stopped by plunging into cryogen.

Mixing time 0.5 ms

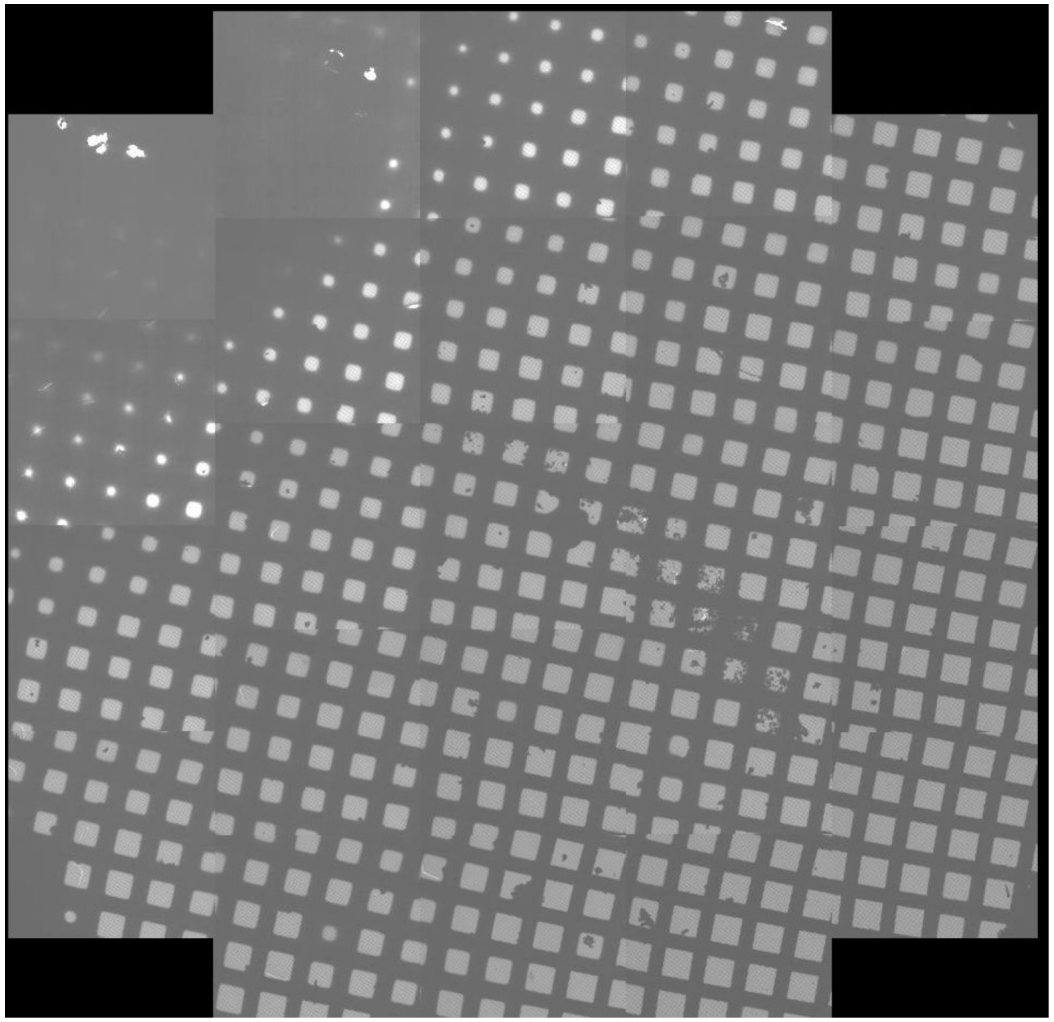
Reaction time 4-500 ms

Plunging time 18 ms

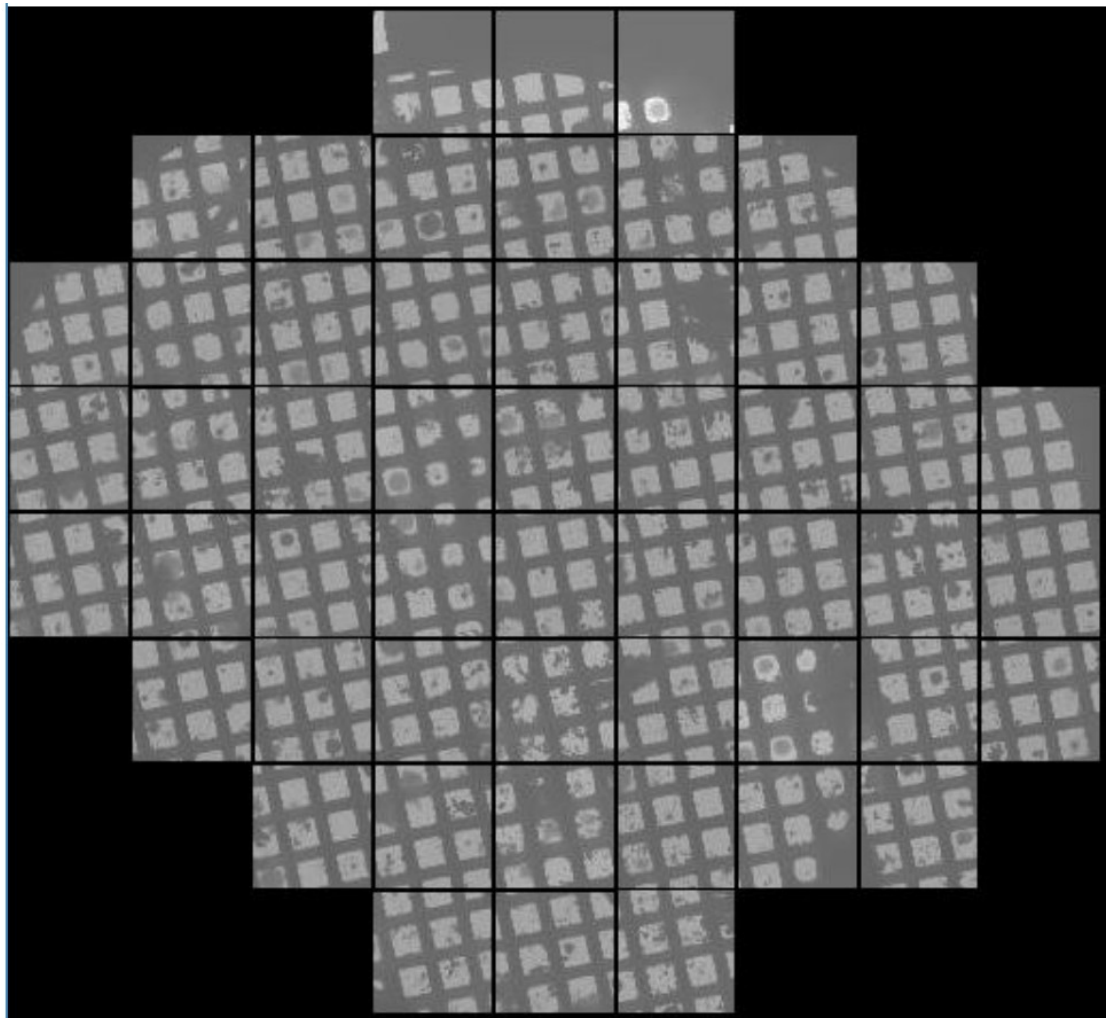
Limitation

- 1. How to get right ice thickness?

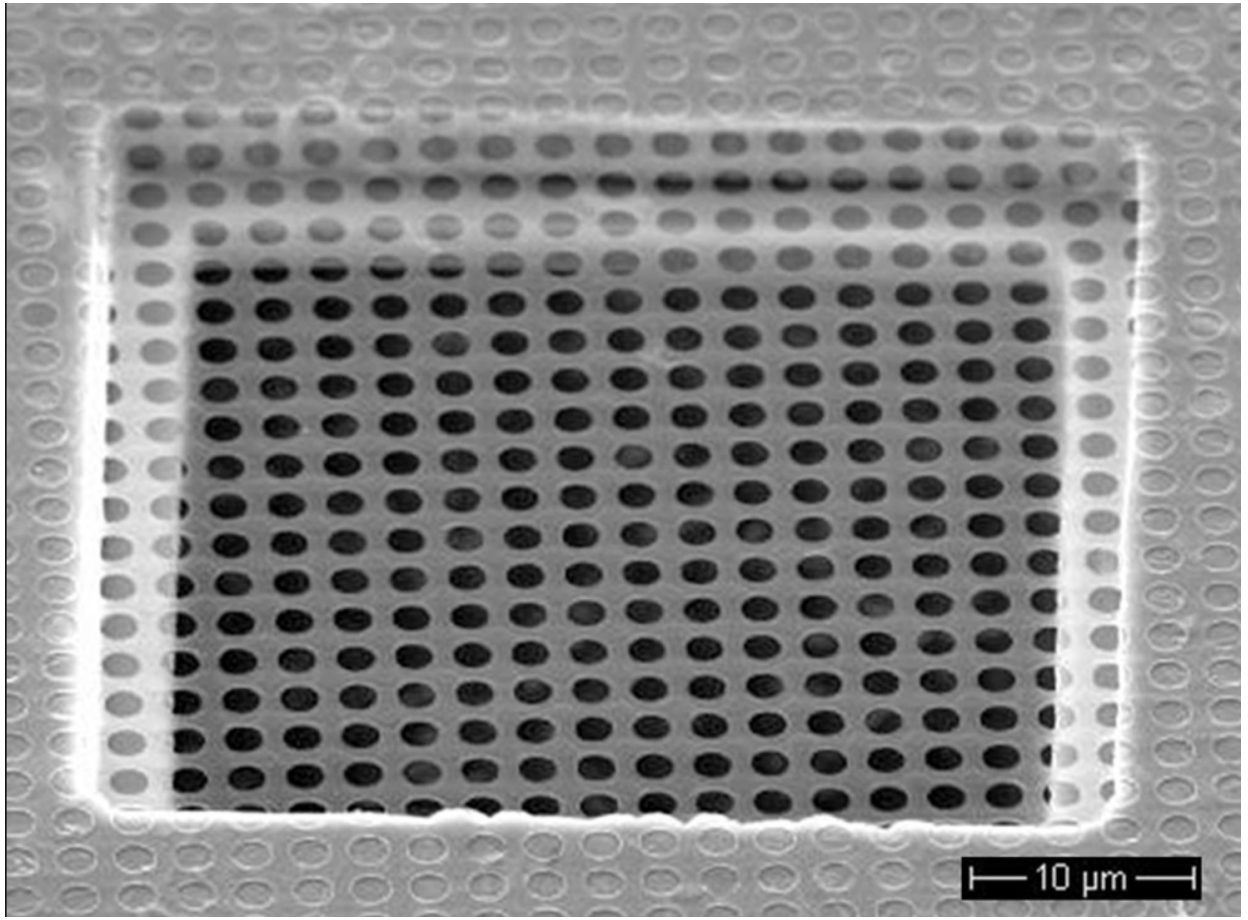
Blotting grid



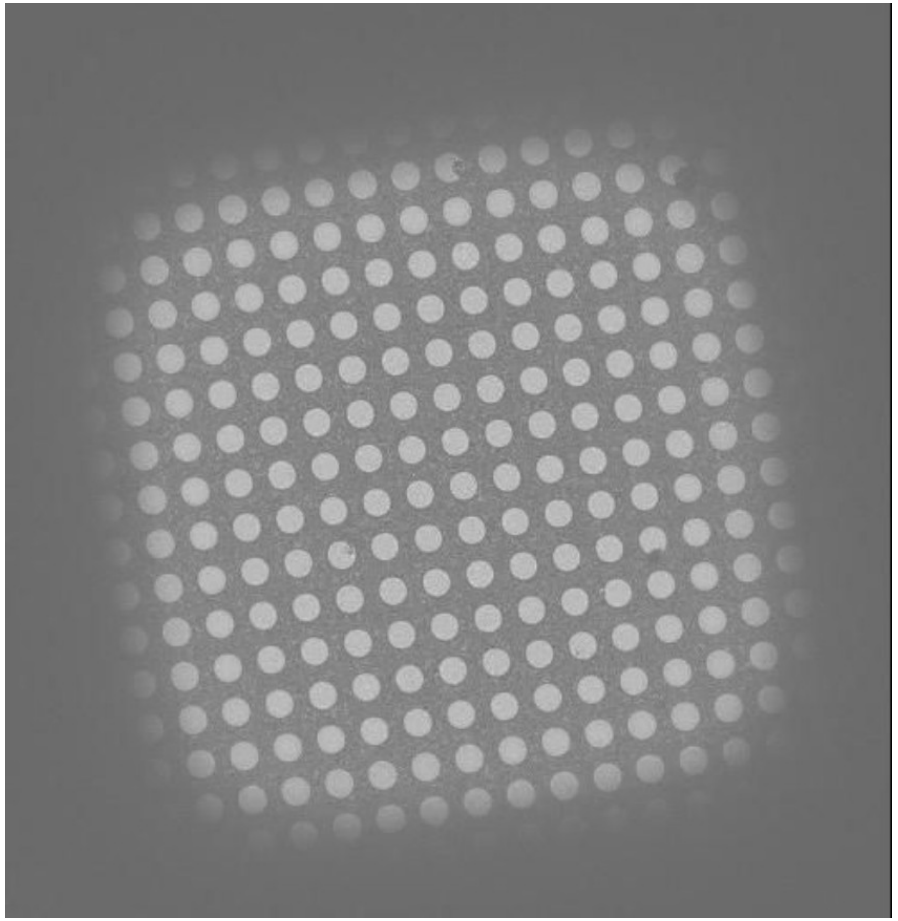
Spraying grid



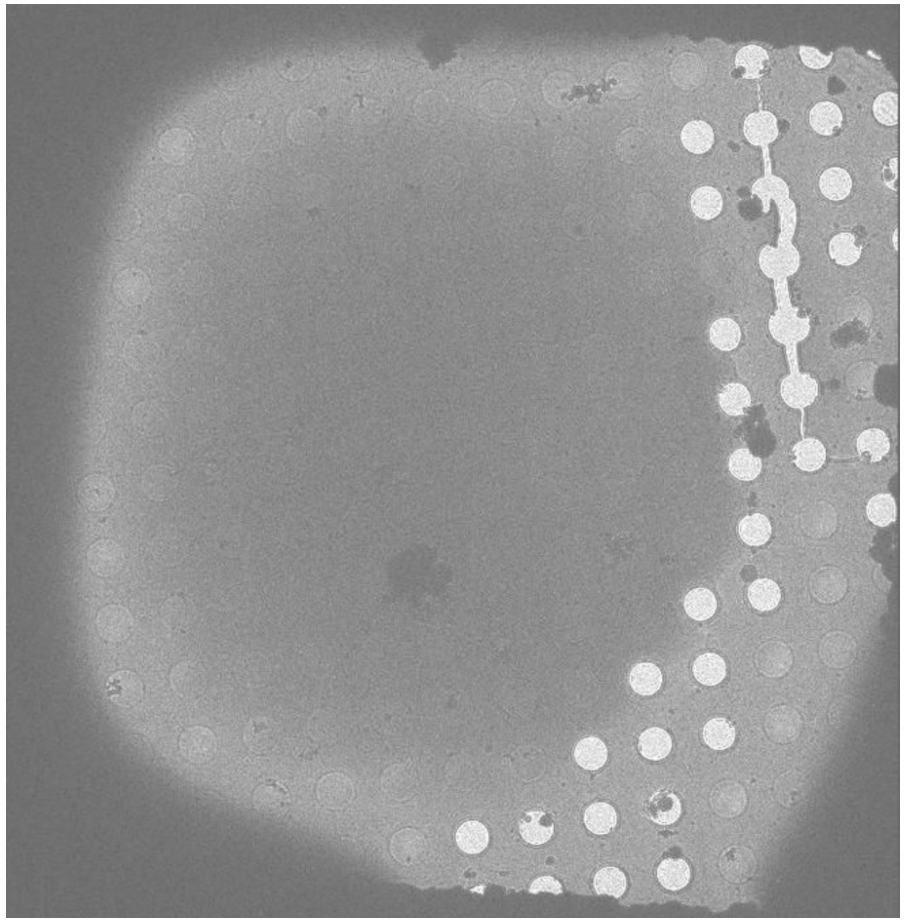
Quantifoil R1.2/1.3 400 mesh



Blotting grid



Spraying grid



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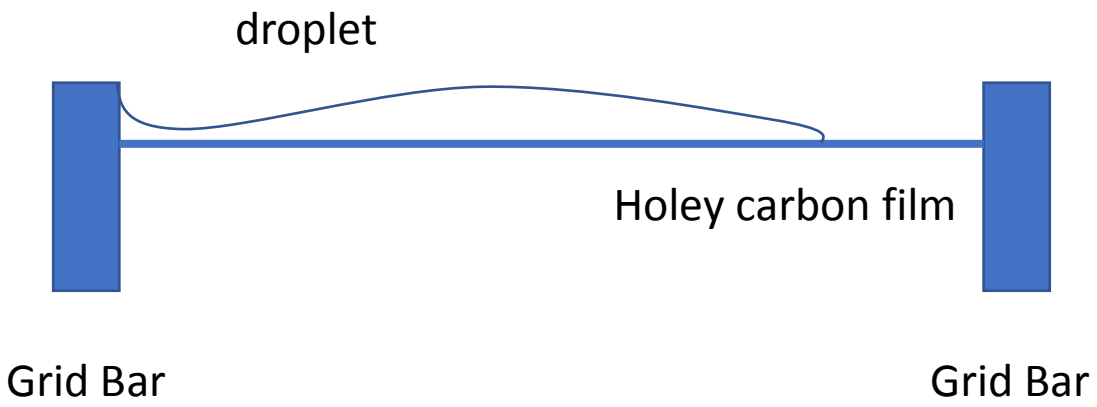

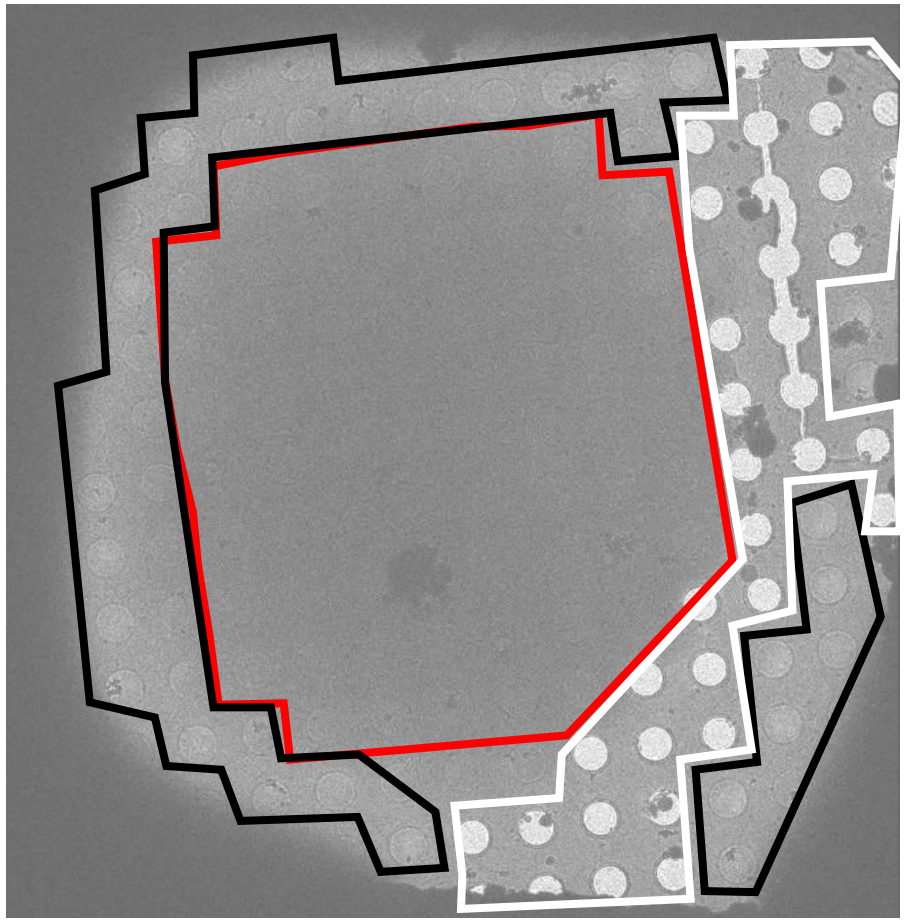

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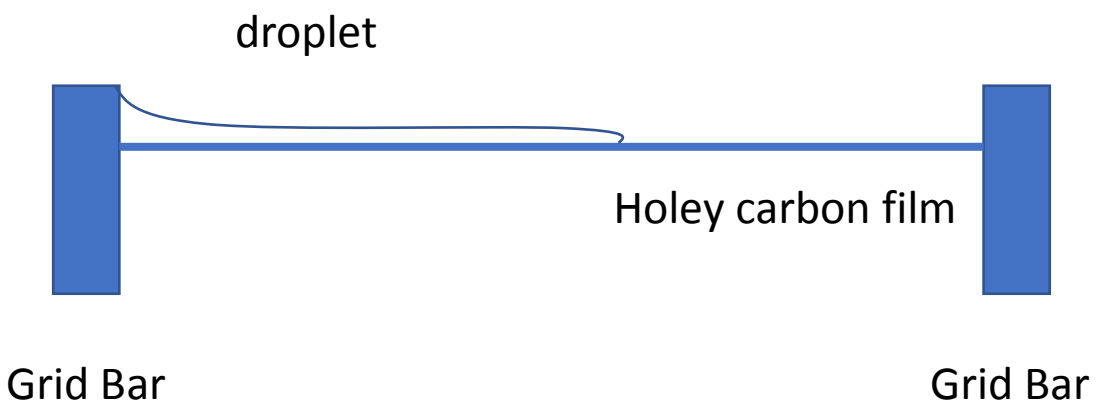
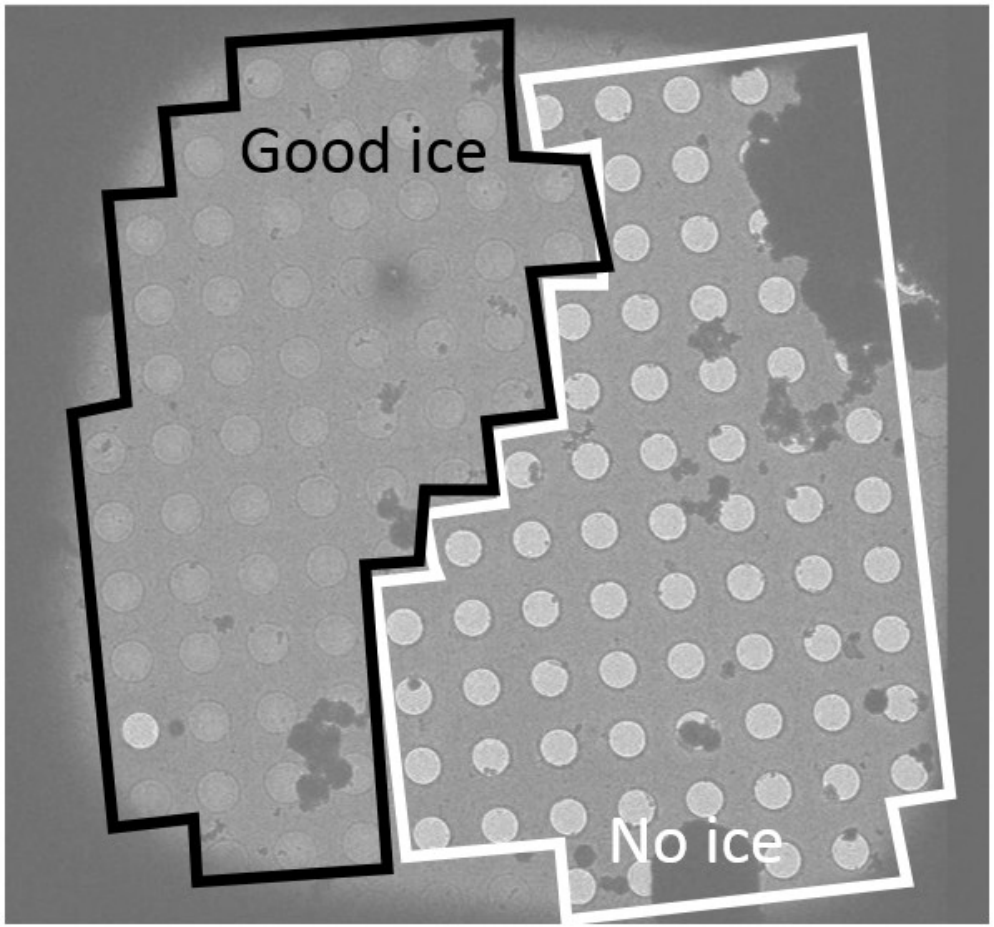
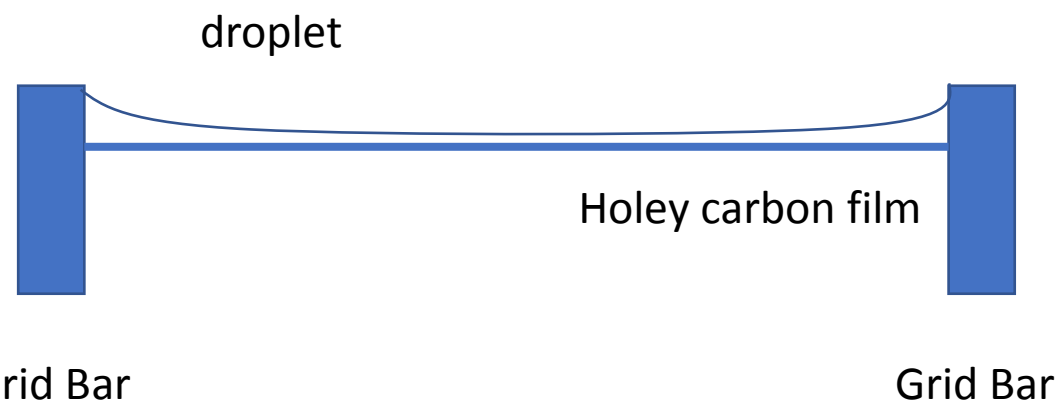
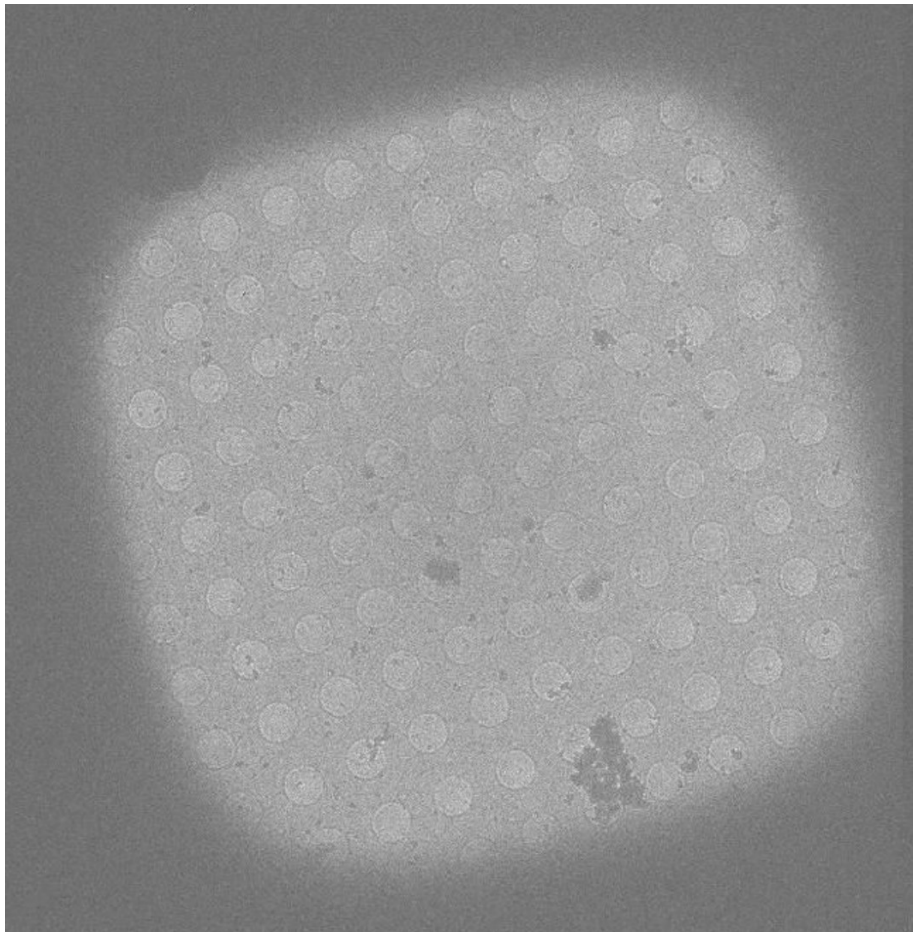
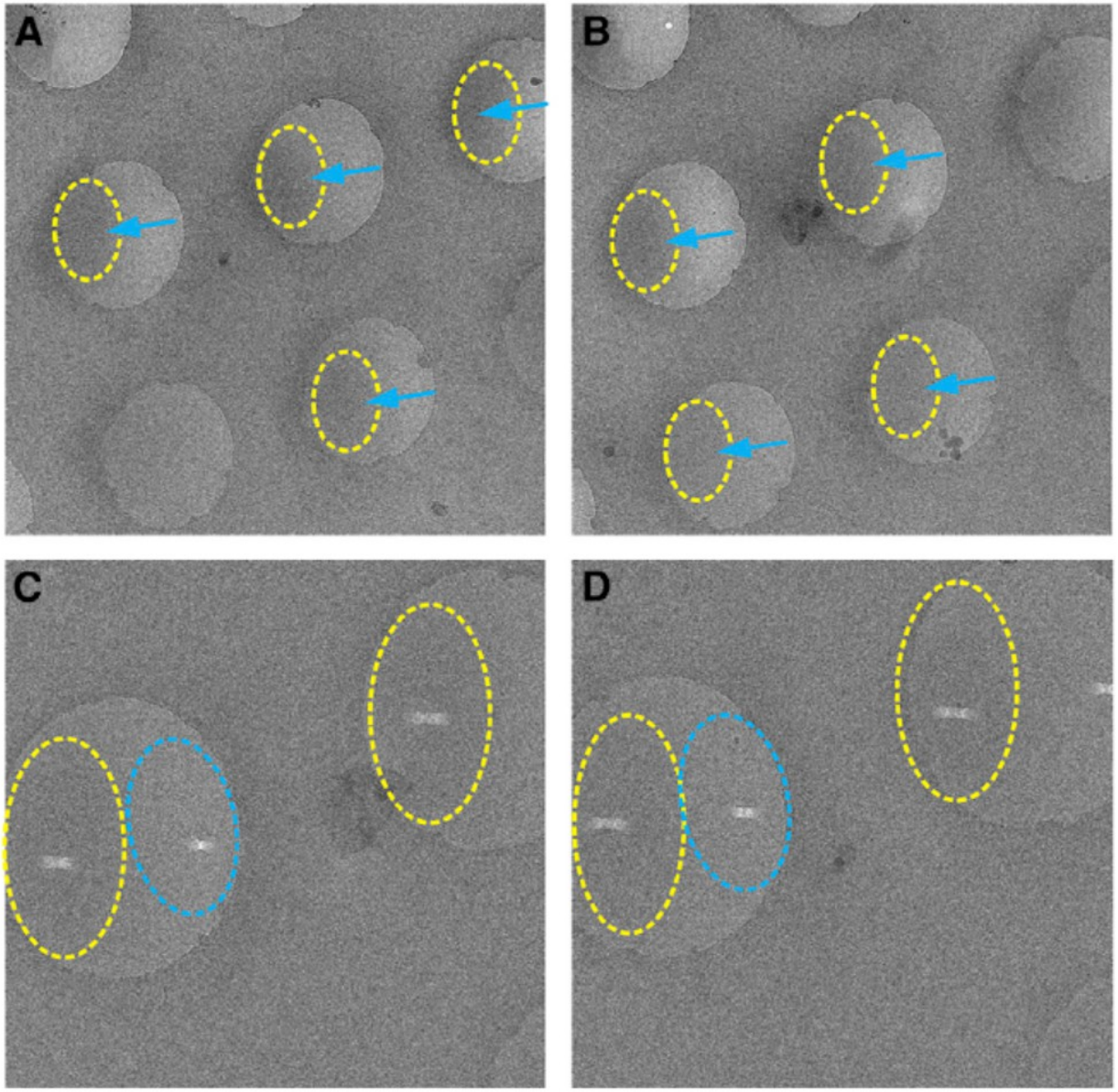




Spraying grid



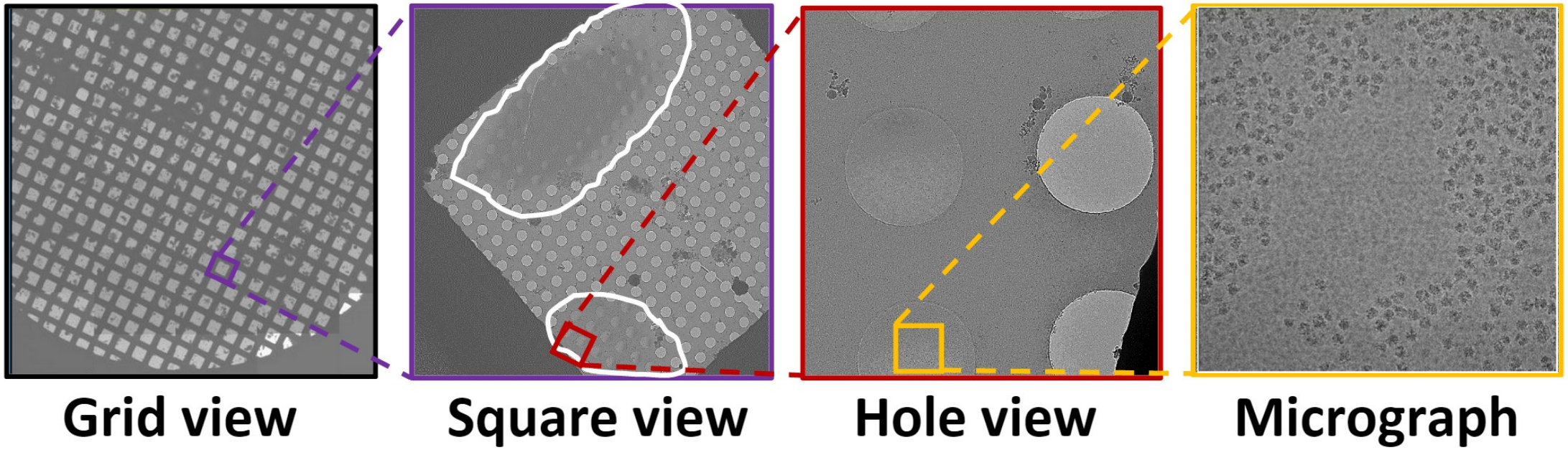




(A and B) The ice thickness is different from the leading to the trailing side of
each hole (blue arrows), which is different from grids obtained by the blotting
method.

(C and D) The ice is thinner on one side than on the other side as indicated by
the different lengths of the tunnels drilled on the two sides. The thicker

Data collection



Mean droplet size – flow rate ratio between liquid and gas

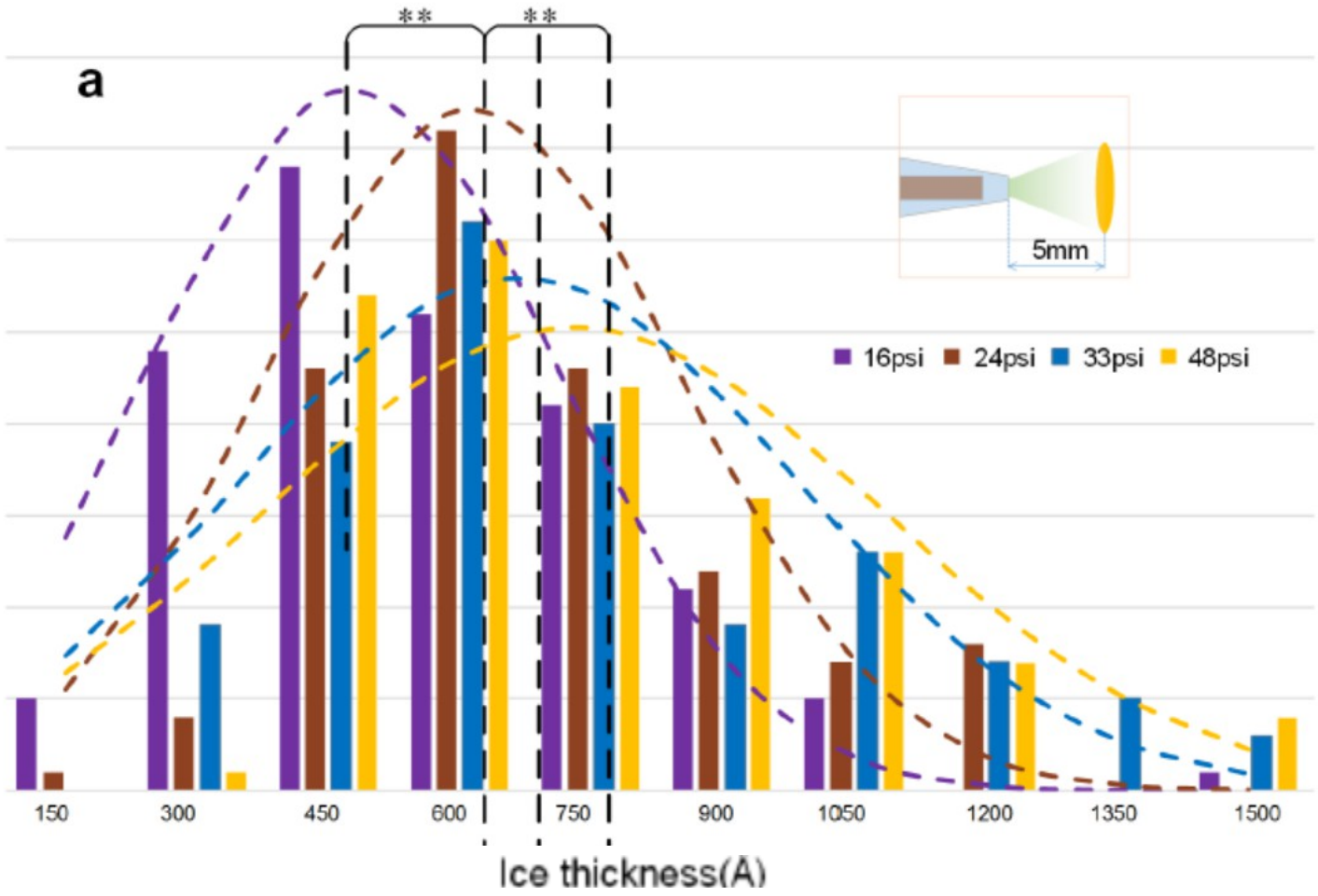
- Diameter: 36.2 to 4.4 µm (Volume : 24.4 pL - 0.044 pL)

Gas pressure: 16 psi to 48 psi

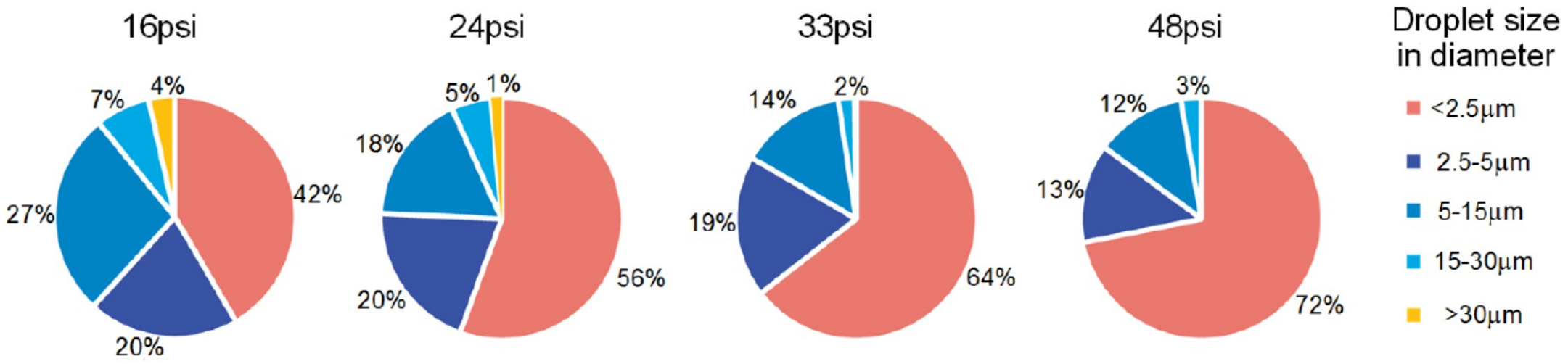
$$\mathrm{SMD} = 0.95\left[\frac{(\sigma_l \dot{m}_l)}{V_r \rho_l^{11/30} \rho_g^{3/10}}\right]\left[1+\frac{\dot{m}_l}{\dot{m}_g}\right]^{17/10} + 0.13\mu_l\left[\frac{D}{\sigma_l \rho_l}\right]^{1/2}\left[1+\frac{\dot{m}_l}{\dot{m}_g}\right]^{17/10}$$

Where *m* is the mass flow rate, and subscripts *g* and *l* denote gas and liquid. Suppose that the solution sprayed is water, the viscosity μ, surface tension σ, density ρ are 0.89 × 10−3 Pa·s, 0.072 N/m, 1 × 103 kg/m3, respectively.

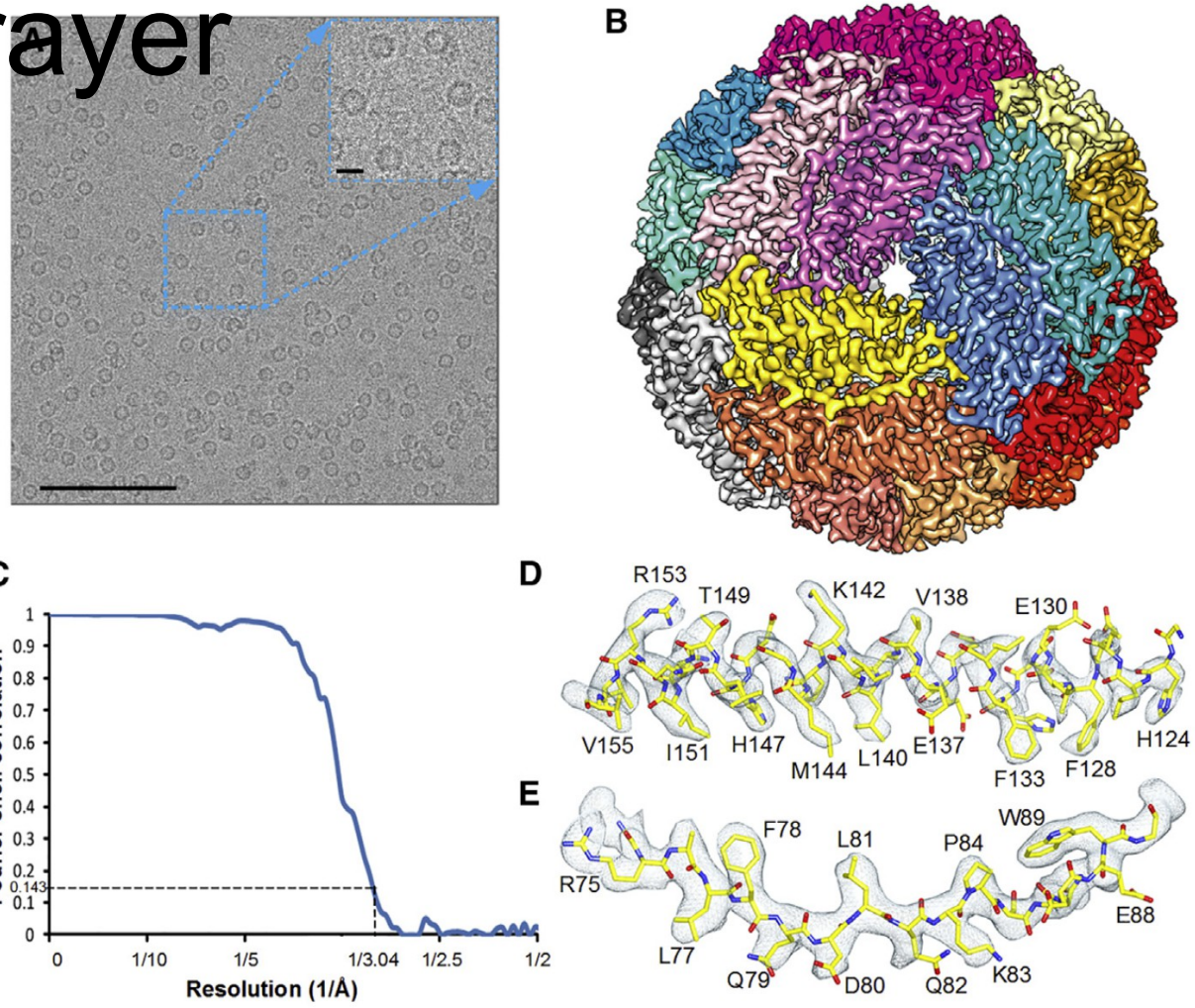
Measurements of Ice Thickness of Droplets Sprayed on the EM Grid



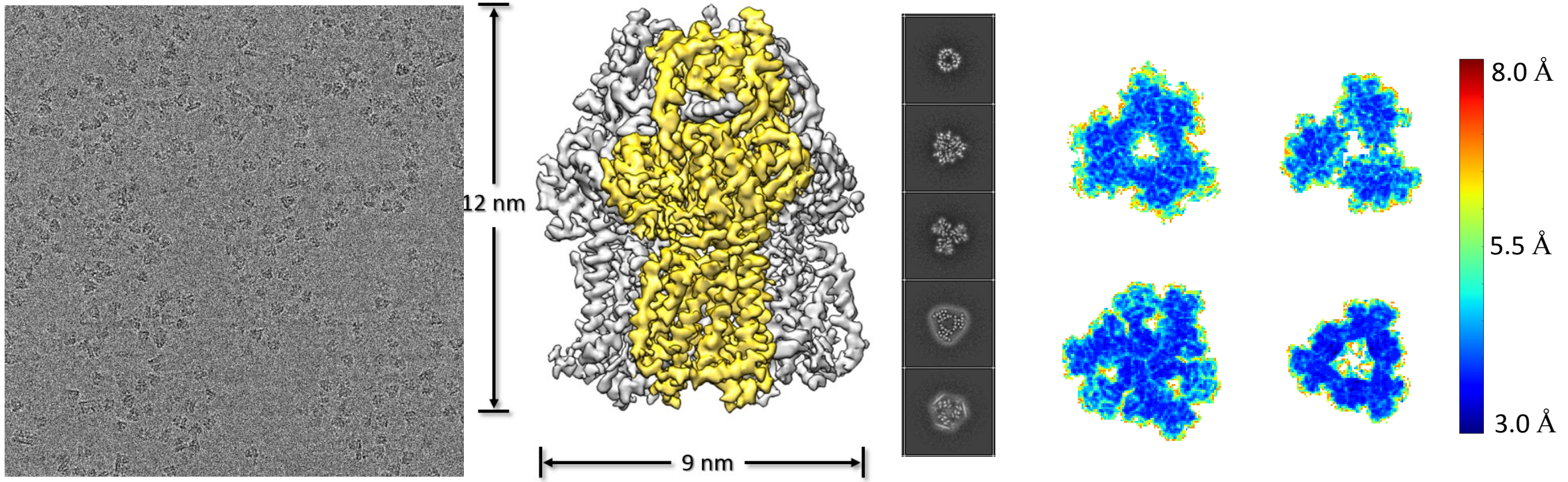
Pie charts illustrating the droplet size distribution under four different spraying conditions.



3.0-Å Resolution Structure of Apoferritin Obtained by Spraying with the Microsprayer

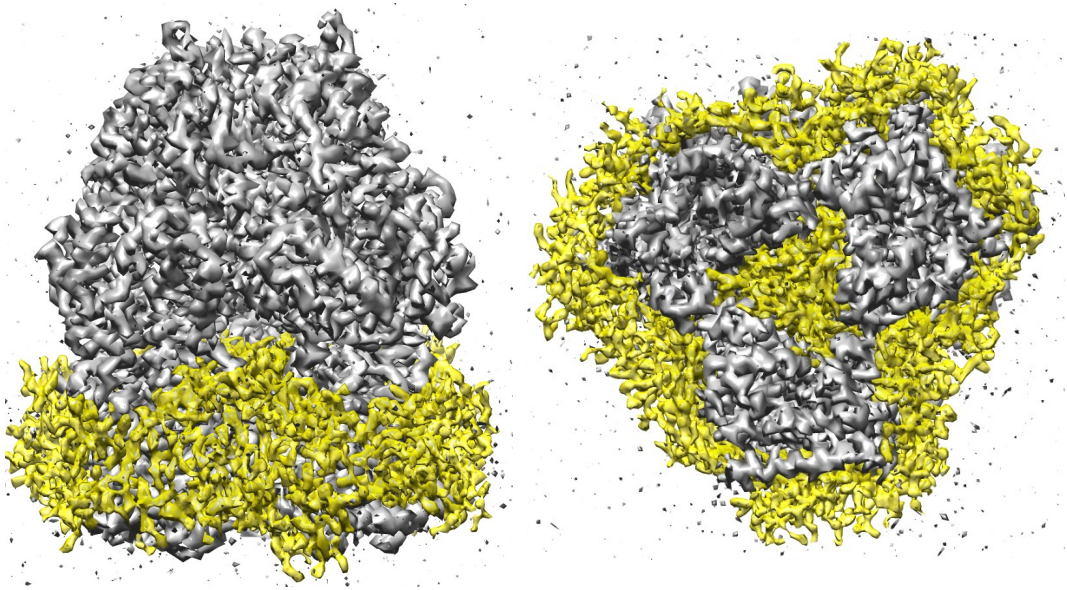


360-kDa membrane protein, AcrB 3.7 Å



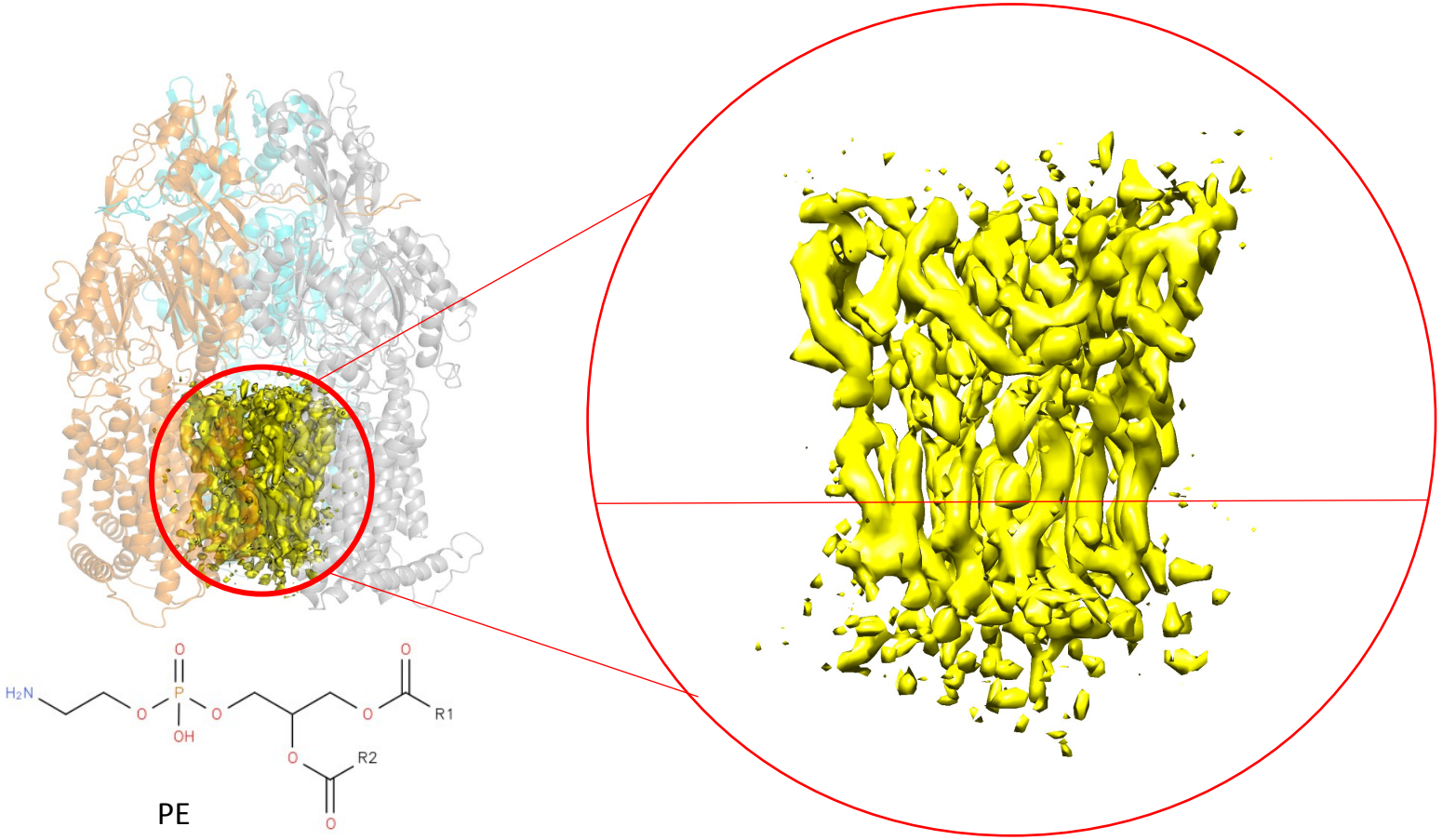
In preparation

3D EM-density (3.2 Å) of AcrB in Native Cell Membrane Nanoparticle



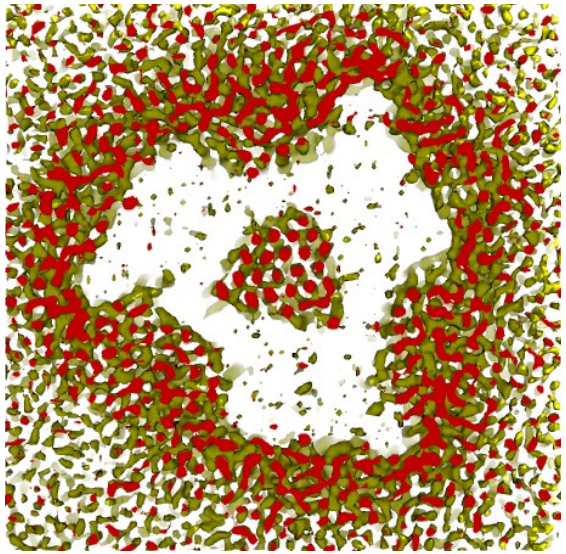
Submitted

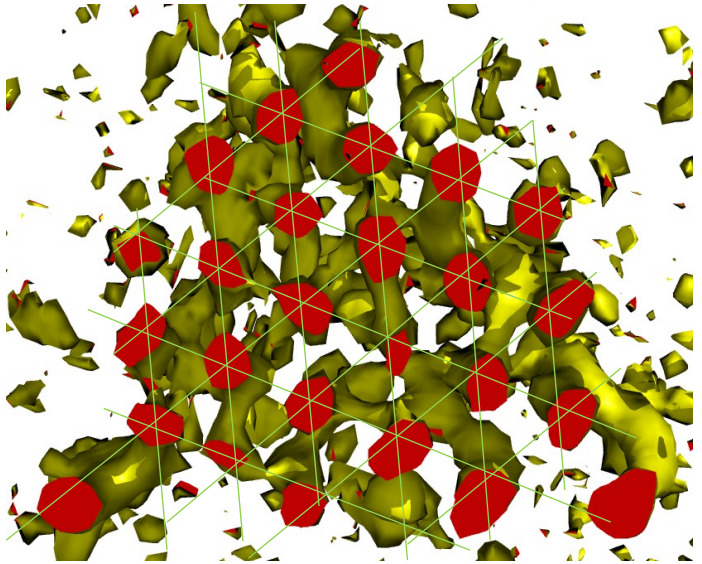
3D EM-density: Native Cell Membrane Bilayer



Submitted

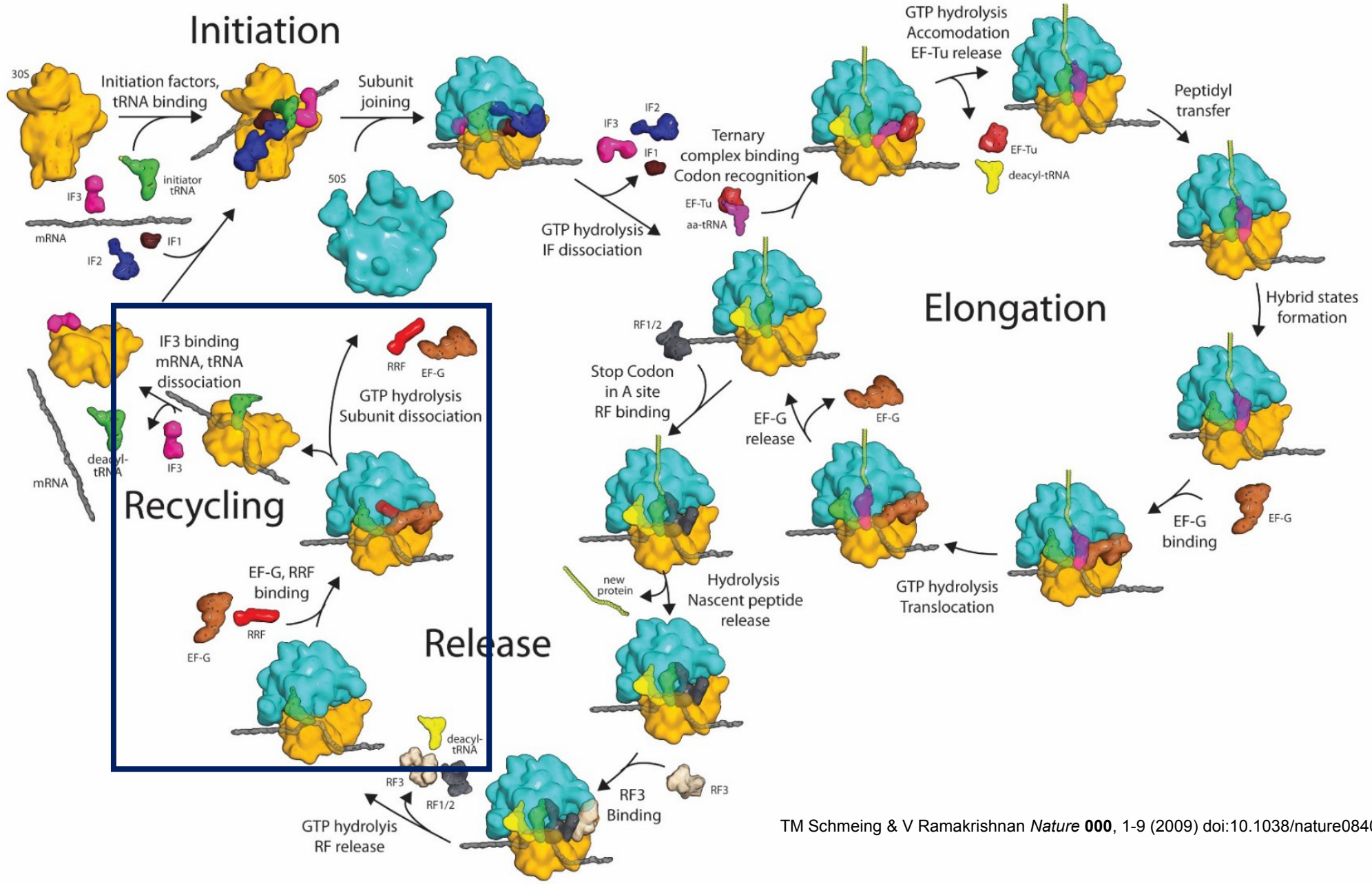
Lipid Belt in Sliced View and Hexagonal Pattern of Lipid Arrangement





Submitted

Overview of translation



TM Schmeing & V Ramakrishnan *Nature* **000**, 1-9 (2009) doi:10.1038/nature08403

The recycling process

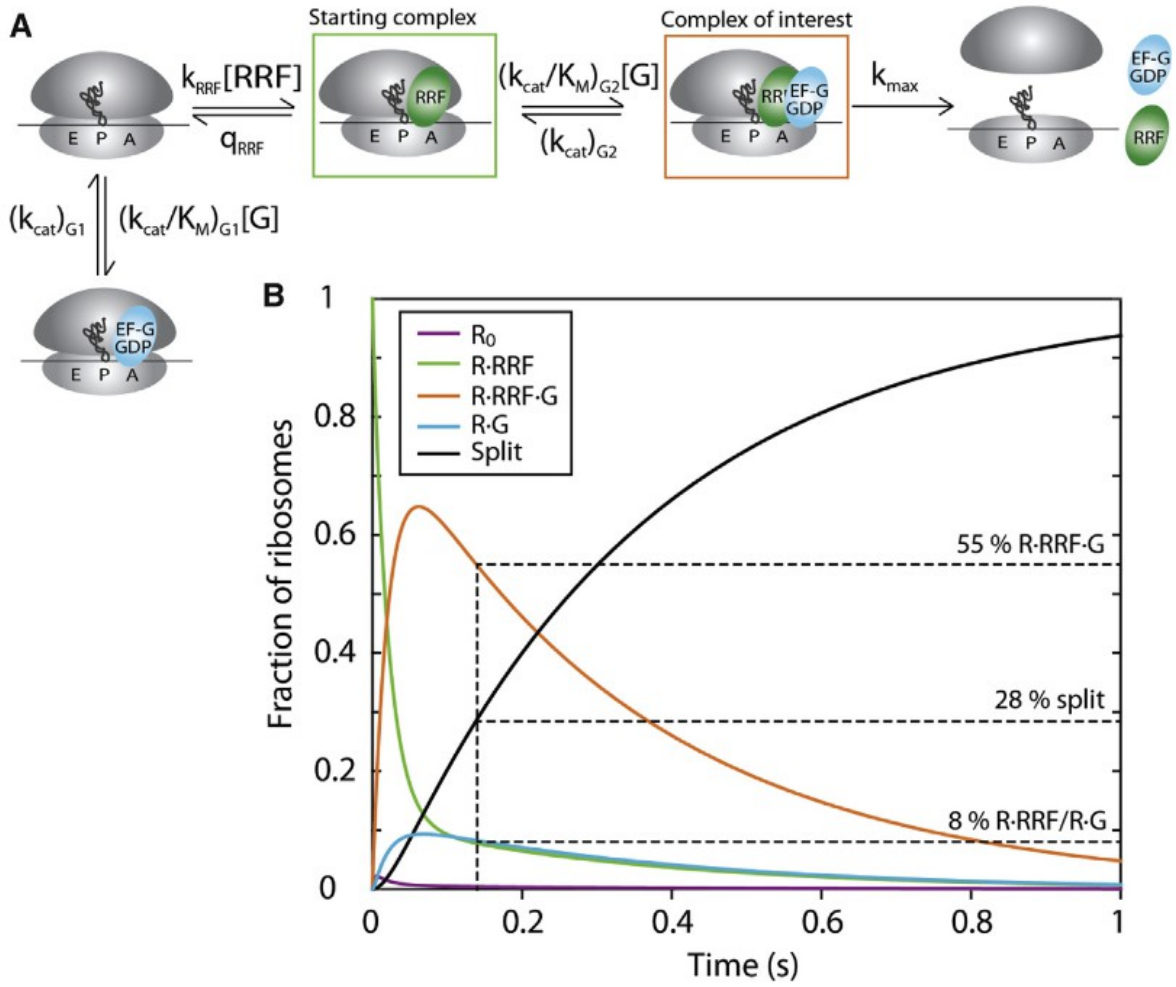
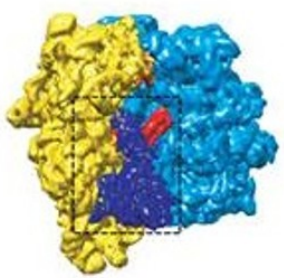
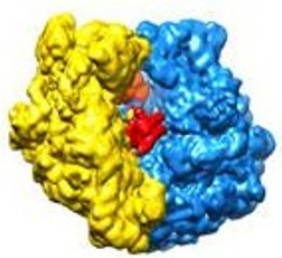


Table 1. List of the Cryo-EM Structures Obtained in this Study

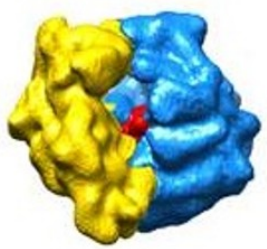
Experiment	Ribosome and Other Components (Rot or Non-rot)	Abbreviation (Resolution, Å)
Control	70S ribosome, RRF, P/E tRNA, mRNA (Rot)	PostTC·$RRF_{control}$ (10)
140 ms	70S ribosome, RRF, P/E tRNA, mRNA (Rot)	PostTC·RRF_{140} (15)
	70S ribosome, RRF, mRNA (Non-rot)	NR-PostTC·RRF_{140} (16)
	70S ribosome, RRF, P/E tRNA, EF-G, mRNA (Rot)	PostTC·RRF·$EF\text{-}G_{140}$ (7.4)
	50S subunit, RRF, EF-G	50S·RRF·$EF\text{-}G_{140}$ (12)
	50S subunit, RRF, EF-G, E tRNA	50S·RRF·EF-G·$tRNA_{140}$ (16)
	30S subunit, P/I tRNA, mRNA	30S·$tRNA_{140}$ (10)
	30S subunit, IF3, mRNA	30S·$IF3_{140}$ (22)
Long incubation	50S subunit, RRF, EF-G	50S·RRF·$EF\text{-}G_{long}$ (14)
	50S subunit, RRF, EF-G, E tRNA	50S·RRF·EF-G·$tRNA_{long}$ (12)
	50S subunit, RRF, E tRNA	50S·RRF·$tRNA_{long}$ (16)
	50S subunit, E tRNA	50S·$tRNA_{long}$ (16)
	30S subunit, IF3, mRNA	30S·$IF3_{long}$ (10)



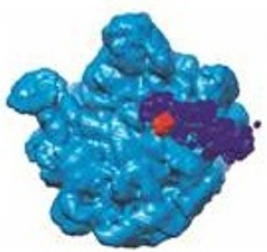
PostTC•RRF•$EF\text{-}G_{140}$ (7.4)



PostTC•$RRF_{control}$ (10)

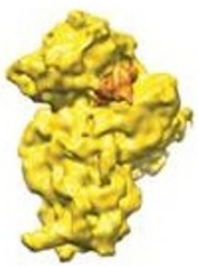


NR-PostTC•RRF_{140}(16)



50S•RRF•$EF\text{-}G_{140}$ (12)

50S•RRF•EF-G•$tRNA_{140}$(16)



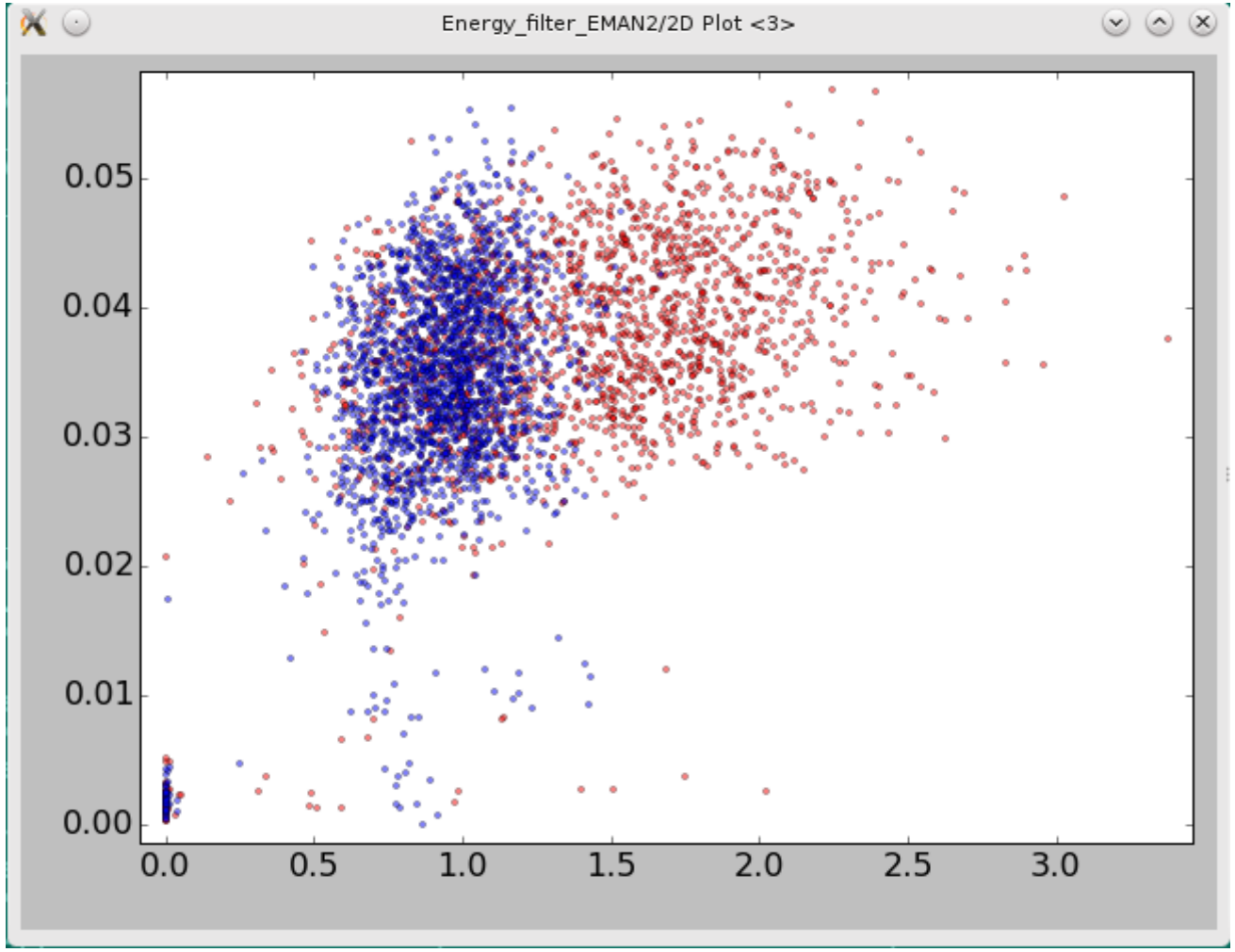
30S•$tRNA_{140}$(10)

30S•$IF3_{long}$ (10)

Next steps

- 1. General application
- 2. Nano-fluidic system (less sample consumption)
- 3. Sub-millisecond system
  - (mixing time < 50 μs, freezing time < 100 μs)

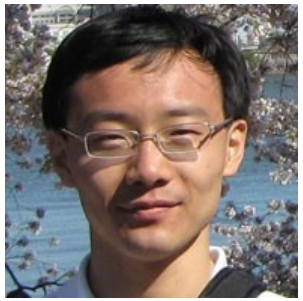
Energy filter 20 eV (red) vs no slit (blue)



Frank Lab Team

Prof. Joachim Frank

Sandip Kaledhonkar



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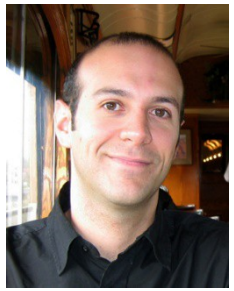
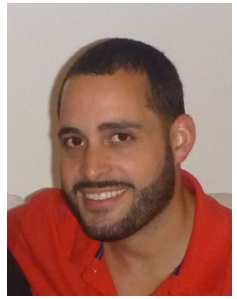
Ming Sun

Bob Grassucci

Gonzalez Lab



Ruben



Kelvin

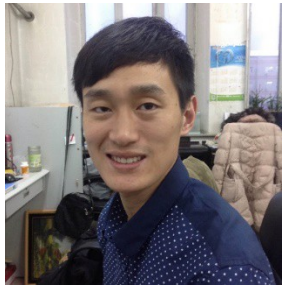
Ehrenberg Lab

Mans

Anneli

3D sprayer Microfluidic device with PDMS

Dr. Qiao Lin



Xiangsong Feng

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New Time-Resolved Machine

Prof. Howard D. White
Eastern Virginia Medical School

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