Microfluidics to produce and manipulate microcrystals

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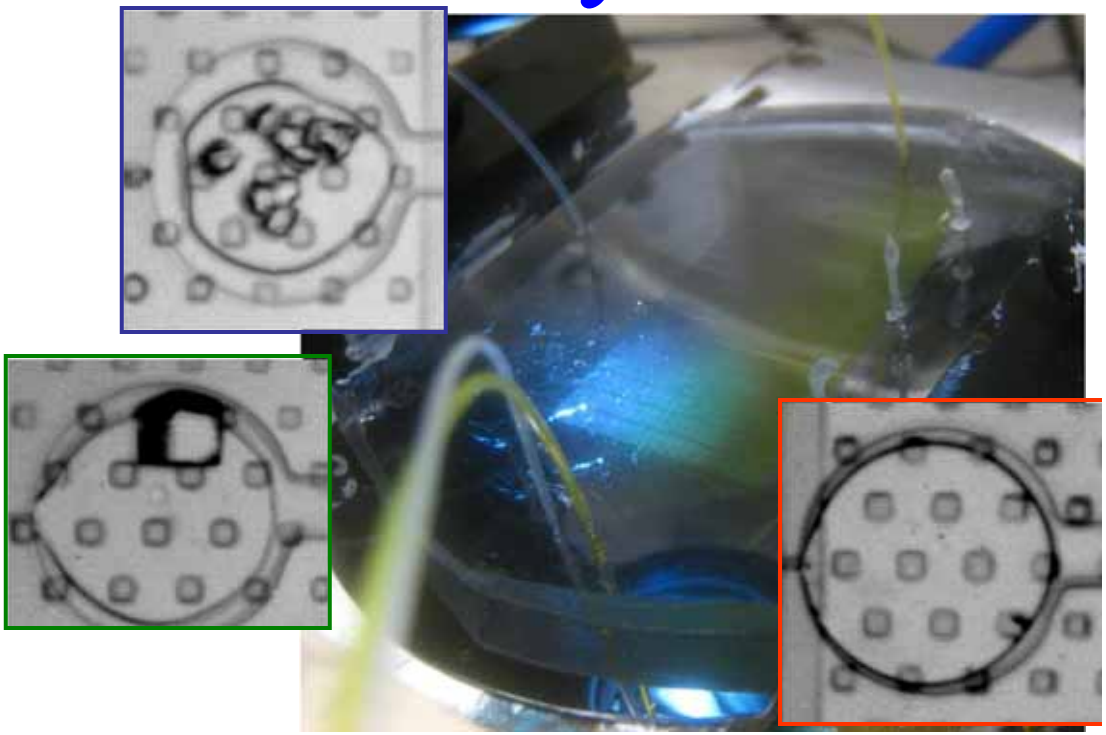


McPherson

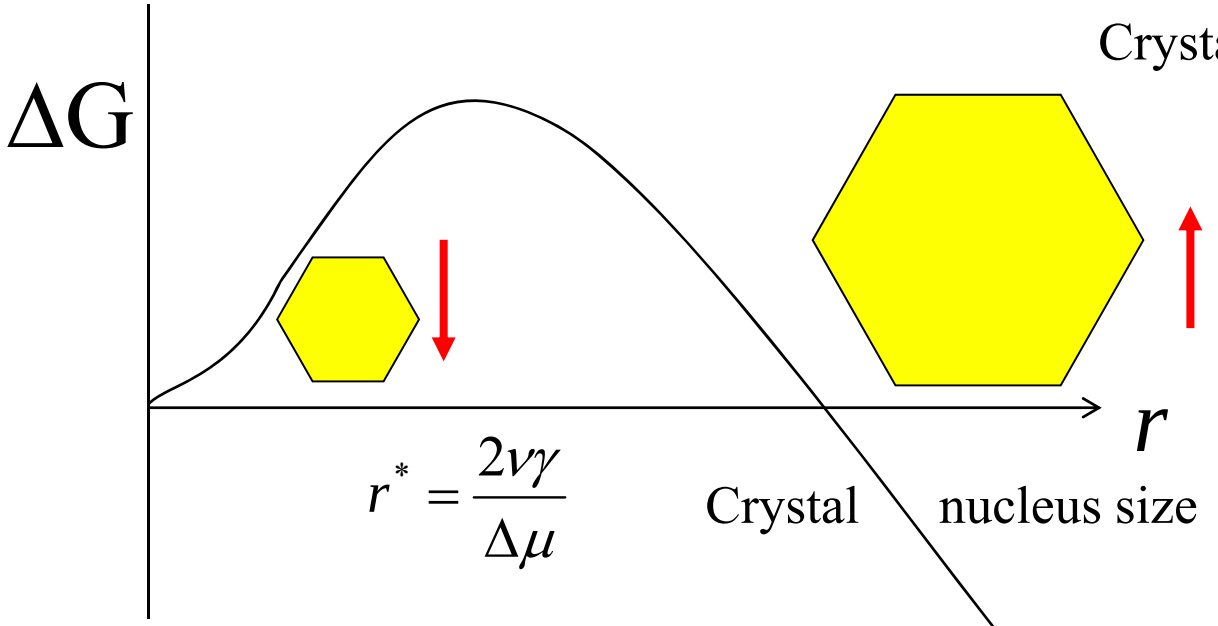
Crystallization of Biological Macromolecules

Crystallization = Formulation + Kinetics

Not only must a precise definition of mother liquor components and their concentration be undertaken, but the method used to grow the crystals, to produce supersaturation, should also be optimized. It is important to remember that the pathway a system follows from a regime of undersaturation to one of supersaturation is critical in determining the point at which nucleation takes place and the conditions under which the nuclei develop into crystals. The physical apparatus or device in which this takes place is a major determinant in this regard.

Nucleation barrier

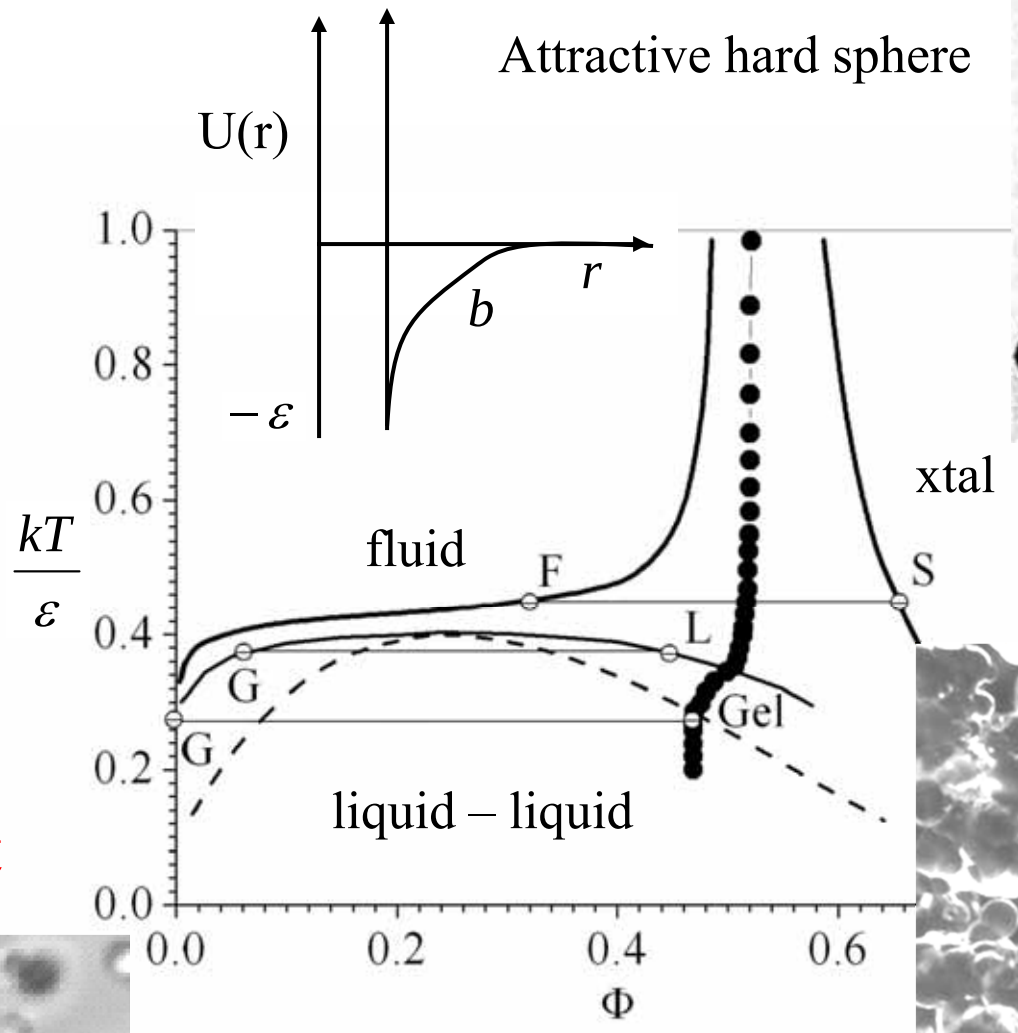
$$\Delta G = -\Delta\mu \frac{1}{\nu}\frac{4\pi r^3}{3} + \gamma 4\pi r^2$$

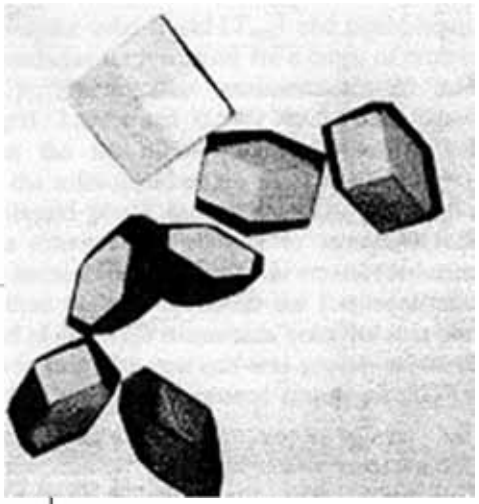


Crystallization rate

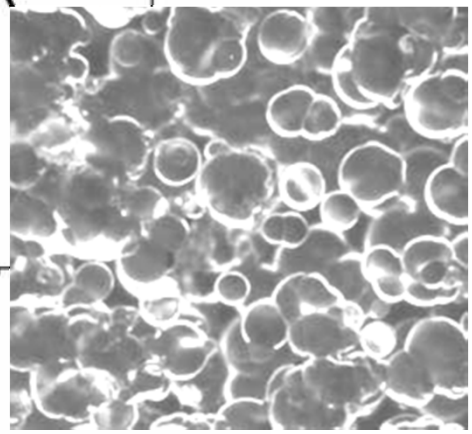
$$\Gamma \propto e^{-\frac{\Delta G^*}{kT}}$$

barrier height $\frac{\Delta G^*}{kT} = \frac{16\pi}{3kT}\frac{\nu^2\gamma^3}{(\Delta\mu)^2} = \frac{16\pi}{3kT}\frac{\nu^2\gamma^3}{(\ln S)^2}$

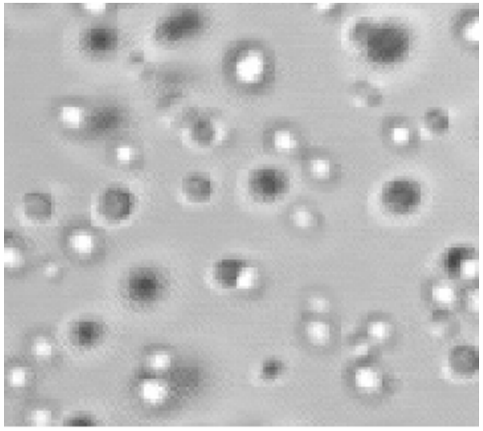




xtal



oiling out



Phase diagram

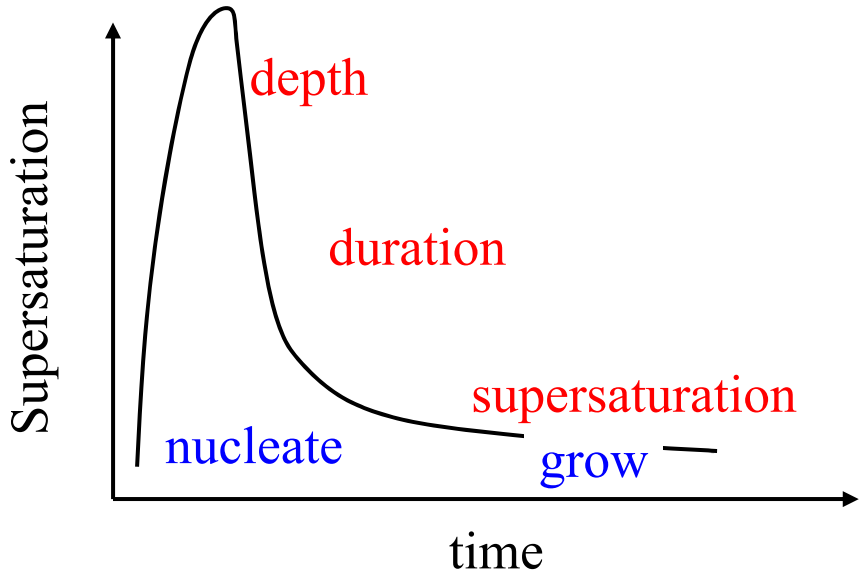
Foffi et al, PRE **65**, 31407 (2002)

Kinetic trap

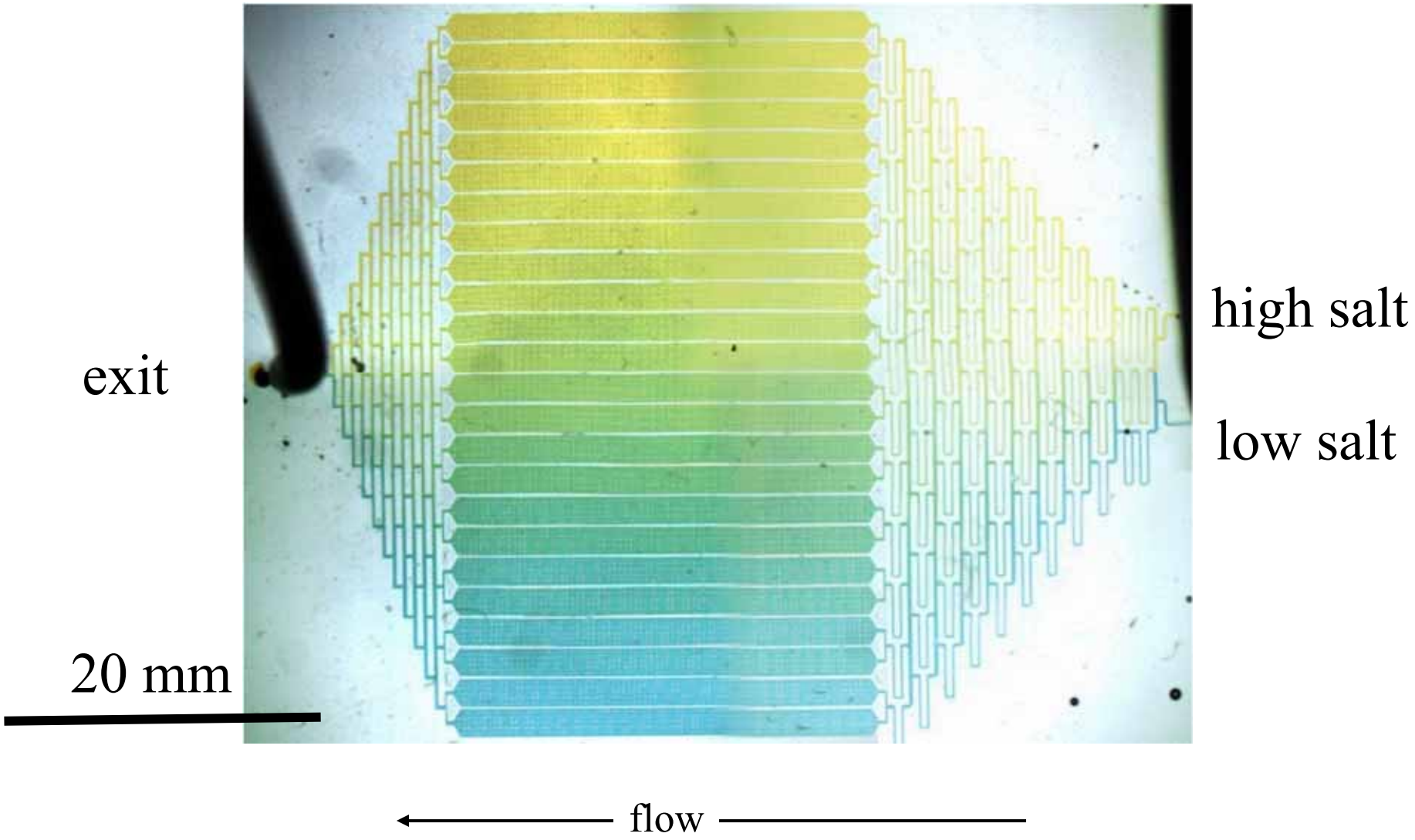
Vary Quench Depth

Nucleate only one crystal

Transform gel to crystal



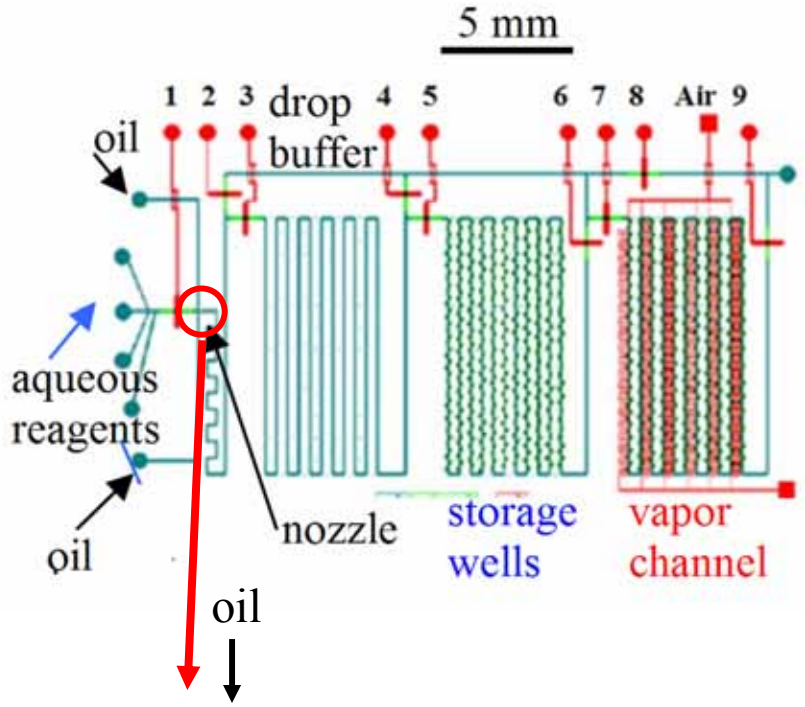
screen supersaturation kinetics



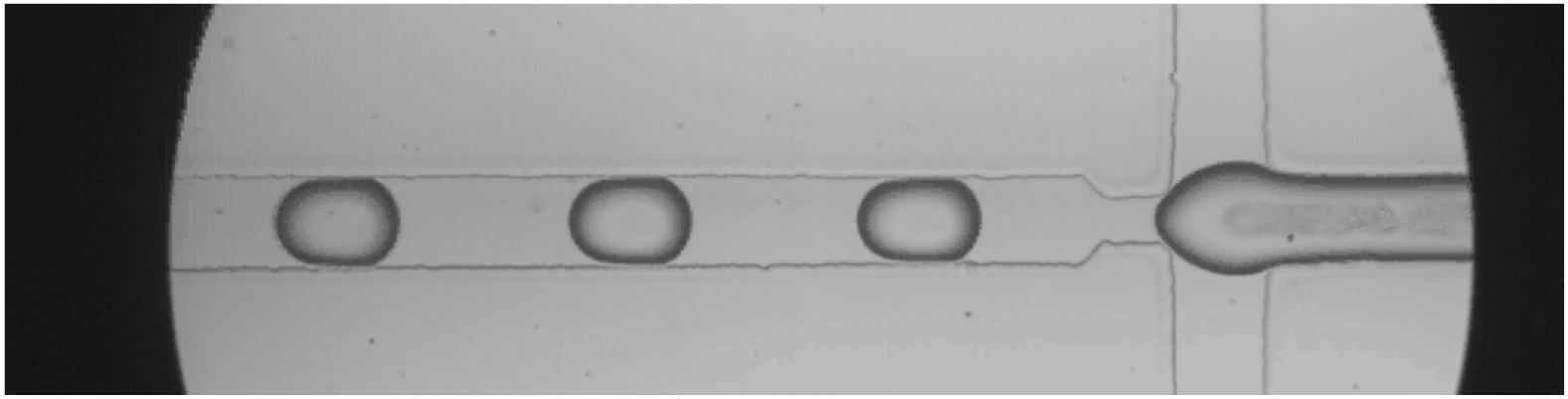
S.K.W. Dertinger, D.T. Chiu, N.L. Jeon, and G.M. Whitesides.
"Generation of gradients having complex shapes using microfluidic networks,"
Analytical Chemistry **73**, 1240-46 (2001).

Create and store microdrops

Create isolated aqueous microdrops of protein solution in an inert oil using flow focusing.



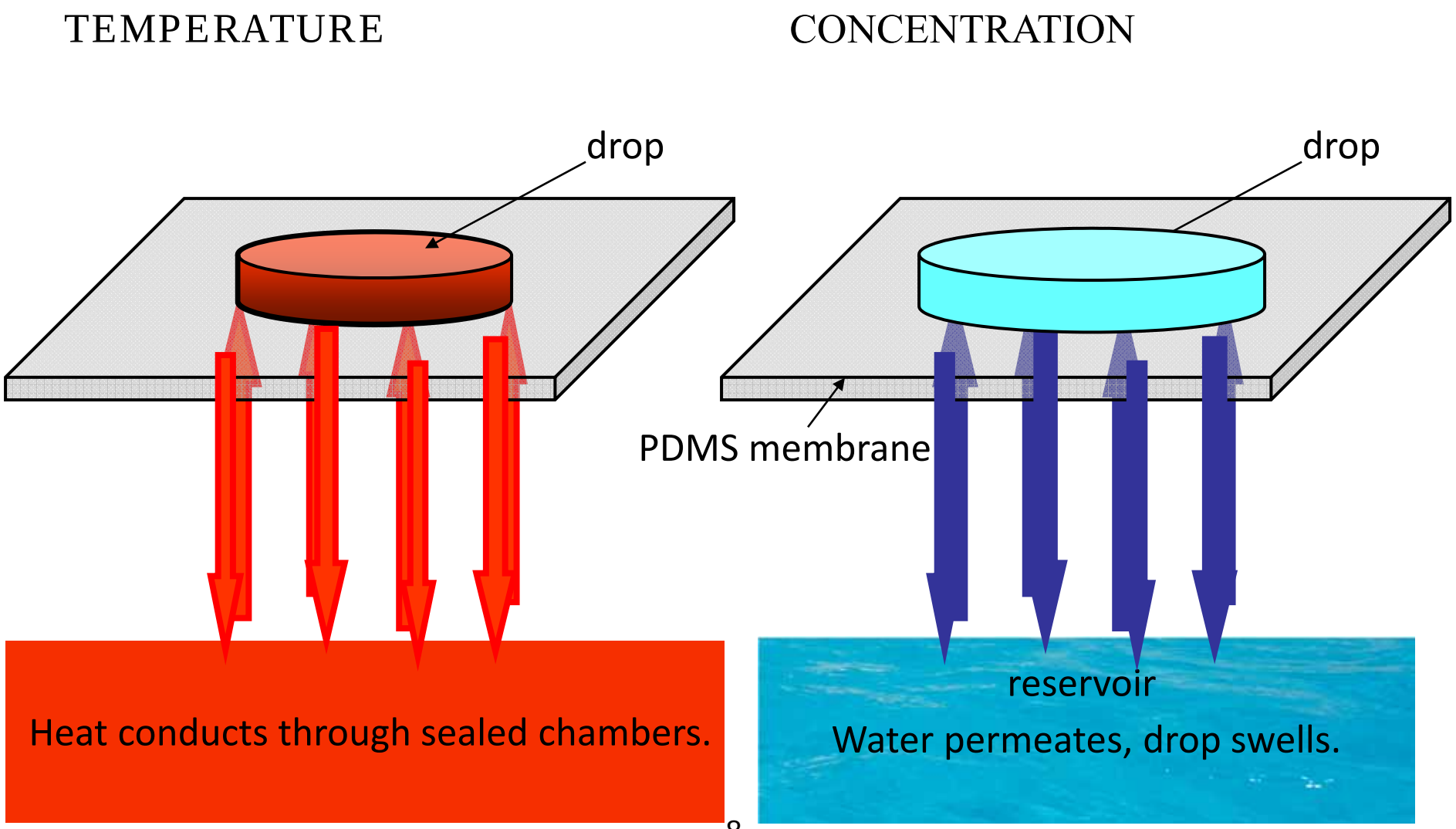
1 – 10 nl drops, 50 micron nozzle
100 micron channel



High speed camera: 5000 frames/sec

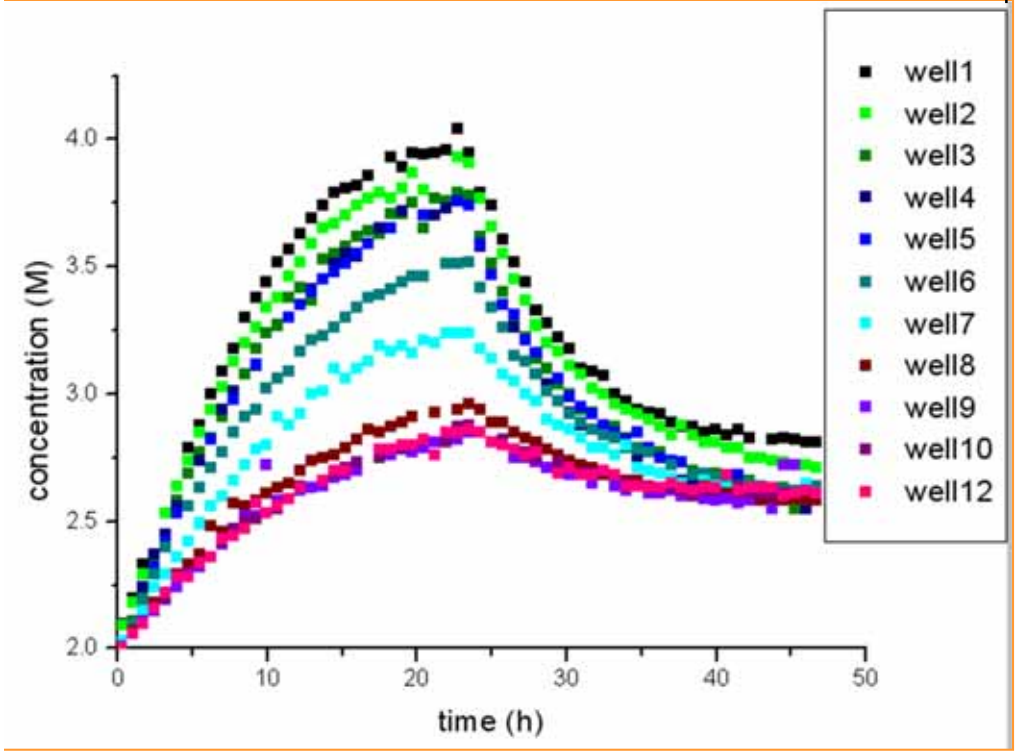
50x time lapse 40 drops / sec

Control temperature and concentration



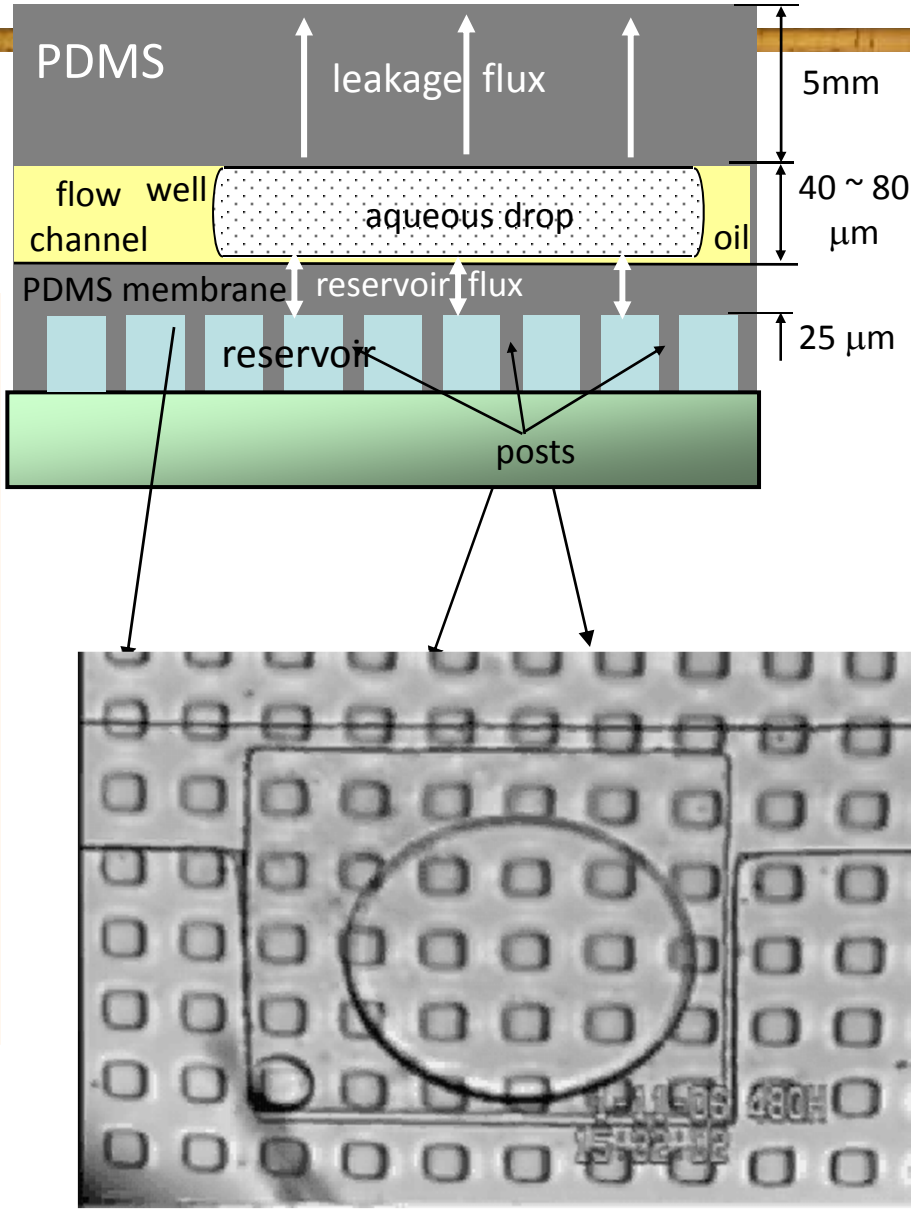
Reversible Permeation

Doyle (05), Leng / Ajdari (06)



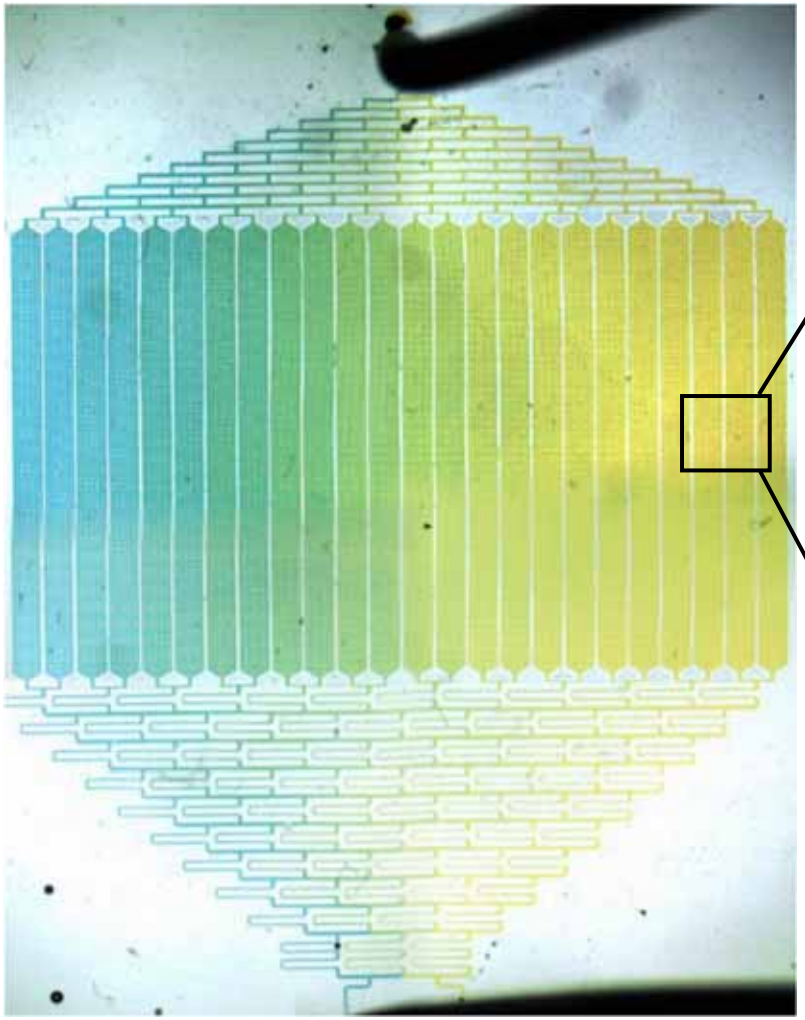
data: Š. Selimović

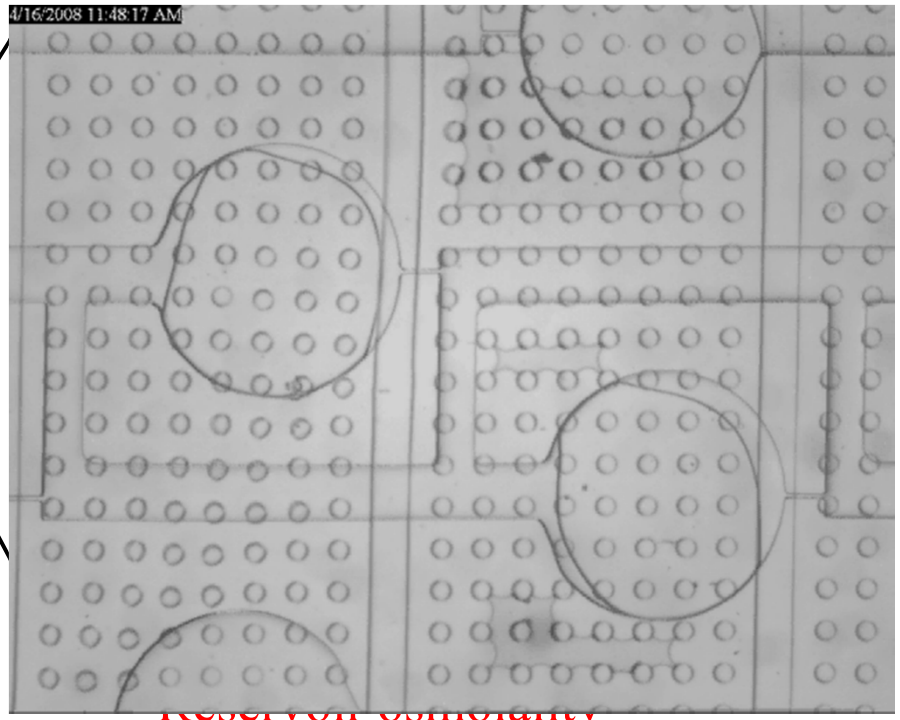
$$\alpha = \frac{A(\infty)}{A_o} = \frac{X_o}{X_\infty}$$

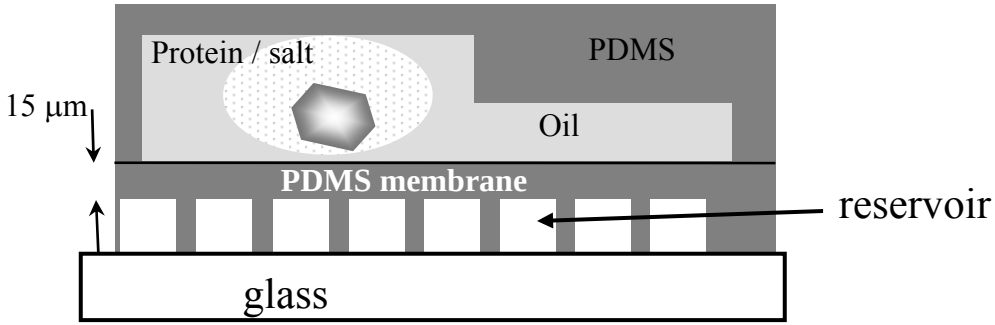


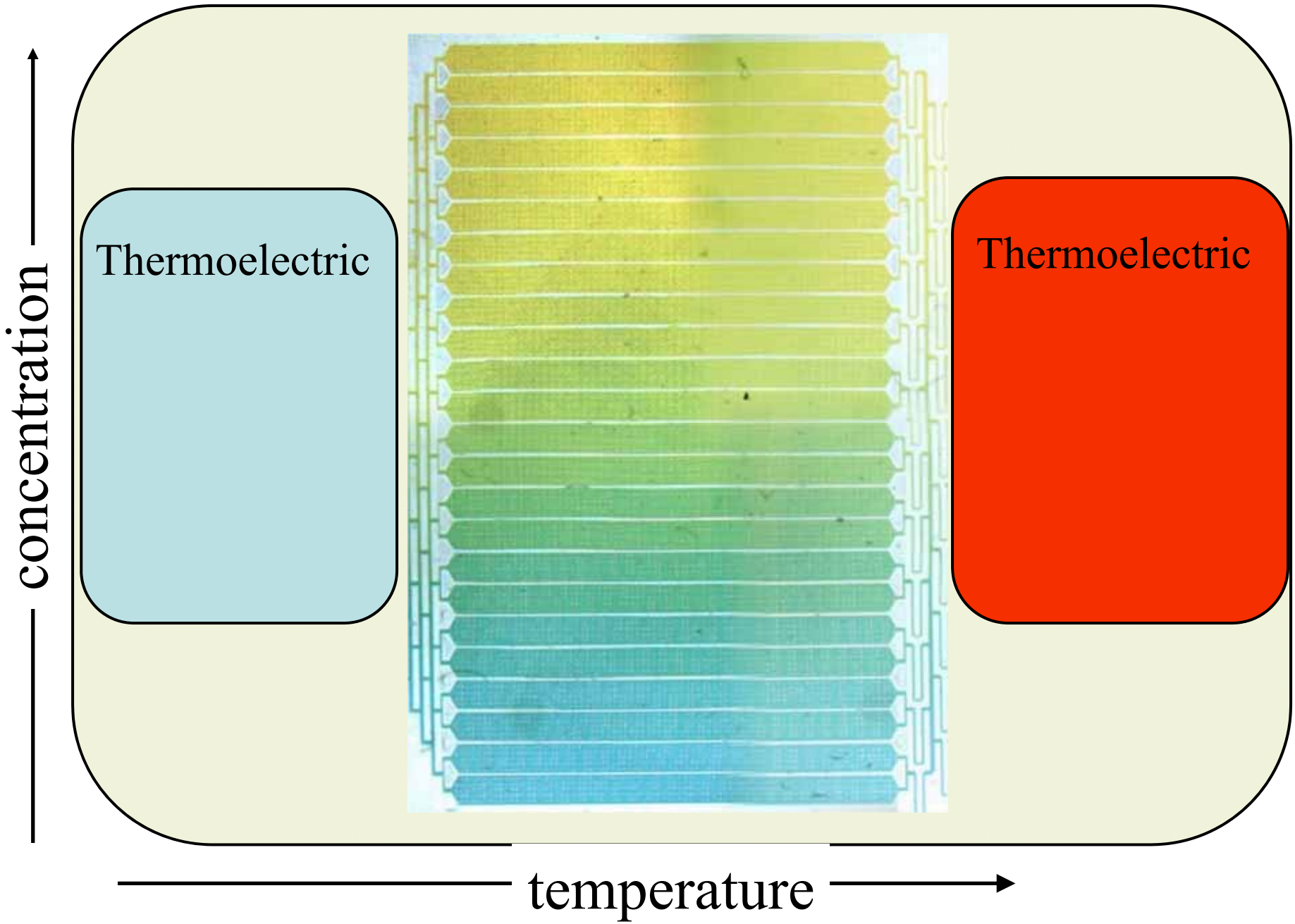
movie: J. Shim

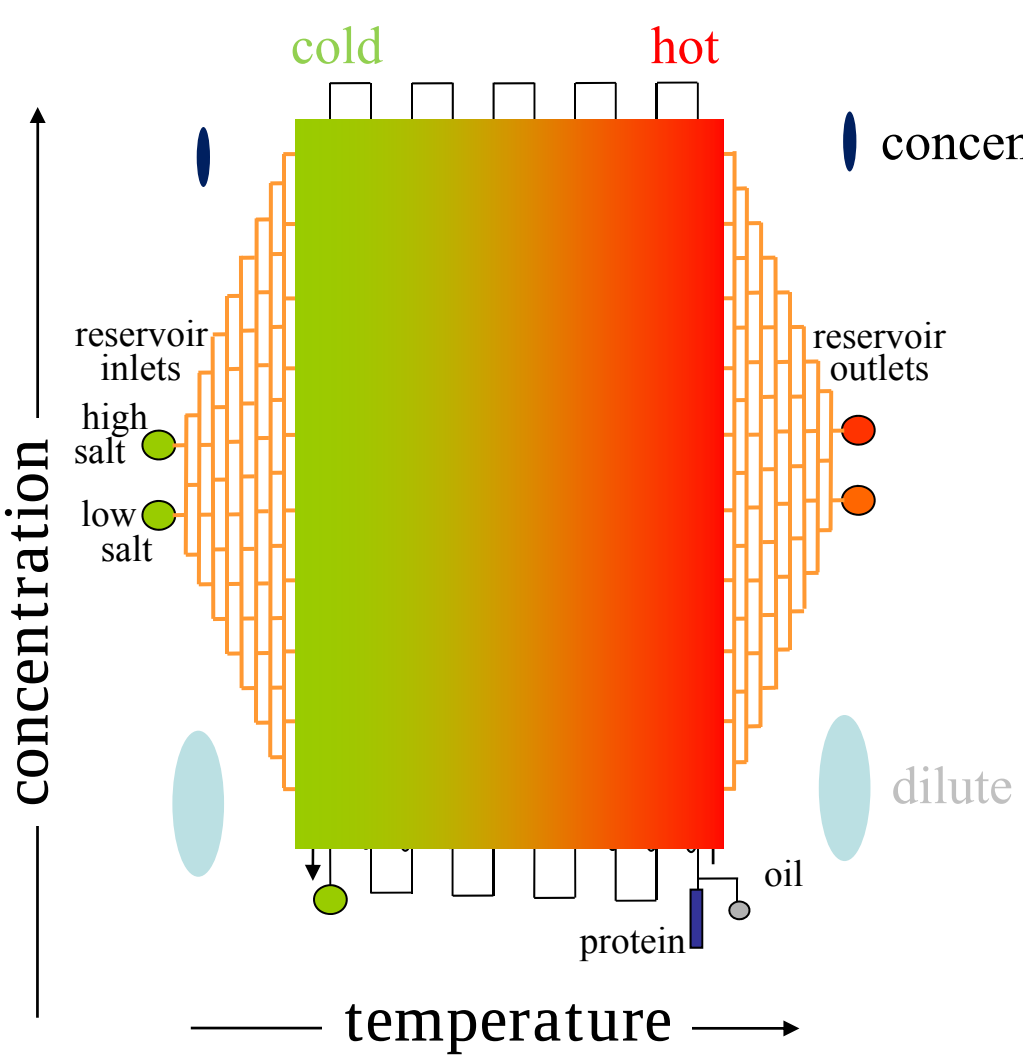
Salt and lysozyme in wells

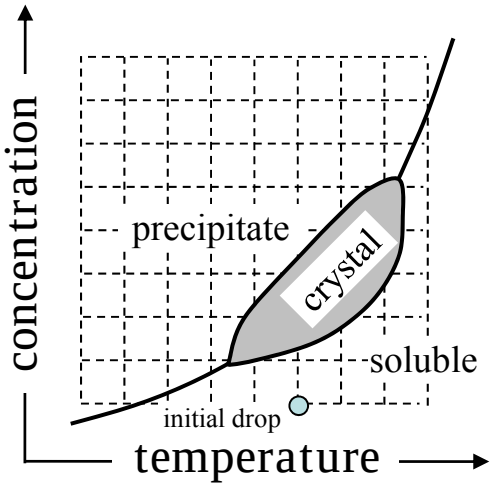


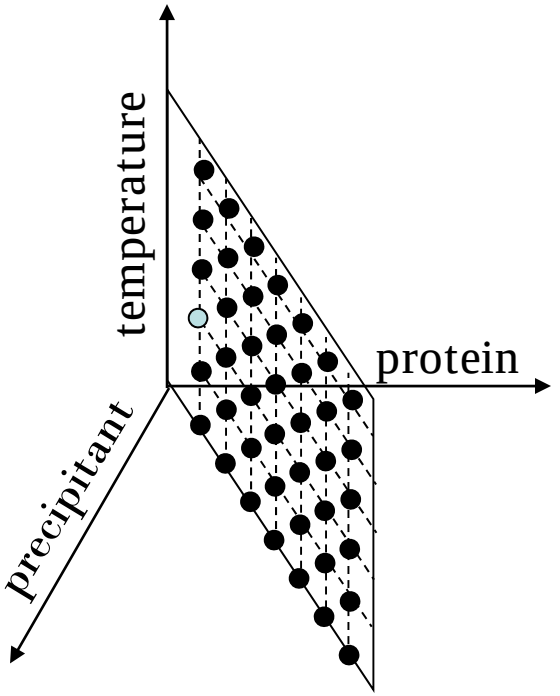





concentration
Thermoelectric
Thermoelectric
temperature

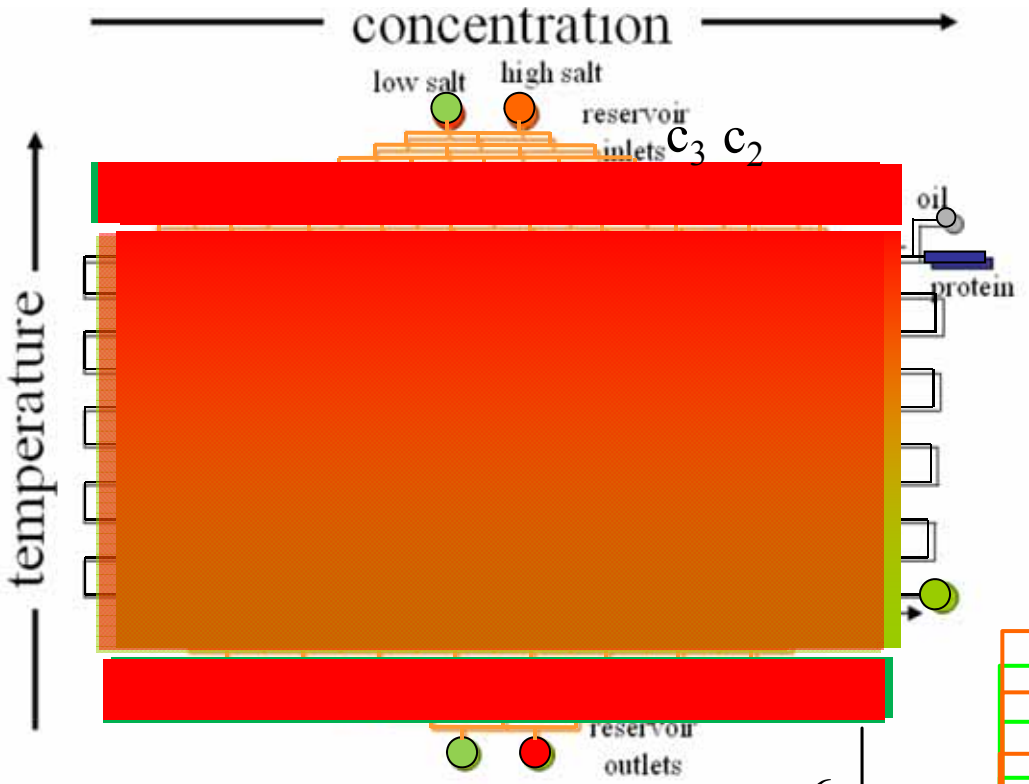


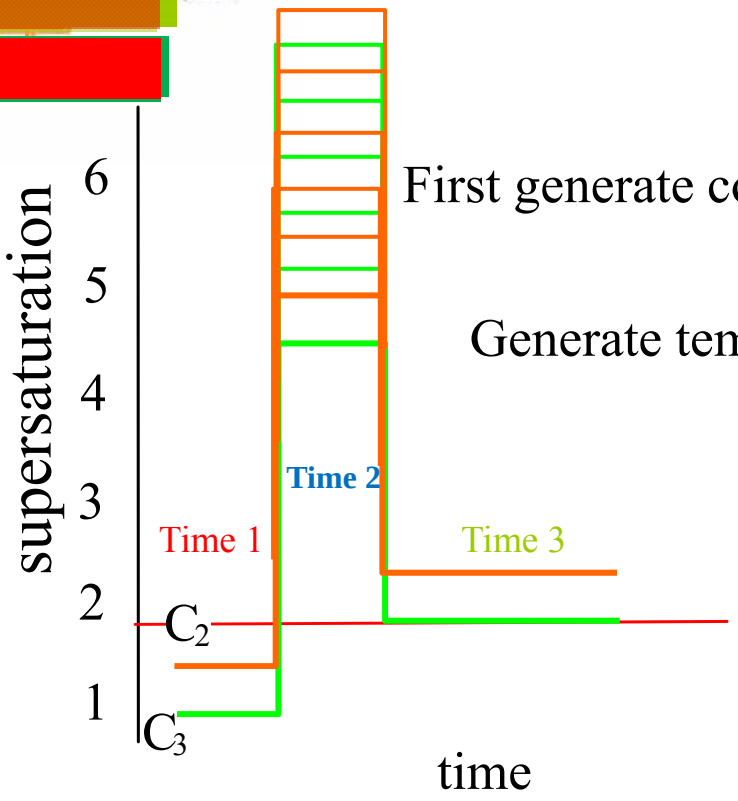




Equilibrium phase behavior

Control kinetics

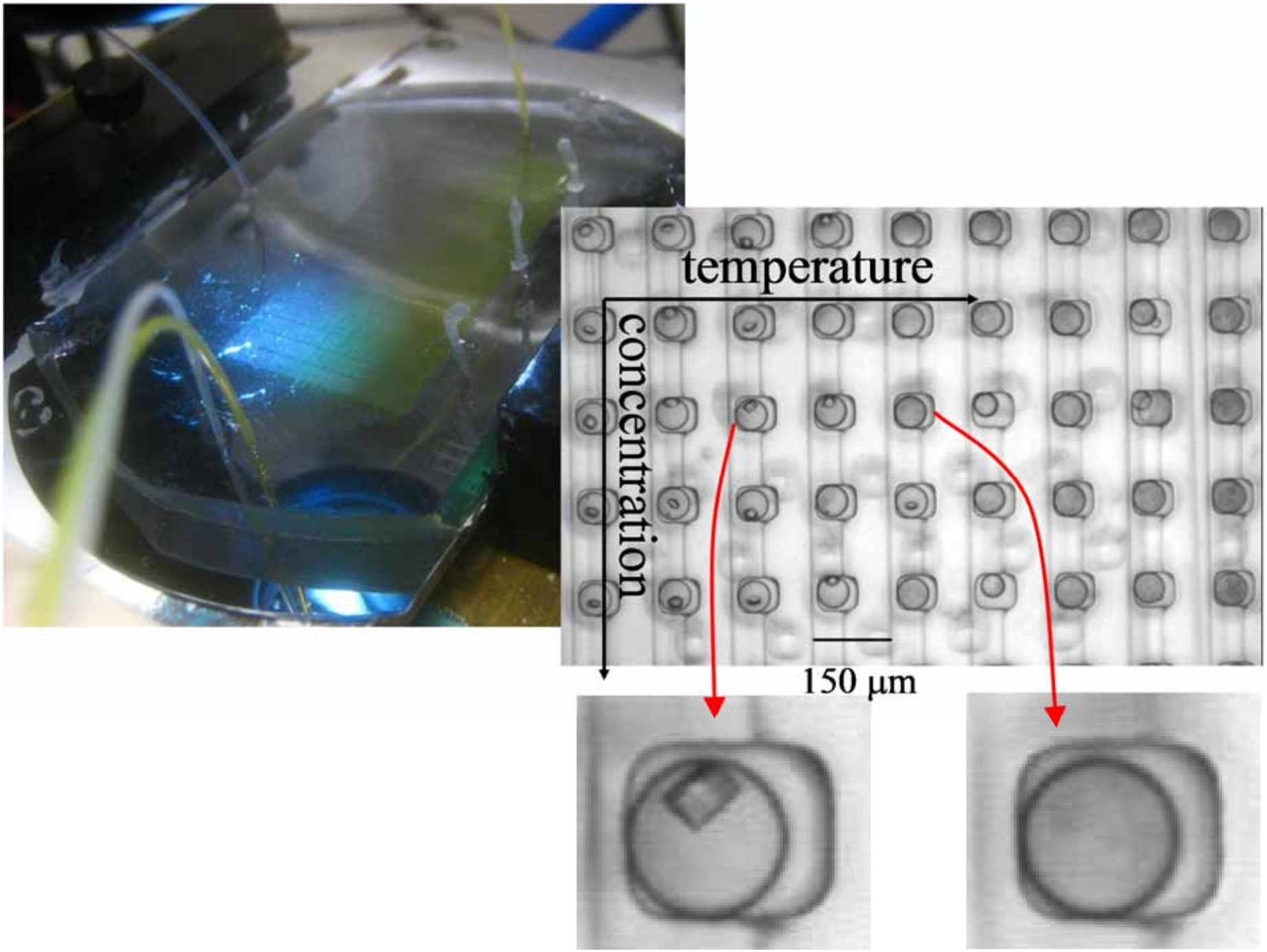




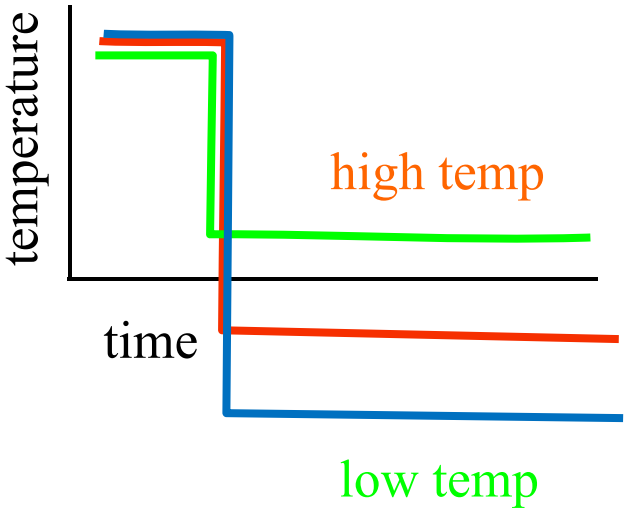
First generate concentration gradient

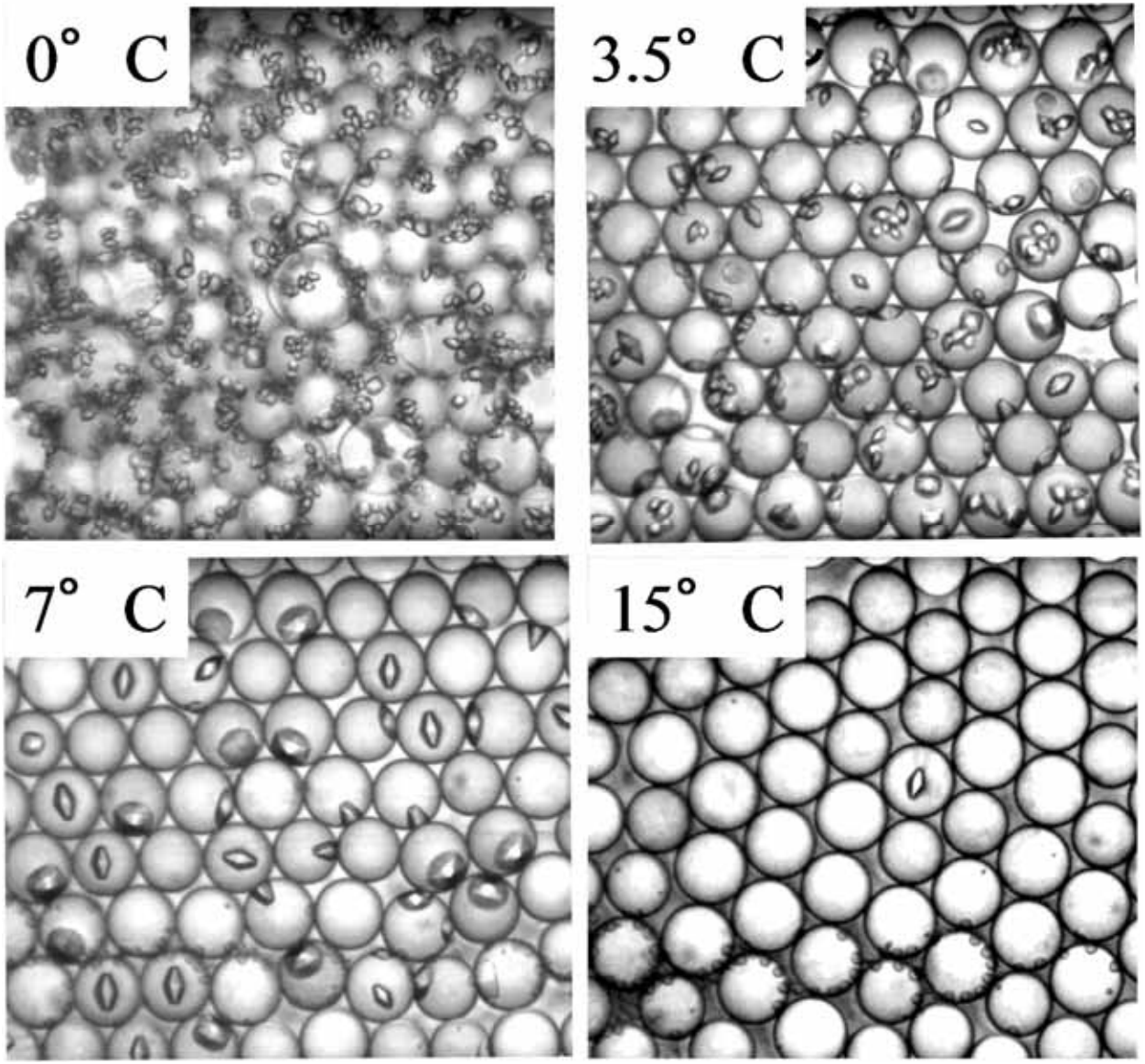
then

Generate temperature gradient


temperature
concentration
150 µm

Control kinetics

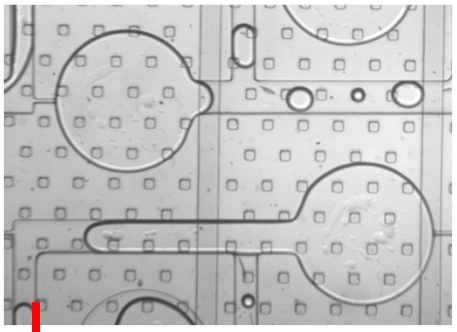




Vary temperature, concentration constant

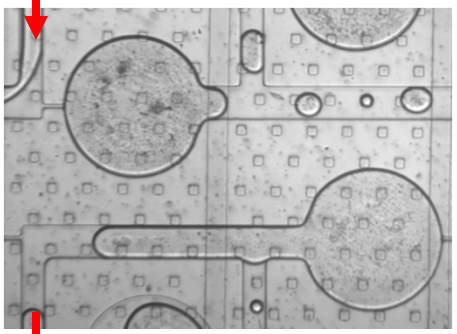
Decoupling Nucleation and Growth (Tempereature Control)

20°C (drop filling)

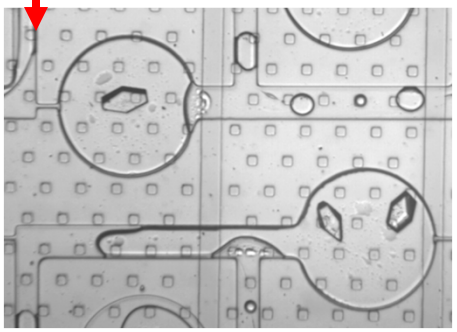


initial

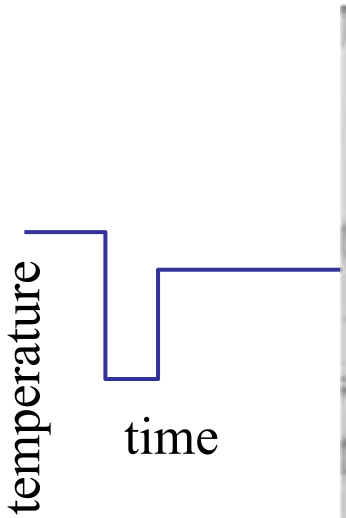
4°C for 0.5 hrs (nucleation)



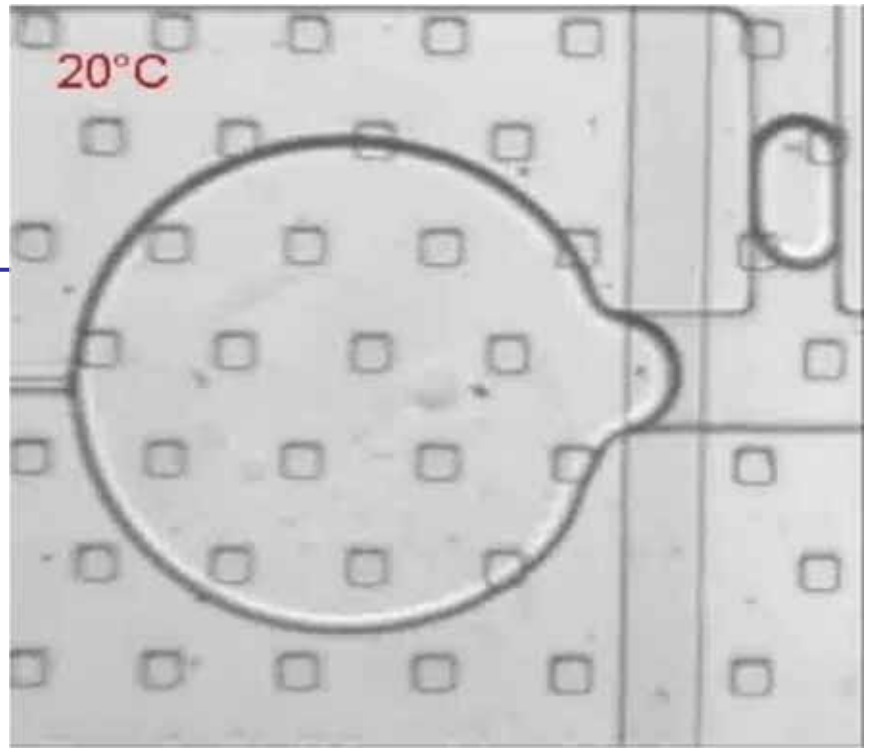
14°C (crystal growth)



final

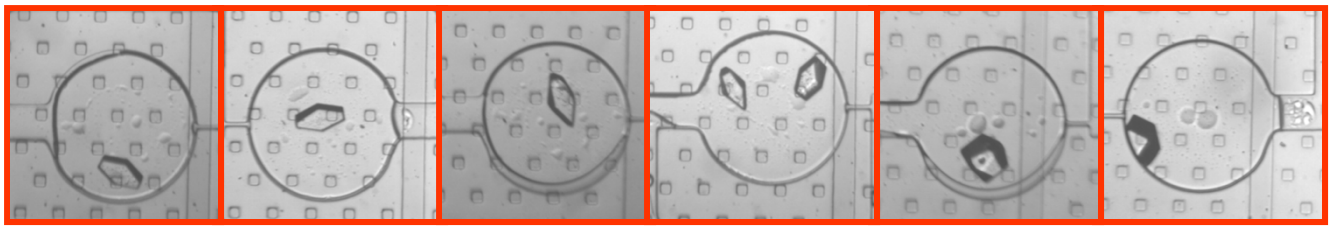


Measured date: Apr 29, 2009
Oil: FC-43, Surfactant: 12% Tridecafluoro-1-Octanol,
Aqueous: Lysozyme 75 mg/ml, 0.05M NaAc, pH 4.5
0.5M NaCl

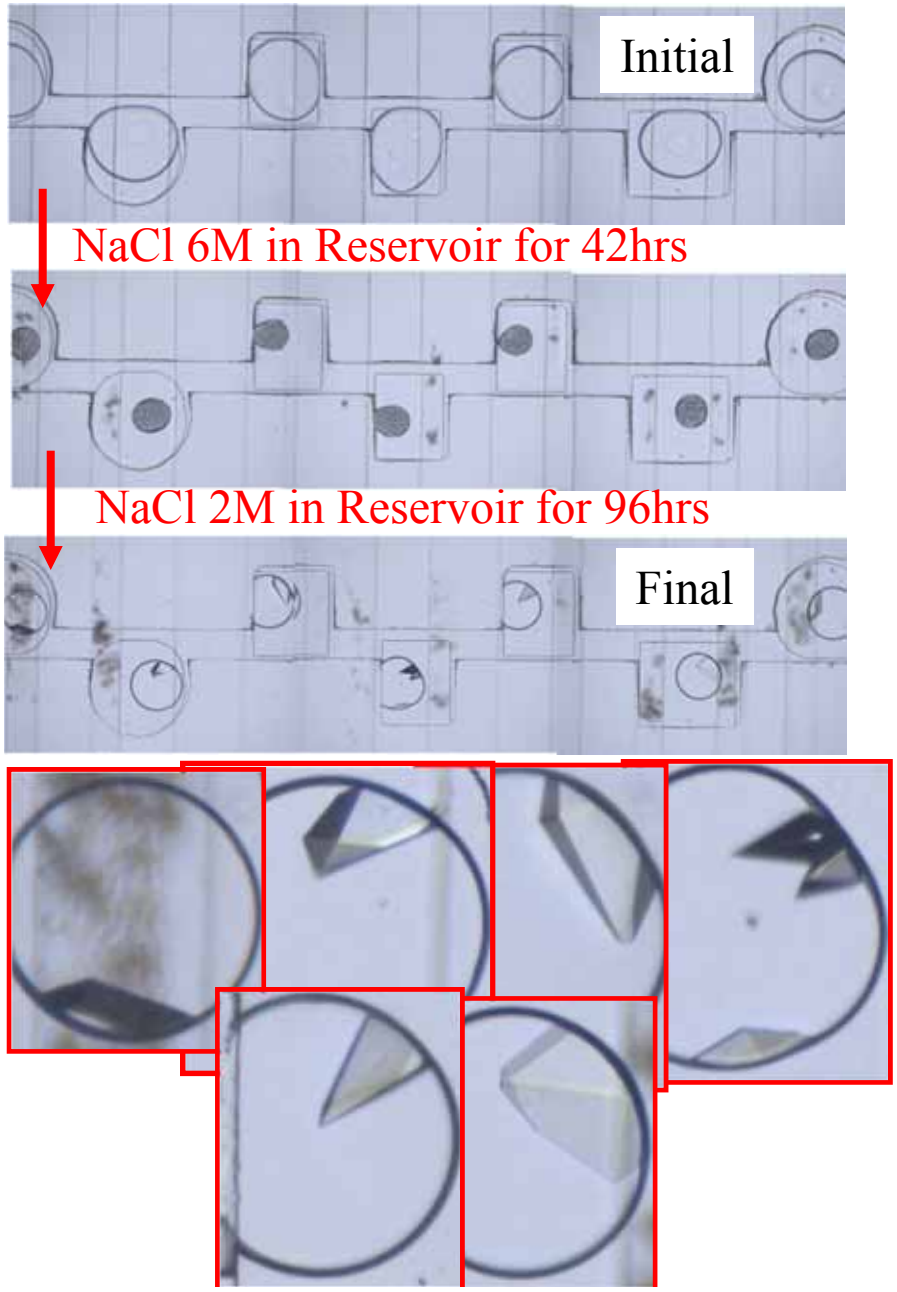


500 um

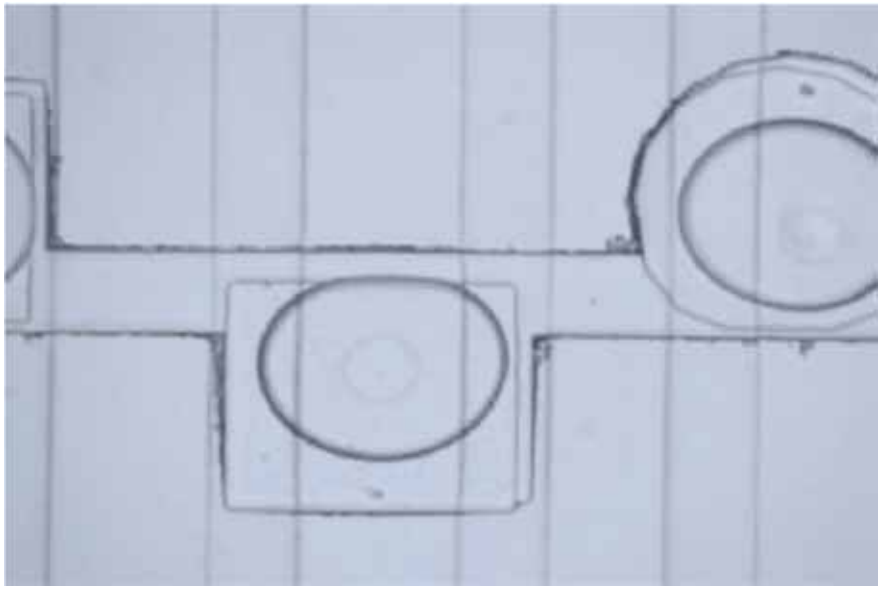
time lapse: 20 hours



Decoupling Nucleation and Growth (Composition Control)



Time lapse: 4 days



300 μm

PEG / lysozyme

PHYSICAL REVIEW E 78, 041402 (2008)

Crystallization and gelation in colloidal systems with short-ranged attractive interactions

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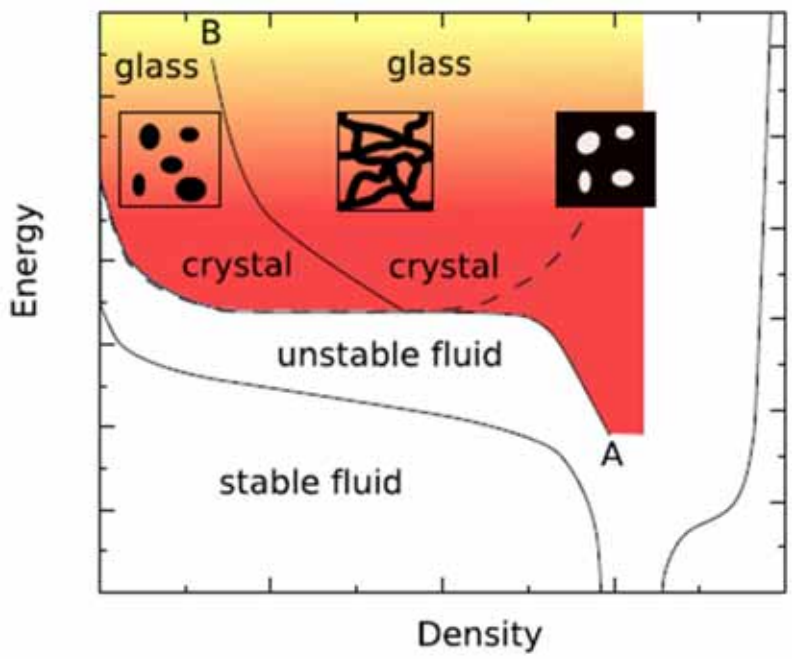
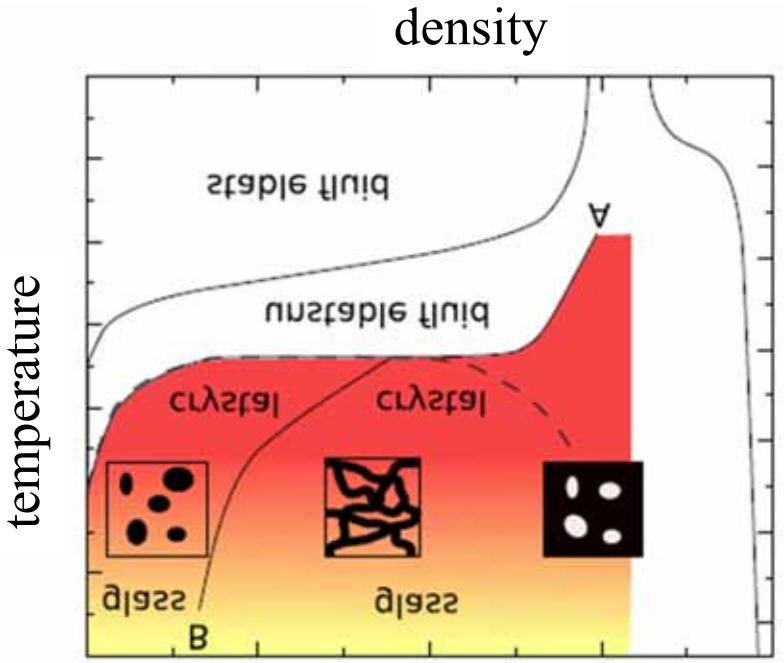
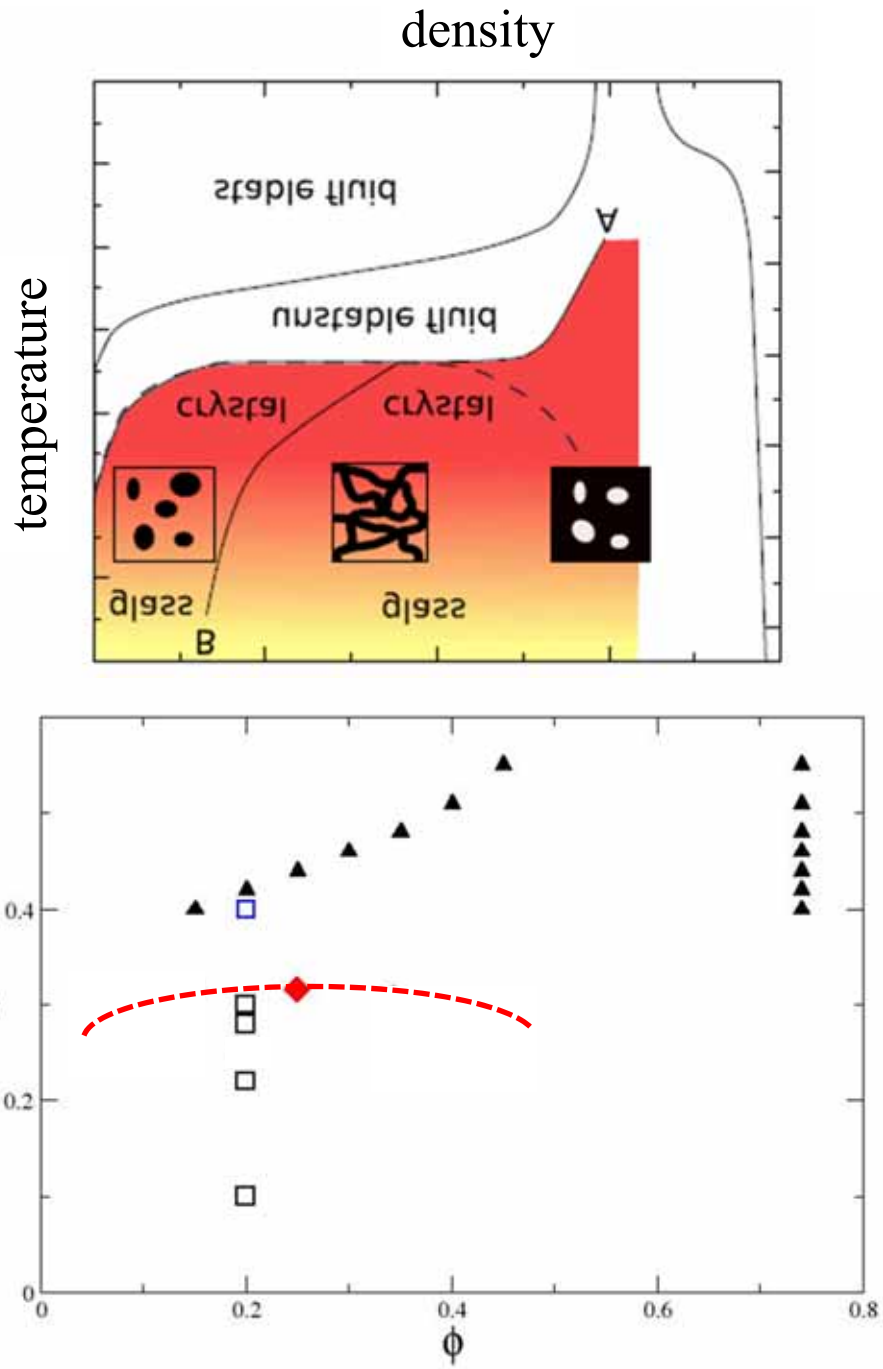
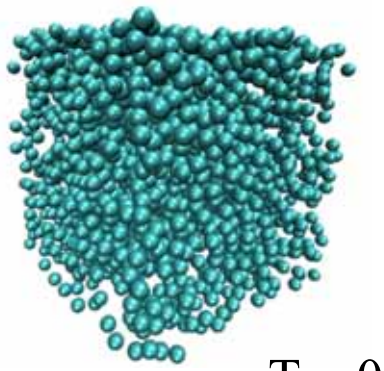
Debye Institute for NanoMaterials Science, Utrecht University, Princetonplein 5, 3584 CC Utrecht, The Netherlands

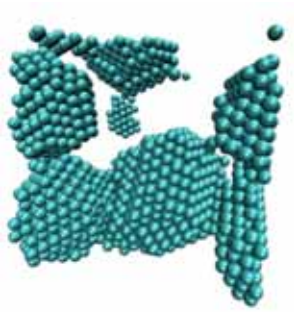




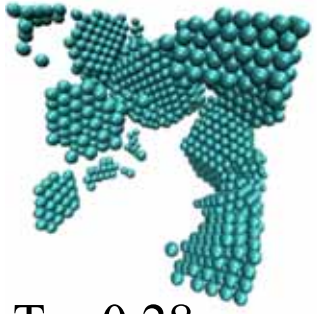




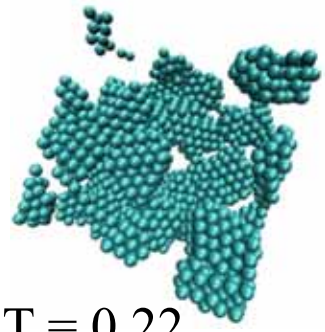
T = 0.4



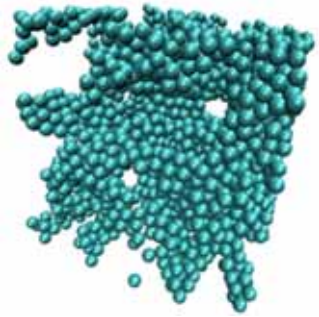
T = 0.3



T = 0.28

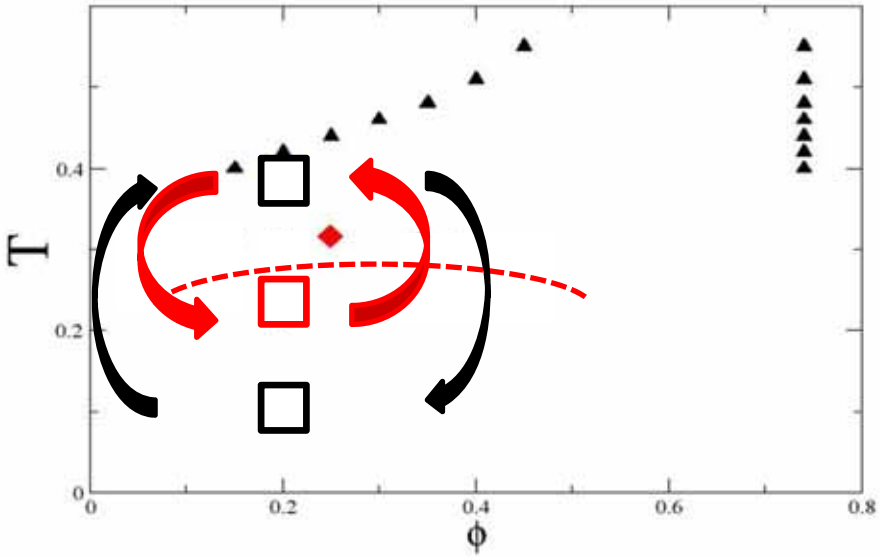


T = 0.22

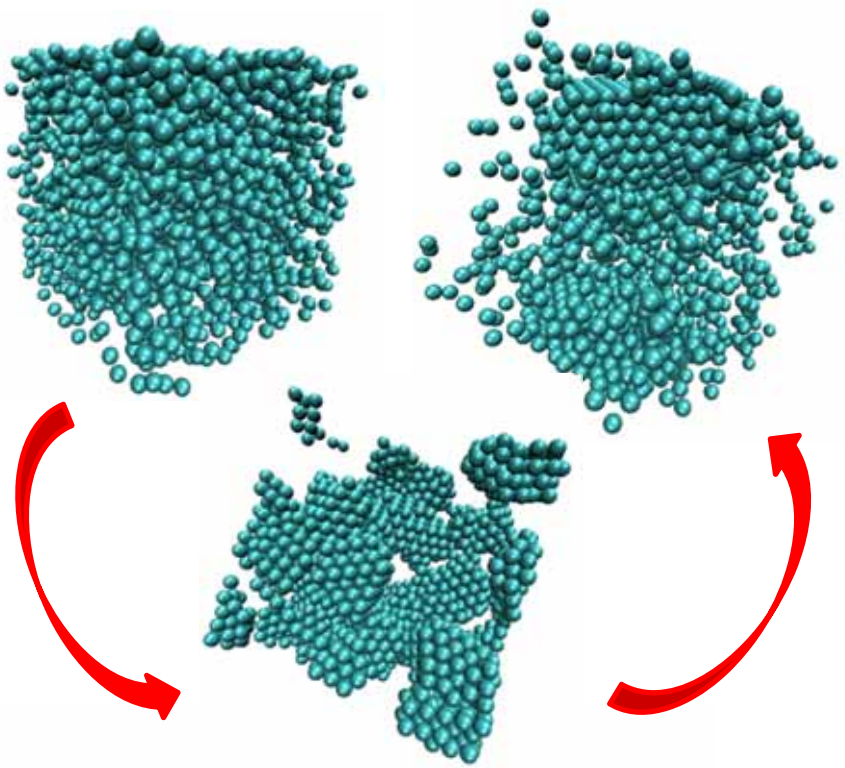


T = 0.1

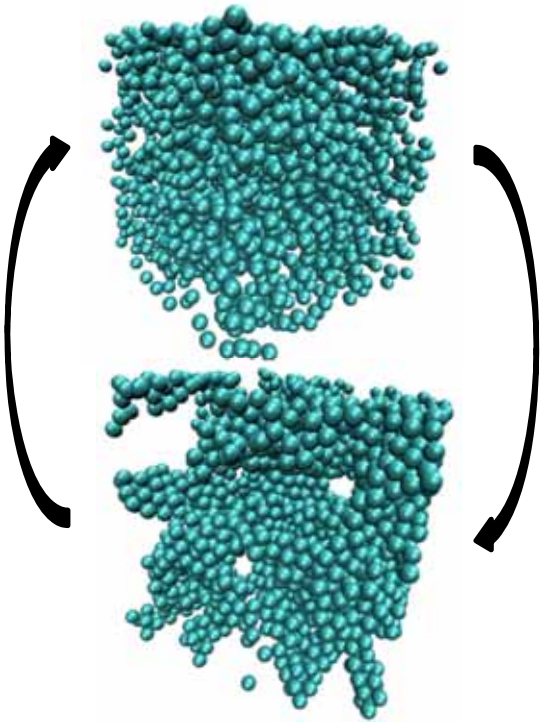
Nicolas Dorsaz



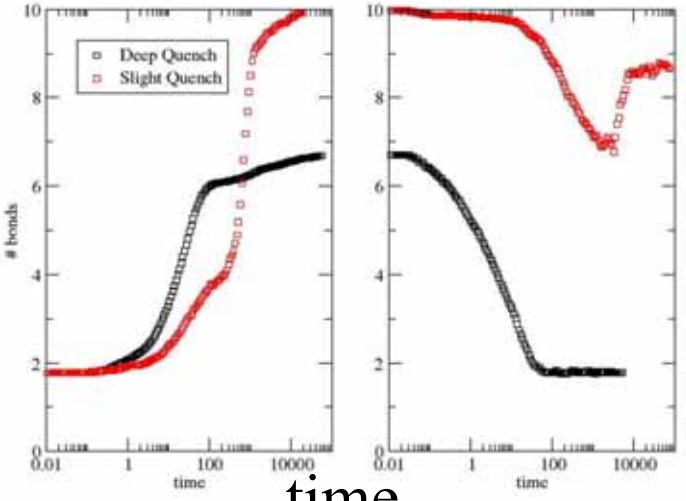
Slight Quench



Deep Quench

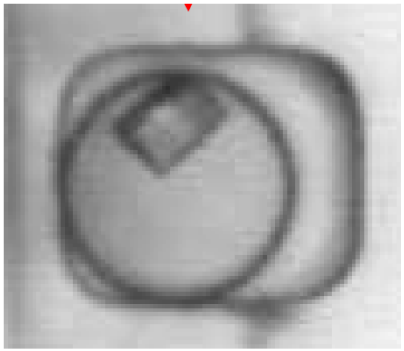


contacts

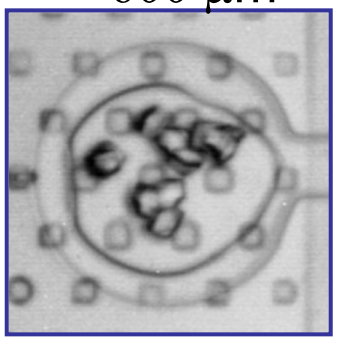


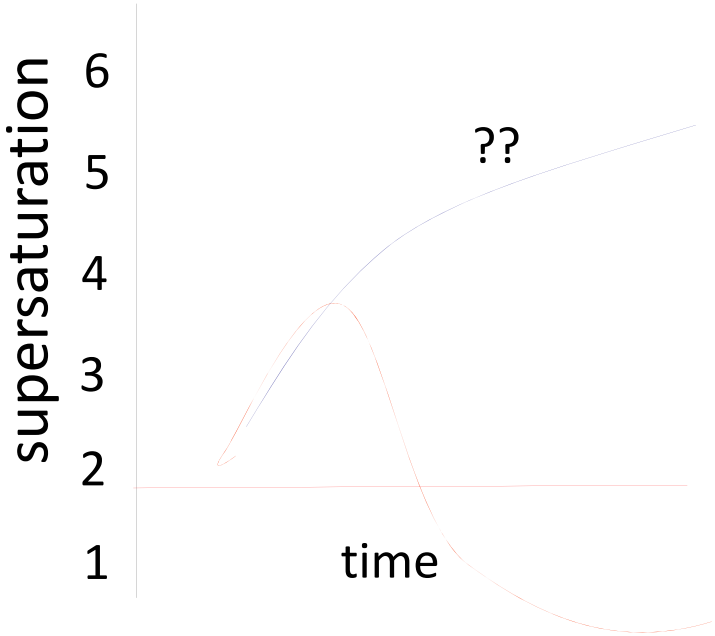
time

60 μm

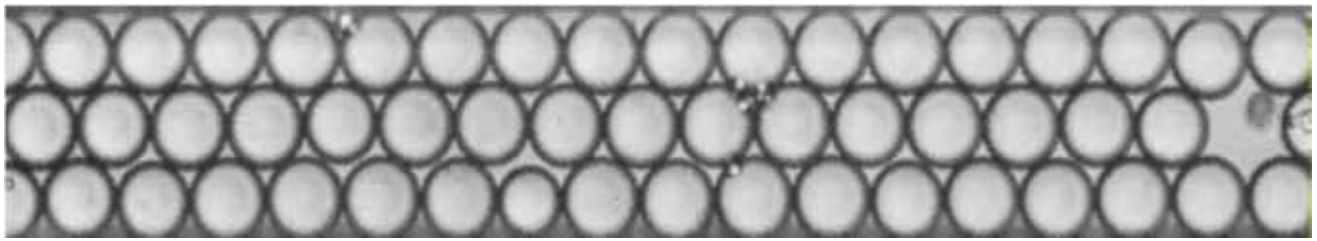


600 μm





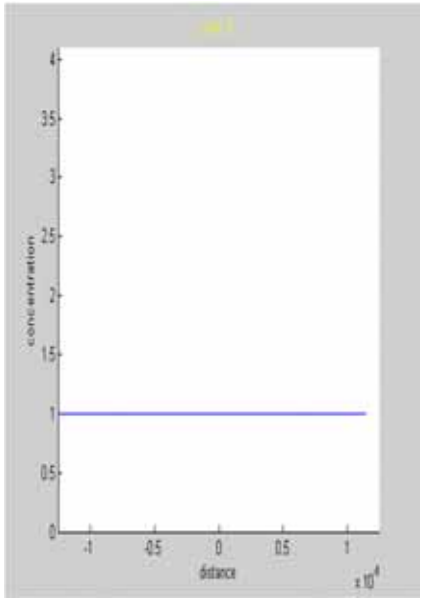
small drop

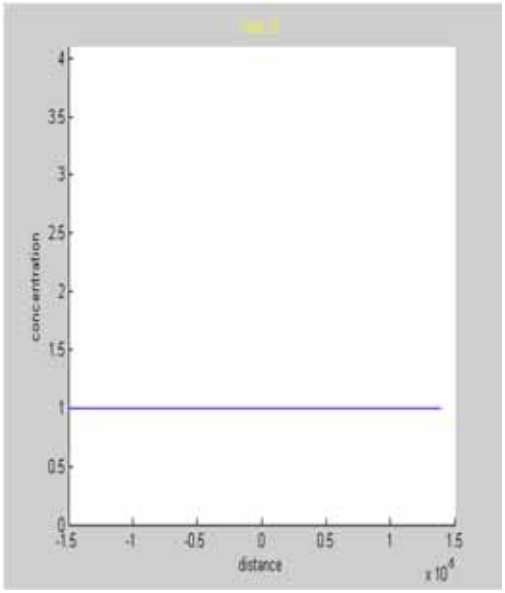


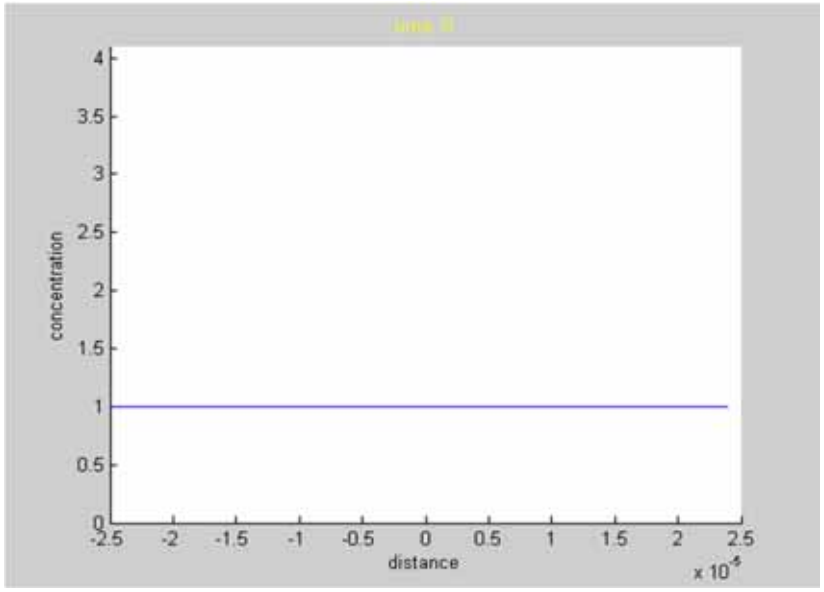
One crystal per drop when growth rate >> nucleation rate

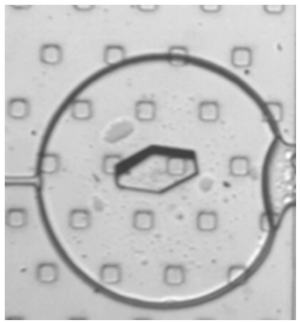
1D crystal nucleation and growth

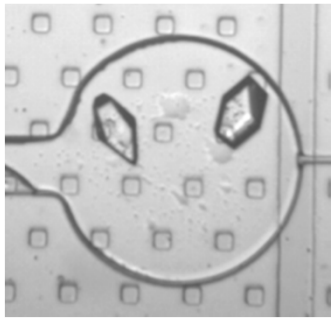
$$\partial c/\partial t = \nabla^2 c \qquad \partial c/\partial t = -v\nabla c$$

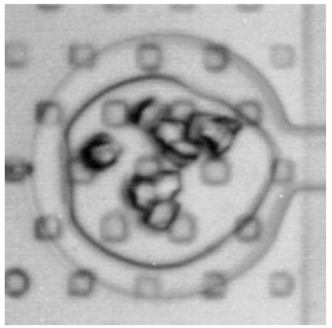




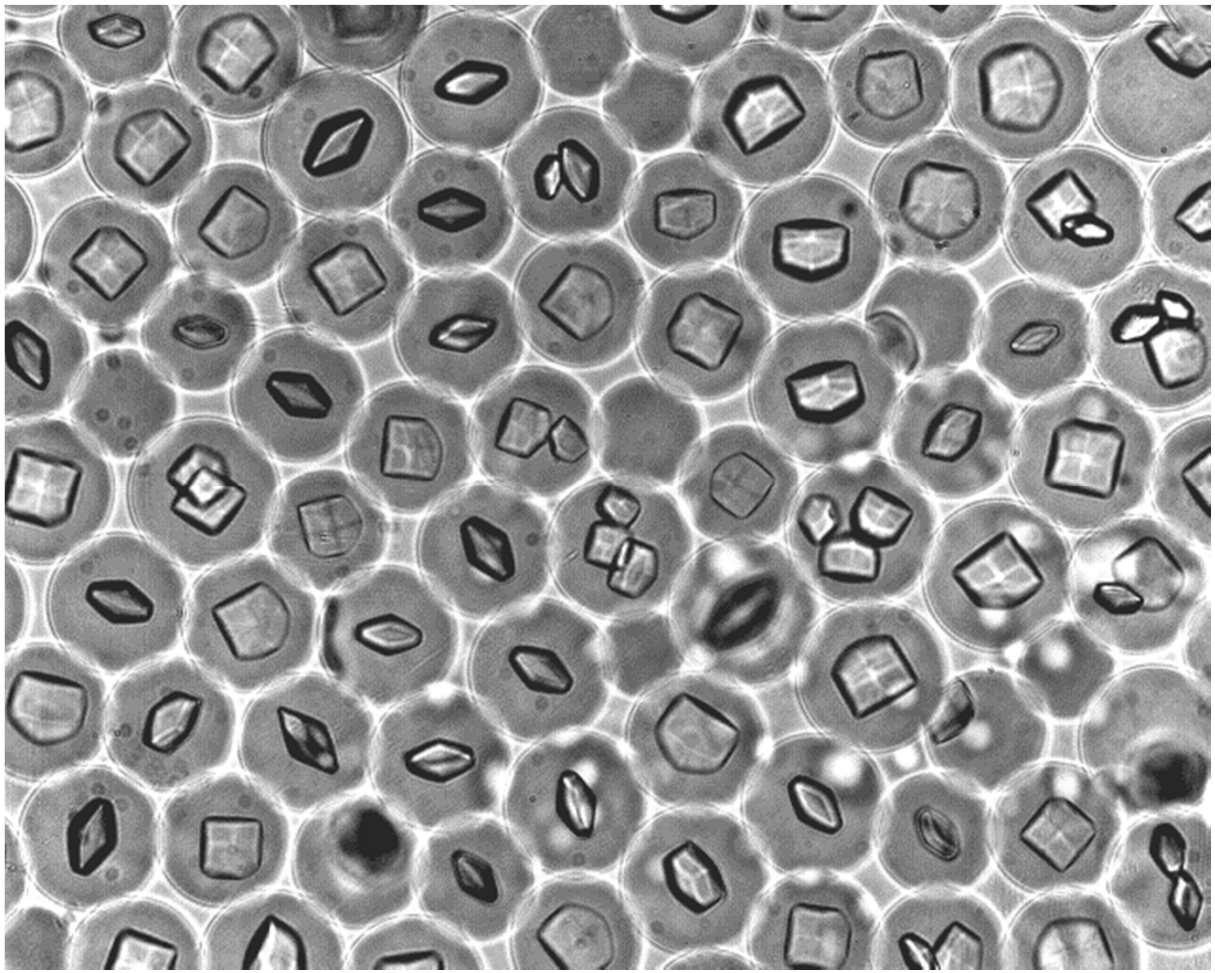






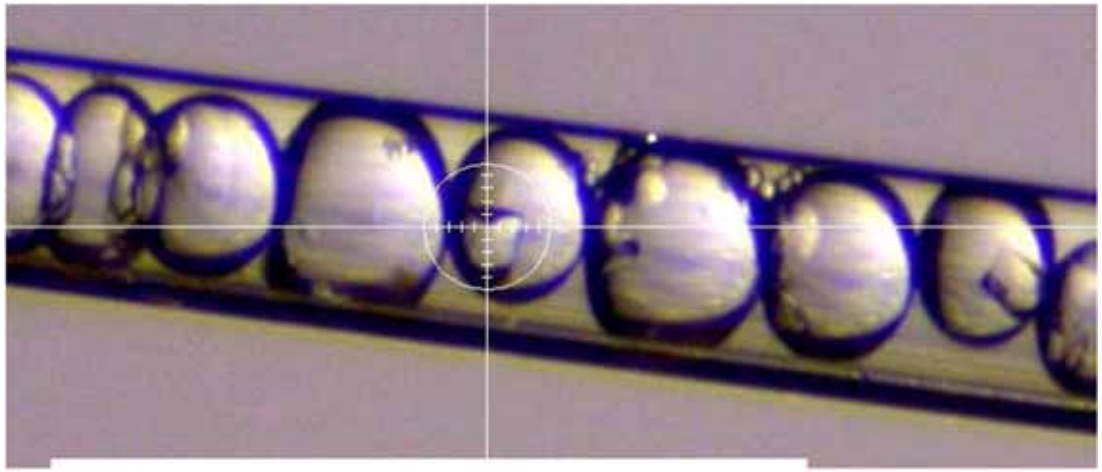


Lysozme.
Emulsion 50 μm does not crystallize at Room T, but bulk does.

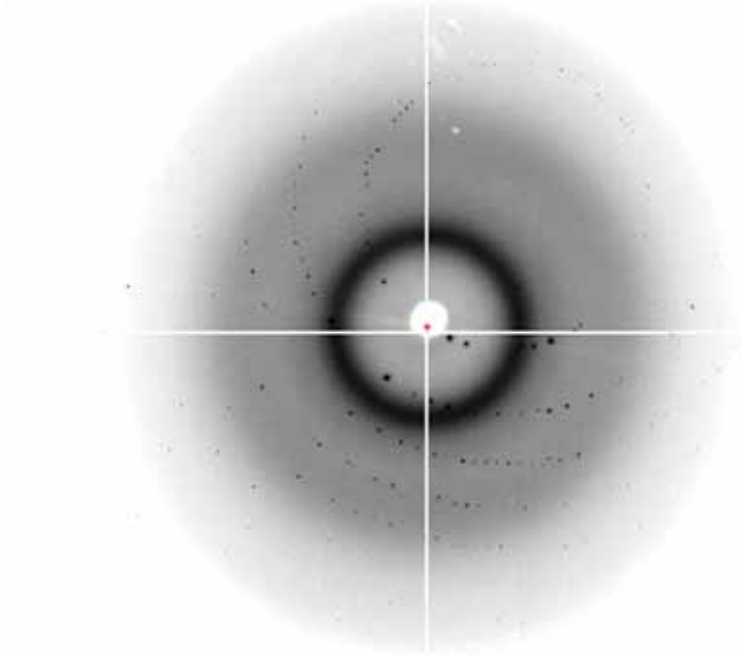


Optimal quench conditions

Shotgun Diffraction (ShDi)



Lysozyme crystal-bearing drops in a thin-walled 200 μm diameter glass capillary, mounted for data collection at CHESS. The central circle is 100 μm across.



Diffraction pattern from the lysozyme crystal above, taken with a 100 μm collimated monochromatic beam at CHESS beam line F1.

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Feasibility of one-shot-per-crystal structure determination using Laue diffraction

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Crystal size is an important factor in determining the number of diffraction patterns which may be obtained from a protein crystal before severe radiation damage sets in. As crystal dimensions decrease this number is reduced, eventually falling to one, at which point a complete data set must be assembled using data from multiple crystals. When only a single exposure is to be collected from each crystal, the polychromatic Laue technique may be pr
owing to its simultaneo
recorded reflections p
solving structures usin
crystals, data were colle
D1 station from groups
of the order of 20–3

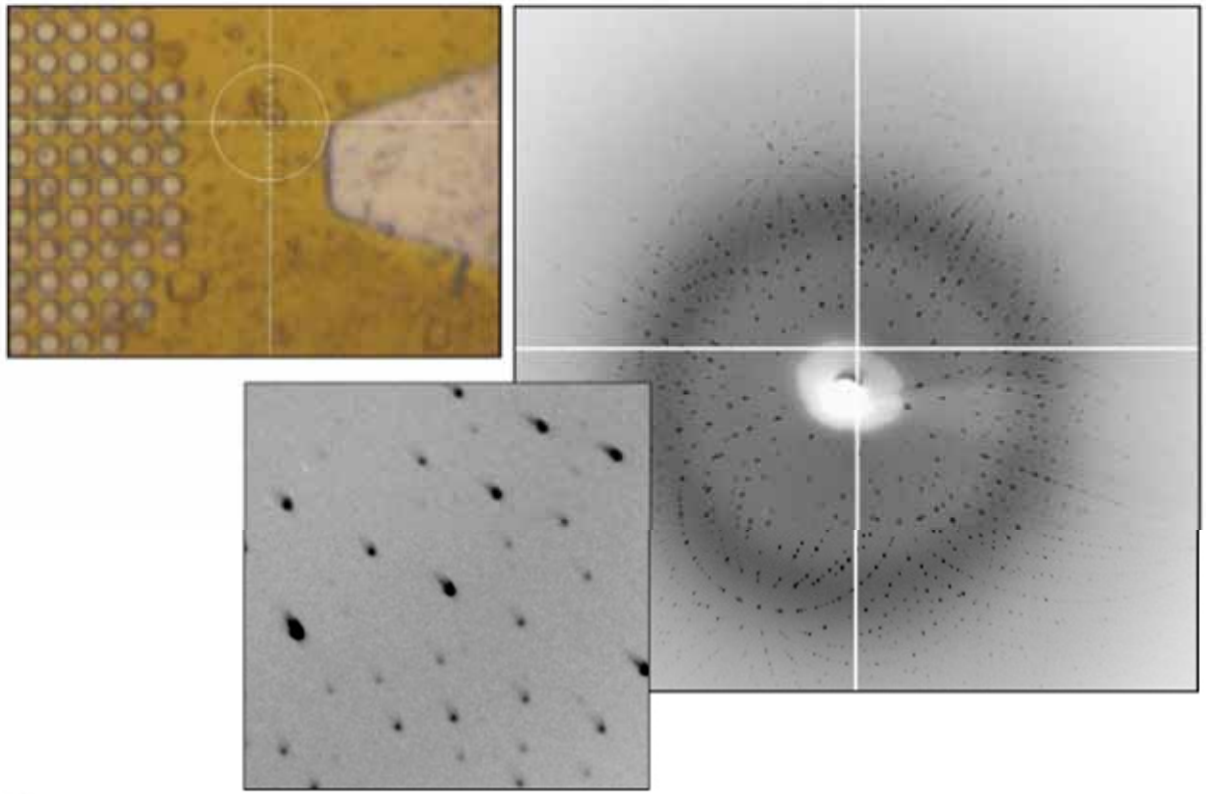

Figure 4
Laue diffraction from a lysozyme crystal. Left: crystal on a MicroMesh mount. The circle around the crystal is ~100 µm in diameter; the crystal is about 30 µm across. Right: diffraction pattern from the crystal with a 10 s exposure time. The inset shows well separated acceptably shaped spots.

PhaseChip

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