Crystallizing Membrane Proteins for Structure-Function Studies Using Lipidic Systems

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Recent Advances in Macromolecular Crystallization (RAMC)

Le Bischenberg Conference Centre, Strasbourg, France

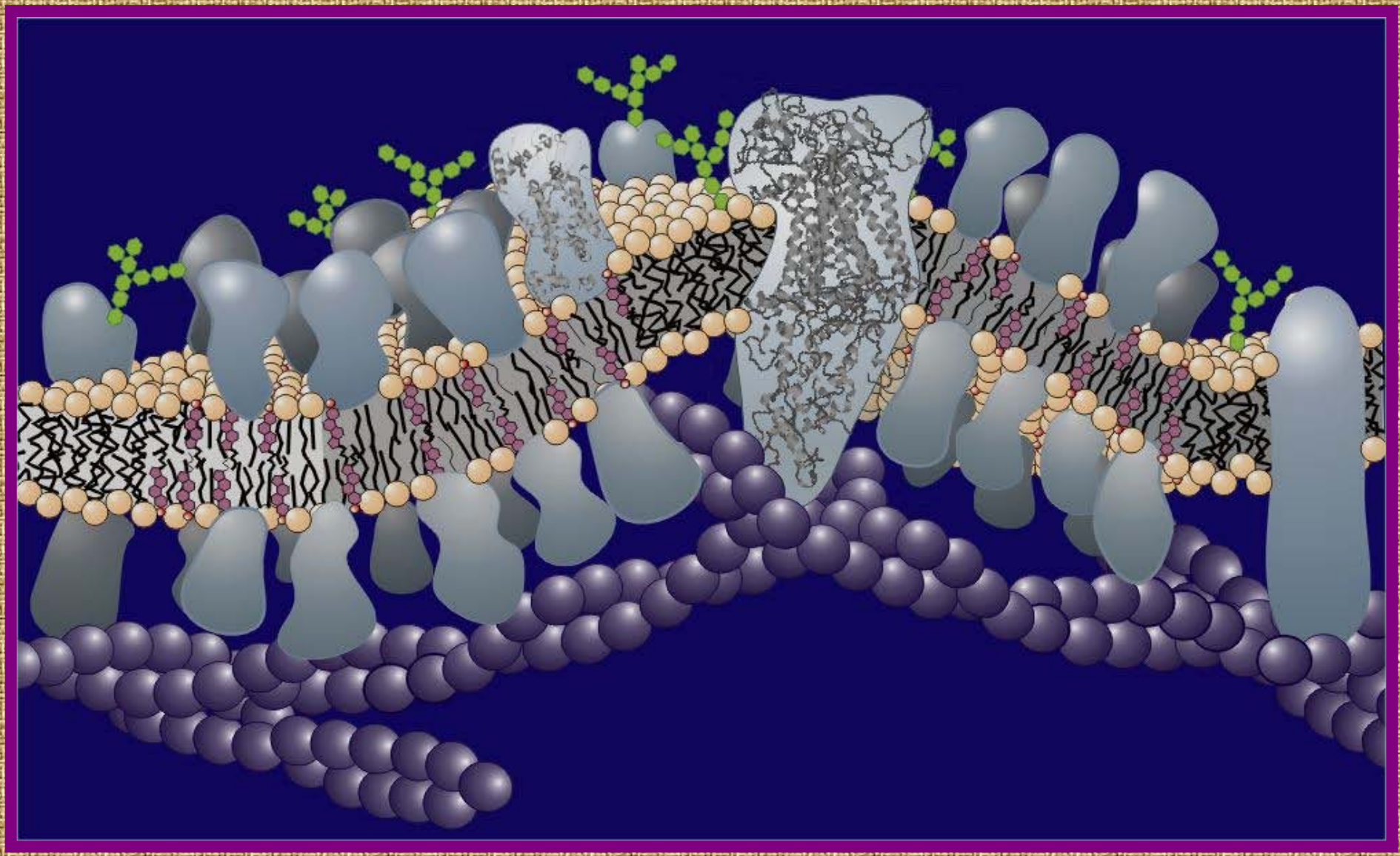
September 11 - 14, 2011

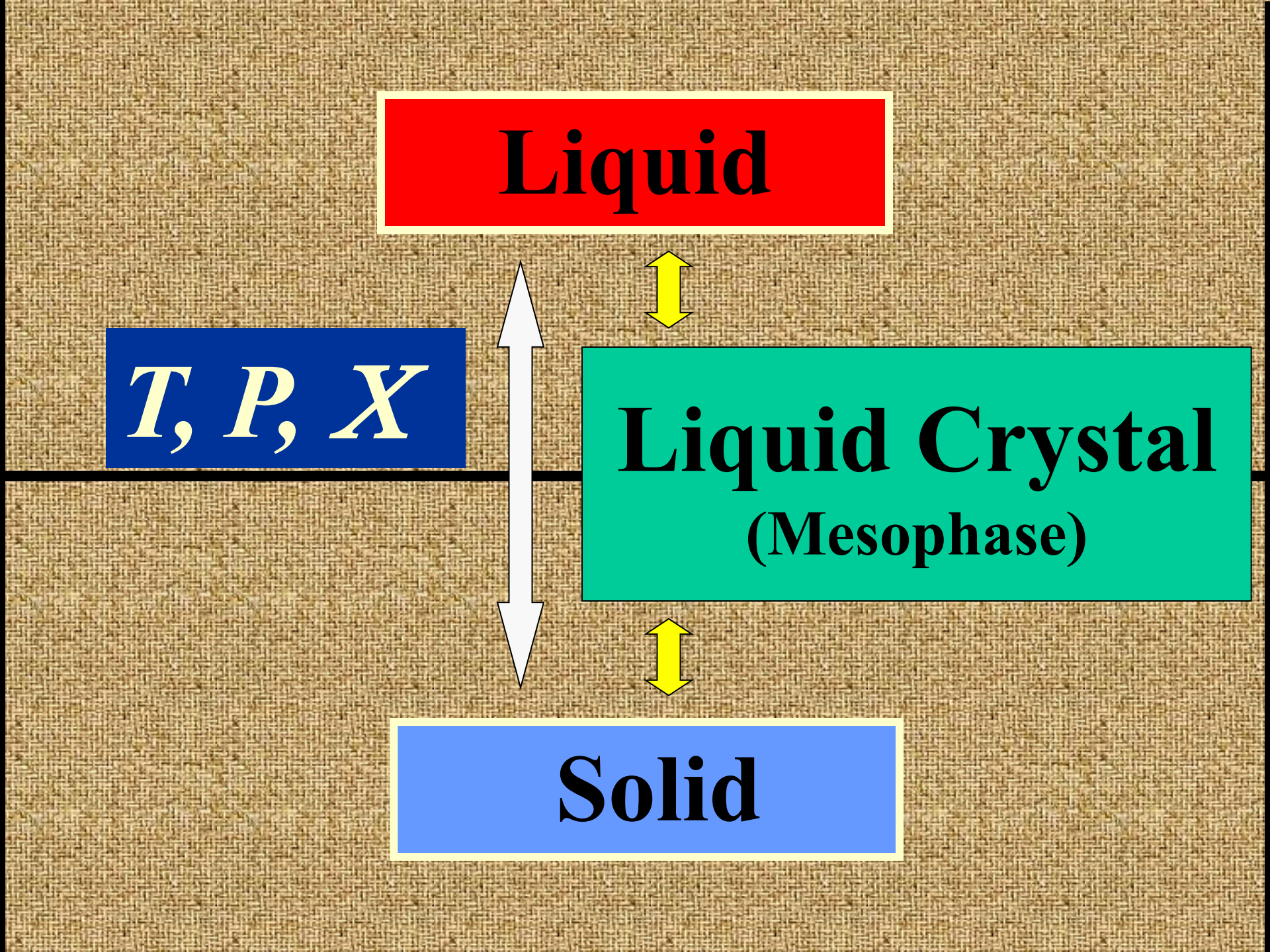
References

- Journal of Structural Biology *142*: 108 – 132
- Annu. Rev. Biophys. *38*: 29 - 51
- Nature Protocols *4*: 706 - 731
- JoVE *45*: id 1712 - online video

O v e r v i e w

- Membranes, Lipids, Phases
- Motivation: Structure - Function
- Membrane Protein Crystallization
- *In meso* Method, Manual Mode - Demo
- *In meso* Method, Robotic Mode

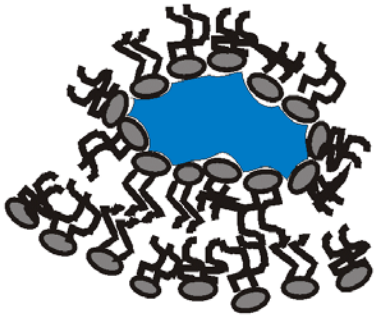



Liquid
T, P, X
Liquid Crystal
(Mesophase)
Solid

LIQUID

LIQUID CRYSTALLINE

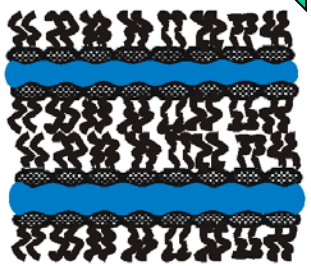
Fluid isotropic
FI



Lamellar crystal
L_c

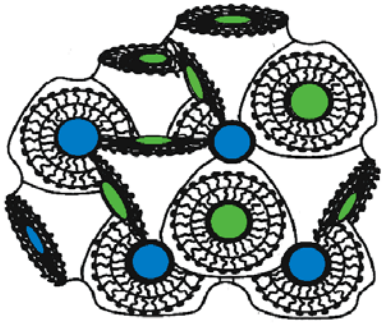
SOLID

Lamellar
liquid crystal
L_α

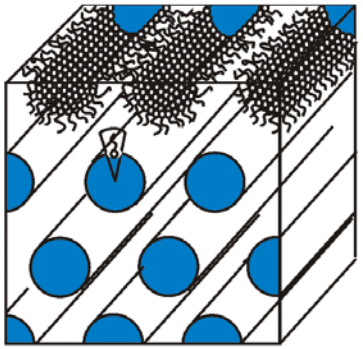


Biomembrane

Cubic
Pn3m

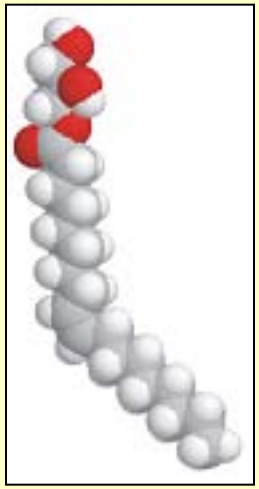


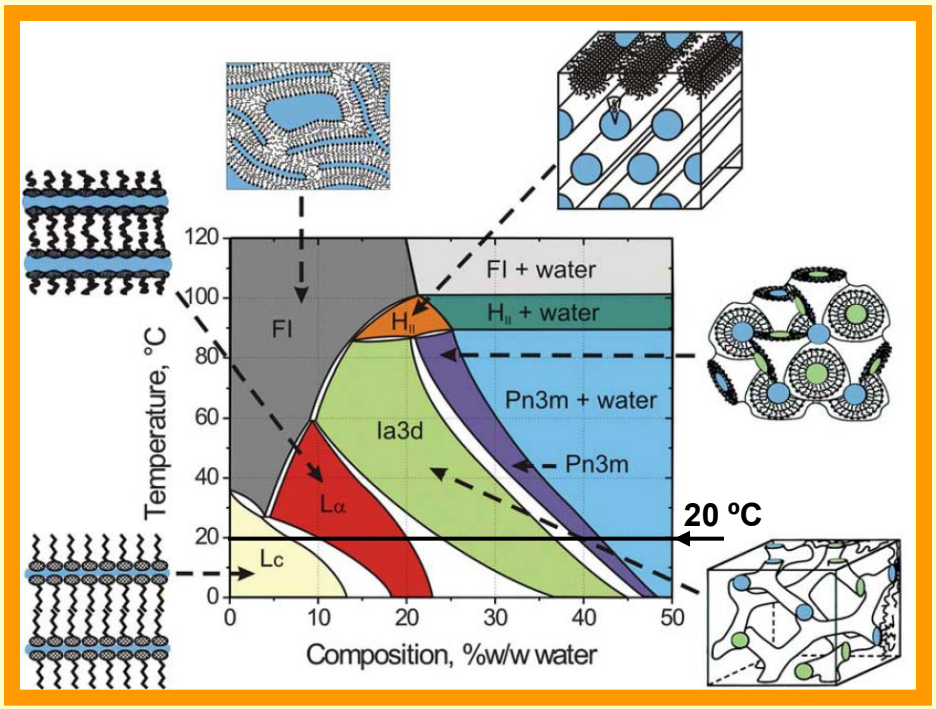
Inverted hexagonal
H_{II}



Lipids. Structure – Function

Structure →





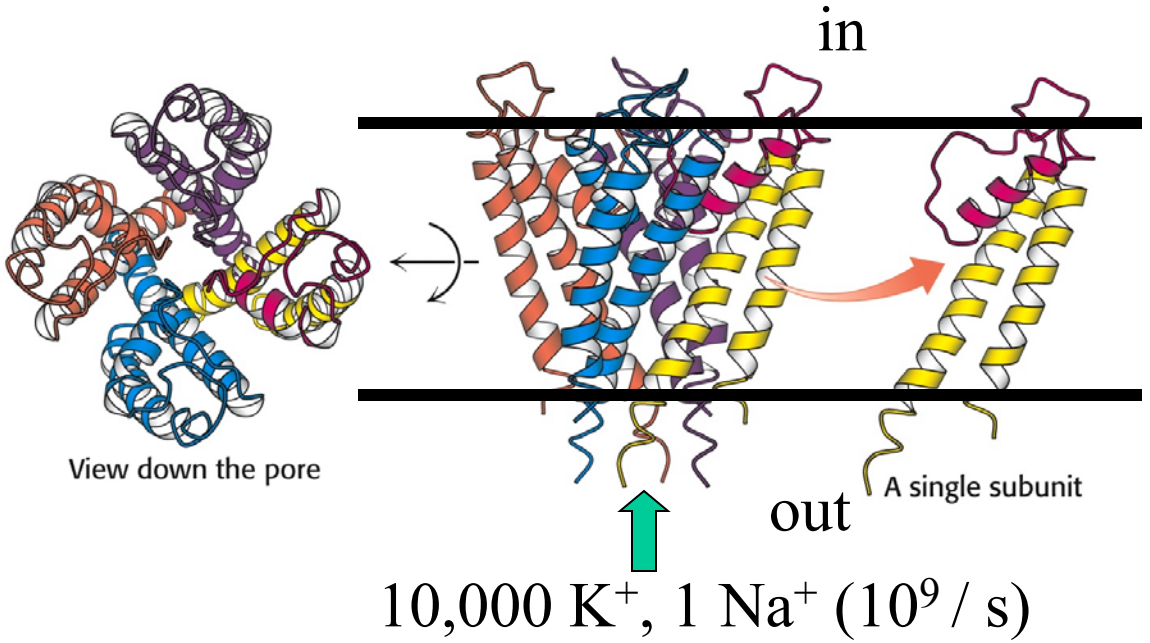
→ Function

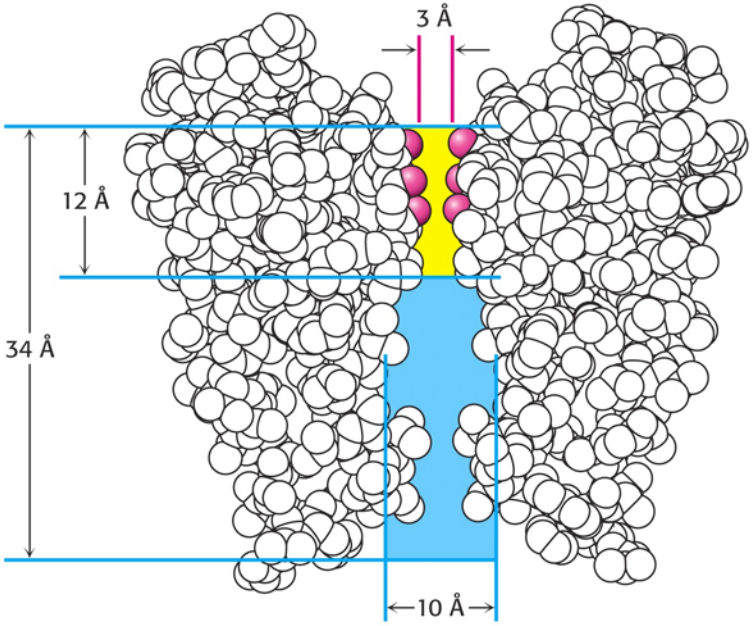
Membrane
Transport
Signaling
Fusion
Energy
Drugs
Foods
Cosmetics
.
.
.
Crystals

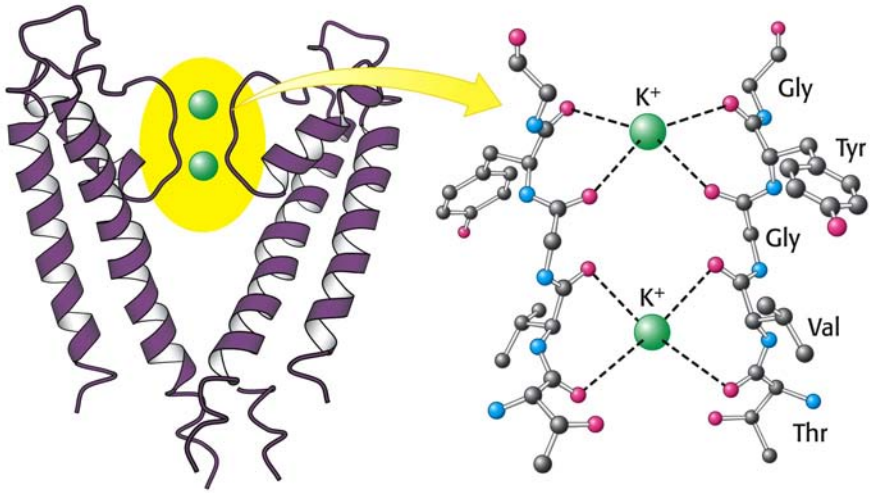
Phase Behavior
Microstructure, Rheology

Knowledge,
Rational Design

Proteins. Form and Function

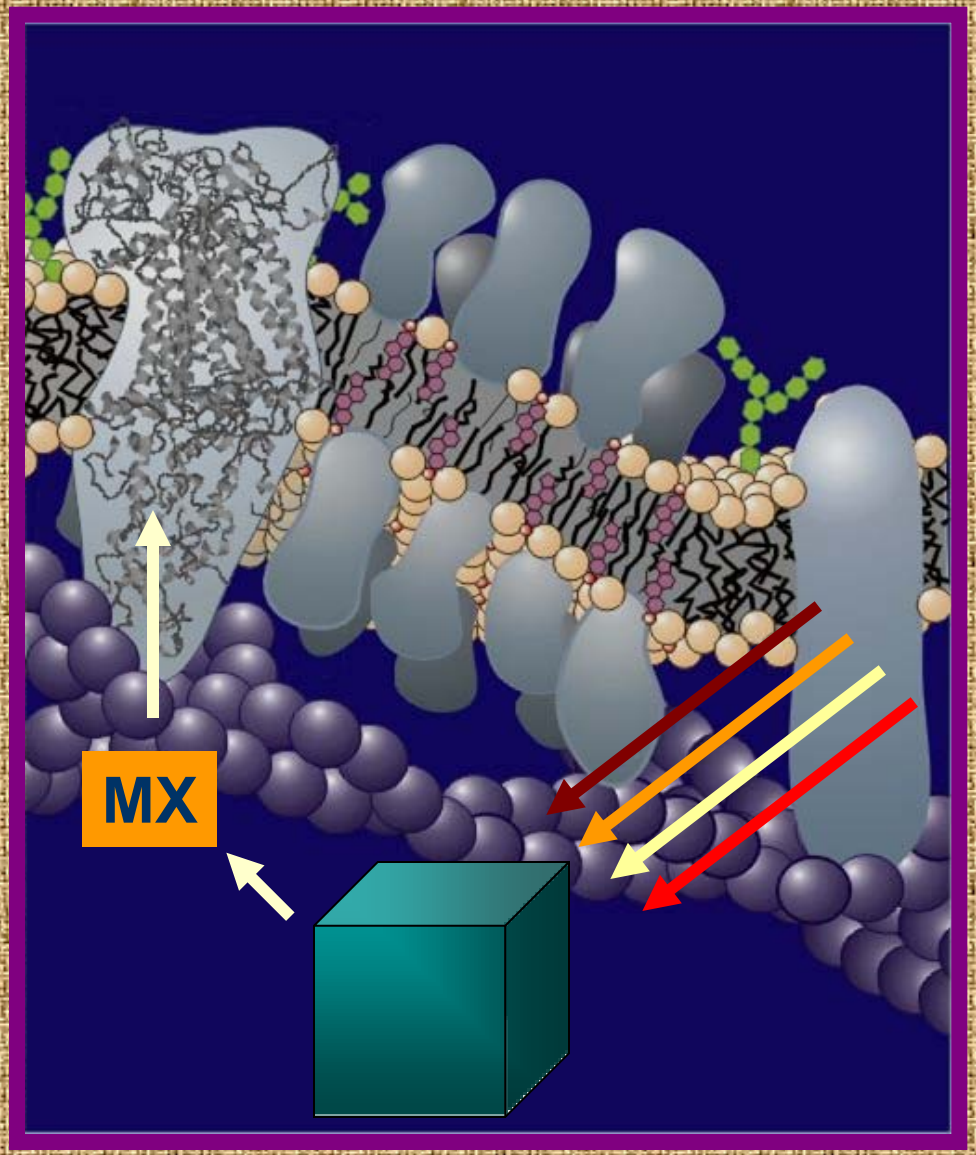






K^+ Channel, Doyle *et al*., 1998. [Stryer *et al*. Biochemistry (2002)]

Structural and Functional Biology of Membranes



Crystallization Methods

Vapor Diffusion
- hanging drop
- sitting drop

Batch

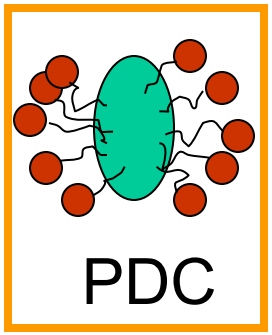
Dialysis

Bilayer Methods
- bicelle
- vesicle fusion
- cubic phase

Evaporation

Interfacial Diffusion

Bicelle Crystallization Method



Protein Solution
(on ice)

10 mg bR/mL

4

1

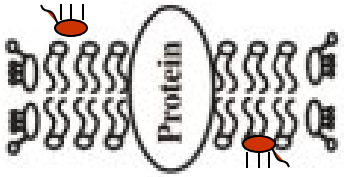
Mix

Lipid/amphiphile
mixture (on ice)

40% DMPC/CHAPSO (3/1)

DMPC vesicles + CHAPSO

Vortex, Sonicate
Thermal cycling

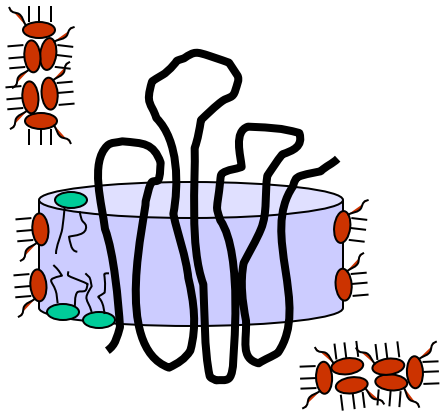


8 mg bR/mL

Precipitant

Homogenous protein/bicelle
mixture (on ice)

Mix



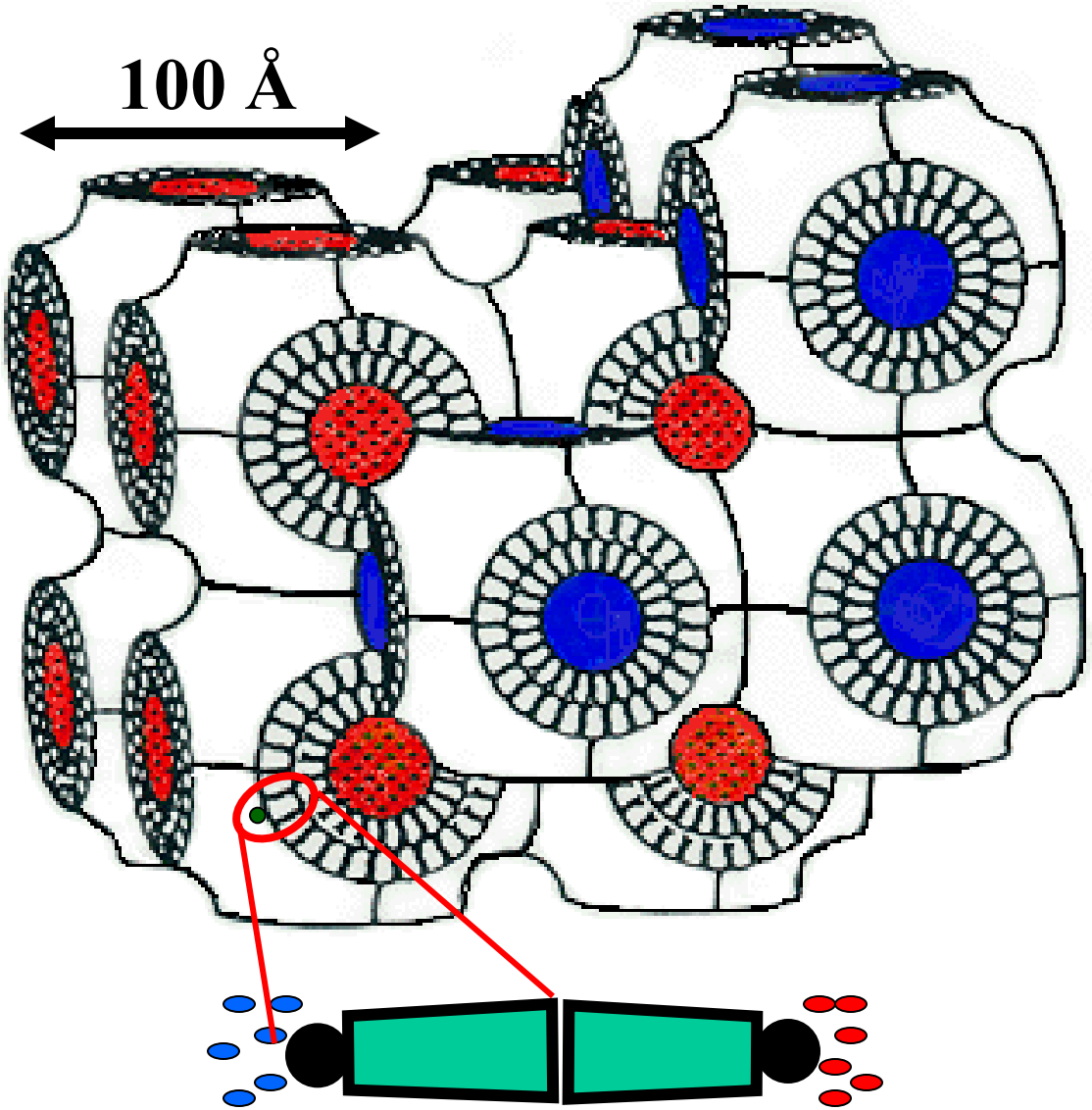
Hanging Drop Vapour Diffusion

Invert coverslip and let warm
to room temperature or to 37°C
to form bicellar, or perforated
lamellar phase.

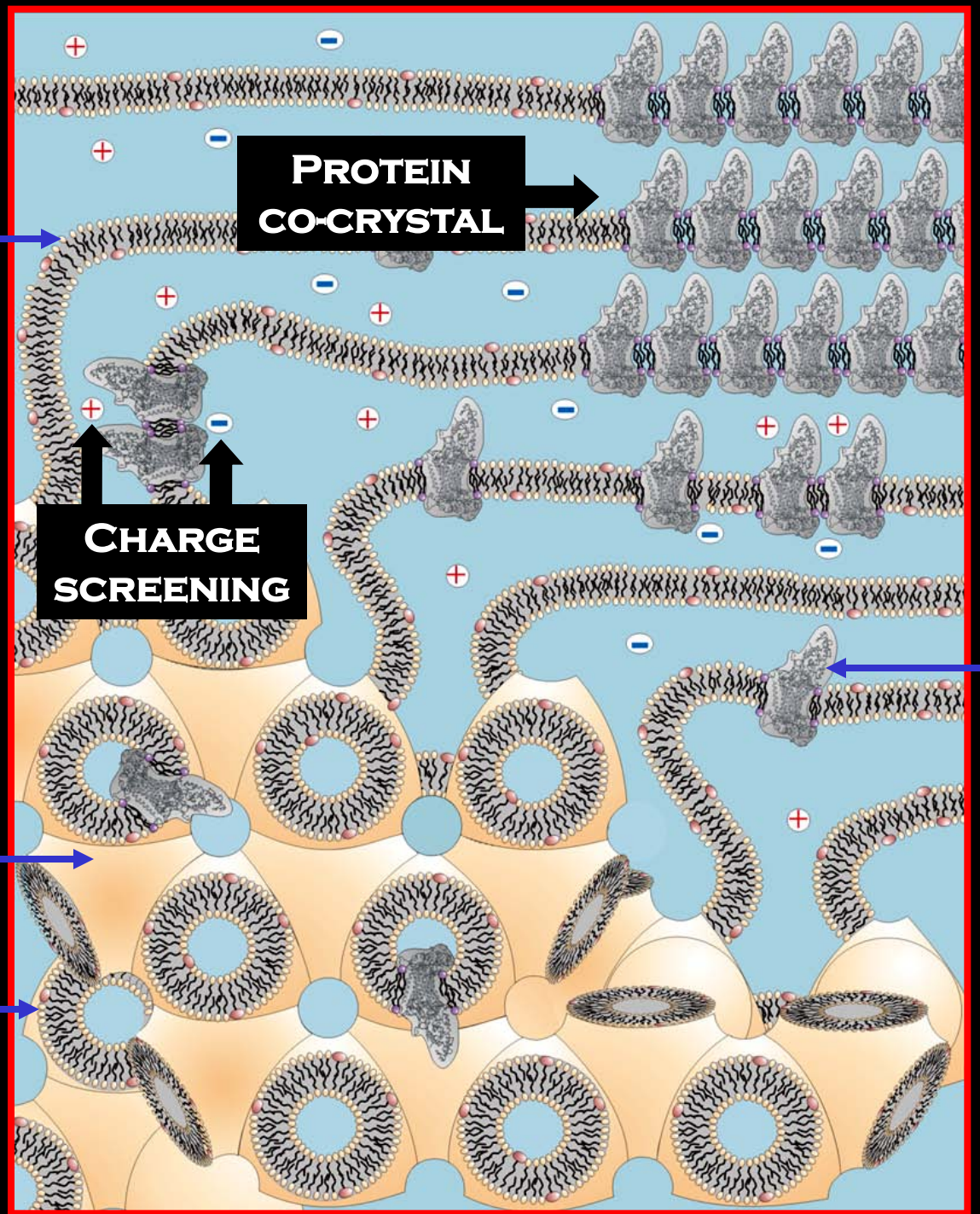
viscous

Faham & Bowie, J. Mol. Biol. 316:1-6, 2002

Cubic Phase




Bacteriorhodopsin crystallized
in lipidic cubic phase
20 µm



Lamellar portal

Crystalline array

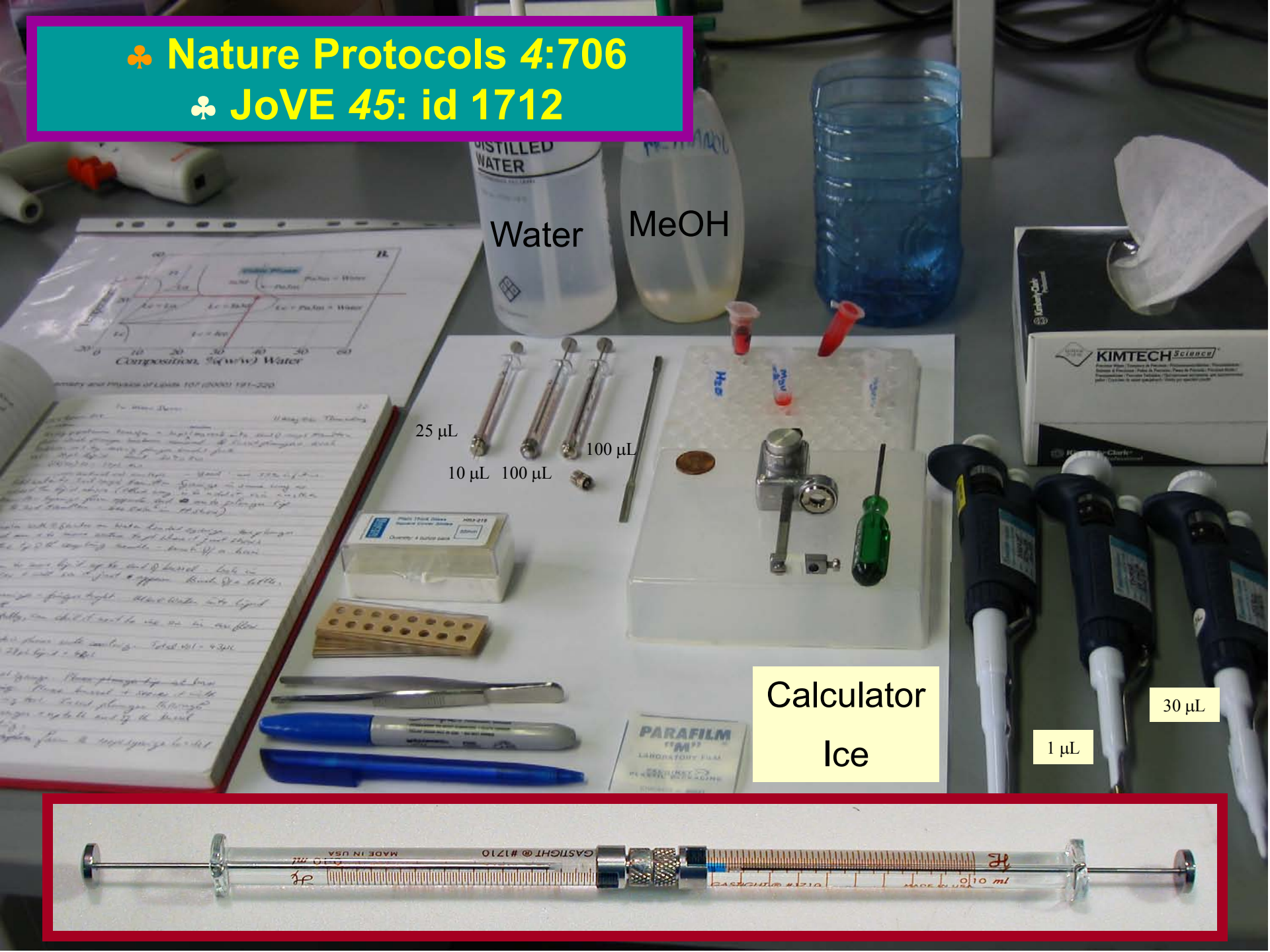
Reconstituted protein

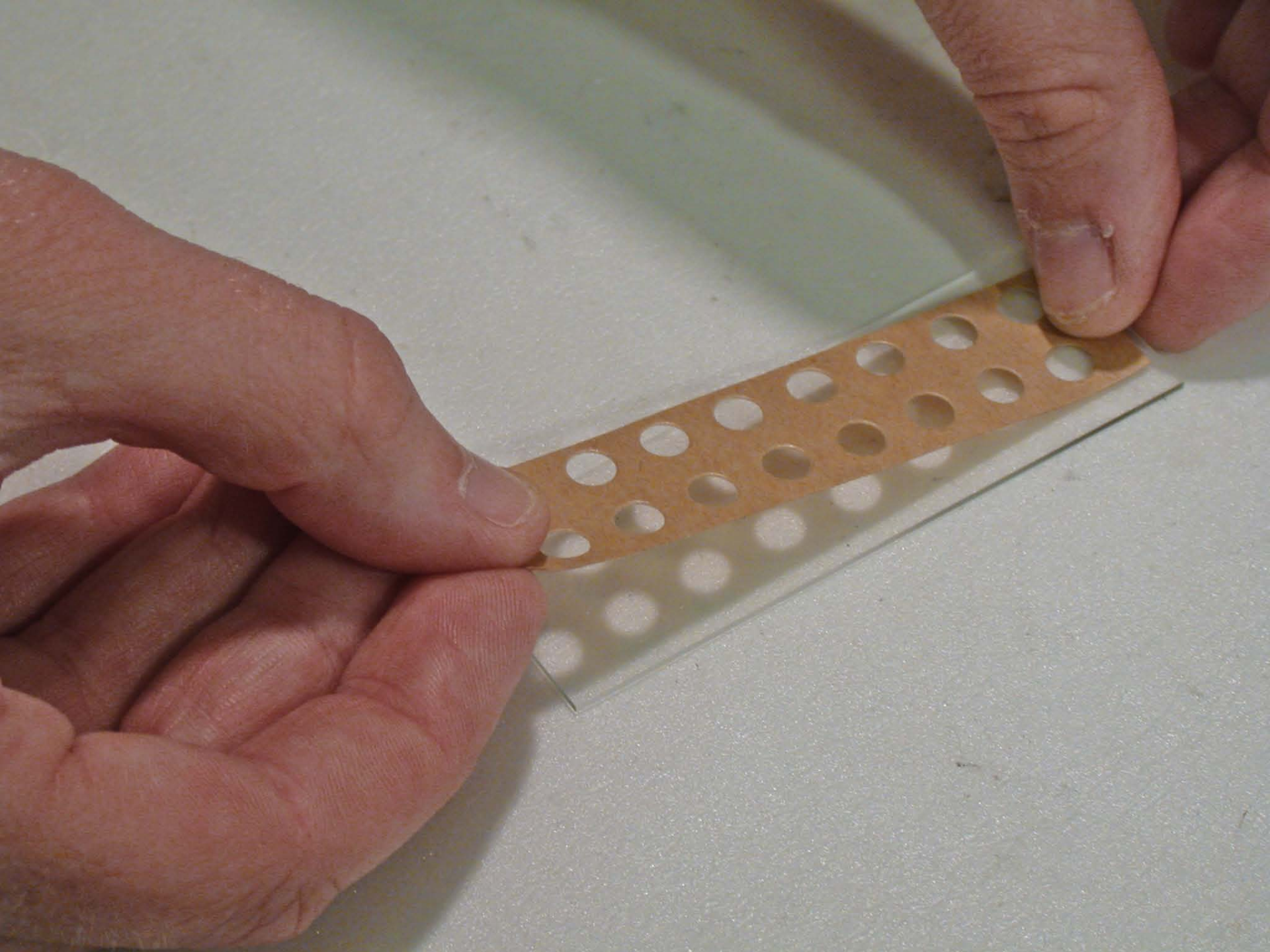
Cubic phase

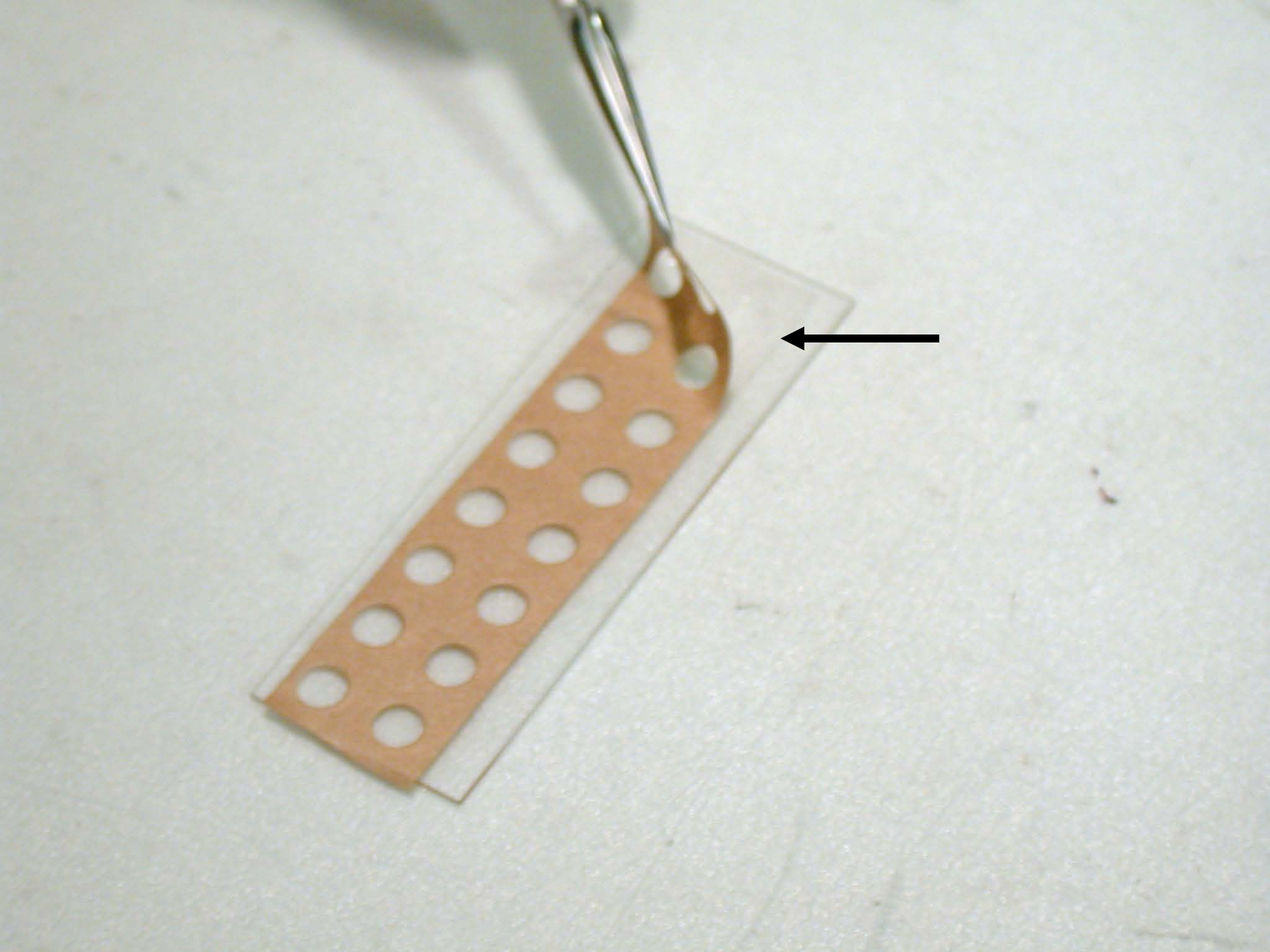
Lipids

J. Struct. Biol. *142*:108-132

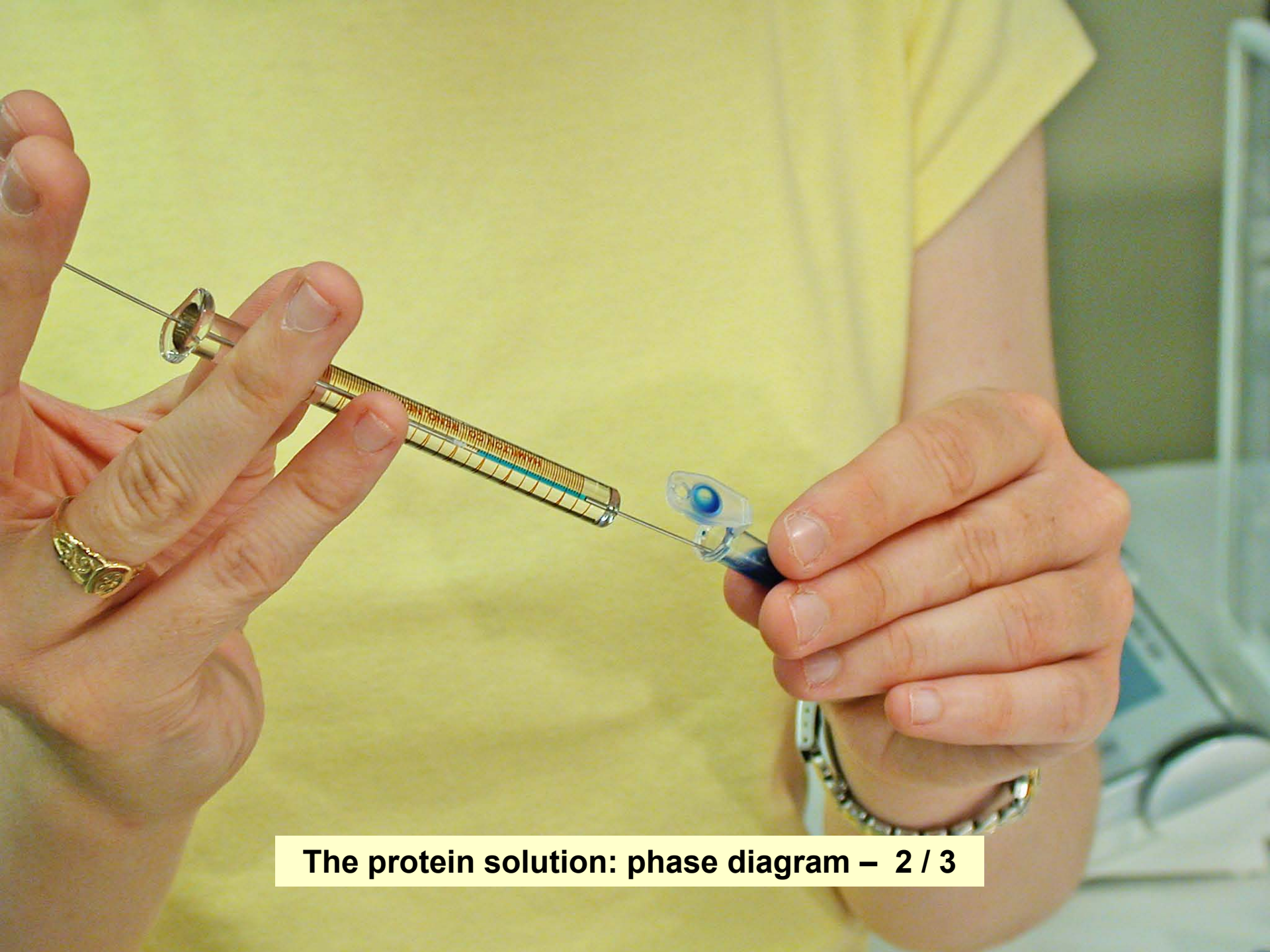
Cryst. Growth Des. *8*:4244-4254


♣ Nature Protocols 4:706
♣ JoVE 45: id 1712
Water
MeOH
25 µL
10 µL
100 µL
100 µL
Calculator
Ice
30 µL
1 µL



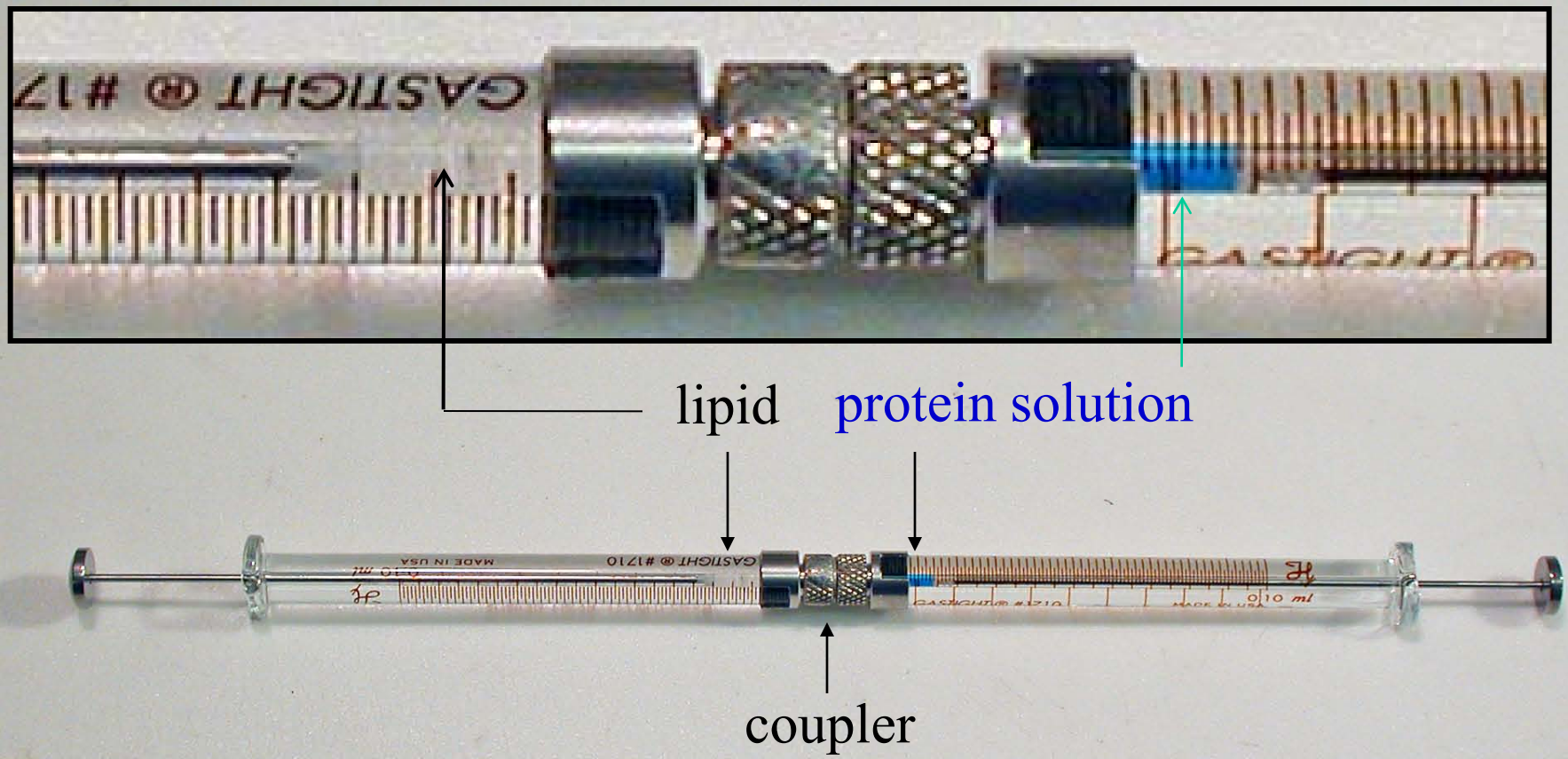


The Lipid: known mass or volume

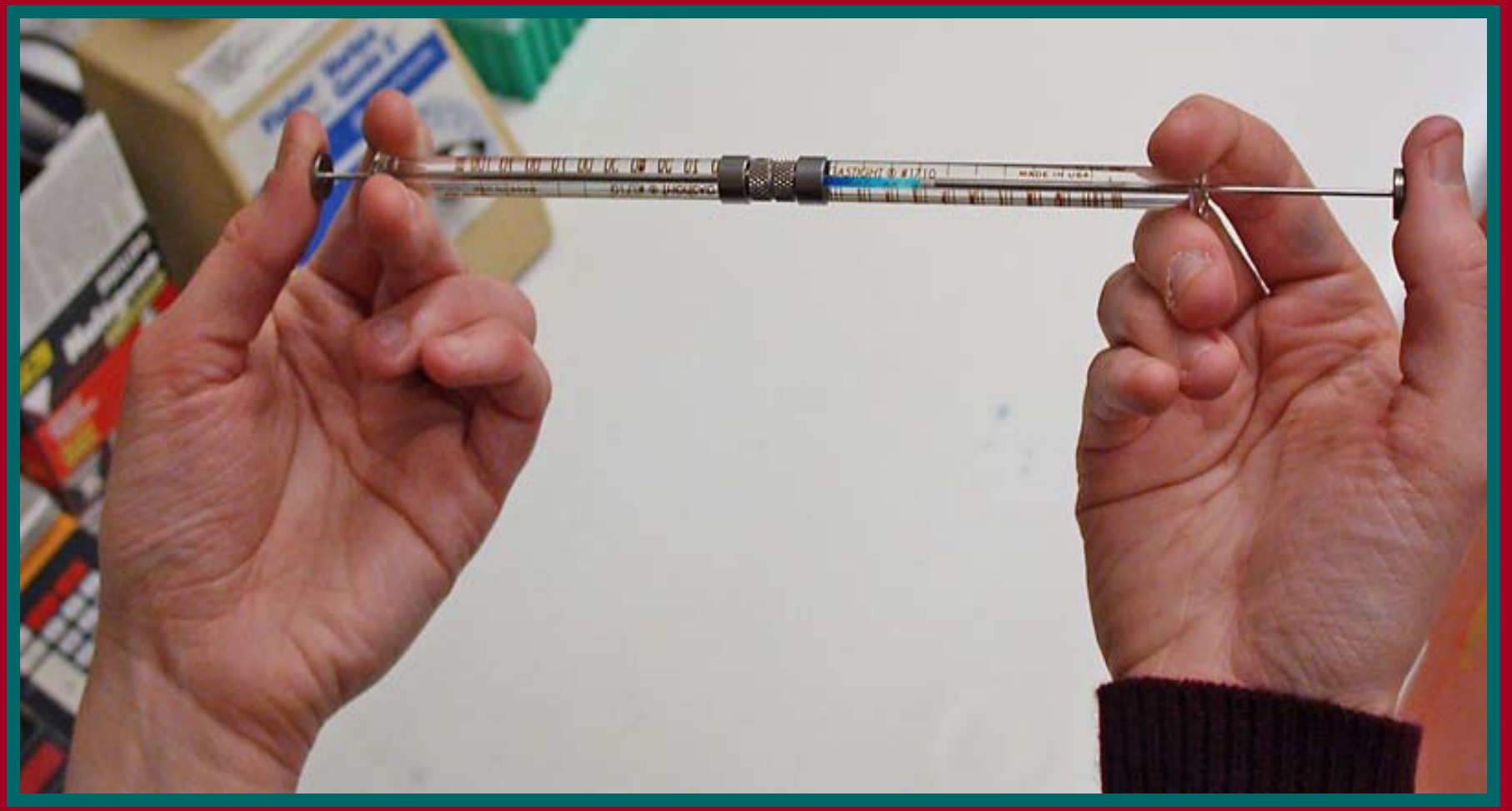


The protein solution: phase diagram – 2 / 3

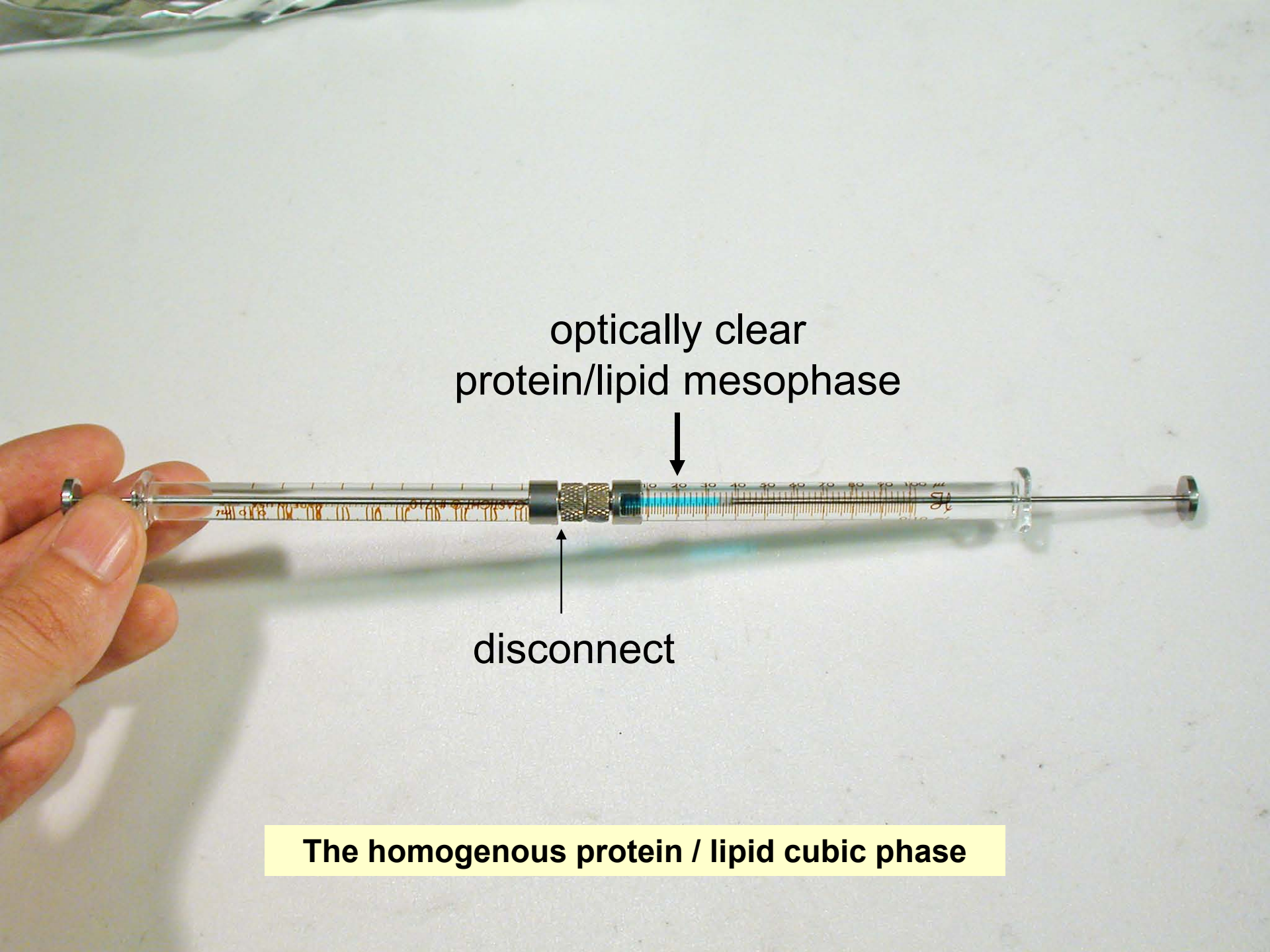

GASTIGHT® #1710
MADE IN USA
0 10 ml



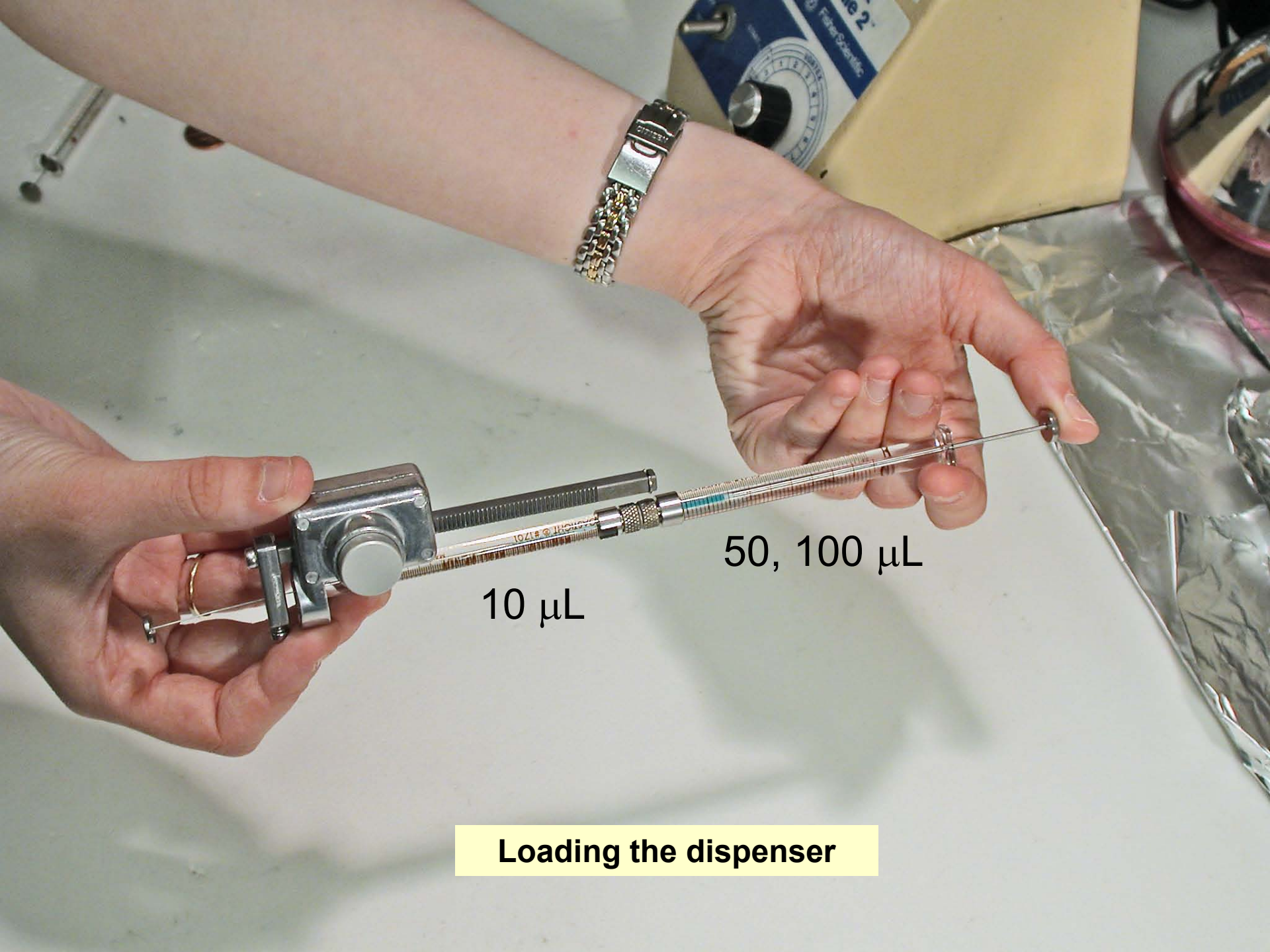
Coupled Syringe Mixer



Mechanically mixing the protein solution and lipid together to form the cubic mesophase

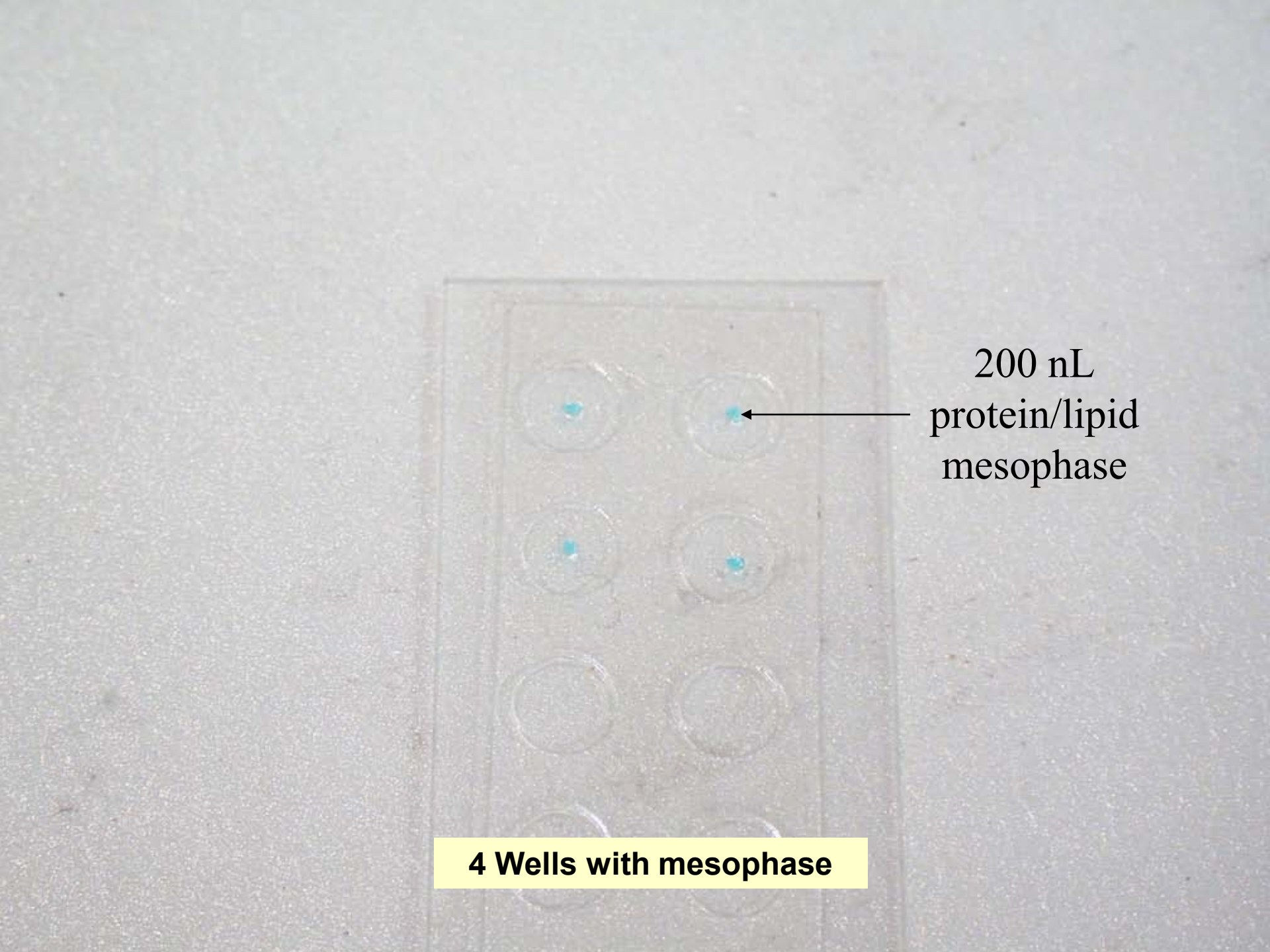


The homogenous protein / lipid cubic phase



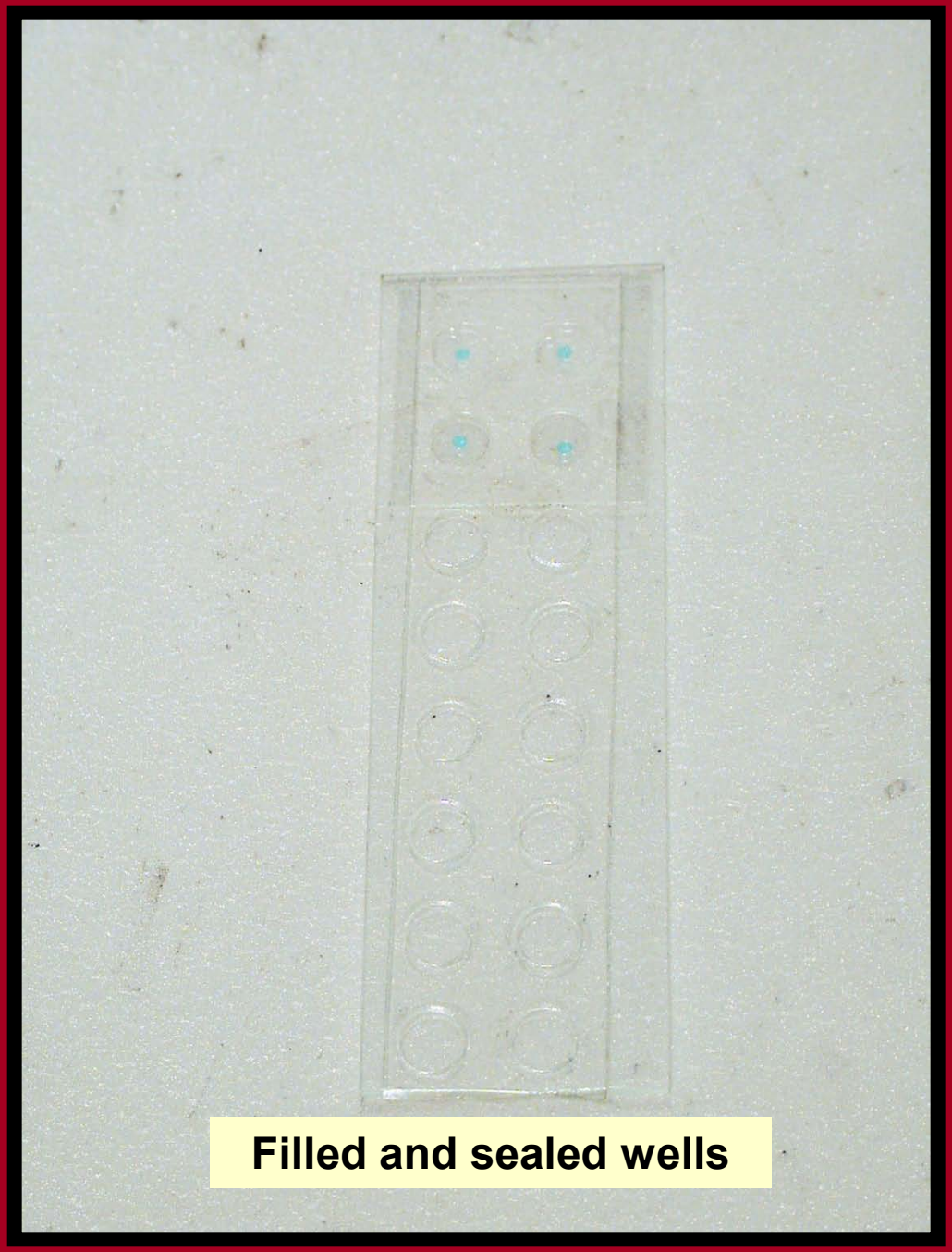
Loading the dispenser

Dispensing the mesophase


200 nL
protein/lipid
mesophase
4 Wells with mesophase

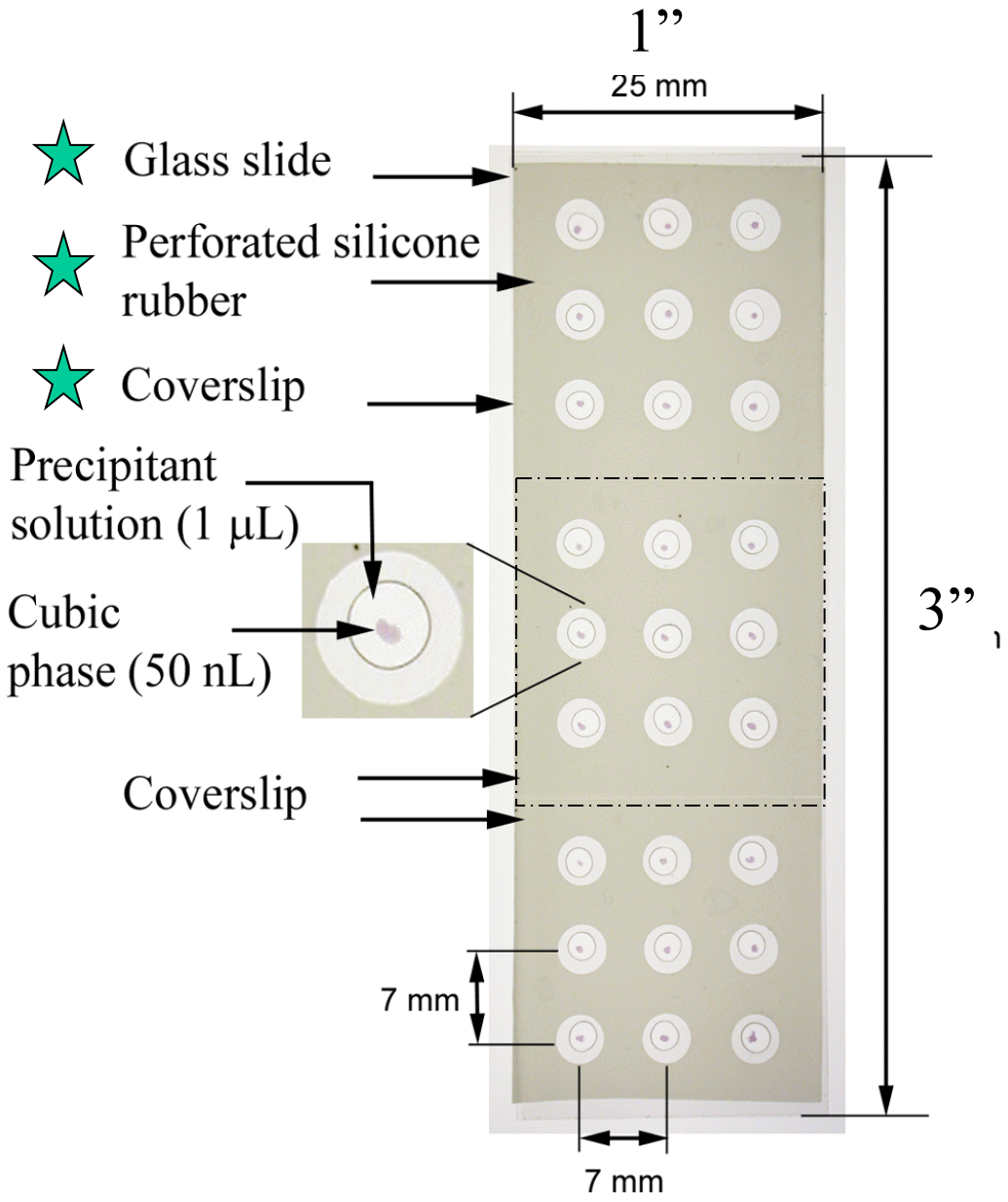
Dispensing 1 μL precipitant solution

Sealing wells with glass coverslip



Filled and sealed wells

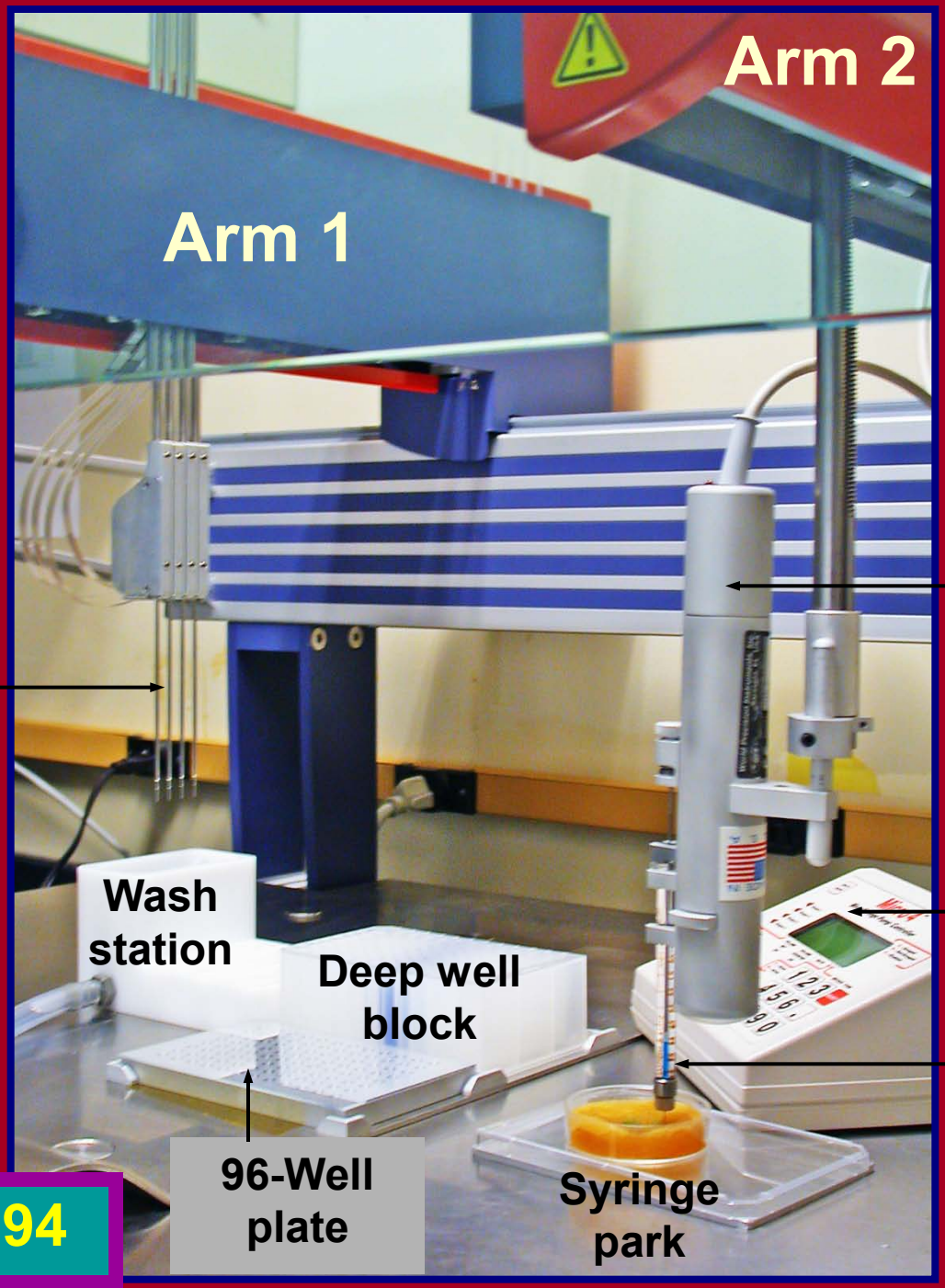
Loaded/sealed 27-well crystallization plate



In Meso Robot

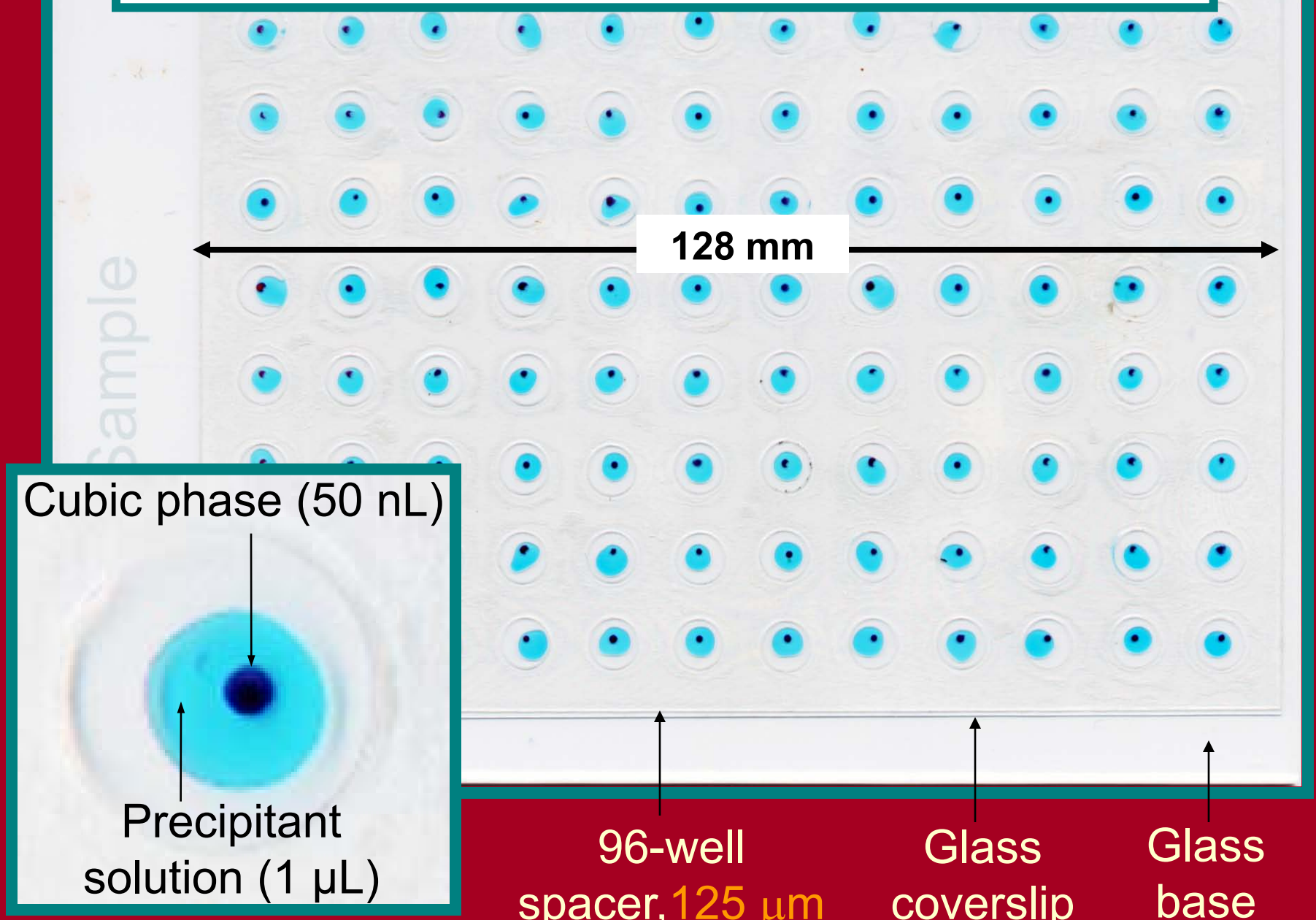
Liquid Handling Robot

Motorized Micro-pump

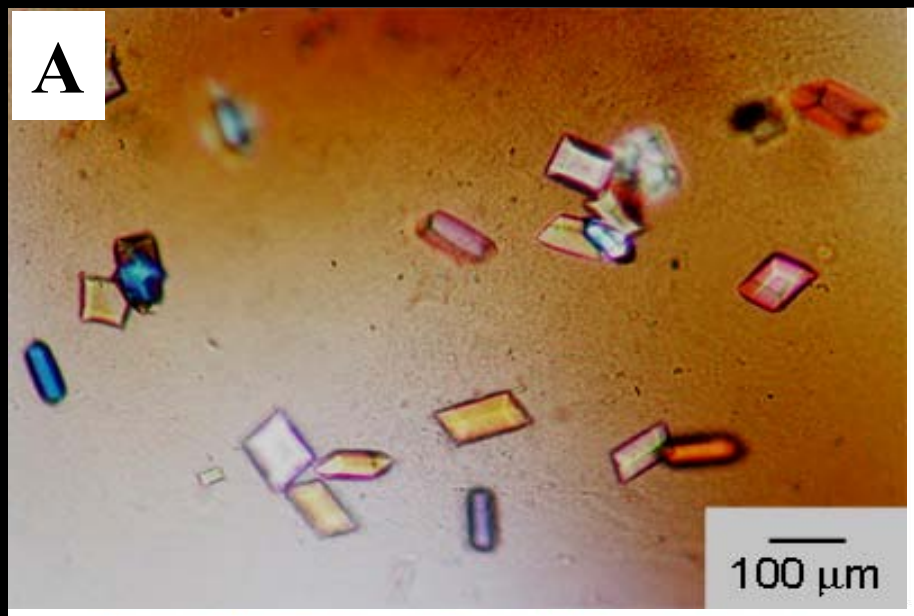


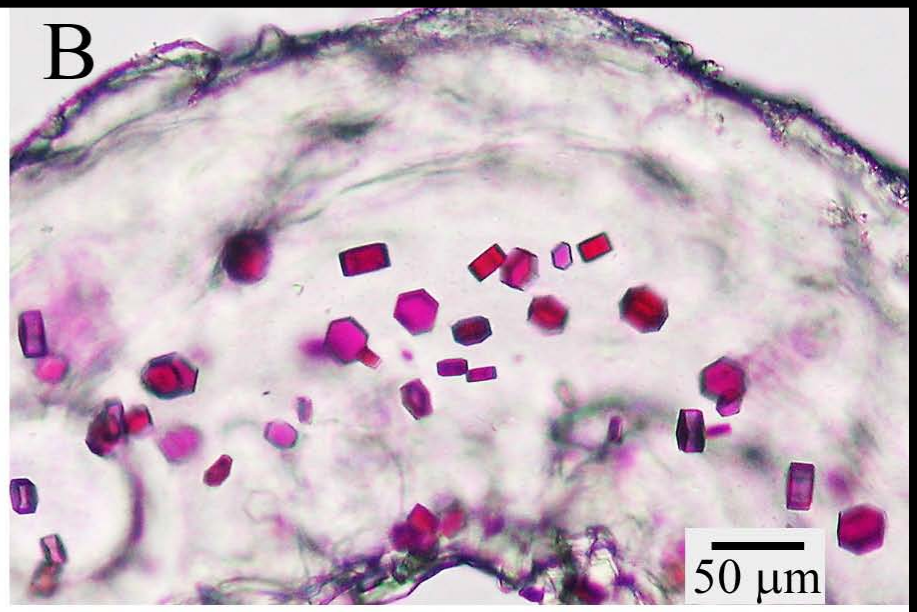
♣ Acta D*60*: 1794

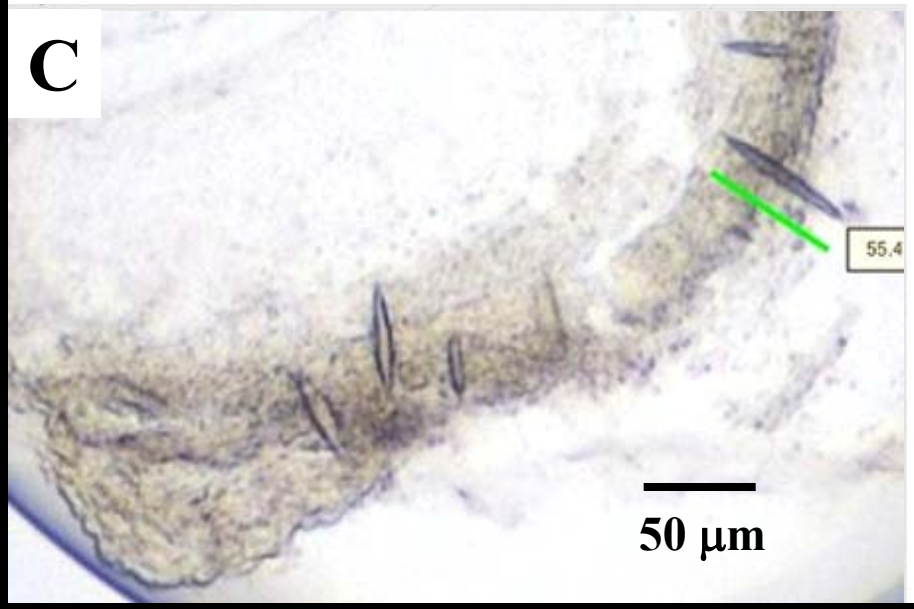
A Fully Loaded and Sealed 96-well Plate – 8 min

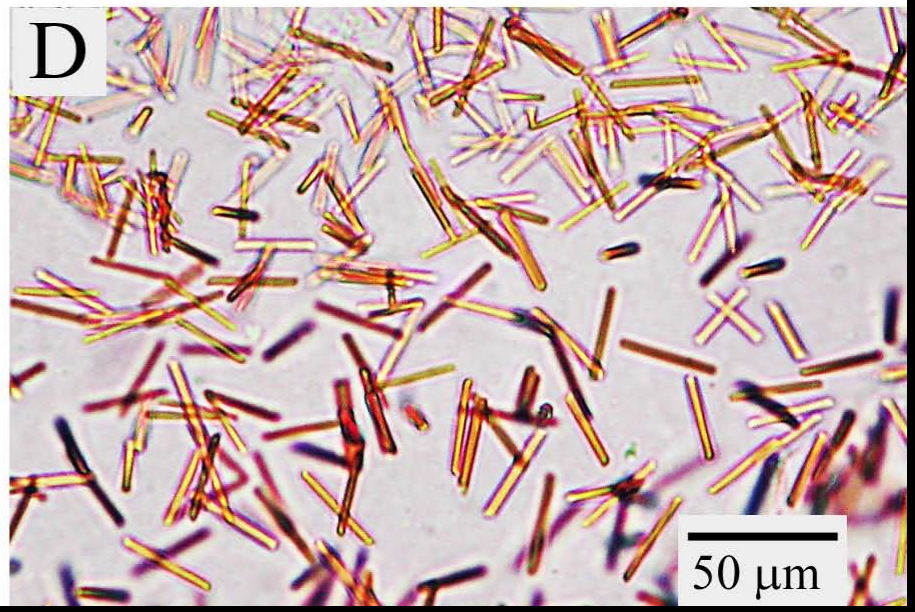


Production Mode









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