

Crystallizing Membrane Proteins for Structure-Function Studies Using Lipidic Systems

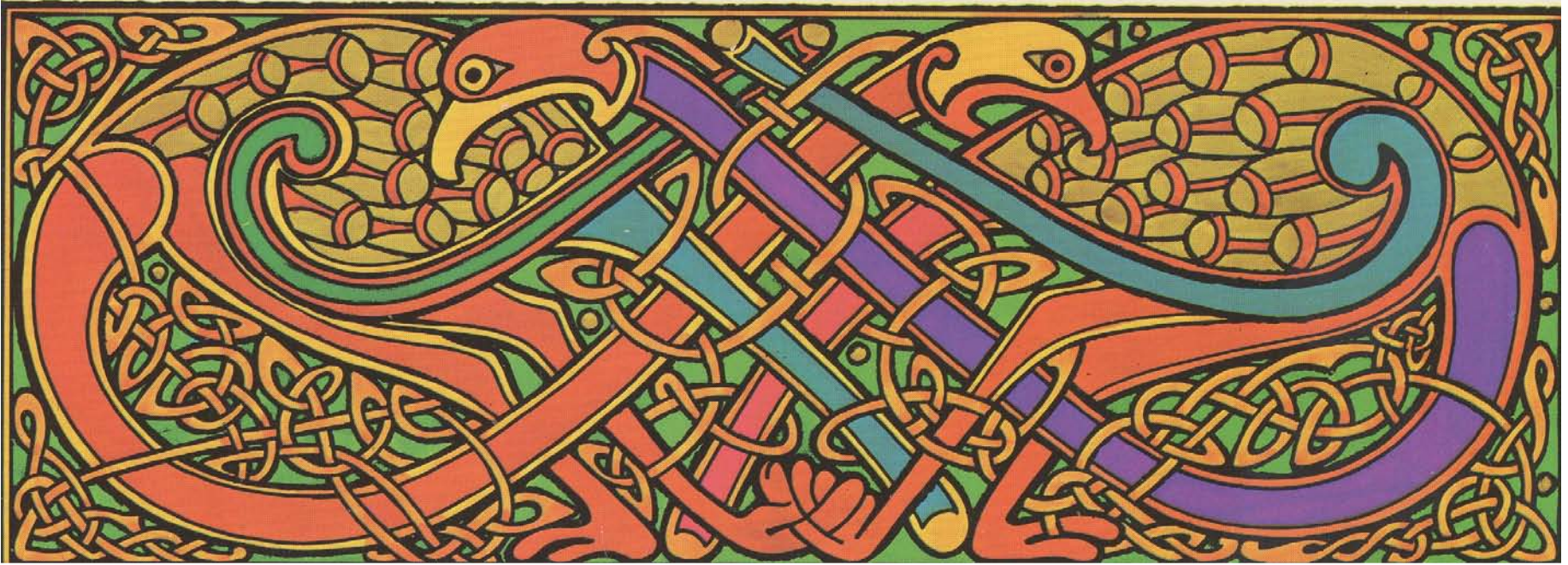
**Martin Caffrey, Membrane Structural and
Functional Biology Group**

Trinity College Dublin, Ireland

Recent Advances in Macromolecular Crystallization (RAMC)

Le Bischenberg Conference Centre, Strasbourg, France

September 11 - 14, 2011

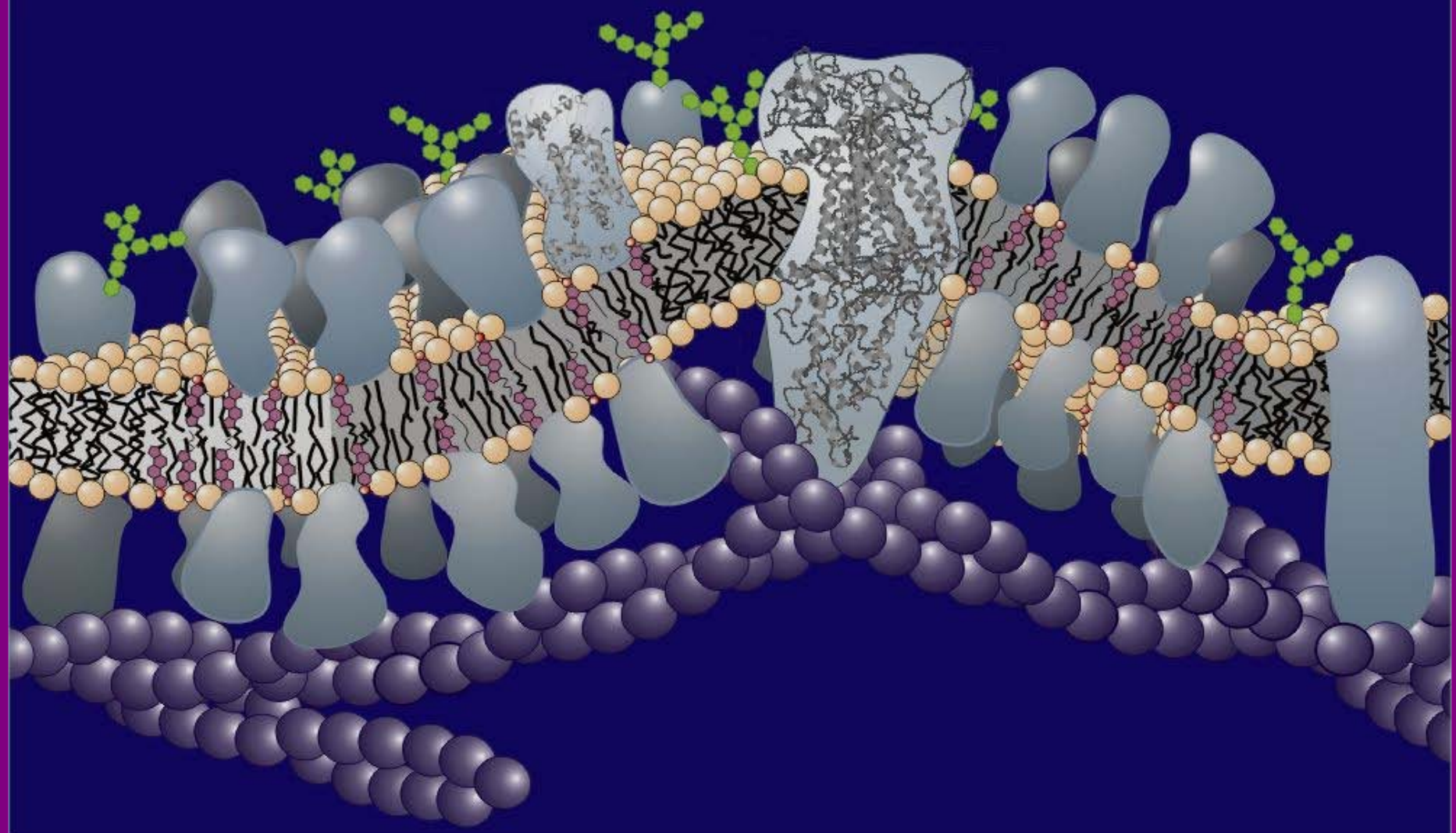


References

- ♣ **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

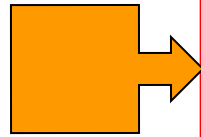
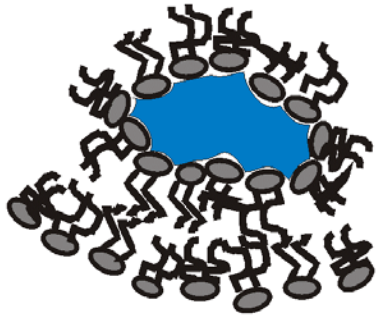
**Liquid Crystal
(Mesophase)**

Solid

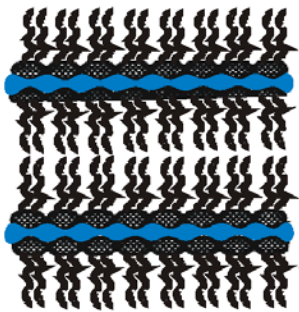


LIQUID

Fluid isotropic
FI

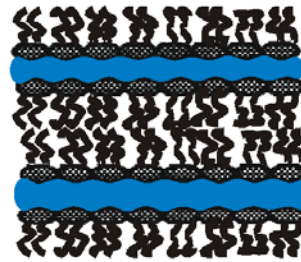


Lamellar crystal
 L_c



LIQUID CRYSTALLINE

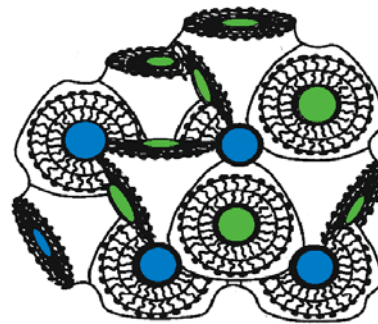
Lamellar
liquid crystal
 L_α



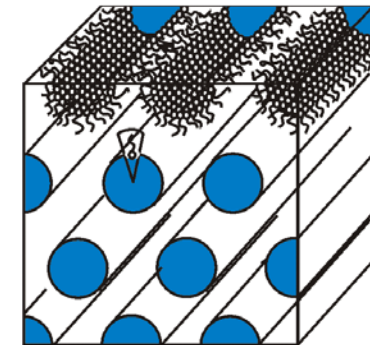
Biomembrane



Cubic
 $Pn3m$



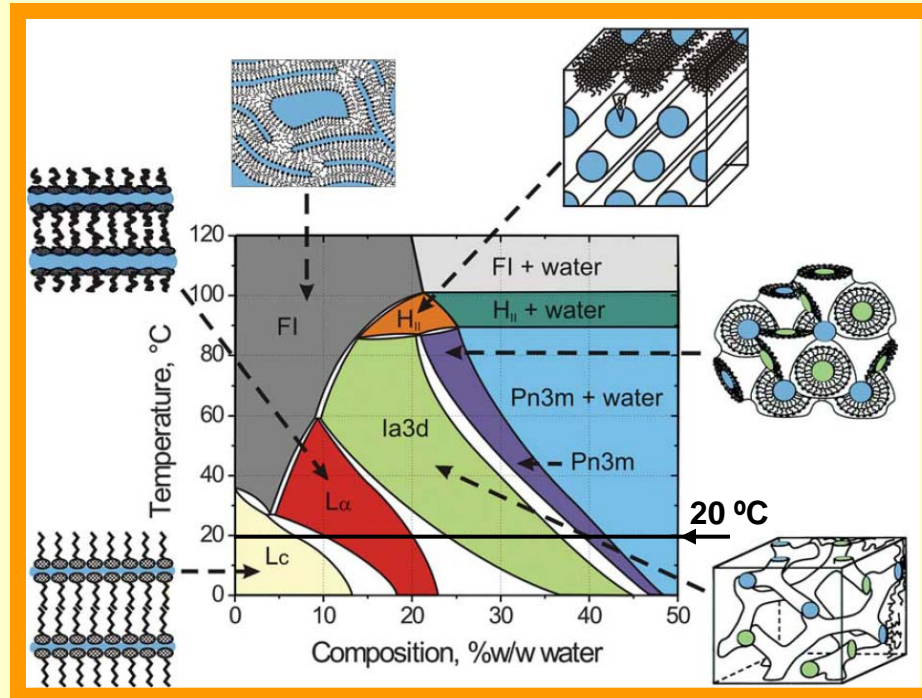
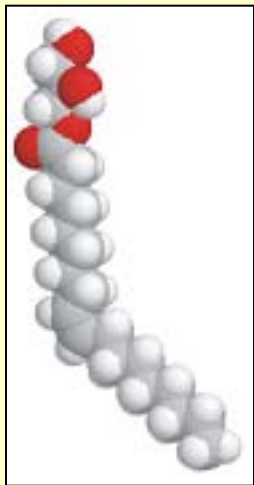
Inverted hexagonal
 H_{II}



SOLID

Lipids. Structure – Function

Structure



Function

Membrane
Transport
Signaling
Fusion
Energy
Drugs
Foods
Cosmetics

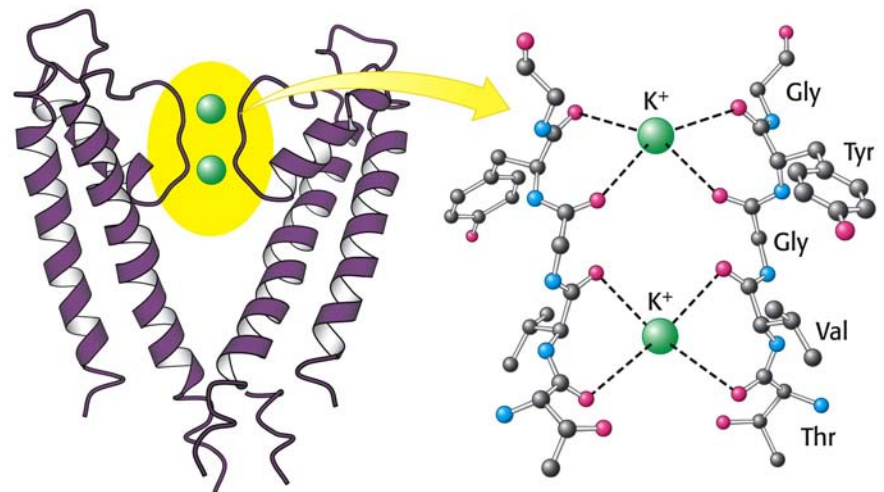
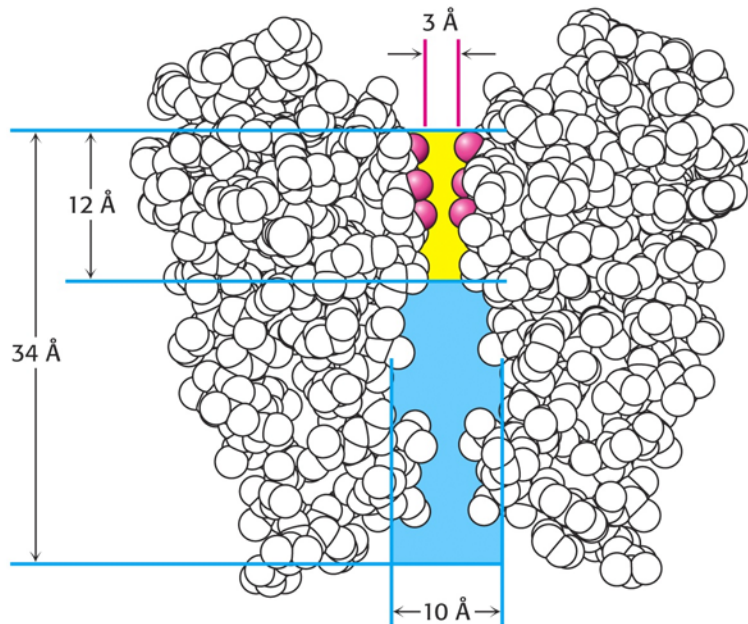
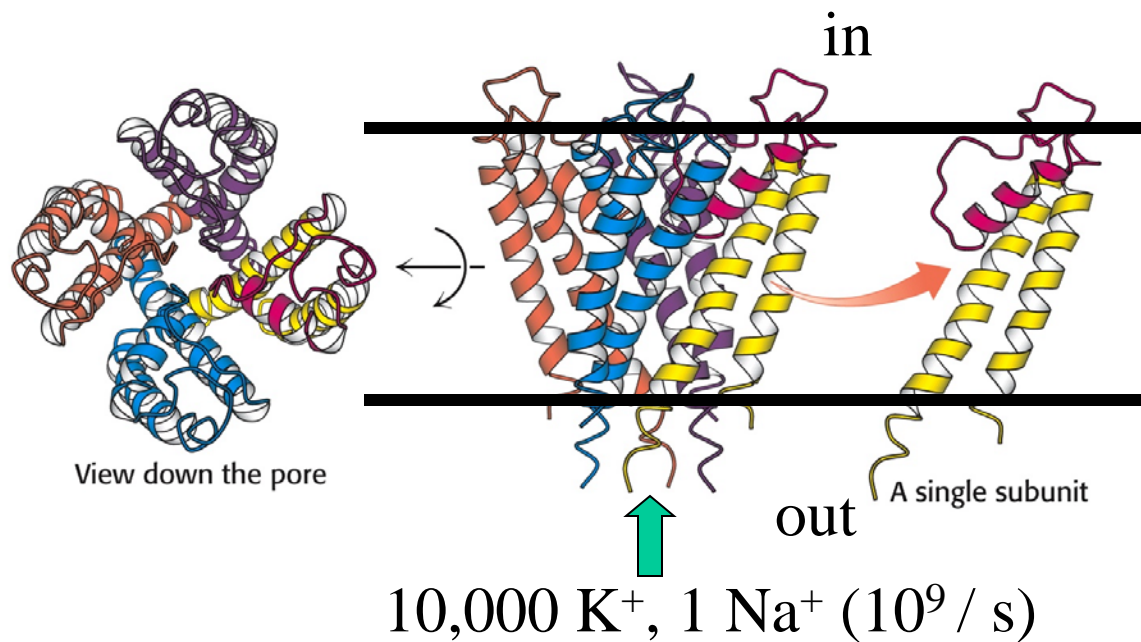
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Crystals

Phase Behavior
Microstructure, Rheology

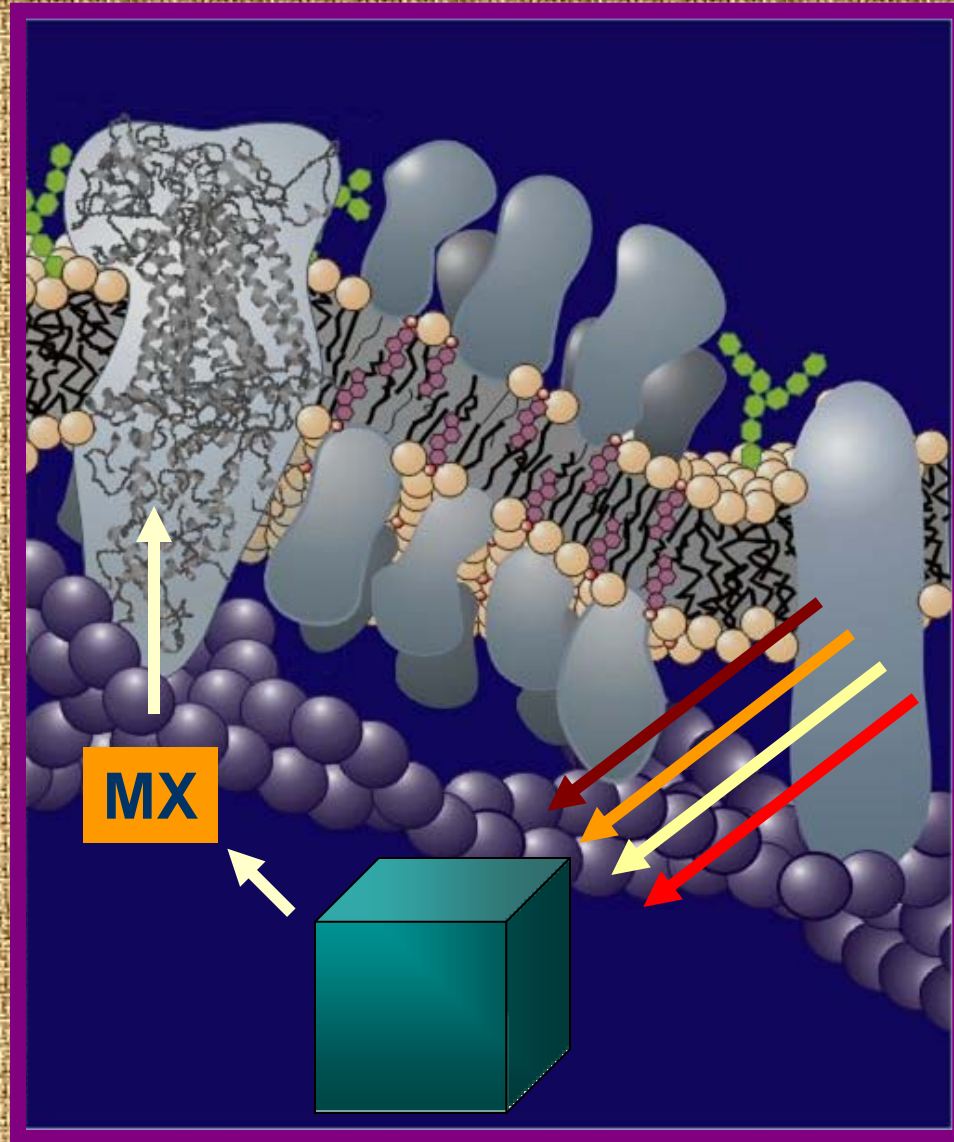
Knowledge,
Rational Design

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

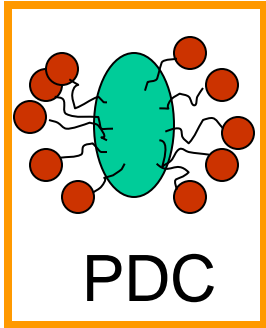
vesicle fusion

cubic phase

Evaporation

Interfacial Diffusion

Bicelle Crystallization Method

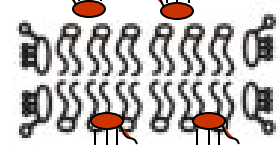


PDC

Protein Solution
(on ice)
10 mg bR/mL



Lipid/amphiphile
mixture (on ice)

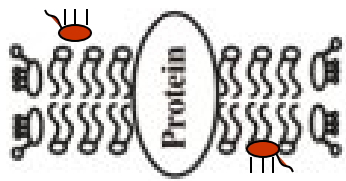


40% DMPC/CHAPSO (3/1)

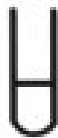
DMPC vesicles + CHAPSO

Vortex, Sonicate
Thermal cycling

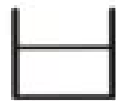
Mix



Homogenous protein/bicelle
mixture (on ice)



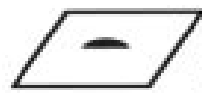
8 mg bR/mL



Precipitant

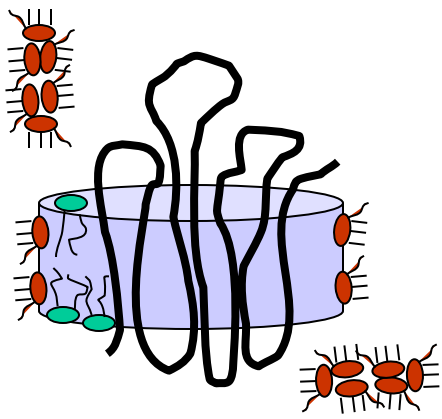
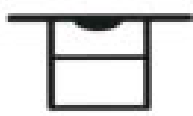
Mix

Hanging Drop Vapour Diffusion



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

viscous



Bacteriorhodopsin crystallized
in lipidic cubic phase



20 μm

Lamellar portal

PROTEIN
CO-CRYSTAL

CHARGE
SCREENING

Crystalline
array

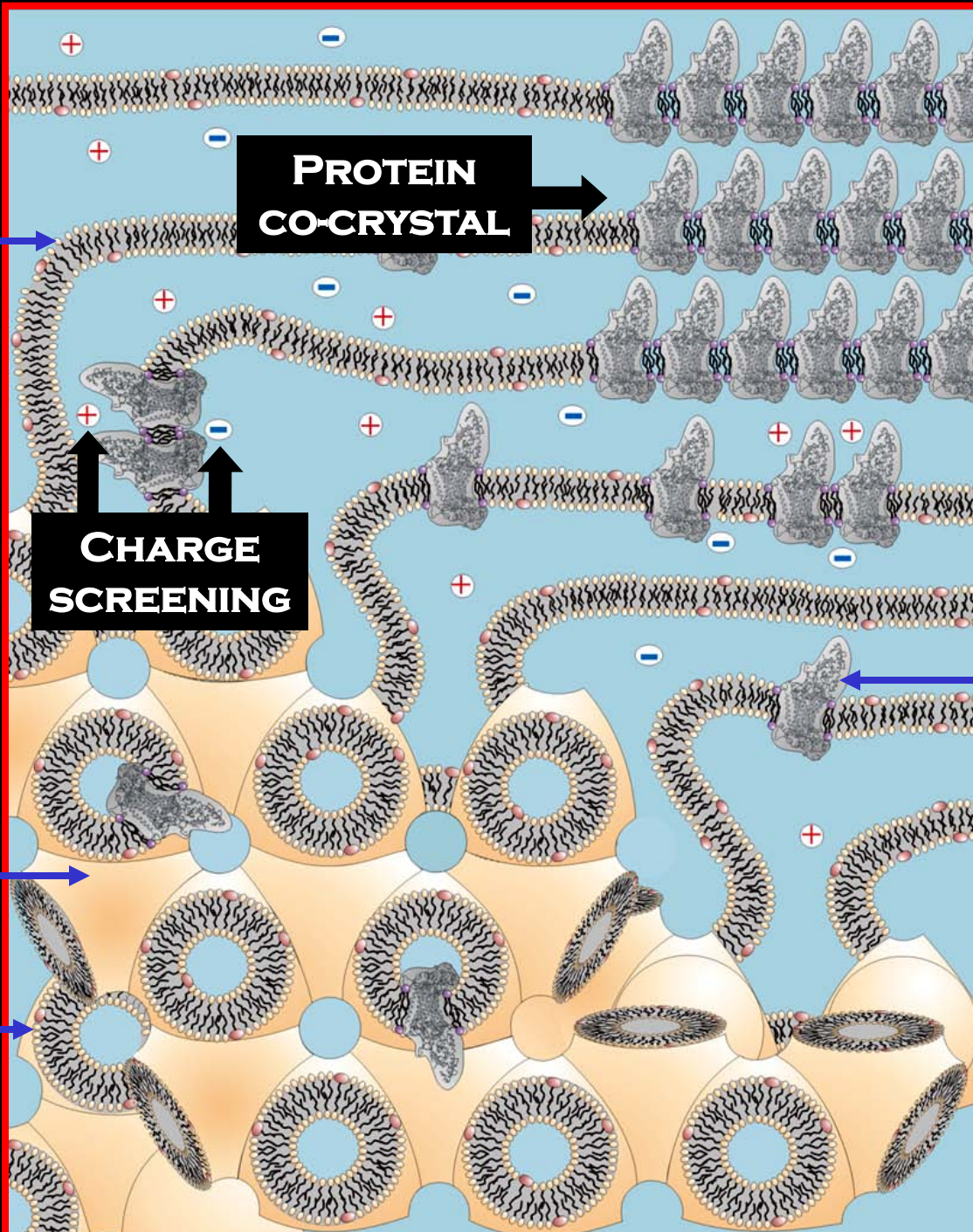
Reconstituted
protein

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 μ L

100 μ L

10 μ L 100 μ L

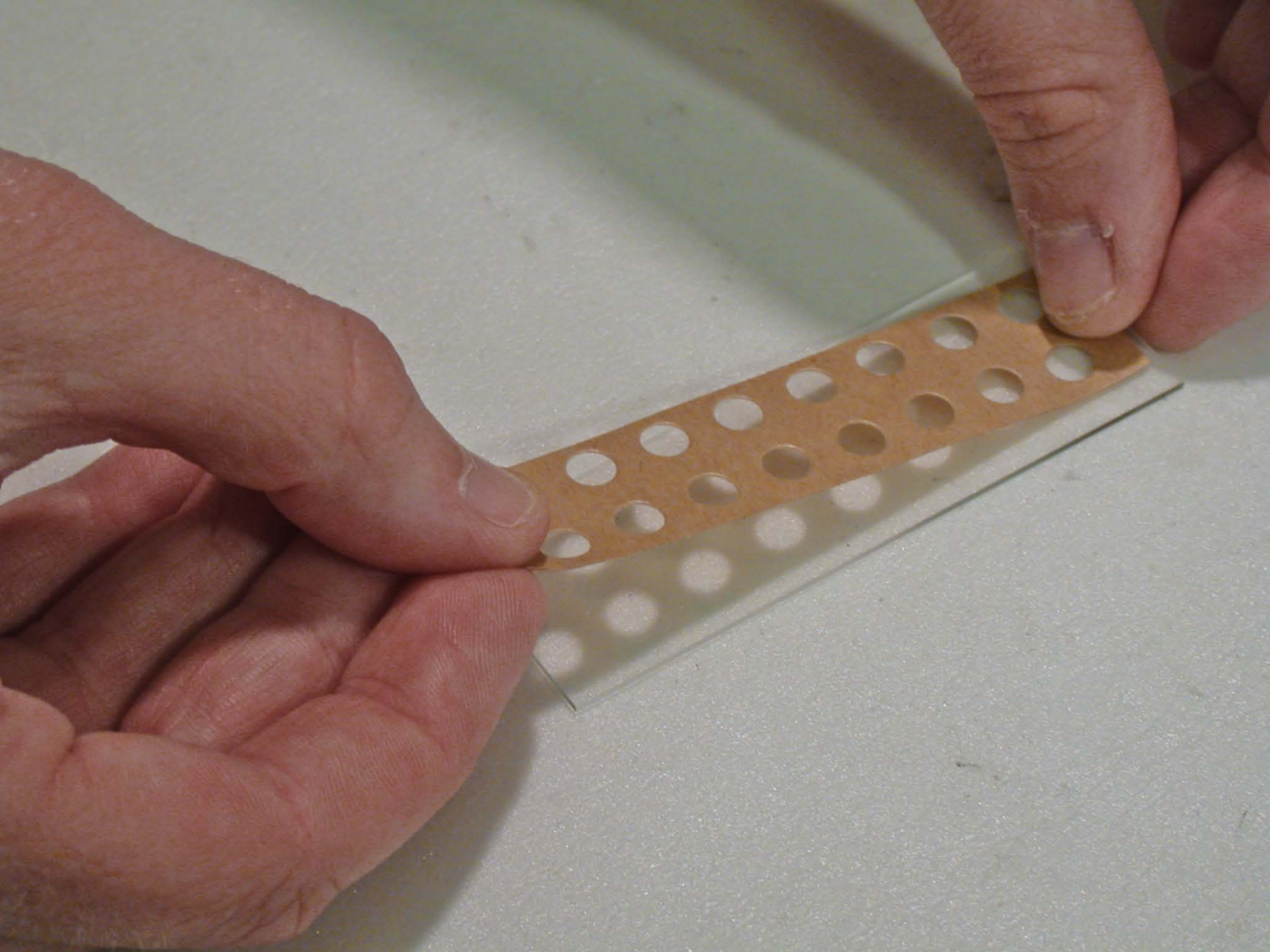
Calculator

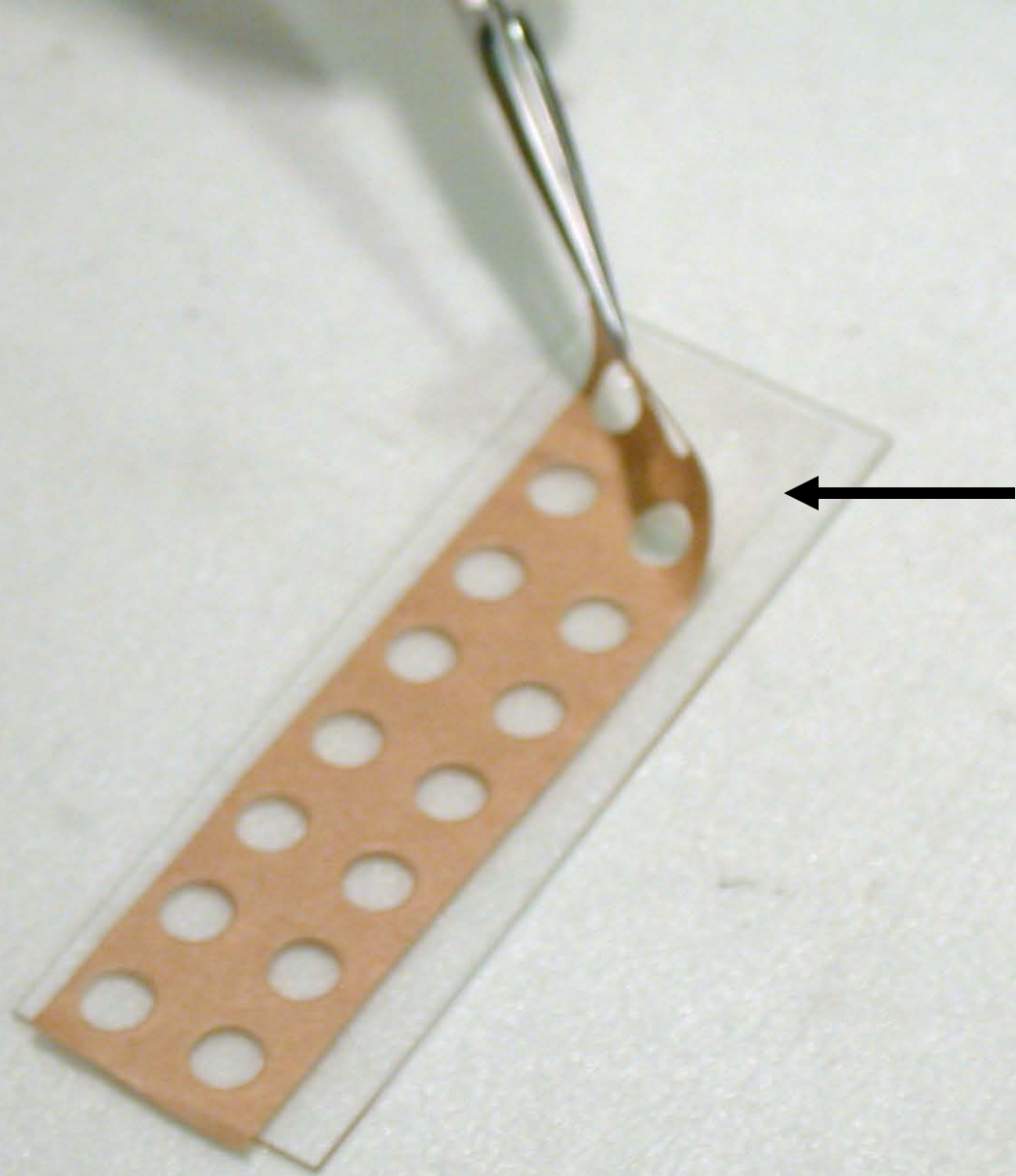
Ice

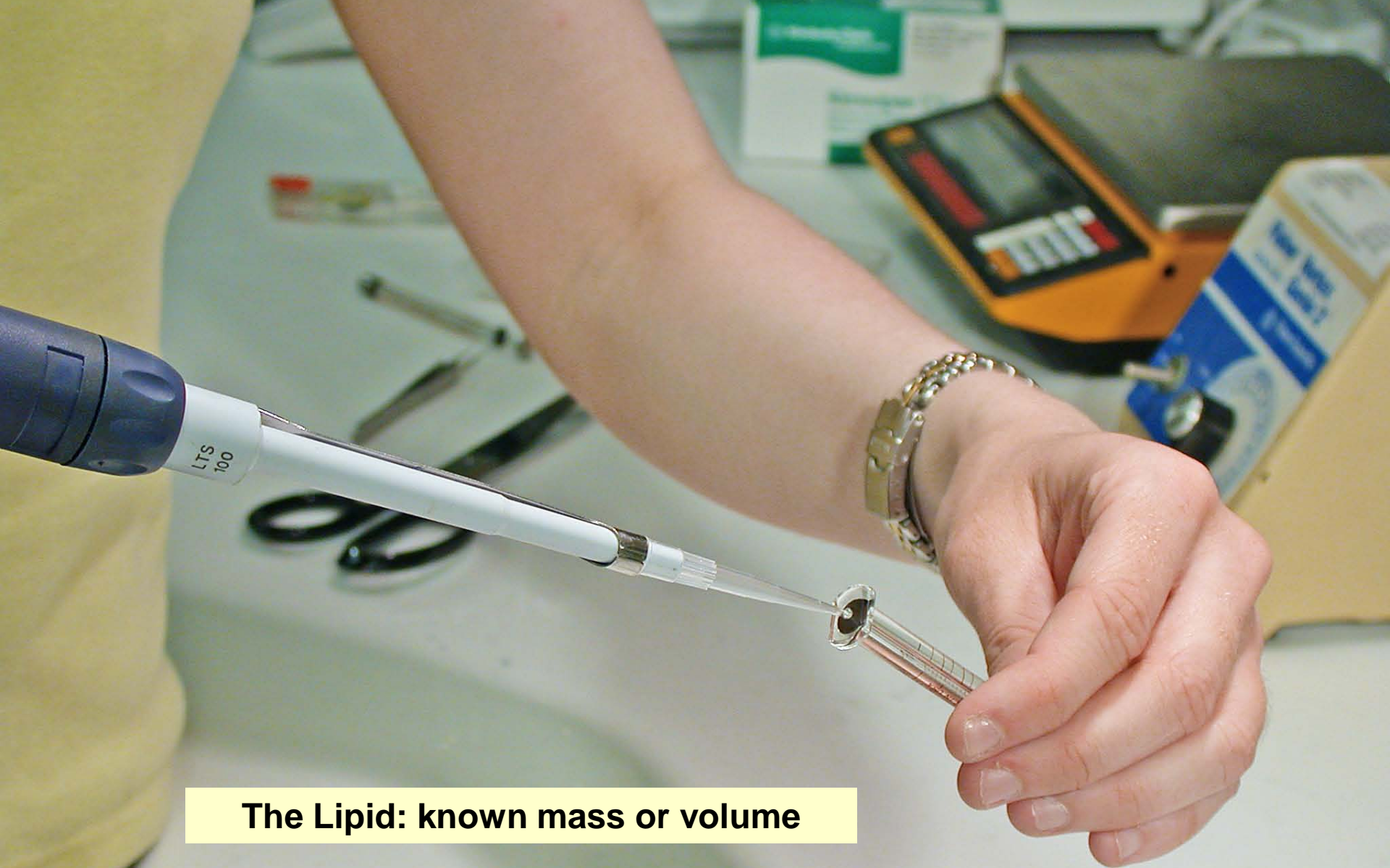
30 μ L

1 μ L



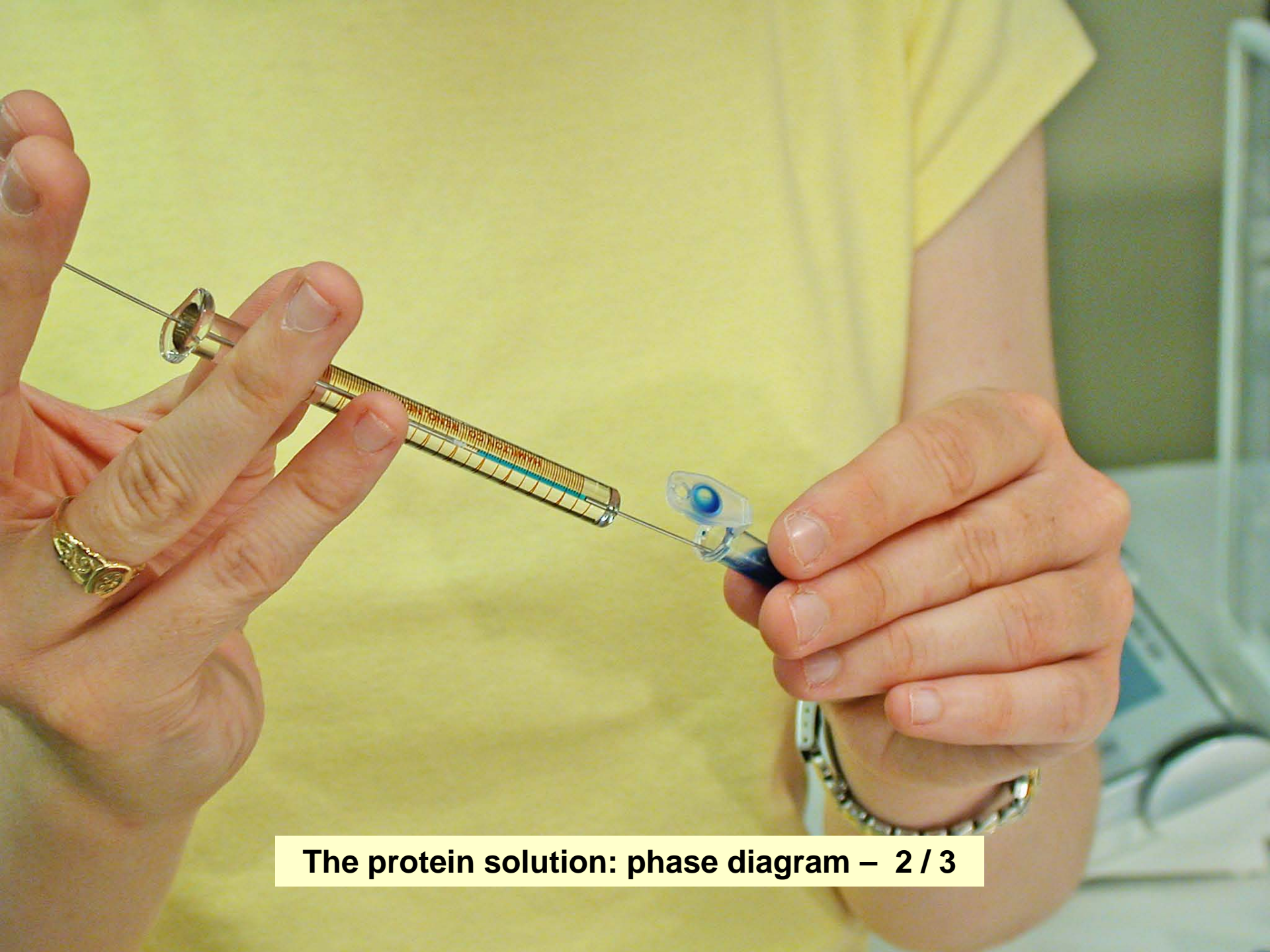






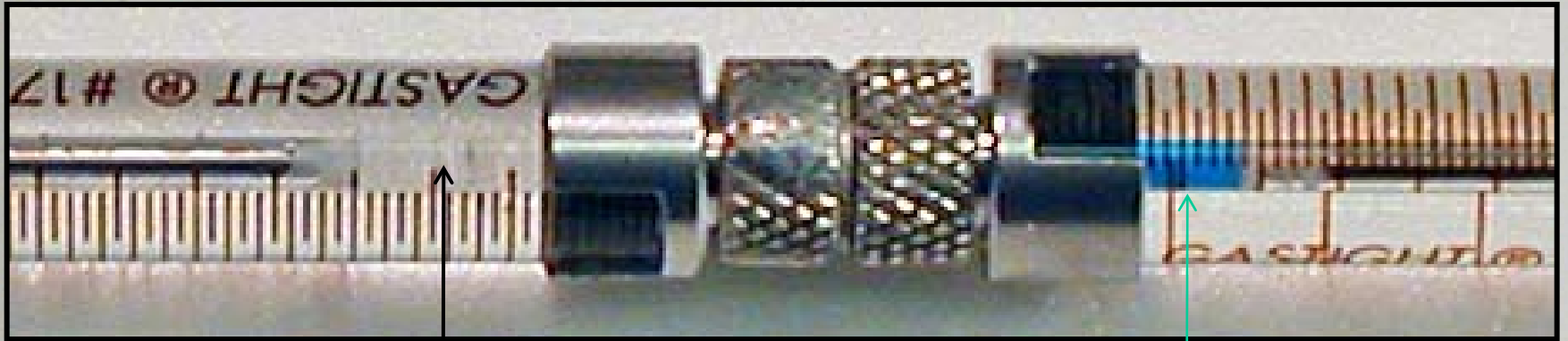
The Lipid: known mass or volume





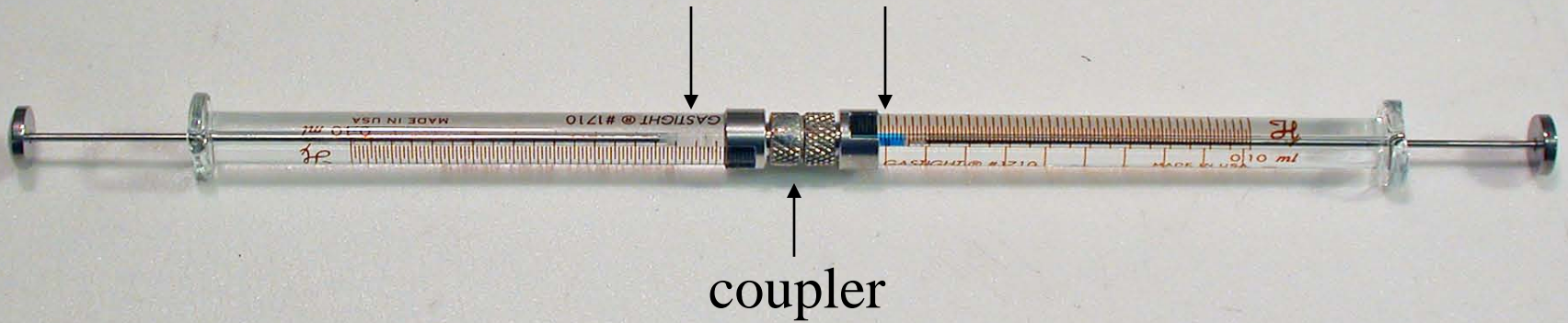
The protein solution: phase diagram – 2 / 3





lipid

protein solution



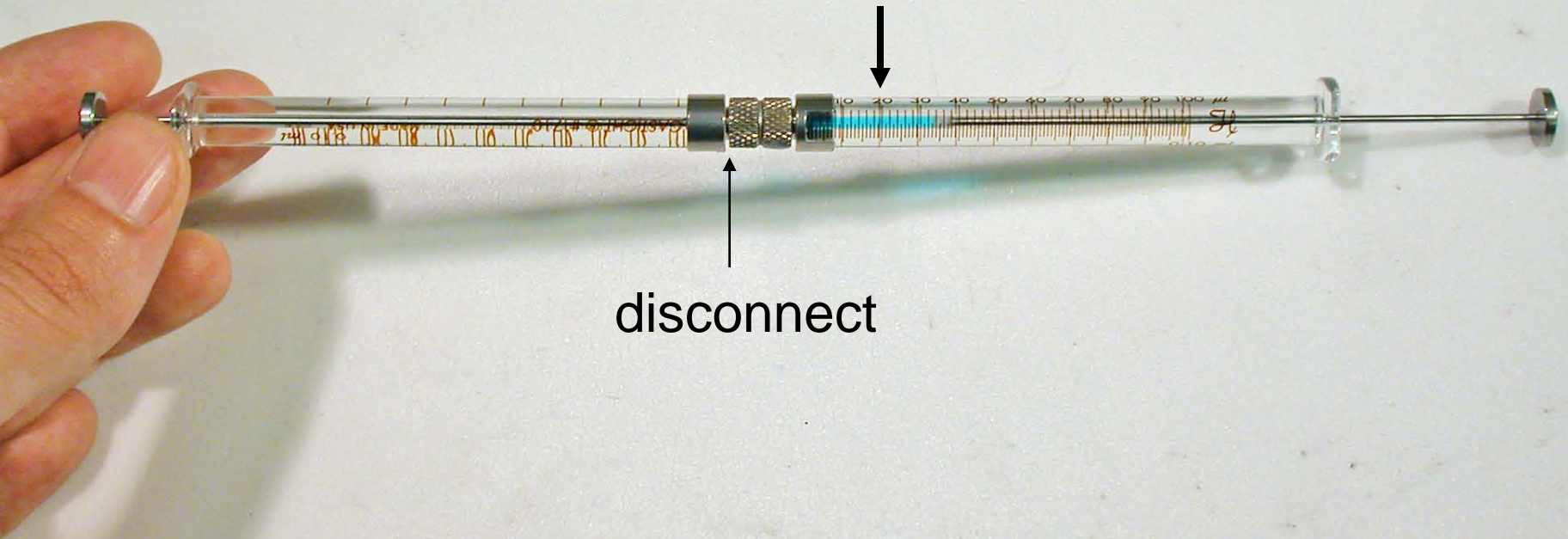
coupler

Coupled Syringe Mixer



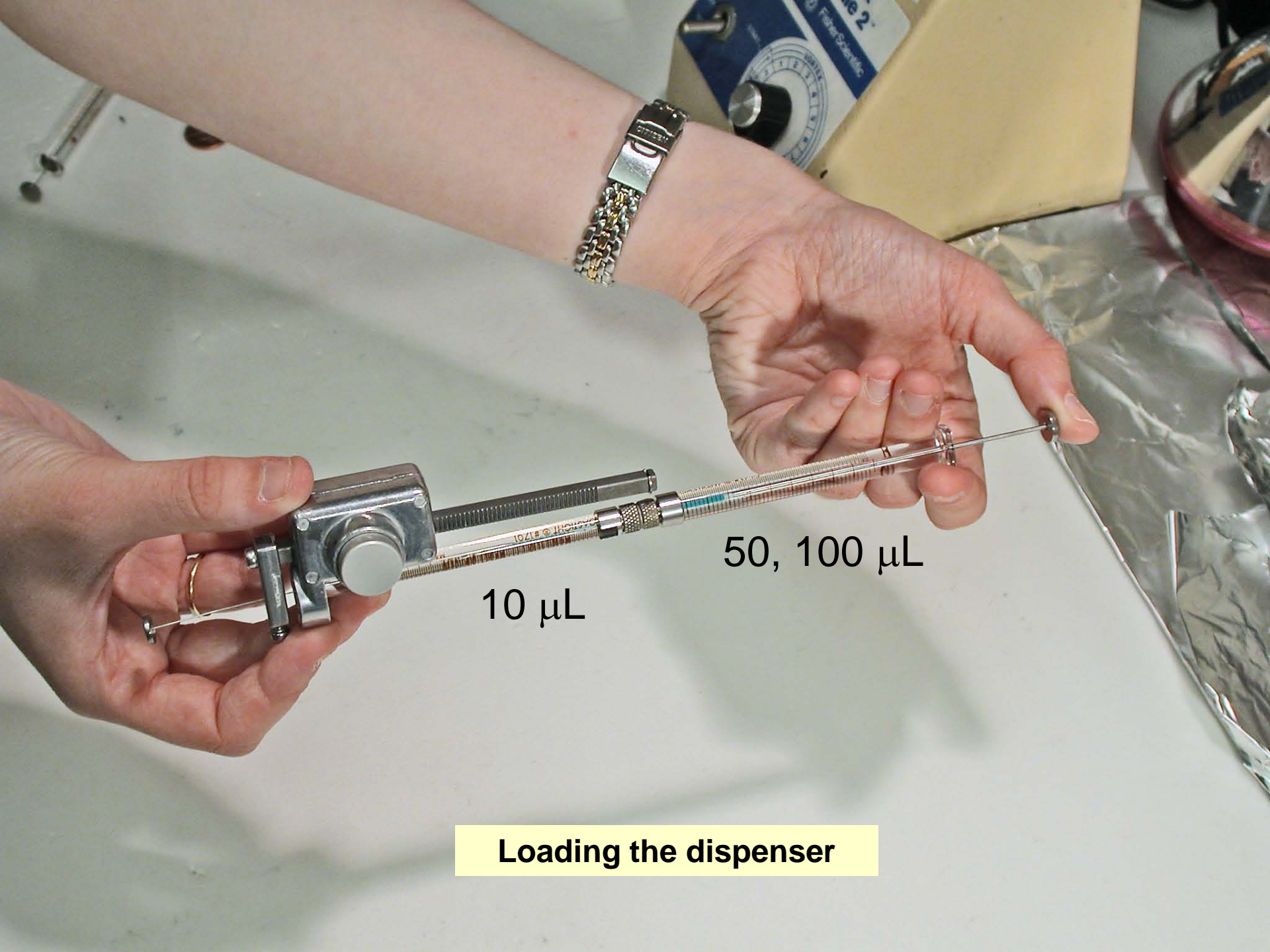
Mechanically mixing the protein solution and lipid together to form the cubic mesophase

optically clear
protein/lipid mesophase



disconnect

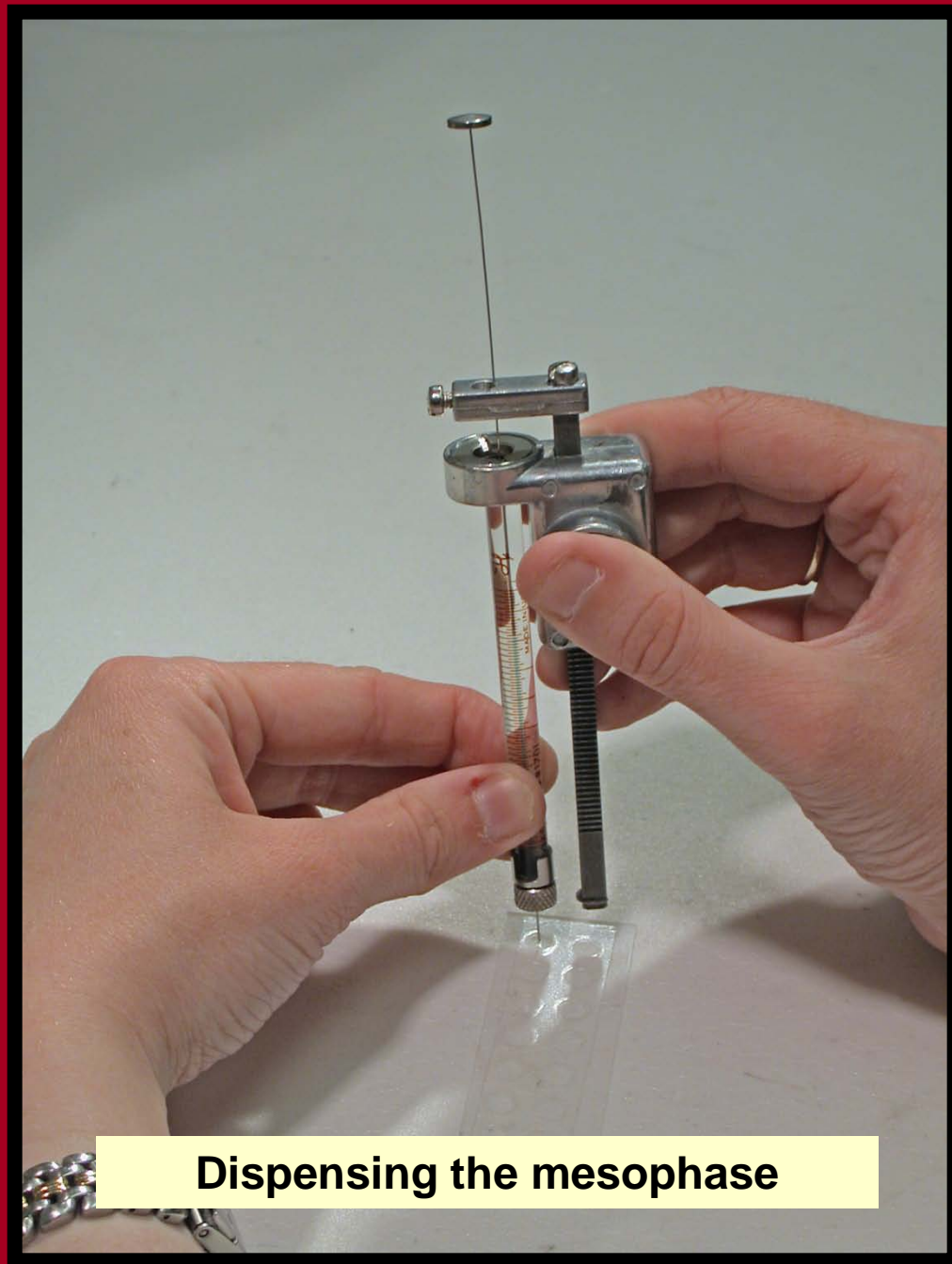
The homogenous protein / lipid cubic phase



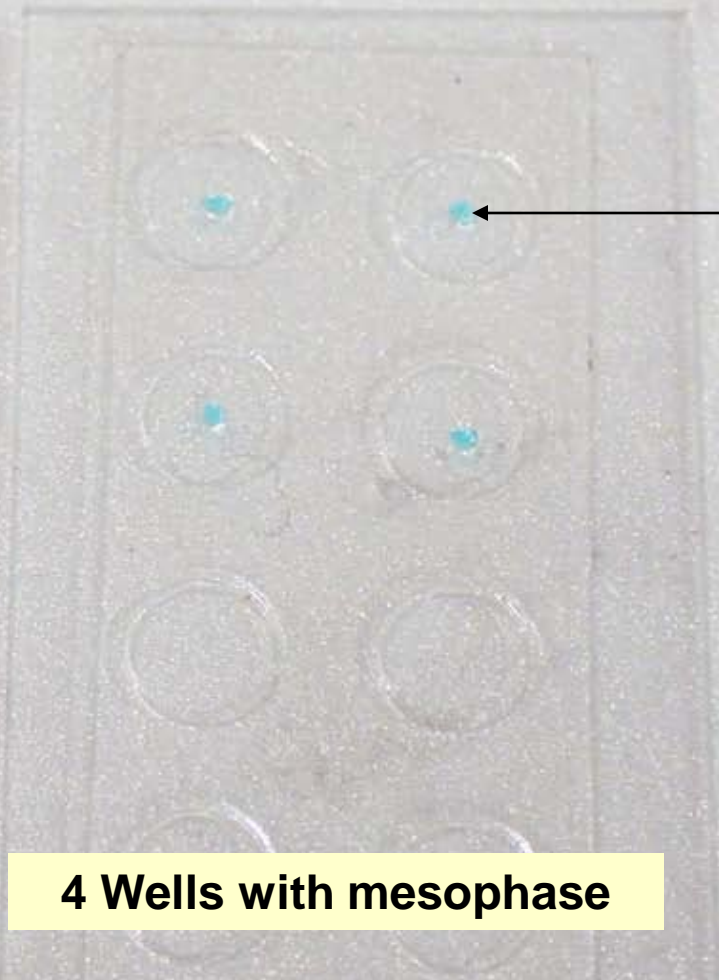
10 μL

50, 100 μL

Loading the dispenser



Dispensing the mesophase



200 nL
protein/lipid
mesophase



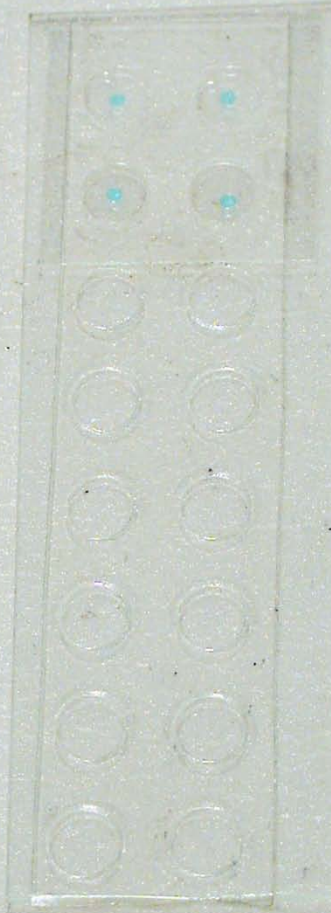
4 Wells with mesophase

**Dispensing 1 μ L
precipitant solution**



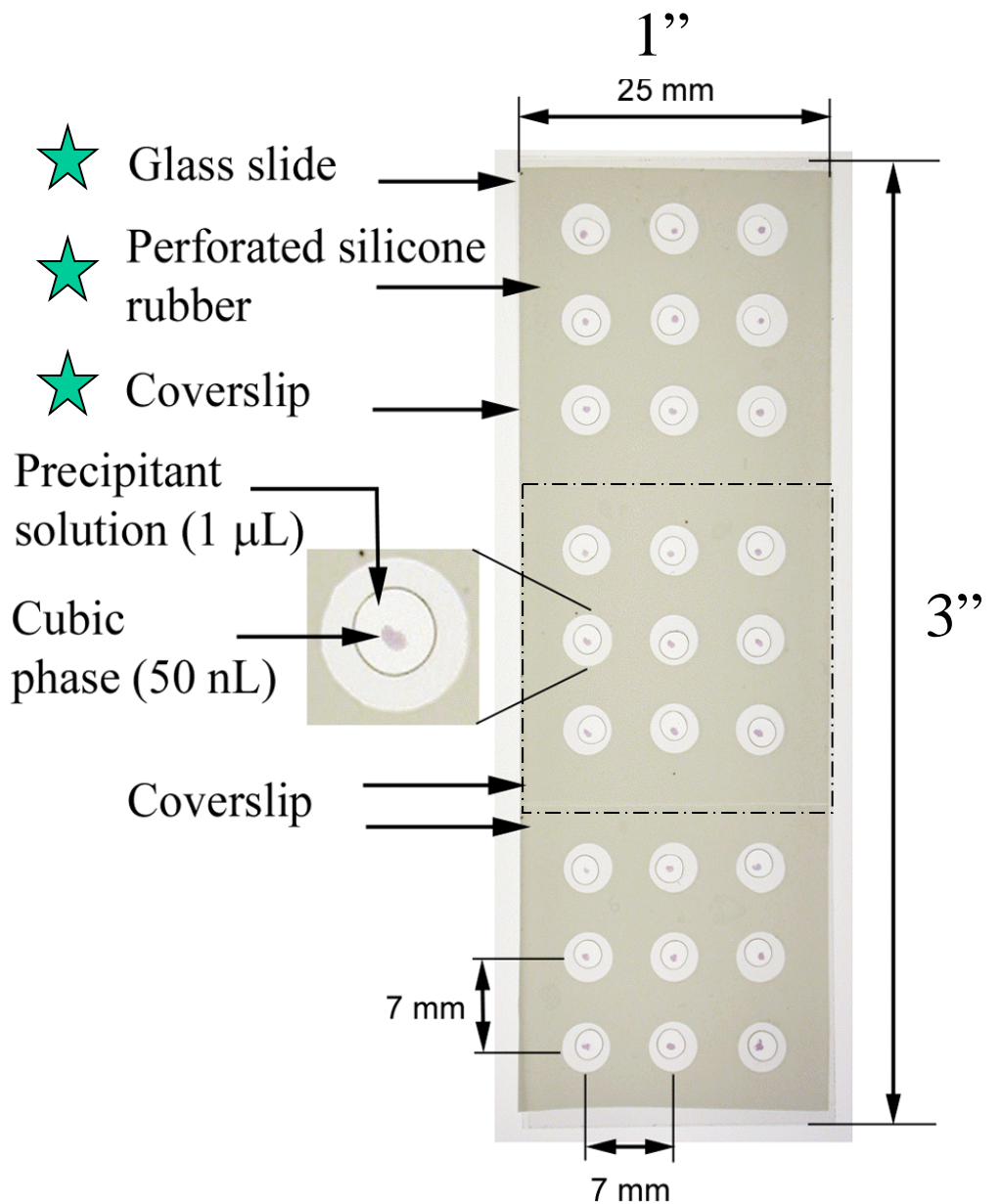


Sealing wells with glass coverslip



Filled and sealed wells

Loaded/sealed 27-well crystallization plate

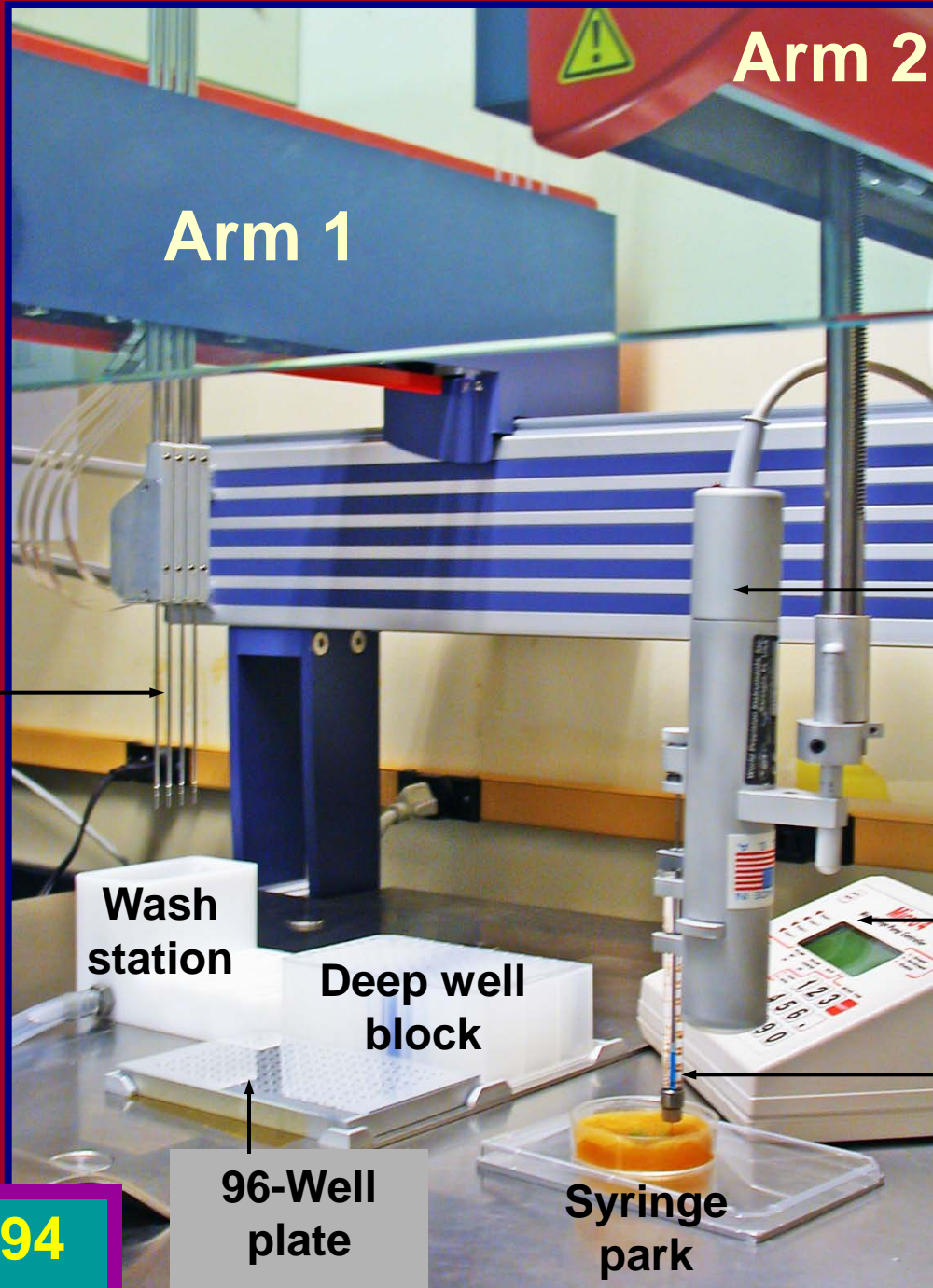


In Meso Robot

Liquid
Handling
Robot

4-Tip
precipitant
solution
dispenser

Motorized
Micro-pump



Arm 1

Arm 2

Protein/lipid
dispenser

Protein/lipid
syringe
controller

Microsyringe

Wash
station

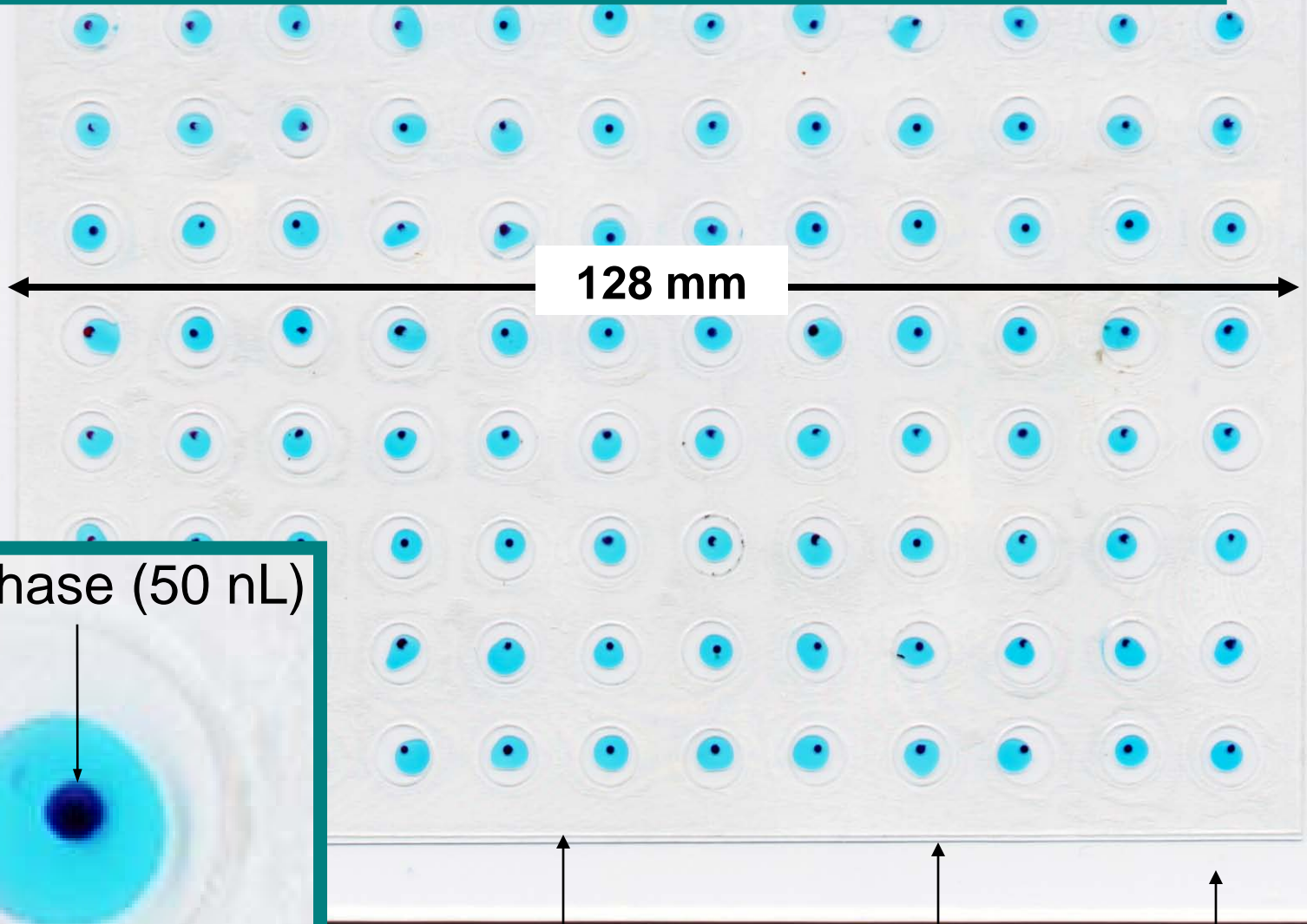
Deep well
block

96-Well
plate

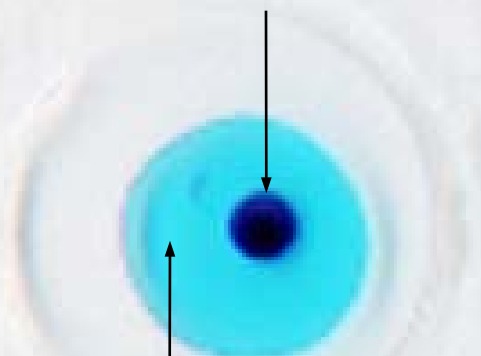
Syringe
park

Acta D60: 1794

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



Cubic phase (50 nL)



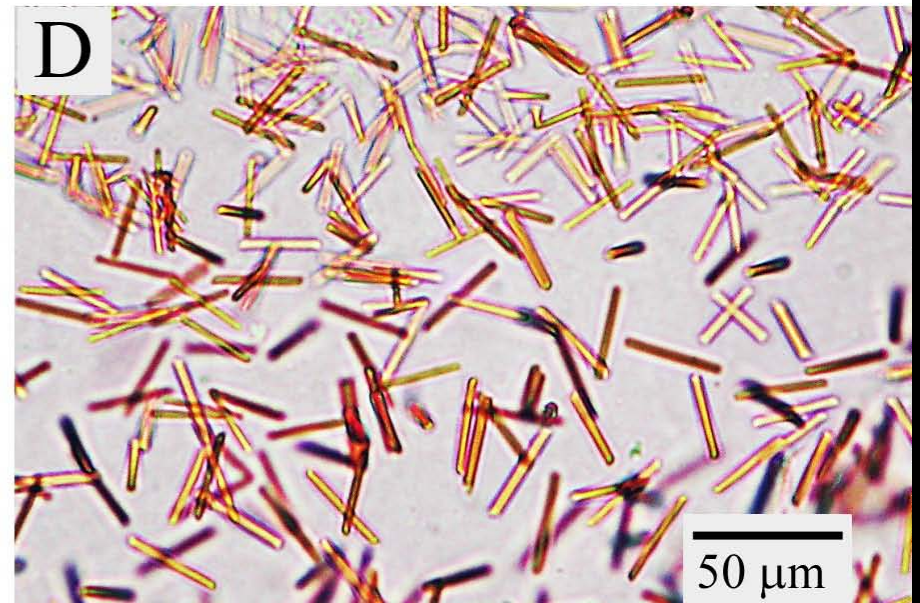
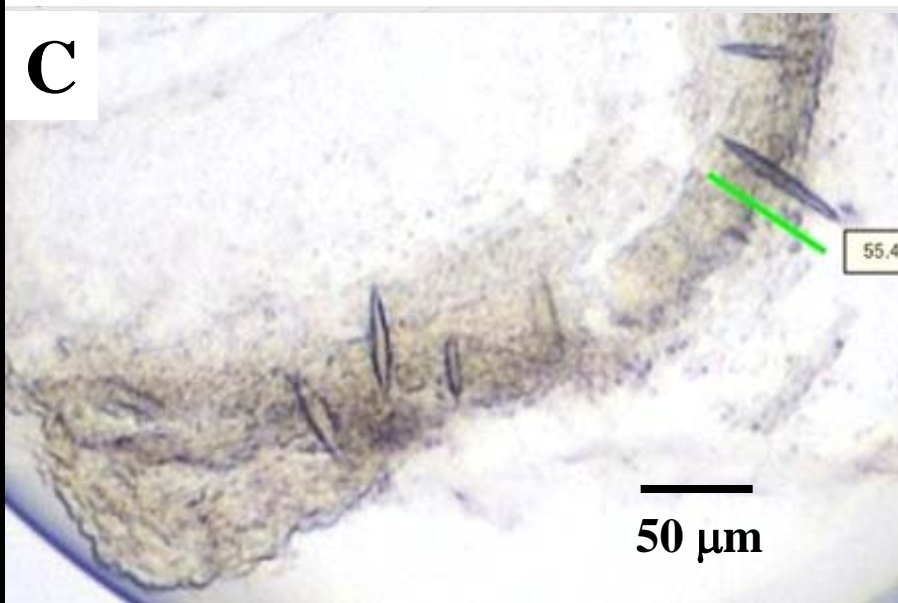
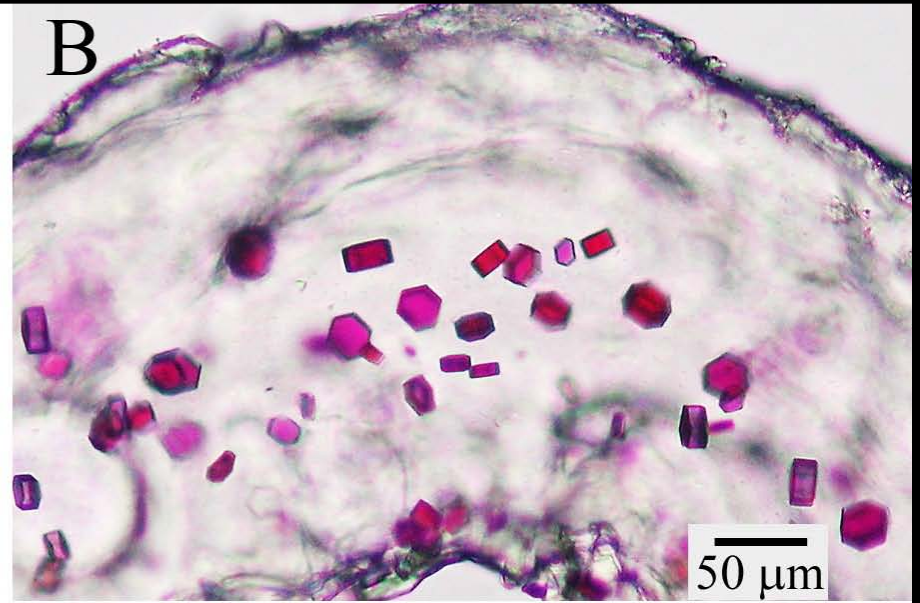
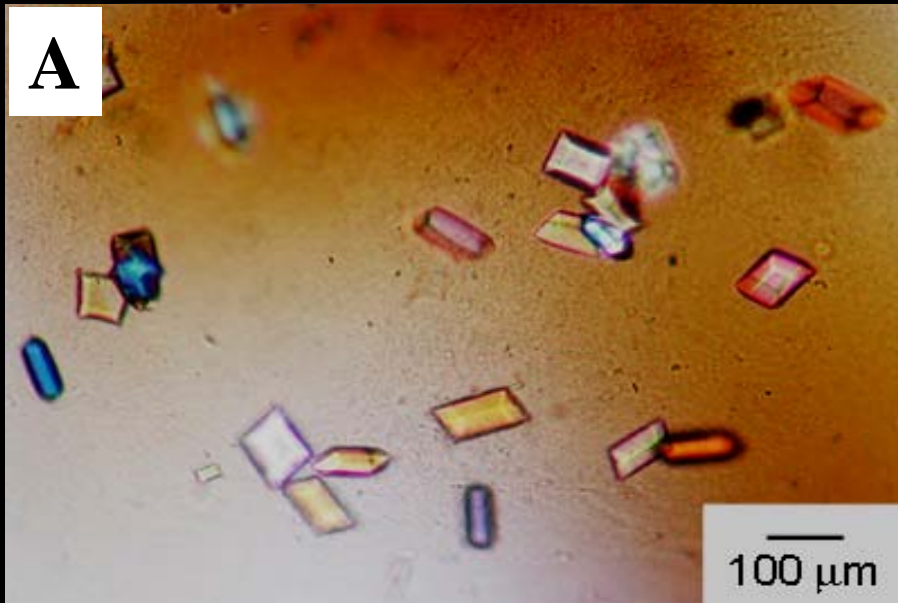
Precipitant
solution (1 μL)

96-well
spacer, 125 μm

Glass
coverslip

Glass
base

Production Mode



ACKNOWLEDGEMENTS

COLLABORATORS

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