

**Membrane (and Soluble) Protein
Crystallization for Structure-Function
Studies Using Lipid Mesophases.
In situ diffraction data collection**

**Membrane Structural and Functional
Biology Group**

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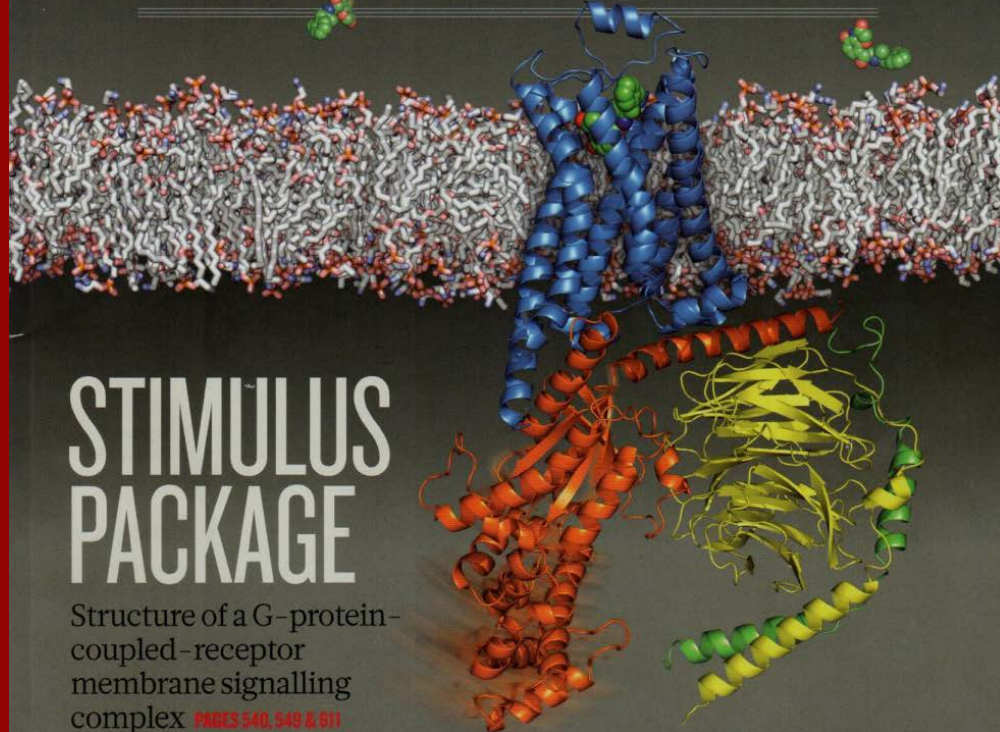
In Situ Serial Crystallography Workshop

Swiss Light Source, Villigen, Switzerland

November 17 - 19, 2015

nature

THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE



THERAPEUTICS

A SUITABLE CASE FOR TREATMENT

Four ways to revitalize the clinical trial

PAGE 526

COSMOLOGY

GENERAL RELATIVITY

Gravitational redshift from galactic clusters is latest test

PAGES 541 & 567

POLICY

REASONS TO BE CHEERFUL

Well-being as an index of social progress

PAGE 532

NATURE.COM/NATURE

29 September 2011 £10

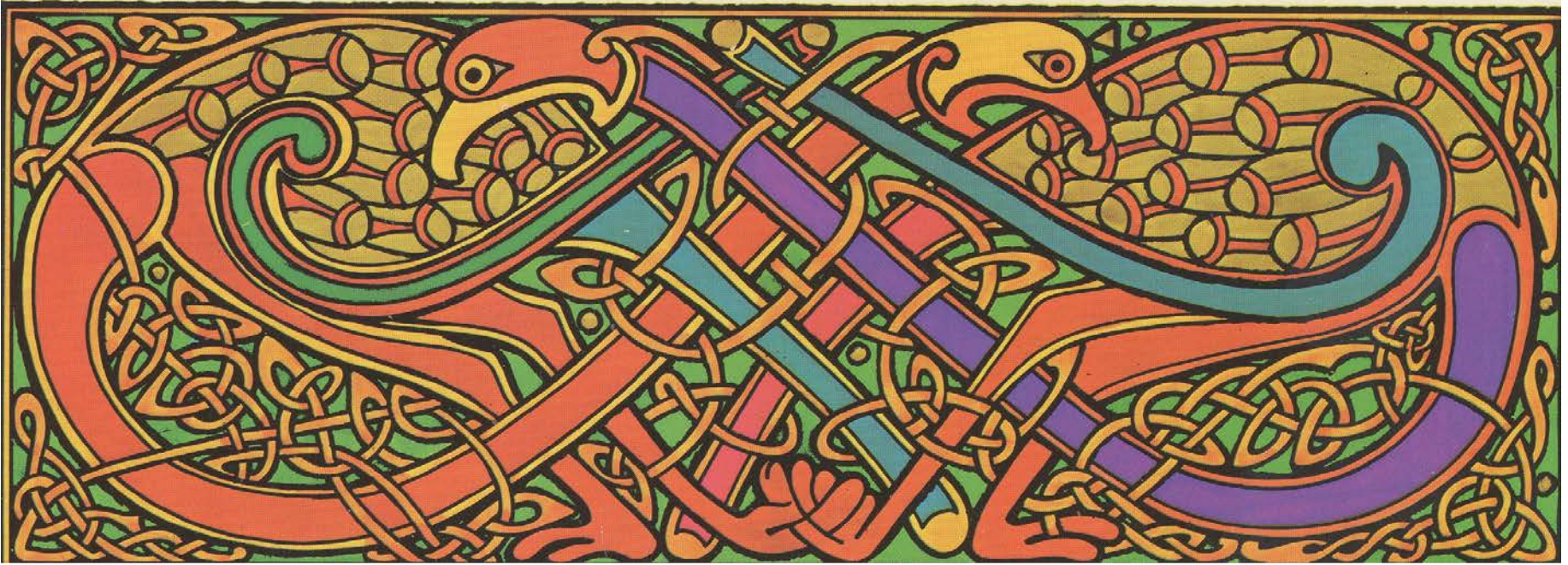
Vol. 477, No. 7366



Nature 477: 549

“This image is a molecular masterpiece ...”

The Royal Swedish Academy of Science. October 10, 2012



Reference Material

- ♣ Slides
- ♣ Acta F71: 3-18. Acta D71:1238-56. Acta D *In press*
- ♣ Nature Protocols 4: 706-31
- ♣ JoVE 45: e1712; 67: e4000; 67: e4001 – Open Access

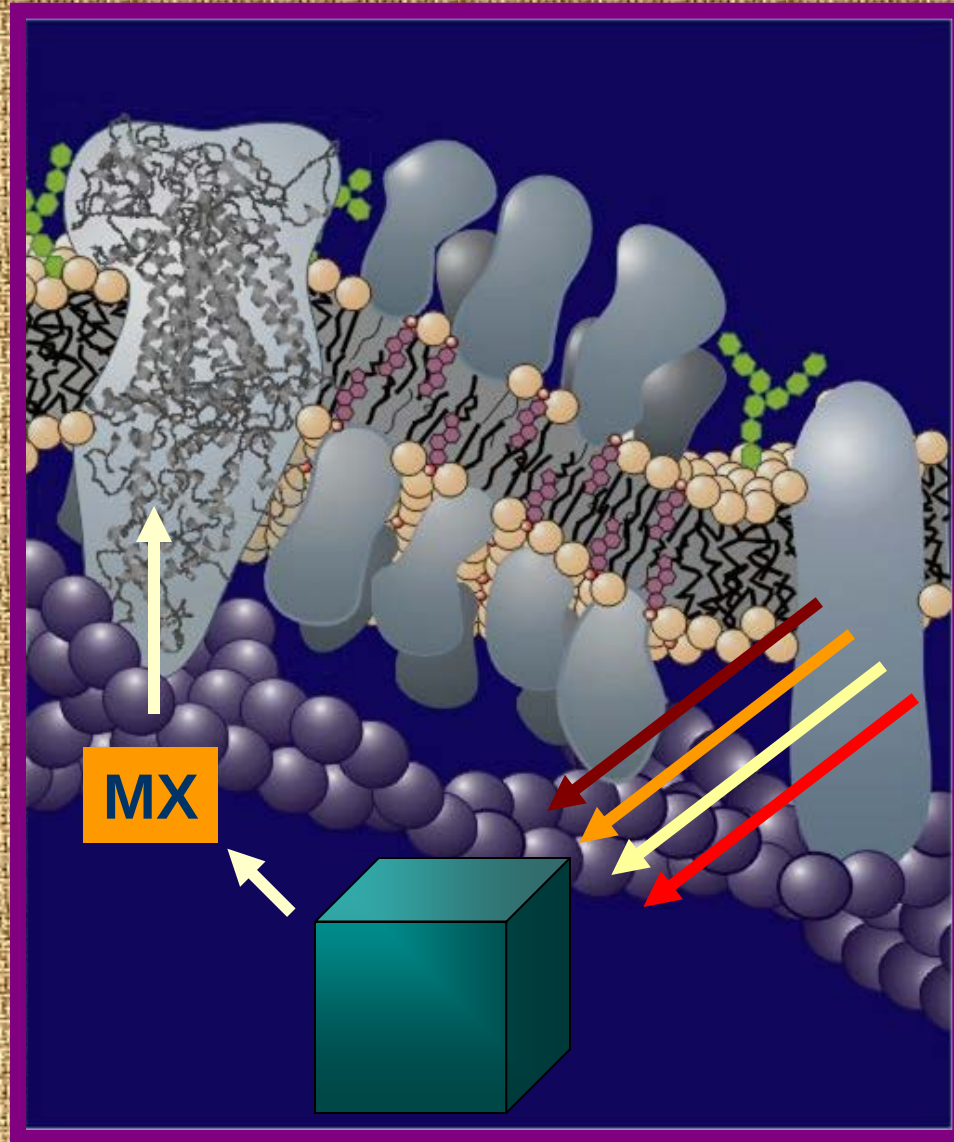
A fast, simple and robust protocol for growing crystals in the lipidic cubic phase

Margaret Aherne, Joseph A. Lyons and Martin Caffrey*

Membrane Structural and Functional Biology Group, Schools of Medicine and Biochemistry and Immunology, Trinity College Dublin, Ireland. Correspondence e-mail: martin.caffrey@tcd.ie

A simple and inexpensive protocol for producing crystals in the sticky and viscous mesophase used for membrane protein crystallization by the *in meso* method is described. It provides crystals that appear within 15–30 min of setup at 293 K. The protocol gives the experimenter a convenient way of gaining familiarity and a level of comfort with the lipidic cubic mesophase, which can be daunting as a material when first encountered. Having used the protocol to produce crystals of the test protein, lysozyme, the experimenter can proceed with confidence to apply the method to more valuable membrane (and soluble) protein targets. The glass sandwich plates prepared using this robust protocol can further be used to practice harvesting and snap-cooling of *in meso*-grown crystals, to explore diffraction data collection with mesophase embedded crystals, and for an assortment of quality control and calibration applications when used in combination with a crystallization robot.

Structural and Functional Biology of Membranes



Crystallization Methods

Vapor Diffusion
hanging drop
sitting drop

Batch
Dialysis

Bilayer Methods

bicelle

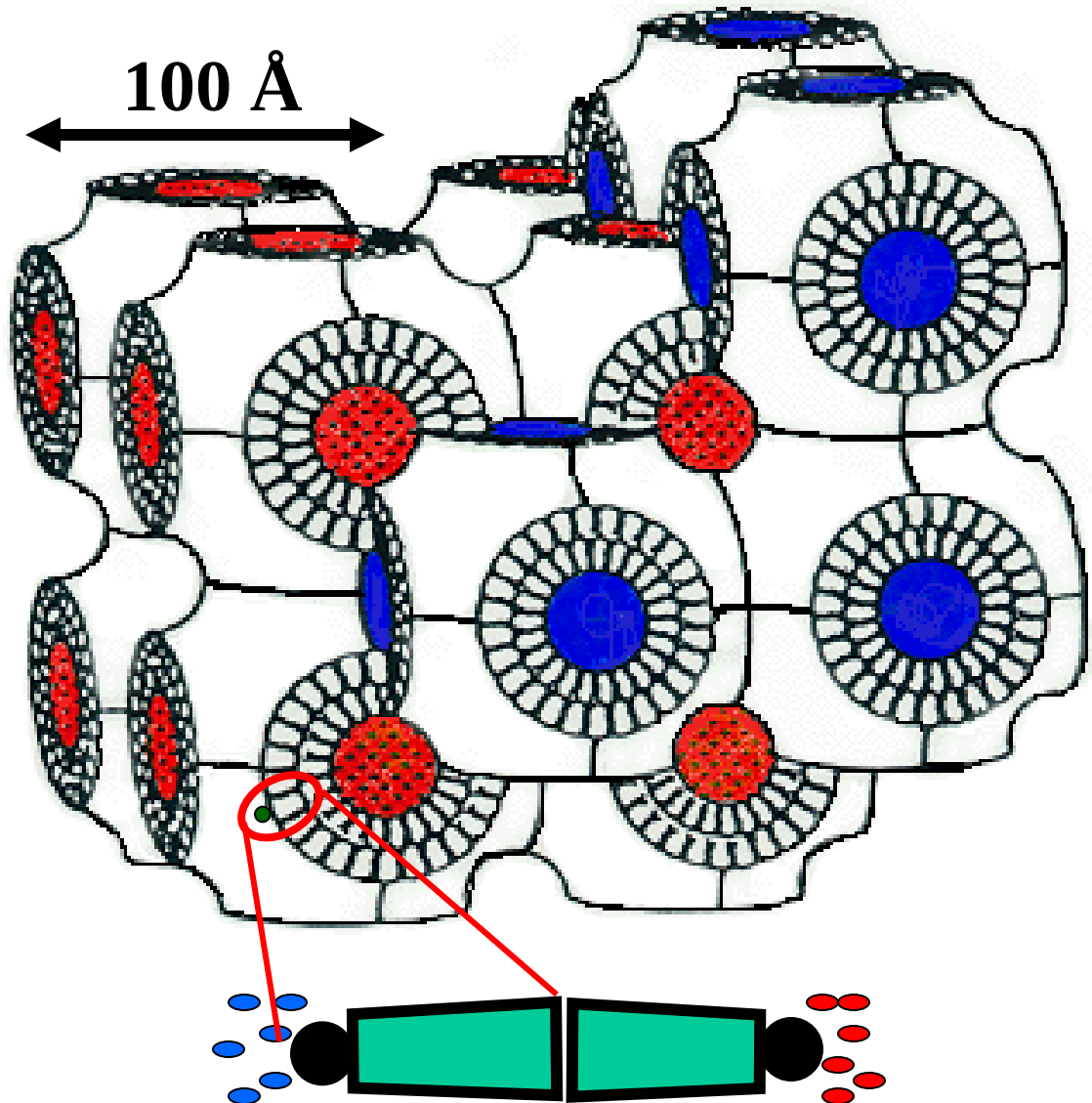
vesicle fusion

cubic phase

Evaporation

Interfacial Diffusion

Cubic Phase



Bacteriorhodopsin crystallized
in lipidic cubic phase



Lamellar
portal

PROTEIN
CO-CRYSTAL

Crystalline
array

CHARGE
SCREENING

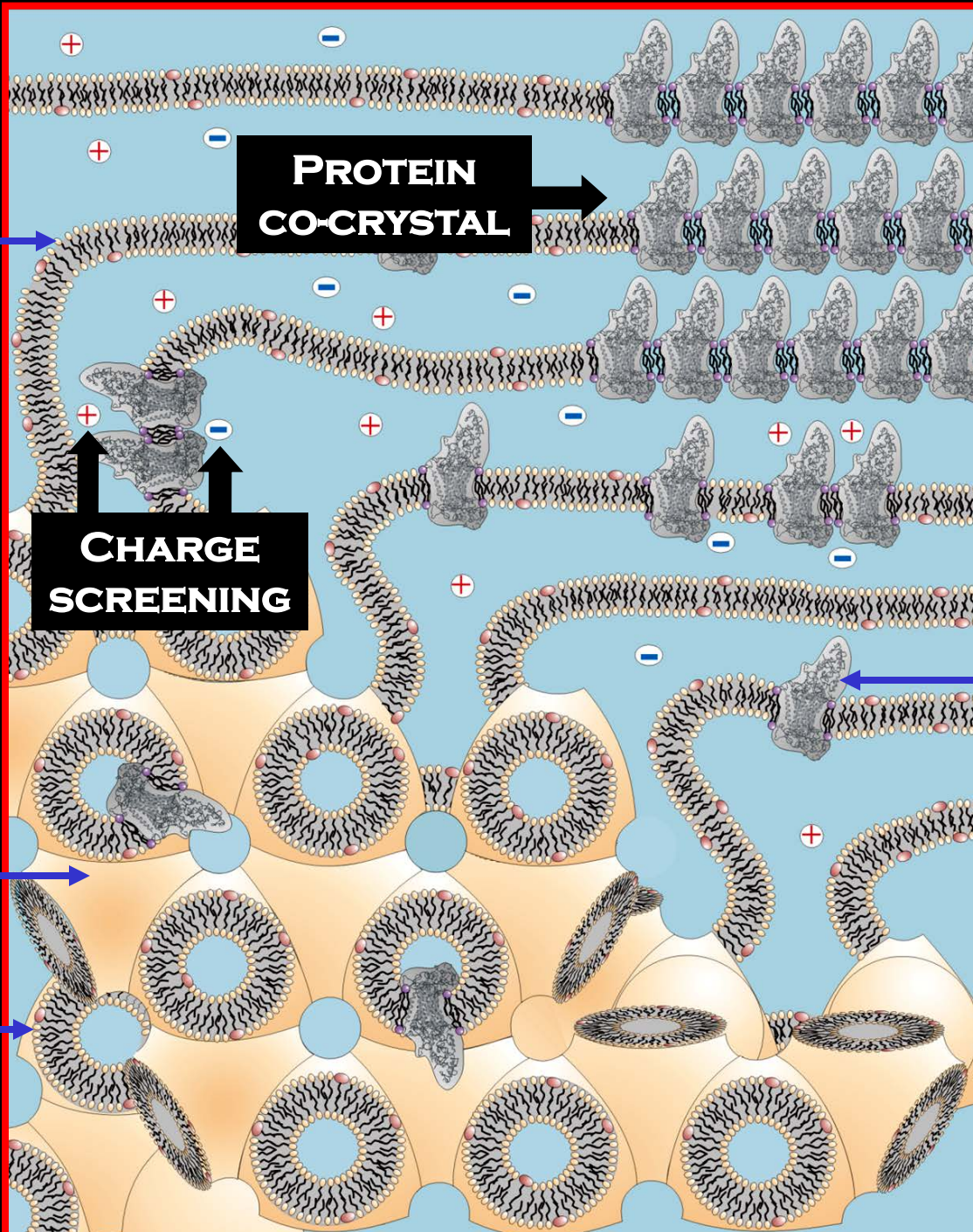
Reconstituted
protein

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

10 μ L 100 μ L

100 μ L

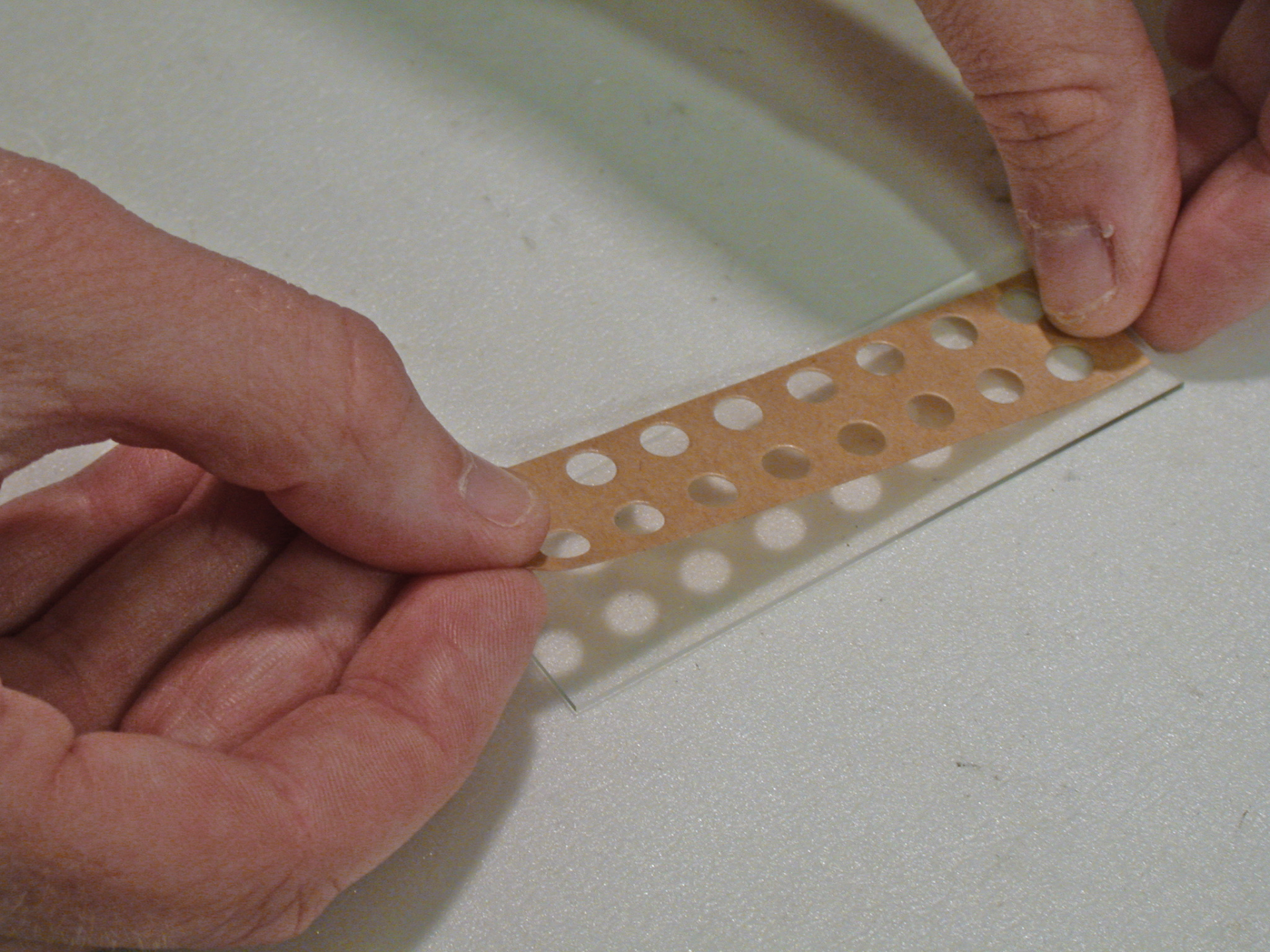
Calculator

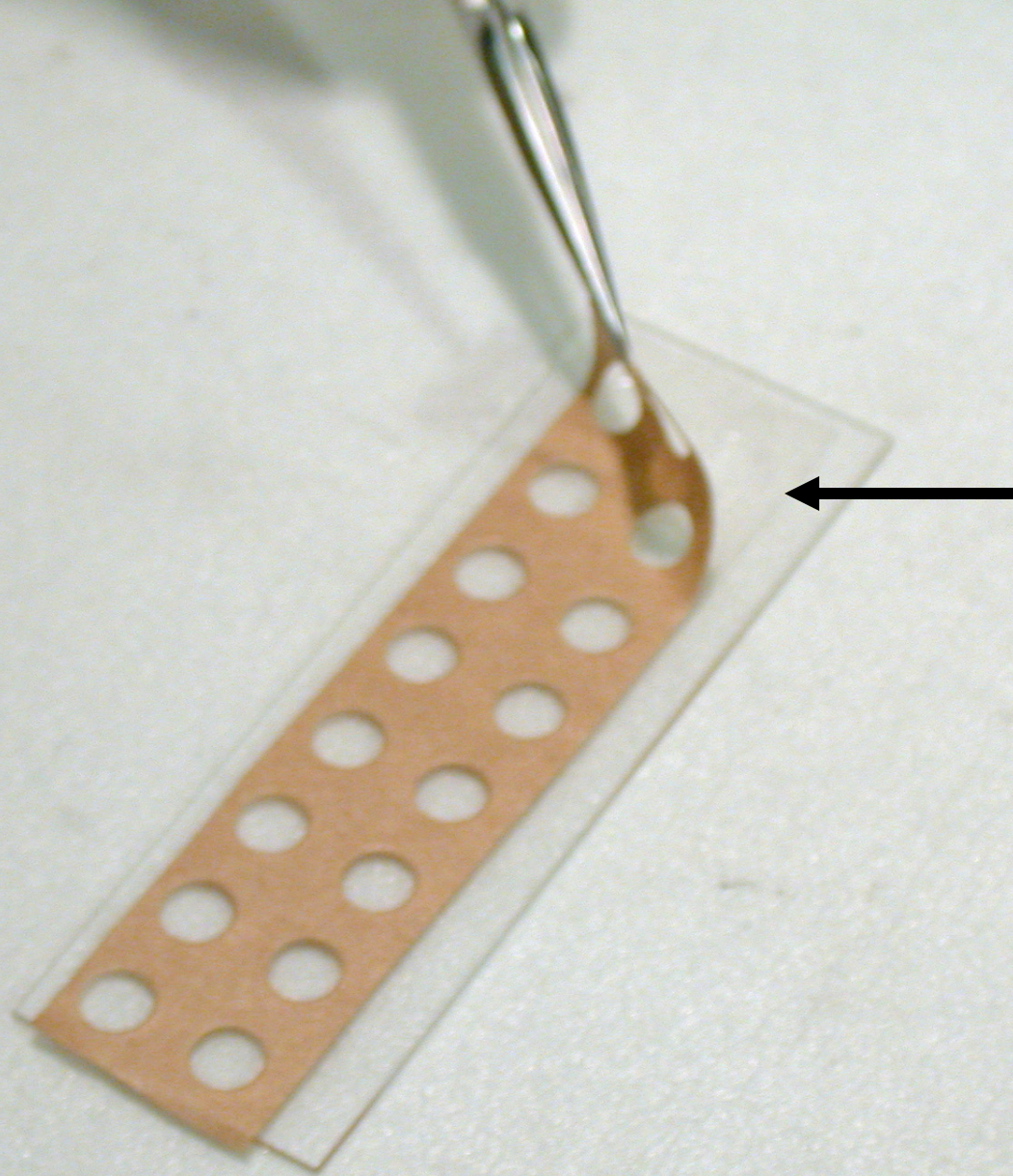
Ice

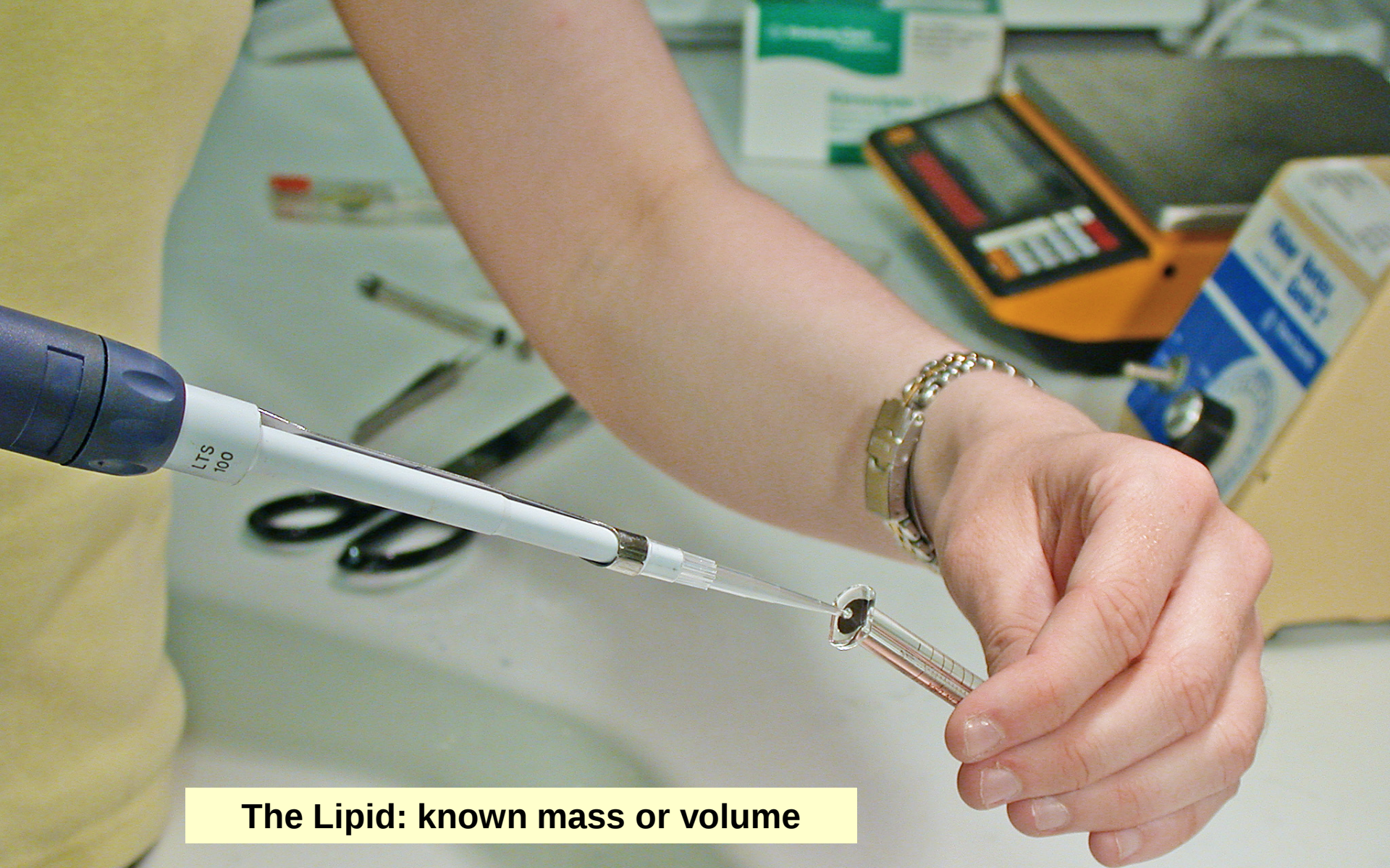
30 μ L

1 μ L



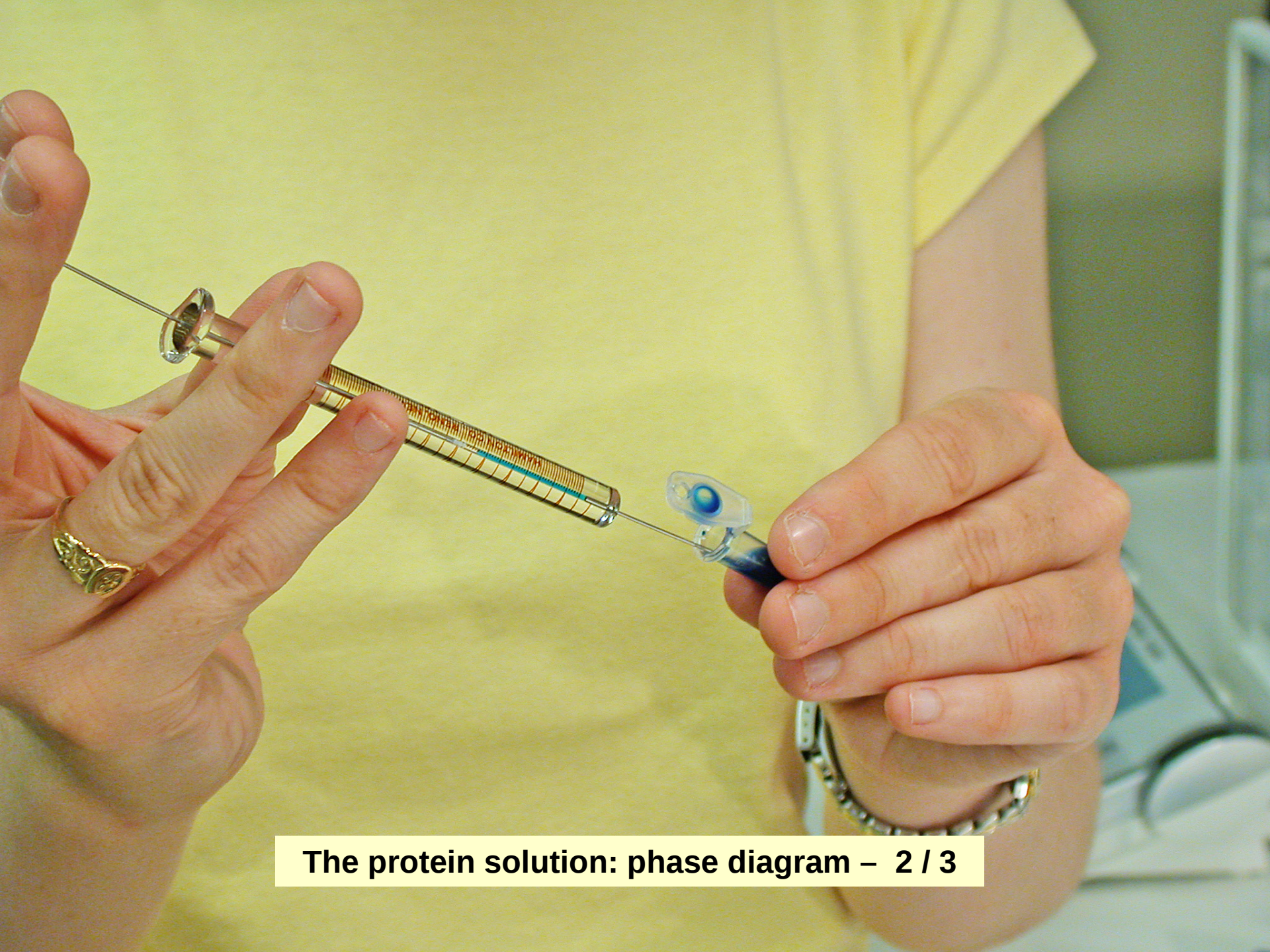






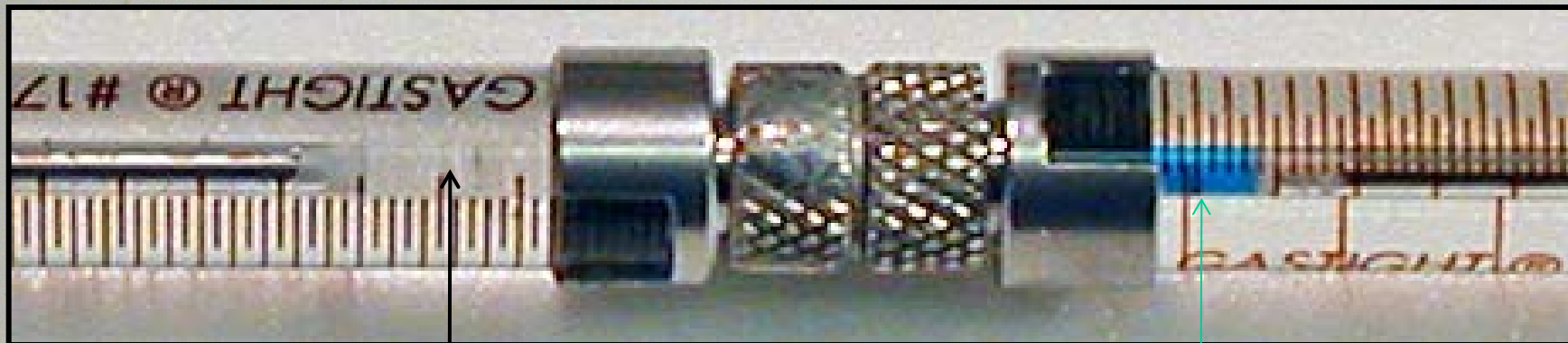
The Lipid: known mass or volume





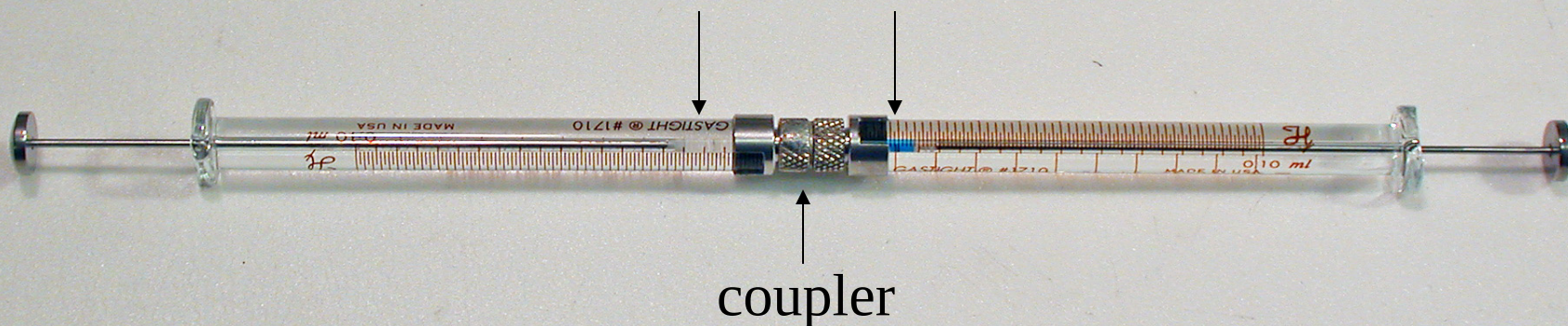
The protein solution: phase diagram – 2 / 3





lipid

protein solution



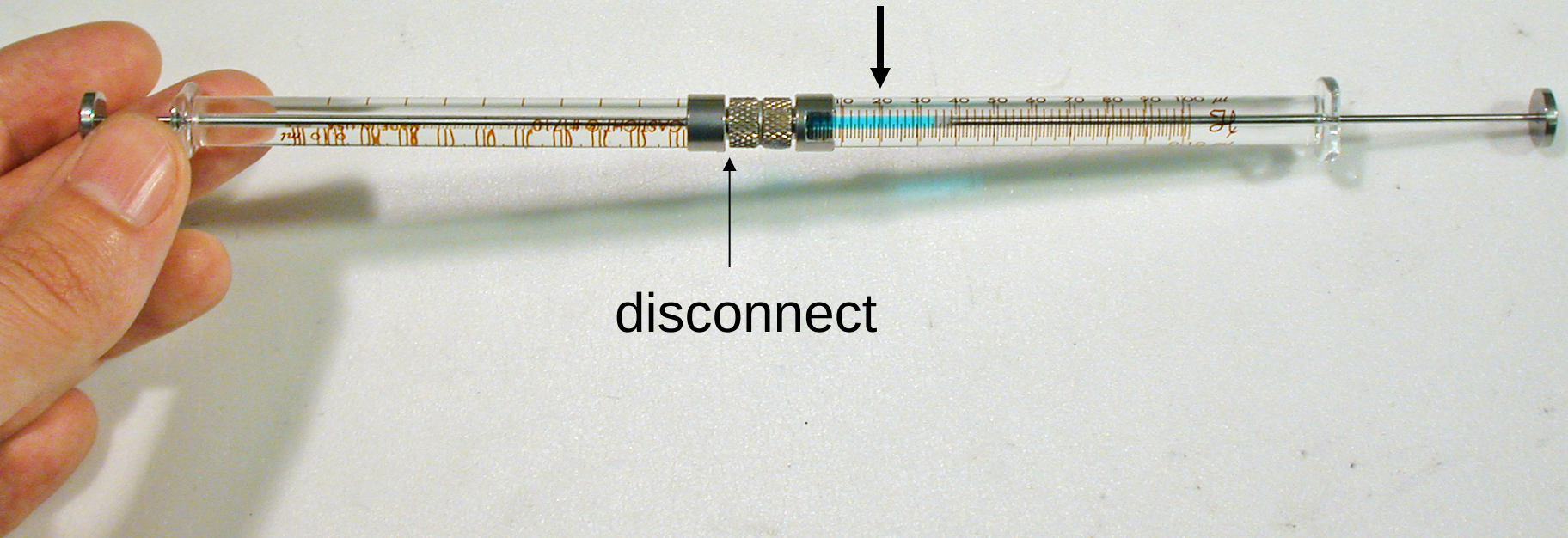
coupler

Coupled Syringe Mixer



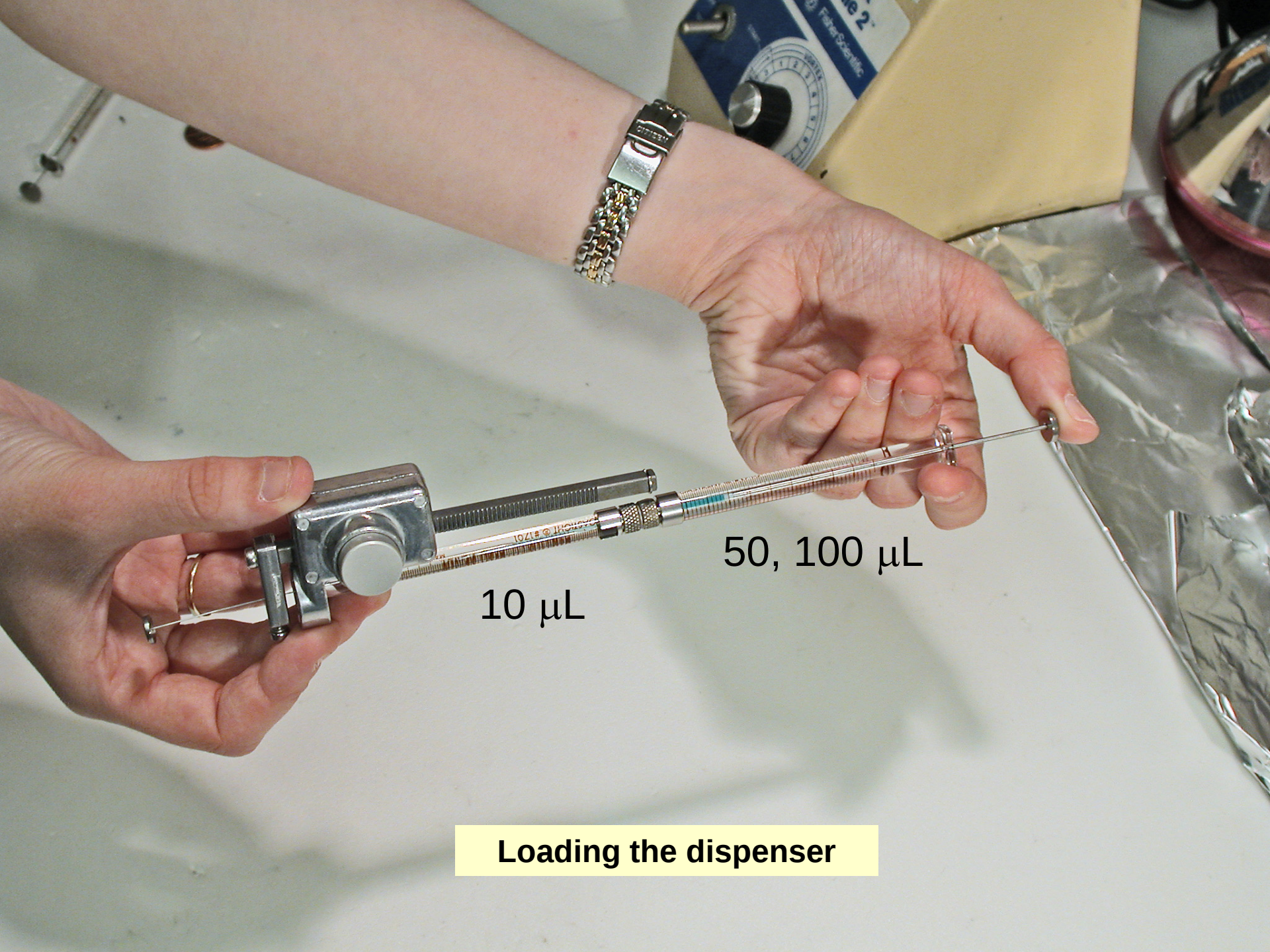
Mechanically mixing the protein solution and lipid together to form the cubic mesophase

optically clear
protein/lipid mesophase



disconnect

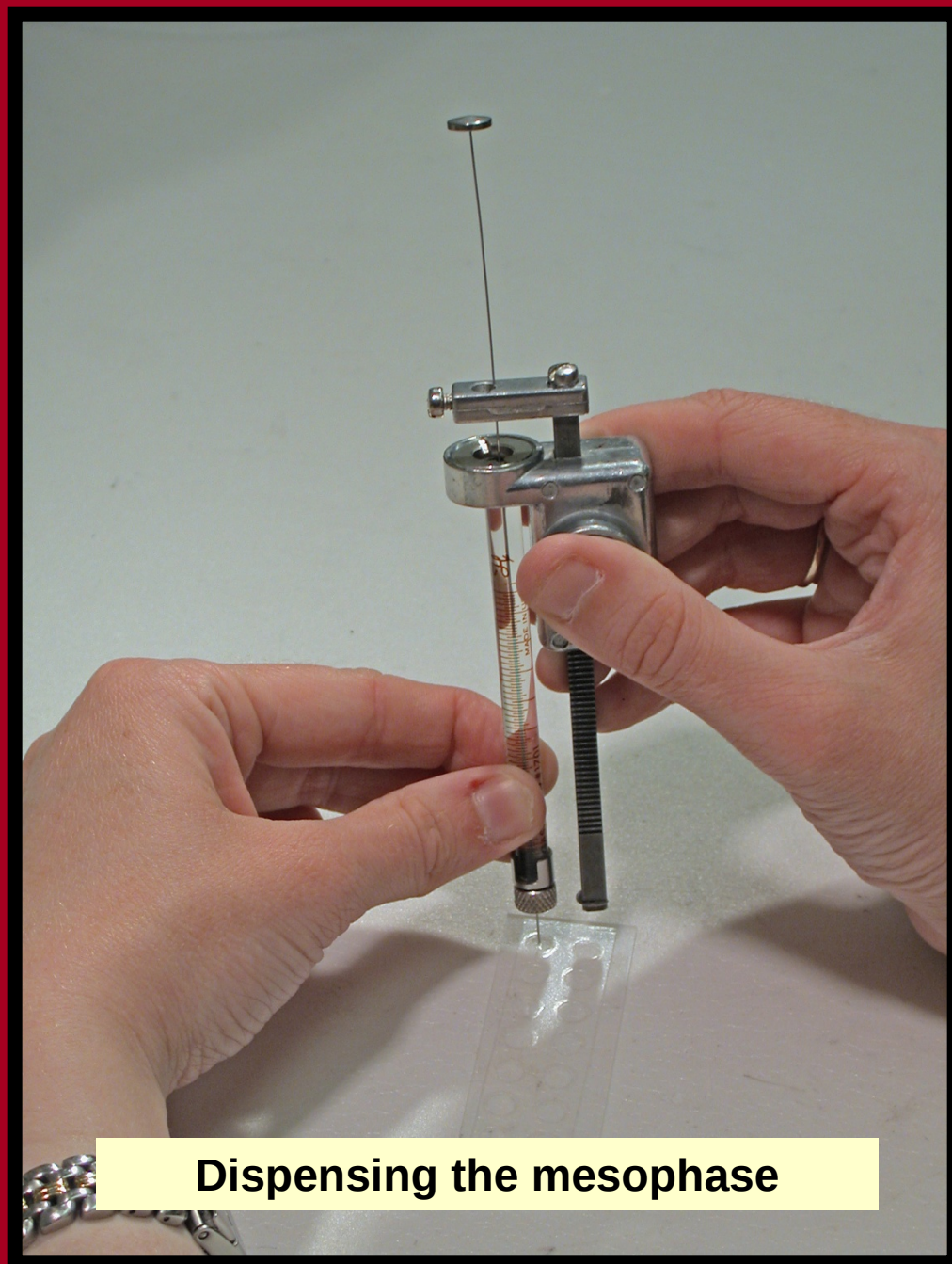
The homogenous protein / lipid cubic phase



10 μL

50, 100 μL

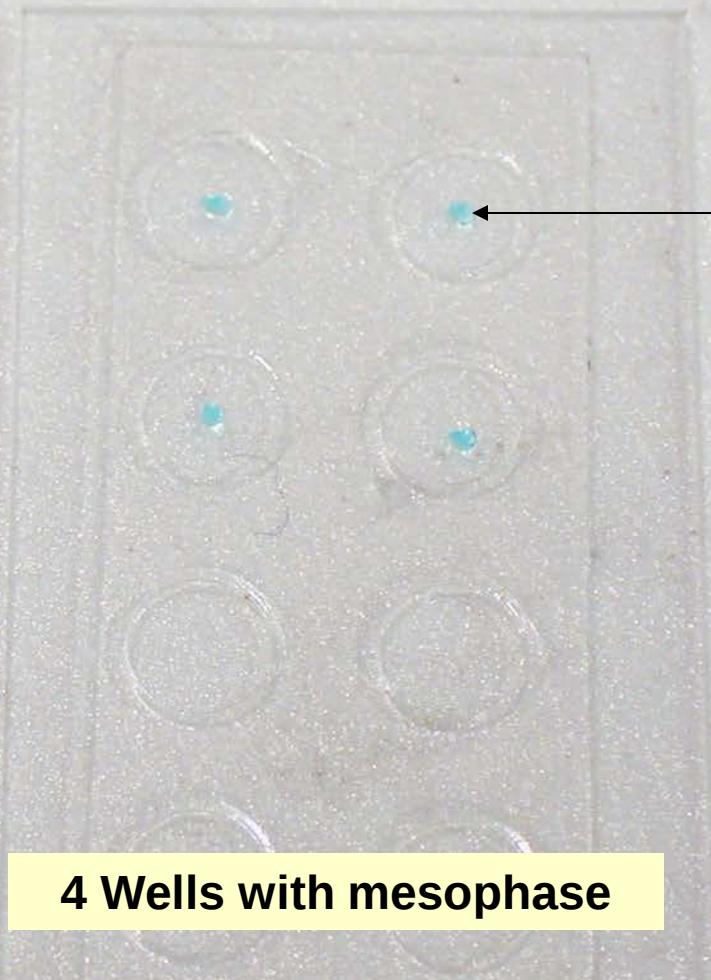
Loading the dispenser



Dispensing the mesophase

200 nL
protein/lipid
mesophase

4 Wells with mesophase

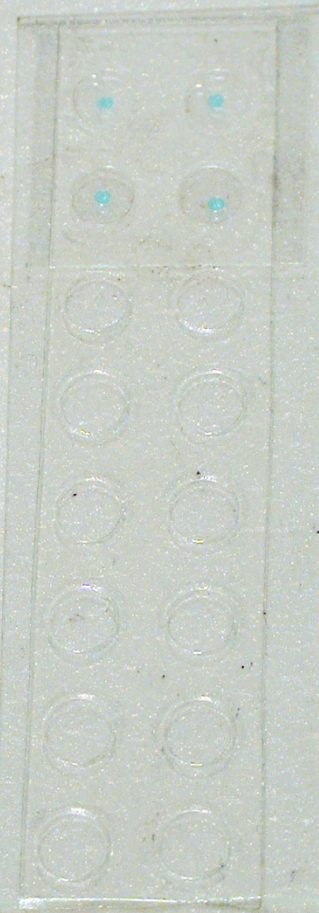


**Dispensing 1 μ L
precipitant solution**



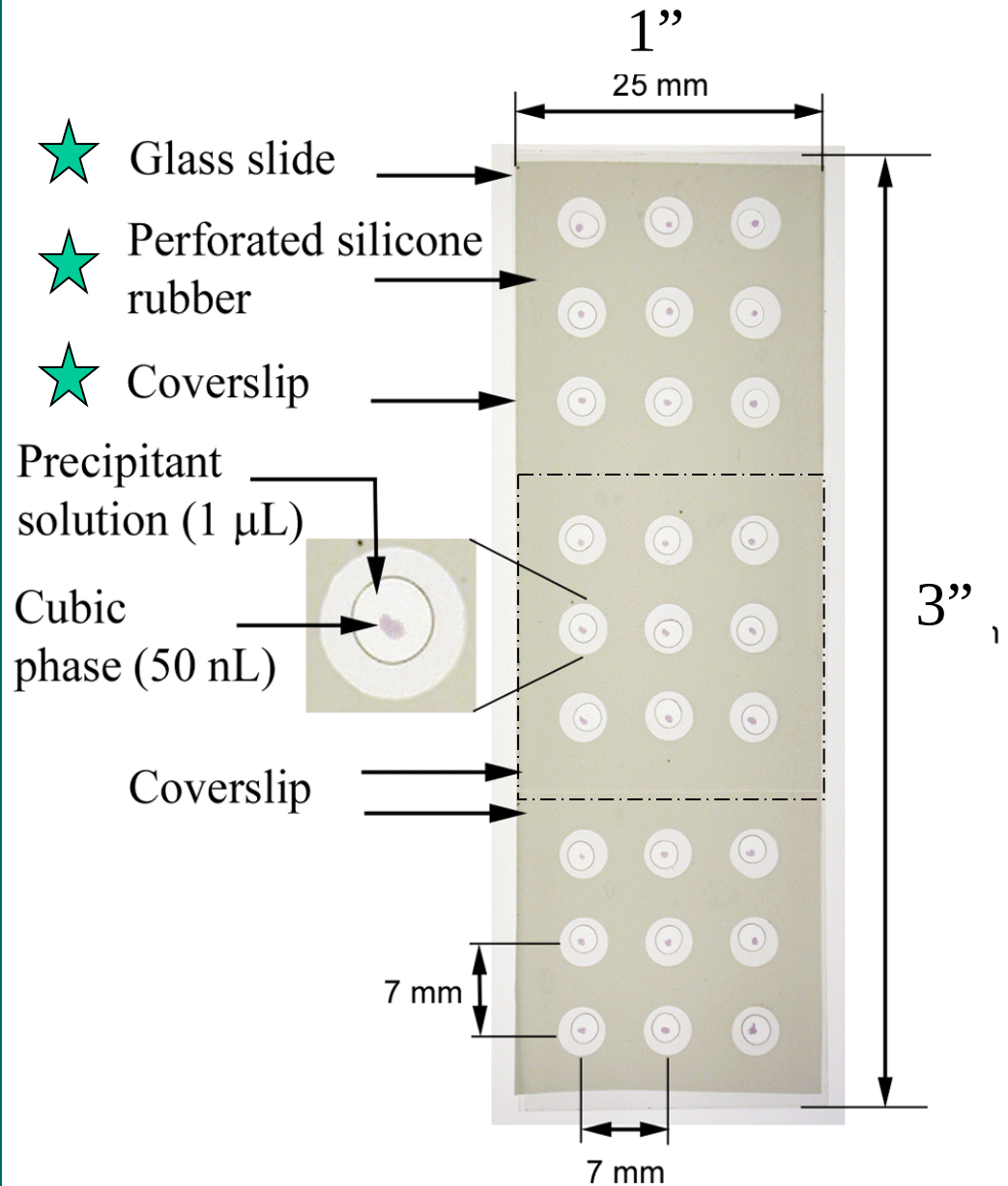


Sealing wells with glass coverslip



Filled and sealed wells

Loaded/sealed 27-well crystallization plate



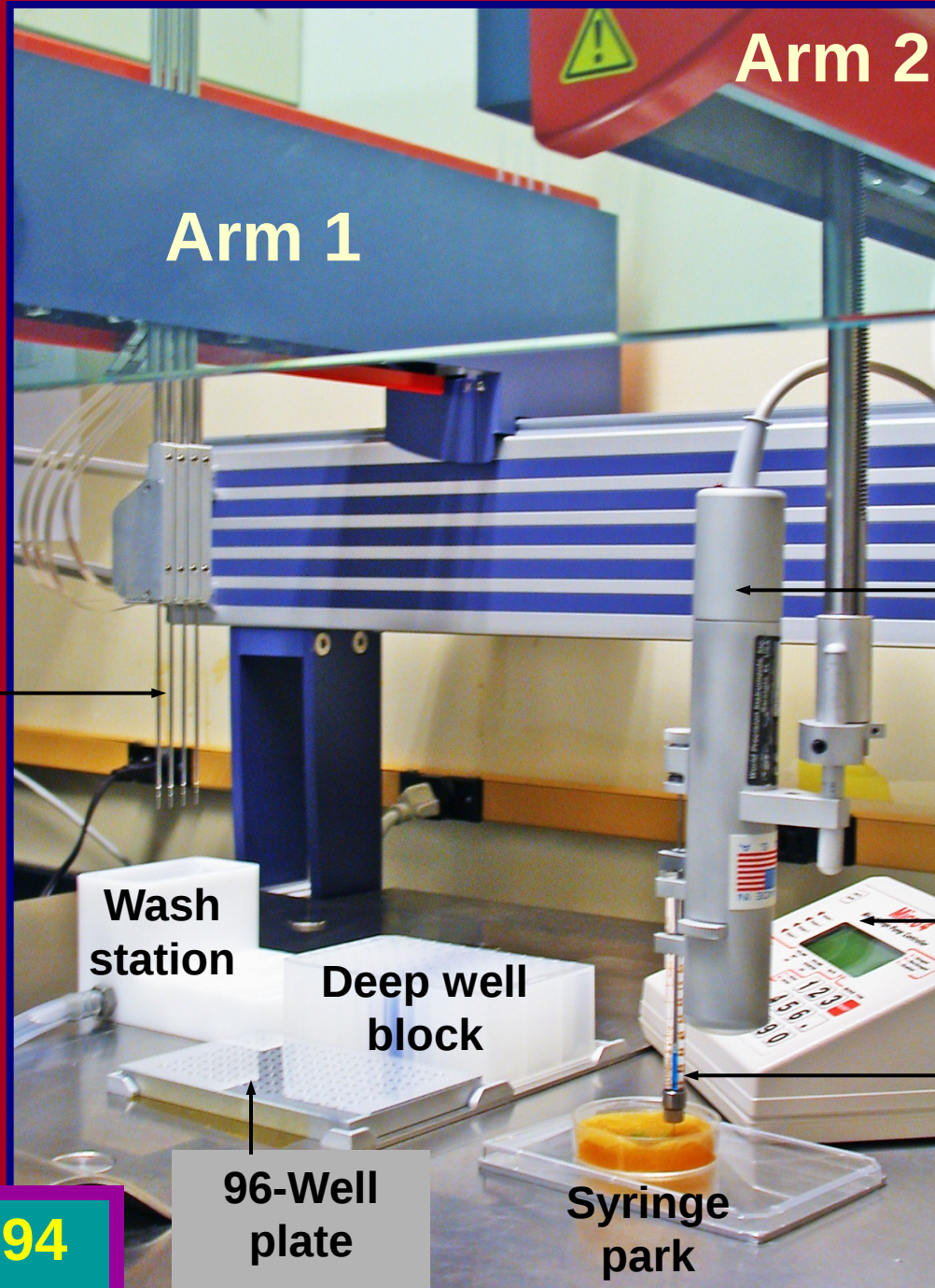
In Meso Robot

Liquid
Handling
Robot

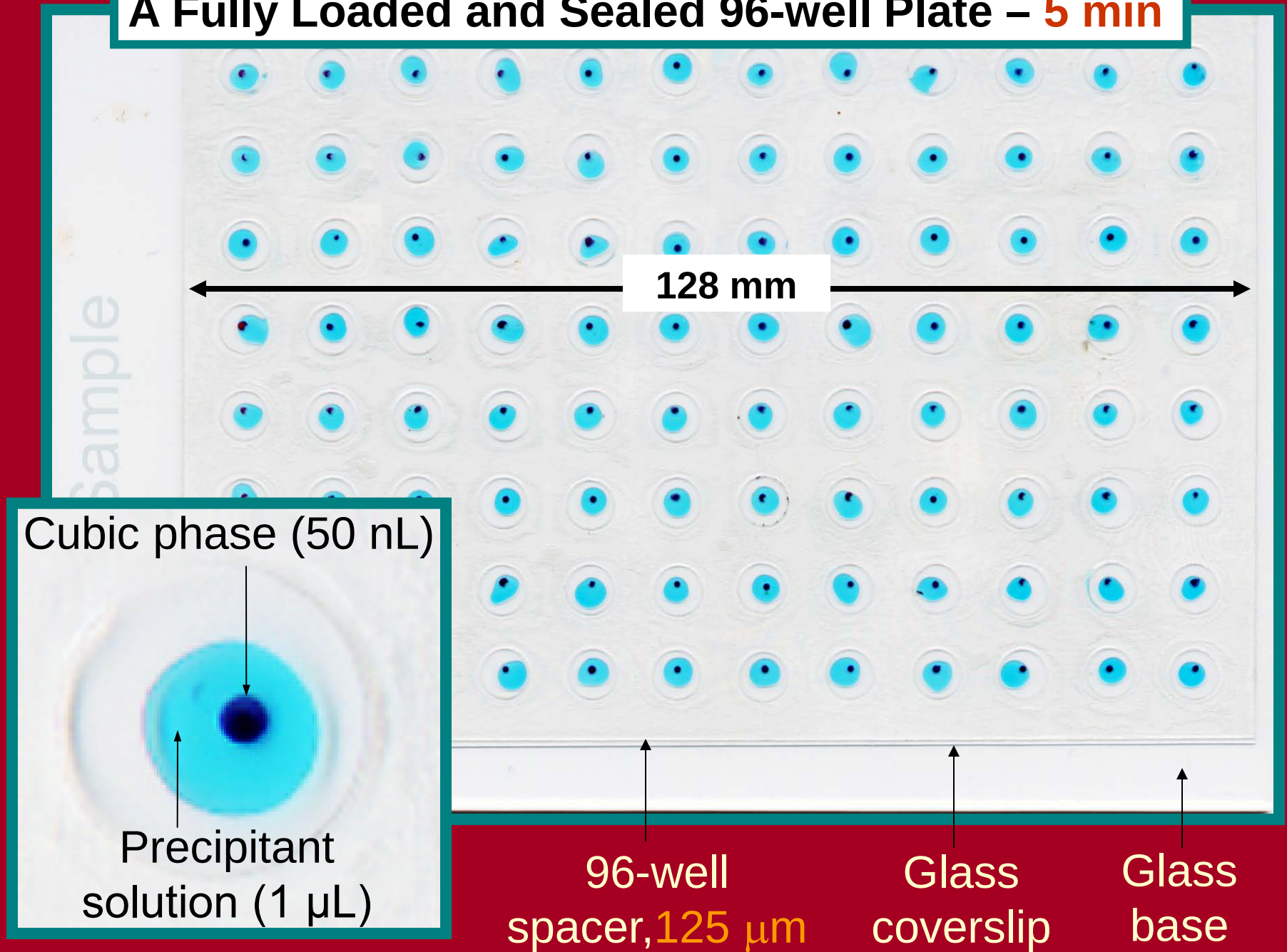
4-Tip
precipitant
solution
dispenser

Motorized
Micro-pump

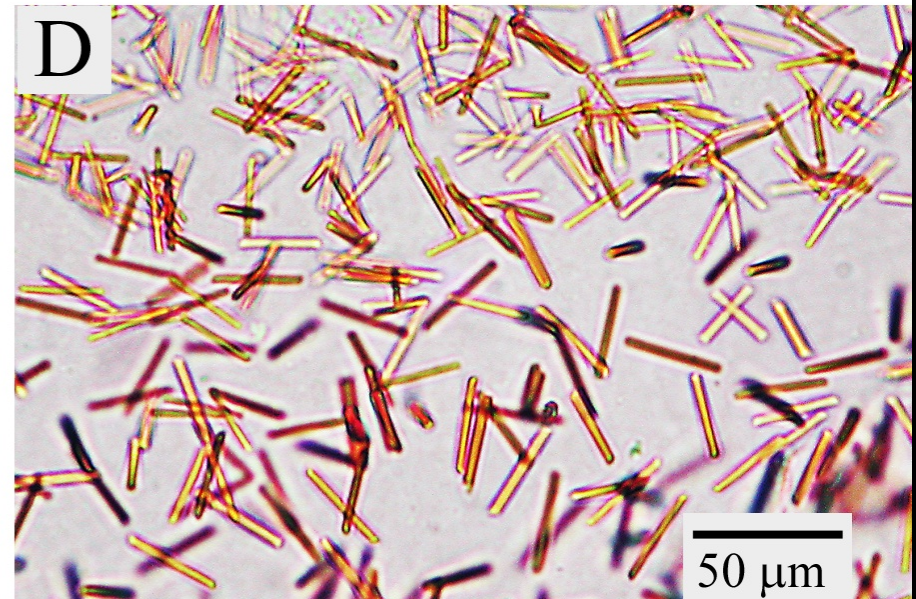
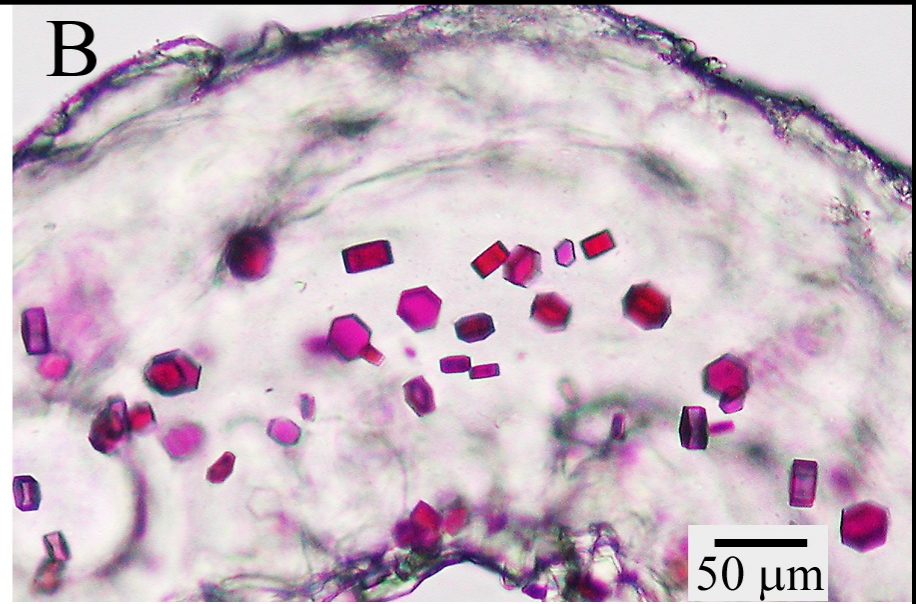
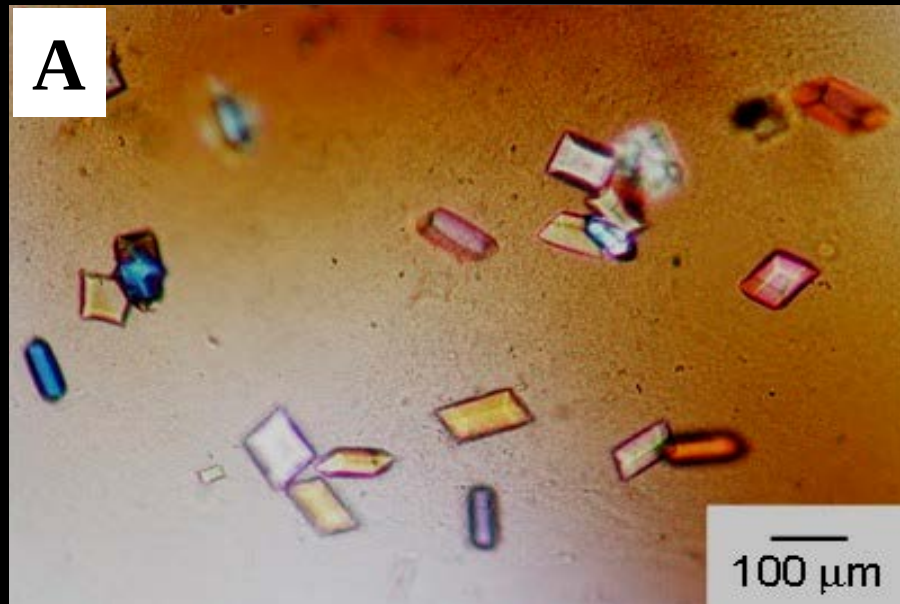
♣ Acta D60: 1794



A Fully Loaded and Sealed 96-well Plate – 5 min

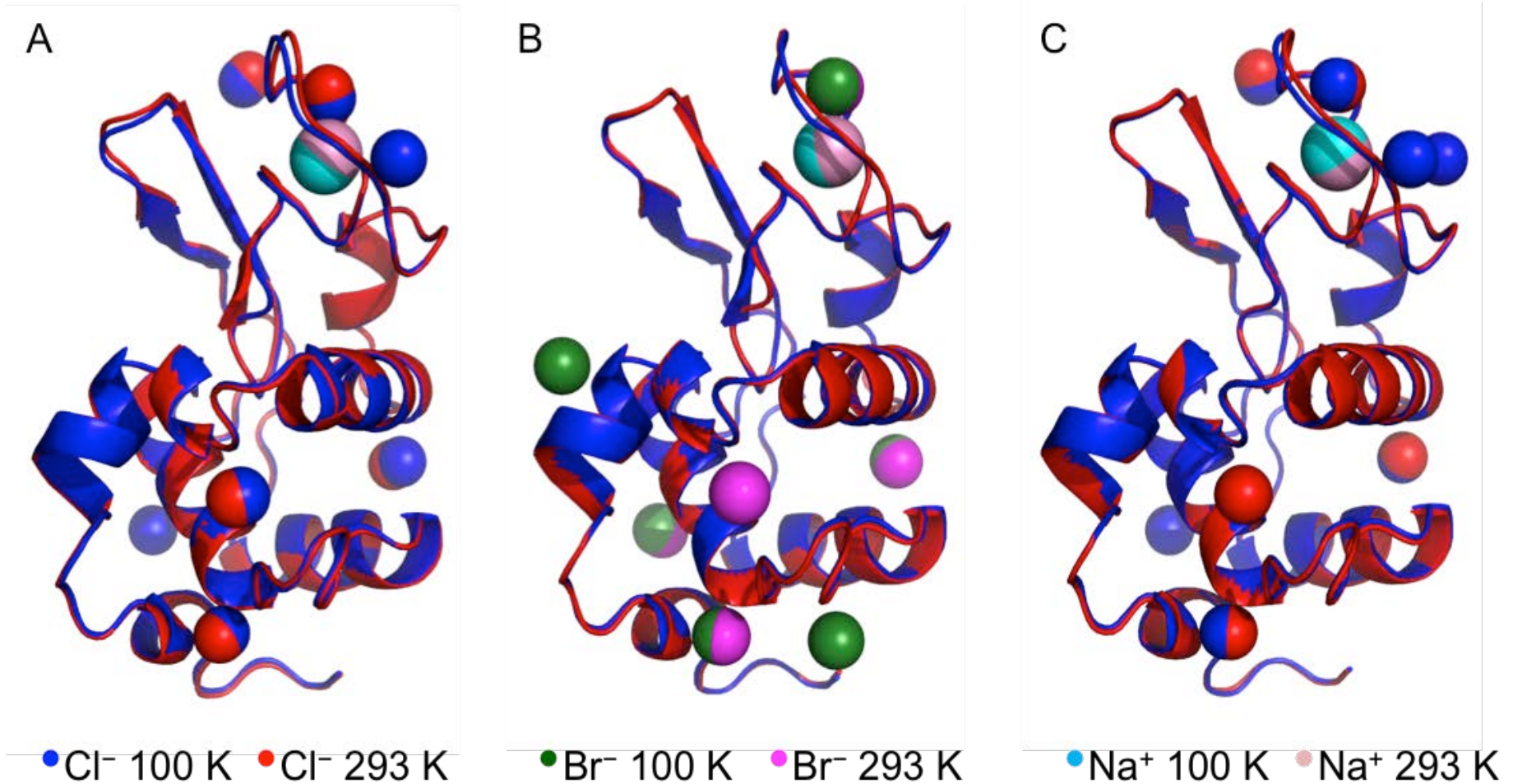


Crystals $\Rightarrow$ Harvest, Snap Cool, MX



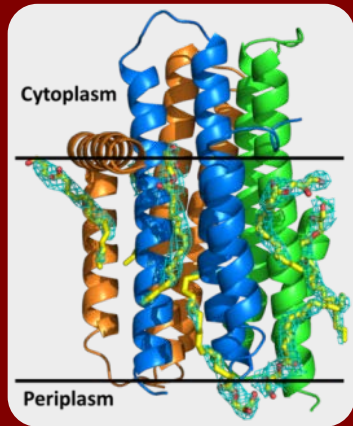
Water-soluble proteins too !

Lysozyme



+ insulin, thaumatin, α -lactalbumin,

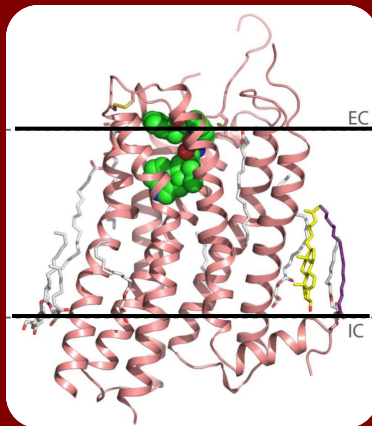
DgkA 2.2 Å



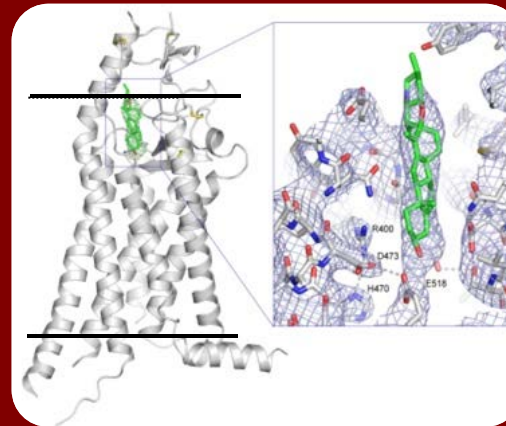
Jet LCP - SFX Works !

♣ Membrane protein structures solved

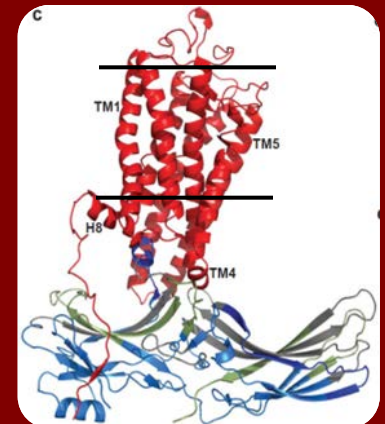
GPCR1 2.8 Å



GPCR2 ~3.2 Å



Rh-Arr ~3.5 Å



♣ Science 342:1521

♣ Nature Commun. 5:3309

♣ Phil. Trans. R. Soc. B 369

♣ Nature 523:561

In Meso In Situ SX

iMiSX

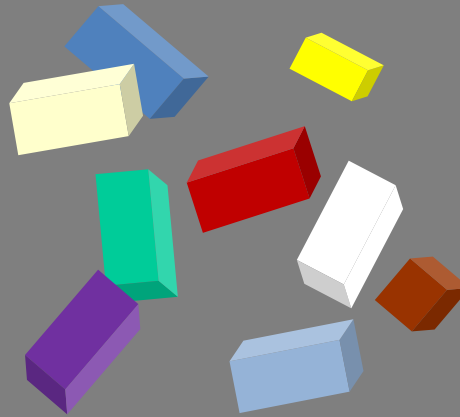
glass

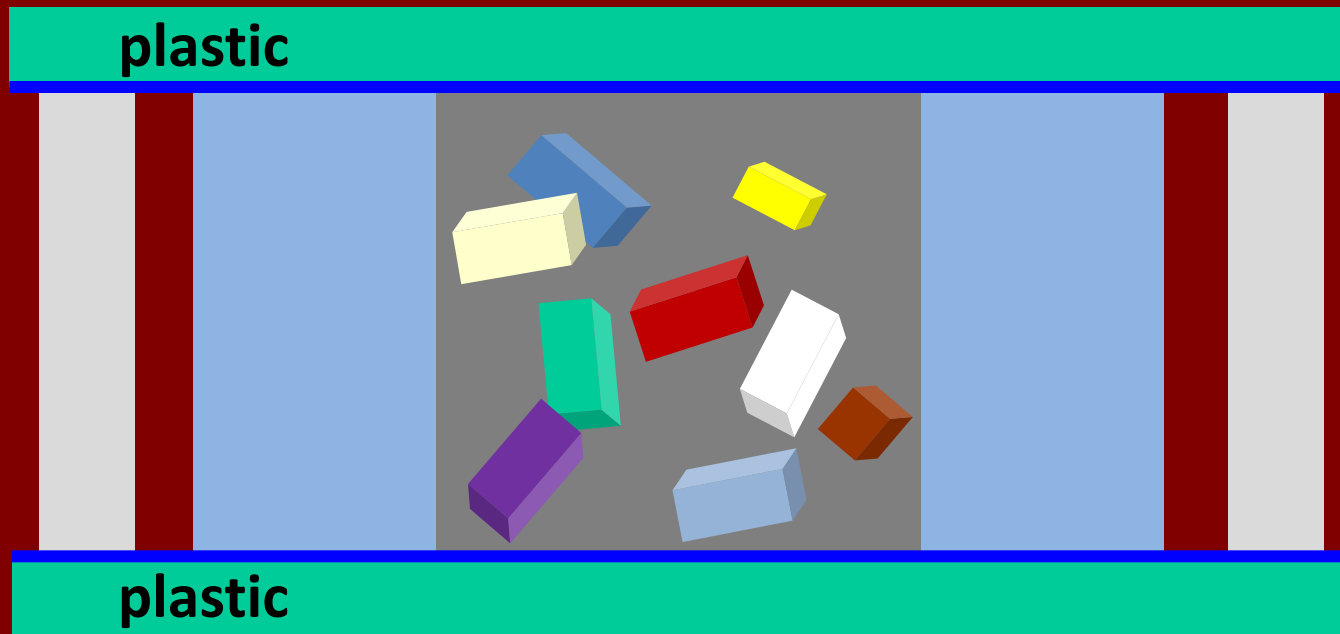
plastic

plastic

glass

Double Sandwich Plate





Inner Sandwich Plate - RT or Cryo