

Crystallization of Membrane Proteins in Lipidic Systems For Structure-Function Studies

**Membrane Structural and Functional
Biology Group**

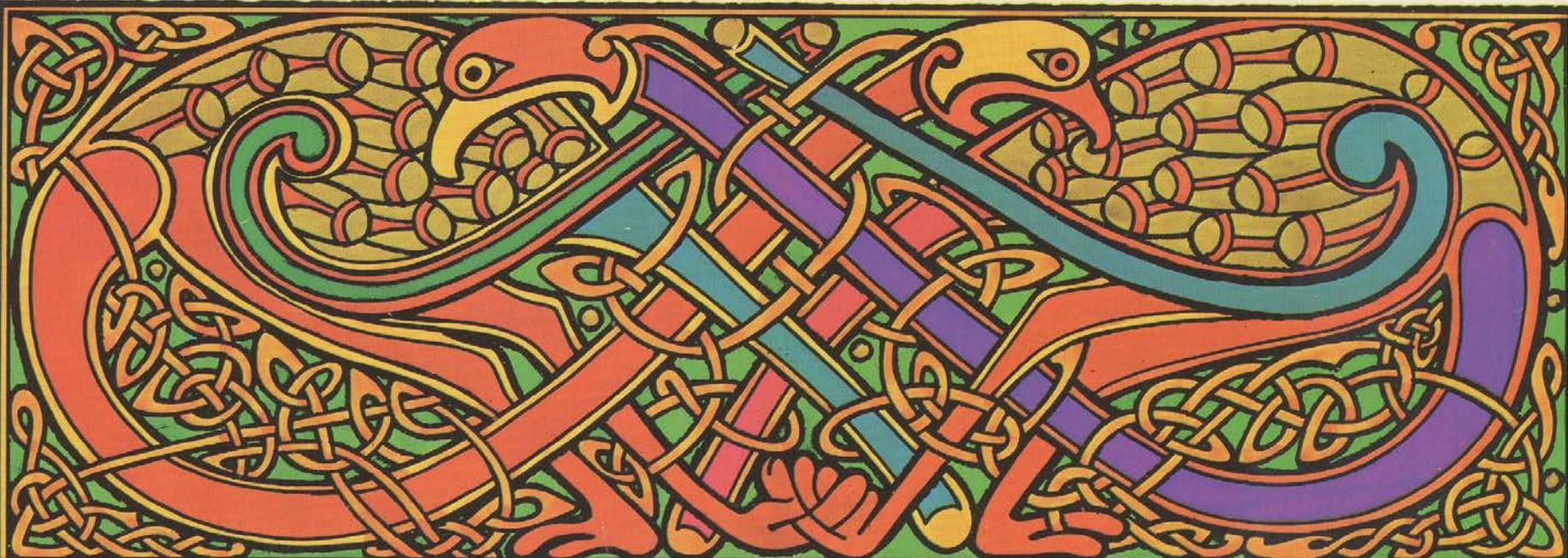
Trinity College Dublin, Ireland

martin.caffrey@tcd.ie

Methods of Structural Biology

**Satellite Workshop to 25th General Assembly and Congress
of IUCr 2021**

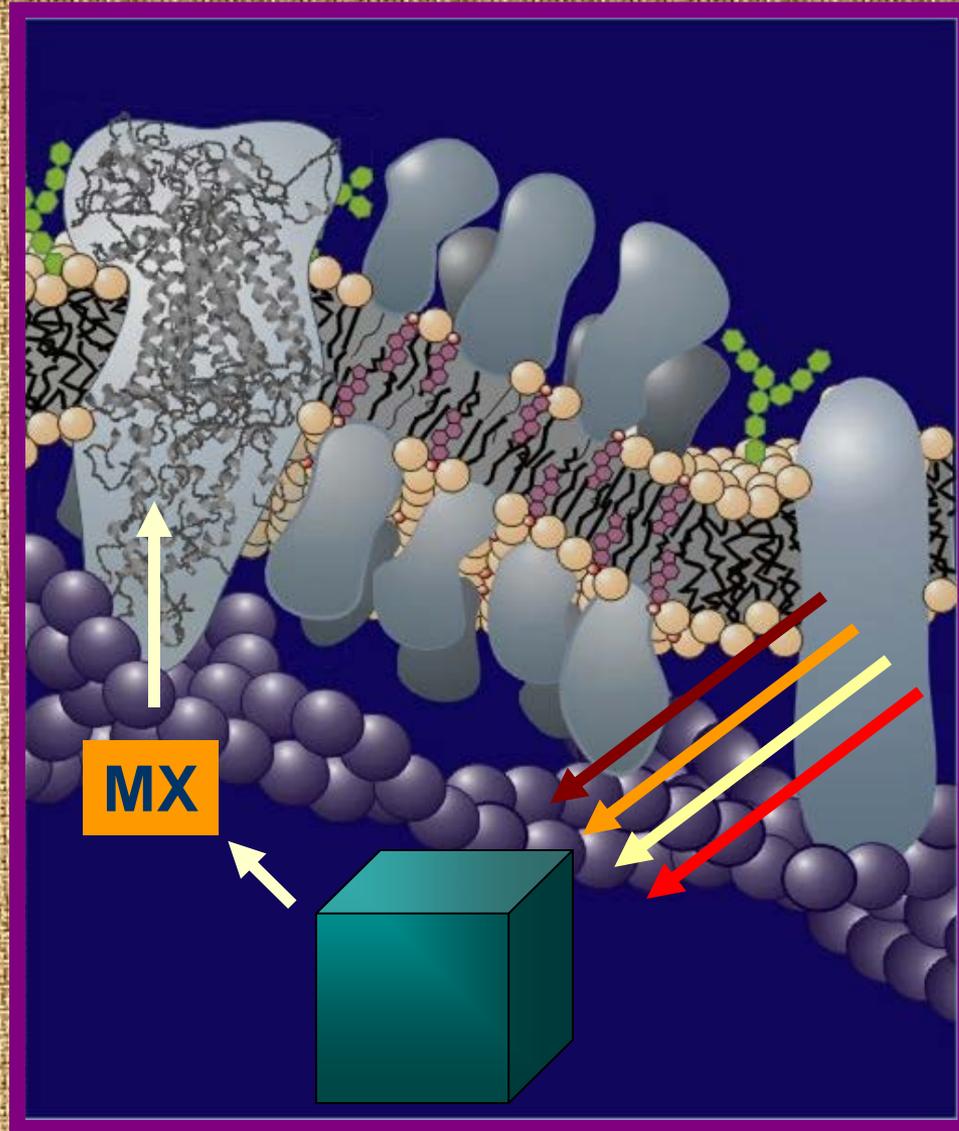
**Institute of Biotechnology of the Czech Academy of Sciences, Biocev,
Prumyslova 595, 252 50 Vestec, Prague, Czech Republic. *August 13, 2021***



Reference Material

- ♣ **Slides**
- ♣ **Acta F71: 3-18. Acta D71:1238-56. Acta D72:93-112**
- ♣ **Nature Protocols 4: 706-31. J. Appl. Cryst. 45:1330-3**
- ♣ **JoVE 45: e1712; 67: e4000; 67: e4001 – Open Access**

Structural and Functional Biology of Membranes



Crystallization Methods

Vapor Diffusion

hanging drop
sitting drop

Batch

Dialysis

Bilayer Methods

bicelle

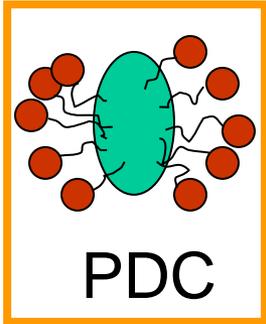
vesicle fusion

cubic phase

Evaporation

Interfacial Diffusion

Bicelle Crystallization Method

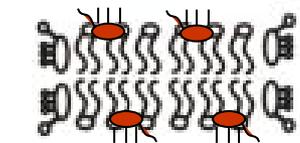


PDC

Protein Solution
(on ice)
10 mg bR/mL



Lipid/amphiphile
mixture (on ice)

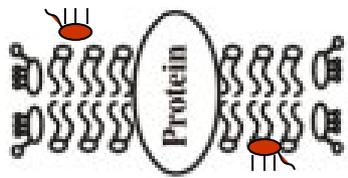


40 % (w/v) DMPC/CHAPSO (3/1 mol)

DMPC vesicles + CHAPSO

Vortex, Sonicate
Thermal cycling

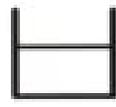
Mix



Homogenous protein/bicelle
mixture (on ice)



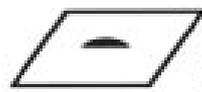
8 mg bR/mL



Precipitant

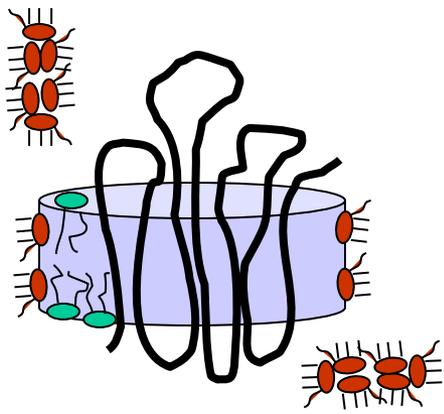
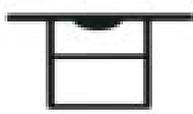
Mix

Hanging Drop Vapour Diffusion

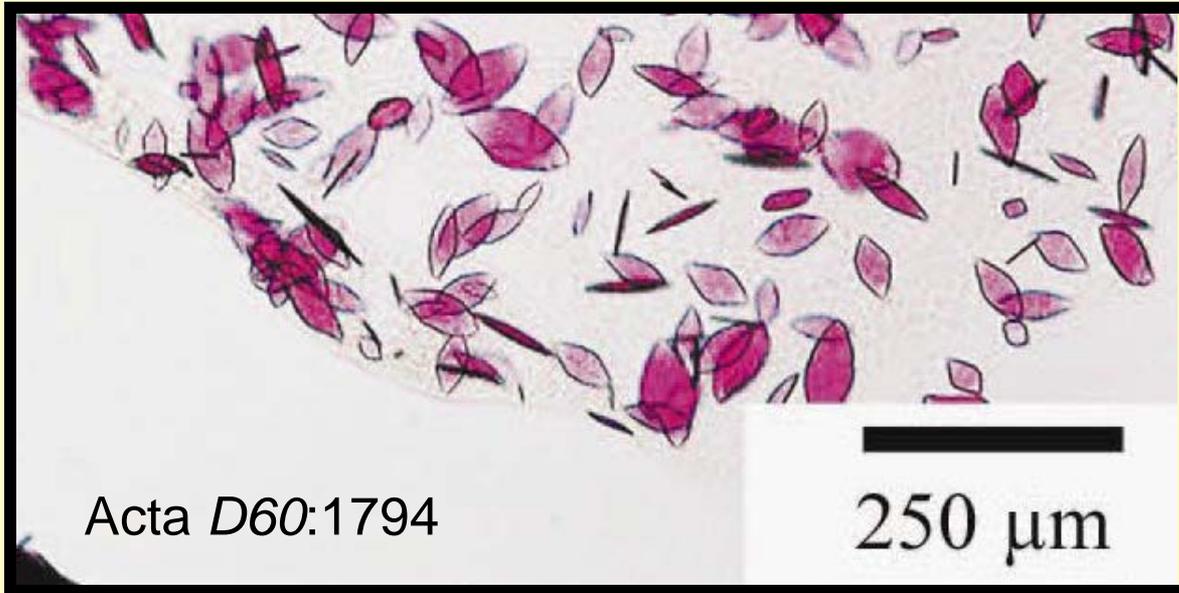
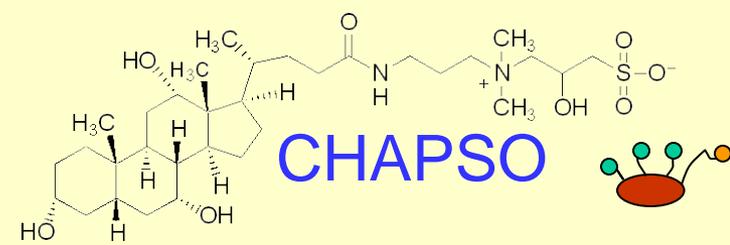


Invert coverslip and let warm
to room temperature or to 37°C
to form bicellar, or perforated
lamellar phase.

viscous



Bicelle Method



Variations

DiC₁₄PC

DiC₁₃PC

CHAPSO

DiC₆PC

Vapour diffusion

Batch

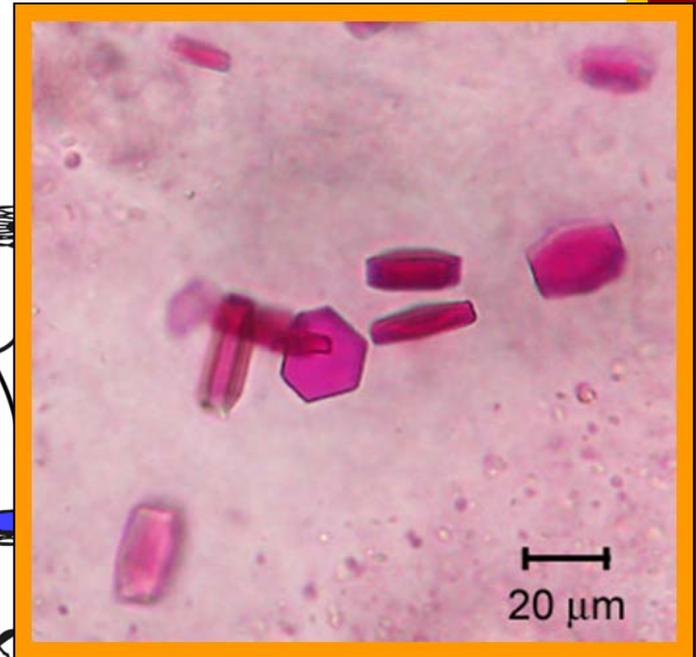
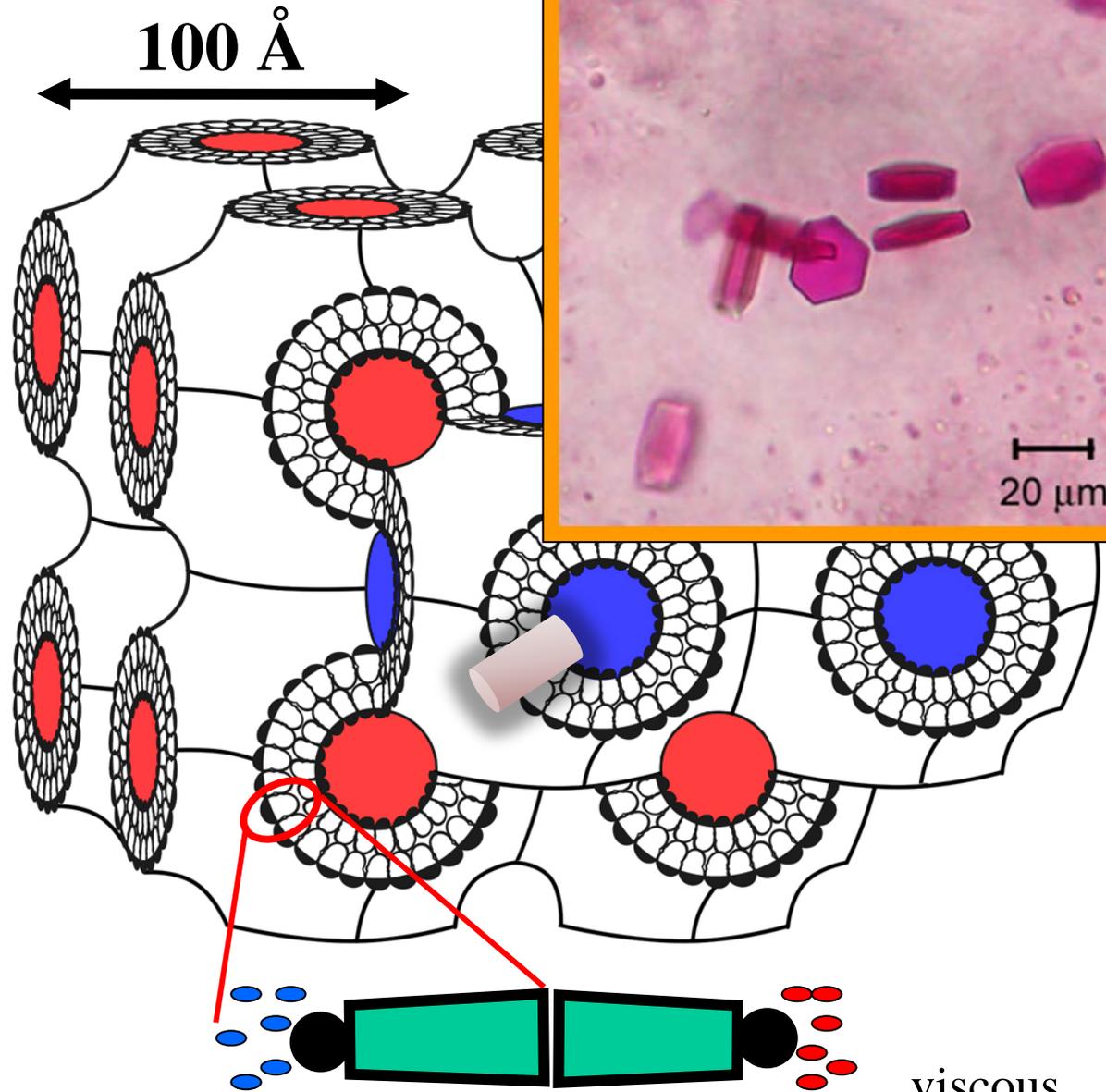
20 °C, 37 °C

www.mpdb.tcd.ie

Bacteriorhodopsin	1.6 – 2.7 Å
Proteorhodopsin	2.3 Å
Xanthorhodopsin	1.9 Å
β ₂ AR-Fab	3.4 / 3.7 Å
VDAC	2.3 Å
Rhomboid protease	1.7 Å

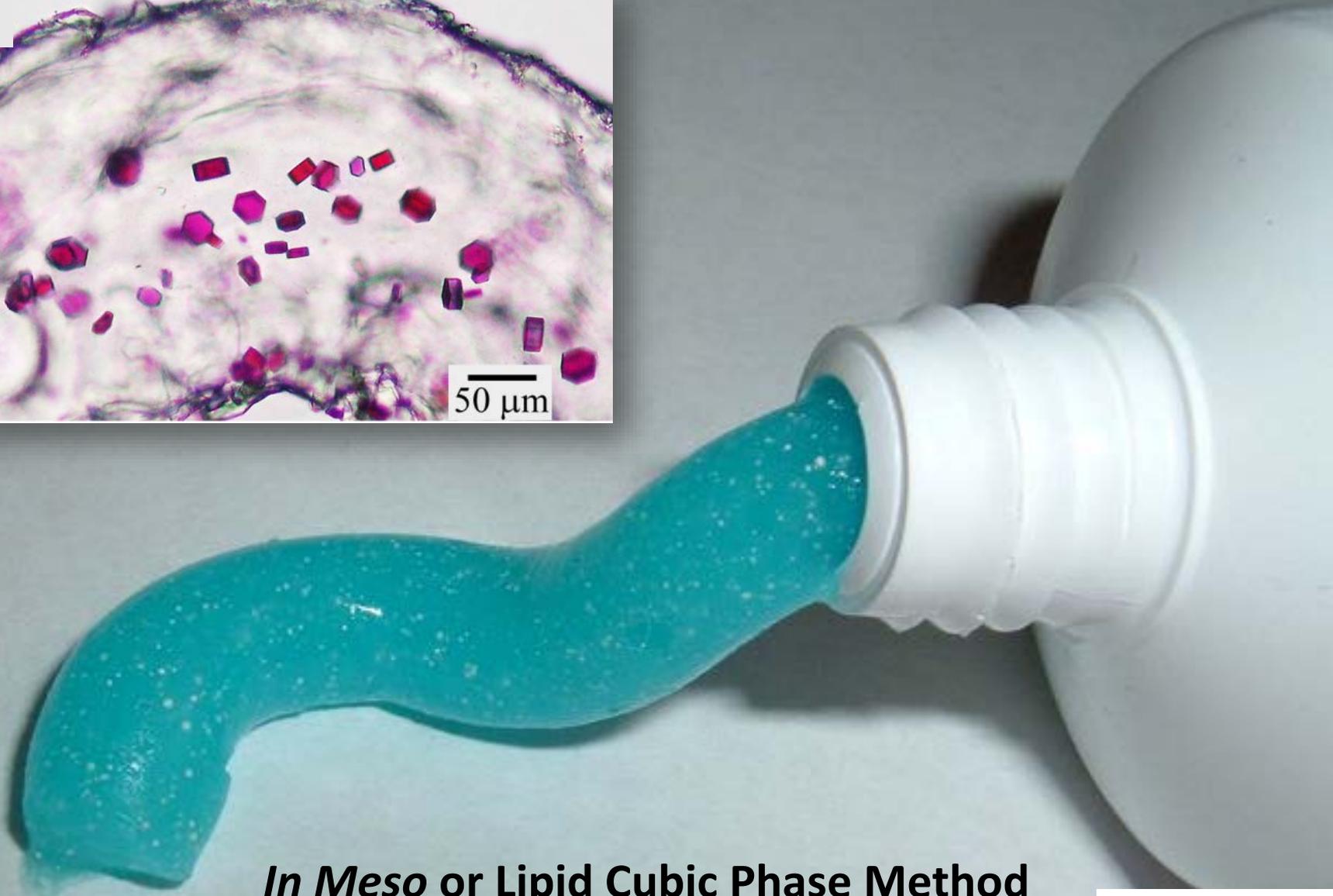
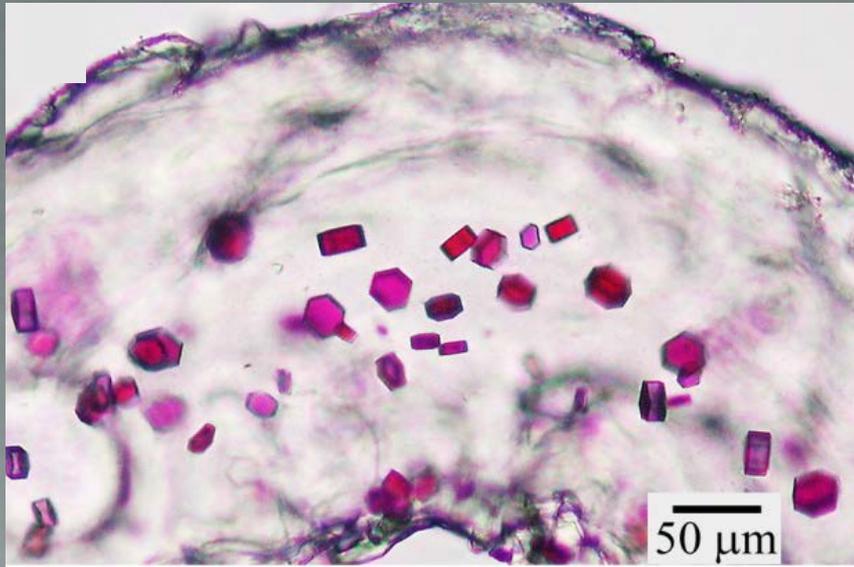
Cubic Phase

Lipid
+
Protein
Soln



viscous

Viscosity



In Meso or Lipid Cubic Phase Method

Mechanism

Lamellar
portal

PROTEIN
CO-CRYSTAL

Crystalline
array

CHARGE
SCREENING

Reconstituted
protein

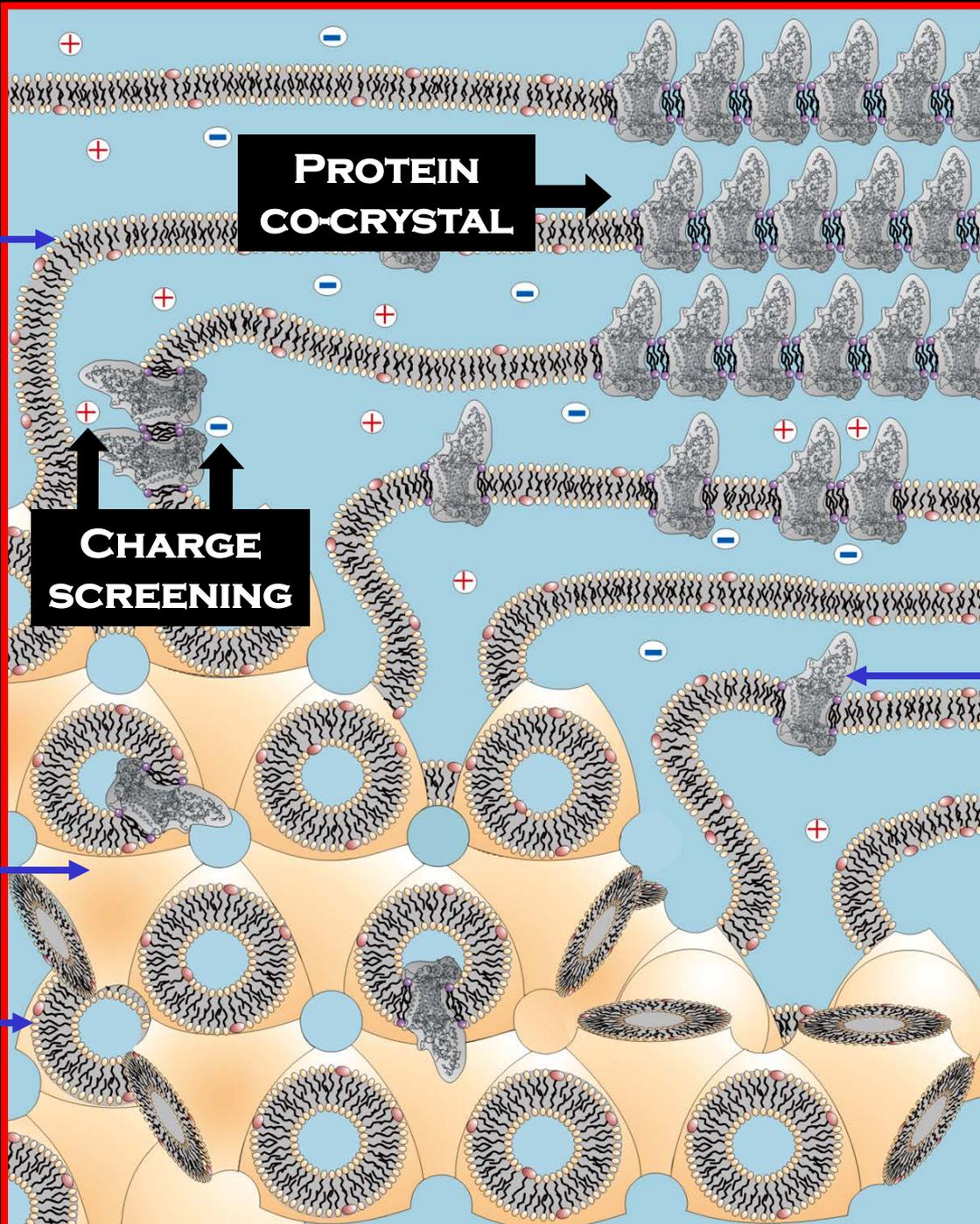
Cubic
phase

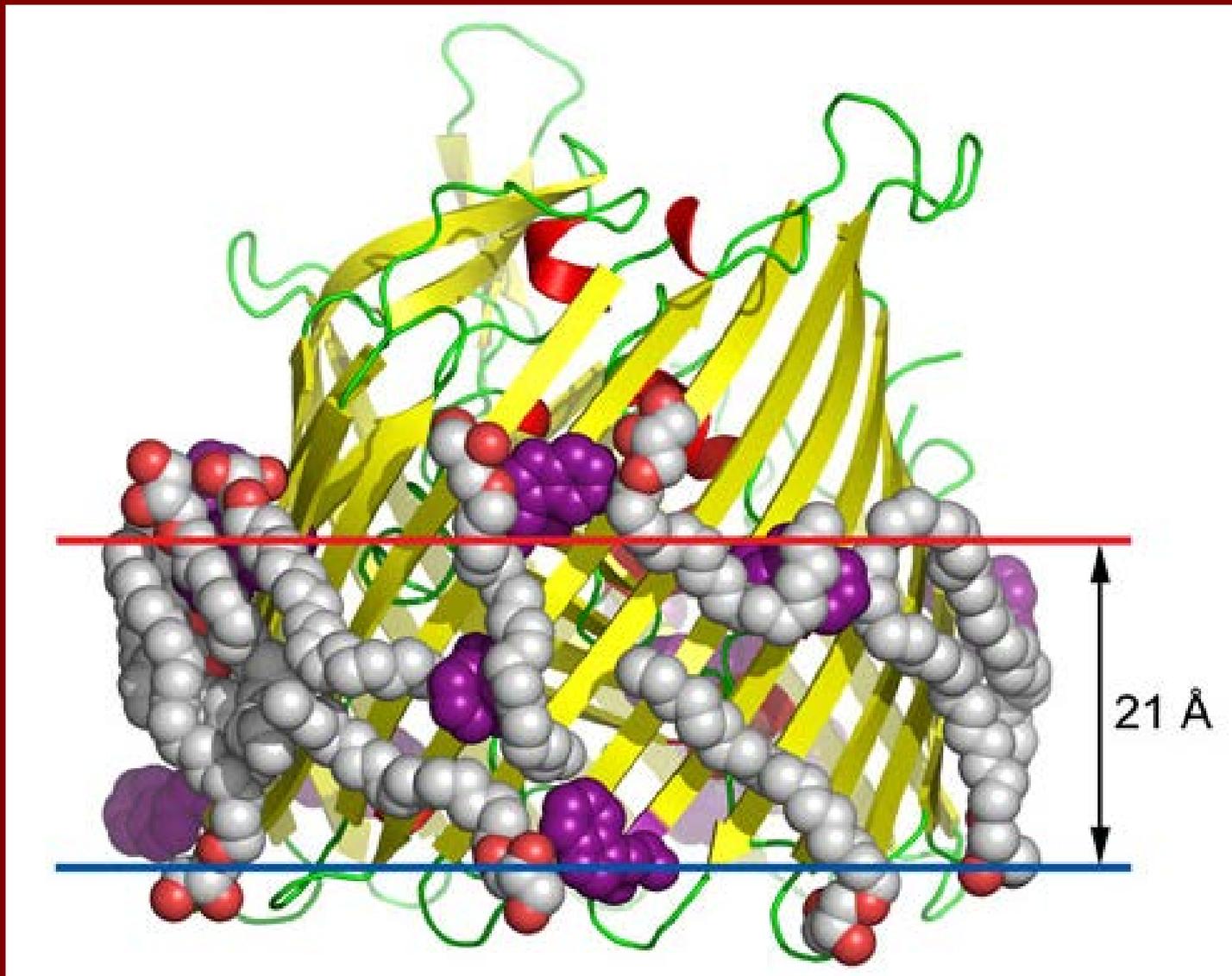
Lipids

J. Struct. Biol.
142:108-132

Cryst. Growth Des.
8:4244-4254

Examples



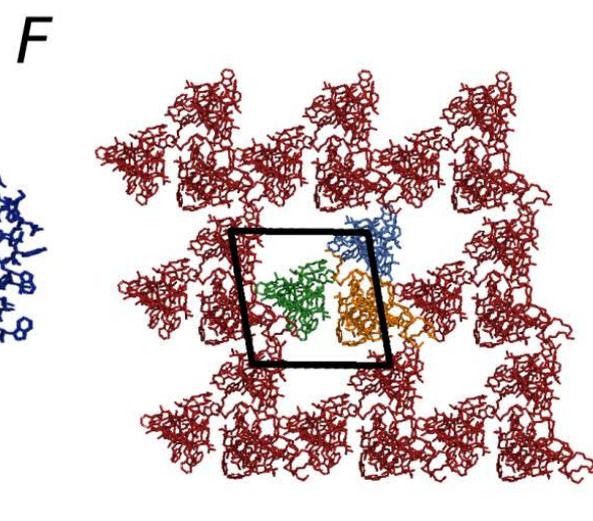
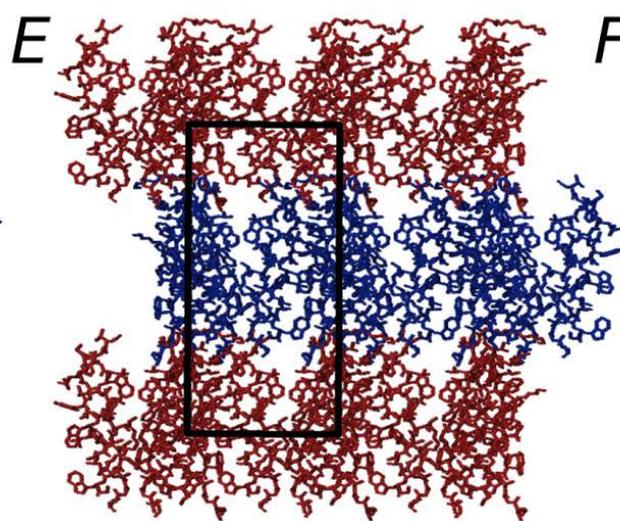
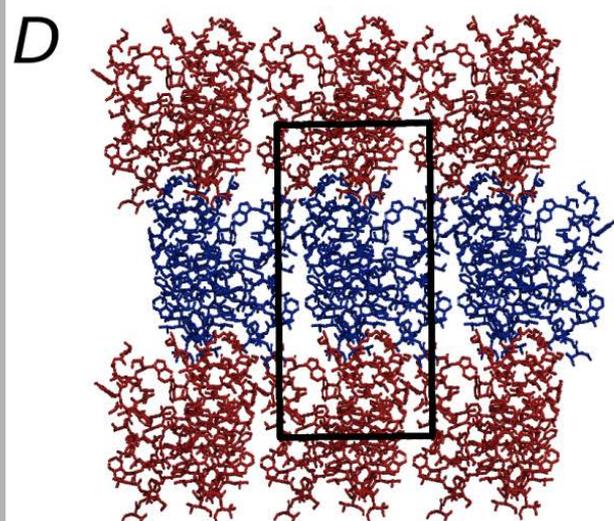
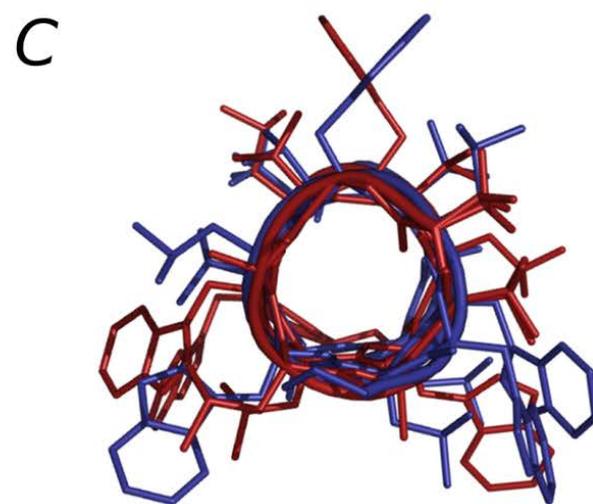
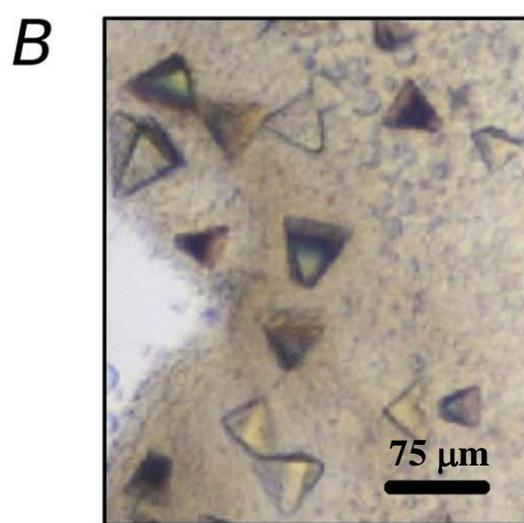
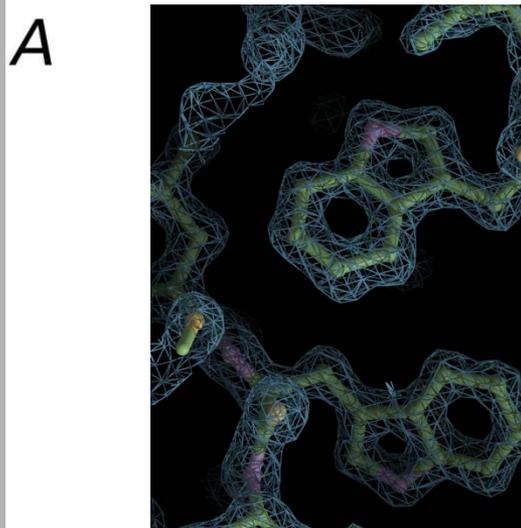


BtuB Receptor / Transporter

*** W. Cramer**

22-stranded β -barrel, 1.95 Å.

J. Mol. Biol. 364:716



Gramicidin
- *B. brevis*, 1.08 Å

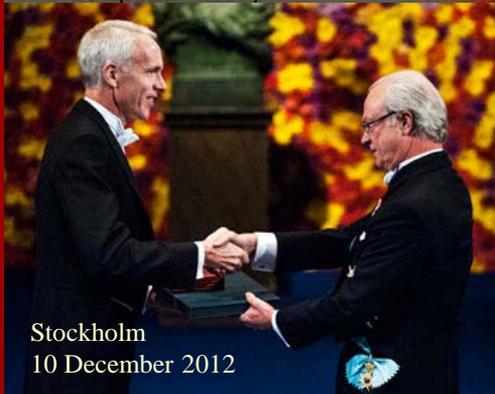
♣ **Biophys. J. 99:L23**
♣ **Cryst. Growth Des. 11:1182**

nature

THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

STIMULUS PACKAGE

Structure of a G-protein-coupled-receptor



Stockholm
10 December 2012

POLICY
REASONS TO BE CHEERFUL
Well-being as an index of social progress
PAGE 532

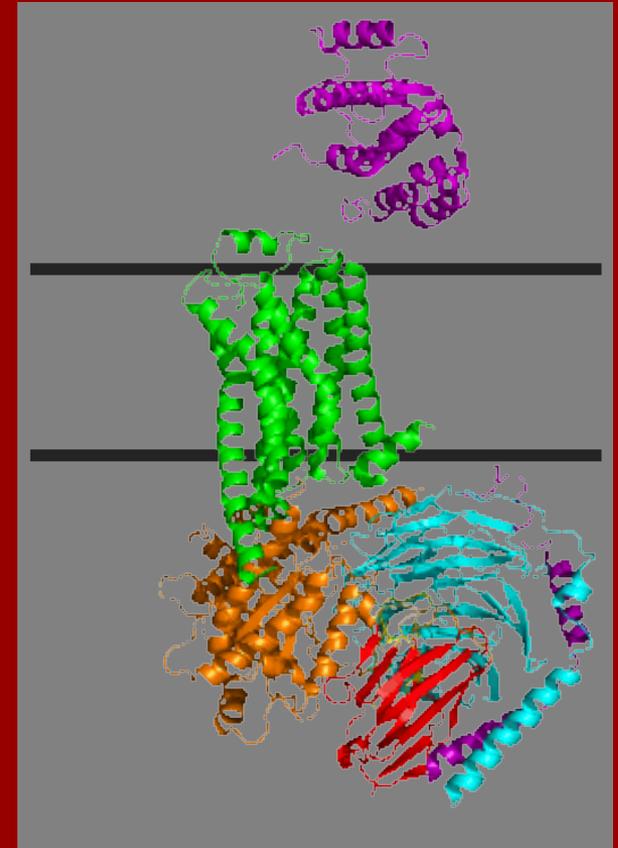
NATURE.COM/NATURE

29 September 2011 £10

Vol. 477, No. 7366



β_2 AR - Gs Complex, 3.2 Å



Rational Approach

- ♣ Host Lipid: 7.7 MAG
- ♣ Additive Lipid: Cholesterol

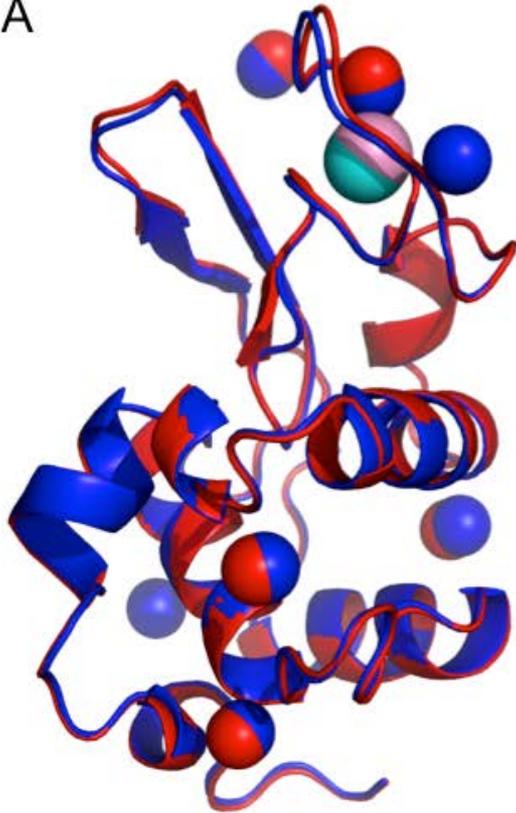
B Kobilka, R Sunahara, *et al.*

Nature 477:549

Water-soluble proteins too !

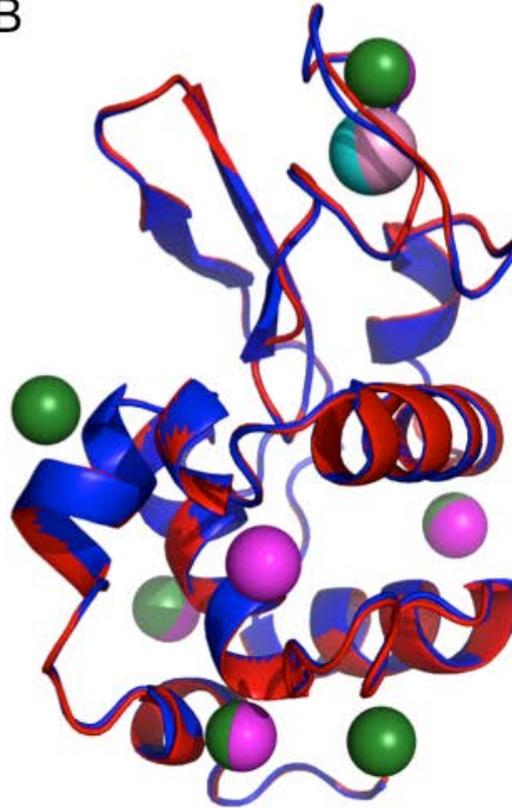
Lysozyme

A



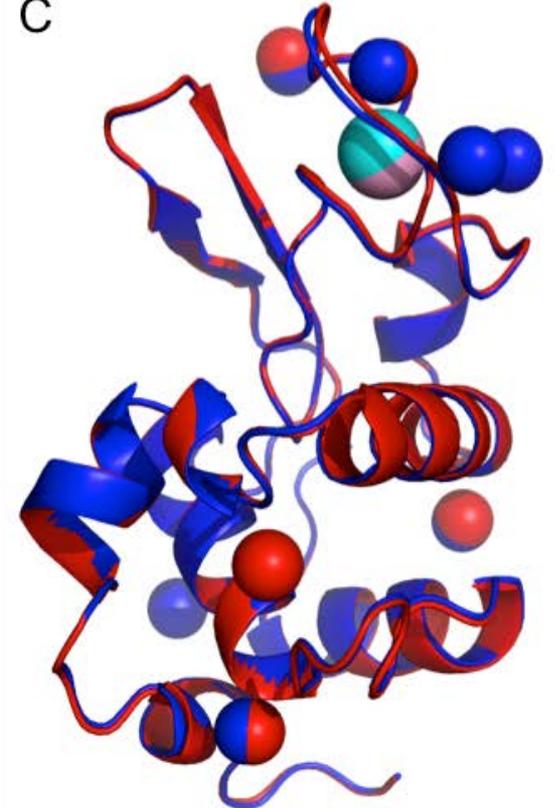
● Cl⁻ 100 K ● Cl⁻ 293 K

B



● Br⁻ 100 K ● Br⁻ 293 K

C



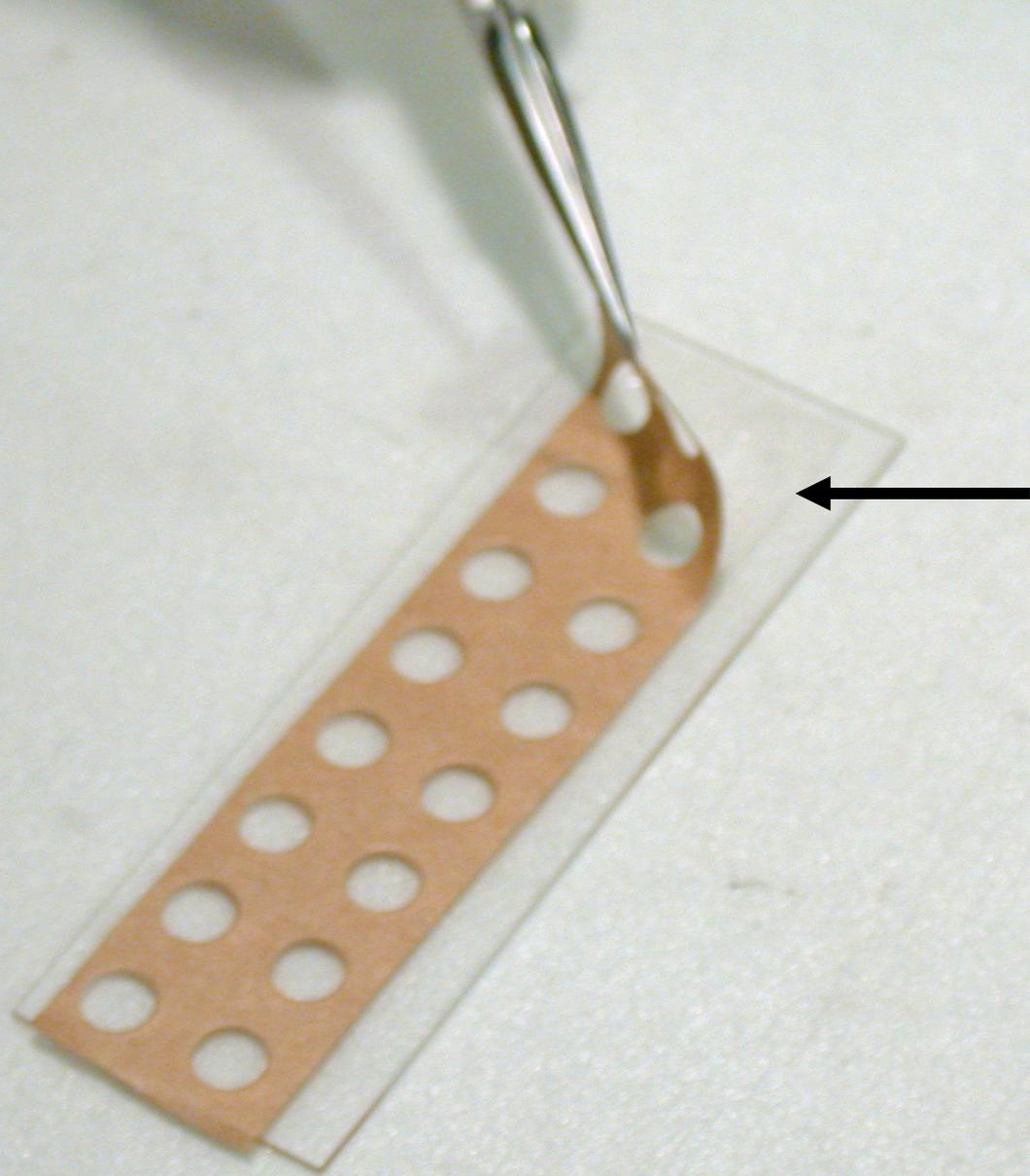
● Na⁺ 100 K ● Na⁺ 293 K

J. Appl. Cryst. 45:1330-1333.

+ insulin, thaumatin, α -lactalbumin,

Step-by-Step
In Meso
Crystallization:
Manual Mode

♣ Nature Protocols 4:706
♣ JoVE 45: id 1712



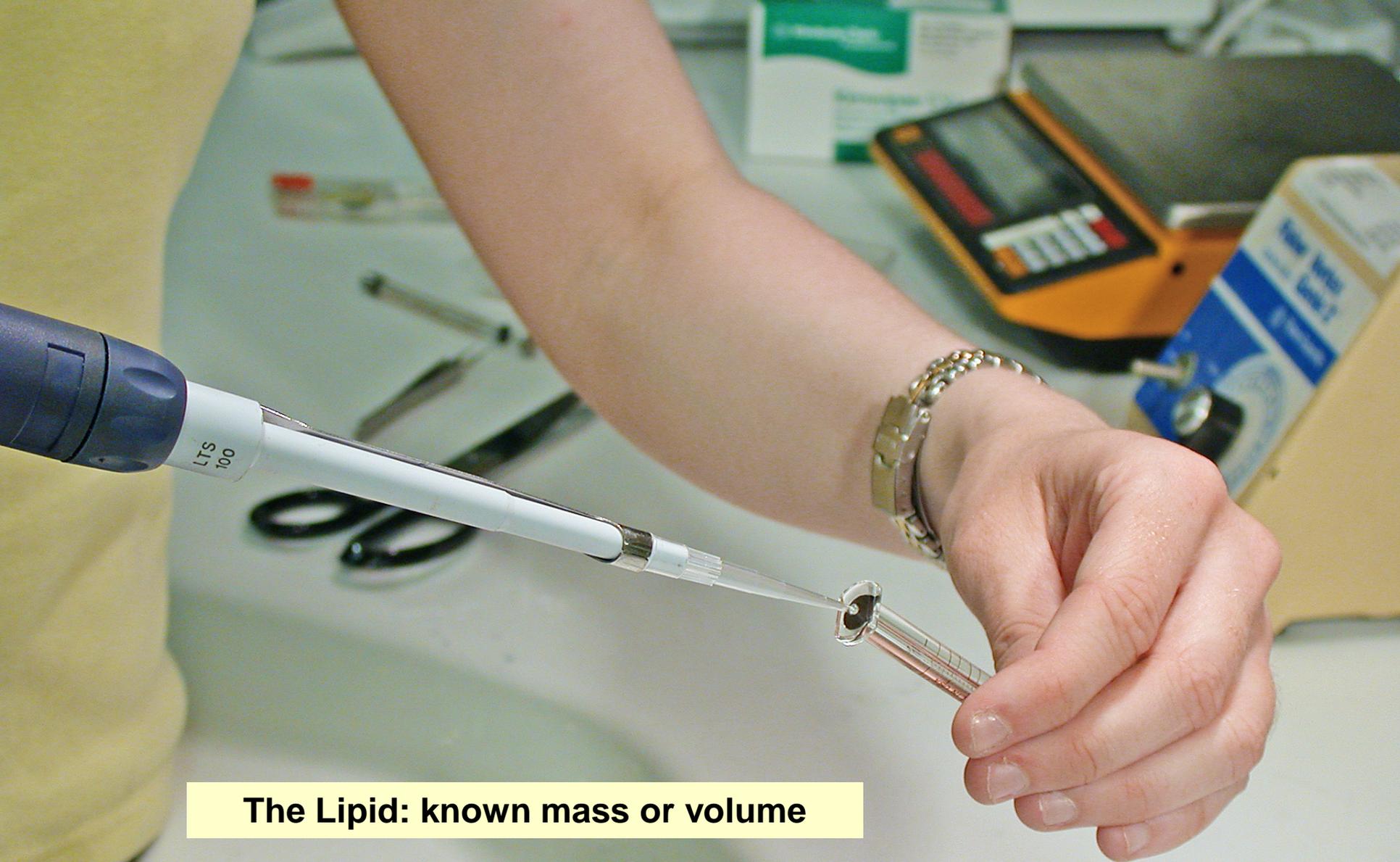


Lipid

Protein Solution

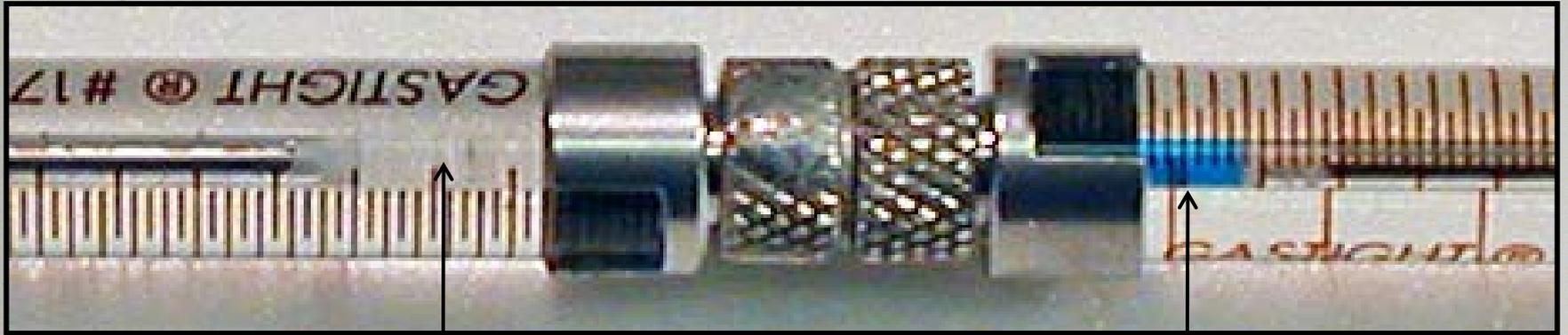


coupled syringe mixer



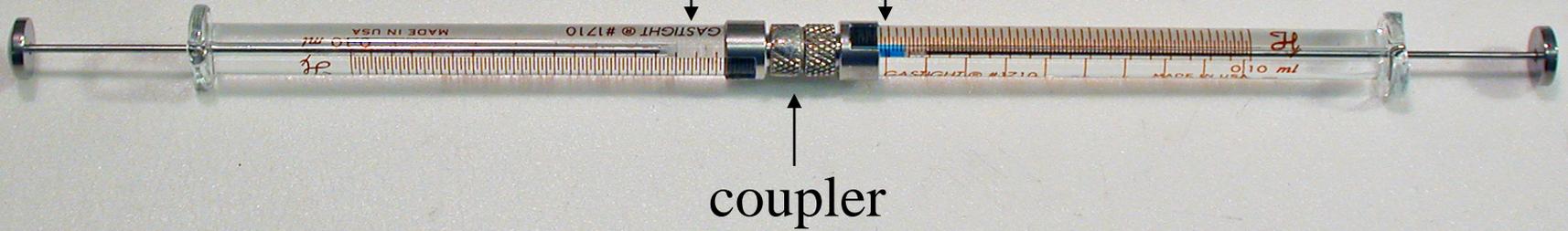
The Lipid: known mass or volume





lipid

protein solution

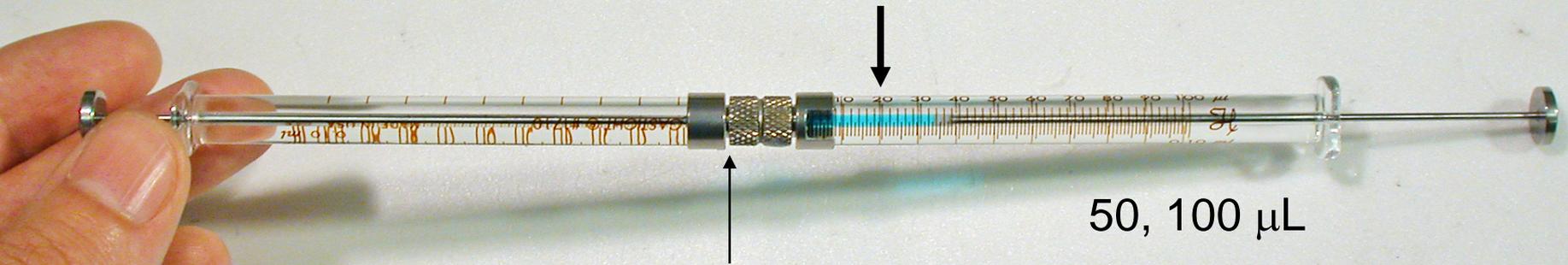


Coupled Syringe Mixer



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

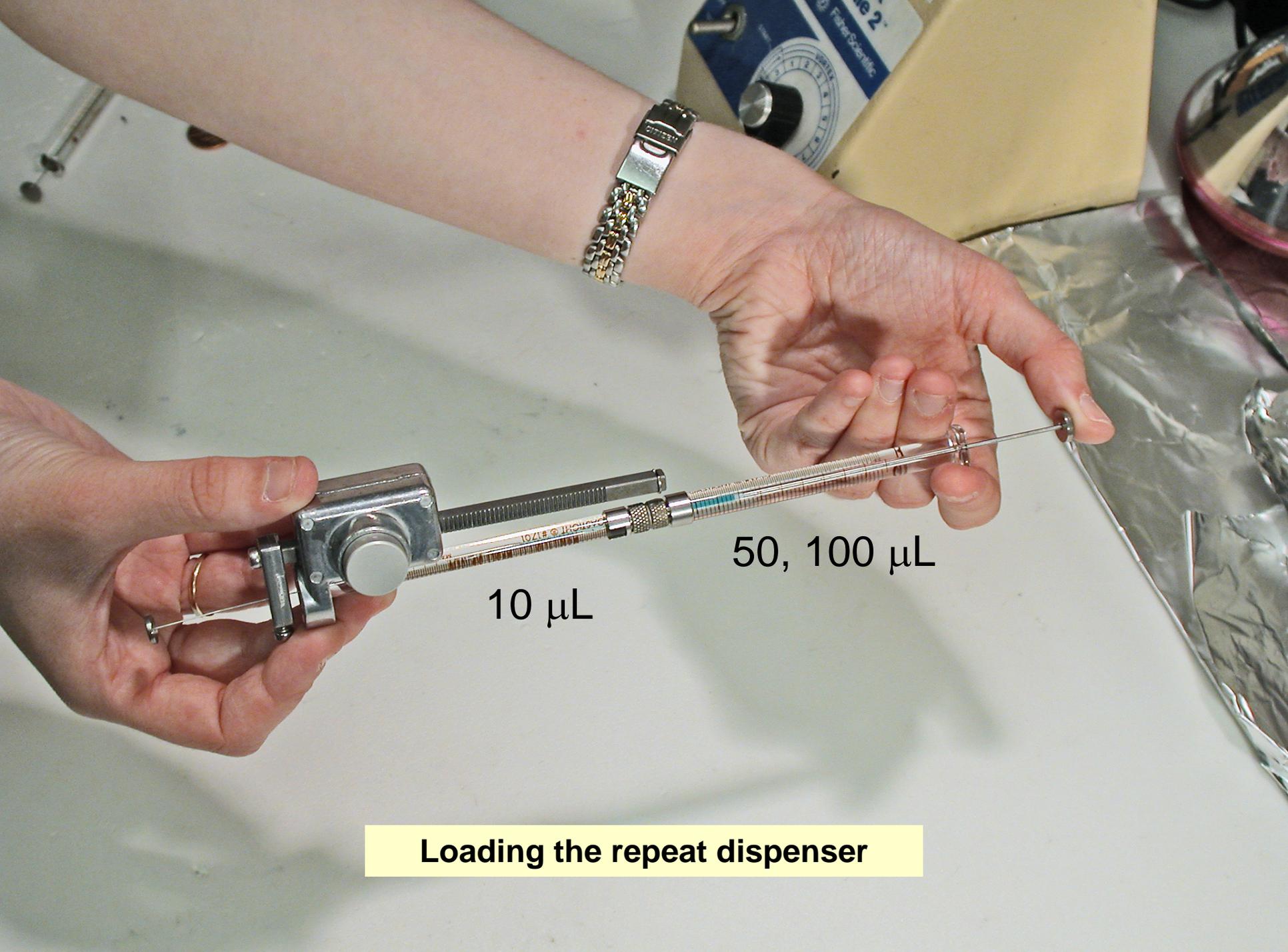
optically clear
protein/lipid mesophase



disconnect

50, 100 μL

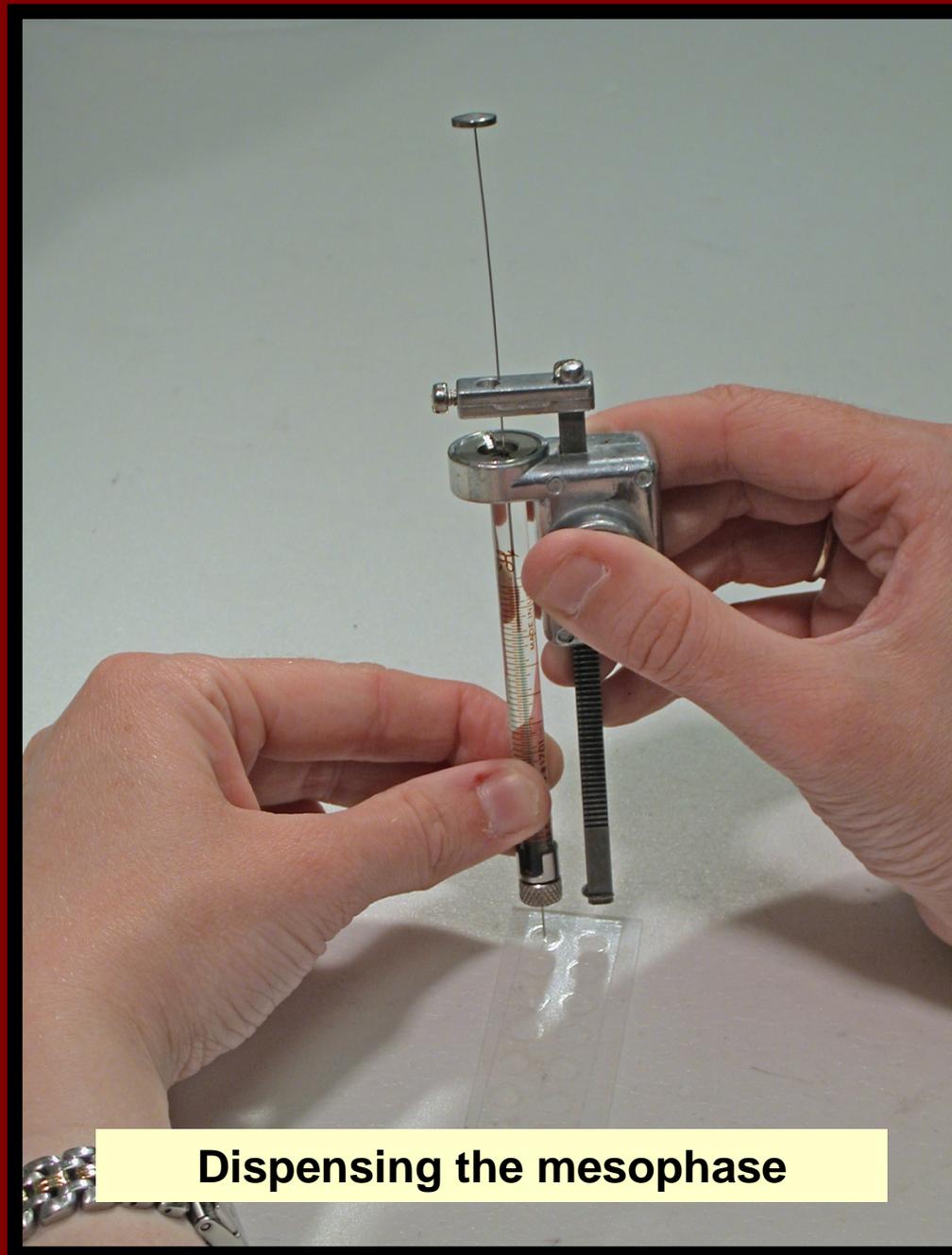
The homogenous protein / lipid cubic phase



10 μL

50, 100 μL

Loading the repeat dispenser

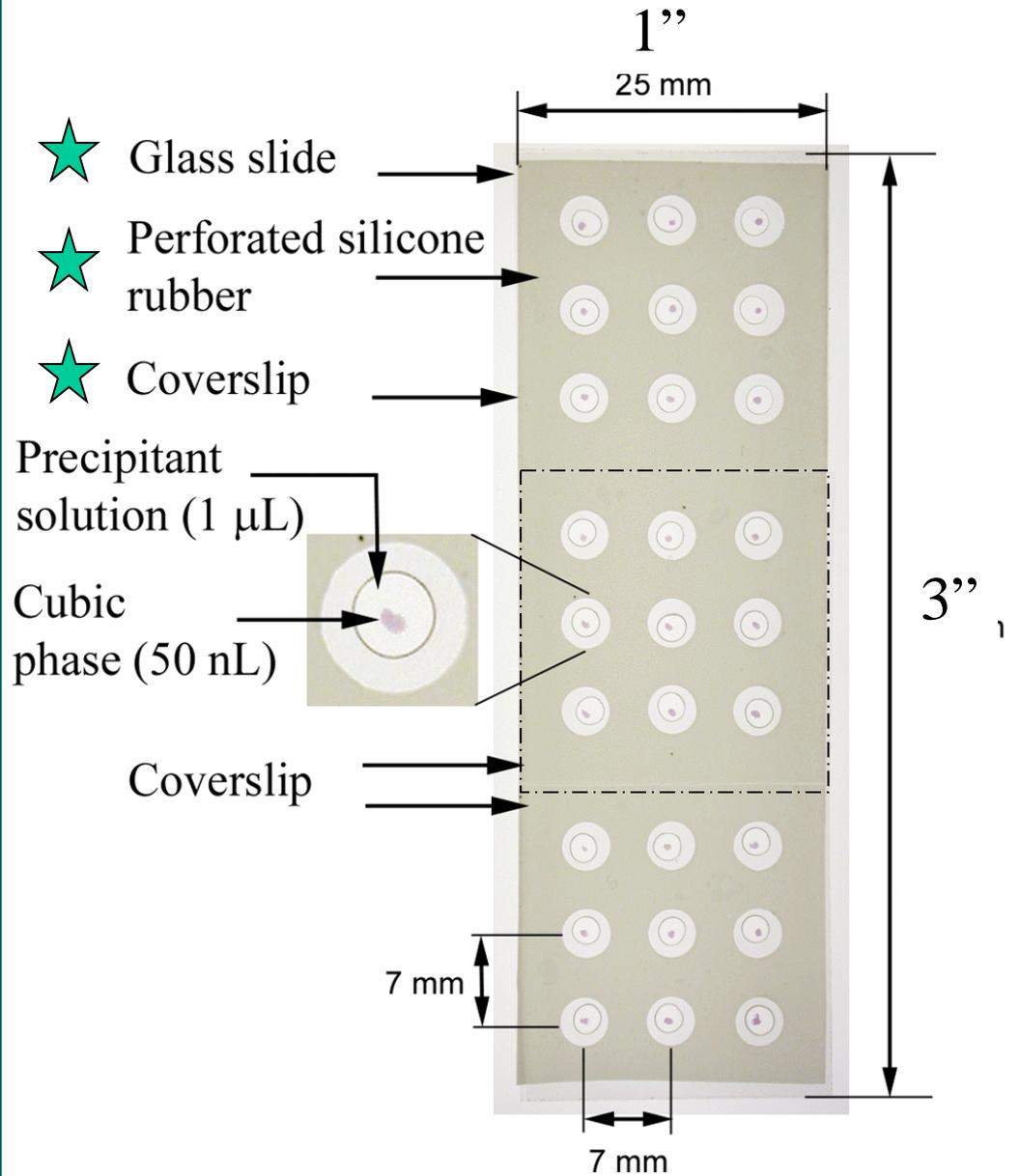


Dispensing the mesophase

**Dispensing 1 μL
precipitant solution**



Loaded/sealed 27-well crystallization plate



Step-by-Step
In Meso
Crystallization:
Robot Mode

- ♣ Nature Protocols 4:706
- ♣ JoVE 67: e4000

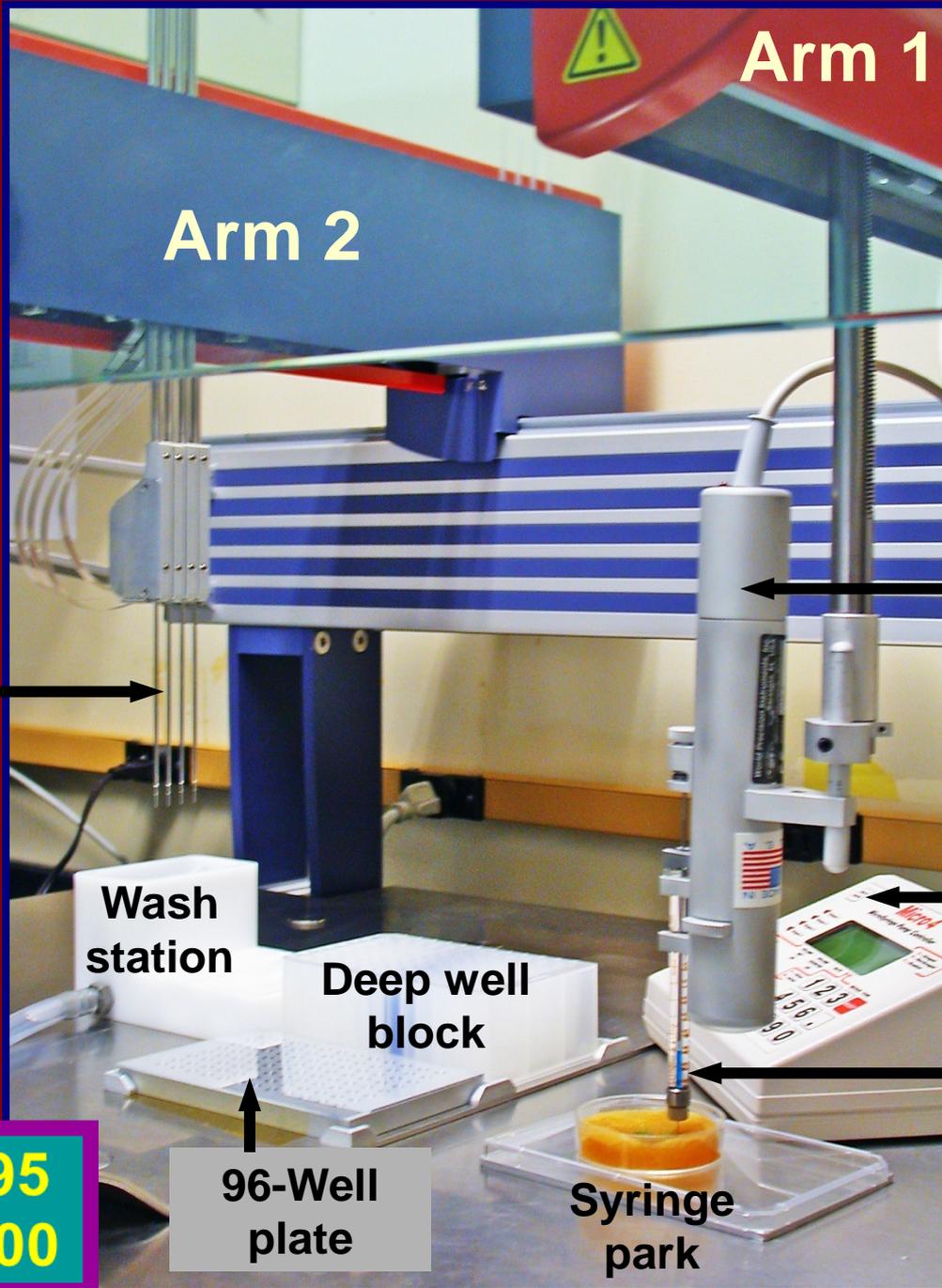
In Meso Robot

Liquid
Handling
Robot

4-Tip
precipitant
solution
dispenser

Motorized
Micro-pump

♣ *Acta D60:1795*
♣ *JoVE 67: e4000*



Arm 2

Arm 1

Vadim Cherezov

Anachem
Douglas
Formulatrix
Gryphon / Art Robbins
TTP Mosquito
Sias
Zinser

Protein/lipid
dispenser

Protein/lipid
syringe
controller

Microsyringe

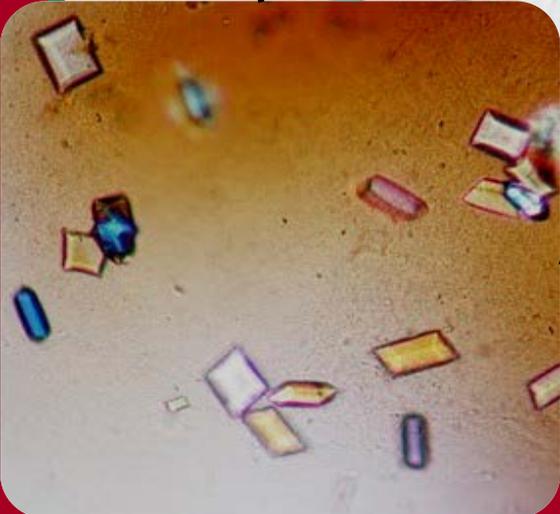
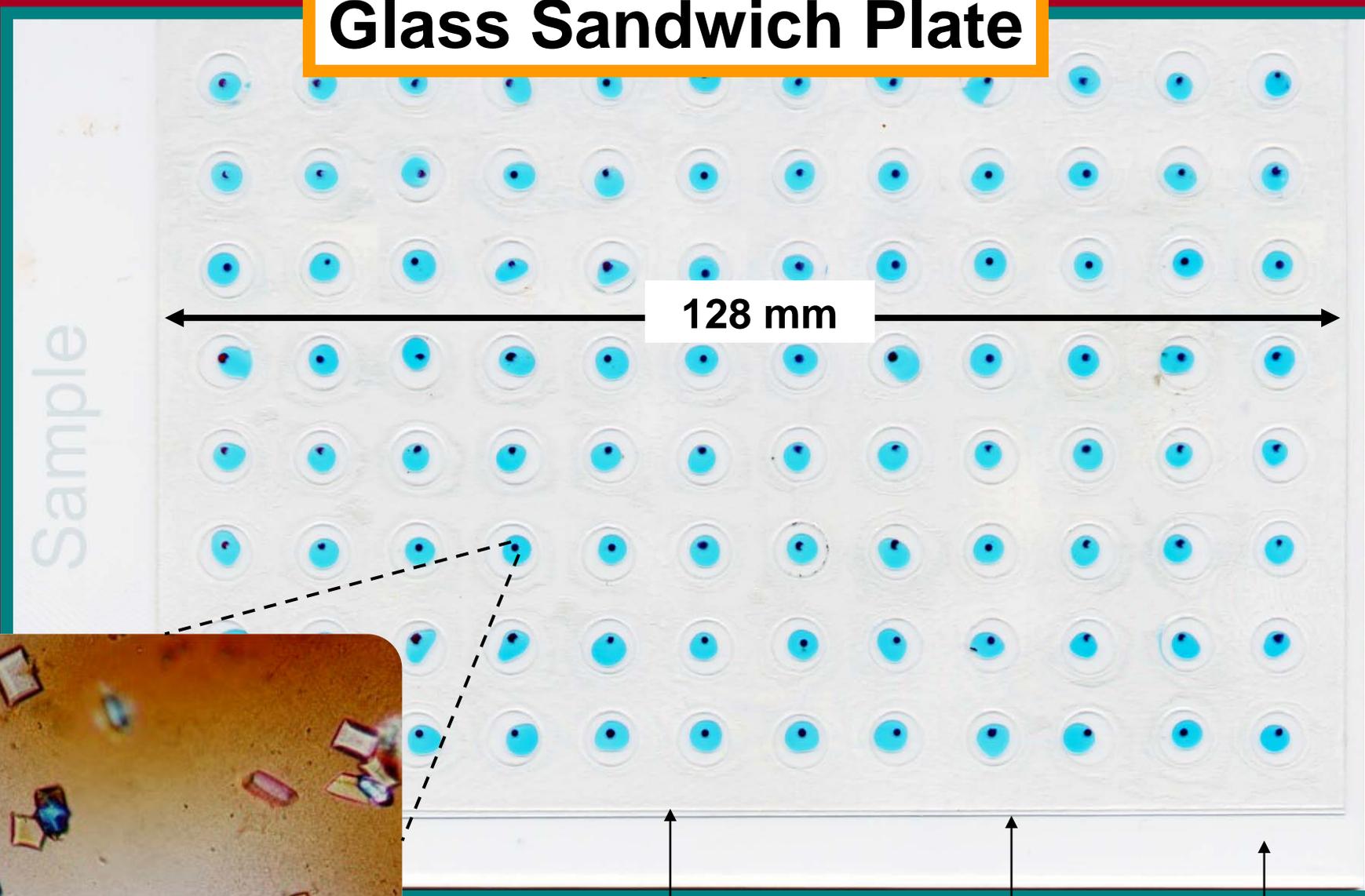
Wash
station

Deep well
block

96-Well
plate

Syringe
park

Glass Sandwich Plate



128 mm

Sample

96-well
spacer, 140 μm

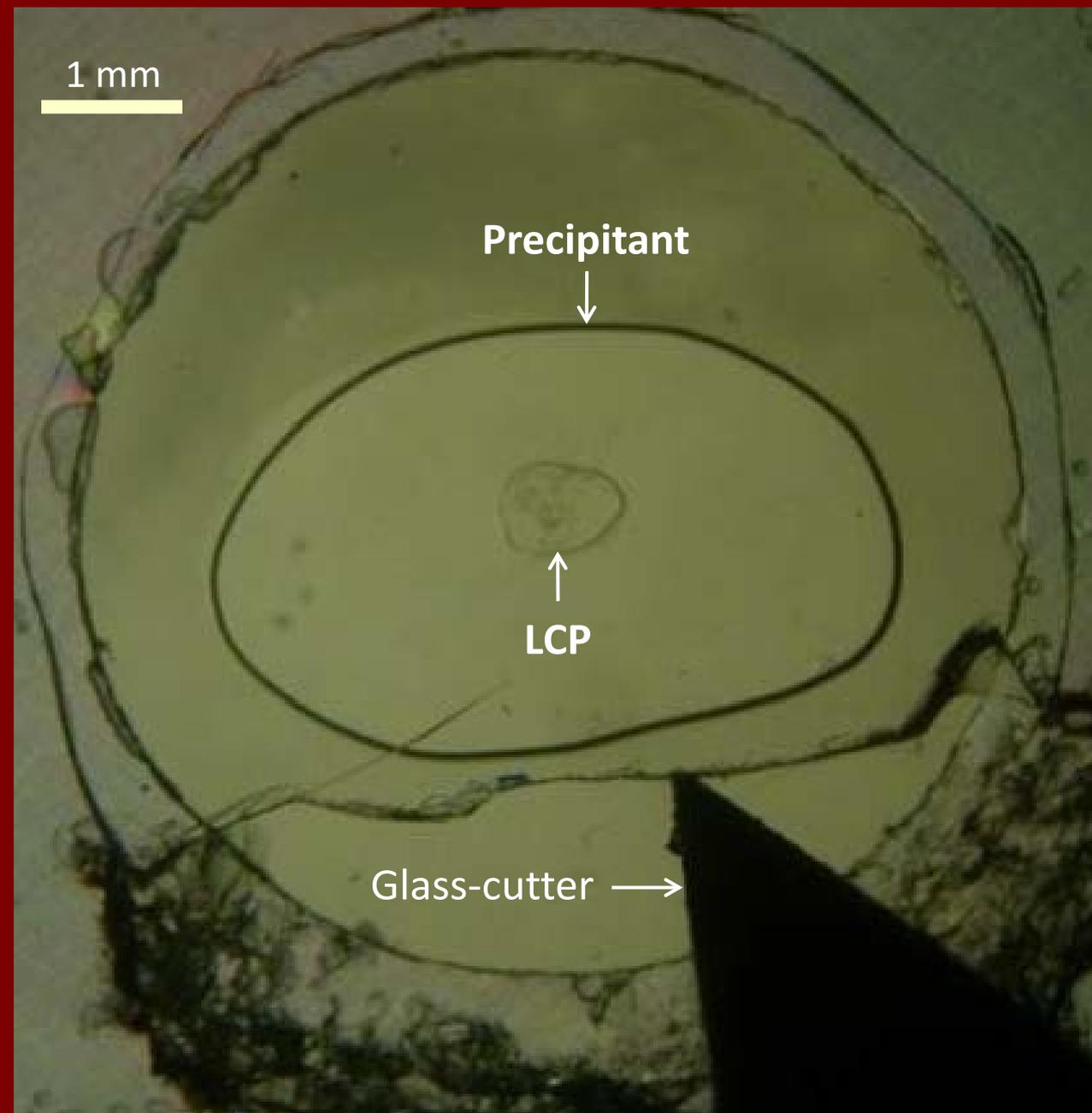
Glass
coverslip

Glass
base

Harvesting Crystals from Glass Sandwich Plates

Glass-cutter

Daunting!



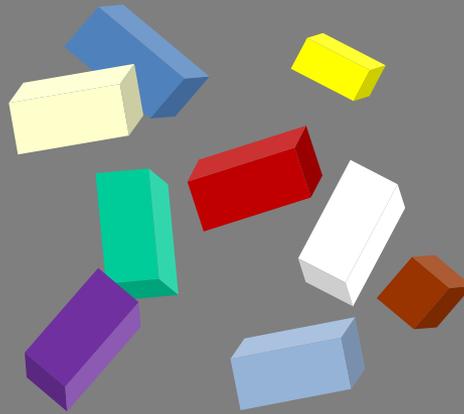
glass

plastic

plastic

glass

Double Sandwich Plate

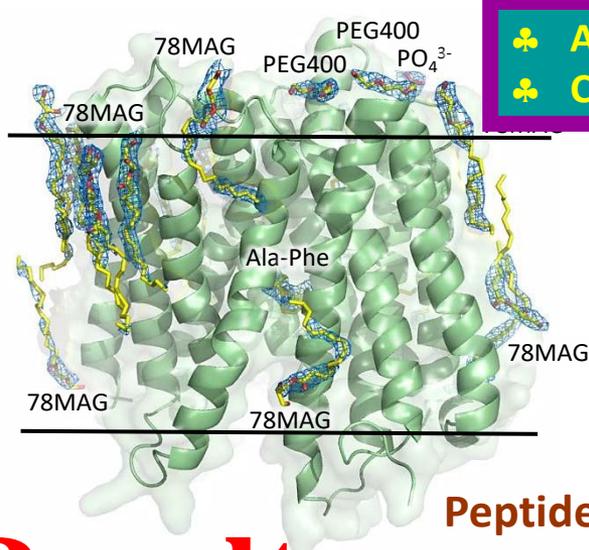
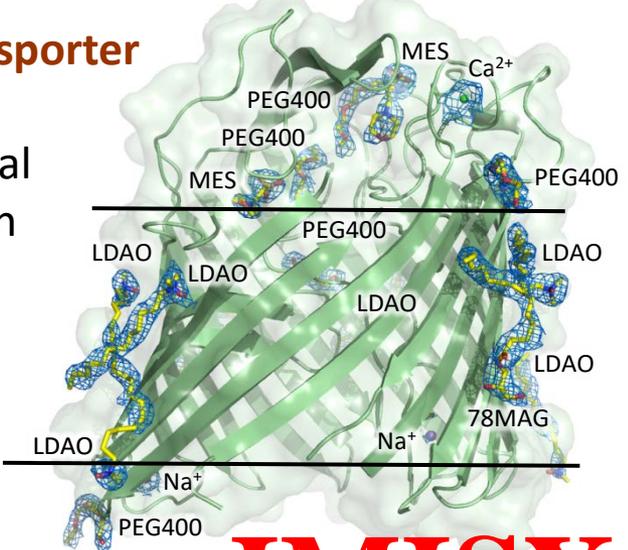


Alginate Transporter

AlgE, 2.4 Å

1 well, 1 crystal

250 ng protein



♣ Acta D72:93, 2016
♣ Comm. Biol. 1:124

Phasing

- ♣ MR
- ♣ Br-SAD
- ♣ S-SAD

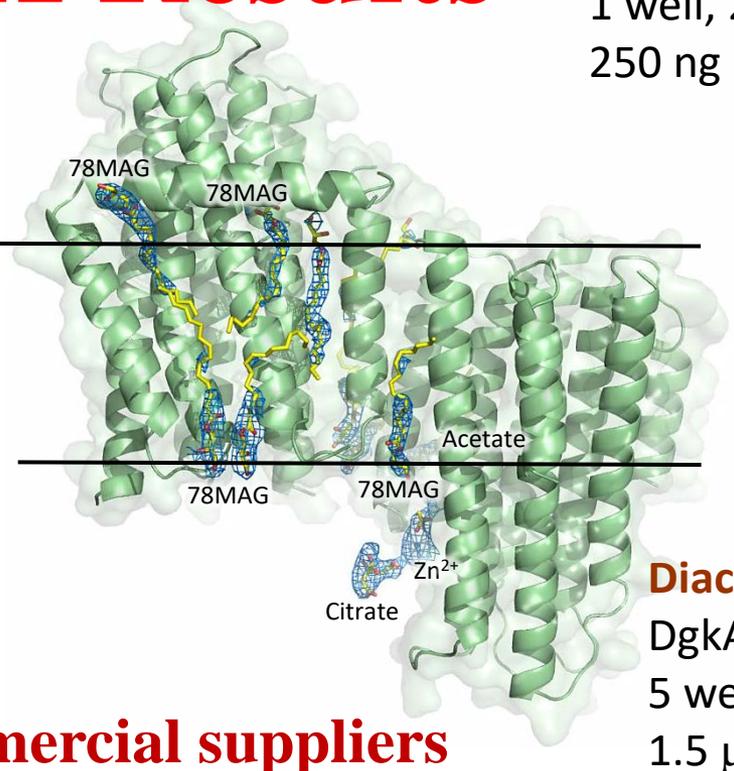
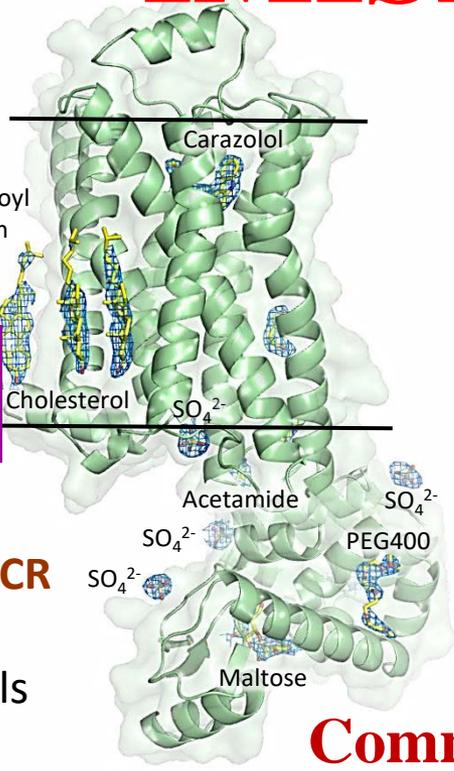
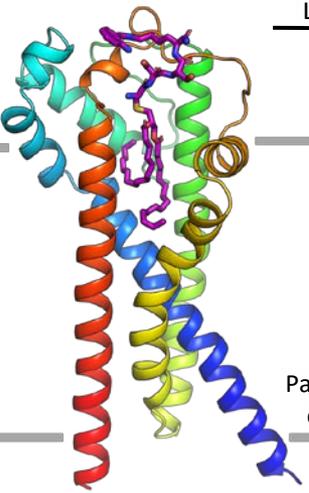
IMISX Results

Peptide Transporter

PepT, 2.4 Å

1 well, 2 crystals

250 ng protein



♣ Nat. Comm. 12:4254, 2021

β₂ Adrenergic GPCR

2.5 Å

1 well, 104 crystals

580 ng protein

Diacylglycerol Kinase

DgkA, 2.8 Å

5 wells, 12 crystals

1.5 μg protein

Commercial suppliers