

**‘Hit and Run’
Serial Femtosecond Crystallography
of Membrane Proteins in the
Lipid Cubic Phase**

Membrane Structural and Functional Biology Group

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**Structural Biology at the ESRF: Past, Present & Future
European Synchrotron Radiation Facility
Grenoble, France**

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COLLEAGUES AND COLABORATORS

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COLLABORATORS WORLDWIDE

BIOCENTRE, BASEL

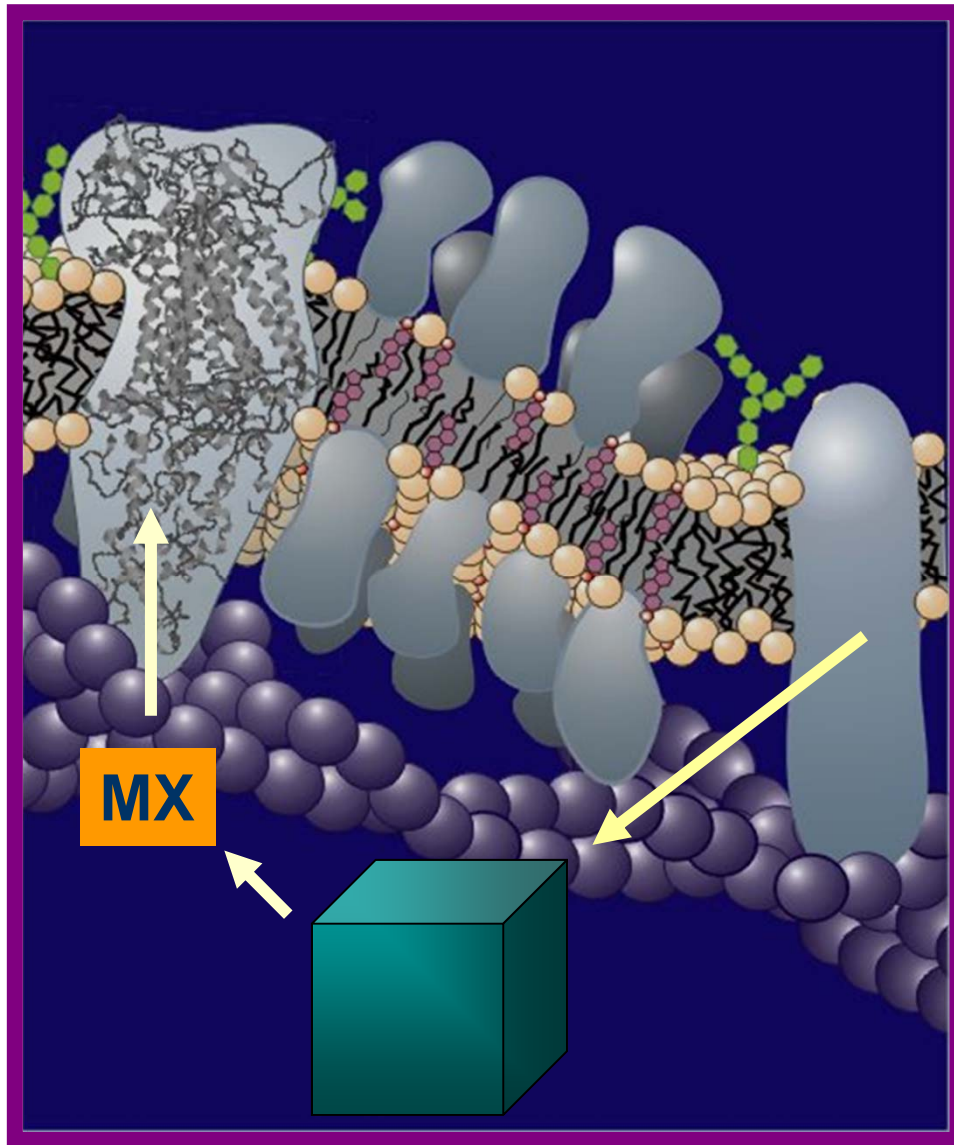
E LANDAU AND J ROSENBUSCH

SUPPORT

NATIONAL INSTITUTES OF HEALTH
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FP7



Structural and Functional Biology of Membranes



MX
Synchrotron Radiation

Solid State
Crystal Contacts

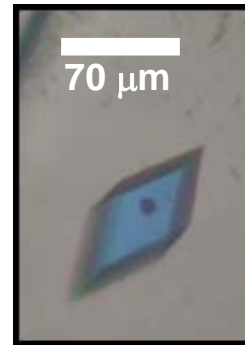
Radiation Damage
Large Crystals
100 K

SFX

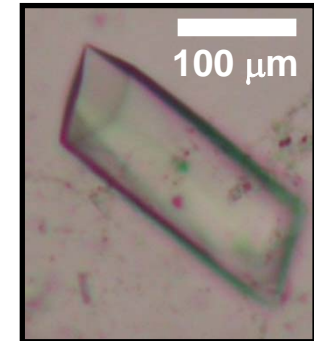
DgkA Structure Determination



9.9 MAG
20 °C



7.8 MAG, 3.0 Å
4 °C



7.8 MAG, 2.1 Å
4 °C

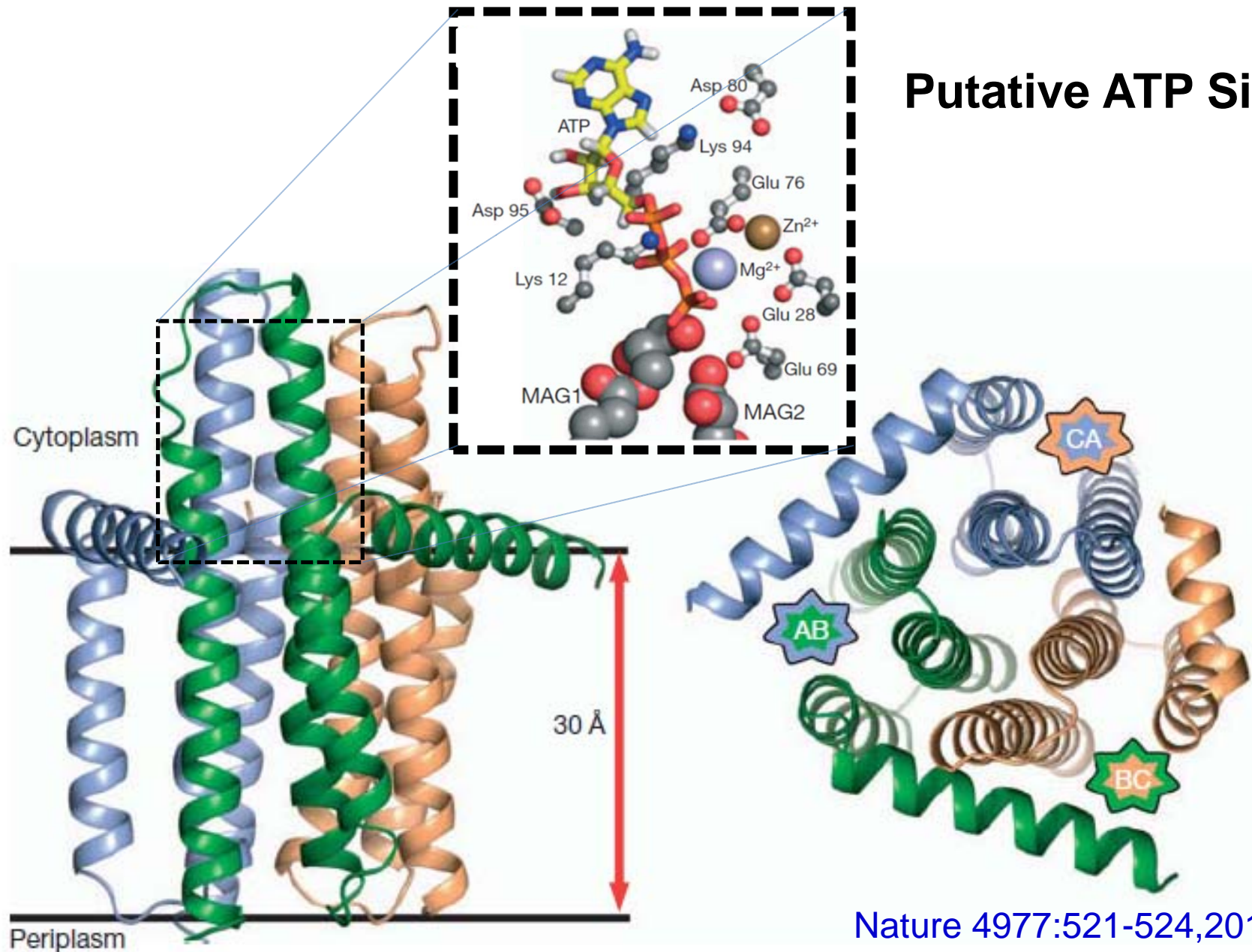
DNA → crystals

Optimize

Phasing

Structure

Diacylglycerol Kinase, 2.05 Å



Nature 4977:521-524,2013

Potential Problems

Radiation damage

100 K

Possible Solution

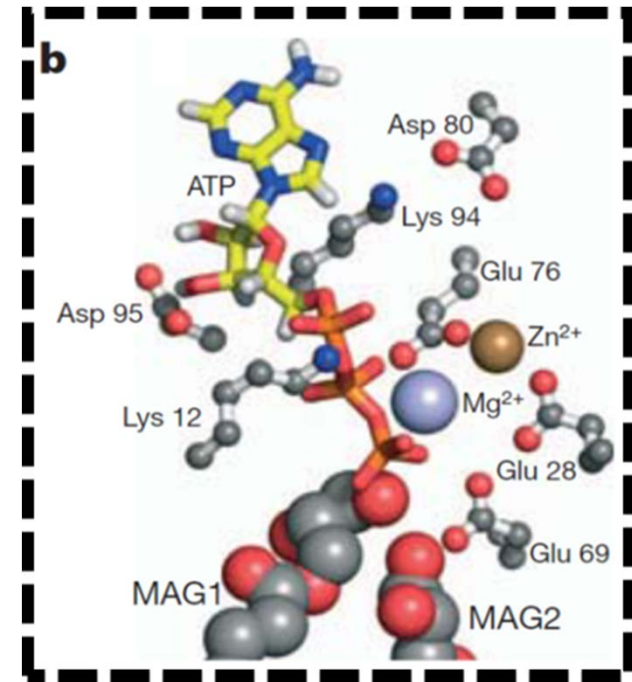
~In situ

Radiation Damage

RT

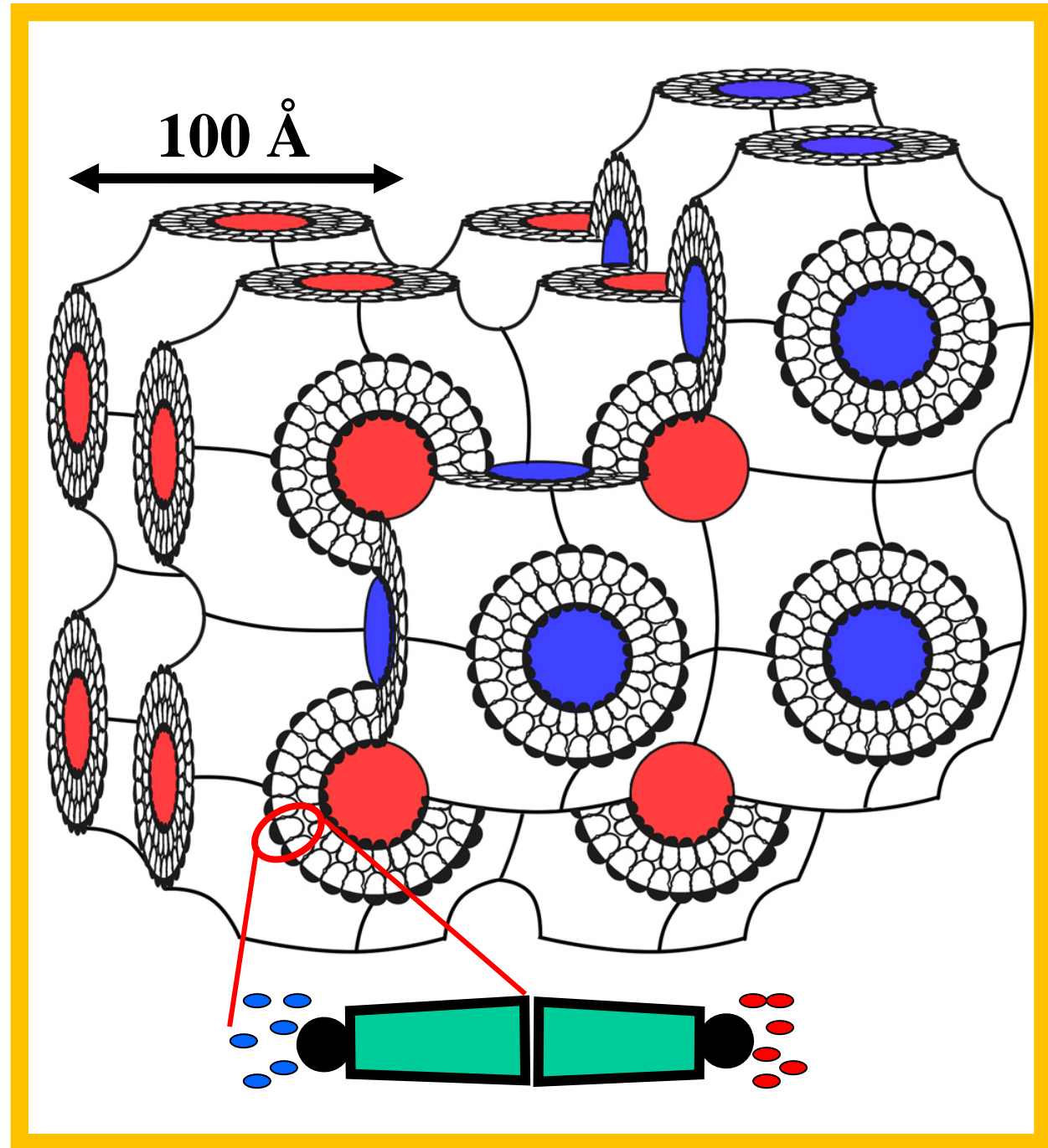
Microcrystals

LCP-SFX



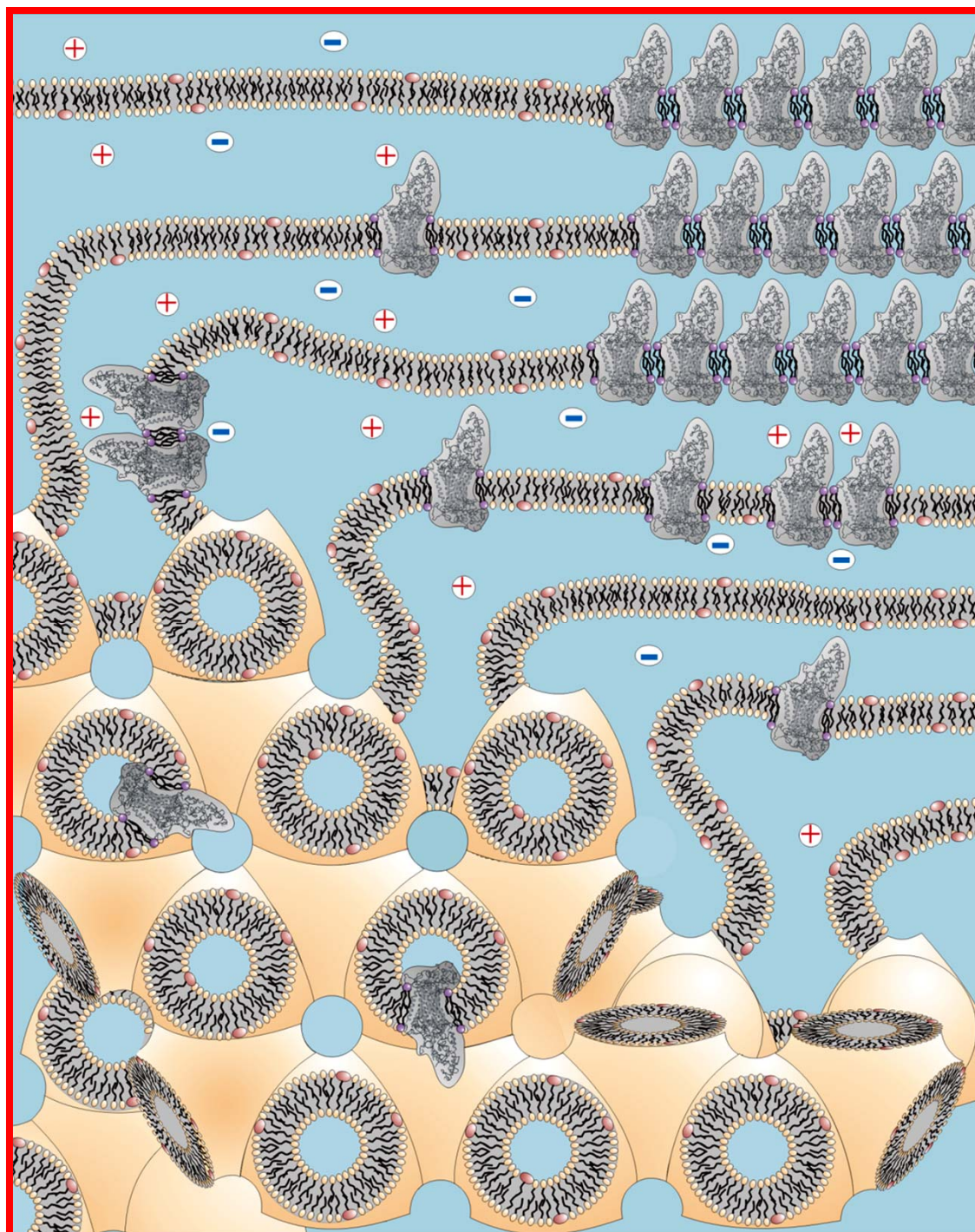
Cubic Phase

Lipid
+
Water



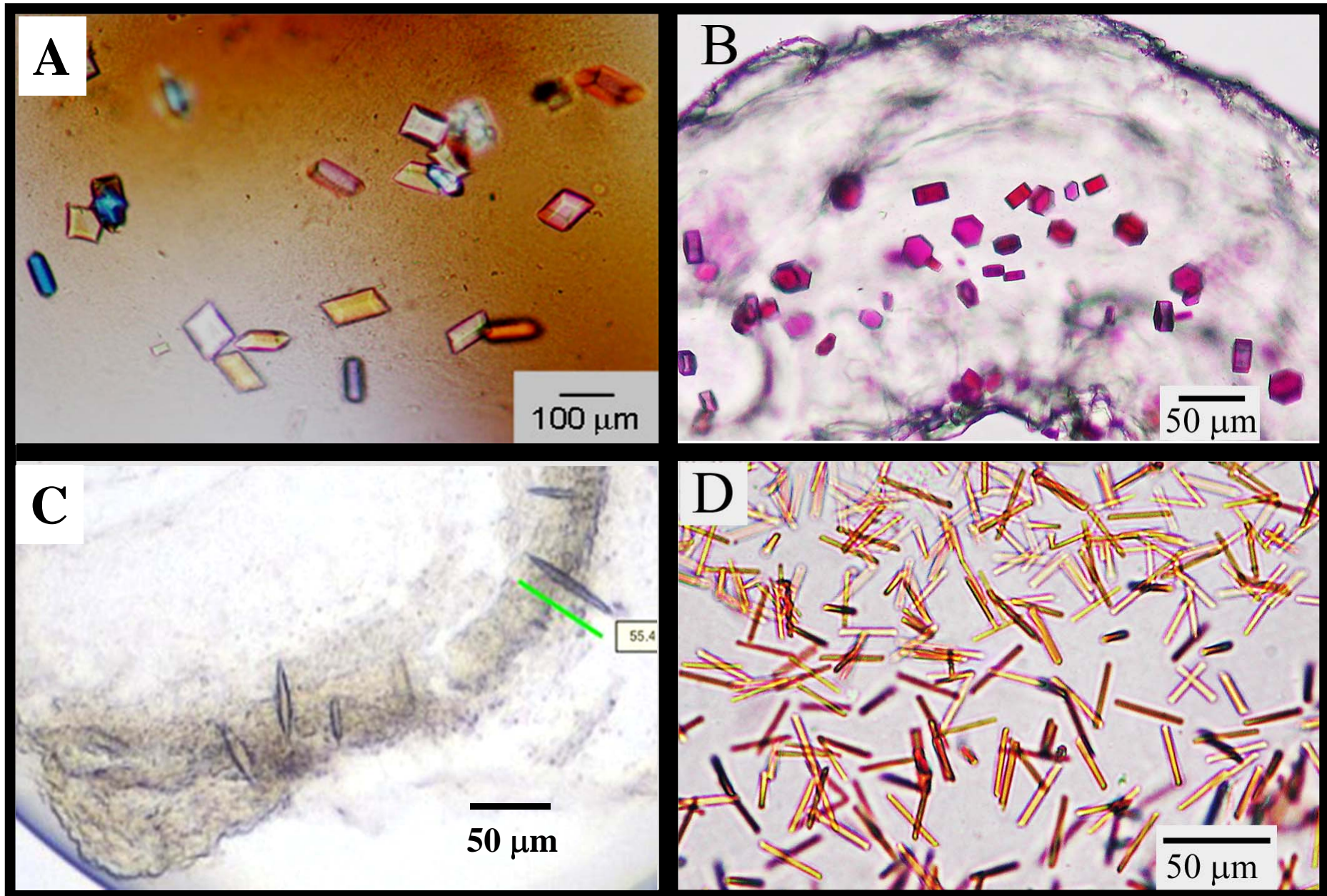
Viscosity



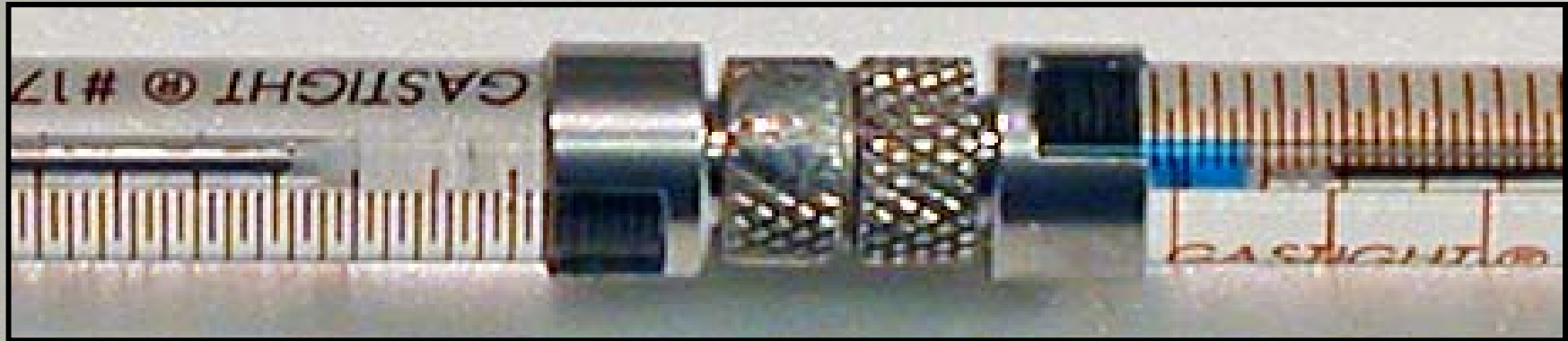


J. Struct. Biol.
142:108-132

Cryst. Growth Des.
8:4244-4254

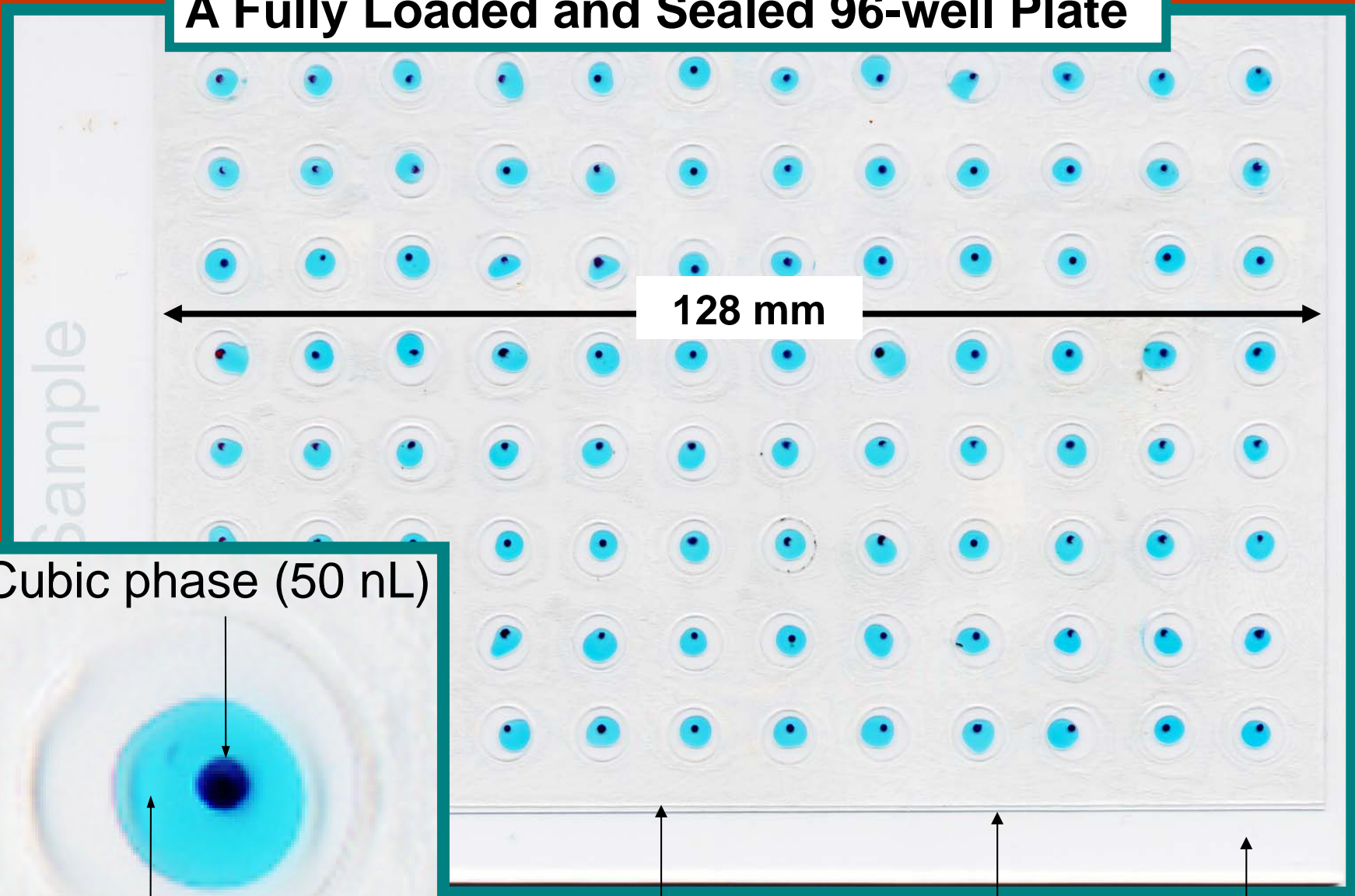


Crystals 162 PDB Records



Coupled Syringe Mixer

A Fully Loaded and Sealed 96-well Plate



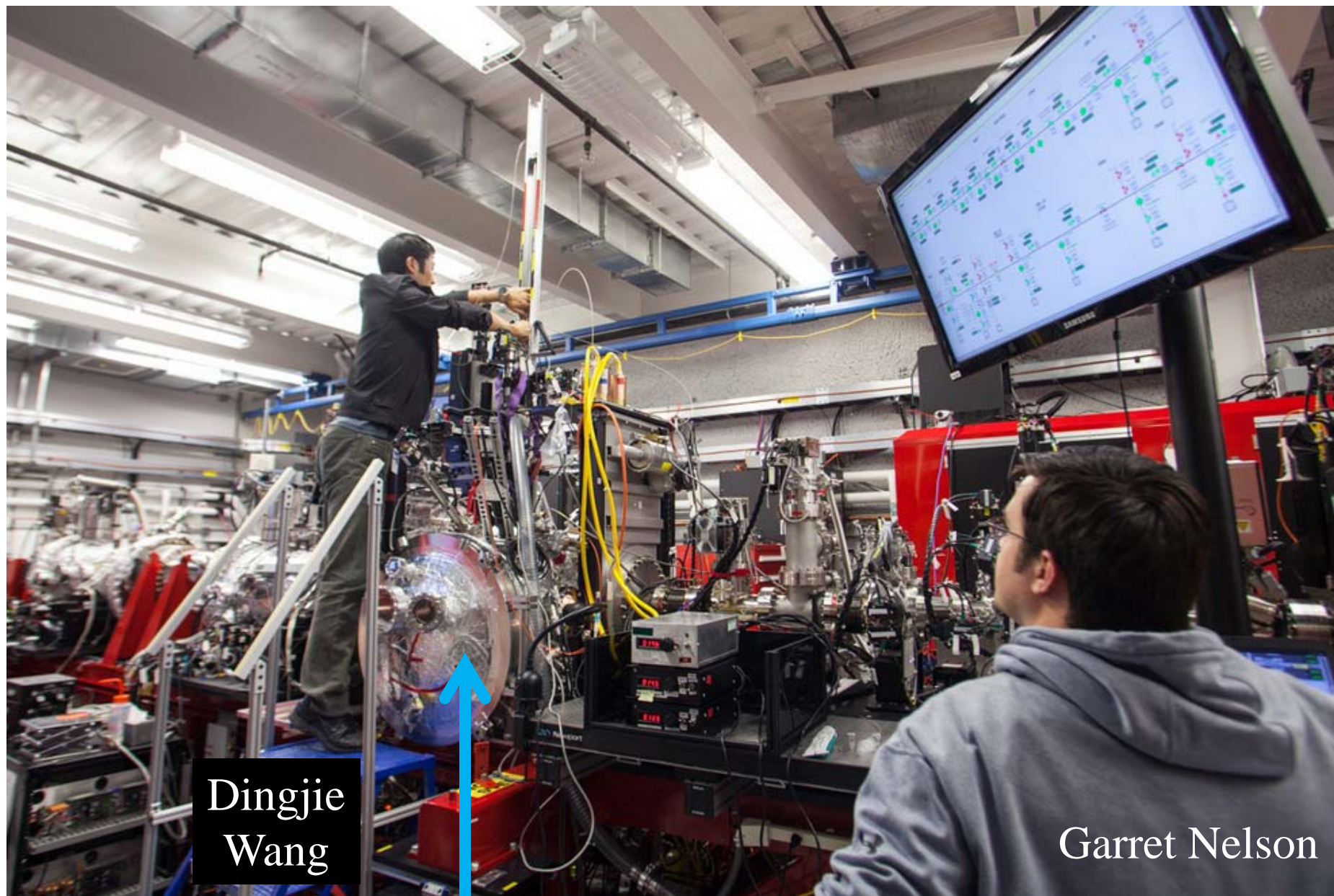
128 mm

Cubic phase (50 nL)

Precipitant solution (1 μ L)

96-well spacer, 125 μ m Glass coverslip Glass base

LCLS CXI Instrument Station

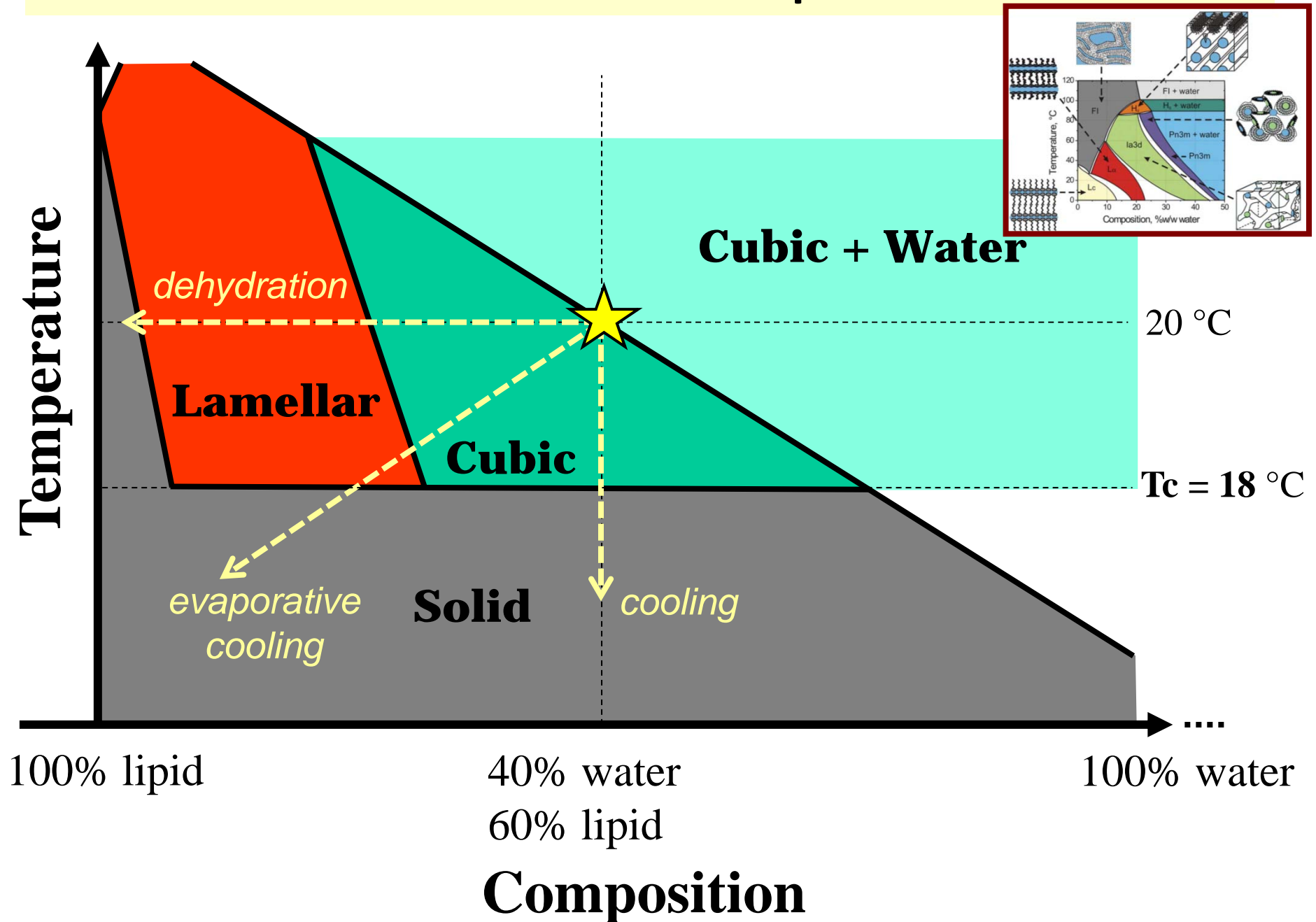


Dingjie
Wang

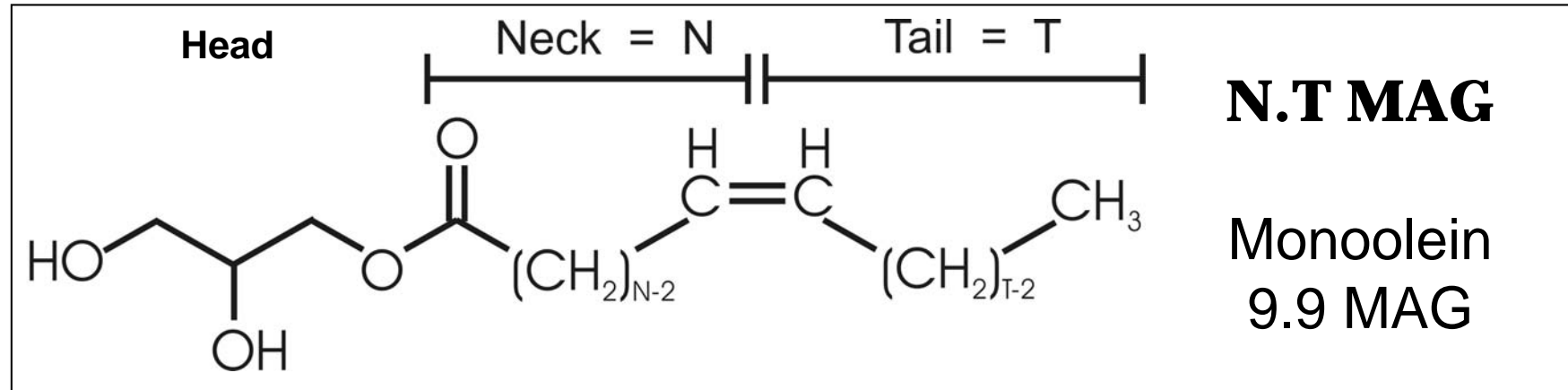
Garret Nelson

Evacuated sample chamber at 20 °C

Phase Behaviour in Evacuated Sample Chamber at 20 °C



Monoacylglycerols (MAG)



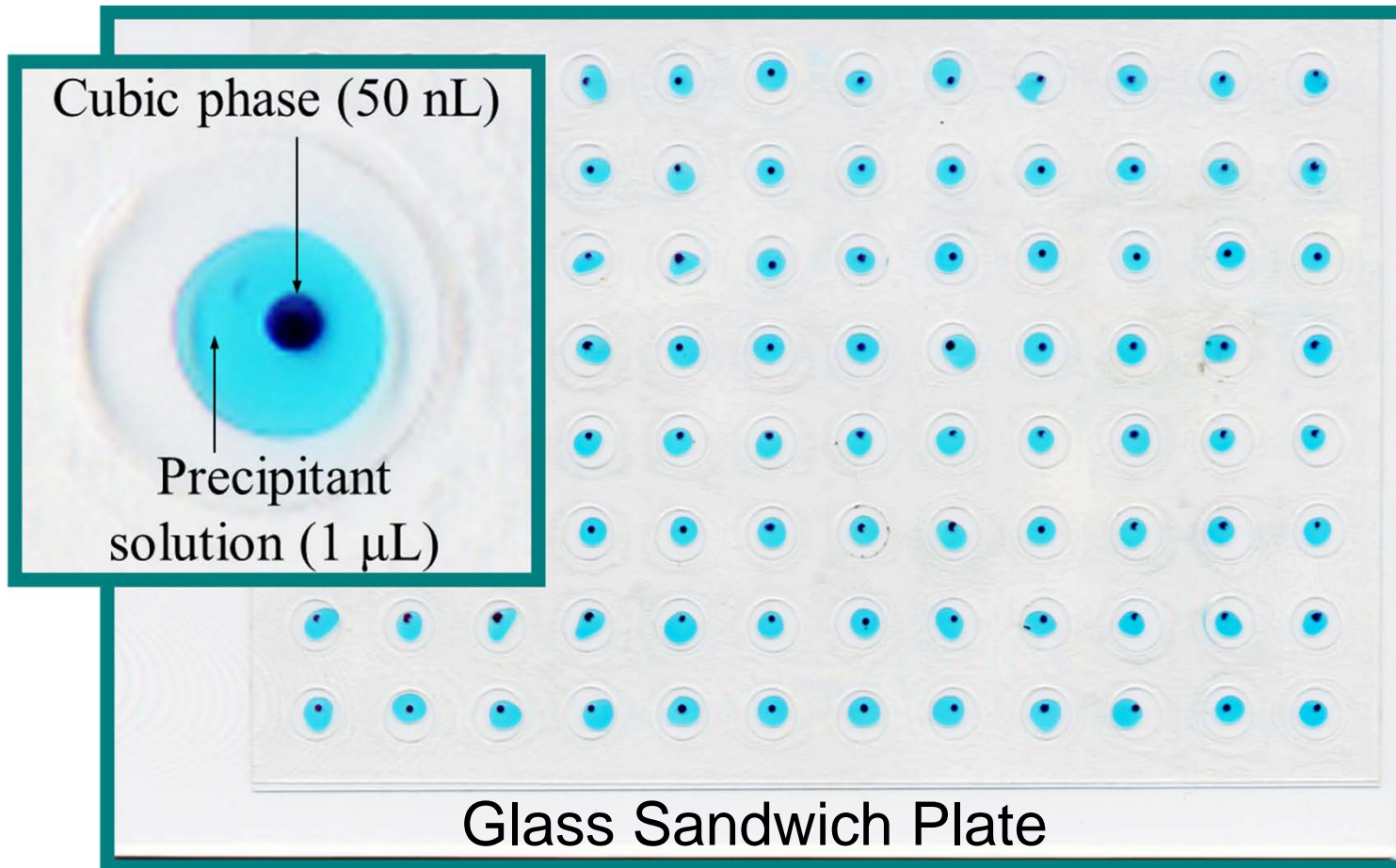
7.9 MAG - Low T_c

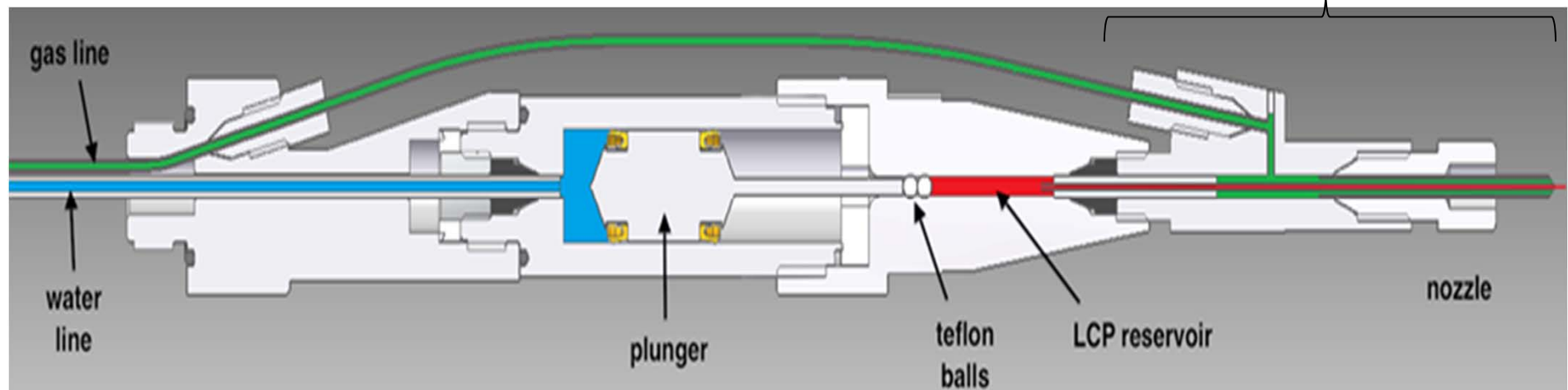
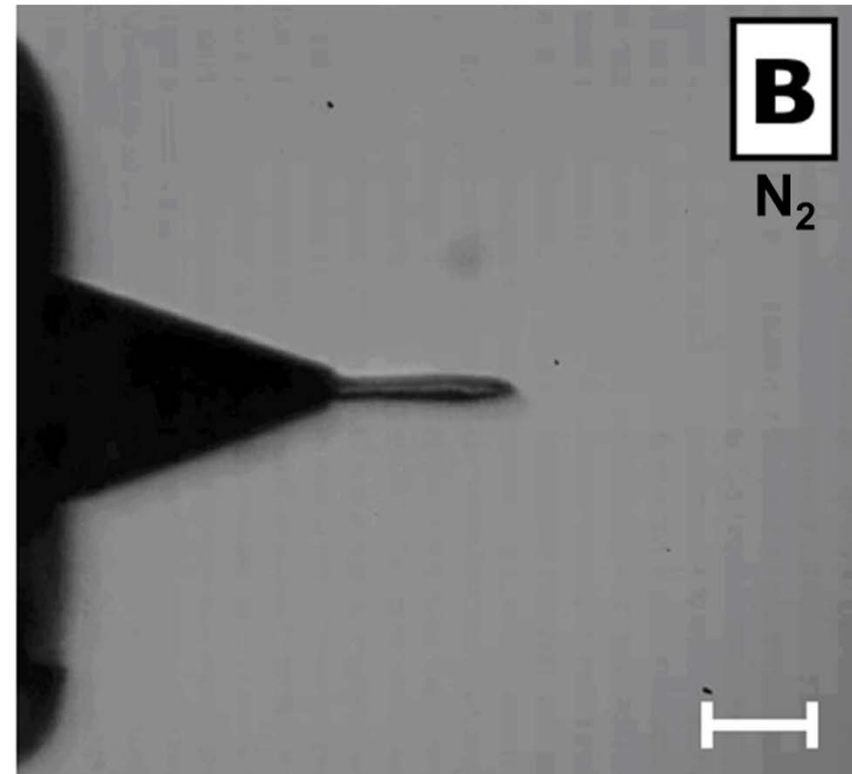
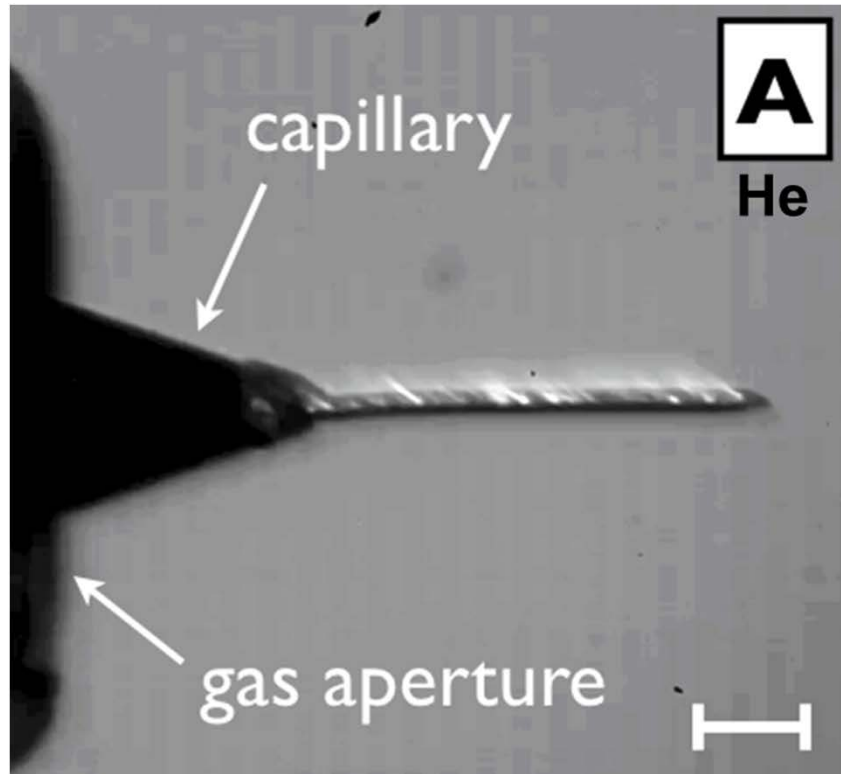
Designed/Synthesized for X_n at Low Temperature

Scaling Up - 1,000-fold

SYN - 50 nL mesophase/well

SFX - 50 μL





LCP Injector, Uwe Weierstall *et al.*

♣ Nature Comm. *In press*

LCP-SFX March 2013 Concept and Method Worked !

4 MP structures solved, microcrystals in LCP

7 shifts, 84 hours total

Record low amounts of protein

No preferential orientation

Experience, Ideas, New Projects

