Selected Topics in Protein Structure and Function

- Structural Determination of Membrane Proteins

Presented by: Jyh-Yeuan (Eric) Lee, Assistant Professor, BMI

Preparatory Readings

- **Lipid cubic phase / bicelle crystallization**
  - doi:10.1107/S2053230X14026843
  - doi:10.1016/j.ymeth.2011.09.020
- **X-ray microdiffraction**
  - doi:10.1039/B618173B
- **Cryo-EM: 2D microcrystals and single particles**
  - doi:10.1016/j.cocis.2018.01.010
  - doi:10.1126/science.aat4346
  - doi:10.1038/nmeth.3700
- **Fusion protein**
  - doi:10.1107/S2053230X15011061

Lecture Outline

1. **Why studying membrane proteins?**

2. **Challenges in membrane protein structural biology**

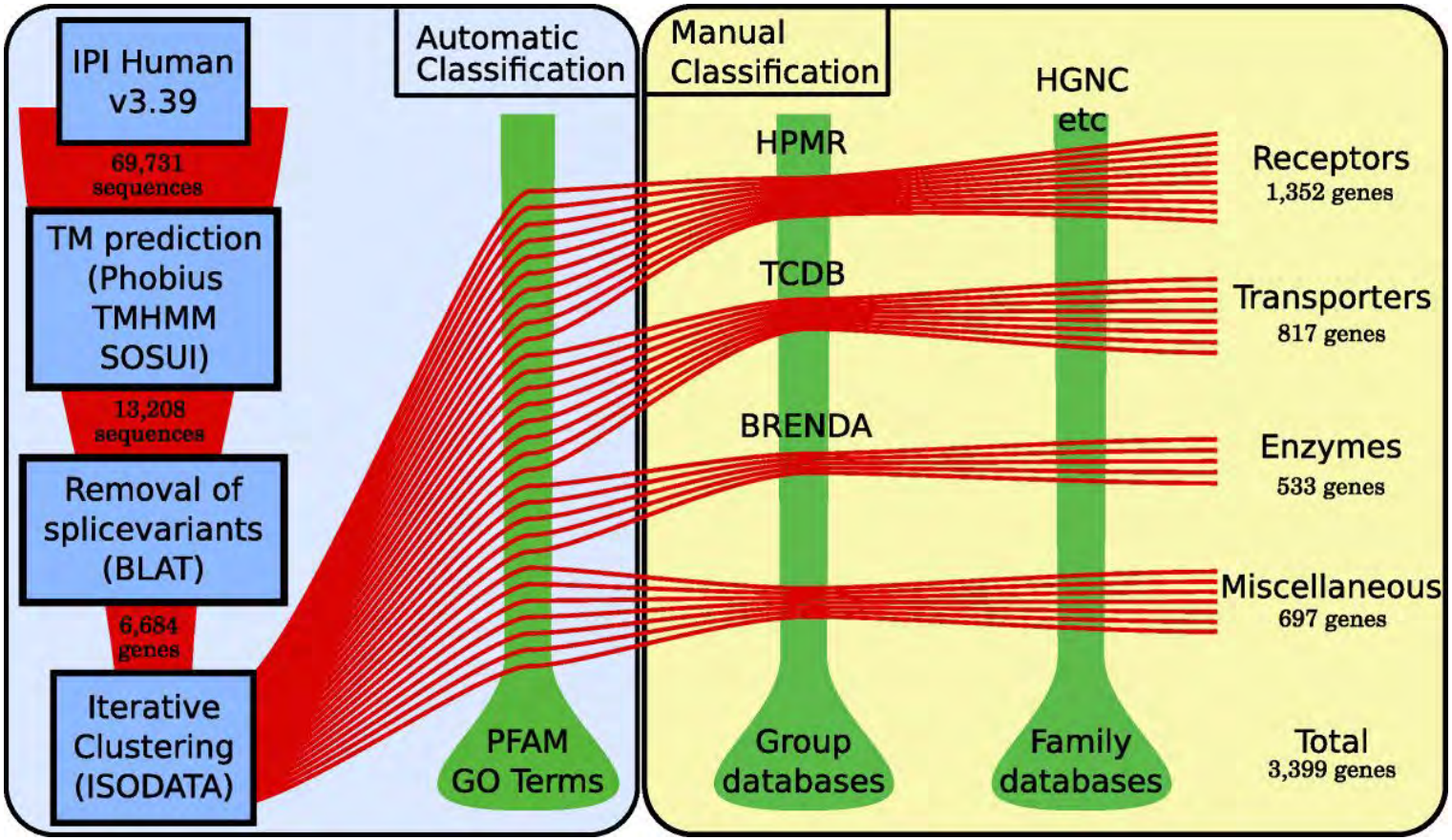
3. **Ways to study membrane protein structures**
   - **X-ray crystallography**
   - **Electron microscopy**
   - **Computer simulation, NMR, mass spec, cross-linking, …**

4. **Strategies in structural determination of membrane proteins**

1. Why studying membrane proteins?

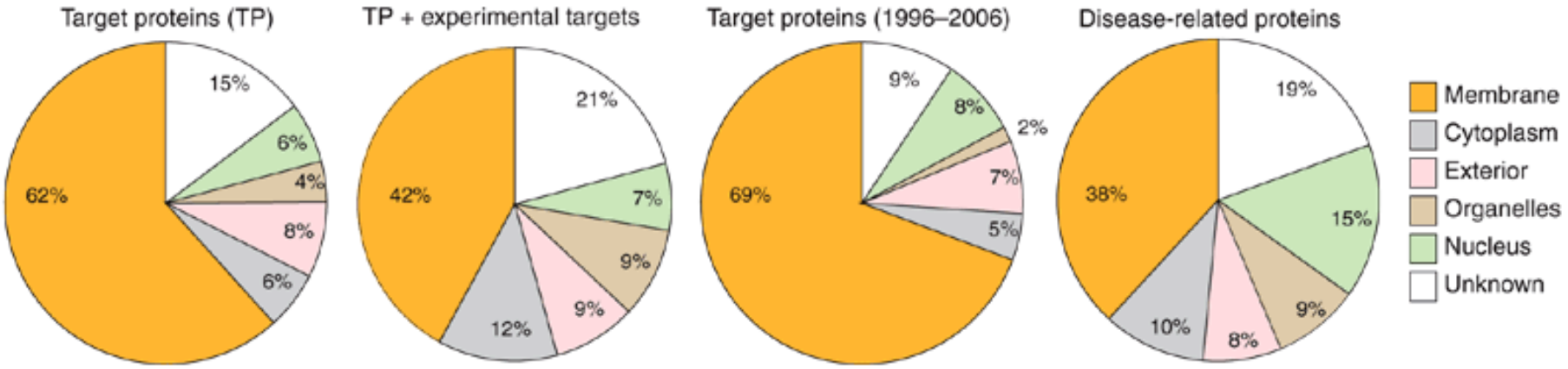
- **Encoded by some 20-30% genes in typical genome.**
- **Major components of the mosaic lipid bilayers in cellular membranes**
- **Mediate cell-to-cell communication and signaling events.**
- **Disruptions or mutations in humans have been implicated in diseases, such as cardiovascular and metabolic diseases, cancer, rare genetic diseases, ...**

Membrane proteome (human)



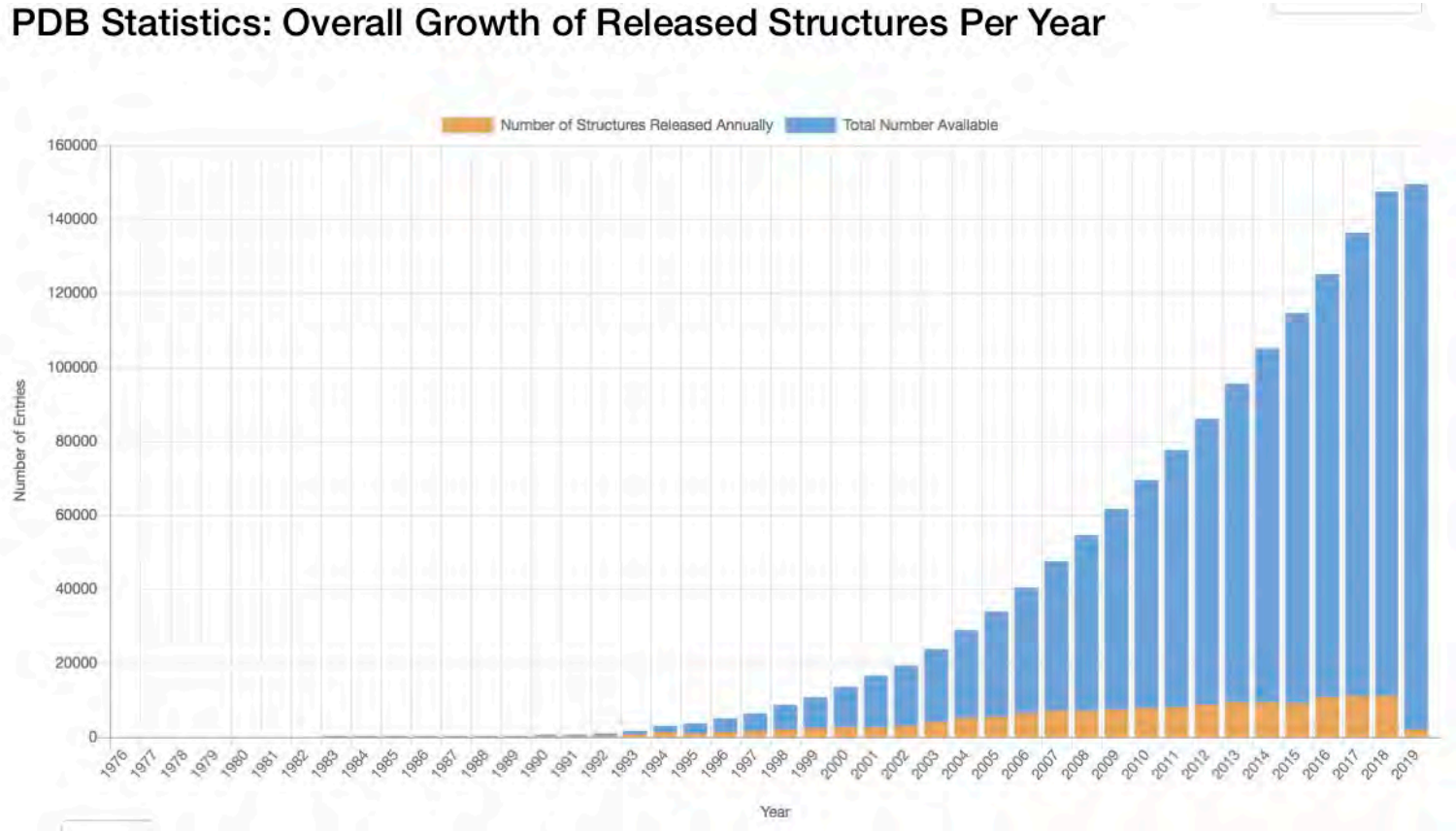
(Almén et al, BMC Biol, 2009)

Half drug targets are membrane proteins.



(Yildirim et al, Nat Biotech, 2007)

Available atomic/near-atomic models of membrane proteins (2019-3-8)



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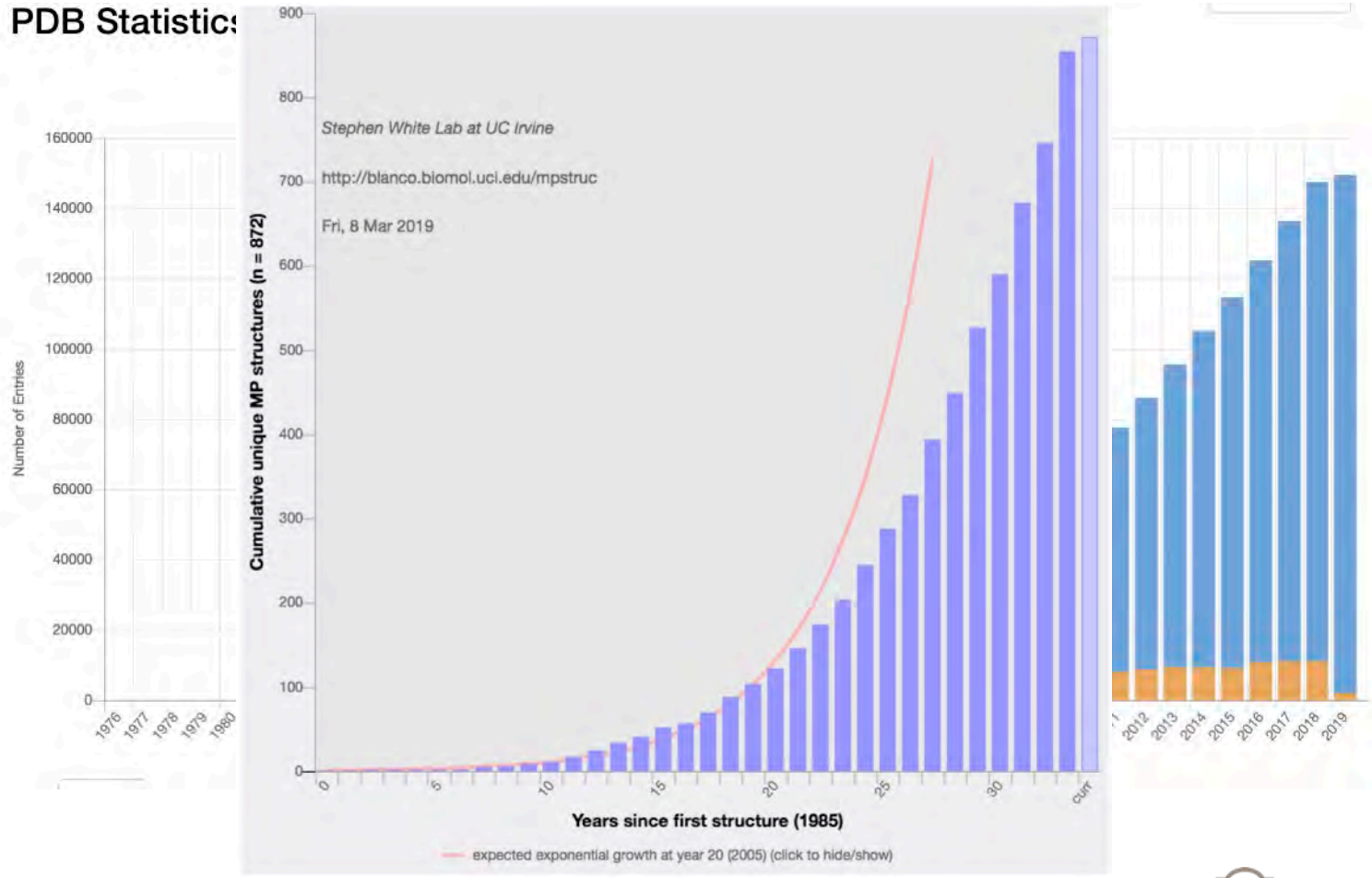


2. Challenges in membrane protein structural biology

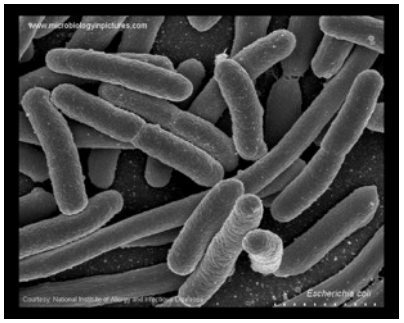
- **Naturally occurred proteins exist in low abundance, with only a few exceptions (*e.g.*, bacteriorhodopsin or aquaporin), and form complexes.**

- ***E. coli* is often not suitable for producing recombinant membrane proteins of eukaryotic origins.**

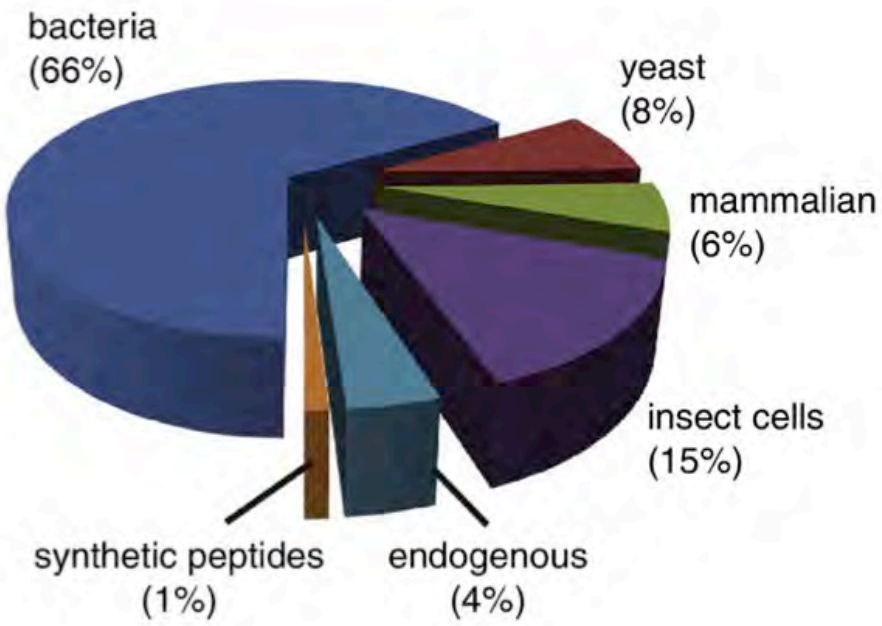
- **No so-called standard protocol of protein extraction, largely due to the complexity of protein-lipid interaction.**

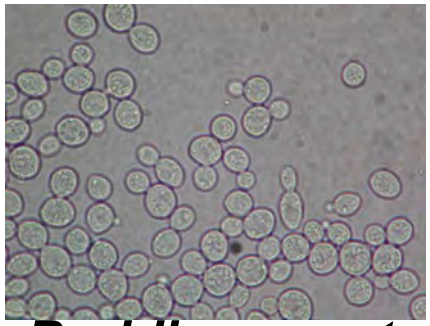
- **Protocols of purification, crystallization, and *in vitro* reconstitution remain empirical for individual cases.**

Choosing the appropriate expression hosts for recombinant proteins

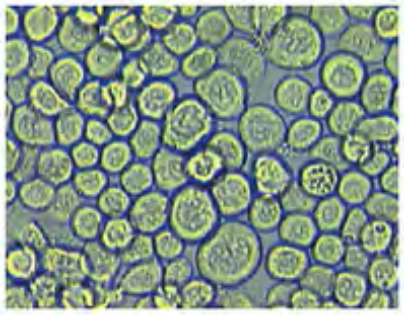


E. coli

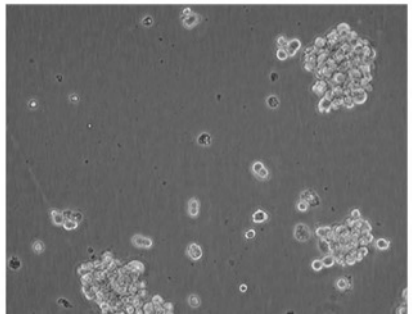




Budding yeast



Sf9 insect cells



HEK 293sus

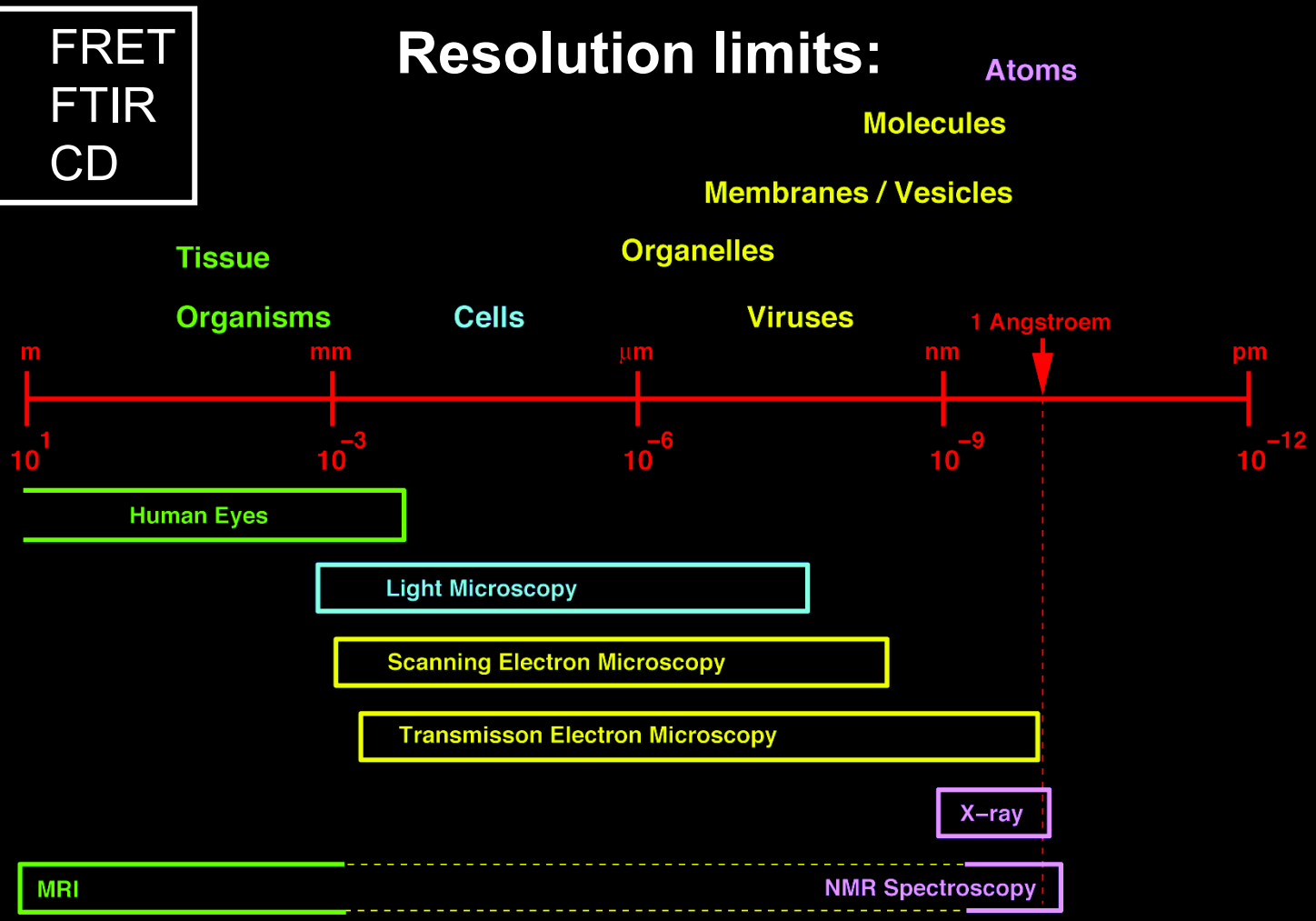
(Zorman et al, Curr Opin Struct Biol, 2015)

Things to consider for membrane protein extraction and purification

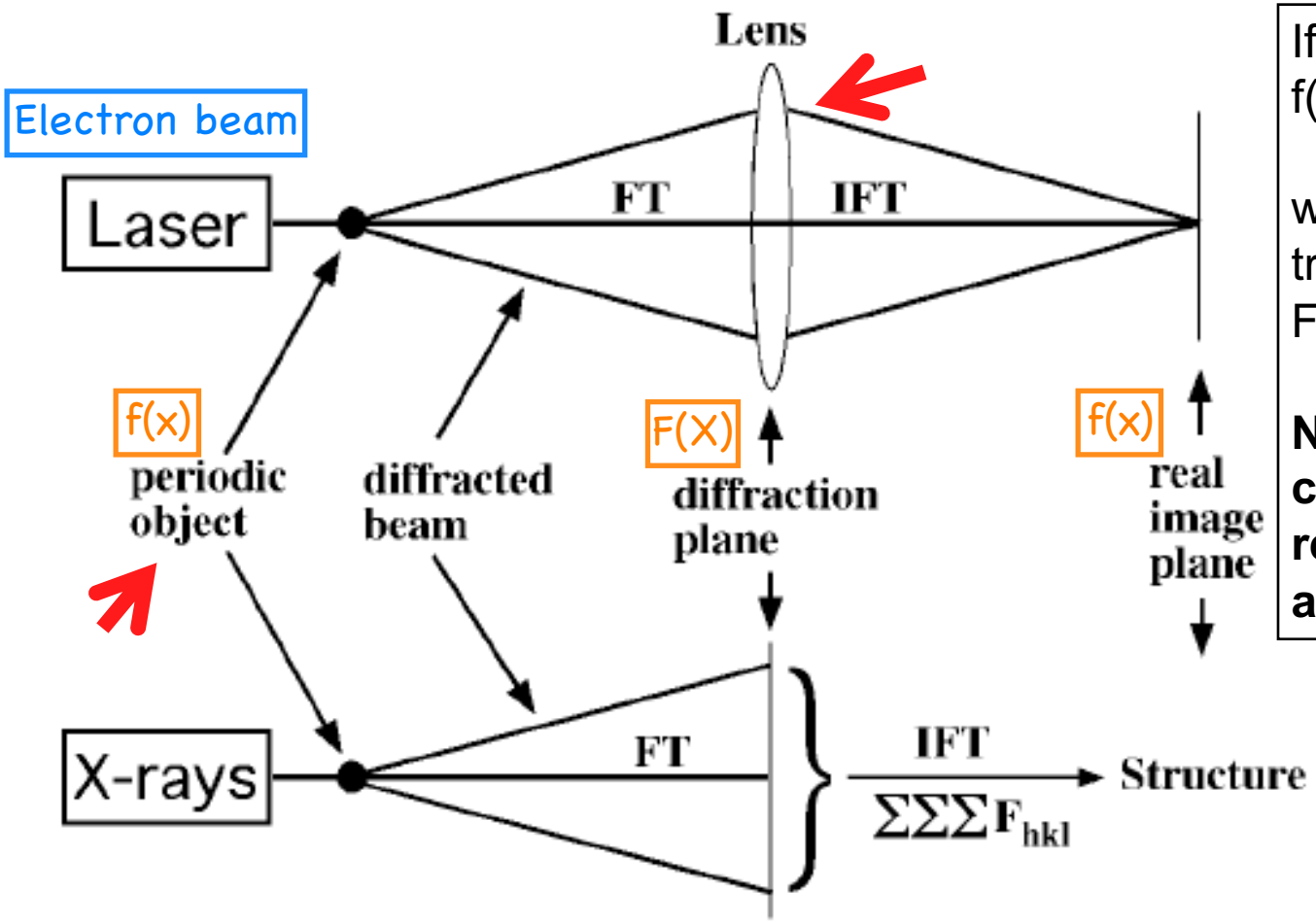
- Cell disruption
- Solubilization agent
  - Detergents
  - Polymers
- Protein engineering
- Column chromatography
- *In vitro* reconstitution

3. Ways to study membrane protein structures: optics & spectroscopy

- FRET
- FTIR
- CD



Optical diffraction & X-ray diffraction



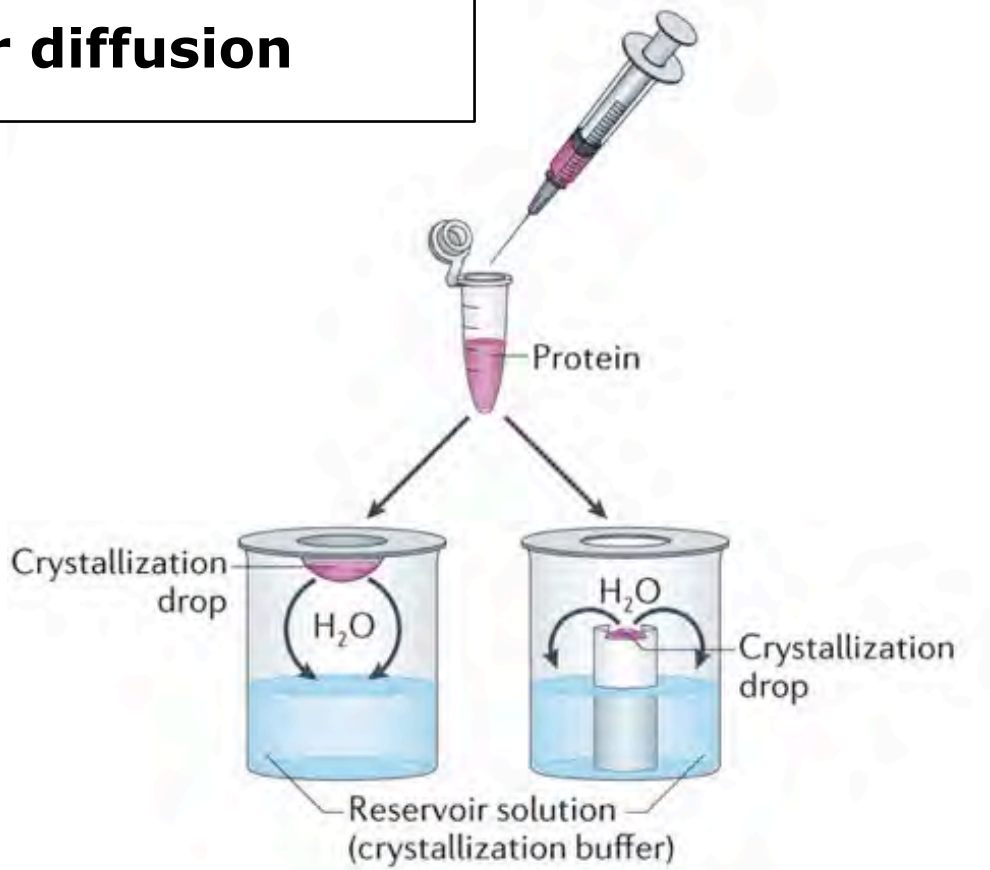
If F(X)=FT[f(x)], then f(x)=IFT[F(X)]

where FT=Fourier transform & IFT=Inverse Fourier transform.

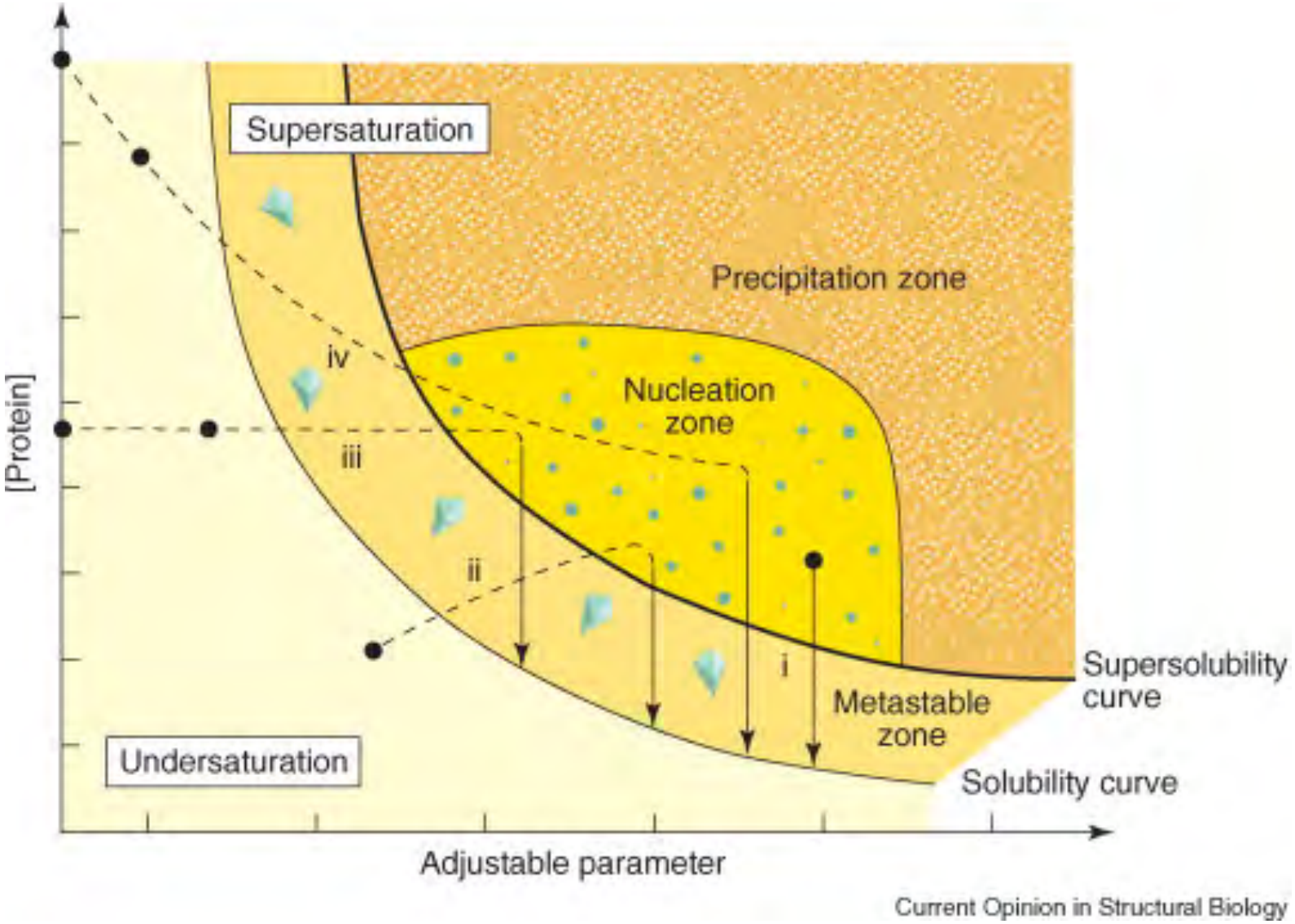
Note: important in X-ray crystallography and 3D reconstruction algorisms.

X-ray crystallography: crystallization

Coarse vapor diffusion



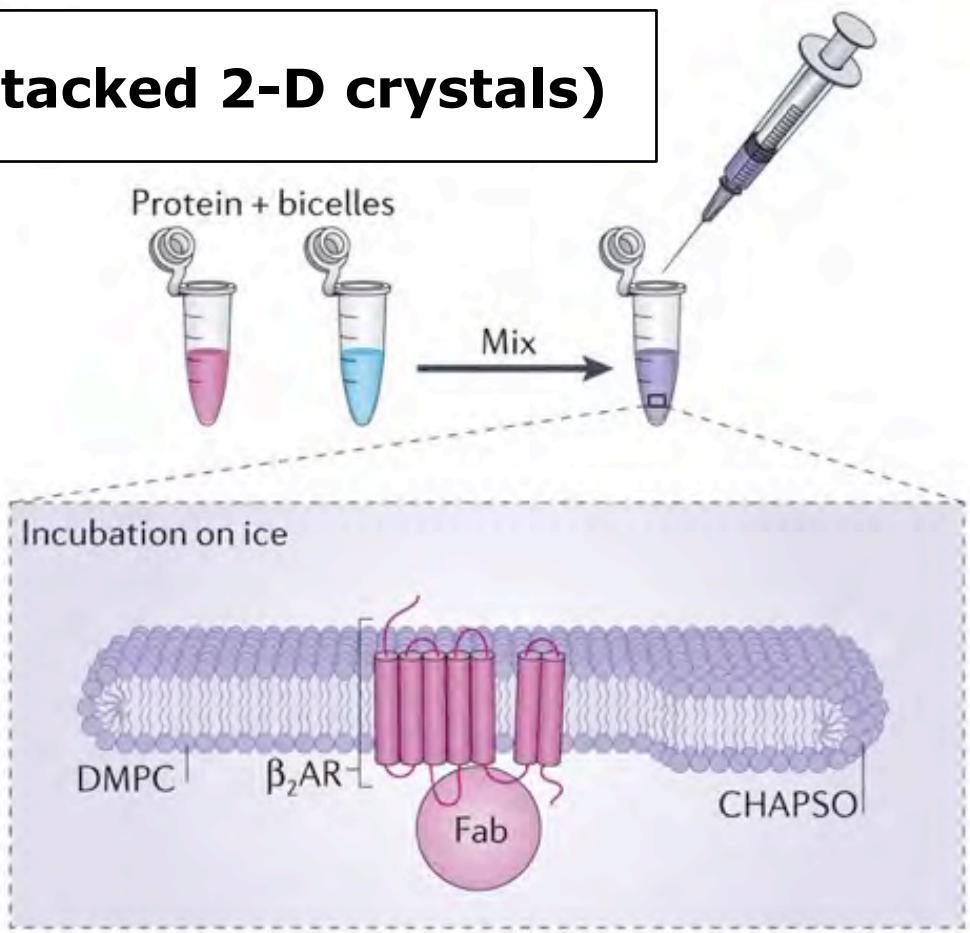
(Ghosh et al, Nature Rev Mol Cell Biol, 2015)



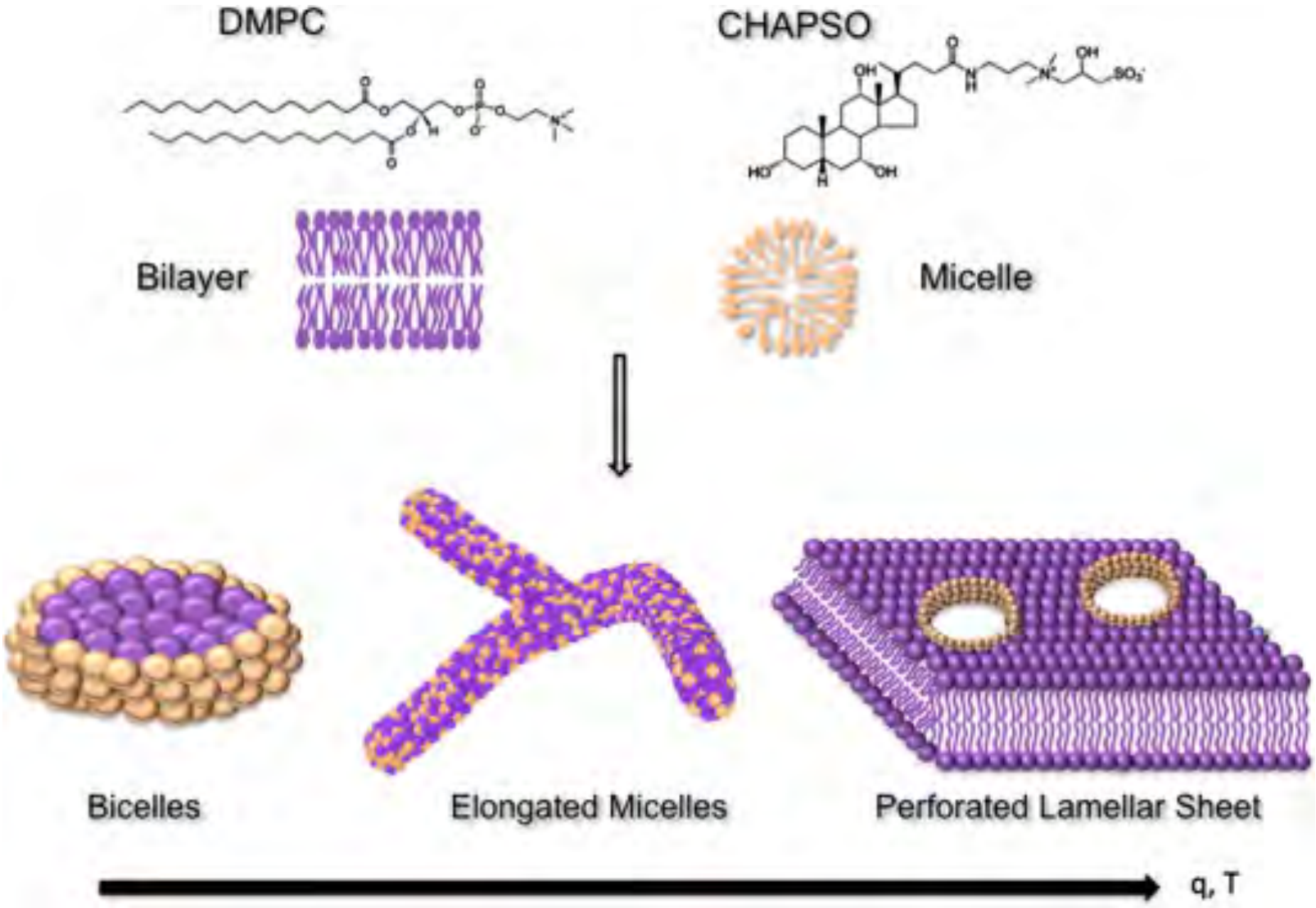
(Naomi Chayen, Curr Opin Struct Biol, 2004)

X-ray crystallography: crystallization

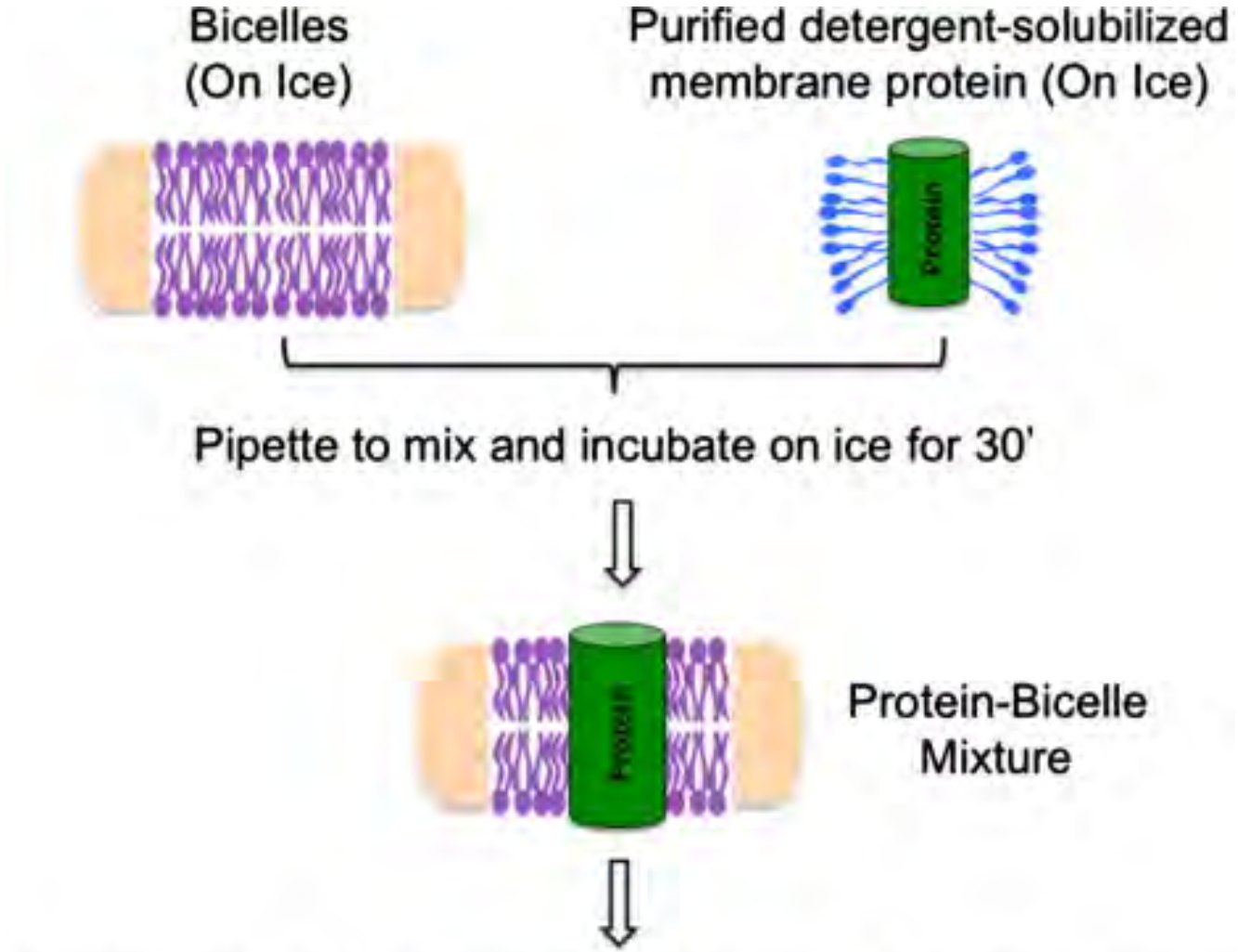
Bicelle (stacked 2-D crystals)



(Ghosh et al, Nature Rev Mol Cell Biol, 2015)

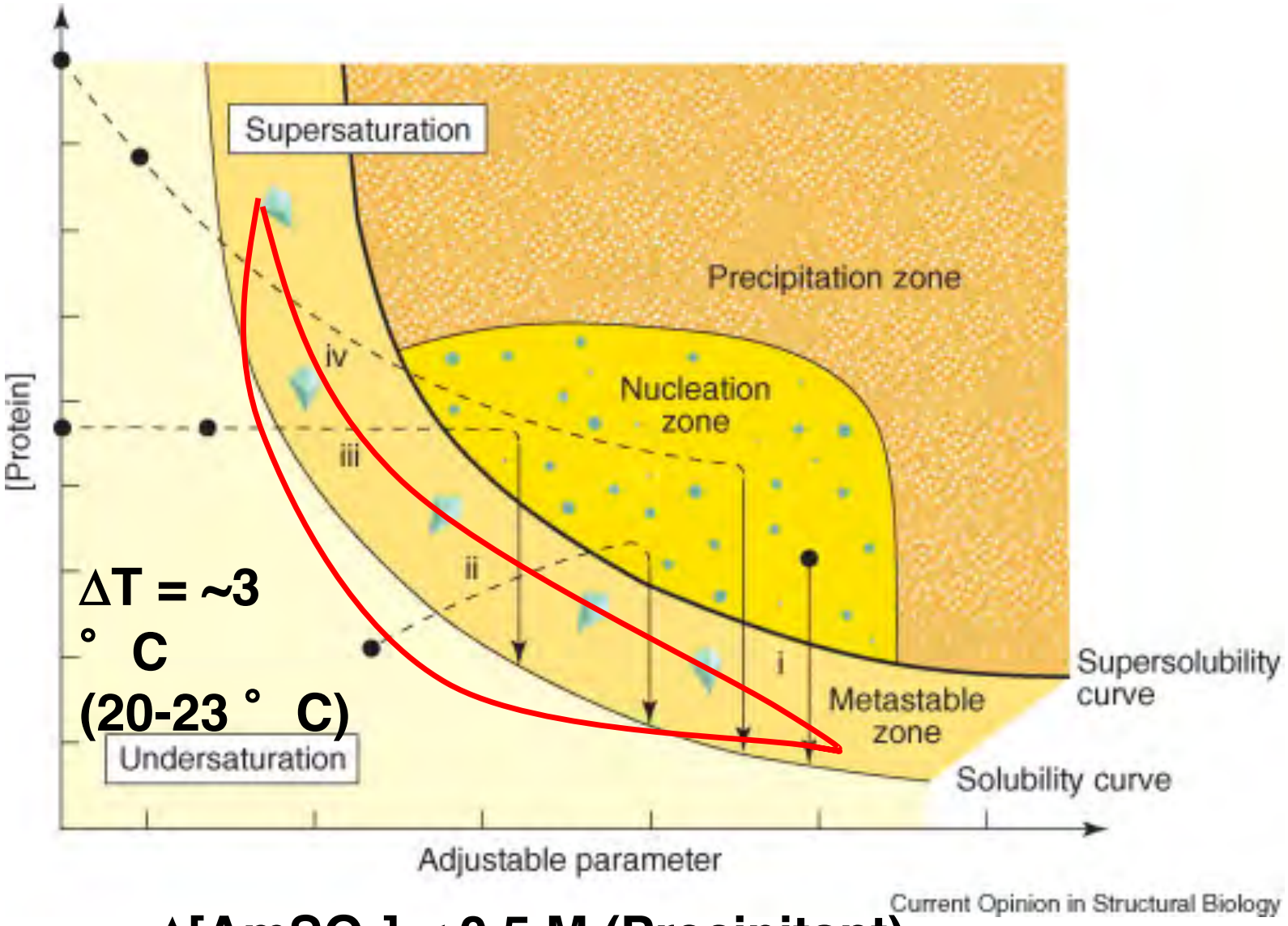


(Ujwal & Bowie, Methods, 2011)



(Ujwal & Bowie, Methods, 2011)

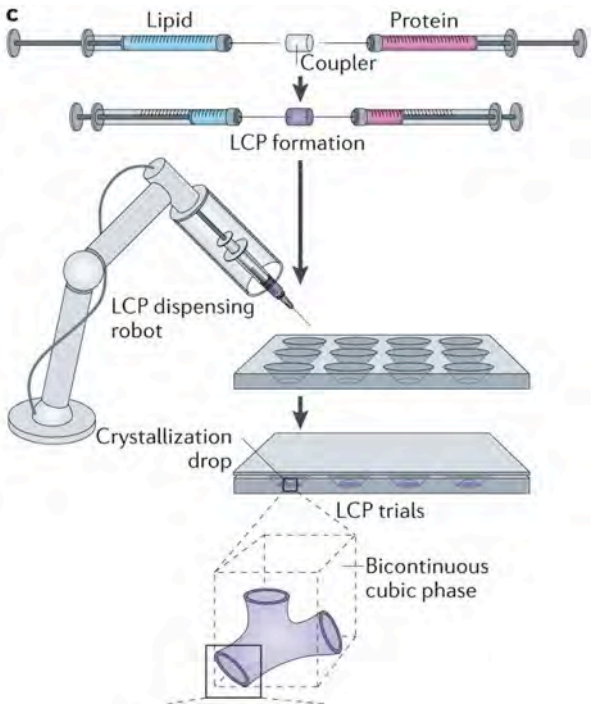
[Protein] = 4-10 mg/mL

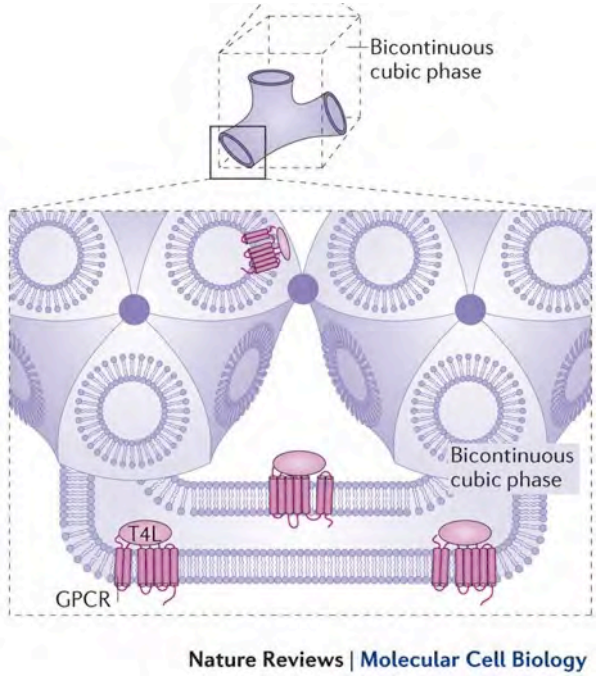


$\Delta[AmSO_4] < 0.5$ M (Precipitant)

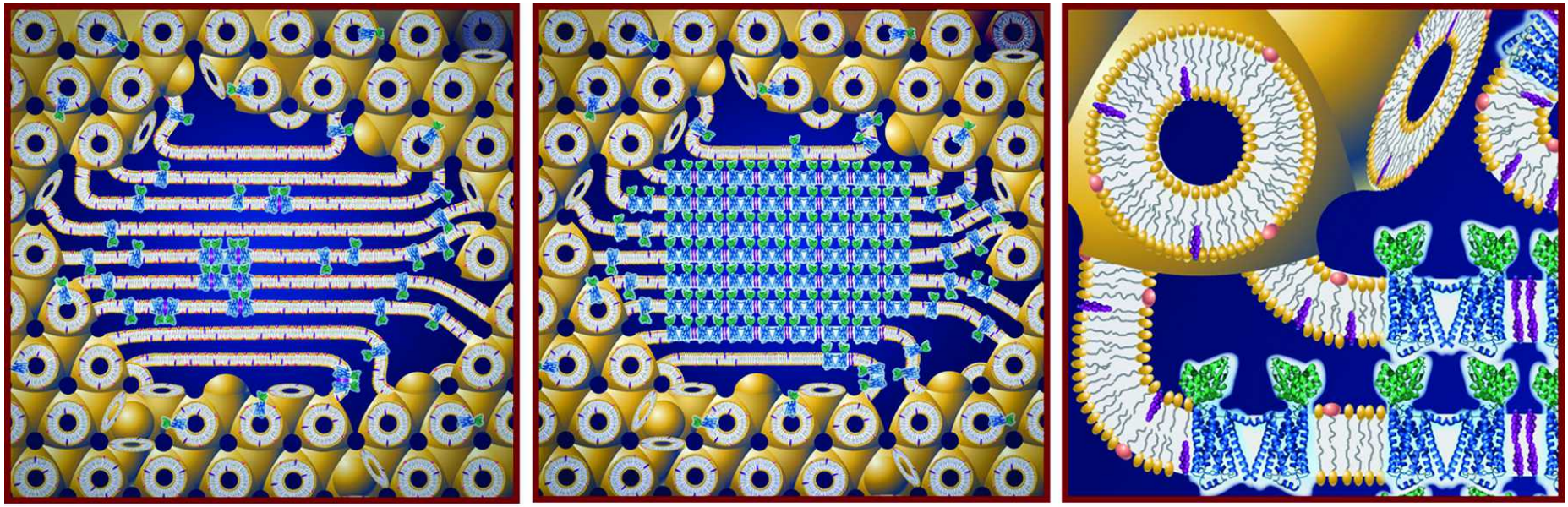
X-ray crystallography: crystallization

***In meso* lipid cubic phase**



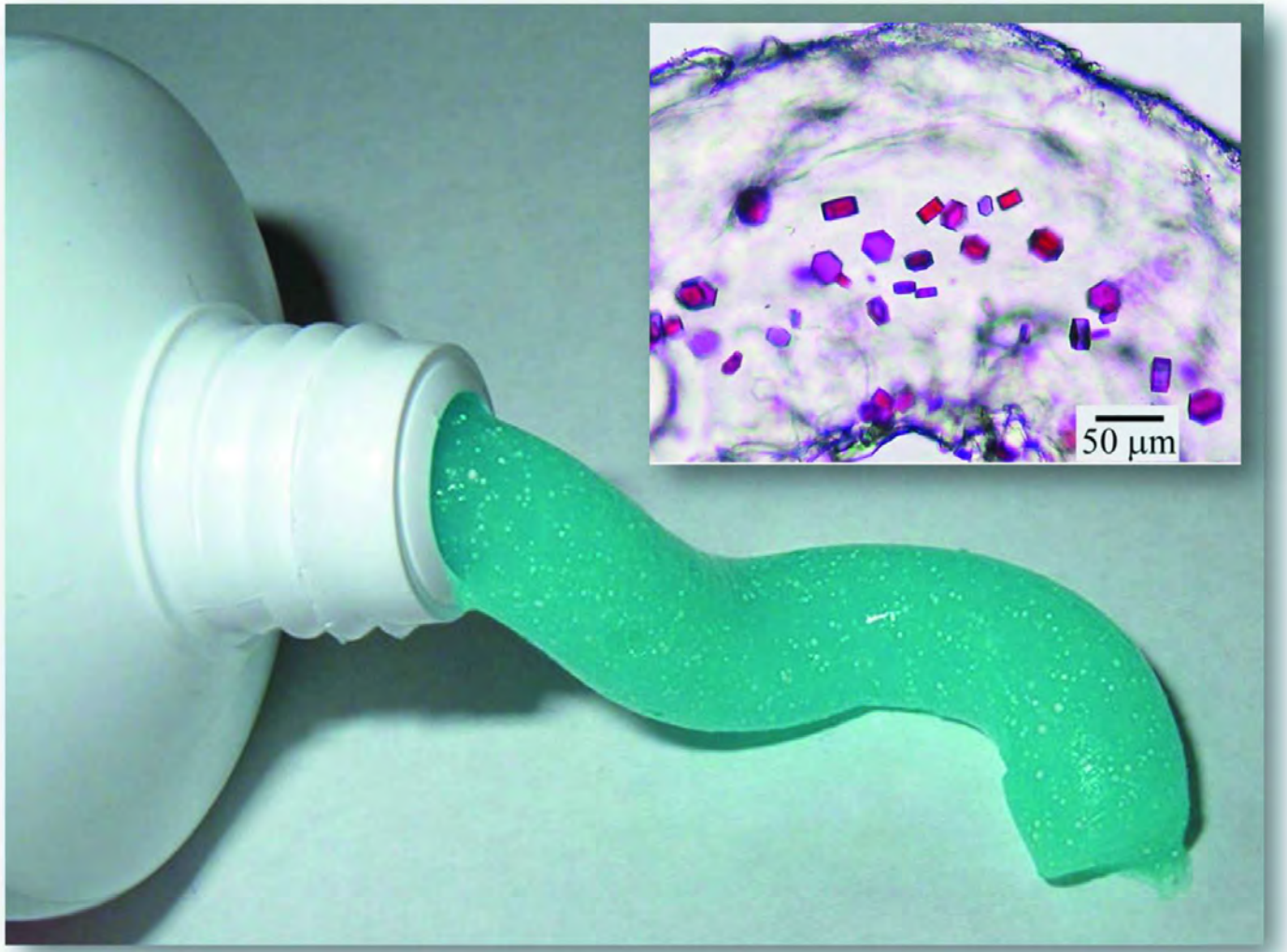


(Ghosh et al, Nature Rev Mol Cell Biol, 2015)

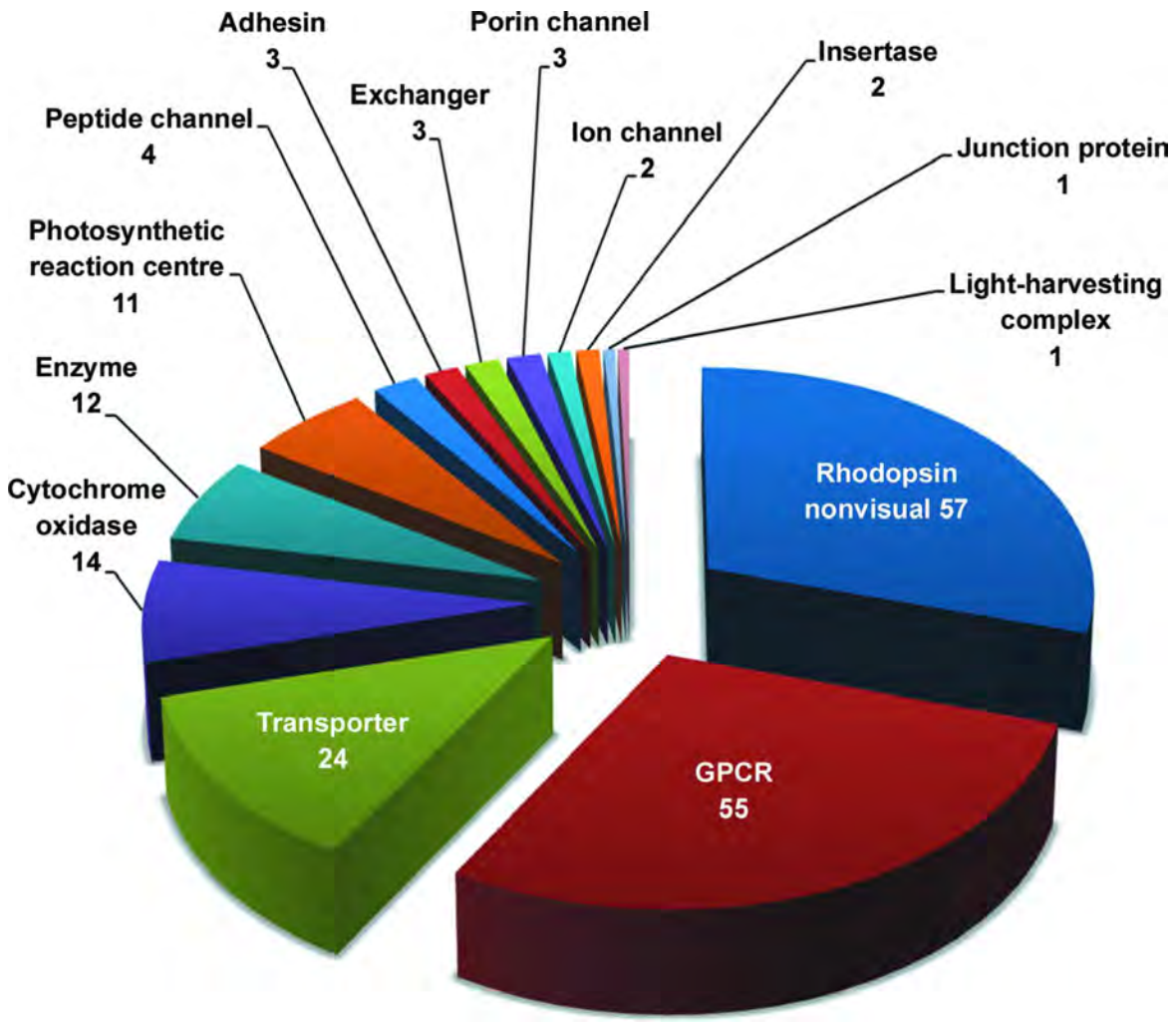


(*a*) (*b*) (*c*)

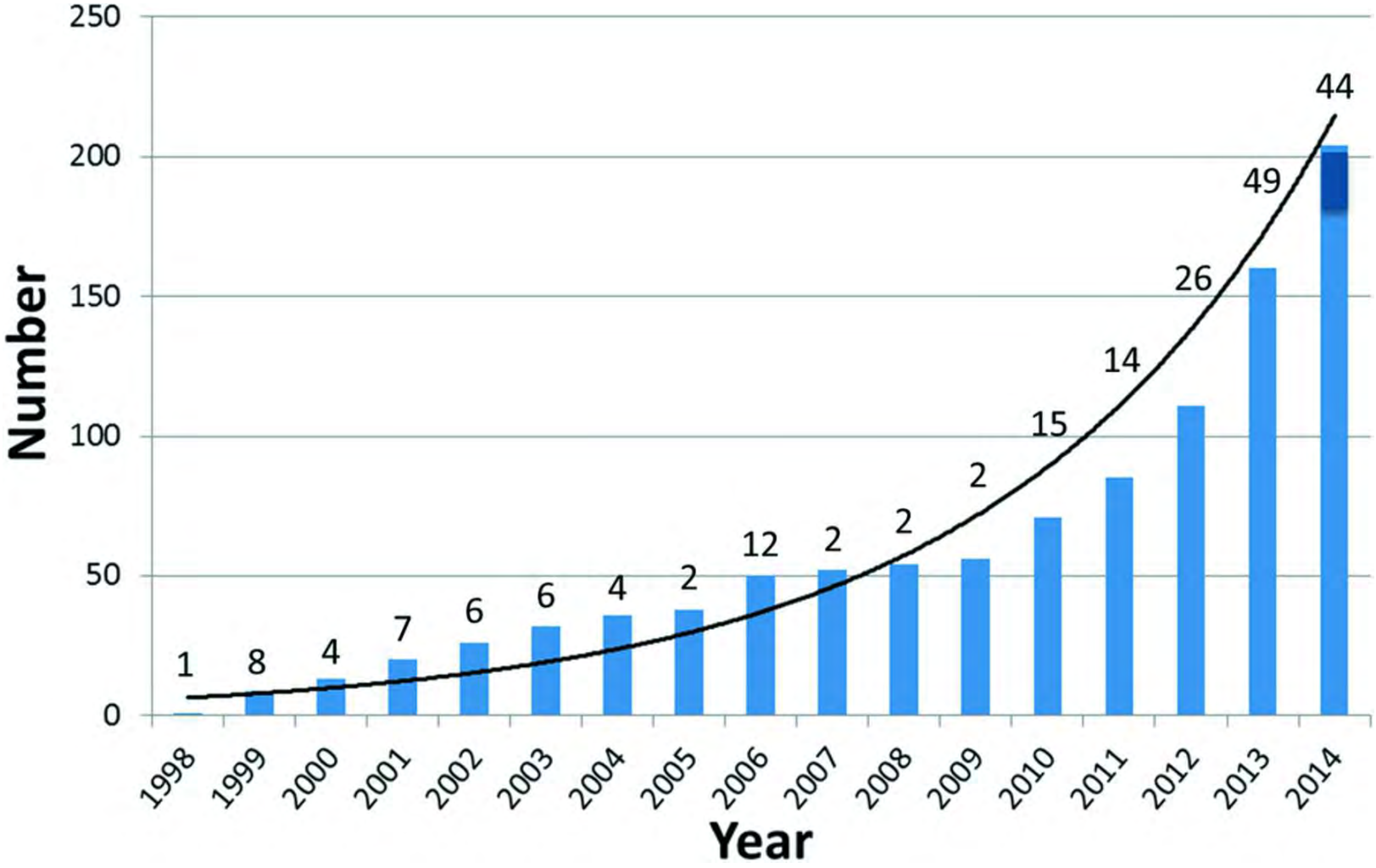
(Caffrey, Acta Cryst F, 2015)



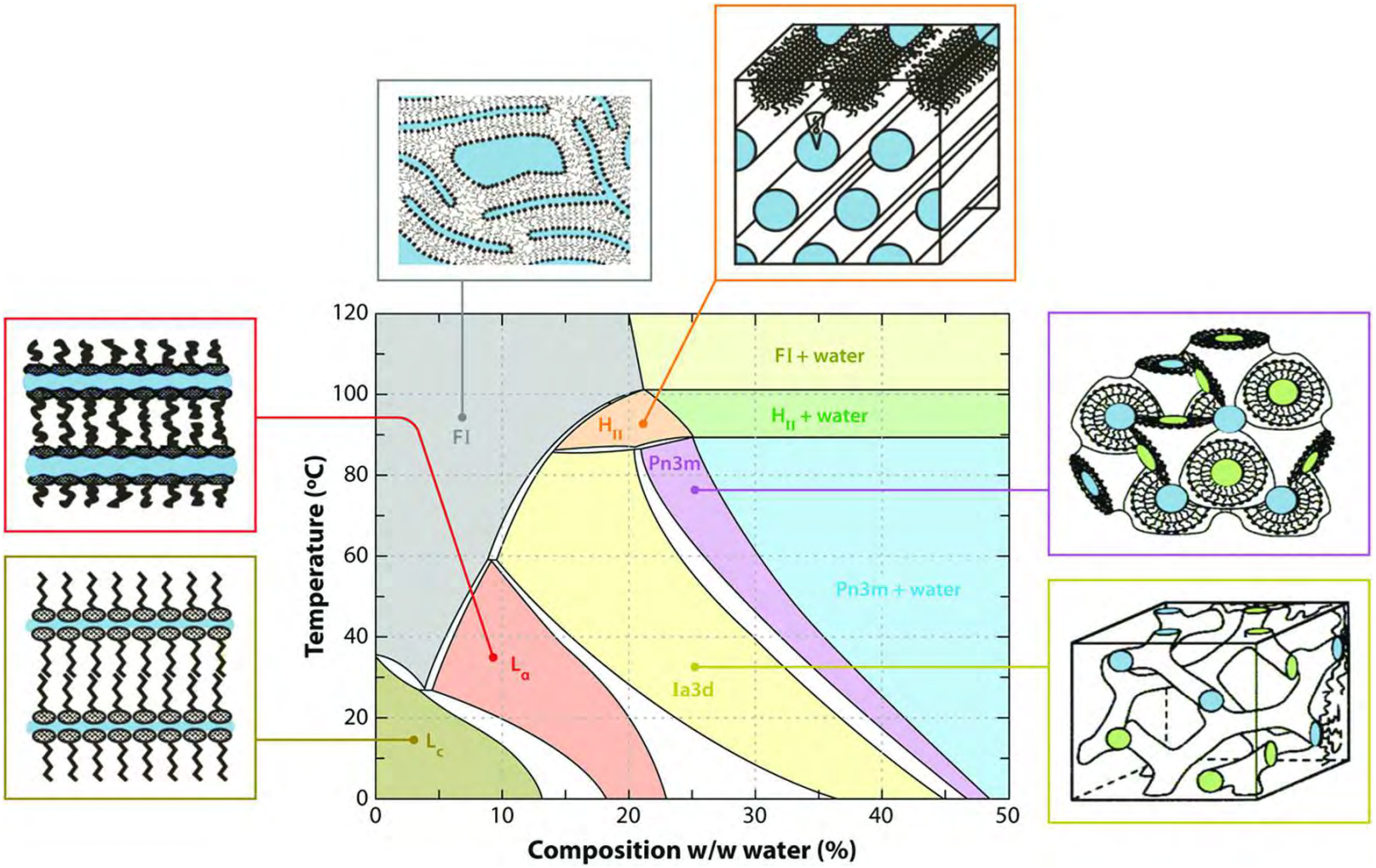
(Caffrey, Acta Cryst F, 2015)



(Caffrey, Acta Cryst F, 2015)



(Caffrey, Acta Cryst F, 2015)

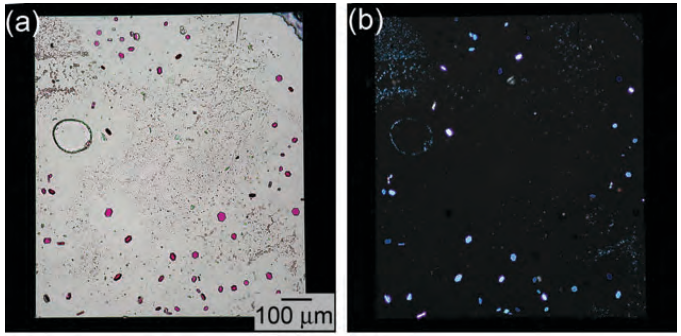


(Caffrey, Acta Cryst F, 2015)

X-ray crystallography: micro-diffraction

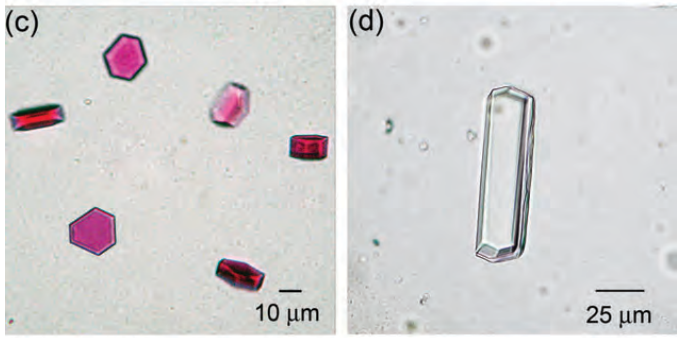
LCP

Bacteriorhodopsin



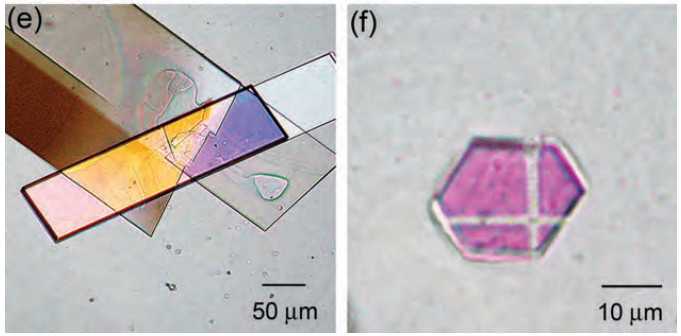
Bacteriorhodopsin (Birefringence)

Bacteriorhodopsin



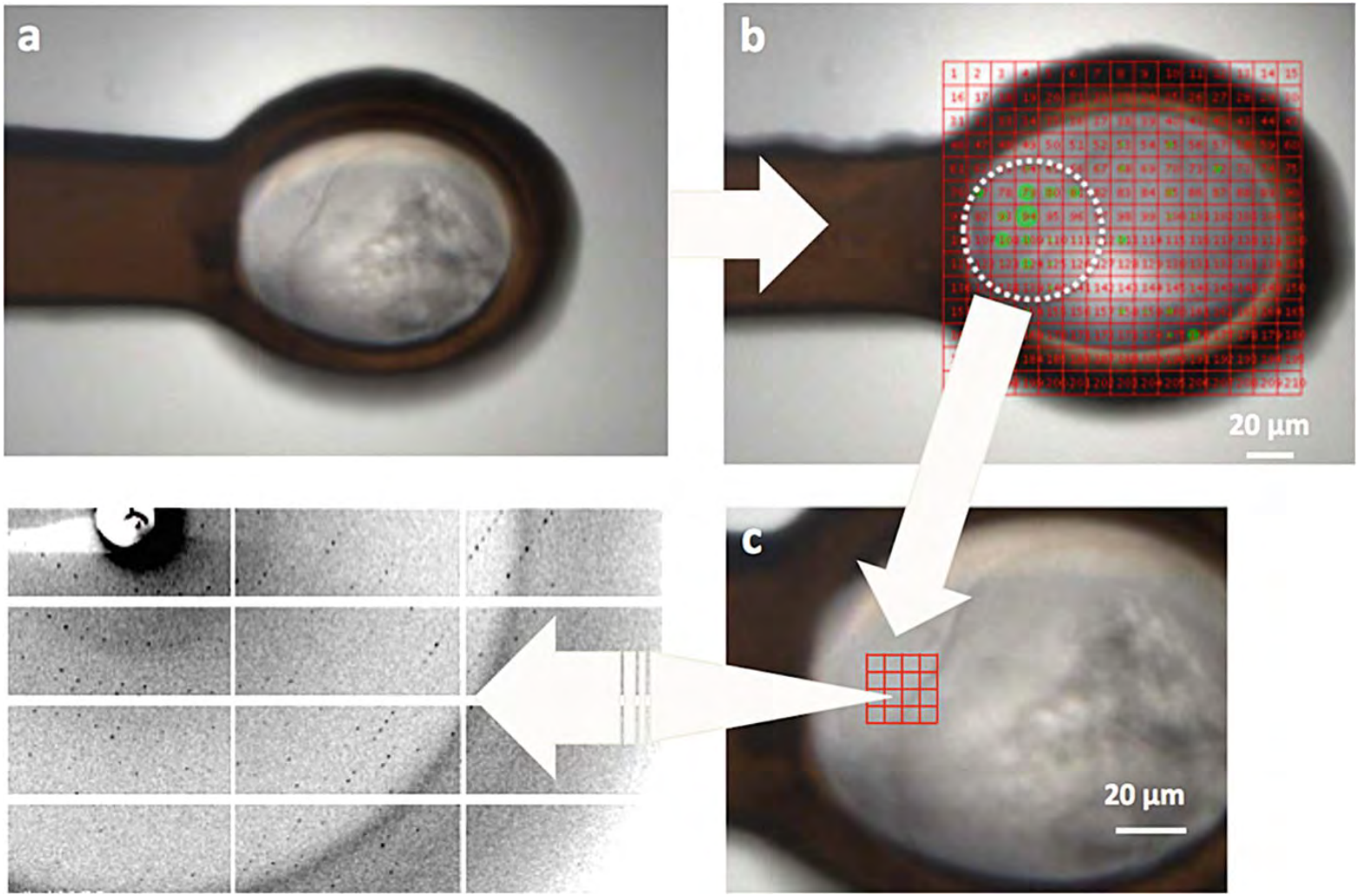
Lysozyme

Cholesterol

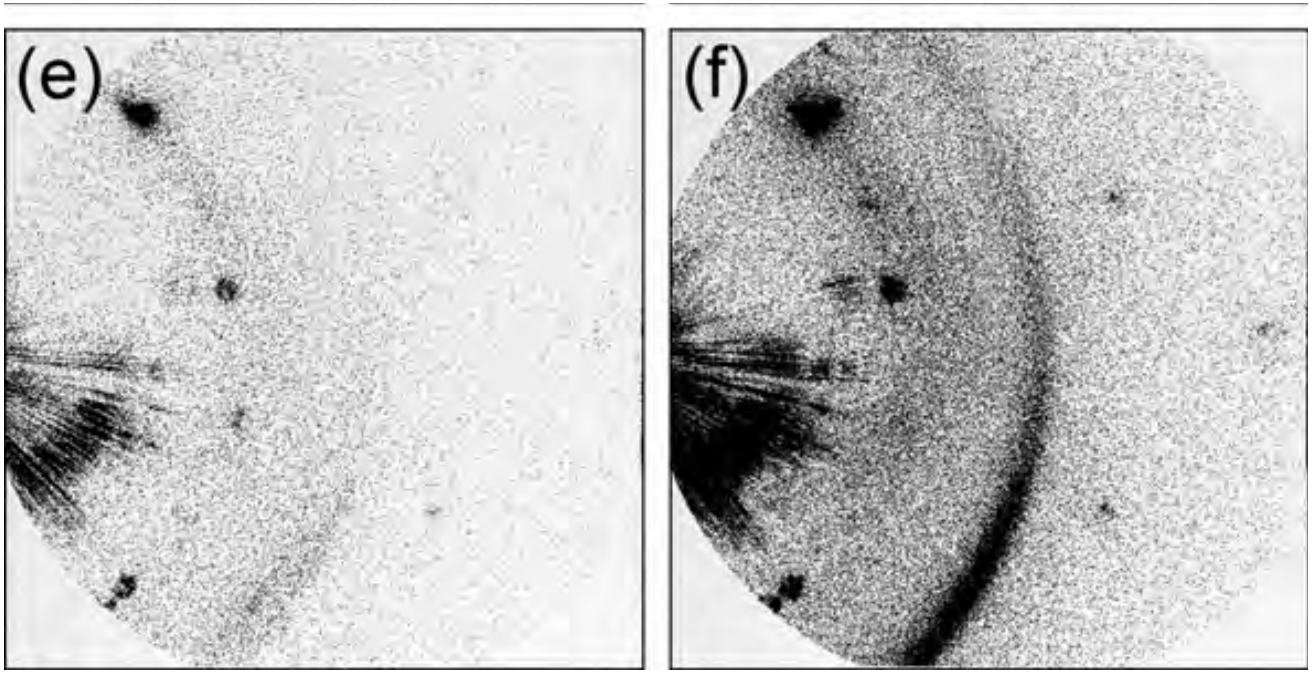


Bacteriorhodopsin (X-ray damaged)

(Cherezov & Caffrey, Faraday Discuss, 2007)



(Warren et al, in "The Next Generation in Membrane Protein Structure Determination", 2016)

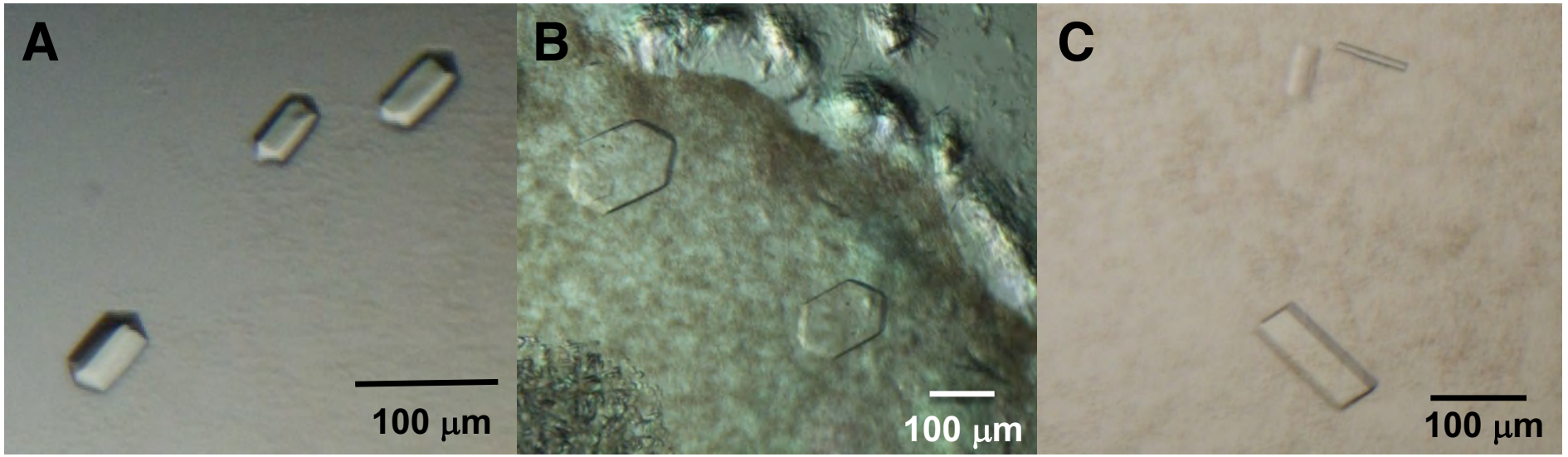


1s exposure **10s exposure**

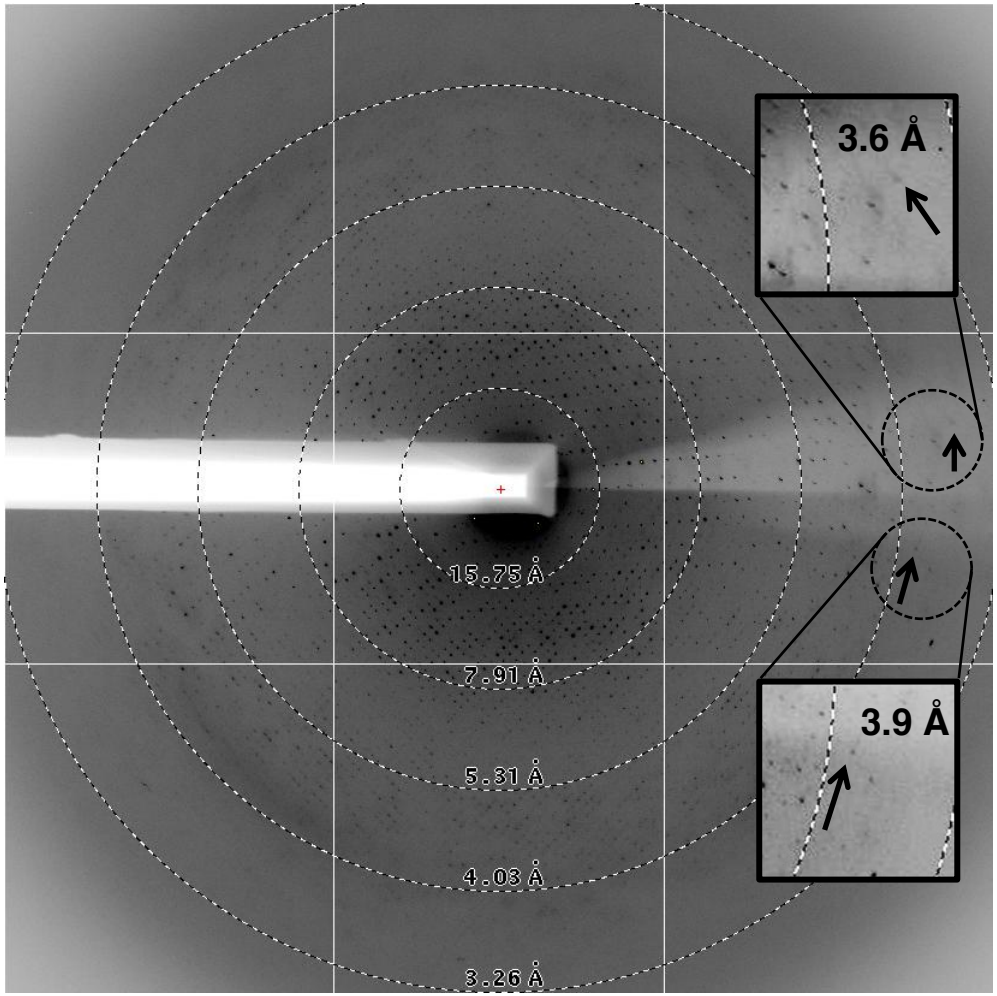
(Cherezov & Caffrey, Faraday Discuss, 2007)

X-ray crystallography: micro-diffraction

Bicelle



50-500 μm	100-300 μm	50-150 μm
25-30 Å	7-10 Å	3.5-4 Å



- Long exposure
  2-5 sec @ APS
  30 sec @ ALS
- Radiation damage
  3-5 frames (< 5°)
- Signal (I/σ = 1-1.5 at 3.9-4Å)

X-ray crystallography: data process

reading from a file: C3_1_0020.x
reading from a file: C3_2_0001.x
reading from a file: C3_2_0002.x
reading from a file: C3_2_0003.x
reading from a file: C3_2_0004.x
reading from a file: C3_2_0005.x
reading from a file: C3_2_0006.x
reading from a file: C3_2_0007.x
reading from a file: C3_2_0008.x
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reading from a file: B3_1_0020.x
reading from a file: B3_2_0001.x
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reading from a file: B3_2_0029.x
reading from a file: B3_2_0030.x

reading from a file: B13_1_0020.x
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reading from a file: B13_1_0031.x
reading from a file: B13_1_0032.x
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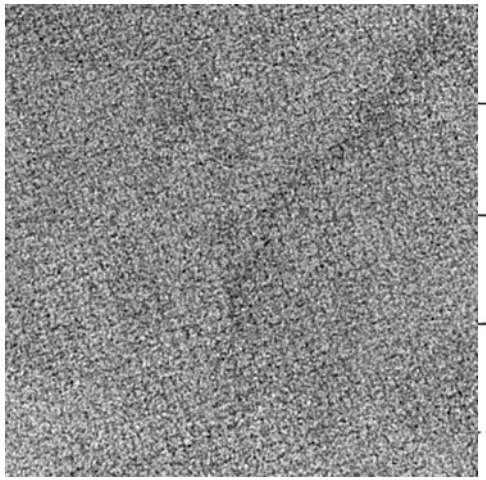
reading from a file: B13_1_0037.x
reading from a file: B13_1_0038.x
reading from a file: B13_1_0039.x
reading from a file: B13_1_0040.x

Cryo-electron microscopy (cryo-EM)

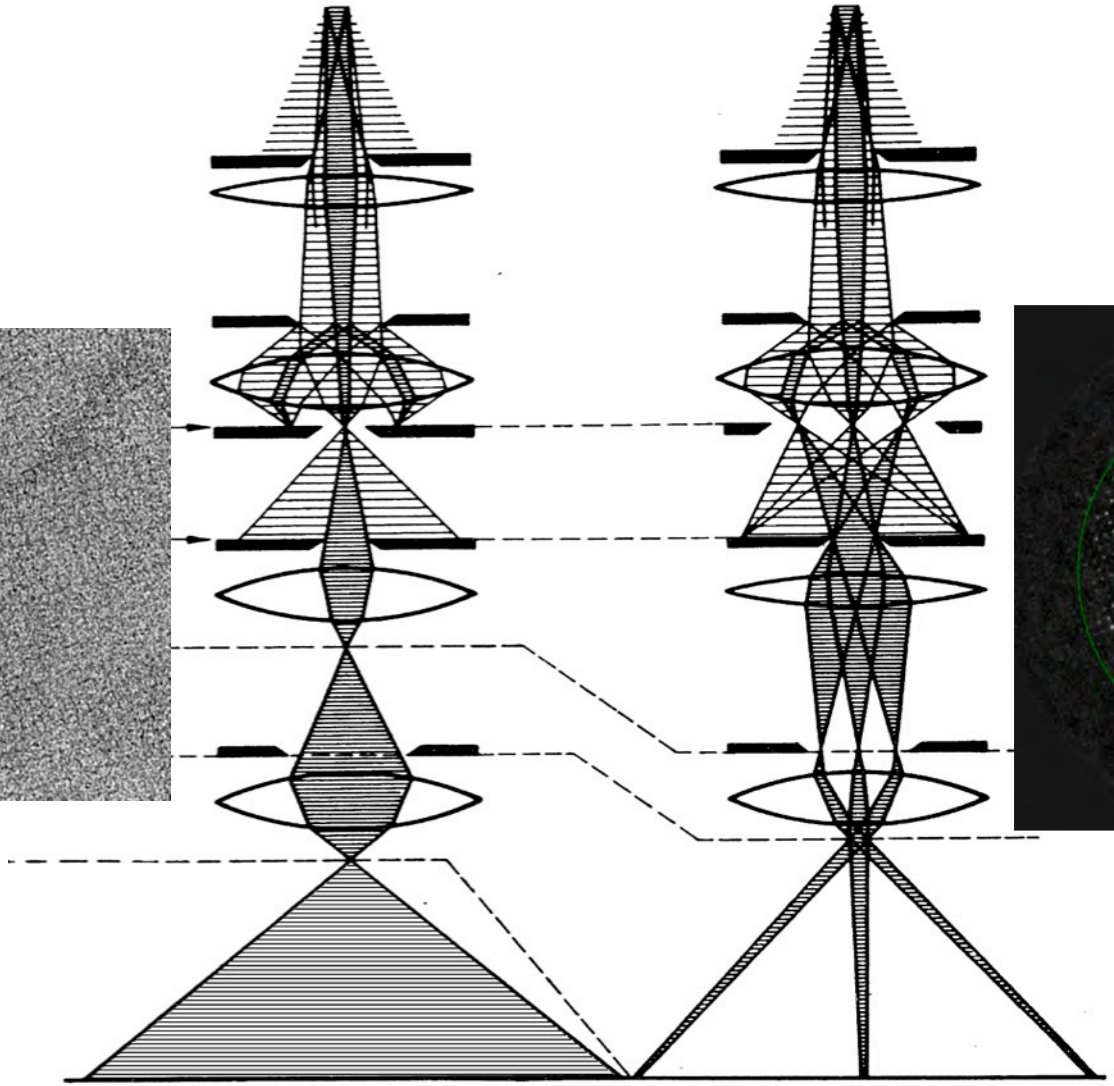
- **Electron microscopy of specimens (usually protein and/or DNA/RNA samples) in their "natural", "hydrated" environment with out artificial contrast.**
- **Specimens are "vitreously" frozen and imaged at $N_{2(l)}$ or $He_{(l)}$ temperature.**
- **Most importantly, NOT cryo-preparation of TEM!**

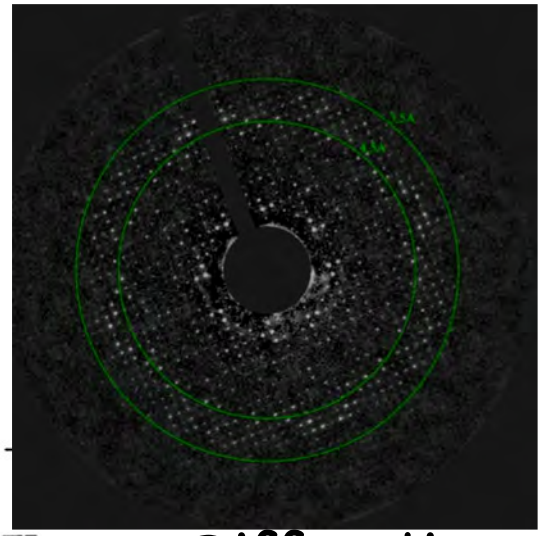
Cryo-electron microscopy (cryo-EM)

- **Electron crystallography**
  - **2-D crystals**
  - **Helical crystals**
- **Single-particle cryo-EM**
- **Electron cryotomography (cryo-ET)**



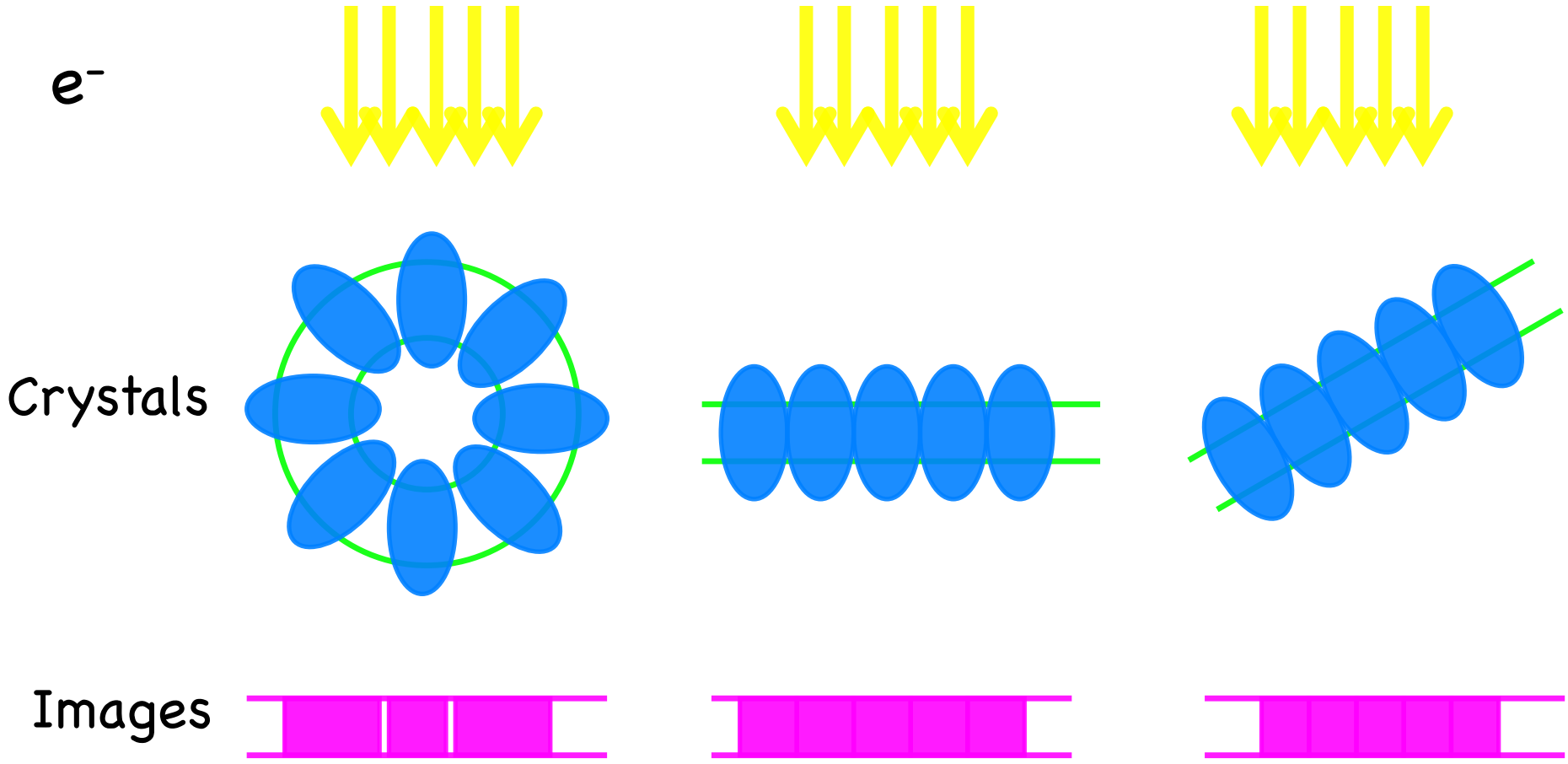
Image

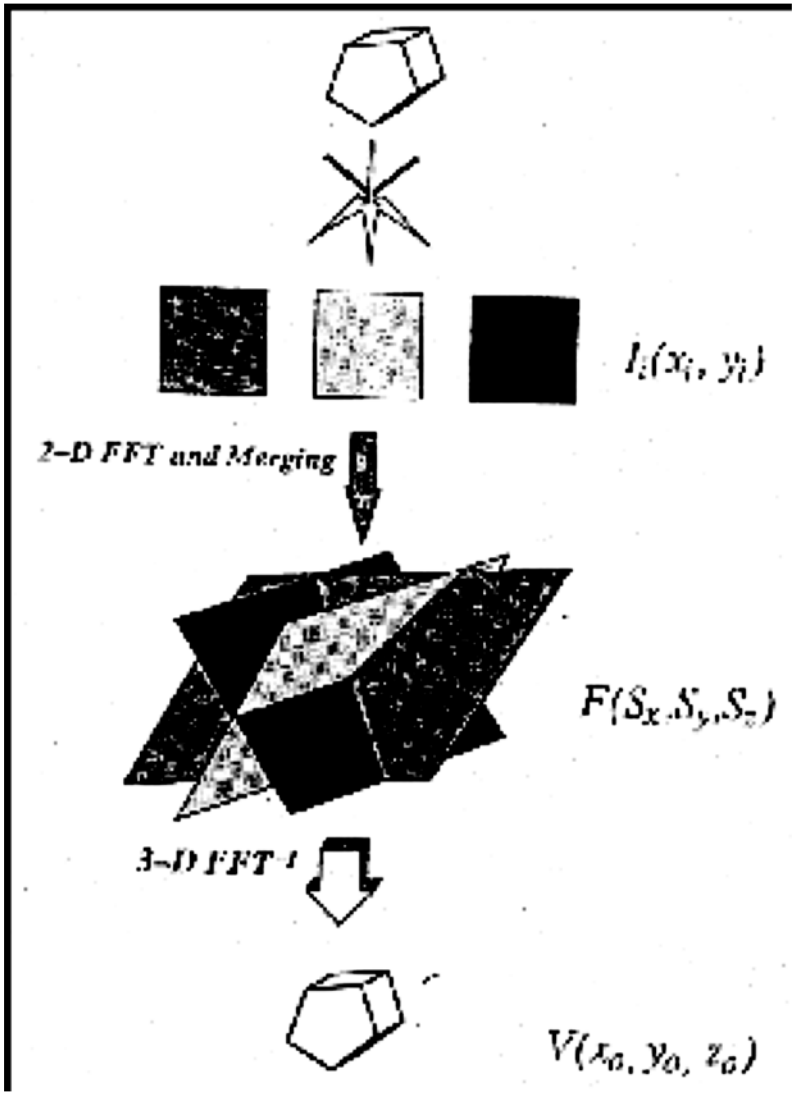




Diffraction

Only 2-D projections are recorded (x,y)!!

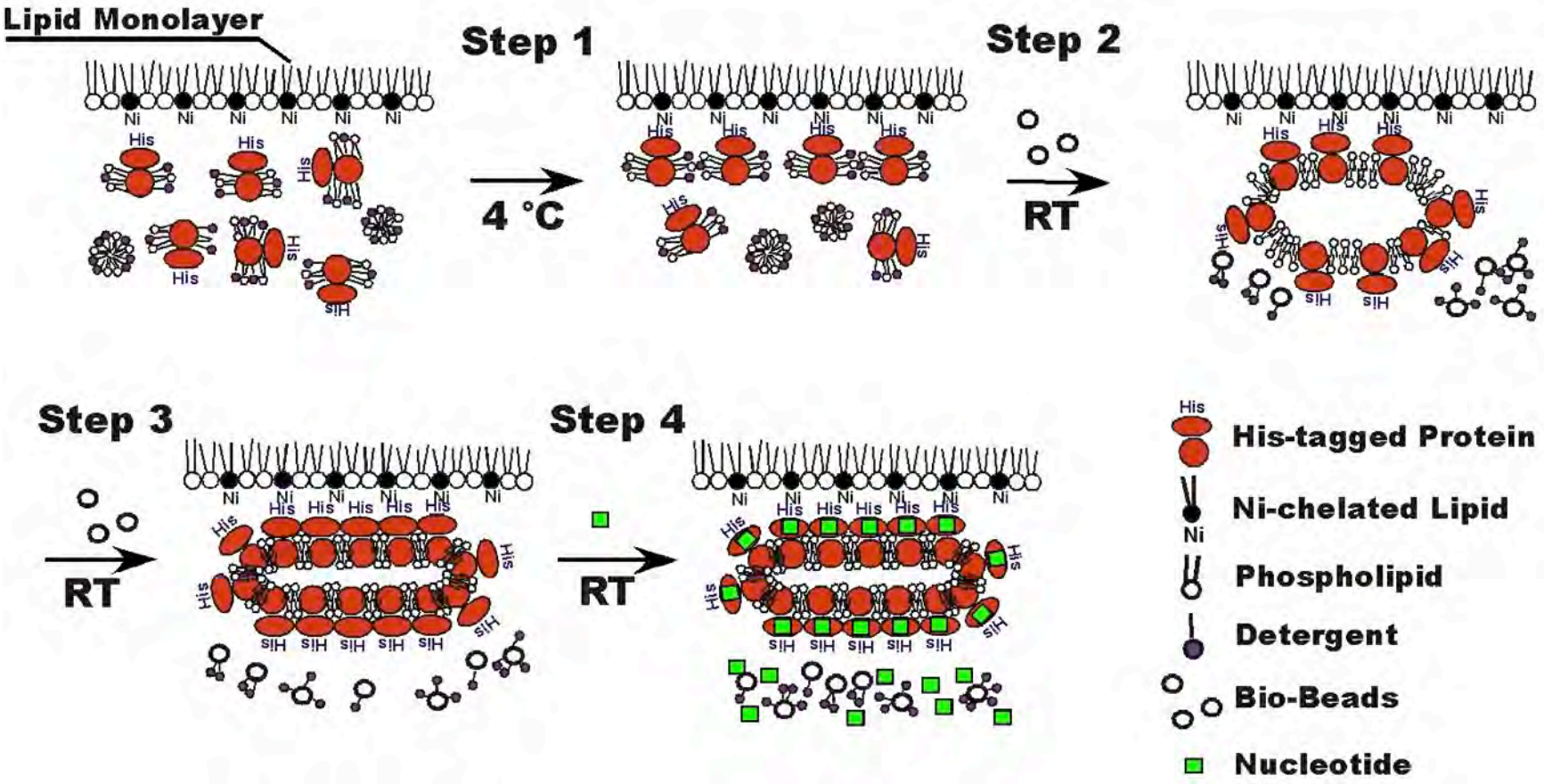




(Amos et al, Prog Biophys Mol Biol, 1983)

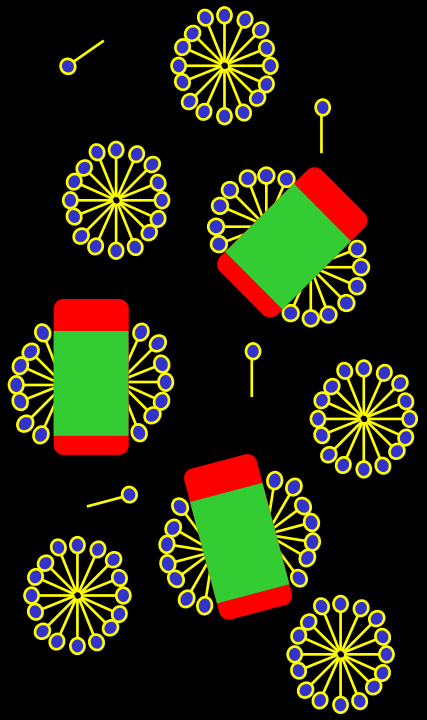
Cryo-EM: 2-D crystals and MicroED

2-D crystallization by lipid-monolayer technique

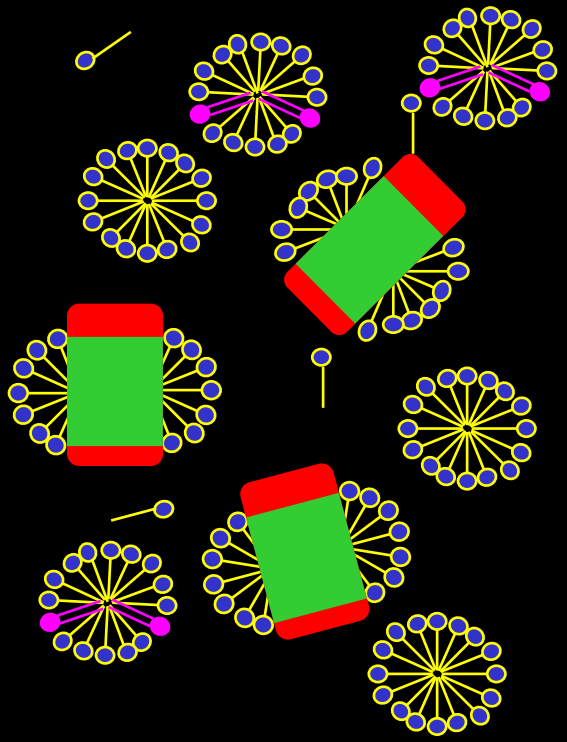


2D crystallization by reconstitution

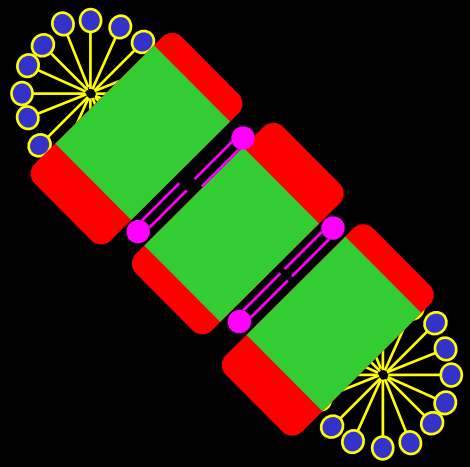
Protein in detergent solution



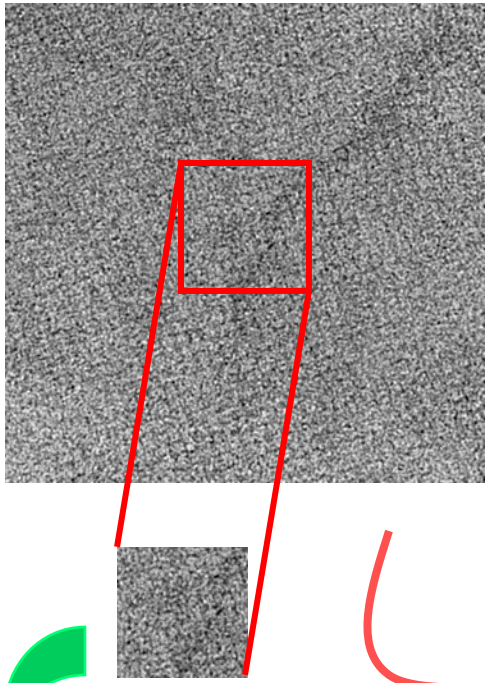
Add lipid



Remove detergent

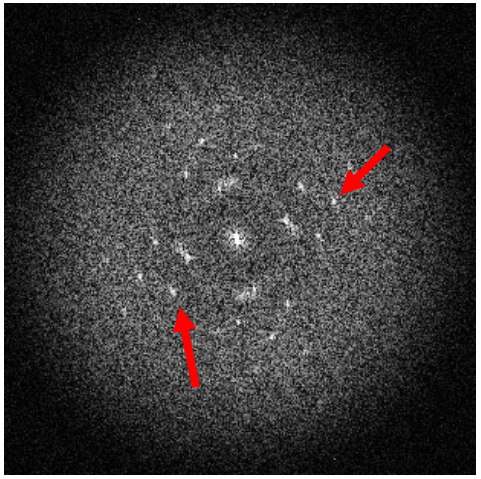


2D crystals!!



FT

IFT



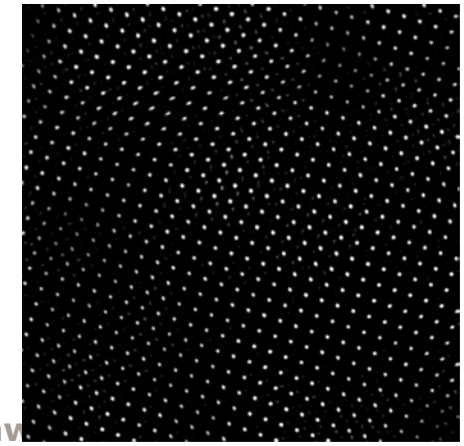
Fourier space

Calculation of Amplitudes & Phases

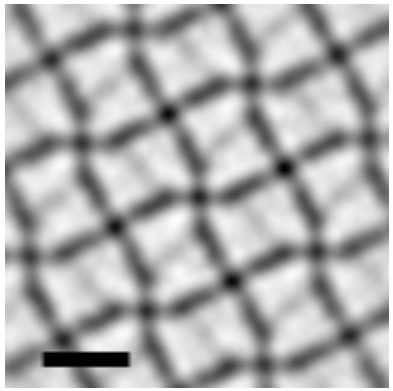
H	K	A	ϕ
1	-3	771.2	-122.9
1	-2	3836.0	-148.3
1	-1	5184.4	9.6
1	0	599.8	-17.9
1	1	5953.2	15.9
2	-7	17.2	83.4
2	-4	41.4	-125.0
2	-3	1485.4	52.6
2	-2	2491.6	-152.5

Correlation Averaging

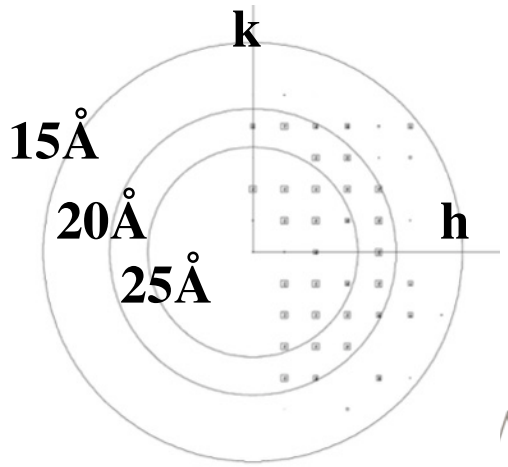
real space



Cross-Correlation Map



S/N-enhanced Image

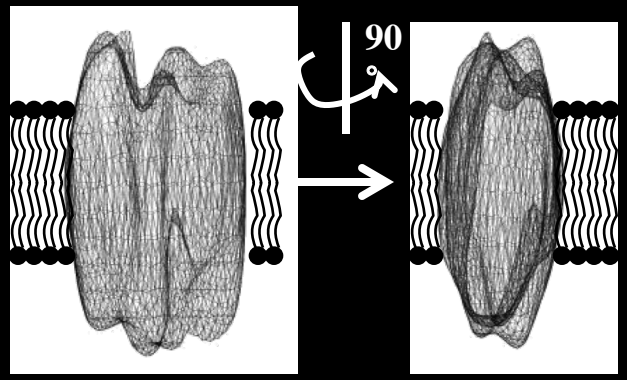


0°

Non-tilting Images

H	K	L	A	ϕ
1	-4	0	402.2	-118.7
1	-3	0	771.2	-122.9
1	-2	0	3836.0	-148.3
1	-1	0	5184.4	9.6
1	0	0	599.8	-17.9
2	-7	0	17.2	83.4
2	-4	0	41.4	-125.0
2	-3	0	1485.4	52.6
2	-2	0	2491.6	-152.5

0° 2D Data (partial)



±70°

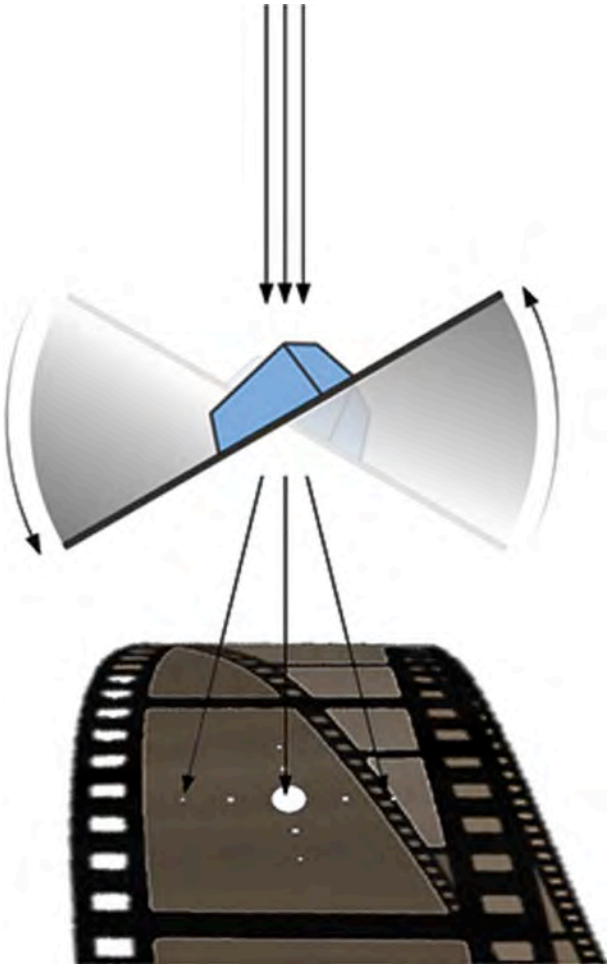
Tilted Images

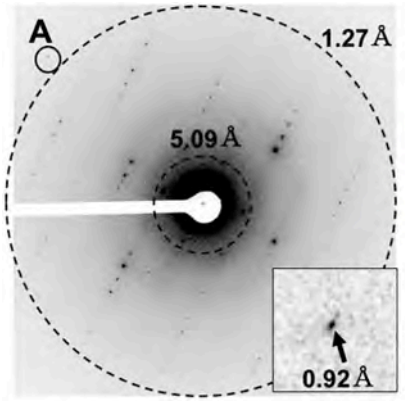
H	K	L	A	ϕ
1	1	0	2529.0	182.4
1	2	0	445.1	14.3
1	3	0	33.5	268.0
1	4	0	16.0	127.6
1	0	0	599.8	-17.9
4	3	0	21.8	208.9
4	4	0	7.6	336.0
5	2	0	5.0	353.0
-6	-1	0	13.9	355.9

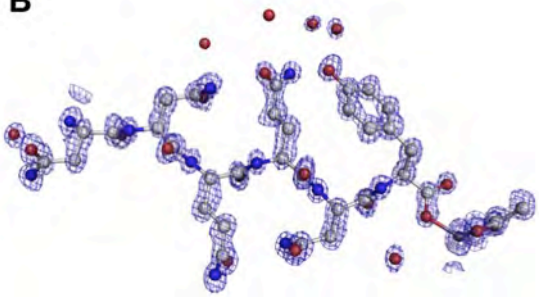
+20° 2D Data (partial)

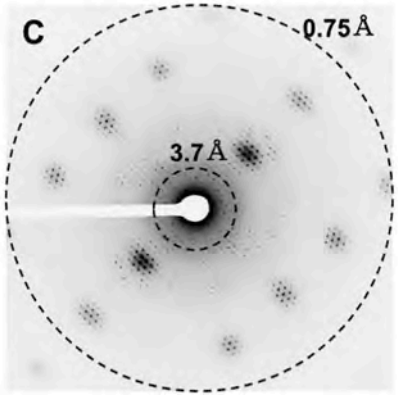
H	K	L	A	ϕ
1	-2	-2	1043.4	138.9
1	-2	-1	1269.8	174.4
1	-2	0	1158.8	171.2
1	-2	1	1016.4	-171.6
2	1	0	1807.7	-3.0
2	2	-1	1127.4	0.5
2	2	0	1253.1	-3.3
2	2	1	1781.4	-0.1
2	3	-2	320.2	-177.2

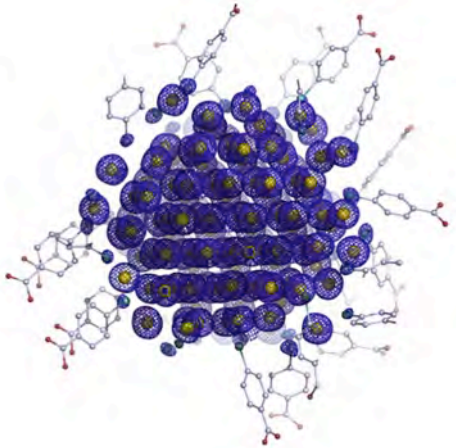
-20° ~ +20° 3D Data (partial)





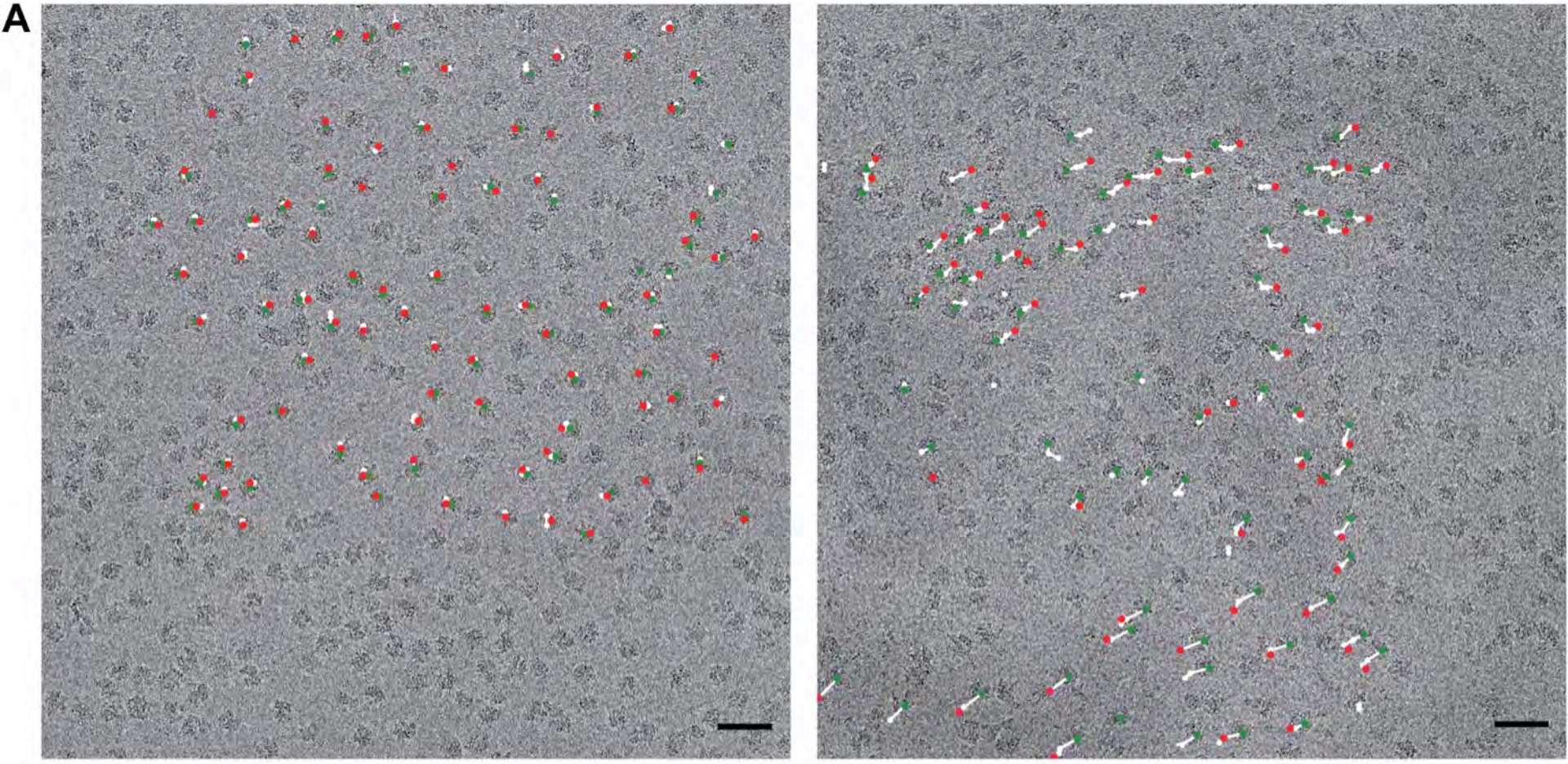




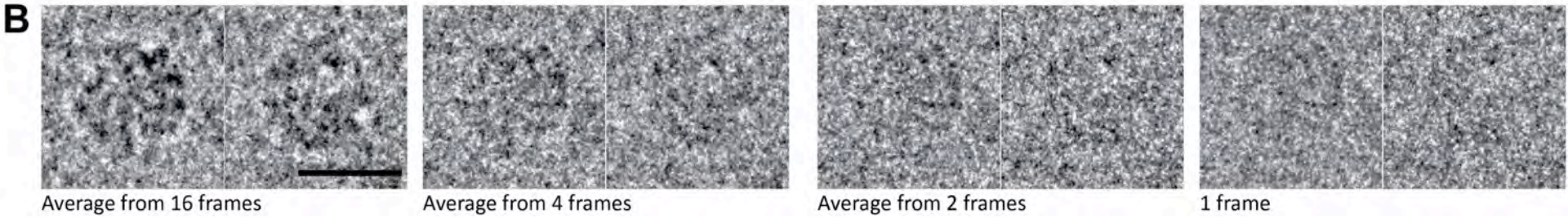


(Martynowycz & Gonen, Curr Opin Colloid Interf Sci, 2018)

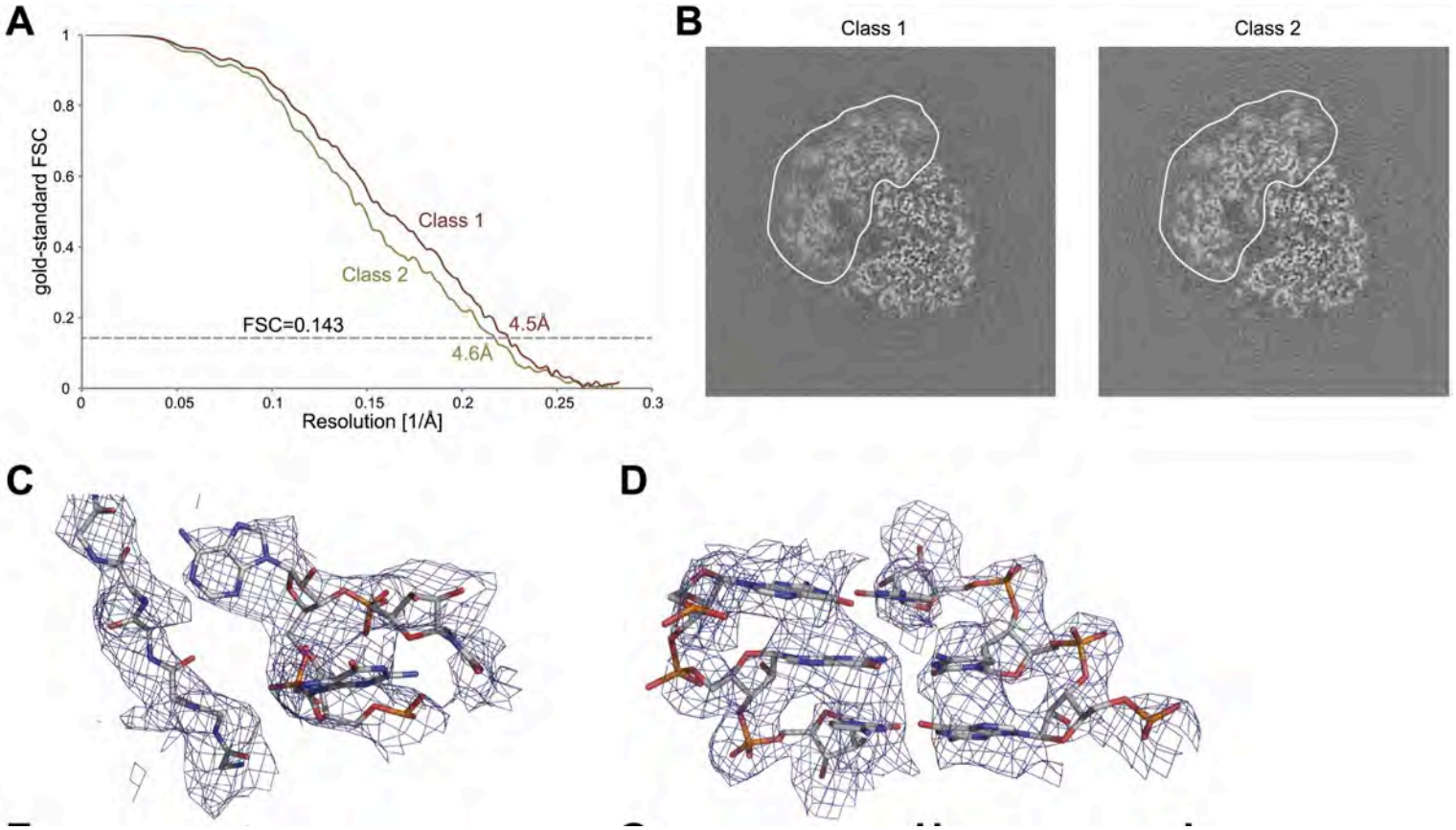
Cryo-EM: Single-particle analysis



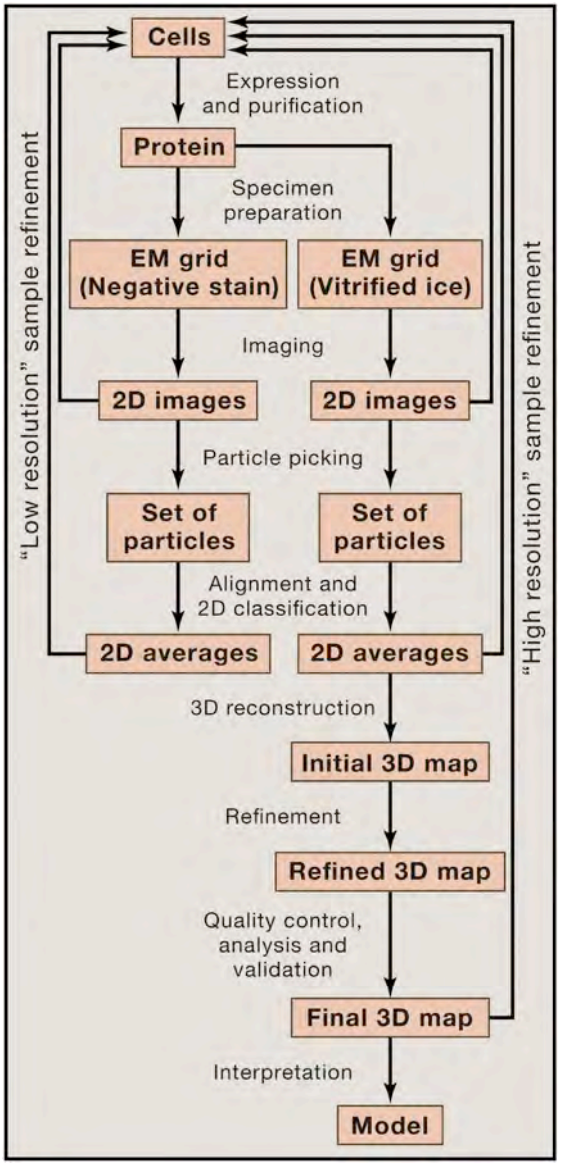
(Bai et al, eLife, 2013)



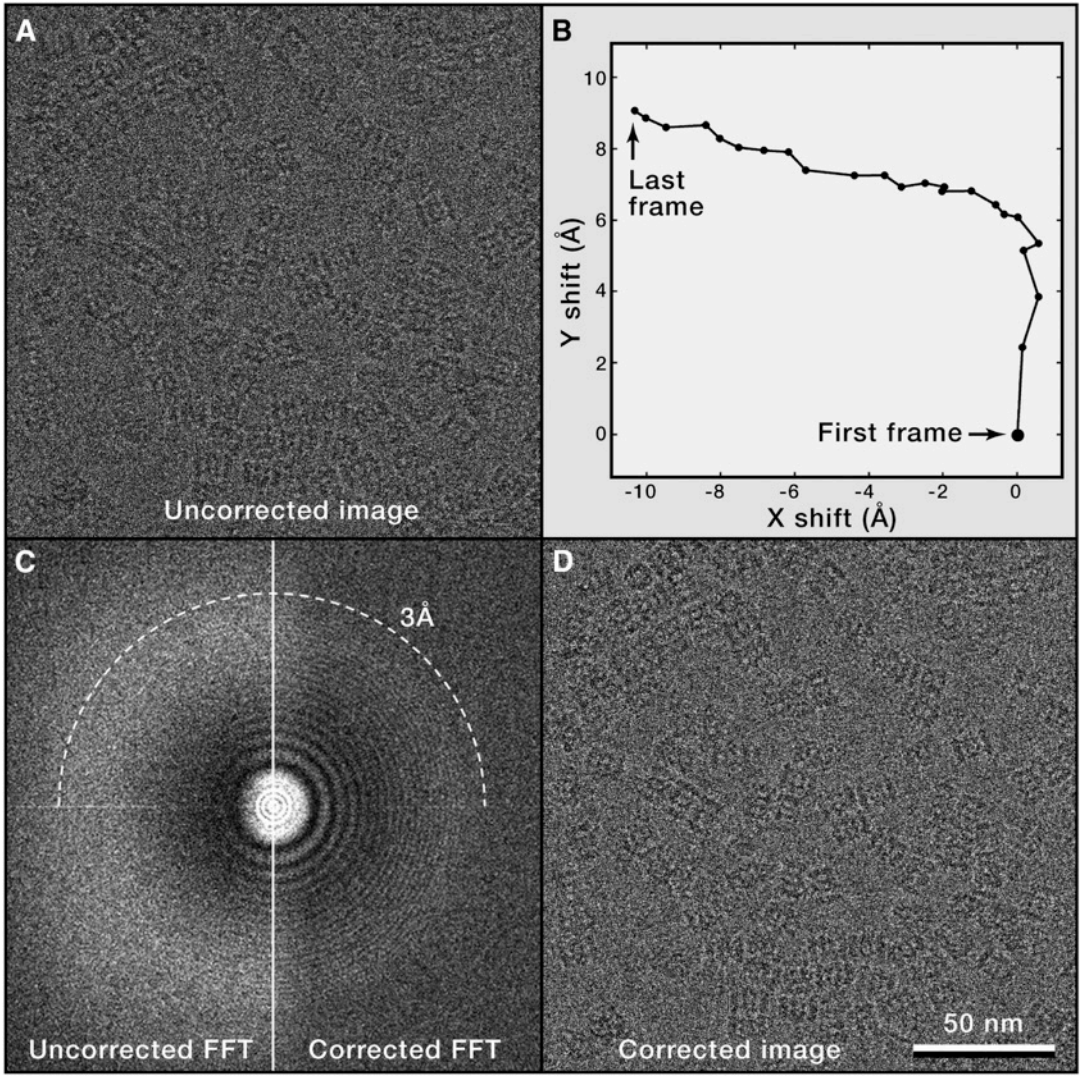
(Bai et al, eLife, 2013)



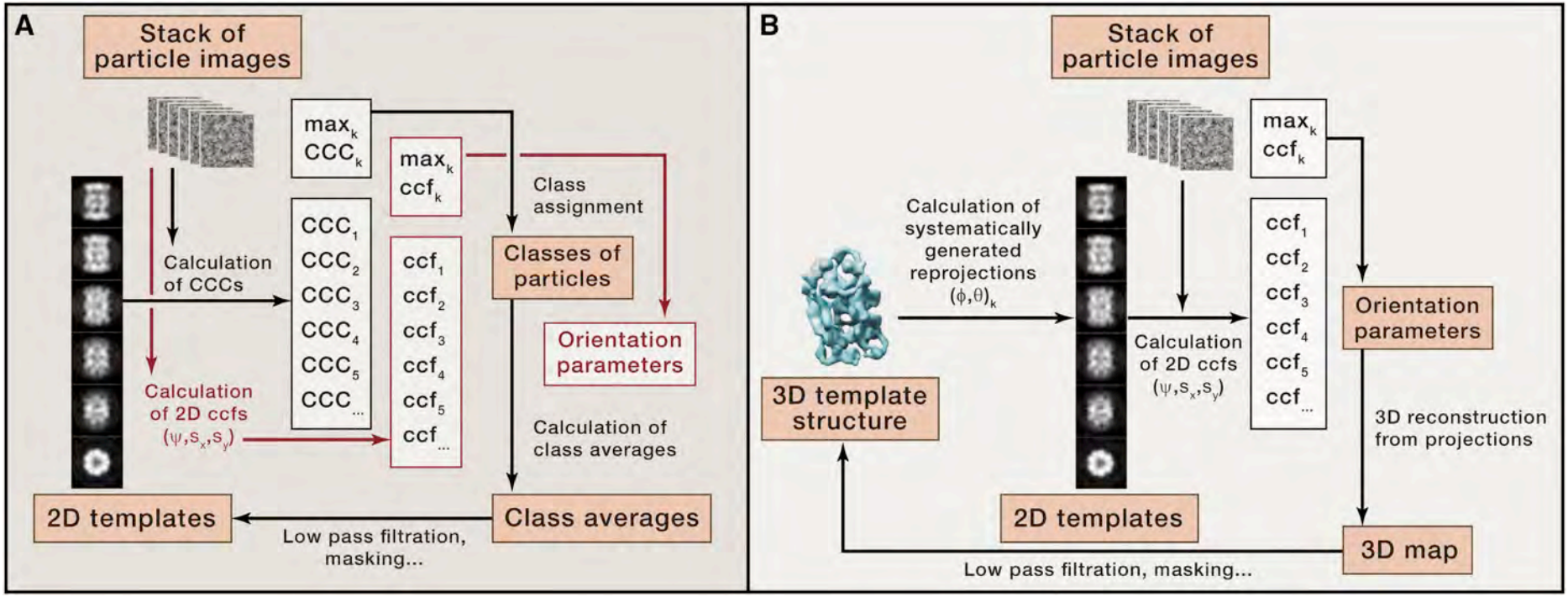
(Bai et al, eLife, 2013)



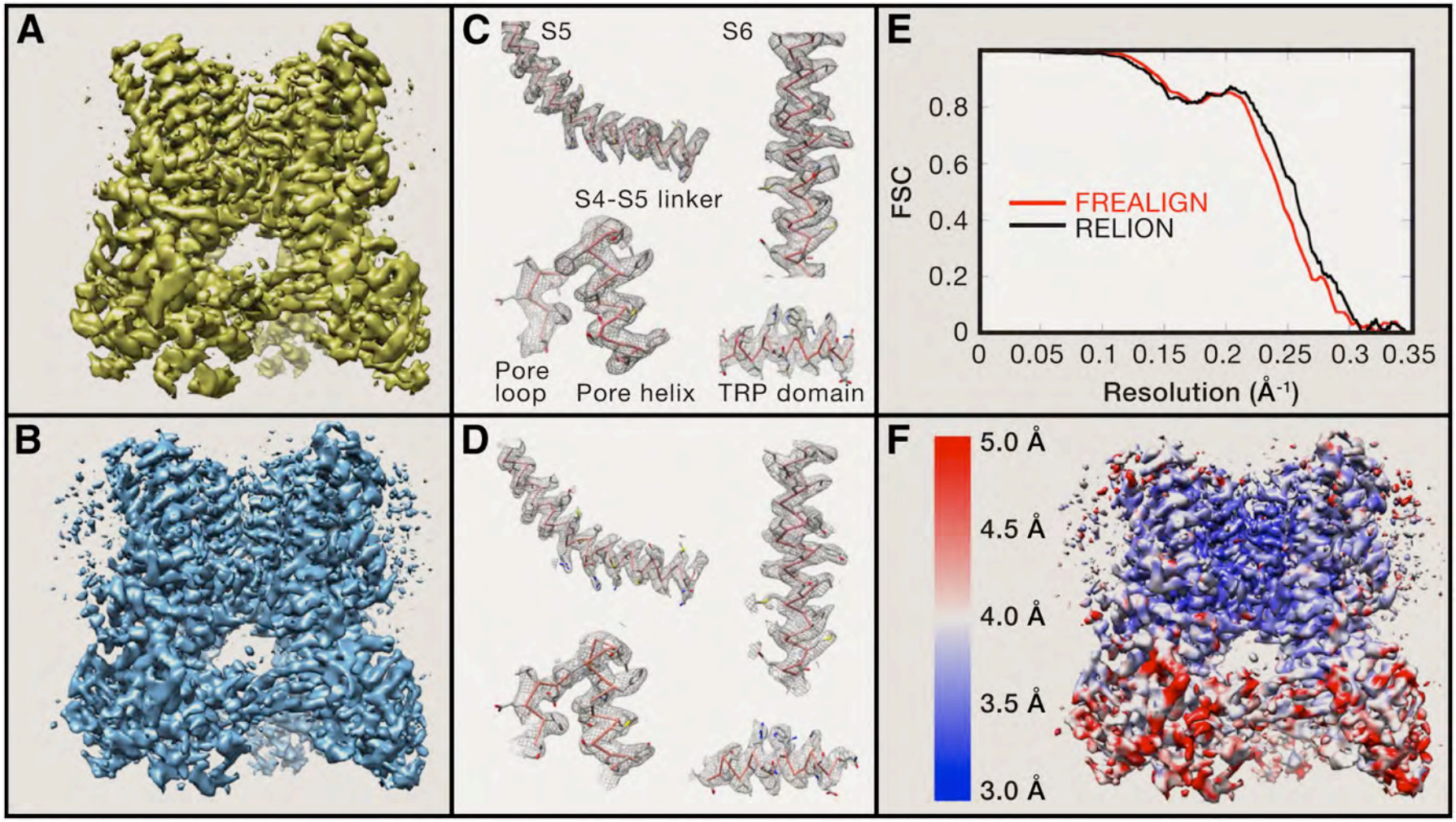
(Cheng et al, Cell, 2015)



(Cheng et al, Cell, 2015)



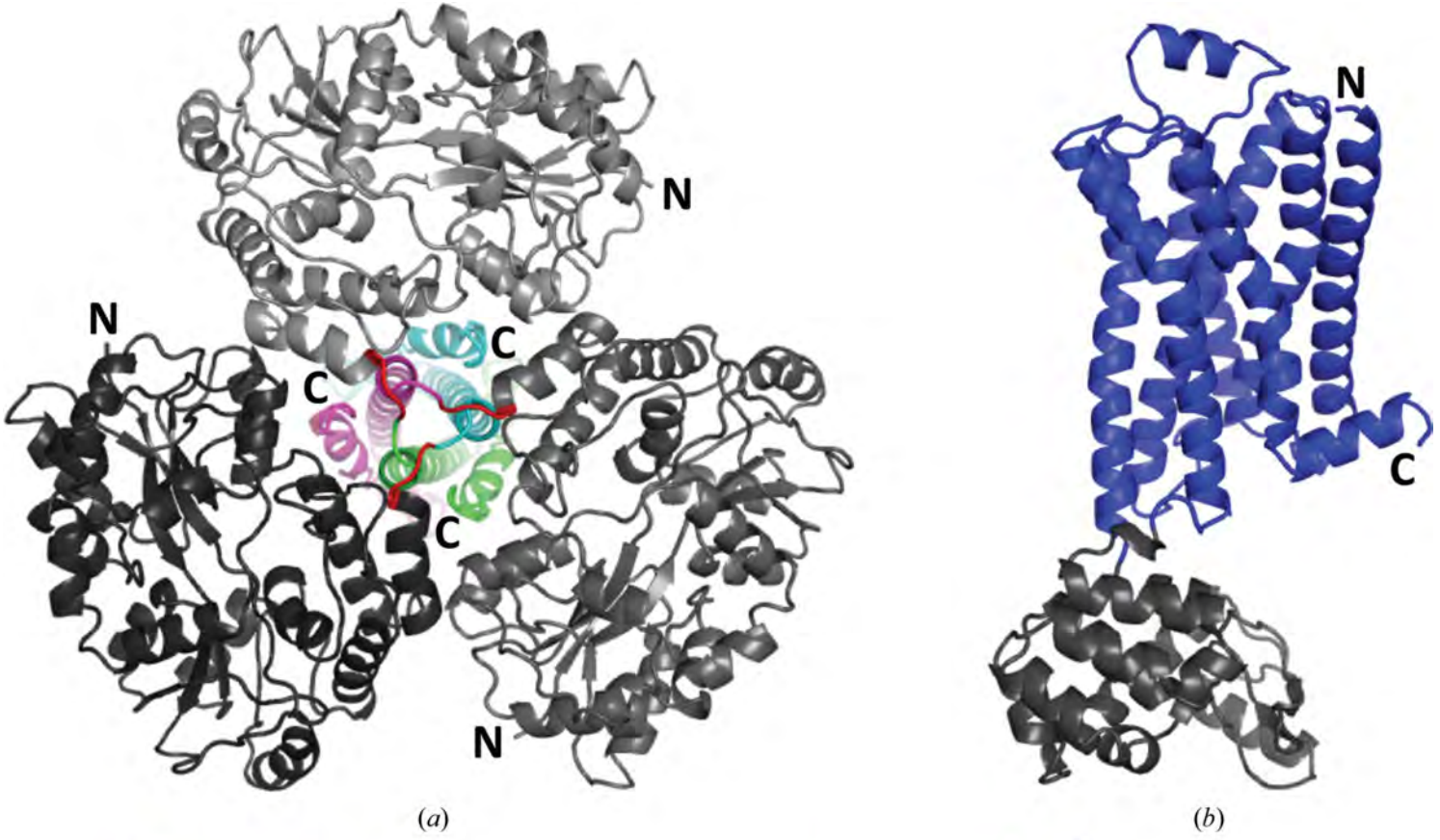
(Cheng et al, Cell, 2015)



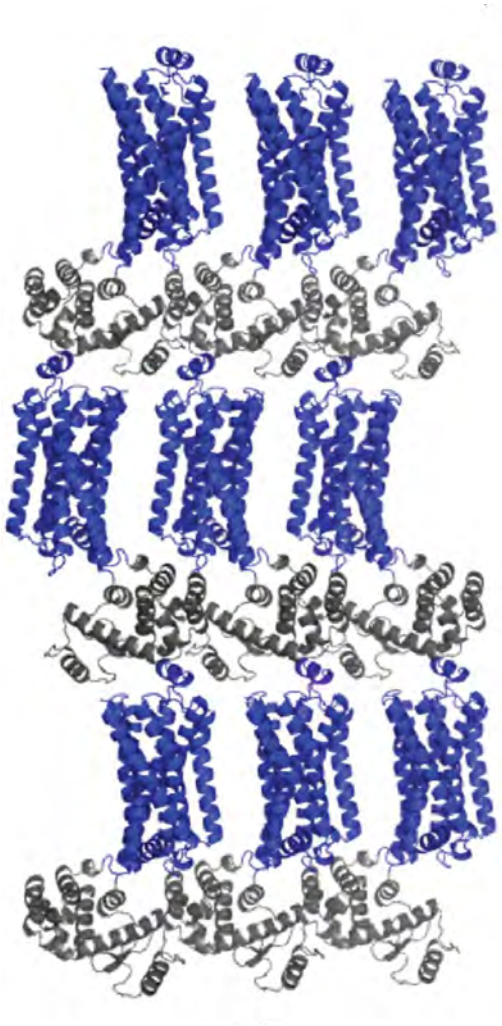
(Cheng et al, Cell, 2015)

4. Strategies in structural determination of membrane proteins

- **Fusion proteins**
- **Antibody**
- **Ligands**
- **Library of small molecules**
- **Protein re-engineering**
- **…**



(Kobe et al, Acta Cryst F, 2015)



(Kobe et al, Acta Cryst F, 2015)

Discussion Topics and Brainstorming

1. How does the use of direct detector benefit structural biology?

1. Why do we need to do motion correction for cryo-EM data?

1. How can we use fluorescent probes to facilitate structural determination?

1. How can we use nanoparticle technology (e.g., nanodiscs, SMA, …) in structural biology?

2. What can we do to examine the dynamic mode of structural biology?