TMM 3102: Protein Structure, Function and Disease

- Integrative Structural Biology: Membrane Proteins (October 14th, 2021)

Jyh-Yeuan (Eric) Lee, Assistant Professor, BMI

Lecture Outline

1. **Why studying membrane proteins?**

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

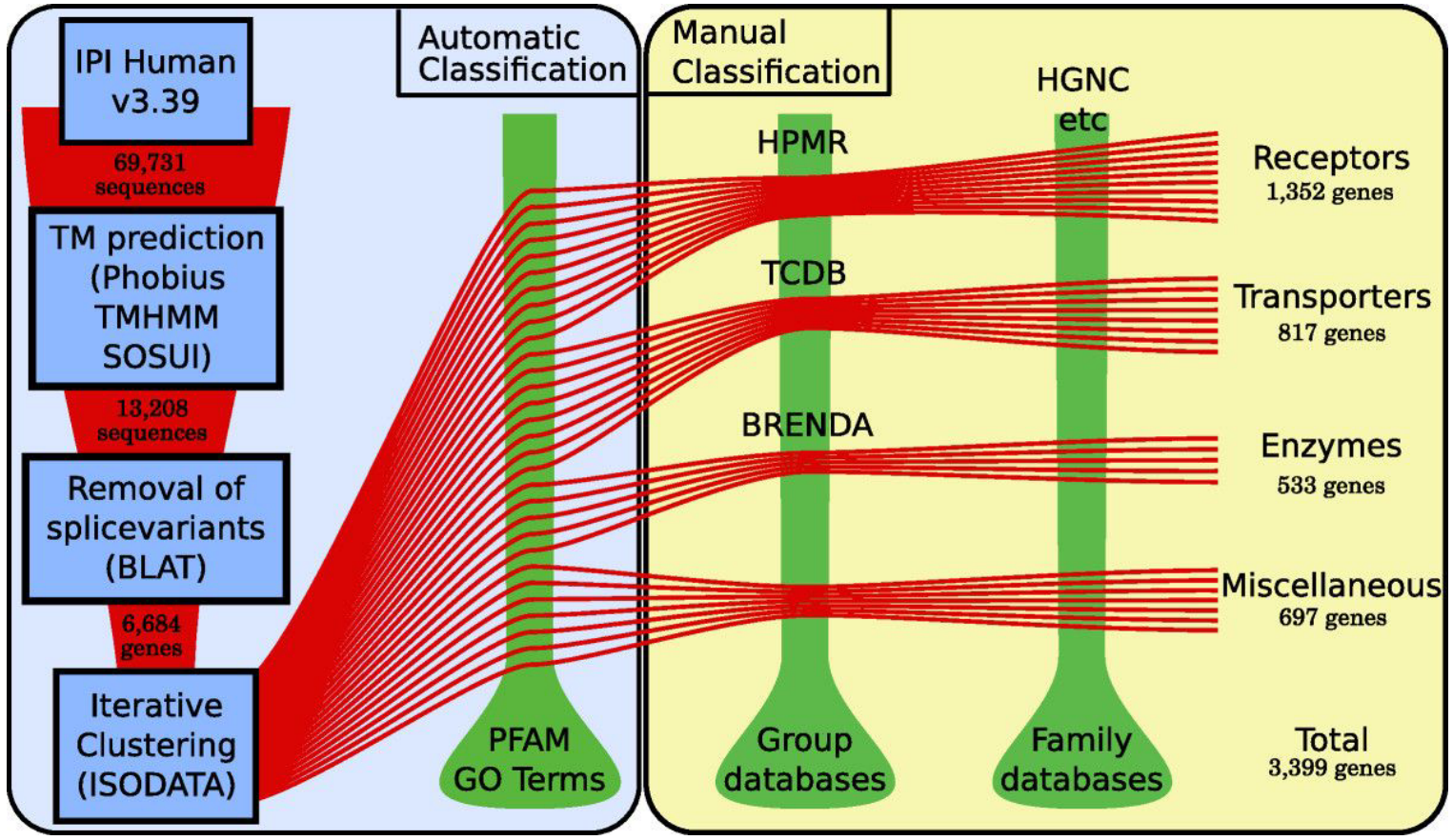
3. **Ways to study membrane protein structures**
   - **X-ray crystallography ✓**
   - **Electron microscopy ✓**
   - **Fluorescence ✓**
   - **Magnetic resonance ✓**
   - **Computer simulation, NMR, mass spec, cross-linking, …**

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

1. Why studying membrane proteins?

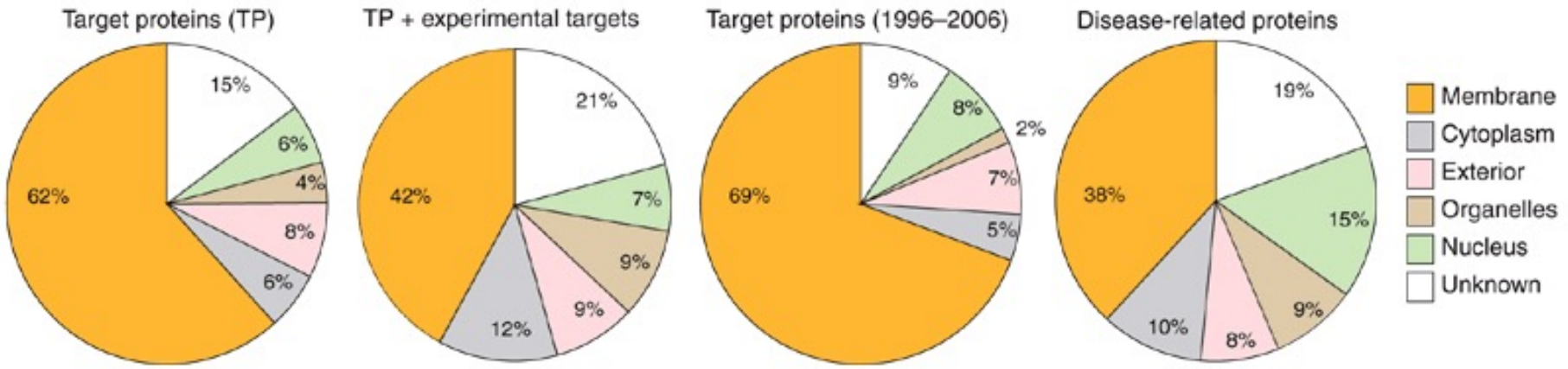
- **Encoded by some 20-30% genes in typical genome.**
- **Major components of the mosaic lipid bilayers in cellular membranes**
- **Mediate cell-to-cell communication and signaling events.**
- **Disruptions or mutations in humans have been implicated in diseases, such as cardiovascular and metabolic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

Membrane proteome (human)



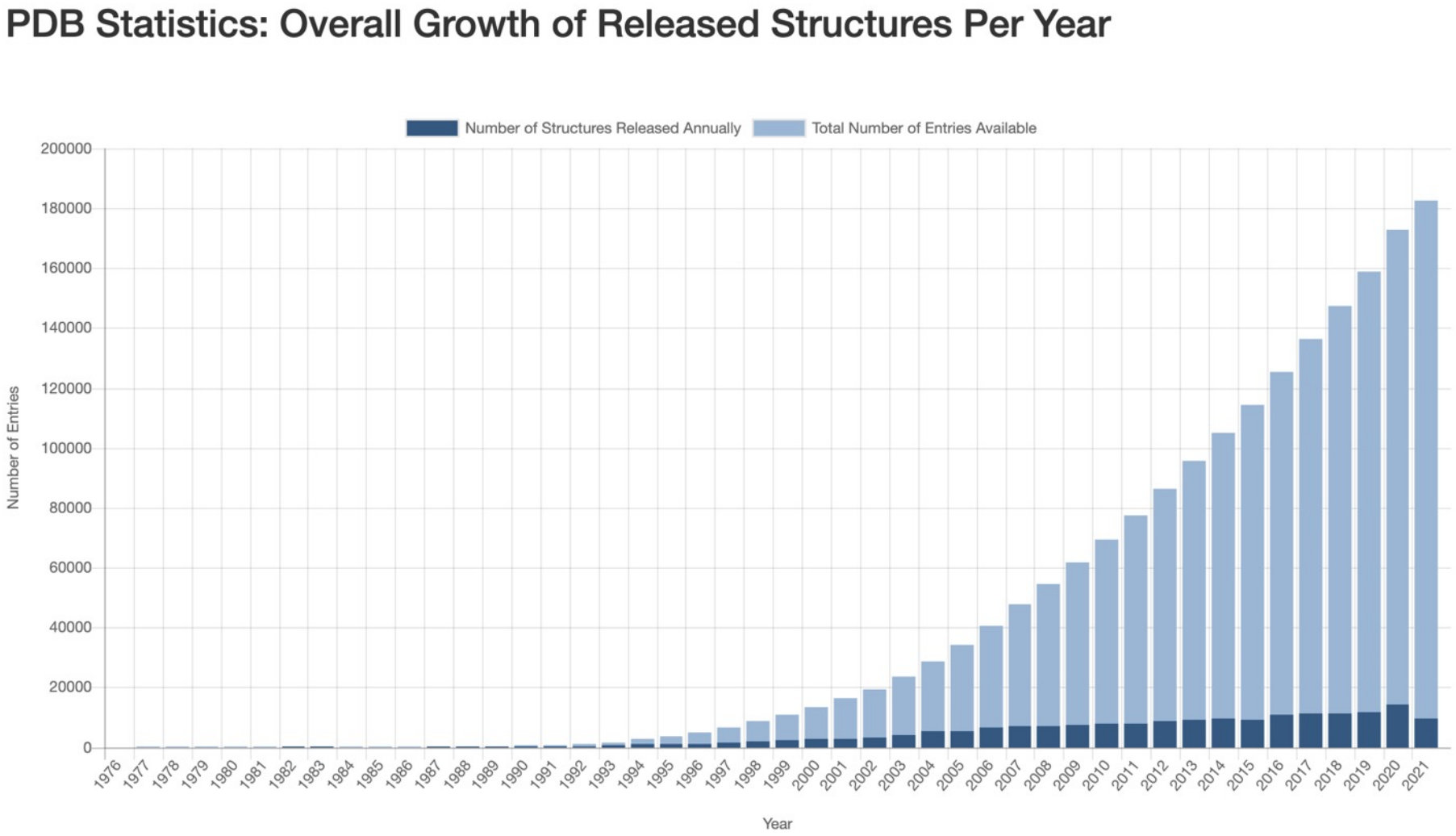
(Almén et al, BMC Biol, 2009)

Half drug targets are membrane proteins.

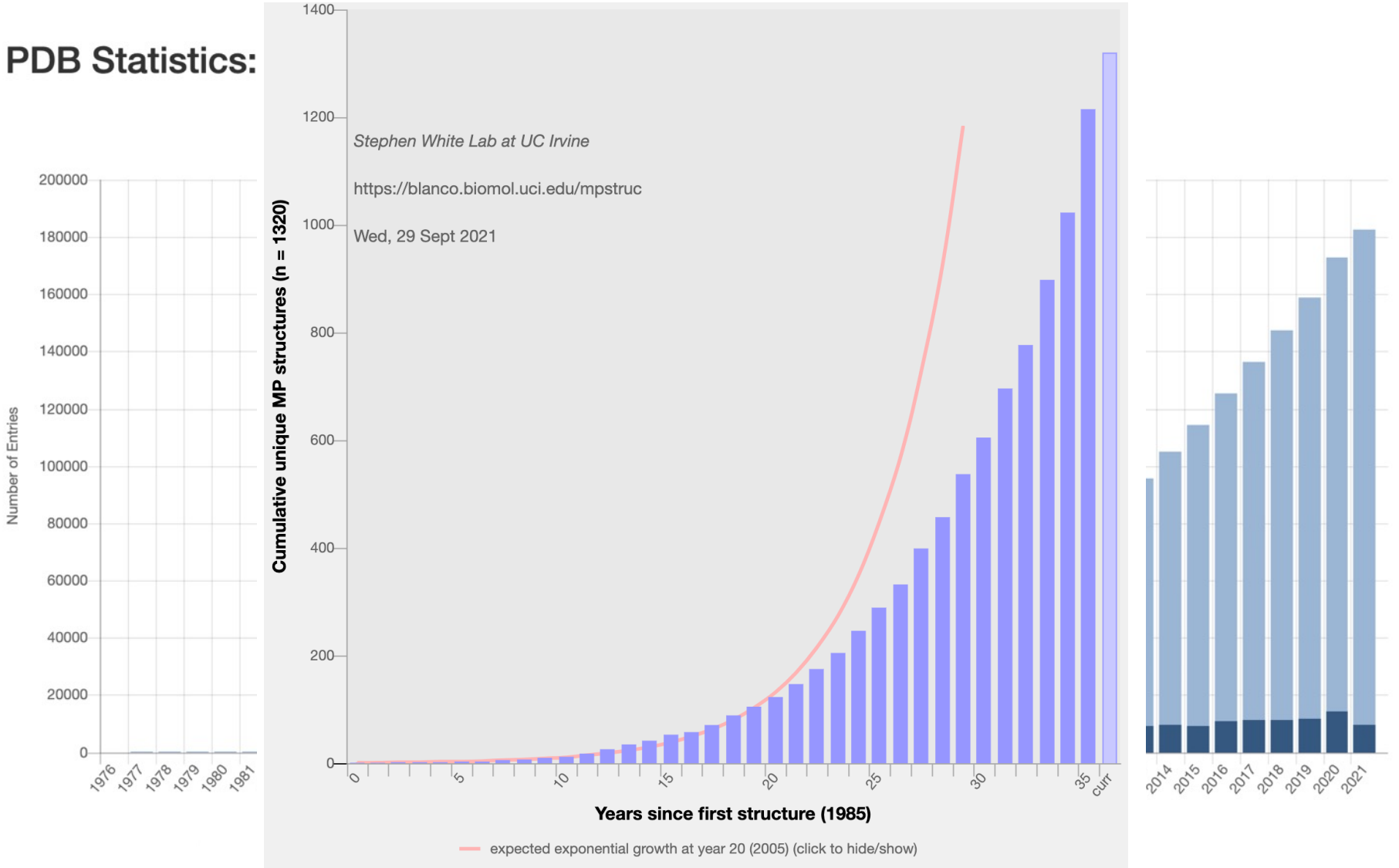


(Yildirim et al, Nat Biotech, 2007)

Available atomic/near-atomic models of membrane proteins (2021-9-29)



Available atomic/near-atomic models of membrane proteins (2021-9-29)



2. Challenges in membrane protein structural biology

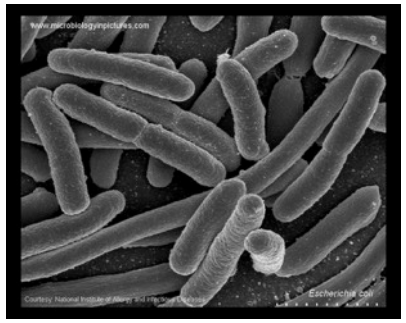
- Naturally occurred proteins exist in **low abundance**, with only a few exceptions (*e.g.*, bacteriorhodopsin or aquaporin), and form complexes.

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

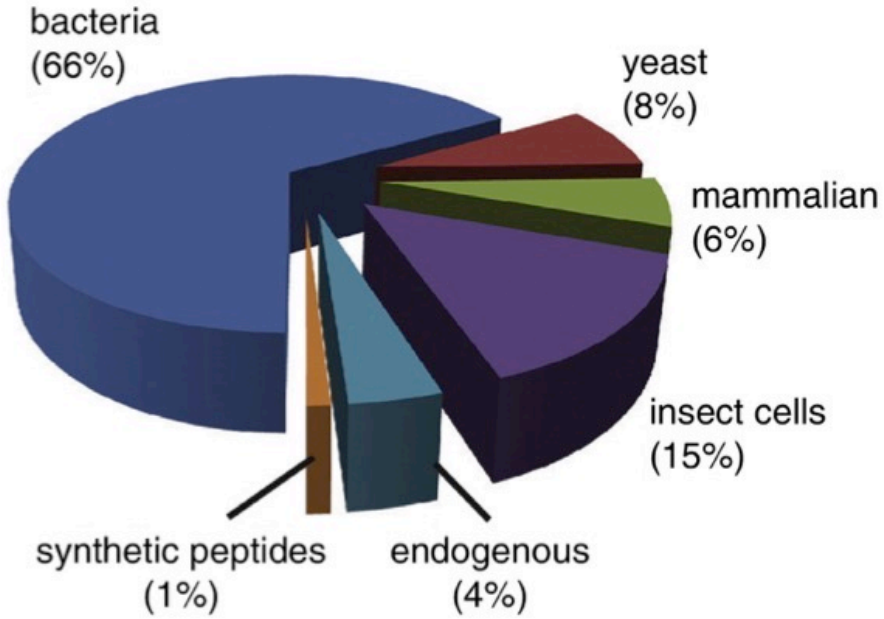
- **No so-called standard protocol** of protein extraction, largely due to the complexity of protein-lipid interaction.

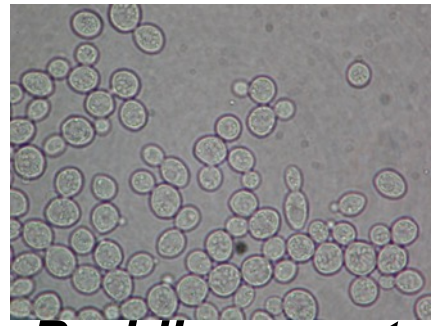
- Protocols of purification, crystallization, and *in vitro* reconstitution remain **empirical** for individual cases.

Choosing the appropriate expression hosts for recombinant proteins

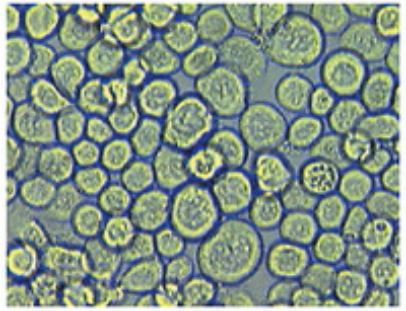


E. coli

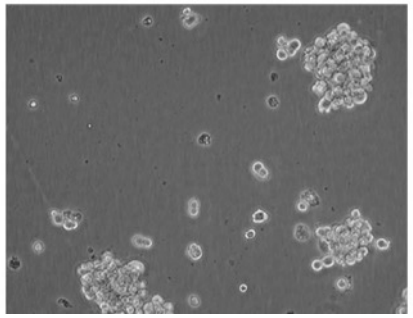




Budding yeast



Sf9 insect cells



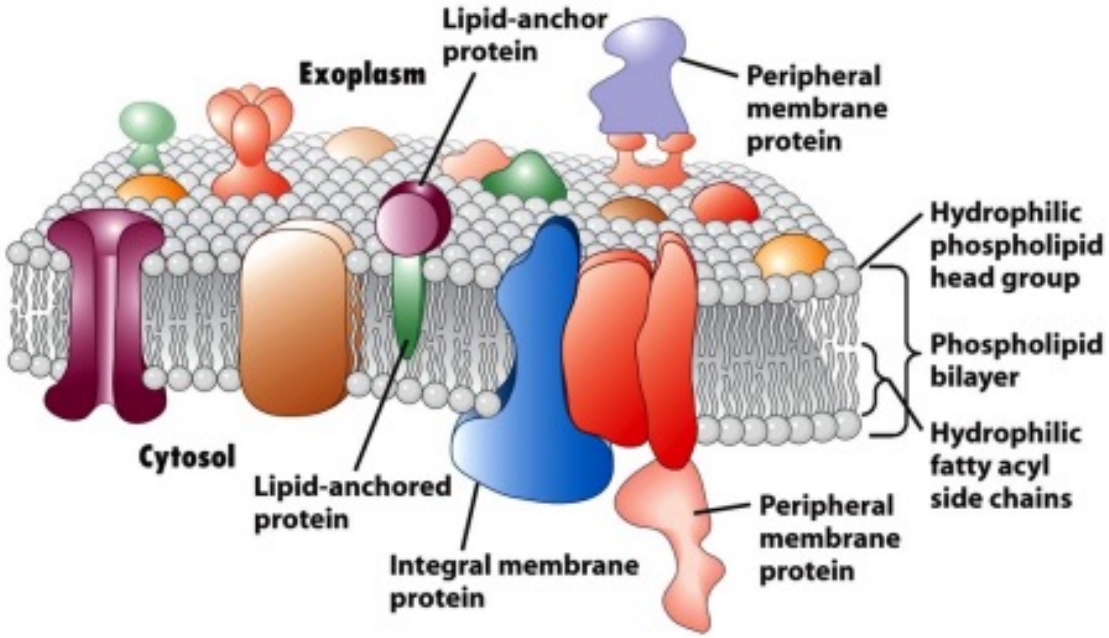
HEK 293sus

(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**

Membrane proteins are present in an anisotropic and hydrophobic environment

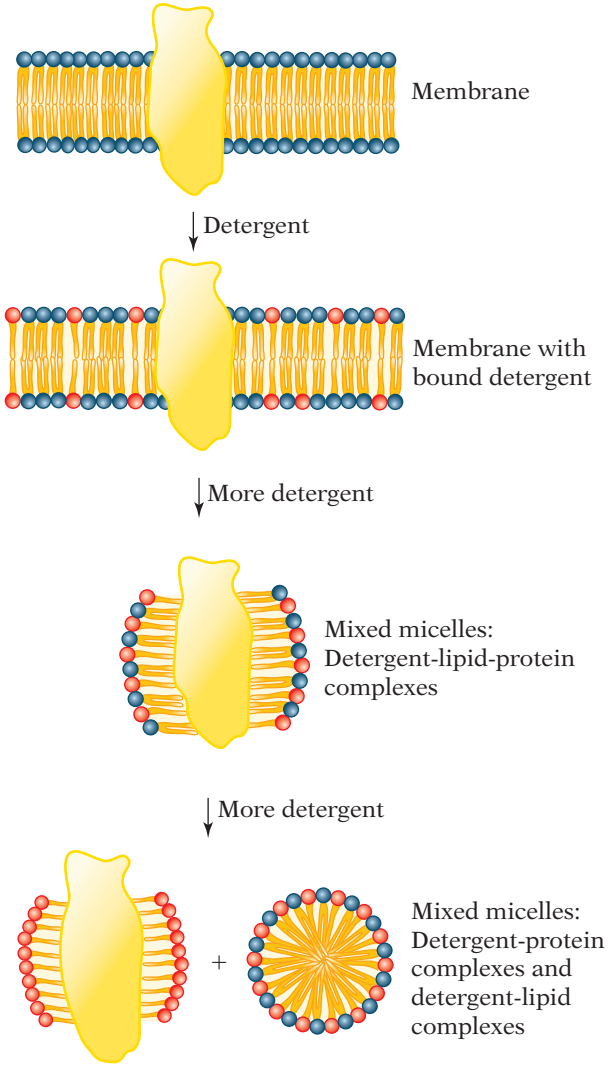


i. Must remove the protein from lipid-rich membrane to separate it from other membrane proteins.

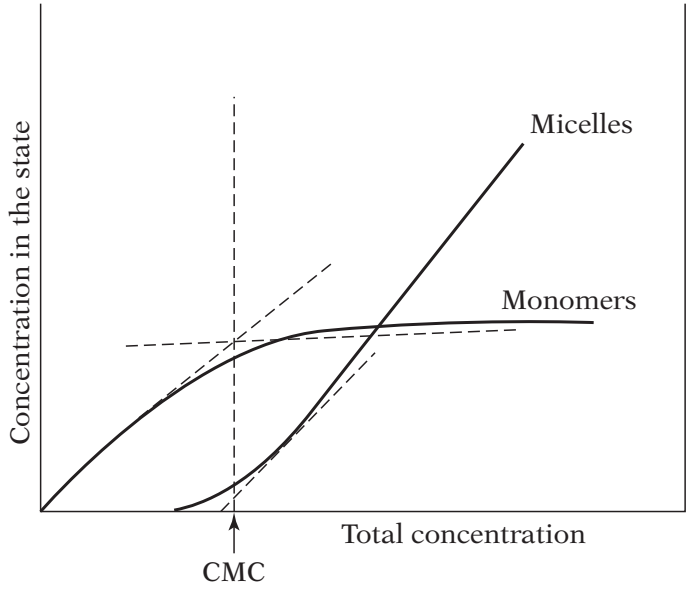
ii. Must be able to stabilize them as single "particles" in an aqueous environment.

(https://www.creative-proteomics.com/services/membrane-proteomics.htm)

Solubilization of membrane proteins using detergents:

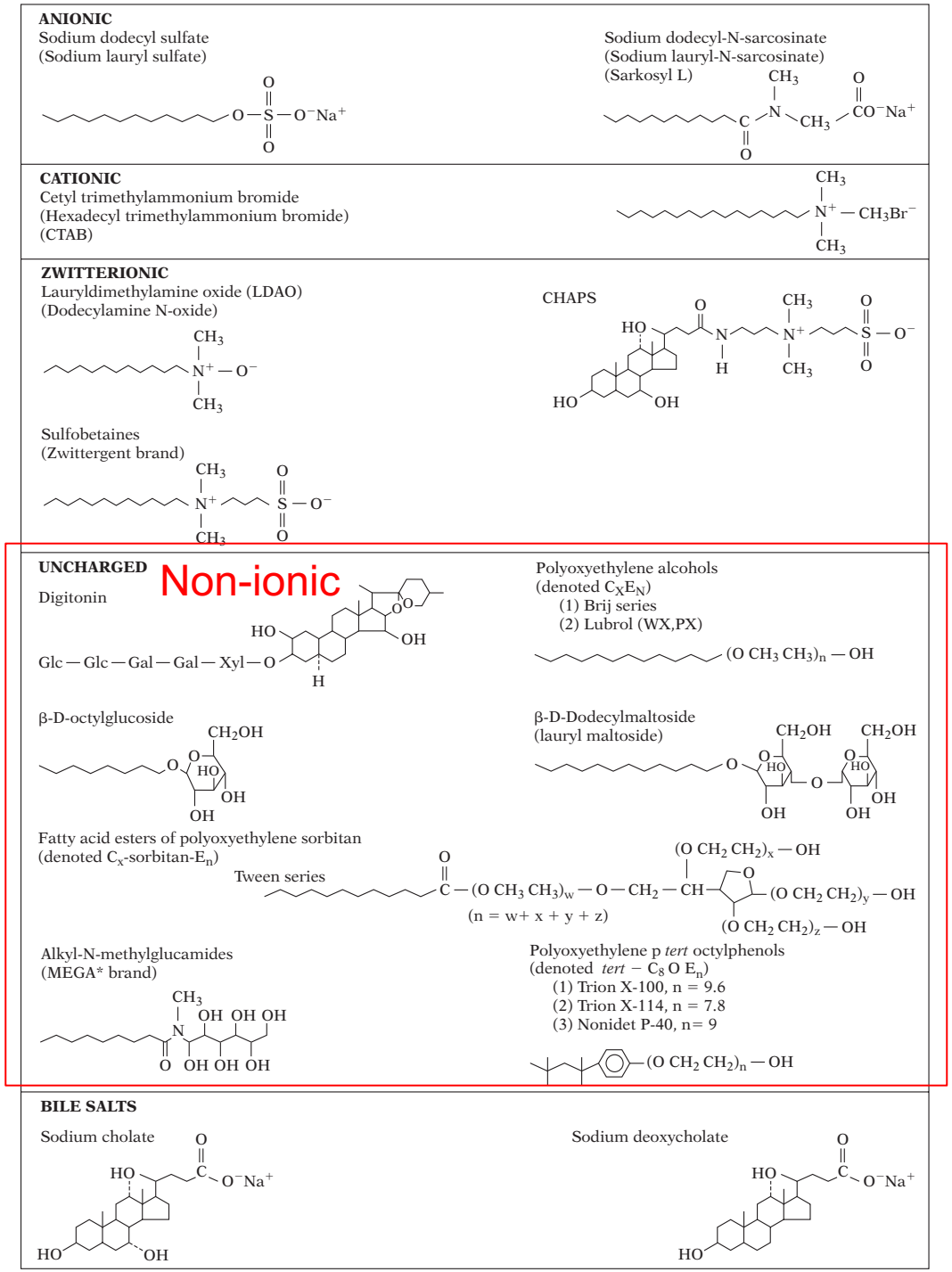


Critical Micellar Concentration (CMC)



3.4. The critical micellar concentration. As detergent (or surfactant) is added to an aqueous solvent, the concentration of dissolved monomers increases until the critical micellar concentration (CMC) is reached. At that concentration, micelles form. Further addition of detergent increases the concentration of micelles without appreciably affecting the concentration of monomers. Redrawn with permission from Helenius, A., and K. Simons, *Biochim Biophys Acta*. 1975, 415:38.

(Mary Luckey, Membrane Structural Biology, 2008)



- Detergent solubilized proteins can be purified and crystallized, <u>but</u> too much detergent or types of detergents may denature the protein.
- Detergents are dynamic and can interfere with the formation of the protein-protein contacts in a crystal.
- You have to find the right detergent (size, shape, charge) and conditions (concentration) that solubilize well and generate homogeneous protein preparation for structural biology.
- <u>Structural biology of membrane proteins is thus a distinct field!</u>

(Mary Luckey, Membrane Structural Biology, 2008)

3. Ways to study membrane protein structures

- X-ray crystallography ✔
- Electron microscopy ✔
- Fluorescence ✔
- Magnetic resonance ✔
- Computer simulation, NMR, mass spec, cross-linking, …

Membrane protein crystallography

Coarse vapor diffusion

i. Transfer purified proteins to a microcentrifuge tube

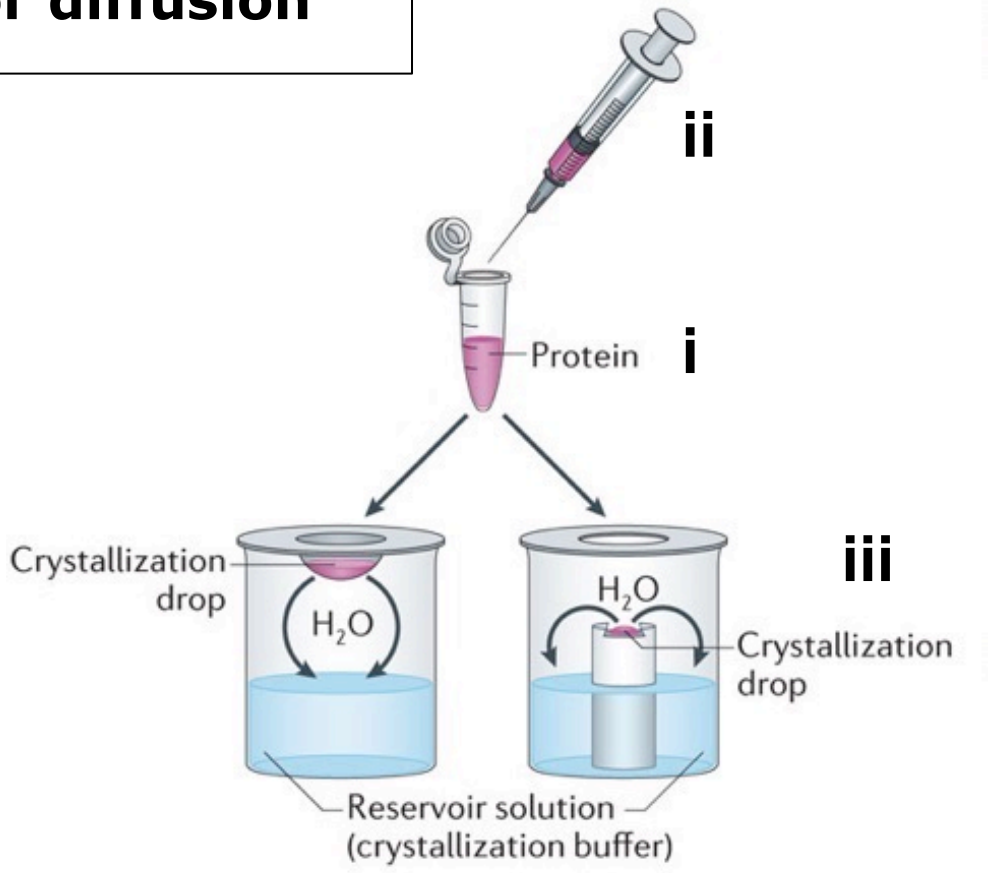
Mix proteins with desired chemicals, other proteins, ligands, etc.

ii. Transfer the protein mixture to a robot-customized syringe

iii. Mix with the reservoir solution (by robot)

Seal each crystallization well

Monitor over time for crystal growth



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

Membrane protein crystallography

Bicelle (stacked 2-D crystals)

i. Transfer purified proteins to a microcentrifuge tube

Mix proteins with desired chemicals, other proteins, ligands, etc.

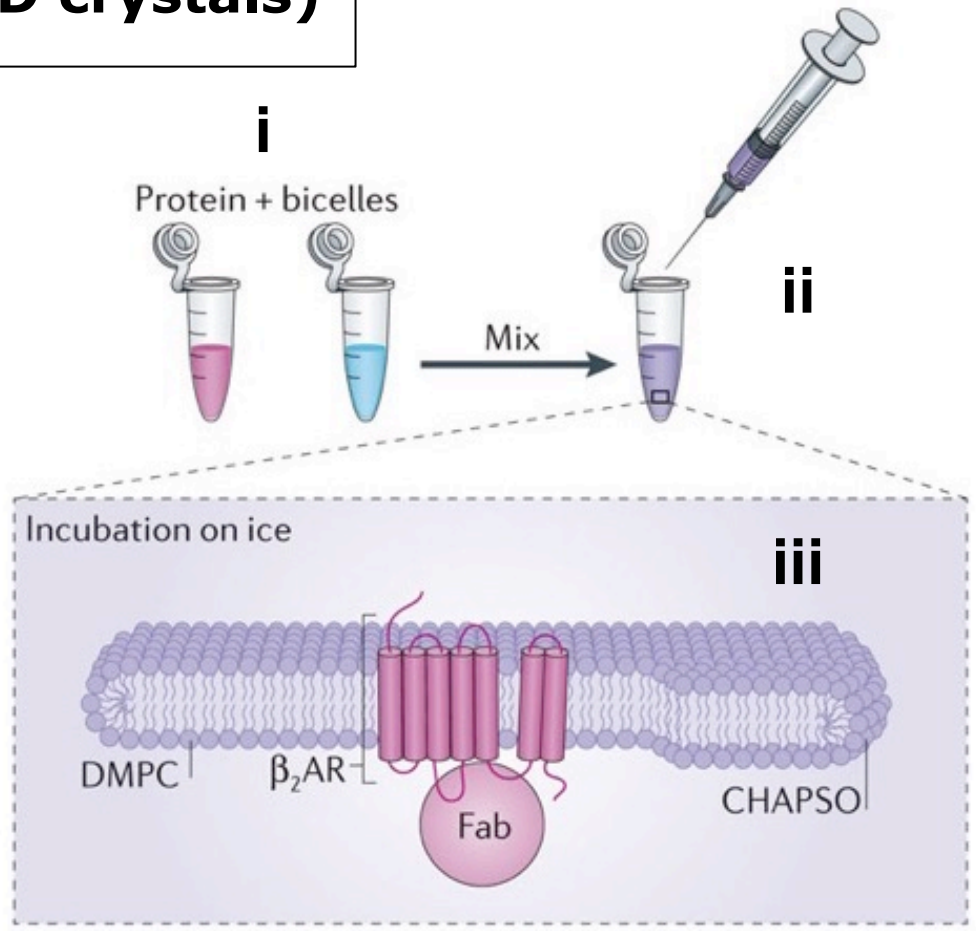
Add bicelle solution to the protein mixture

ii. Transfer the protein mixture to a robot-customized syringe

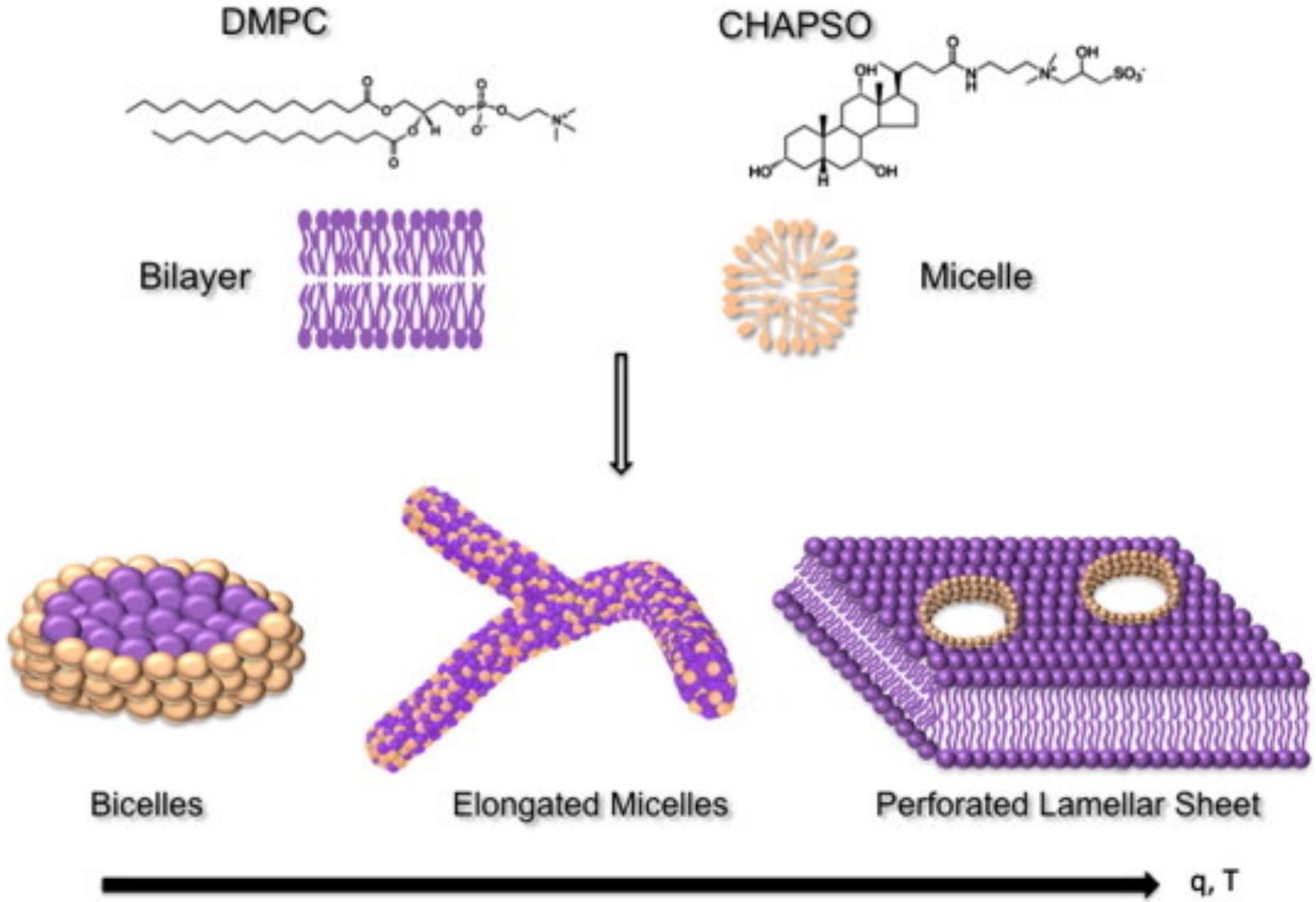
iii. Mix with the reservoir solution (by robot)

Set up hanging-drop crystal trays and seal each crystallization well

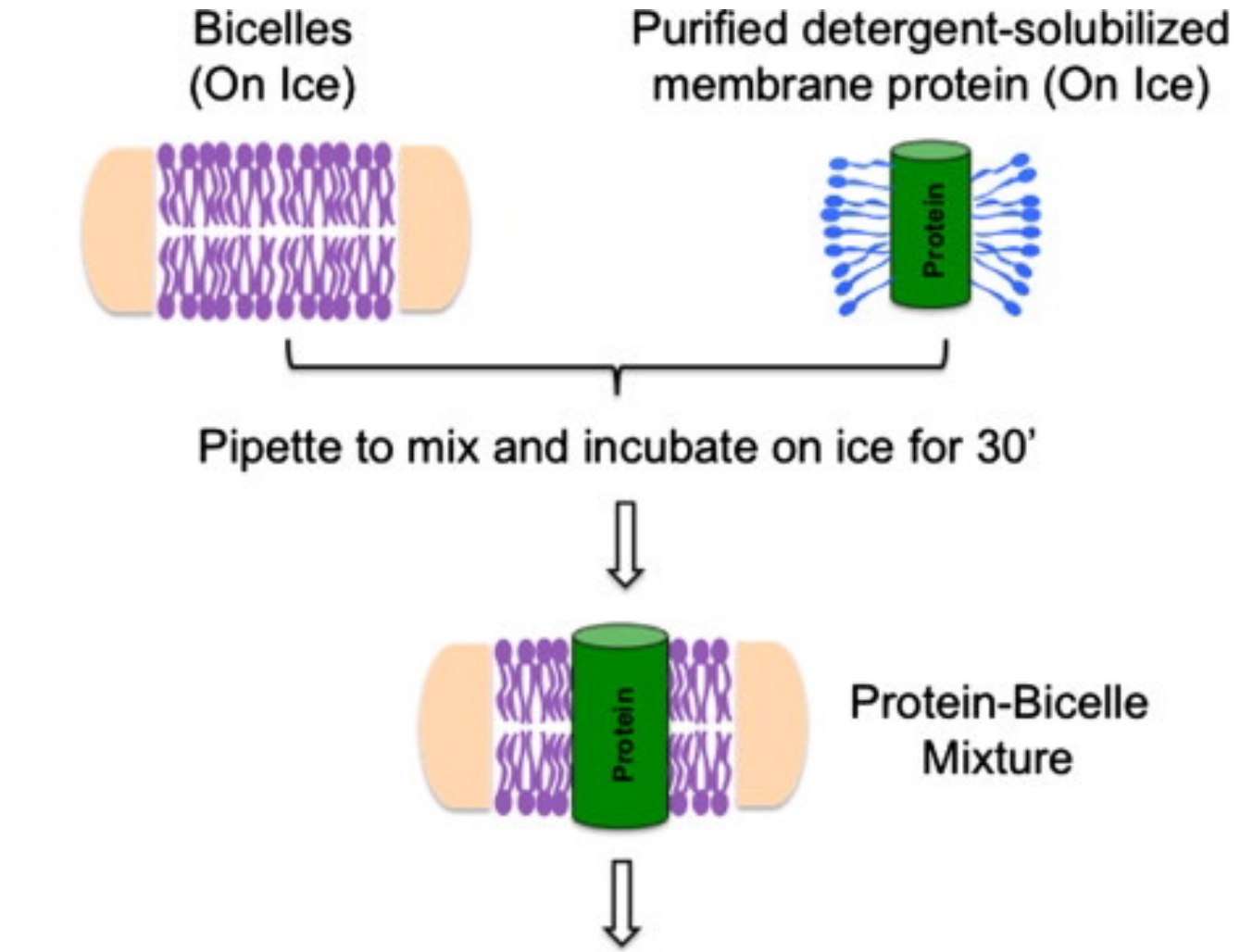
Monitor over time for crystal growth



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



(Ujwal & Bowie, Methods, 2011)



(Ujwal & Bowie, Methods, 2011)

Membrane protein crystallography

In meso lipid cubic phase (LCP)

i. Mix proteins with desired chemicals, other proteins, ligands, etc.

   Transfer the protein mixture to a robot-customized syringe

   Transfer pre-made LCP lipids (usually monoolein ± cholesterol) to another robot-customized syringe.

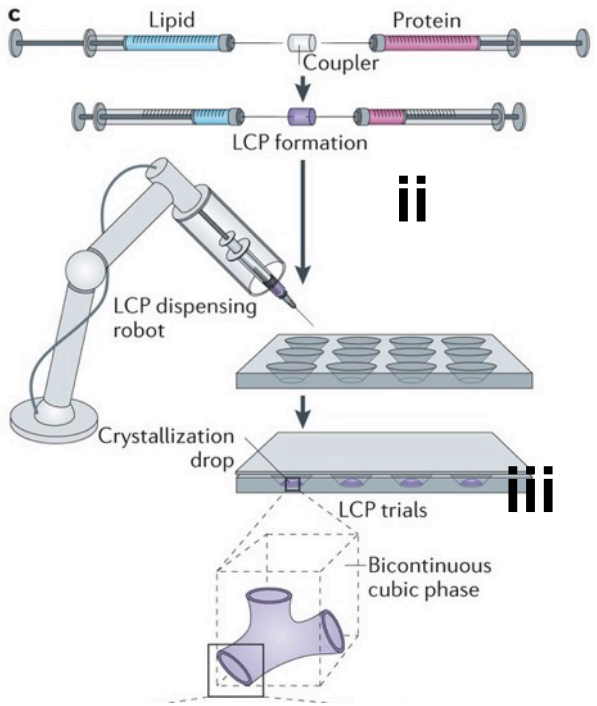
   Mix proteins and the lipids until the mixture shows no cloudiness

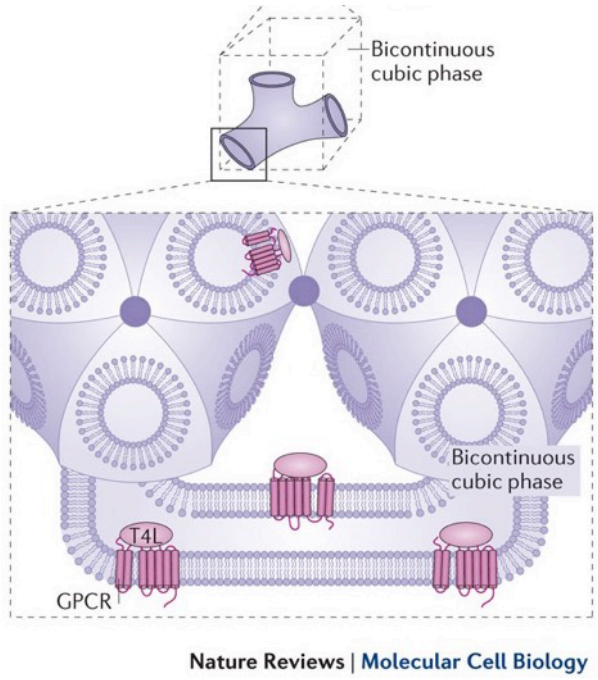
ii. Mix with the reservoir solution on sandwich plates (by robot)

   Seal the LCP sandwich plates

   Monitor over time for crystal growth (usually at 20°C - room temperature)

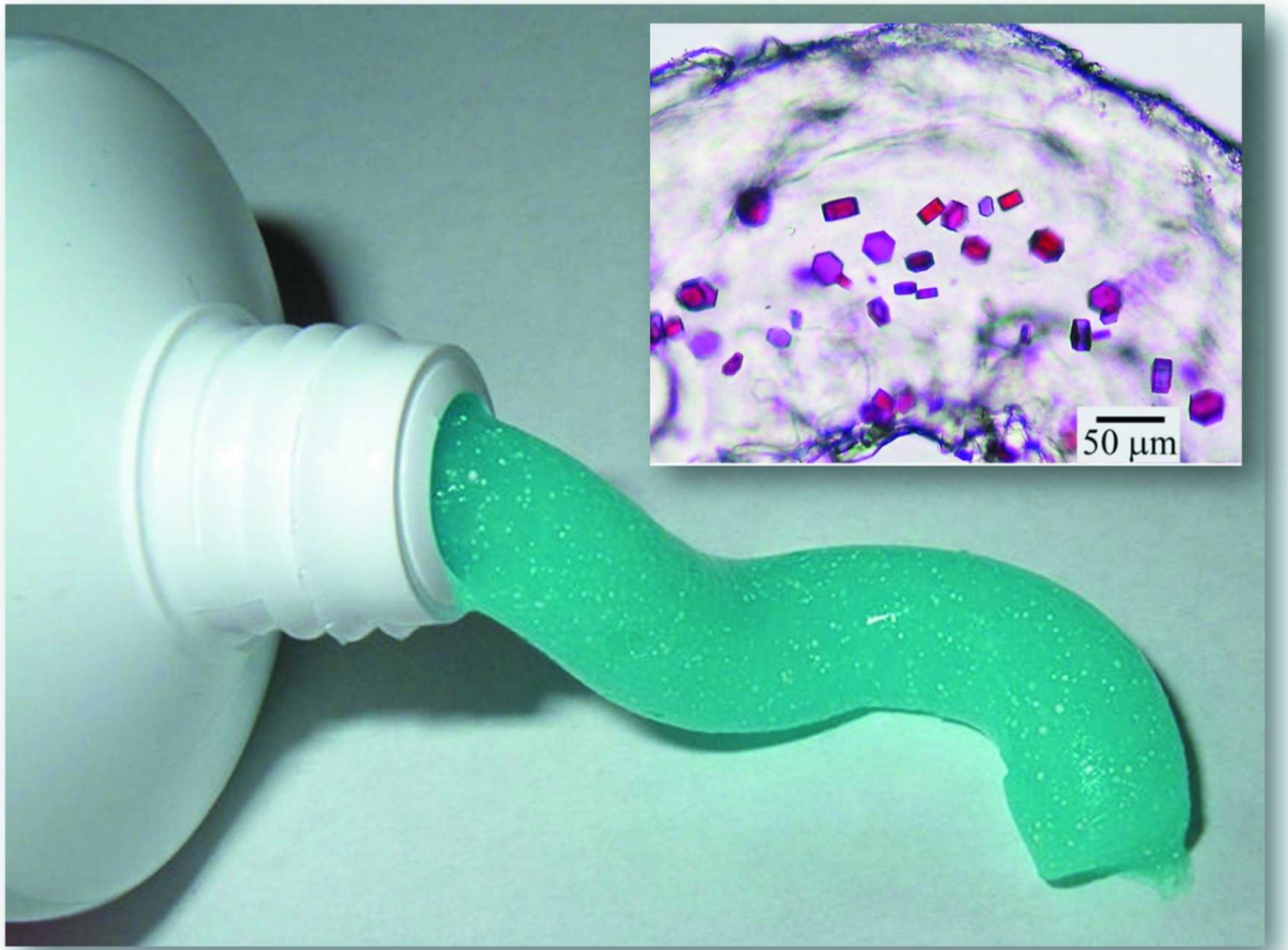
i



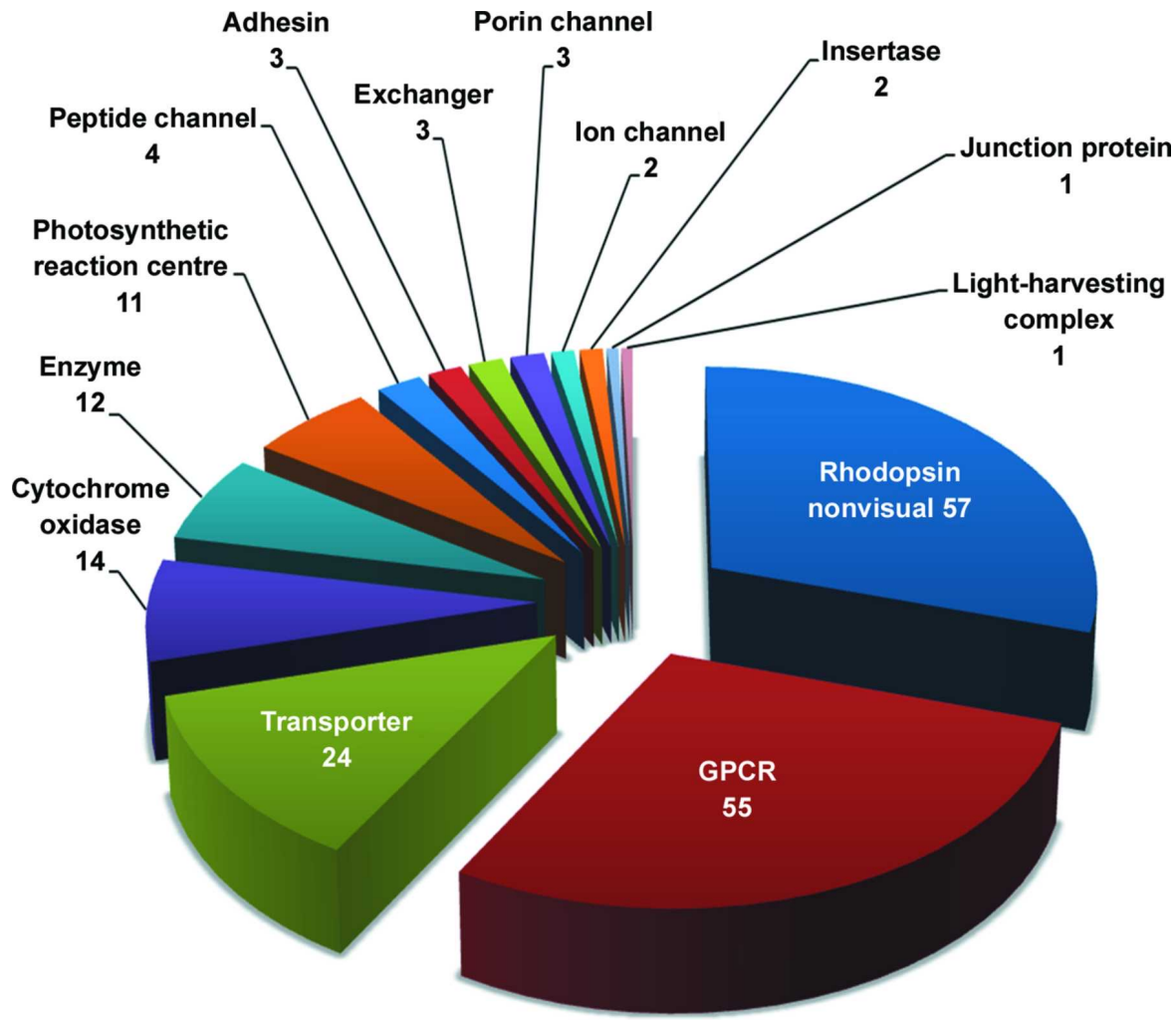


iv

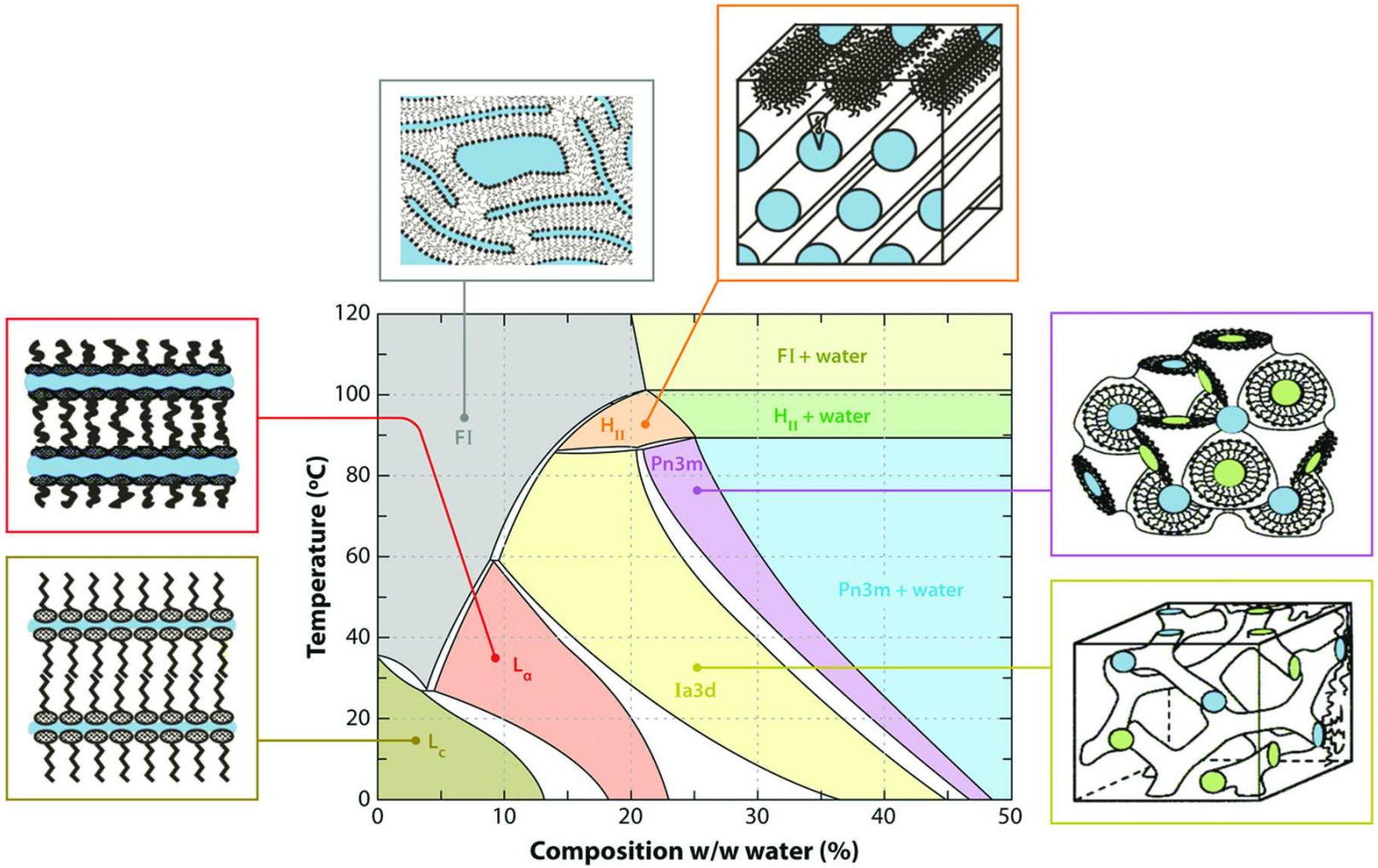
(Ghosh et al, Nature Rev Mol Cell Biol, 2015)



(Caffrey, Acta Cryst F, 2015)



(Caffrey, Acta Cryst F, 2015)

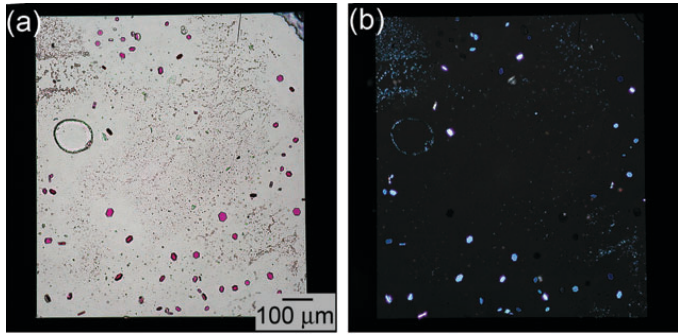


(Caffrey, Acta Cryst F, 2015)

X-ray crystallography: micro-diffraction

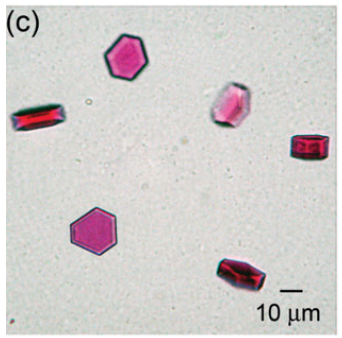
LCP

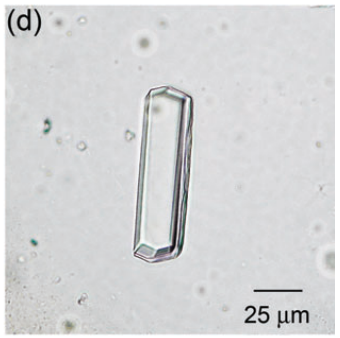
Bacteriorhodopsin



Bacteriorhodopsin
(Birefringence)

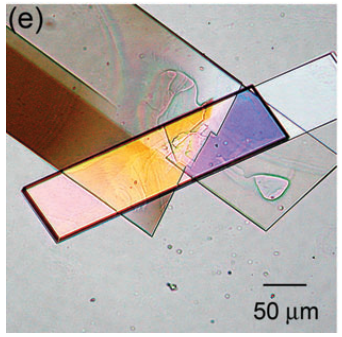
Bacteriorhodopsin

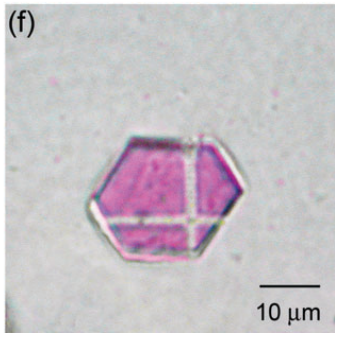




Lysozyme

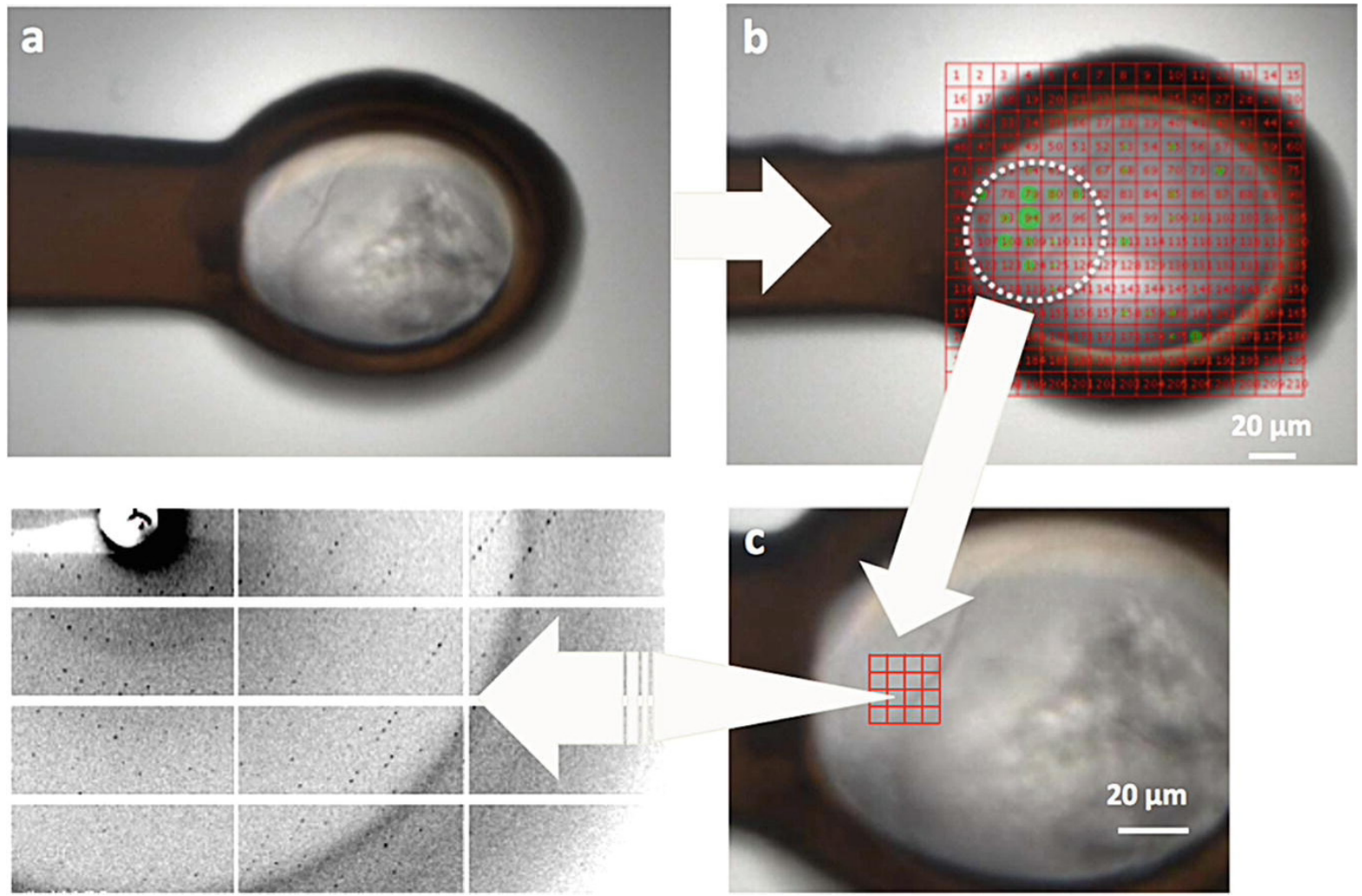
Cholesterol



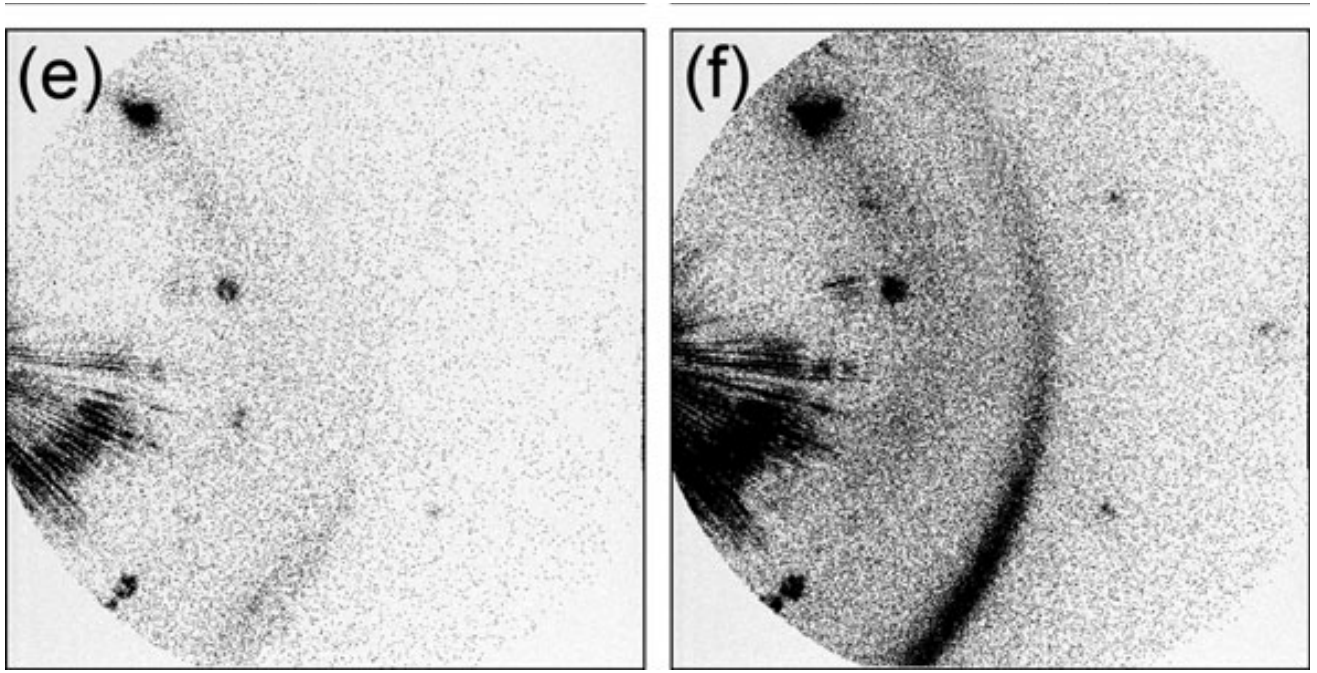


Bacteriorhodopsin
(X-ray damaged)

(Cherezov & Caffrey, Faraday Discuss, 2007)



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

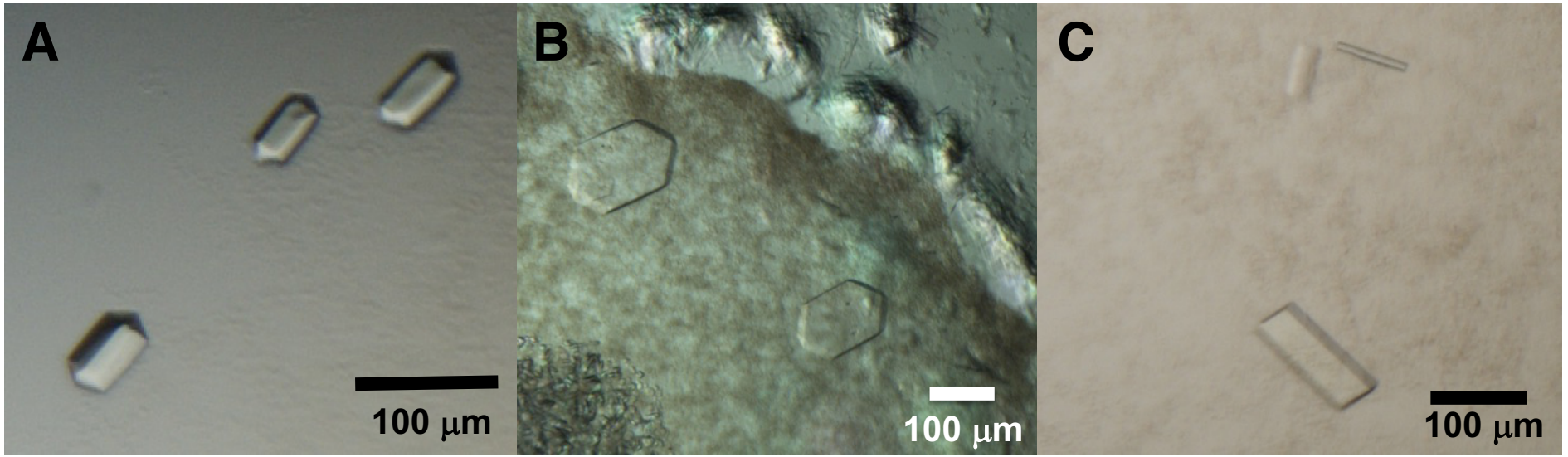


1s exposure **10s exposure**

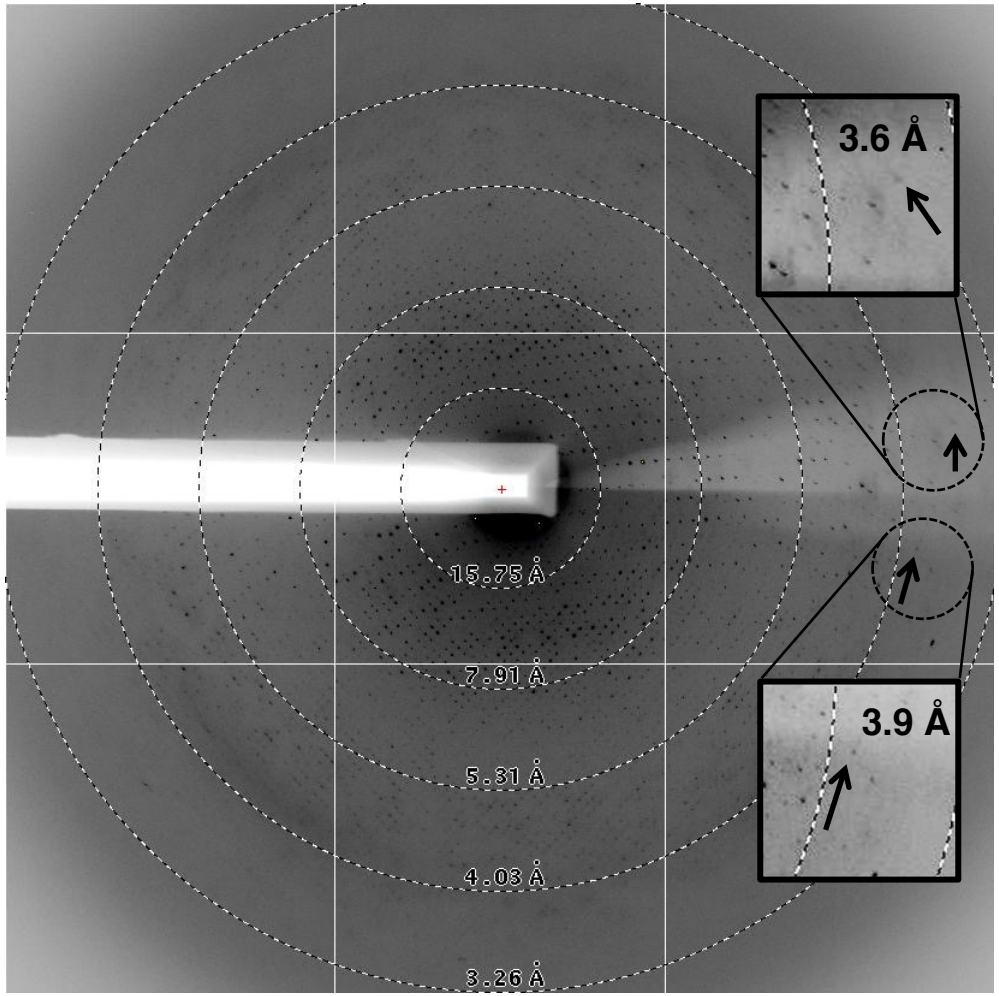
(Cherezov & Caffrey, Faraday Discuss, 2007)

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)

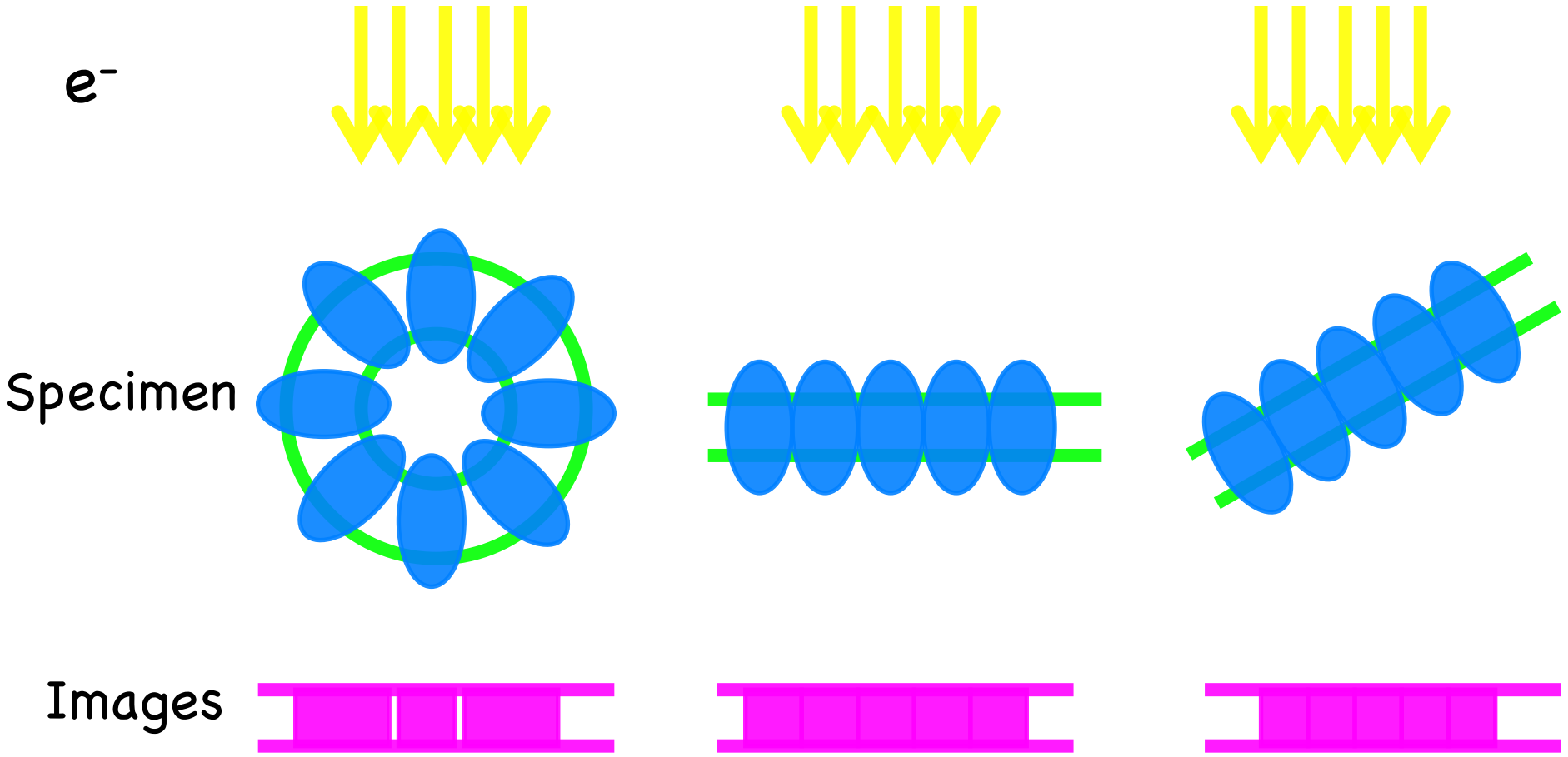
Cryo-electron microscopy (cryo-EM)

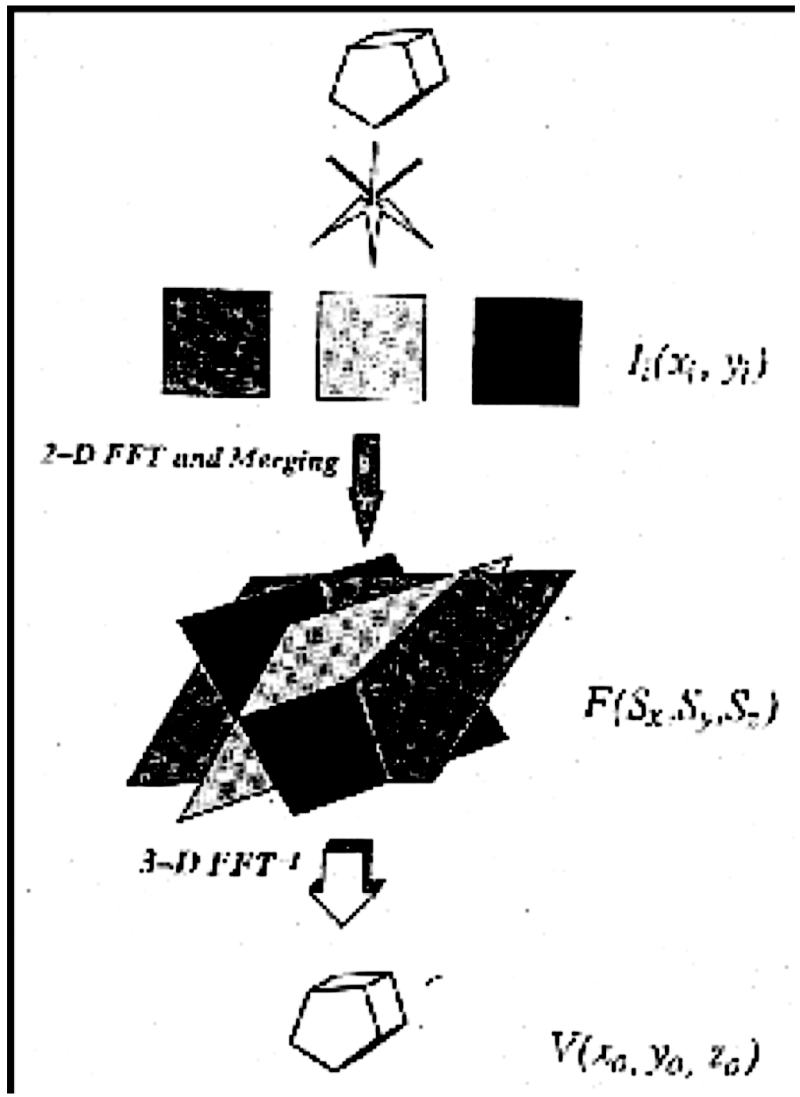
- **Electron crystallography ✓**
  - **2-D crystals / MicroED**
  - **Helical crystals**

- **Single-particle cryo-EM ✓**

- **Cryo-electron tomography (cryo-ET)**

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





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

Microelectron Diffraction (MicroED) & Electron Crystallography

Cryo-EM applications in protein structural biology:

2) Microelectron diffraction:
(need to generate protein crystals, but much smaller than that for X-ray crystallography)

a. Prepare purified protein samples, crystallize and and freeze them.

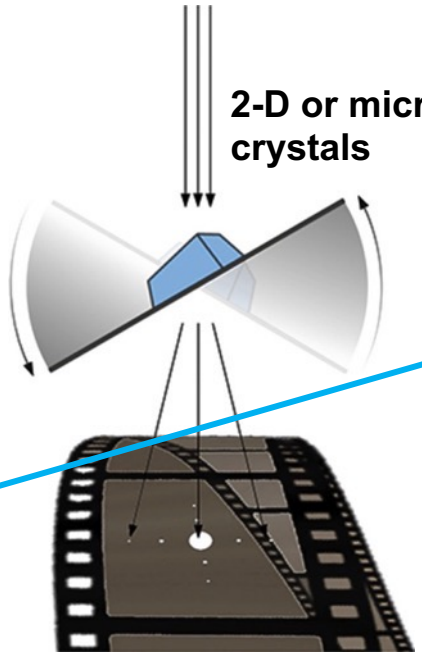
b. Take electron micrographs of protein crystals and diffraction images.

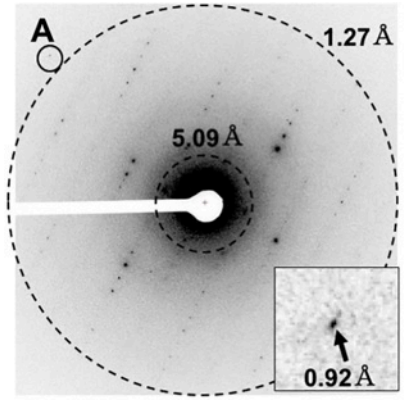
c. Use diffractions to establish the amplitude information and images to find the phase information, then calculate the structural factors to generate the electron clouds for the target proteins.

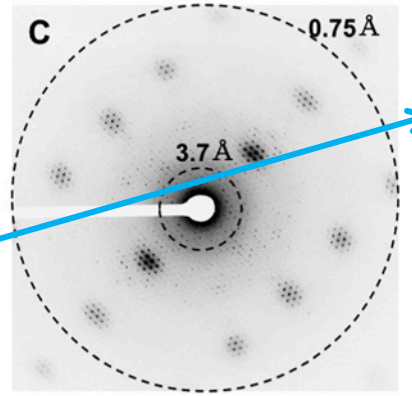
d. Model building like that in X-ray crystallography.

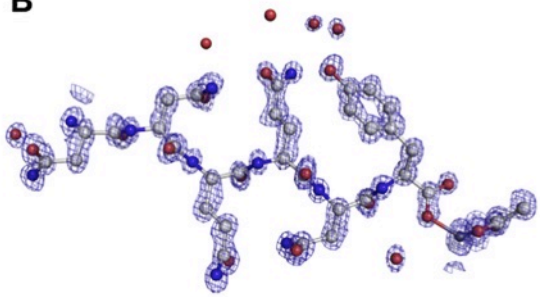
Samples

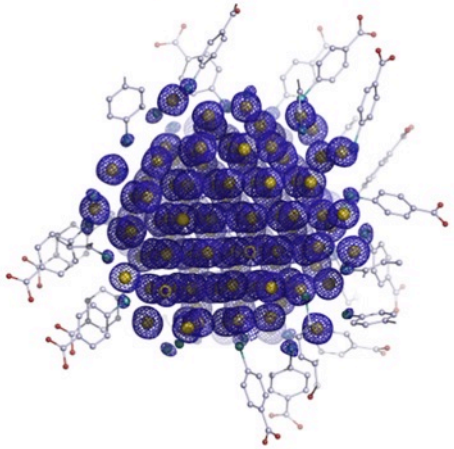
2-D or micro-crystals



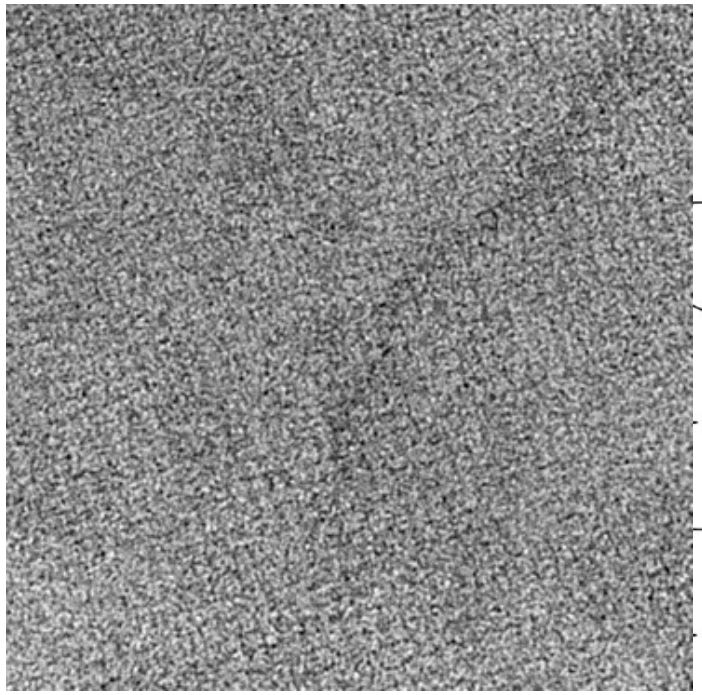




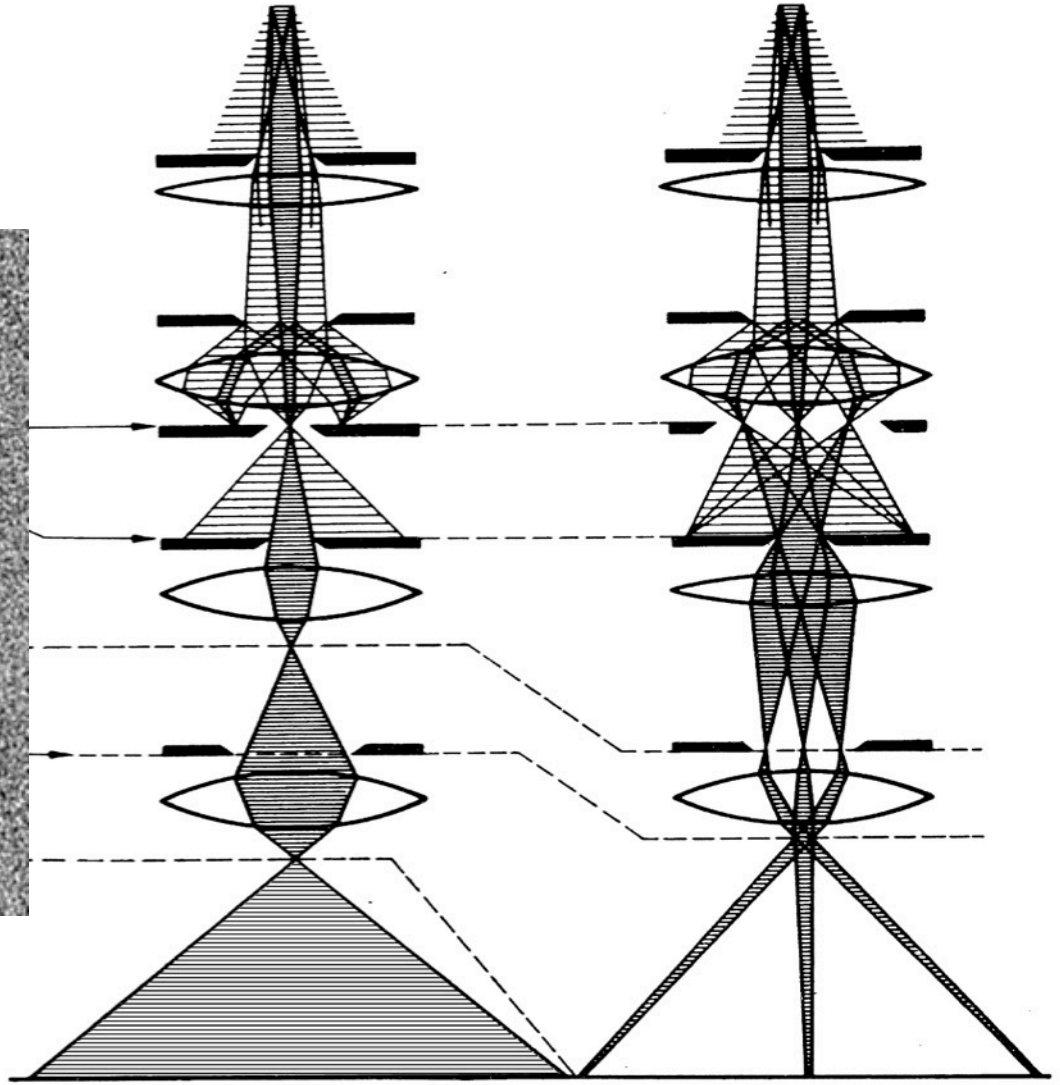


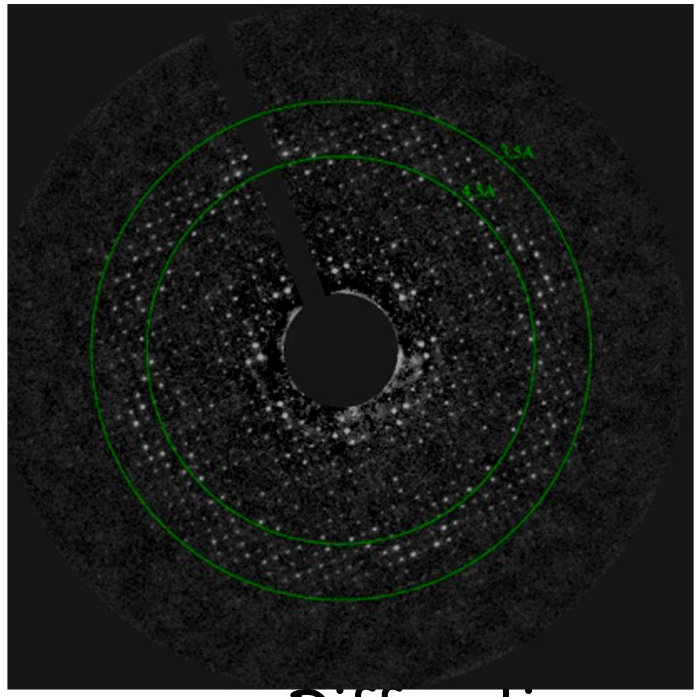


(Martynowycz & Gonen, Curr Opin Colloid Interf Sci, 2018)

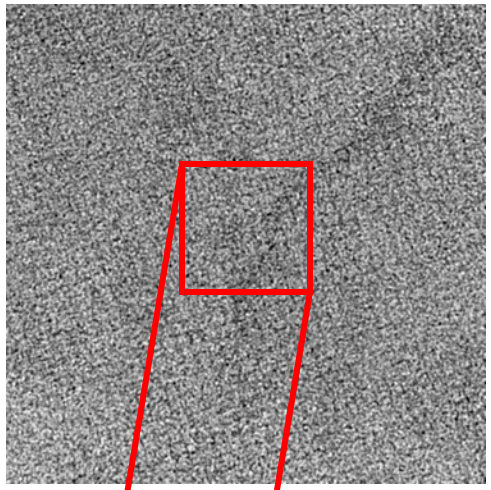


Image



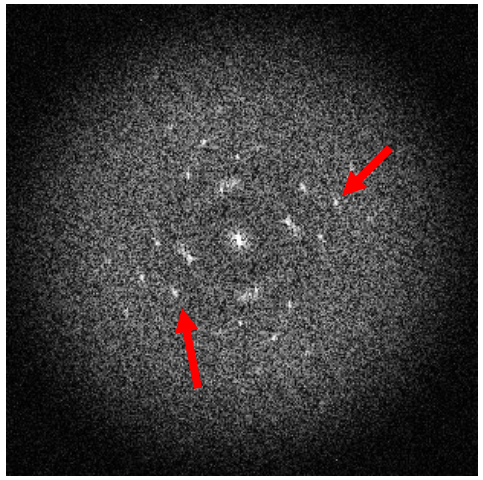


Diffraction



FT

IFT



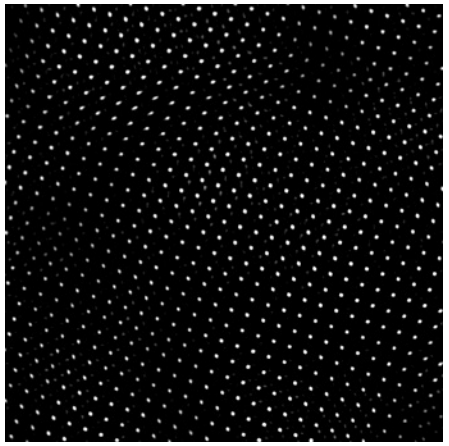
Fourier space

Calculation of Amplitudes & Phases

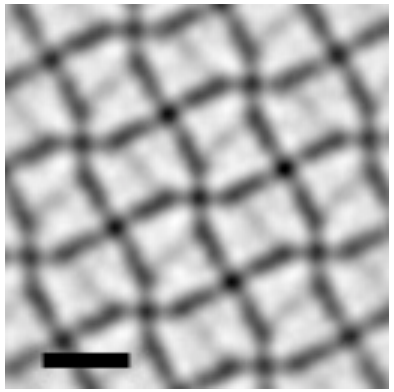
H	K	A	ϕ
1	-3	771.2	-122.9
1	-2	3836.0	-148.3
1	-1	5184.4	9.6
1	0	599.8	-17.9
1	1	5953.2	15.9
2	-7	17.2	83.4
2	-4	41.4	-125.0
2	-3	1485.4	52.6
2	-2	2491.6	-152.5

Correlation Averaging

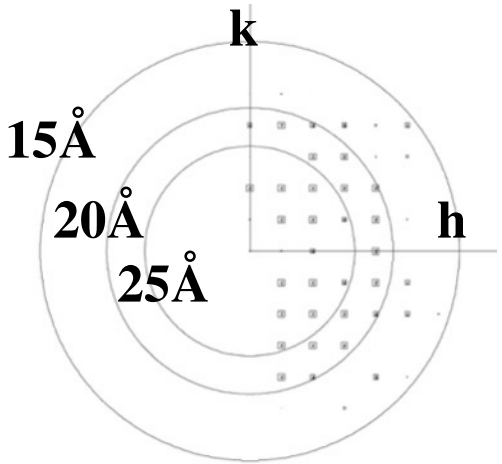
real space



Cross-Correlation Map



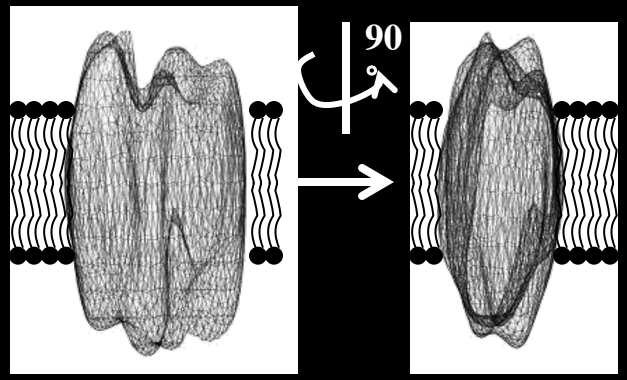
S/N-enhanced Image

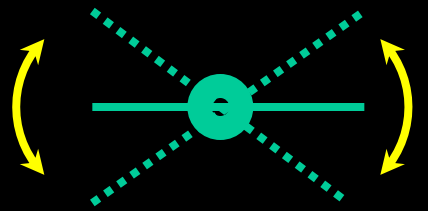


Non-tilting Images

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)





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)

Single-Particle Analysis (SPA)

Cryo-EM applications in protein structural biology:

1) Single particle analysis:
(no need to generate protein crystals)

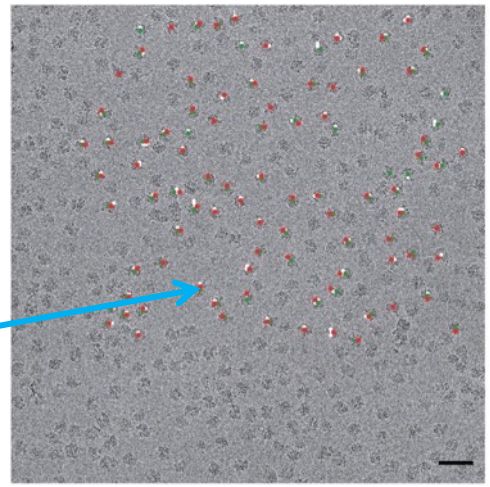
a. Prepare purified protein samples and freeze them.

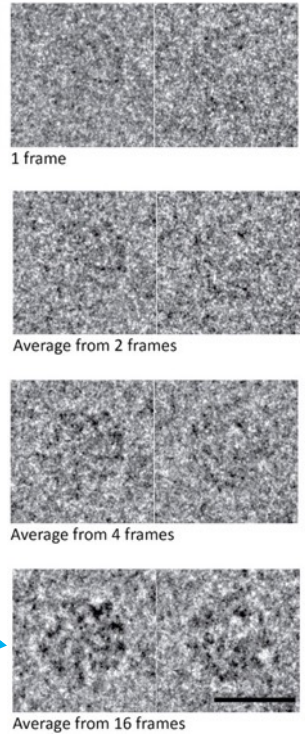
b. Take electron micrographs of isolated protein particles.

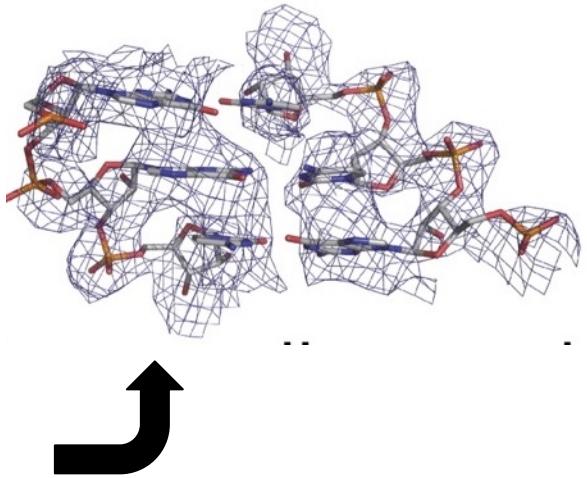
c. Collect several of “identical” images and add altogether to enhance the signal of the objects (darker area)

d. Then place amino acid models in the electron density.

Samples







(Bai et al, eLife, 2013)

A. Raw image data typically seen from a cryo-EM experiments. Protein particles are usually shown as the dark objects. This image shows particles of a proteasome complex.

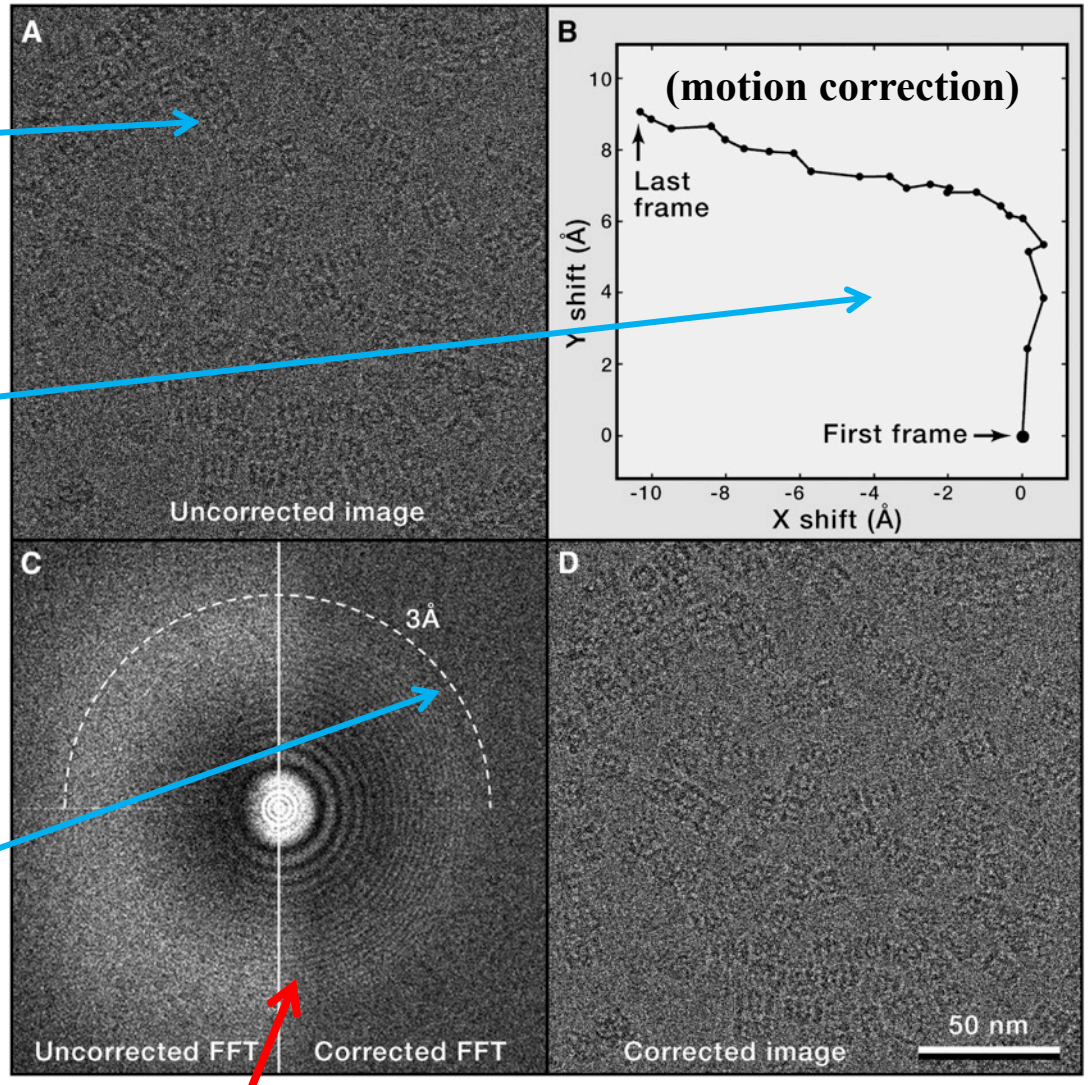
B. However, often time, each particle looks “blurred”, largely because of **image drifting during the picture taking, as well as the microscopic movement of protein molecules in the ice**. ”Motion correction” is thus necessary to help enhance the image quality, i.e., making the images sharper.

C. We use power spectrum to evaluate the quality of an image. Power spectrum can be seen as a theoretical diffraction pattern of the image in A.

D. Corrected and sharpened images from A. As indicated in C, the corrected picture clearly reveals potential information that can be resolved as better as 3Å.

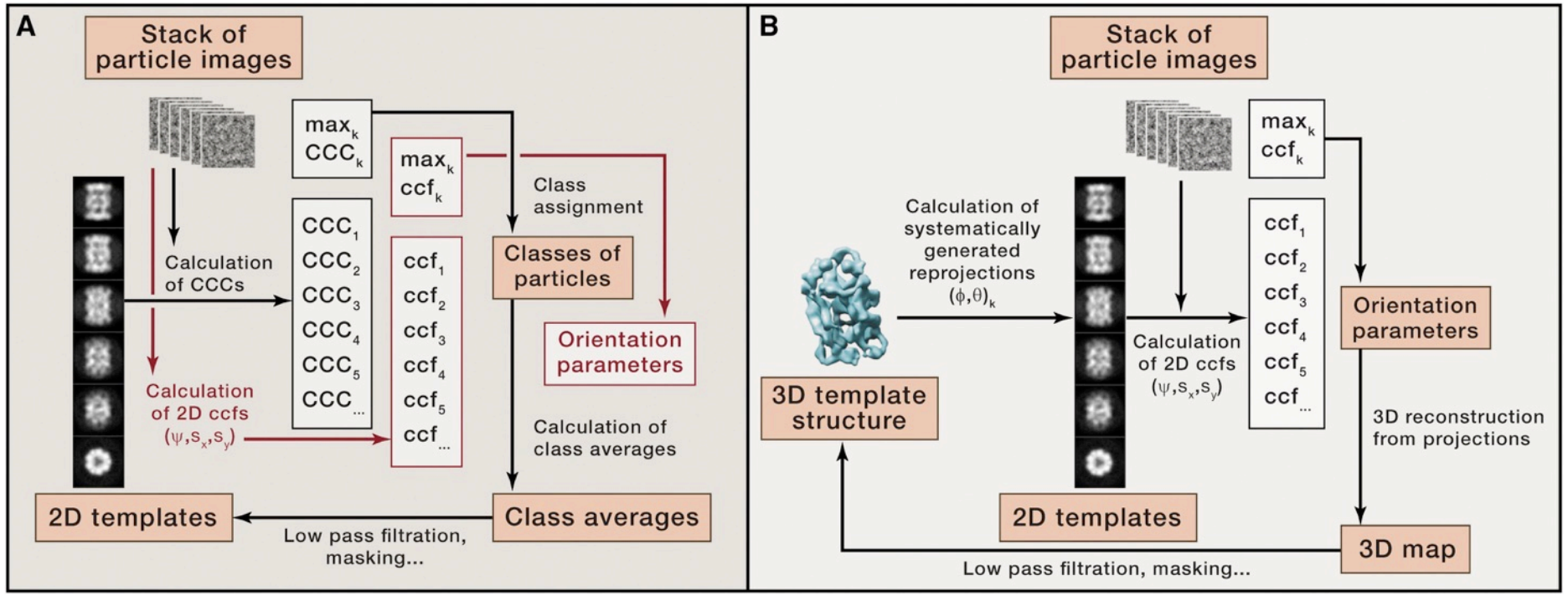
Image film

Power spectrum (Computed diffraction)

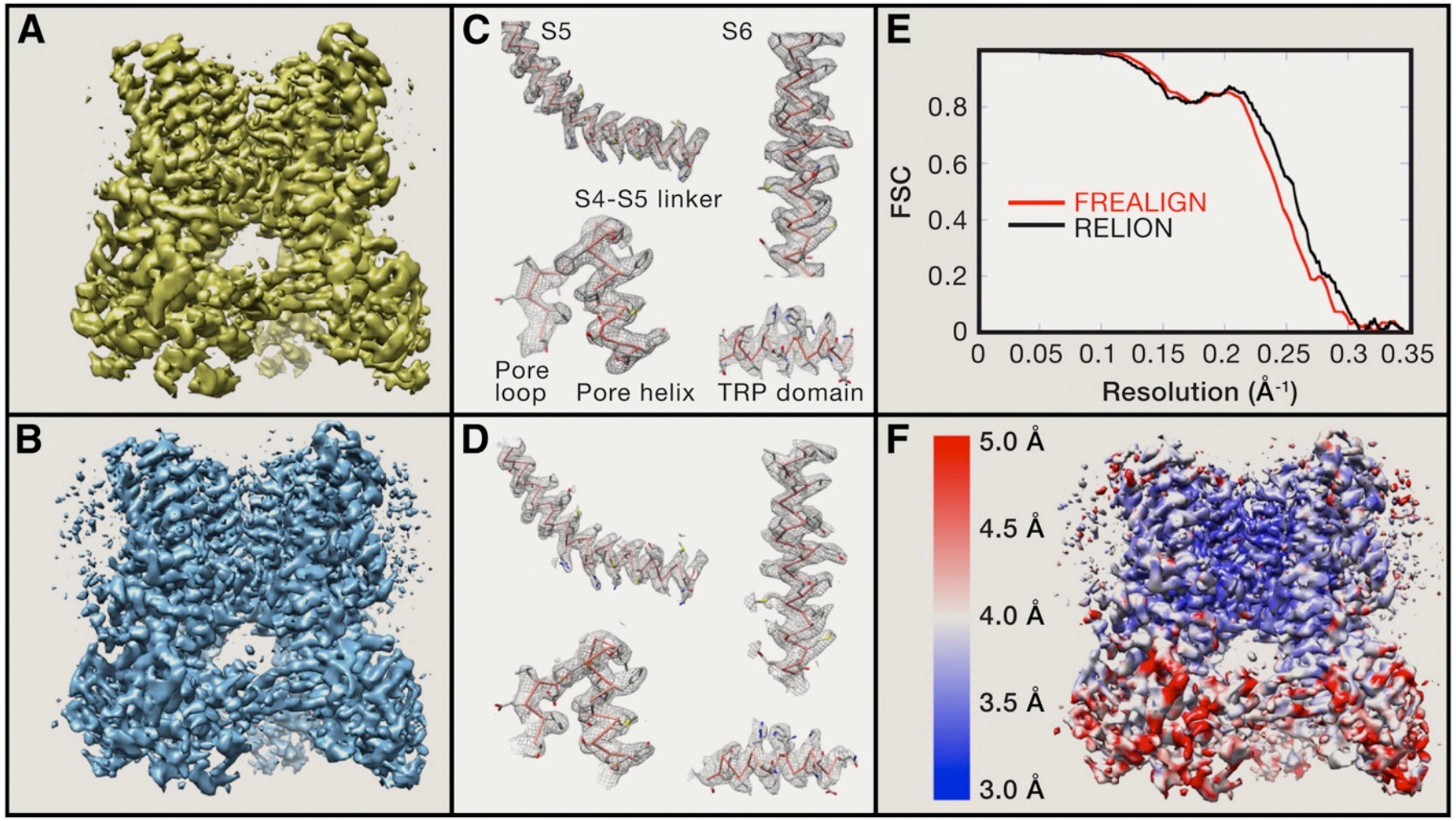


(Cheng et al, Cell, 2015)

What’s this?



(Cheng et al, Cell, 2015)



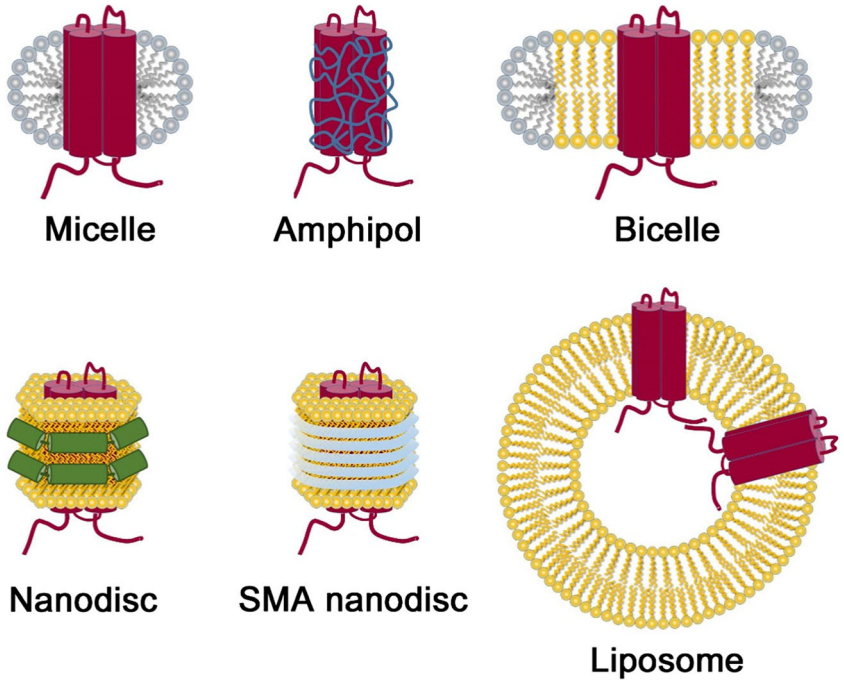
(Cheng et al, Cell, 2015)

EM Samples: Membrane Proteins

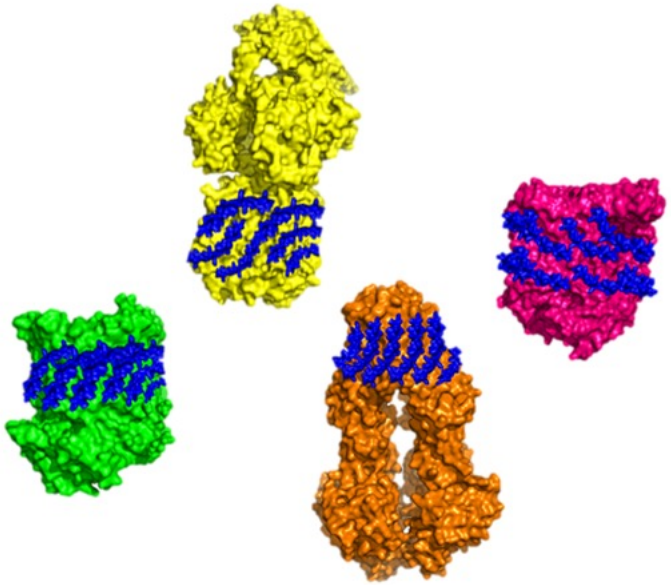
- Use of detergents: starting with the one used for purification, *e.g.*, DDM.
  - Mild non-ionic
  - Amphipols

- Nanoparticles: a membrane-mimetic scaffold that stabilizes proteins in the native lipid-bilayered environment.
  - MSP-nanodiscs (MSP: membrane scaffold protein)
  - SMA nanodiscs (SMA: styrene–maleic acid)
  - Bicelles
  - Peptidiscs

EM Samples: Membrane Proteins



(Mio & Sato, Biophys Rev, 2018)



Peptidisc

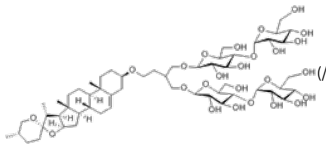
(Carlson et al, eLife, 2018)

EM Samples: Membrane Proteins

DETERGENTS FOR CRYO-EM

The following detergents have been sucessfully used in the Cryo-EM studies of membrane proteins. Want to learn more? Check out our compilations of membrane protein strucutures for 2016(/Landing/2016/Cryo-EM-Update-Sept16), 2017(/Landing/2017/Cryo-EM-Update-Oct17), and 2018(/Landing/2018/Cryo-EM-Update-Oct18).

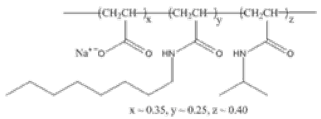
GDN101 - GDN(/PRODUCTS/SPECIALTY-DETERGENTS-PRODUCTS/COMPLEX/GDN101)



(/Products/Specialty-Detergents-Products/COMPLEX/GDN101)

Digitonin is commonly used for Cryo-EM, but there are many drawbacks including batch-to-batch variability and solubility. GDN has been shown to be an effective drop-in substitute for Digitonin which is being used in a number of recent structures.

A835 - AMPHIPOL A8-35(/PRODUCTS/SPECIALTY-DETERGENTS-PRODUCTS/AMPHIPOL/A835)

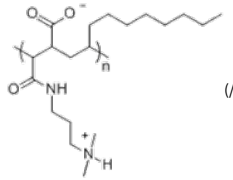


x ~ 0.35, y ~ 0.25, z ~ 0.40

(/Products/Specialty-Detergents-Products/AMPHIPOL/A835)

First described in 1996 by Jean-Luc Popot, amphipols are a class of polymers that can stabilize membrane proteins in a detergent-free, aqueous solution. To date, there have been over 20 Cryo-EM structures of membrane proteins determined using Amphipol A8-35.

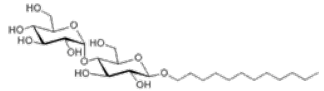
P5008 - AMPHIPOL PMAL-C8(/PRODUCTS/SPECIALTY-DETERGENTS-PRODUCTS/AMPHIPOL/P5008)



(/Products/Specialty-Detergents-Products/AMPHIPOL/P5008)

In recent years, PMAL-C8 has been gaining traction for use in Cryo-EM(/Landing/2018/PMAL-July18) with a number of unique structures published. PMAL amphipols are zwitterionic, and contain repeating units of a carboxyl, ammoniumamidate, and alkyl chain.

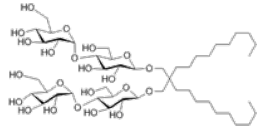
D310 - DDM(/PRODUCTS/DETERGENTS/MALTOSIDES/D310)



(/Products/Detergents/MALTOSIDES/D310)

The most commonly used detergent in membrane protein crystallization, Dodecyl Maltoside (DDM), has also been used in the Cryo-EM structures of a number proteins. DDM is also often used as a mixture with Cholesteryl Hemisuccinate (CHS)(/Products/Detergents/MALTOSIDES/10-1-DDM-CHS-Pre-Made-Solution).

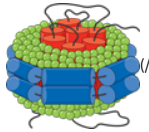
NG310 - LMNG(/PRODUCTS/DETERGENTS/NG-CLASS/NG310)



(/Products/Detergents/NG-CLASS/NG310)

Due to its very low CMC, the concentration of LMNG in the buffer can often be reduced to low concentrations, reducing the amount of free detergent micelles, and reducing background. Like DDM, LMNG is often used as a mixture with Cholesteryl Hemisuccinate (CHS) (/Products/Detergents/NG-CLASS/LMNG-CHS-Pre-Made-Solution).

LIPID NANODISCS(/PRODUCTS/LIPIDS/LIPIDS)



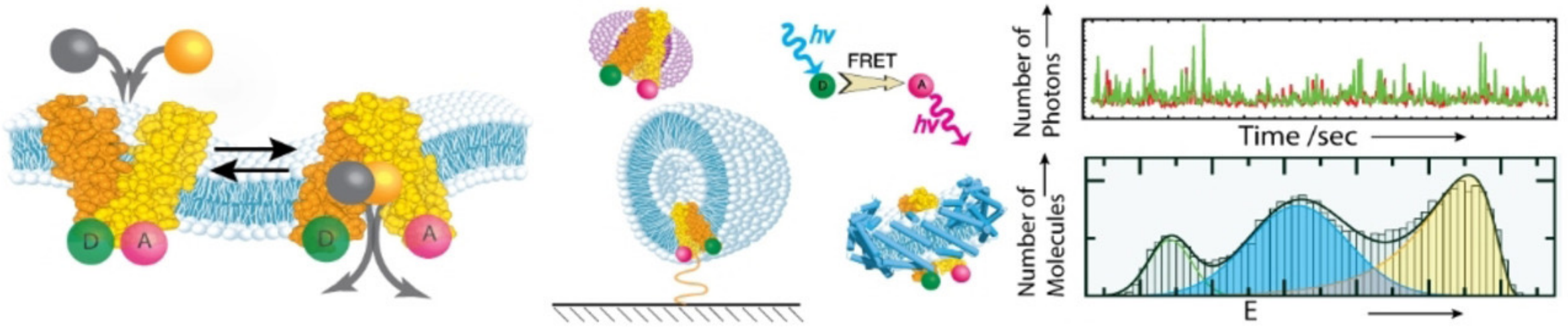
(/Products/Lipids/LIPIDS)

Lipid nanodiscs allow for the reconstitution of a detergent solubilized membrane protein into a lipid environment, and are being increasingly used in Cryo-EM. Anatrace offers a full selection of the lipids commonly used in nanodisc reconstitution.

(Anatrace, Inc.)

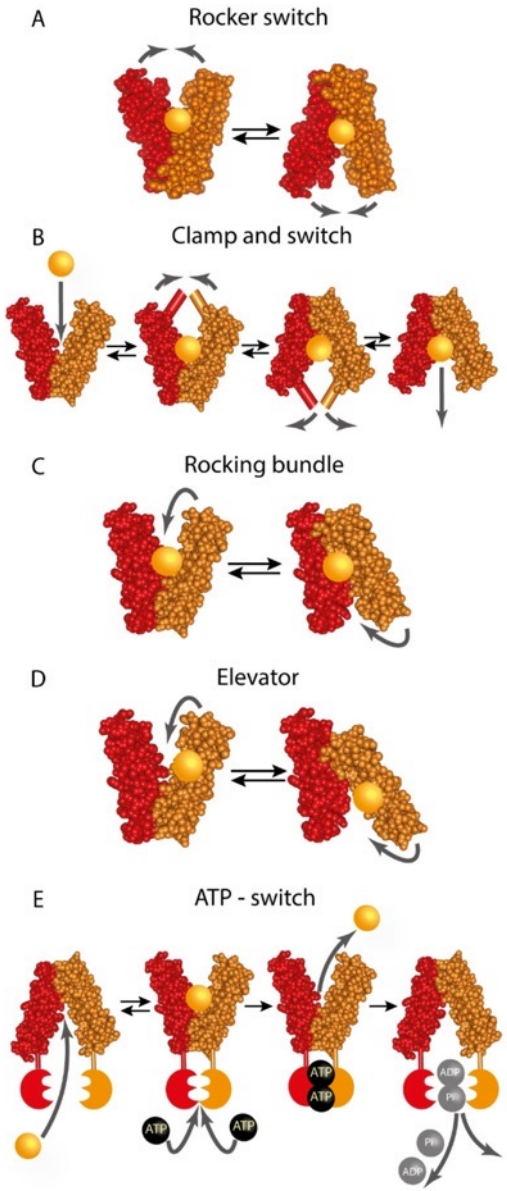
Fluorescence as a mean to study membrane protein structures:

Fluorescence Resonance Energy Transfer (FRET)



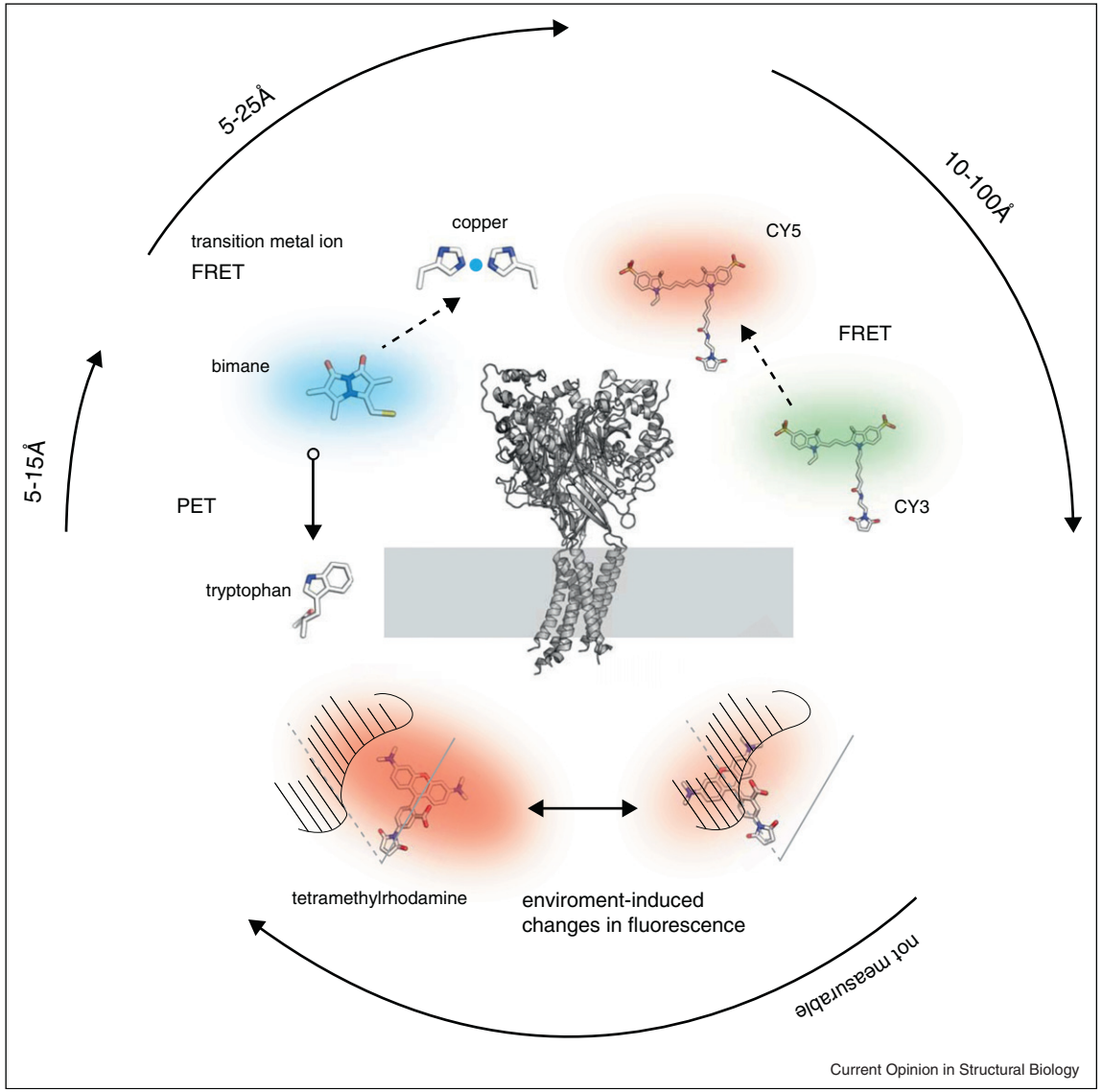
(Bartels et al, ChemBioChem, 2021)

Transporters



(Bartels et al, ChemBioChem, 2021)

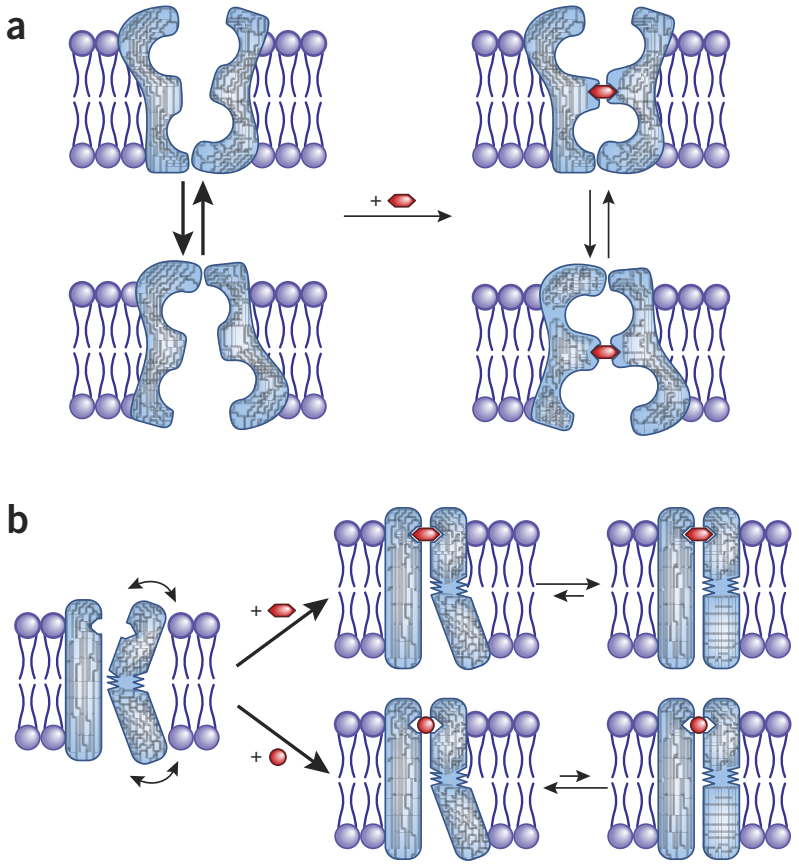
Ion channels / Receptors



(Taraska, Curr Opin Struct Biol, 2012)

Magnetic resonance as a mean to study membrane protein structures:

Nuclear magnetic resonance (NMR): function and dynamics



Transporters

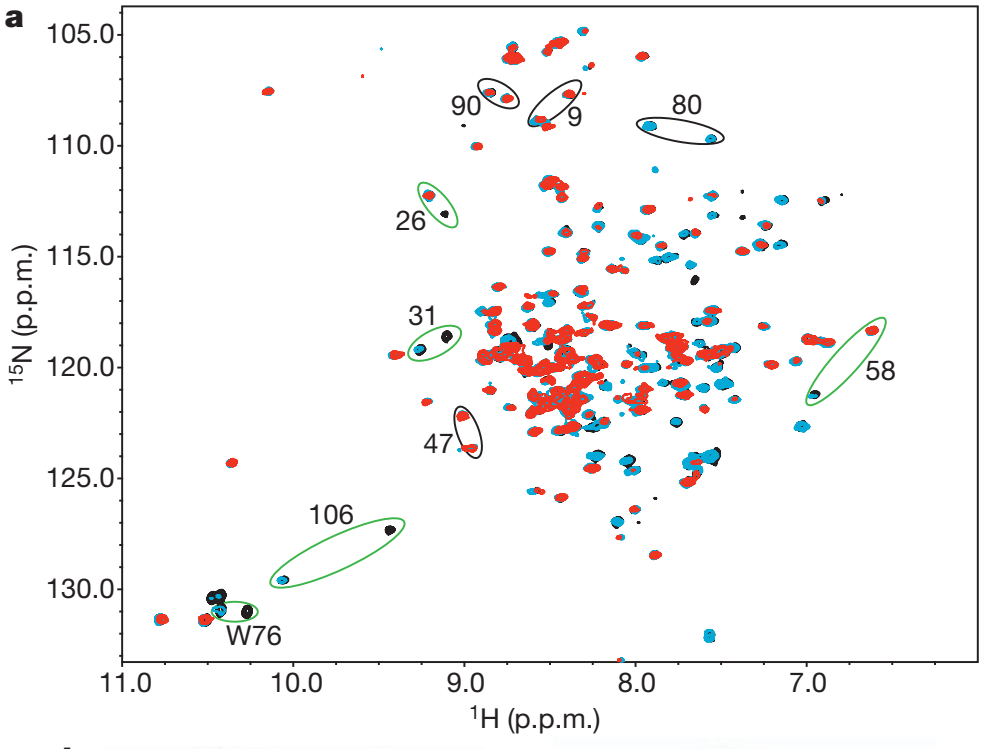
Receptors

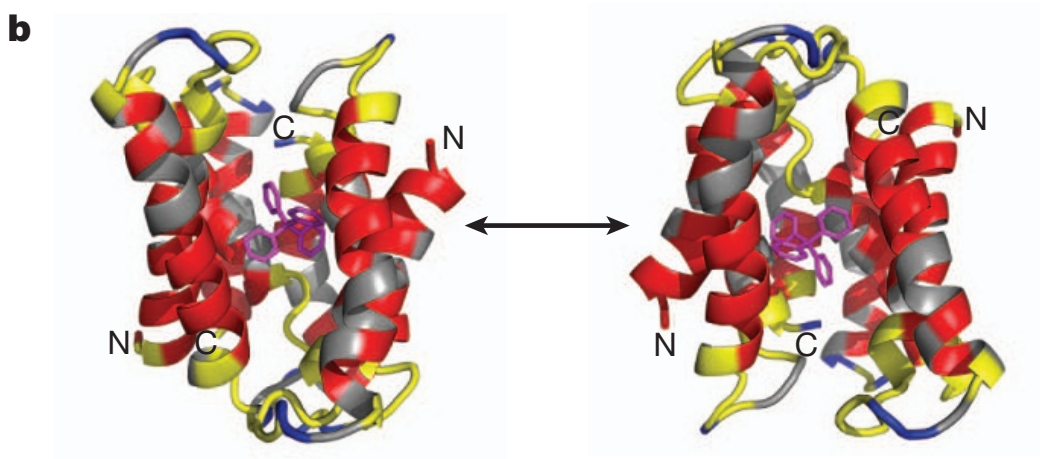
(Liang & Tamm, Nat Struct Mol Biol, 2016)

Magnetic resonance as a mean to study membrane protein structures:

Nuclear magnetic resonance (NMR): function and dynamics

Asymmetric water accessibility in *E coli* multidrug transporter EmrE



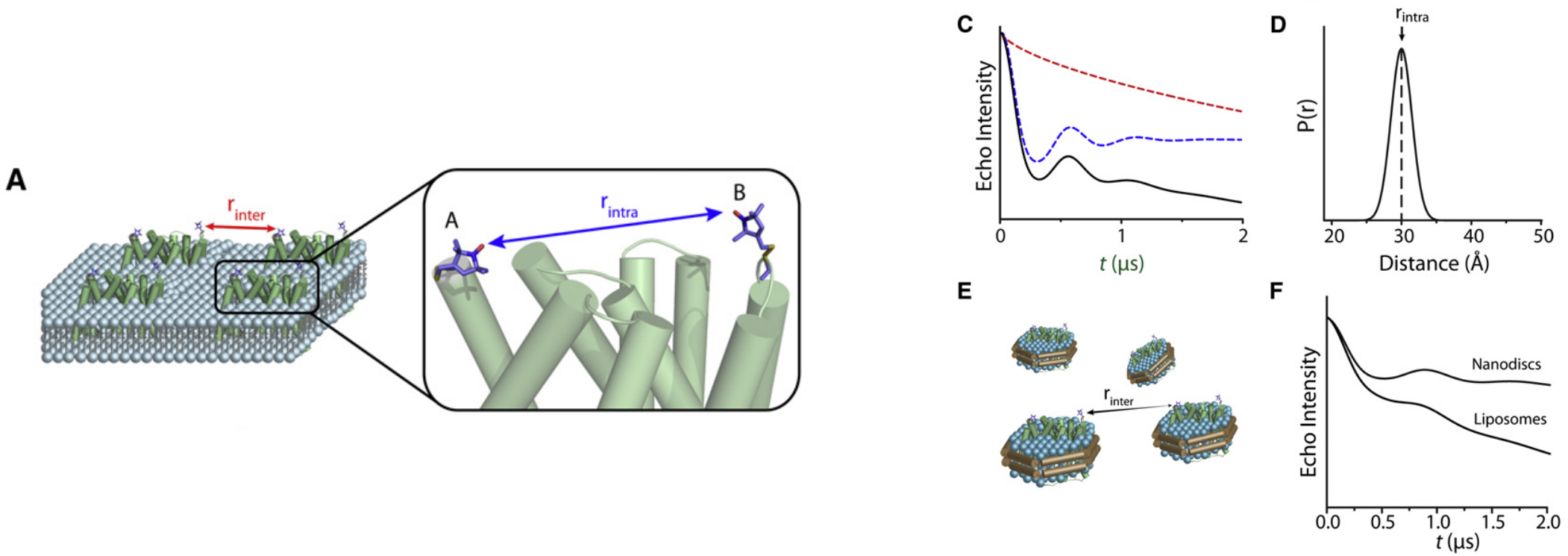


(Morrison et al, Nature, 2012)

Magnetic resonance as a mean to study membrane protein structures:

Spin-labeled Electron paramagnetic resonance (EPR) spectroscopy

Distance Distribution

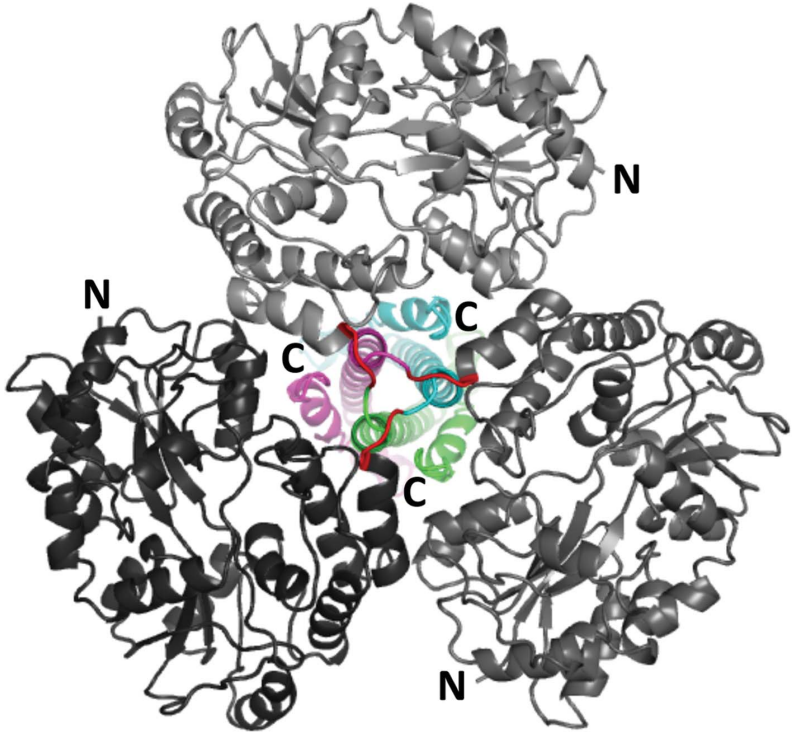


(Mchaourab et al, Structure, 2011)

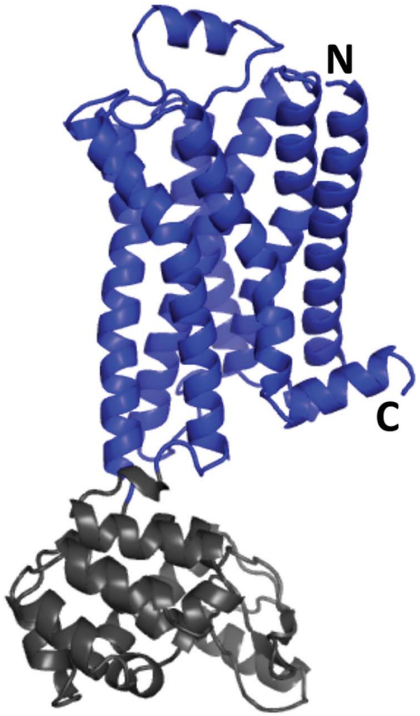
4. Other strategies in studying membrane protein structures

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

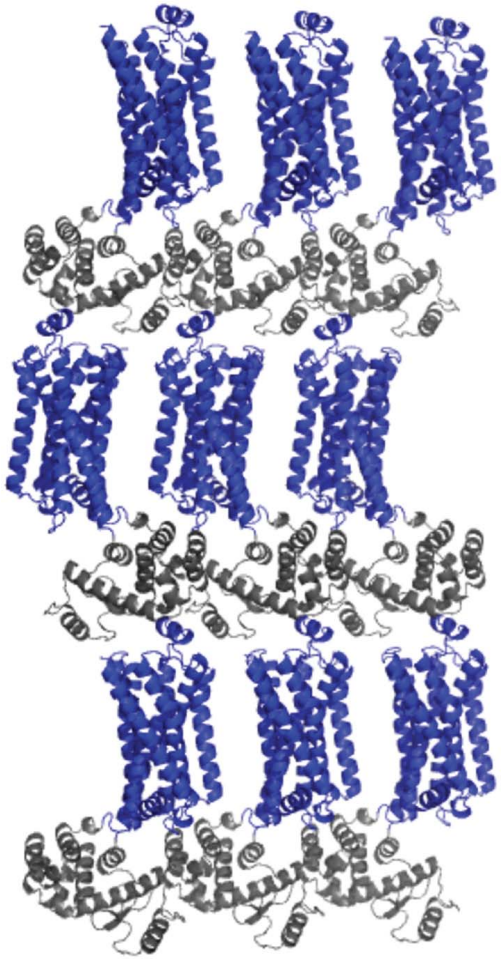
Fusion protein strategy:



(a)



(b)



(Kobe et al, Acta Cryst F, 2015)