From electron crystallography to single particle cryoEM

Nobel Lectures in Chemistry

8th December 2017

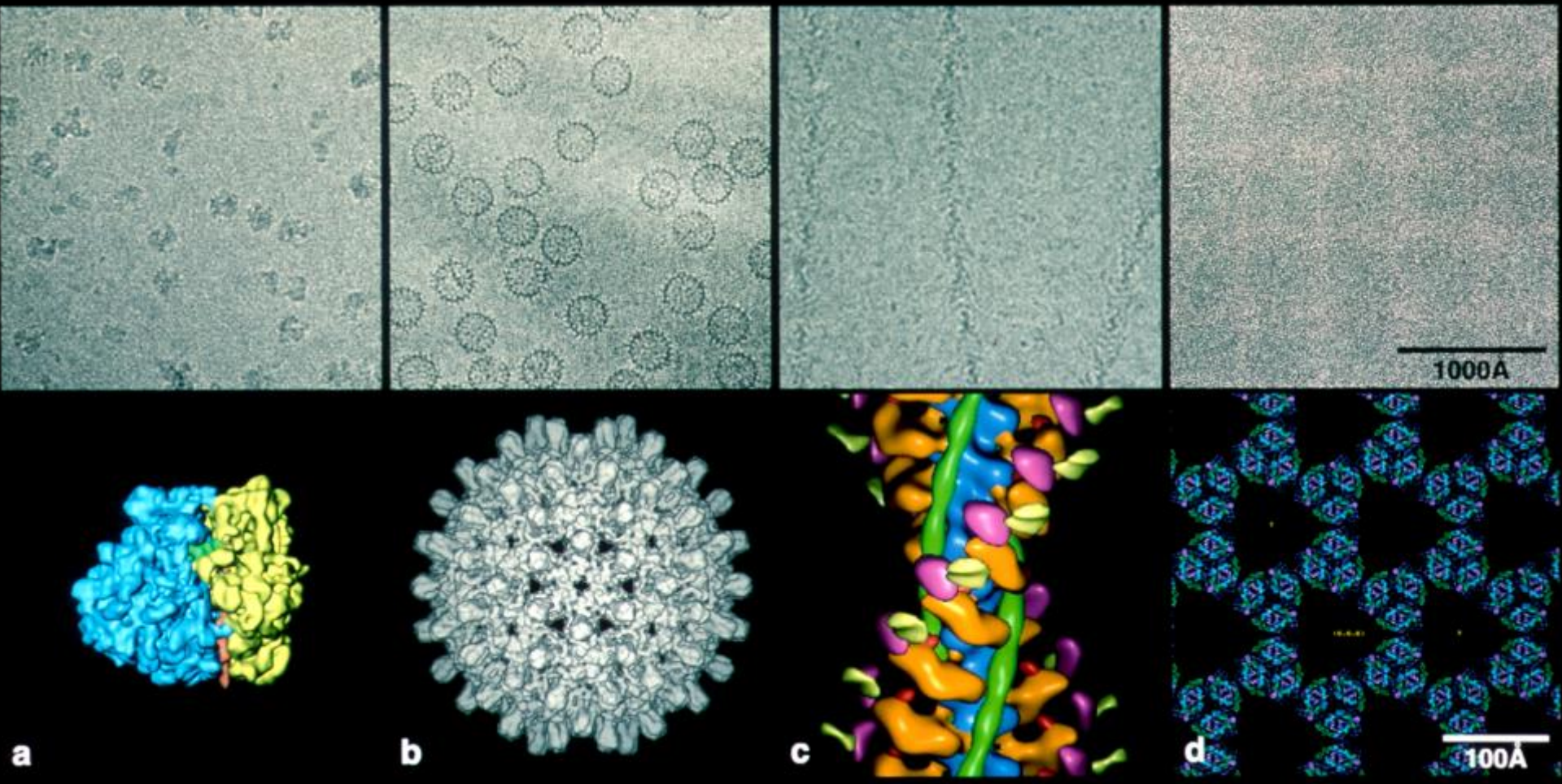
Richard Henderson

GORDON RESEARCH CONFERENCE
Hawthorne College
THREE DIMENSIONAL ELECTRON
MICROSCOPY OF MACROMOLECULES
Wah Chiu, chairman
P.N.T. Unwin, vice chairman
July 8-12, 1985
Achber Studio, Laconia, N.H.

From Baker & Henderson (2001) Int.Tab.Cryst.Vol.F, on-line (2006), revised (2011)

What is electron cryomicroscopy? - CryoEM comes in several flavours

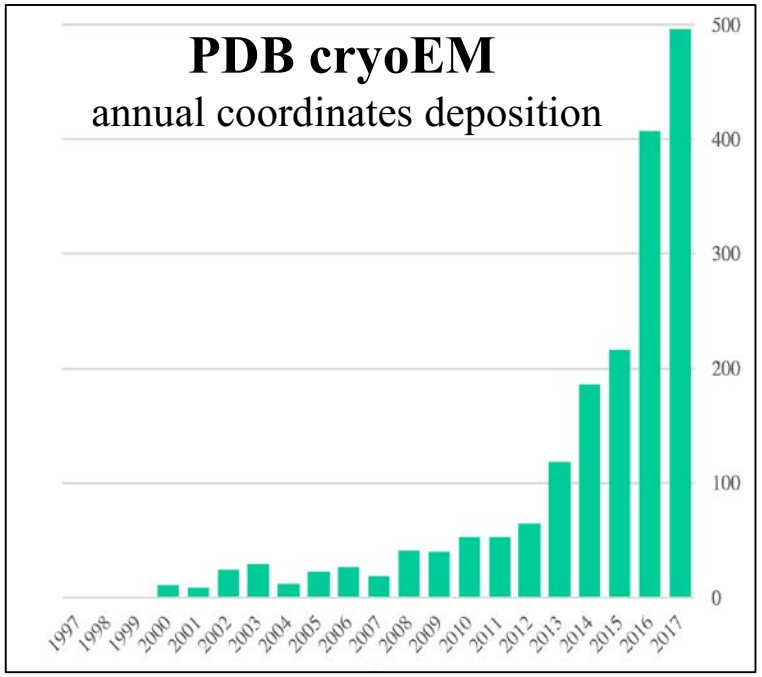
70S ribosome 11 Å | hepatitis B cores 7 Å | decorated actin 30 Å | LHC II 3.4 Å



Gabashvili (2000) | Boettcher (1997) | Milligan (~1995) | Kuhlbrandt (1994)


Three Dimensional Electron Microscopy
Gordon Research Conference
June 11-16, 2017
Les Diablerets Conference Center, Les Diablerets, Switzerland
Chair: John Briggs
Vice Chair: John Rubinstein
VICTORIA
Les Diablerets
GLACIER 3000
LES DIABLERE

Growth in cryoEM community



3dem mailing list

Started in 1995 by Ross Smith at NYU
transferred to NCMIR at UCSD in 2000
Gina Sosinsky, now Guy Perkins
Current list has **3202** subscribers

Gordon Research Conference on 3dem

started by Wah Chiu & Nigel Unwin in 1985, held every year rotating between USA, Europe & Asia

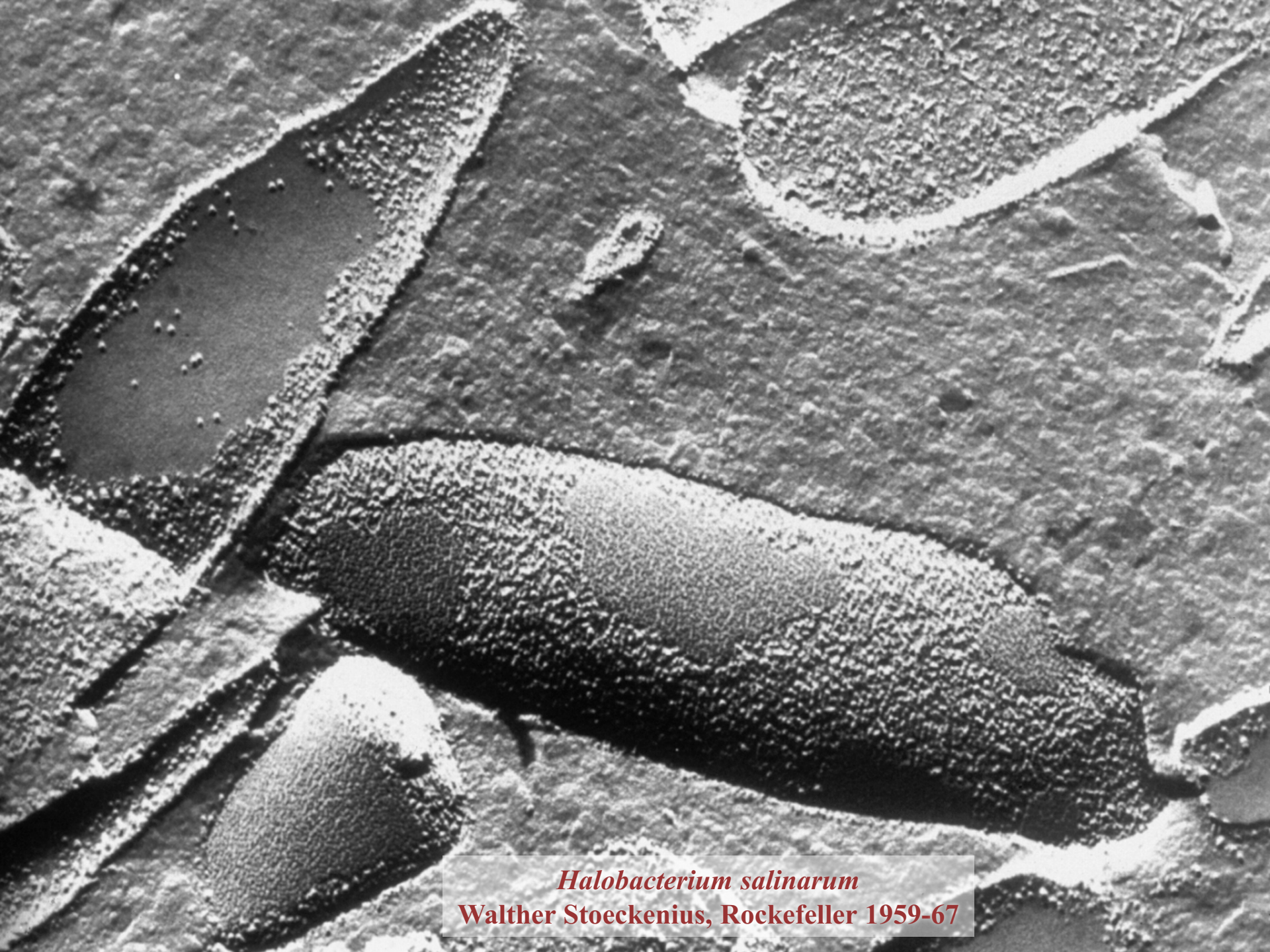
EMDB

(Electron Microscopy Data Bank) for deposition of cryoEM maps (5400)

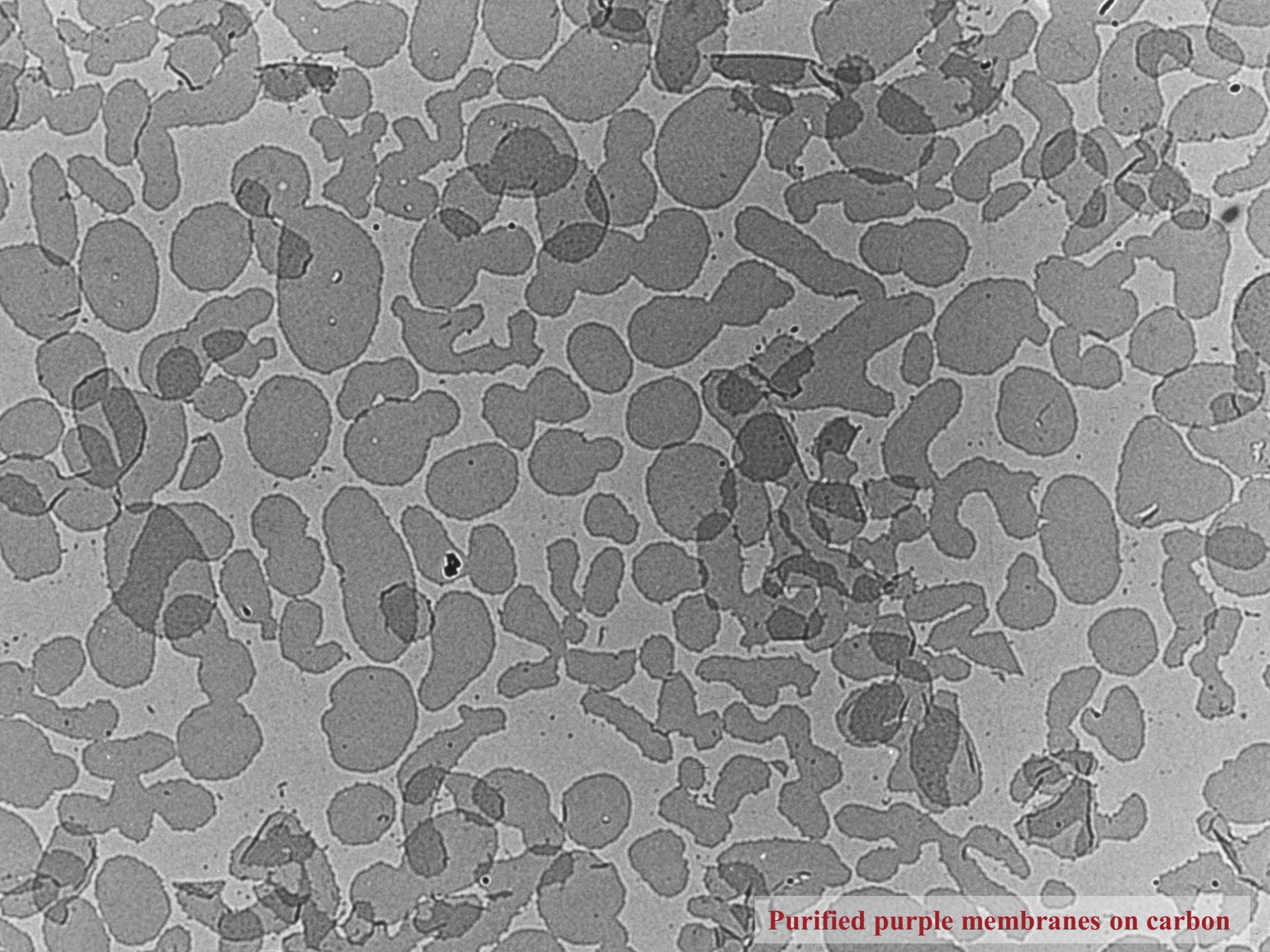
PDB (Protein Data Bank) for coordinate depositions (less)

Outline

1. X-ray diffraction, electron crystallography (diffraction, microscopy)
2. Bacteriorhodopsin at 7Å, then 3.5Å, refinement & kinetics
3. Single particle cryoEM - blobology then “resolution revolution”



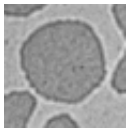
Halobacterium salinarum
Walther Stoeckenius, Rockefeller 1959-67



Purified purple membranes on carbon

X-ray powder diffraction pattern of oriented pellet of purple membrane 1973

bacteriorhodopsin projection

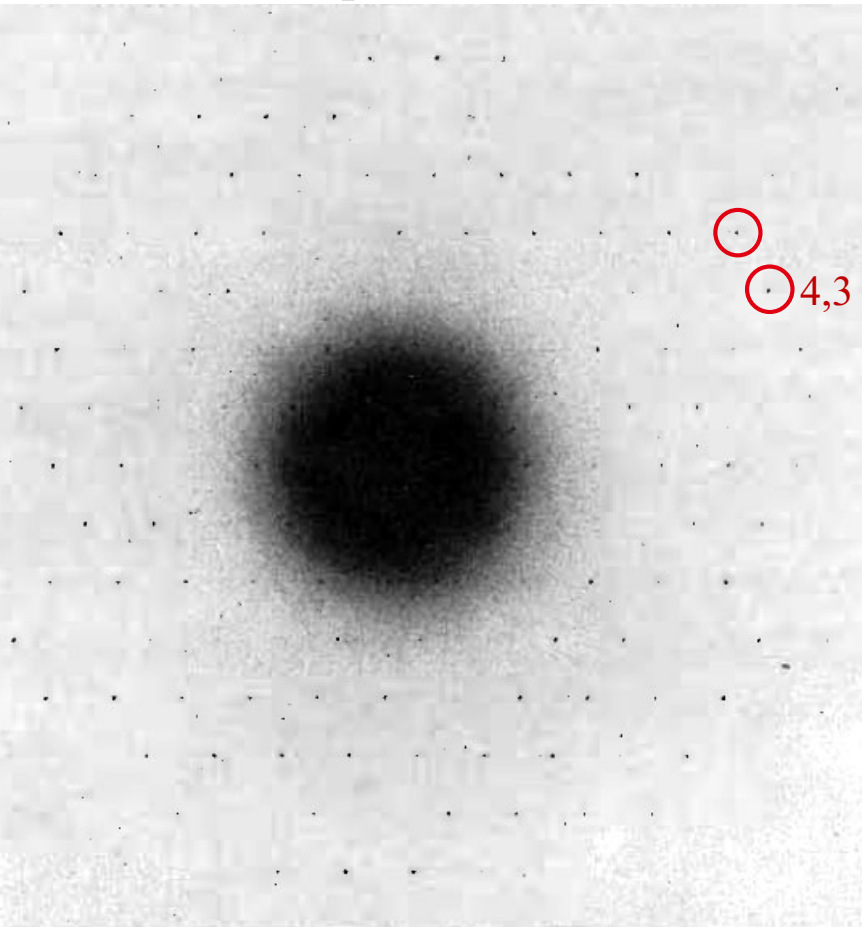


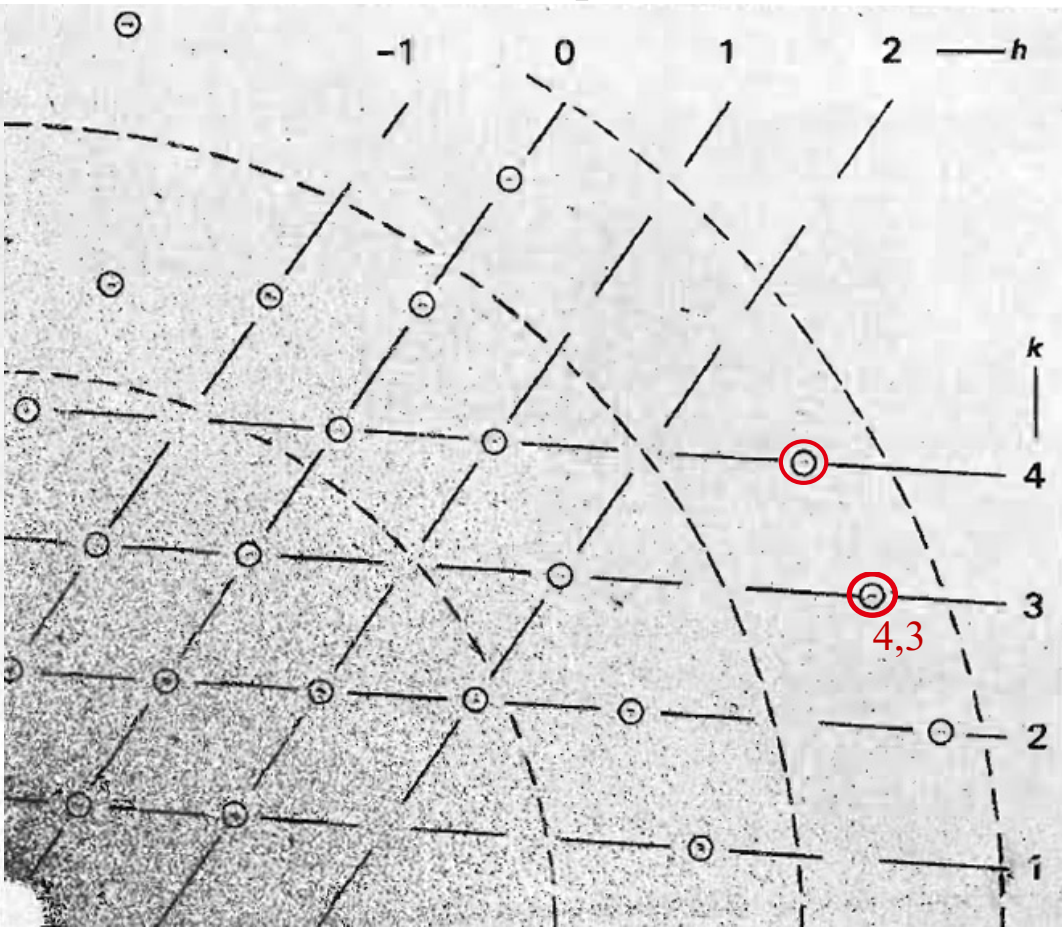
glucose embedding, no cryoEM

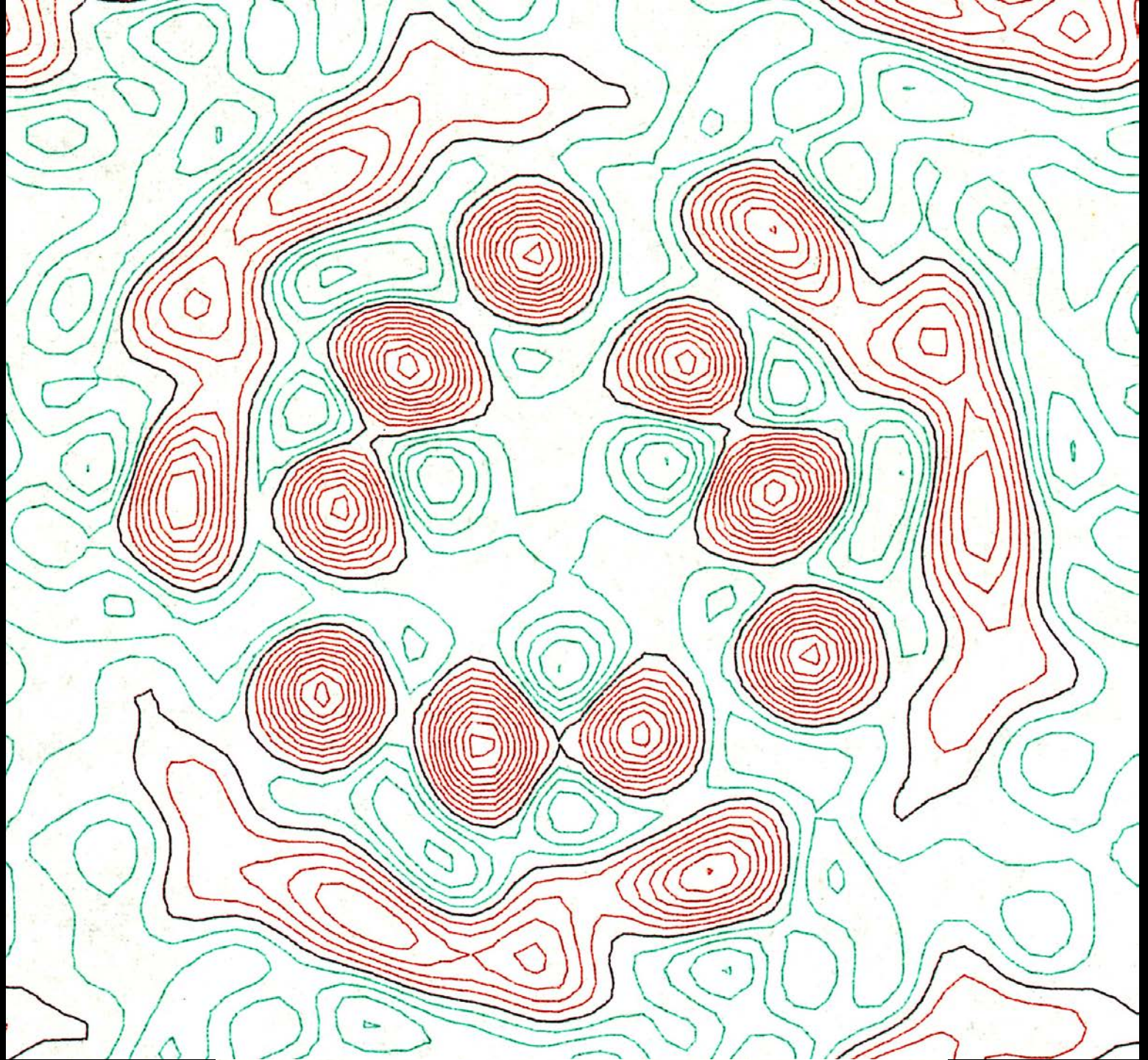
Unwin & Henderson JMB, 1975

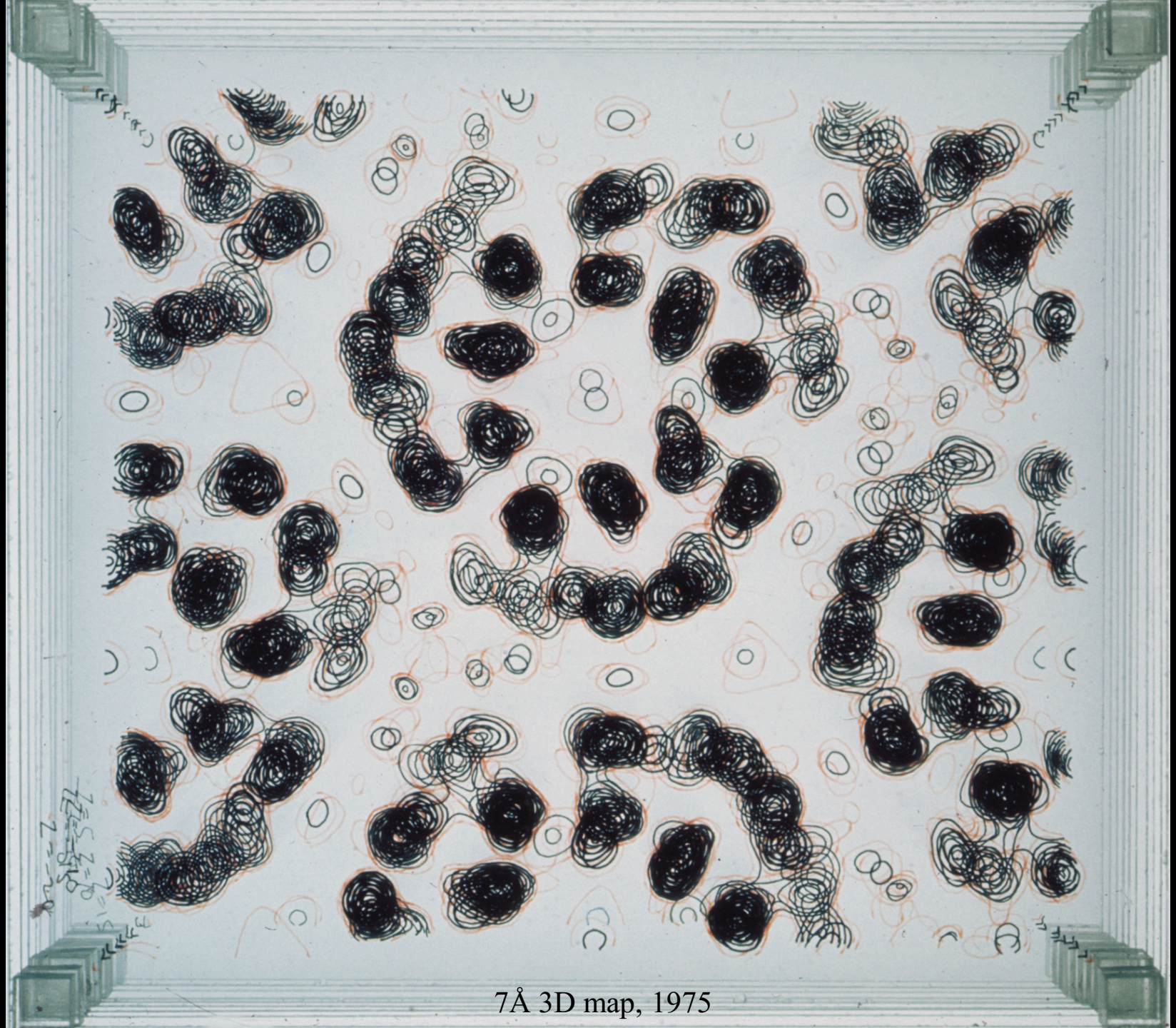
electron diffraction
(amplitudes)

optical diffraction of electron micrograph
(FFT --> phases)

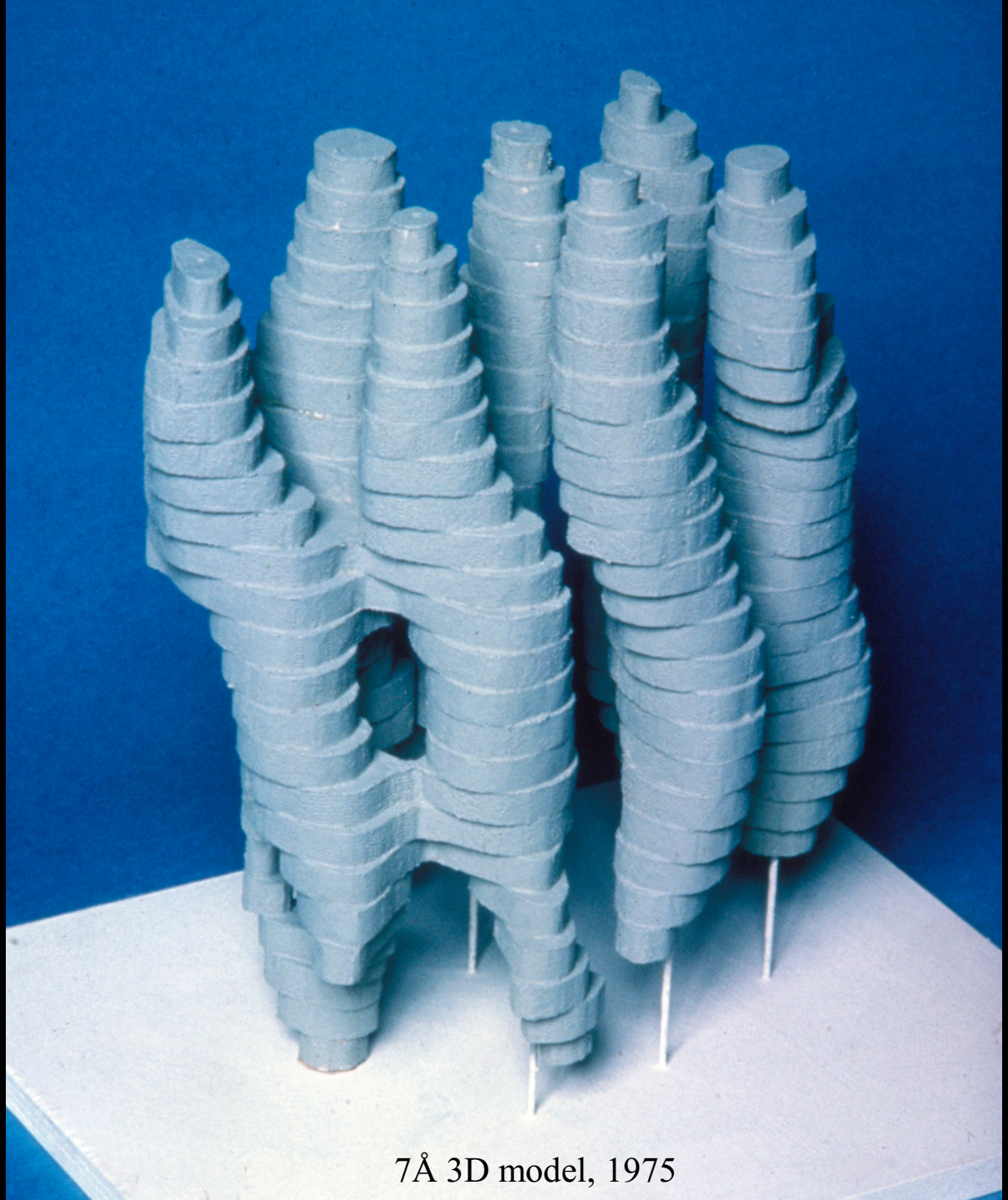








7Å 3D map, 1975



7Å 3D model, 1975

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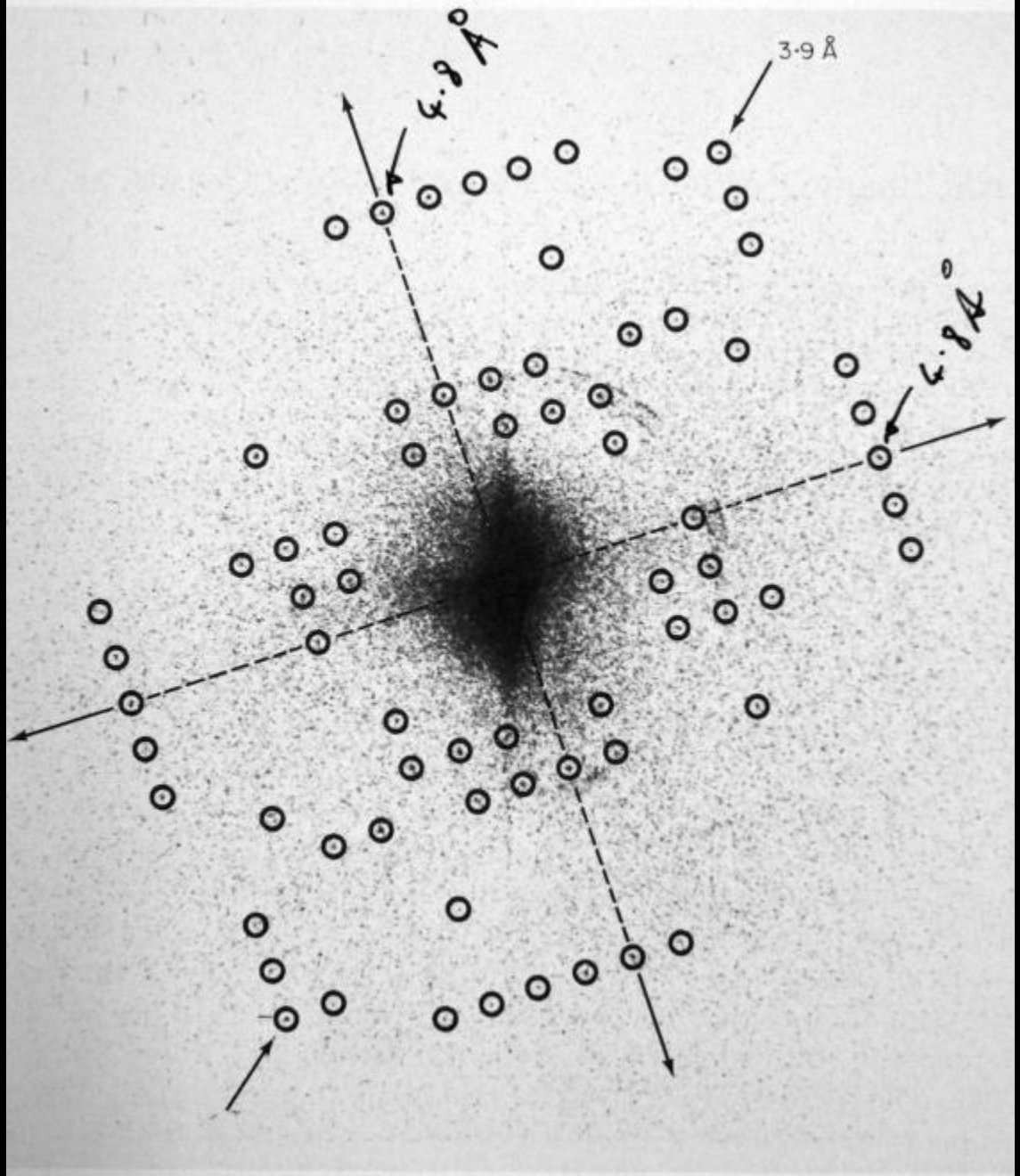
Faruqi CCD detectors

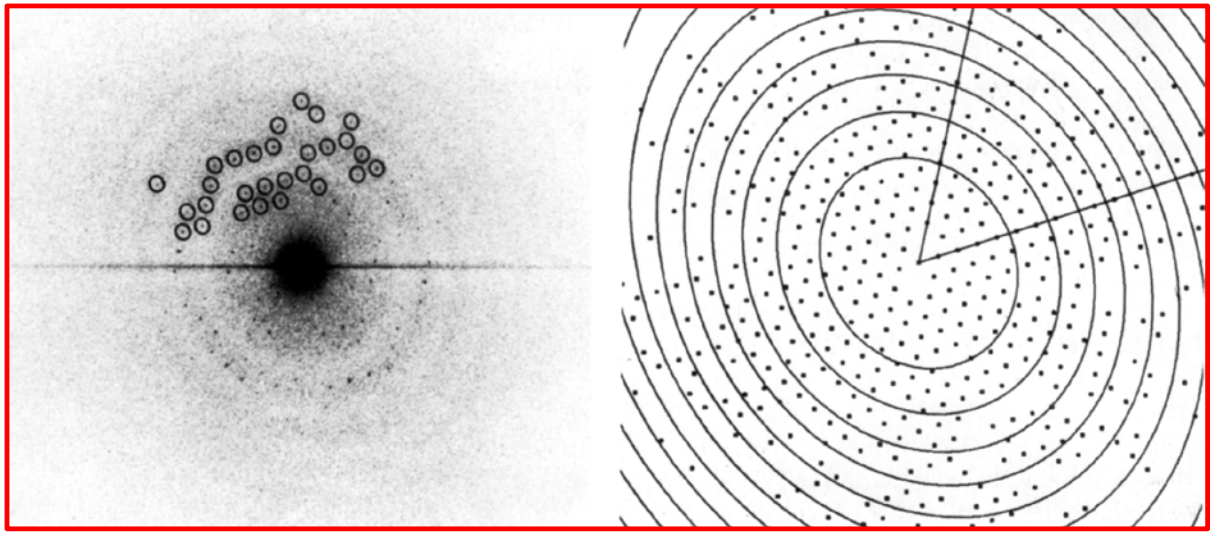


Optical diffractogram of a medium low dose (10 electrons/Å^2) image of crotoxin

Jeng, Chiu, Zemlin & Zeitler, 1984 JMB
Crotoxin thin 3D xtals

Solving bacteriorhodopsin structure at high resolution

High-resolution cryoEM imaging



heavy atom derivatives

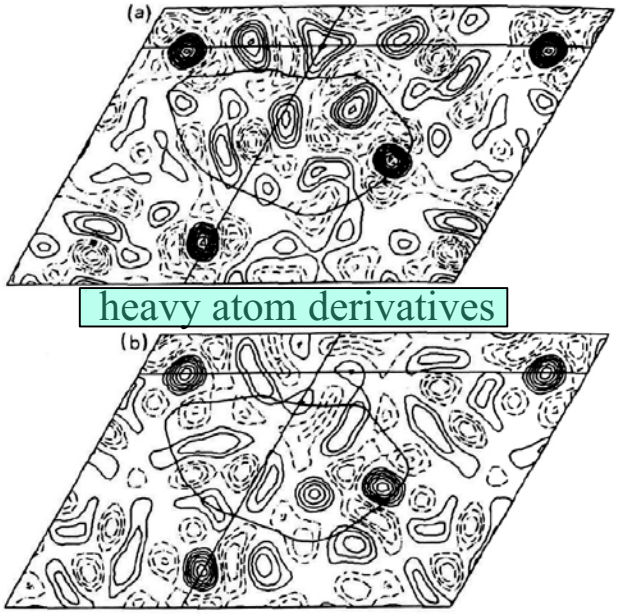


Fig. 5. PTA derivative difference maps at 6Å resolution. a) Projection map; several peaks can be seen within the protein boundary. b) Section of 3-D map near the cytoplasmic surface of the membrane, showing one of the major sites located in 3-D.

molecular replacement

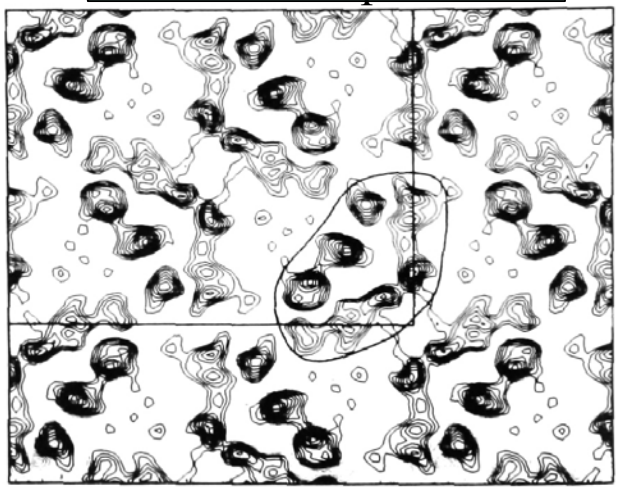


Fig. 4. The final averaged map for the $p22_12_1$ (small cell) structure. Fourier components are included to a resolution of 3·3 Å. Phases are heavily weighted towards the observed structure out to about 6 Å, and determined entirely by molecular replacement between 6 and 3·3 Å.

Model building: With this approach, a simple atomic model is used to generate approximate phases to high resolution, which are then refined by successive approximation using reciprocal space refinement and search procedures together with difference Fourier methods to follow progress in real space. In our case, our initial model was seven straight lengths of α-helical polyalanine about 25-28 amino acids in length. Bending and kinking of these helices is then allowed followed by addition of lipid molecules. Finally side chains are introduced into the body of the protein and attempts made to interpret the resulting maps in terms of the known amino acid sequence of the protein.

The path from 7 Å resolution to 3.5 Å and atomic model

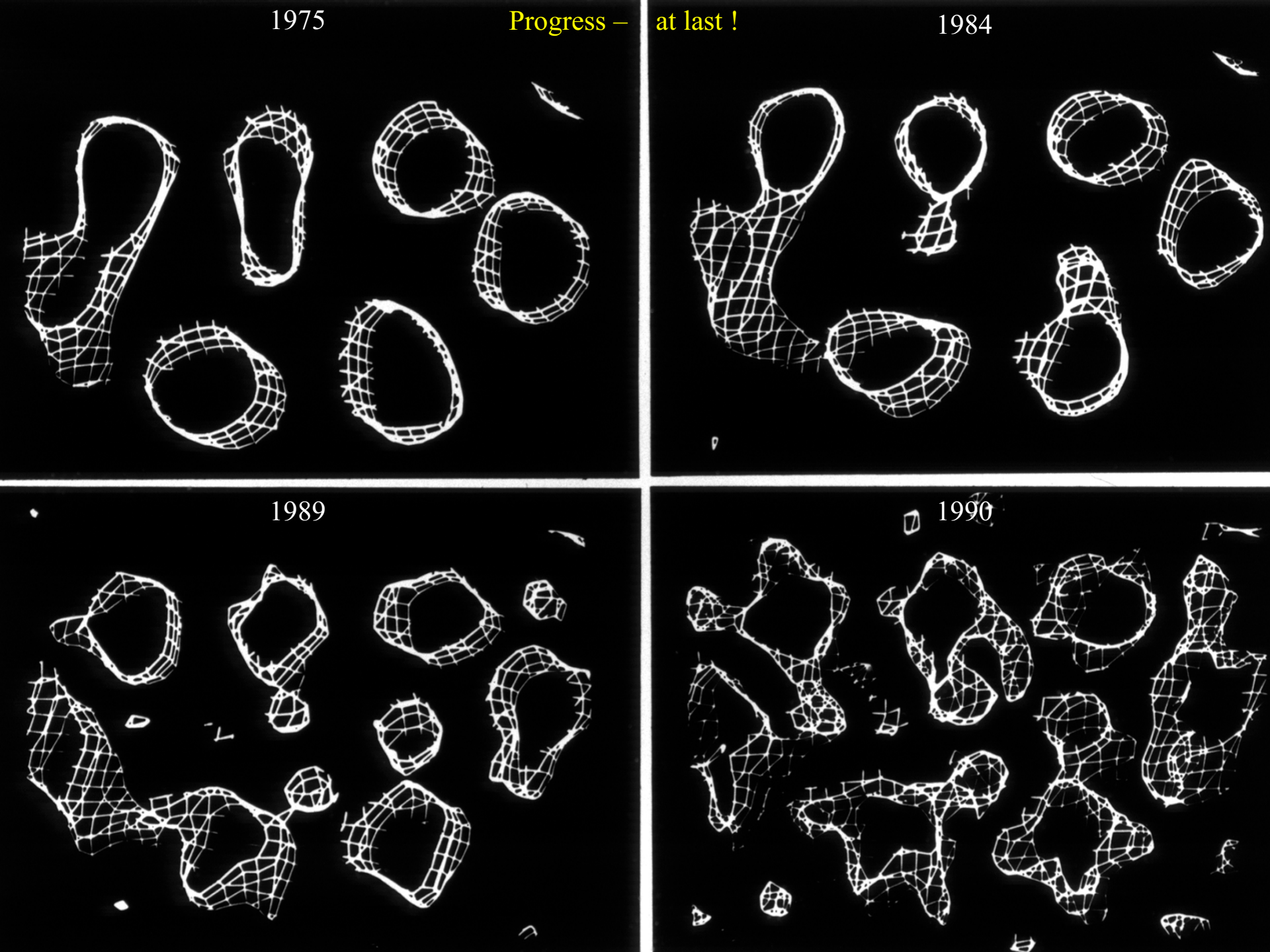
- Cooling specimen to liq. N_2 or liq. He temperature reduces the effects of radiation damage and gives 4- to 5-fold increase in diffraction
- Very few electron microscopes were stable enough in 1980s to achieve imaging with 3.5 Å resolution using cold stages
- Collaborations with and travelling to three different labs were essential:

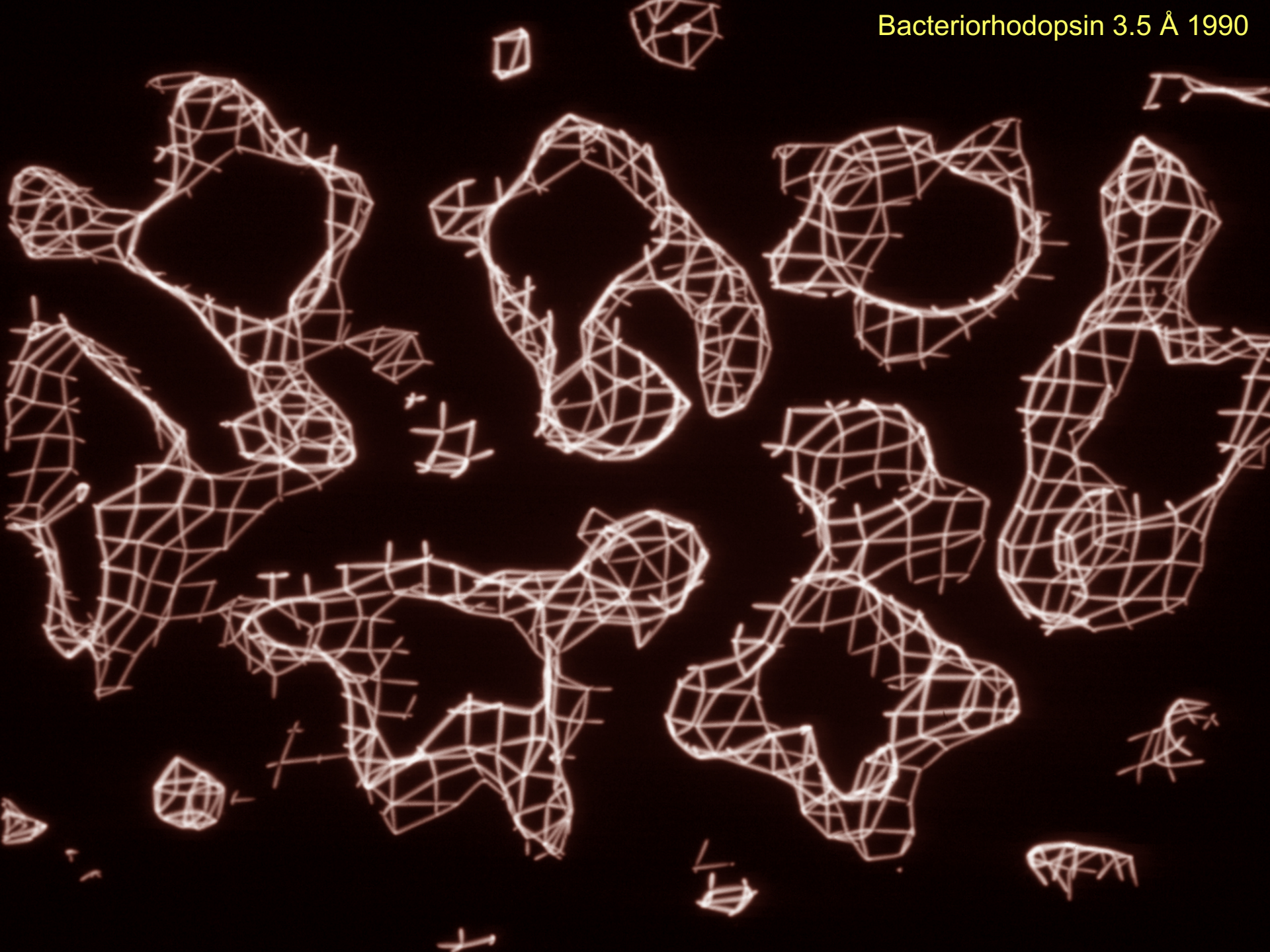
  Lepault/Dubochet at EMBL

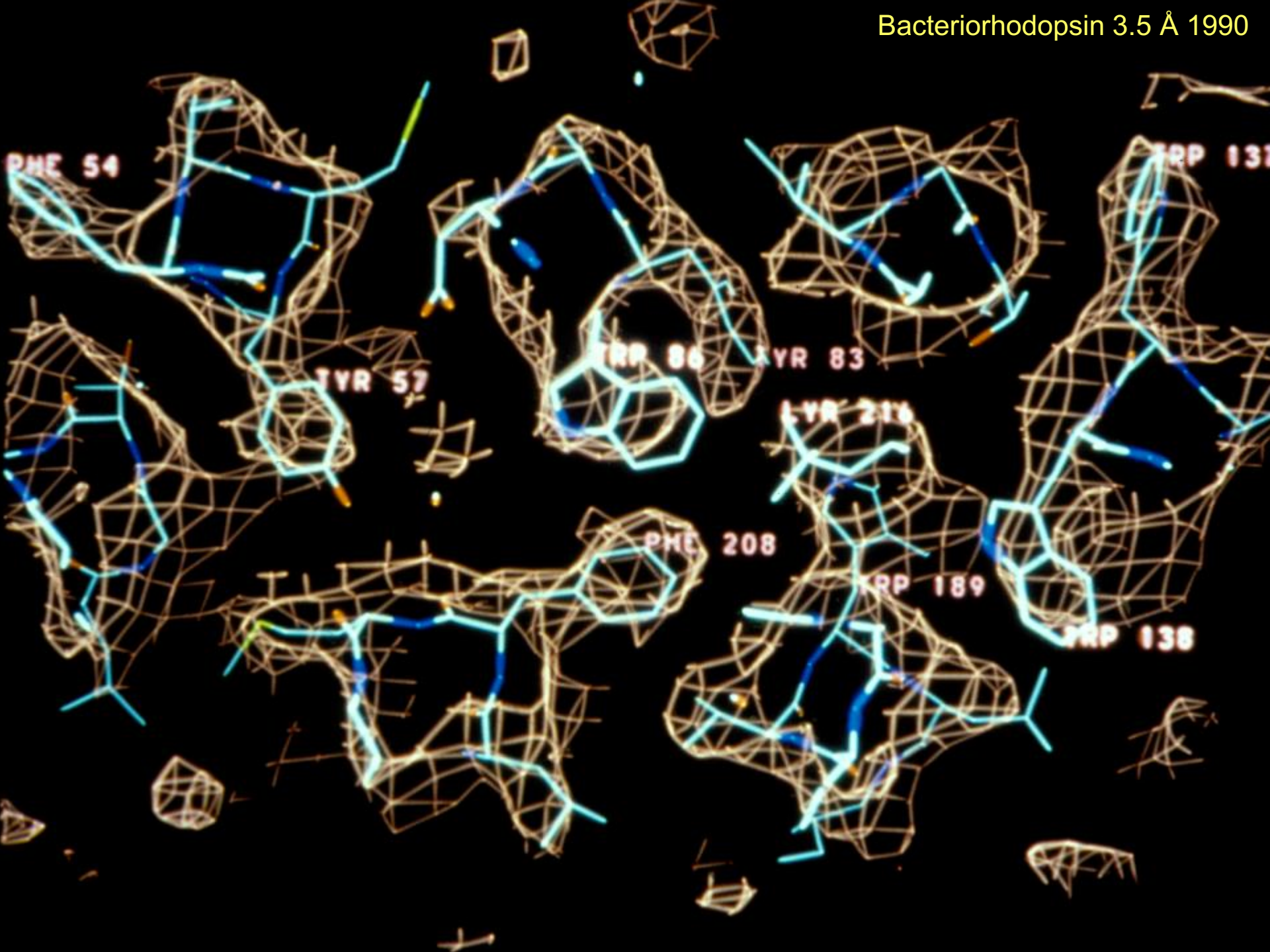
  Zemlin/Beckmann/Zeitler at Fritz-Haber-Institute in Berlin

  Downing/Glaeser at Berkeley

- Beam tilt was a key feature that required computational correction
- Correcting for defocus gradient when tilting specimen was another technical challenge
- Finally, 70 images allowed a map to be calculated, adequate to build an atomic model
- Refinement by Niko Grigorieff + increase to 100 images with 30 more from Ken Downing
- Yoshi Fujiyoshi independently determined the structure with an improved map
- All subsequent X-ray structures used the cryoEM coordinates for molecular replacement


1975
Progress –
at last !
1984
1989
1990


Bacteriorhodopsin 3.5 Å 1990


Bacteriorhodopsin 3.5 Å 1990
PHE 54
TYR 57
TRP 86
TYR 83
TYR 216
PHE 208
TRP 189
TRP 138

Outline

1. X-ray diffraction, electron crystallography (diffraction, microscopy)
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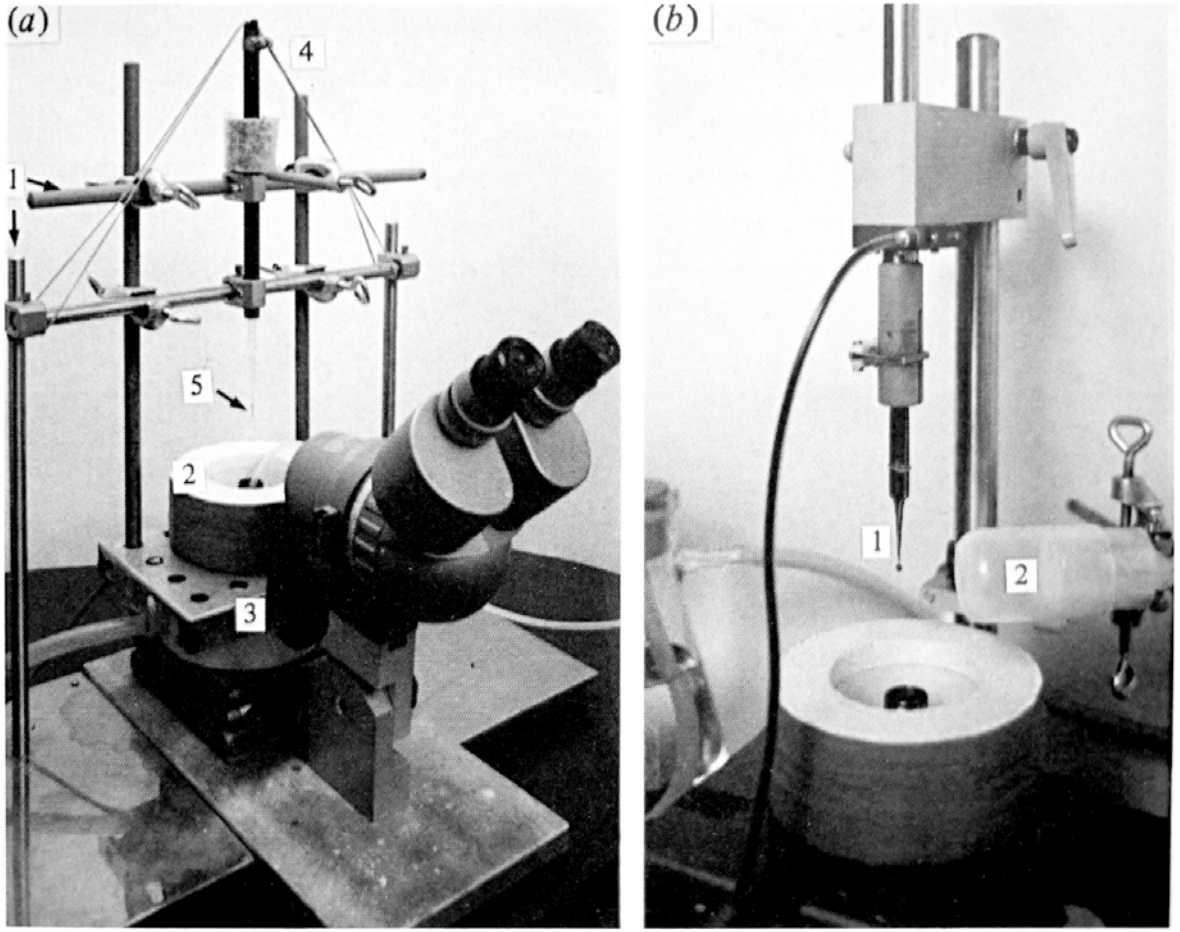
From Dubochet et al, QRB (1988)



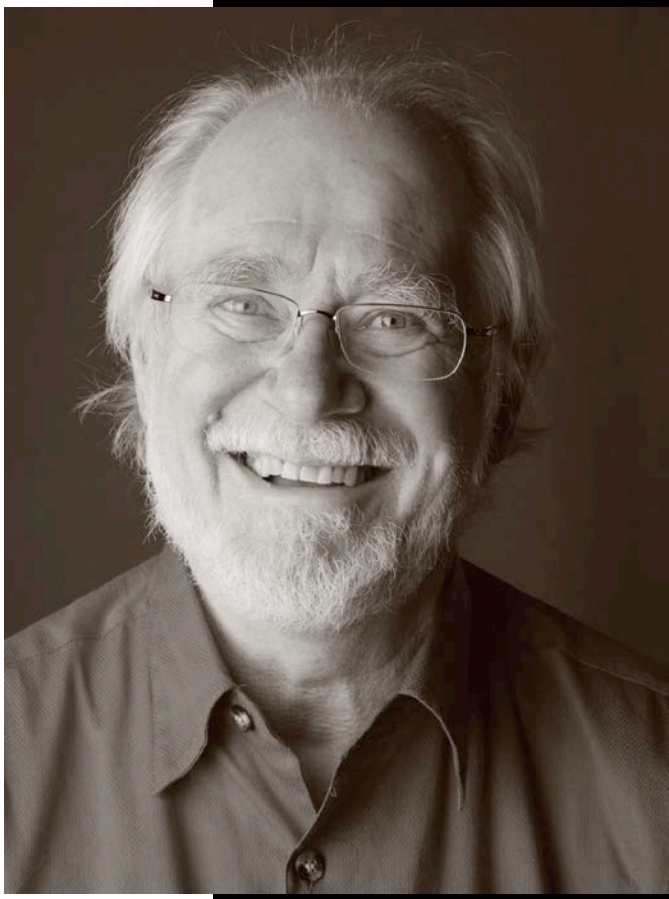
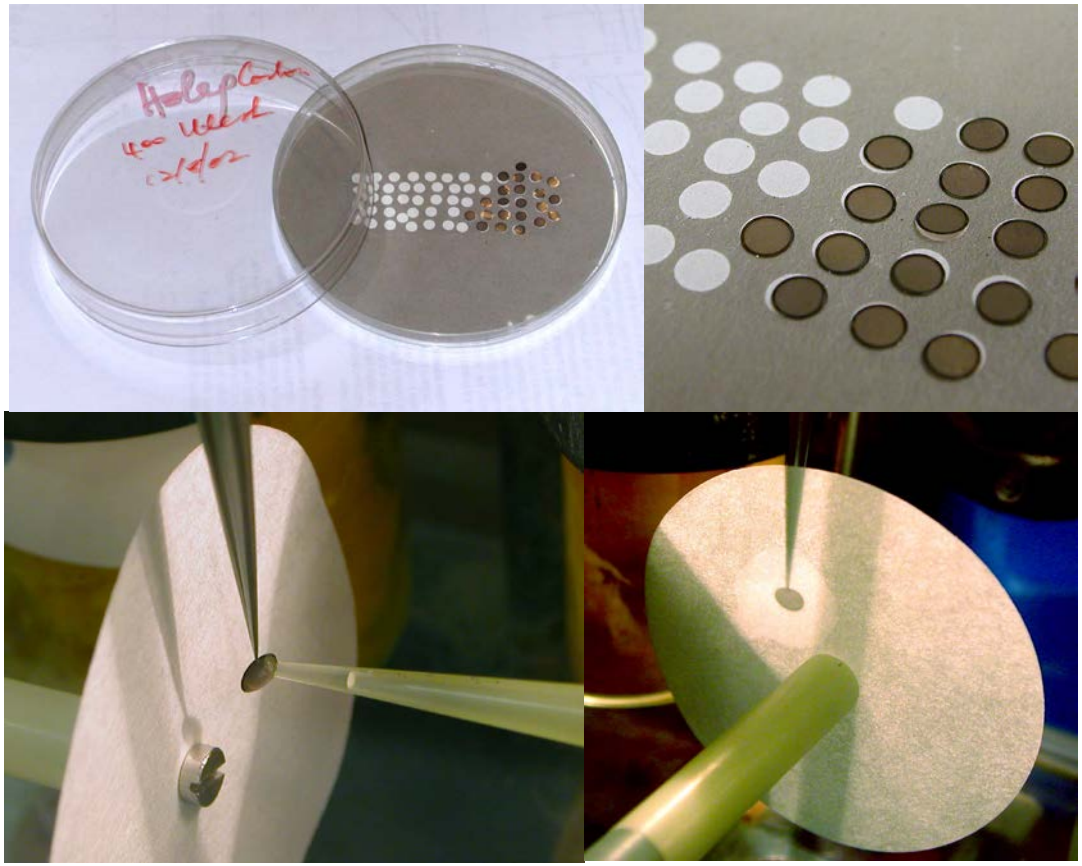
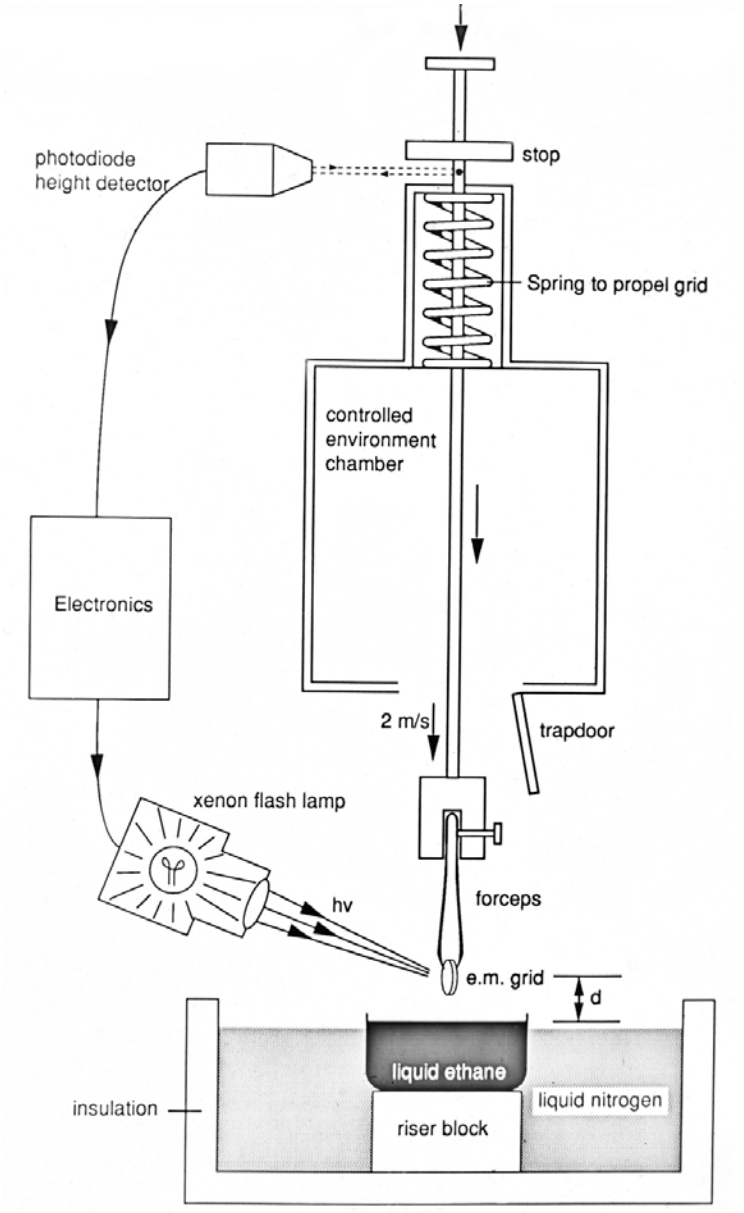


Fig. 43. Plunger for freezing. (*a*) Simple apparatus equipped here for freezing bulk specimens. (1) Retort stand clamps; (2) liquid nitrogen and ethane dewars; (3) water-driven magnet; (4) plunger with elastic band propulsion; (5) specimen support. (*b*) A more elegant freezing apparatus equipped for preparing thin vitrified layers of suspensions. (1) Tweezer holding the specimen support grid; (2) humidified air outlet.

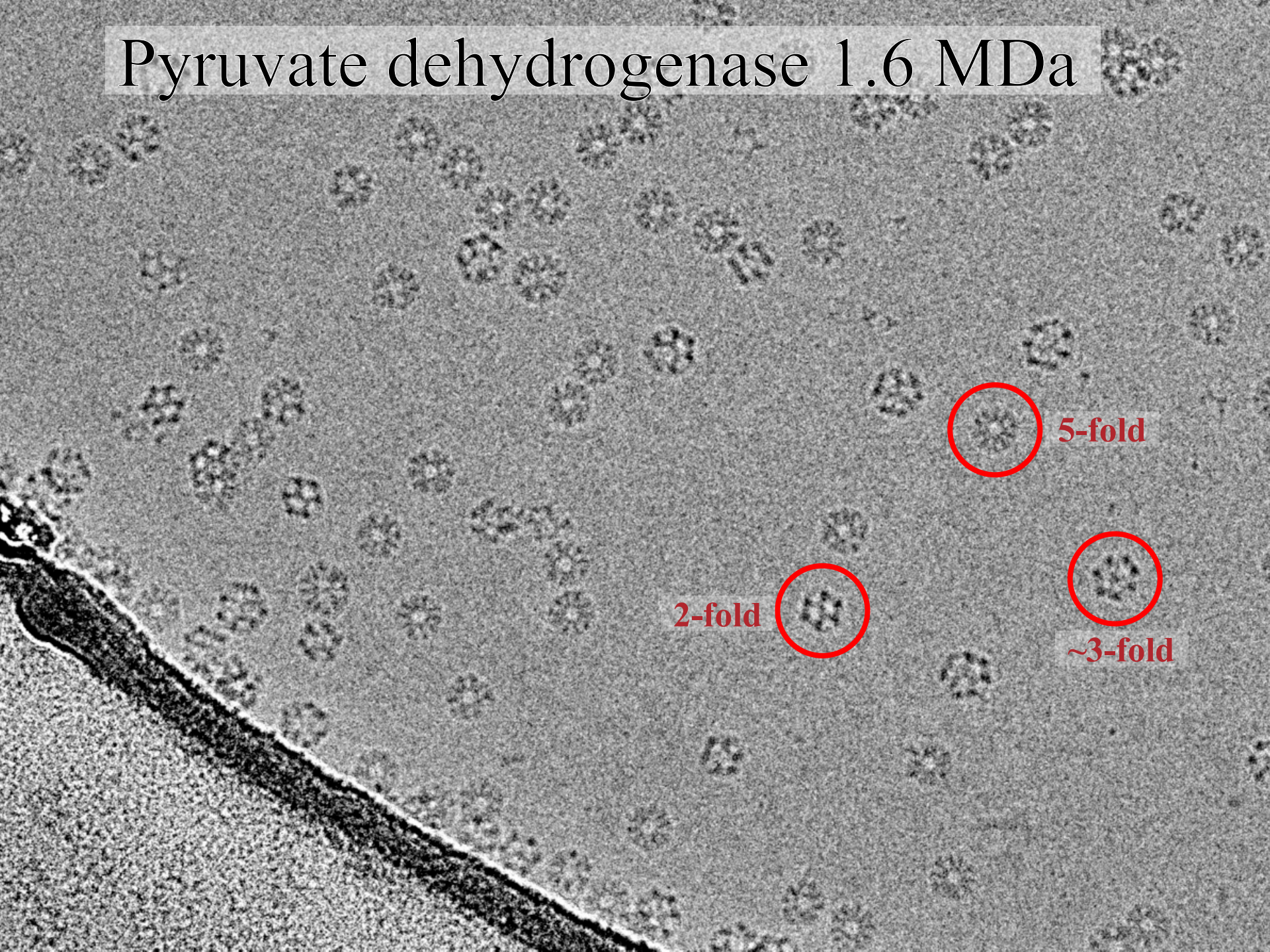
Preparing a cryoEM grid using the method of Adrian, McDowell & Dubochet





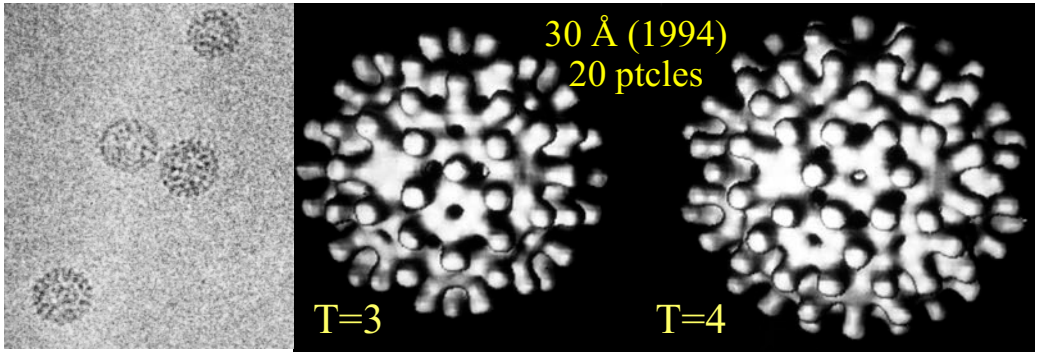
Controlled environment plunge-freeze apparatus of Ishi Talmon (1988)

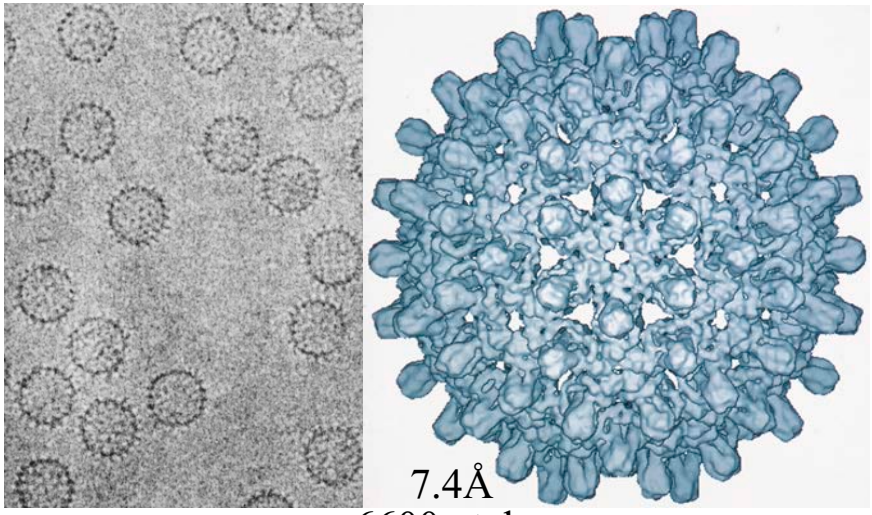
Pyruvate dehydrogenase 1.6 MDa



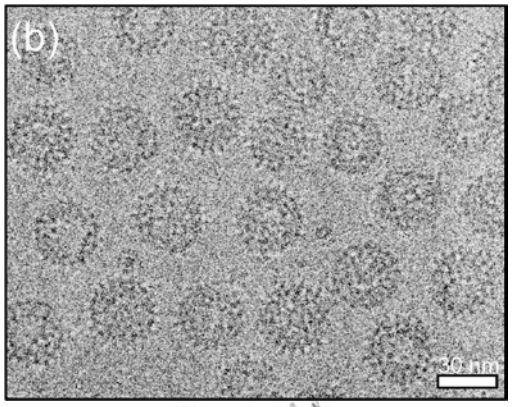
hepB virus cores (1997)
first sub-nm single particle structure

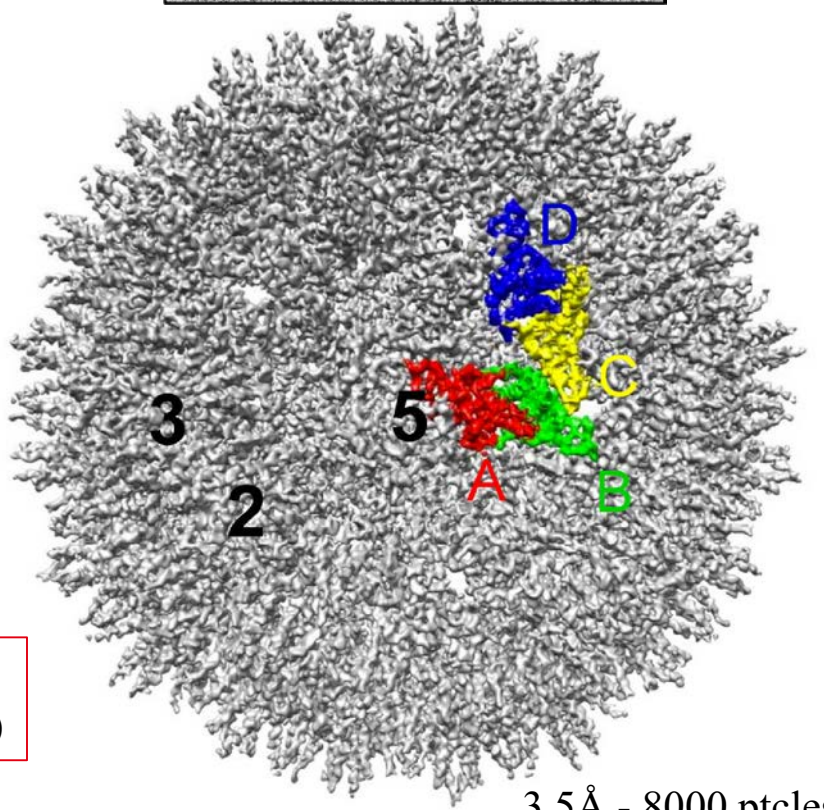
hepB most recent structure
Yu, Jin, Jih, Shih & Zhou (2013)





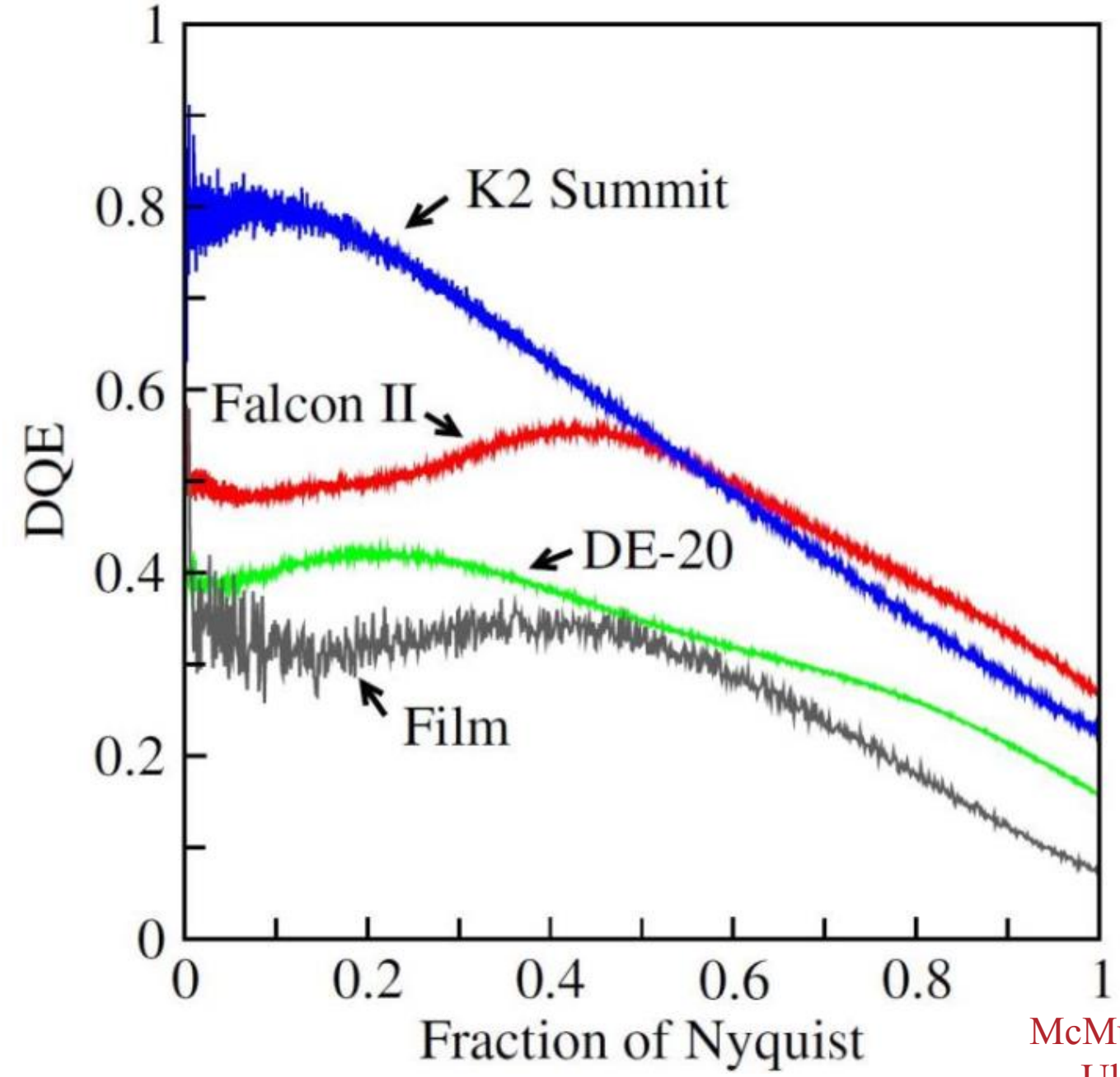
Boettcher, Wynne
& Crowther (1997)



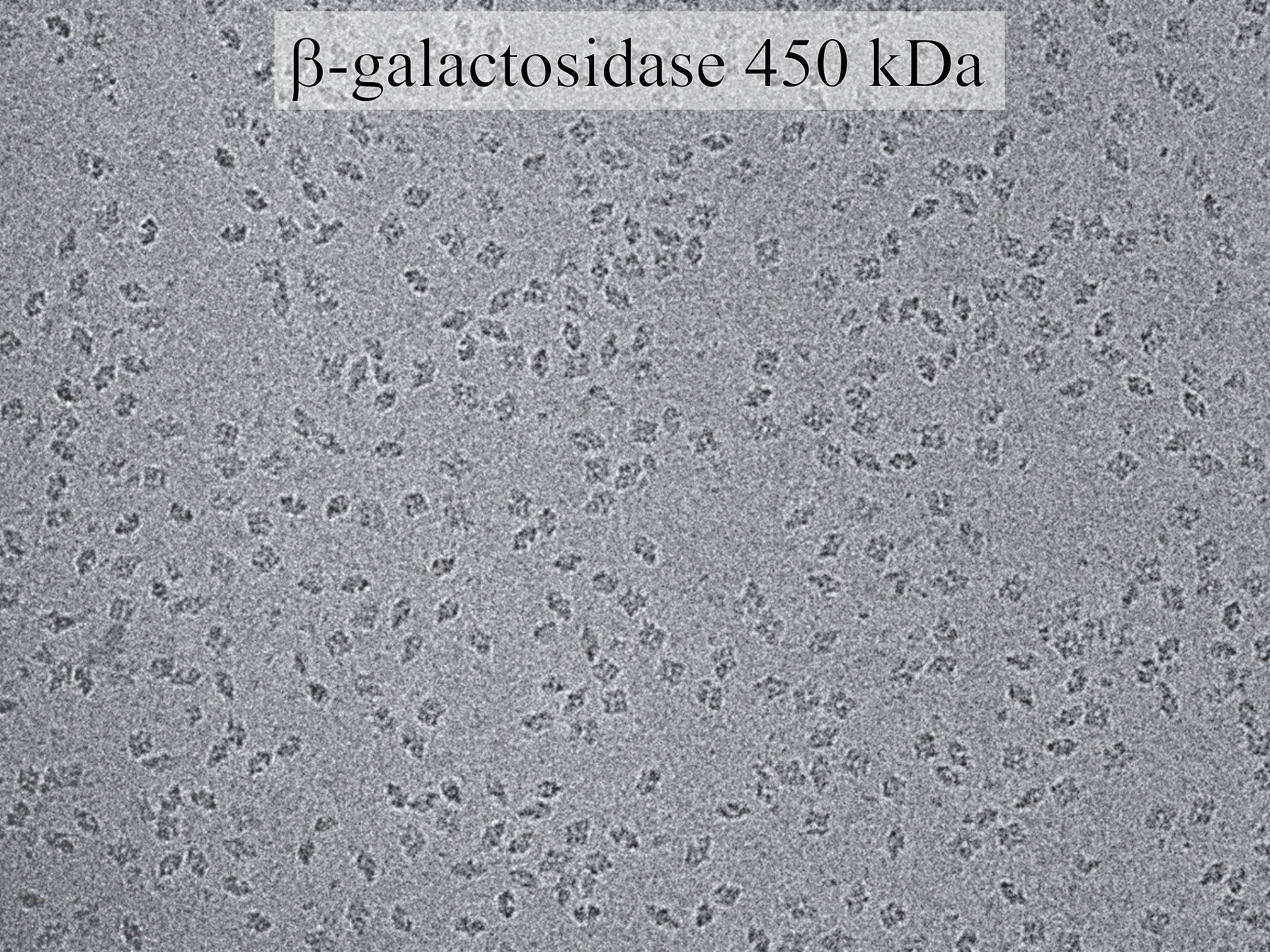


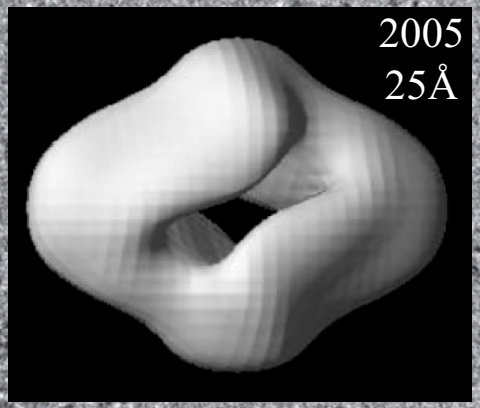
3.5Å - 8000 ptcles

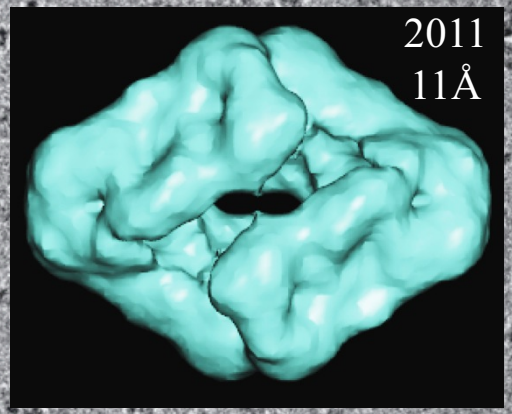
Comparison of 300keV DQE of direct electron detectors versus film

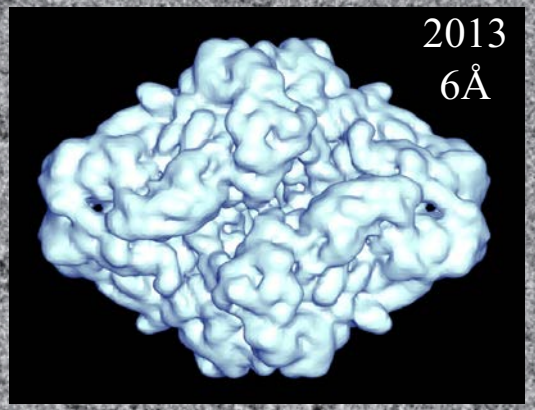


McMullan et al (2014)
Ultramicroscopy

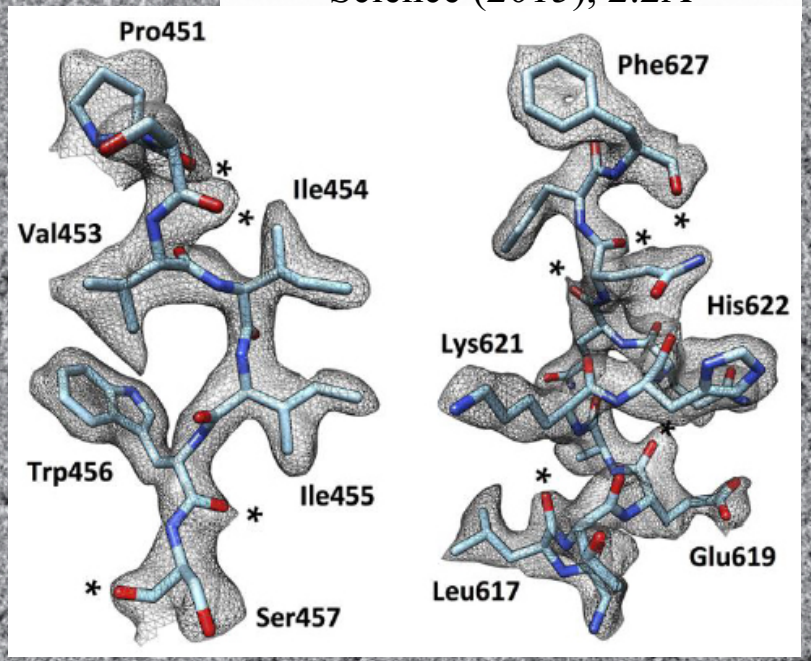

β-galactosidase 450 kDa



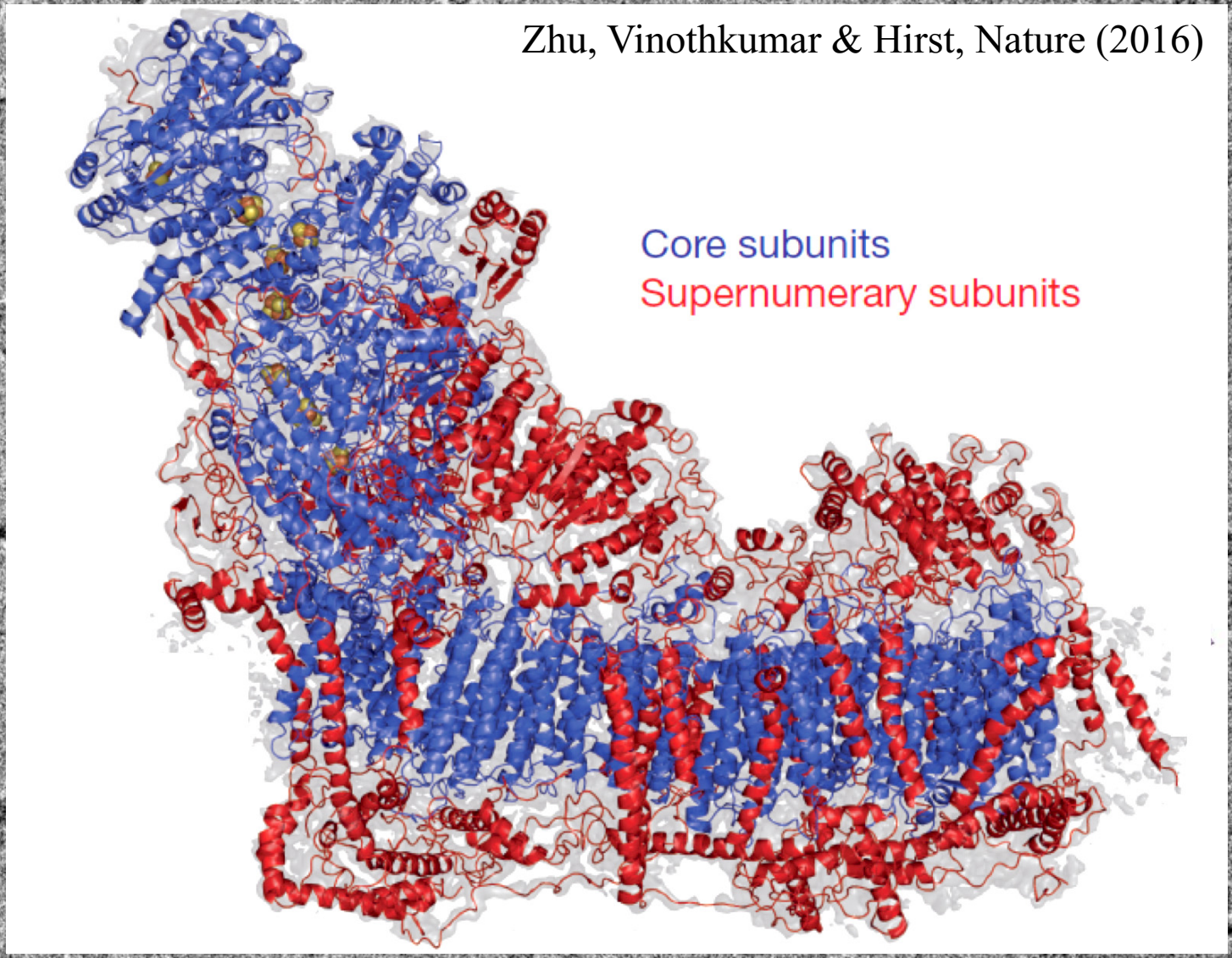


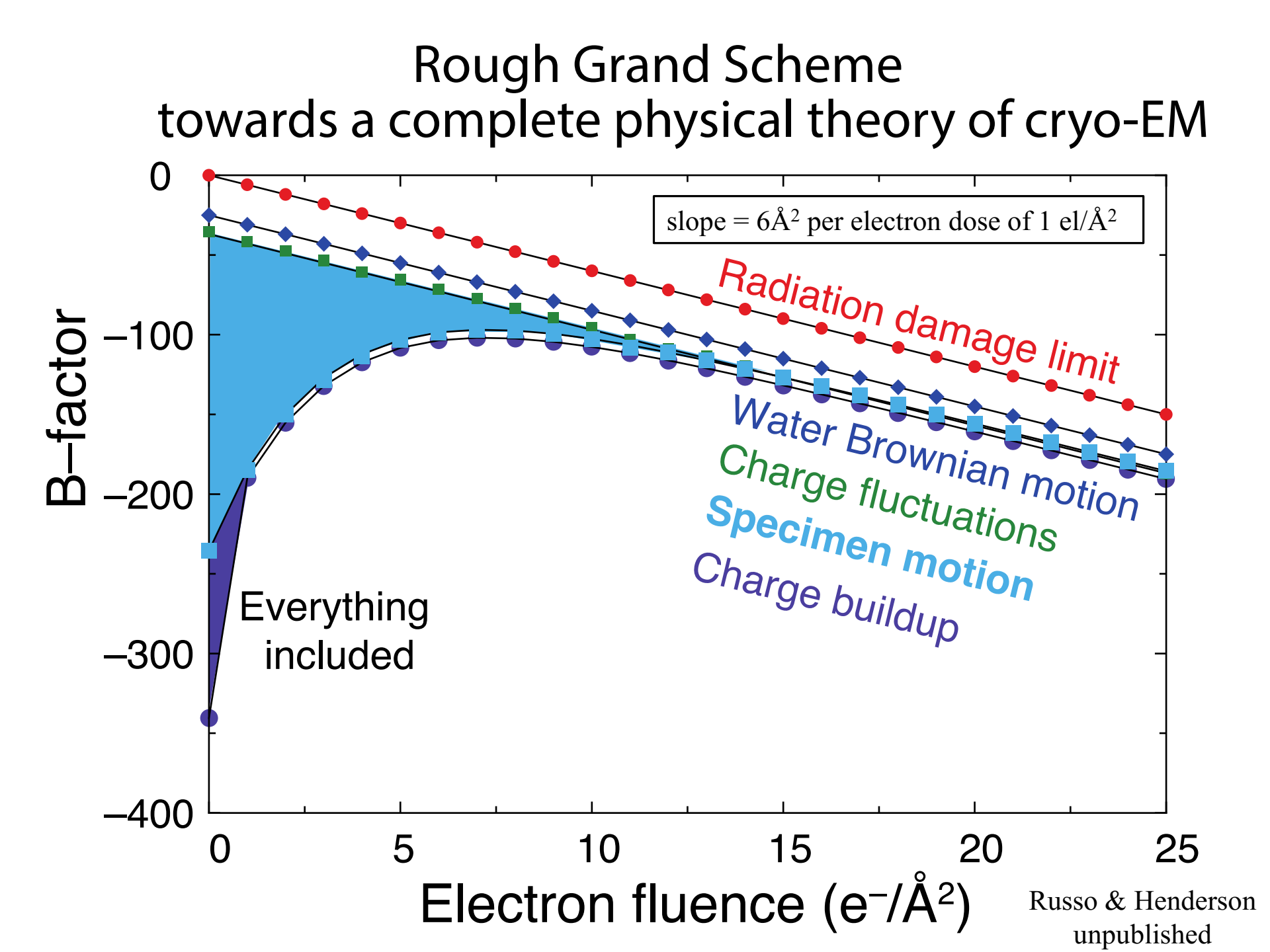


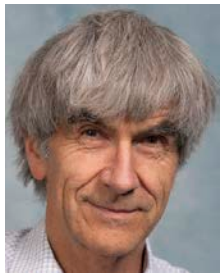
Bartesaghi et al & Subramaniam
Science (2015), 2.2Å



Mitochondrial Complex I 900 kDa



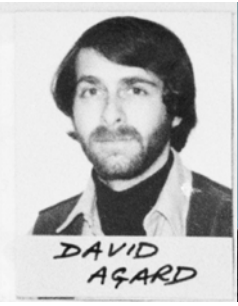

Rough Grand Scheme
towards a complete physical theory of cryo-EM
slope = 6Å² per electron dose of 1 el/Å²
Radiation damage limit
Water Brownian motion
Charge fluctuations
Specimen motion
Charge buildup
Everything
included
B–factor
0
–100
–200
–300
–400
0
5
10
15
20
25
Electron fluence (e⁻/Å²)
Russo & Henderson
unpublished



Nigel Unwin
bacteriorhodopsin 7Å

Colleagues in rough chronological order

bacteriorhodopsin 3.5Å using cryoEM





Joyce Baldwin Tom Ceska David Agard Bob Glaeser Jean Lepault Fritz Zemlin Erich Beckmann Ken Downing





detector & microscope
technical developments

Wasi Faruqi Greg McMullan Renato Turchetta Nicola Guerrini Shaoxia Chen






single
particle
cryoEM

Sriram Subramaniam Niko Grigorieff Peter Rosenthal Chris Russo

Jacqueline Milne John Rubinstein Vinothkumar

With many thanks to current and previous colleagues

- Electron crystallography — Nigel Unwin, Tom Ceska, Joyce Baldwin, David Agard, Jean Lepault, Fritz Zemlin, Erich Beckmann, Ken Downing, Bob Glaeser, Niko Grigorieff, Sriram Subramaniam
- Single particle cryoEM — Niko Grigorieff, Sriram Subramaniam, Jacqueline Milne, Peter Rosenthal, John Rubinstein, Vinothkumar
- Detector development — Wasi Faruqi, Greg McMullan, Renato Turchetta, Nicola Guerrini, Shaoxia Chen
- Grand scheme — Chris Russo