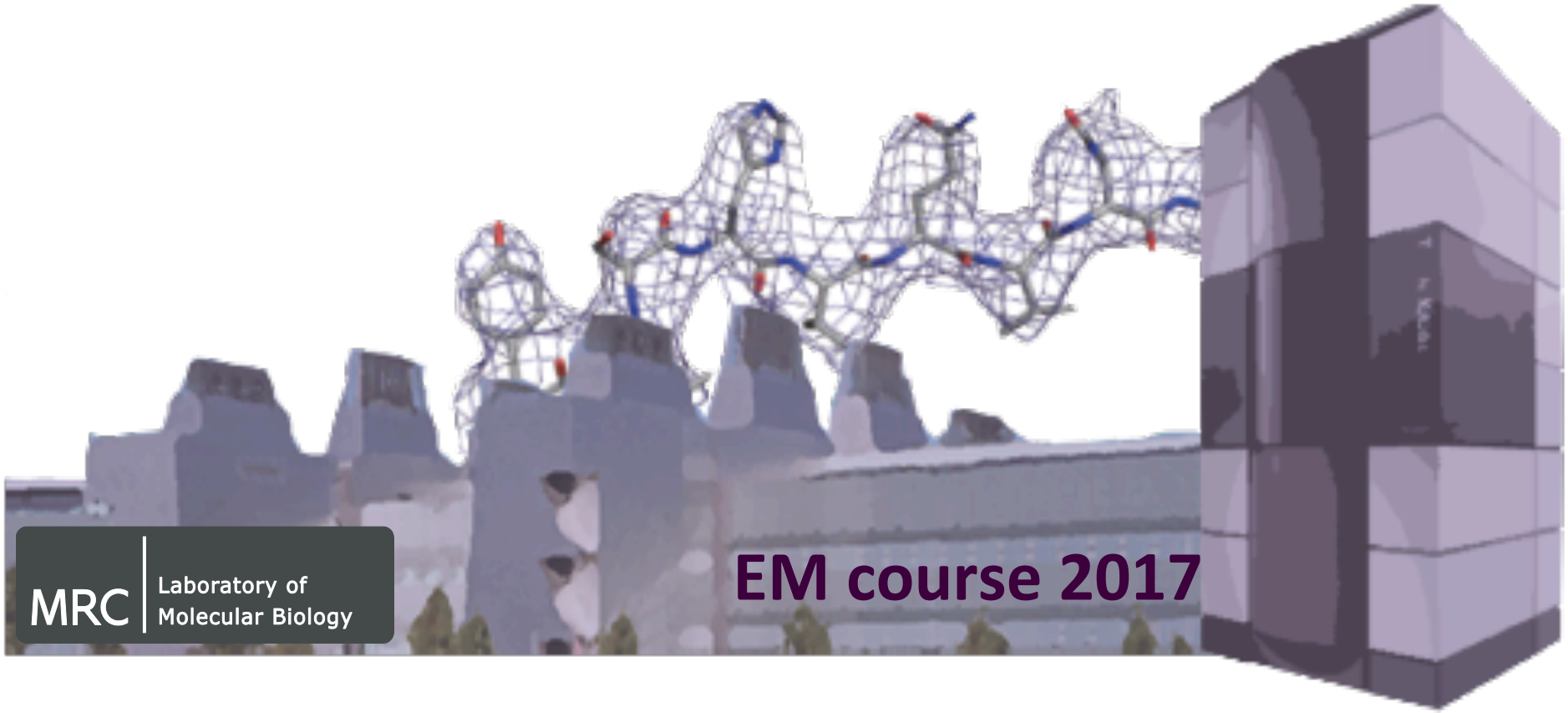
1. Past,Present & Future of Cryo-EM

Richard Henderson



Key concepts

Many people, ideas and technical advances have contributed

- Radiation damage makes individual images of molecules too noisy (averaging)
- Frozen specimens instead of environmental cells allow electron transmission
- Rapid plunge-freezing is a simple method to prepare thin specimens
- Electron microscopes (EMs) need a bright coherent source (FEG)
- EMs need very stable cold stages that do not drift or vibrate
- EMs need a very high vacuum to avoid specimen contamination
- Detectors with a high detective quantum efficiency are essential
- Computer programs need alignment algorithms that use all available information

Major milestones in single particle cryoEM

- Development of plunge-freezing - Dubochet et al, 1982
- Microscope technology - FEGs, stable cold stages, better vacuum

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- Detector technology - film, phosphor-CCD, direct electron detectors
- Computation - Spider, Imagic, EMAN, Frealign, Xmipp, Relion

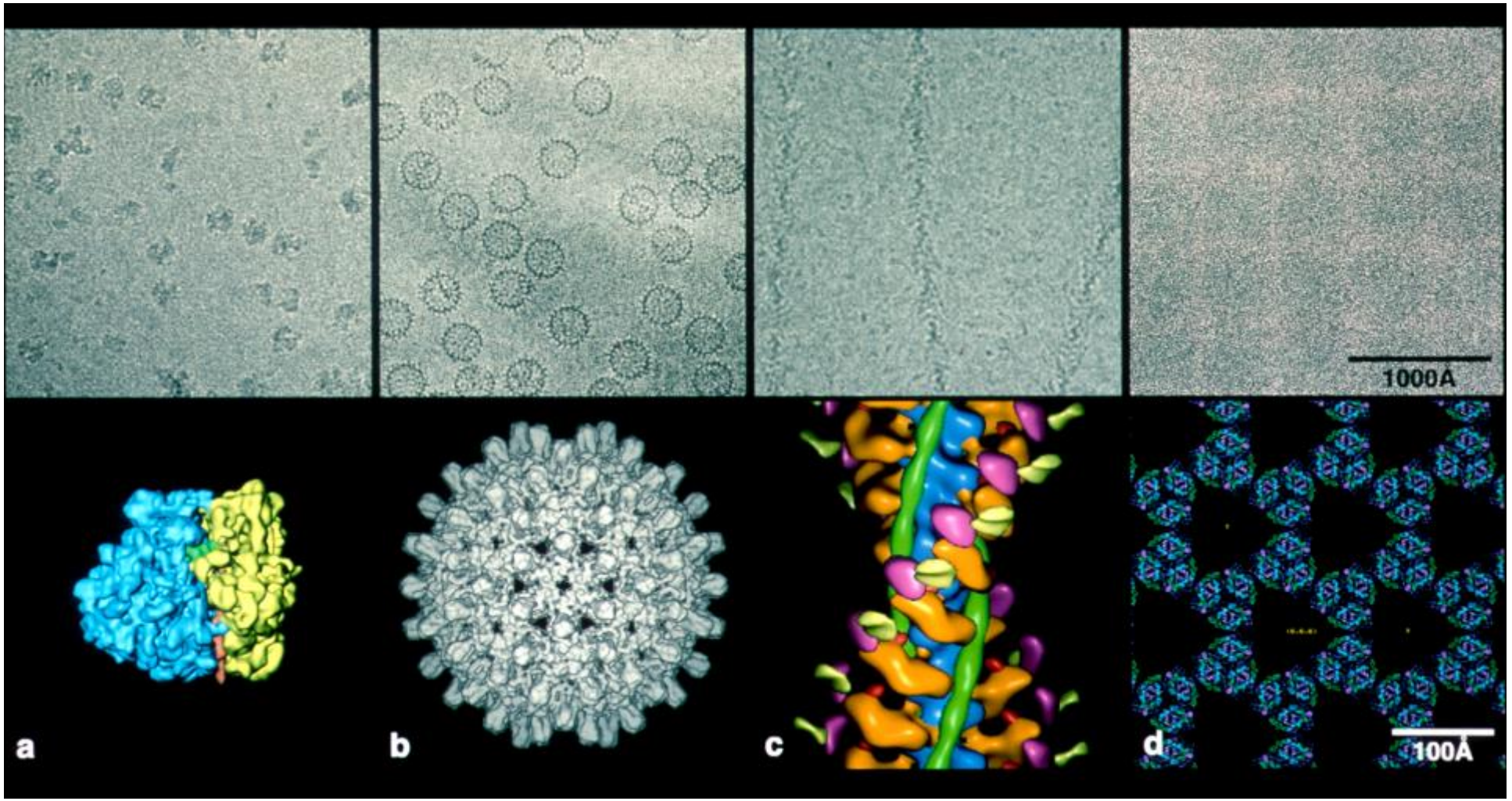
Topics

- Earliest history – DeRosier & Klug, Glaeser, Dubochet (Kellenberger)
- Theory - Crowther, Frank, Henderson
- Helical, icosahedral, 2D crystal – symmetrical structures
- Particles without symmetry – ribosome, Frank (Hoppe), van Heel (van Bruggen)

- Technology – FEG, vacuum, stability, automation
- Detectors – film, CCD, CMOS, hybrid pixel
- Computer programs – Spider, Imagic, EMAN, Xmipp, Relion, Simple/Prime, BSOFT, SPARX

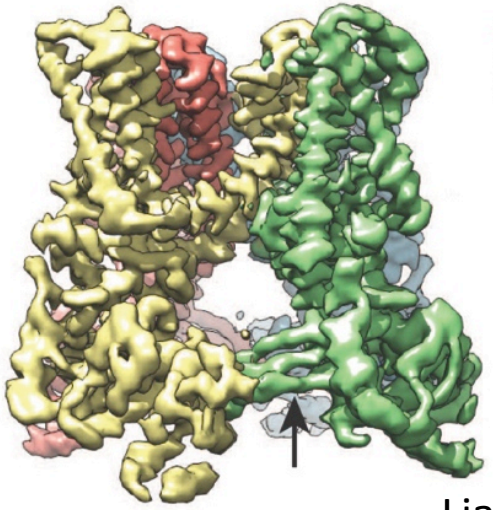
From Baker & Henderson (2001) Int.Tab.Cryst.Vol.F, on-line (2006), revised (2011)

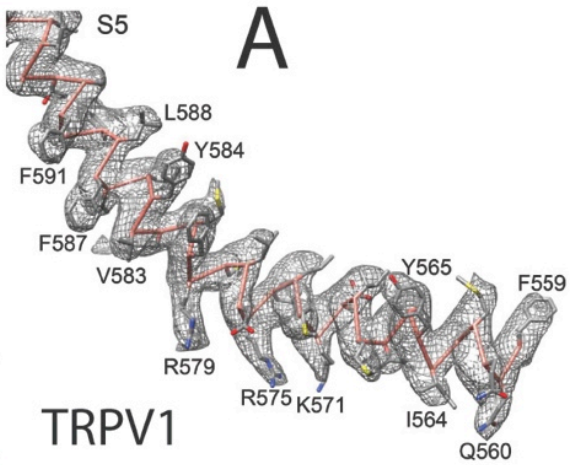
70S ribosome 11.5 Å | hepatitis B cores 7 Å | decorated actin 30 Å | LHC II 3.4 Å

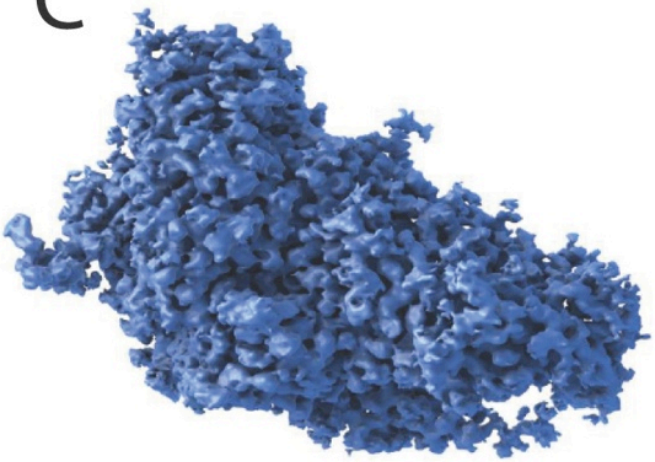


Gabashvili et al, 2000 | Böttcher et al, 1997 | Milligan, ~2000 | Kühlbrandt et al, 1994

Three recent smaller structures from cryoEM **(MW 170-450 kDa)**

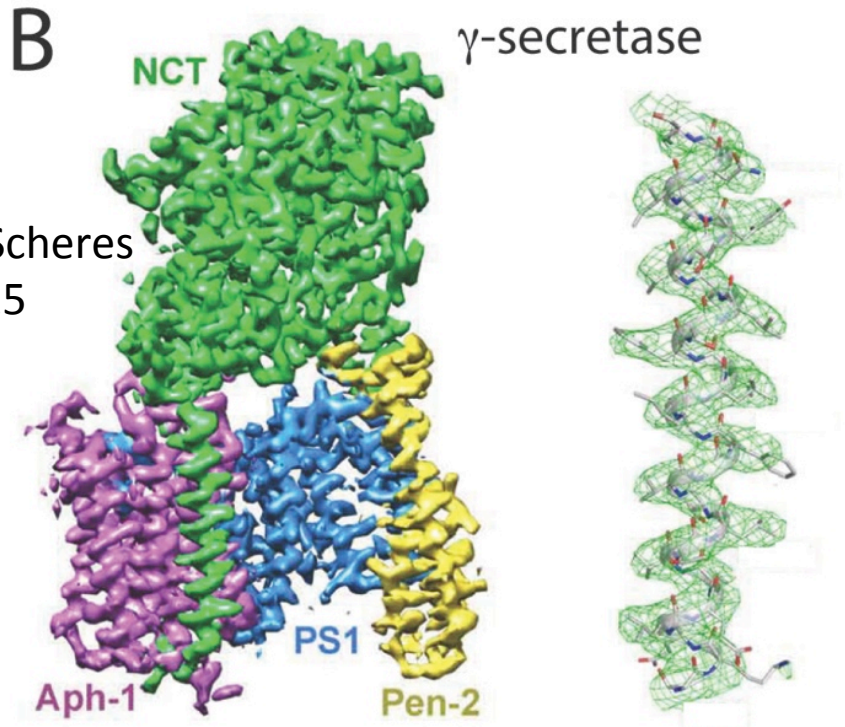


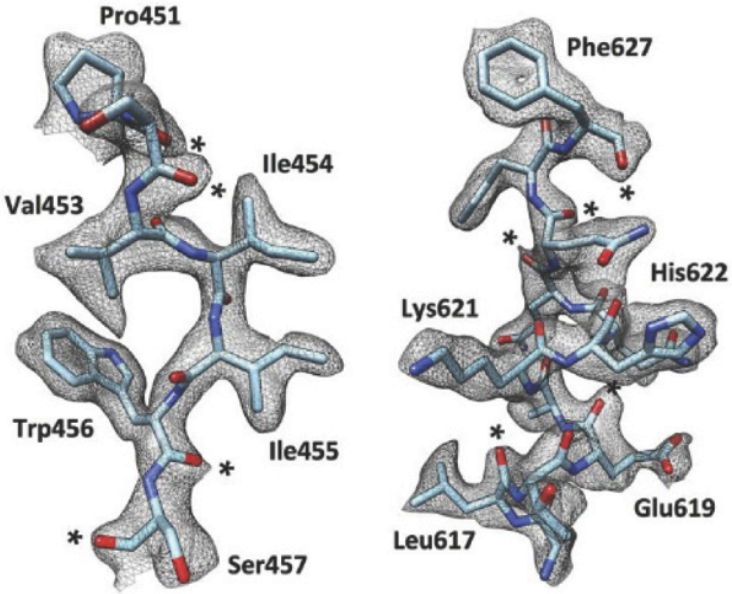




Liao et al & Cheng, 2013

Bai et al, Scheres & Shi, 2015





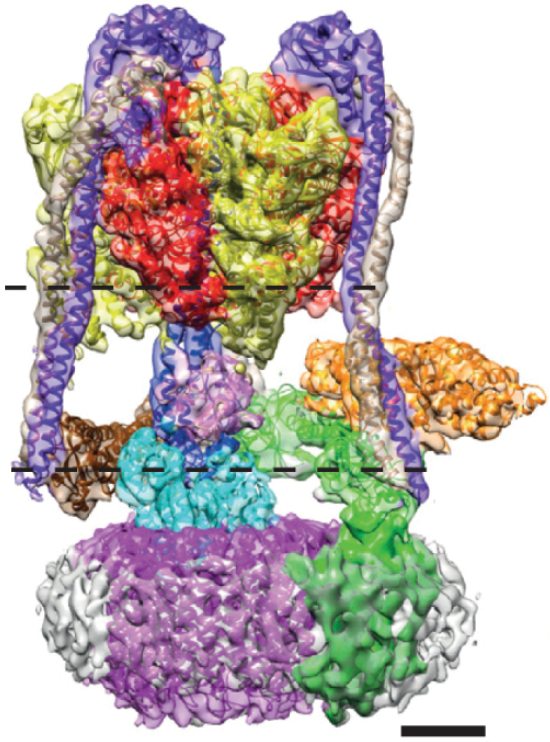
β-galactosidase

Bartesaghi et al & Subramaniam, 2015

Three recent membrane protein structures from cryoEM

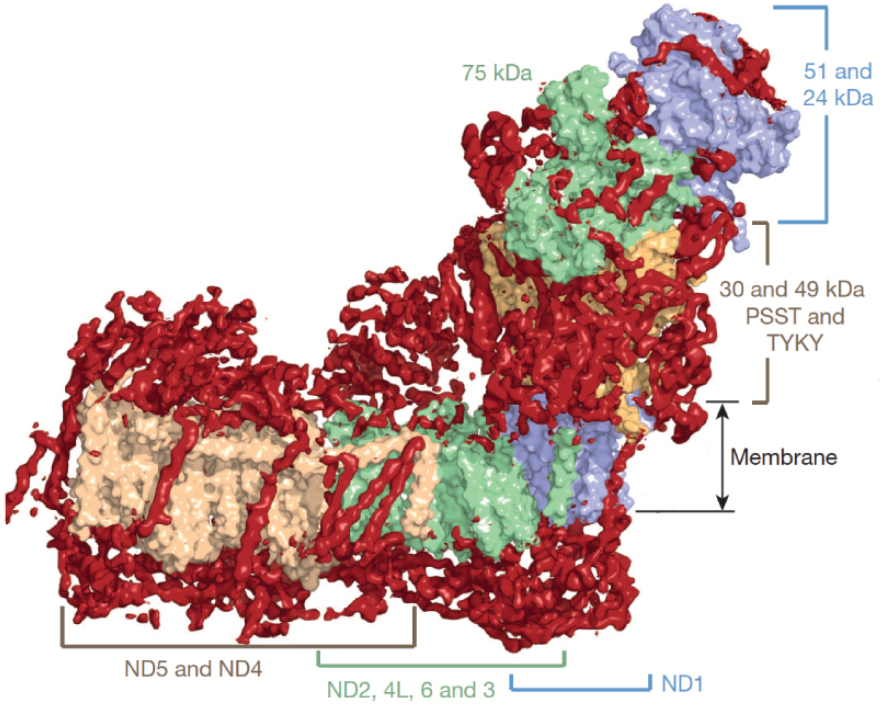
(MW 450-900 kDa)

A V-type ATPase



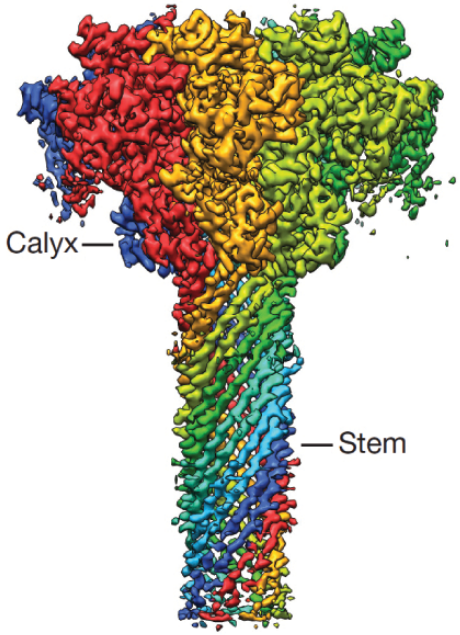
Zhao, Benlekbir & Rubinstein, 2015

B Complex I



Vinothkumar, Zhu & Hirst, 2014

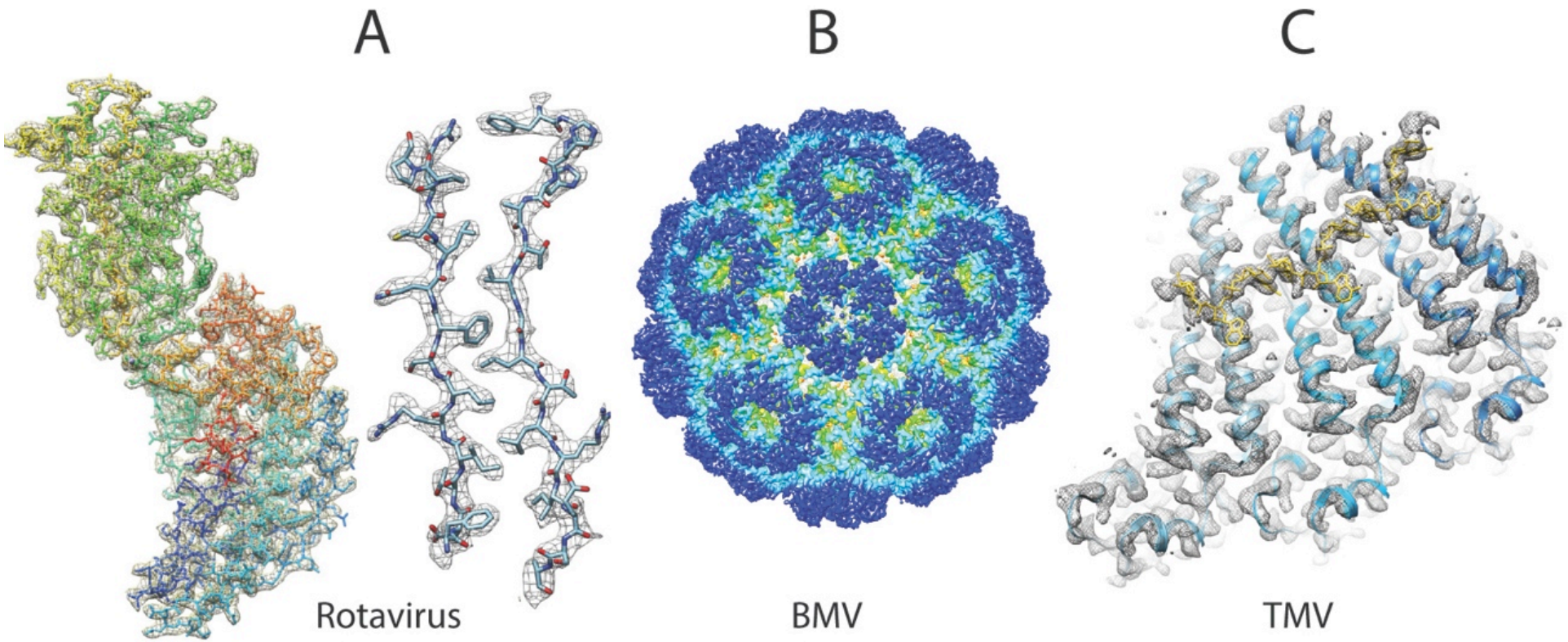
C Anthrax pore



Jiang, Pentelute, Collier & Zhou, 2015

Three recent membrane virus structures from cryoEM

(virus MW 11-60 MDa)

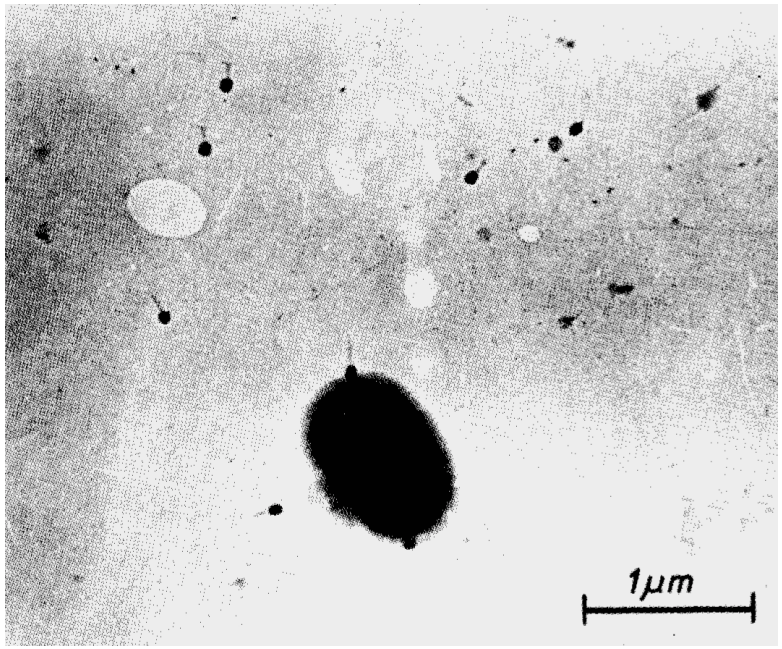


Grant & Grigorieff, 2015

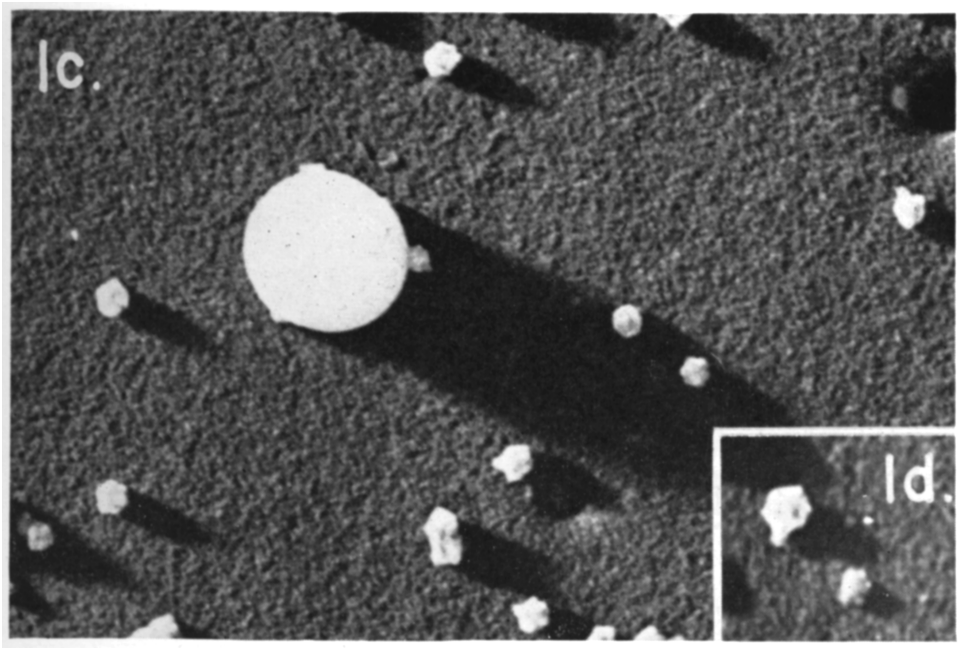
Wang et al & Chiu, 2014

Fromm et al & Sachse, 2015

Earliest EM of biological samples

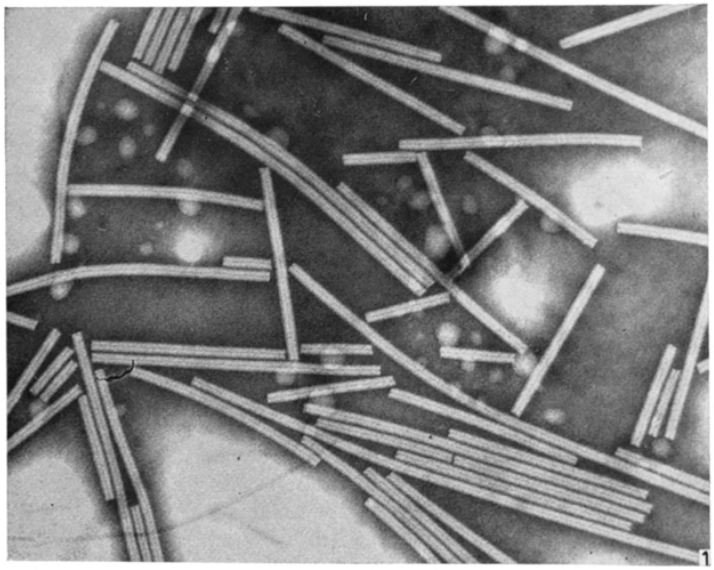


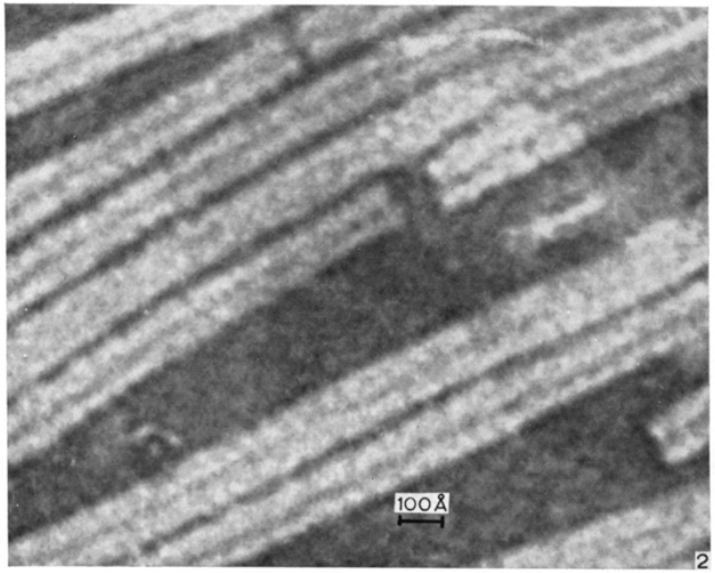
Helmut Ruska 1942 T4



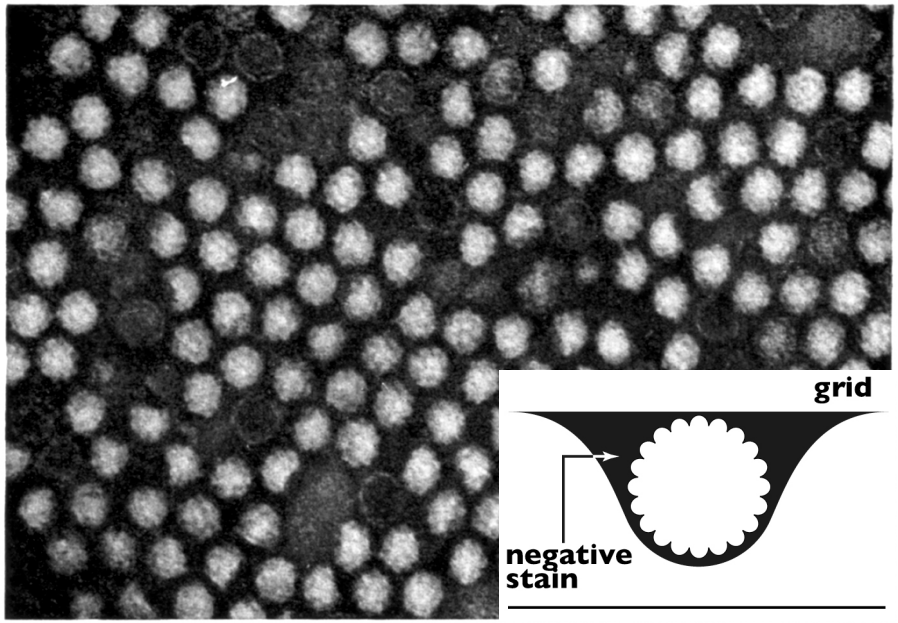
Fraser & Williams 1953 T3, T7

Introduction of negative staining

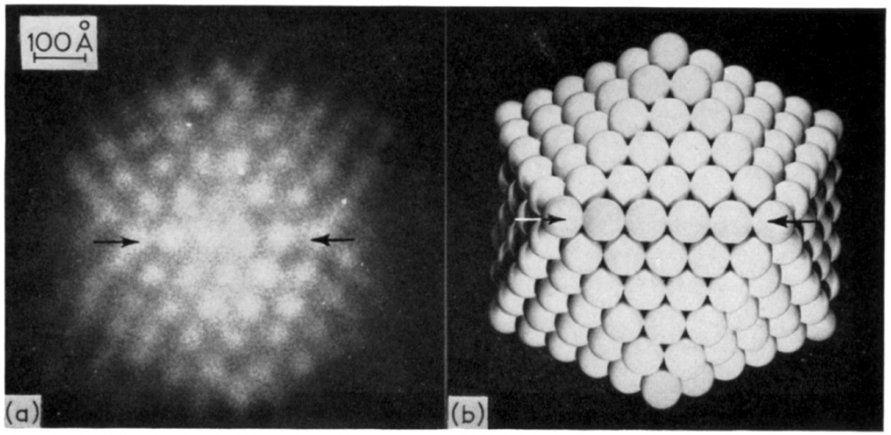




Brenner & Horne 1959 TMV



Huxley & Zubay 1960 TYMV



Horne *et al*. 1959 adenovirus

First 3D structure from electron micrographs

(*Reprinted from Nature, Vol. 217, No. 5124, pp. 130–134, January 13, 1968*)

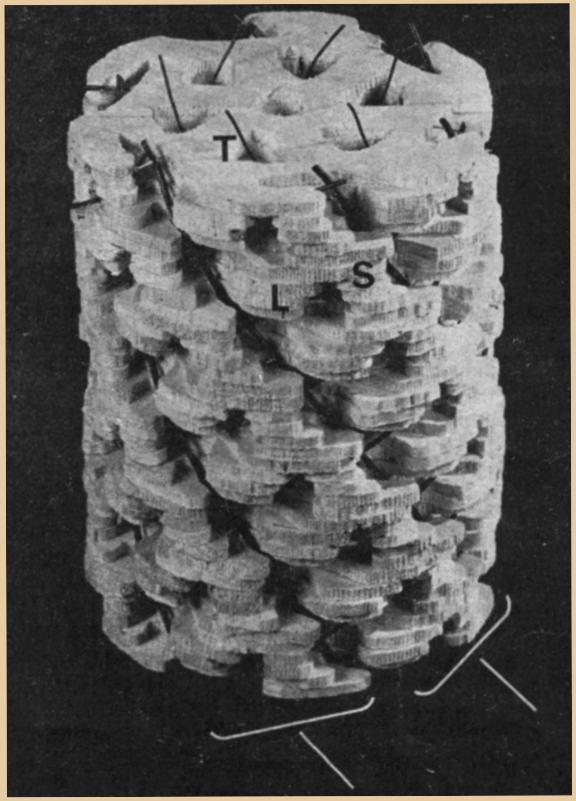
Reconstruction of Three Dimensional Structures from Electron Micrographs

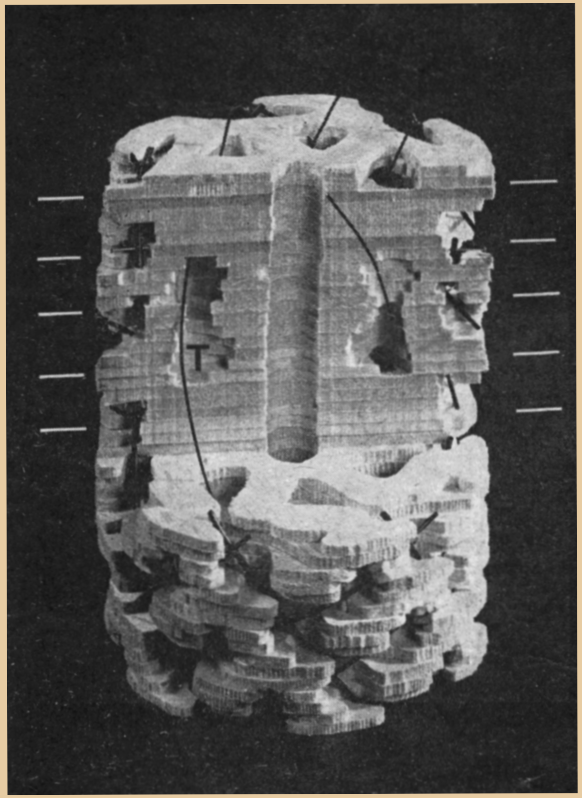
by

D. J. DE ROSIER
A. KLUG

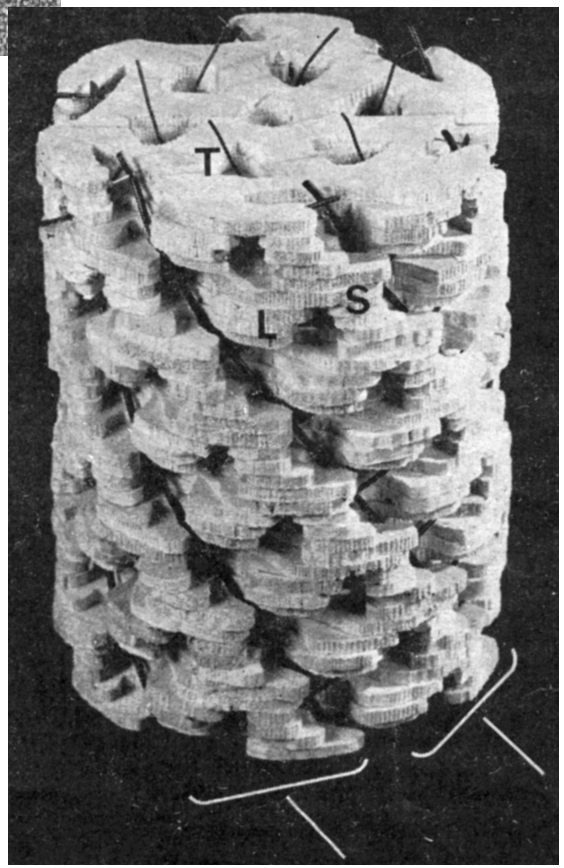
MRC Laboratory of Molecular Biology,
Hills Road, Cambridge

General principles are formulated for the objective reconstruction of a three dimensional object from a set of electron microscope images. These principles are applied to the calculation of a three dimensional density map of the tail of bacteriophage *T4*.

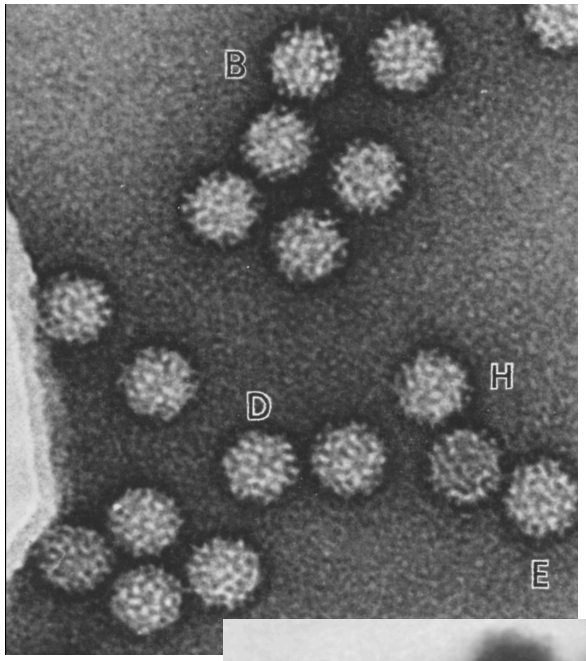


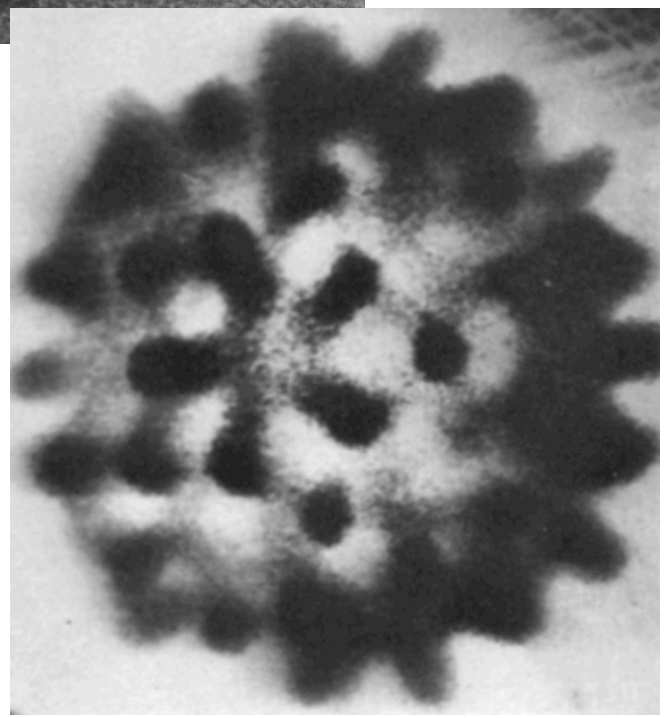


From helical 3D in 1968 to icosahedral 3D in 1970



DeRosier & Klug 1968 T4 tail





Crowther *et al*. 1970 TBSV

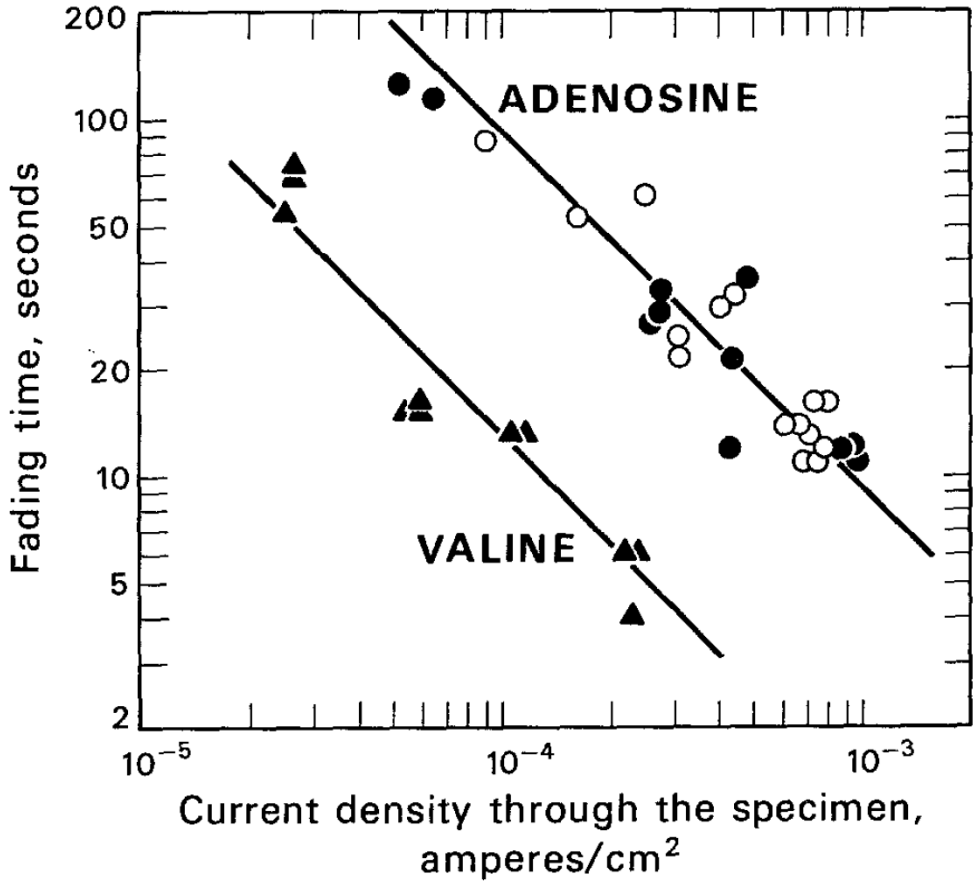
J. ULTRASTRUCTURE RESEARCH 36, 466–482 (1971)

Limitations to Significant Information in Biological Electron Microscopy as a Result of Radiation Damage[1]

ROBERT M. GLAESER

Division of Medical Physics and Donner Laboratory, University of California, Berkeley, California 94720

Received October 23, 1970, and in revised form January 22, 1971



Dose for fading at 20 degC corresponds to 1el/Å^2 for valine, 10 for adenine

"This analysis suggests that spatial superposition of statistically noisy images might be a possible method for obtaining high resolution images with low amounts of radiation damage."

Taylor & Glaeser (1974) Science 186, 1036-1037

Electron Diffraction of Frozen, Hydrated Protein Crystals

Abstract. *High-resolution electron diffraction patterns have been obtained from frozen, hydrated catalase crystals to demonstrate the feasibility of using a frozen-specimen hydration technique. The use of frozen specimens to maintain the hydration of complex biological structures has certain advantages over previously developed liquid hydration techniques.*

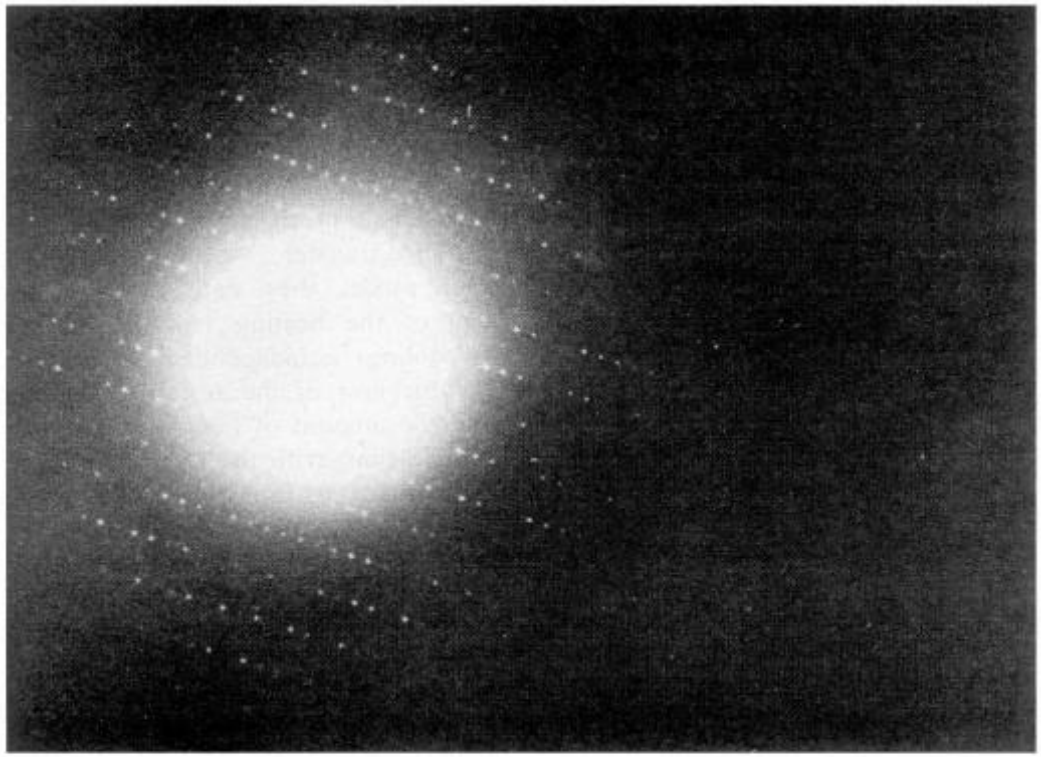
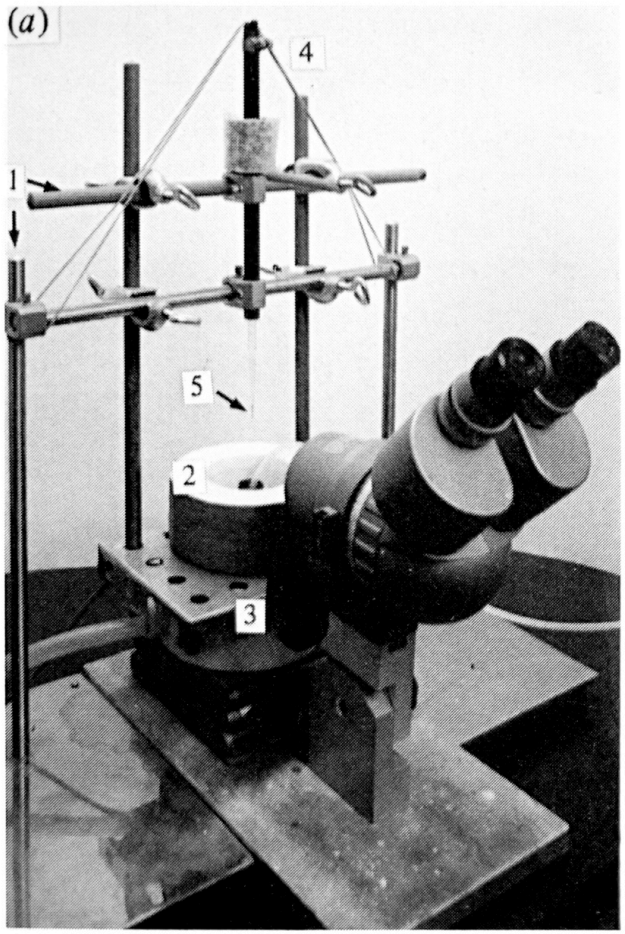
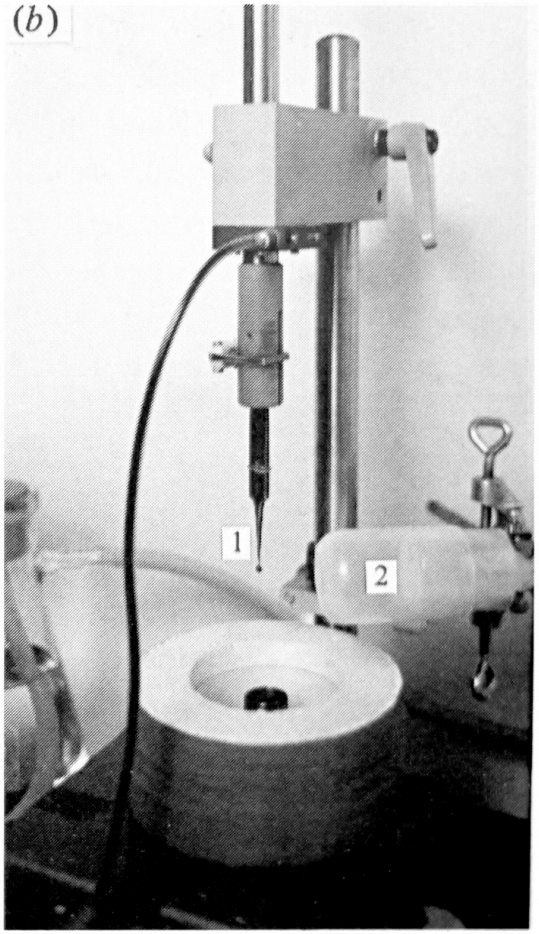


Fig. 1. Electron diffraction pattern of a catalase crystal which was frozen in liquid nitrogen and observed on a specimen stage cooled with liquid nitrogen. The resolution of the photographic reproduction is 4.5 Å, although that of the diffraction pattern on the original plate was 3.4 Å.

From Dubochet et al, QRB (1988)





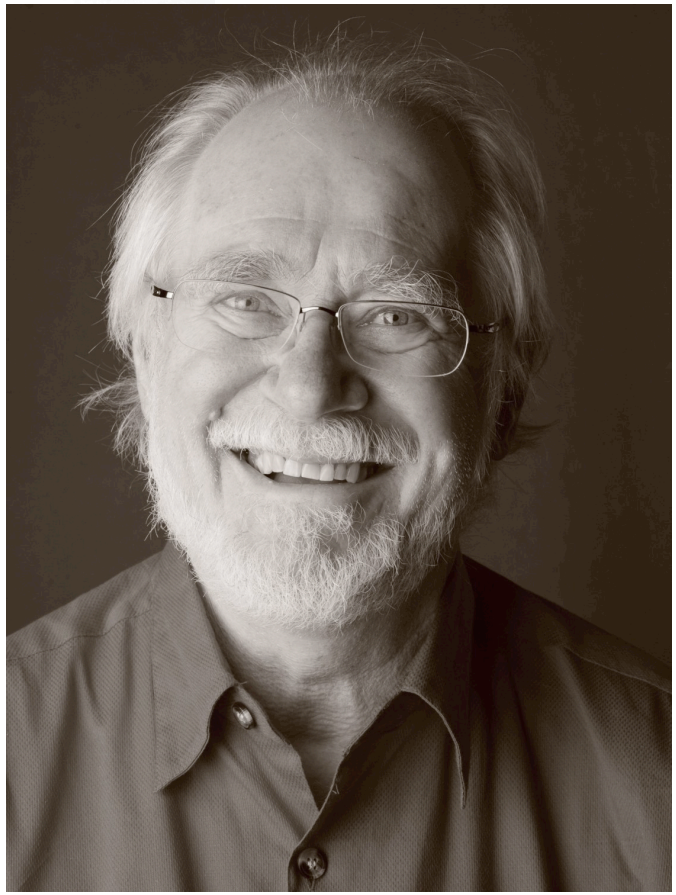


Fig. 43. Plunger for freezing. (*a*) Simple apparatus equipped here for freezing bulk specimens. (1) Retort stand clamps; (2) liquid nitrogen and ethane dewars; (3) water-driven magnet; (4) plunger with elastic band propulsion; (5) specimen support. (*b*) A more elegant freezing apparatus equipped for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

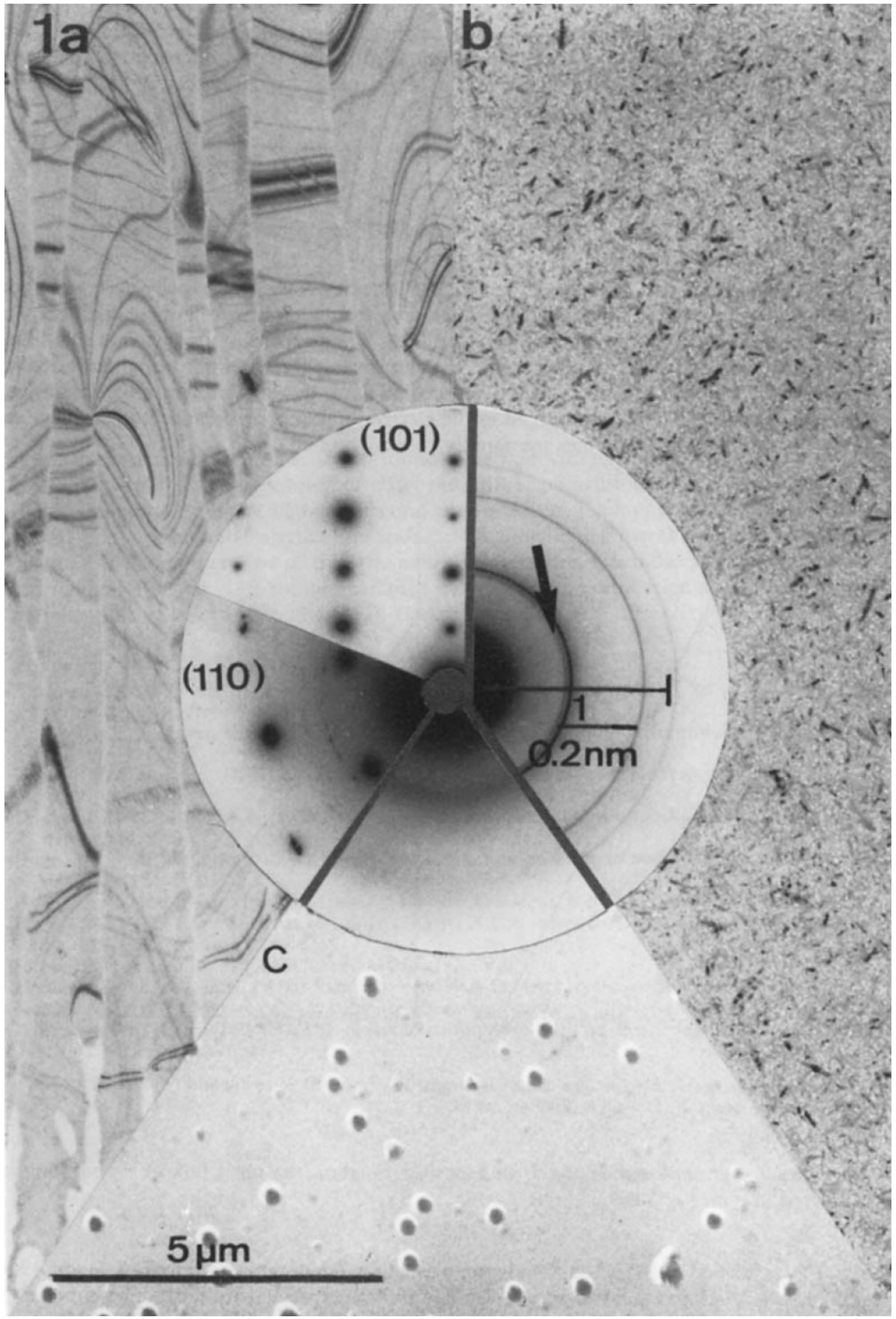
Dubochet et al (1982) J.Micros.

Hexagonal, cubic and vitreous ice

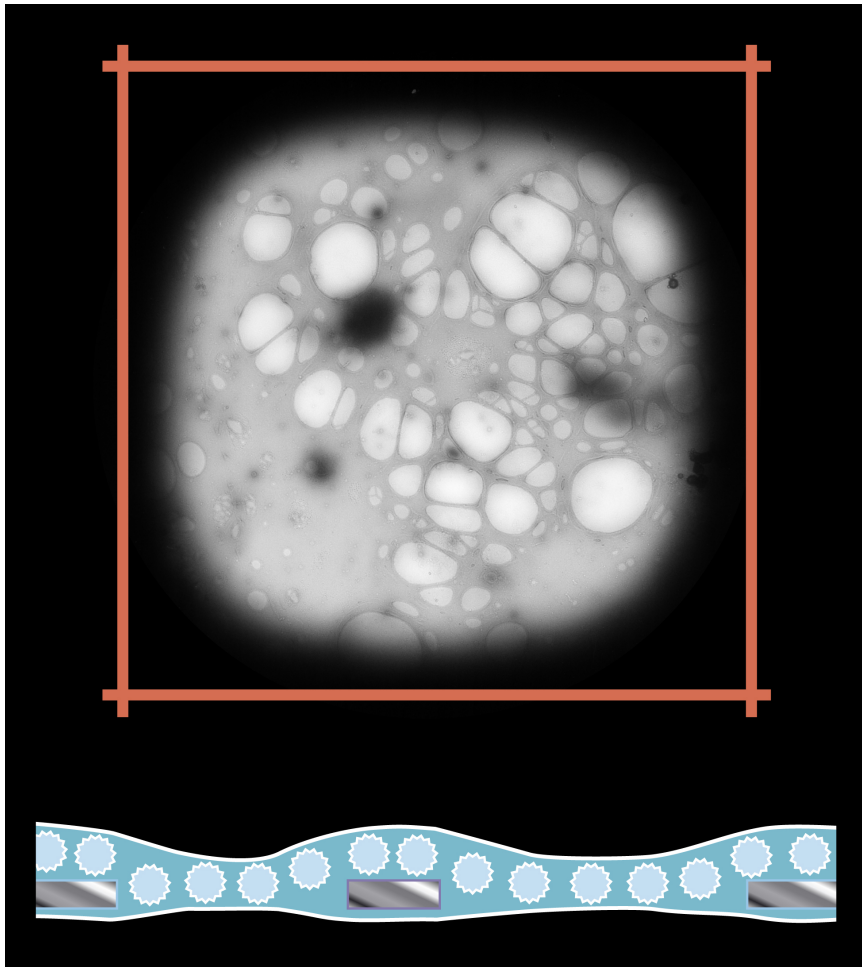
(a) Ih obtained by rapid freezing of a thin water layer spread on a carbon film. The thickness of the layer shown on the micrograph is 50-80 nm. The diffractograms which are taken from other specimens show the (110) and (101) planes.

(b) Ic obtained by warming a layer of Iv. The small contribution of the (100) form of Ih has been marked on the diffractogram (arrow). The Ic layer is approximately 70 nm thick.

(c) Iv obtained by deposition of water vapour in the electron microscope on a film supporting polystyrene spheres. The layer is approximately 70 nm thick.



Plunge-freeze method of Dubochet

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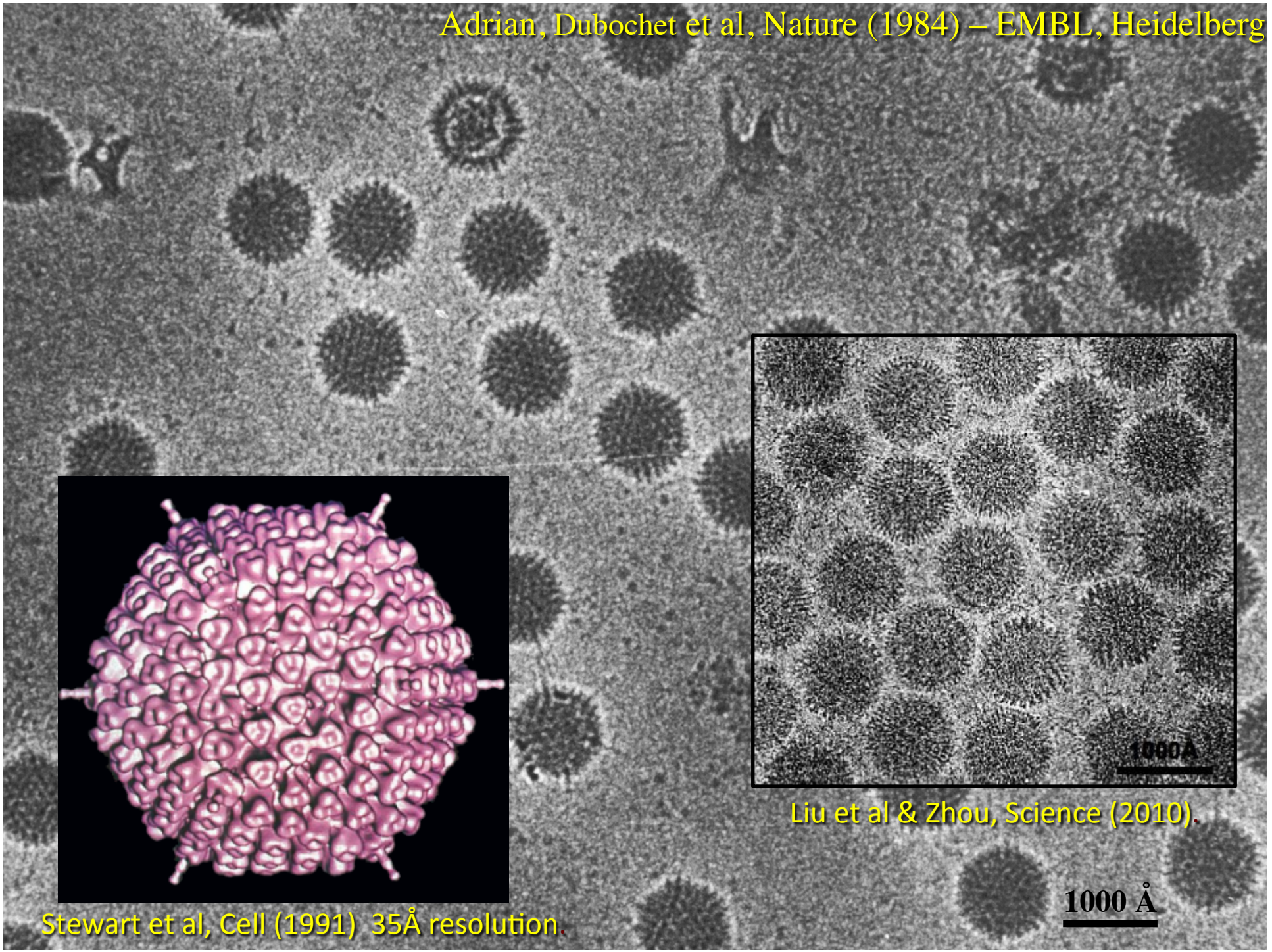
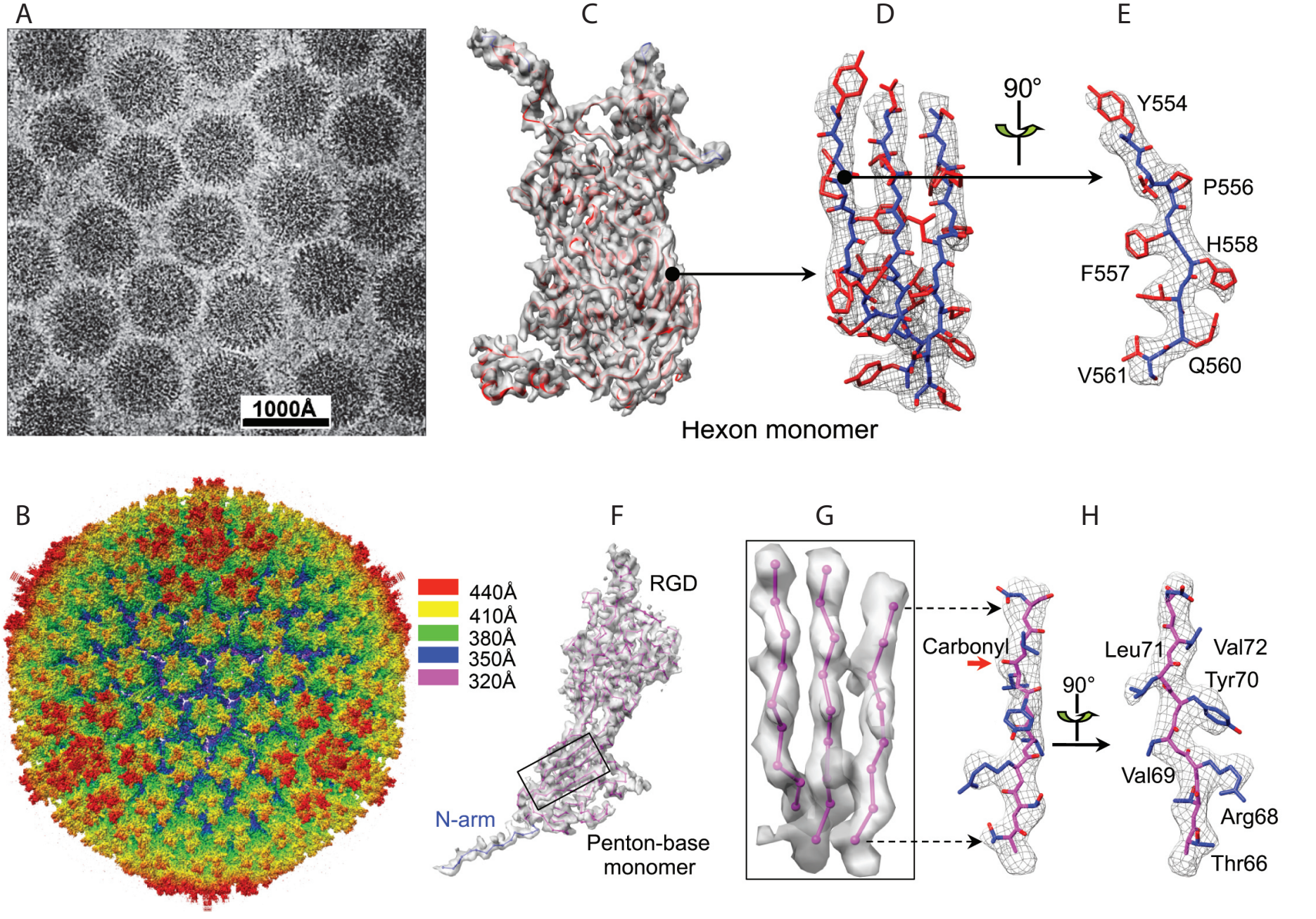
VITROBOT
Please check that blotting pads rotate after each blot.
PLEASE SWITCH OFF AFTER USE
FEI
VITROBOT

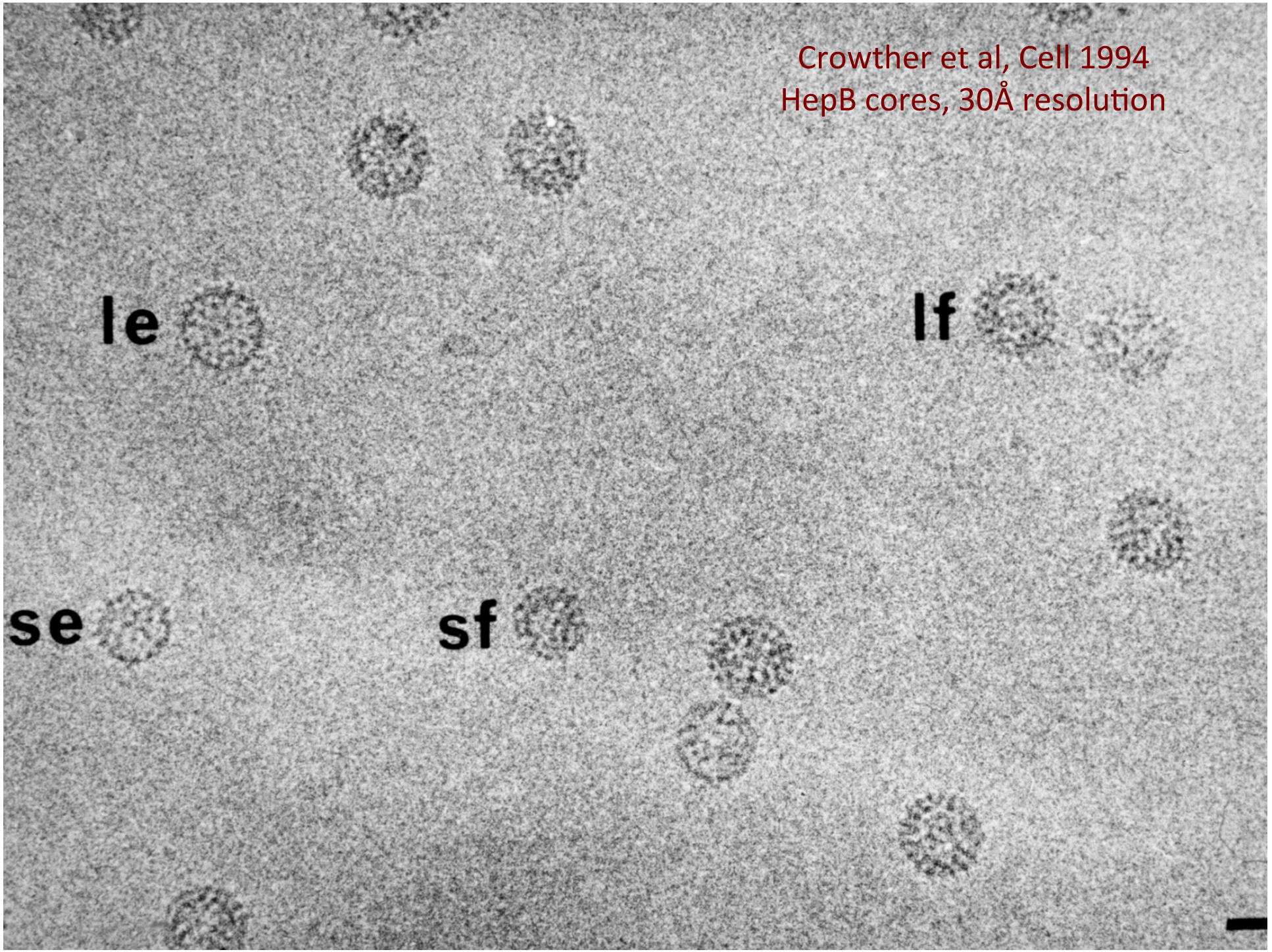

Adrian, Dubochet et al, Nature (1984) – EMBL, Heidelberg
1000Å
Liu et al & Zhou, Science (2010).
1000 Å
Stewart et al, Cell (1991) 35Å resolution.

Atomic structure of human adenovirus by Cryo-EM

Liu et al & Zhou, Science (2010), JMB (2011).

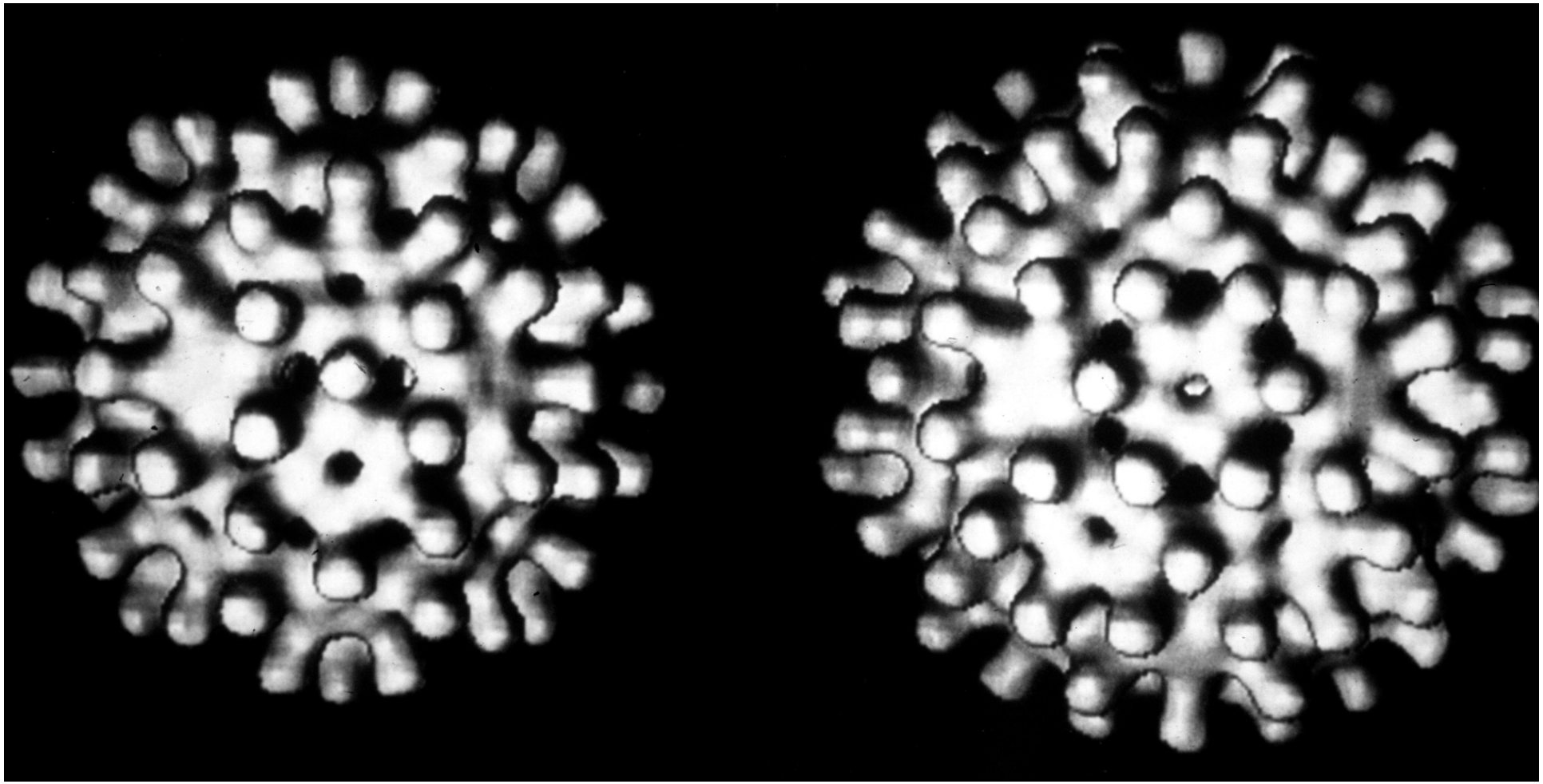
3.6Å resolution
300keV on film

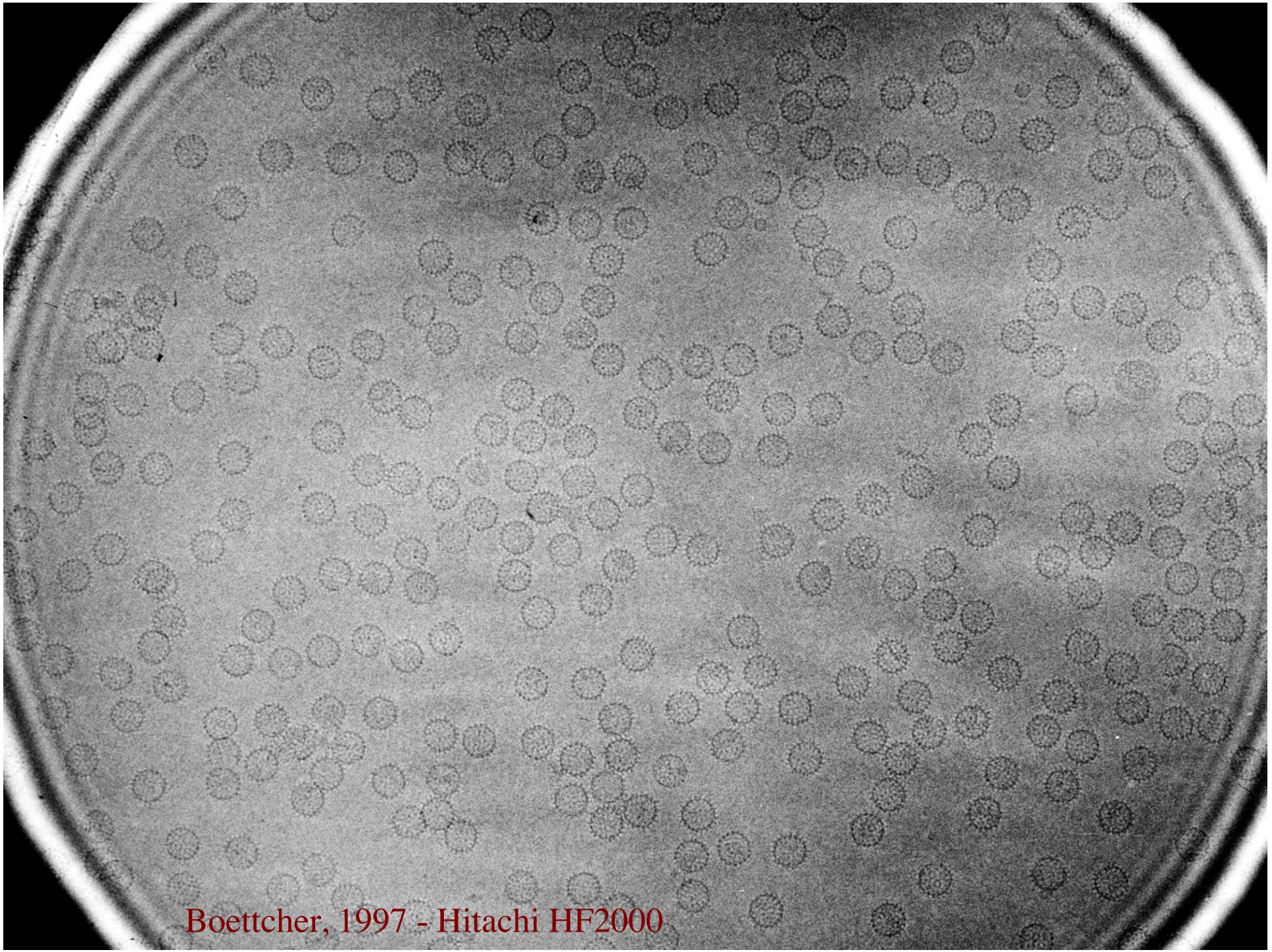



Crowther et al, Cell 1994
HepB cores, 30Å resolution
le
lf
se
sf

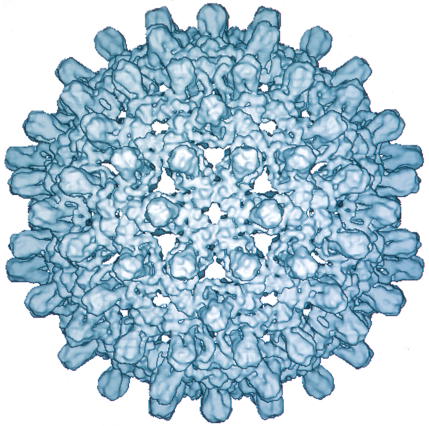
T=3

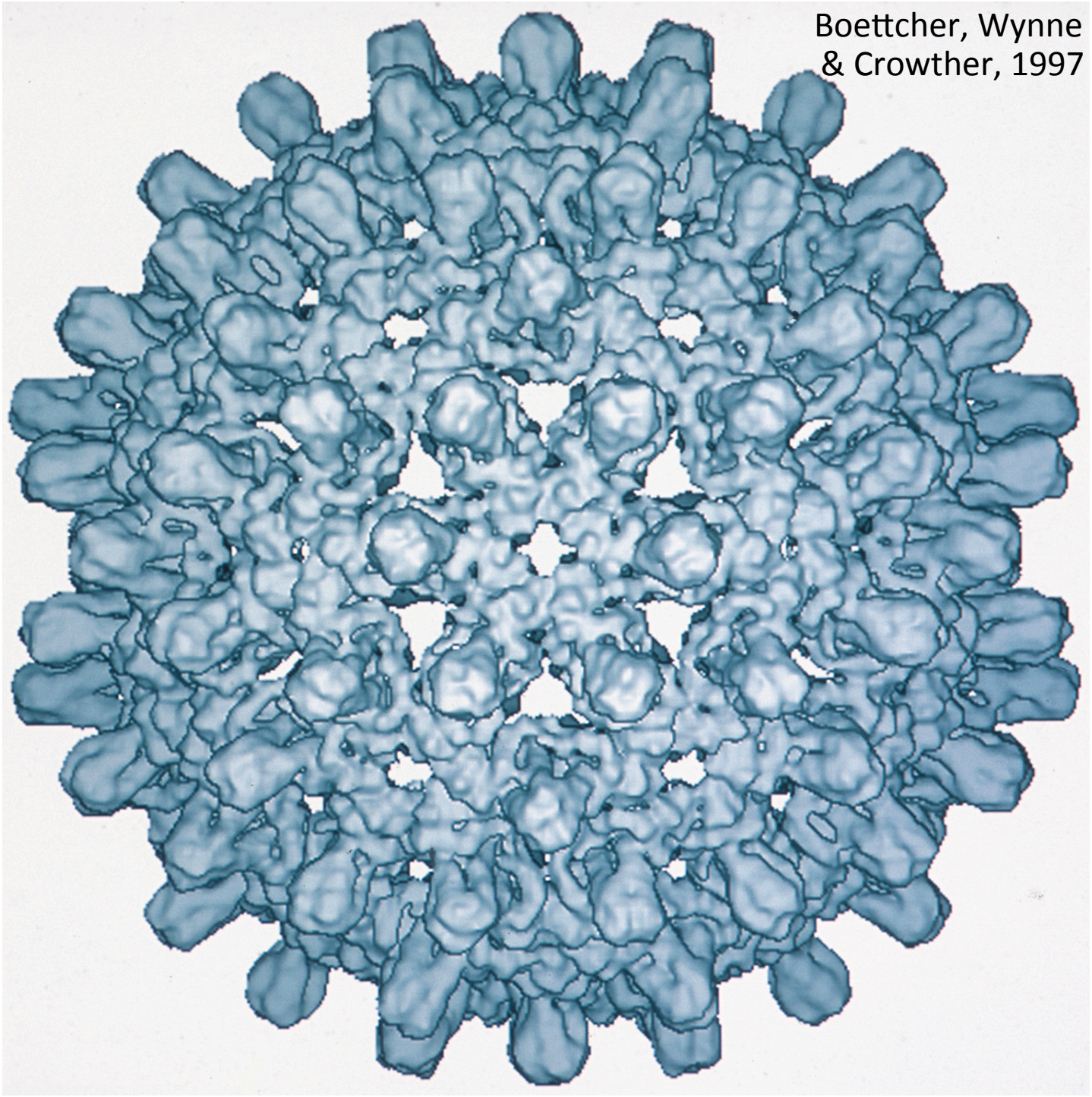
T=4




Boettcher, 1997 - Hitachi HF2000

Boettcher, Wynne & Crowther, 1997
7.4Å resolution, first sub-nm single particle structure




Boettcher, Wynne
& Crowther, 1997

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        10         20         30         40
MDIDPYKEFG ATVELLSFLP SDFFPSVRDL LDTASALYRE
        50         60         70         80
ALESPEHCSP HHTALRQAIL CWGELMTLAT WVGVNLEDPA
        90         100        110        120
SRDLVVSYVN TNMGLKFRQL LWFHISCLTF GRETVIEYLV
        130        140        150        160
SFGVWIRTPP AYRPPNAPIL STLPETTVVR RRGRSPRRRT
        170        180
PSPRRRRSQS PRRRRSQSRE SQC
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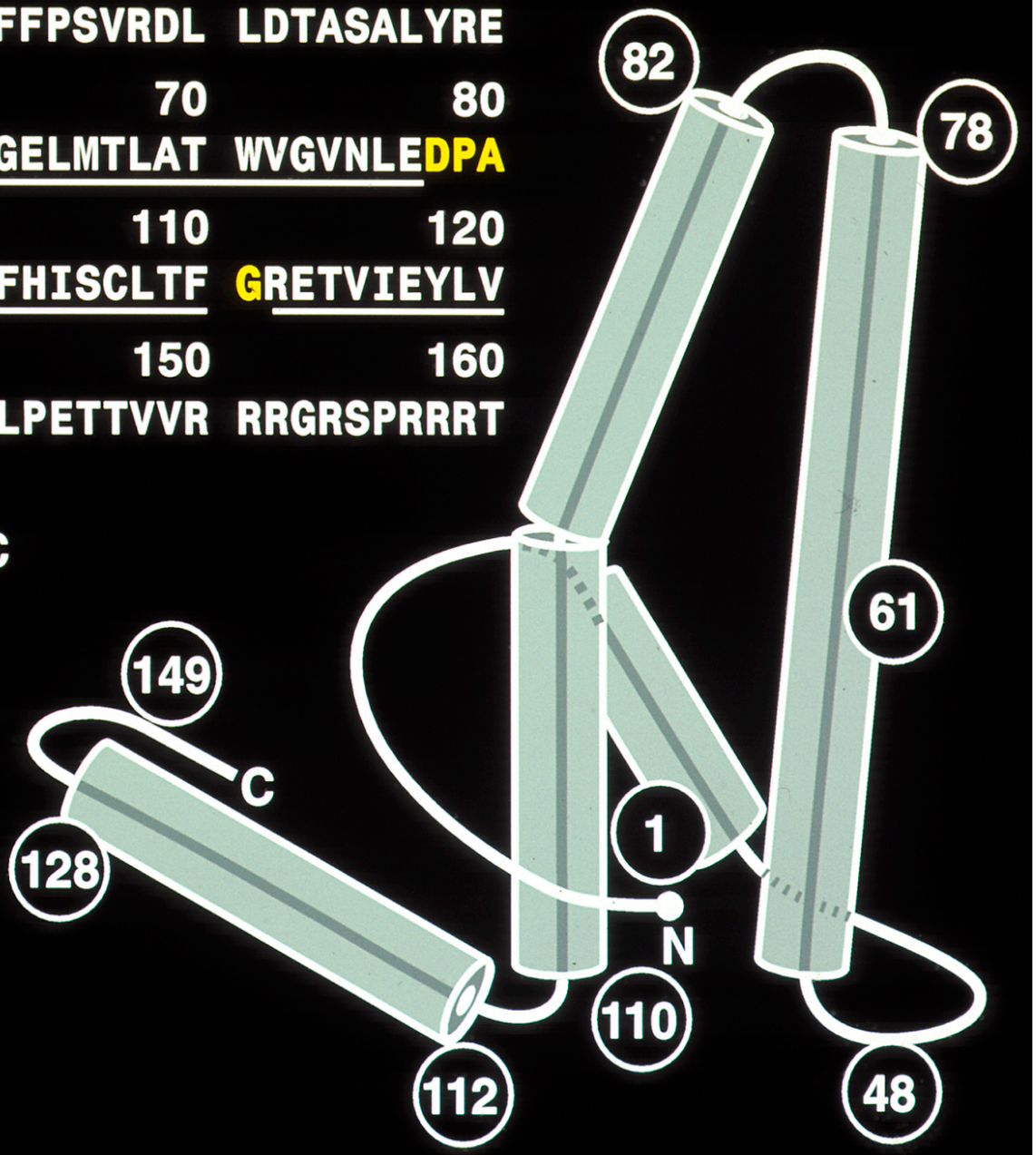


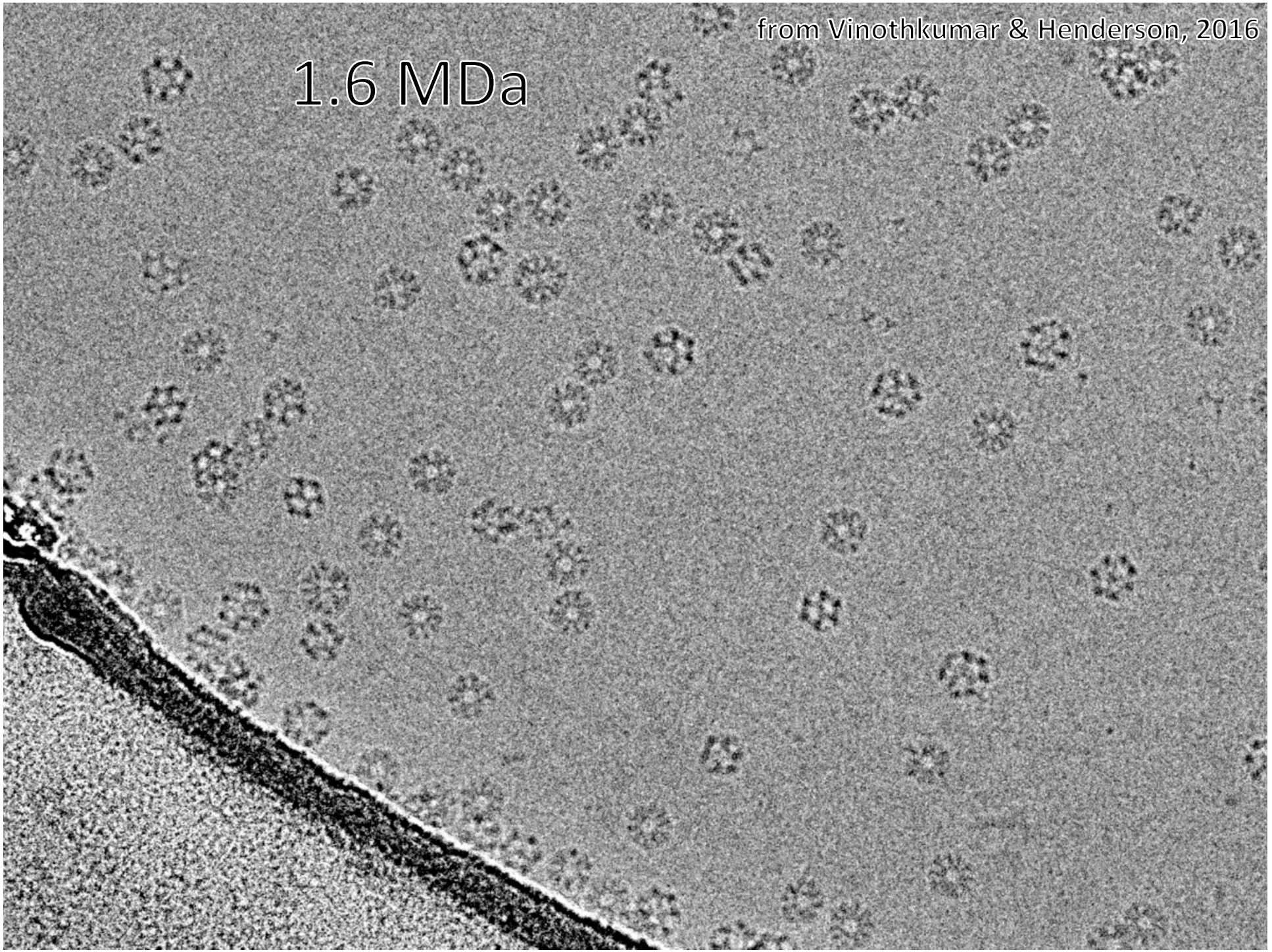
Boettcher, Wynne & Crowther, 1997

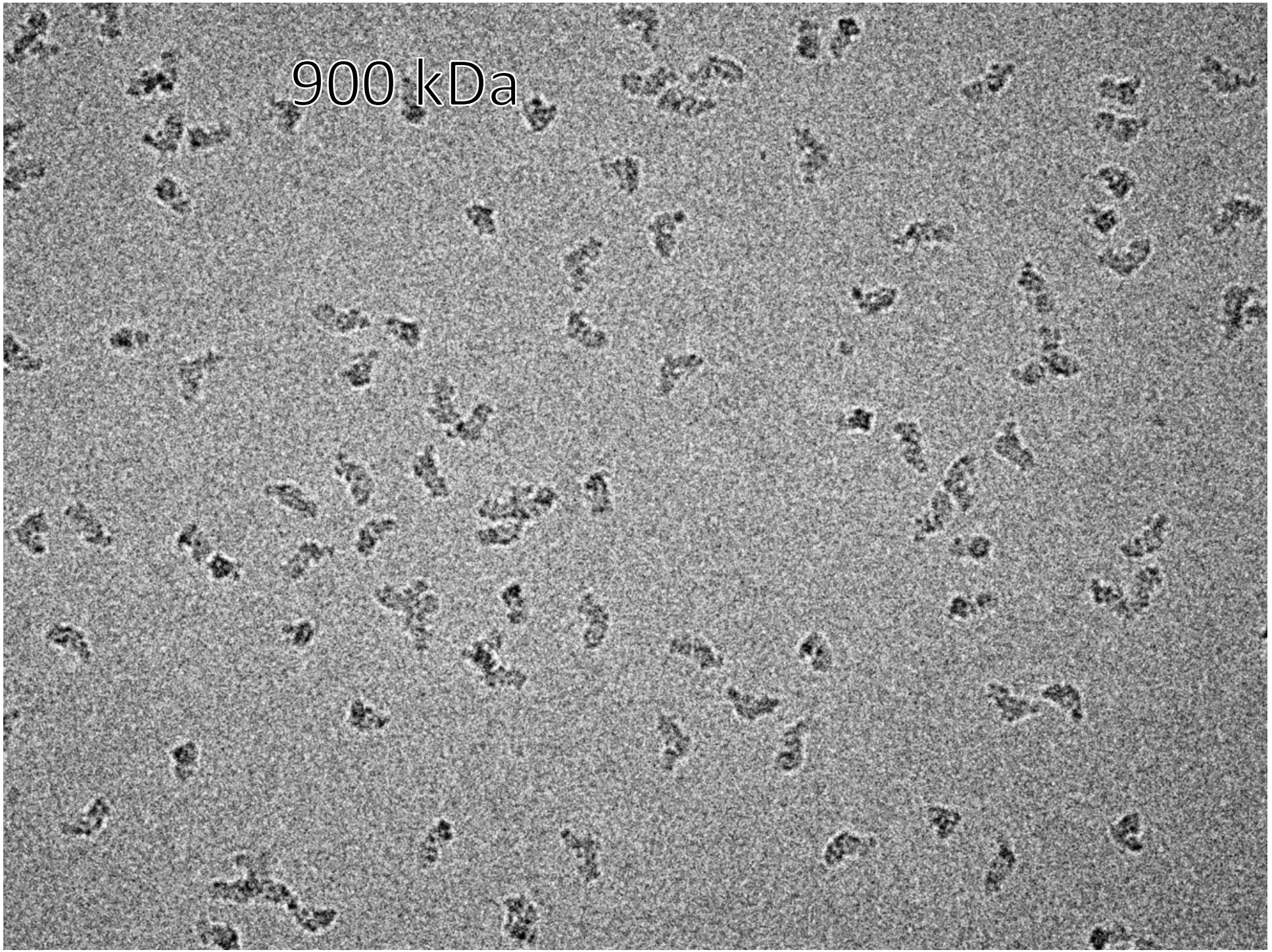
Henderson (1995) QRB **28**, 171-93, Potential and limitations of neutrons, electrons & X-rays for atomic resolution microscopy

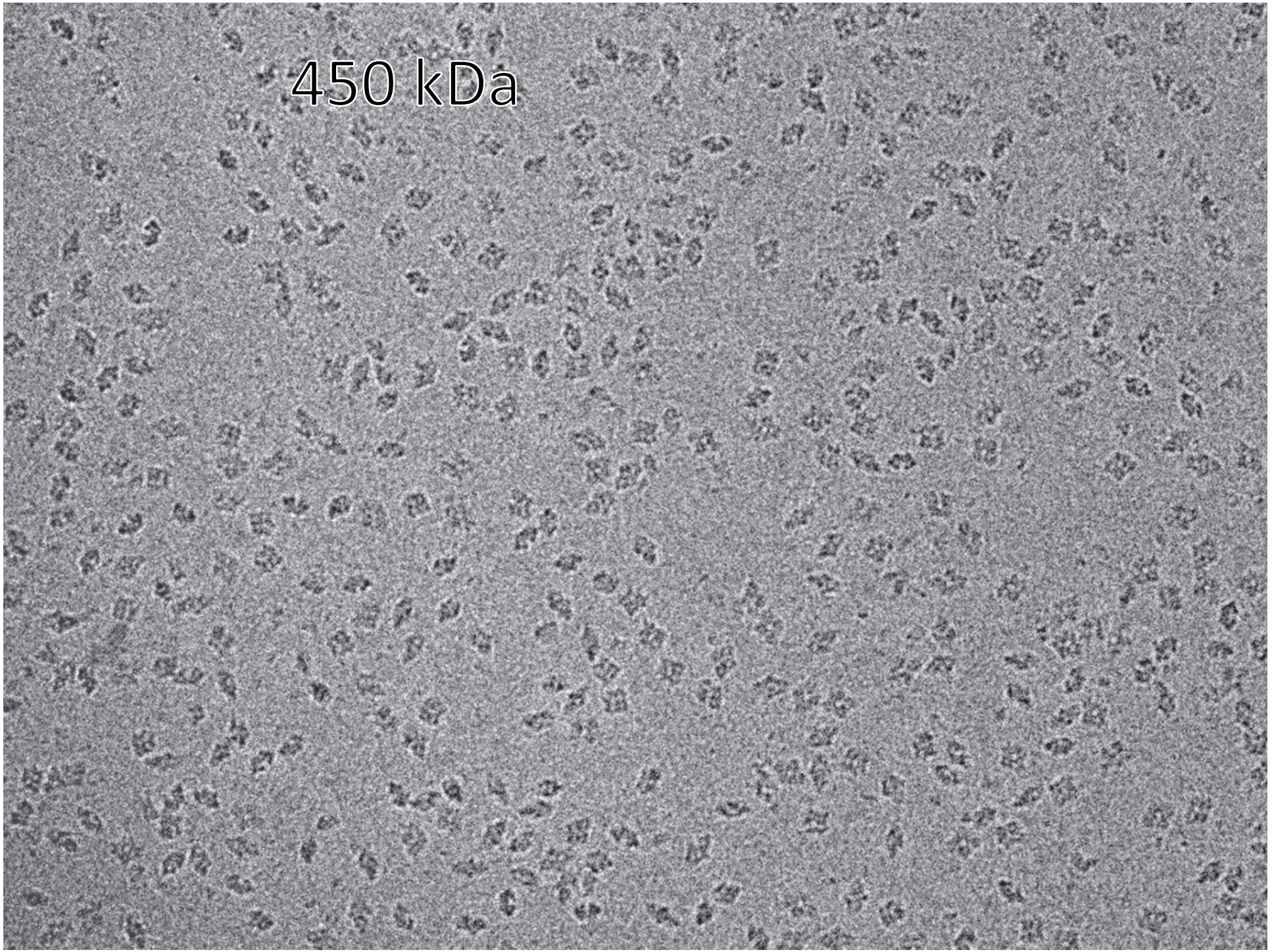
TABLE 2

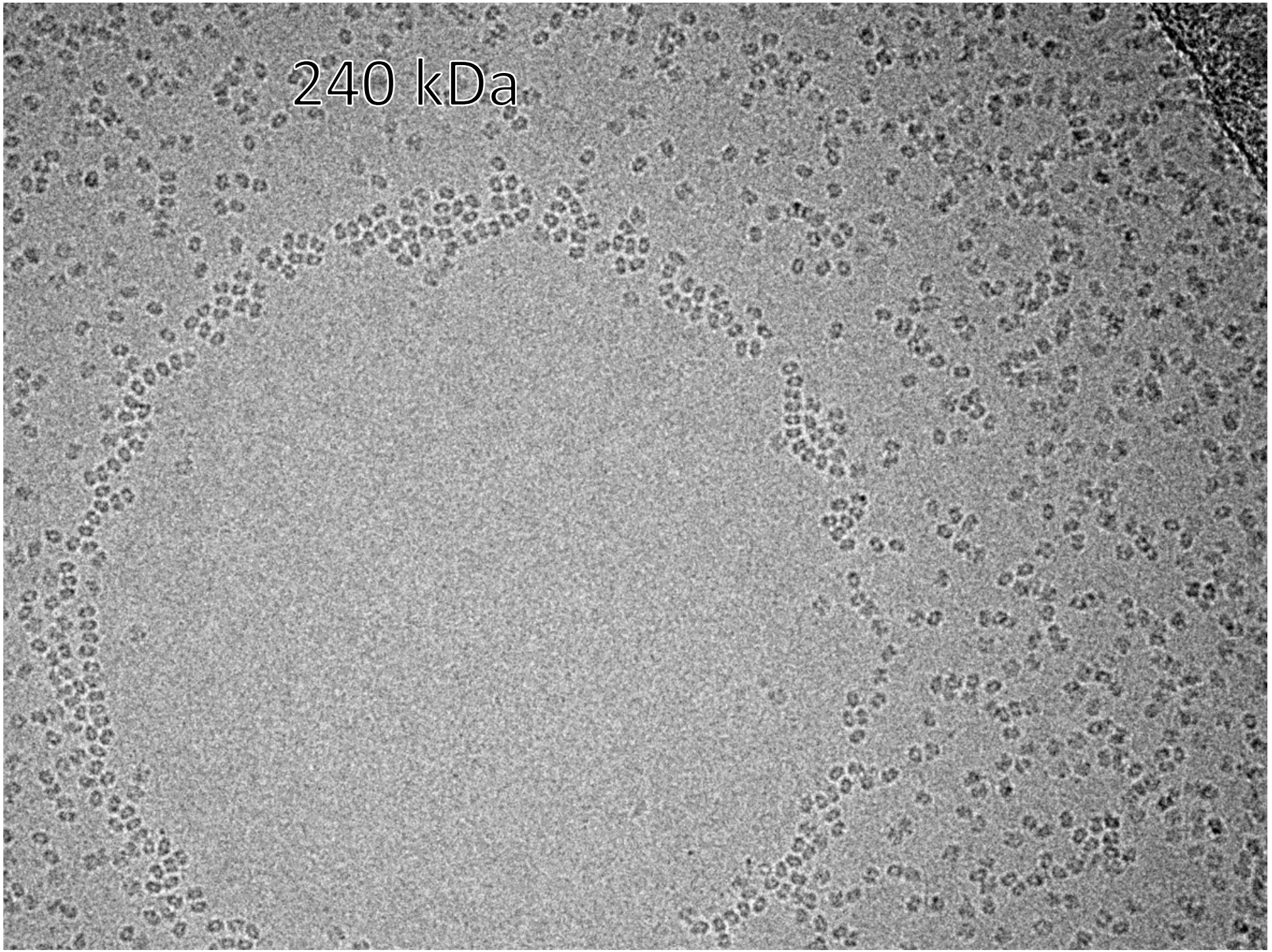
Type of molecule	Approx. M.W. (Daltons)	D (Å)	N_c, number of carbon atom equivalents	N_s, number of unique diffraction spots to resolution of d = 3Å in projection	f, fraction of electrons elastically scattered out to 3Å resolution	$\langle I_{OBS} \rangle$ / Io	$\langle F_{OBS} \rangle$ / Fo	Phase contrast = total image fractional contrast = signal	Fractional noise level in pixel of dimension $\left(\frac{d}{2}\right)^2$ = 1.5Å x 1.5Å	Can single molecule be detected? How many times > noise	Multiple of sigma expected within unit cell at random	Multiple of sigma expected within entire volume of 5 parameter space at random	Can single molecule alignment be carried out in practice?	Minimum number of images needed for structure with average Fourier component to be >3 σ in projection	Total number of images in 3D x $[\frac{\pi R}{d}]$ De Rosier & Klug (1967)
large virus	300M	900	25,000,000	141,371	0.0520	0.184×10^{-6}	0.429×10^{-3}	0.322	0.30	644	5.2	8.5	yes	13	12600
small virus	11M	300	936,000	15,707	0.0173	0.552×10^{-6}	0.743×10^{-3}	0.186	0.30	124	4.8	7.7	yes	40	12600
ribosome	3.3M	200	277,000	6,981	0.0115	0.827×10^{-6}	0.910×10^{-3}	0.152	0.30	68	4.7	7.5	yes	60	12600
	1.4M	150	117,000	3,926	0.0087	1.103×10^{-6}	1.050×10^{-3}	0.132	0.30	44	4.6	7.3	yes	80	12600
multimeric enzyme	420K	100	35,000	1,745	0.0058	1.654×10^{-6}	1.286×10^{-3}	0.107	0.30	24	4.4	7.1	possibly	120	12600
	180K	75	14,600	981	0.0043	2.206×10^{-6}	1.485×10^{-3}	0.093	0.30	16	4.2	6.8	possibly	160	12600
	52K	50	4,330	436	0.0029	3.309×10^{-6}	1.819×10^{-3}	0.076	0.30	8.4	4.1	6.7	possibly	240	12600
small protein	18K	35	1,500	213	0.0020	4.727×10^{-6}	2.174×10^{-3}	0.064	0.30	4.9	3.9	6.3	no	345	12600
very small protein	7K	25	540	109	0.00144	6.618×10^{-6}	2.572×10^{-3}	0.054	0.30	3.0	3.5	5.9	no	480	12600
equation	(1)	-	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(13)	(16)	(17)	(10)	(11)
relation to D	$0.418 \times D^3$	D	$0.0346 \times D^3$	$0.01745 \times D^2$	$5.7 \times 10^{-5} \times D$	$1.654 \times 10^{-4} \times D^{-1}$	$0.0128 \times D^{-\frac{1}{2}}$	$0.0107 \times D^{\frac{1}{2}}$	-	$0.02388 \times D^{\frac{1}{2}}$				$12087 \times D^{-1}$	-
dependence on resolution d	-	-	-	$\alpha\ 1/d^2$	-	-	-	$\alpha\ 1/d$	$\alpha\ 1/d$	$\alpha\ 1/d$				-	$38,000/d$

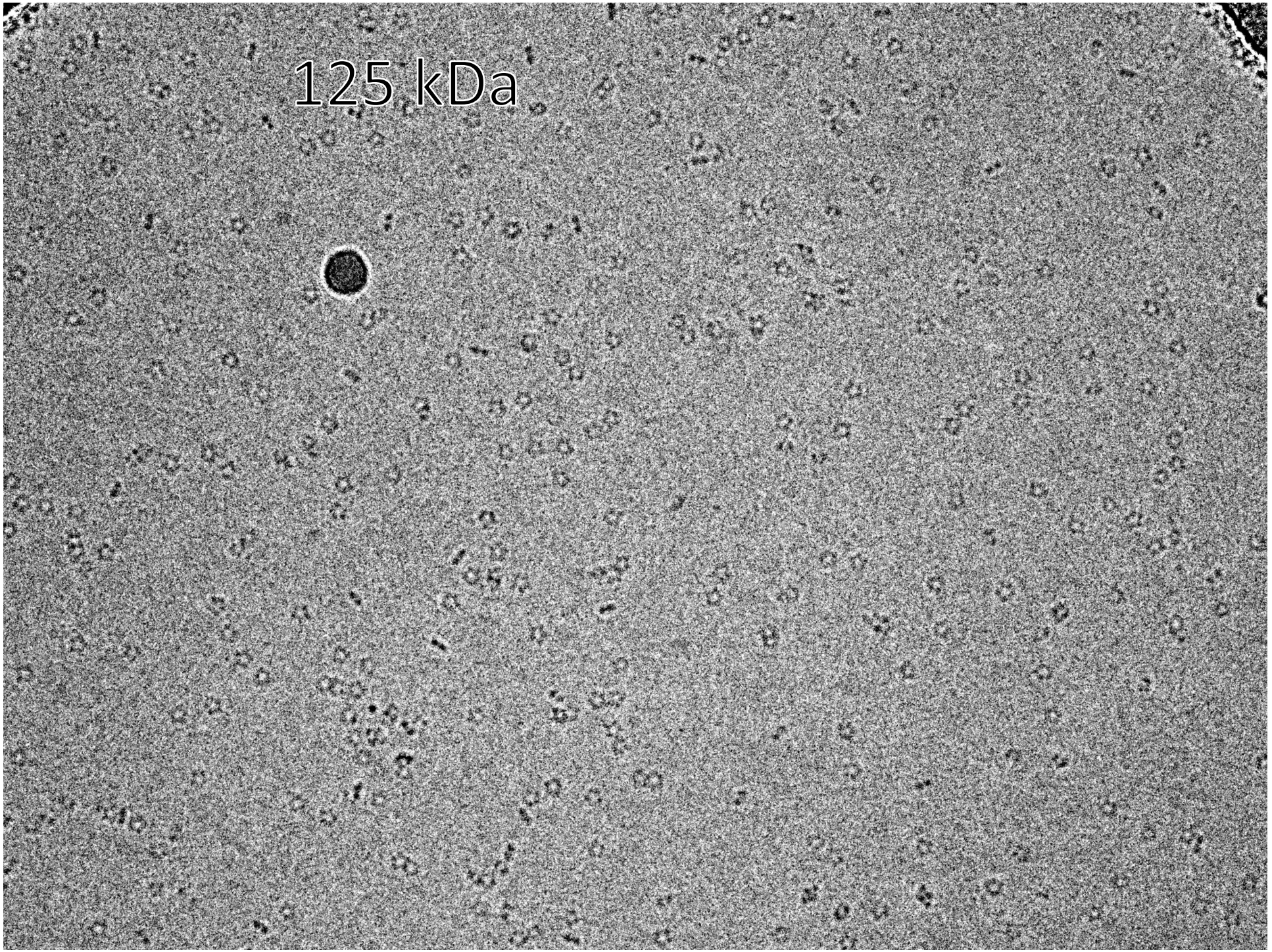
Parameters in electron microscopy of single protein molecules or molecular assemblies. To simplify the presentation, it is assumed that the molecules are arranged in a closely-packed 2-dimensional crystal with a square unit cell as shown in Fig. 3. The formulae used to derive Table 2 are given in the Appendix.

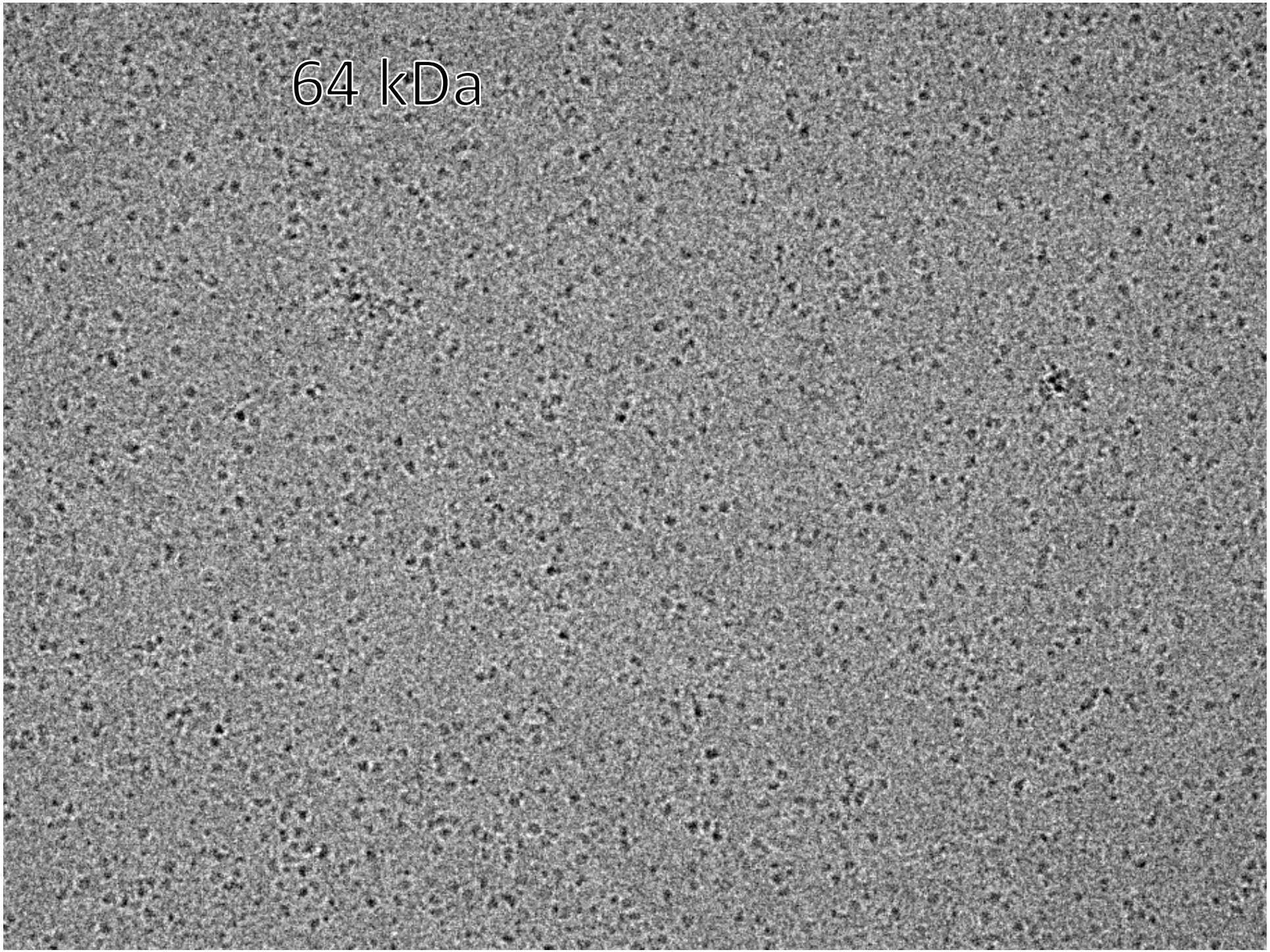

1.6 MDa
from Vinothkumar & Henderson, 2016

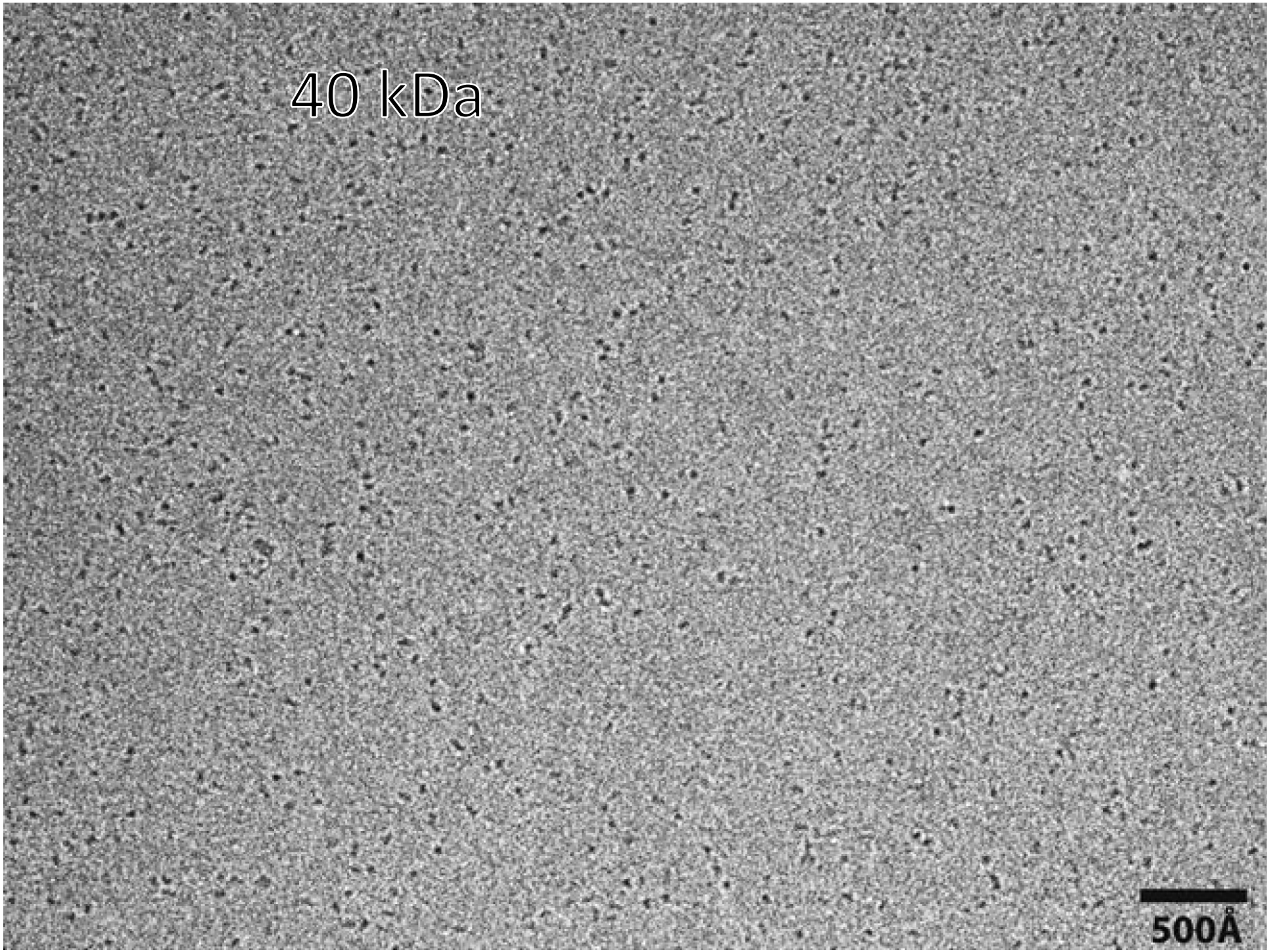

900 kDa

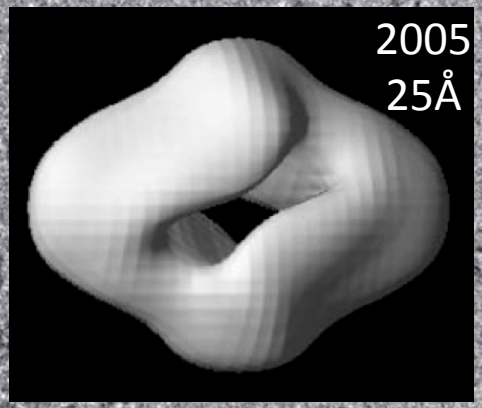

450 kDa

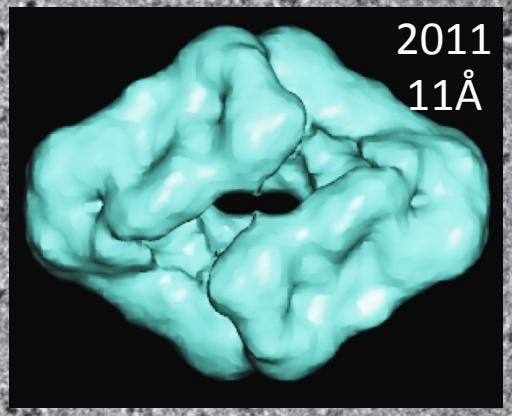

240 kDa

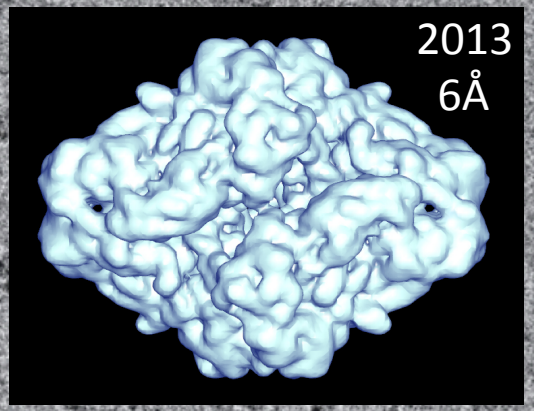

125 kDa

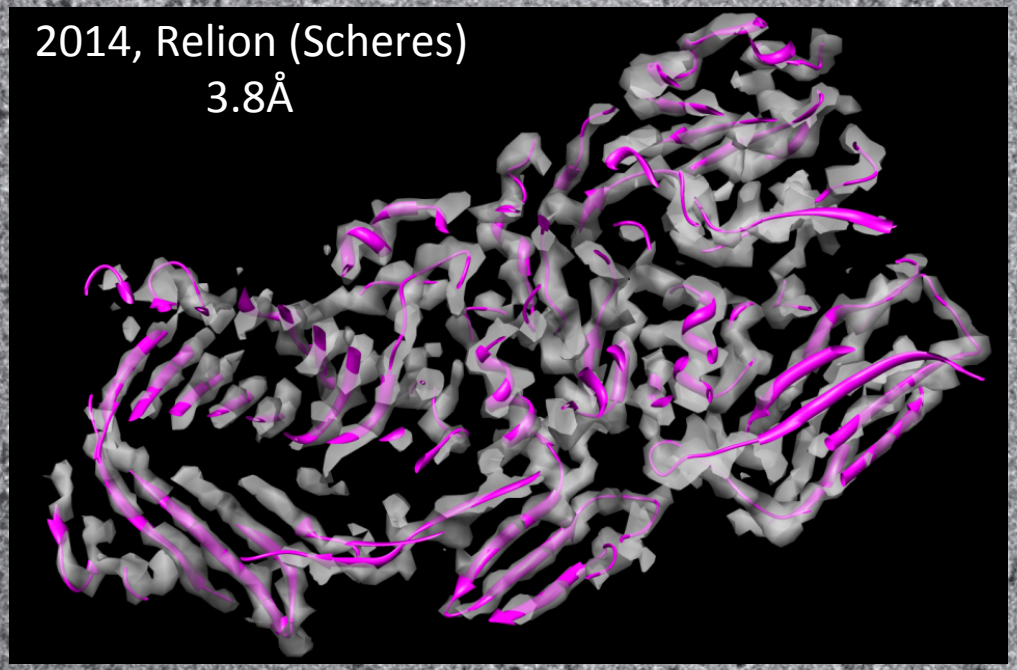

64 kDa


40 kDa
500Å

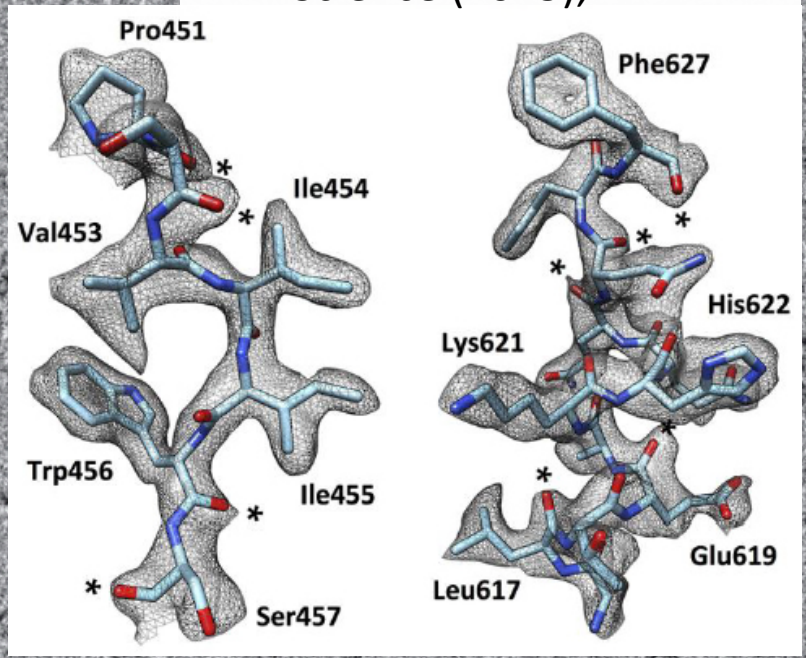


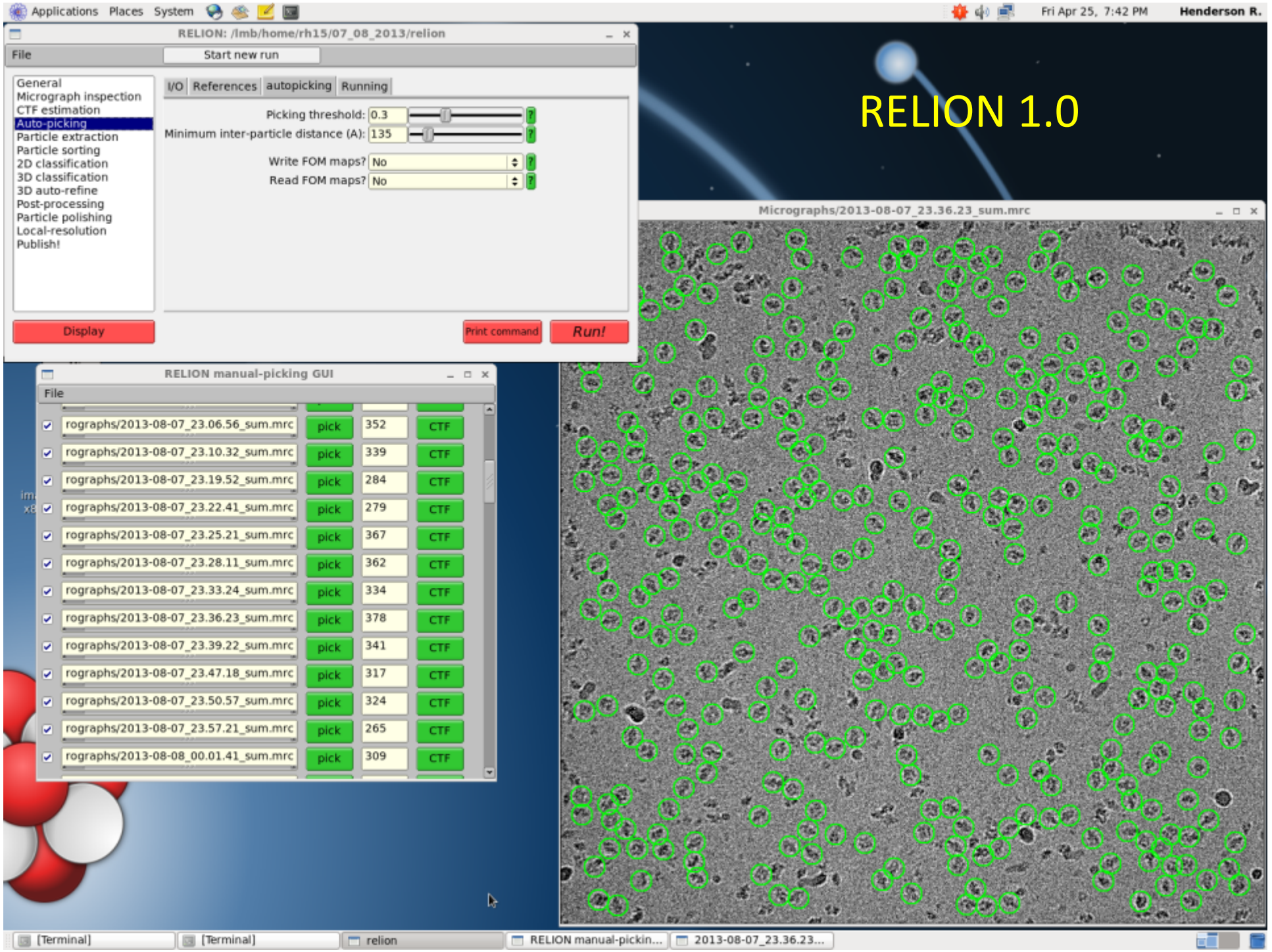






Bartesaghi et al+Subramaniam
Science (2015), 2.2Å




Applications Places System
Fri Apr 25, 7:42 PM Henderson R.
RELION: /lmb/home/rh15/07_08_2013/relion
File
Start new run
General
Micrograph inspection
CTF estimation
Auto-picking
Particle extraction
Particle sorting
2D classification
3D classification
3D auto-refine
Post-processing
Particle polishing
Local-resolution
Publish!
I/O
References
autopicking
Running
Picking threshold: 0.3
Minimum inter-particle distance (A): 135
Write FOM maps? No
Read FOM maps? No
Display
Print command
Run!
RELION 1.0
Micrographs/2013-08-07_23.36.23_sum.mrc
RELION manual-picking GUI
File
rographs/2013-08-07_23.06.56_sum.mrc pick 352 CTF
rographs/2013-08-07_23.10.32_sum.mrc pick 339 CTF
rographs/2013-08-07_23.19.52_sum.mrc pick 284 CTF
rographs/2013-08-07_23.22.41_sum.mrc pick 279 CTF
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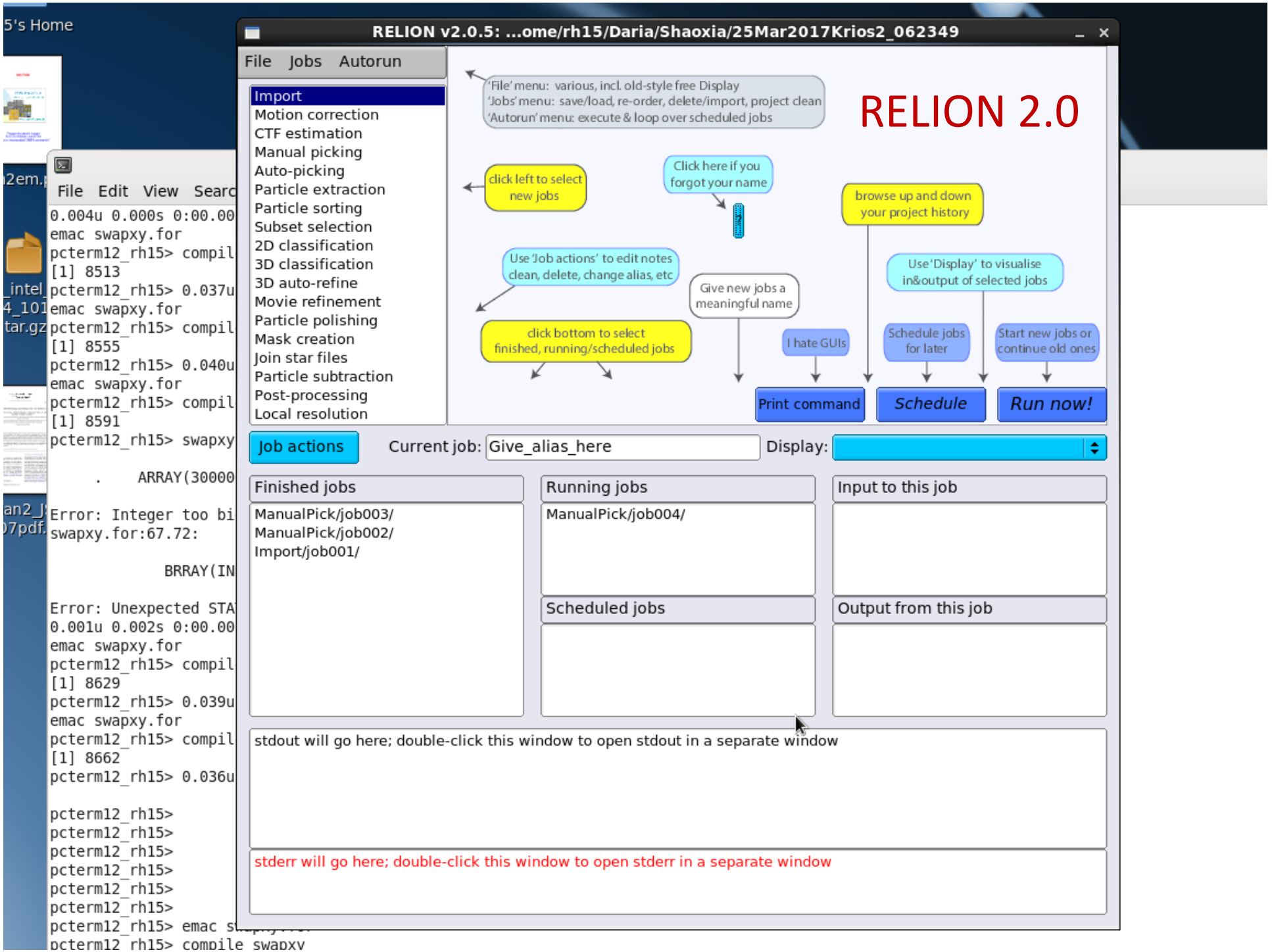
rographs/2013-08-07_23.28.11_sum.mrc pick 362 CTF
rographs/2013-08-07_23.33.24_sum.mrc pick 334 CTF
rographs/2013-08-07_23.36.23_sum.mrc pick 378 CTF
rographs/2013-08-07_23.39.22_sum.mrc pick 341 CTF
rographs/2013-08-07_23.47.18_sum.mrc pick 317 CTF
rographs/2013-08-07_23.50.57_sum.mrc pick 324 CTF
rographs/2013-08-07_23.57.21_sum.mrc pick 265 CTF
rographs/2013-08-08_00.01.41_sum.mrc pick 309 CTF
[Terminal]
[Terminal]
relion
RELION manual-pickin...
2013-08-07_23.36.23...


RELION v2.0.5: ...ome/rh15/Daria/Shaoxia/25Mar2017Krios2_062349
File Jobs Autorun
Import
Motion correction
CTF estimation
Manual picking
Auto-picking
Particle extraction
Particle sorting
Subset selection
2D classification
3D classification
3D auto-refine
Movie refinement
Particle polishing
Mask creation
Join star files
Particle subtraction
Post-processing
Local resolution
'File' menu: various, incl. old-style free Display
'Jobs' menu: save/load, re-order, delete/import, project clean
'Autorun' menu: execute & loop over scheduled jobs
RELION 2.0
click left to select new jobs
Click here if you forgot your name
browse up and down your project history
Use 'Job actions' to edit notes clean, delete, change alias, etc
Give new jobs a meaningful name
Use 'Display' to visualise in&output of selected jobs
click bottom to select finished, running/scheduled jobs
I hate GUIs
Schedule jobs for later
Start new jobs or continue old ones
Print command
Schedule
Run now!
Job actions
Current job: Give_alias_here
Display:
Finished jobs
ManualPick/job003/
ManualPick/job002/
Import/job001/
Running jobs
ManualPick/job004/
Input to this job
Scheduled jobs
Output from this job
stdout will go here; double-click this window to open stdout in a separate window
stderr will go here; double-click this window to open stderr in a separate window
File Edit View Searc
0.004u 0.000s 0:00.00
emac swapxy.for
pcterm12_rh15> compil
[1] 8513
pcterm12_rh15> 0.037u
emac swapxy.for
pcterm12_rh15> compil
[1] 8555
pcterm12_rh15> 0.040u
emac swapxy.for
pcterm12_rh15> compil
[1] 8591
pcterm12_rh15> swapxy
. ARRAY(30000
Error: Integer too bi
swapxy.for:67.72:
BRRAY(IN
Error: Unexpected STA
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[1] 8629
pcterm12_rh15> 0.039u
emac swapxy.for
pcterm12_rh15> compil
[1] 8662
pcterm12_rh15> 0.036u
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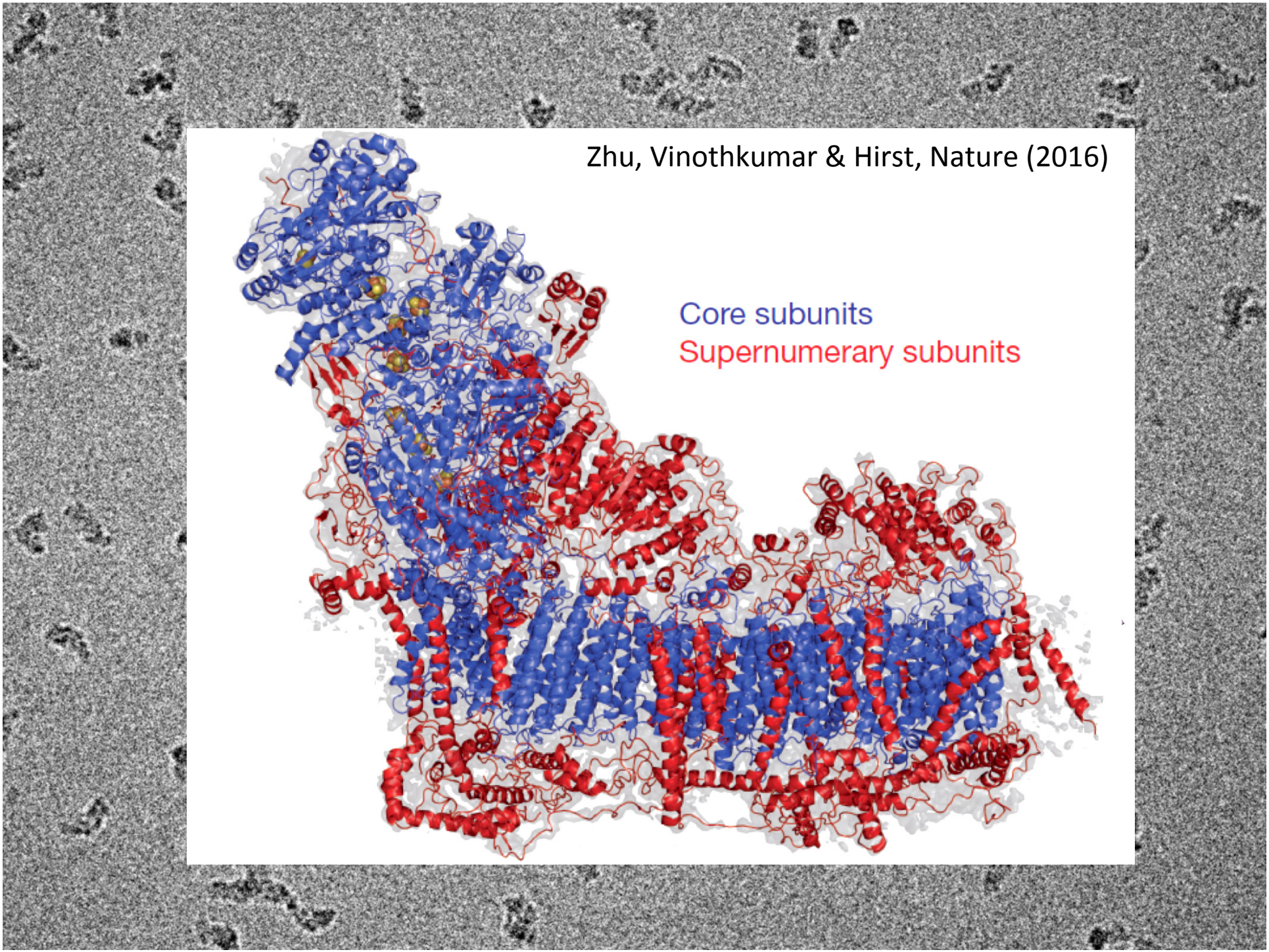
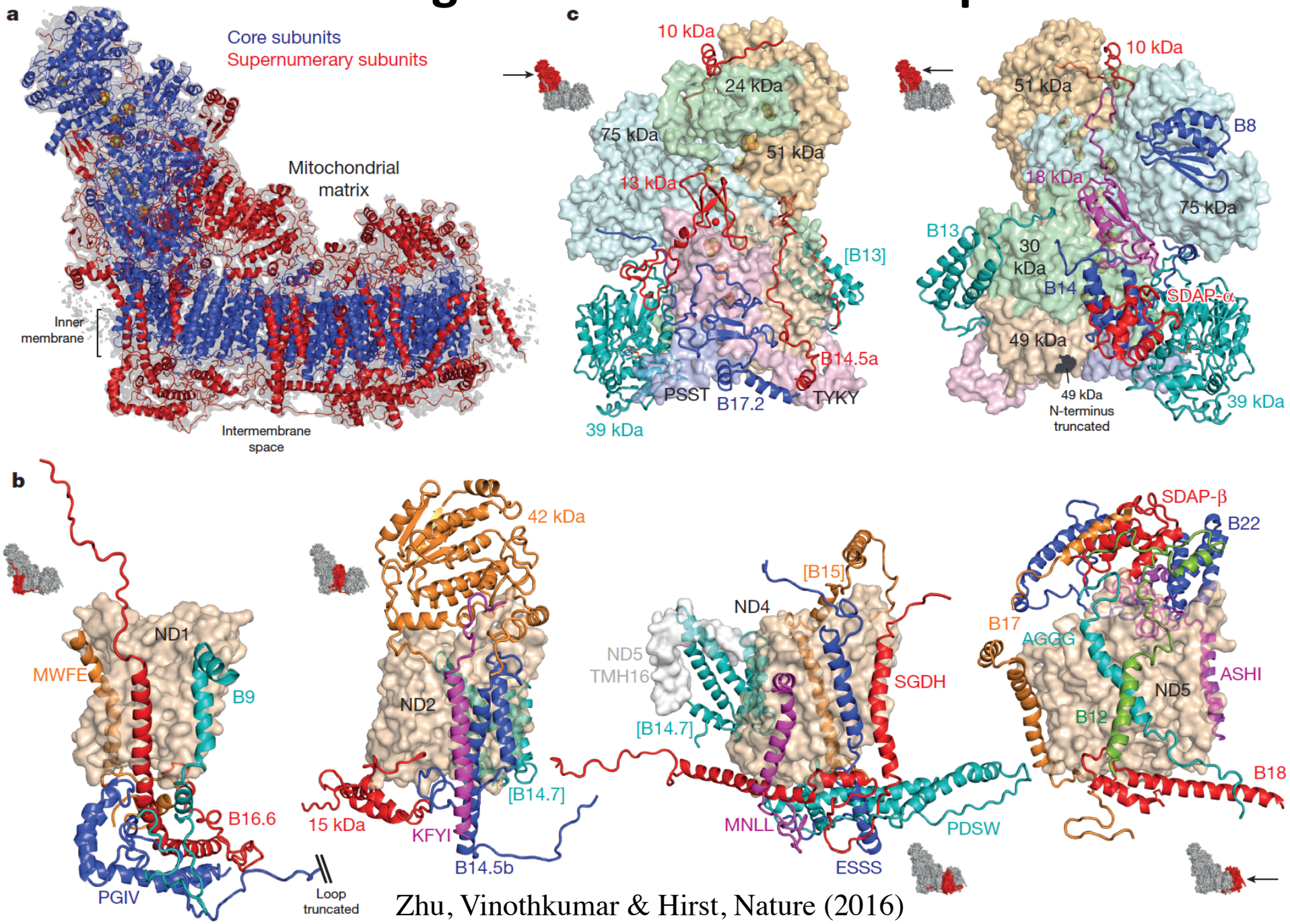
pcterm12_rh15> compile swapxy

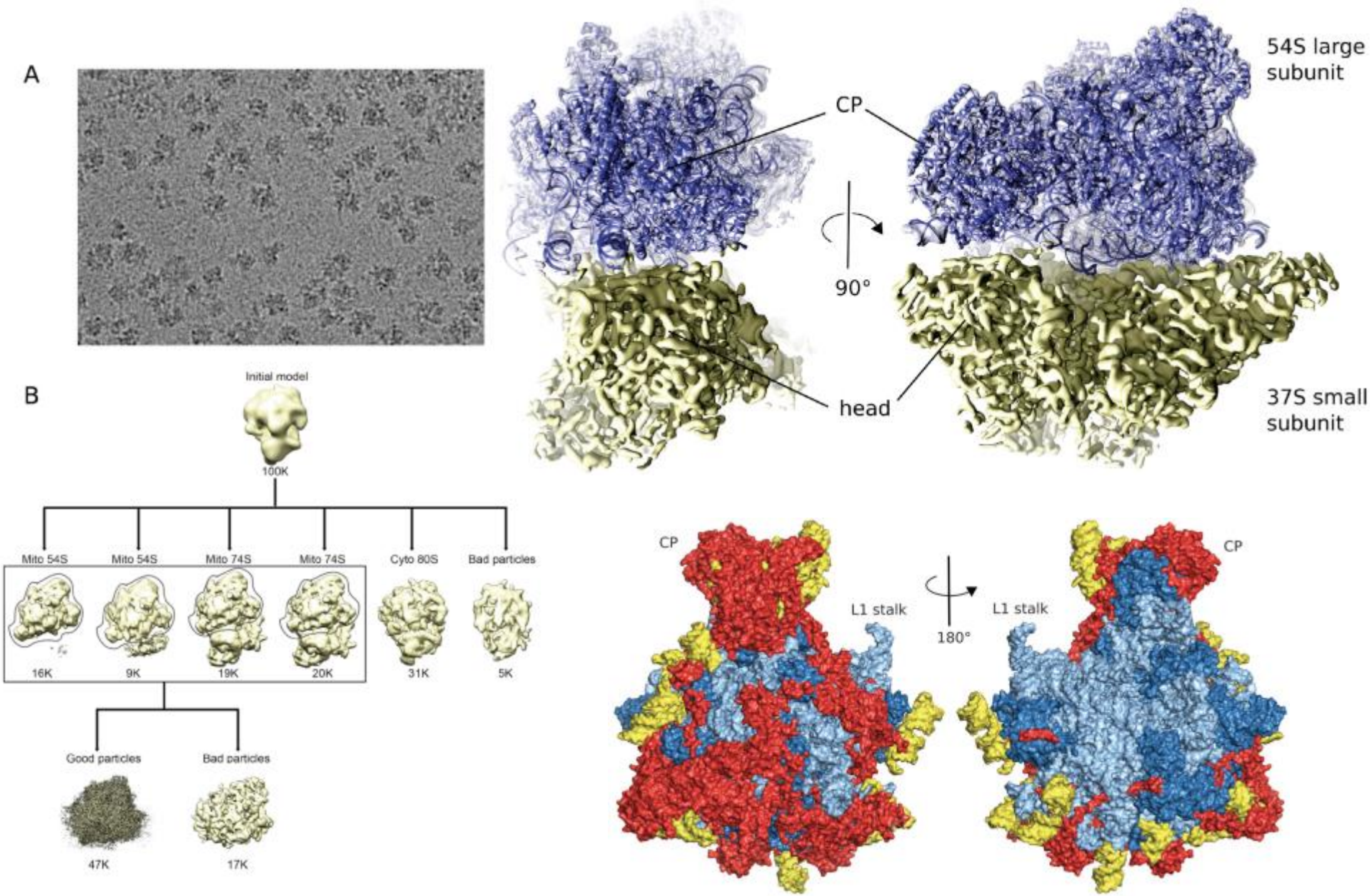

Zhu, Vinothkumar & Hirst, Nature (2016)
Core subunits
Supernumerary subunits

Subunit assignment of bovine Complex I

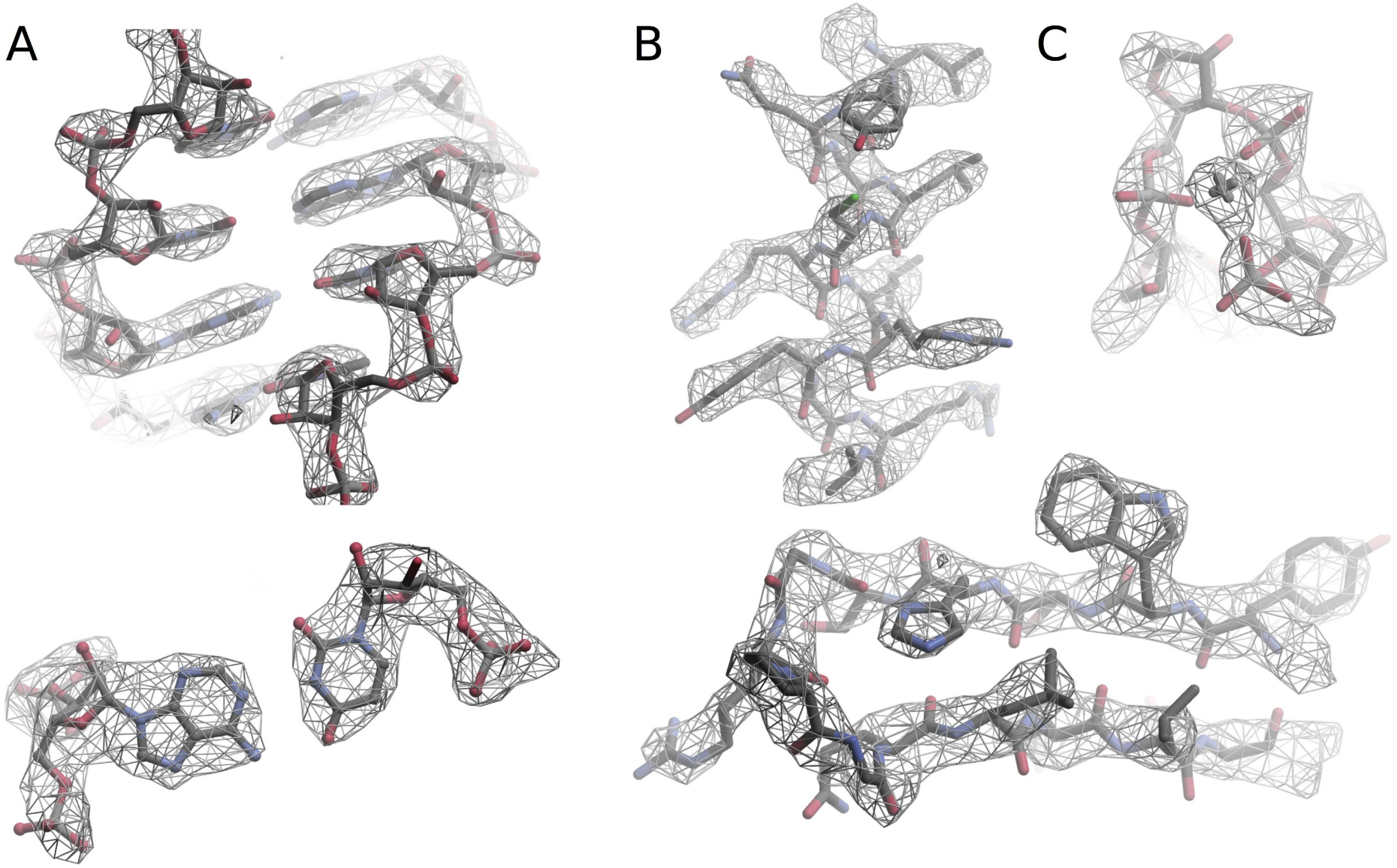


Zhu, Vinothkumar & Hirst, Nature (2016)

3.1 Å mitochondrial ribosome map 300keV, Falcon, Amunts et al, Science **343**, 1485-1489 (2014)
(groups of Scheres & Ramakrishnan)

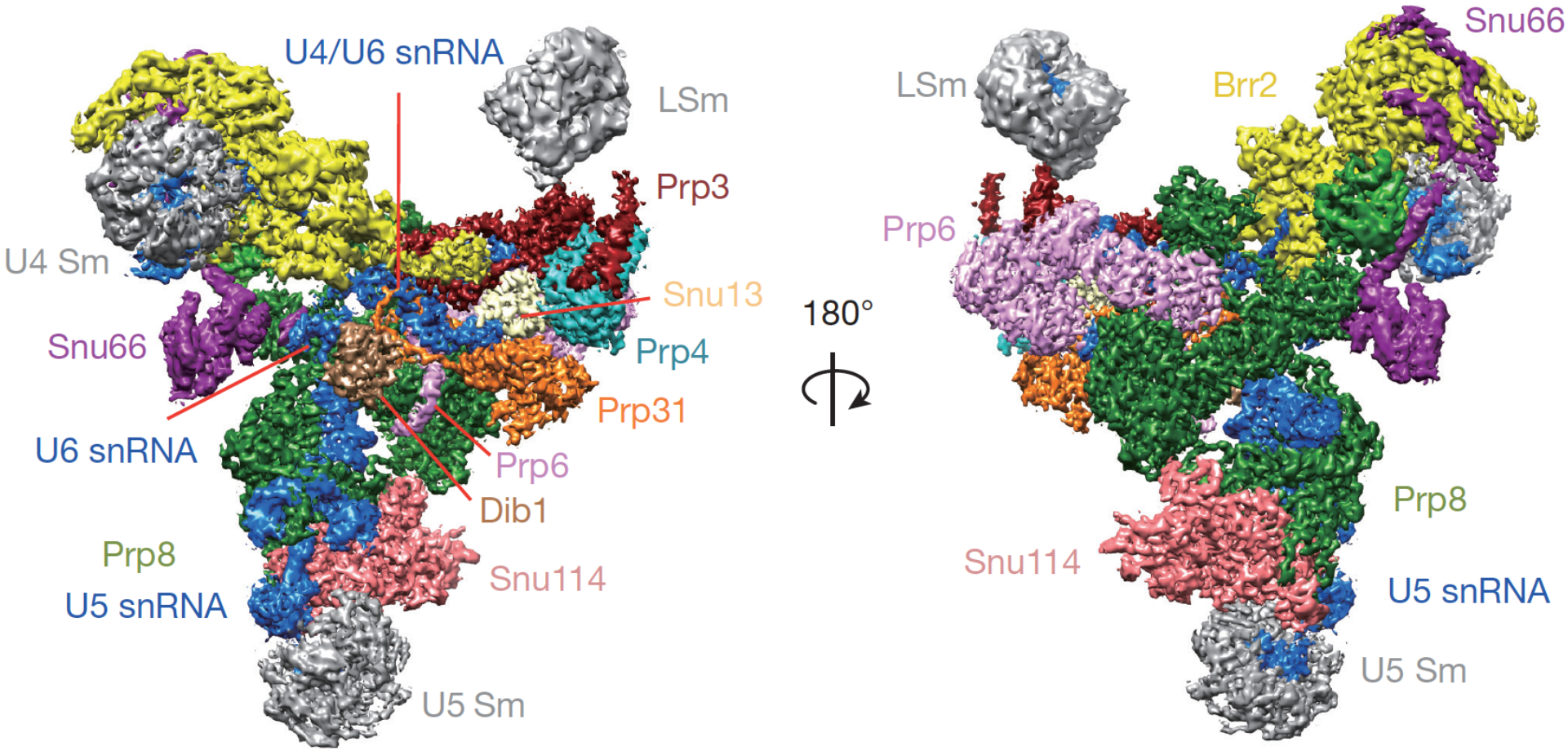


3.1 Å mitochondrial ribosome map 300keV, Falcon, Amunts et al, Science **343**, 1485-1489 (2014)
(groups of Scheres & Ramakrishnan)



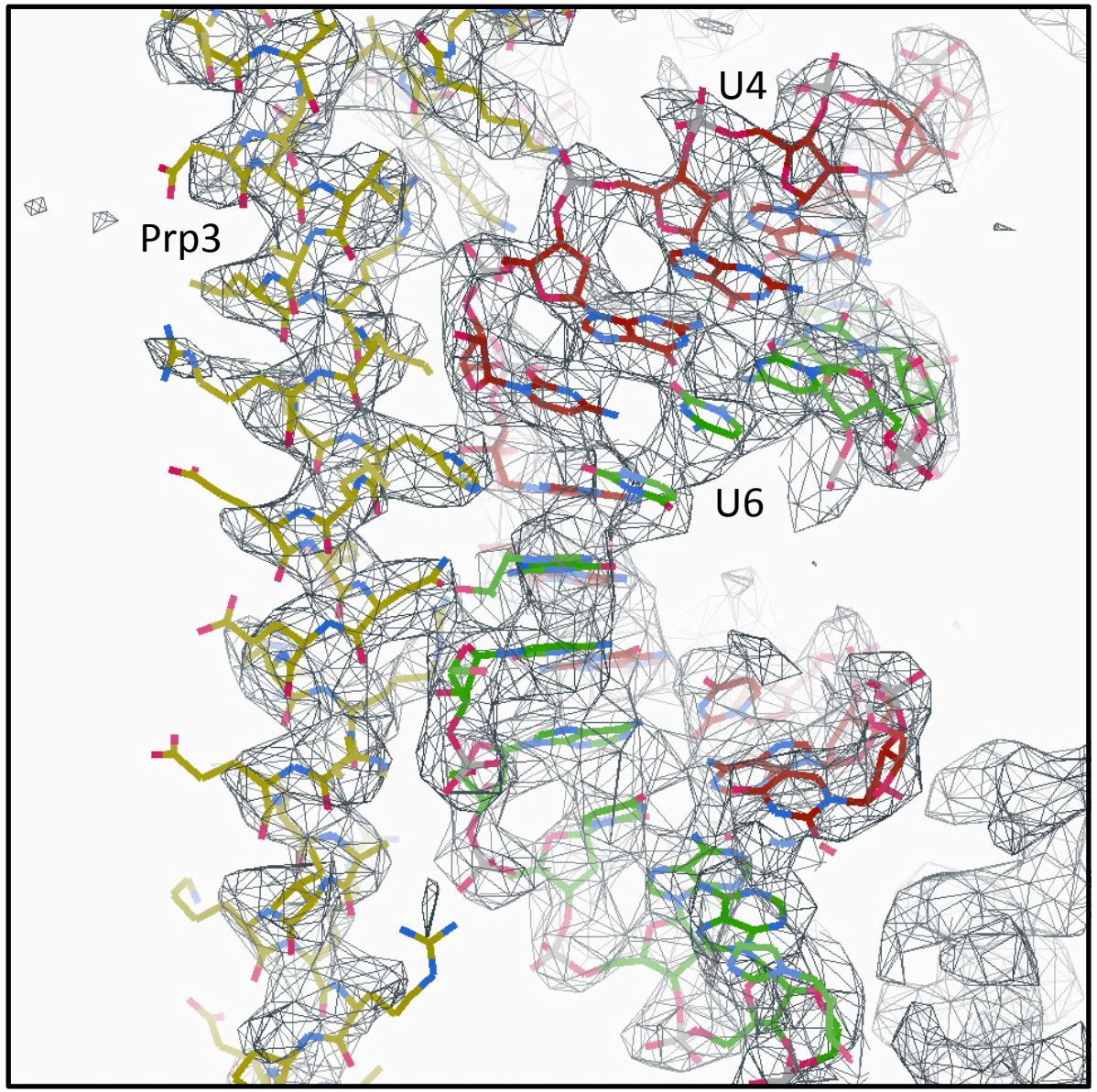
Cryo-EM structure of the yeast U4/U6.U5 tri-snRNP at 3.7 Å resolution

Nguyen et al & Nagai, Nature **530** 298-302 (2016)

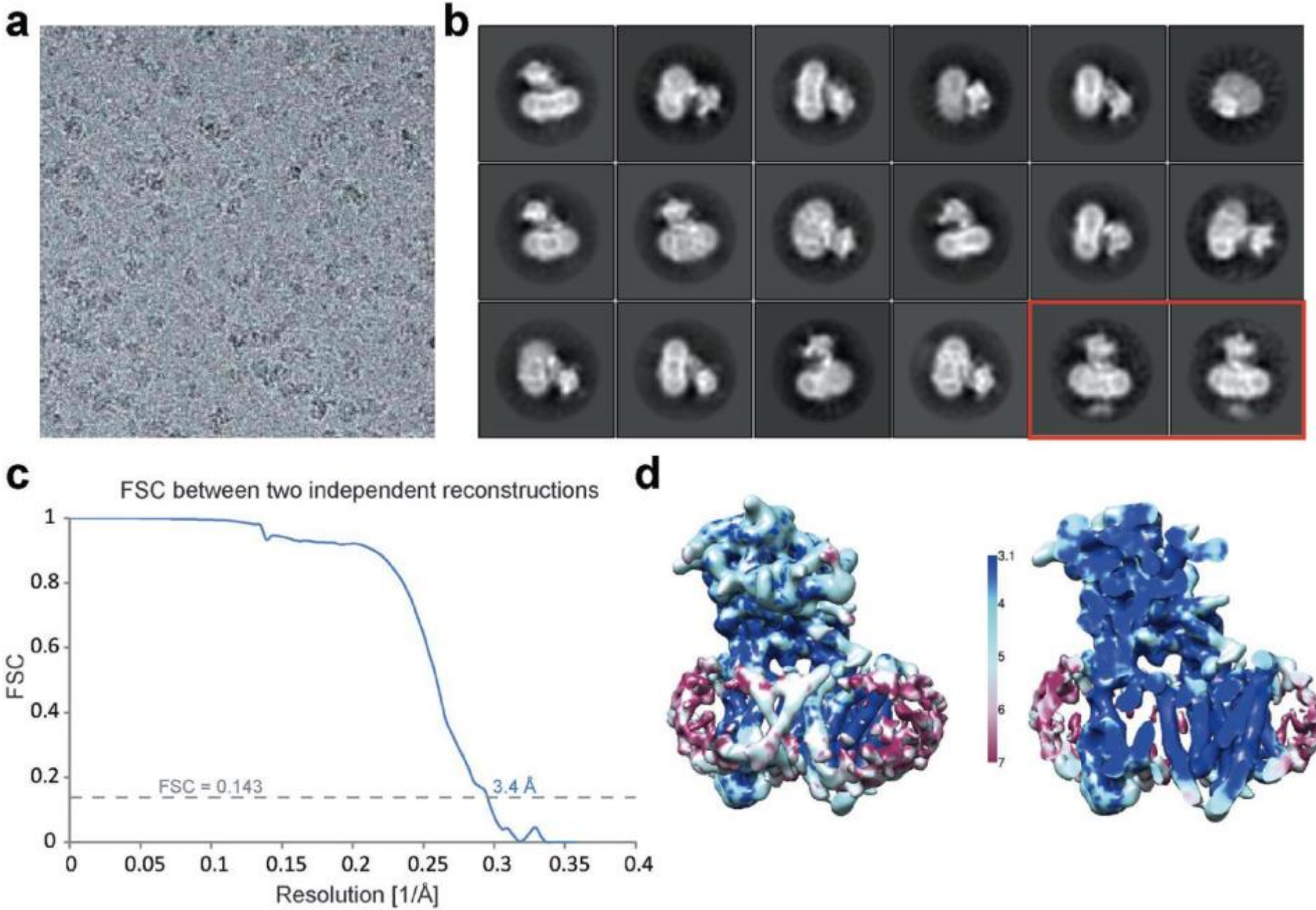


Cryo-EM structure of the yeast U4/U6.U5 tri-snRNP at 3.7 Å resolution

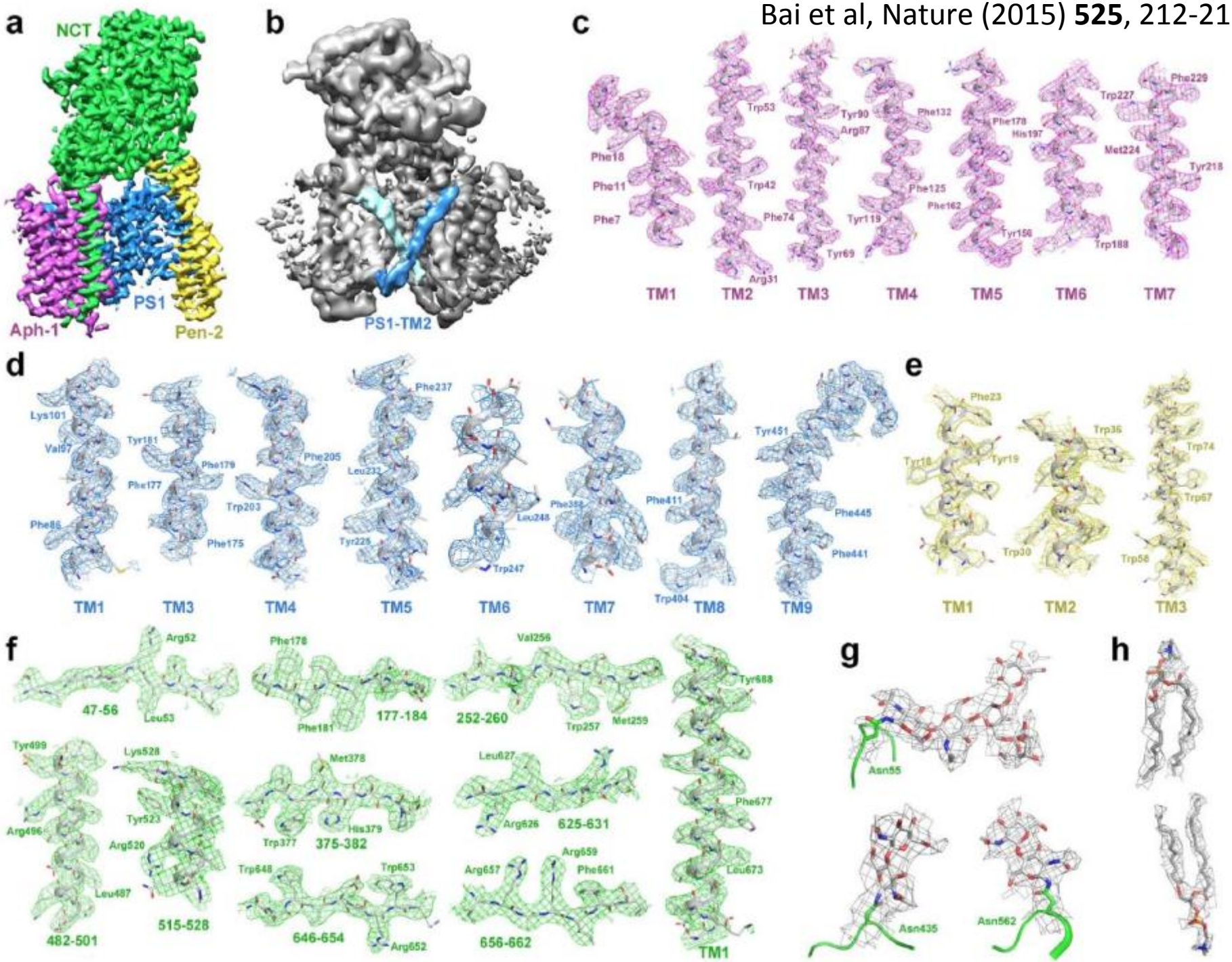
Nguyen et al & Nagai, Nature **530** 298-302 (2016)



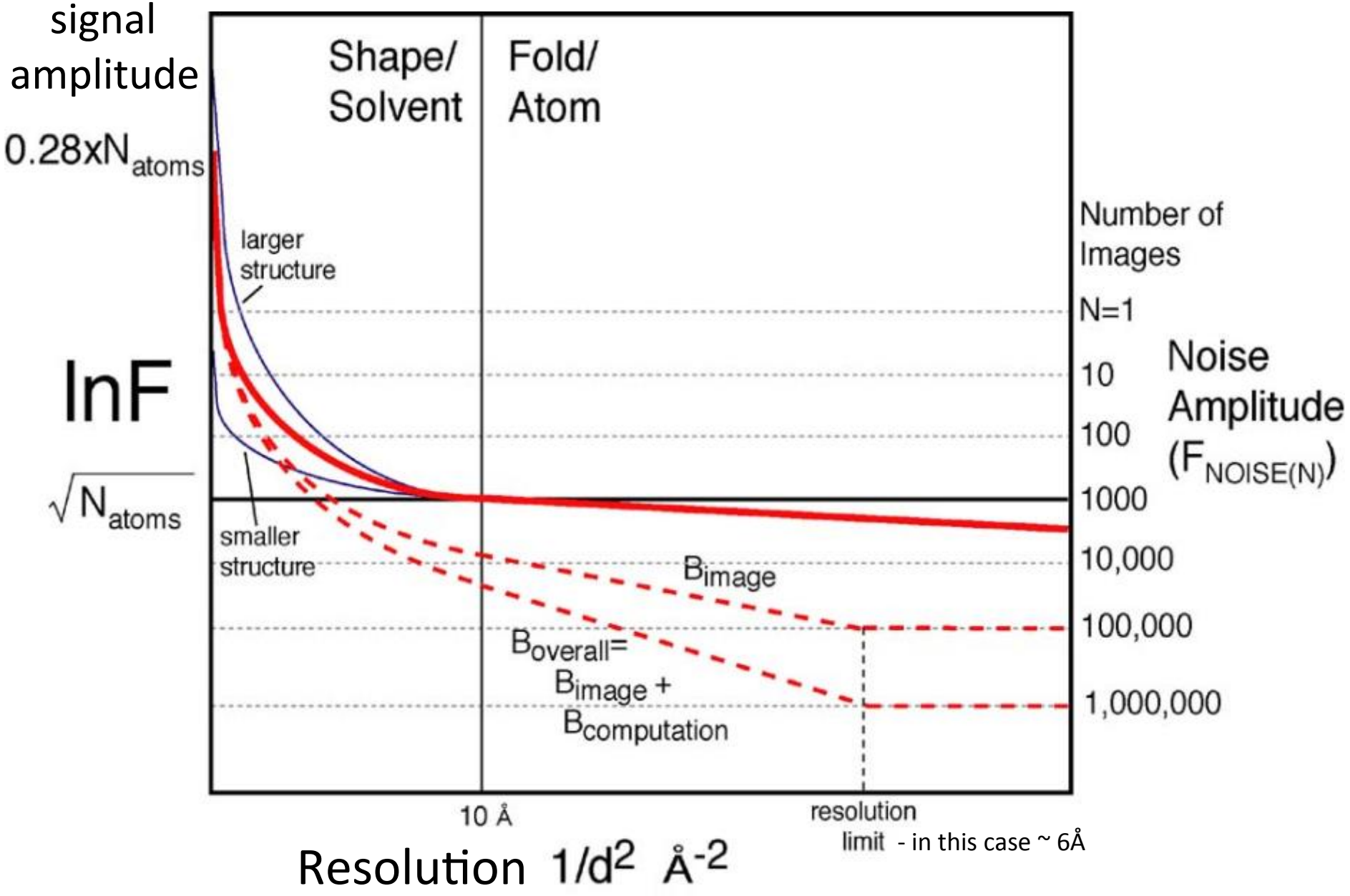
Gamma-secretase Bai et al, Nature (2015) **525**, 212-217



Bai et al, Nature (2015) **525**, 212-217

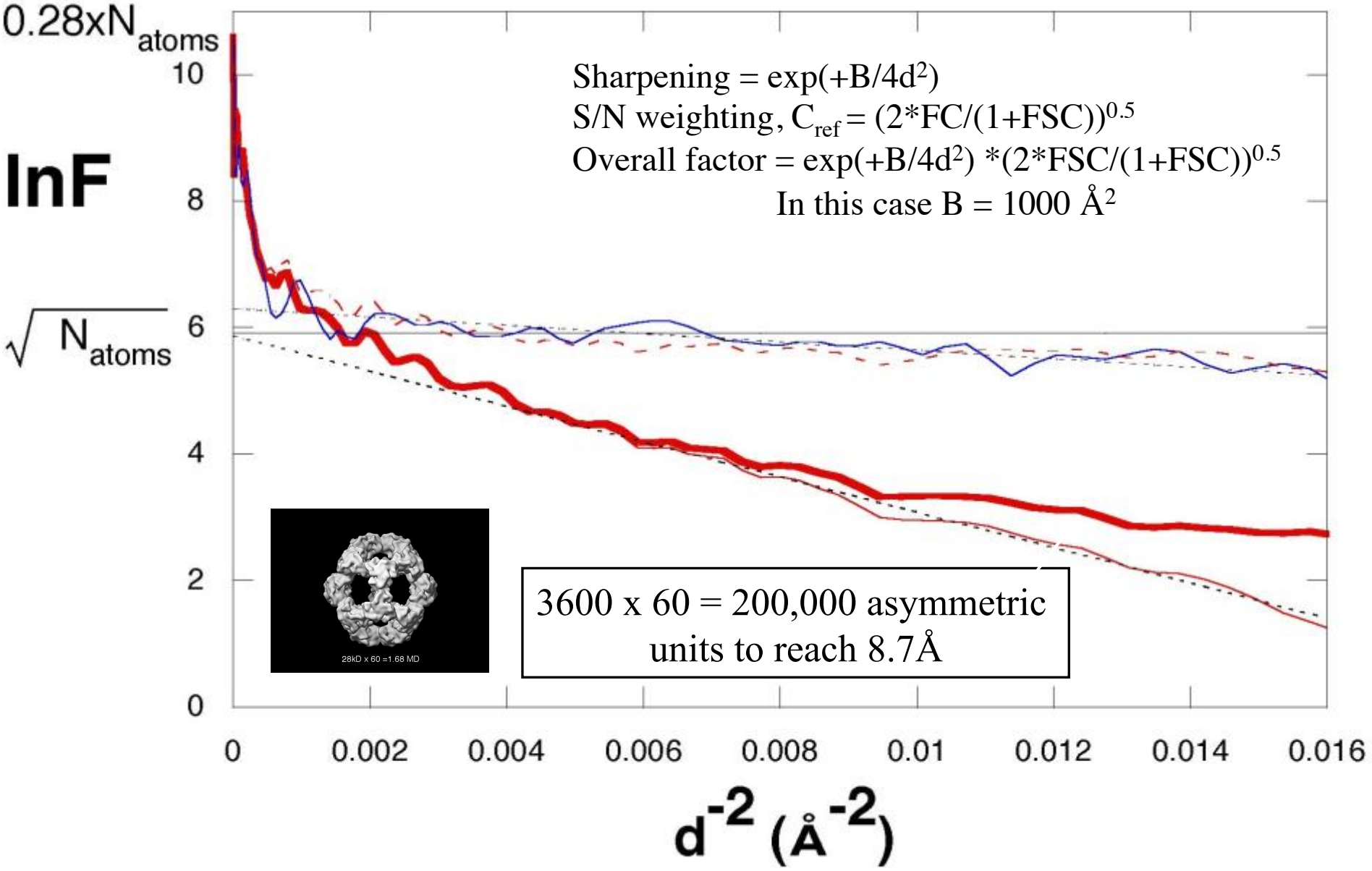


Theory – single particles in ice



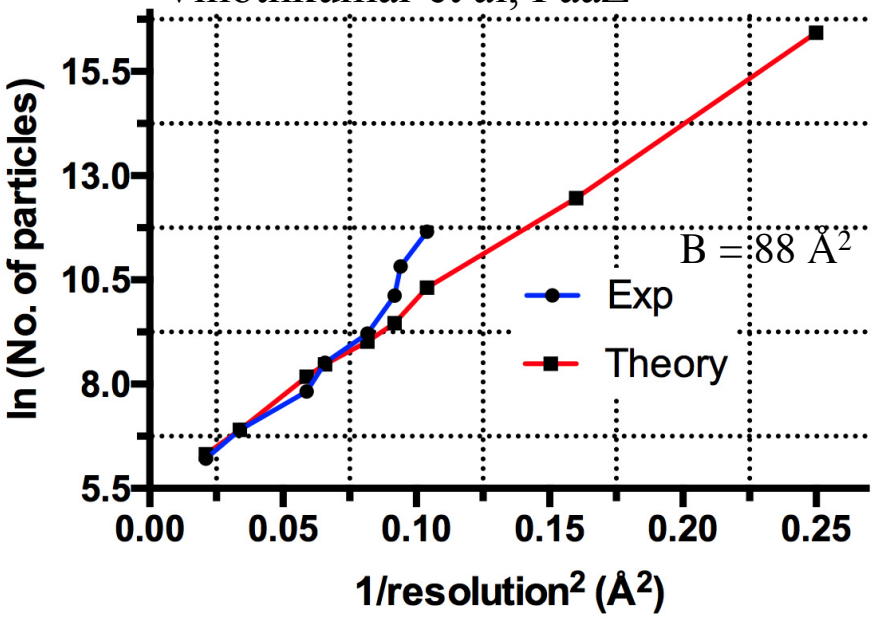
Experimental data

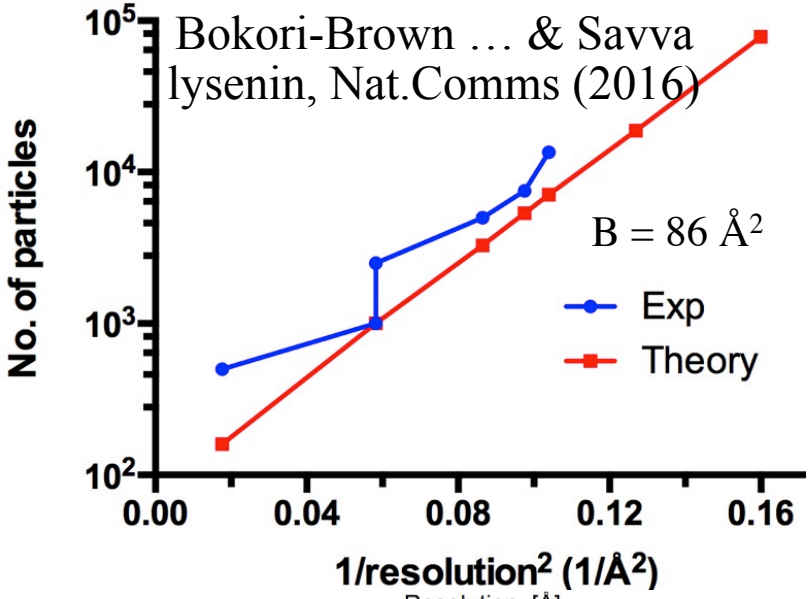
Rosenthal (2003) JMB **333**, 225-36
Fernandez (2008) JSB **164**, 170-5



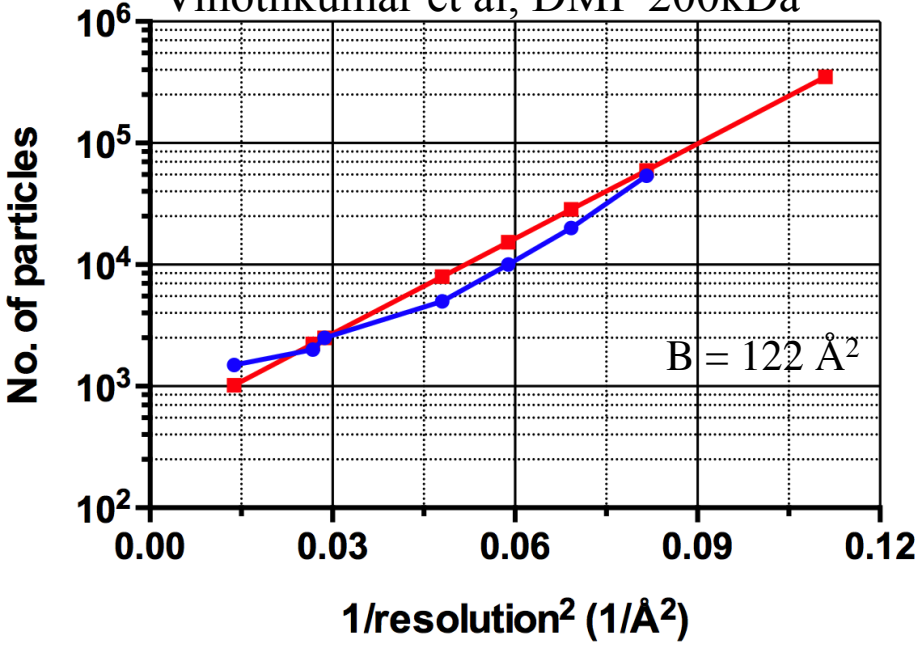
Resolution vs. number of particles

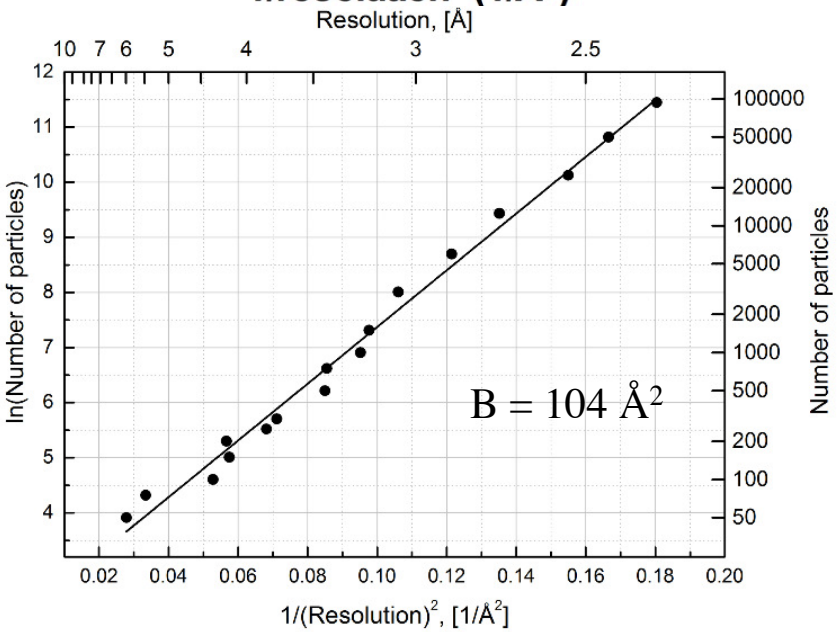
Vinothkumar et al, PaaZ





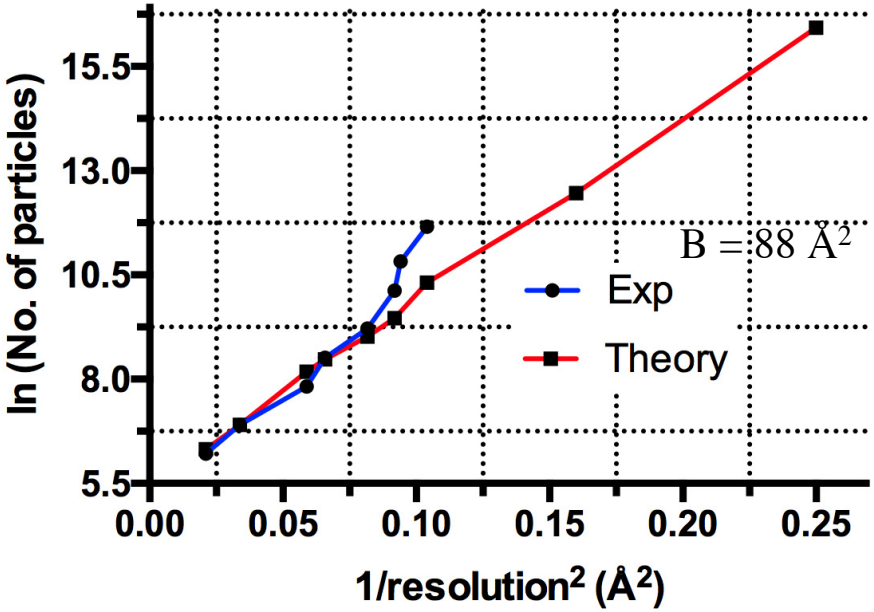
Vinothkumar et al, DMF 200kDa





Danev et al, eLife 2017

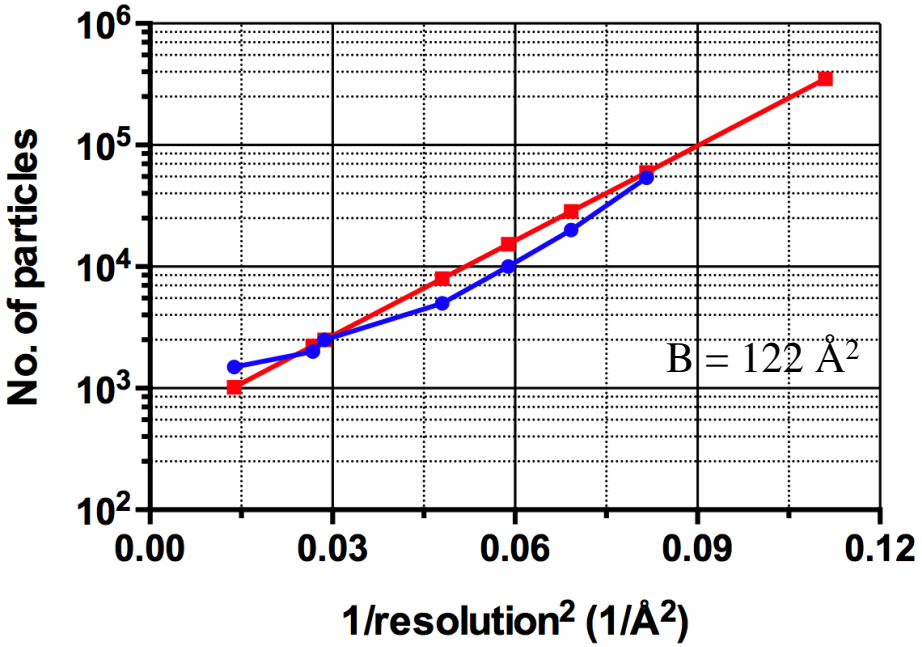
Resolution vs. number of particles

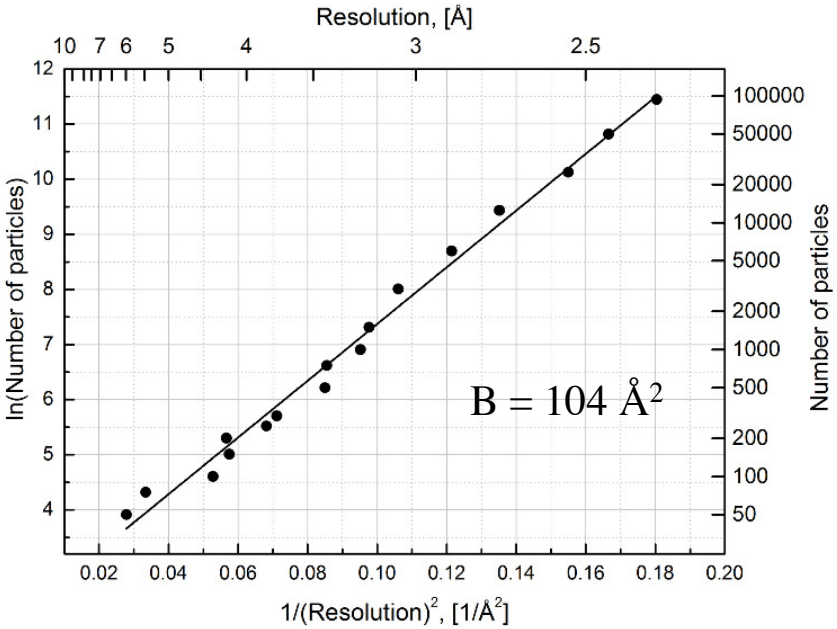


Plot of number of particles versus resolution should be linear if a single B-factor describes the average structure factor.

These three examples needed ~5000 asymmetric units to reach 4Å resolution, ~1500 to reach 6Å, 800 to 10Å.

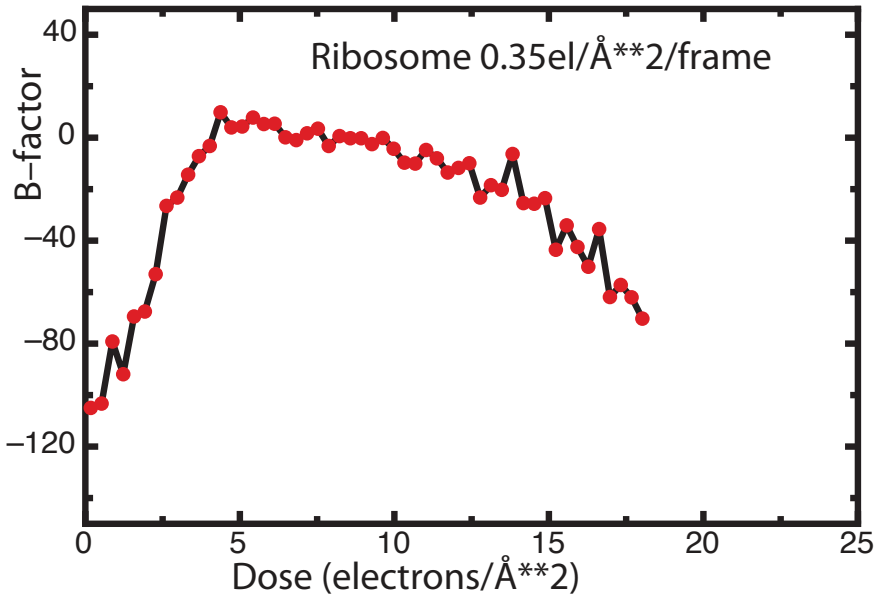
Reducing B-factor is key to high resolution without unrealistic numbers of particles

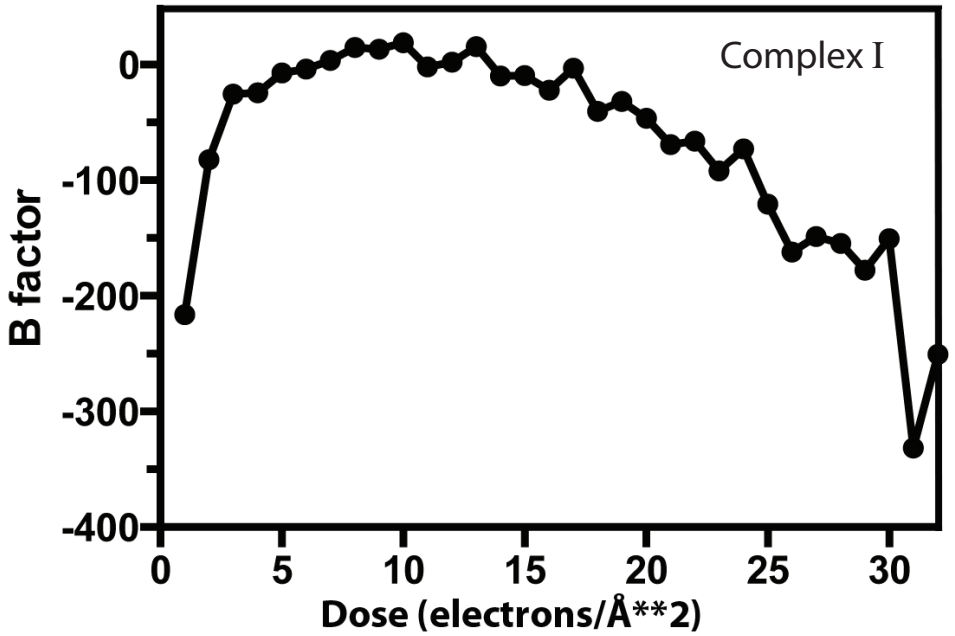


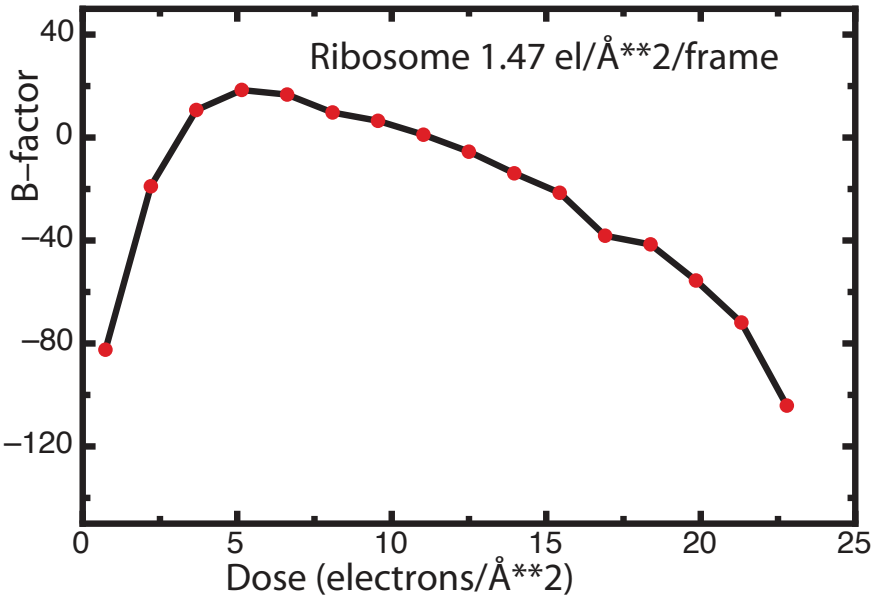


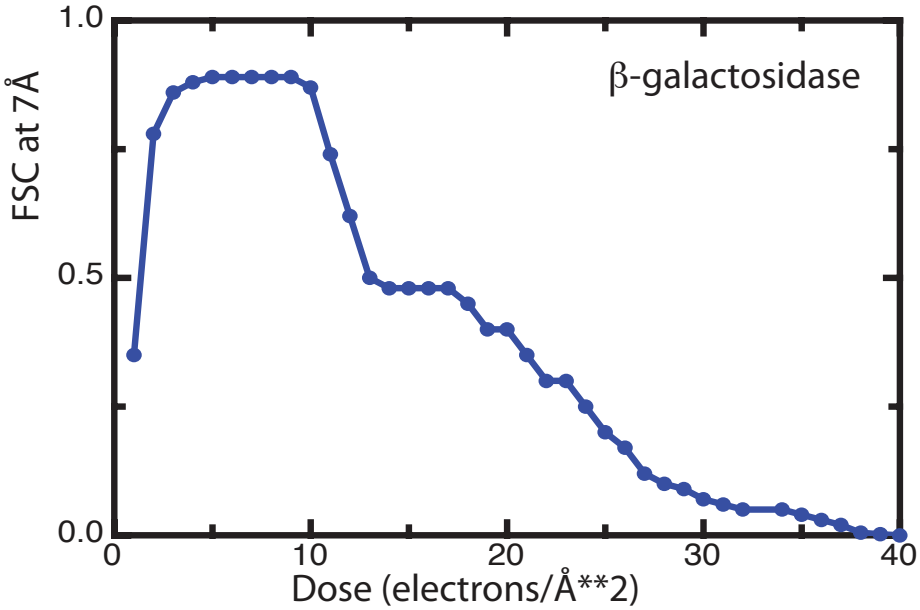
Danev et al, eLife 2017

Information content in movie frames from 4 different datasets

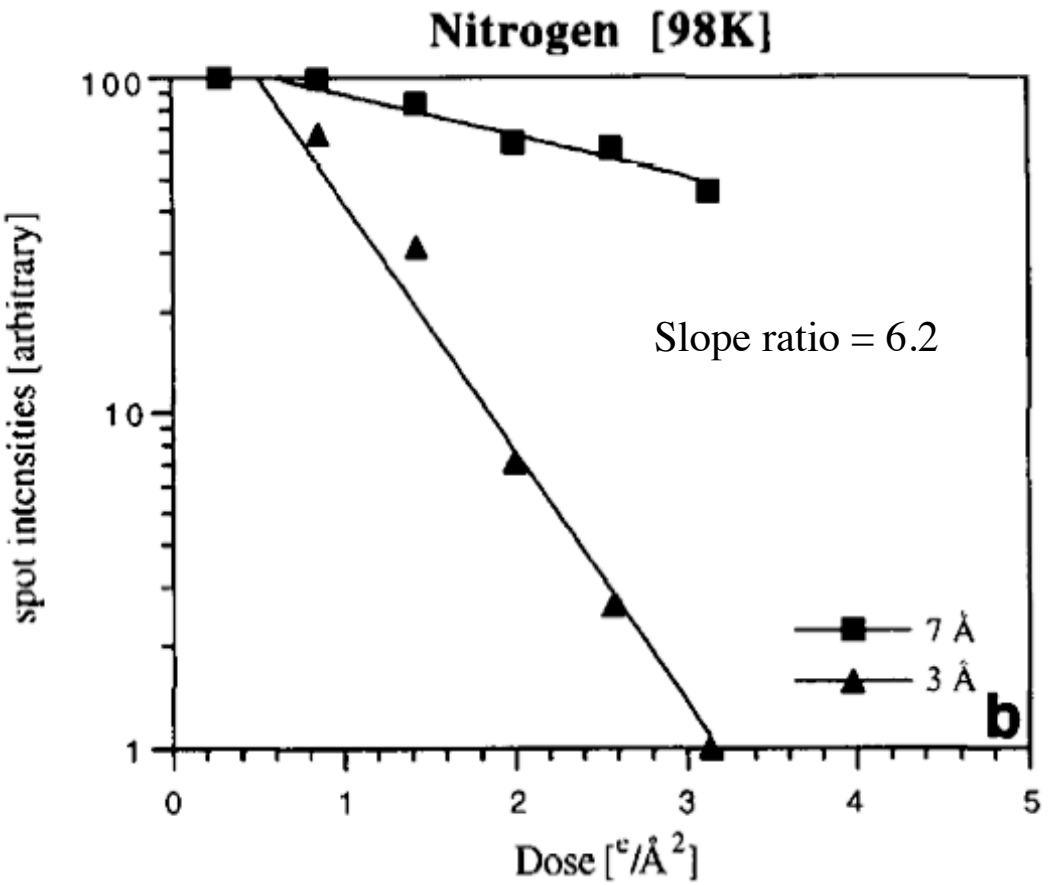








Stark, Zemlin & Boettcher (1996) Ultramicroscopy



Conclusions

- 3Å data is more radiation sensitive than 7Å data by a factor of 6.2x.

- This translates into a B-factor due to radiation damage of B = 90Å^2 at 98K.

Radiation damage measured by critical dose Ne, corrected to 300keV electron energy

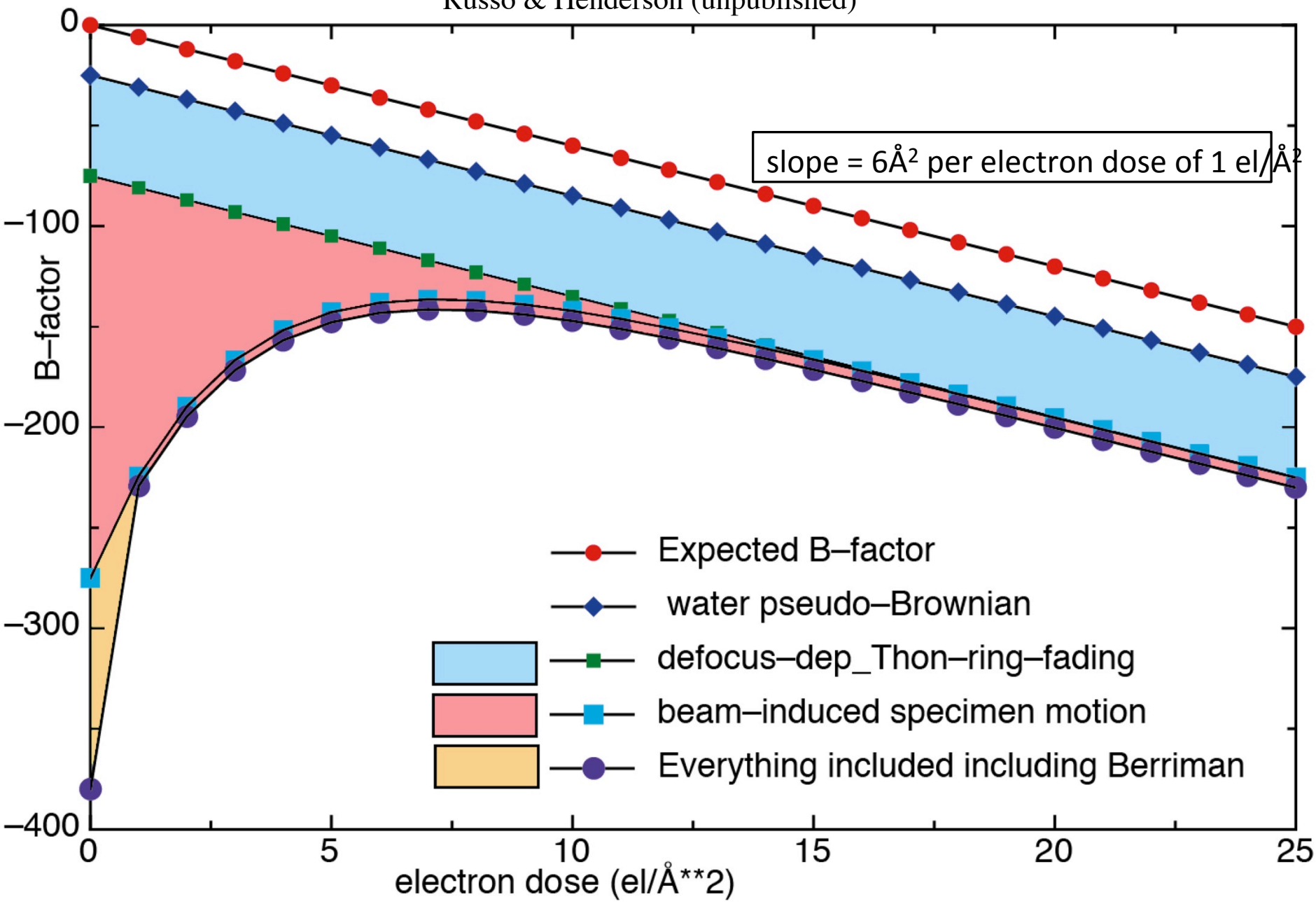
Stark outlier **Fujiyoshi outlier**

The correction is 2.03x for 100keV, 1.25x for 200keV, 0.86x for 400keV electrons. The uncorrected number is shown in brackets.

Publication	Low resolution (7Å)			High resolution (4Å)			Ratio (7ÅRes/3ÅRes)		
	Ne (RT) 7Å	Ne (N_2) 7Å	Ne (He) 7Å	Ne (RT) 4Å	Ne (N_2) 4Å	Ne (He) 4Å	RT	N_2	He
Siegel 1972 paraffin C32				6 (2.2-4.4)	14 (5-9)	18 (6-13)			
Hayward 1979, bR	2.6 (1.3)	20 (10)	-	1.0 (0.5)	6.0 (3)		2.6	3.3	
Unwin 1975, bR+cat	1.2 (0.6)	-	-	-	-	-			
Stark 1996, bR	2.5 (1.2)	9.3 (4.5)	16.0 (8.0)	-	1.6 (0.8)	4.0 (2.0)		5.8	4.0
Brink+Chiu 1991, paraffin	-	-	-	6.4 (2.5-4)	10 (10-14), 16	18 (7.5-14)			
Glaeser 2011, paraffin	-	-	-	~3 (~1.3)	-	-			
Russo &H, 2016, paraffin					9	16.5			
Baldwin 1984, bR	1.03 (0.5)	-	-	0.2 (0.1)	-	-	5.2		
Glaeser 1971, valine	-	-	-	0.8 (0.4)	-	-			
Glaeser 1971, adenine	-	-	-	6.0 (3)	-	-			
Fujiyoshi, 1998 cat+tRNA	(0.5-4)	(2)	(5-60)						
Fujiyoshi 2016, AQP0		~35	~90		~15	~45		2.3	2.0
Grant 2015, rotavirus image	-	19	-	-	12	-		1.6	
Baker 2010, catalase image	-	6.8 (5.5)	-	-	5.8 (4.6)	-		1.2	
Richard's consensus	2	11.0	16.0	0.75-2.5	2.0-6.0	4.0-10			
ΔB-factor RNA ($3Å^2/elÅ^{-2}$)	-	22	-	-	6.0	-			
ΔB-factor prot. ($6Å^2/elÅ^{-2}$)	-	11	-		4.0	-			

Rough grand scheme showing the potential for further improvements in cryoEM

Russo & Henderson (unpublished)



Types of electron phase plate

electrostatic
Zernike, Boersch einzel lens w. 3 electrodes (Majorovits et al & Schroeder, 2007)
Anamorphic (Rose)
Biased drift tube (Cambie et al, 2013)

Refractive index shift
Thin (carbon) film with hole (Danev & Nagayama)

Ponderomotive retardation
Laser cavity (Glaeser, 2013; Rev.Sci.Instr. Table w. ~15 different image methods)

Other electrostatic
Gold-plated spider silk, Unwin, 1973
Hole free phase plate (hfpp) Malac et al & Egerton, 2012
Volta phase plate (VPP) Danev et al, 2014; heat 200ºC

Danev et al (2017) eLife 6:e23006, Using Volta phase plate with defocus, Fig. 1.

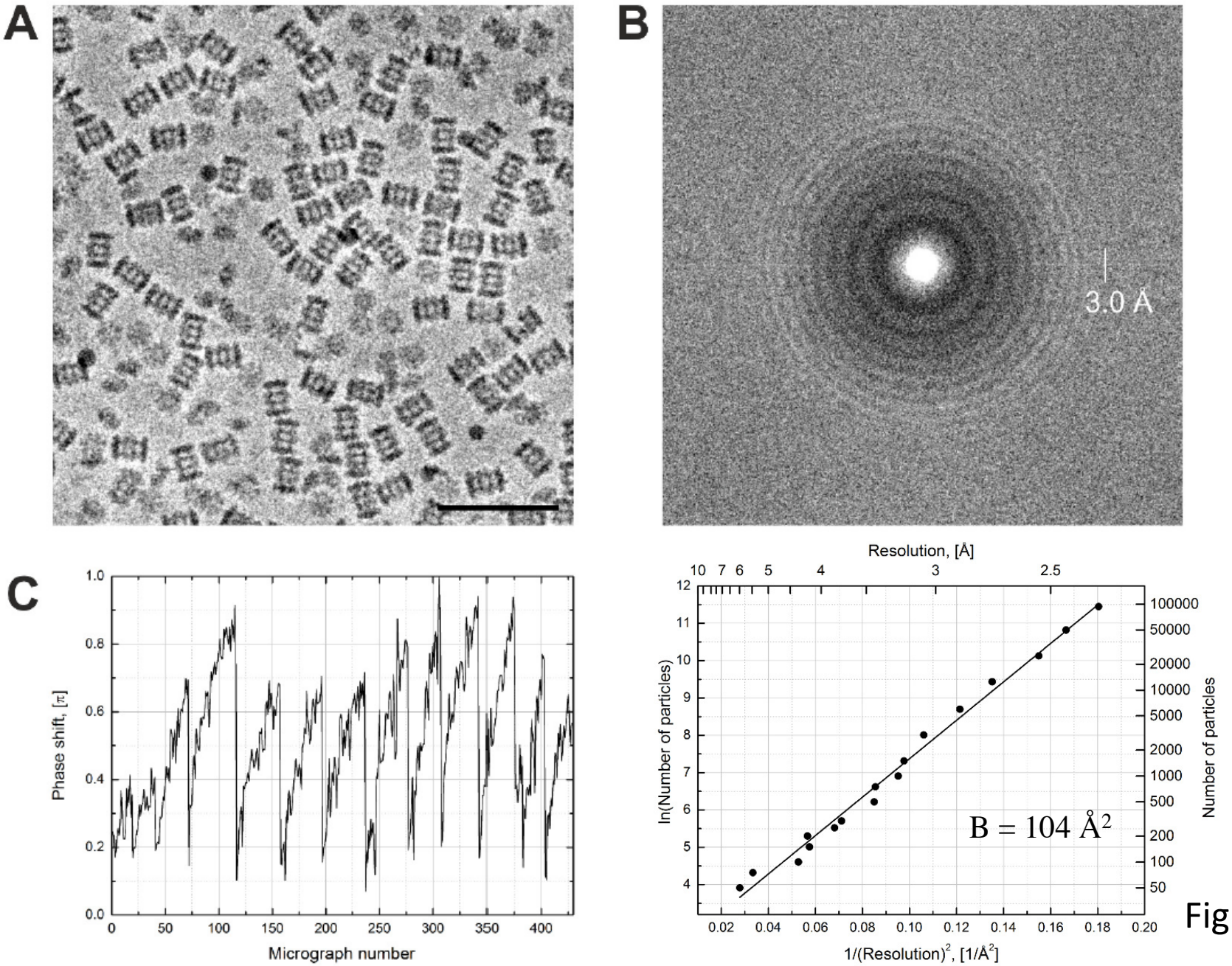
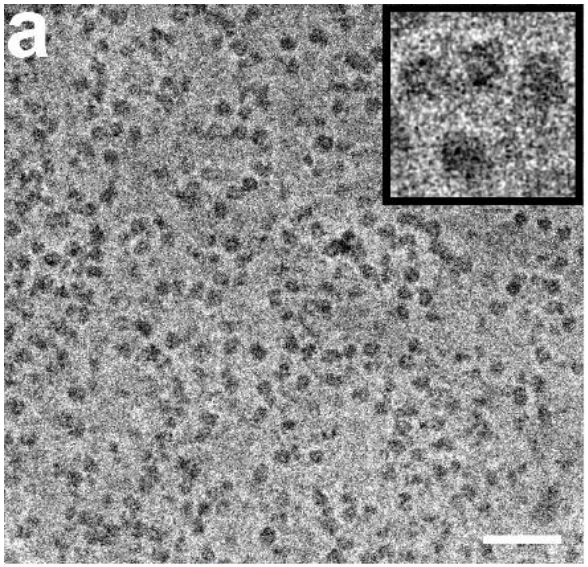
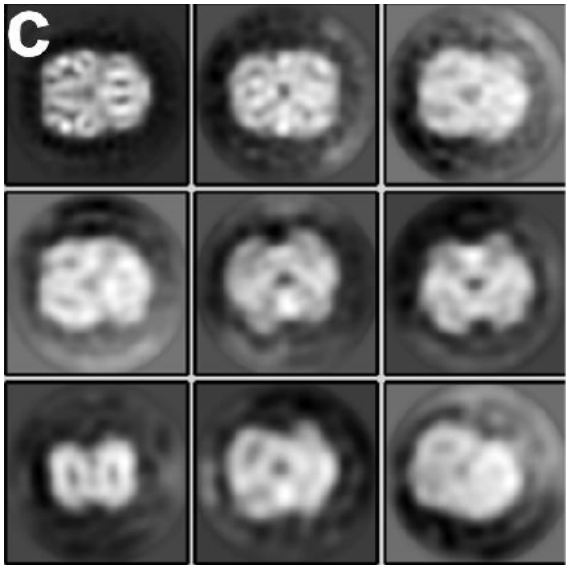
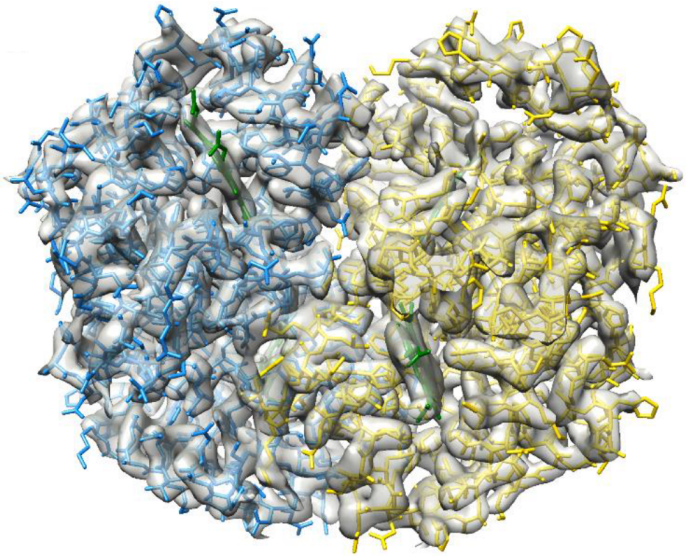


Fig.3F

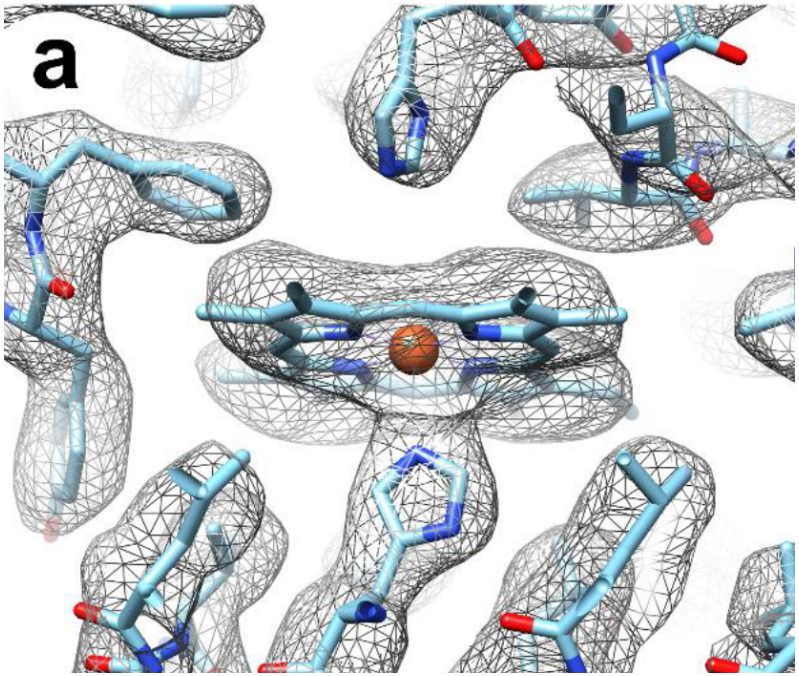
Khoshouei, Radjainia, Baumeister & Danev (2016) BioarXiv http://dx.doi.org/10.1101/087841

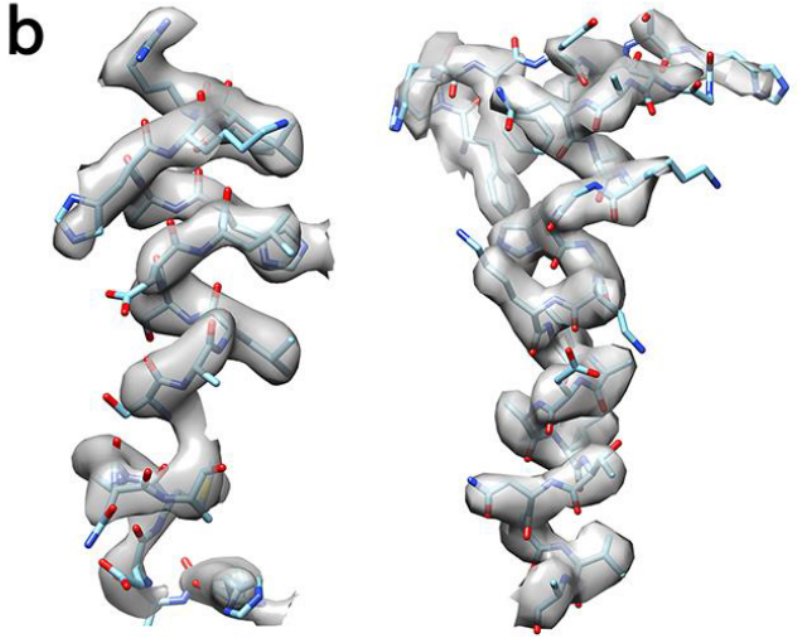






Human haemoglobin by cryoEM

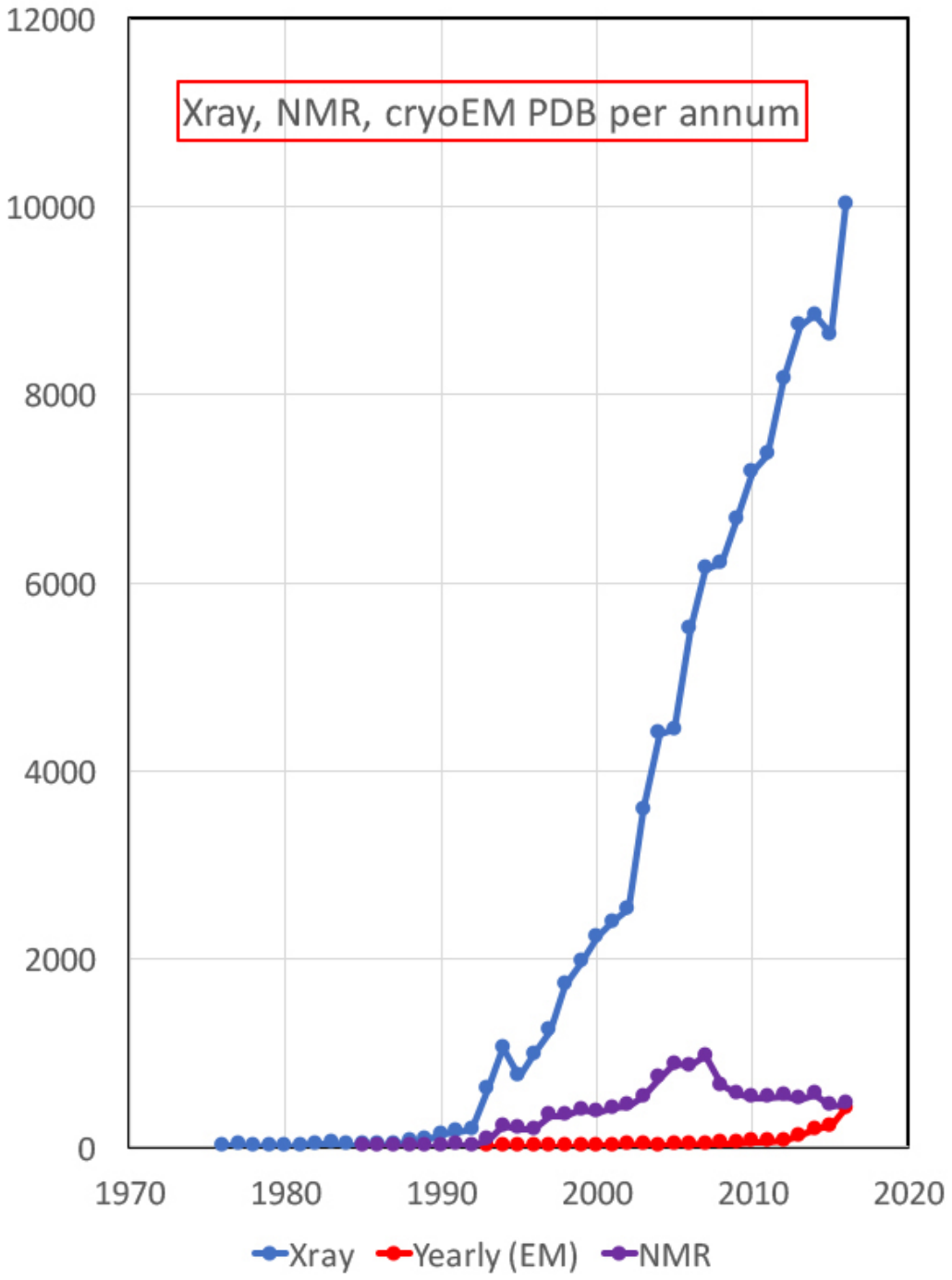




Statistics from Protein Data Bank (PDB)

Total coordinates deposited
128,000

Yearly depositions	Xray	EM	NMR
2017	1600	81	55
2016	10019	408	457
2015	8637	216	436
2014	8847	186	554
2013	8734	118	506
2012	8167	65	537
2011	7364	53	523
2010	7179	54	520
2009	6669	40	567
2008	6204	41	653
2007	6148	19	965



Looking forward

Improving high-end cryoEM

- Faster detectors with 100% DQE
- Stable phase plate with 90° phase shift
- Minimise beam-induced motion
- Keep 4K helium possibilities in mind

Reducing the entry cost of cryoEM - diagnostic tool

- Use 100keV with FEG
- 100% DQE big pixel detector

Fantastic progress in structural biology in the last 100 years

Foundations

1911 Rutherford model of atom with tiny nucleus and surrounding electrons
1913 atomic numbers for elements (Moseley)
1923 periodic table of elements in its current 18-column form (Deming)

Pioneering information

1950 DNA chemical structure (Todd et al, Nobel prize 1957)
1953 DNA double helix (Watson & Crick, Franklin & Gosling, Wilkins et al; Nobel prize 1962)
1954 insulin primary sequence of first protein (Sanger, Nobel prize 1958)
1960 myoglobin 3D atomic structure (Kendrew & Perutz, Nobel prize 1962)
1973 3D structure of tRNA nucleic acid adapter molecule (Kim & Rich; Klug et al)
1975 Sanger DNA sequencing method (Nobel prize 1980)

Consolidation

1980-90 gene sequences (cDNA) for many important proteins
2000 first human genome sequence identifies all 25,000 human proteins (HUGO)

Deluge

2017 genome sequences for thousands of organisms; plants, animals, bacteria, extinct species
2017 human genome sequences from thousands of individuals
2017 3D structures of 128,000 macromolecules or complexes in PDB

Future

2025 ? all structures in biology determined and downloadable from databanks
2025 ? genome sequences for anyone who wants to know about their own genetics (ethical concerns)
2025 ? pharmaceutical drugs developed for most protein targets (agonists & antagonists)

Overview references

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