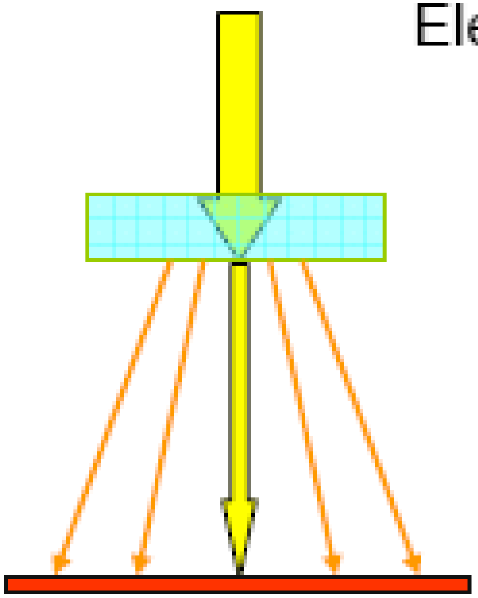
Historical Milestones in Transmission Electron Microscope



o.lambert@cbmn.u-bordeaux.fr

Nobel prizes

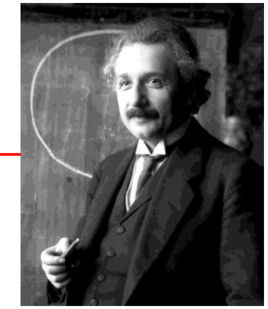
Physics

1929

Louis De Broglie

For his discovery of the wave nature of electrons

1921



Albert Einstein

for his services to Theoretical Physics

1986

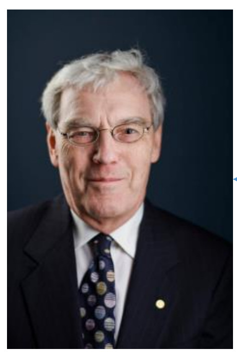
Ernst Ruska **(1906-1988) designed the first TEM in 1931**

Chemistry

2017

Jacques Dubochet

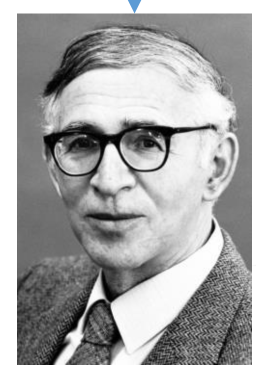
Joachim Frank



Richard Henderson

for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution

1982



Aaron Klug

development of crystallographic electron microscopy and his structural elucidation of nucleic acid-protein complexes

Magnetic Electron Microscope

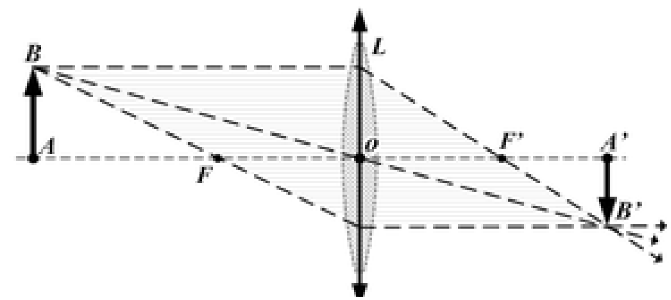
Ernst Ruska and Max Knoll (1931)

18e — Development of Cathode rays

1897 — J.J. Thomson **Electron discovery**

Wikipedia tube de Crookes

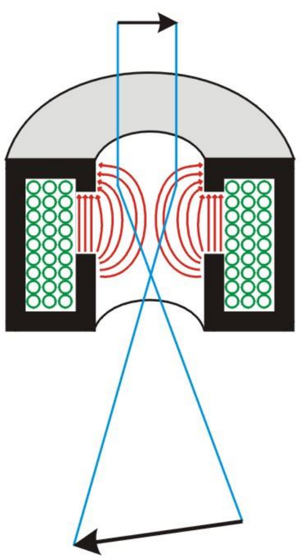
1926 — Hans Busch theoretical discovery: a short magnetic coil can concentrate an electron beam as a glass lens does on light rays



$$\frac{1}{d_{object}} + \frac{1}{d_{image}} = \frac{1}{f} \qquad M = \frac{d_{image}}{d_{object}}$$

Engineer for development of cathode-ray oscillographs

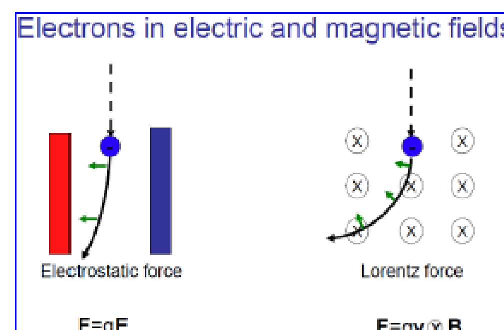
Magnetic electron lens



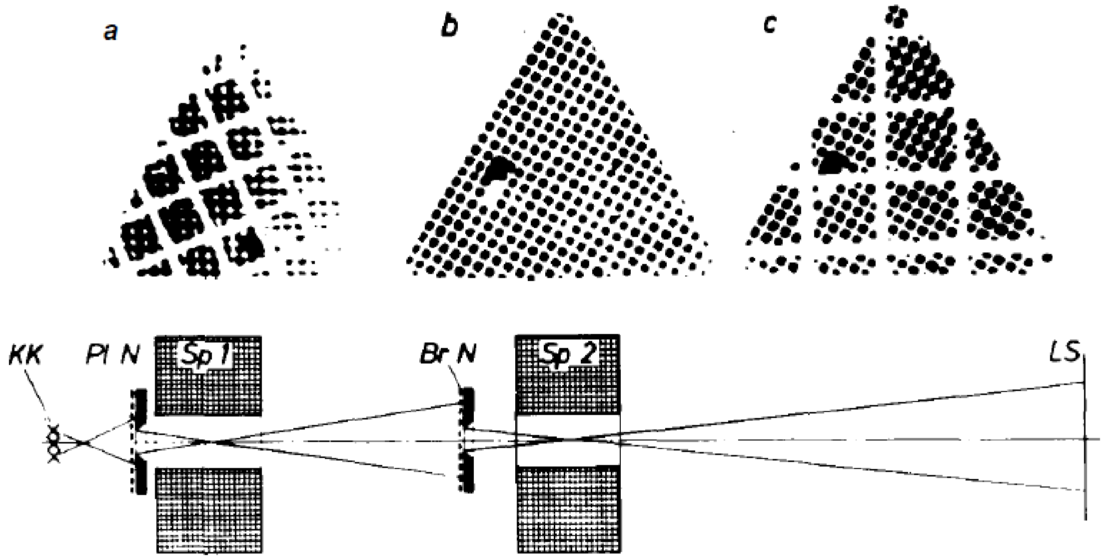
1/ change the trajectories of electron beam
2/ control the focal length by means of the coil current (Busch eq)

$$f = KV_r / (N \cdot I)^2$$

where
- f = the focal length of the lens
- K = a constant
- V_r = the accelerating voltage, relativistically corrected
- $N \cdot I$ = the number of ampere turns in the excitation coils



First images of irradiated specimens by electron beam and magnetic lens (as for light and glass lens)



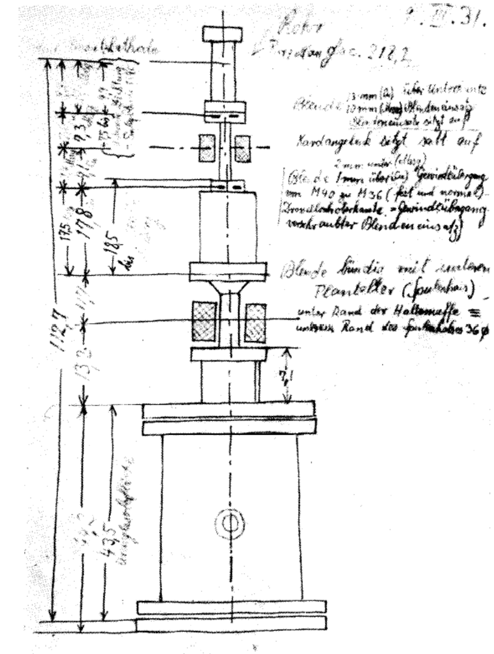


Fig. 3: First experimental proof (7 April 1931) that speciemens (aperture grids) irradiated by electrons can be imaged in magnified form not only in one but also in more than one stage by means of (magnetic) electron lenses.
(U = 50 kV). [8].

a) one-stage image of the platinum grid in front of coil 1 by coil 1; M = 13 x

b) one-stage image of the bronze grid in front of coil 2 by coil 2; M = 4.8 x

c) two-stage image of the platinum grid in front of coil 1 by coil 2; M = 17.4 X together with the one-stage image of the bronze grid in front of coil 2 by coil 2; M = 4.8 x

kk Cold cathode; Pt N Platinum grid; Sp 1 coil 1;
Br N Bronze grid; Sp 2 coil 2; LS Fluorescent screen

Resolution

Ernst Abbe criteria Resolution of light microscope limit ~ $\lambda/2$ ($d = 0{,}61.\lambda/n.\sin\alpha$)

1924 Louis de Broglie **Duality wave length-particle**

A particle with a mass m and a speed v is characterized by a wave length λ:

$$\lambda = h/(m.v)$$

A electron submitted to electric field with a tension V

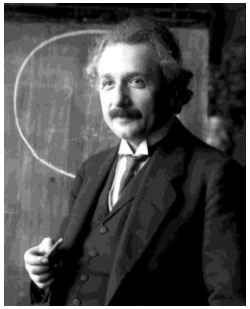
$$Ec = 1/2\ mv^2 = e.V$$

$$\lambda = h\ /(2e.m.V)^{1/2} \approx 12{,}25/\ \sqrt{V}\ \text{Å}$$

For high tension, $v > c\ 3\ 10^8$ m/s

At 75 kV and aperture of 20 mRad
λ = 0.04 Å
Resolution limit=2.2 Å

Albert Einstein (relativistic effects on electron)



Correction for the mass $m_1 = m_0\ /(1 - v^2/c^2)^{1/2}$

$$\boxed{\lambda = \frac{1.23}{\sqrt{V + 10^{-6}V^2}}\ nm}$$

Image formation

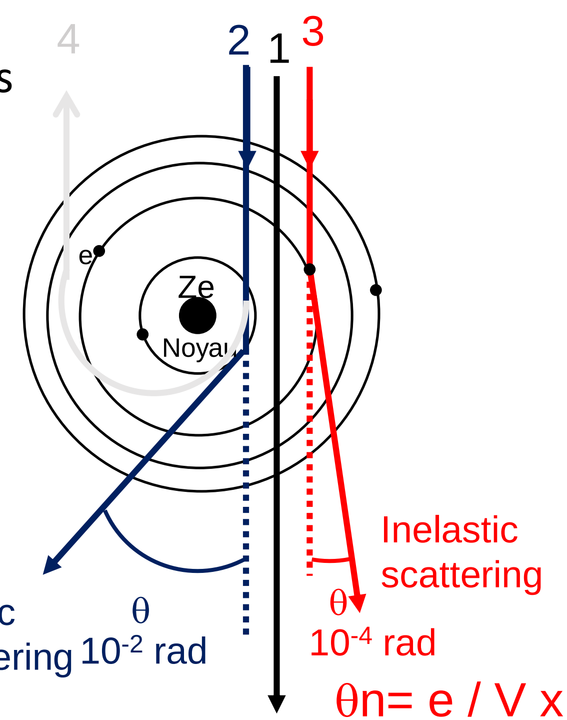
1924 Louis de Broglie **Duality wave length-particle**

A particle with a mass m and a speed v is characterized by a wave length λ:

$$\lambda = h/(m.v)$$

$$\lambda = h/(2e.m.V)^{1/2} \approx 12{,}25/\sqrt{V}$$

Electron incident



Strong interaction with the nucleus

$\theta e = Z\ e\ /\ V\ x\ r_n$

$\theta n = e\ /\ V\ x\ r_e$

Di=20/Z De

high radiation damage, especially for light atoms, e.g. C:
3 electrons scattered inelastically per elastic event

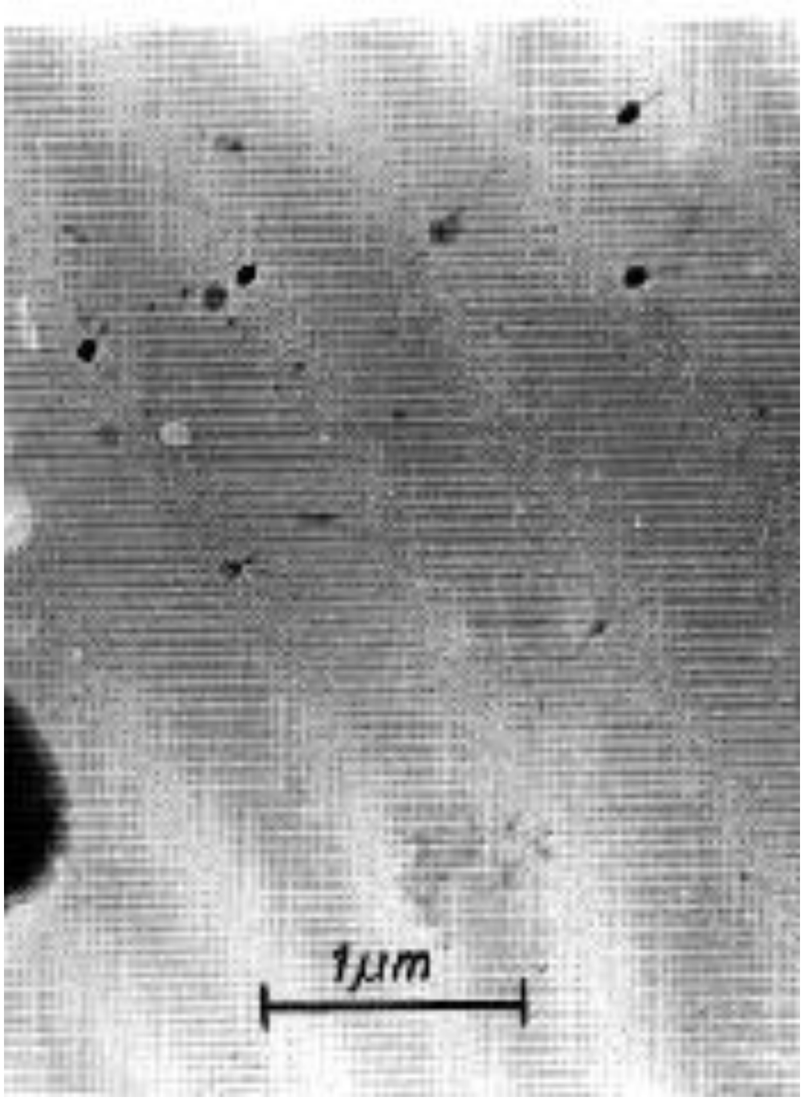
Only elastically scattered electrons contribute to the theoretical image intensity

Inelastically scattered electrons produced an unwanted background

Electron microscopy measures the Coulomb potential distribution
≠ x-ray crystallography determines the electron density

First images of biological specimens with a light microscope

Bacteriophages.
(Helmut Ruska, 1941-1942)

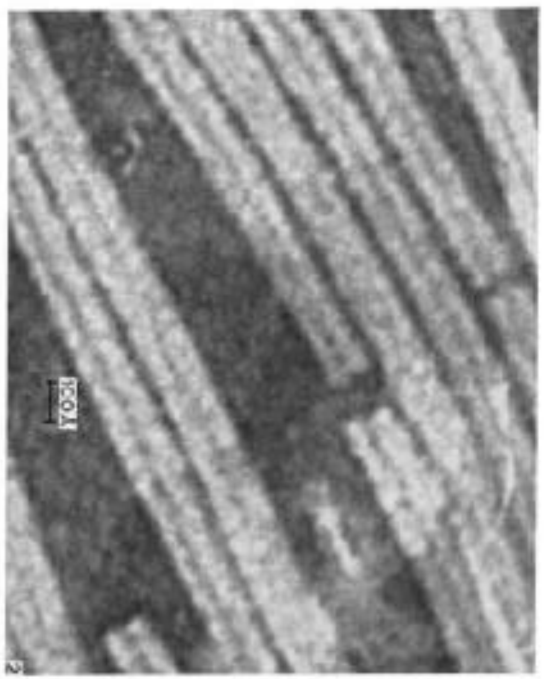


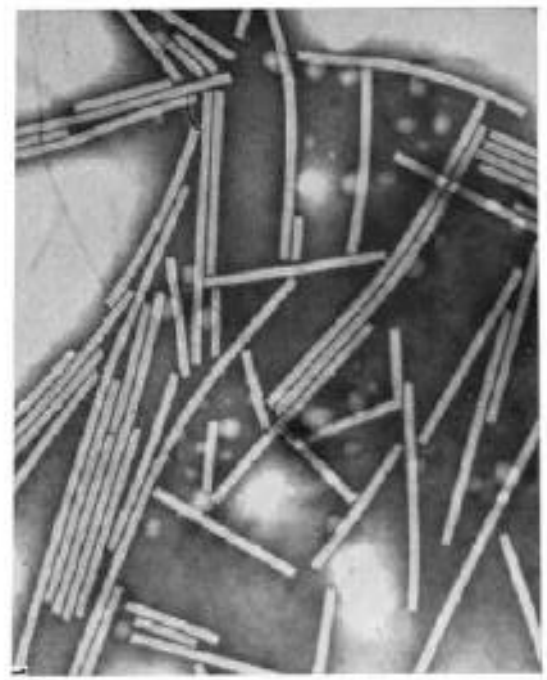
Biochim Biophys Acta. 1959 Jul;34:103-10.

A negative staining method for high resolution electron microscopy of viruses.

BRENNER S, HORNE RW.

PMID: 13804200 [PubMed - indexed for MEDLINE]

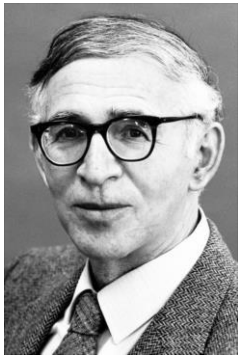




Tobacco mosaic virus
embedded in phosphotungstate

Image processing

1982



Aaron Klug

1922 Sir Lawrence Bragg Xray crystallography
1962 Max Perutz John Kendrew First structure of proteins

1954-1962 Xray crystallography on TMV

1962 (Cambridge) turn to electron microscopy because it produces IMAGE

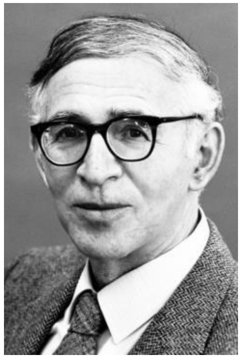
Limitation

1/ Preparation artefact, radiation damage
2/ Artificial means of contrat enhancement
3/ image formed depends on operating condition, focussing, aberrations

4/ Large depth of defocus: all features along the direction of view are superimposed in the image

Raw image is not easily interpretable without procedures for image processing

1982



Aaron Klug

3D reconstruction

3 Dimensional reconstruction of an object from a set of 2D projections

130 NATURE, VOL. 217, 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*.

Tail of bacteriophage T4

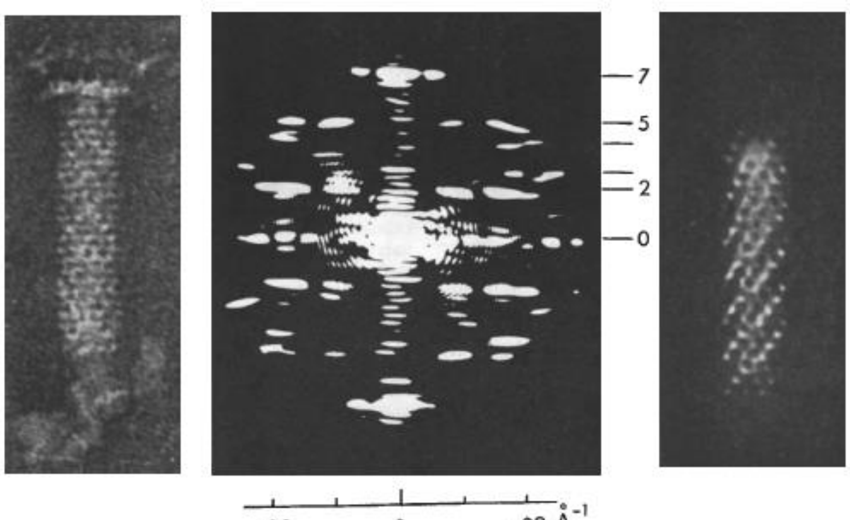
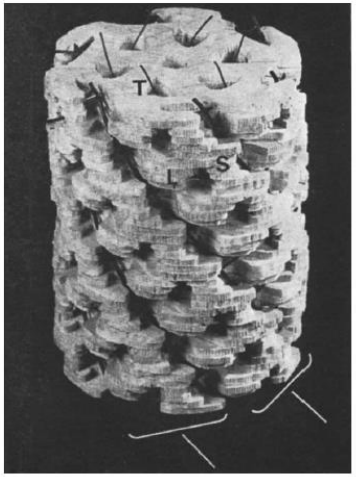


Fig. 1 Fig. 2 Fig. 3

Fig. 1. Electron micrograph of a tail of bacteriophage *T4*, negatively stained with uranyl formate. (× 500,000.)

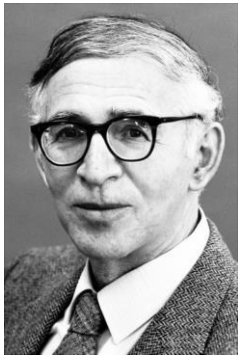
Fig. 2. Optical diffraction pattern of the phage tail image in Fig. 1. Stronger exposures show that the order in the micrograph extends to spacings of about 25 Å. The strong meridional peak on the seventh layer line arises from the spacing of 38 Å between annuli. The layer lines are approximately equally spaced at the orders of an approximate repeat of 7 × 38 = 266 Å. The helical selection rule for the diffraction pattern is $l = 2n + 7m$, the only permitted values of n being those which are multiples of six.

Fig. 3. Optically filtered image of phage tail in Fig. 1, admitting only the diffracted rays corresponding to the far side of the particle. The dominant features are two distinct sets of oblique striations, which correspond to two distinct sets of helical lines running along two cylindrical surfaces of different diameter, and thus correspond to features at different depths in the particle.



Resolution ~ 30 Å

1982



Aaron Klug

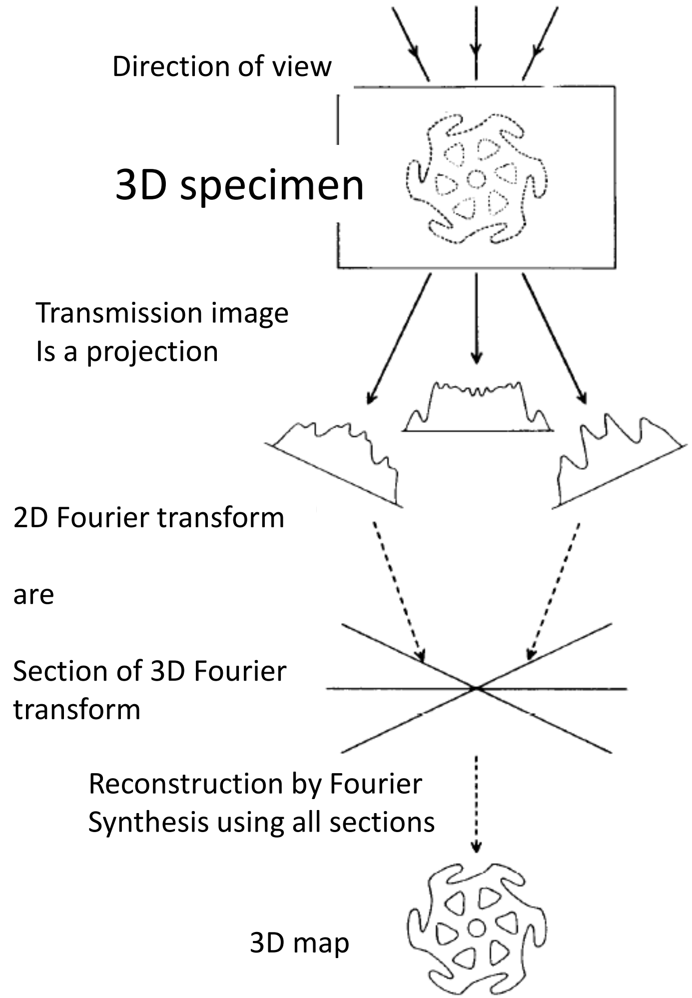
3D reconstruction

3 Dimensional reconstruction of an object from a set of 2D projections

DeRosier and Klug Nature 1968

The Projection-Slice Theorem

- The Fourier Transform of a 2D projection of a 3D object is a central slice through the 3D Fourier Transform of that object
- The vector normal to that slice corresponds to the projection direction



Richard Henderson

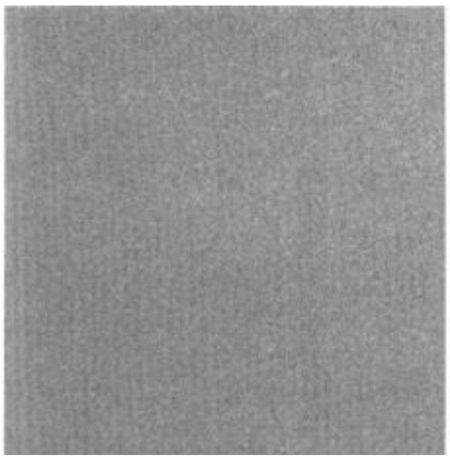
Three-dimensional model of purple membrane obtained by electron microscopy

R. Henderson & P. N. T. Unwin 1975

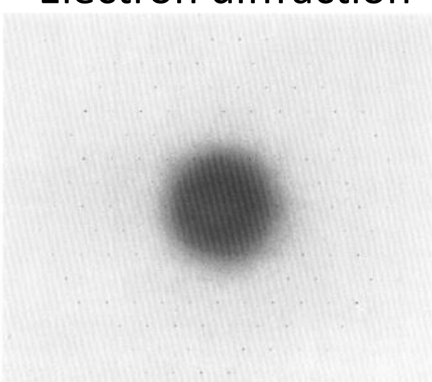
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

7 A resolution, embedded in glucose 0.5%

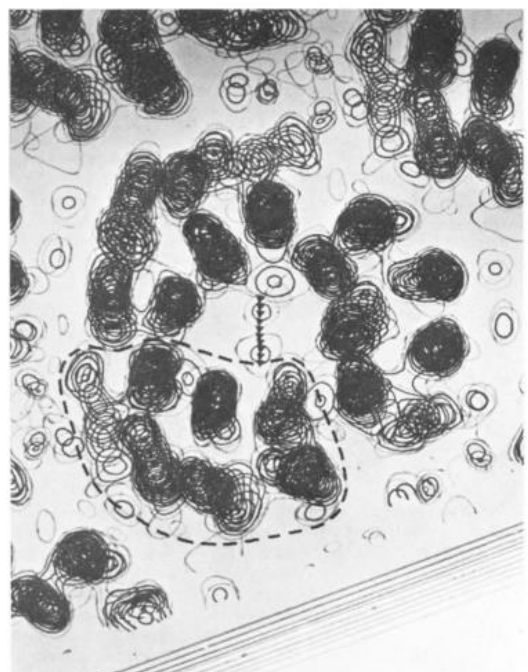
image

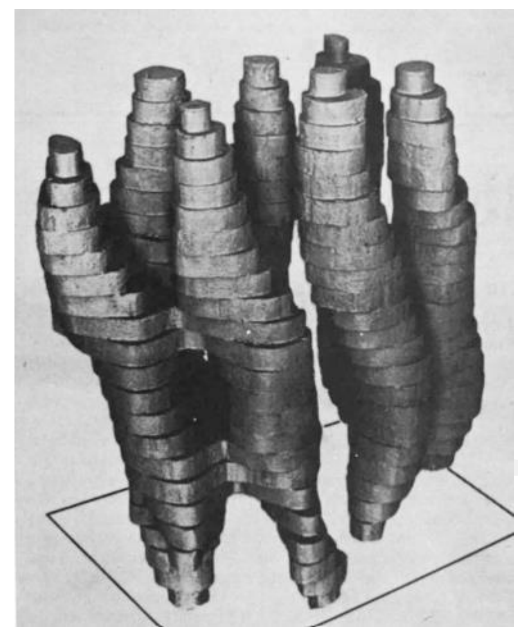


Electron diffraction



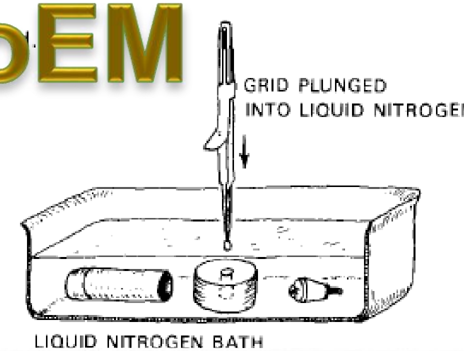
Three-dimensional potential map





The early era of cryoEM

JOURNAL OF ULTRASTRUCTURE RESEARCH 55, 448–456 (1976)



Electron Microscopy of Frozen Hydrated Biological Specimens

KENNETH A. TAYLOR AND ROBERT M. GLAESER

Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720

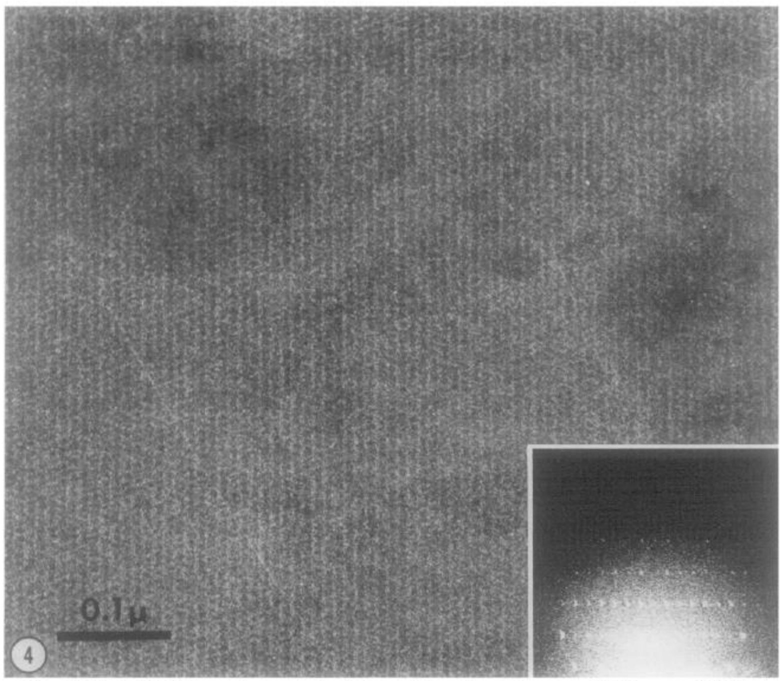


FIG. 4. Bright field image of an unstained frozen hydrated catalase crystal. The optical diffraction pattern in the insert indicates the presence of the (6,0) reflection at 11.5 Å resolution.

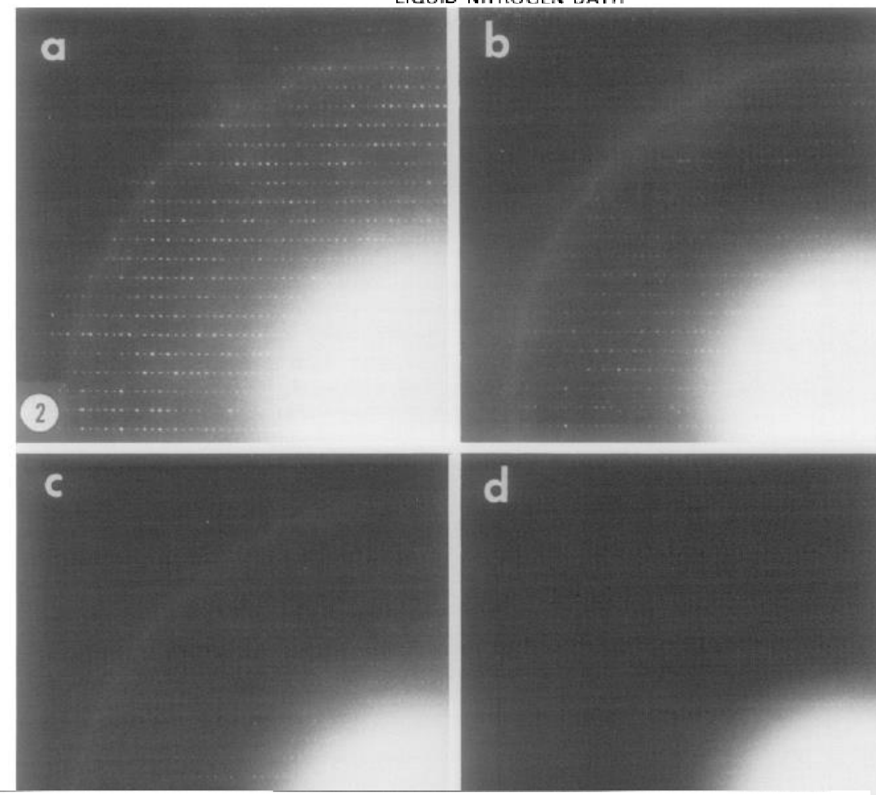


FIG. 2. Changes in the electron diffraction pattern of frozen, hydrated catalase crystals resulting from radiation damage. (a) The initial diffraction pattern extends to 2.8 Å. The ring present in the pattern at a spacing of 3.67 Å is due to ice condensed on the surface of the specimen from water vapor present in the column of the microscope. (b) The pattern recorded after an exposure of 2.5 electrons/Å^2. (c) The pattern recorded after an exposure of 5.0 electrons/Å^2. (d) The pattern recorded after an exposure of 11 electrons/Å^2; the diffraction pattern still extends to 8.5 Å resolution.

LOW DOSE

Sample Vitrification

Jacques Dubochet

Journal of Microscopy, Vol. 124, *Pt* 3, *December* 1981, *pp. RP3–RP4.*
Rapid Publication accepted 9 *November* 1981

VITRIFICATION OF PURE WATER FOR ELECTRON MICROSCOPY

J. Dubochet and A.W. McDowall
European Molecular Biology Laboratory (EMBL)
Postfach 10.2209, D-6900 Heidelberg, F.R.G.

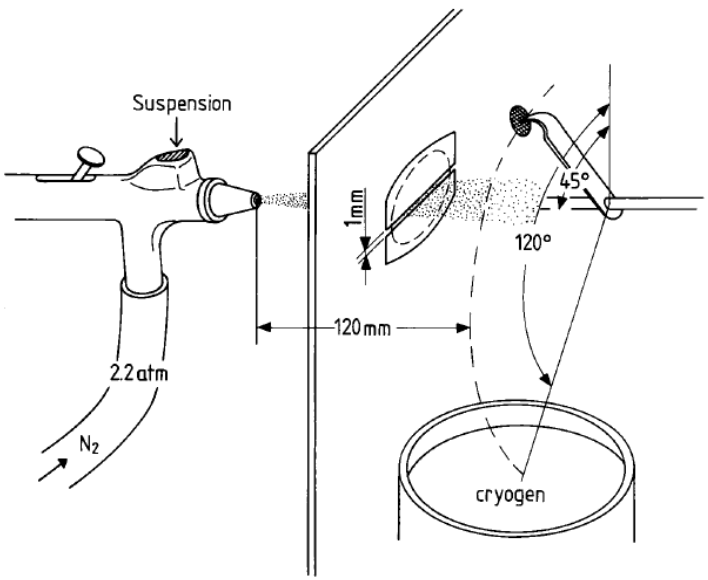


Fig. 3. Schematic of spray freezing apparatus.

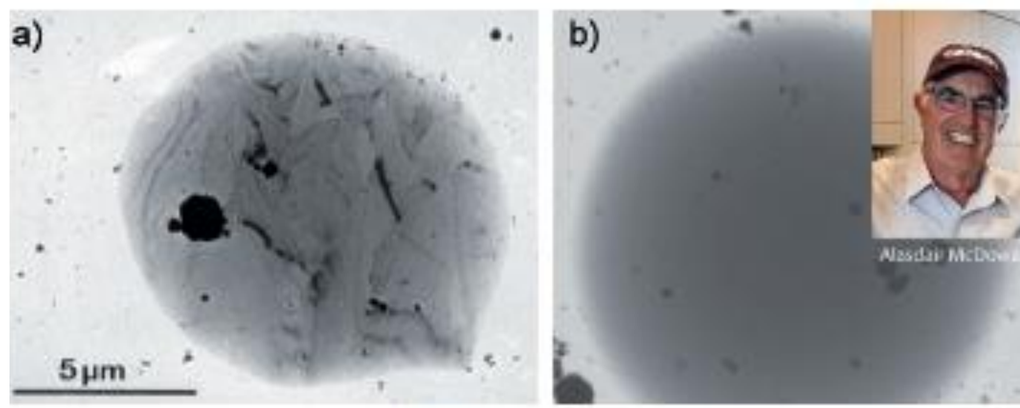


Figure 4. a) Frozen ice microdroplet. b) Vitrified water microdroplet.

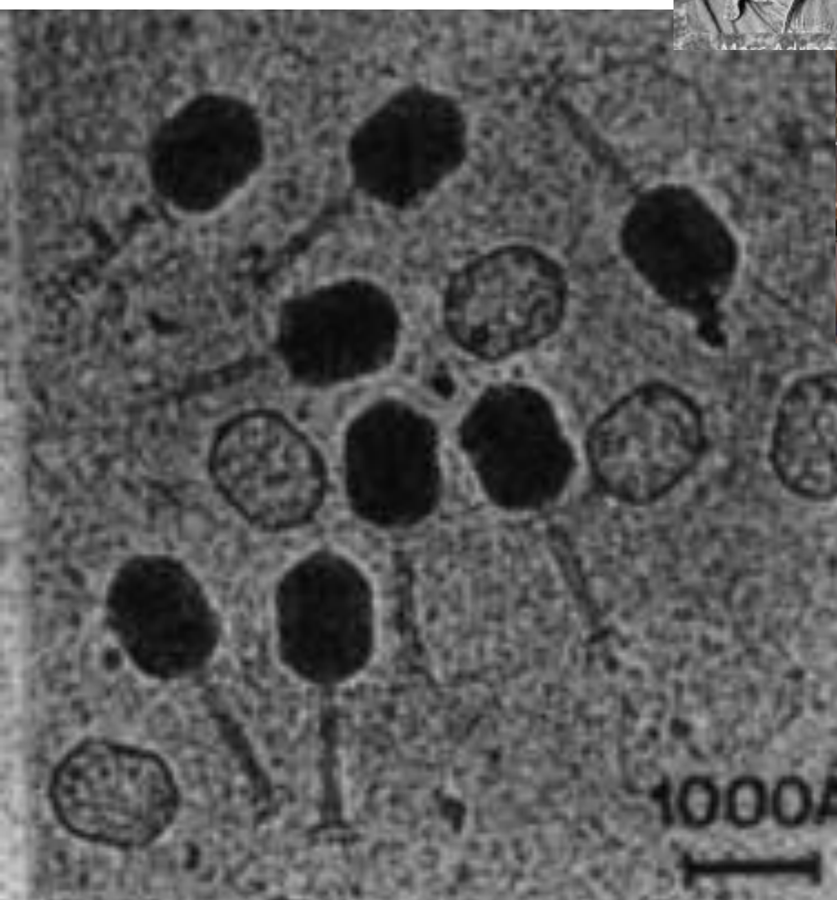
Cryo-electron microscopy of viruses

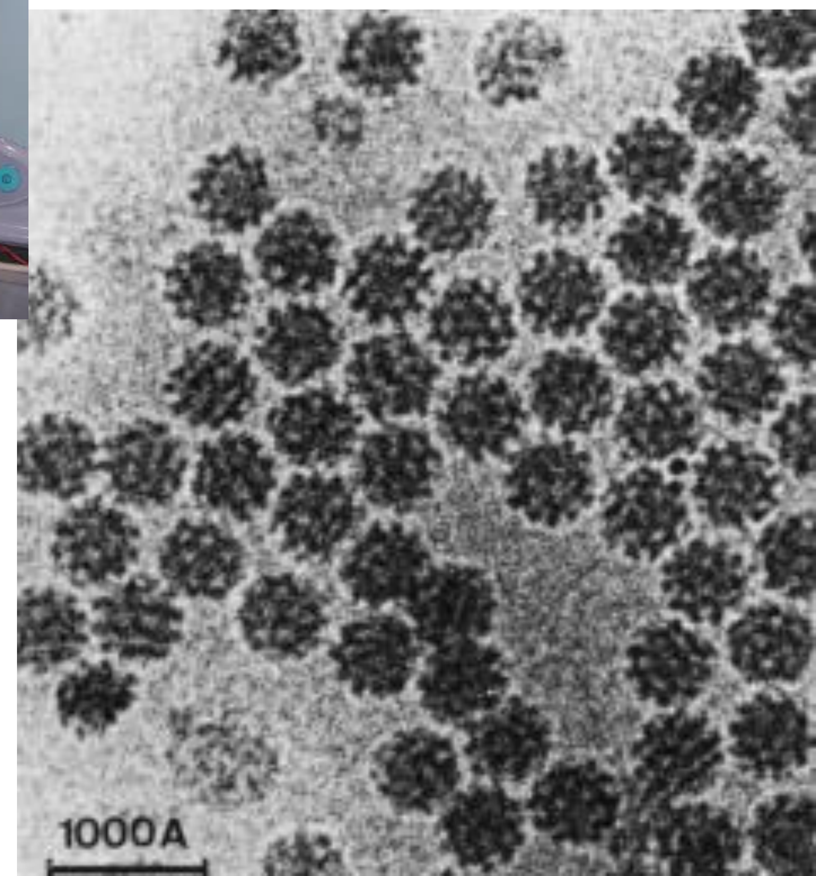
Marc Adrian, Jacques Dubochet, Jean Lepault & Alasdair W. McDowall

European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, FRG

Bacteriophage T4

Semliki forest virus





Single particle Reconstruction

Joachim Frank
Nobel Lecture

Ultramicroscopy 1 (1975) 159–162

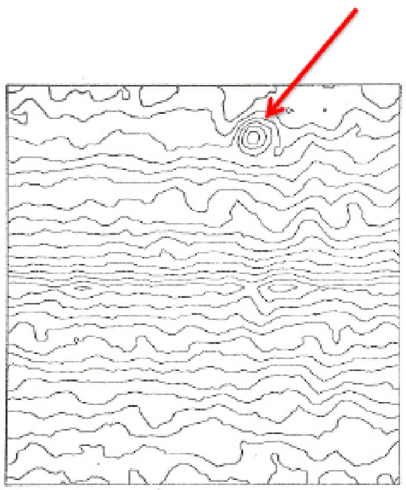
SHORT NOTE

AVERAGING OF LOW EXPOSURE ELECTRON MICROGRAPHS OF NON-PERIODIC OBJECTS

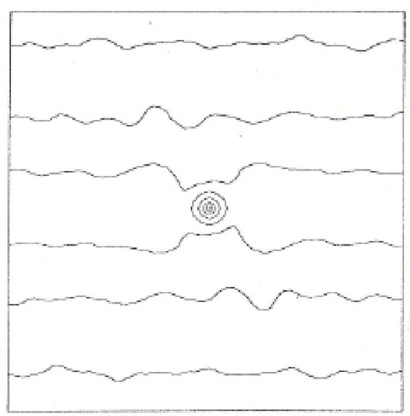
Joachim FRANK *
The Cavendish Laboratory, Free School Lane, Cambridge CB2 3RQ, UK

Received 20 October 1975

Peak



Cross-correlation function



Autocorrelation function

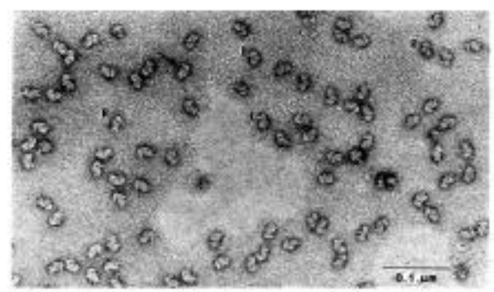
J. Frank, Ph.D. thesis 1970

Computer averaging of electron micrographs of 40S ribosomal subunits

J Frank, A Verschoor and M Boublik

Computer Averaging of Electron Micrographs of 40*S* Ribosomal Subunits

Abstract. *An enhanced lateral view of the 40S ribosomal subunit of HeLa cells has been obtained by computer averaging of single particles visualized in the electron microscope. Application of crystallographic criteria to independent averages shows that the reproducibility of the result is comparable to that obtained for thin, stained protein crystals by conventional Fourier filtration methods.*



A

B C D E

Frank et al. Science 1981

- ALIGN & AVERAGE
- ESTIMATE RESOLUTION
- SORT/CLASSIFY
- FIND ANGLES
- RECONSTRUCT

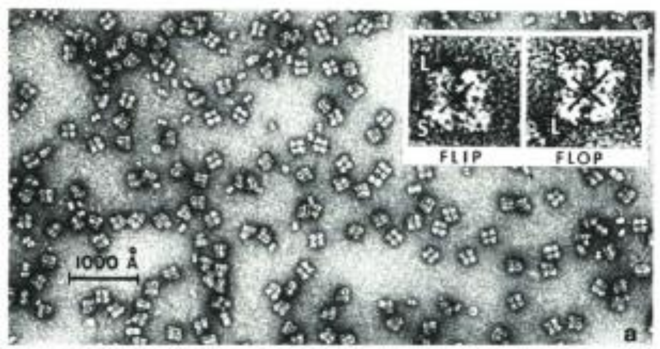
USE OF MULTIVARIATE STATISTICS IN ANALYSING THE IMAGES OF BIOLOGICAL MACROMOLECULES

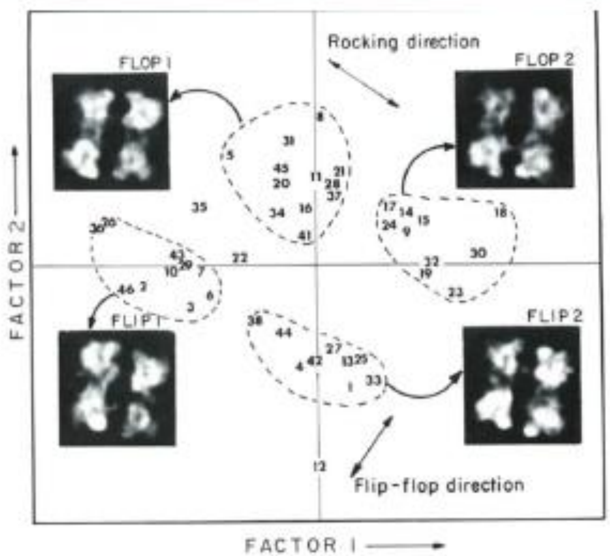
Marin VAN HEEL
Biochemisch Laboratorium der Rijksuniversiteit Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands

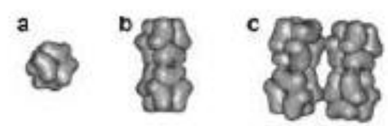
and

Joachim FRANK
Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201, USA

Received 5 March 1981

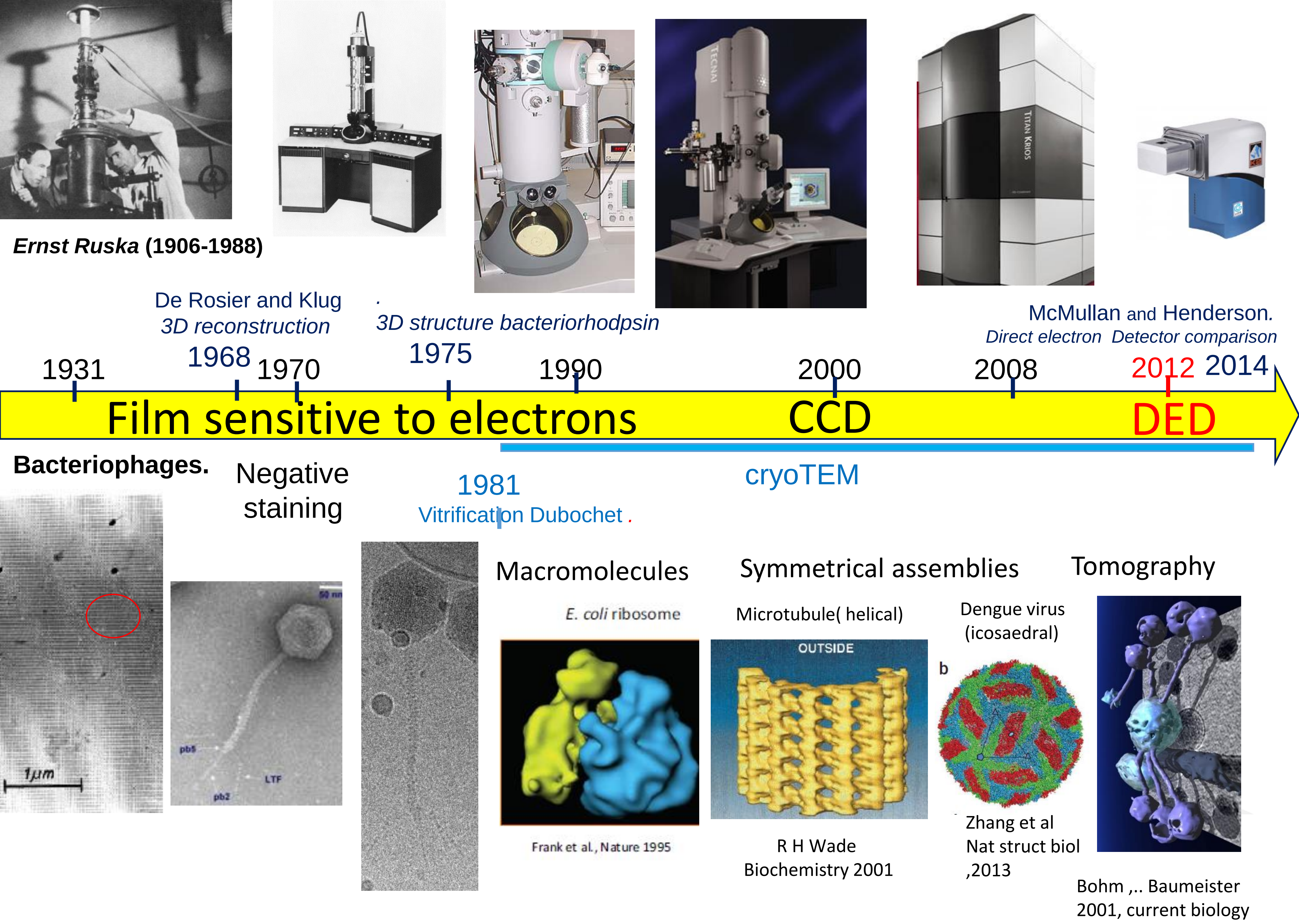






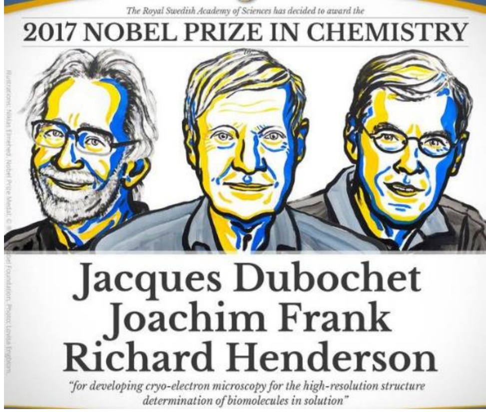
Limulus polyphemus hemocyanin

Van Heel and Frank, Ultramicroscopy 1981



Ernst Ruska (1906-1988)
De Rosier and Klug
3D reconstruction
3D structure bacteriorhodpsin
McMullan and Henderson.
Direct electron Detector comparison
1931
1968
1970
1975
1990
2000
2008
2012
2014
Film sensitive to electrons
CCD
DED
Bacteriophages.
Negative staining
1981
Vitrification Dubochet .
cryoTEM
Macromolecules
Symmetrical assemblies
Tomography
E. coli ribosome
Microtubule(helical)
Dengue virus (icosaedral)
OUTSIDE
b
1µm
pb5
LTF
pb2
Frank et al., Nature 1995
R H Wade Biochemistry 2001
Zhang et al Nat struct biol ,2013
Bohm ,.. Baumeister 2001, current biology

CryoEM revolution in Structural Biology



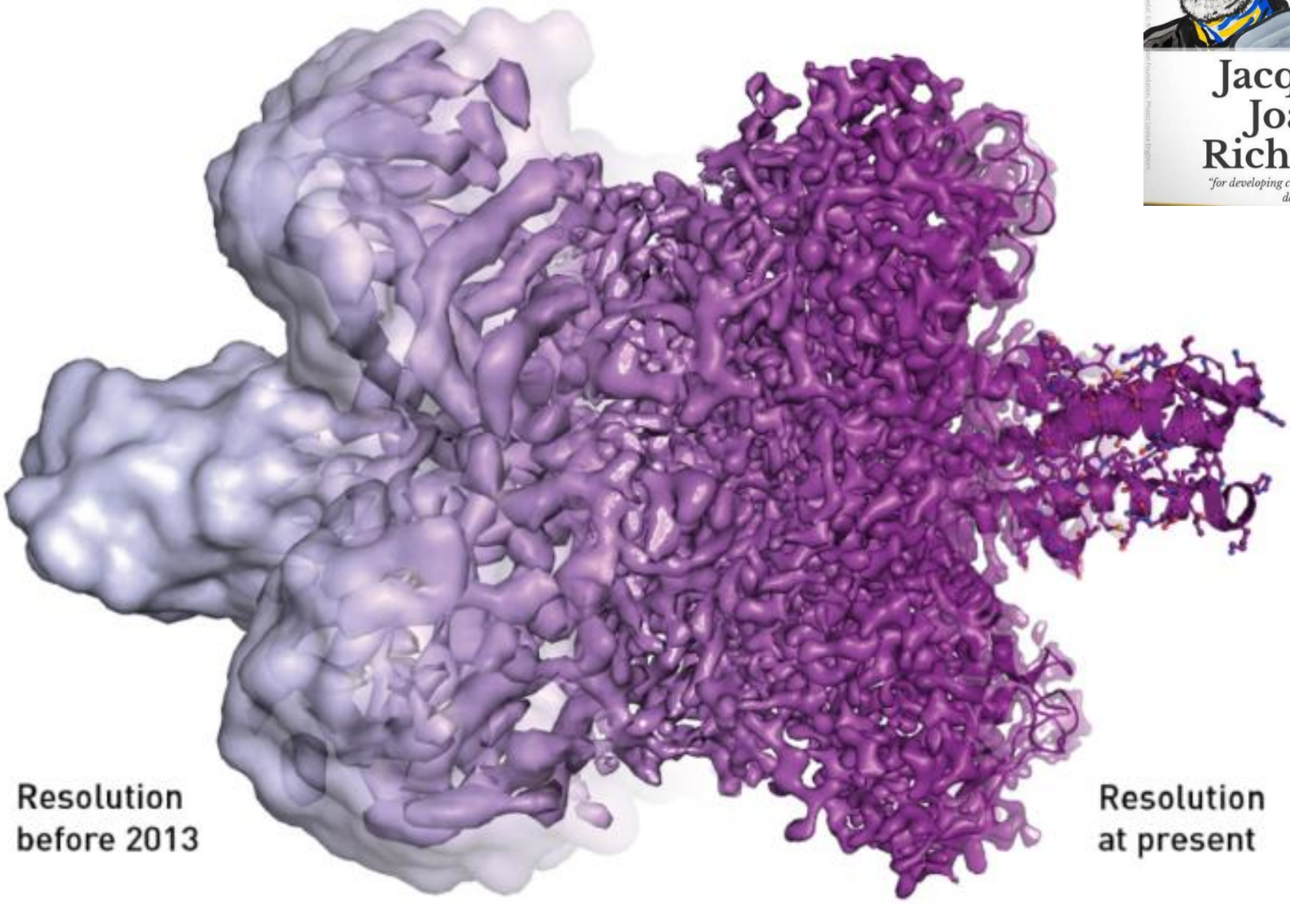
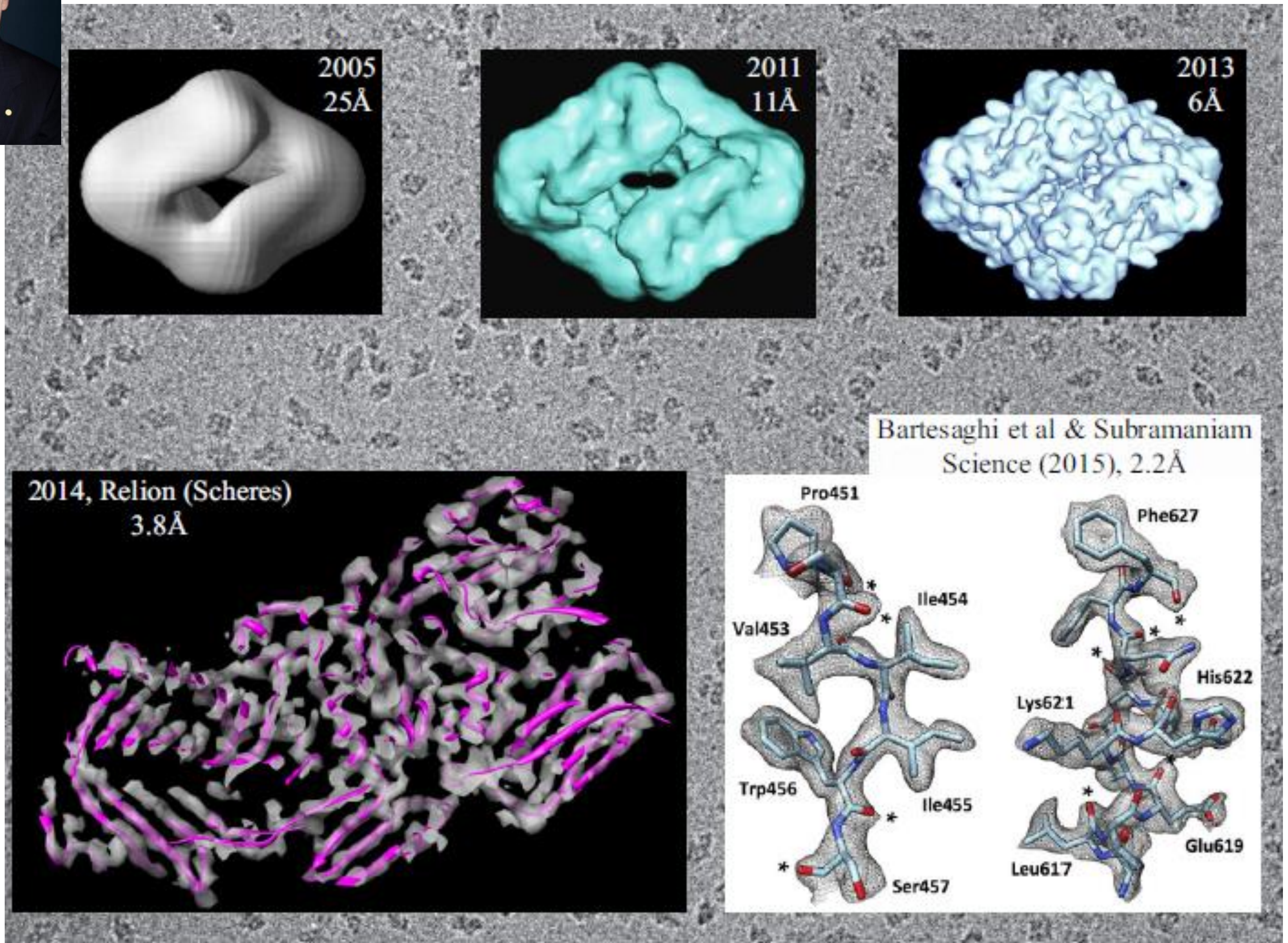
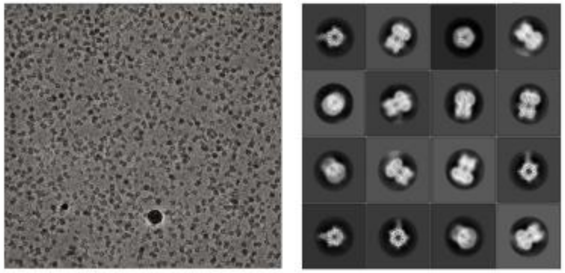


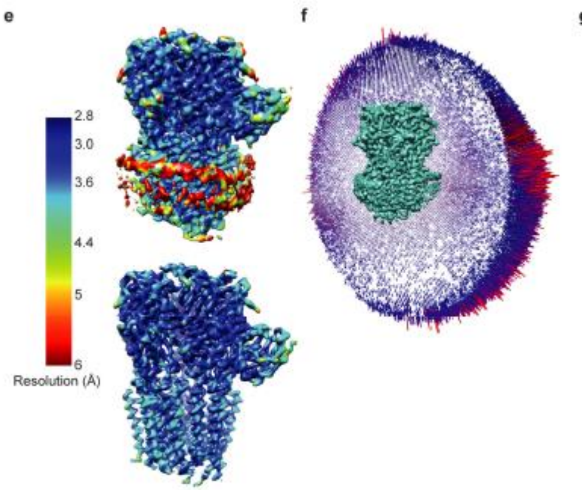
Illustration by Martin Högbom/The Royal Swedish Academy of Science

Richard Henderson
Nobel Lecture

β Galactosidase 450kDa







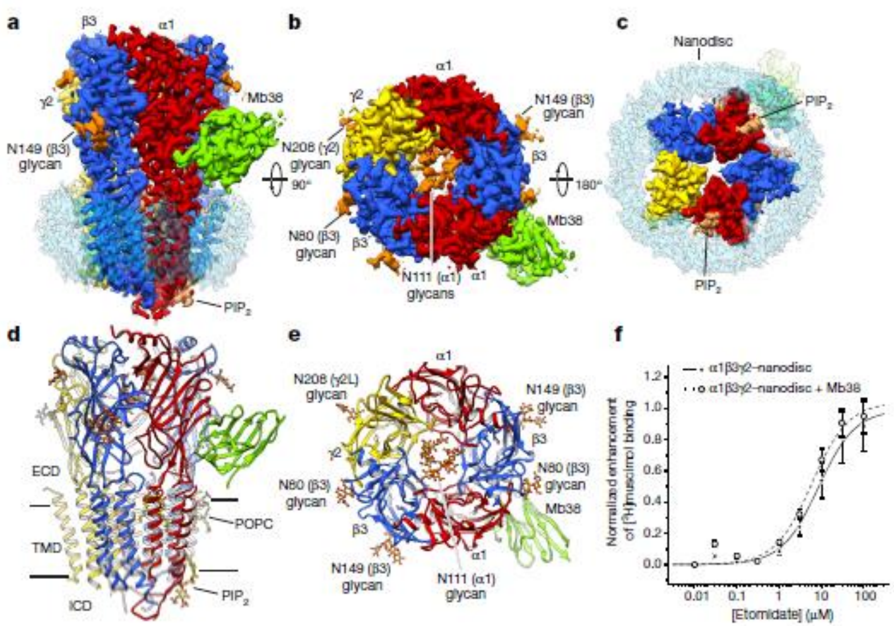
LETTER

https://doi.org/10.1038/s41586-018-0833-4

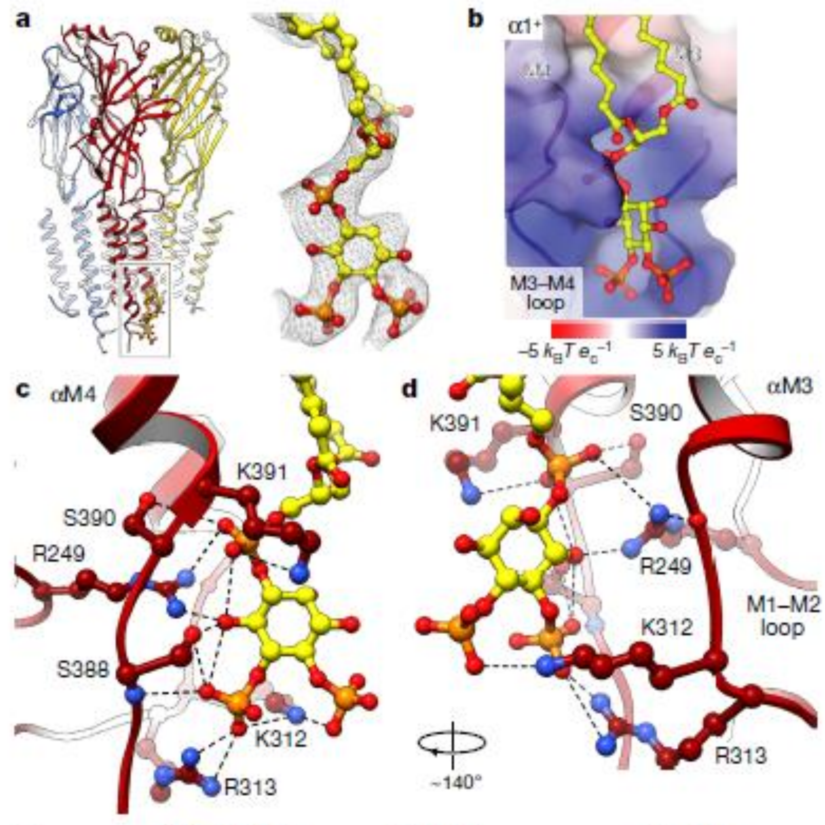
Cryo-EM structure of the human α1β3γ2 GABA$_A$ receptor in a lipid bilayer

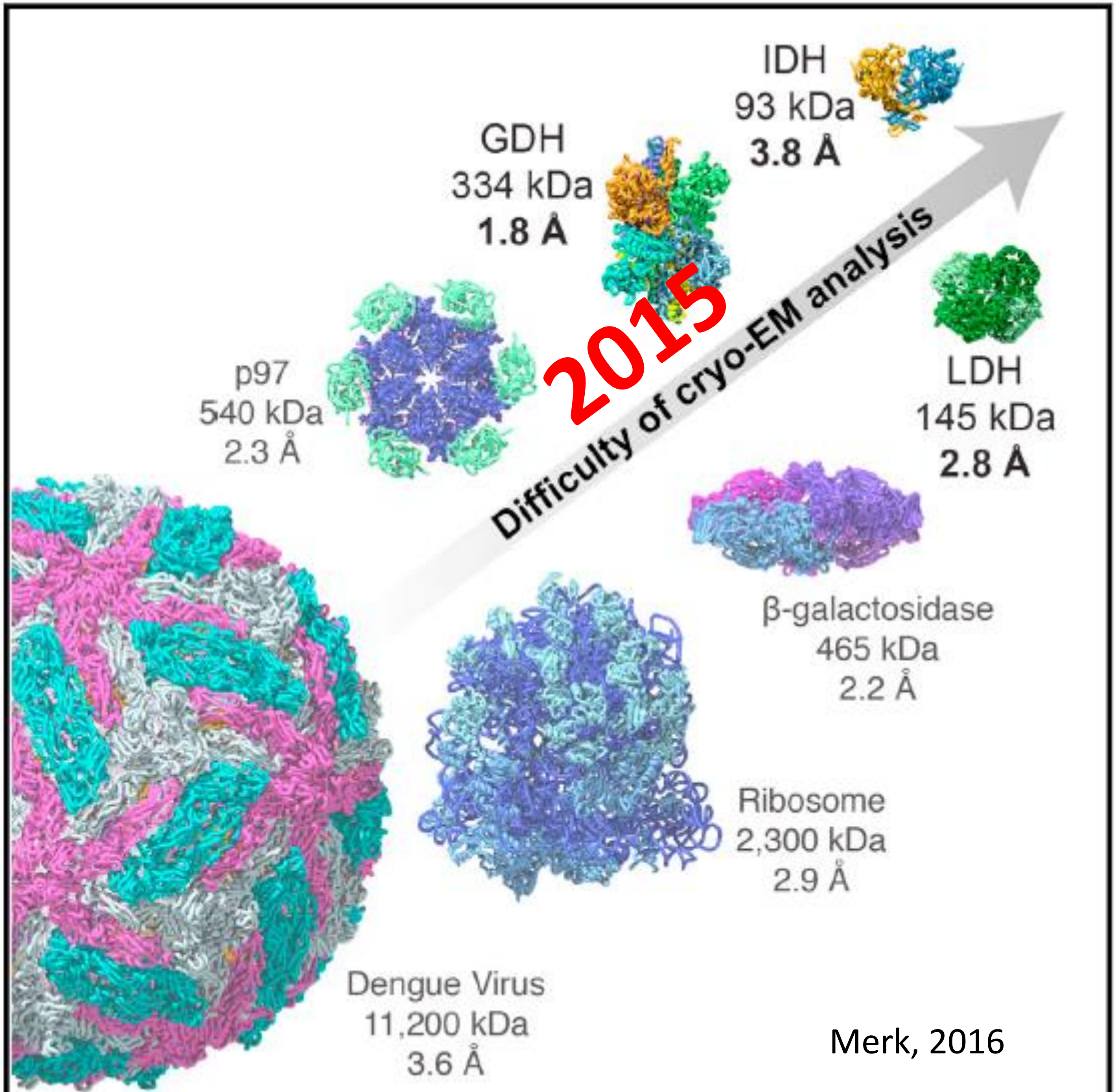
Duncan Laverty[1]*, Rooma Desai[2], Tomasz Uchański[3,4], Simonas Masiulis[1], Wojciech J. Stec[2], Tomas Malinauskas[5], Jasenko Zivanov[1], Els Pardon[3,4], Jan Steyaert[3,4], Keith W. Miller[2]* & A. Radu Aricescu[1,5]*

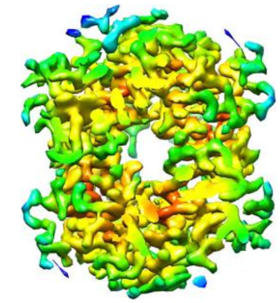
516 | NATURE | VOL 565 | 24 JANUARY 2019



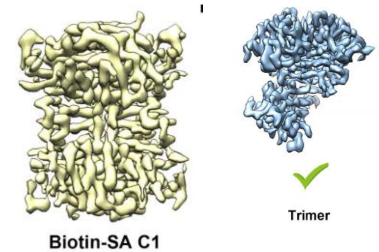
PIP2 bound in α1 subunit





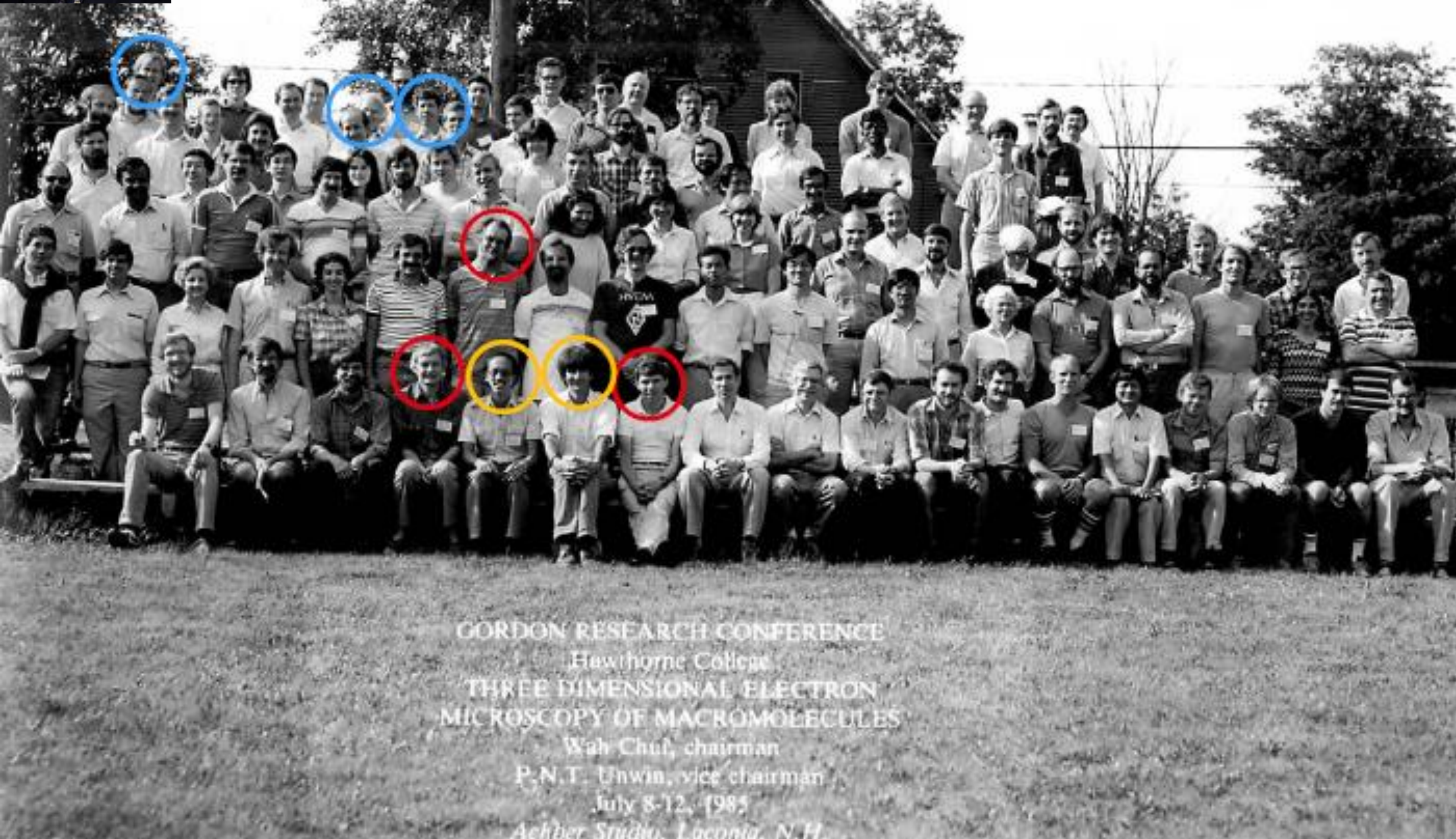


Hemoglobin
64 kDa 3.2 A
R Danev, 2017



Streptavidin
52 kDa 3.2 A
H-W Wang, 2018
BioRxiv

Richard Henderson
Nobel Lecture



Richard Henderson
Nobel Lecture

X-RAY

NMR

Cryo EM

Chemistry

Structural biology