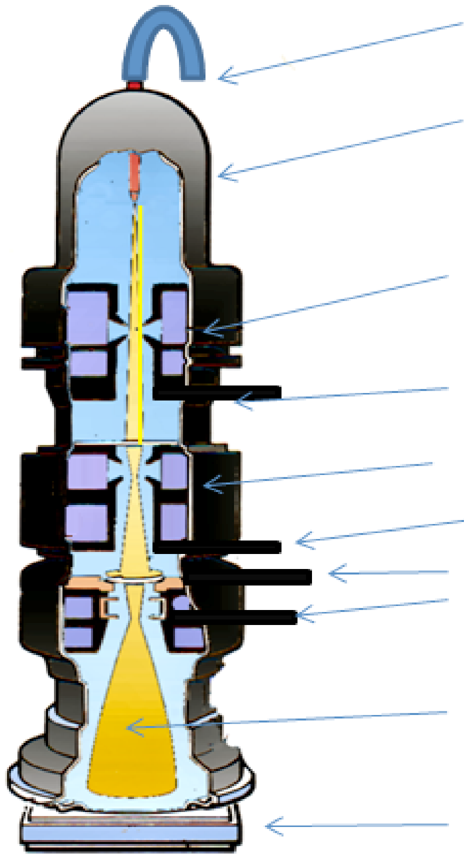
Single-Particle Reconstruction
Story in a Sample

Joachim Frank

Department of Biochemistry and Molecular Biophysics
and Department of Biological Sciences
Columbia University



High voltage

Electron gun

First condenser lens

Condenser aperture

Second condenser lens

Condenser aperture

Specimen holder and air-lock

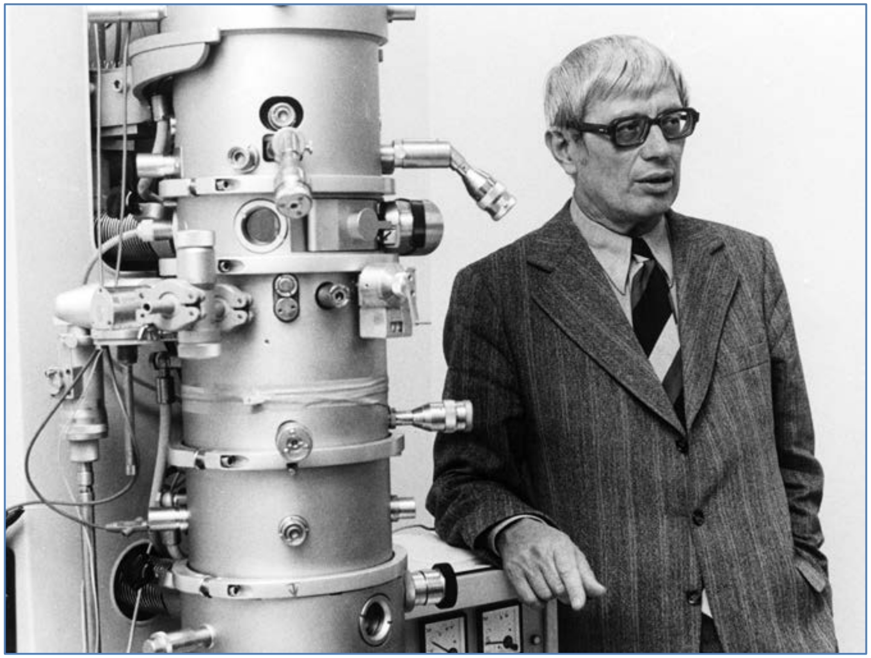
Objective lenses and aperture

Electron beam

Fluorescent screen and camera

Transmission Electron Microscope

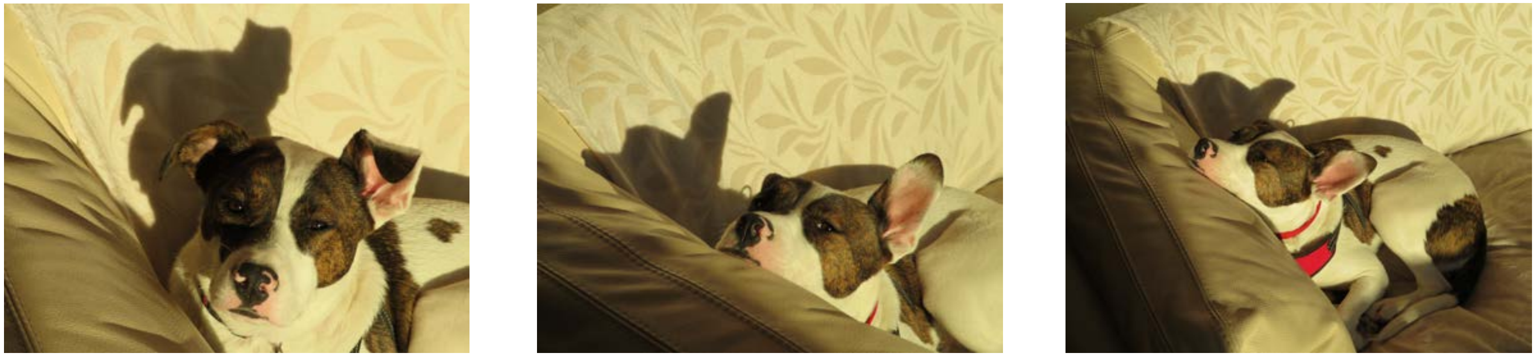
Drawing by Graham Colm, courtesy of Wikimedia Commons



Archives of the Max Planck Society, Berlin

Walter Hoppe (March 21, 1917 – November 3, 1986)

TO CAPTURE A THREE-DIMENSIONAL OBJECT . . .



. . . MULTIPLE VIEWS ARE NEEDED

THREE-DIMENSIONAL RECONSTRUCTION:
HELICAL SYMMETRY

Pioneering work: 3D reconstruction of bacteriophage tail using the Fourier-Bessel approach, 1968

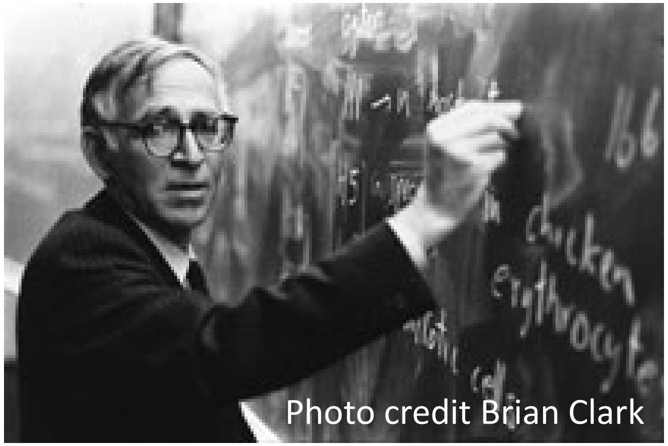
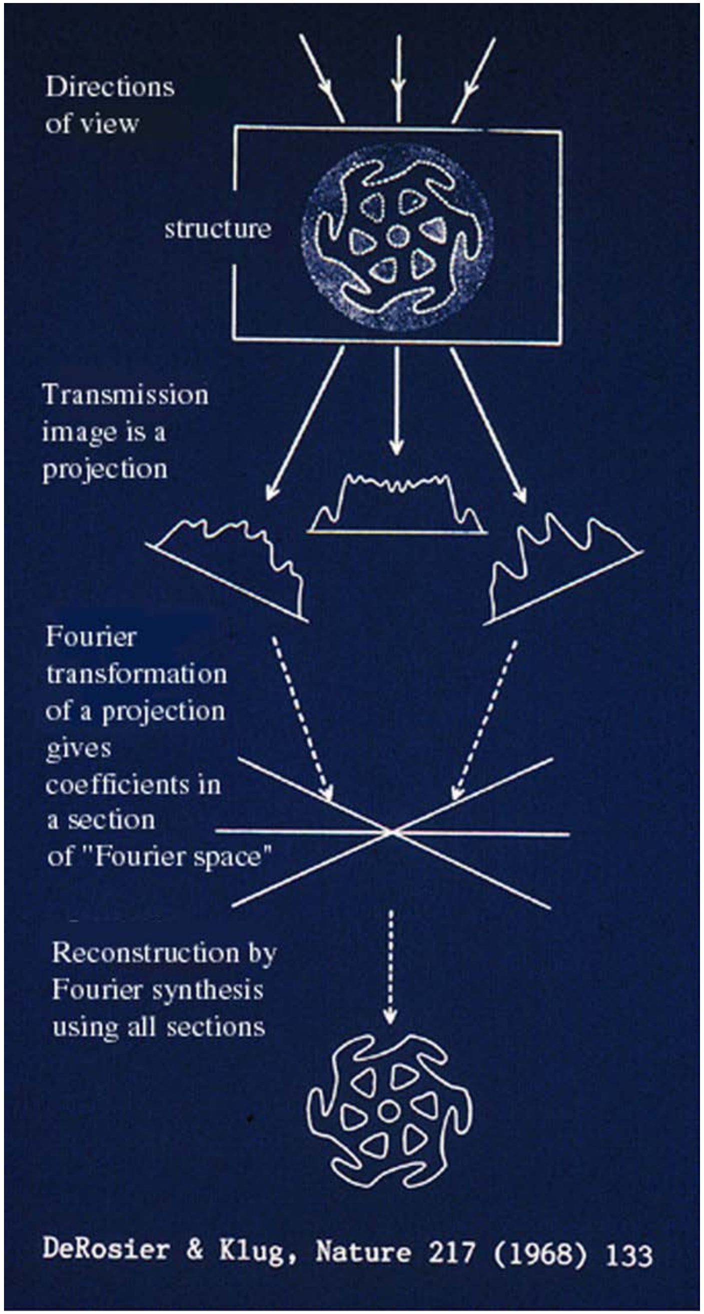


Photo credit Brian Clark

Aaron Klug David

DeRosier



Directions of view

structure

Transmission image is a projection

Fourier transformation of a projection gives coefficients in a section of "Fourier space"

Reconstruction by Fourier synthesis using all sections

DeRosier & Klug, Nature 217 (1968) 133

THREE-DIMENSIONAL RECONSTRUCTION: VIRUSES WITH ICOSAHEDRAL SYMMETRY

Tony Crowther

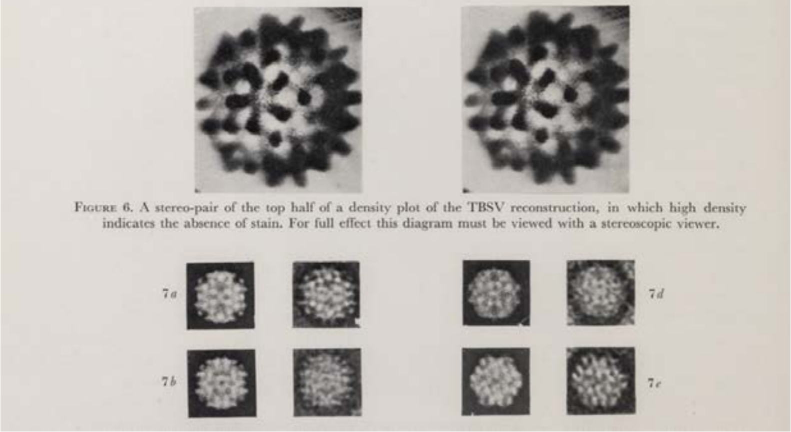
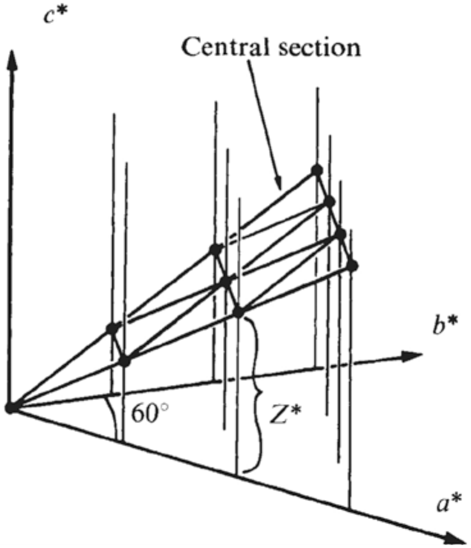


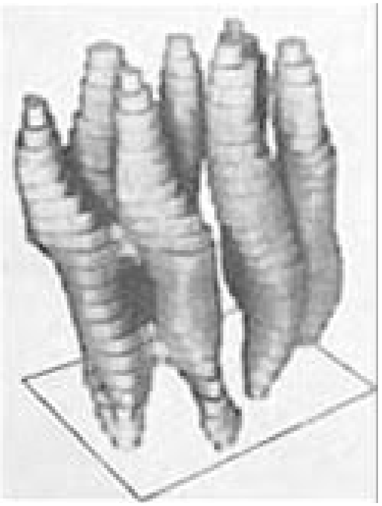
FIGURE 6. A stereo-pair of the top half of a density plot of the TBSV reconstruction, in which high density indicates the absence of stain. For full effect this diagram must be viewed with a stereoscopic viewer.

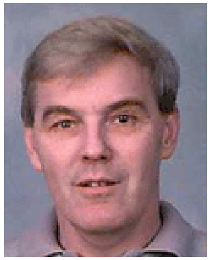
R. A. Crowther, Phil. Trans. Roy. Soc. 1971

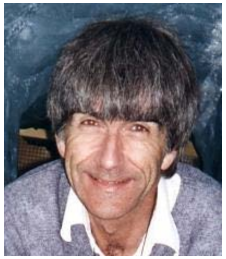
Alpbach, site of workshops on protein X-ray crystallography organized by Walter Hoppe and Max Perutz

THREE-DIMENSIONAL RECONSTRUCTION: TWO-DIMENSIONAL CRYSTAL (PURPLE MEMBRANE PROTEIN)

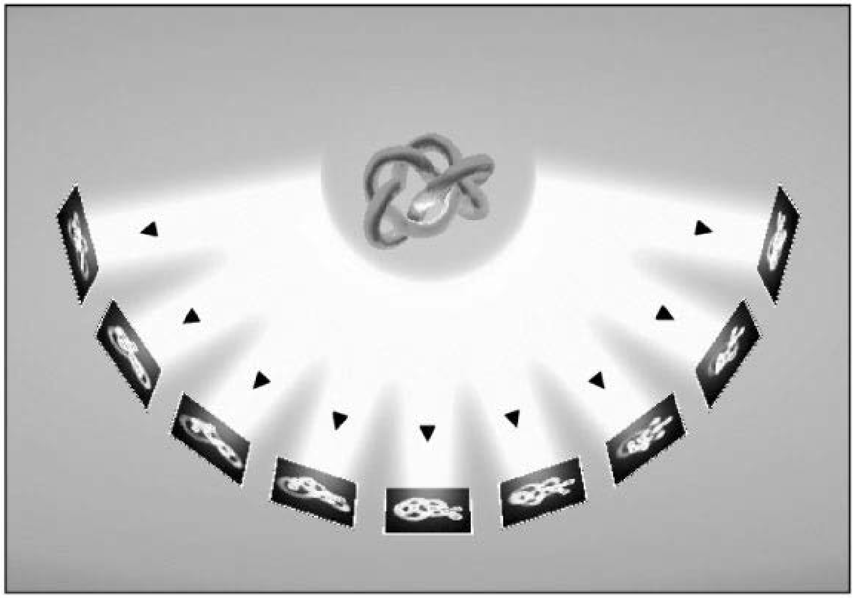




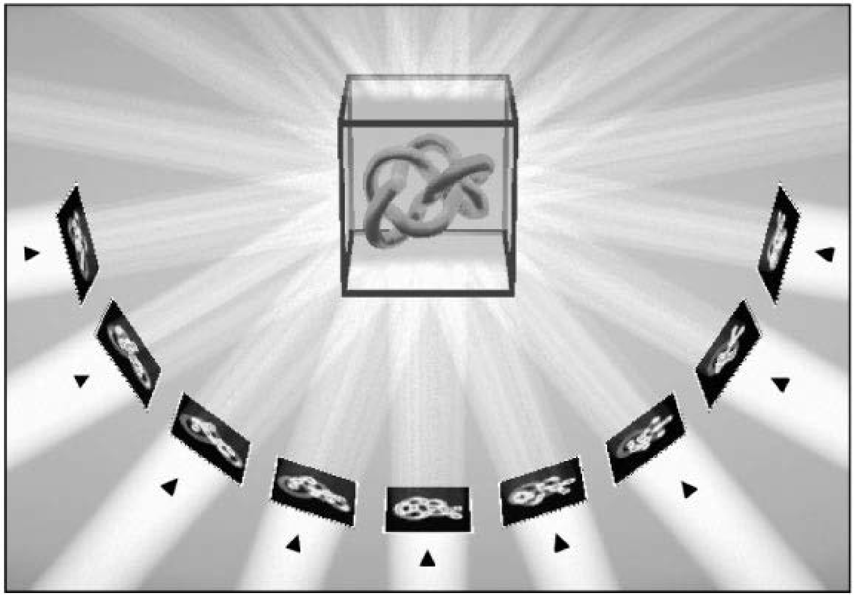




Henderson and Unwin, Nature 1975



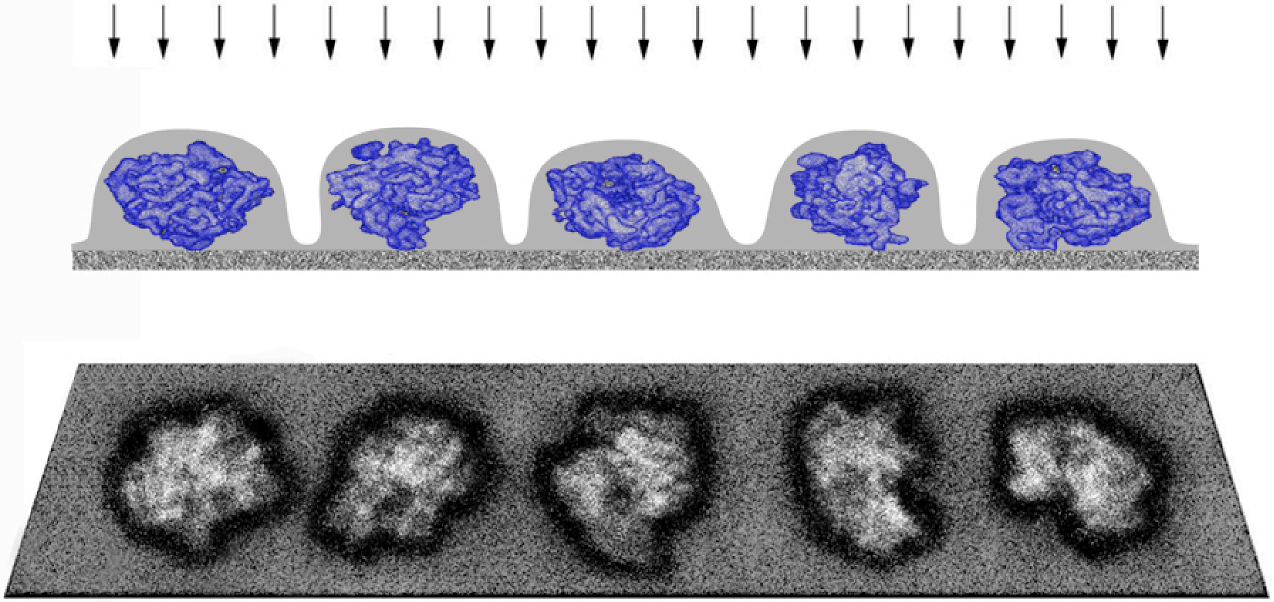
Molecule captured in different views

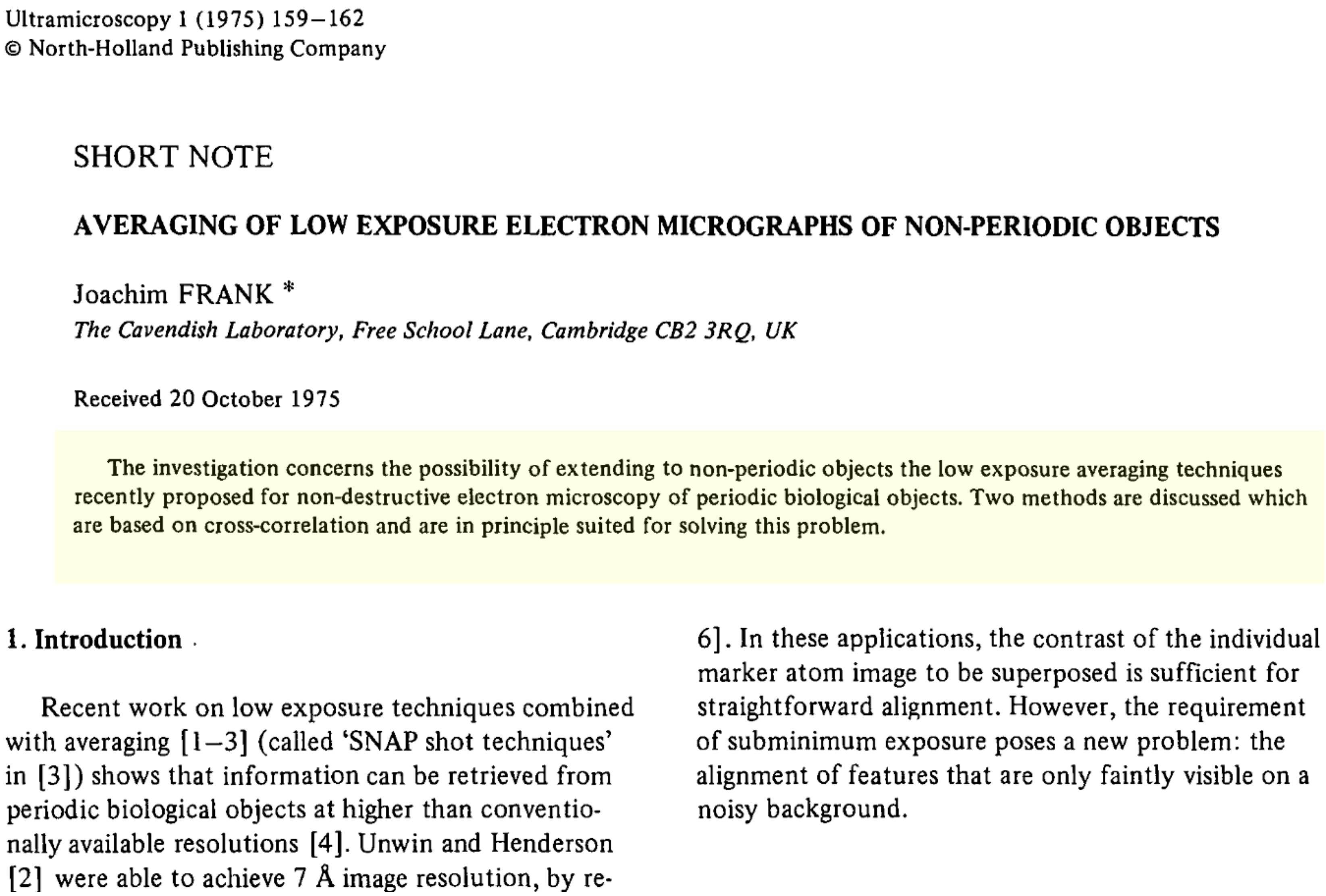


Three-dimensional reconstruction produces a 3D image, or density map

Sali et al. Nature 2003

SINGLE-PARTICLE PROJECTIONS – MOLECULES NEGATIVELY STAINED





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

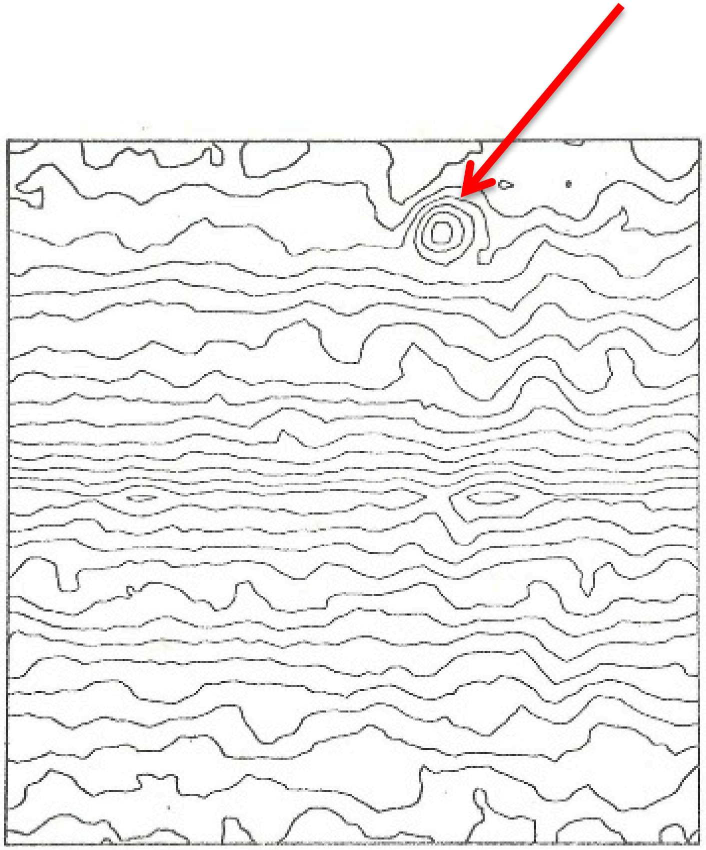
The investigation concerns the possibility of extending to non-periodic objects the low exposure averaging techniques recently proposed for non-destructive electron microscopy of periodic biological objects. Two methods are discussed which are based on cross-correlation and are in principle suited for solving this problem.

1. Introduction

Recent work on low exposure techniques combined with averaging [1–3] (called ‘SNAP shot techniques’ in [3]) shows that information can be retrieved from periodic biological objects at higher than conventionally available resolutions [4]. Unwin and Henderson [2] were able to achieve 7 Å image resolution, by re-

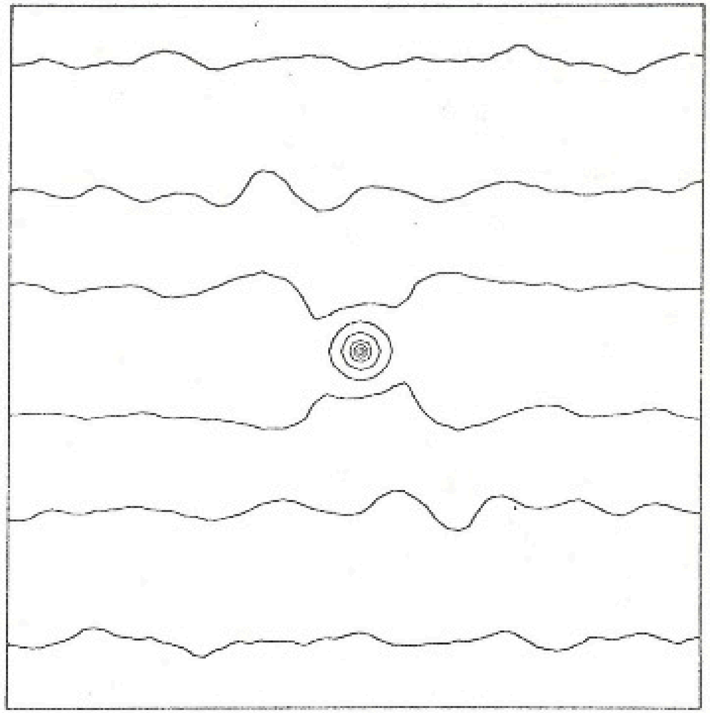
6]. In these applications, the contrast of the individual marker atom image to be superposed is sufficient for straightforward alignment. However, the requirement of subminimum exposure poses a new problem: the alignment of features that are only faintly visible on a noisy background.

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



Peak

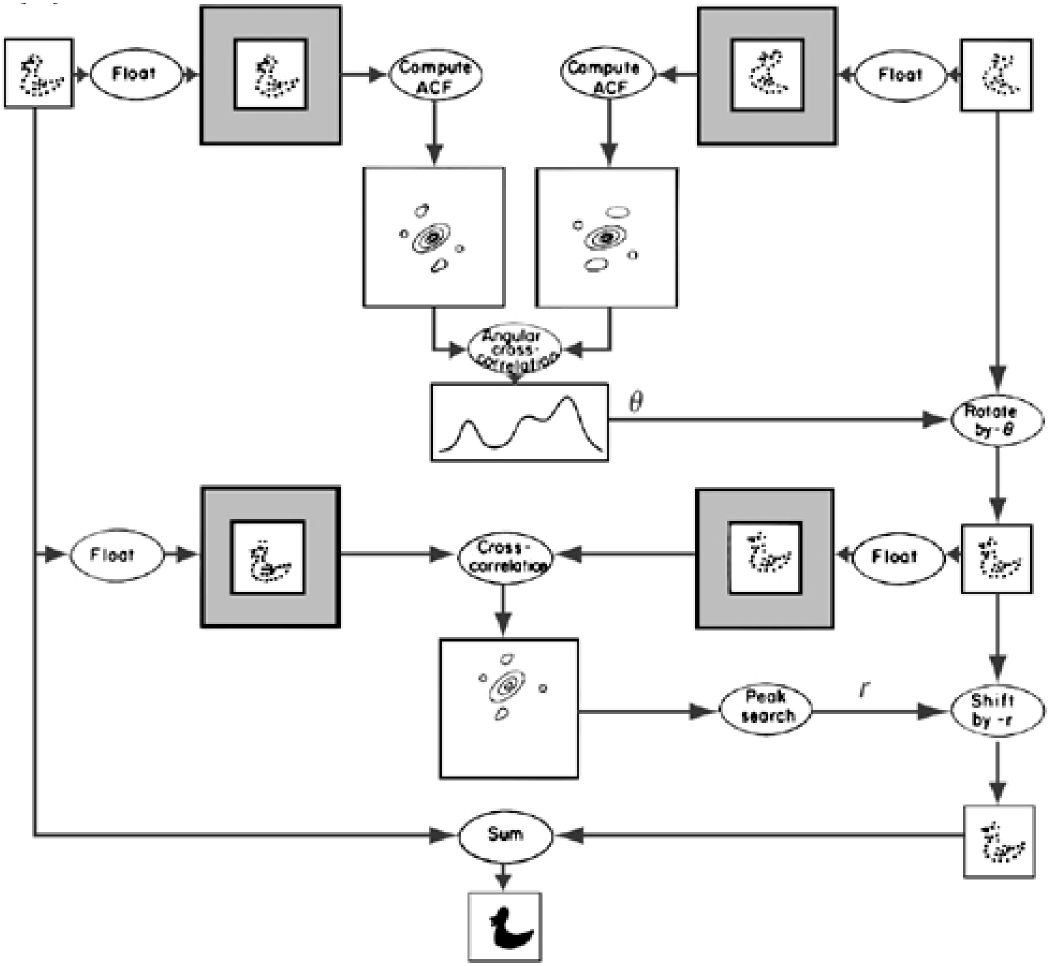
Cross-correlation function



Autocorrelation function

J. Frank, Ph.D. thesis 1970

SIMULTANEOUS SHIFT AND ROTATION ALIGNMENT



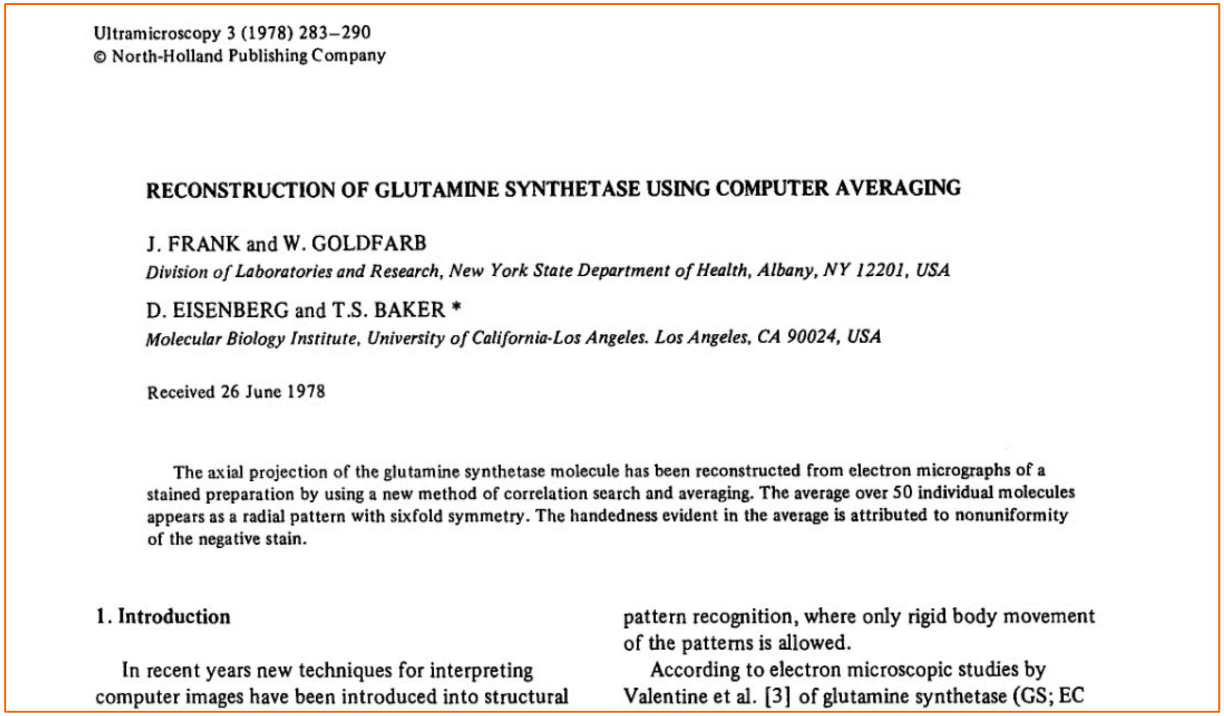
CONDITIONS FOR ALIGNMENT OF TWO IMAGES OF A MOLECULE OF SIZE *D*

$$\boxed{D \geq \frac{3}{c^2 d p_{crit}}}$$

PARTICLE SIZE > 3/[CONTRAST^2 x RESOLUTION (in Å) x CRITICAL ELECTRON DOSE]

Saxton & Frank, Ultramicroscopy 1977

GLUTAMINE SYNTHETASE



Ultramicroscopy 3 (1978) 283–290
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RECONSTRUCTION OF GLUTAMINE SYNTHETASE USING COMPUTER AVERAGING

J. FRANK and W. GOLDFARB
Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201, USA

D. EISENBERG and T.S. BAKER *
Molecular Biology Institute, University of California-Los Angeles. Los Angeles, CA 90024, USA

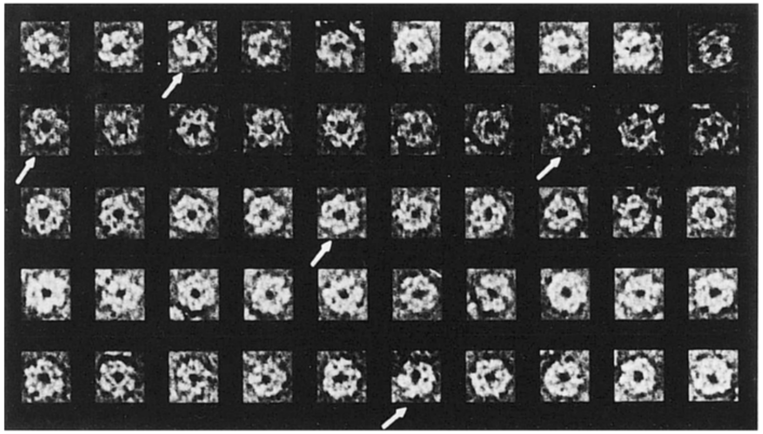
Received 26 June 1978

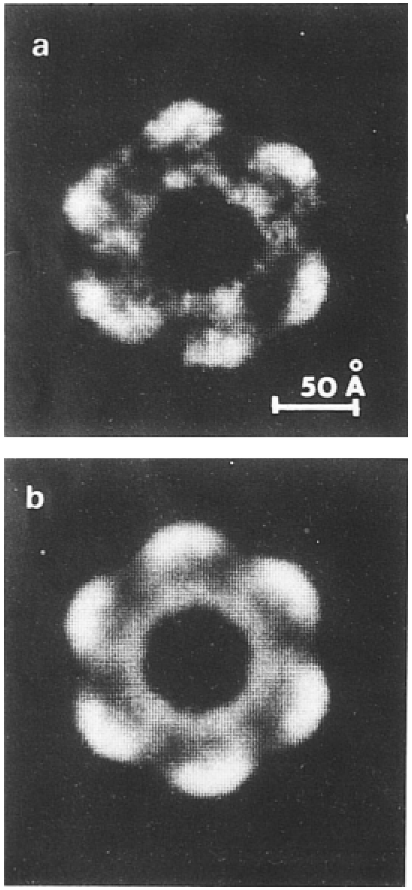
The axial projection of the glutamine synthetase molecule has been reconstructed from electron micrographs of a stained preparation by using a new method of correlation search and averaging. The average over 50 individual molecules appears as a radial pattern with sixfold symmetry. The handedness evident in the average is attributed to nonuniformity of the negative stain.

1. Introduction

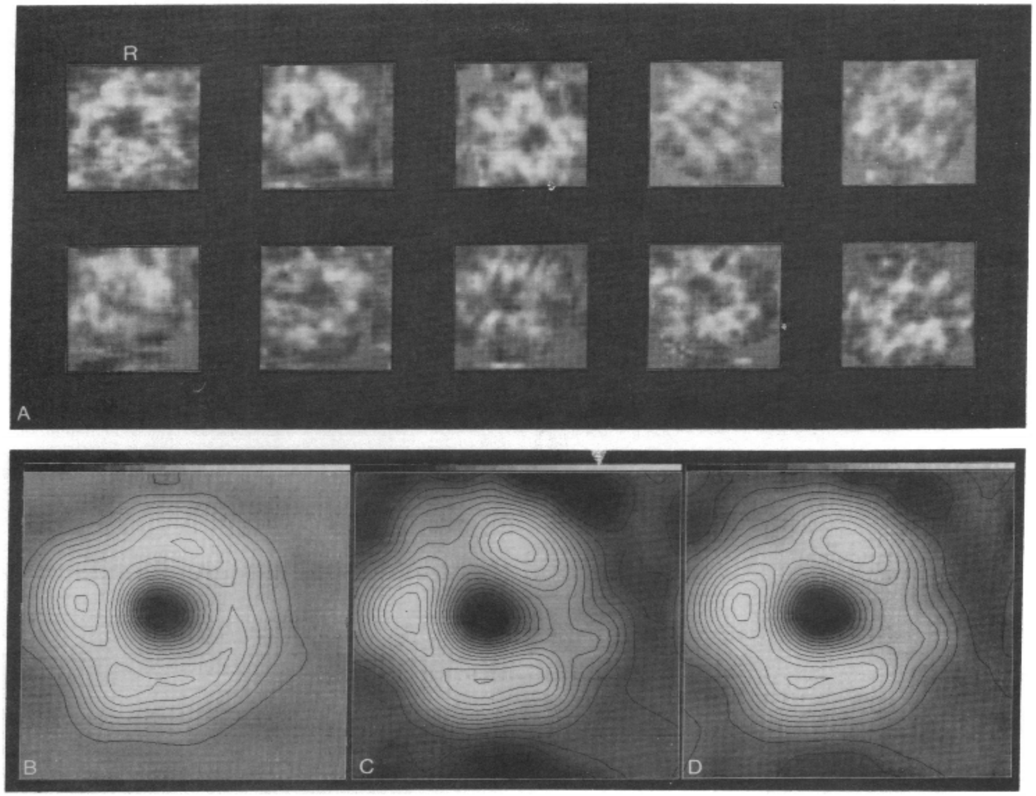
In recent years new techniques for interpreting computer images have been introduced into structural pattern recognition, where only rigid body movement of the patterns is allowed.

According to electron microscopic studies by Valentine et al. [3] of glutamine synthetase (GS; EC



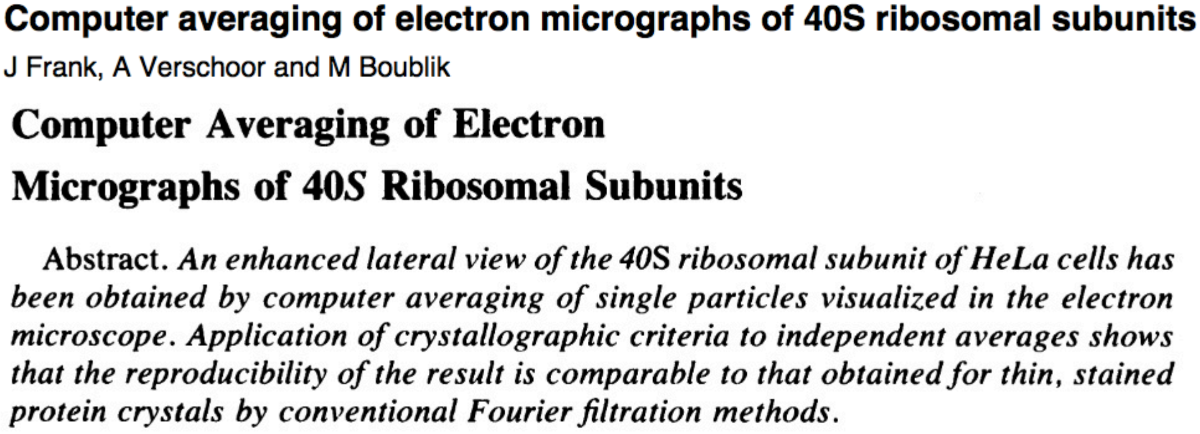


ACETYLCHOLINE RECEPTOR



Zingsheim et al., Proc. Natl. Adad. Sci. USA 1980

40S RIBOSOMAL SUBUNIT FROM HELA CELLS

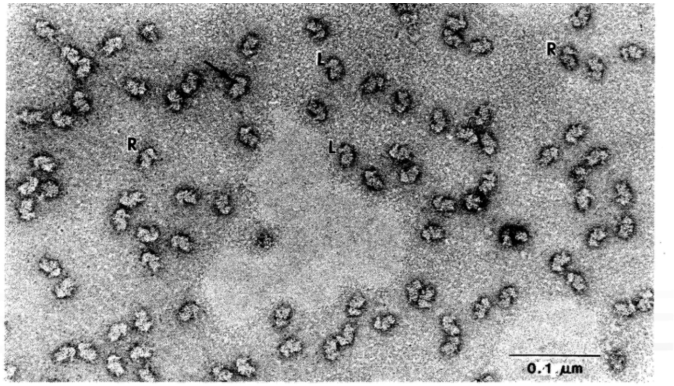


Computer averaging of electron micrographs of 40S ribosomal subunits

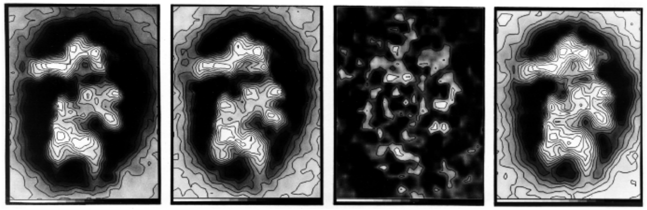
J Frank, A Verschoor and M Boublik

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.*



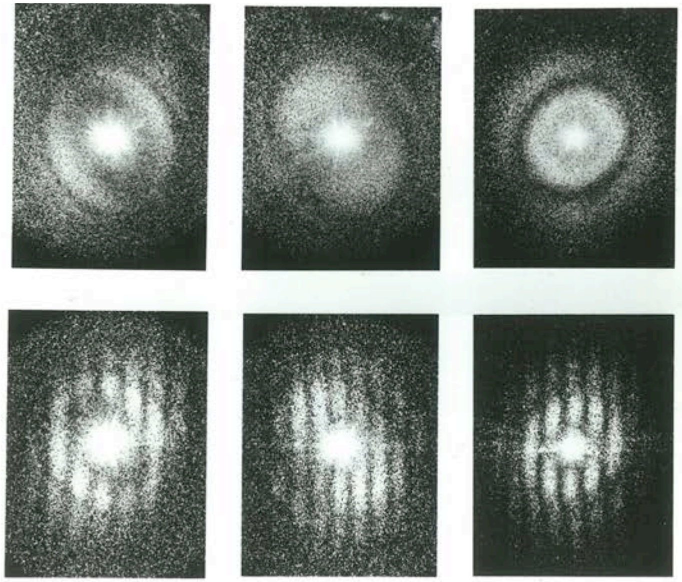
A



B C D E

Frank et al. Science 1981

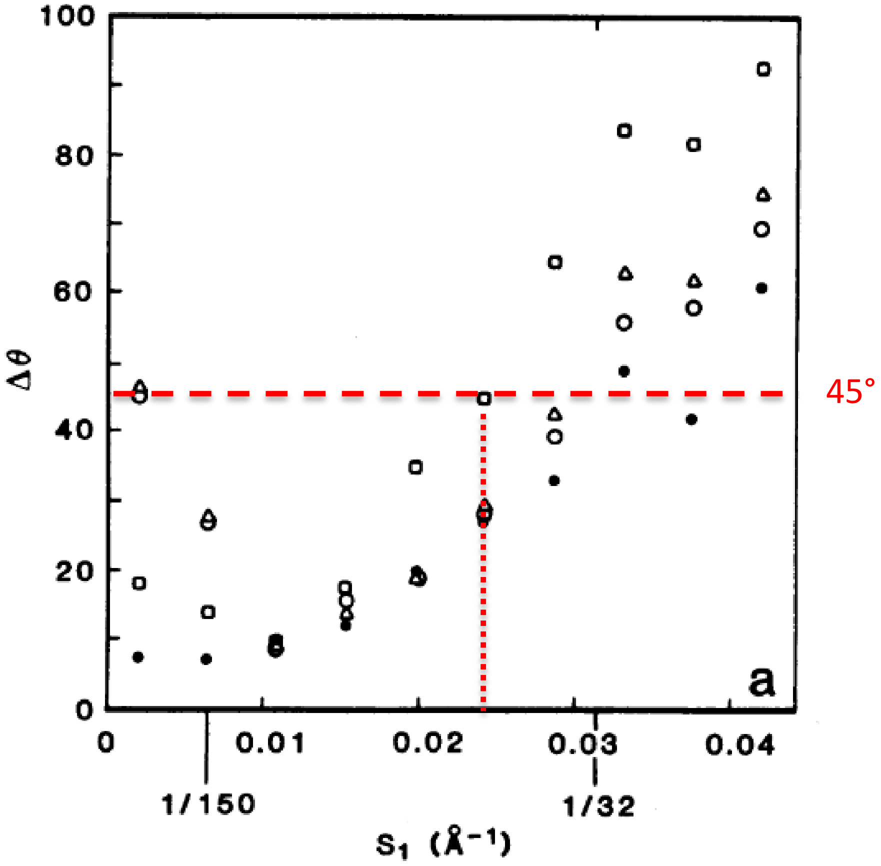
RESOLUTION = EXTENT OF REPRODUCIBILITY IN FOURIER SPACE BY OPTICAL DIFFRACTION



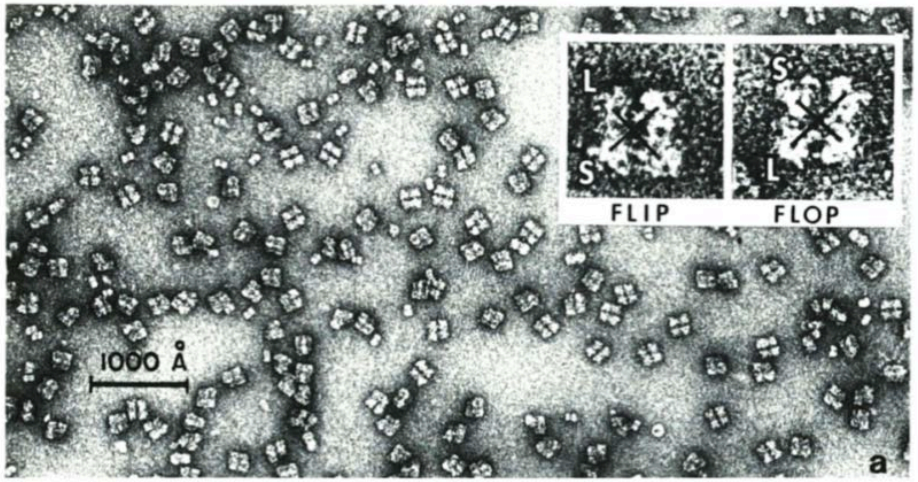
J. Frank, Ph.D. thesis 1970

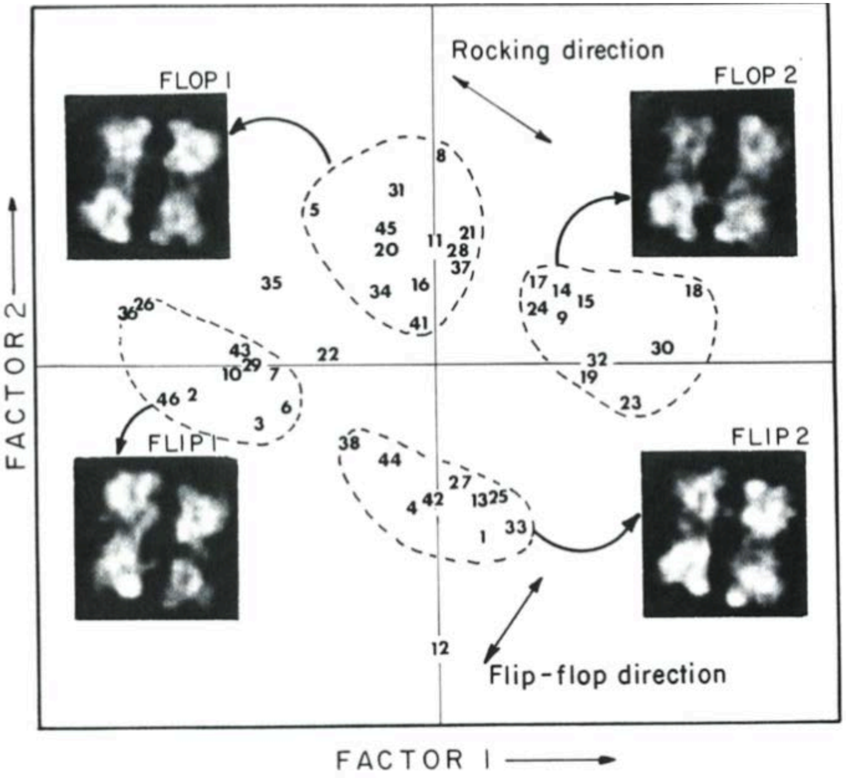
RESOLUTION = EXTENT OF REPRODUCIBILITY IN FOURIER SPACE USING THE COMPUTER

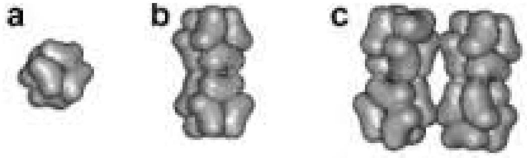
Differential Phase Residual



J. Frank et al., Science 1981

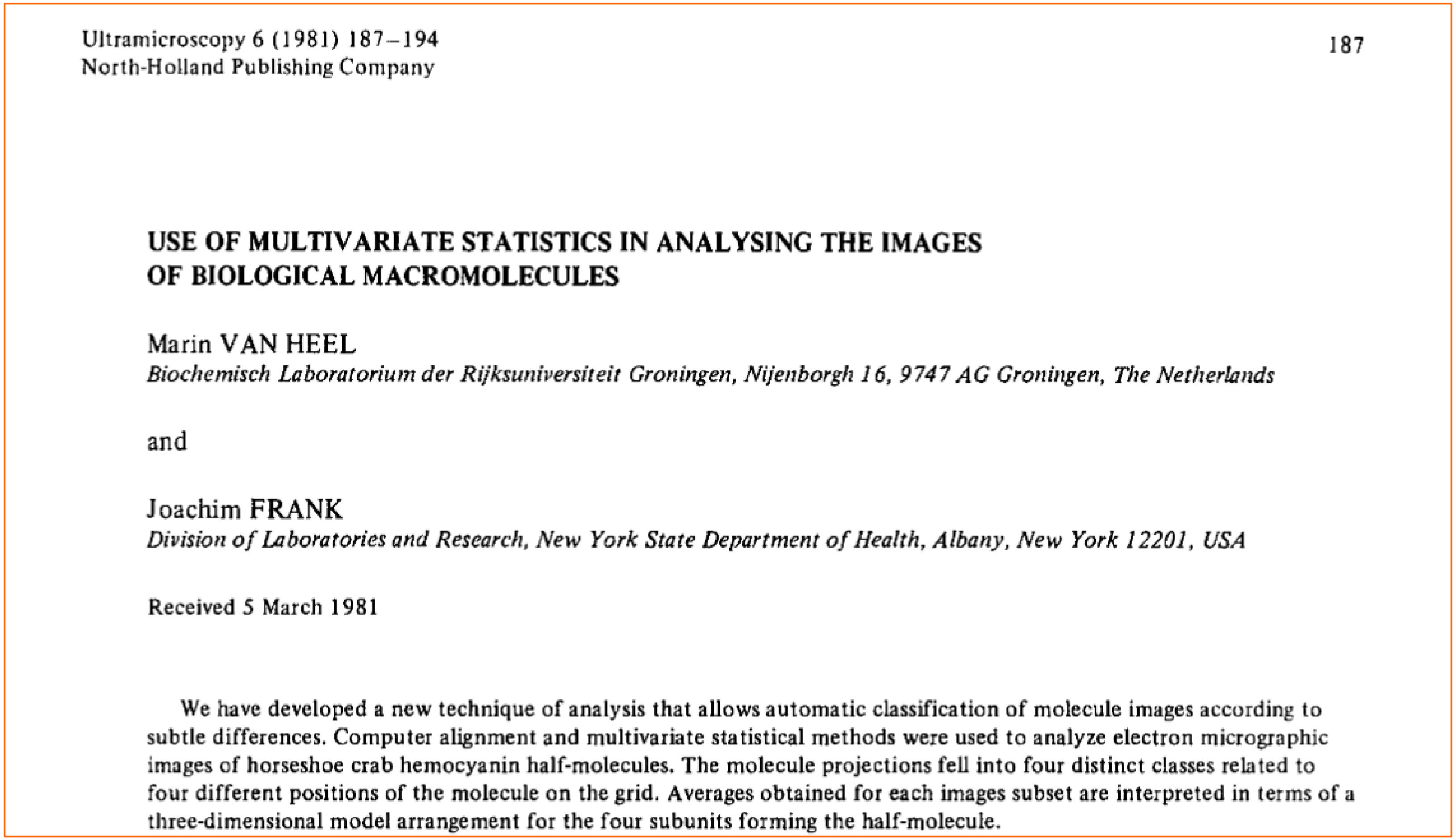






Limulus polyphemus hemocyanin

Van Heel and Frank, Ultramicroscopy 1981



Ultramicroscopy 6 (1981) 187–194
North-Holland Publishing Company

USE OF MULTIVARIATE STATISTICS IN ANALYSING THE IMAGES OF BIOLOGICAL MACROMOLECULES

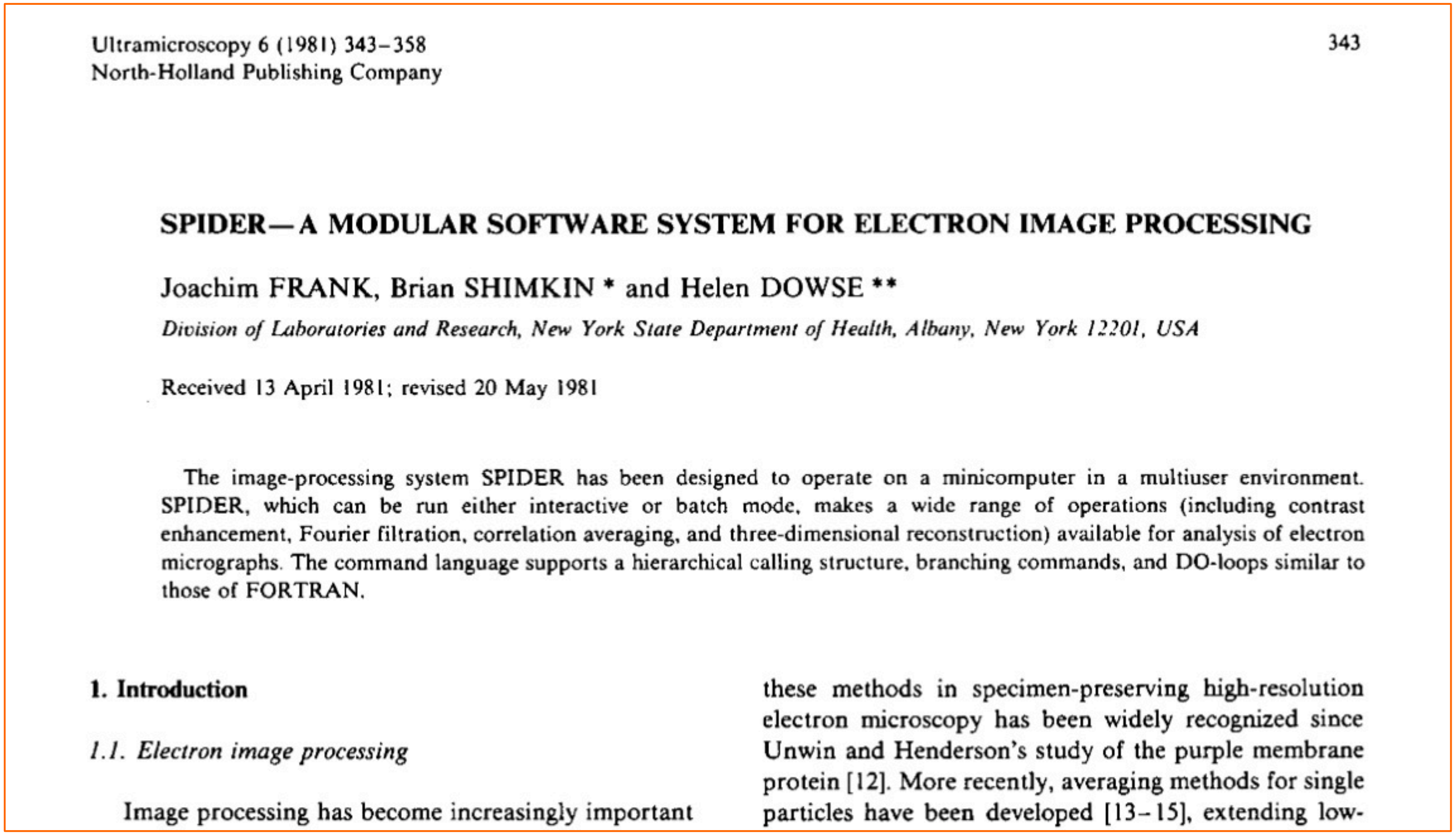
Marin VAN HEEL
Biochemisch Laboratorium der Rijksuniversiteit Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands

and

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

Received 5 March 1981

We have developed a new technique of analysis that allows automatic classification of molecule images according to subtle differences. Computer alignment and multivariate statistical methods were used to analyze electron micrographic images of horseshoe crab hemocyanin half-molecules. The molecule projections fell into four distinct classes related to four different positions of the molecule on the grid. Averages obtained for each images subset are interpreted in terms of a three-dimensional model arrangement for the four subunits forming the half-molecule.



Ultramicroscopy 6 (1981) 343–358
North-Holland Publishing Company

SPIDER—A MODULAR SOFTWARE SYSTEM FOR ELECTRON IMAGE PROCESSING

Joachim FRANK, Brian SHIMKIN * and Helen DOWSE **

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

Received 13 April 1981; revised 20 May 1981

The image-processing system SPIDER has been designed to operate on a minicomputer in a multiuser environment. SPIDER, which can be run either interactive or batch mode, makes a wide range of operations (including contrast enhancement, Fourier filtration, correlation averaging, and three-dimensional reconstruction) available for analysis of electron micrographs. The command language supports a hierarchical calling structure, branching commands, and DO-loops similar to those of FORTRAN.

1. Introduction

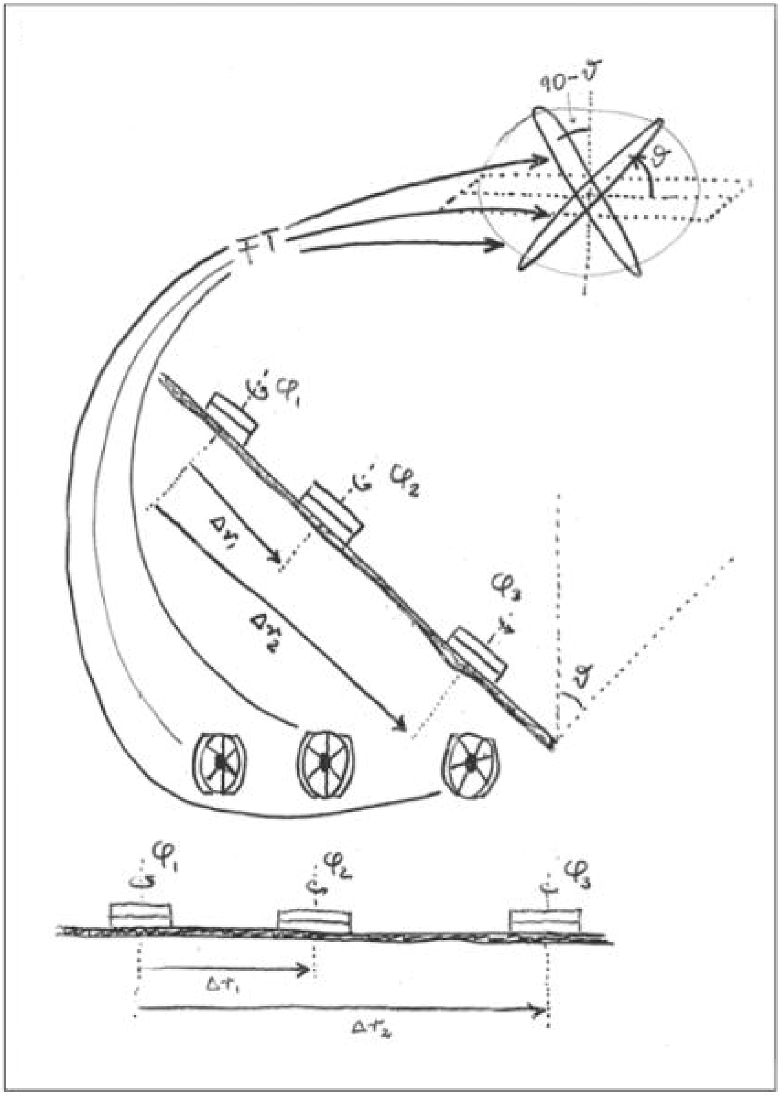
1.1. Electron image processing

Image processing has become increasingly important

these methods in specimen-preserving high-resolution electron microscopy has been widely recognized since Unwin and Henderson's study of the purple membrane protein [12]. More recently, averaging methods for single particles have been developed [13–15], extending low-

Jet Propulsion Laboratory, Pasadena, California

RANDOM-CONICAL RECONSTRUCTION – PRINCIPLE

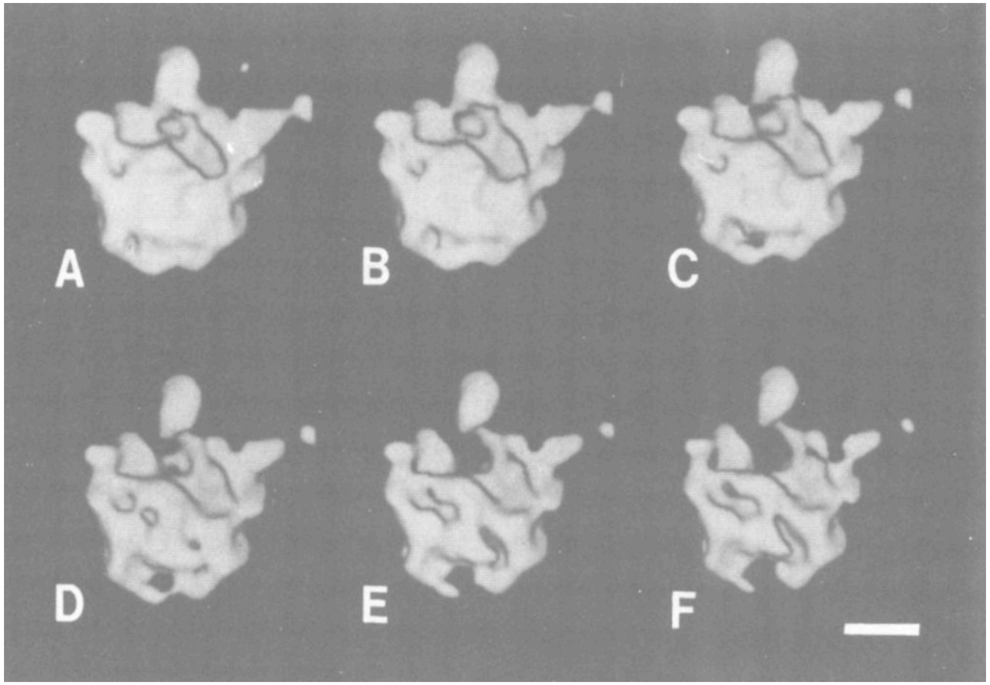


J. Frank, overhead 1979

RANDOM-CONICAL RECONSTRUCTION – PRINCIPLE (FANCY VERSION)

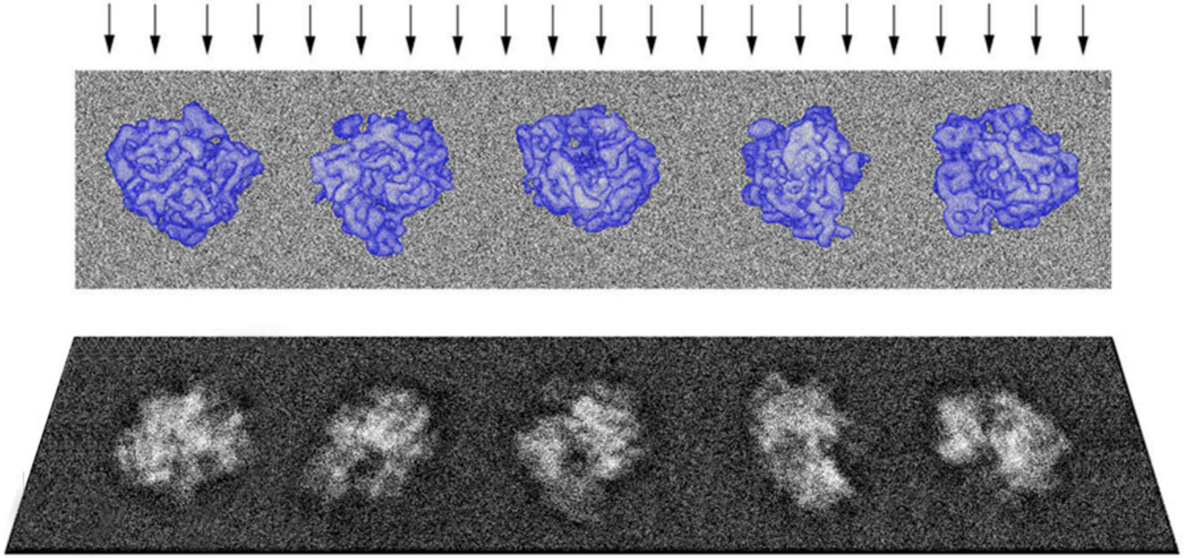
J. Frank, American Scientist 1998

RECONSTRUCTION OF 50S RIBOSOMAL SUBUNIT FROM *E. COLI* RIBOSOME

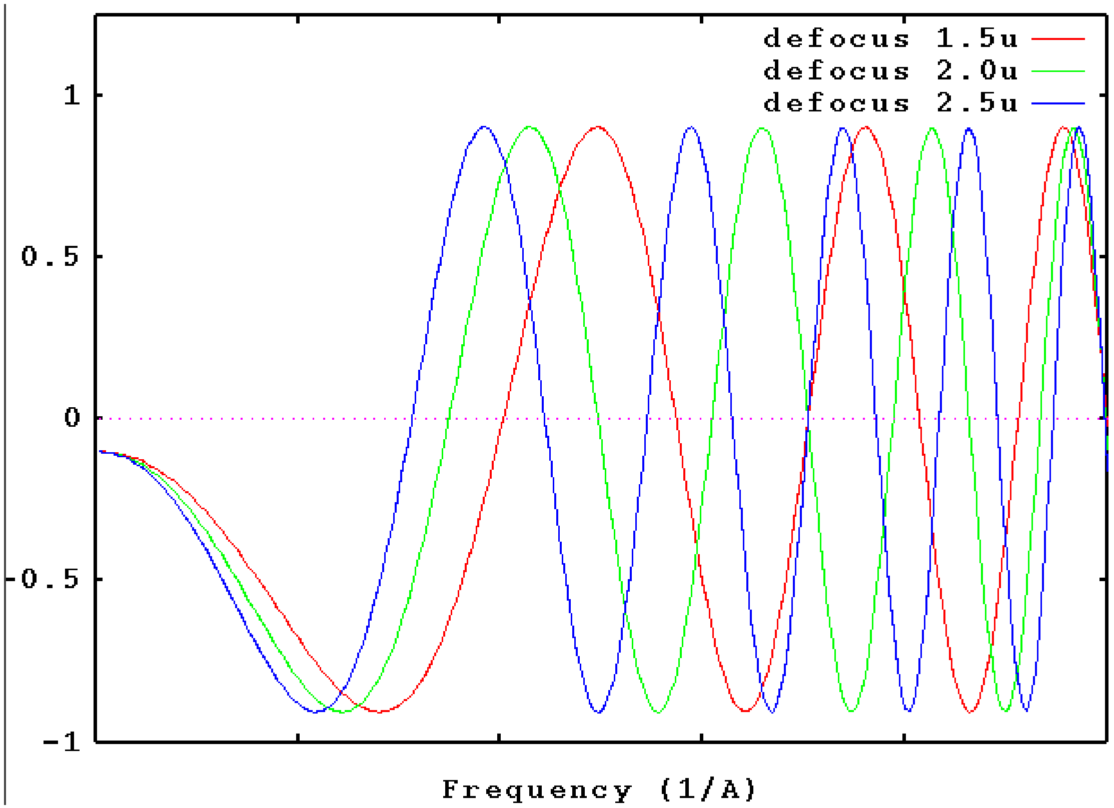


Radermacher et al., EMBO J. 1987

SINGLE-PARTICLE PROJECTIONS – MOLECULES EMBEDDED IN ICE



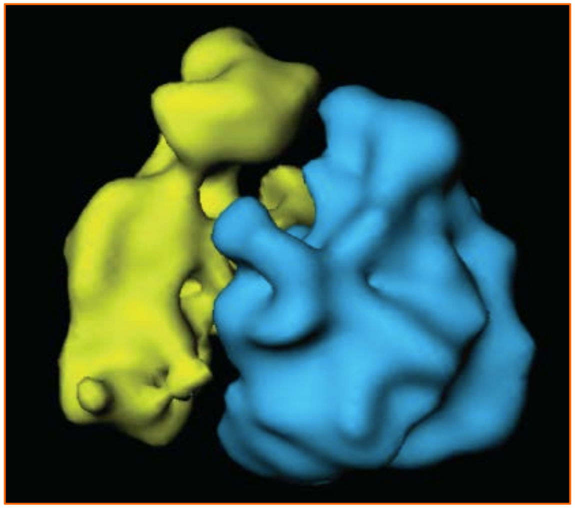
CONTRAST TRANSFER FUNCTION CORRECTION FROM DEFOCUS SERIES



$$F(\mathbf{k}) = \sum_{n=1}^{N} W_n(k) F_n(\mathbf{k}) \qquad W_n(k) = \frac{SNR_n(k) H_n^*(k)}{\sum_{n=1}^{N} SNR_n(k) \, | H_n(k) |^2 + 1}$$

Penczek et al., Scanning Microscopy 1997

E. coli ribosome

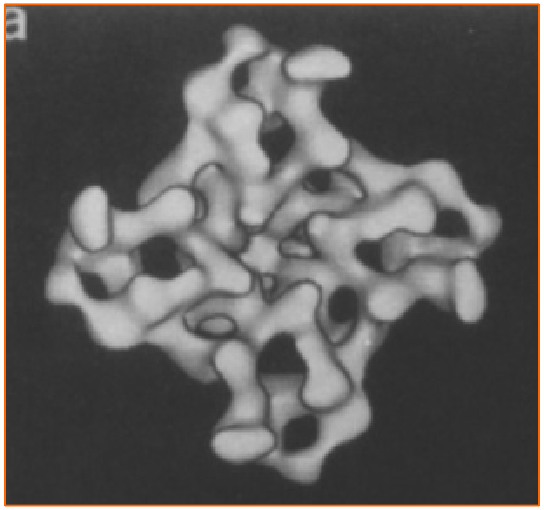


Frank et al., Nature 1995

Octopus hemocyanin

Lambert et al., 1994

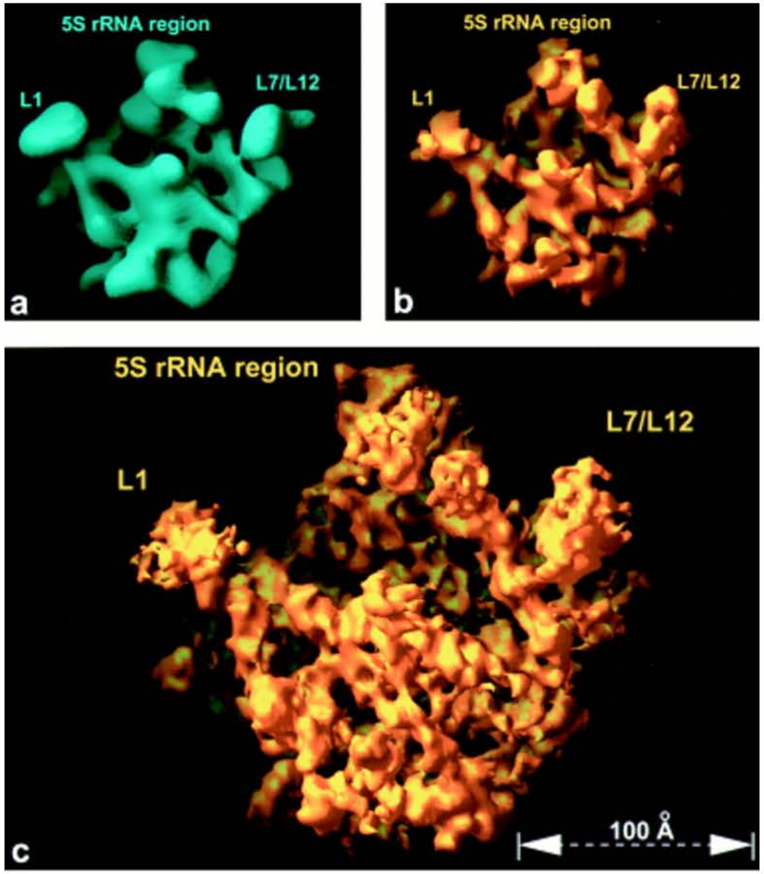
Calcium Release Channel



Radermacher et al., 1994

CRYO-EM RECONSTRUCTION ASSISTING IN PHASING THE X-RAY STRUCTURE

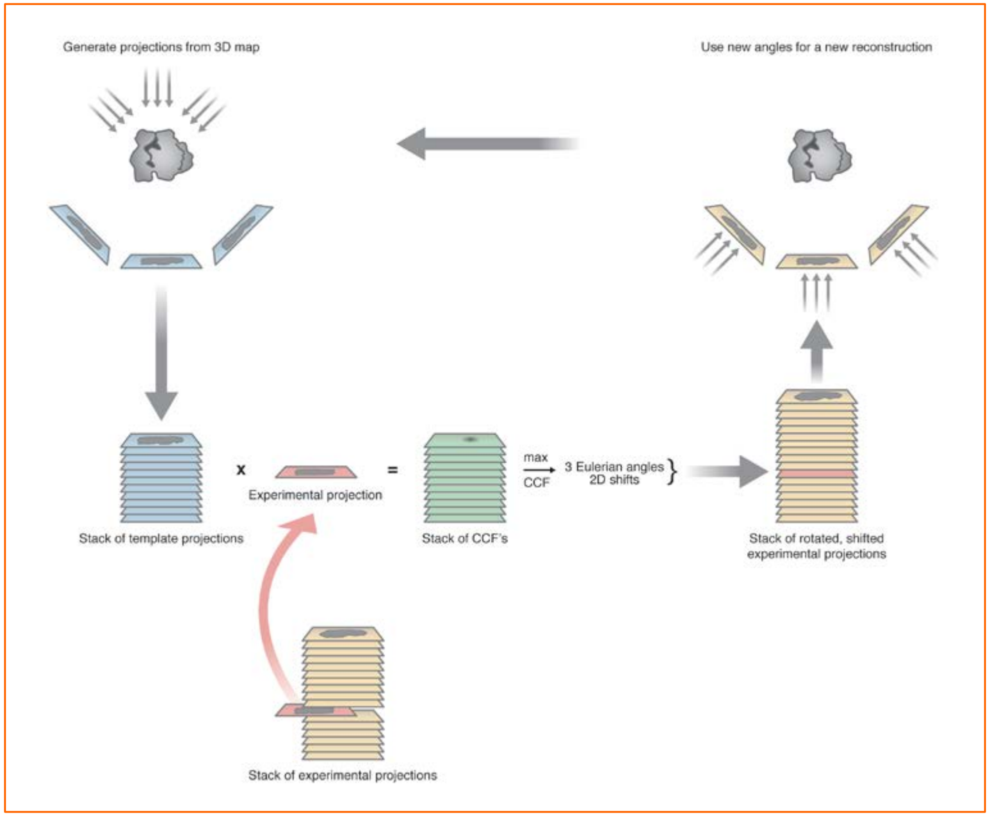
Cryo-EM



X-ray

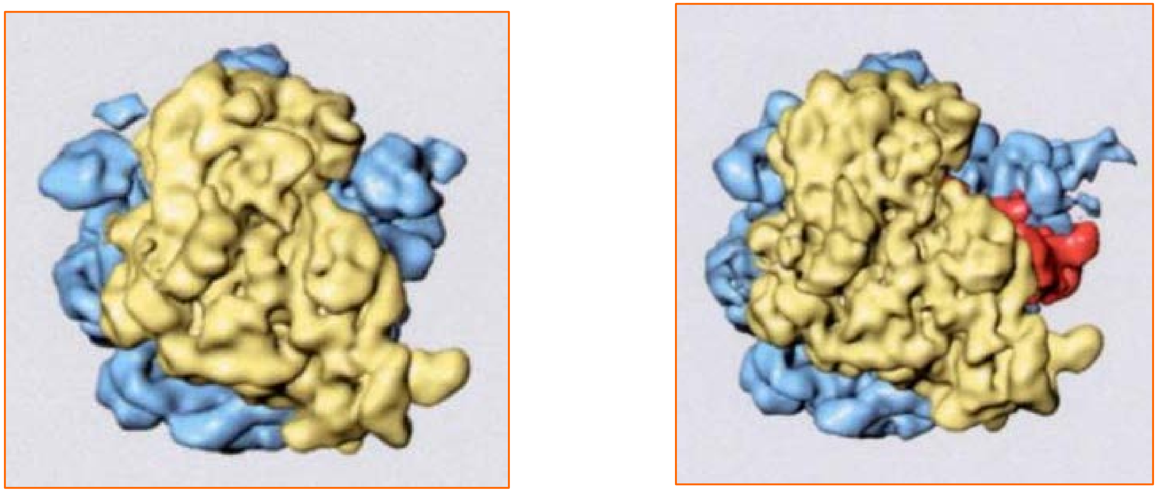
Ban et al., Cell 1998

ANGULAR REFINEMENT (3D PROJECTION MATCHING)



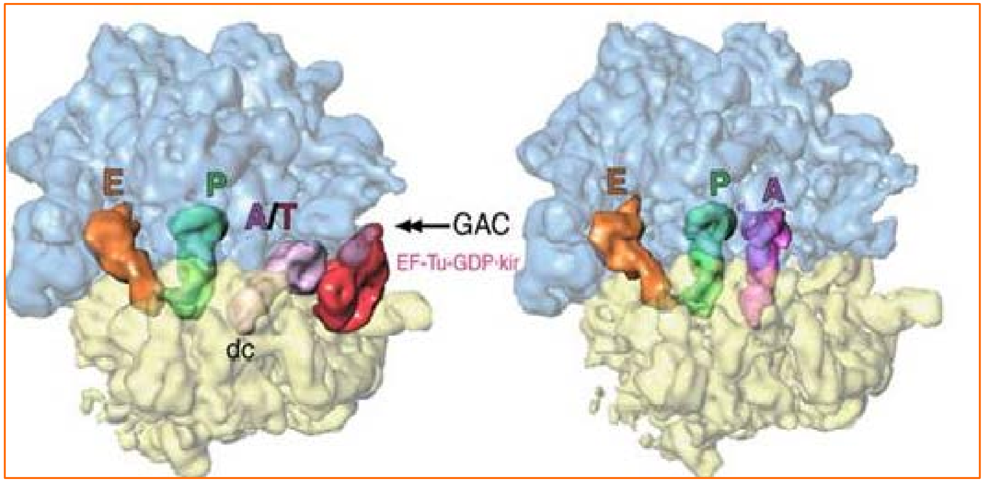
J. Frank, Molecular Machines in Biology, Ch. 2, Cambridge University Press 2011

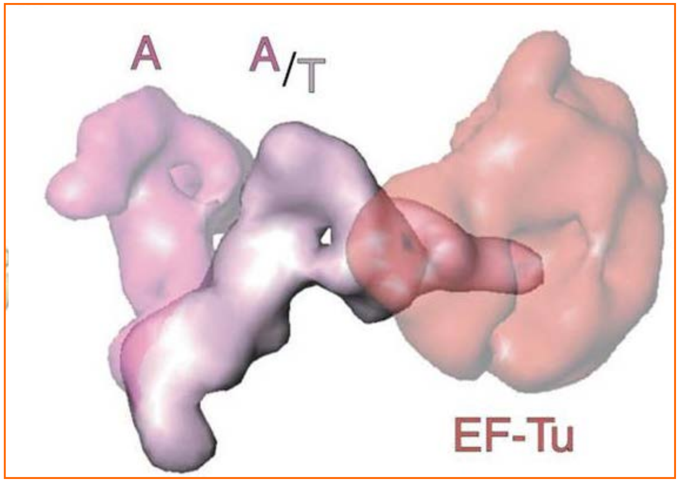
RATCHET-LIKE INTERSUBUNIT MOTION Frank & Agrawal Nature 2000



Valle et al., Cell 2003

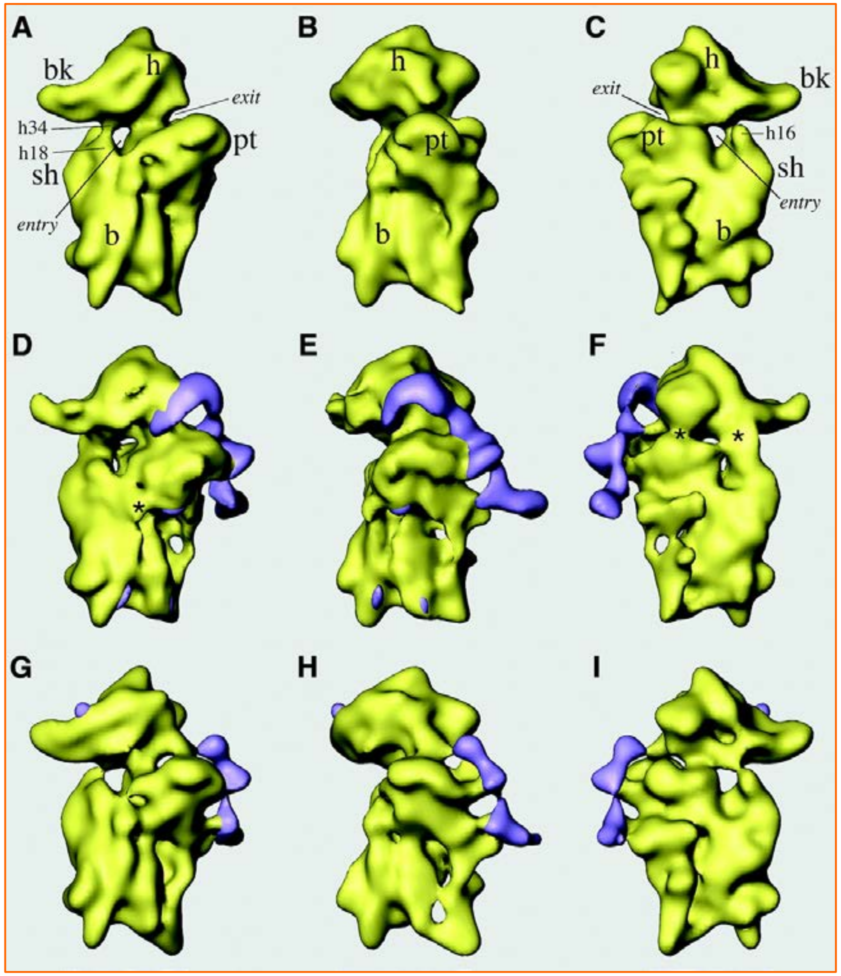
tRNA AS A MOLECULAR SPRING DURING DECODING





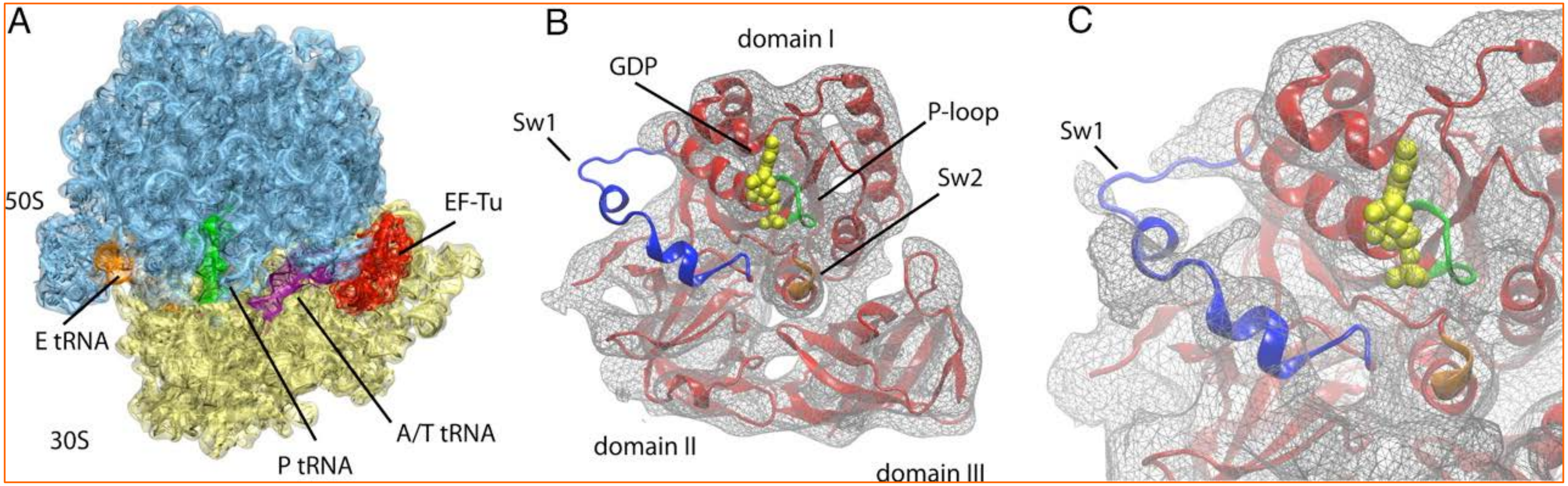
Valle et al., Nat. Struct. Biol. 2003

HEPATITIS C VIRUS IRES INVADING THE HOST RIBOSOME



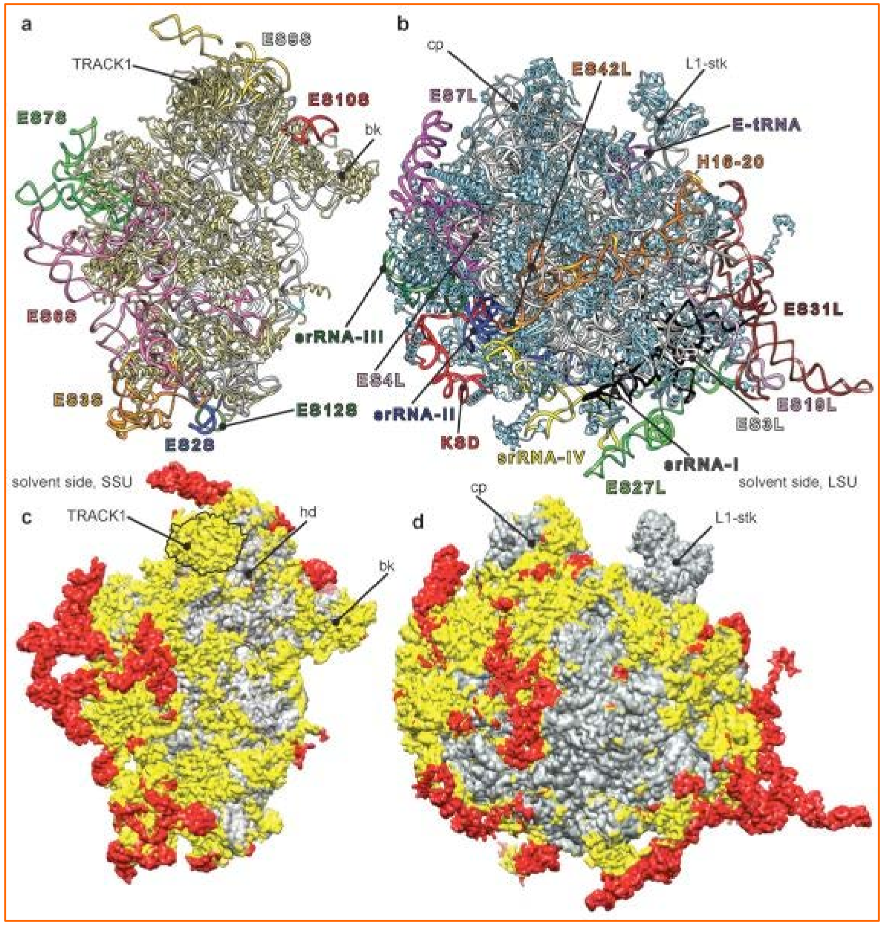
Spahn et al., Science 2001

ATOMIC MODELS THROUGH FLEXIBLE FITTING I



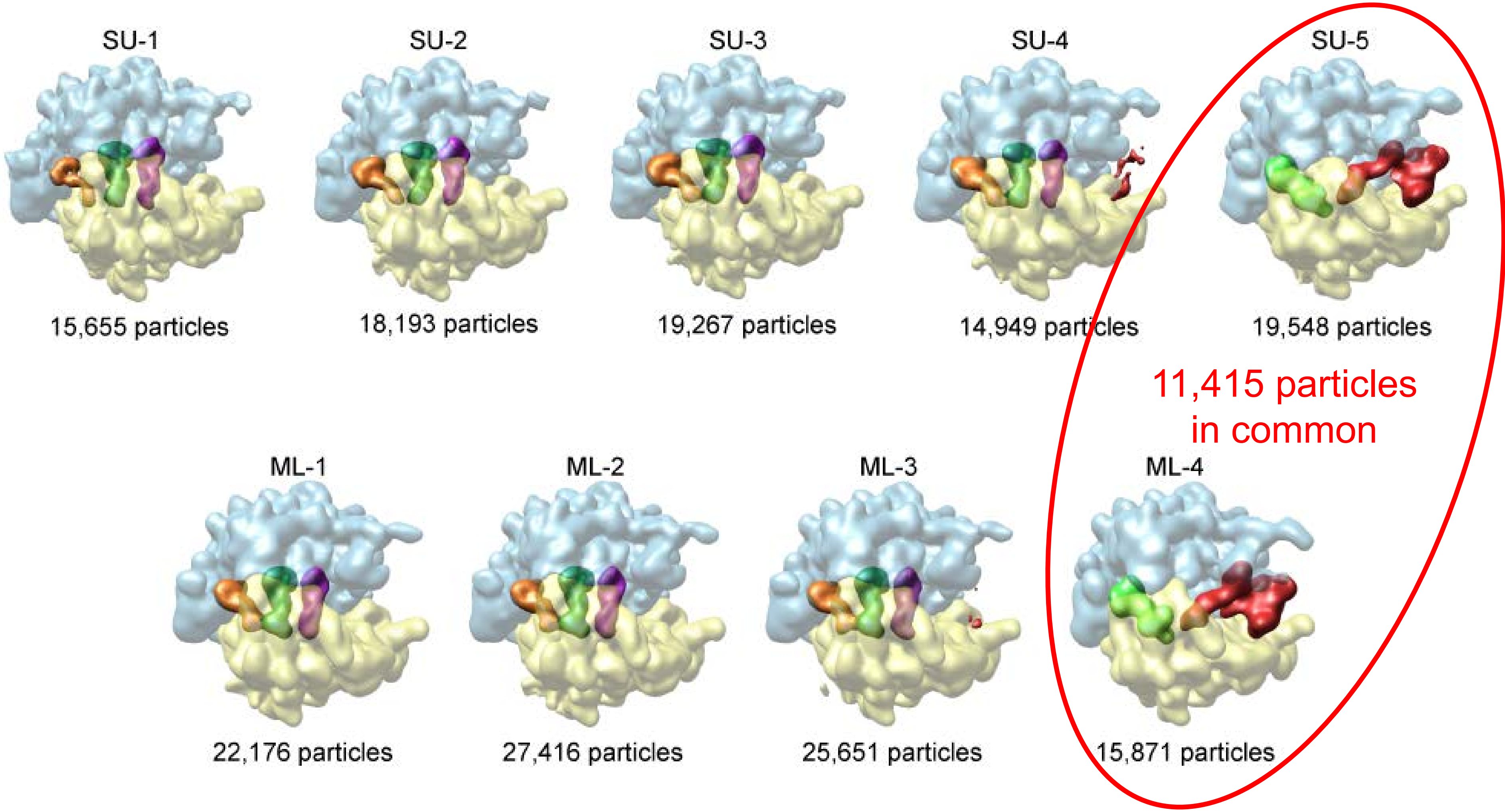
Villa et al., PNAS 2009

ATOMIC MODELS THROUGH FLEXIBLE FITTING II



Hashem et al., Nature 2013

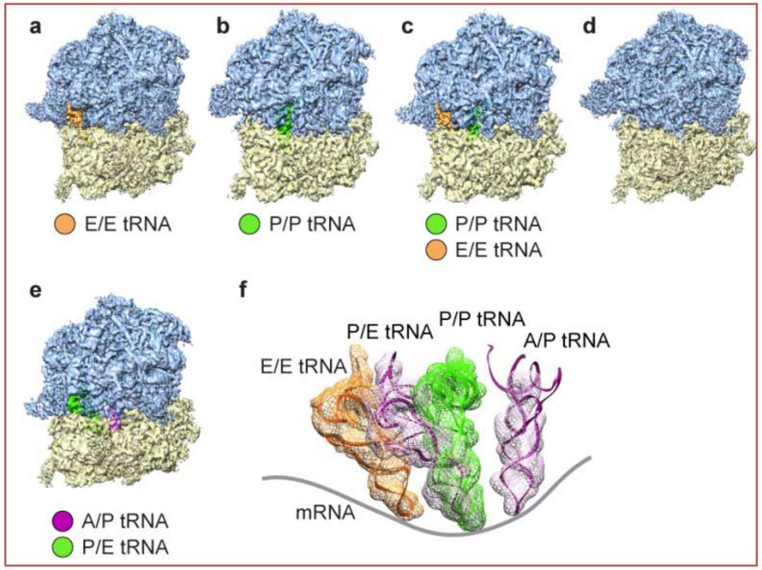
Top: classes derived by supervised classification
Bottom: classes derived by Maximum Likelihood classification



Scheres et al., Nature Methods 2007

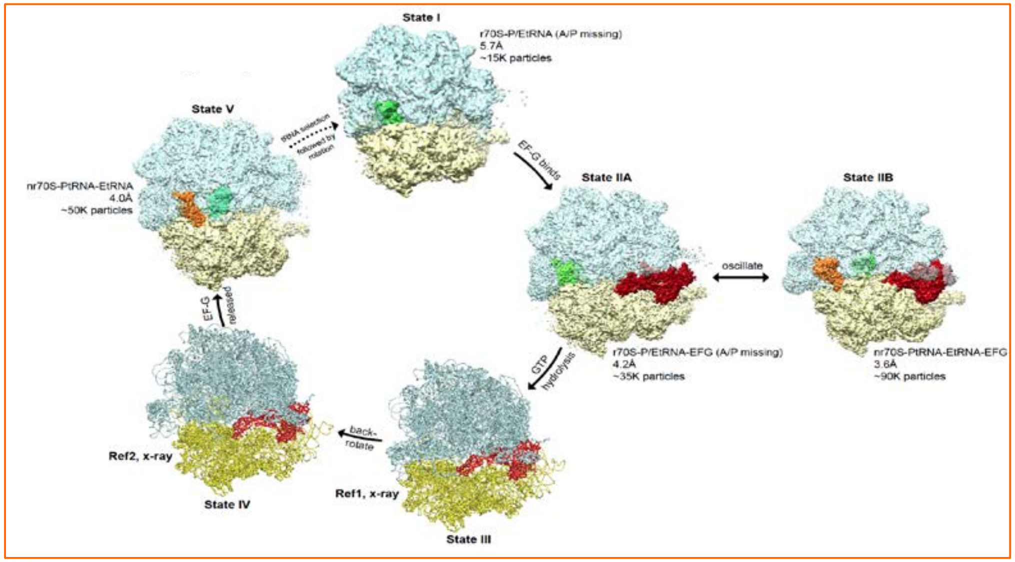
“STORY IN A SAMPLE”

Plasmodium falciparum ribosomes purified from cell extracts

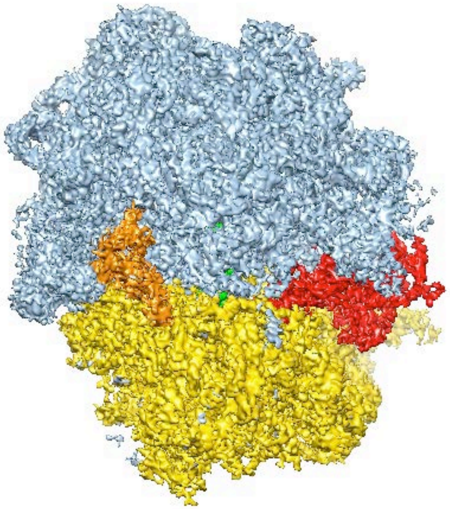


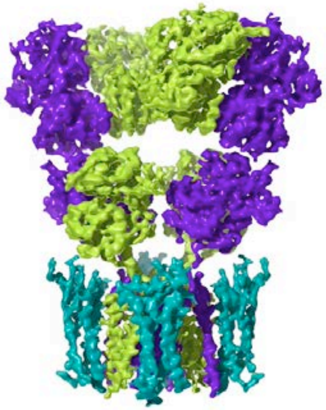
Sun et al., Nucl. Acid Res. 2015

EF-G mutant binding to “PRE” complex



Adapted from Li et al., Science Adv. 2015





T. cruzi ribosome
Liu et al.,
PNAS 2016

Calcium release channel
Des Georges et al.,
Cell 2017

AMPA receptor
Twomey et al.,
Nature 2017

Wadsworth Center, Albany, New York State Department of Health

Department of Biomedical Sciences, University at Albany

Since 2008: Department of Biochemistry and Molecular Biophysics, and Department of Biological Sciences, Columbia University

Funding:

National Institute of General Medical Science, NIH

National Science Foundation

Howard Hughes Medical Institute