Single-particle Cryo-EM -- Visualization of Biological Molecules in their Native States

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Crowded environment inside a cell, simulated on the Stampede supercomputer by Michael Feig of Michigan State University. MD simulation shows a few ns of dynamics in atomistic detail of bacterial cytoplasm.

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Molecular Machines in the Cell

- Molecular machines: many molecules act in concert, in a *processive* way
- We wish to know the structures of all components but also the way they interact dynamically
- **Reductionism**: we study a subsystem in isolation (*in vitro*), hoping to approximate the processes in the environment of the cell
ATP Synthase: makes ATP
Proteasome: recycles proteins
RNA Polymerase: copies DNA → mRNA
Ribosome: makes proteins
Chaperone: folds proteins
Dynein: transports molecules
Spliceosome: edits mRNA
Flagella motor: rotates flagella
MULTIPLE STATES OF A MOLECULAR MACHINE: THE RIBOSOME

Schmeing and Ramakrishnan, Nature 2009
X-ray Crystallography

• Crystal: many copies of the molecule arranged in regular order.

• *Exposure to X-ray beam → diffraction pattern → structure determination.*

• X-ray beam must be high-intensity, crystal must be almost perfect.

• *To date ~ 140,000 structures solved by X-ray crystallography, available in public databanks.*

• Crystal packing → molecules not visualized in all conformations/binding states that important for function.

• *Many molecules do not form highly ordered crystals.*

• Sample quantity can be a big issue, as well.

Max Perutz and John Kendrew with a model of hemoglobin, 1962

http://www.mfpl.ac.at/vips/max-f-perutz/
Electron Microscopy

- Electron microscopy can be used to solve molecular structures, as well.
- *Projection images formed at very high magnification, e.g. 30,000 x.*
- To reconstruct an object, many different views must be collected.
- *Sample must be very thin, electrons are readily absorbed by matter.*
- BUT: Electrons strongly damage the molecules -- need for low dose! 10-20 electrons/square Angstrom.
- *Images are very noisy (shot noise)*
THREE-DIMENSIONAL RECONSTRUCTION: STRUCTURES WITH HELICAL SYMMETRY (negative staining used)  
Pioneering work: 3D reconstruction of a bacteriophage tail using the Fourier-Bessel approach, 1968  
Application of the Projection-Slice Theorem

Aaron Klug and David DeRosier, LMB/MRC Cambridge
THREE-DIMENSIONAL RECONSTRUCTION: VIRUSES WITH ICOSAHEDRAL SYMMETRY
(negative staining used)

Tony Crowther

R. A. Crowther, Phil. Trans. Roy. Soc. 1971
THREE-DIMENSIONAL RECONSTRUCTION: STRUCTURES THAT FORM 2D CRYSTALS (glucose embedding used)

Richard Henderson and Nigel Unwin

Purple membrane Protein

Bacteriorhodopsin

Electron dose is spread over many repeats of the molecule in the crystal
WHY CRSTALS?
3D Reconstruction of Asymmetrical Molecules by Electron Tomography

- Electron Tomography of single molecules
- Examples: fatty acid synthetase and ribosome
- BUT: Accumulated electron exposure exceeded 1000 e⁻/Å²
WHY CRYSTALS?
3D Reconstruction of Asymmetrical Molecules by Single-Particle Techniques – the Concept

- Single-particle techniques: structural information from images of single (i.e., unattached) molecules in many copies.
- *Molecules are free to assume all naturally occurring conformations.*
- Molecules are randomly oriented.
- *A single snapshot may already give us hundreds of particle views.*
- As we collect more snapshots, more orientations will be covered, until we have enough for reconstructing the molecule in three dimensions.
EM images can be aligned to within better than 3 Angstrom!

Cross-correlation function of 2 successive micrographs of the same carbon film

J. Frank, Ph.D. thesis 1970
Dissertation at Technical University Munich, published in 2019, 49 years after completion

“Analysis of high-resolution electron micrographs using image difference and reconstruction methods”
SHORT NOTE

AVERAGING OF LOW EXPOSURE ELECTRON MICROGRAPHS OF NON-PERIODIC OBJECTS

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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.
“If such methods (i.e., for averaging data from arrays of identical objects that are not periodic) were to be perfected, then, in the words of one scientist, “the sky would be the limit.”

Arthur L. Robinson, Science 192 (1976) 360-363
CONDITIONS FOR ALIGNMENT OF TWO IMAGES
OF A MOLECULE OF SIZE $D$

\[
D \geq \frac{3}{c^2 dp_{\text{crit}}}
\]

PARTICLE SIZE $> 3 / \left[ \text{CONTRAST}^2 \times \text{RESOLUTION (in Å)} \times \text{CRITICAL ELECTRON DOSE} \right]$

Saxton & Frank, Ultramicroscopy 1977
Devil in the detail – *Problems to be solved:*

- ALIGN IMAGES
- ESTIMATE RESOLUTION OF AVERAGE
- SORT/CLASSIFY IMAGES
- FIND PROJECTION ANGLES
- RECONSTRUCT IN 3D
SPIDER -- Modular image processing program

Toronto EM conference abstract 1978
Ultramicroscopy 1981

Some of the operations
(out of hundreds):

AC -- autocorrelation
CC – cross-correlate 2 images
FT -- Fourier transform
RT -- rotate
SH -- shift
WI -- window

“WORKBENCH” FOR PROCESSING IMAGES
Alignment and averaging

Proof of concept

40S subunits of HeLa (human) Ribosomes

Frank et al., Science 1981
Problem of heterogeneity: molecules are in different orientations and conformations

L and R views (flip and flop) of HeLa ribosomes

flip and flop views of hemocyanin

Frank et al., Science 1981

N. Boisset, thesis 1987
Multivariate analysis of aligned molecule images

FLIP/FLOP and Rocking positions

Hemocyanins of Arthropods are oligomers of a basic unit

dodecamer

Van Heel and Frank, Ultramicroscopy 1981
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
The 50S ribosomal subunit as a contour stack in 3D

First 3D Reconstruction using Single Particle Reconstruction
Nobel Museum, Stockholm
Frozen-hydrated specimen / freeze-plunging / vitreous ice / cryo-EM

Molecules embedded in vitreous ice

Robert Glaeser 1976
Jacques Dubochet 1981
Plunge-freezer
Plunge-Freezer

manual

automated, climatized
ribosomes, recorded on film
Iterative angular refinement

J. Frank, in *Molecular Machines in Biology* 2011
E. coli ribosome

Frank et al., Nature 1995

Octopus hemocyanin

Lambert et al., 1994

Calcium Release Channel

Radermacher et al., 1994
Elongation Cycle (for adding each amino acid)

Decoding

Translocation
DISCOVERY OF RATCHET-LIKE MOVEMENT DURING TRANSLOCATION

Frank and Agrawal, Nature 2000; Valle et al., Cell 2003
MAXIMUM LIKELIHOOD METHODS OF CLASSIFICATION


“STORY IN A SAMPLE” -- intermediate states in the ratchet-like motion and hybrid tRNA positions in the absence of EF-G

Class | 1 | 2 | 3 | 4 | 5 | 6

Agirrezabala et al., PNAS 2012
MILESTONES IN SINGLE-PARTICLE RECONSTRUCTION

1975 Concept

1978 Alignment via CCF Resolution estimation

1981 Multivariate statistical analysis 2D classification

1986 Determine orientation 3D reconstruction

1996 CTF correction via Wiener filter

2007 Max likelihood 3D classification

2013
Best resolution from recording on film: 5.5Å
New era (since 2012): *New single-electron detecting cameras*

Detection Quantum Efficiency (DQE):
(how good is the recording device in capturing every single electron?)

Ribosomes, recorded on K2 GATAN direct electron detection camera
Elongation Factor G mutant H94A bound to the ribosome

nr 70S--P-E
nr 70S--EF-G—P-E
r 70S—EF-G—P/E
r 70S—P/E

50,000  90,000  35,000  15,000

Example for maximum likelihood 3D classification
Multiple states in the same sample

Li et al., Science Advances 2015
T. cruzi ribosome large subunit at 2.5 Å
Liu et al., PNAS 2016
AMPA Receptor
Twomey et al., Nature 2017
Ryanodine Receptor
Zalk et al., Nature 2015
Des Georges et al., Cell 2016

~30Å

Des Georges et al., Cell 2016
3.6Å
Molecular Structure of the Human CFTR Ion Channel. Liu et al. Cell 2017
Open and Closed states of mutant 508del-hCFTR

Voribiev, Wang, & Yang et al.
Dr. Frank

I want to add to the chorus of congratulations that you have no doubt received since the Nobel was announced.
I have a special reason to send a thank you as well, my seven year old grandson Jackson was diagnosed with CF as a newborn in 2010. I have attached news that we received this week regarding his 10 months on combination therapy.
Just wanted to share this as a small expression of my (and Jackson’s) gratitude.

Ralph

Ralph T. Wynn, MD, FACR
Associate Clinical Professor, Director of Breast Imaging
Department of Radiology, Columbia University Medical Center
Conclusion -- Single-particle cryo-EM: A new era in structural biology

- No need for crystals!
- *Very small sample quantity needed*
- Resolution in the 3-4 Å range now routinely achievable
- *Multiple structures retrieved from the same sample → clues on function*
- Molecules in close-to-native conditions
- *Solving structures of membrane proteins much easier than with X-ray crystallography*
- Huge expansion of structural data base relevant for Molecular Medicine
Impact in Biology and Molecular Medicine

The number of molecules with relevance to human health that can now be investigated is enormous. Among these:

1) Transmembrane proteins with particular biomedical significance, such as ion channels and receptors

   CFTCR
   Zhang & Chen
   Cell 2016

   Pain receptor
   Zubcevic et al.
   NSMB 2016

1) Large molecular assemblies such as the spliceosome, which edits the genetic transcript

   Spliceosome
   Zheng et al.
   Cell 2017
Thanks to students (too numerous to list) and to my collaborators . . . . . . .

Special thanks to my wife, Carol Saginaw, for 37 years of unwavering support!