An Introduction to electron cryo-microscopy, and how I cold nuked my sample into insights about ribosome function

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The Central Dogma

DNA → Transcription → mRNA → Translation → Protein

DNA → Information → mRNA → Translation → Protein

Complex Molecular Machine
~55 polymers
> 100’000 atoms per complex
The ribosome is a complex molecular machine that adopts various states during its function. Snapshots of these states allow us to gain insights into how it functions, such as how tRNA moves through the ribosome, how chemistry is facilitated, how the ribosome selects the correct tRNA... Some of these states are very hard to visualize, as they are transient.
Electron Microscopes

Philips CM300

FEI F30

Courtesy of Alexis Rohou
Similarities with Light Microscopy

Composed of the same basic parts:

“Light” Source
Condenser lenses
Sample holder
Objective lens
Projector lenses
Camera

Designed to record an image of a sample

From: Principles and Practice of Electron Microscope Operation. Agar, Alderson & Chescoe

Inspired/Stolen from Nikolaus Grigorieff
Dissimilarities with Light Microscopy

**Lenses**
Magnetic lenses vs. glass lenses
Different aberrations

**Magnification/Resolving Power**
Related to the wavelength of light
~ 100 nm for a light microscope
Better than 0.1 nm for a TEM

**Mechanism of Image formation**
Phase contrast, amplitude contrast

From: Principles and Practice of Electron Microscope Operation. Agar, Alderson & Chescoe
Dissimilarities with light microscopy you might actually care about

**Vacuum**
Samples in EM must be “dry”
Dry samples are usually dead

**Radiation Damage**
Electrons are ionizing radiation
Severely limits the dose allowed

From: Principles and Practice of Electron Microscope Operation. Agar, Alderson & Chescoe

Inspired/Stolen from Nikolaus Grigorieff
Dissimilarities with light microscopy you might actually care about

InGaAs (a=5.87 Å)

2.94 Å

Length scales studied by EM and other imaging methods

Scales

- Naked Eye
- Light Microscopy
- Electron Cryo-Microscopy
- X-Ray Crystallography
- NMR

- 1 cm
- 1 mm
- 100 µm
- 10 µm
- 1 µm
- 100 nm
- 10 nm
- 1 nm
- 0.1 nm (1 Å)

- Plant cell
- Animal cell
- Bacterium
- Virus ribosome
- Globular protein
- Small molecule
- Atom

- Papillomavirus
- 80S Ribosome
- E. coli Cl⁻ Transporter

Stolen from Nikolaus Grigorieff
Length scales studied by EM and other imaging methods

Stolen from Nikolaus Grigorieff
Sample Preparation: Holey Grids and Plunge Freezing

Kasas et al., J. Micros. 211(1):48  (Stolen from Alexis Rohou)
Imaging & Reconstruction
Imaging & Reconstruction
Imaging & Reconstruction

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantage</th>
<th>Problem</th>
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<tbody>
<tr>
<td><strong>Diffraction (X-ray, electrons)</strong></td>
<td>Fast data collection</td>
<td>Crystals needed</td>
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<tr>
<td></td>
<td>Atomic resolution</td>
<td>Large amounts of protein needed</td>
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<td></td>
<td>No weight limit</td>
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<tr>
<td><strong>NMR</strong></td>
<td>No crystals needed</td>
<td>Weight limit $\approx 50 \text{ kD}$</td>
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<tr>
<td></td>
<td>Fast data collection</td>
<td>Large amounts of protein needed</td>
</tr>
<tr>
<td></td>
<td>Atomic resolution</td>
<td></td>
</tr>
<tr>
<td><strong>Single particle electron microscopy</strong></td>
<td>No crystals needed</td>
<td>Hard to get atomic resolution</td>
</tr>
<tr>
<td></td>
<td>Can get data from <em>heterogeneous samples</em></td>
<td>Slow data collection</td>
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<tr>
<td></td>
<td>No upper weight limit</td>
<td>Lower weight limit $\approx 200 \text{ kD}$</td>
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<tr>
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<td>Little protein needed</td>
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Courtesy of Niko
Structure of the ribosome with elongation factor G trapped in a pre-translocation state
The 70S Ribosome

Tertiary structures of the 30S (A) and 50S (B) subunits, seen from the interface side

30S: 1 RNA, 1522 nt
20 proteins

50S: 2 RNA, 2893 nt, 120 nt
33 proteins

The 70S Ribosome

3 tRNA binding sites at the interface: A (Acceptor), P (Peptidyl), E (Exit)
1 mRNA binding site on the 30S subunit

Translation

Aminoacyl-tRNA binding

Peptide-bond formation

Translocation

GTP

Elongation factor G

GDP + P_i

tRNA dissociation
Critical step during elongation
Move peptidyl tRNA from A to P site
Move deacylated tRNA from P to E site

Spontaneous function of the ribosome
Accelerated 4-5 order of magnitude by EF-G

Move 2 tRNA 20-30 Angstroms
Maintain the reading frame
Allow for in vivo elongation rate of 15-20 a.a./s
What is the role of EF-G?
Antibiotics in Translocation

Pre-translocation

EF-G binding and unlocked-state formation

GTP hydrolysis

30S head movement (spectinomycin)
A/P state formation (viomycin) and P_i release (thiostrepton)

EF-G engages the A site, followed by translocation (viomycin)

EF-G release (fusidic acid) and relocking

Post-translocation

Munro, JB. et al. 2010. *NSMB*. 17(12):1470-7
Steric clash of EF-G with A site in Post-translocation state.

Antibiotics in Translocation

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Post-translocation

Munro, JB. et al. 2010. *NSMB*. 17(12):1470-7
The Raw Data

1.35 Million particles, collected on a Titan Krios Microscope at 300 kV.
The Expectation-Maximization algorithm

Images → Reconstruct → References

Compare images to maps, obtaining the maximum likelihood estimate of the new parameters for each image with each reference.

New particle orientations, occupancies

Particle orientations, occupancies

Iterate with new parameters
The Results

I. 3 tRNA (26.7%)
II. EF-G, P & E tRNA (13.4%)
III. EF-G, P site tRNA (6.8%)
IV. A/P & P/E tRNA (3.5%)
V. EF-G, A/P & P/E tRNA (2.4%)
View of EF-G with pre and post-translocation 50S subunits aligned shows a rotation around the immobile SRL

View of EF-G with domains I-II aligned shows movement of domains III-IV-V relative to domains I-II

Conformational Changes of EF-G
EF-G binding induces a new tRNA hybrid state

EF-G: Pre-translocation
A/A: Classical pre-translocation state
A/P*: EF-G Bound, pre-translocation state
A/P*: No EF-G bound, pre-translocation state
P/P: Classical Post-translocation State
Schematic of EF-G catalyzed translocation
Schematic of EF-G catalyzed translocation
Our Collaborators

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U. Rochester Med. Center
EF-G complexes
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