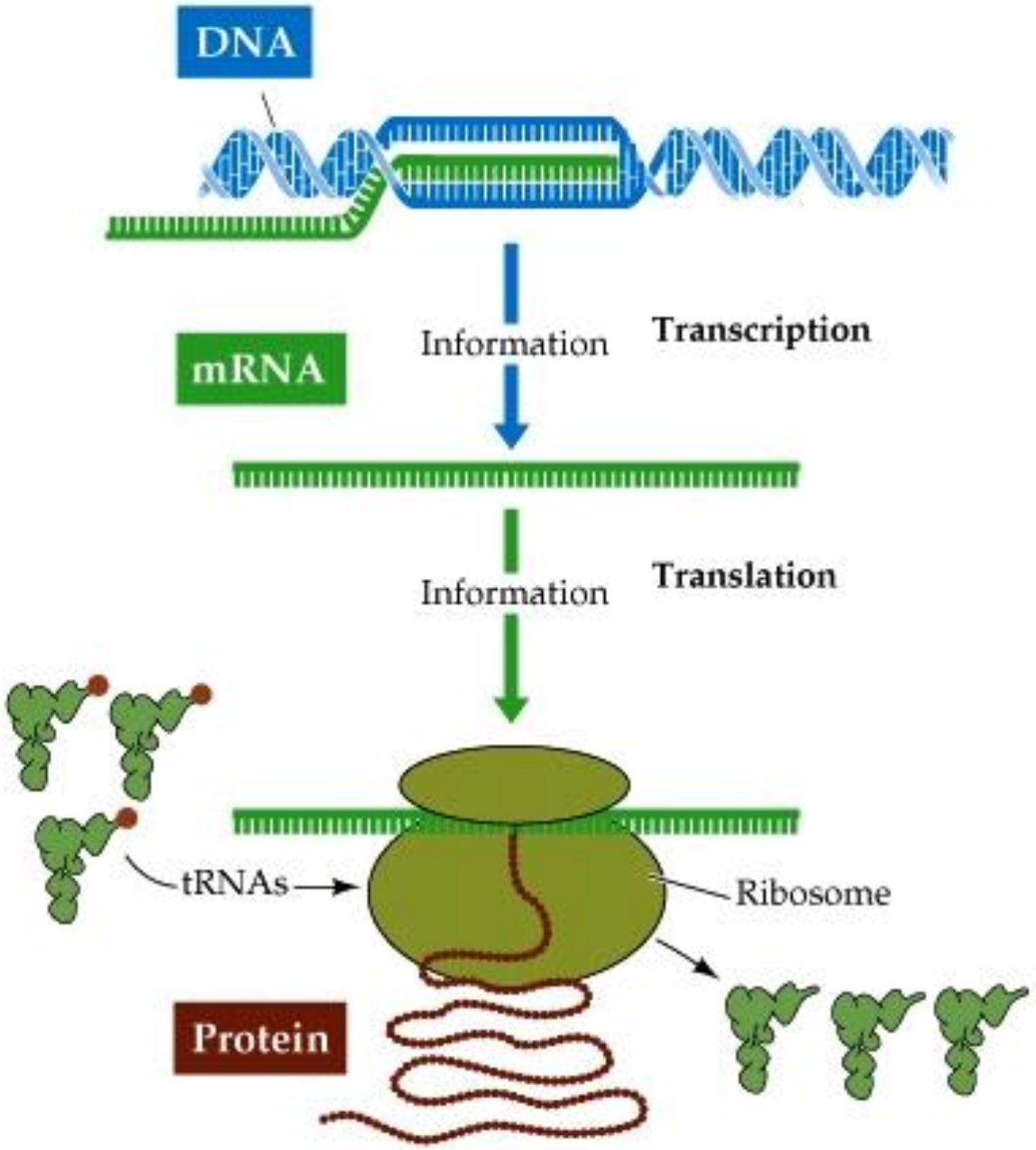
An Introduction to electron cryo-microscopy, and how I cold nuked my sample into insights about ribosome function

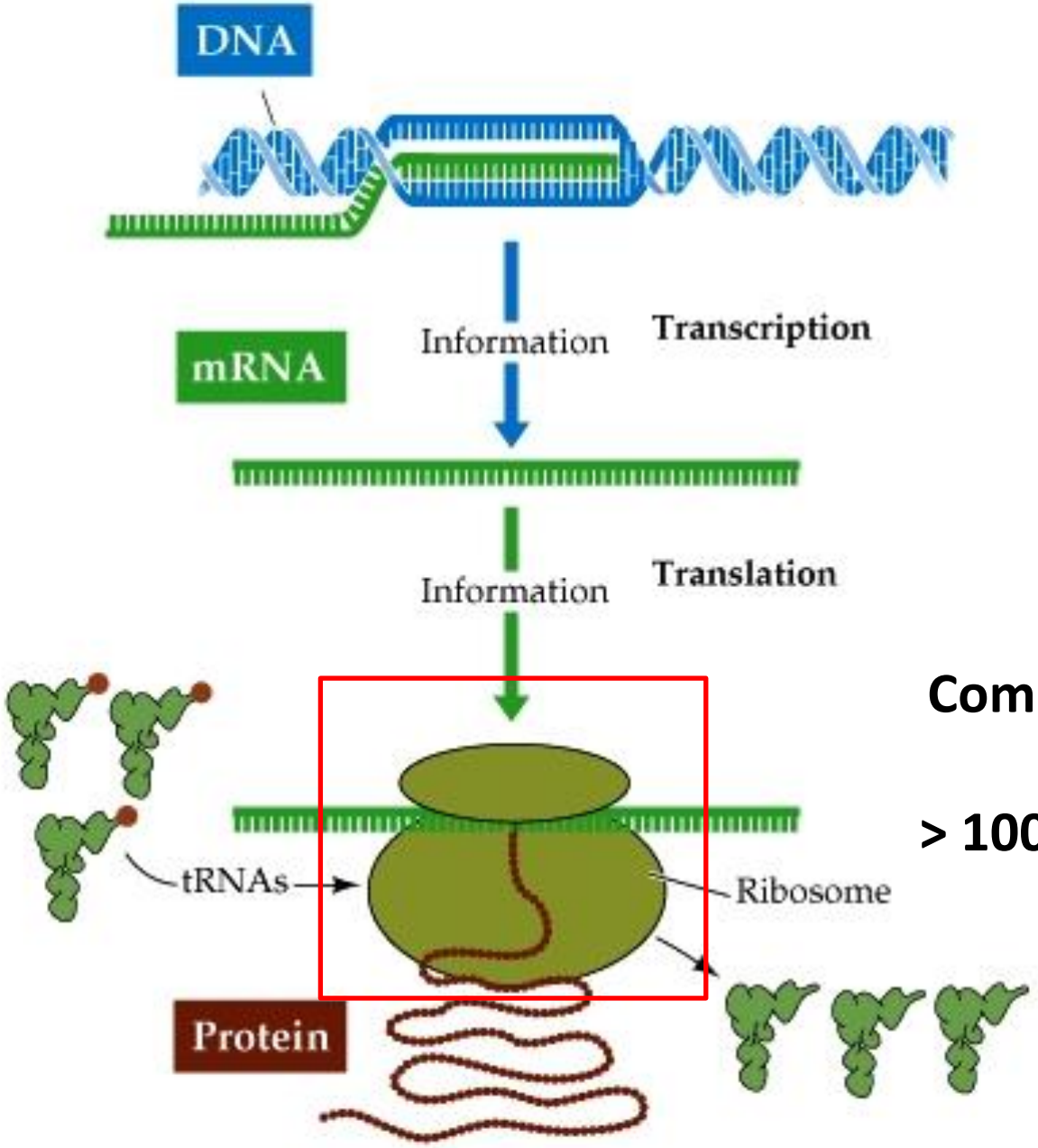
Axel Brilot

January 9th, 2014

The Central Dogma

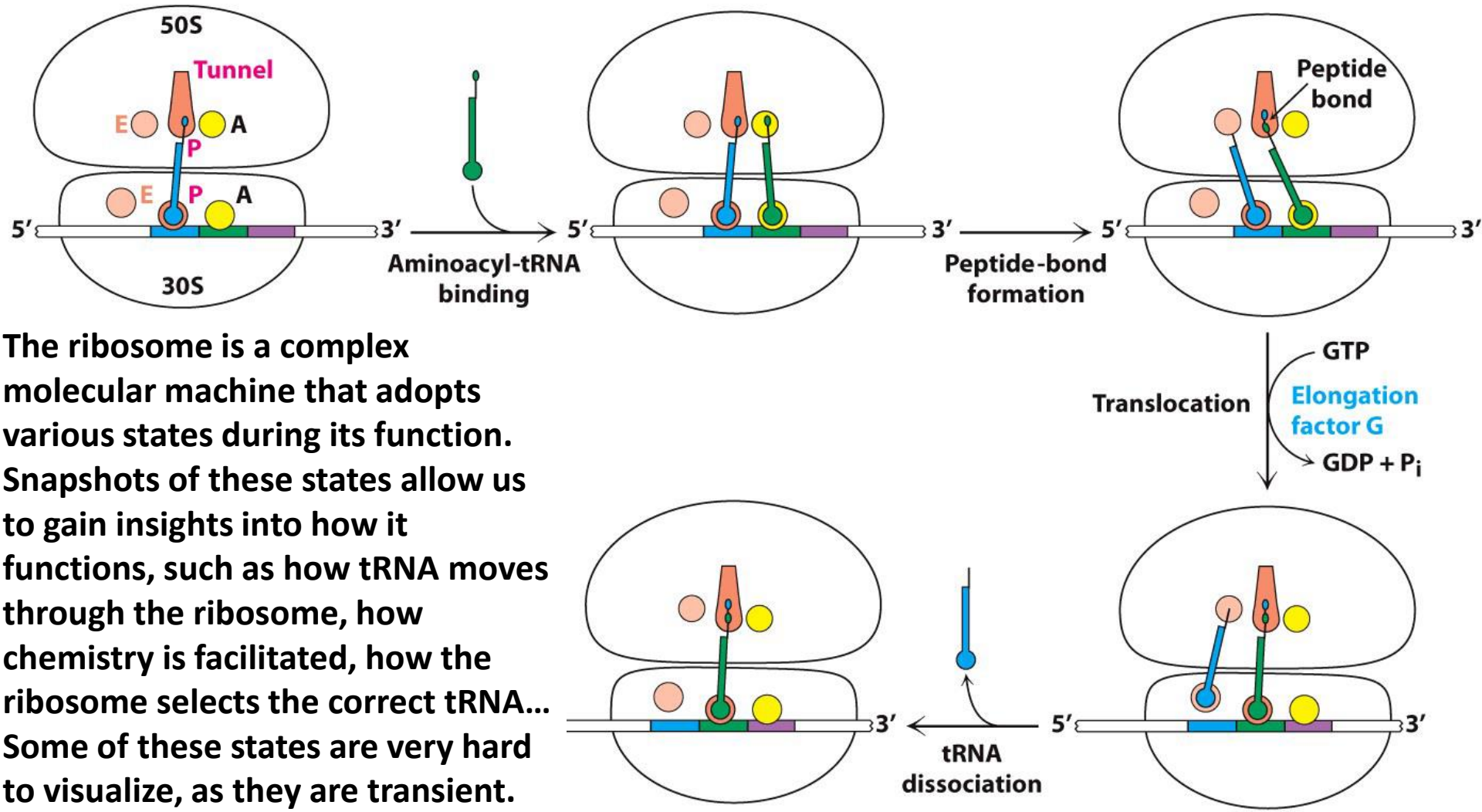


The Central Dogma



Complex Molecular Machine
~55 polymers
> 100’000 atoms per complex

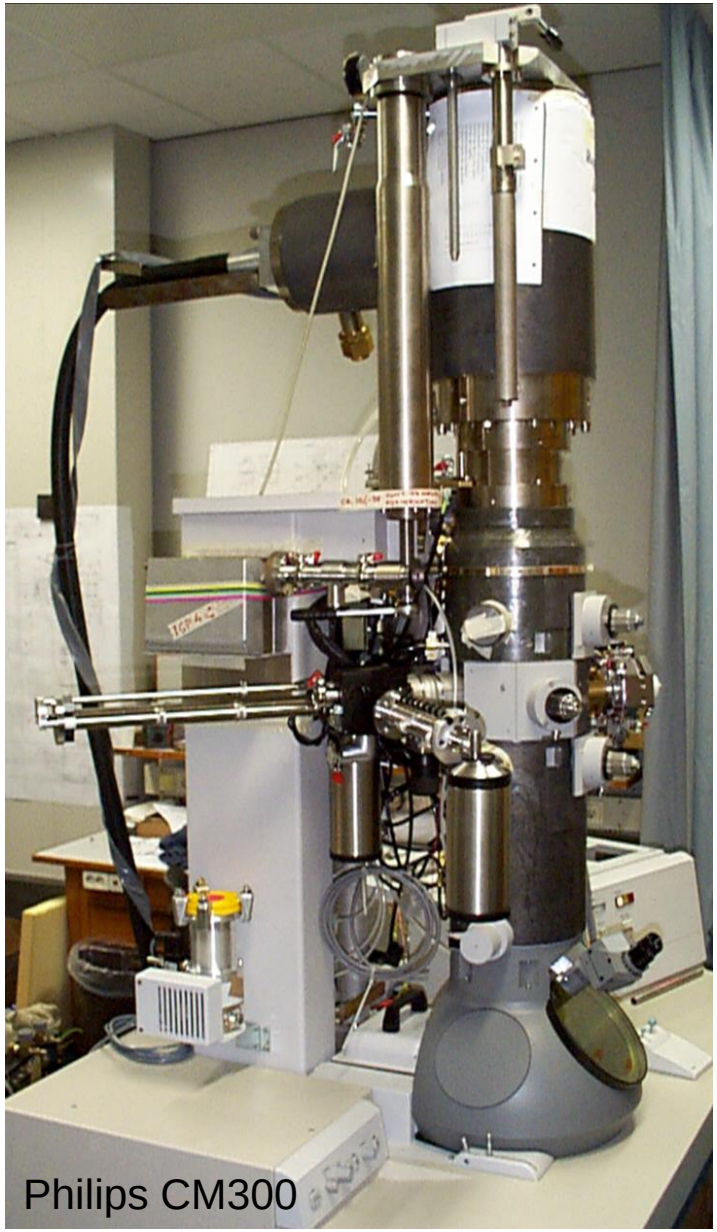
Why Structure?



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.

Figure 40.7
Biochemistry: A Short Course, Second Edition

Electron Microscopes



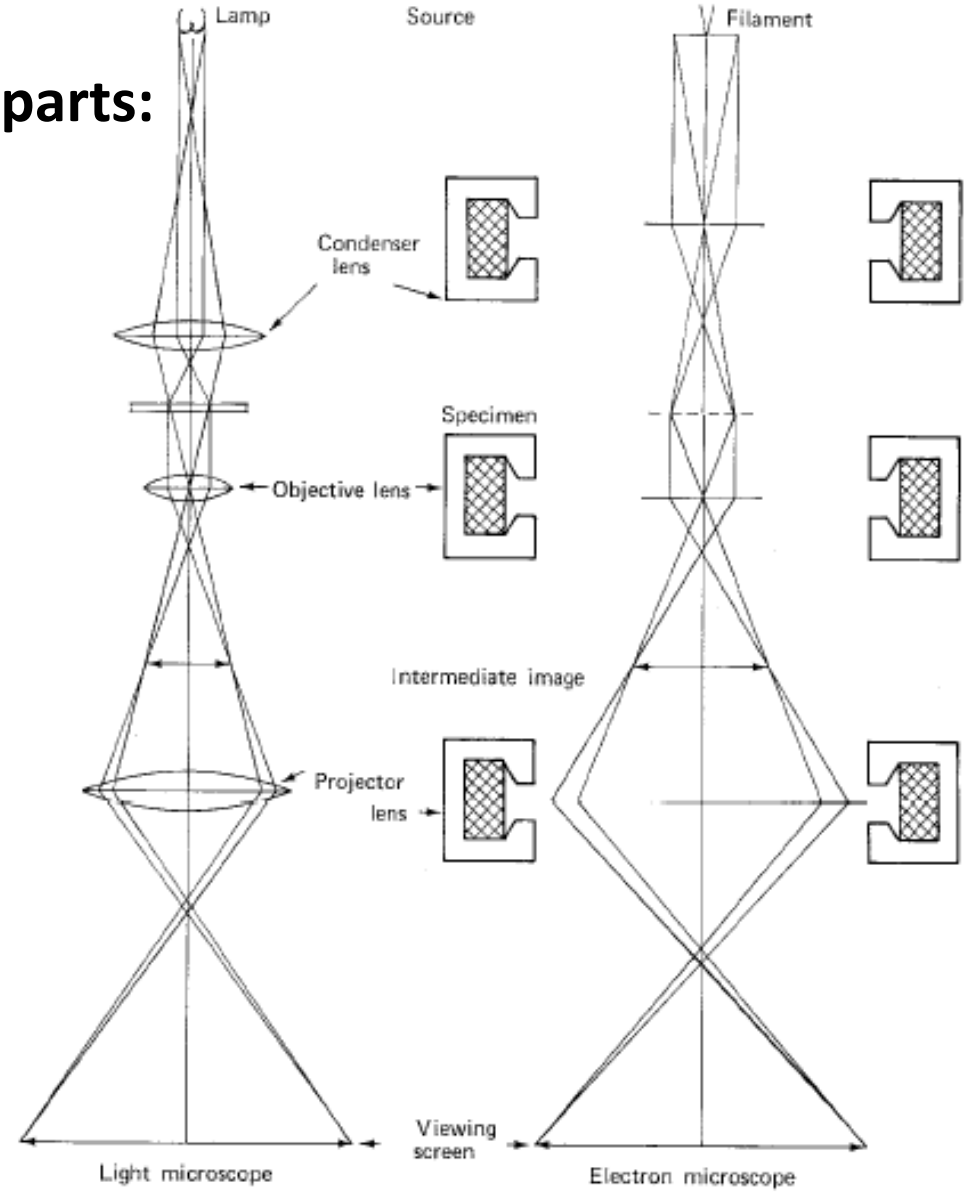
Courtesy of Alexis Rohou

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

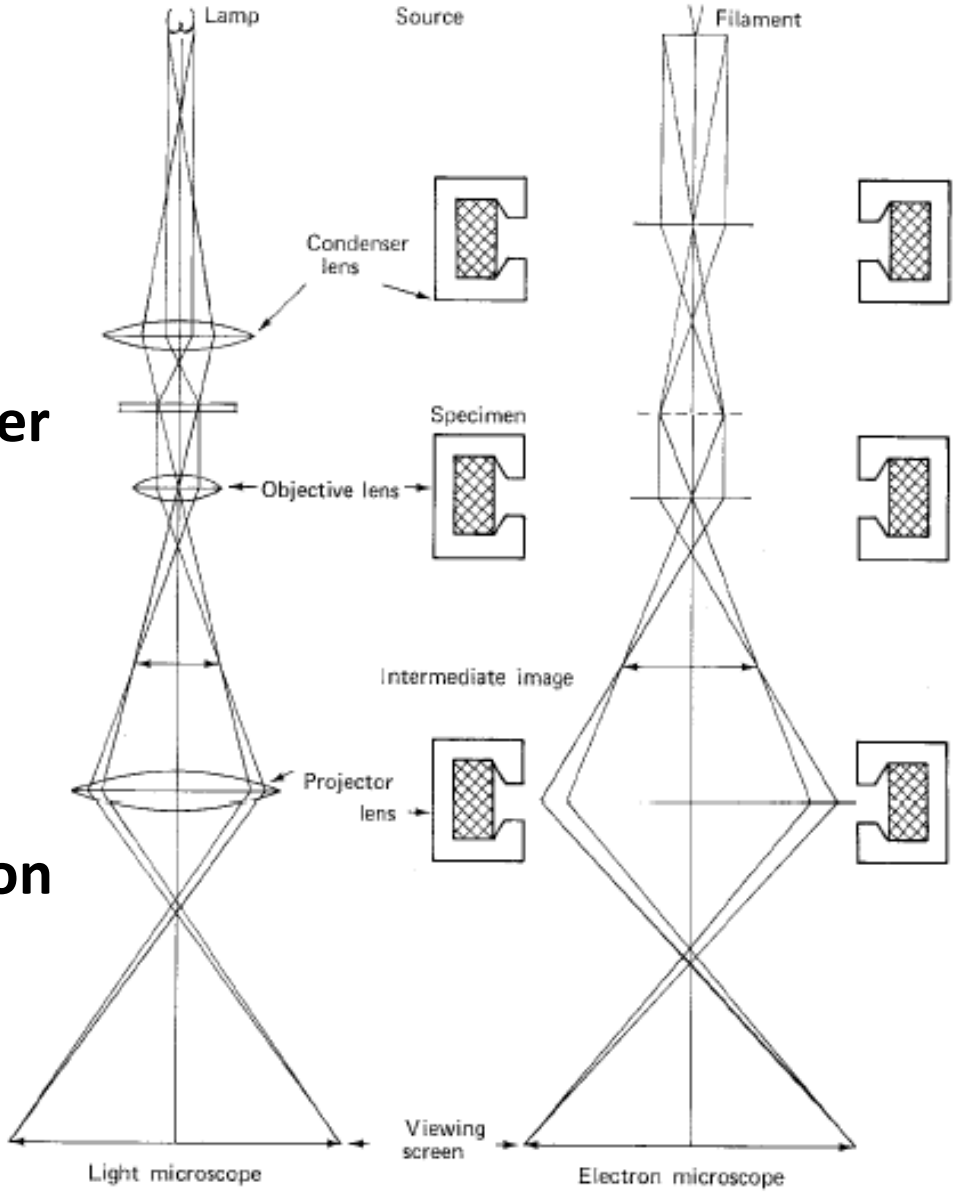
Magnetic lenses vs. glass lenses
Different aberrations

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

Inspired/Stolen from Nikolaus Grigorieff

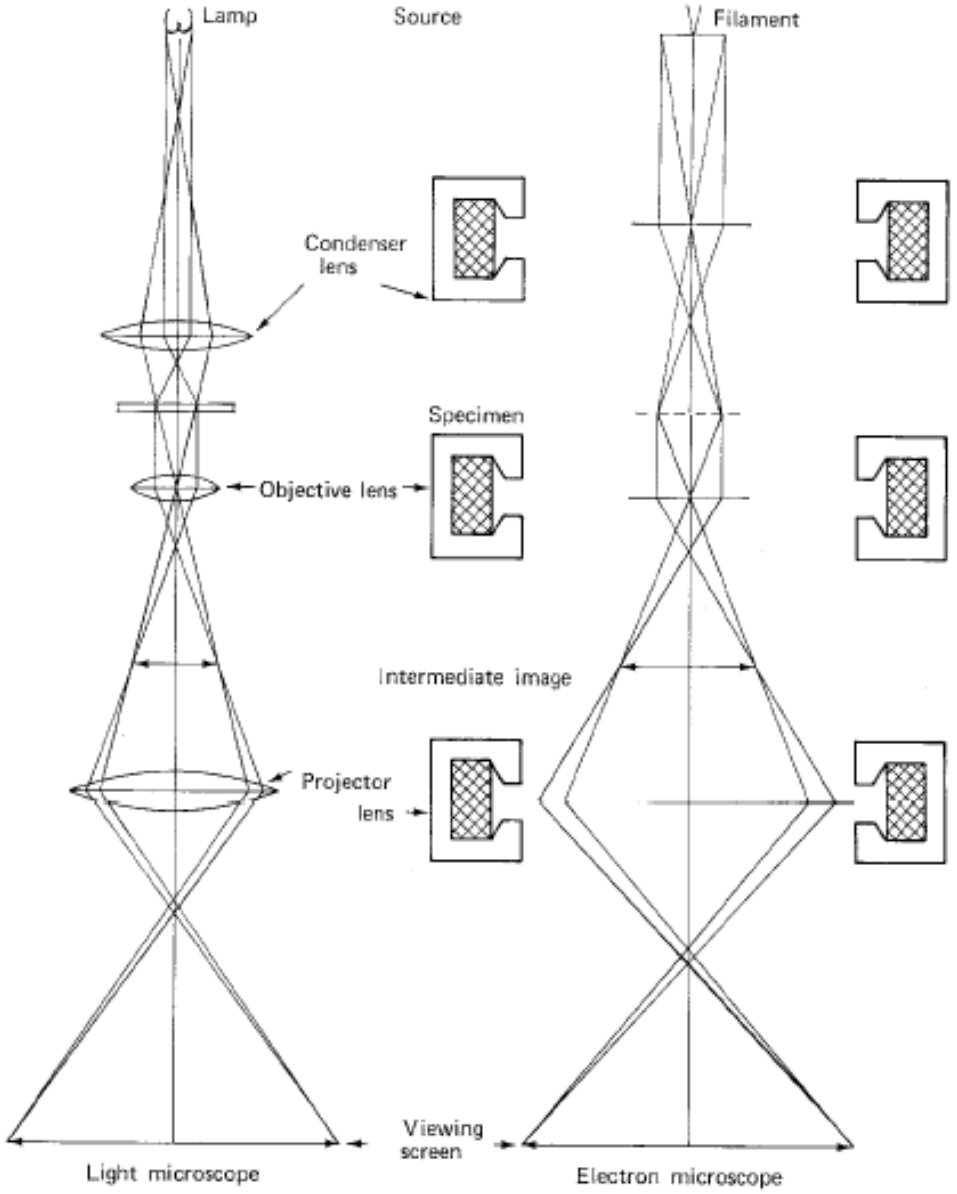
Dissimilarities with light microscopy you might actually care about

Vacuum

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

Radiation Damage

Electrons are ionizing radiation
Severely limits the dose allowed



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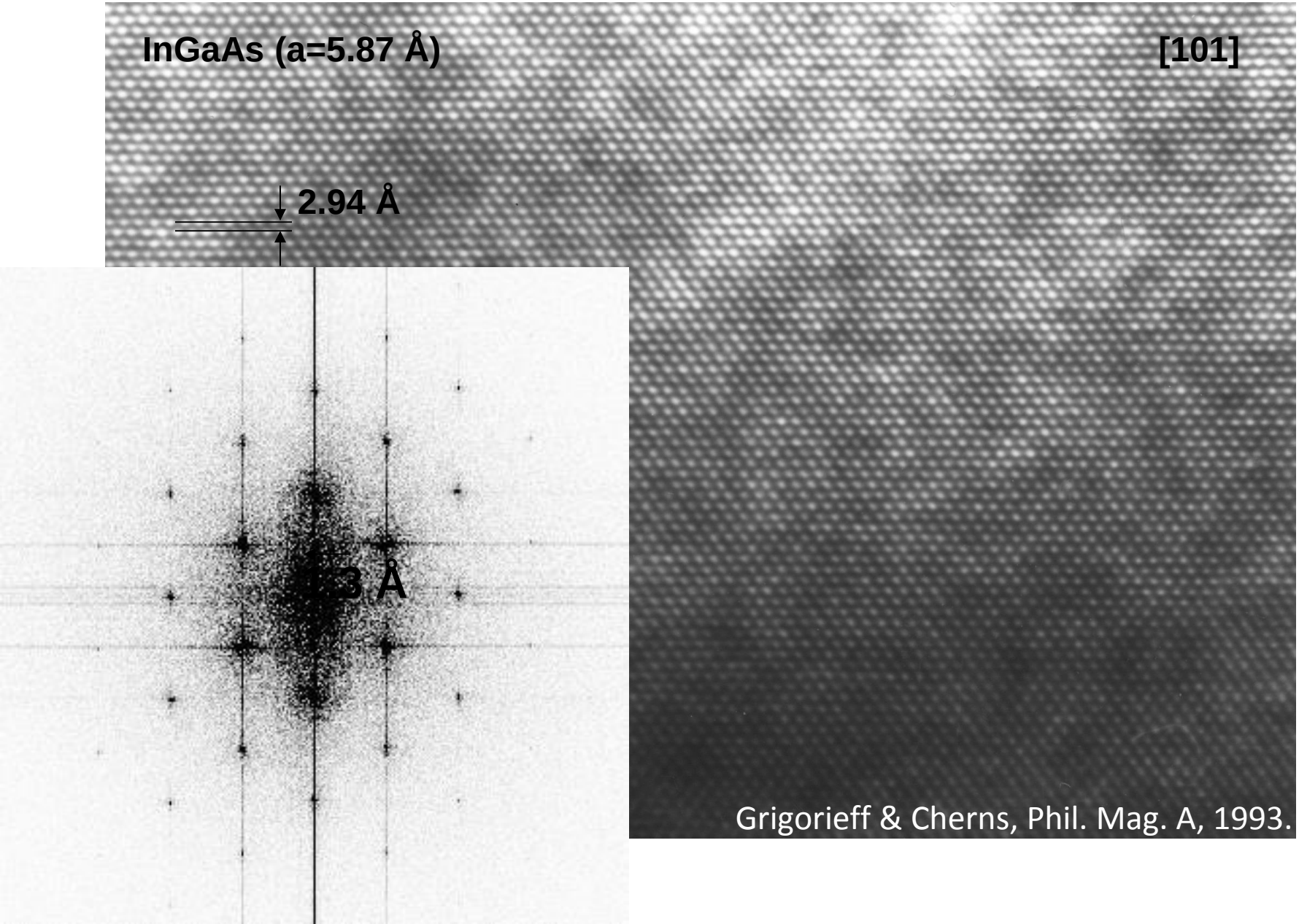
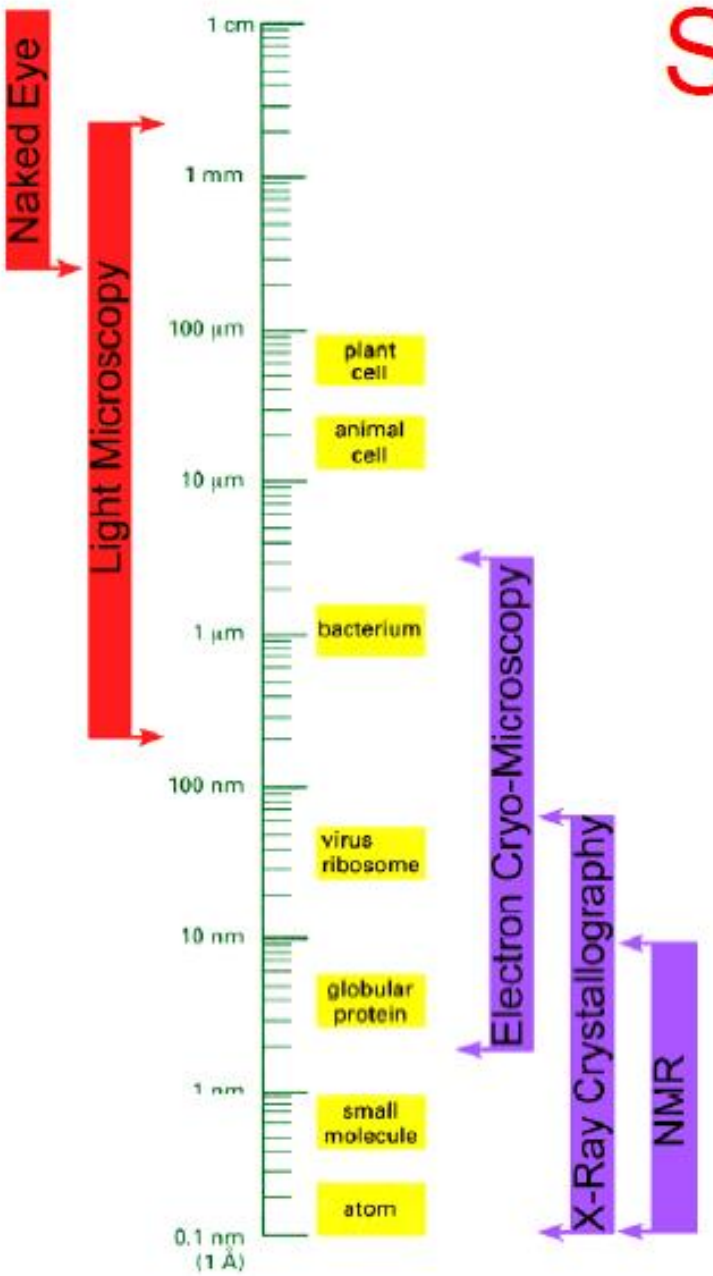
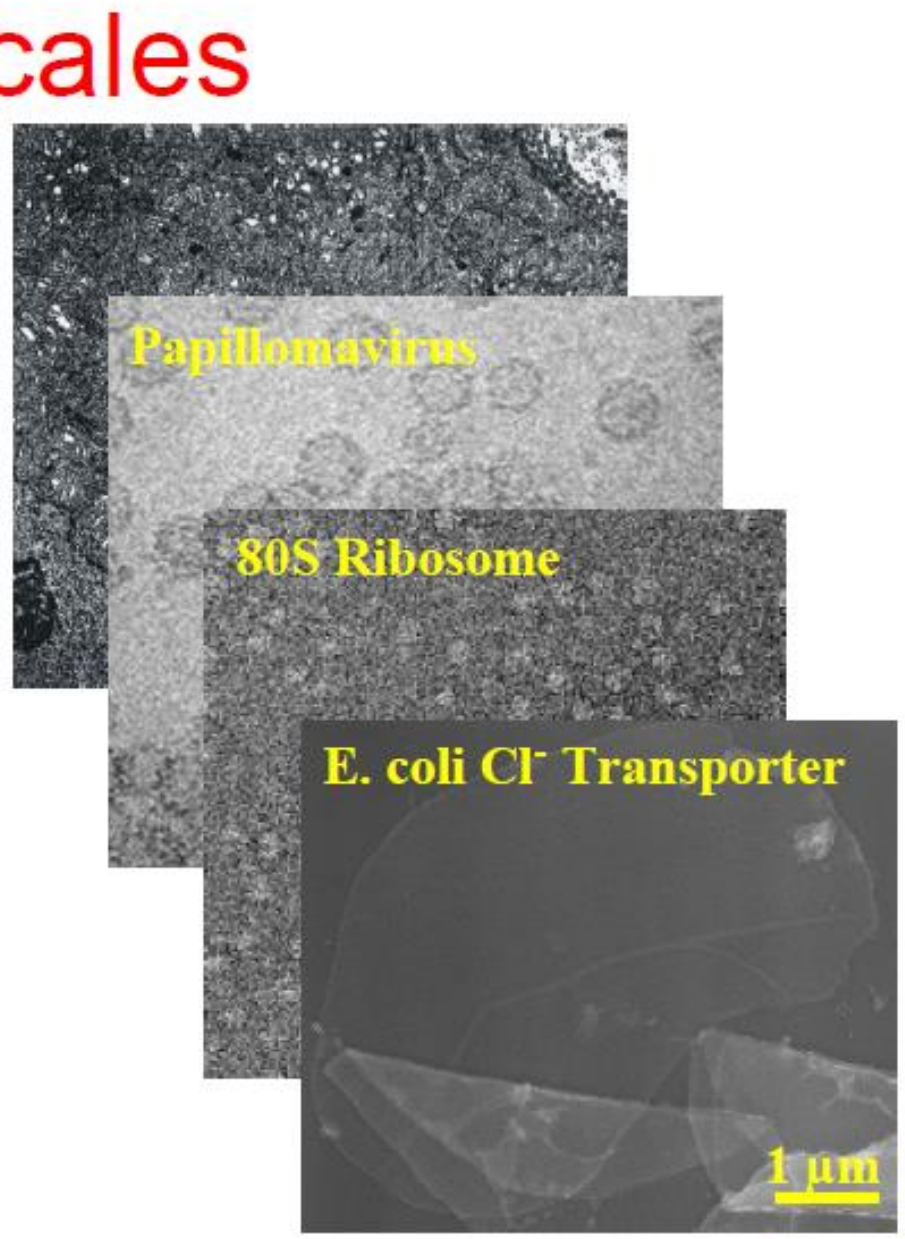
Dissimilarities with light microscopy you might actually care about



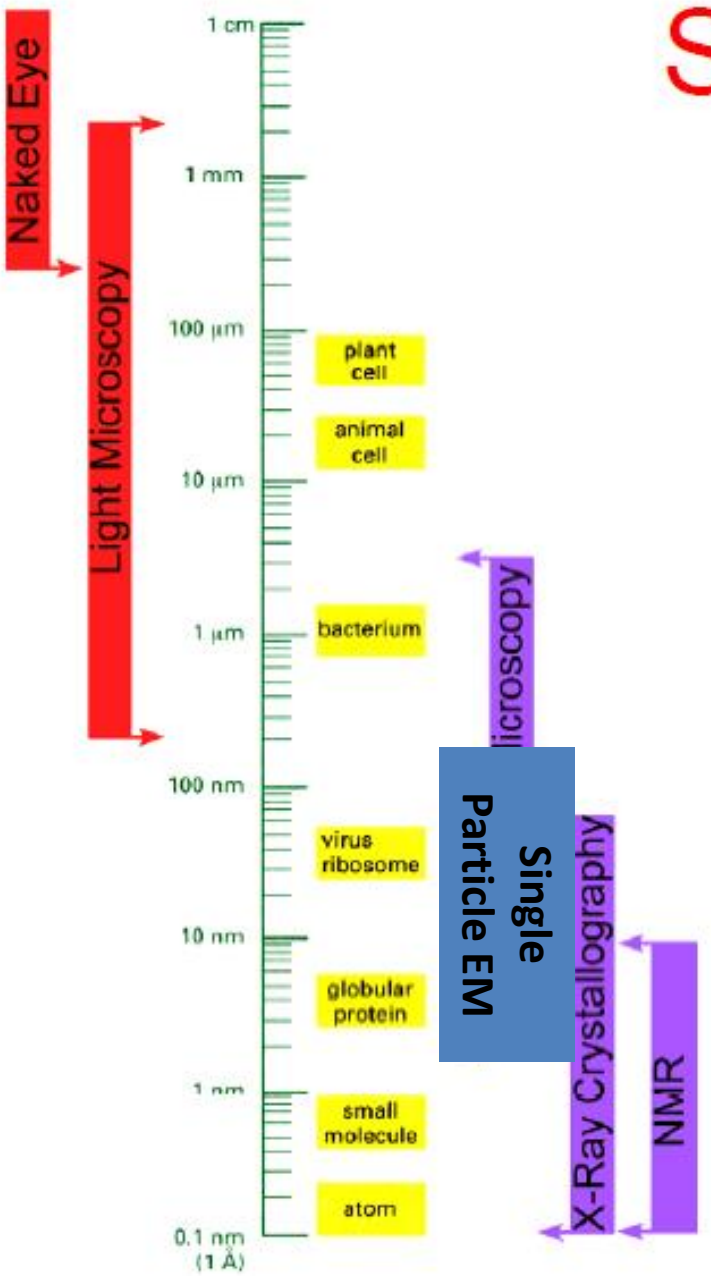
Length scales studied by EM and other imaging methods

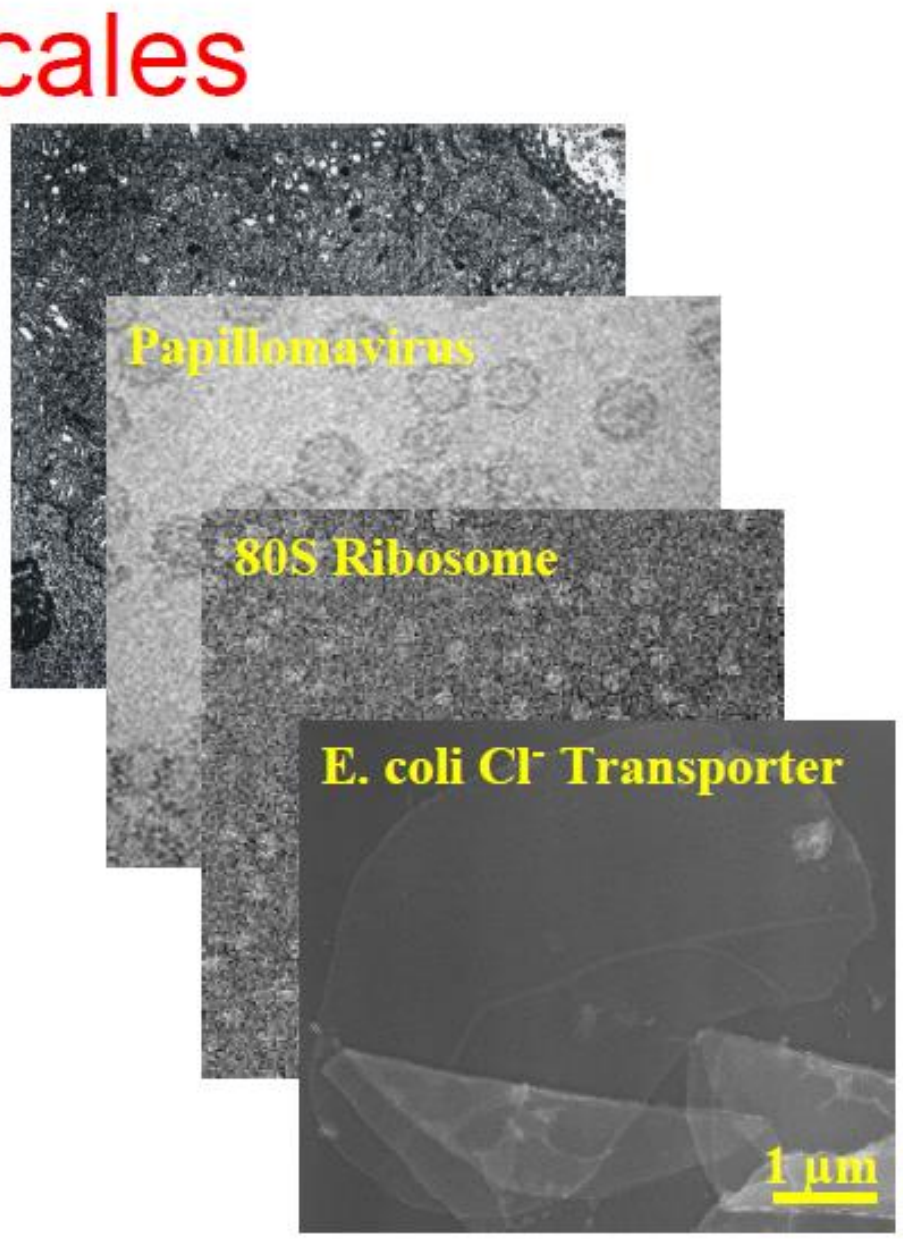




Stolen from Nikolaus Grigorieff

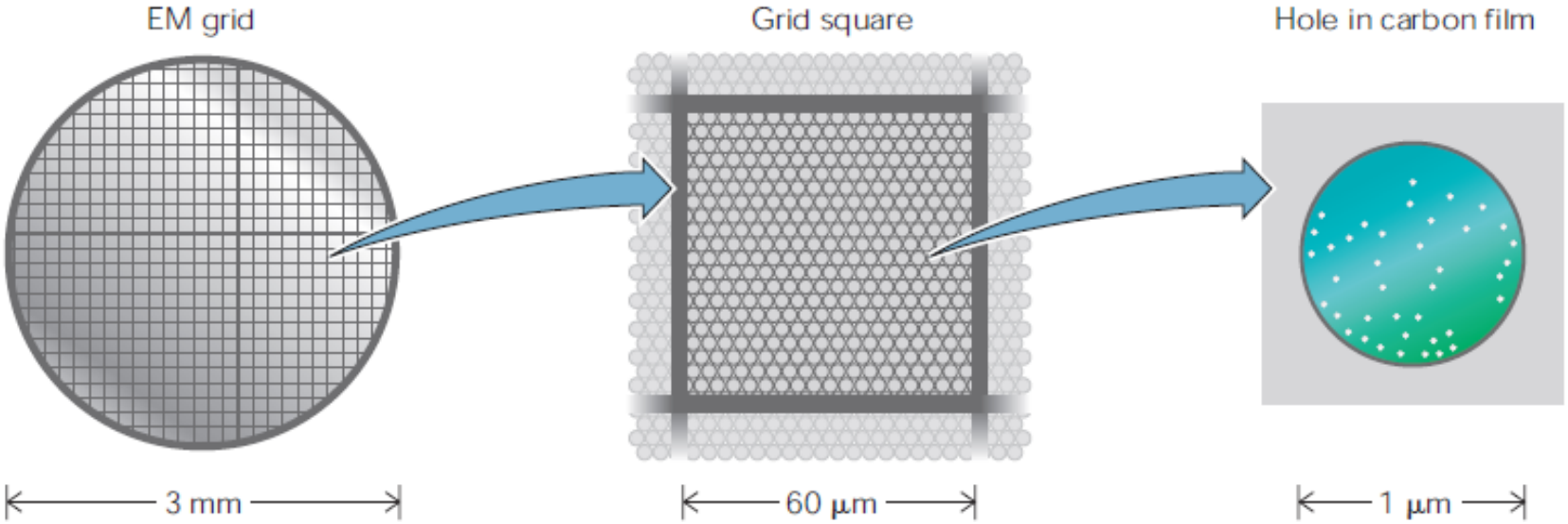
Length scales studied by EM and other imaging methods



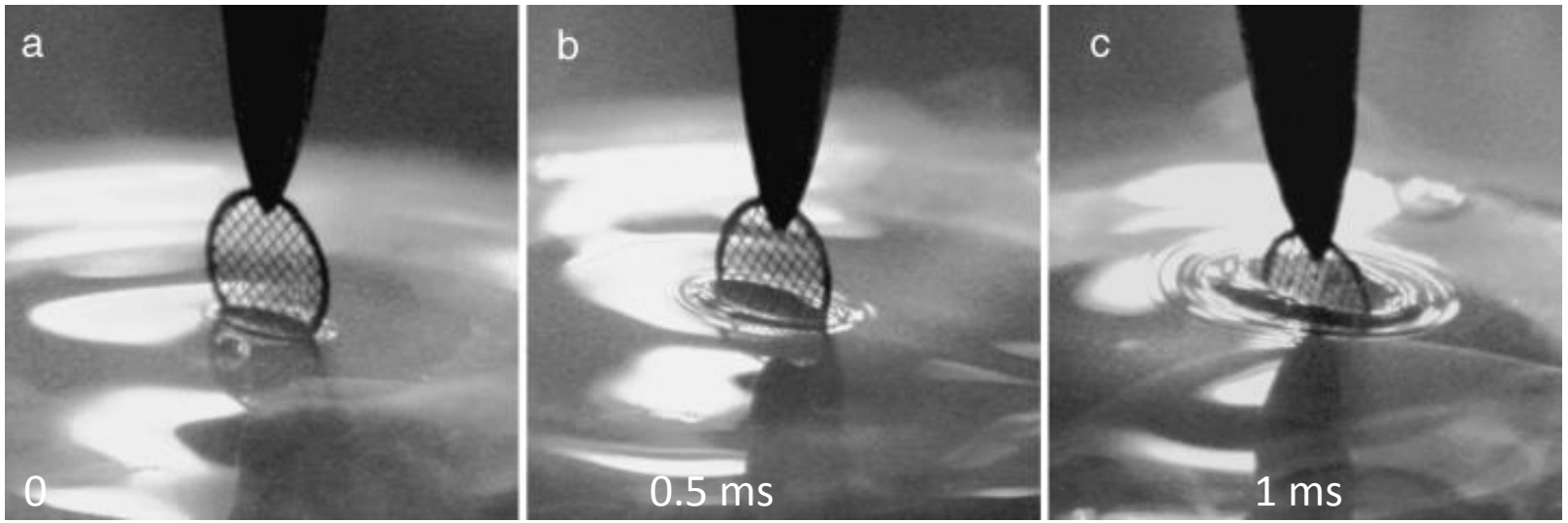


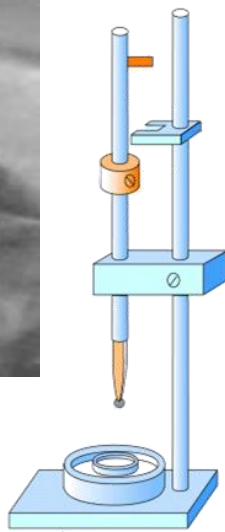
Stolen from Nikolaus Grigorieff

Sample Preparation: Holey Grids and Plunge Freezing



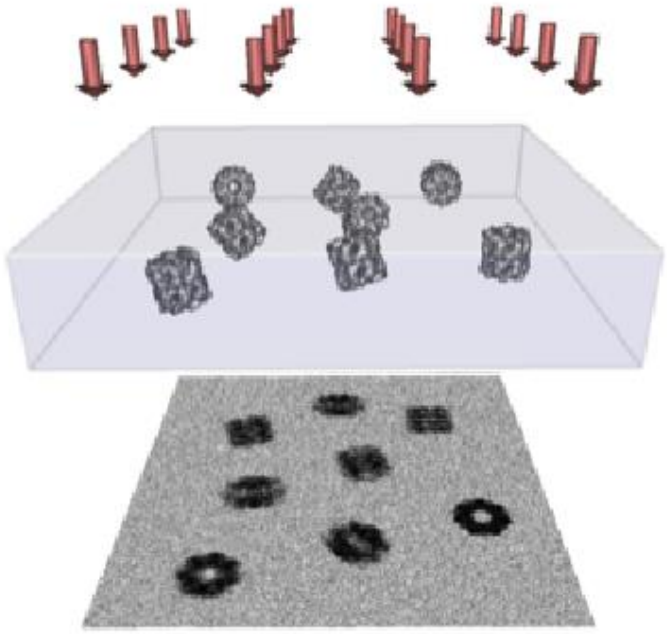
Wang & Sigworth, Physiology, 2009



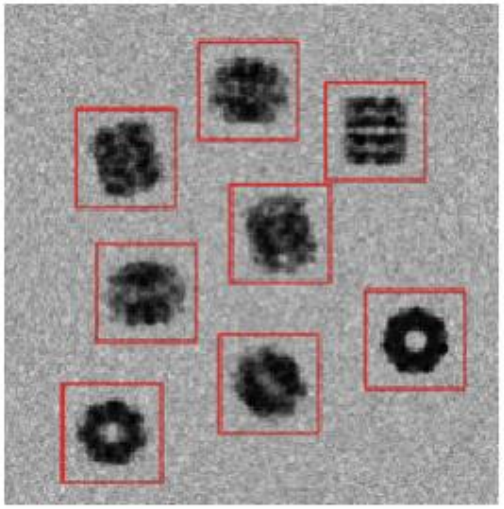


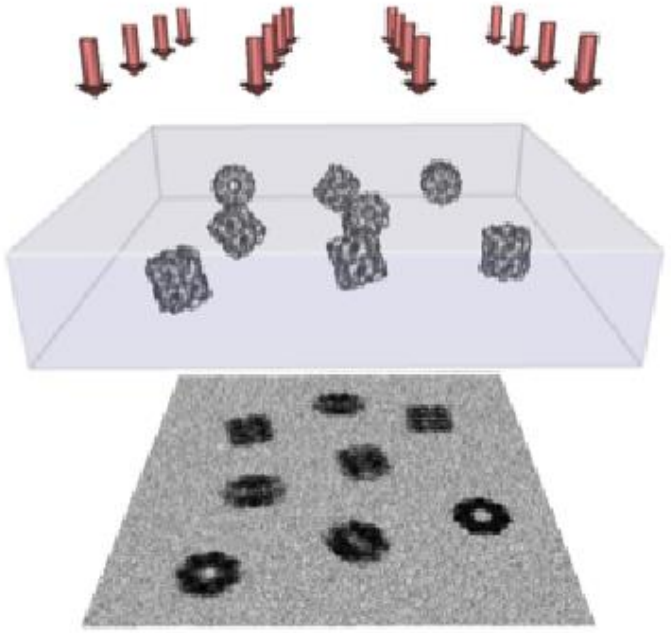
Kasas *et al.*, J. Micros. 211(1):48 (Stolen from Alexis Rohou

Imaging & Reconstruction



Imaging & Reconstruction





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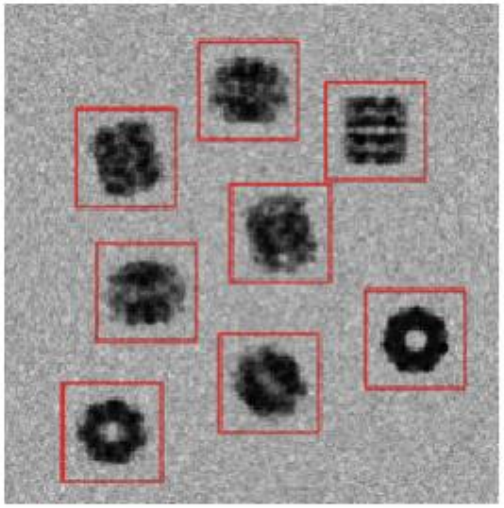
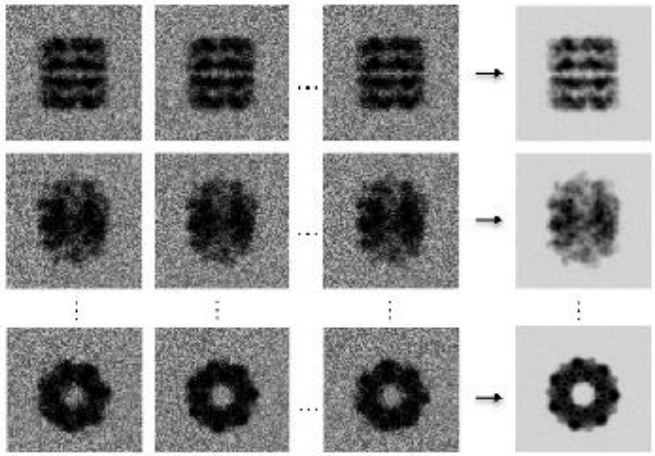
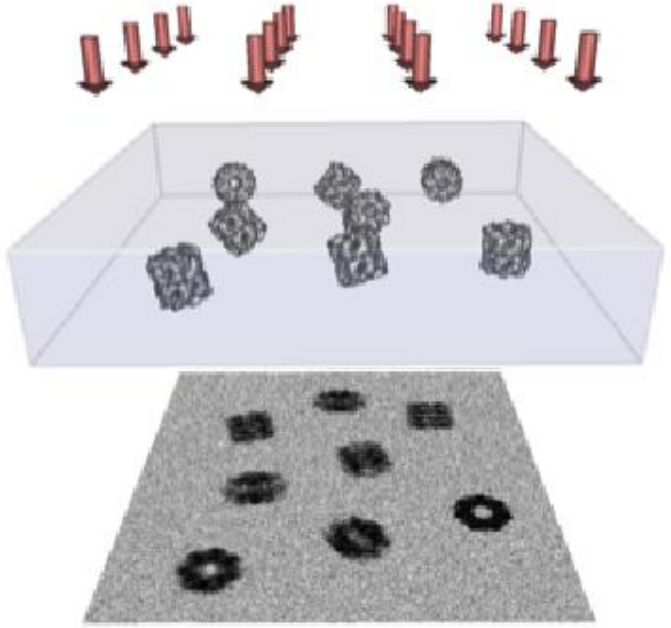
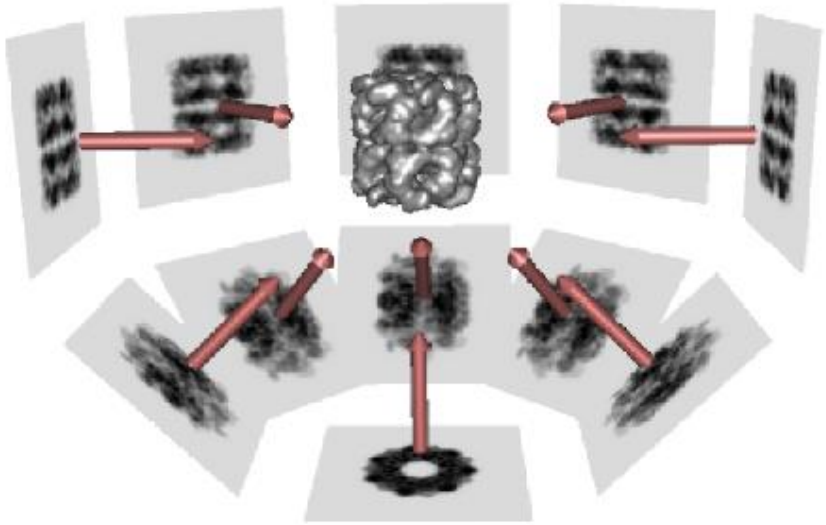








(Figures from Greg Pintilie, MIT,
http://people.csail.mit.edu/gdp/index.html)

Why Single-Particle EM?

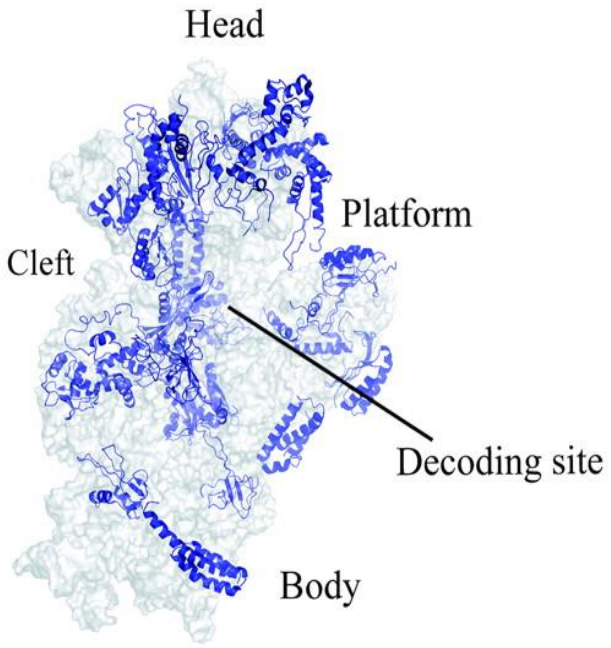
Technique	Advantage	Problem
Diffraction (X-ray, electrons)	Fast data collection Atomic resolution No weight limit	Crystals needed Large amounts of protein needed
NMR	No crystals needed Fast data collection Atomic resolution Protein dynamics	Weight limit ≈ 50 kD Large amounts of protein needed
Single particle electron microscopy	No crystals needed *Can get data from heterogeneous samples* No upper weight limit Little protein needed	Hard to get atomic resolution Slow data collection Lower weight limit ≈ 200 kD

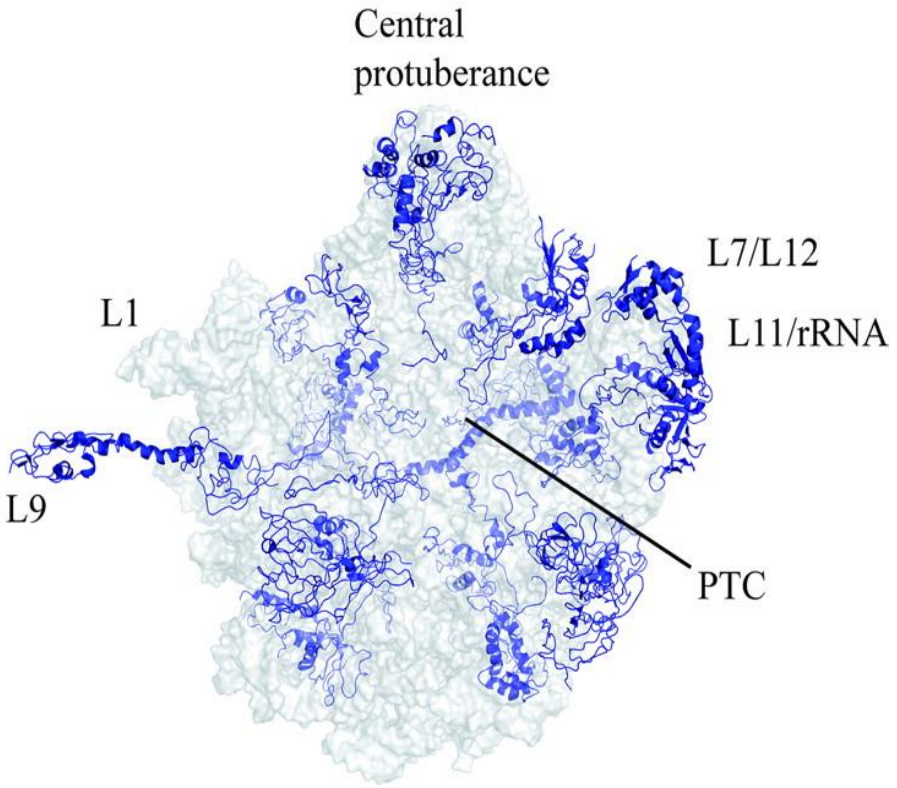
Courtesy of Niko

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



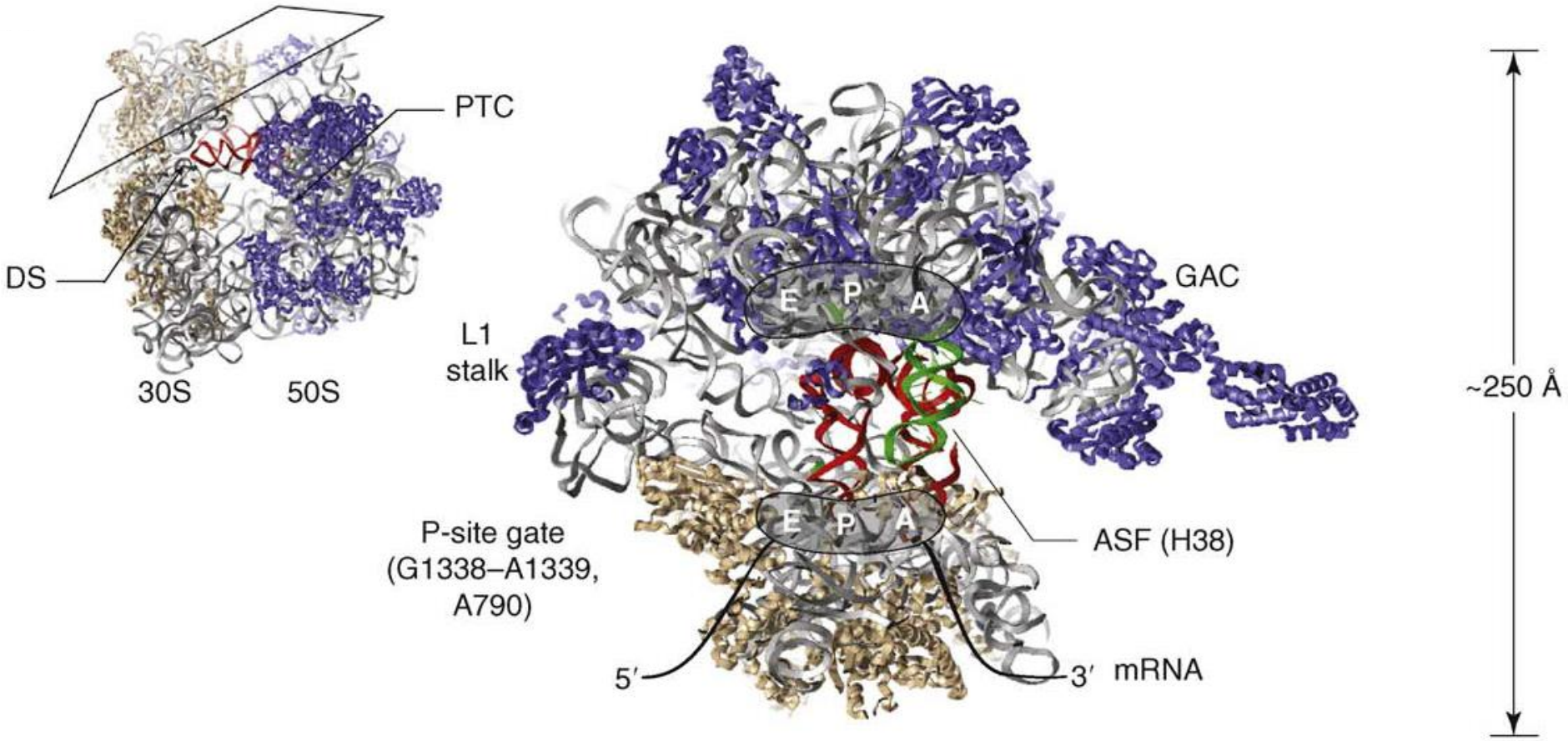


30S: 1 RNA, 1522 nt
20 proteins

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

Kaczanowska, M. et al. 2007. *Microbiol. Mol. Biol. Rev*. 71(3):477-494

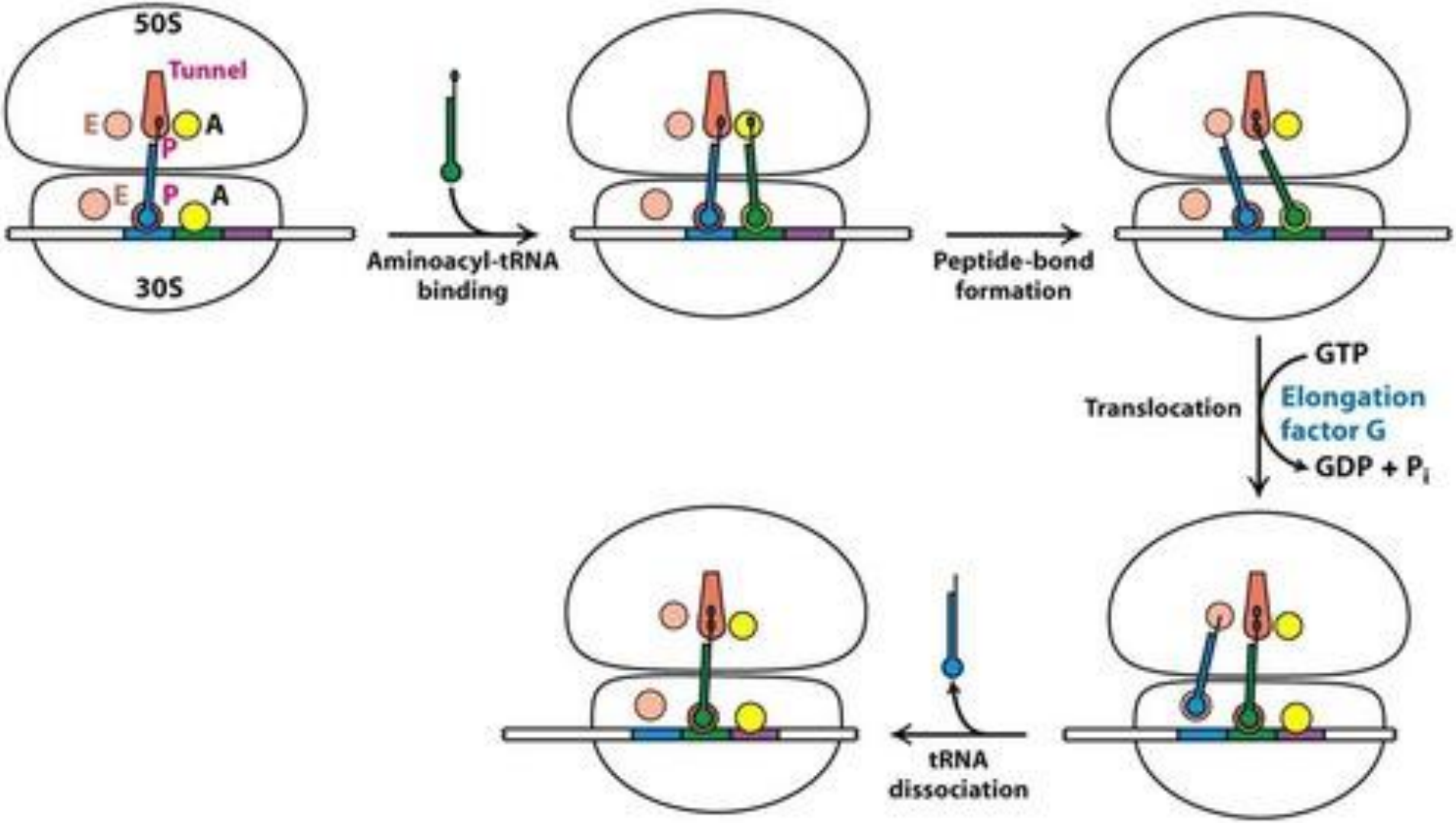
The 70S Ribosome



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

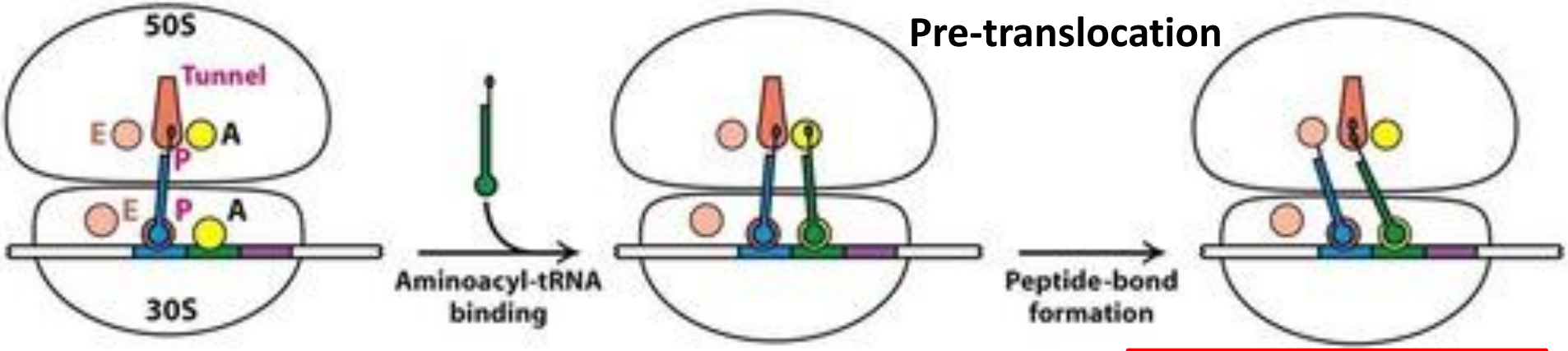
Munro, JB. et al. 2007. *TiBS*. 71(3):477-494

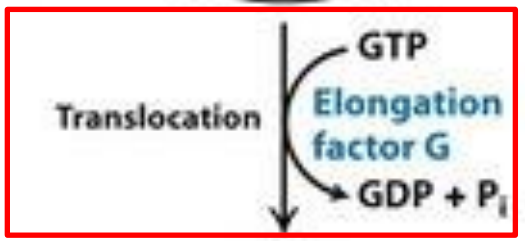
Translation

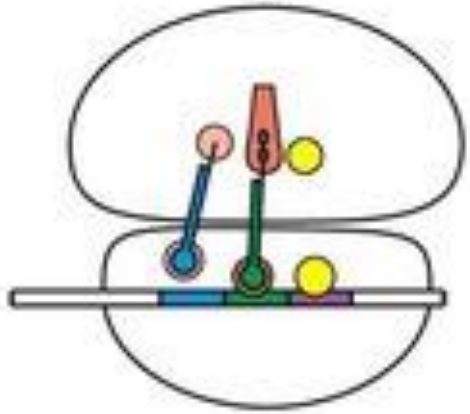


Stolen brom Biochemistry, Seventh Edition, 2012, Freeman and Company.

Translation







Post-translocation

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

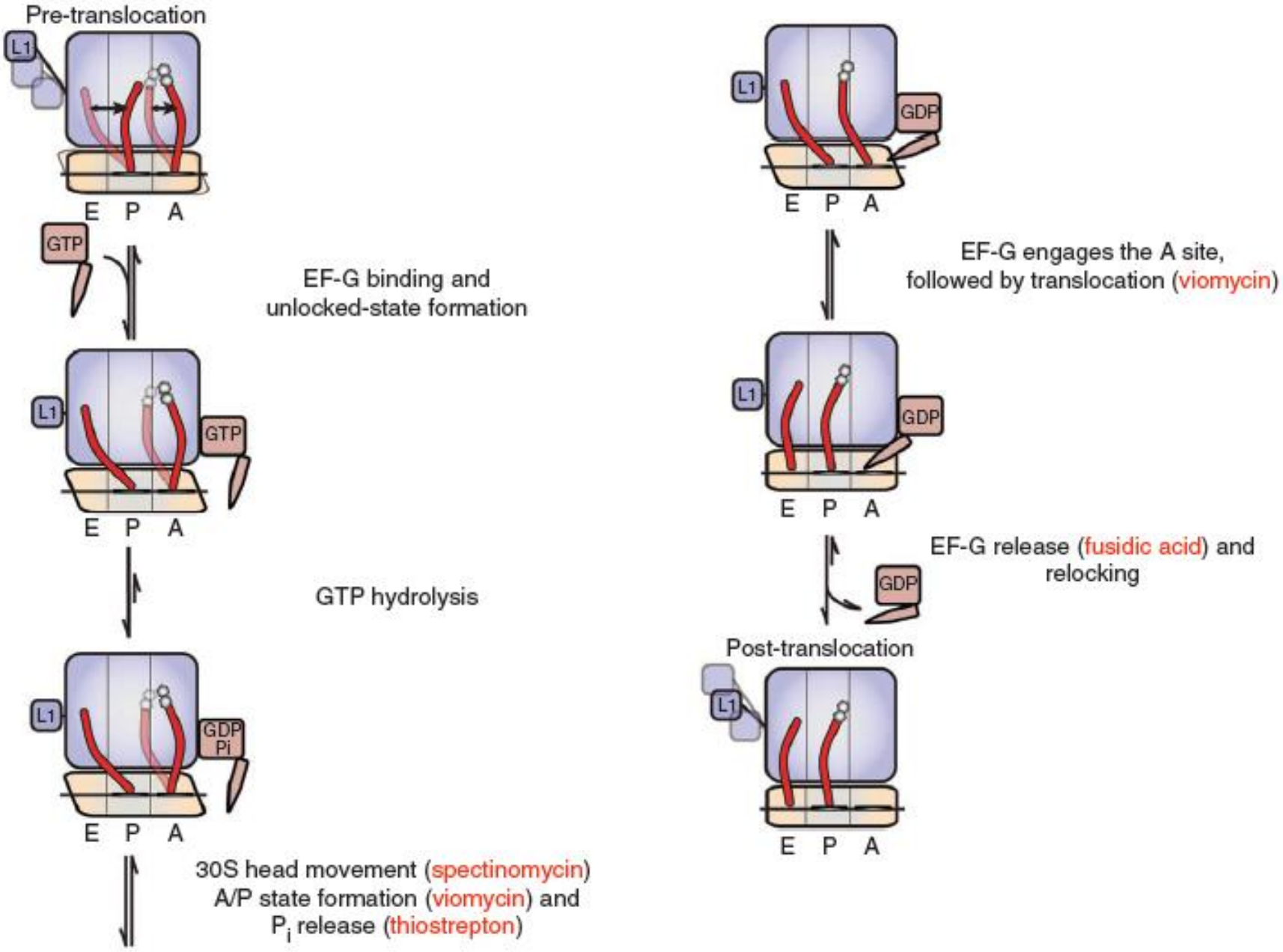
Spontaneous function of the ribosome
Accelerated 4-5 order of magnitude by EF-G

Move 2 tRNA 20-30 Angstroms
Maintain the reading frame
Allow for in vivo elongation rate of 15-20 a.a./s

Stolen brom Biochemistry, Seventh Edition, 2012, Freeman and Company.

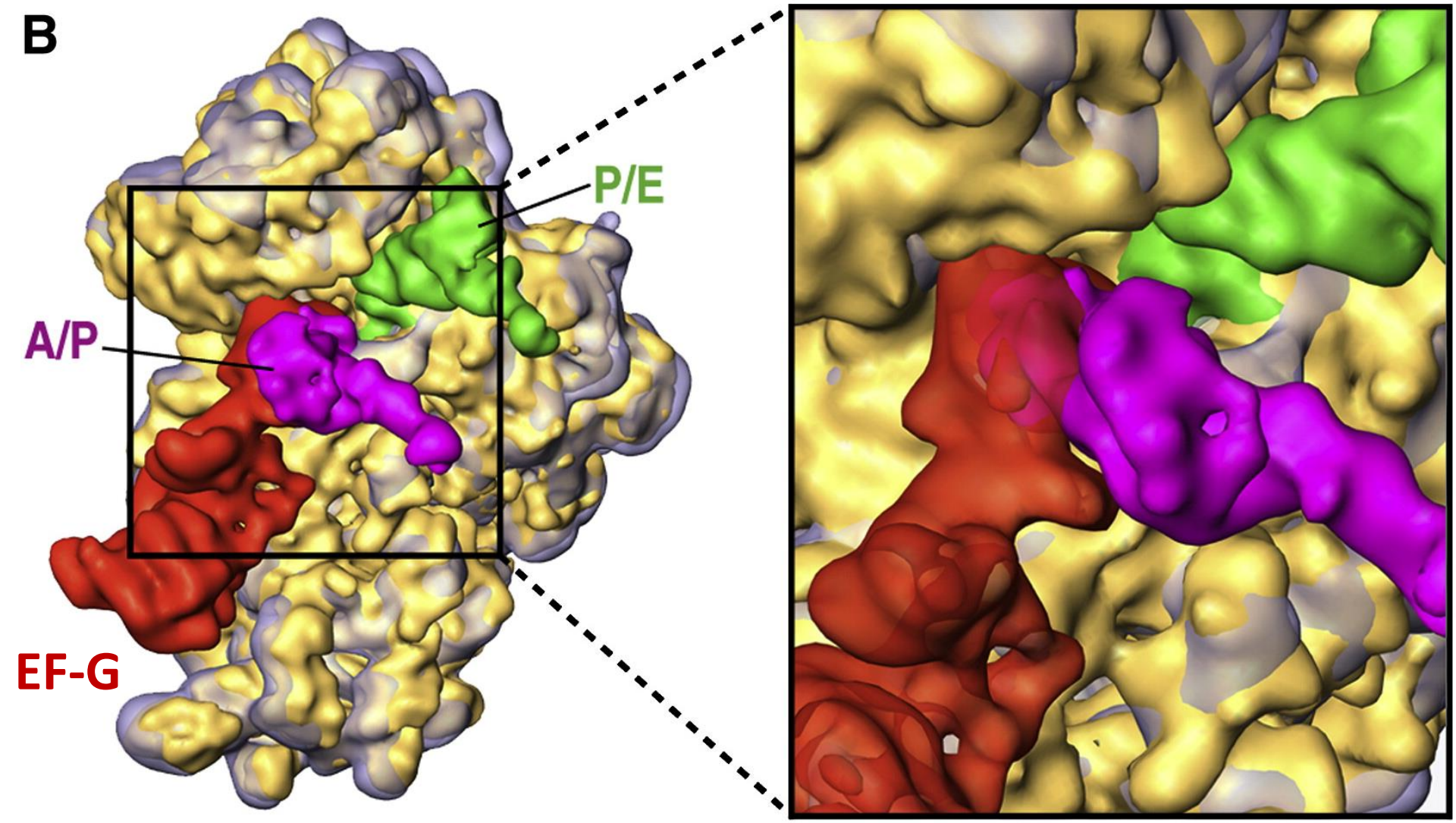
What is the role of EF-G?

Antibiotics in Translocation



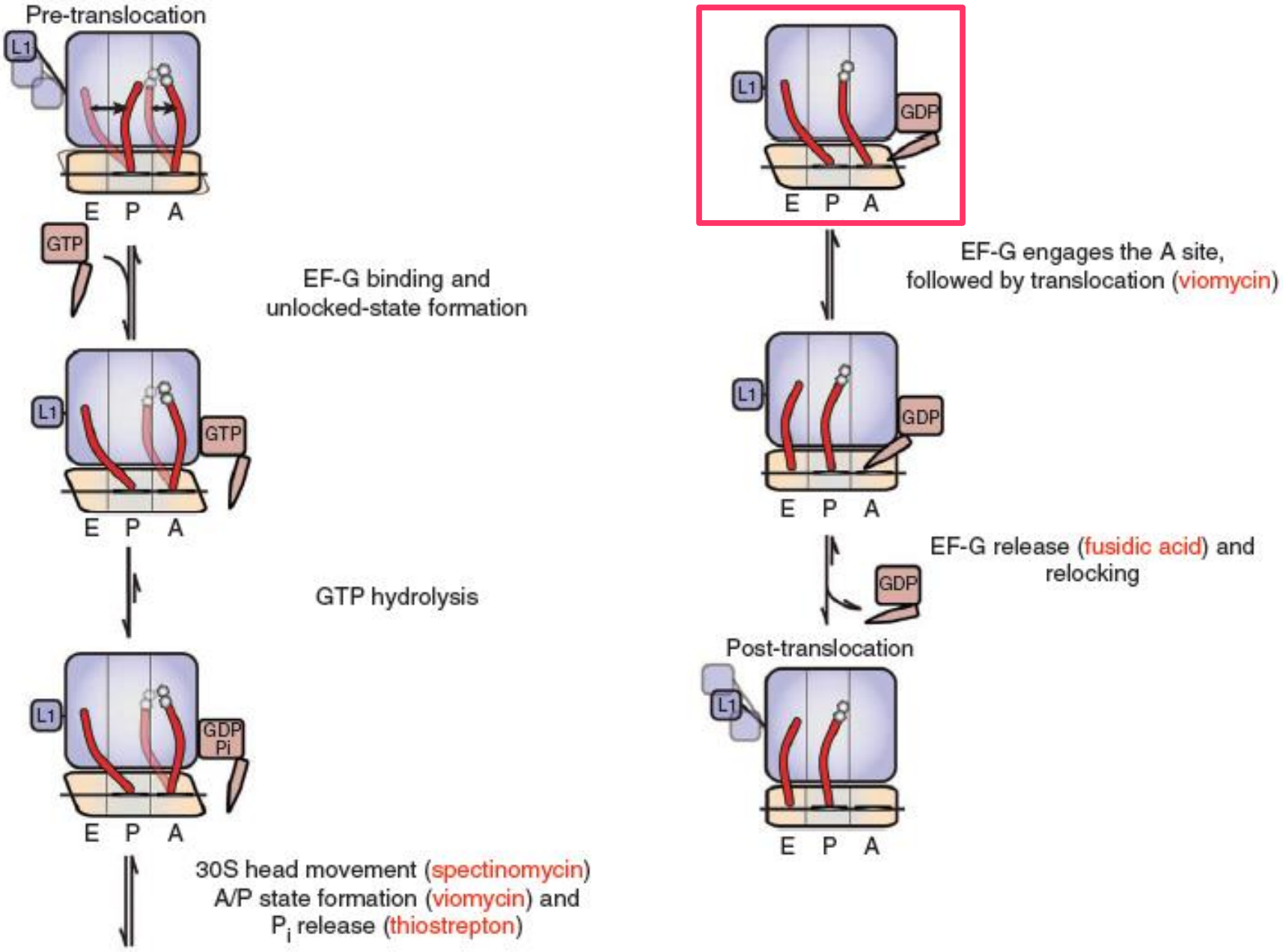
Munro, JB. et al. 2010. *NSMB.* 17(12):1470-7

Steric clash of EF-G with A site in Post-translocation state.



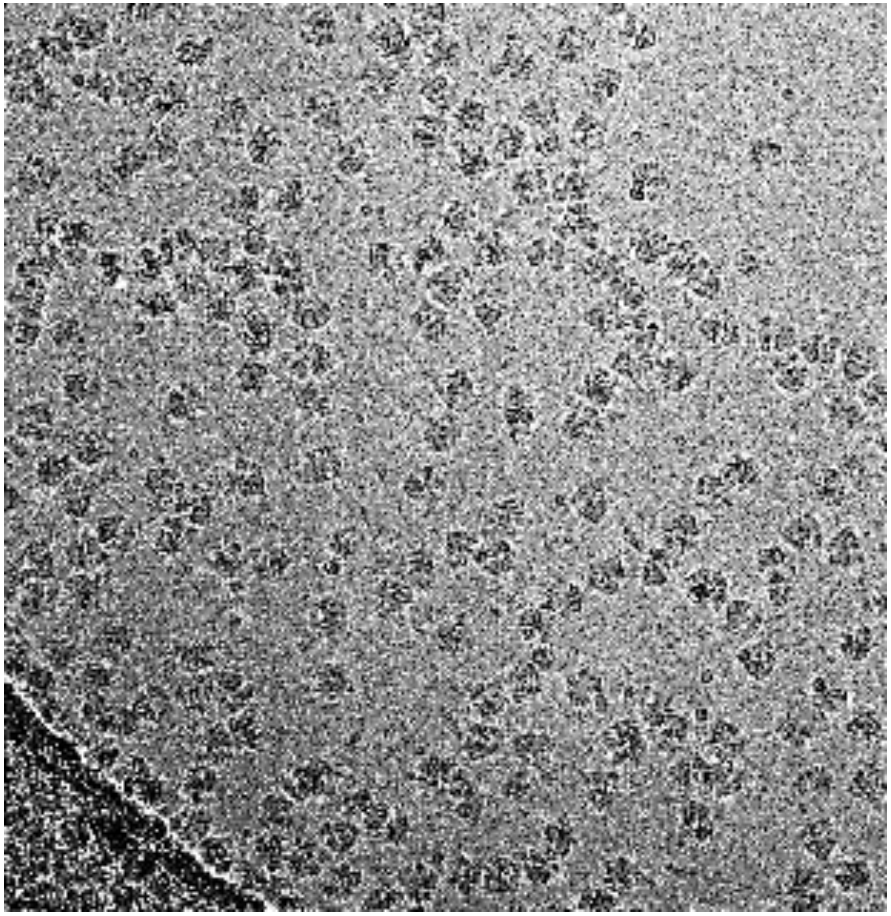
Agirrezabala, X. et al. 2009. *Q. Rev. Biophys.* 42(3):159-200

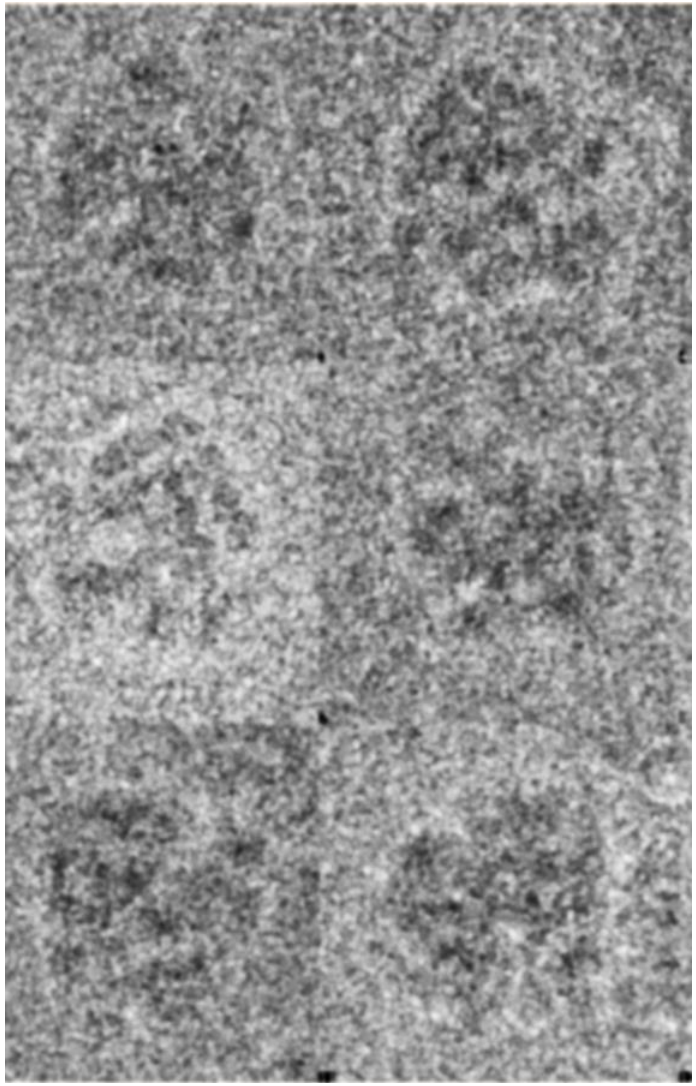
Antibiotics in Translocation



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

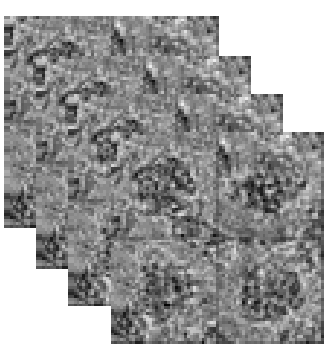
The Raw Data





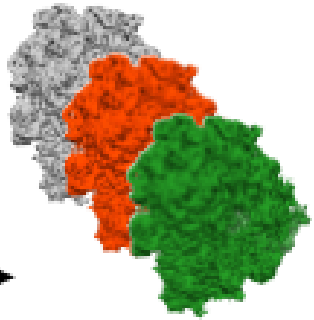
1.35 Million particles, collected on a Titan Krios Microscope at 300 kV.

The Expectation-Maximization algorithm



Images

Reconstruct



References

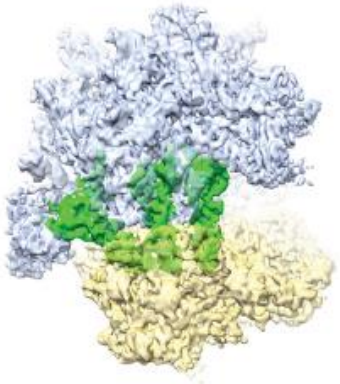
Compare images to maps, obtaining the maximum likelihood estimate of the new parameters for each image with each reference

New particle orientations, occupancies

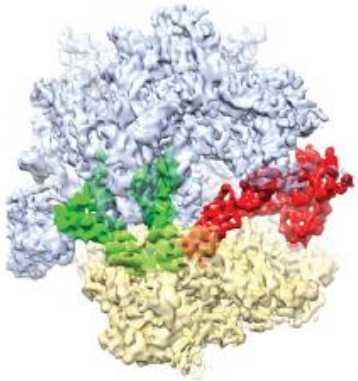
Particle orientations, occupancies

Iterate with new parameters

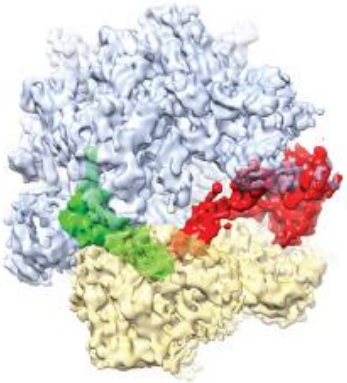
The Results



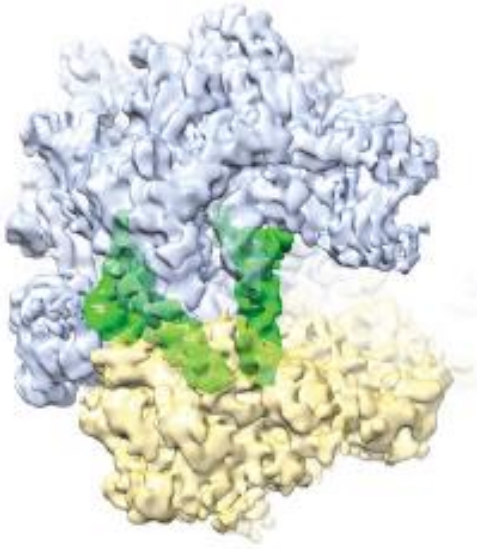
I. 3 tRNA
(26.7%)



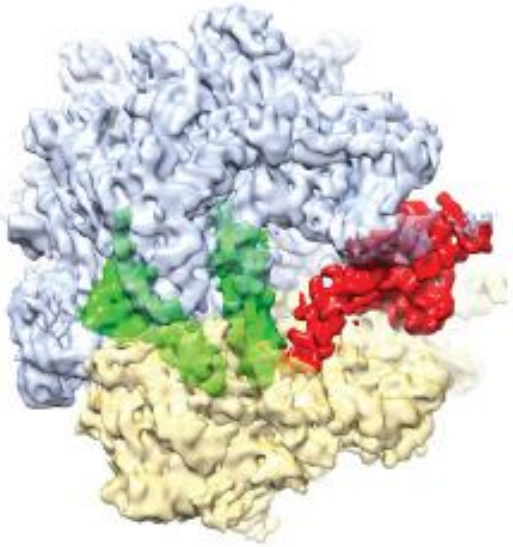
II. EF-G, P & E tRNA
(13.4%)



III. EF-G, P site tRNA
(6.8%)

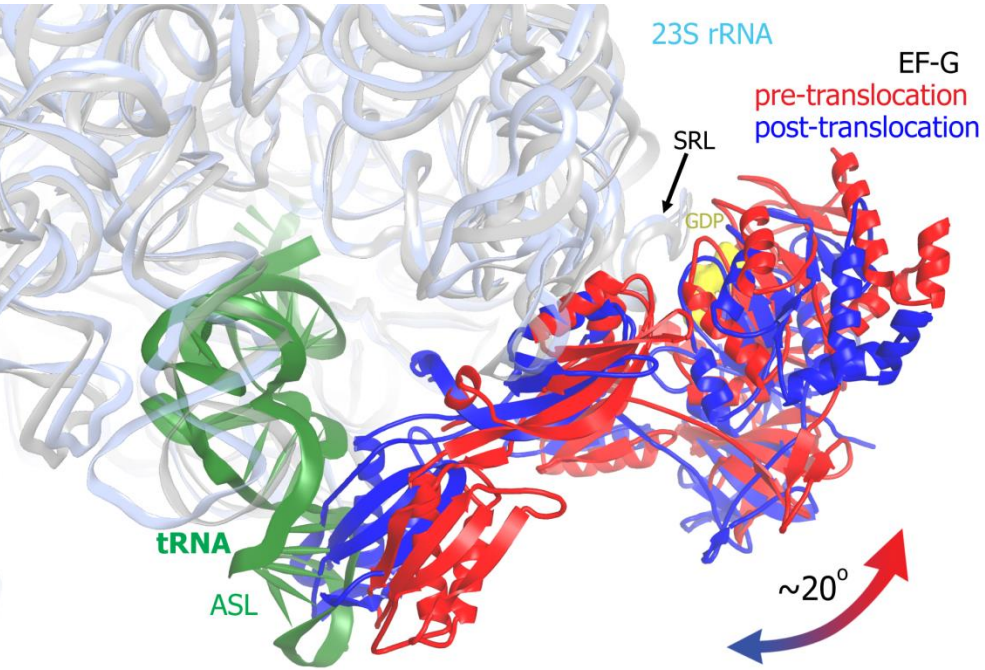


IV. A/P & P/E tRNA
(3.5%)

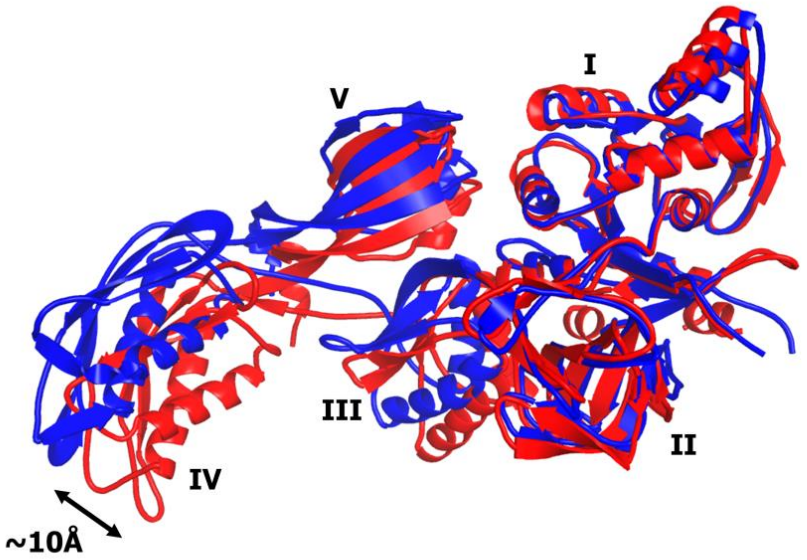


V. EF-G, A/P & P/E tRNA
(2.4%)

Conformational Changes of EF-G

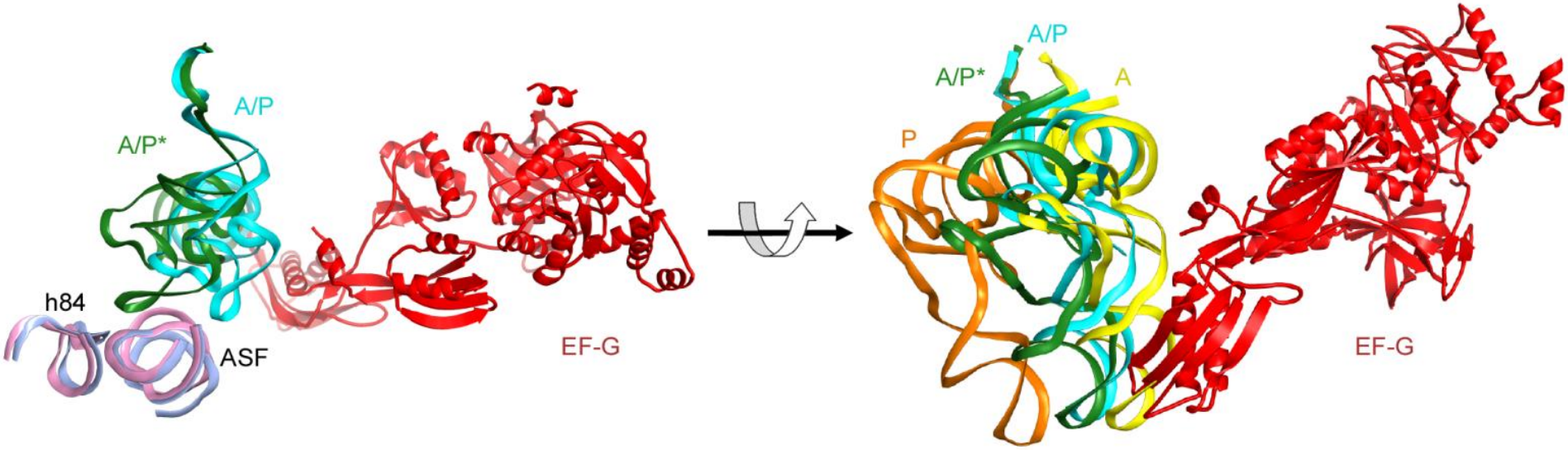


View of EF-G with pre and post-translocation 50S subunits aligned shows a rotation around the immobile SRL



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

EF-G binding induces a new tRNA hybrid state



EF-G: Pre-translocation

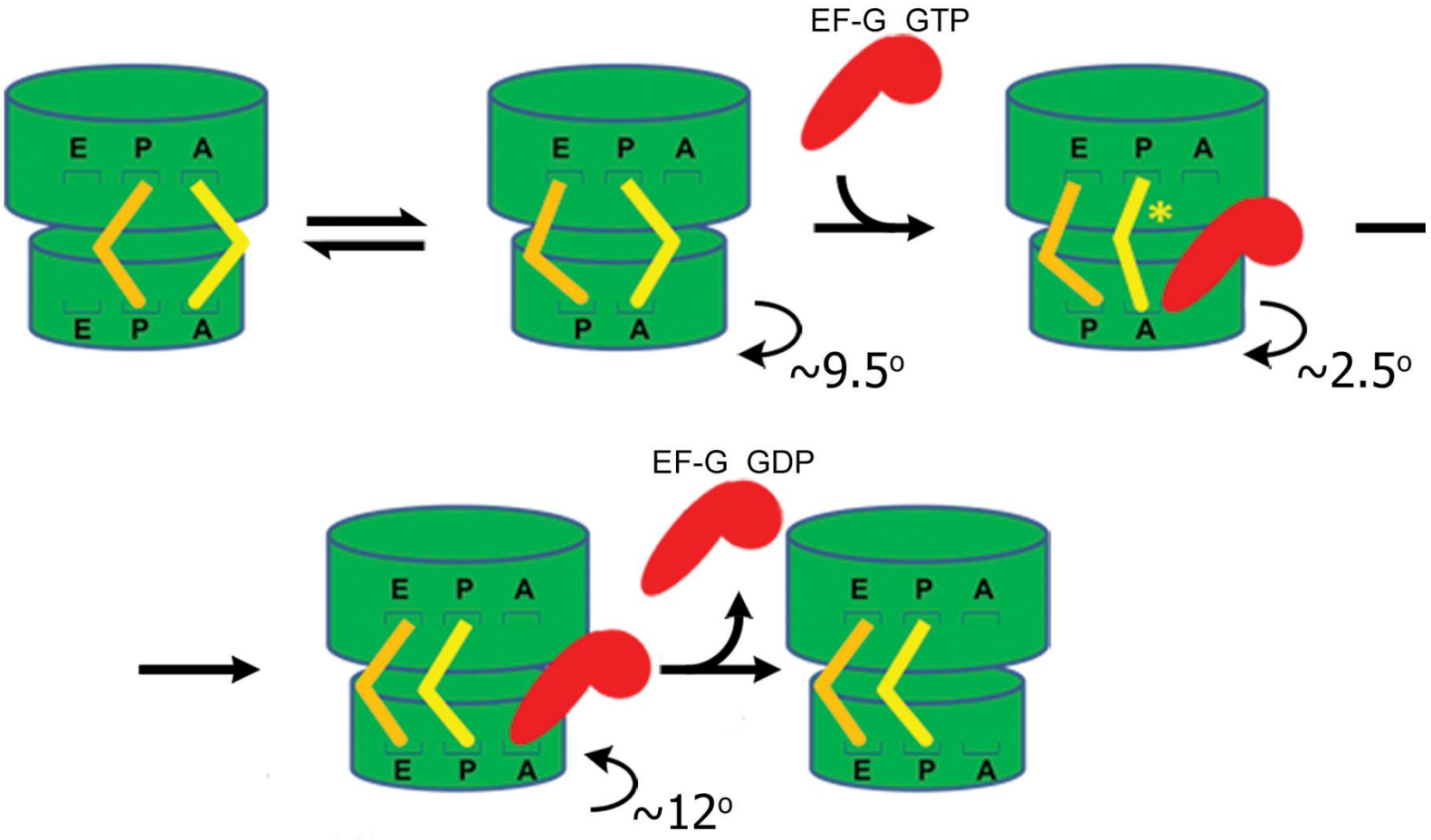
A/A: Classical pre-translocation state

A/P*: EF-G Bound, pre-translocation state

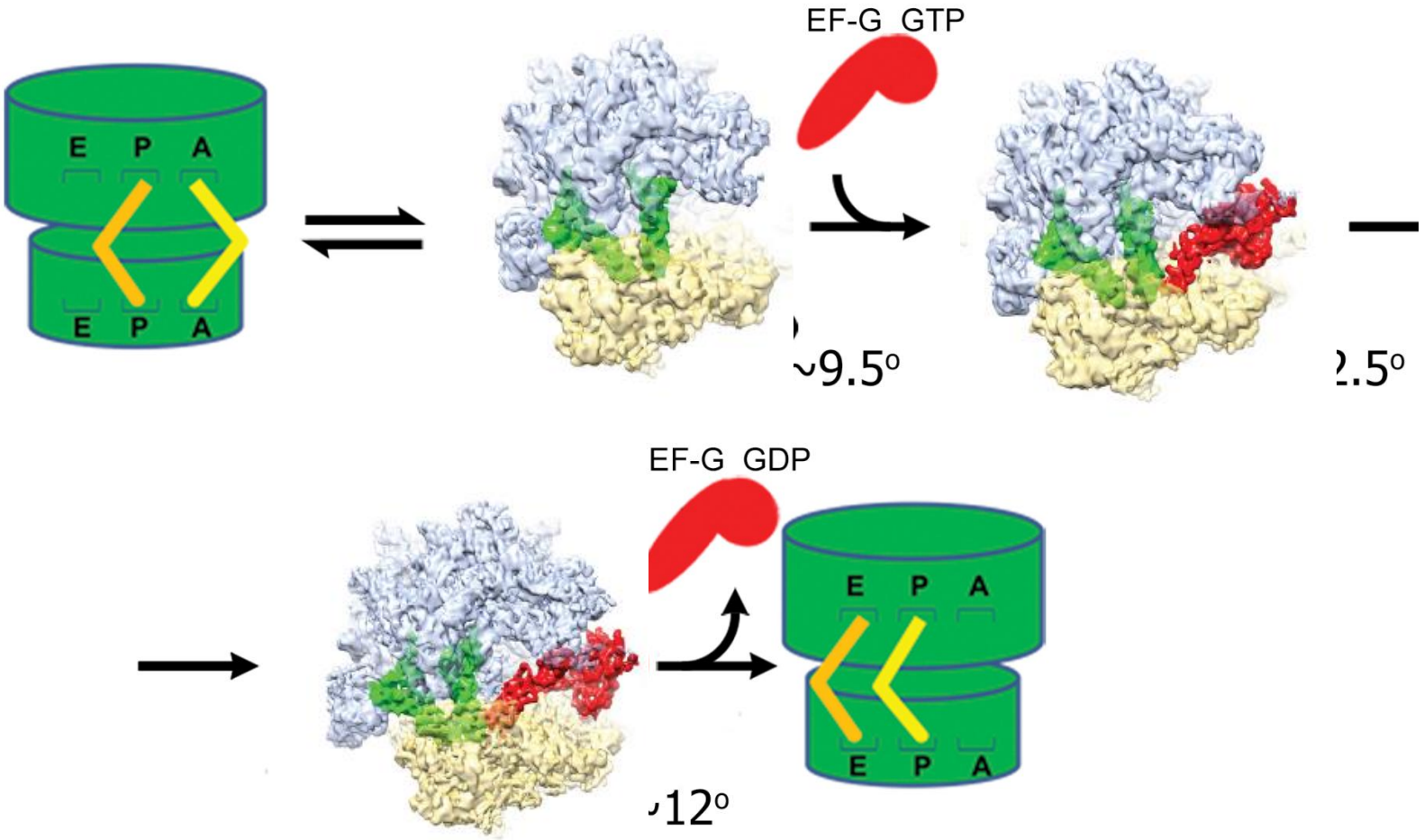
A/P*: No EF-G bound, pre-translocation state

P/P: Classical Post-translocation State

Schematic of EF-G catalyzed translocation



Schematic of EF-G catalyzed translocation



Our Collaborators

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EF-G complexes

Acknowledgments

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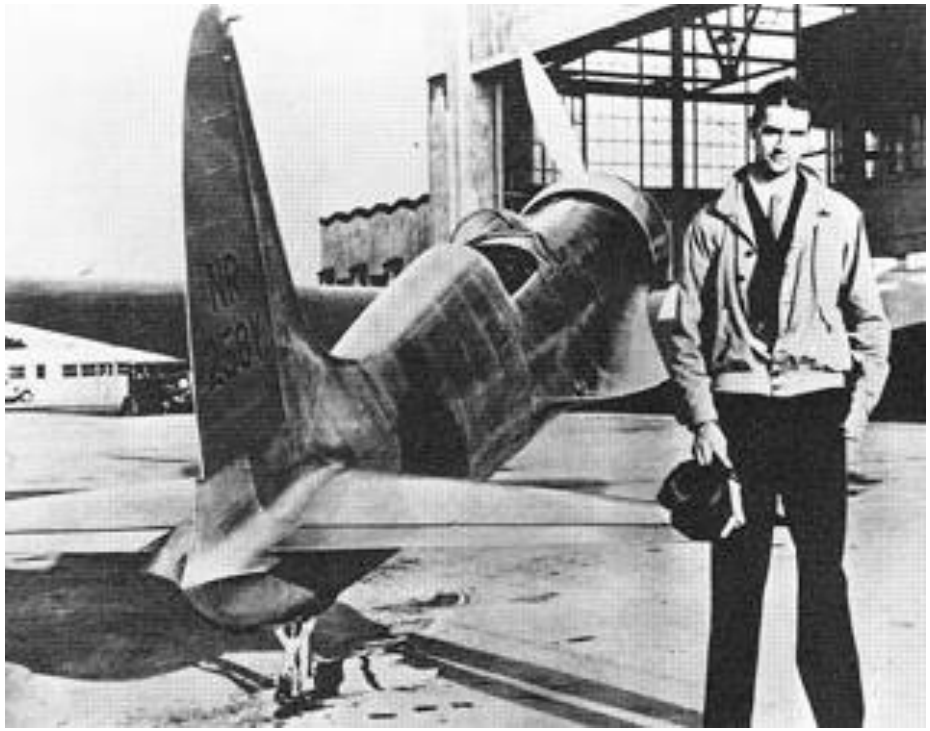
Grigorieff Group
Chen Xu
Alexis Rohou
Mike Rigney

Janelia Farm
Zhiheng Yu
Jason De La Cruz

Funding



HHMI NSERC CRSNG