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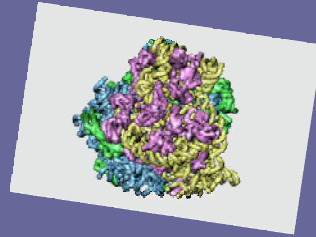
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What Makes It Tick?

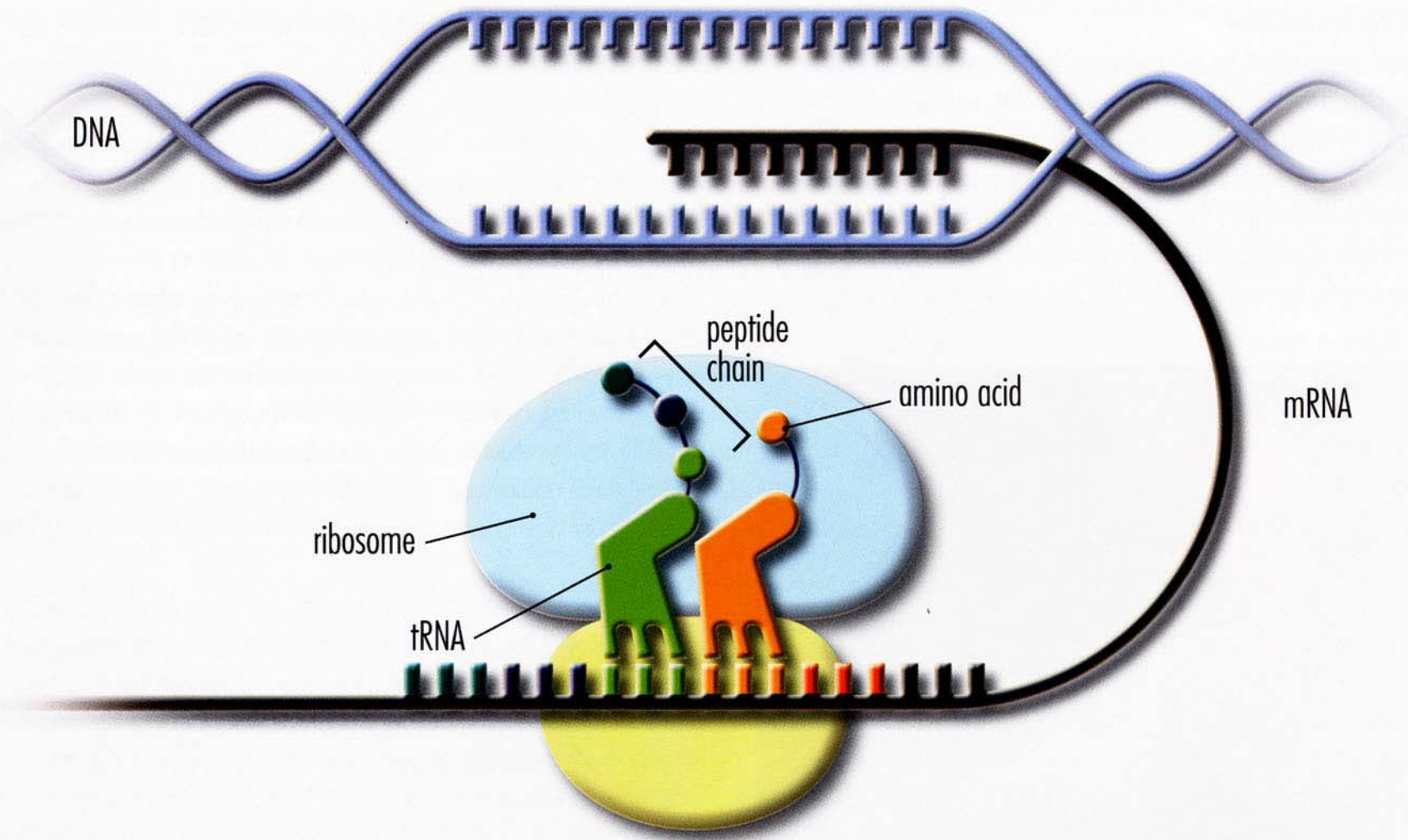
Dynamics of the Ribosome as Inferred from Cryo-electron Microscopy



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Supported by HHMI, NIH R01 GM55440, and NIH R37 GM29169



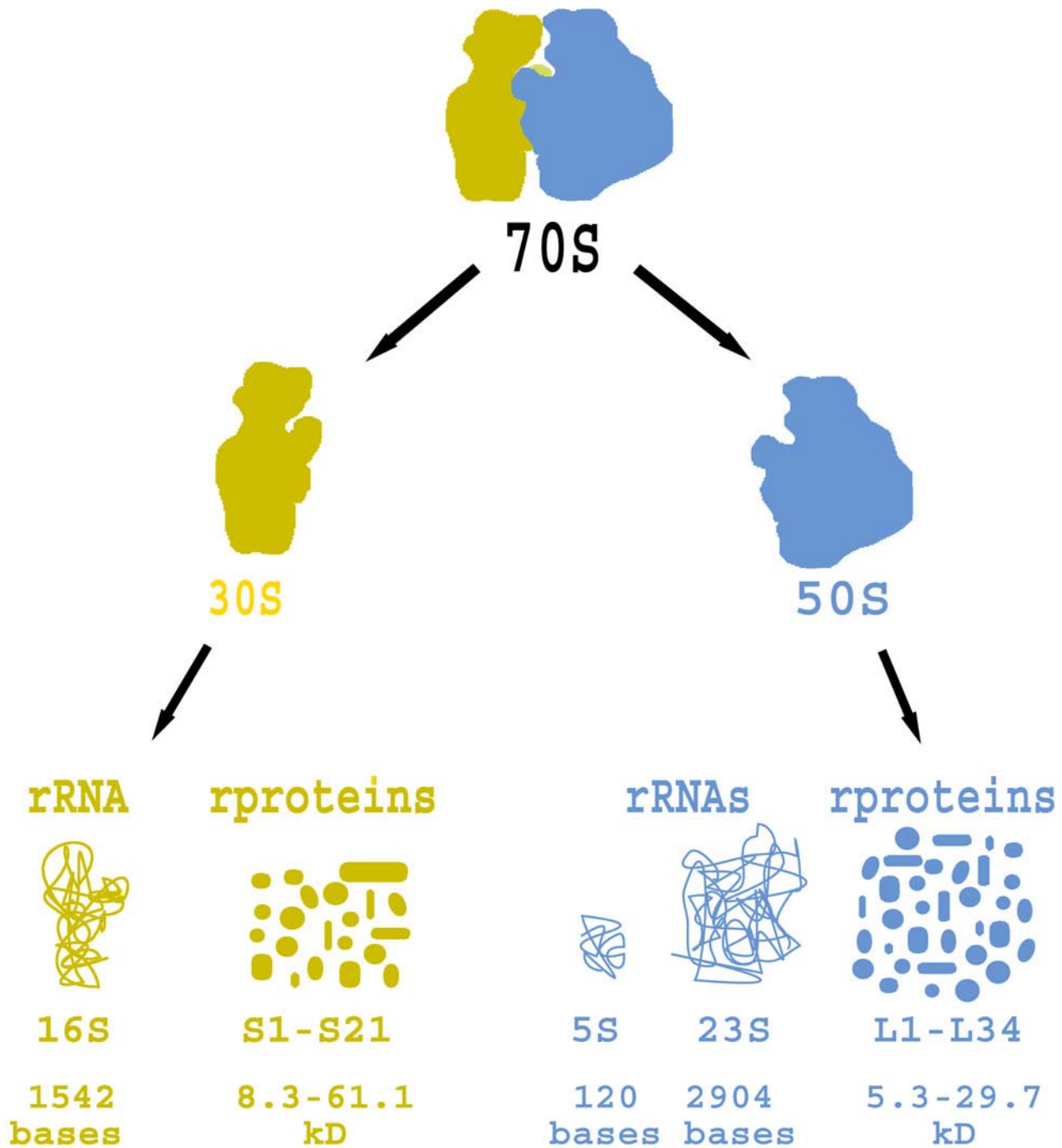
Translation

The Players:

- Ribosome – the “machine”
- mRNA – carries the message
- tRNA – a single lookup of a 20-word dictionary: recognizes a codon with its “anticodon” end, and carries the corresponding amino acid on its CCA end
- Elongation factor Tu – helps tRNA go into the ribosome
- Elongation factor G – moves things (tRNAs and mRNA) along

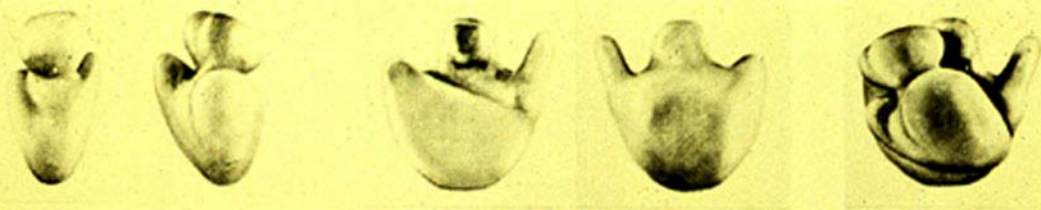
The Ribosome

- Makes proteins according to genetic instructions
- Core region (intersubunit space) highly conserved throughout evolution
- 10,000 – 200,000 ribosomes per cell
- composed of 3 RNAs and 51 proteins (prokaryotic)
- two subunits: small and large
- division of labor:
 - small subunit: decoding
 - large subunit: links up the amino acids to form protein (“peptidyl transfer”)

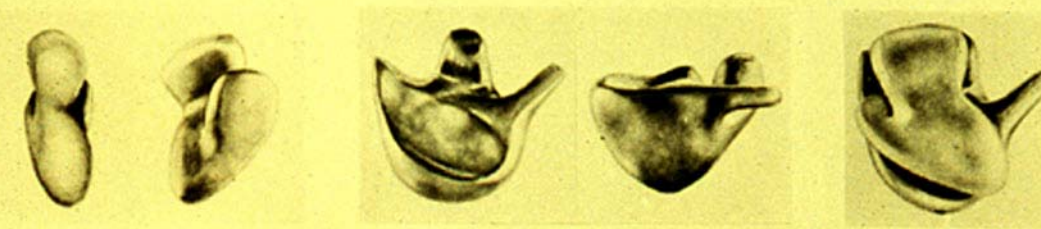


Electron Microscopy of Ribosomes

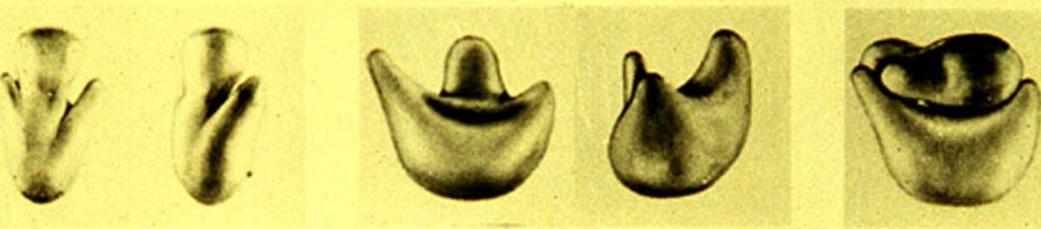
- *EM of negatively stained ribosomes:*
H. Huxley, ~1960
- *Location of proteins, by immuno-electron microscopy:*
J. Lake; M. Stöffler-Meilicke, early 1980s
- *Location of proteins, by neutron scattering:*
P. Moore et al., 1980s
- *Exit site of polypeptide chain, by immuno-EM:*
J. Lake, 1981
- *Exit tunnel of eukaryotic ribosomes, by electron crystallography of 2D crystals:*
R. Milligan and P.N.T. Unwin, 1986
- *Exit tunnel of prokaryotic ribosomes, by electron crystallography of 2D crystals:*
A. Yonath et al. 1987
- *Cryo-EM single-particle reconstruction of E. coli ribosome:*
J. Frank et al., 1991



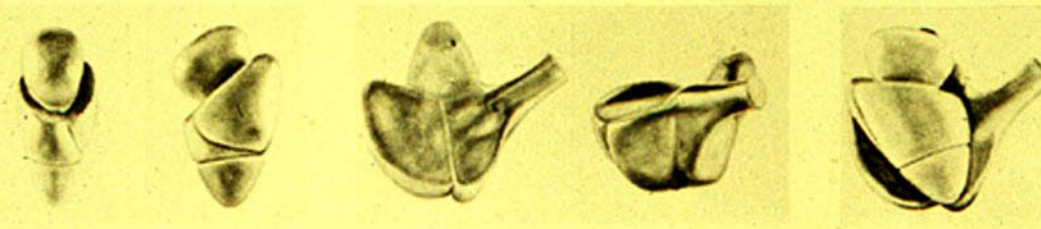
Stöffler



Lake



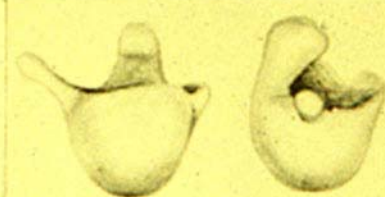
Boublik



Vasiliev

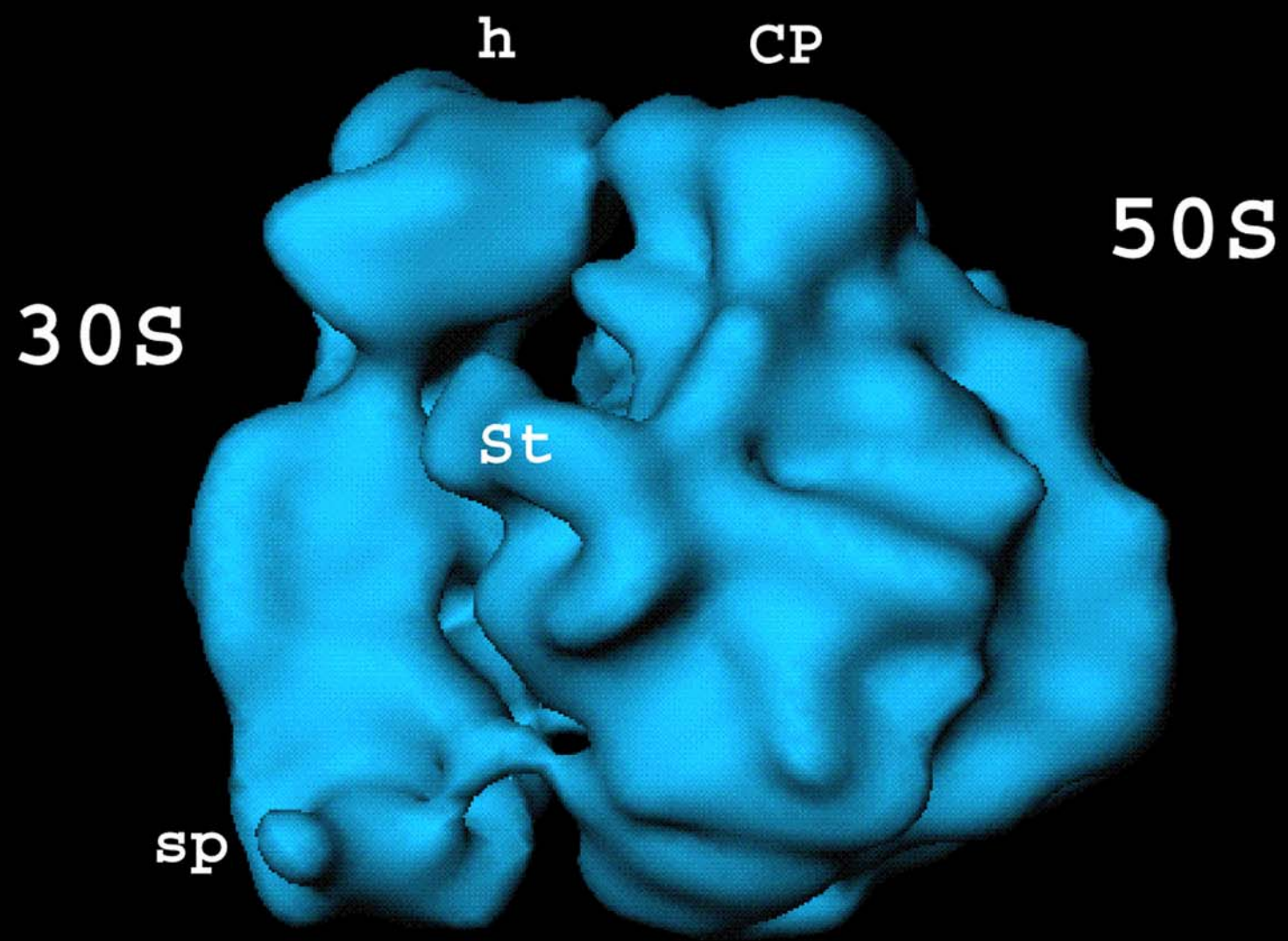


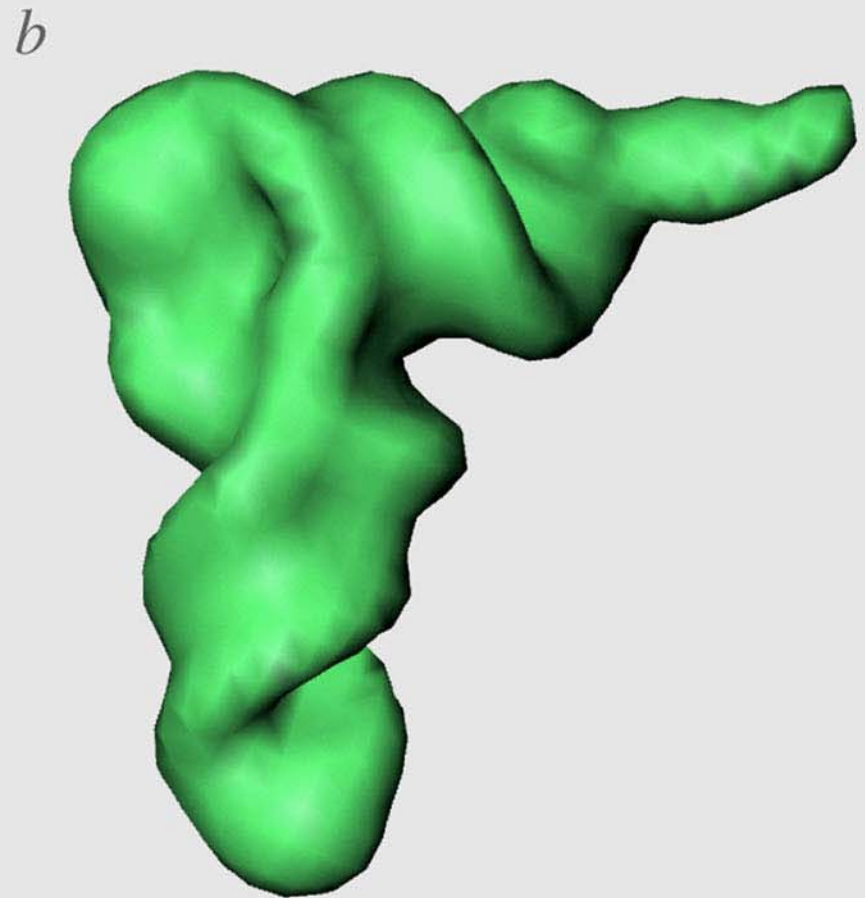
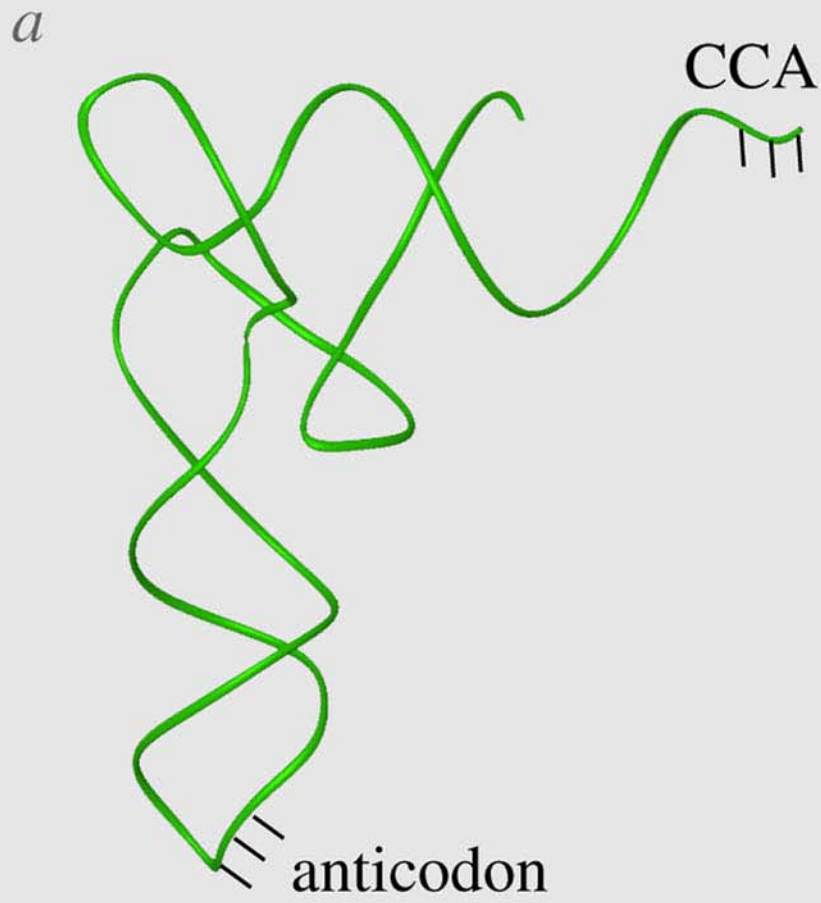
Korn



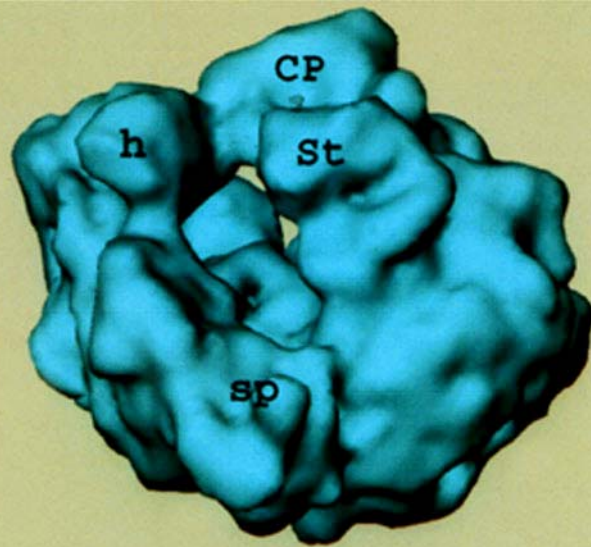
Spieß

E. coli
30 S
50 S
70 S

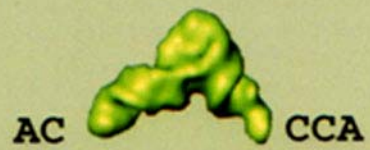




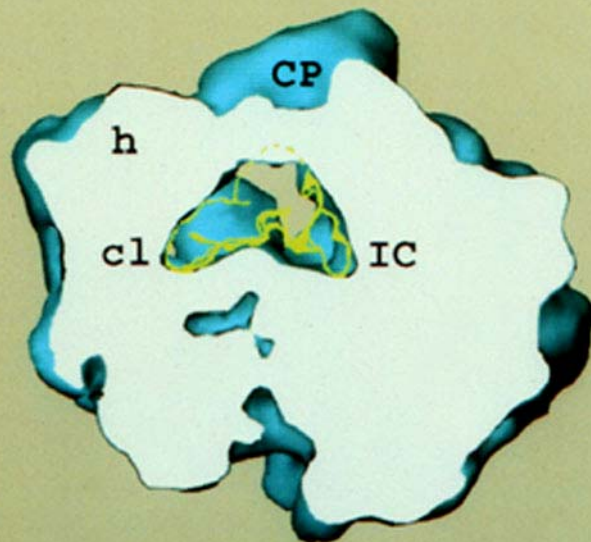
a



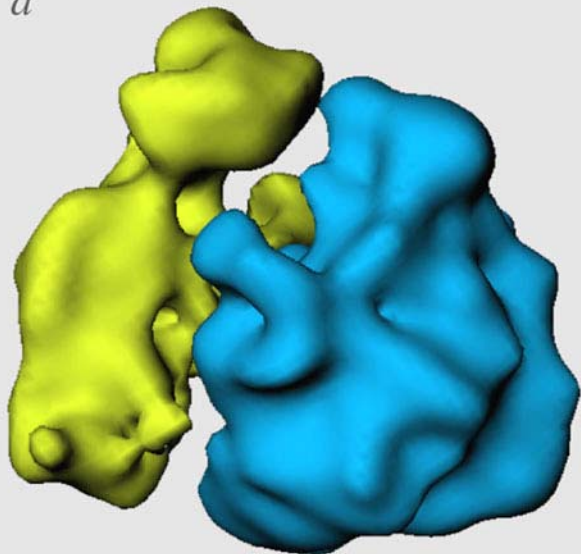
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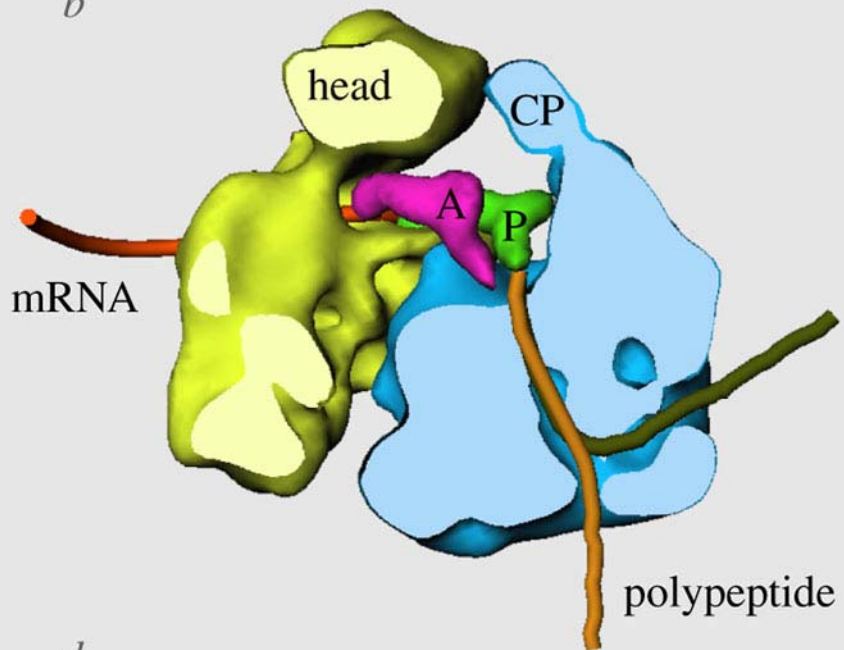
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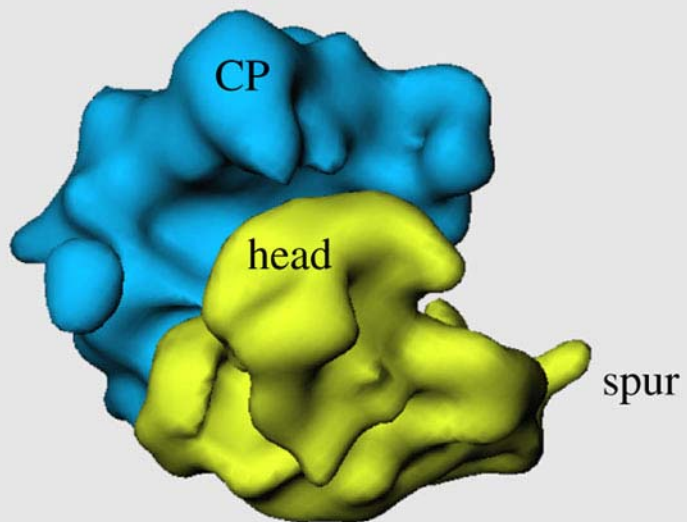
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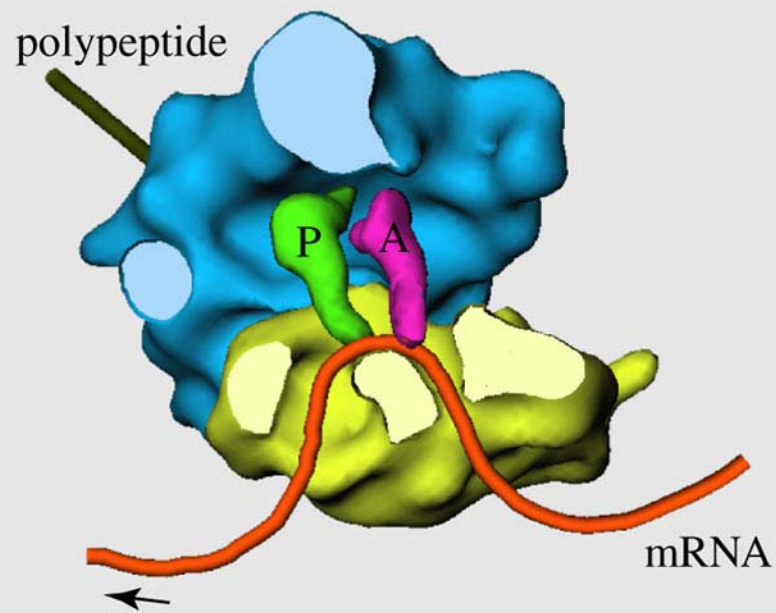
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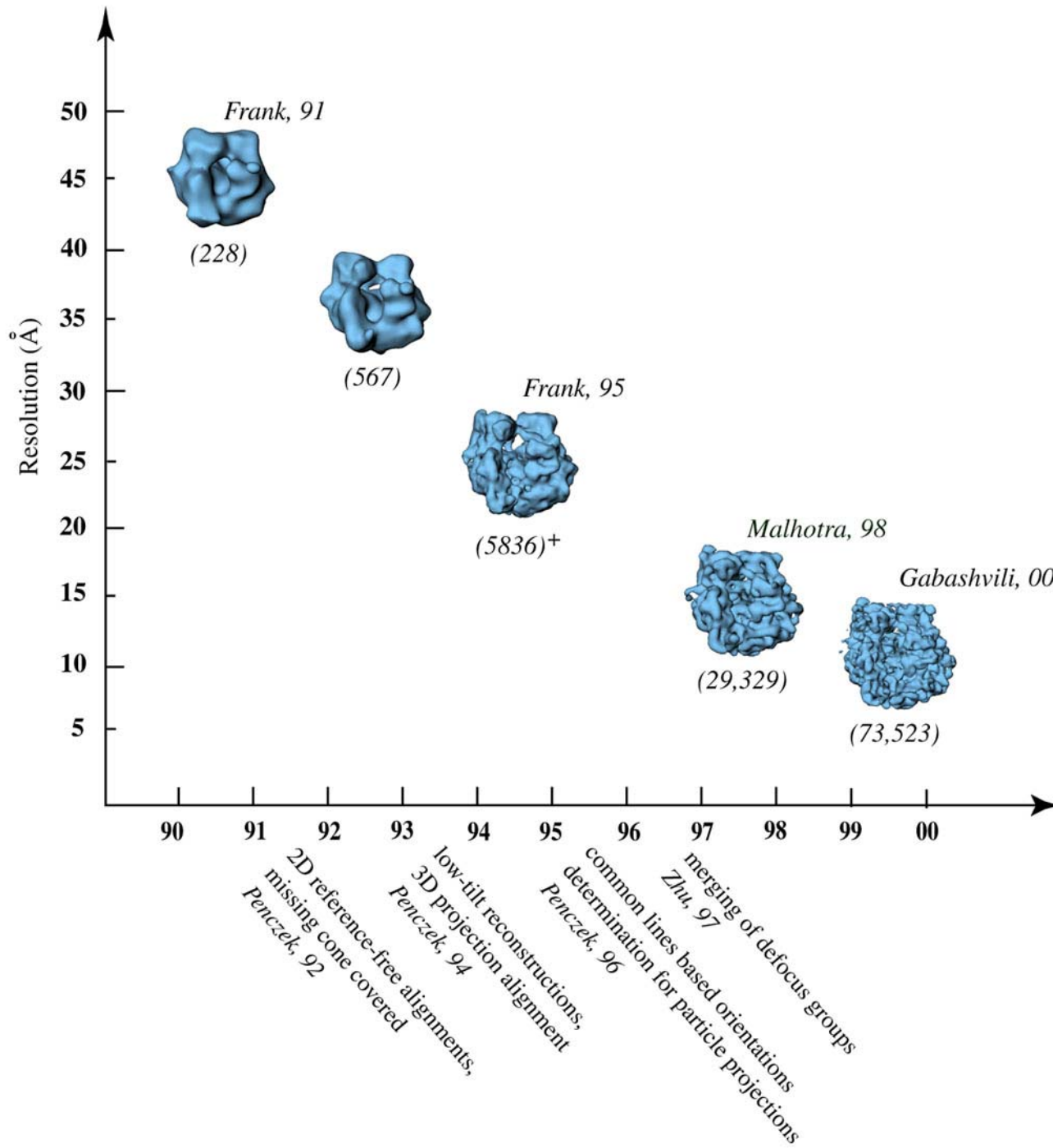


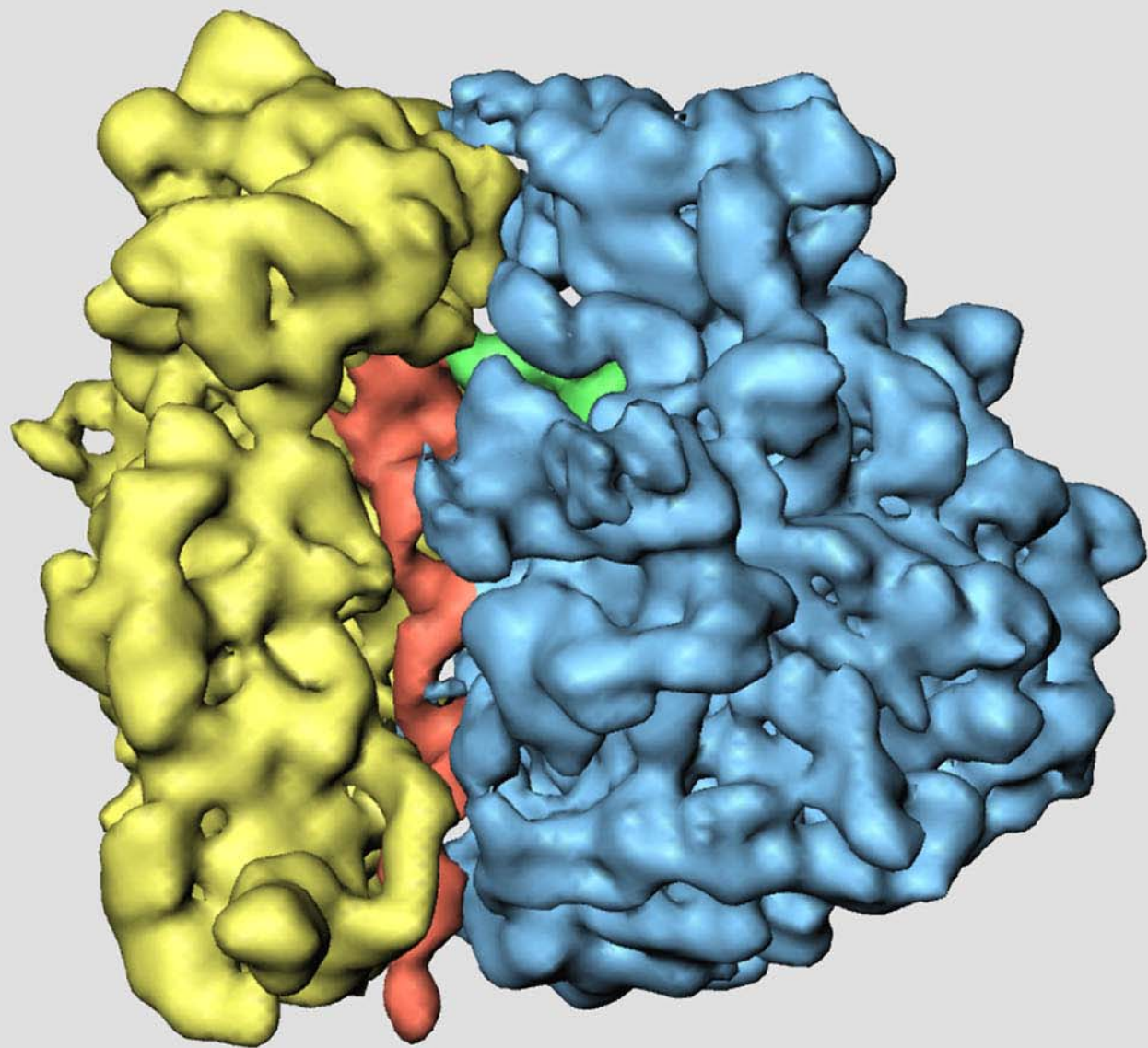
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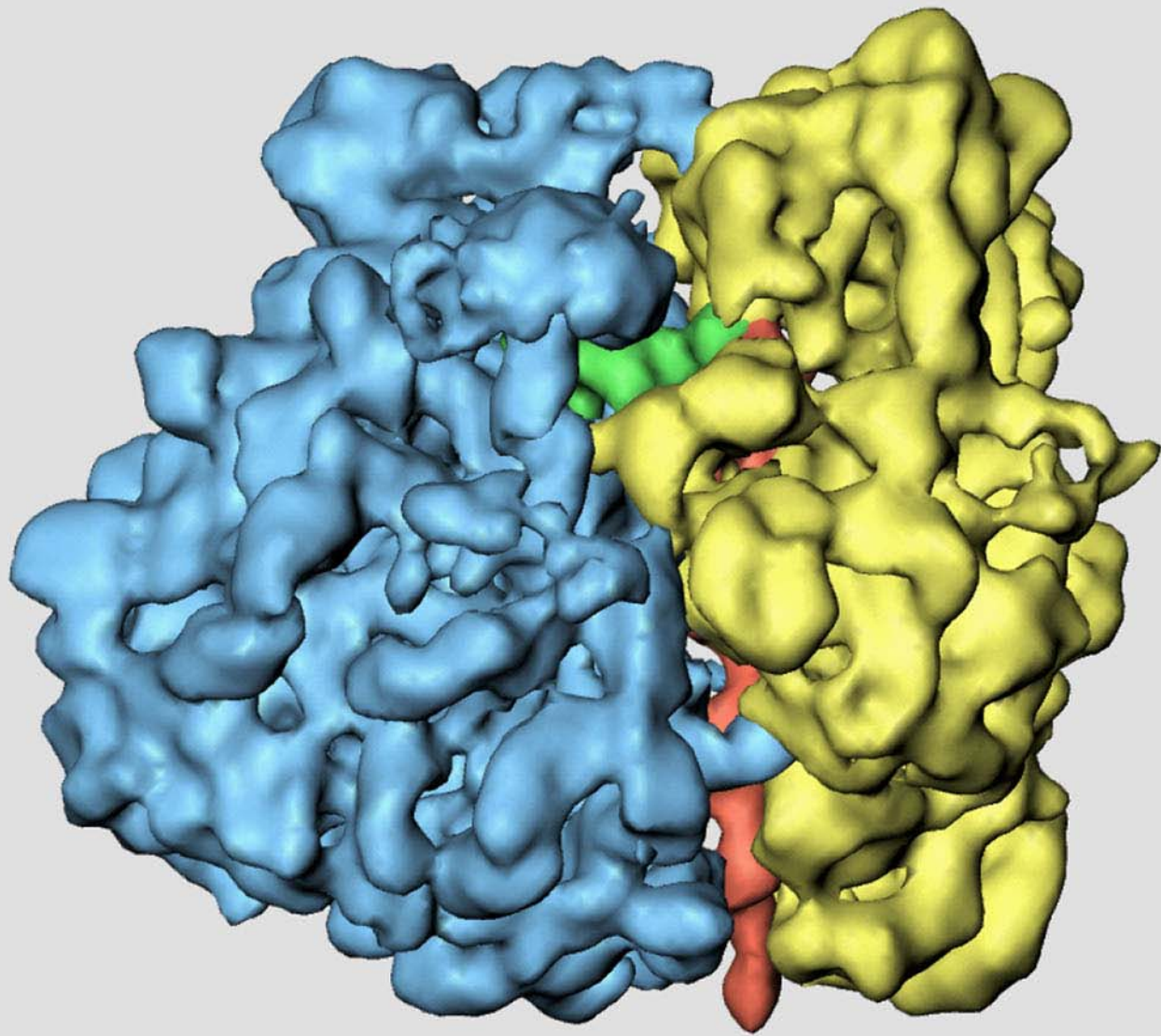


d

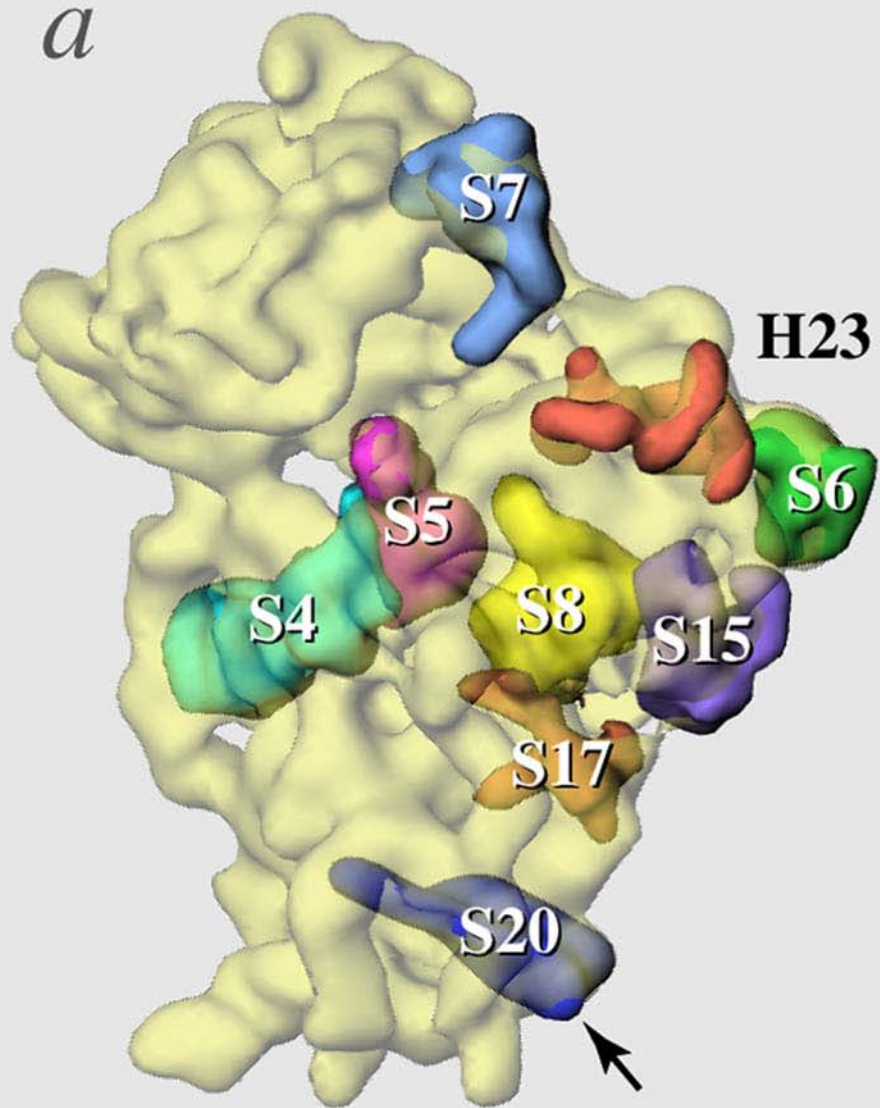




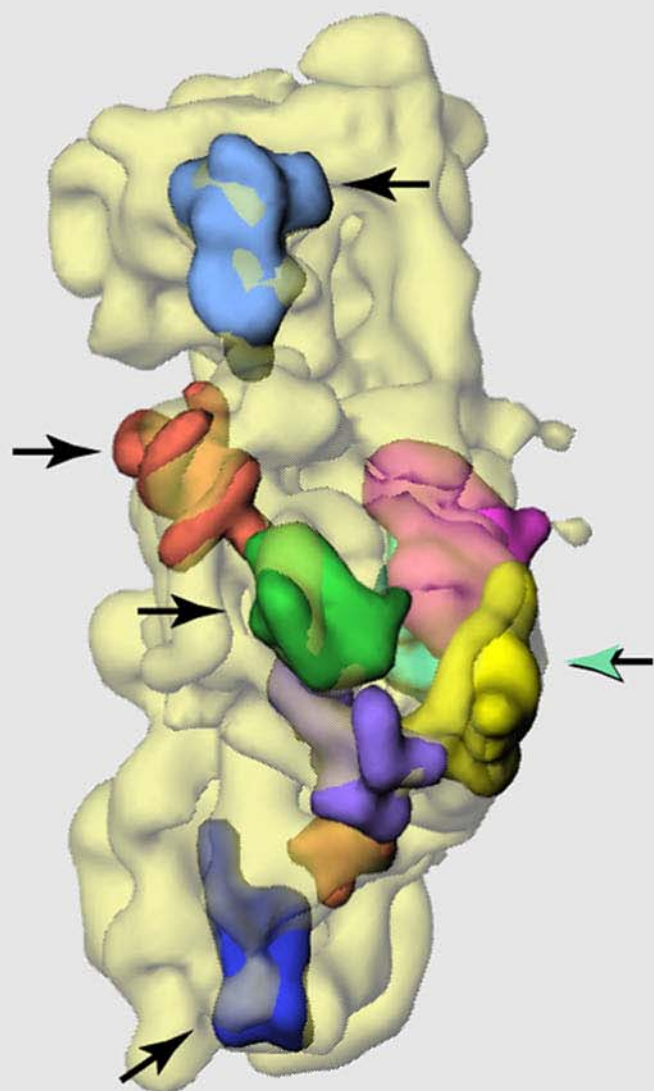




a



b



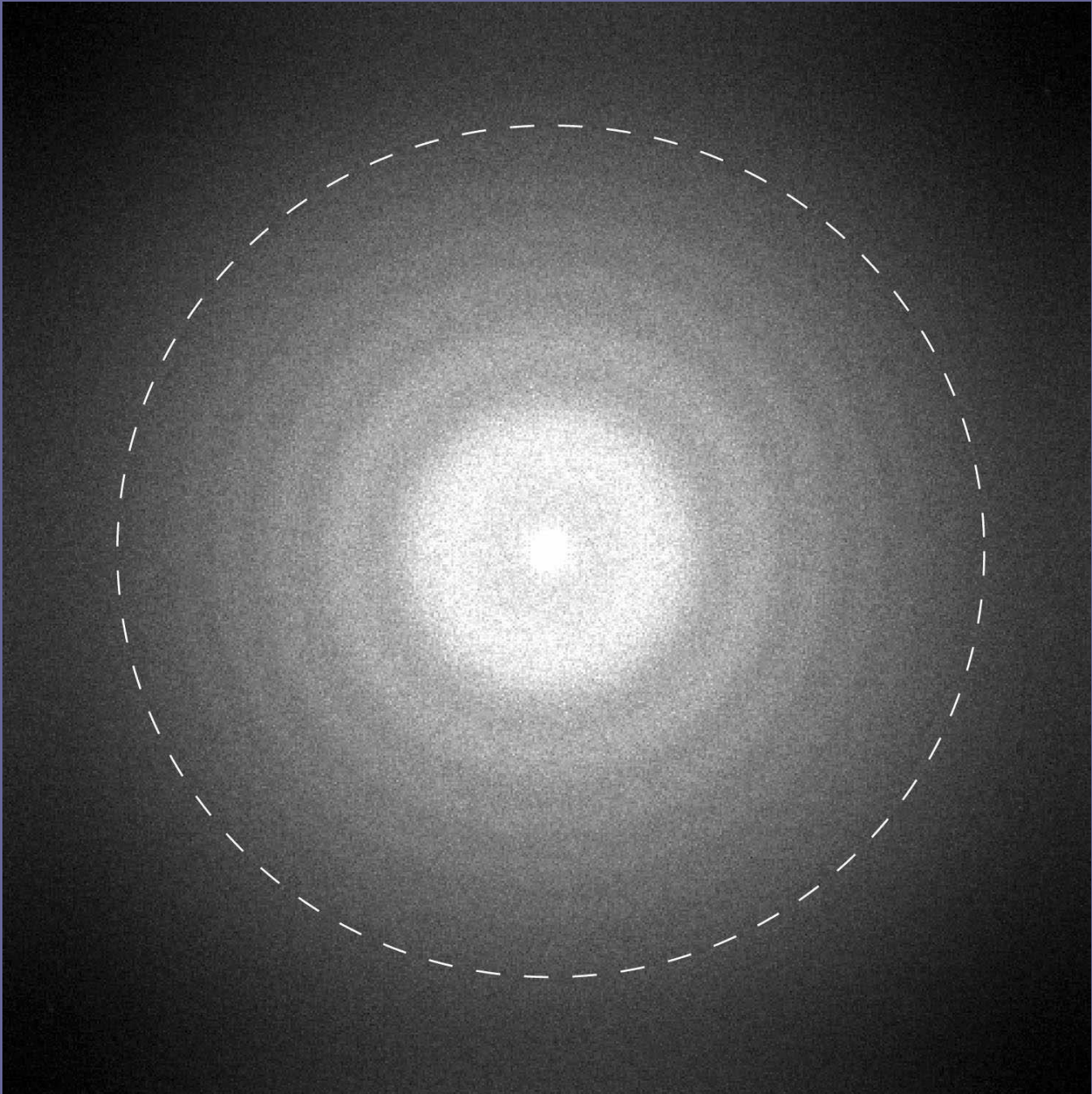
E. coli ribosome at 8.7 Å resolution (FSC=0.5)

C.M.T. Spahn, R.A. Grassucci, K.H. Nierhaus, J. Frank

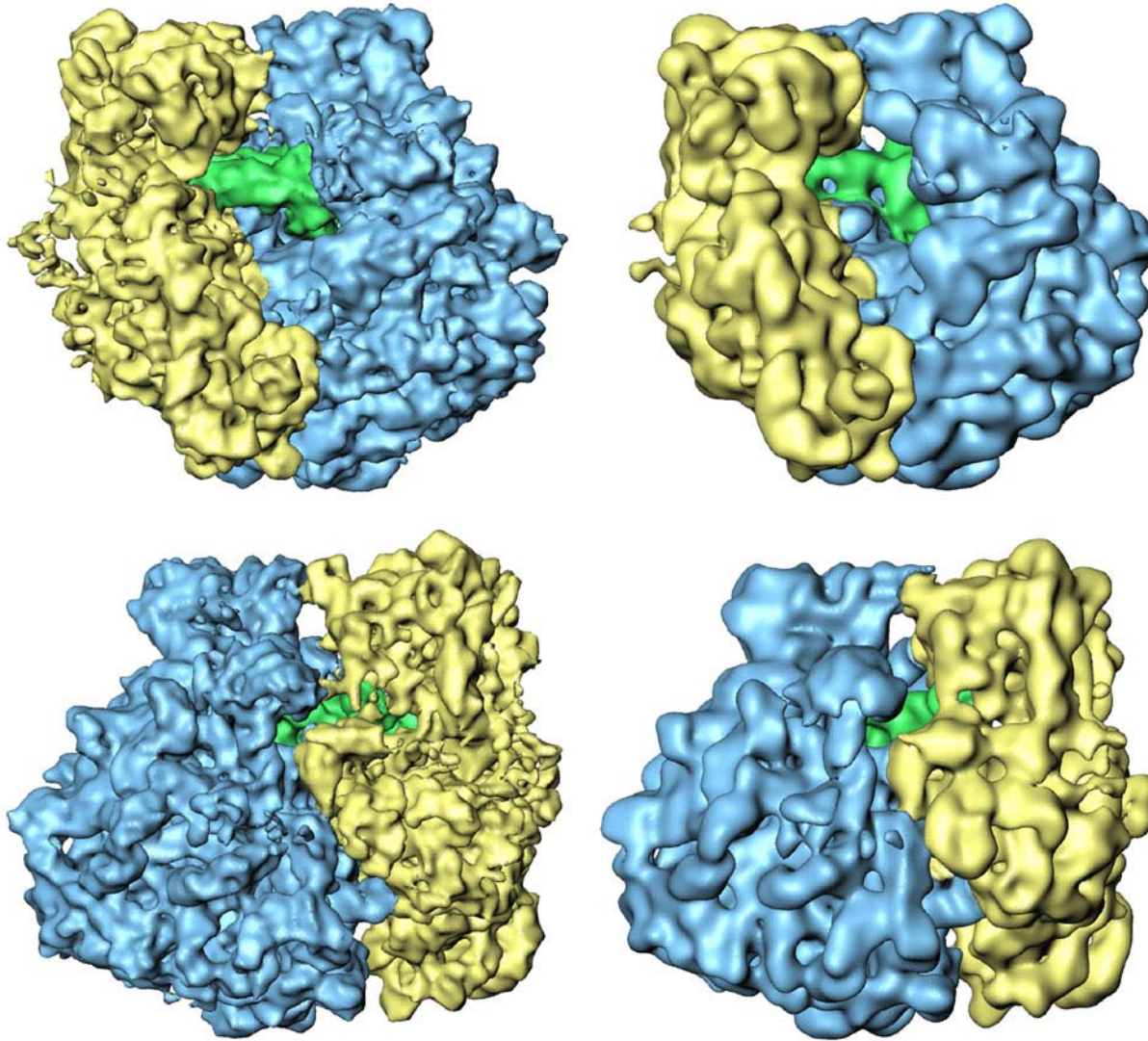
- Phe-tRNA^{phe} in A site;
- AcPhe-N-tRNA^{phe} in P site;
- programmed with poly-U.

Complex is stalled in pre-translocational state, prior to peptide transfer.

- 110,000 particles.
- 77% of the data collected on Philips/FEI Tecnai F30, rest on F20.
- Reconstruction was amplitude-corrected using X-ray solution scattering data.
- Both A- and P-site tRNA are ~100% occupied



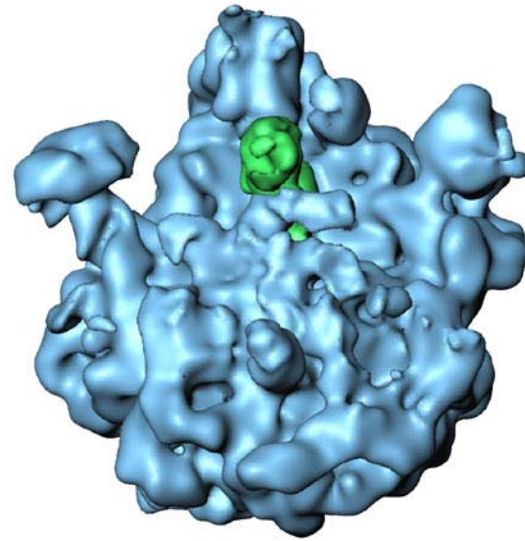
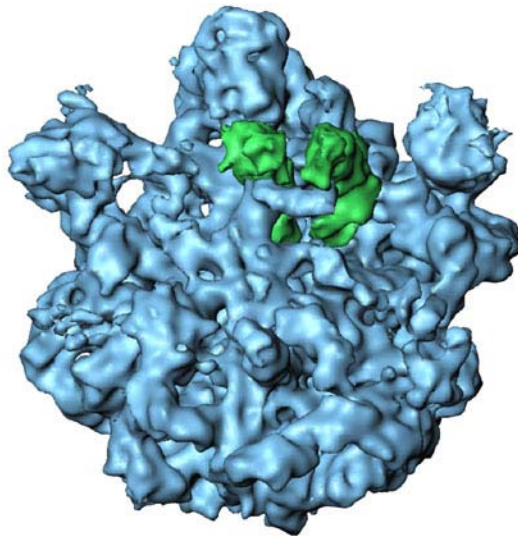
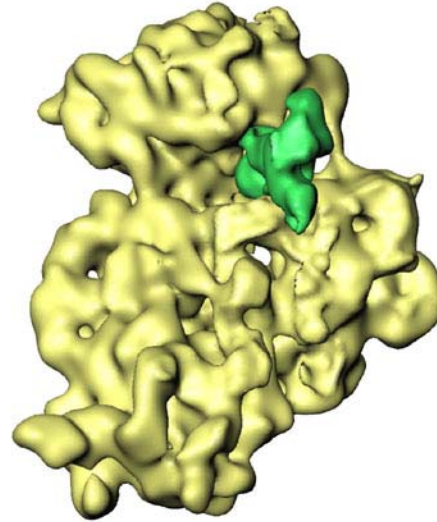
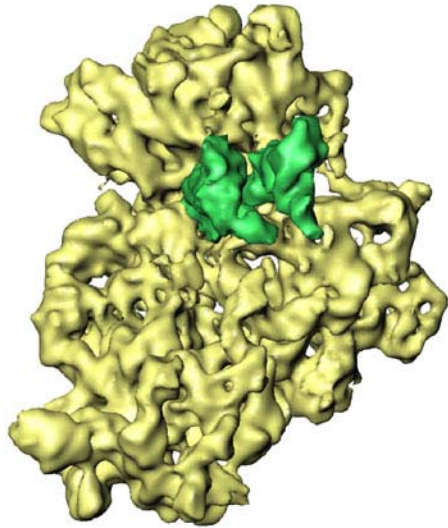
70S ribosome from *E. coli*



A + P-site tRNA
110,000 particles
8.7Å

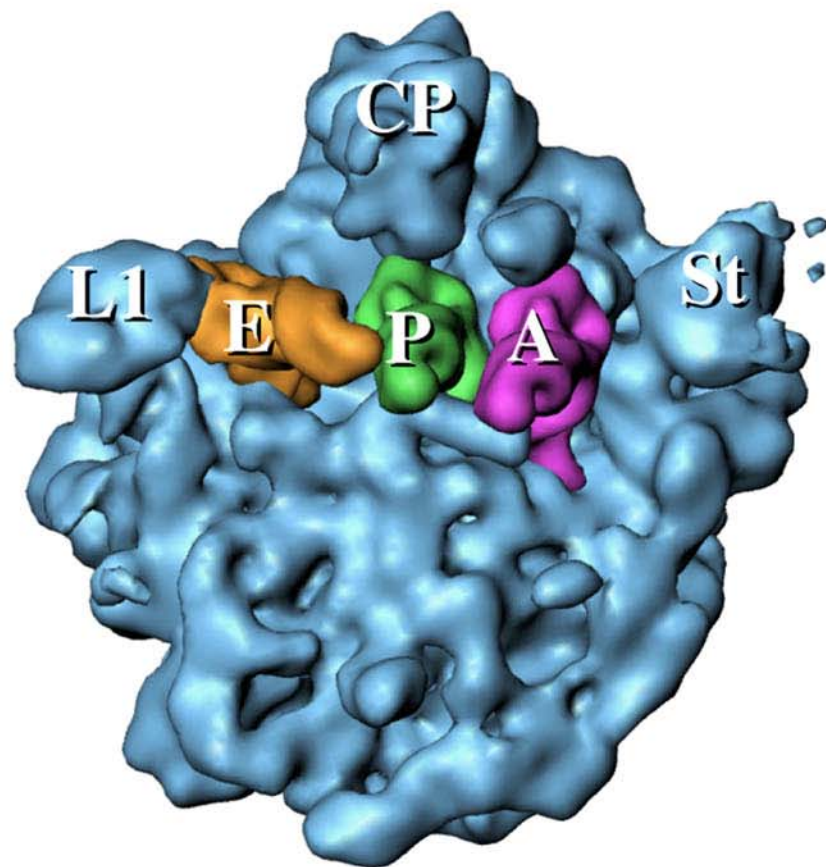
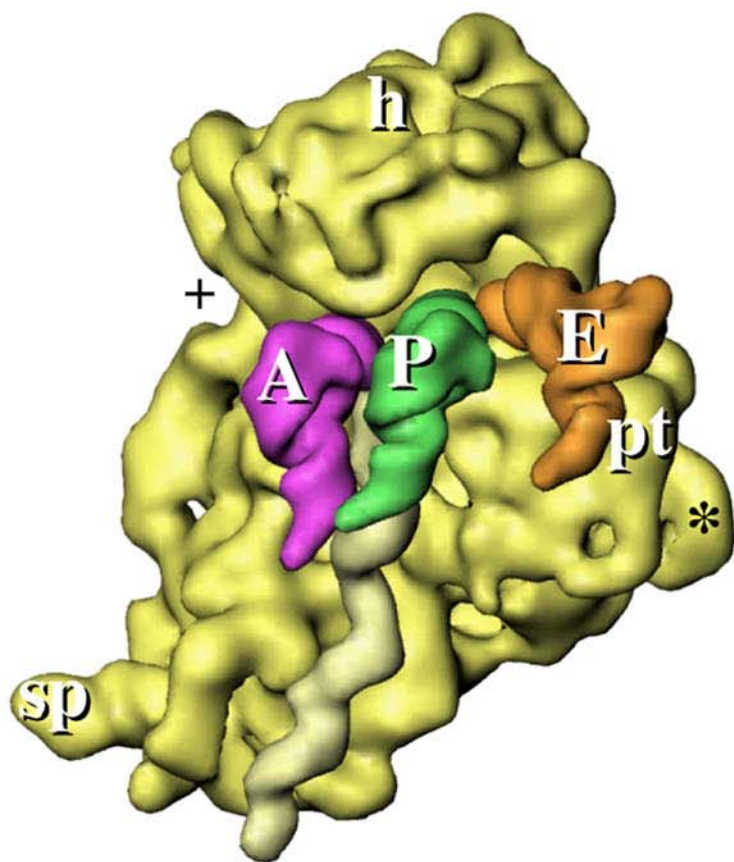
P-site tRNA
73,000 particles
11.5Å

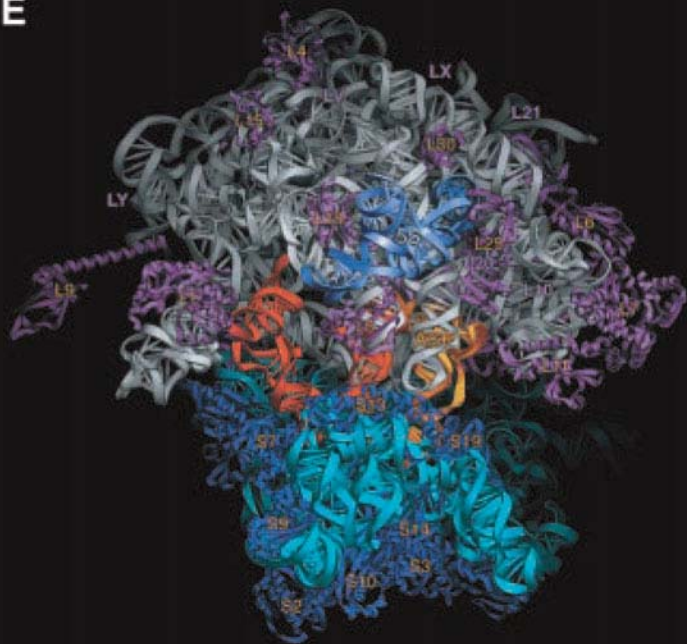
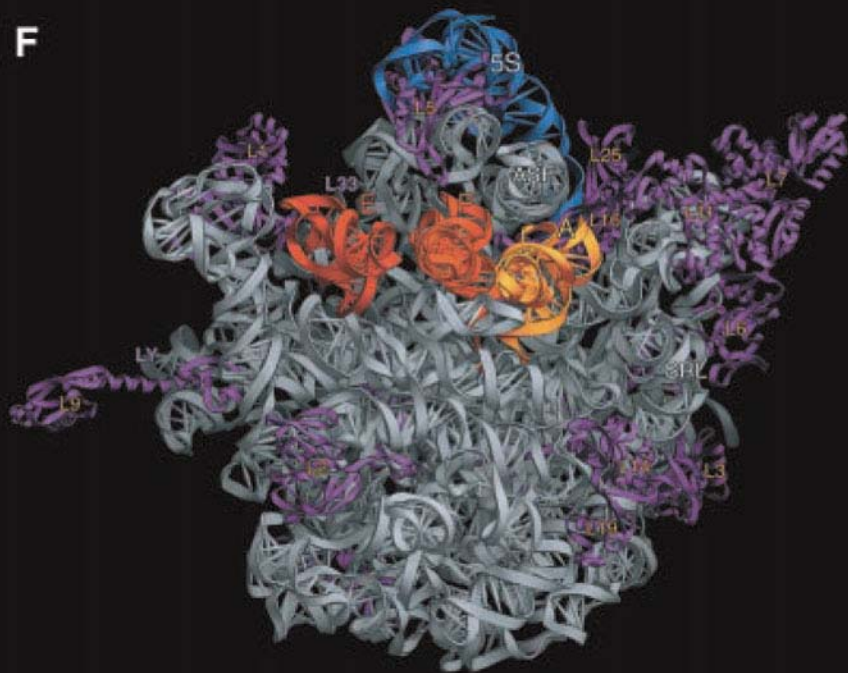
30S and 50S ribosomal subunits from *E. coli*

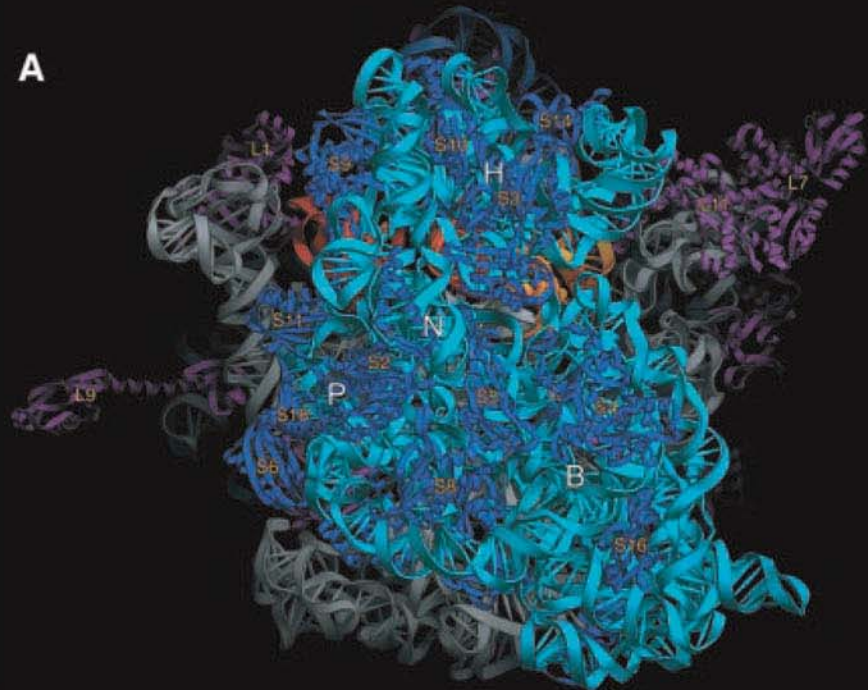
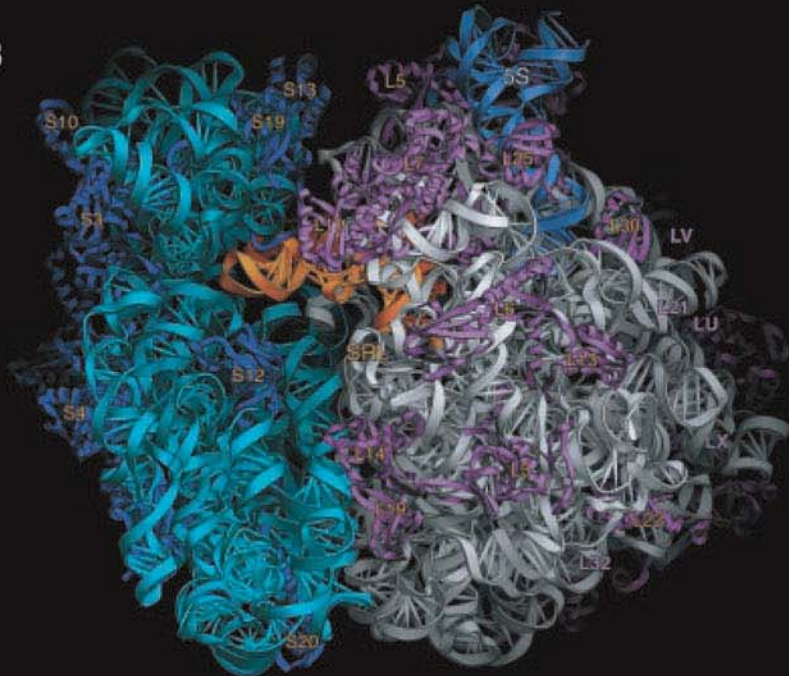
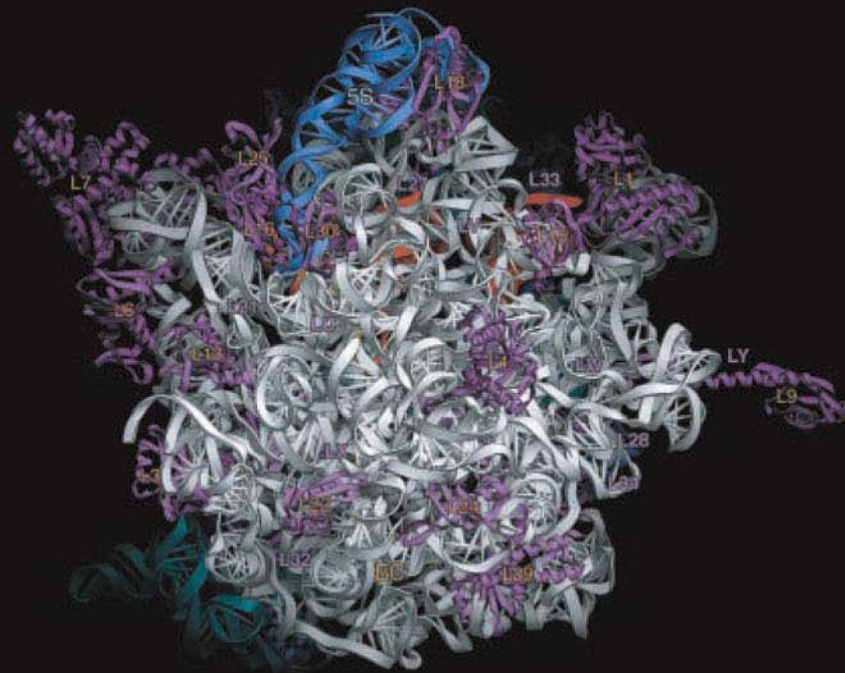
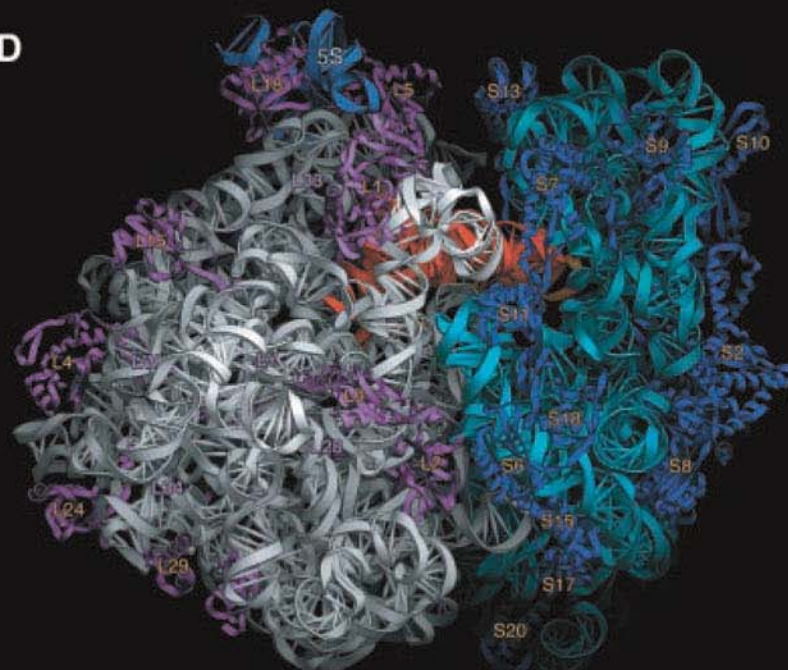


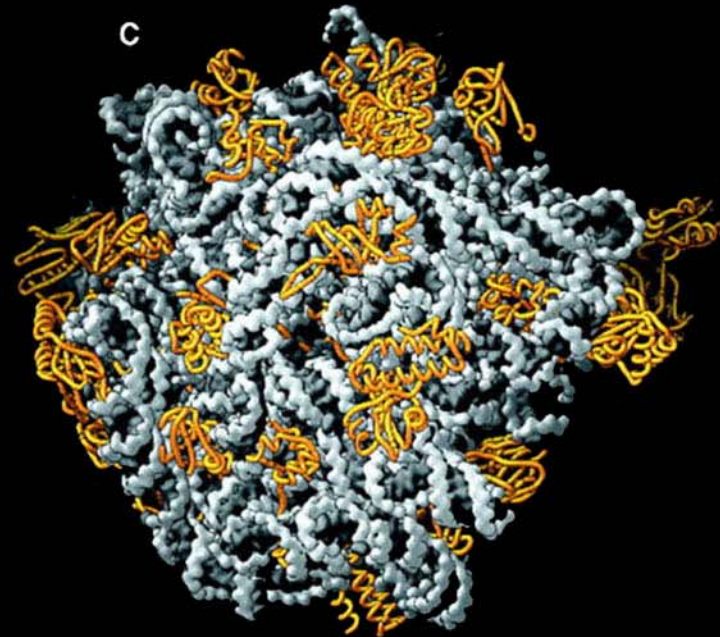
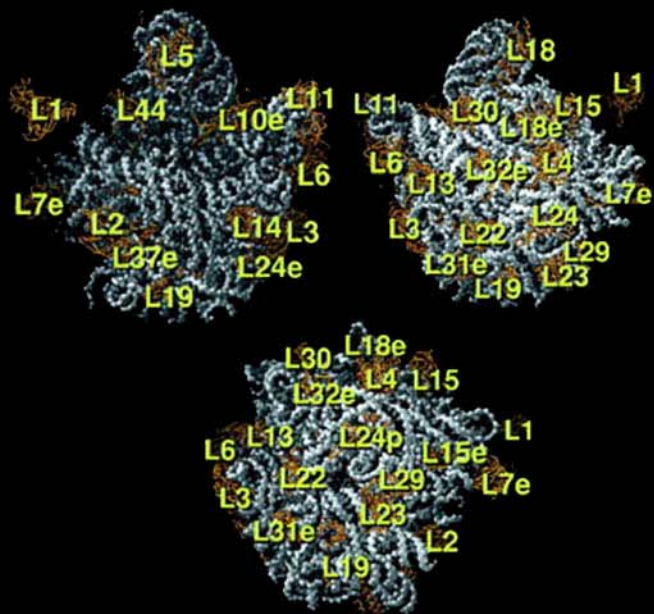
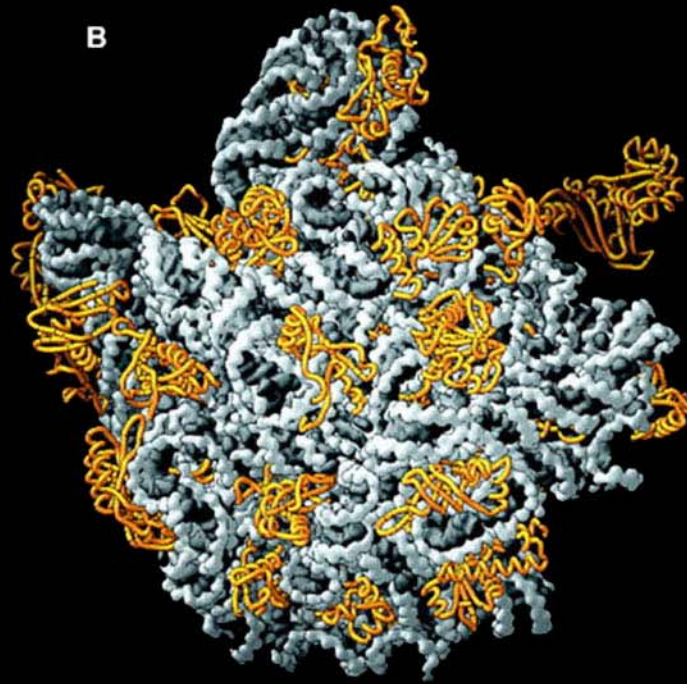
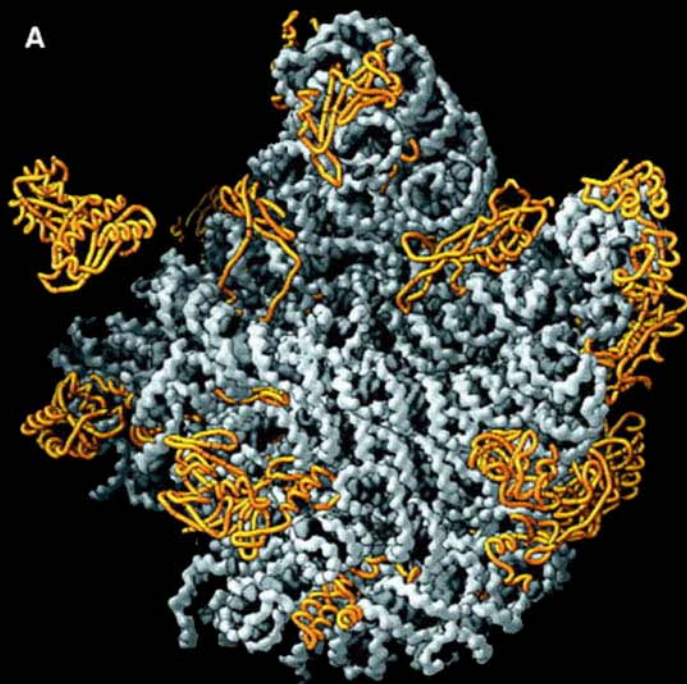
A + P-site tRNA
110,000 particles
8.7Å

P-site tRNA
73,000 particles
11.5Å



E**F****G**

A**B****C****D**



What did we learn from the X-ray structure?

- Catalytic domain of large subunit (peptidyltransferase center) is free of protein
- Proteins are mostly peripherally located, mainly on the solvent-accessible side
- But many proteins have unstructured “tentacles” that are intertwined with rRNA in the interior. Purpose: aiding in association, and possible regulatory role
- A-minor motif: minor groove interactions between rRNA helices involving adenines. Apparently contribute to stability of ribosome.
- Two adenines “read” the shape of the helix formed by mRNA and tRNA in the codon-anticodon interaction.

Bridges between 30S and 50S

mostly RNA

16 Contacts (Gabashvili et al., 2000)

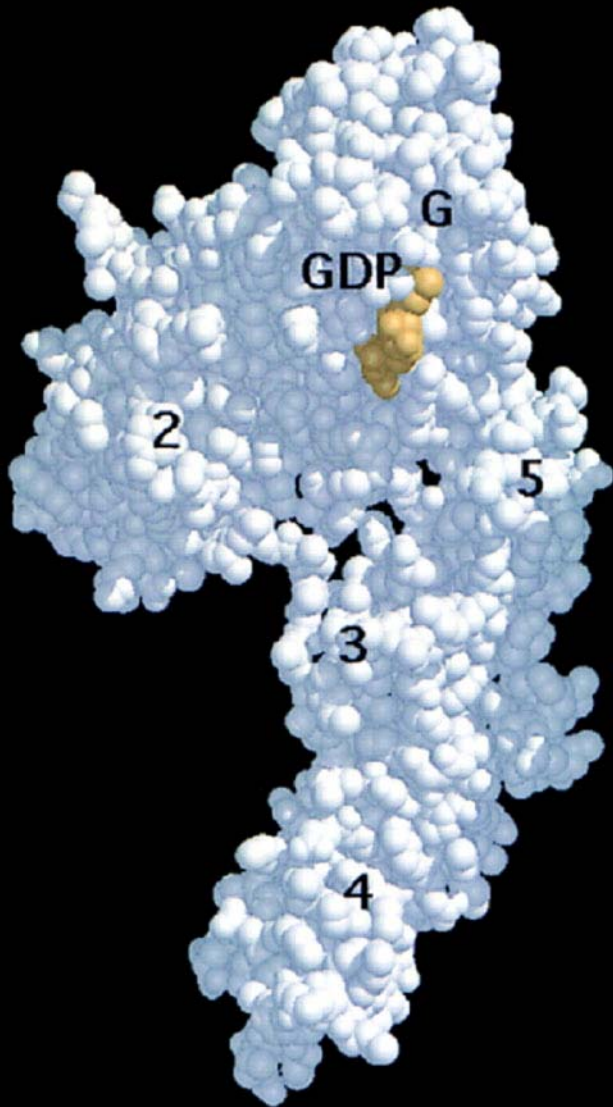
30 Contacts (Yusopov et al., 2001)

- put subunits in register
- provide flexible connection
- conformational signaling
- active role in subunit movement

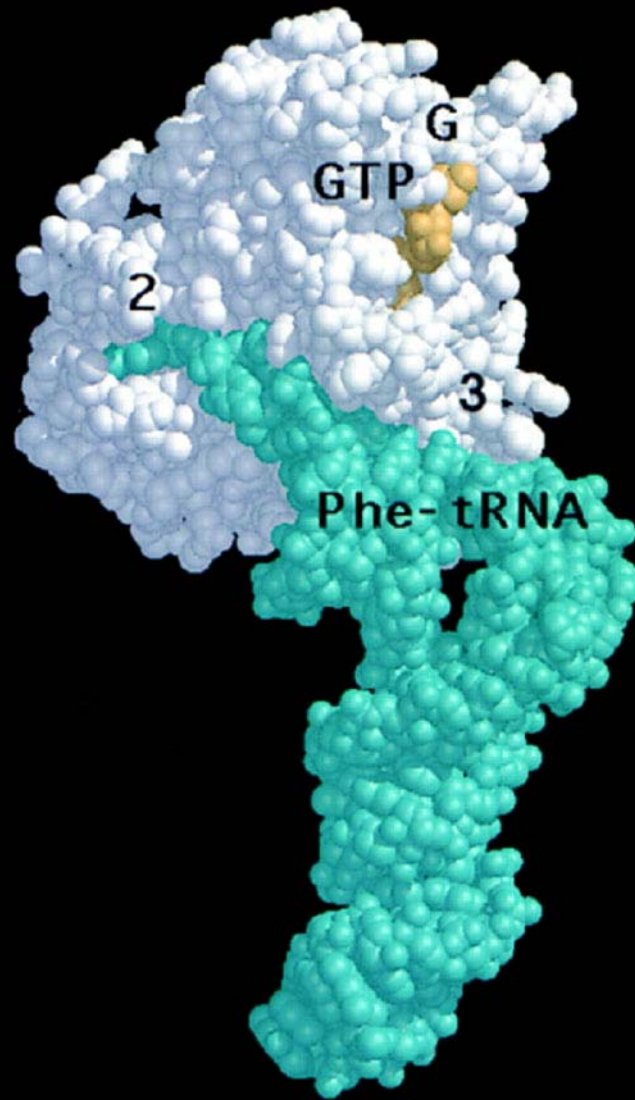
The Steps of Translation

- *Initiation*: mRNA associates with small subunit with the help of initiation factors; fMet-tRNA joins in; the complex is recognized by the large subunit => association of small+large subunit into translating ribosome.
- *Elongation*: for each codon of the mRNA, one cycle (“elongation cycle”) takes place, consisting of
 - (i) EF-Tu dependent decoding and tRNA accommodation
 - (ii) peptidyl transfer = transfer of the peptide from the P-site tRNA to the amino acid carried by the A-site tRNA, and
 - (iii) EF-G dependent translocation of tRNAs and mRNA.
- *Termination*: upon recognition of a stop codon by one of the release factors, the release factor binds tightly to the ribosome and cleaves the polypeptide chain off the P-site tRNA.
- *Recycling*: the post-termination complex is dissociated into its components with the aid of a recycling factor (RRF) and EF-G

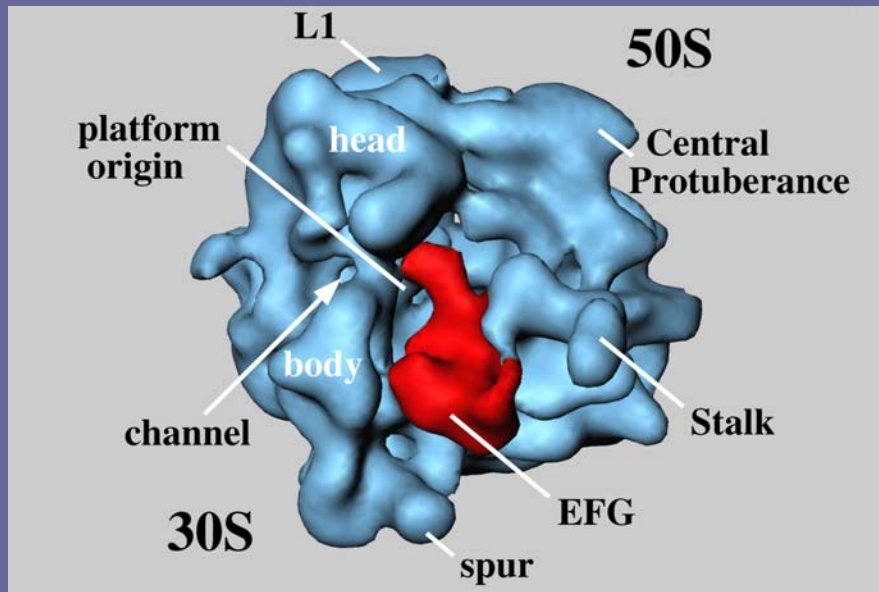
EF-G



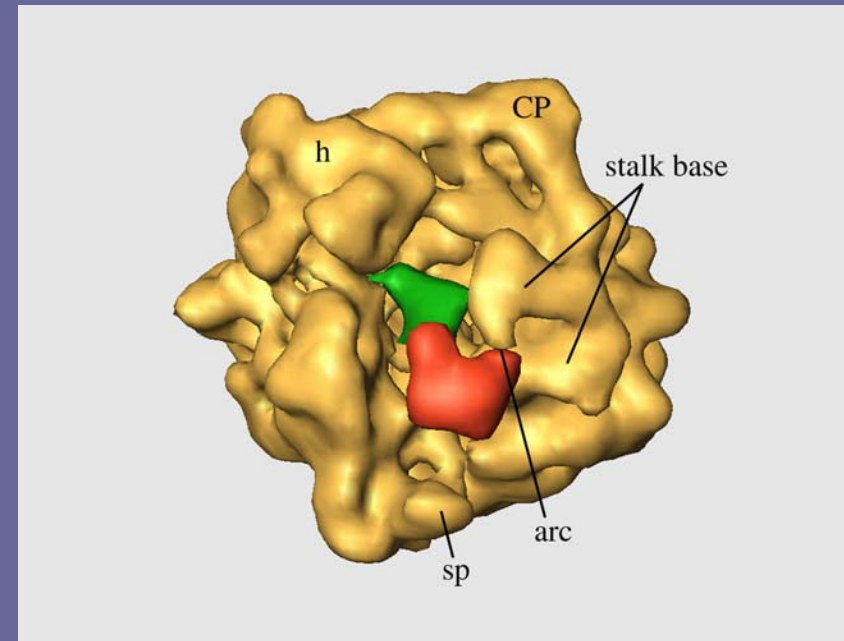
EF-Tu
ternary complex



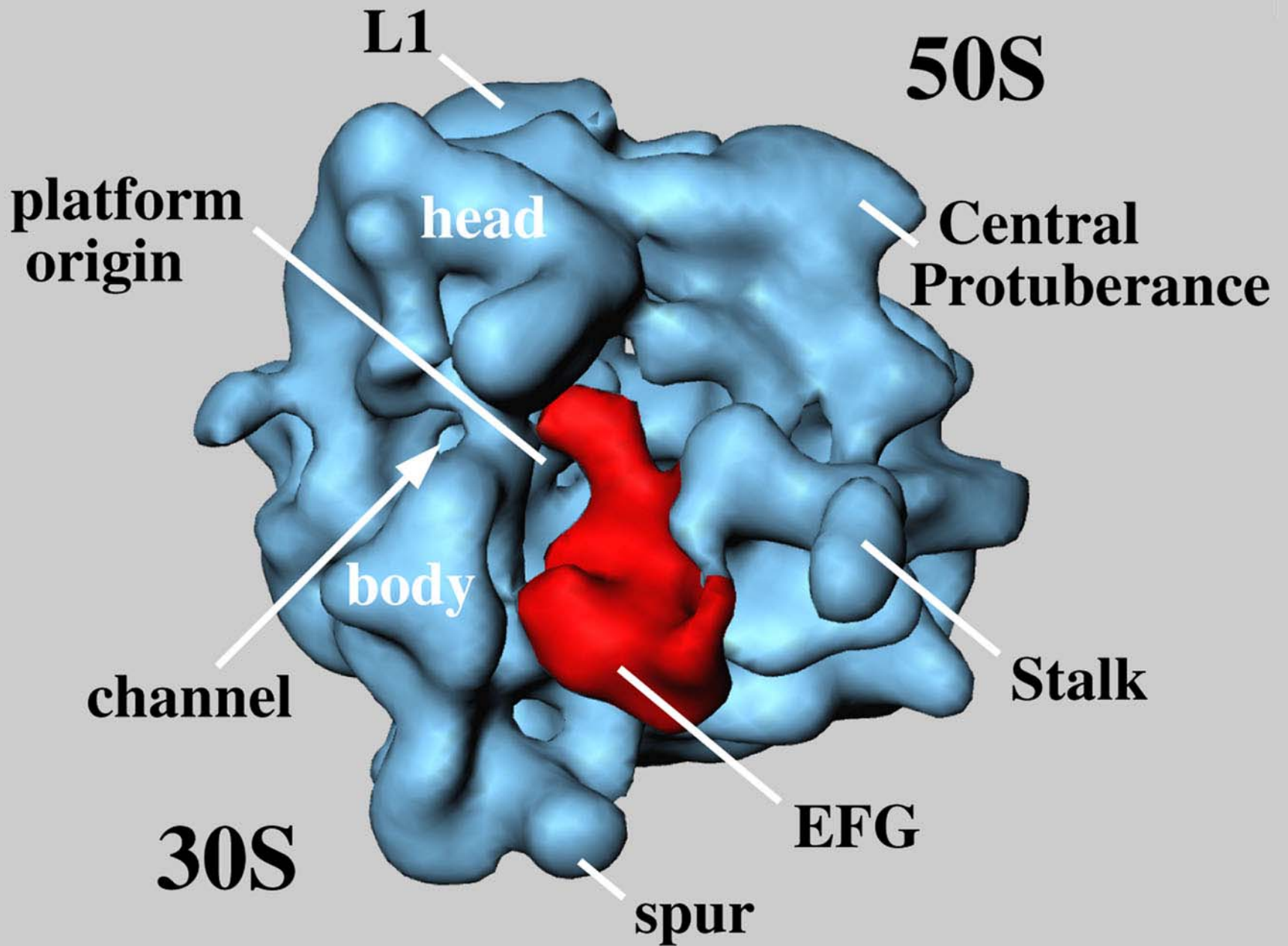
Implications of Structural Mimicry



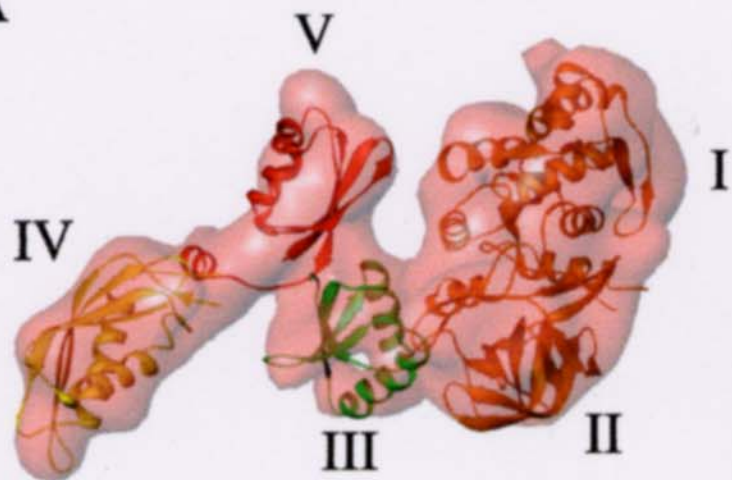
Agrawal et al., PNAS 1998
EF-G•GDP, fusidic acid



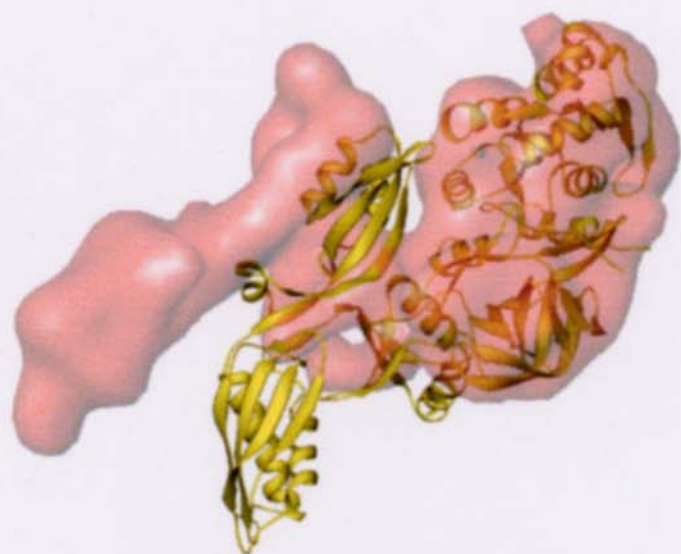
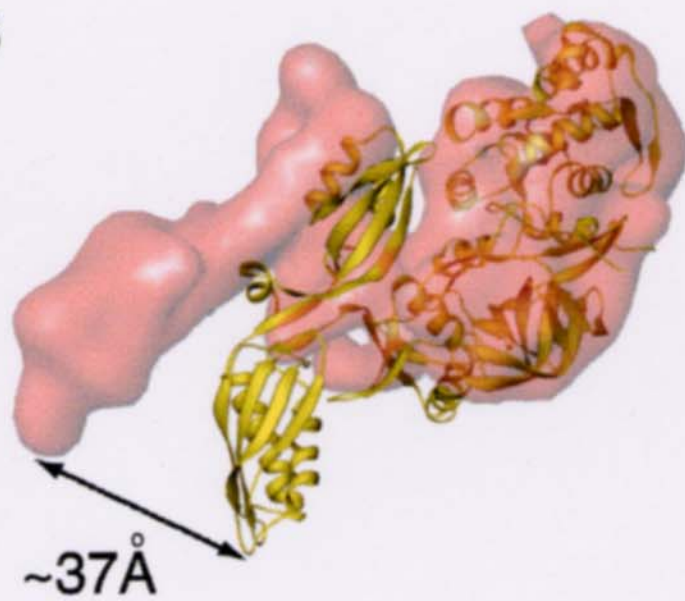
Stark et al., Nature 1997
ternary complex, kirromycin

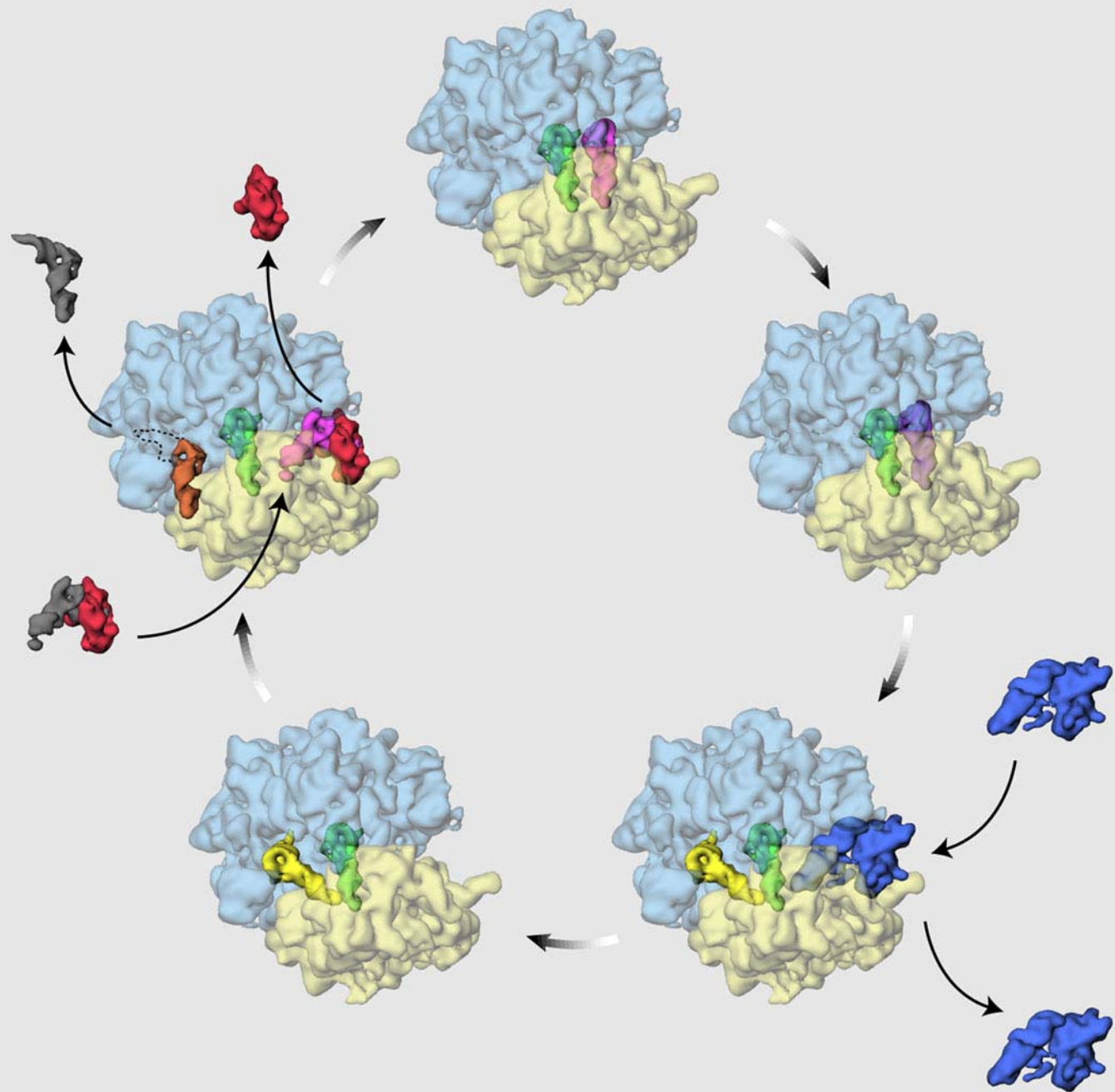


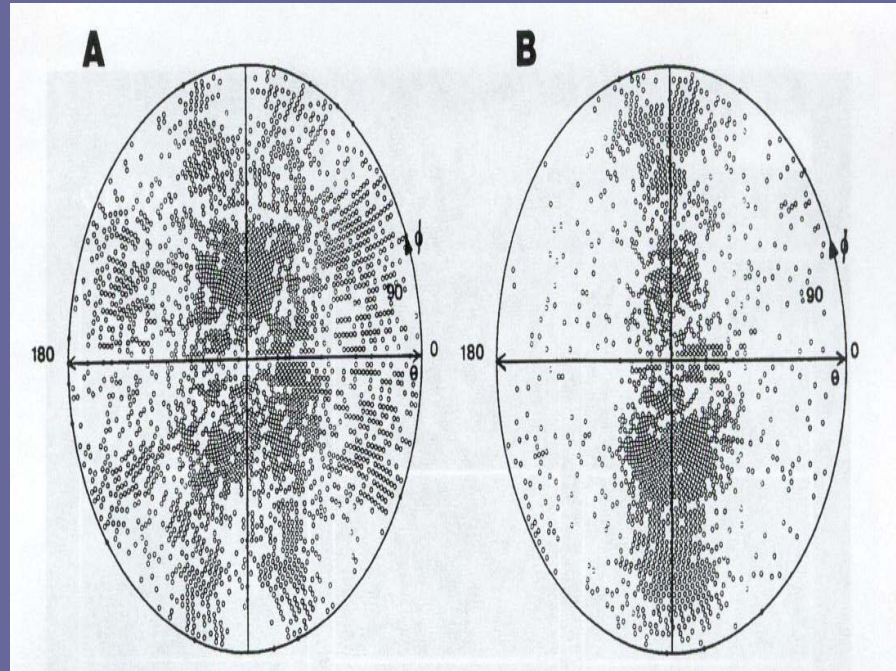
A



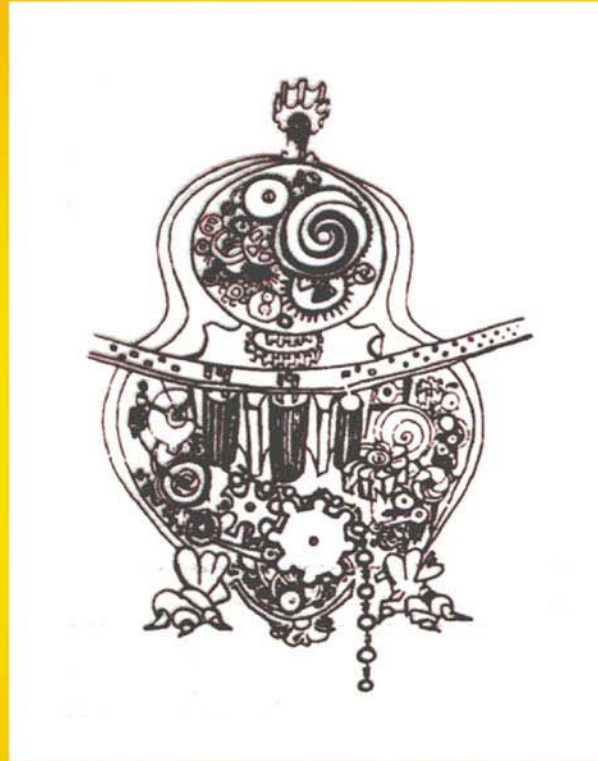
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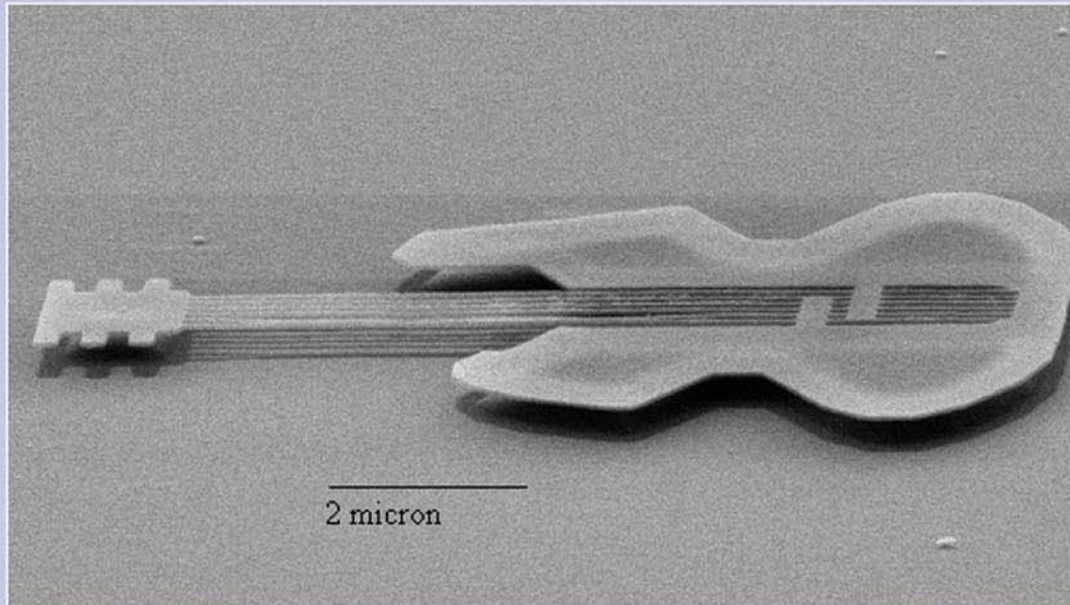




The Ribosome ...



a clockwork?



... or a machine with modes of motions intrinsic to its architecture?

Dynamics of ribosome and its binding to ligands can be inferred from 3D cryo-EM images of the ribosome in different states, trapped by . . .

1. use of antibiotics (e.g., fusidic acid, thiostrepton, kirromycin)
2. non-hydrolyzable GTP analogs

Advantage: easy to use

Disadvantage: time points are pre-ordained

3. physical methods: spray-freezing or use of caged compounds

Advantage: possible to choose any time point.

Disadvantage: experimentally demanding

tRNA and mRNA Translocation

Sequence of Events:

1) Binding of Elongation Factor G complexed with GTP

-----*GTP nonhydrolyzable analog*-----

2) Translocation part I

3) GTP hydrolysis

4) Translocation part II

-----*fusidic acid*-----

5) Release of EF-G•GDP

Spontaneous tRNA and mRNA Translocation

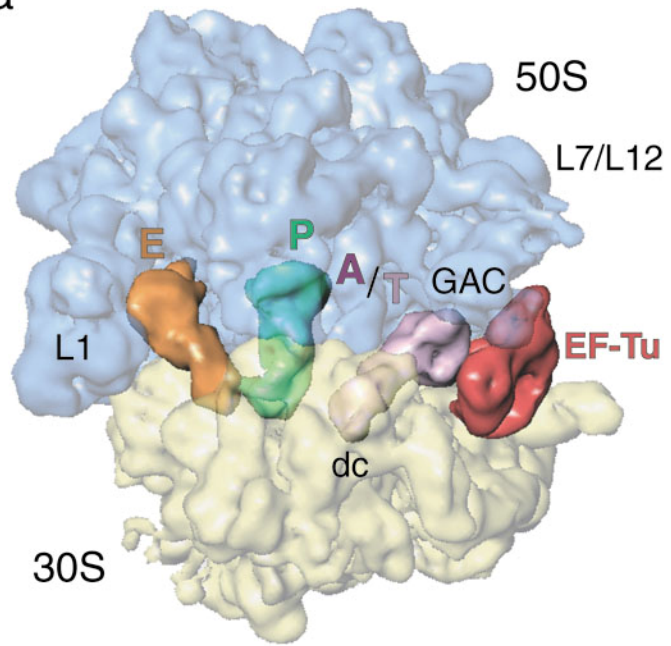
- Rate much lower than with EF-G
- Accelerated with antibiotic sparsomycin
- Intrinsic property of the ribosome
- Suggests existence of two states (“pre-” and “post-translocational”) separated by a low energy barrier

Decoding and Accommodation

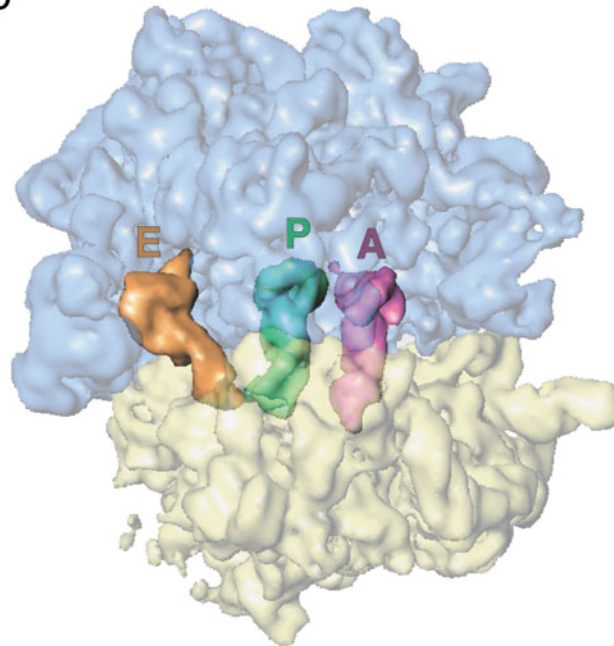
Sequence of events (cognate case):

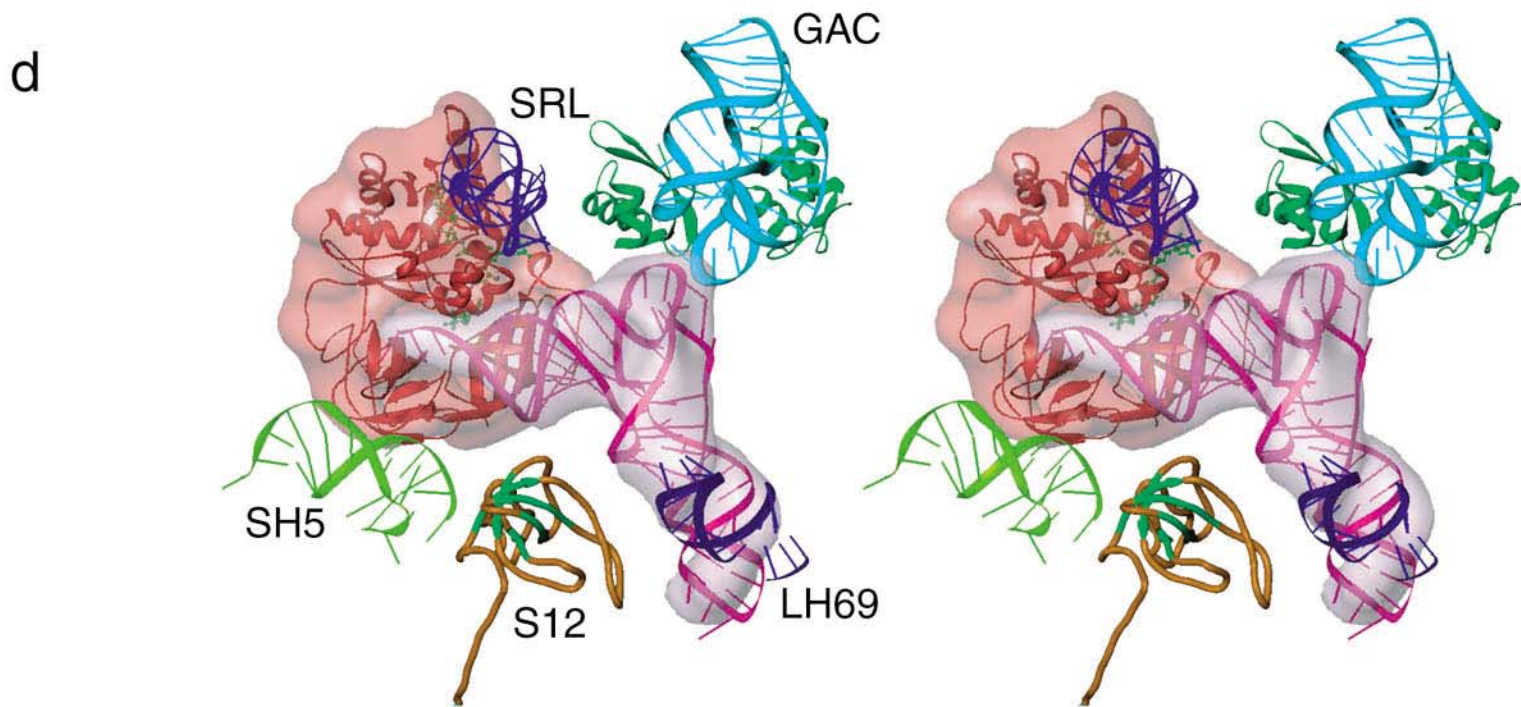
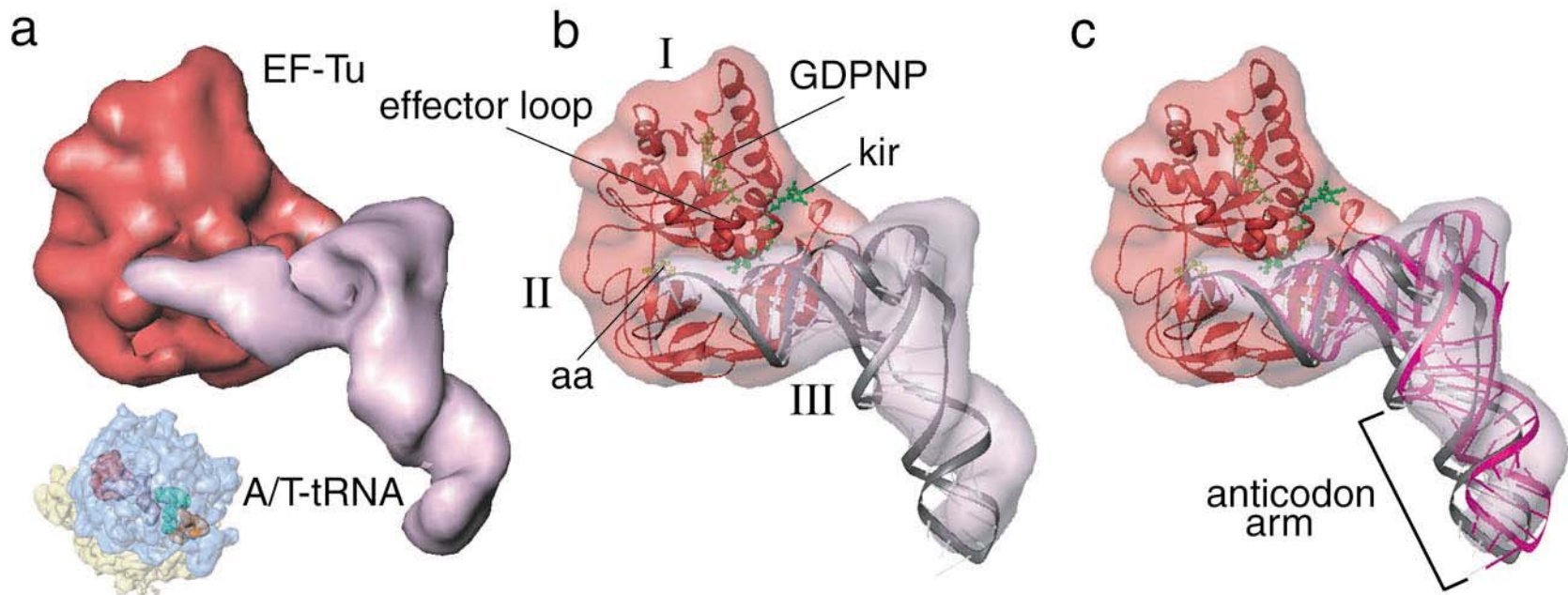
- 1) Initial binding of the ternary complex
aa-tRNA•EF-Tu•GTP
- 2) Codon recognition (codon-anticodon pairing)
-----*GTP nonhydrolyzable analog*-----
- 3) GTP hydrolysis
- 4) EF-Tu changes conformation
-----*kirromycin*-----
- 5) Release of EF-Tu•GDP
- 6) Accommodation of tRNA in the A site

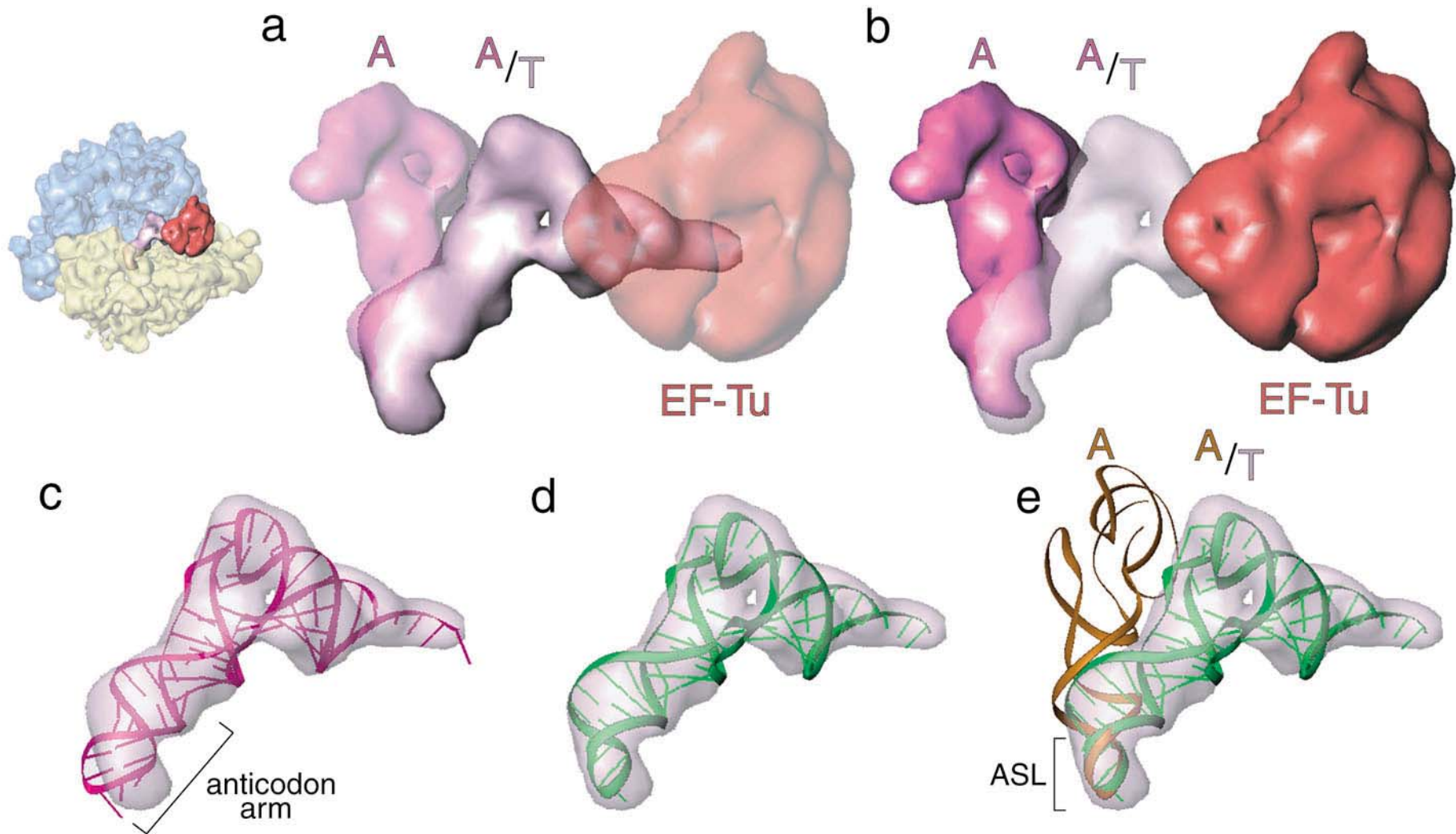
a

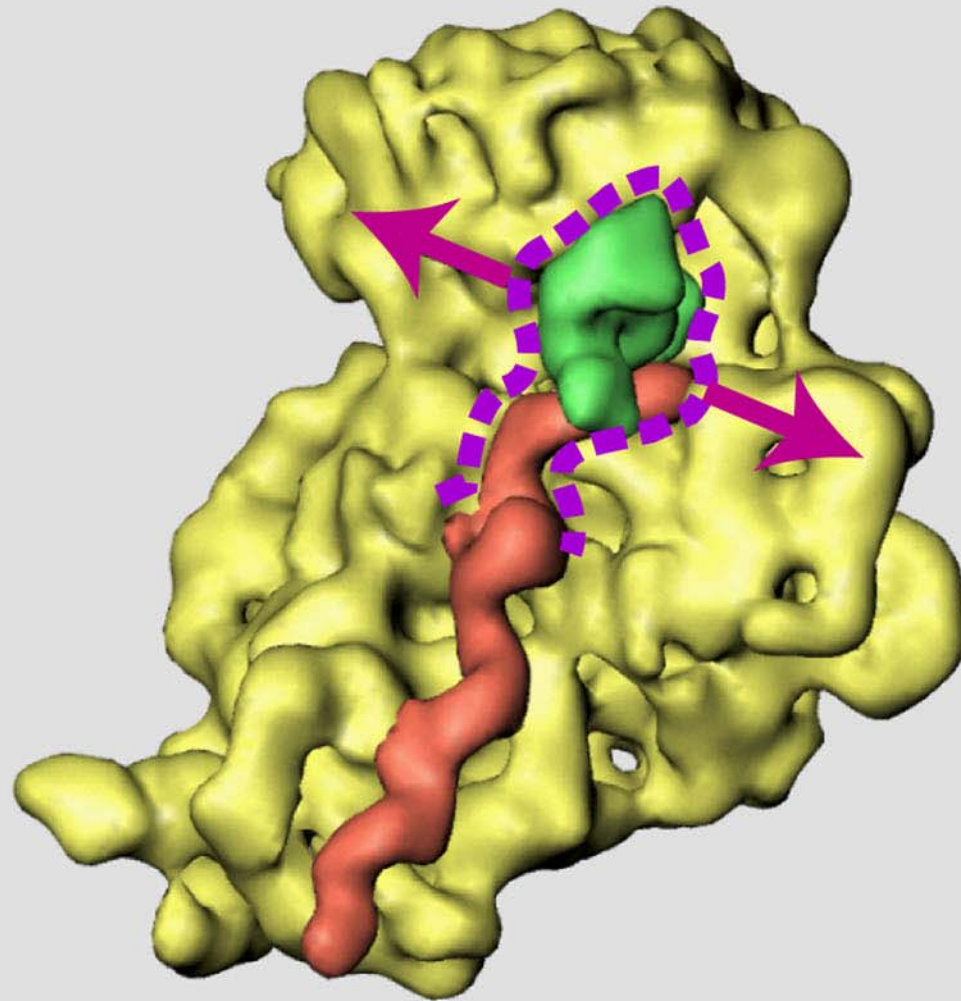


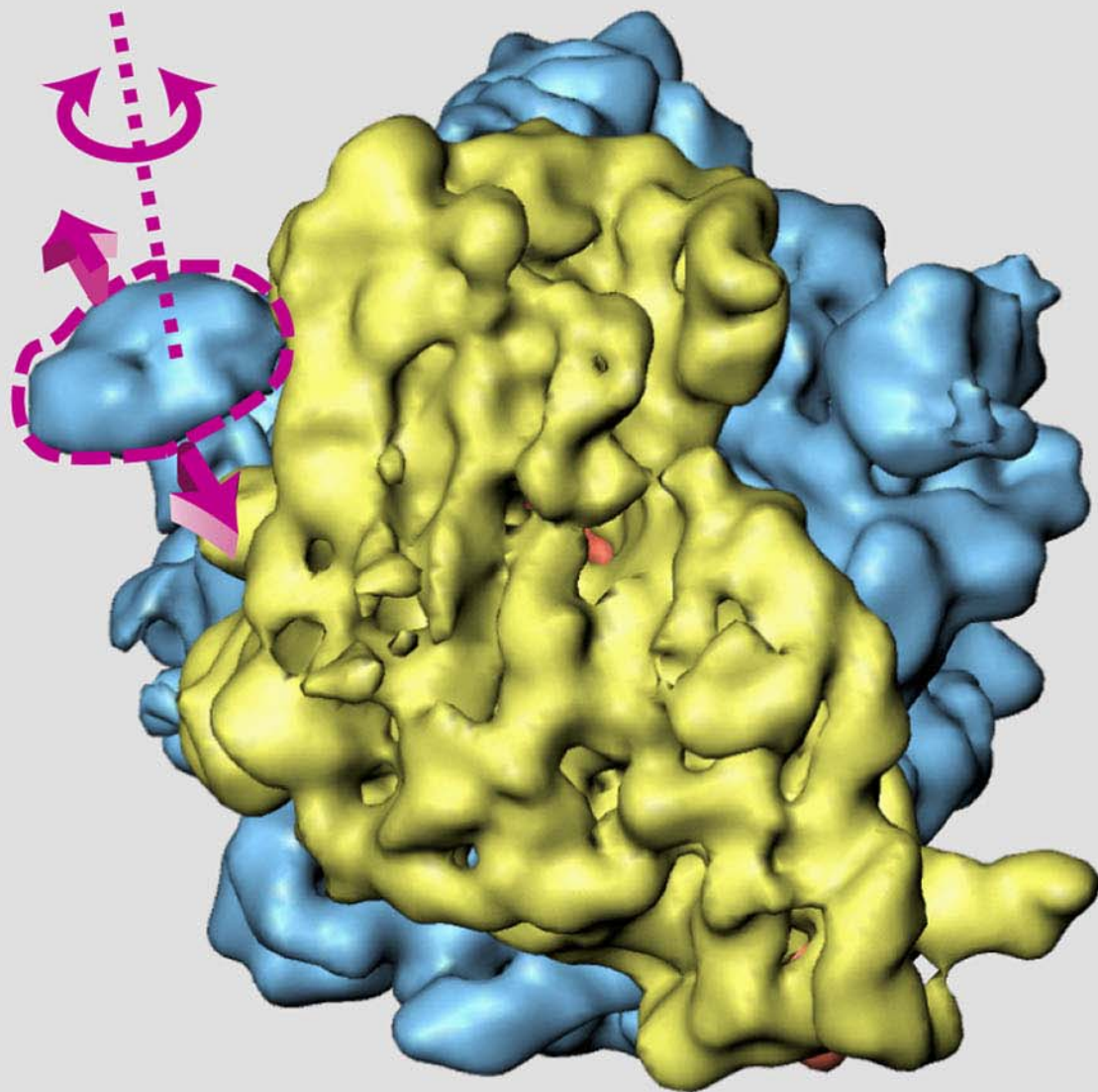
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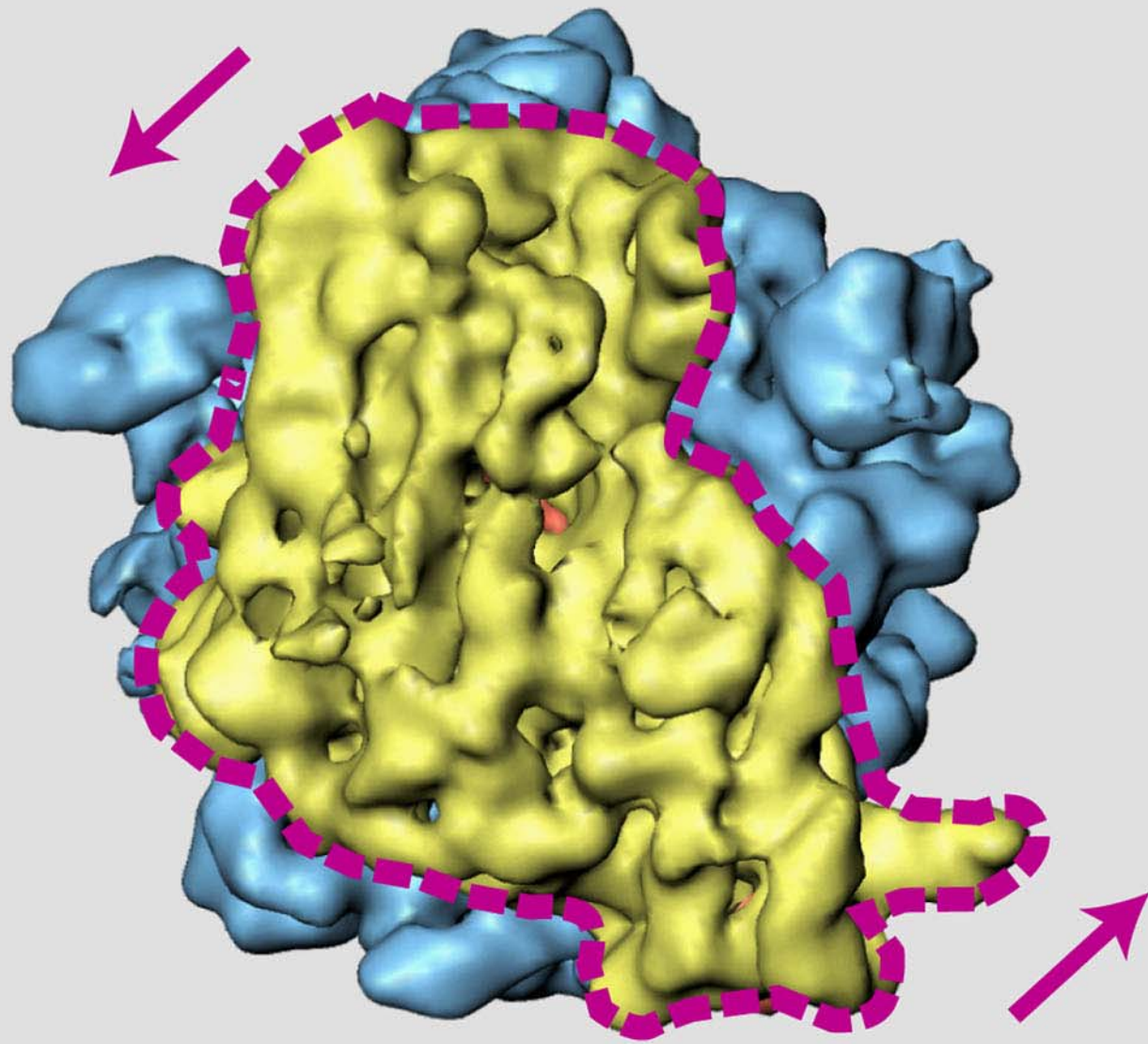






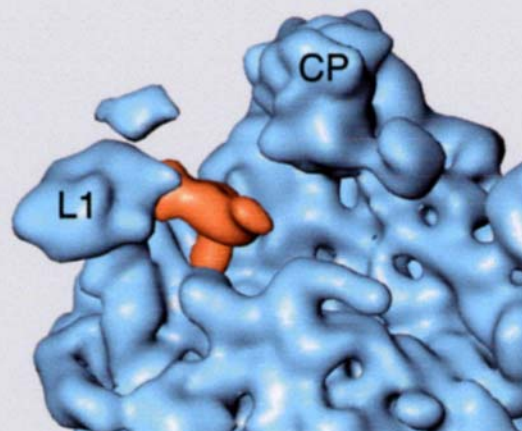
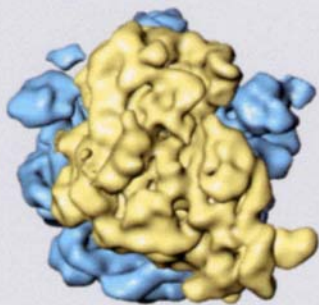
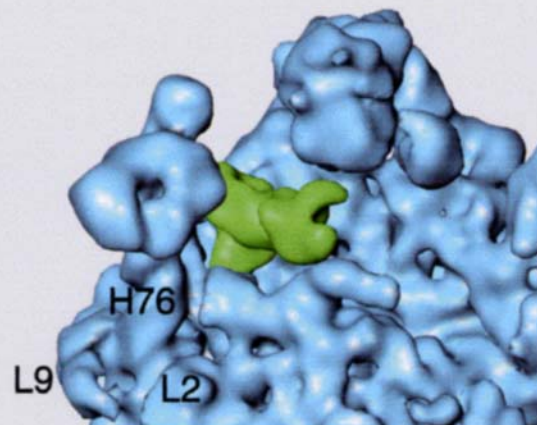
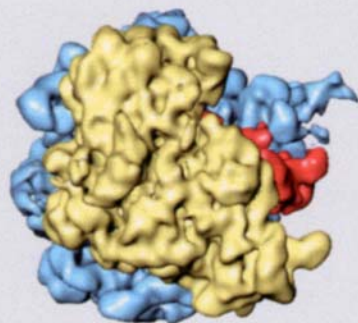
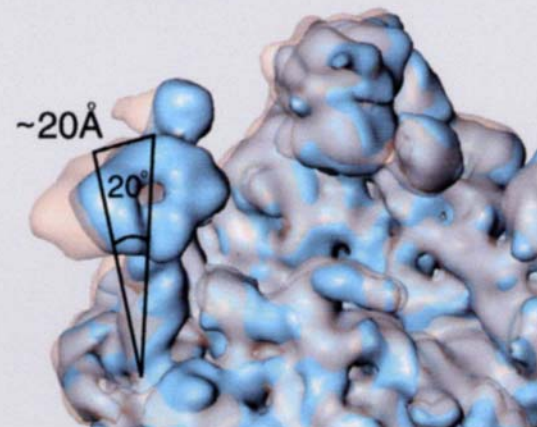






The Ratchet Motion

- counter-clockwise motion of 30S relative to 50S subunit;
- Part I of translocation of tRNA-mRNA complex (moves mRNA into the “right” direction);
- observed upon binding of EF-G (GTP state, or GDP state stabilized with fusidic acid), but not with EF-Tu;
- only observed when P-site tRNA is deacylated (ribosome in the “unlocked” state);
- goes hand in hand with internal reorganization of 30S (e.g., mRNA channels expand/contract) and flexing motion of L1 stalk of 50S subunit.
- other binding events triggering the ratchet motion: RF3, RRF, and EF2 (= equivalent of EF-G in eukaryotes)

A**D****B****E****C****F****G**

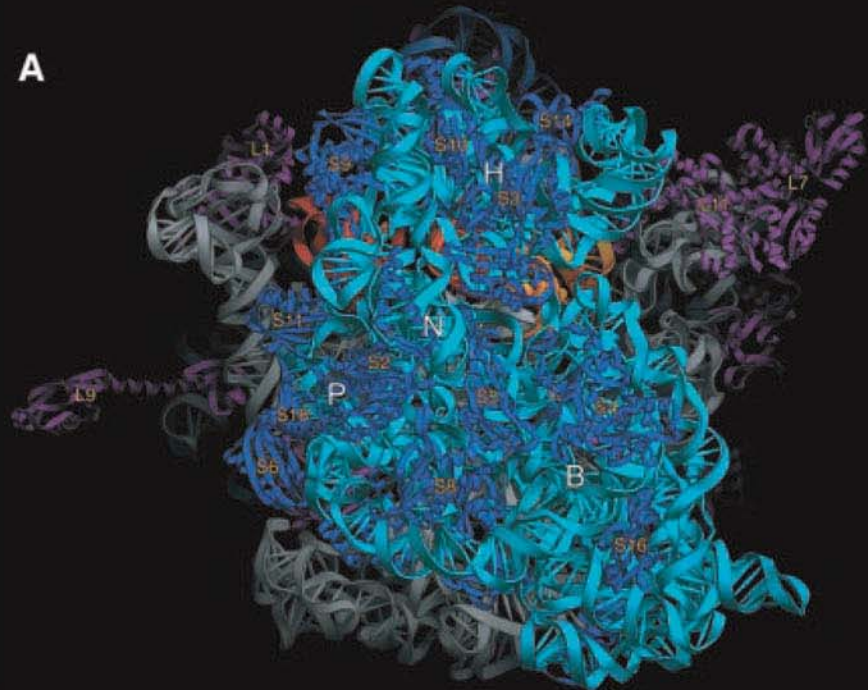
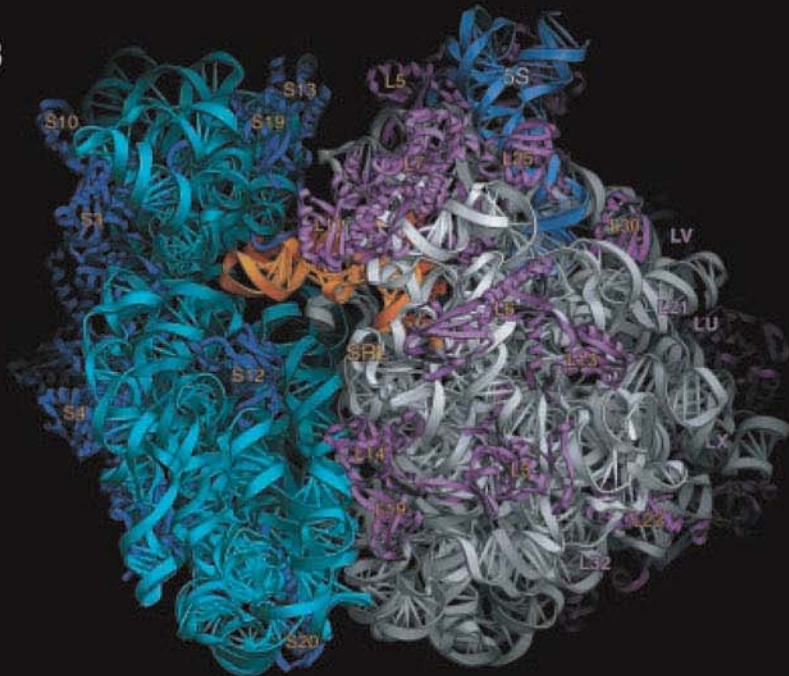
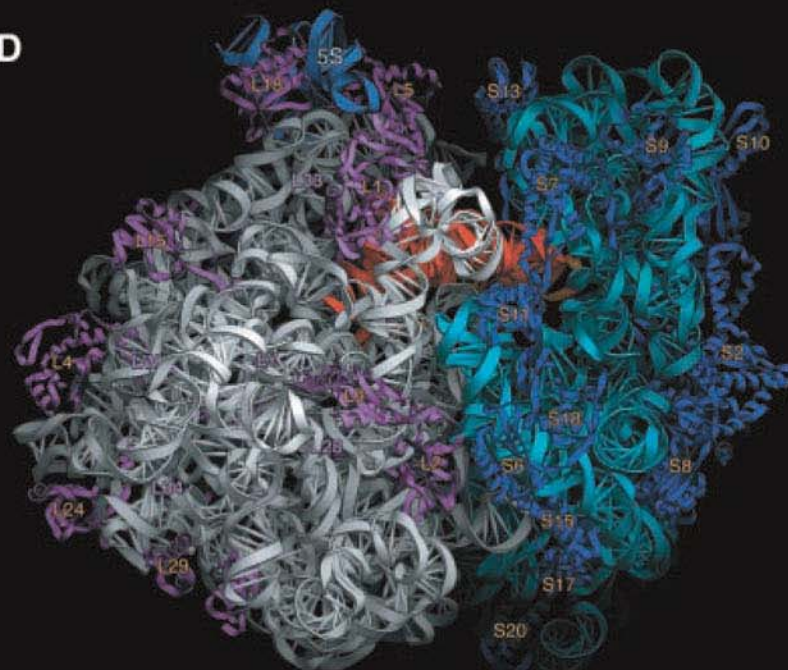
**Conformational Changes in
the *E. coli* Ribosome Triggered
by EF-G Binding**

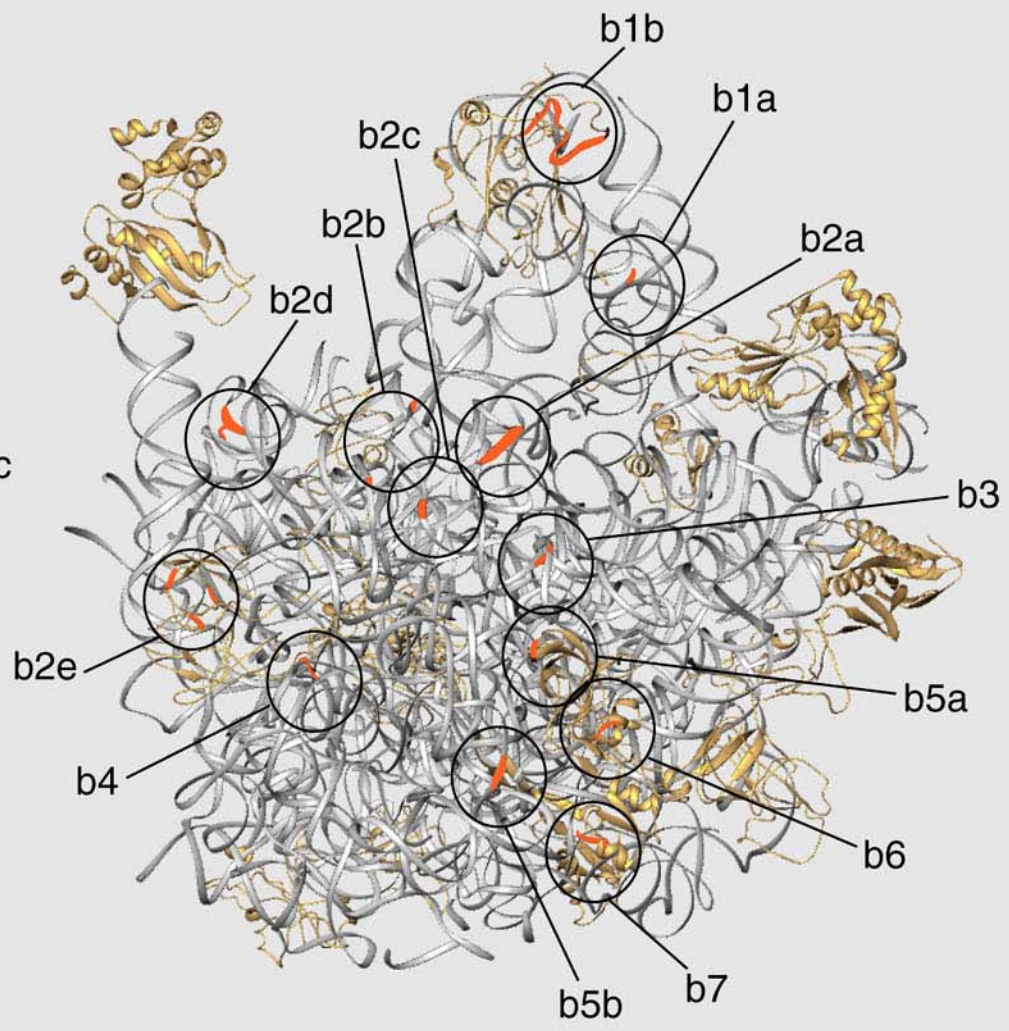
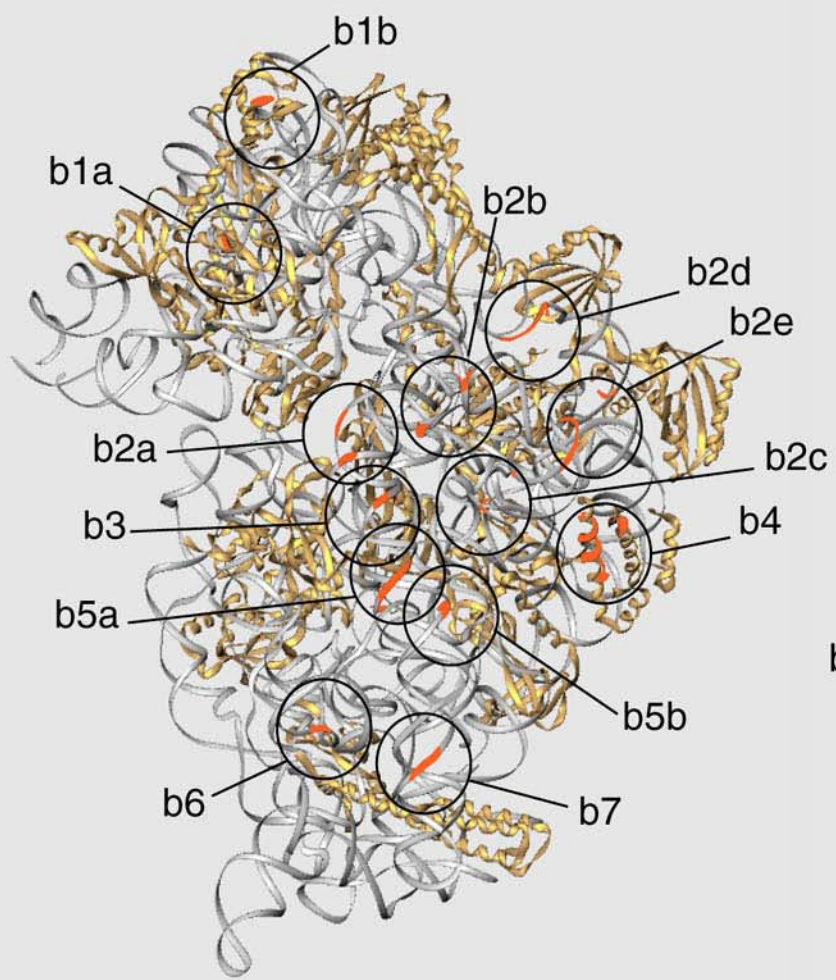
**Mikel Valle
Joachim Frank**

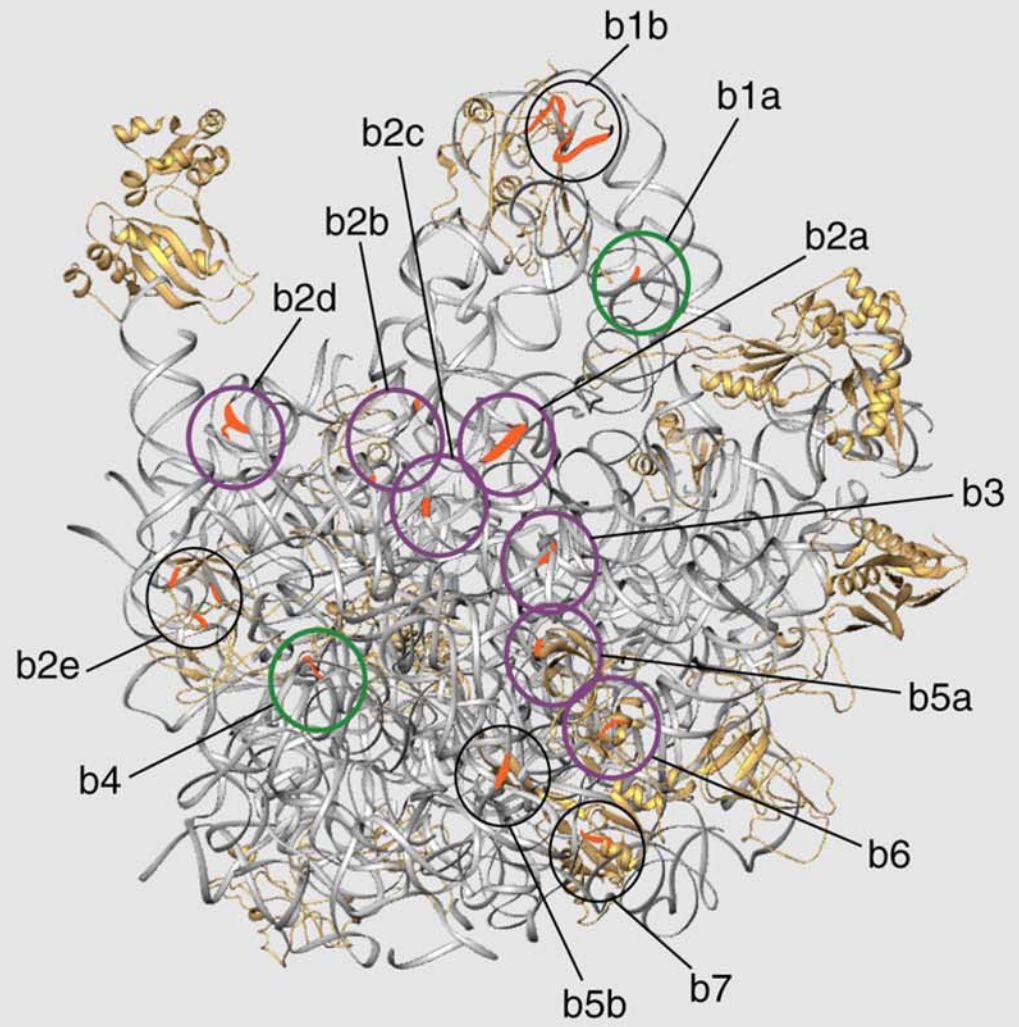
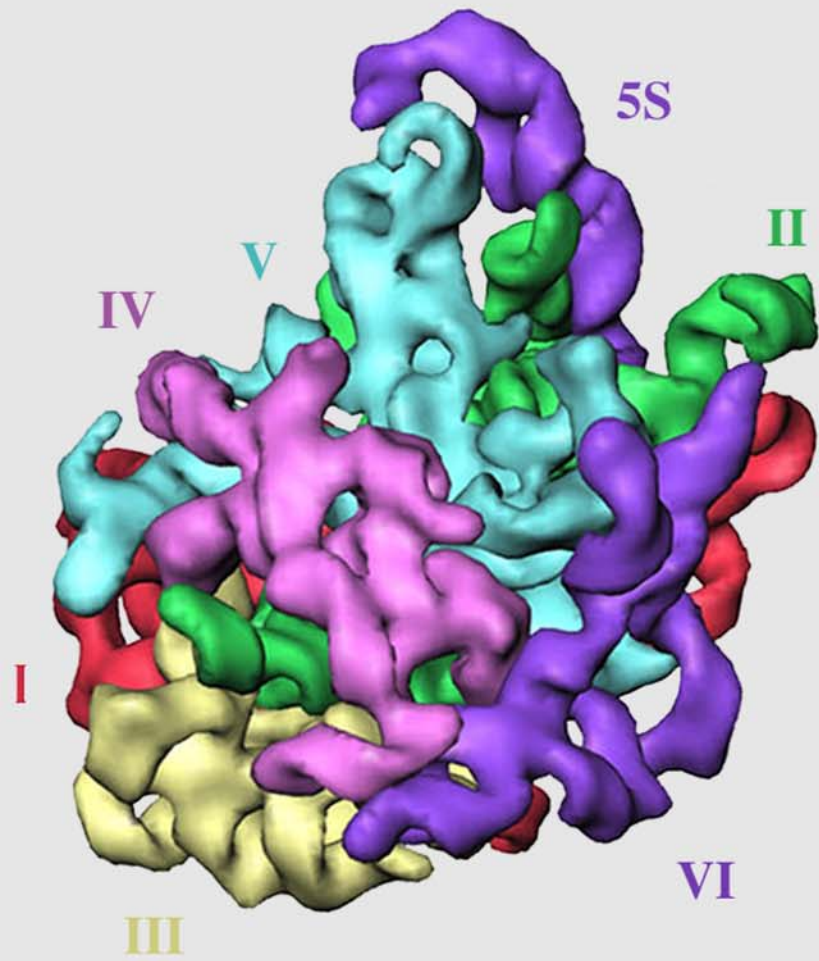
animation

Amy Heagle Whiting

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A**B****C****D**





Real-Space Refinement Using RSRef (Chapman et al., 1995) and TNT (Tronrud et al., 1987)

$$R = R_\rho + R_{\text{geom}} \longrightarrow \min$$

$$R_\rho(P, S, k) = \sum_{xyz} \{ [S \rho_o(xyz) + k] - \rho_c(P, xyz) \}^2$$

atomic parameters

observed density

scaling parameter

scaling parameter

calculated density



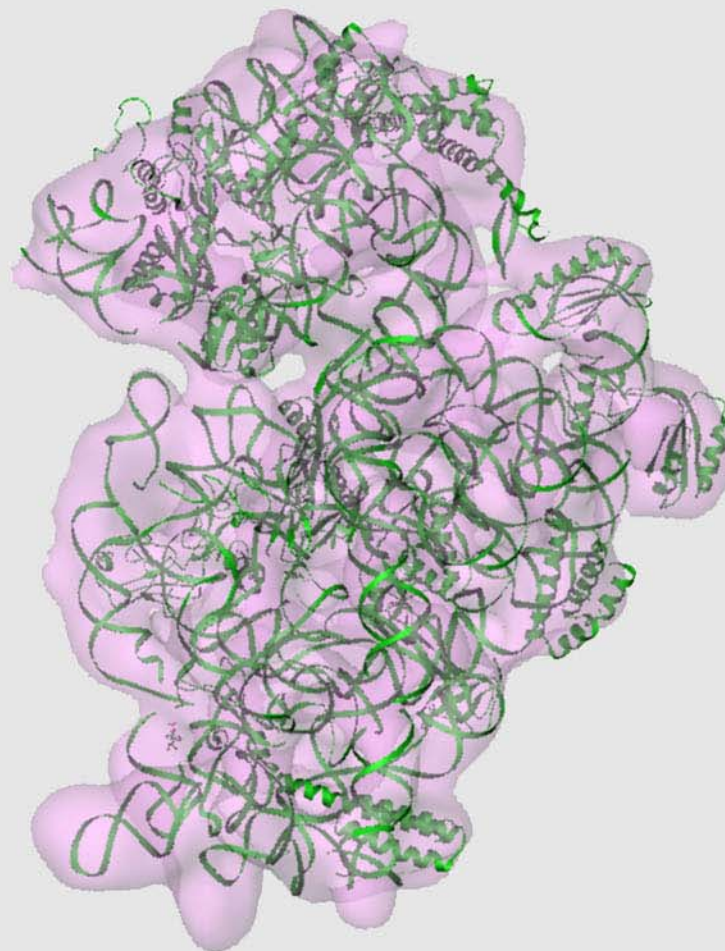
Initiation-like complex

Resolution at 11.5Å

Cross-correlation = 0.70

R-factor = 0.22

#poor vdW = 2760



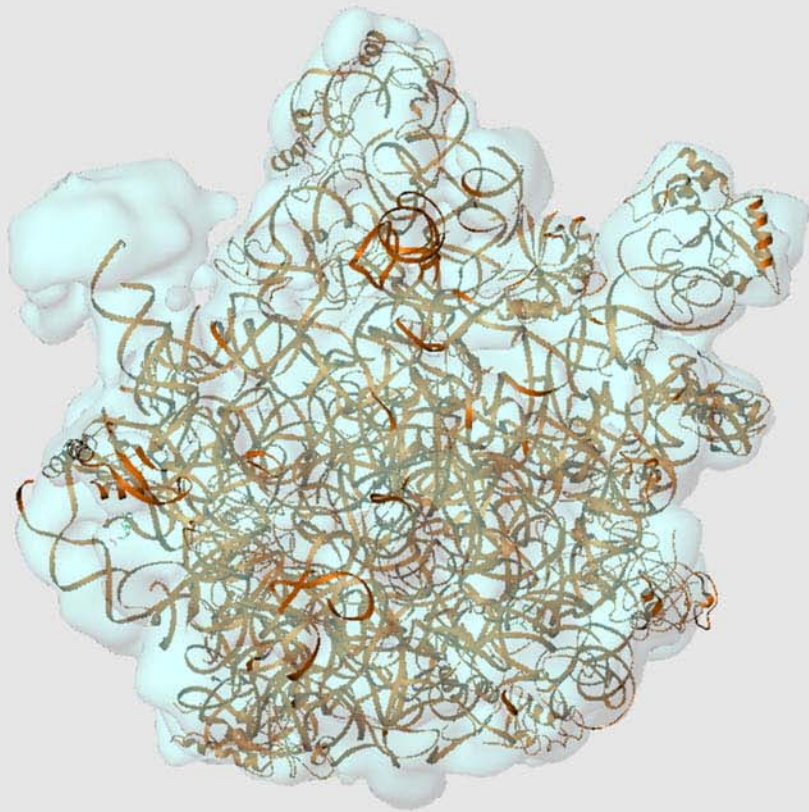
EF-G · GMPP(CH₂)P - bound complex

Resolution at 12.3Å

Cross-correlation = 0.67

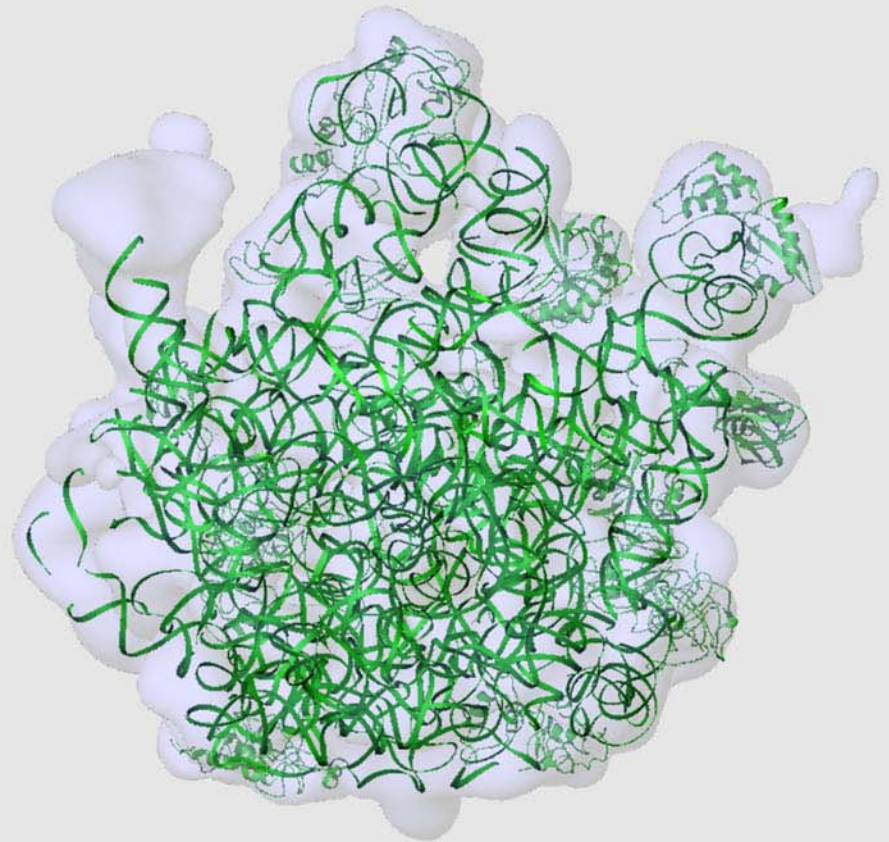
R-factor = 0.25

#poor vdW = 2822



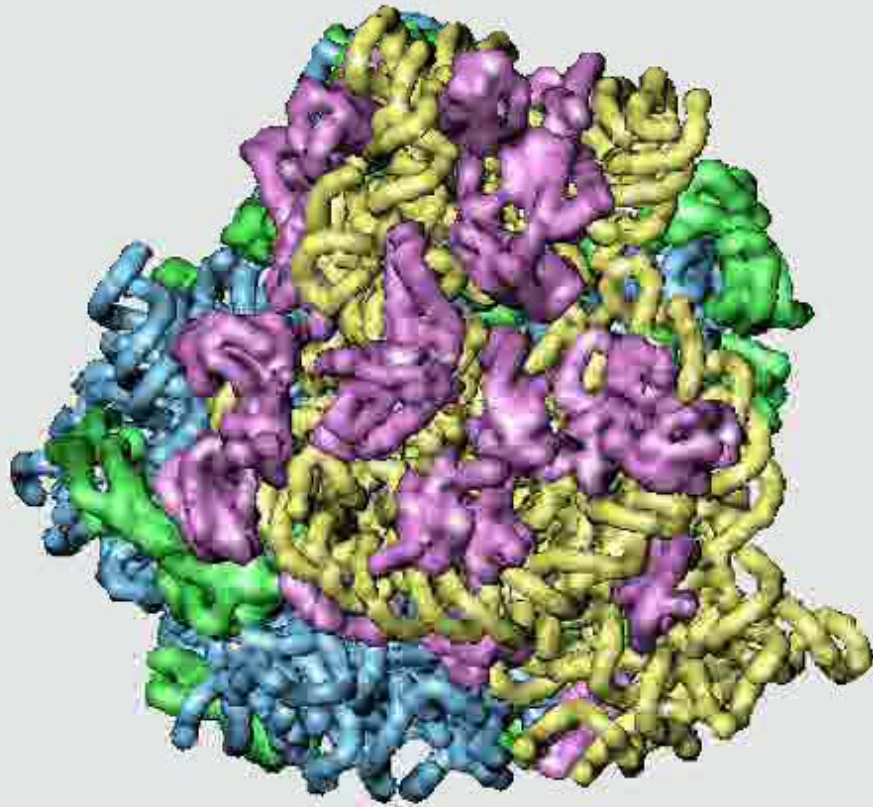
Initiation-like complex

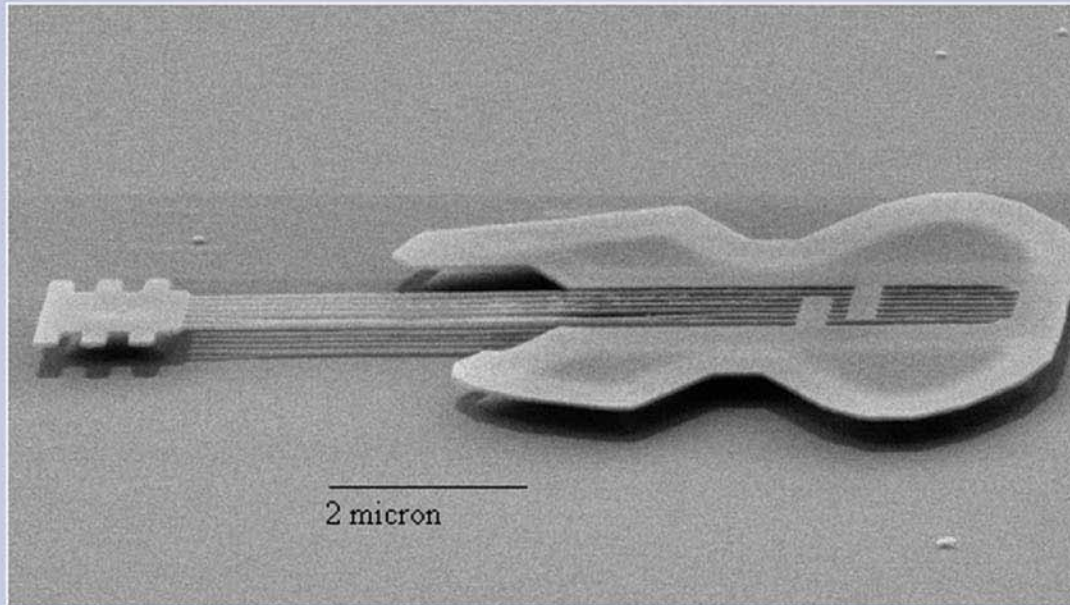
Resolution at 11.5Å
Cross-correlation = 0.70
R-factor = 0.22
#poor vdW = 2760



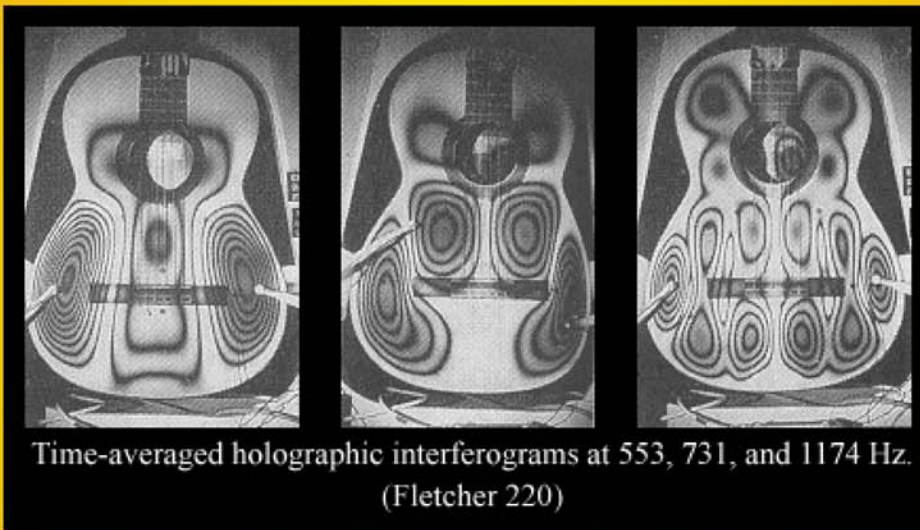
EF-G · GMPP(CH₂)P - bound complex

Resolution at 12.3Å
Cross-correlation = 0.67
R-factor = 0.25
#poor vdW = 2822

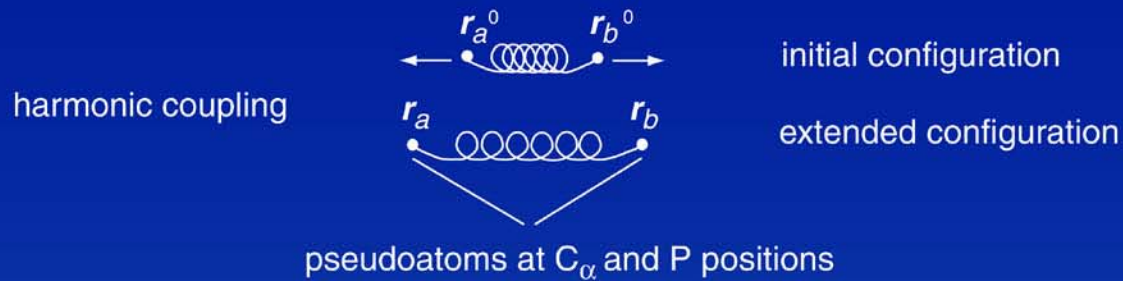




... or a machine with modes of motions intrinsic to its architecture?



Normal Mode Analysis



$$U(\mathbf{r}_a, \mathbf{r}_b) = \begin{cases} \frac{k}{2} (|\mathbf{r}_a - \mathbf{r}_b| - |\mathbf{r}_a^0 - \mathbf{r}_b^0|)^2 & \text{for } |\mathbf{r}_a^0 - \mathbf{r}_b^0| < R_C \\ 0 & \text{for } |\mathbf{r}_a^0 - \mathbf{r}_b^0| > R_C \end{cases}$$

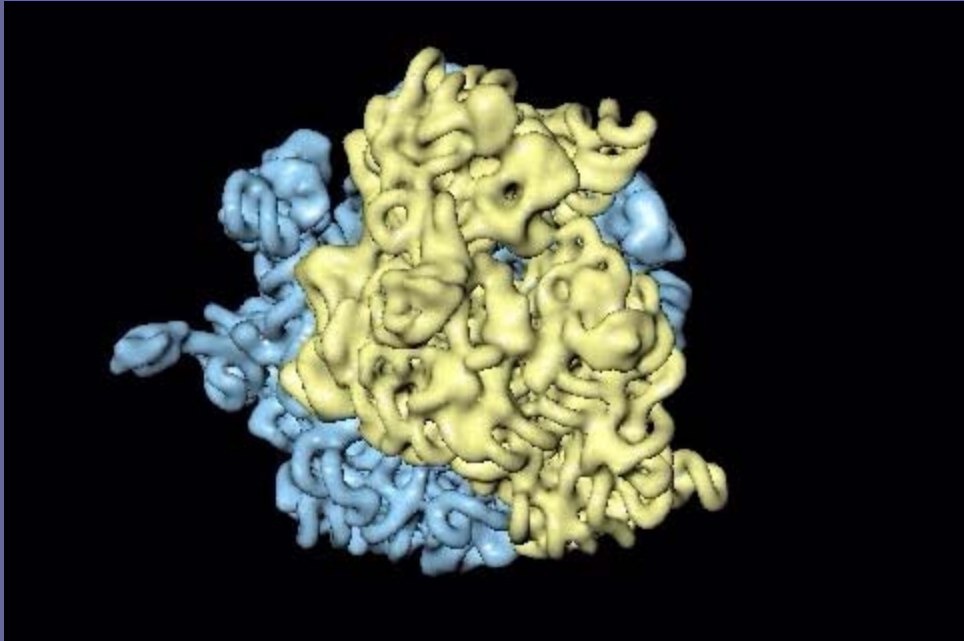
k = empirical constant

R_C = spatial constant

$$U_{system} = \sum_{(a,b) \in S} U(\mathbf{r}_a, \mathbf{r}_b).$$

Hessian: $3N \times 3N$ matrix of 2nd derivatives of U_{system} with respect to mass-weighted coordinates.

Normal modes: diagonalization of Hessian.



Conclusions

- During the elongation cycle, the ribosome undergoes large conformational changes, prompted alternately by the binding of EF-G or the ternary complex with cognate or near-cognate tRNA.
- EF-G, EF-Tu, and the tRNA undergo changes, as well.
- The ribosome structure is affected in its entirety.
- The large-scale conformational changes, and the underlying structural reorganization, can be studied by cryo-EM, and “re-molding” X-ray structures into the cryo-EM maps.
- The observed coordinated motions can perhaps be understood on the basis of the dynamic properties of the entire mechanical system.
- The existence of factor-free translation suggests that the conformational changes, such as the ratchet motion, involve low-energy barriers that can be overcome by thermal motion.

Contributors

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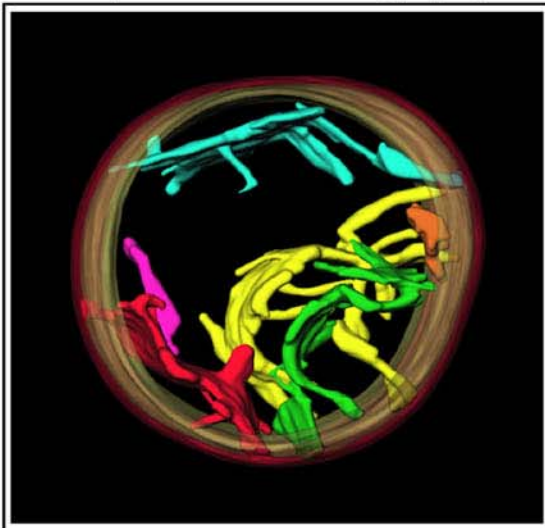
Collaborating Groups:

Charles Brooks III, Scripps Institute
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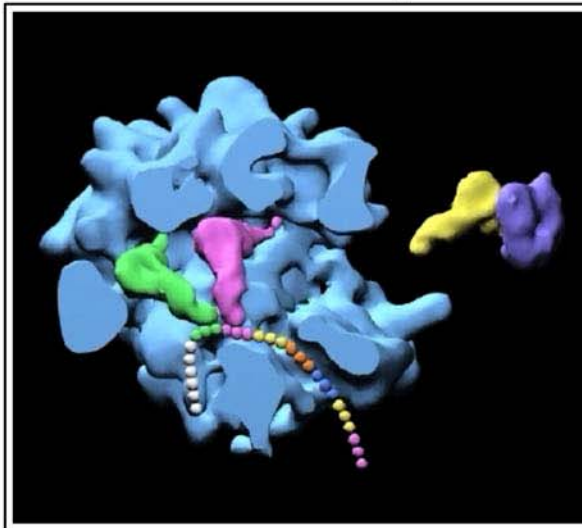
Steven Harvey, Georgia Tech
Knud Nierhaus, Max Planck, Berlin
Poul Nissen, University of Aarhus, Denmark
Venki Ramakrishnan, MRC, Cambridge
Andrej Sali, UCSF

RVBC Core Projects:

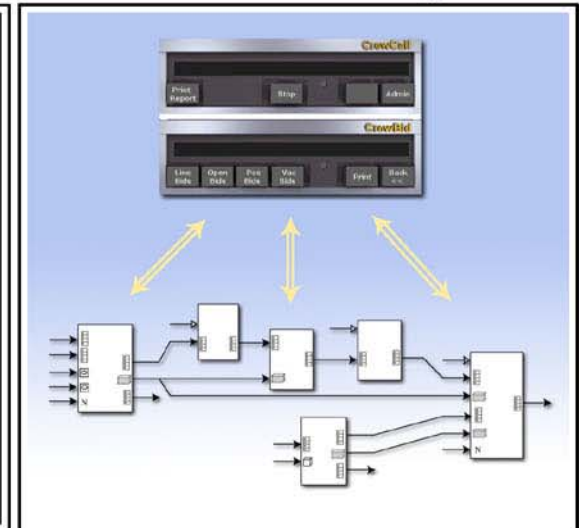
Cryo-electron Tomography

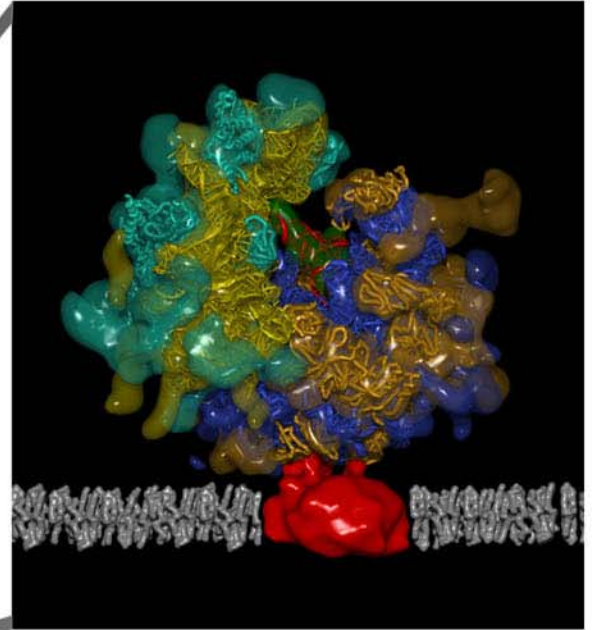
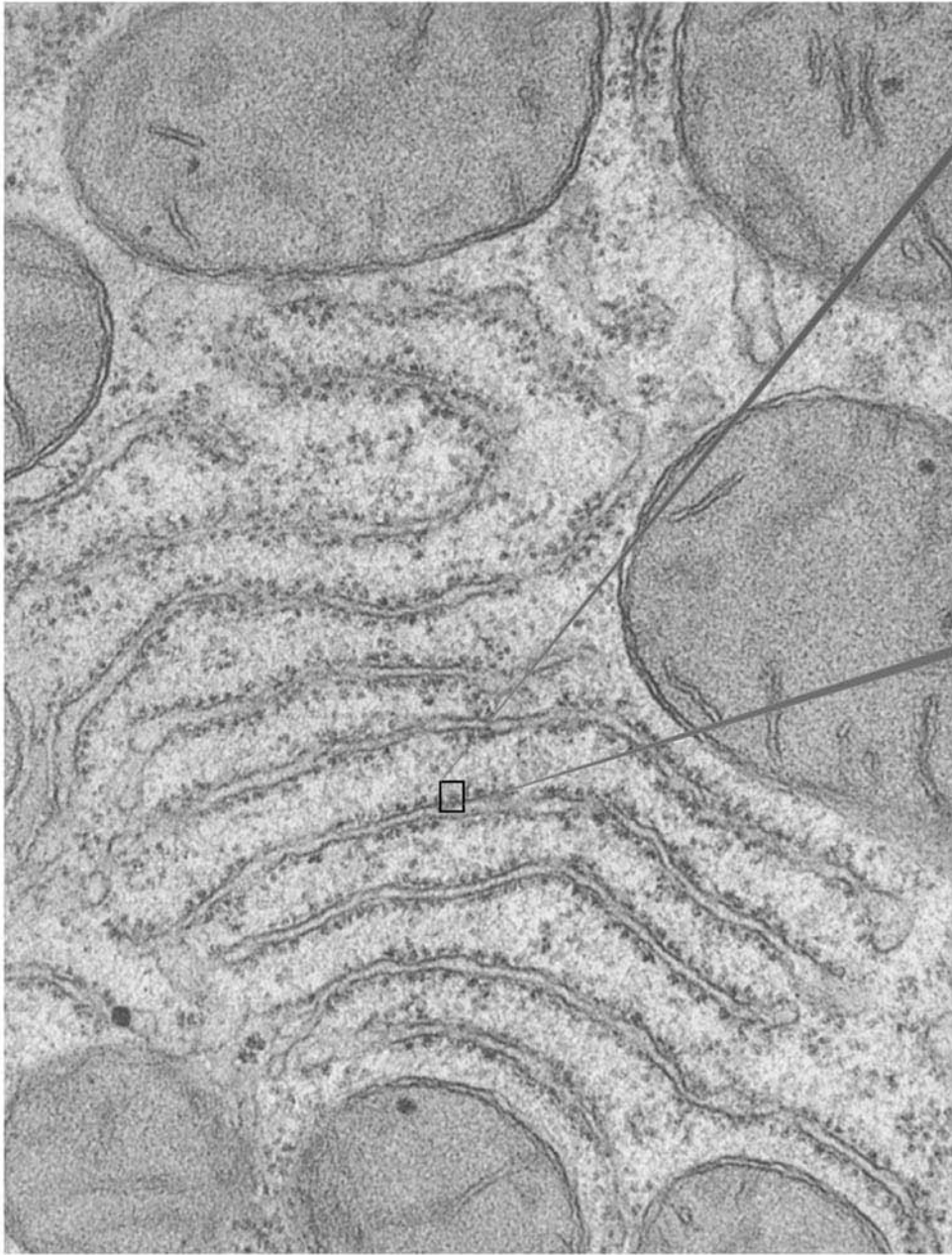


Time-Resolved Cryo-EM



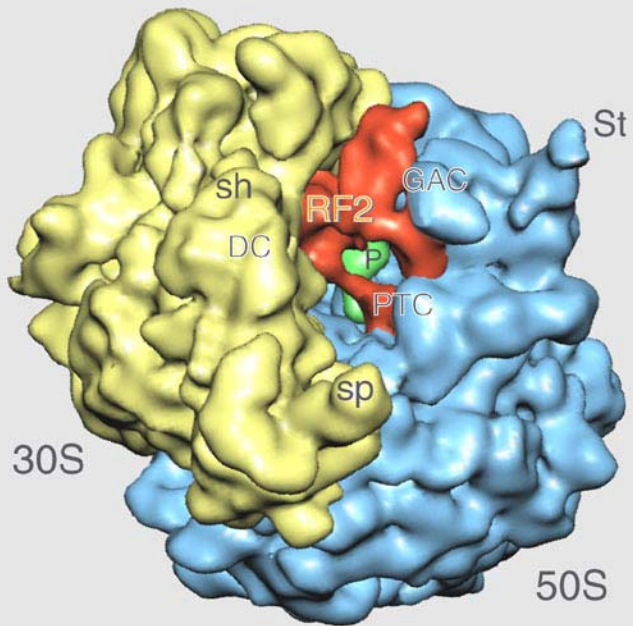
Reconstruction Engine



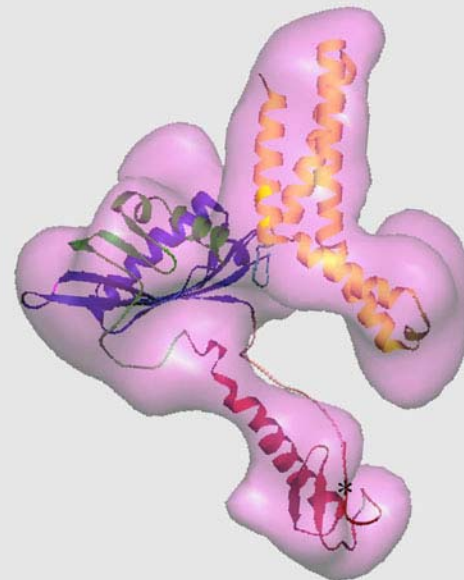
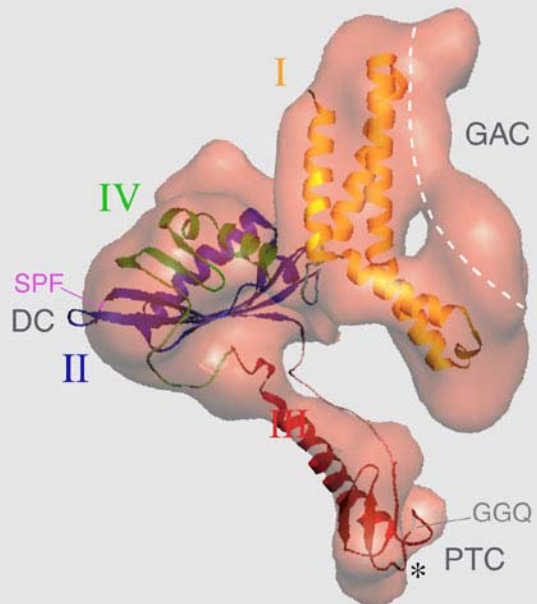
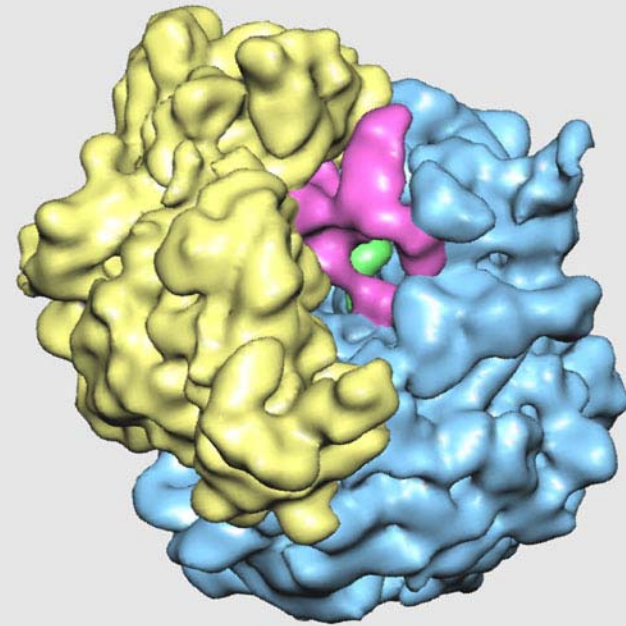


D.W Fawcett, The Cell, W.B. Saunders Company, 1966

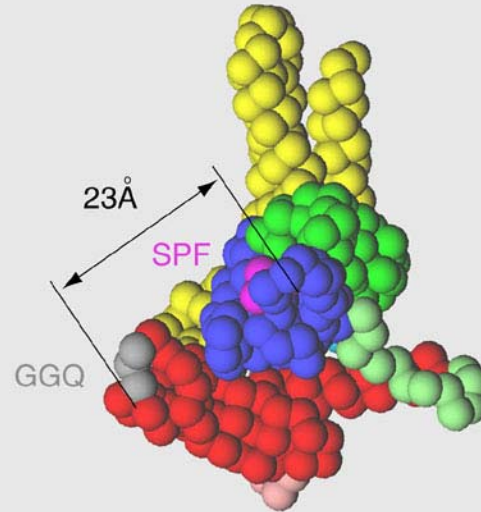
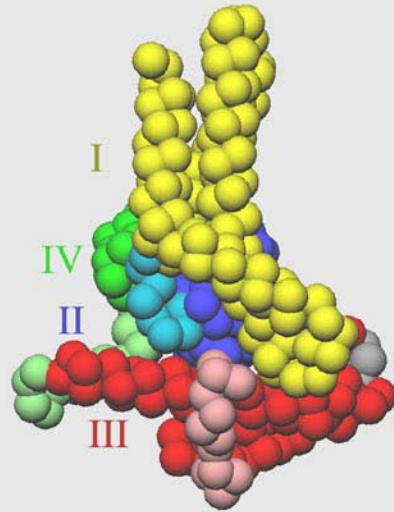
RF2 mutant (GAQ)



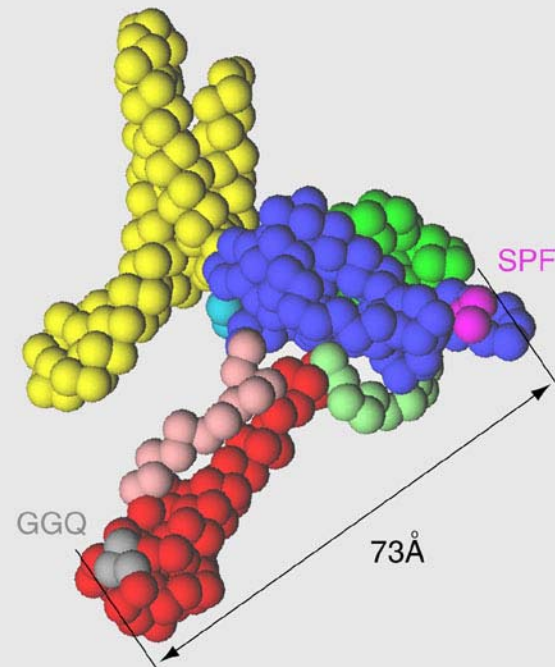
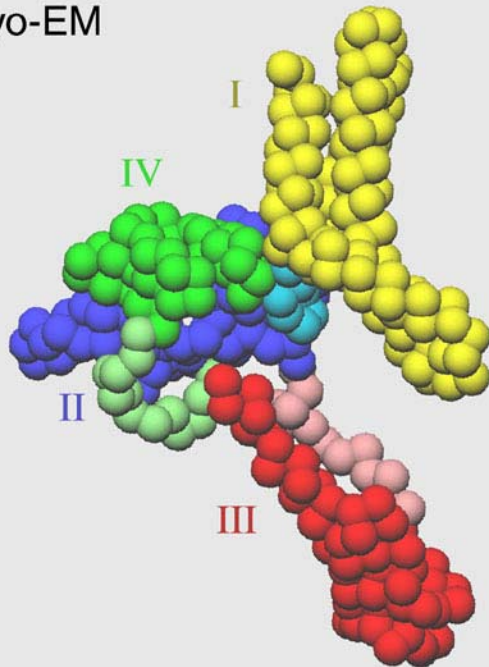
wild type (GGQ)



X-ray

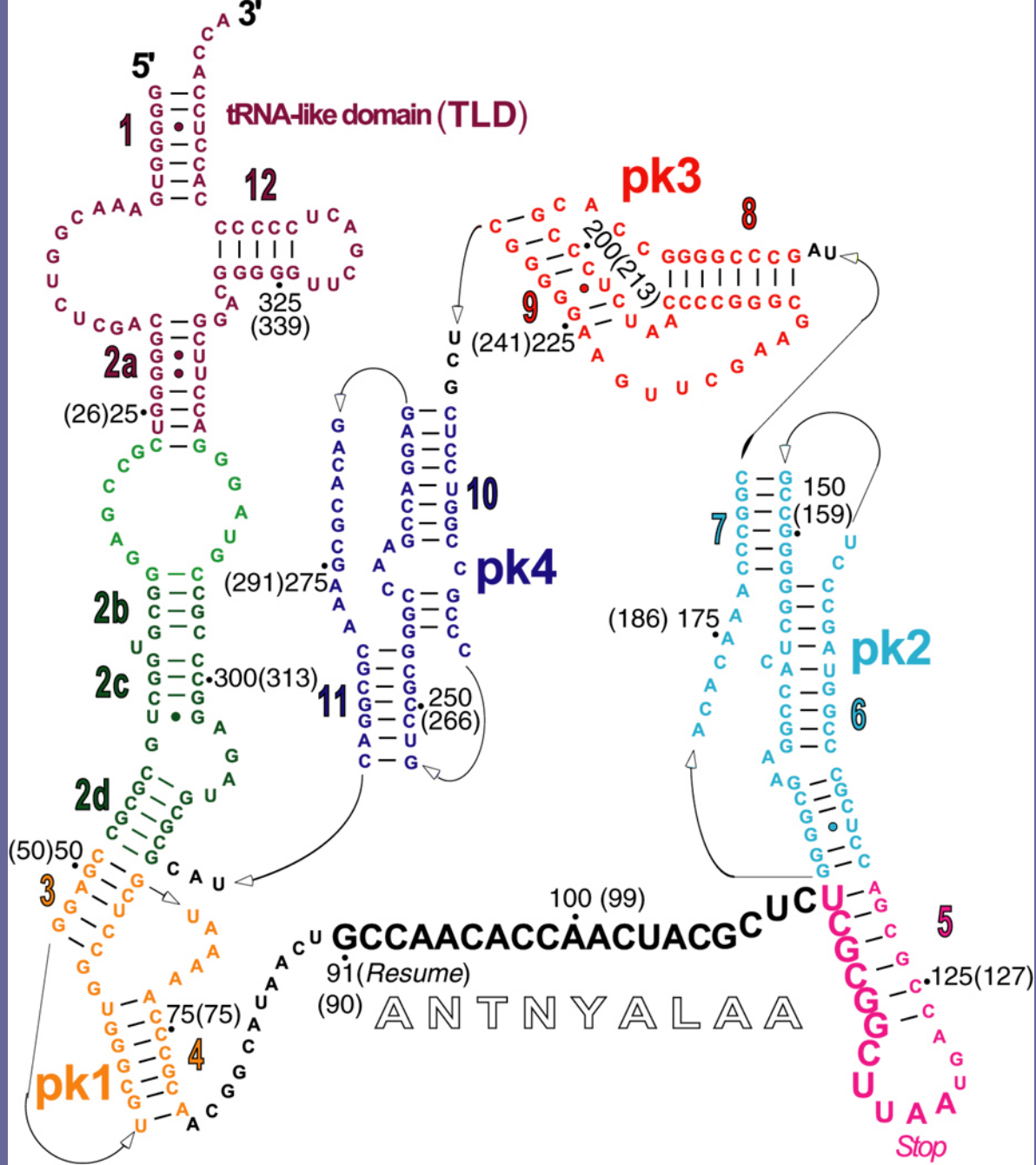


Cryo-EM

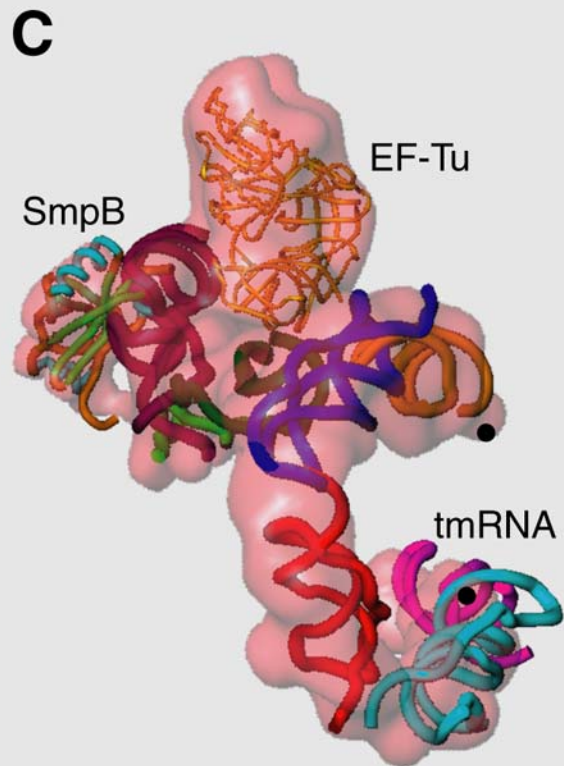
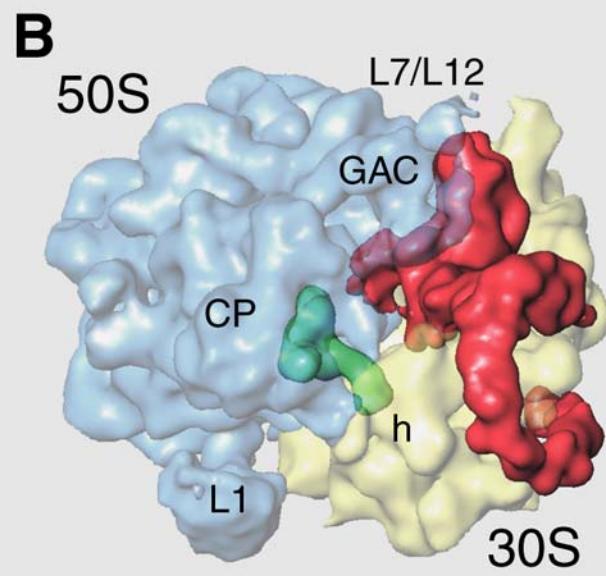
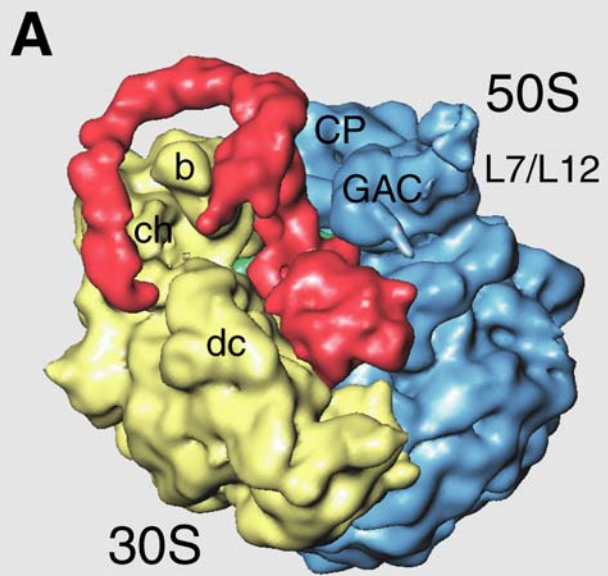


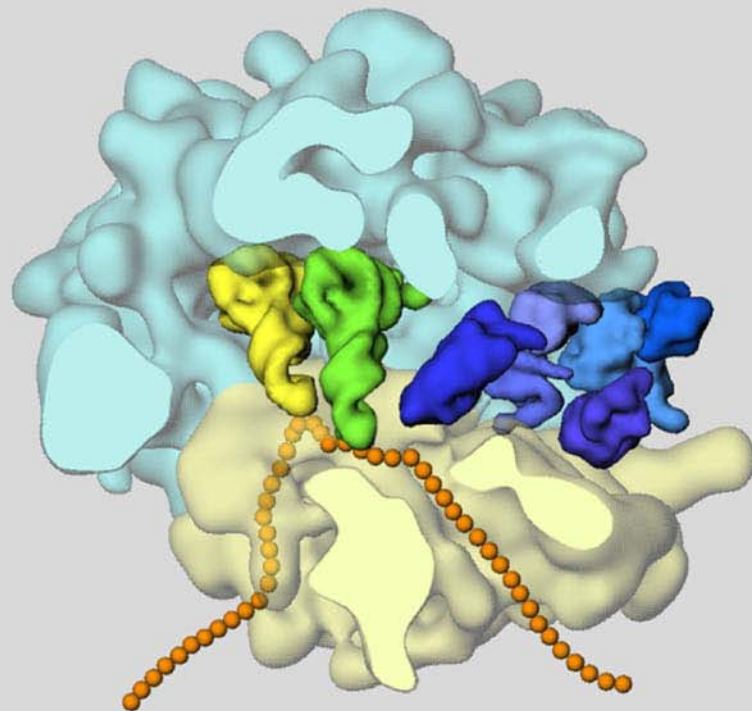
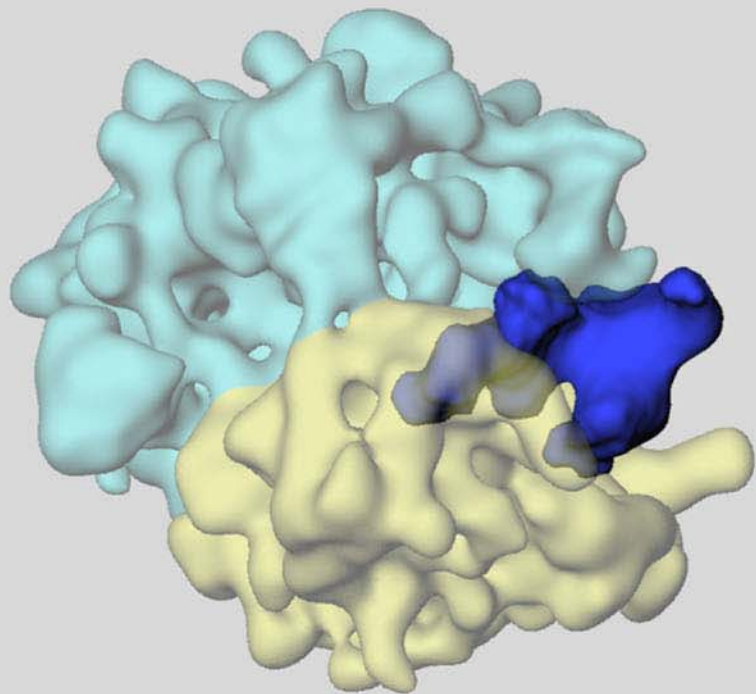
70S•EF-Tu•GDP•tmRNA•SmpB

- When no stop codon is encountered, or mRNA is otherwise defective, the ribosome recruits tmRNA.
- Rescue mechanism unique to prokaryotes, prevents formation of toxic proteins and ensures orderly termination and recycling
- tmRNA includes (i) a tRNA-like domain (TLD), (ii) an ORF coding for a signal sequence to be tagged to the polypeptide chain, marking it for degradation, (iii) a stop codon.
- The TLD is inserted into ribosomal A site just like a tRNA, in the form of a ternary complex with EF-Tu and GTP.
- SmpB acts as a helper protein.



tmRNA (Thermus thermophilus)





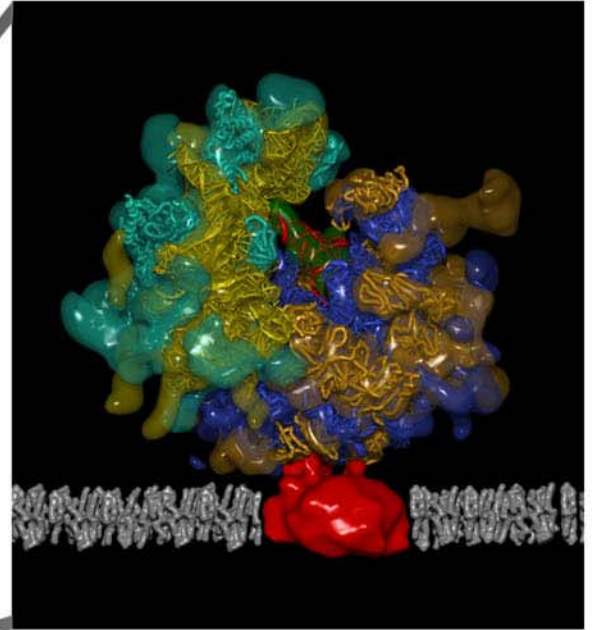
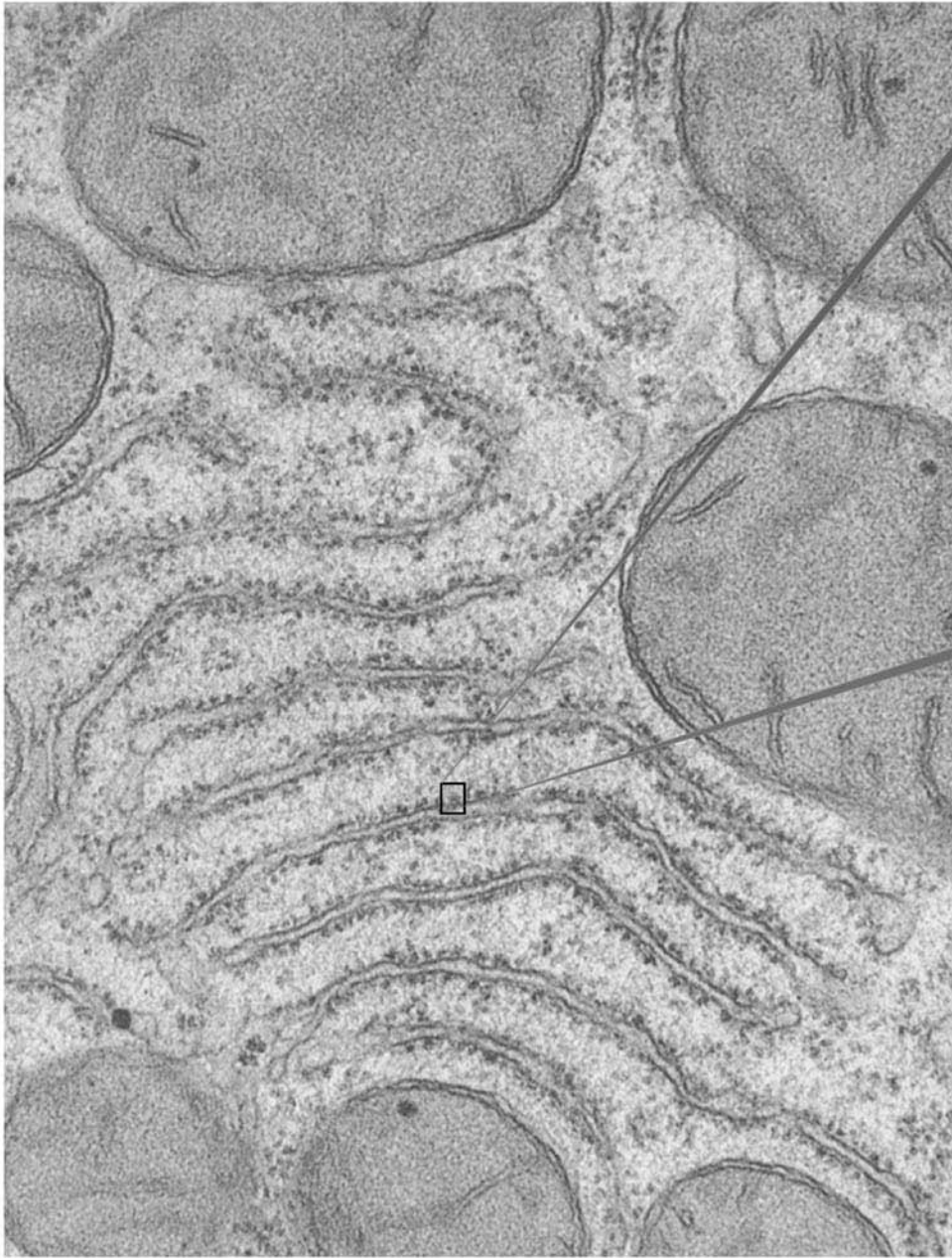
Conclusions: 2) Role of Cryo-EM in Studying Translation

Past/Present:

- First maps showing detailed morphology: bridges, mRNA channel, peptide tunnel
- Instrumental in low-resolution phasing of first X-ray maps
- Interaction with tRNAs, EF-G, EF-Tu ternary complex, and release factor RF2
- First 3D scheme of the elongation cycle
- Conformational changes of EF-G, ternary complex, and RF2
- Dynamics of the ribosome as a series of snapshots
- Resolution good enough to guide fitting of X-ray structures

Future:

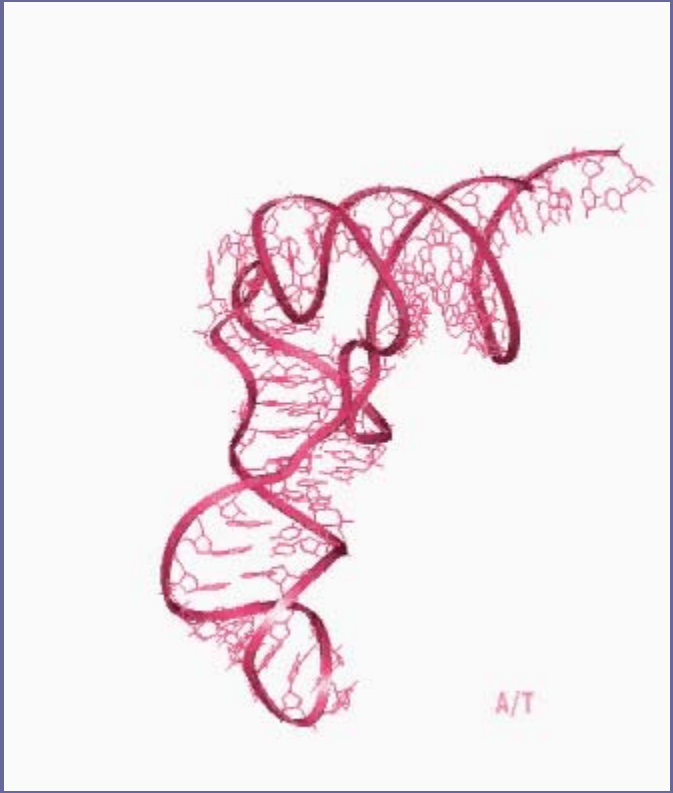
- Integration of software from X-ray and EM communities. Fitting of dynamically changing structures. Searching for motifs.
- Develop true time-resolved techniques
- Routine achievement of 7Å resolution?



D.W Fawcett, The Cell, W.B. Saunders Company, 1966

Scheme of Alternate Factor Binding to Ribosome in Alternate Conformations

- 1) Factor A binds to ribosome in State I
- 2) GTP hydrolysis
- 3) Ribosome goes into State II, causing release of Factor A
- 4) Factor B binds to ribosome in State II
- 5) GTP hydrolysis
- 6) Ribosome goes into State I, causing release of Factor B
- 1) ...



Aminoacyl-tRNA acts as a flexible molecular spring during codon recognition and accommodation

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animation

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