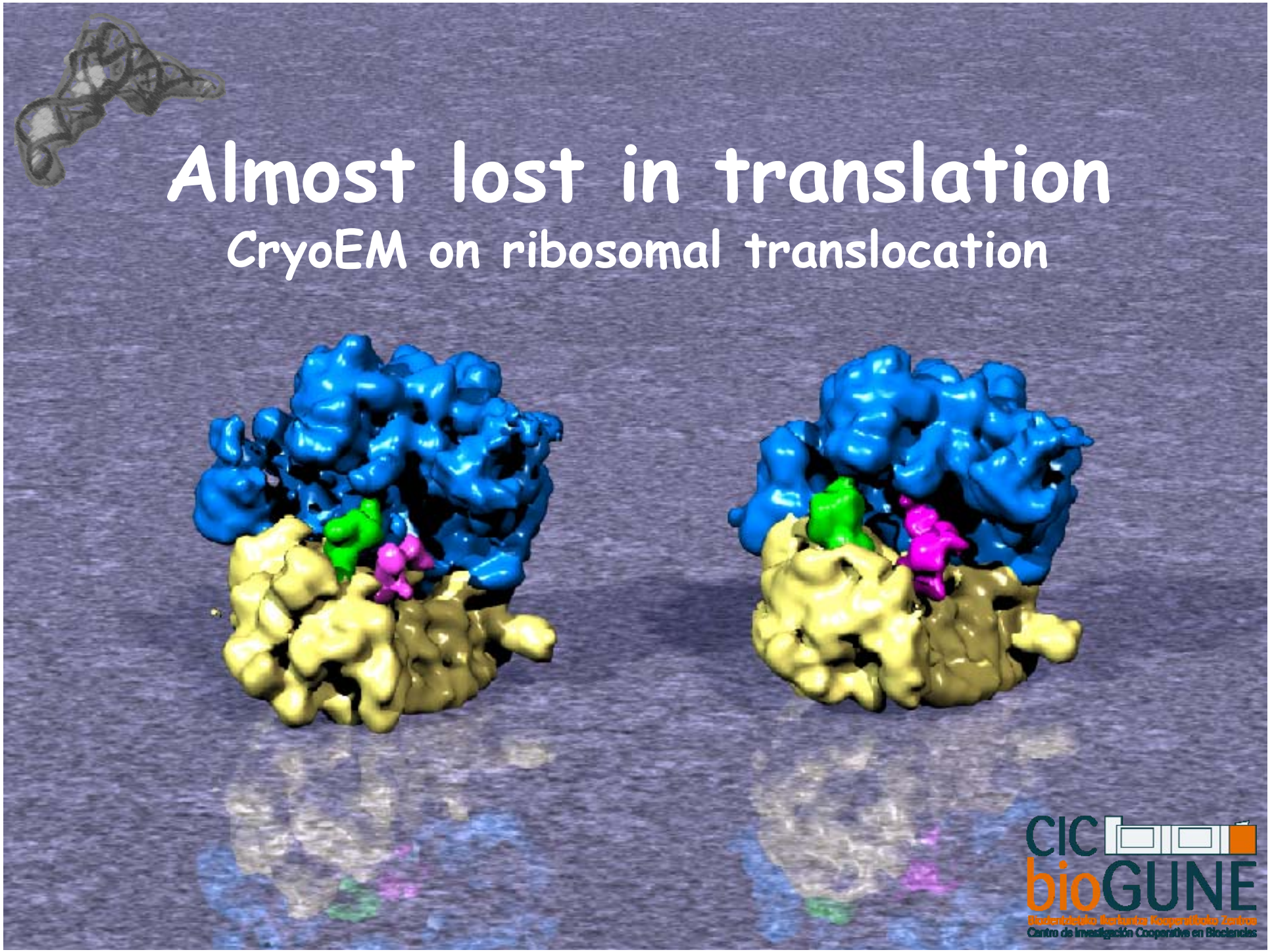
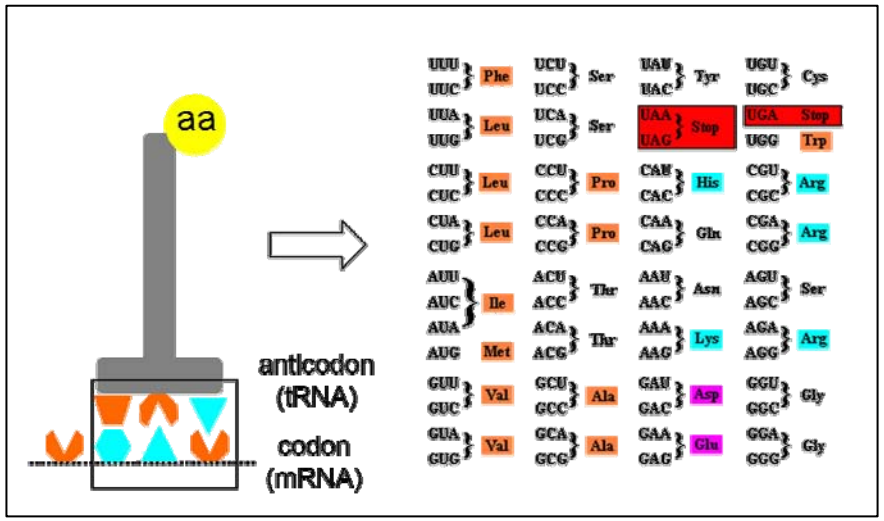
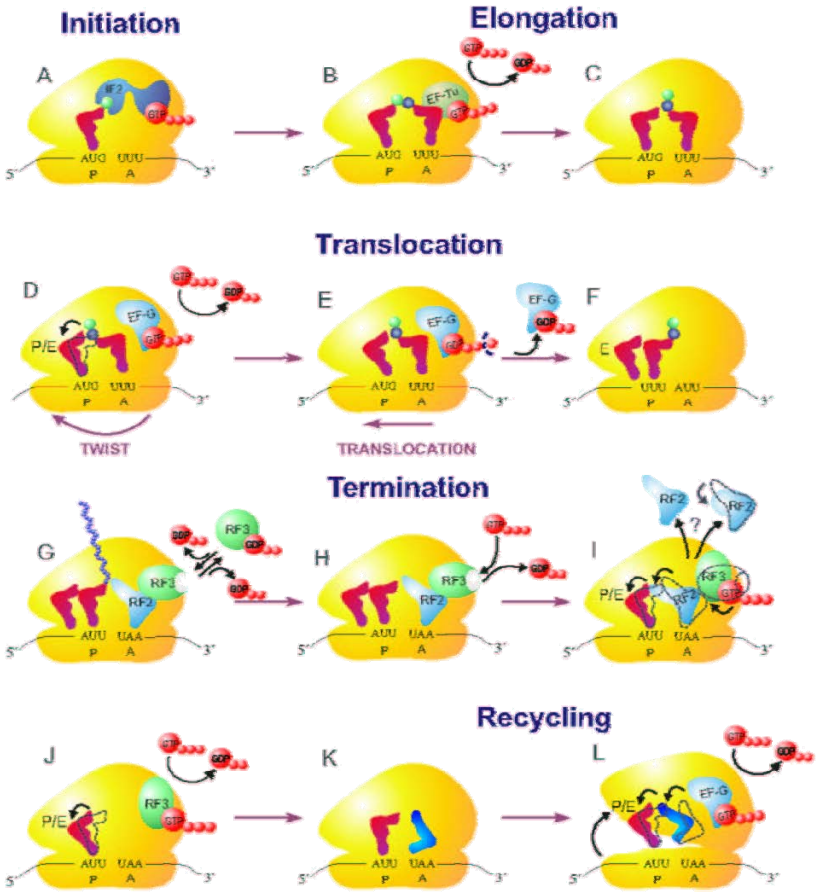

Almost lost in translation
CryoEM on ribosomal translocation
CIC
bioGUNE
Centro de Investigación Cooperativa en Biociencias

The ribosome and translation





- Elongation 1 aa/60 mS
- Average protein (250 aas) 15 s
- Error rate aa 10^{-3}-10^{-4}
- Early termination 10-20%

Ribosomal structure

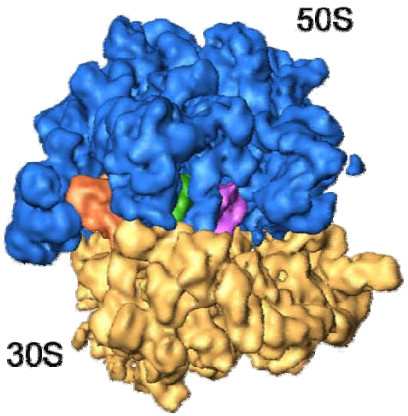
- **Two subunits**

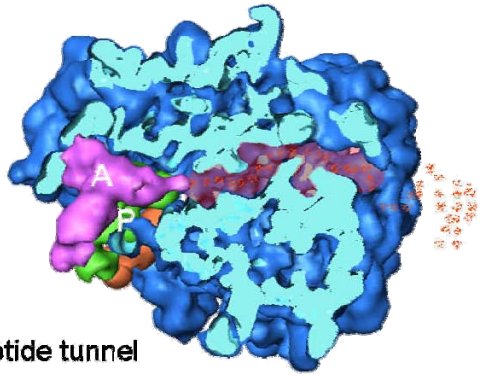
Large (50S) peptide synthesis

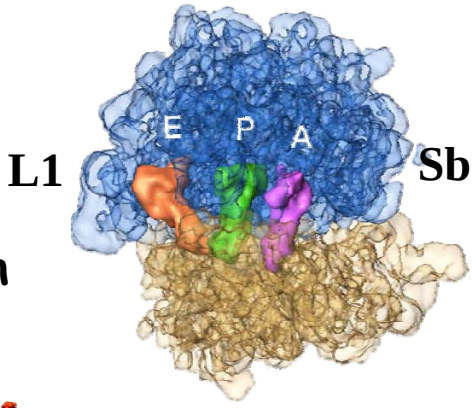
Small (30S) mRNA reading

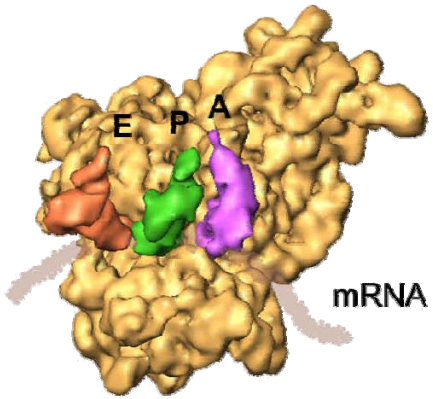
50S 23S rRNA (~2900 Nu)
5S rRNA (~120 Nu)
~40 proteins

30S 16S rRNA (~1540 Nu)
~30 proteins







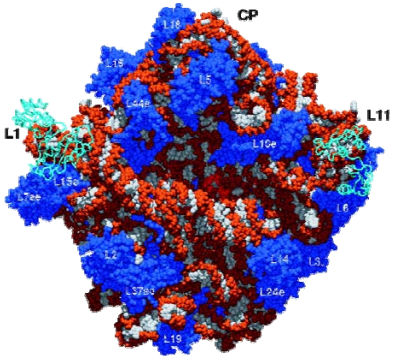


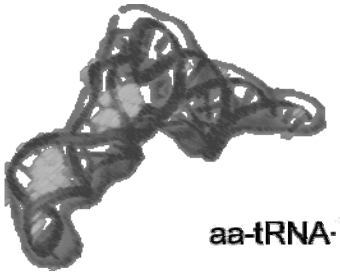
- **Three tRNA binding sites in both subunits**

A (aminoacyl-tRNA)

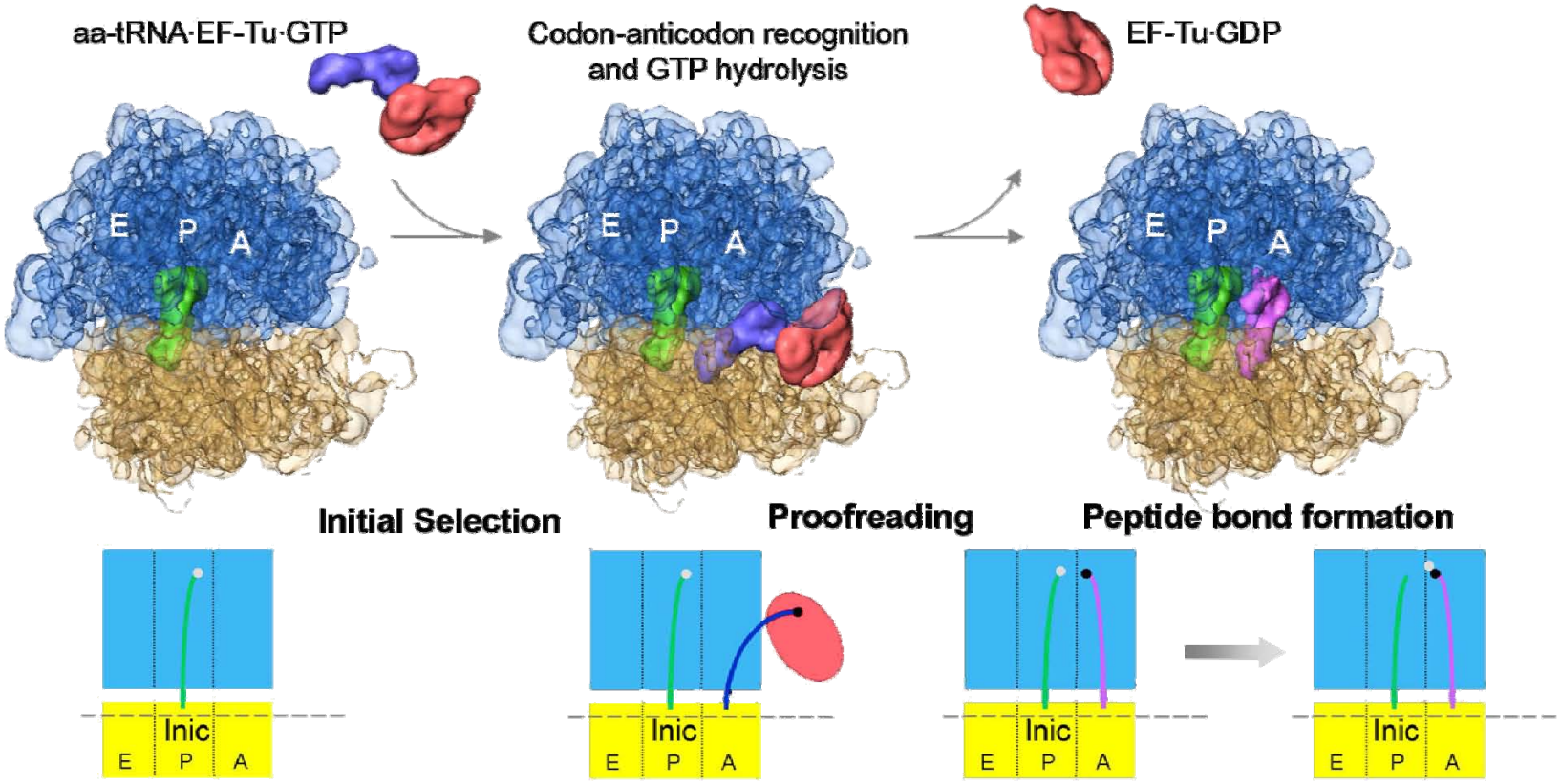
P (peptidyl-tRNA)

E (exit-tRNA)



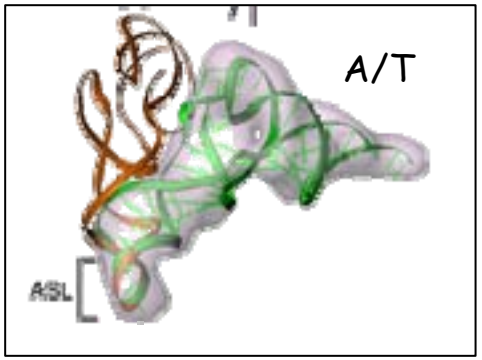


Elongation. New amino acid added.

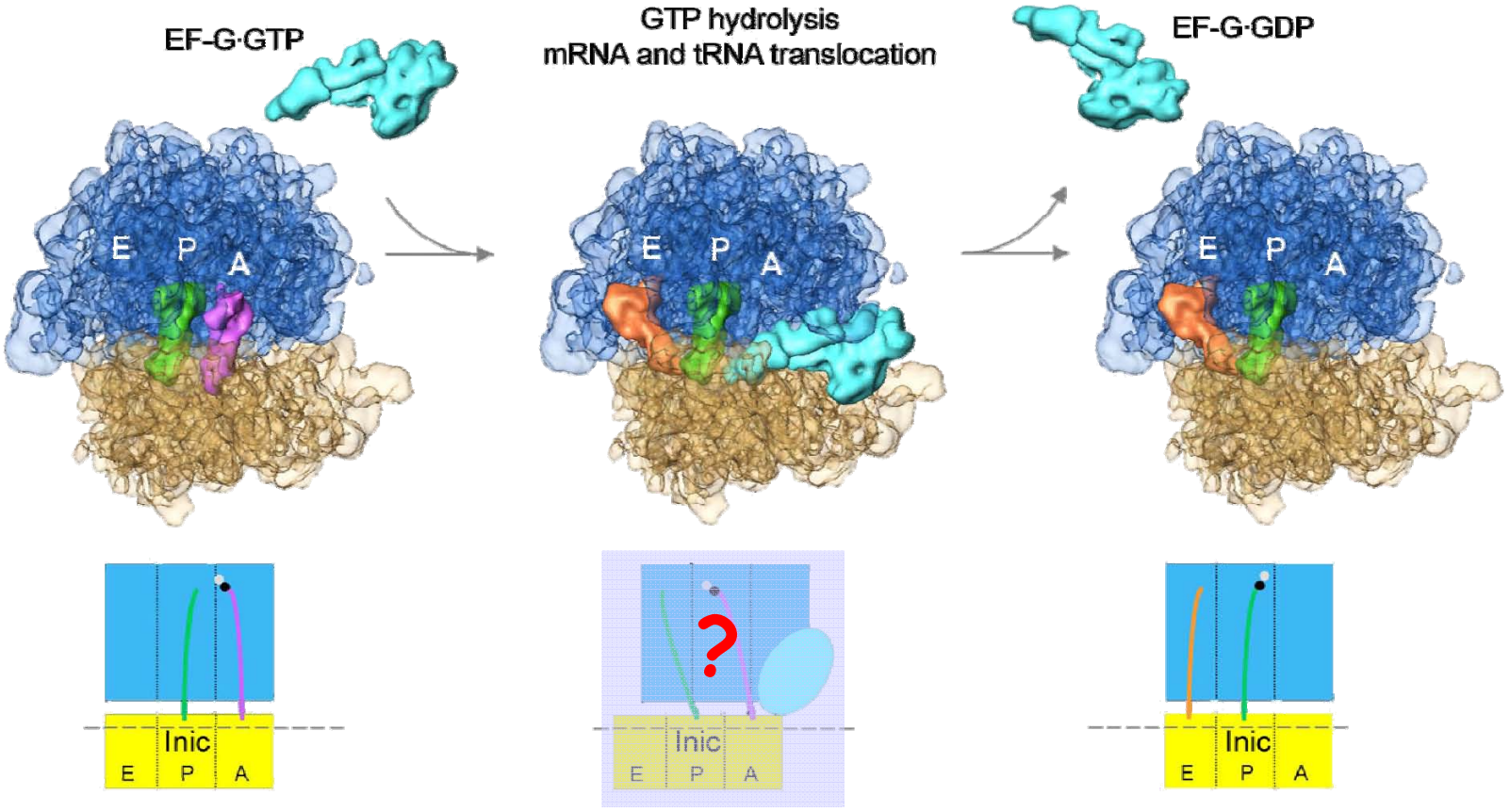


Valle, M., J. Sengupta, N.K. Swami, R.A. Grassucci, N. Burkhardt, K.H. Nierhaus, R.K. Agrawal, and J. Frank. (2002) EMBO J *21*, 3557-3567.

Valle, M., A. Zavialov, W. Li, S.M. Stagg, J. Sengupta, R.C. Nielsen, P. Nissen, S.C. Harvey, M. Ehrenberg, and J. Frank (2003). Nat. Struct. Mol. Biol. *10*, 899-906.



Translocation by EF-G

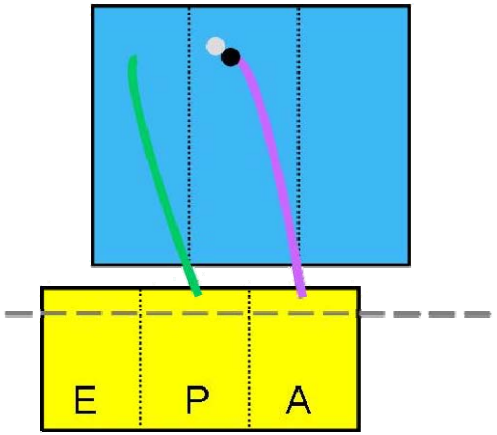


Pretranslocation **Posttranslocation**

Ribosome and factor conformational changes
Measure codons in mRNA (?)

A hybrid for translocation

- Universal design into two subunits
- One subunit at a time
- Anchor and keep reading frame
- Chemical-footprinting



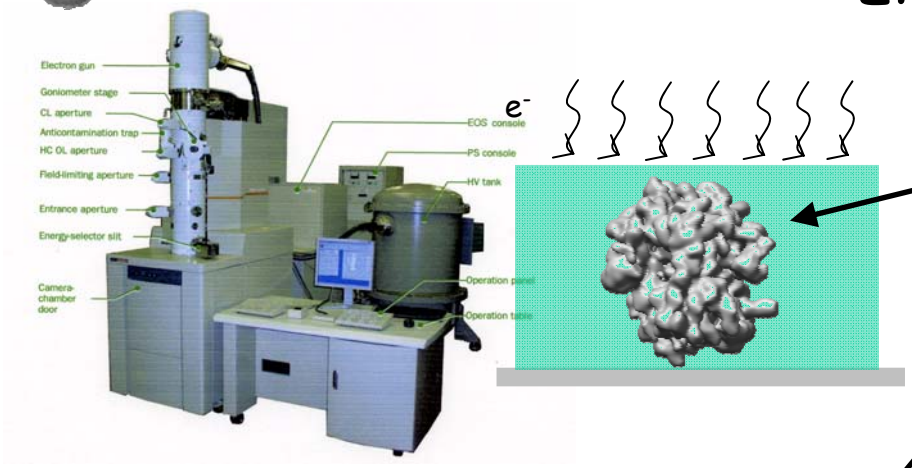
Bretscher, M.S. (1968). Nature *218*, 675-677.

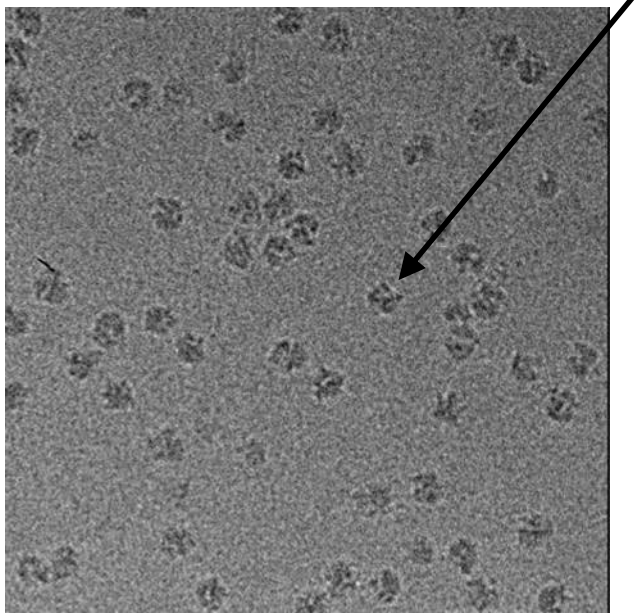
Spirin, A.S. (1969). Cold Spring Harb Symp 34, 197-207.

Moazed, D., and Noller, H.F. (1989). Nature *342*, 142-148.

CryoEM of ribosomes

EM of single particles (2D images)



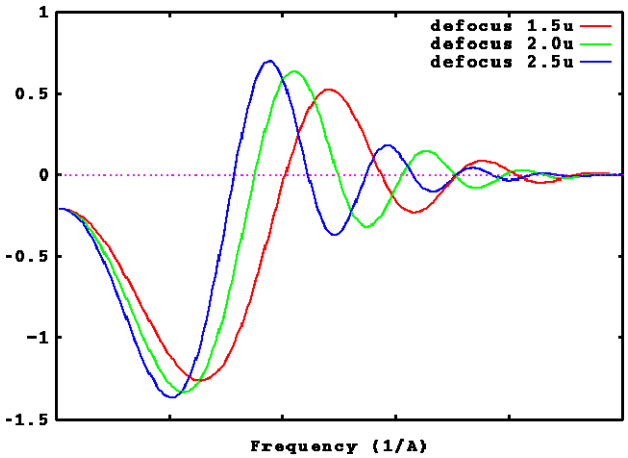


$$i(\mathbf{r}) = o(\mathbf{r}) * h(\mathbf{r})$$

h(r) point spread function

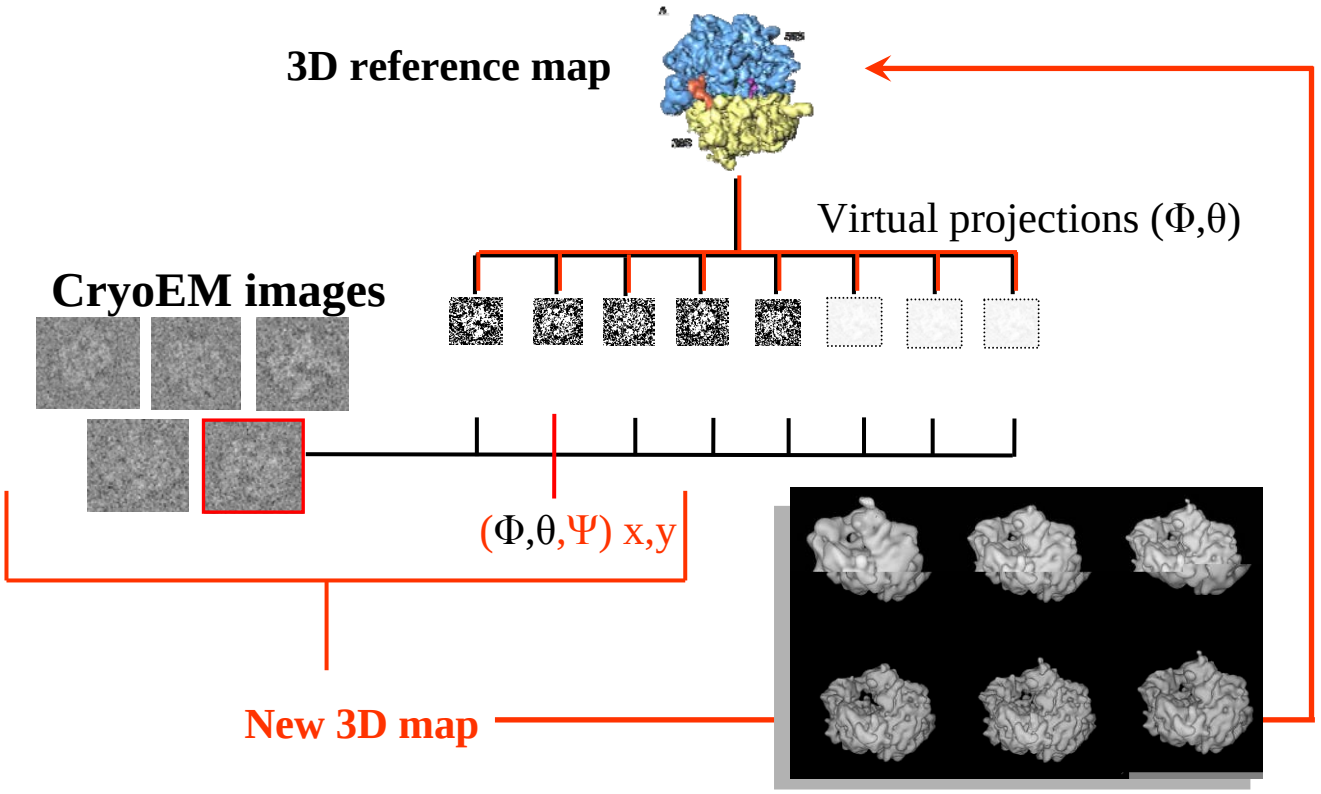
$$I(\mathbf{k}) = O(\mathbf{k})\, H(\mathbf{k})$$

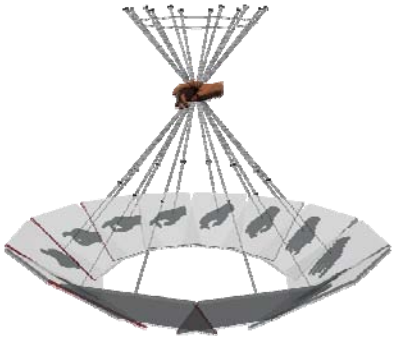
H(k) Contrast Transfer Function



CryoEM of ribosomes

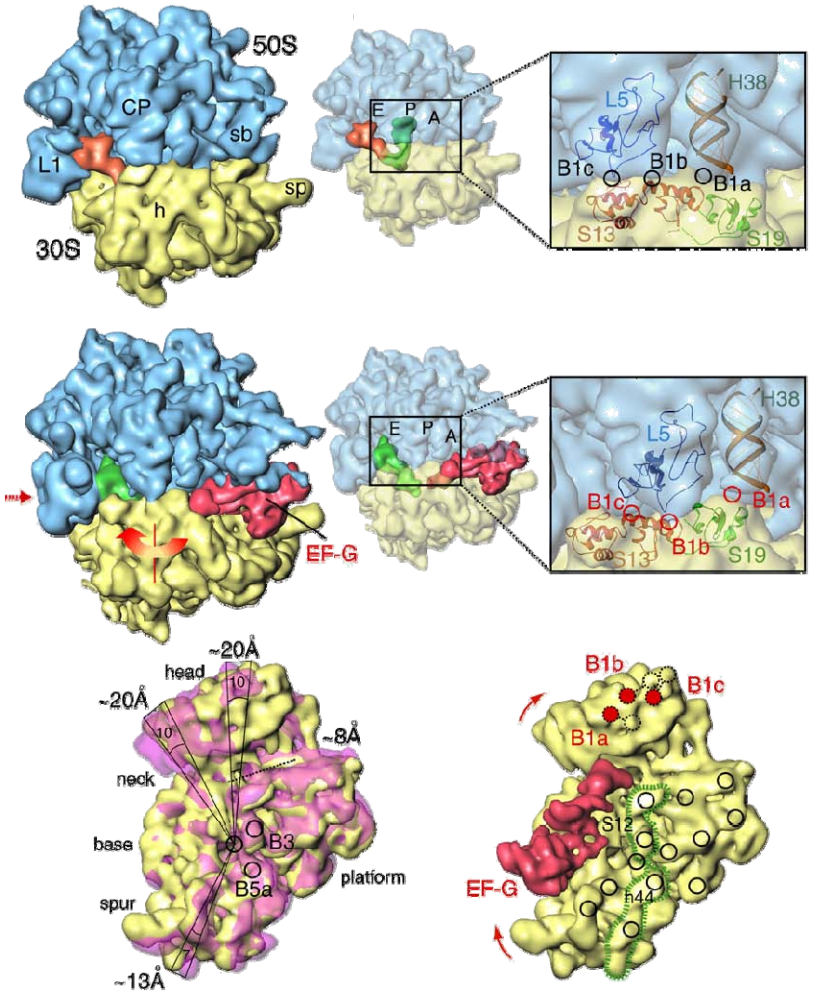
From 2D images to 3D map

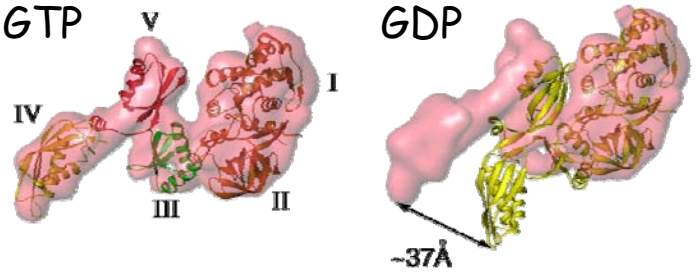




Translocation seen by CryoEM

EF-G and ribosome interaction



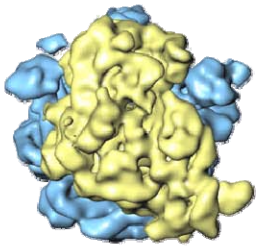


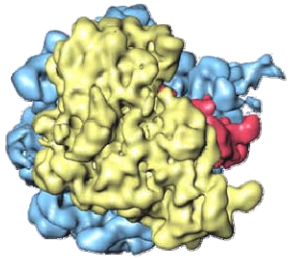
Valle, M., Zavialov, A., Sengupta, J., Rawat, U., Ehrenberg, M., and Frank, J. (2003). Cell 114, 123-134.

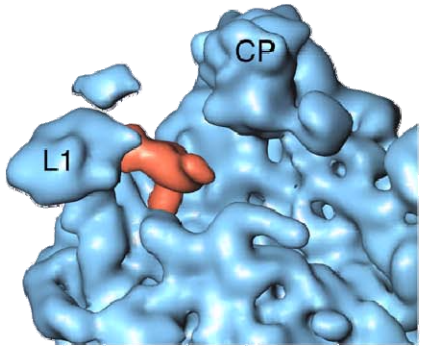
Translocation by CryoEM

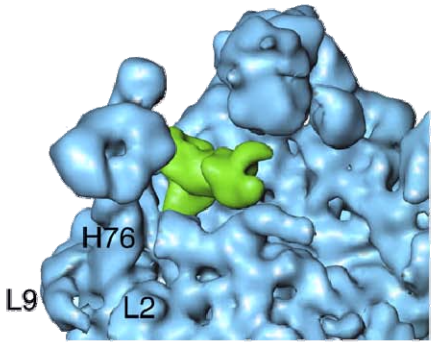
L1 stalk moving

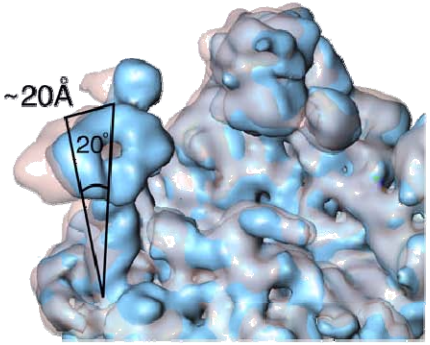
the tRNA out

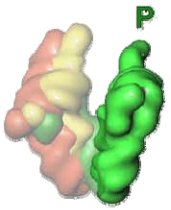


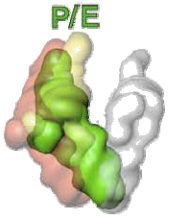


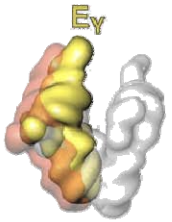


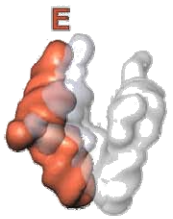








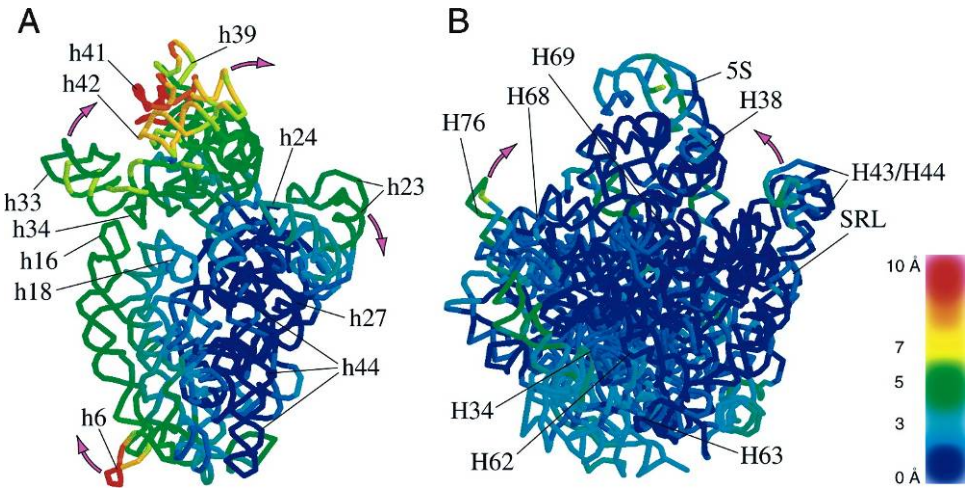




Valle, M., Zavialov, A., Sengupta, J., Rawat, U., Ehrenberg, M., and Frank, J. (2003). Cell 114, 123-134.

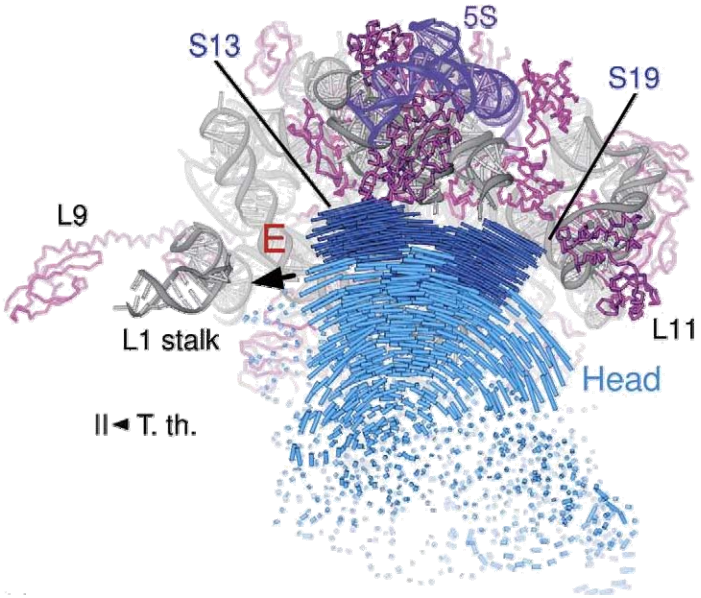
At atomic level

Flexible fitting



Gao, H., J. Sengupta, M. Valle, A. Korostelev, N. Eswar, S.M. Stagg, P. Van Roey, R.K. Agrawal, S.C. Harvey, and A. Sali. (2003) Cell. *113*, 789-801.

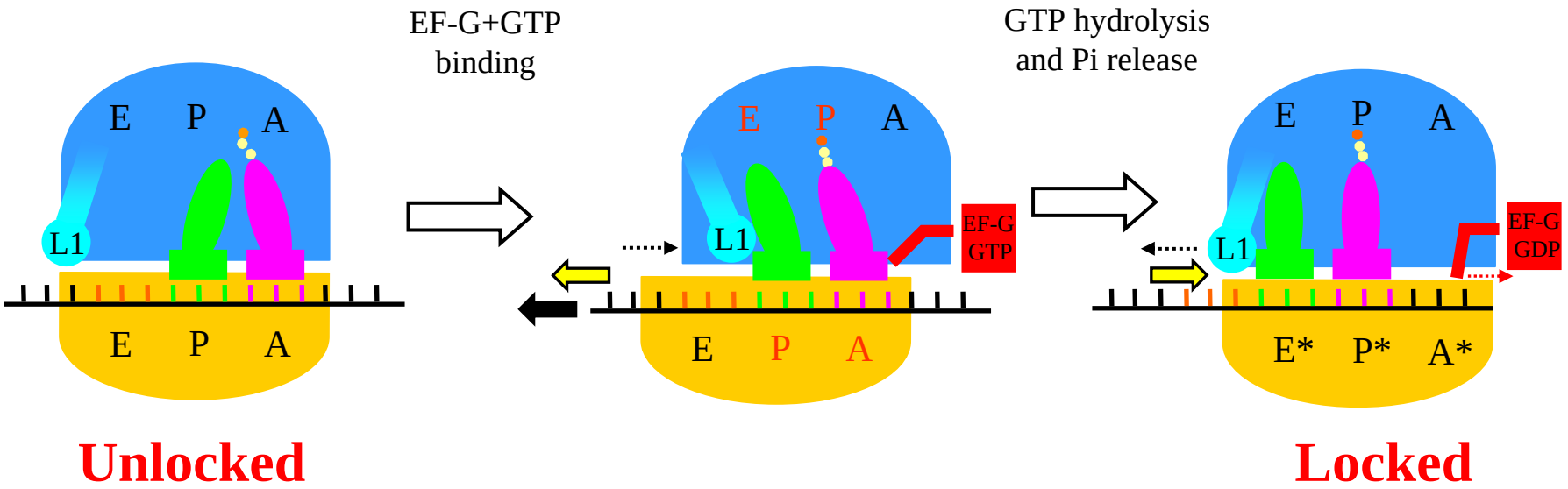
Xray structures



Schuwirth BS, Borovinskaya MA, Hau CW, Zhang W, Vila-Sanjurjo A, Holton JM, Cate JH (2005) Science*310*,827-834.

Translocation by CryoEM

Update for translocation model

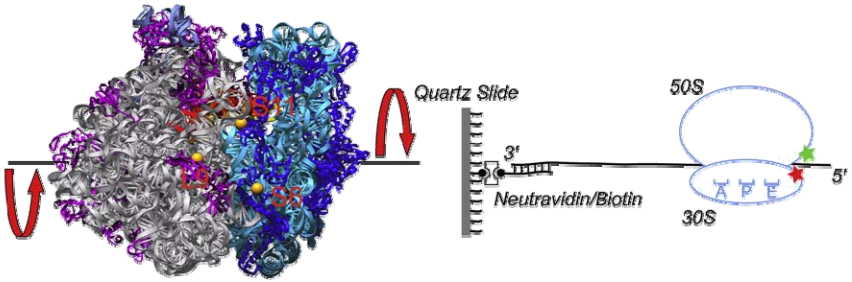


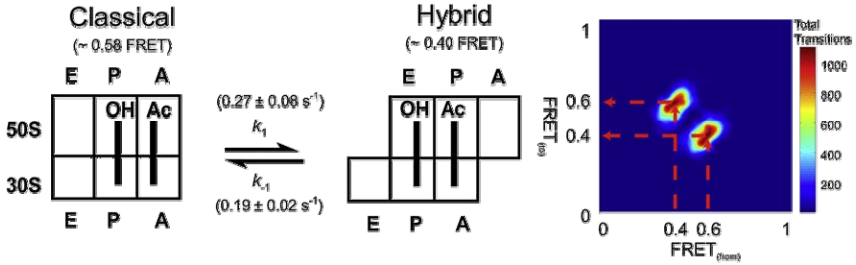
- Translocation on the 50S first (hybrid tRNAs)
- Relative rotation between subunits
- L1 stalk holds the mRNA-(tRNAs) complex
- EF-G changes recover ground state

A new twist on translocation

Single molecule FRET

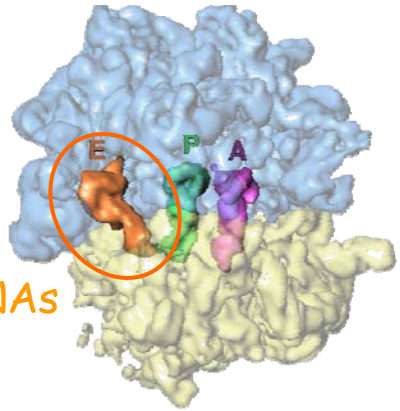
- Spontaneous rotation without EF-G





Cornish, P.V., Ermolenko, D.N., Noller, H.F., and Ha, T. (2008). Molecular cell *30*, 578-588.

Munro, J.B., Altman, R.B., O'Connor, N., and Blanchard, S.C. (2007). Molecular cell *25*, 505-517.



Deacylated tRNAs

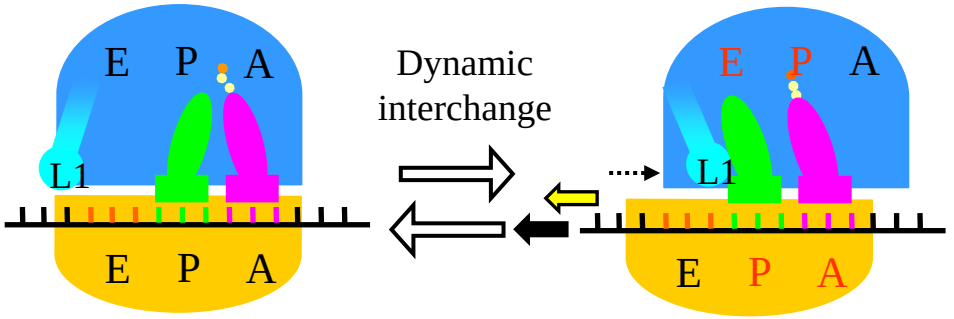
- Previous cryoEM of pretranslocation

Valle, M., A. Zavialov, W. Li, S.M. Stagg, J. Sengupta, R.C. Nielsen, P. Nissen, S.C. Harvey, M. Ehrenberg, and J. Frank (2003). Nat. Struct. Mol. Biol. *10*, 899-906.

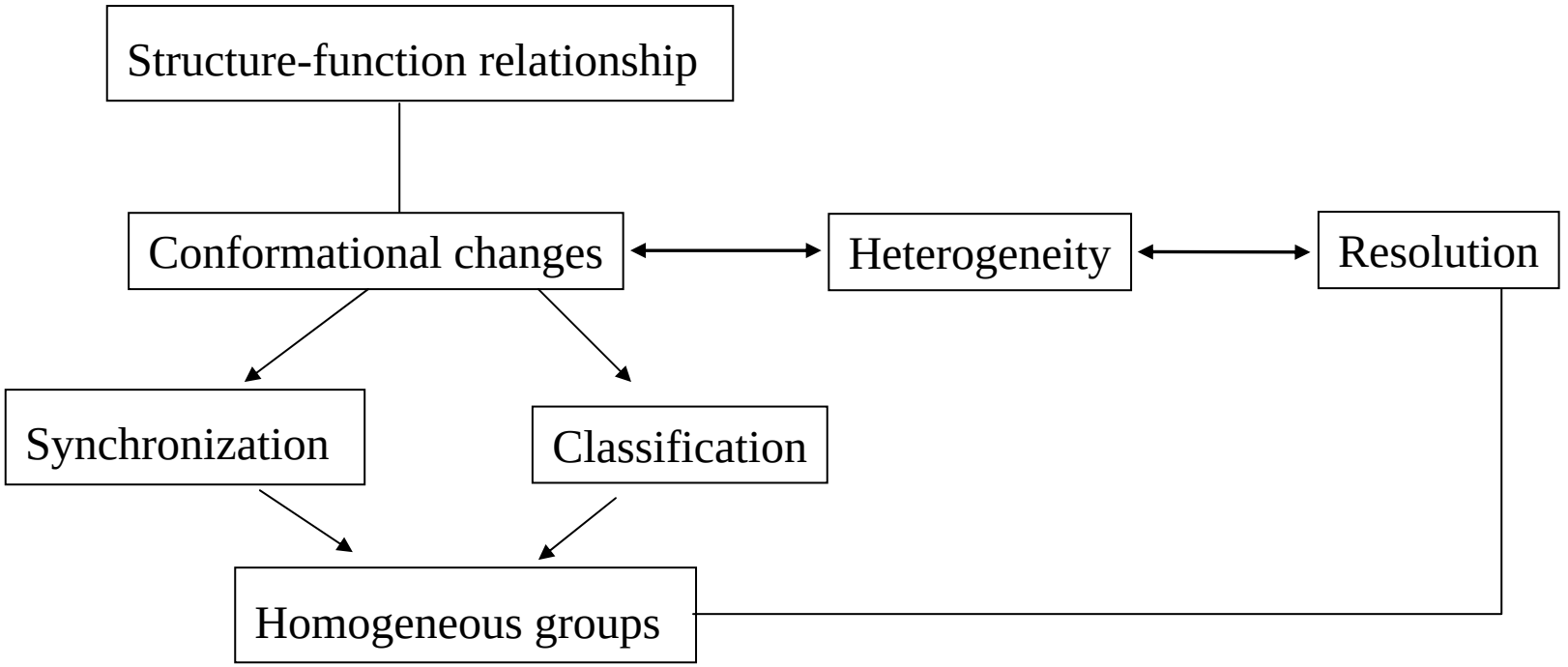
So …, translocation must be revisited

What a "CryoEM-ist" would say

Pseudo-native state
No spatial restrictions (close-packing)
Conformational changes
Dynamic reactions



Paradox: polymorphism vs resolution



Dealing with heterogeneity

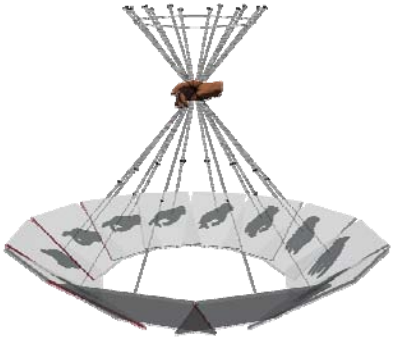
Classification: separation of different 3D objects

2D variability sources

Intra-population (3D orientation)

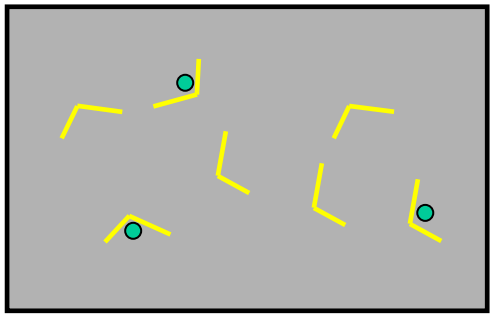
↕

Inter-population

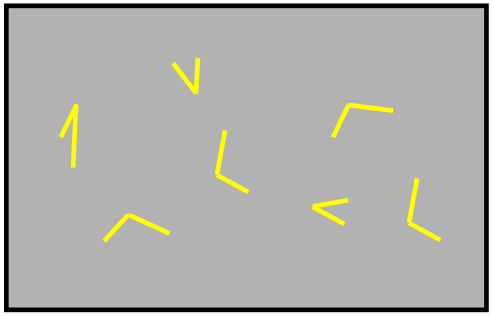


Inter-population: scenarios

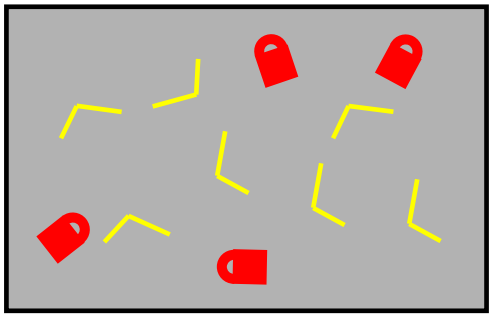
Ligand



Conformation



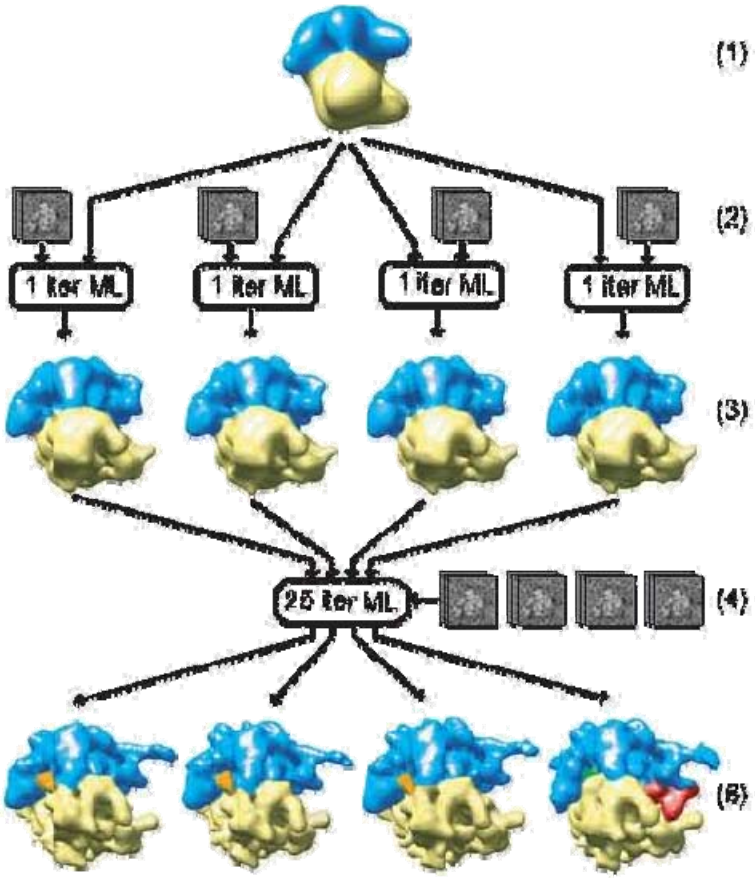
Nature



Similarity with known references (real or simulated)

A new tool was developed

Classification



Maximum-likelihood based

Unsupervised

- random initial seeds
- low resolution initial model

Probability-weighted assignments

- all the classes
- all the projections

Likelihood optimization

- most likely set of parameters

Iterative

- classification
- reconstruction

Scheres, S.H., Gao, H., Valle, M., Herman, G.T., Eggermont, P.P., Frank, J., and Carazo, J.M. (2007). Nature methods *4*, 27-29.

A new tool was developed

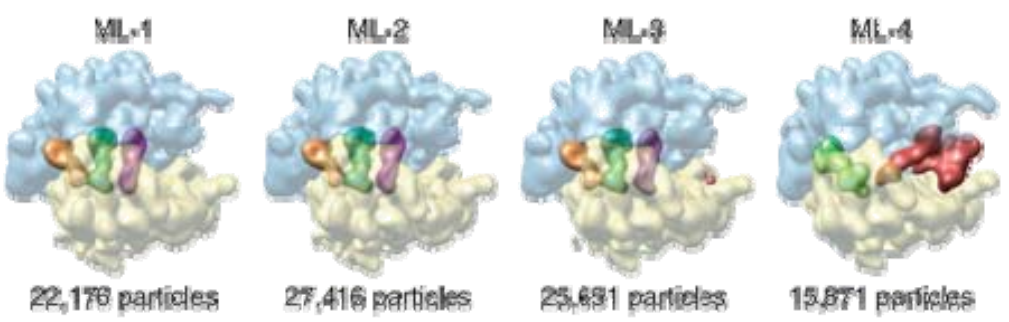
Classification

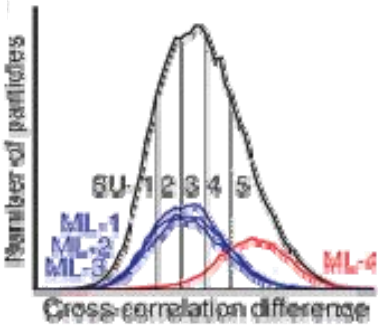
Conformational changes ⟷ Heterogeneity

Conformational change + occupancy (EF-G) Initial 91114 particles

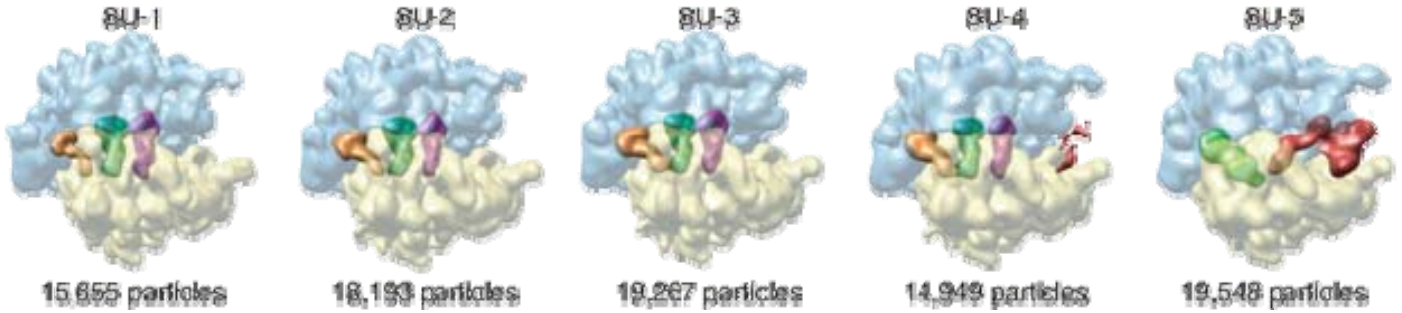
ML3D

Unsupervised





Supervised

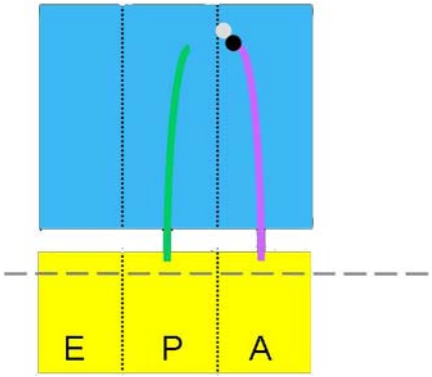


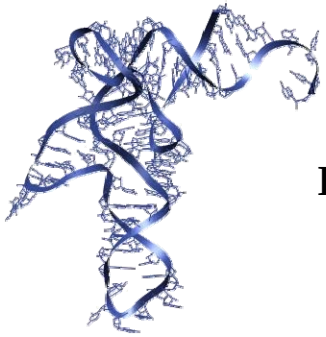
Scheres, S.H., Gao, H., Valle, M., Herman, G.T., Eggermont, P.P., Frank, J., and Carazo, J.M. (2007). Nature methods *4*, 27-29.

Pretranslocation revisited by CryoEM

E.coli 70S ribosomes

70S · mRNA· fMet-tRNA · Leu-tRNA^{fMetLeu}





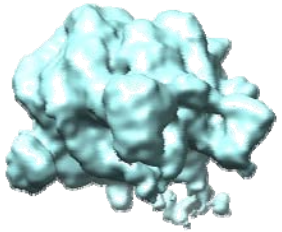
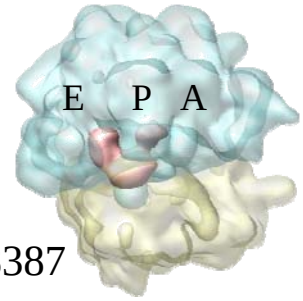
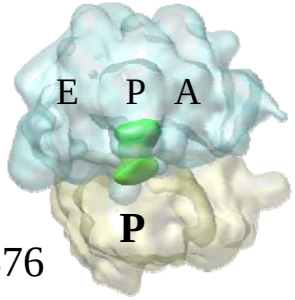
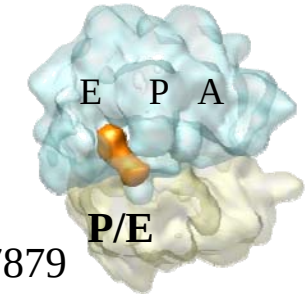
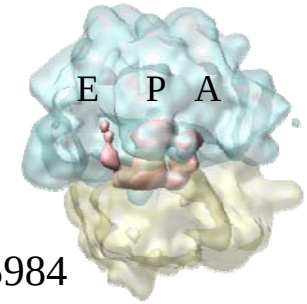
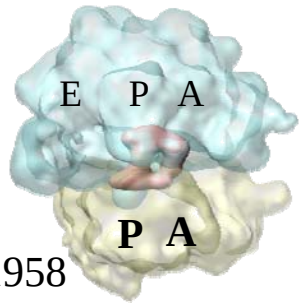
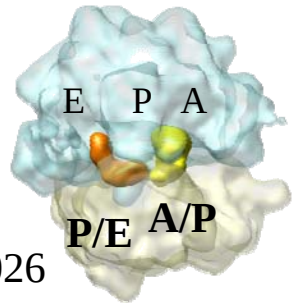
Leu-tRNA

Variables Mg++ Association between ribosomal subunits
Spermine Structure and stability of nucleic acids

ML3D classification

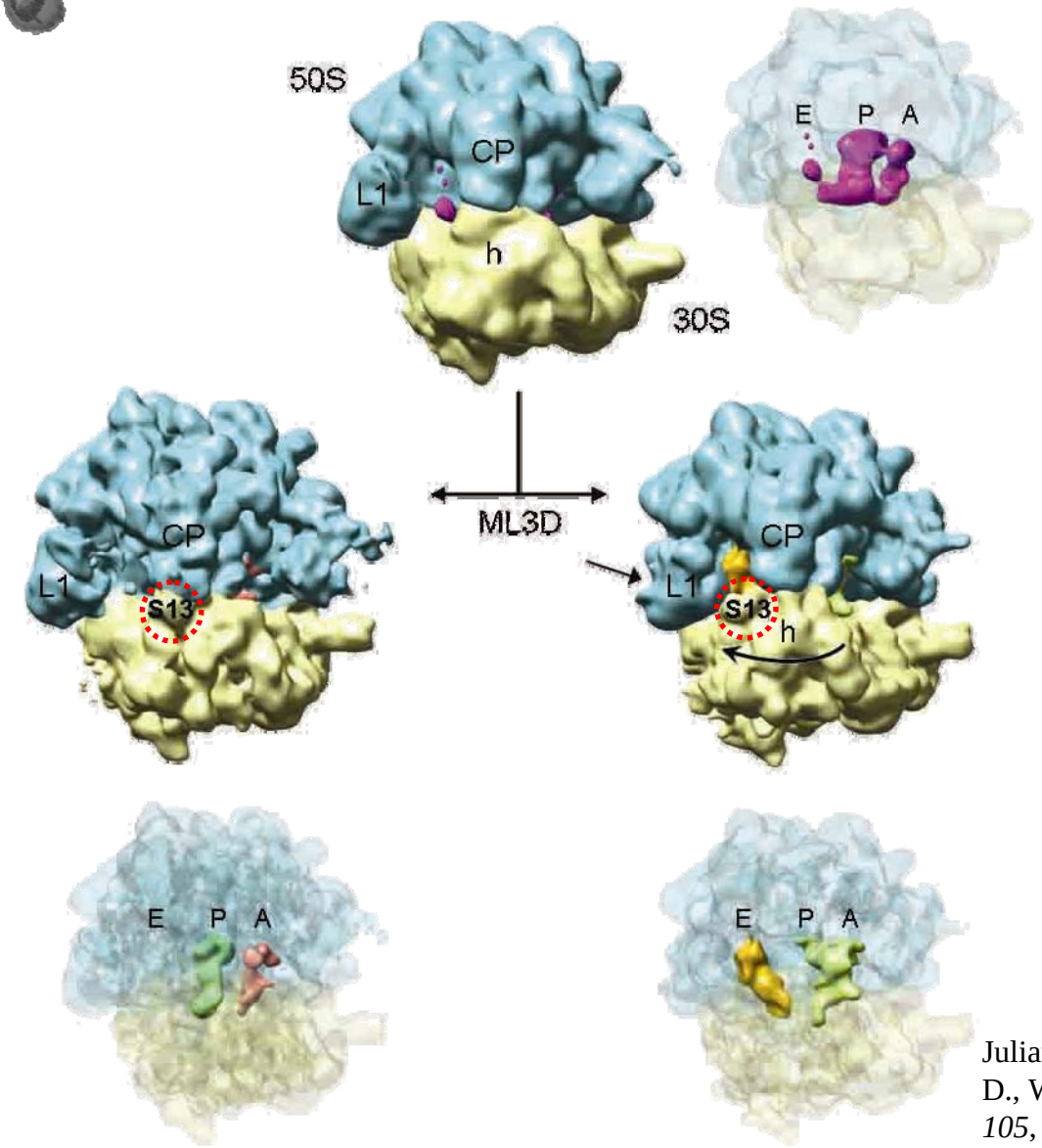
Pretranslocation revisited by CryoEM

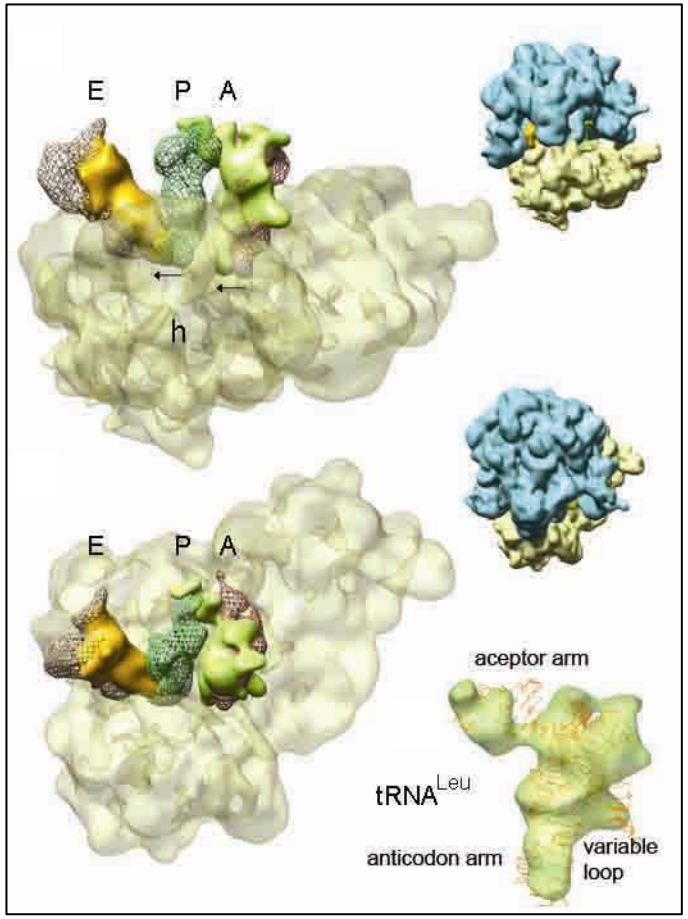
Preliminary screening

	Full set	ML3D Class 1	ML3D Class2
$MgCl_2$ 0 mM Spermine 0 mM	 18400	—	—
$MgCl_2$ 20 mM Spermine 0 mM	 18387	 4576	 7879
$MgCl_2$ 20 mM Spermine 2 mM	 15984	 11958	 4026

Pretranslocation revisited by CryoEM

Scaling up the positive



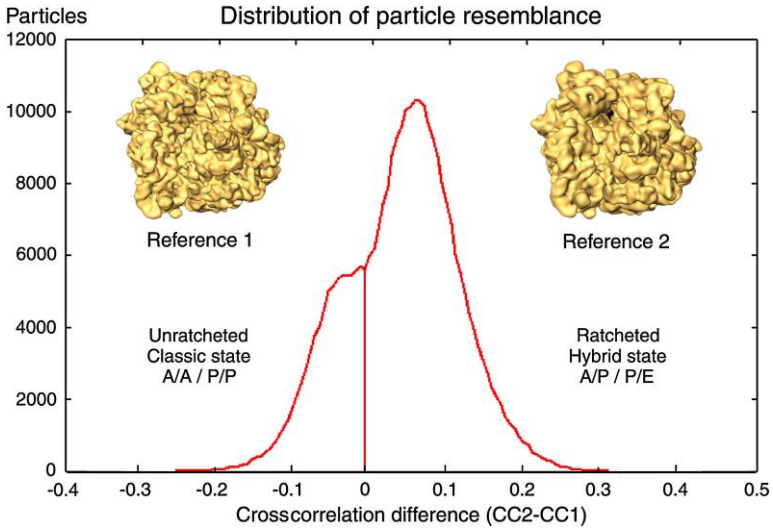


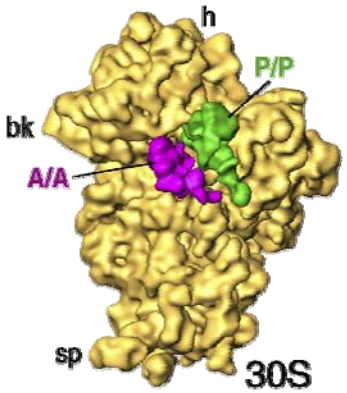
Julian, P., Konevega, A.L.K., Scheres, S.H.S., Lazaro, M., Gil, D., Wintermeyer, W., Rodnina, M.V., and Valle (2008). PNAS *105*, 16924-16927.

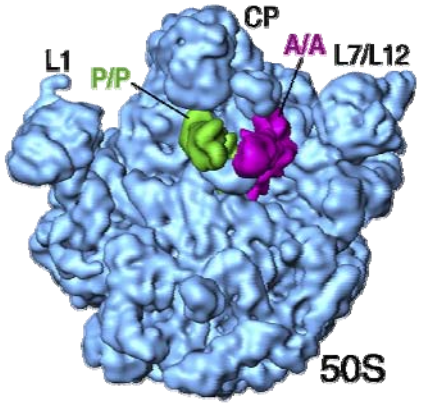
Pretranslocation revisited by CryoEM

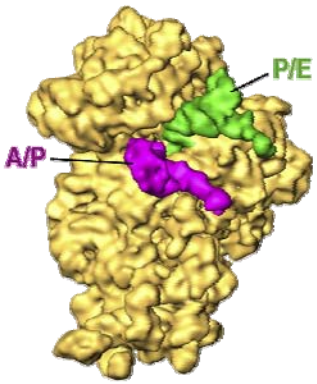
At the same time

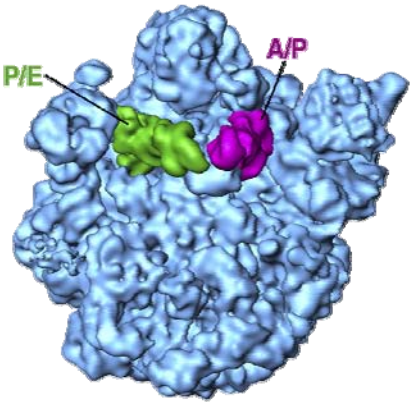
Supervised classification





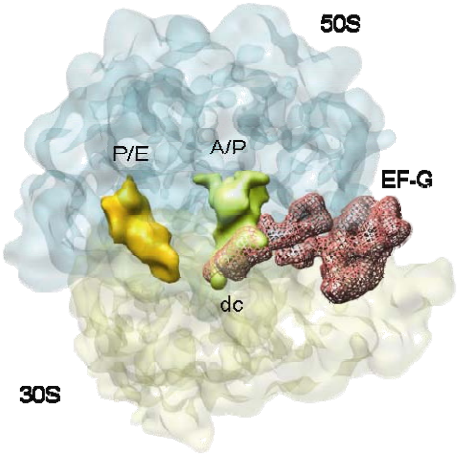




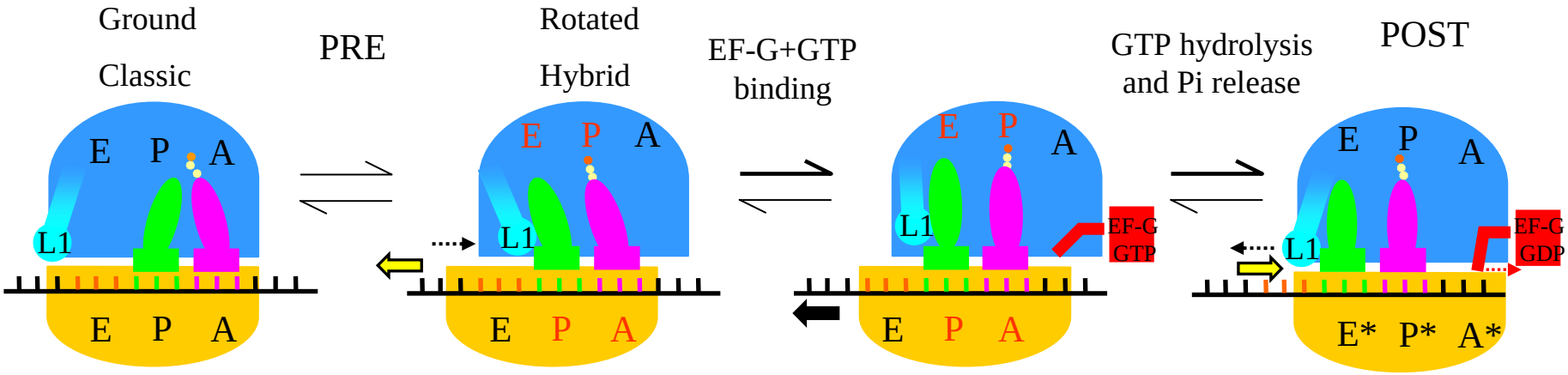


Agirrezabala, X., Lei, X., Brunelle, J., Ortiz-Meoz, R., Green, R. and Frank, J. (2008). Mol. Cell *32*, 190-197.

A model for translocation



The role of EF-G



Unlocked

Rotation between subunits

Classic/Hybrid positions for tRNA

L1 stalk

EF-G binds A site

Movement of mRNA-tRNAs

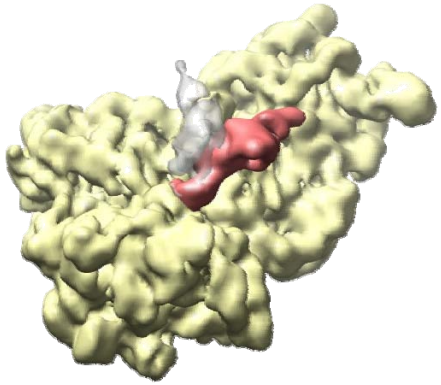
EF-G changes

Reverse rotation

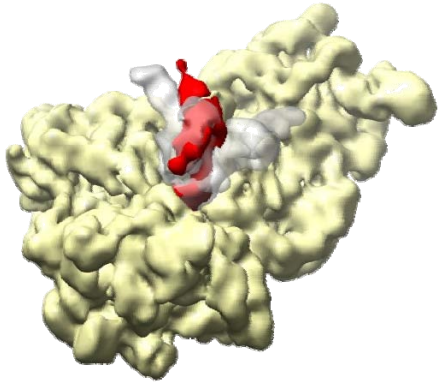
Back to locked state

The path of tRNAs through the ribosome

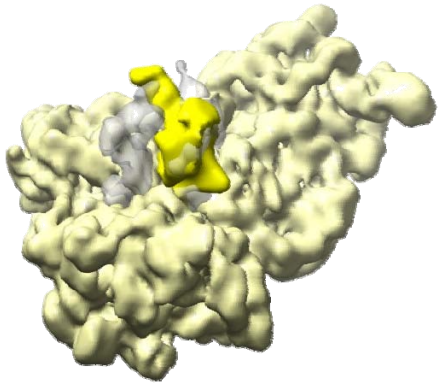
A/T



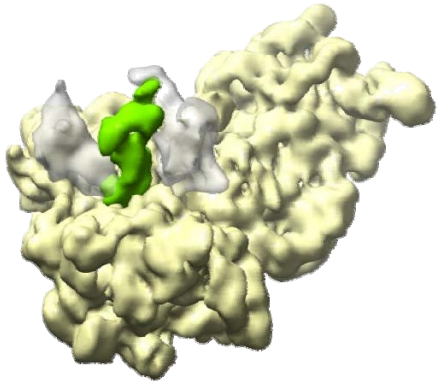
A



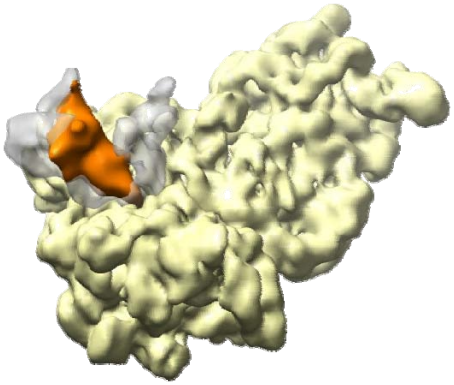
A/P



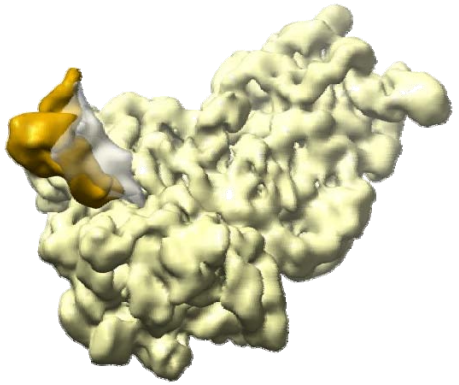
P



P/E



E



Labs on it

Jayati Sengupta
Wen Li
Urmila Ramat
Joachim Frank
Wadsworth Center/Columbia Univ.

Andrey Zavialov
Mans Ehrenberg
Biomedical Center Uppsala

Andrey Konevega
Marina Rodnina
Max Plank Institute ofr Biophysical Chemistry
Wolfgang Wintermeyer
University of Witten/Herdecke

Sjors Scheres
José María Carazo (CNB-CSIC)

Patricia Julián
Melisa Lázaro
Gorka Lasso
Xabier Aguirrezabala
David Gil
Mikel Valle
Structural Biology Unit (CICbioGUNE)
(2007. Xray/NMR/EM)