

Modeling of Cryo-EM Maps

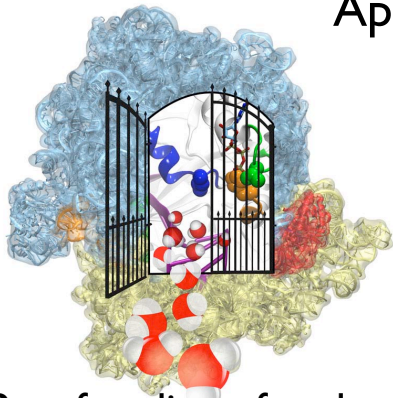
Workshop

Baylor College of Medicine

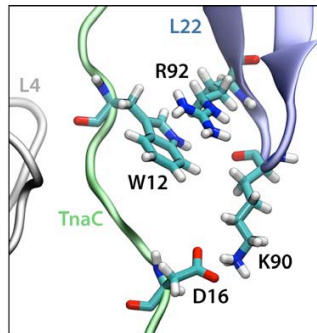
Klaus Schulten, U. Illinois at Urbana-Champaign

Molecular Modeling Flexible Fitting 3:

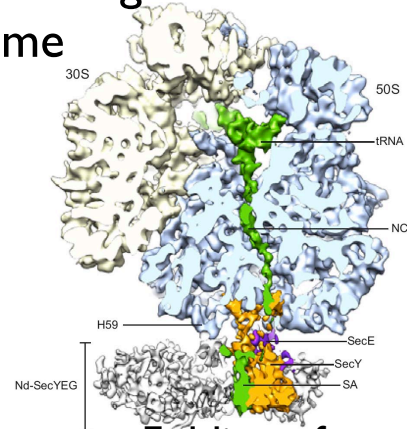
Application to Ribosome



Proofreading of codon recognition



Gene regulation through nascent chain



Folding of nascent protein

Ribosome structure employed in MDFF calculations

- E. coli ribosome (PDB 2I2U/2I2V [1])
- Completed parts: all missing residues
- A-site finger based on PDB 1TWB [2]
- L1-stalk homology model based on PDB 1MZP [3]
- L10: homology model based on PDB 1ZAV [4]
- L12: PDB: 1RQU [5]
- L10(L12)4 interactions: based on PDB 1ZAV [4]
- anti-Shine-Dalgarno region: based on PDB 2HGP [6]
- tRNA-Phe: PDB 2OW8 [7]
- path of the mRNA within the ribosome: based on PDB 2HGP [6]

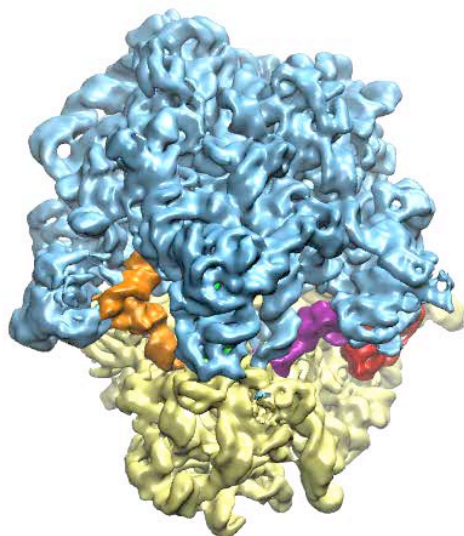
1. V. Berk, W. Zhang, R. D. Pai, and J. H. D. Cate, PNAS, **103**, 15830-15834 (2006).
2. C. S. Tung and K. Y. Sanbonmatsu, Biophys. J., **87**, 2714-2722 (2004).
3. A. Nikulin, I. Eliseikina, S. Tishchenko, N. Nevskaya, N. Davydova, O. Platonova, W. Piendl, M. Selmer, A. Liljas, D. Drygin, R. Zimmermann, M. Garber, and S. Nikonov, Nat. Struct. Biol., **10**, 104-108 (2003).
4. M. Diaconu, U. Kothe, F. Schlunzen, N. Fischer, J. M. Harms, A. G. Tonevitsky, H. Stark, M. V. Rodnina, and M. C. Wahl, Cell, **121**, 991-1004 (2005).
5. E. V. Bocharov, A. G. Sobol, K. V. Pavlov, D. M. Korzhnev, V. A. Jaravine, A. T. Gudkov, and A. S. Arseniev, J. Biol. Chem., **279**, 17697-17706 (2004).
6. G. Yusupova, L. Jenner, B. Rees, D. Moras, and M. Yusupov, Nature, **444**, 391-394 (2006).
7. A. Korostelev, S. Trakhanov, M. Laurberg, and H. F. Noller, Cell, **126**, 1-13 (2006).



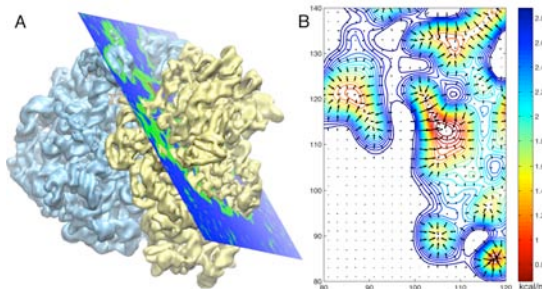
Eduard Schreiner

Ribosome structure determination by MDFF

Cryo-EM map of the *E. coli* ribosome at 6.7-Å resolution



The Molecular Dynamics Flexible Fitting Method



- [1] Trabuco et al. *Structure* (2008) 16:673-683.
- [2] Villa et al. *PNAS* (2009) 106:1063-1068.
- [3] Sener et al. *Chem Phys* (2009) 357:188-197.
- [4] Trabuco et al. *Methods* (2009) 49:174-180.
- [5] Hsin et al. *Biophys J* (2009) 97:321-329.
- [6] Gumbart et al. *Structure* (2009) In press.
- [7] Seidelt et al. *Science* (2009) 326: 1412-1415.
- [8] Becker et al. *Science* (2009) 326: 1369-1373.

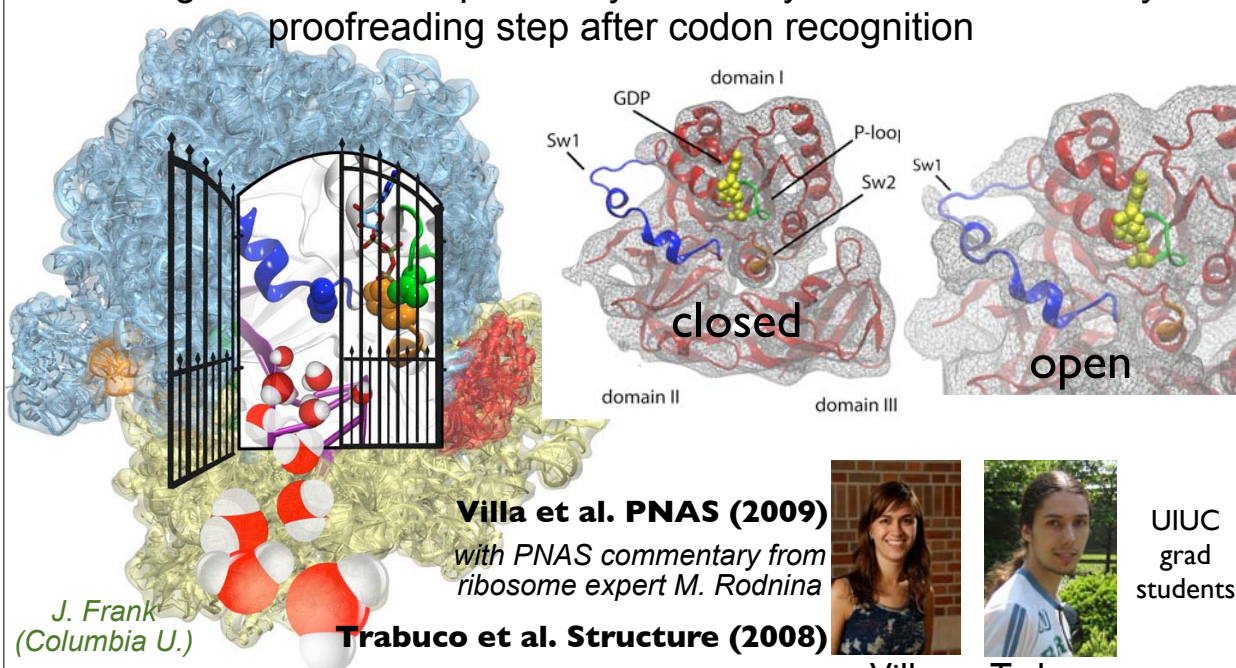


Villa et al. *PNAS* (2009) 106:1063-1068.



Discovery Through the Computational Microscope

Gating mechanism of protein synthesis by the ribosome as key proofreading step after codon recognition



J. Frank
(Columbia U.)

Villa et al. PNAS (2009)
with PNAS commentary from
ribosome expert M. Rodnina

Trabuco et al. Structure (2008)

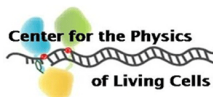


Villa



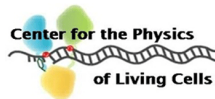
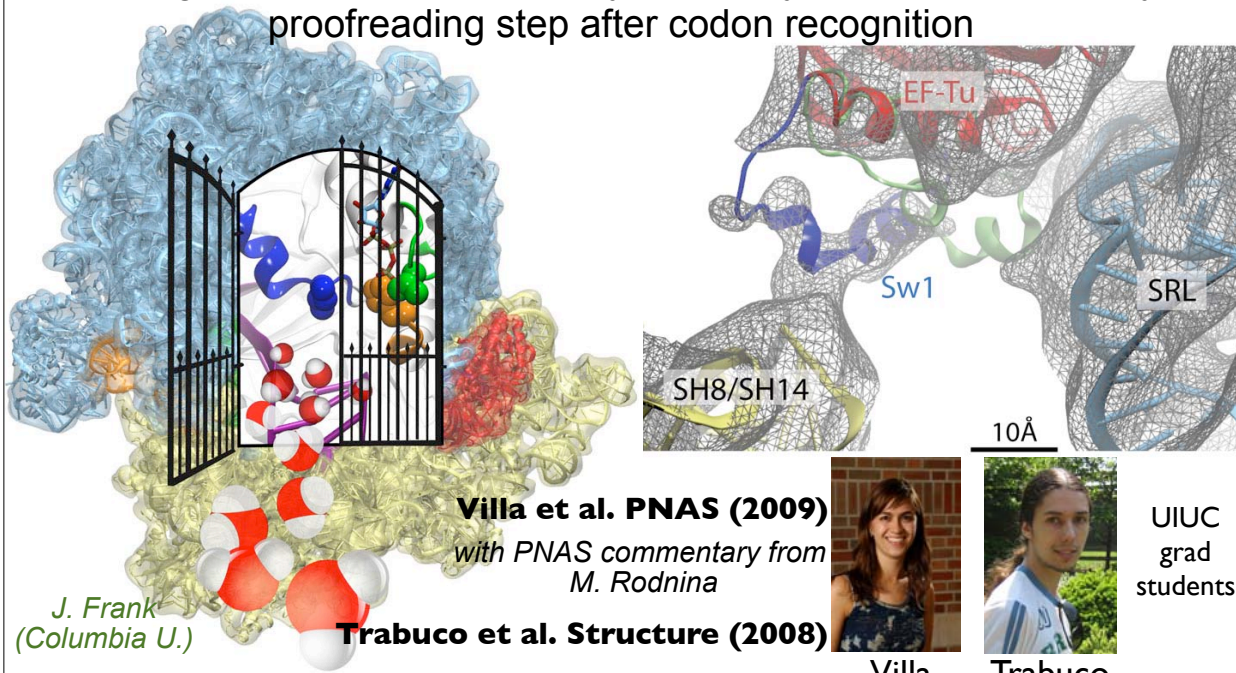
Trabuco

UIUC
grad
students



Successes of Computational Microscope Prototype

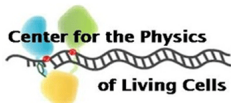
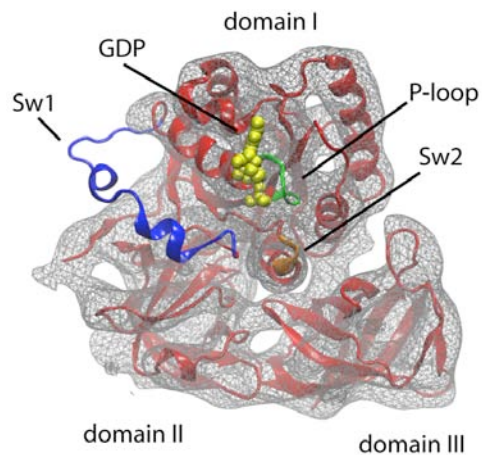
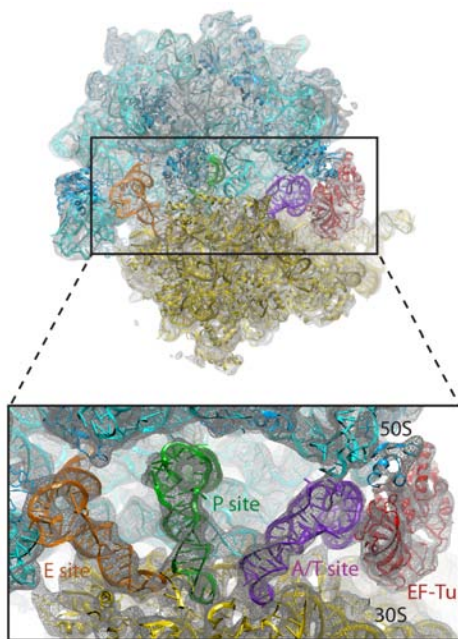
Gating mechanism of protein synthesis by the ribosome as key proofreading step after codon recognition



Mechanism of GTPase activation in EF-Tu

6.7-Å map of the *E. coli* ribosome bound to a ternary complex

Collaboration with J. Frank (HHMI, Columbia U.)



Villa et al. *PNAS* (2009) 106:1063-1068.



Seeing Nascent Proteins in the Ribosome

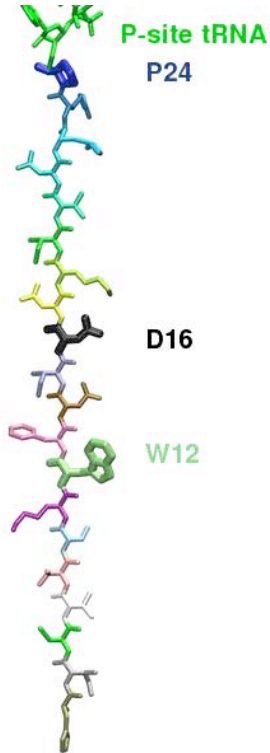


Leonardo Trabuco

TnaC peptide

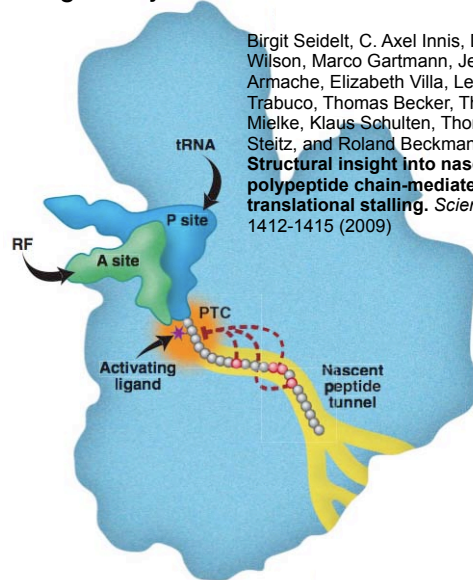


Eduard Schreiner



Simulated ribosome system

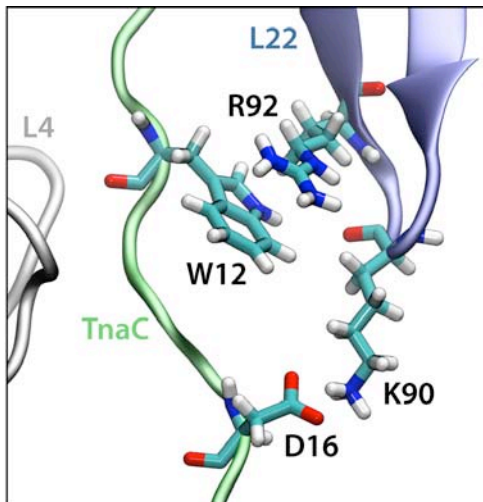
Expression of the tryptophanase (*trnA*) operon in *E. coli*, responsible for Trp degradation, is controlled by the regulatory nascent chain TnaC



Regulatory nascent chain

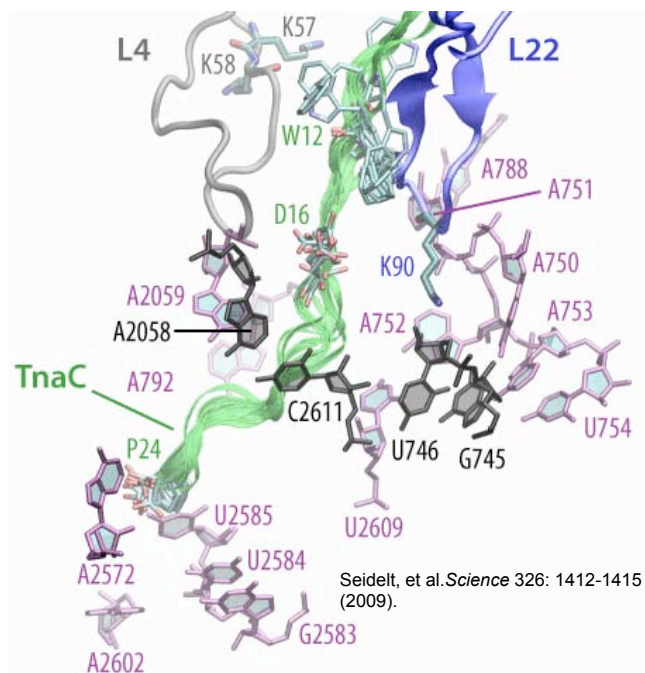
Birgit Seidelt, C. Axel Innis, Daniel N. Wilson, Marco Gartmann, Jean-Paul Armache, Elizabeth Villa, Leonardo G. Trabuco, Thomas Becker, Thorsten Mielke, Klaus Schulten, Thomas A. Steitz, and Roland Beckmann. **Structural insight into nascent polypeptide chain-mediated translational stalling.** *Science* 326: 1412-1415 (2009)

Seeing Nascent Proteins in the Ribosome



- W12 is recognized via a cation- π interaction
- D16 is recognized via a salt bridge
- Mutations I19R and V20R may prevent stalling by displacing A2609 (23S rRNA)

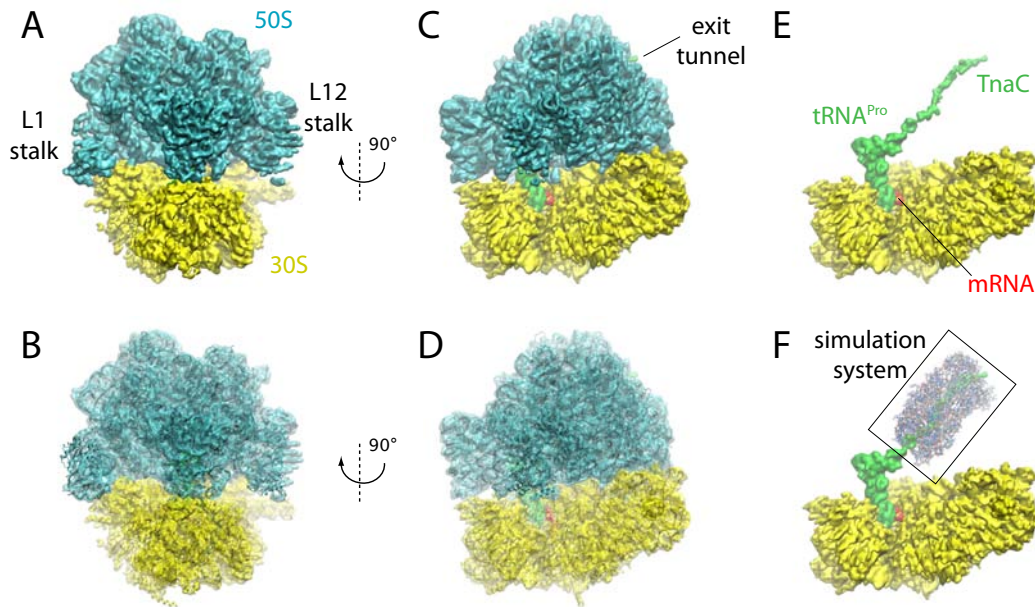
Molecular dynamics simulation



Seidelt, et al. *Science* 326: 1412-1415 (2009).

Structure of TnaC in ribosome
(EM density interpreted through ensemble of structures)

MD simulations of TnaC in the exit tunnel



MD simulations of TnaC in the exit tunnel

System preparation

- Cut exit tunnel (within 20Å of TnaC)
- Restrain backbone atoms at the system boundaries
- Add Mg²⁺ ions with cionize (GPU version) [1]
- Complete Mg²⁺ solvation with mgSolvate.tcl [2]
- Solvate with DOWSER (VMD plugin) [3]
- VMD solvate
- VMD autoionize

Simulation setup

- System size: ~125,000 atoms
- MD package: NAMD 2.7
- Force field: CHARMM27 with CMAP
- p = 1 bar; T = 310K
- Time step: 2 fs
- Short-range cutoff: 8.0 Å
- PME with pencil decomposition
- 18 NCSA Abe nodes (144 cores): ~12.5 ns/day
- 10 simulations x 180 ns = 1.8 μs: ~0.5 M SUs

[1] Stone et al. *J Comp Chem* (2007) 28:2618-2640.

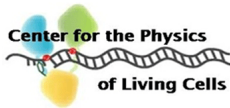
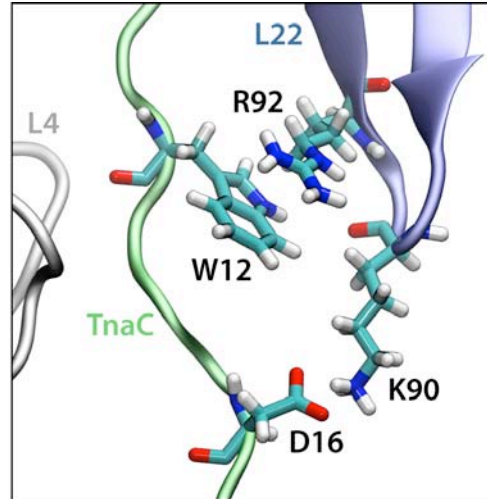
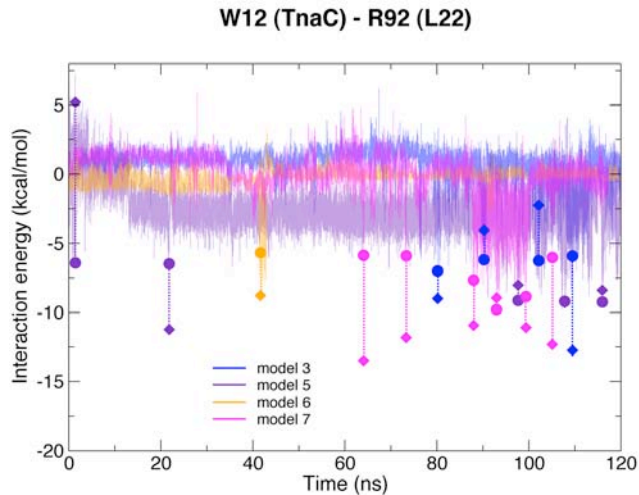
[2] Eargle et al. *J Mol Biol* (2008) 377:1382-1405.

[3] Gumbart et al. *Structure* 17: 1453-1464 (2009).

Trabuco et al., submitted.

Recognition of TnaC by the exit tunnel

- Critical W12 is recognized via cation- π interaction (diamonds correspond to quantum-mechanical interaction energies).
- Critical D16 is recognized via a salt bridge

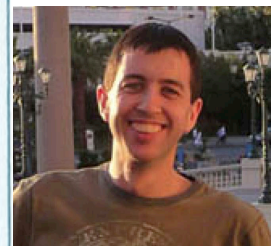
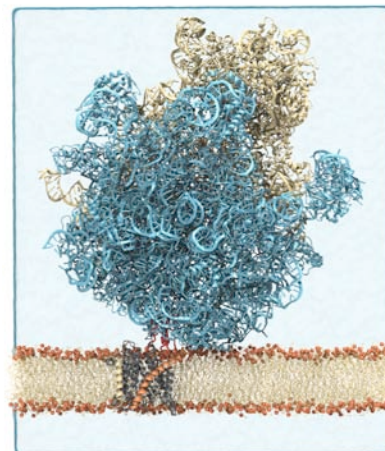
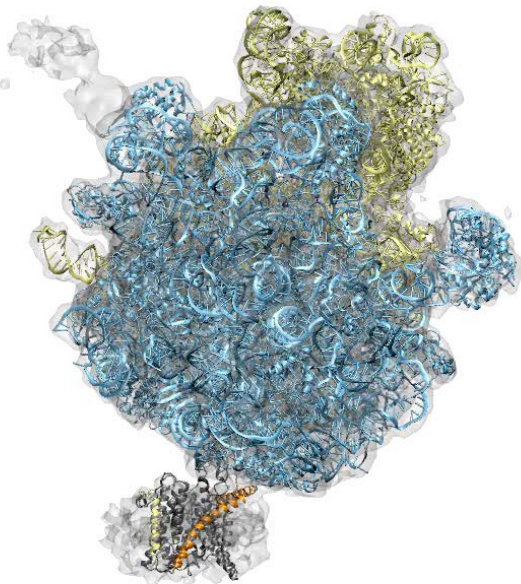


Trabuco et al. (2009) submitted.

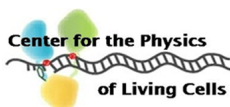


Regulation of the protein-conducting channel

- Atomic model of 70S•SecY complex derived from cryo-EM [1] using MDFF
- Simulated 70S•SecY plus water and membrane (2.7 million atoms) for a total of ~50ns



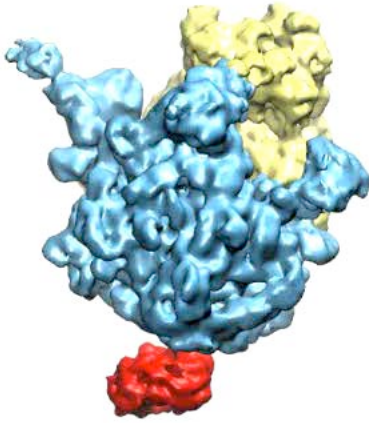
James Gumbart



[1] Menetret et al. *Mol Cell* (2007) 28:1083-1092.
Gumbart et al. *Structure* (2009) 17:1453-1464.



Modeling a ribosome-channel complex

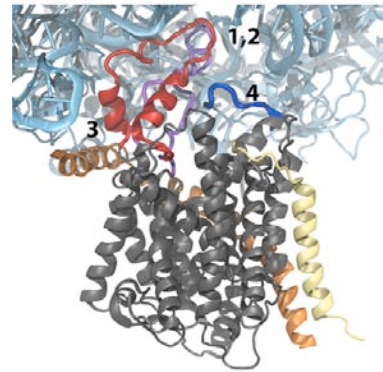
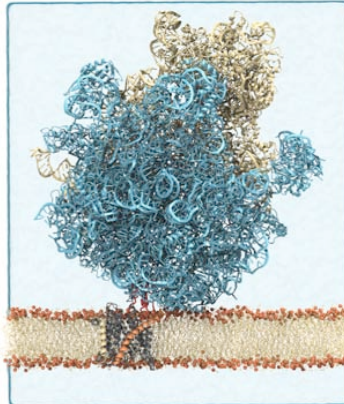


- Ribosome-SecY channel complex: known only from low-resolution density maps (grey outline)
- Used MD Flexible Fitting to fit atomic structures to map

2.7 million atoms simulated in total for nearly 50 ns

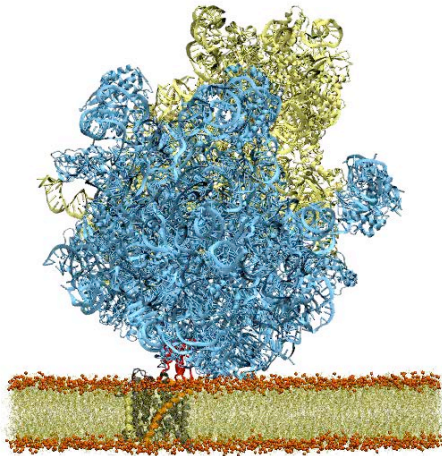
James Gumbart, Leonardo G. Trabuco, Eduard Schreiner, Elizabeth Villa, and Klaus Schulten. Regulation of the protein-conducting channel by a bound ribosome. *Structure* 17: 1453-1464 (2009).

Thomas Becker, Elisabet Mandon, Shashi Bhushan, Alexander Jarasch, Jean-Paul Armache, Soledad Funes, Fabrice Jossinet, James Gumbart, Thorsten Mielke, Otto Berninghausen, Klaus Schulten, Eric Westhof, Reid Gilmore, and Roland Beckmann. Structure of monomeric yeast and mammalian Sec61 complexes interacting with the translating ribosome. *Science* 326: 1412-1415 (2009).



Simulations reveal atomic-scale interactions that maintain complex

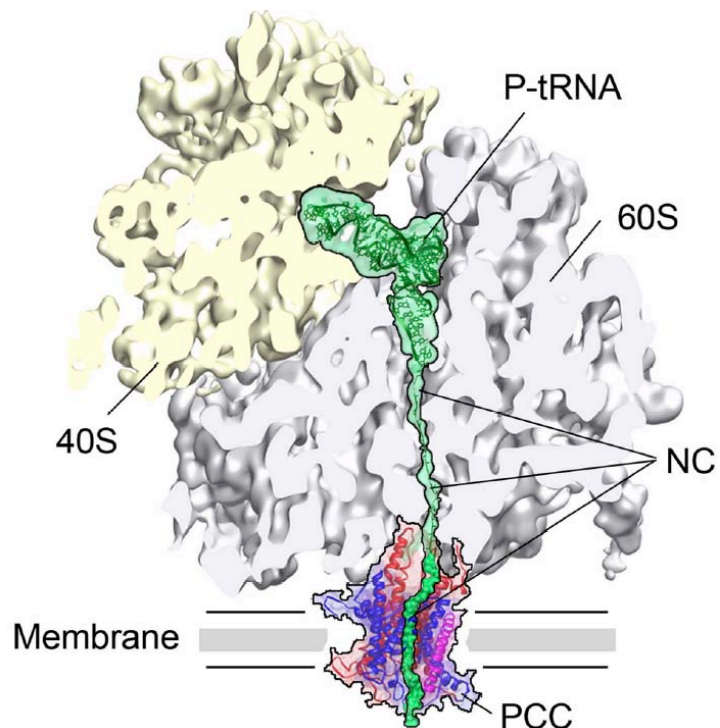
Modeling a ribosome-channel complex



2.7 million atoms simulated in total for nearly 50 ns

James Gumbart, Leonardo G. Trabuco, Eduard Schreiner, Elizabeth Villa, and Klaus Schulten. Regulation of the protein-conducting channel by a bound ribosome. *Structure* 17: 1453-1464 (2009).

Thomas Becker, Elisabet Mandon, Shashi Bhushan, Alexander Jarasch, Jean-Paul Armache, Soledad Funes, Fabrice Jossinet, James Gumbart, Thorsten Mielke, Otto Berninghausen, Klaus Schulten, Eric Westhof, Reid Gilmore, and Roland Beckmann. Structure of monomeric yeast and mammalian Sec61 complexes interacting with the translating ribosome. *Science* 326: 1412-1415 (2009).

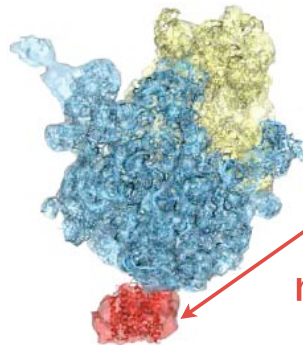
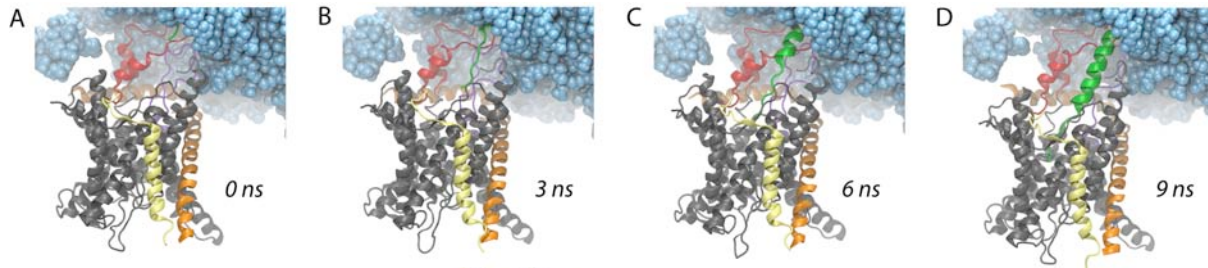


Simulations reveal atomic-scale interactions that maintain complex

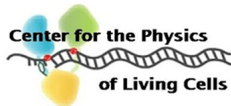
Peptide translocation from ribosome to channel

- A polypeptide (Ala₂₆) could be translocated from the exit tunnel to the SecY channel using steered MD with minimal disruption of ribosome-channel interactions

Gumbart et al. *Structure* (2009) 17:1453-1464.

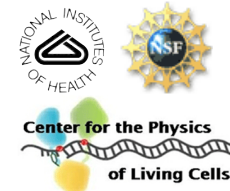


but Sec YEbeta
poorly resolved
due to use
of detergents;
replaced by nanodisc



Acknowledgements

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Keiichi Namba (Osaka U.)
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Elizabeth
Villa



James
Gumbart



Eduard
Schreiner



Leonardo
Trabuco

