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Supplementary Materials for

Ribosome rearrangements at the onset of translational bypassing

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This PDF file includes:

SUPPLEMENTARY MATERIALS

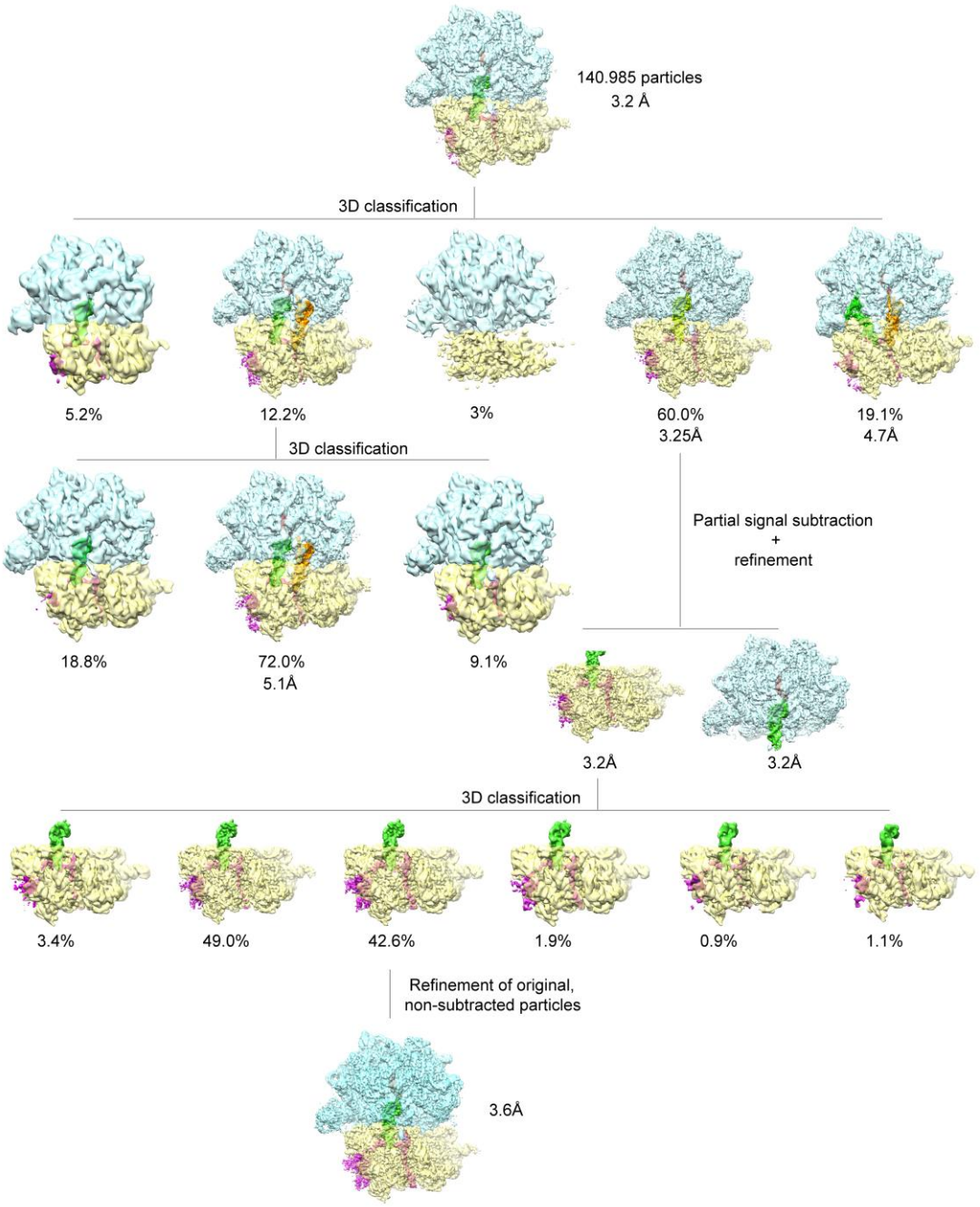
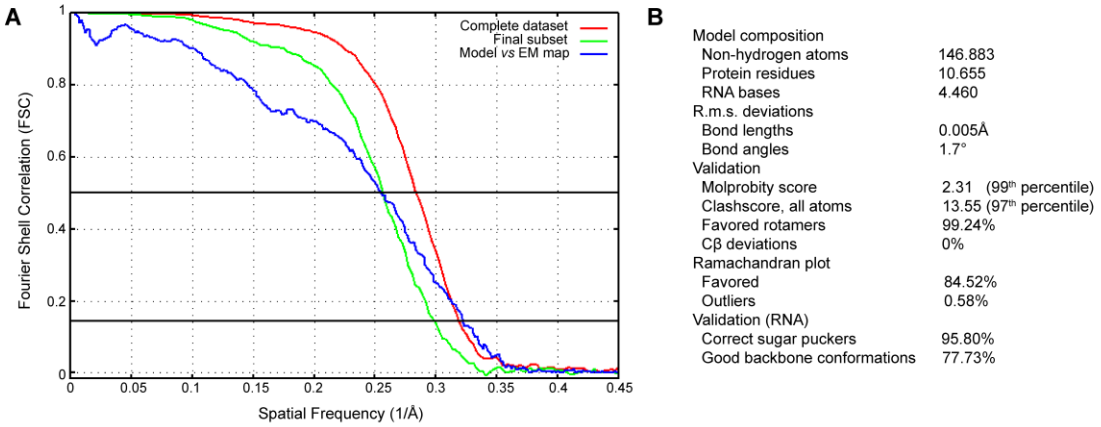


fig. S1. Overview of particle classification and structure determination. Cryo-EM densities showing ribosomal 30S (yellow) and 50S (blue) subunits, A-site tRNA (orange), P-site tRNA (green), nascent peptide (red) and mRNA (magenta). See Experimental procedures for details.



B

Model composition	
Non-hydrogen atoms	146.883
Protein residues	10.655
RNA bases	4.460
R.m.s. deviations	
Bond lengths	0.005Å
Bond angles	1.7°
Validation	
Molprobity score	2.31 (99th percentile)
Clashscore, all atoms	13.55 (97th percentile)
Favored rotamers	99.24%
Cβ deviations	0%
Ramachandran plot	
Favored	84.52%
Outliers	0.58%
Validation (RNA)	
Correct sugar puckers	95.80%
Good backbone conformations	77.73%

fig. S2. Fourier shell correlation curves and model statistics. The FSC (Fourier Shell Correlation) curve from the complete dataset is shown in red, the curve for the final subset is shown in green. The curve for the final model versus the map is shown in blue. The resolution was estimated using the FSC calculated between fully independent half-sets (the so-called "gold standard"). The 0.5 and 0.143 cutoffs in the FSC are highlighted. The table to the right shows the model quality statistics.

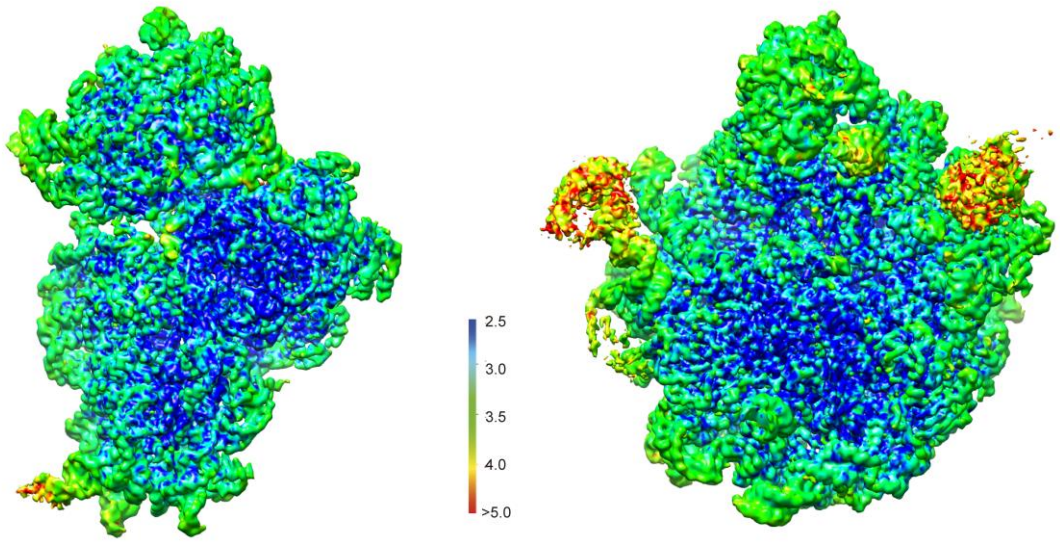


fig. S3. Local resolution. The local resolution for the reconstructed cryoEM map. Scale bar shows colour scale with resolution in Å.

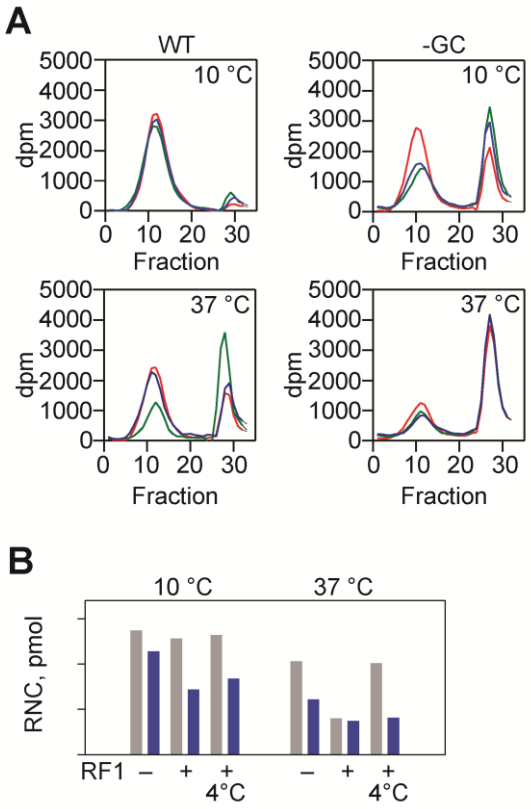


fig. S4. Effect of the A-site hairpin on the stability of peptidyl-tRNA binding and on the action of RF1. Translation was carried out for 20 min at 10°C or 37 °C. Ribosome-nascent-chain complexes (RNC) were isolated by gel filtration on a Biosuite 450 HR 8 µm column. The amount of stop-peptide that is released from the ribosome or remains bound to the ribosomes as peptidyl-tRNA was determined by radioactive counting using the N-terminal [^{3}H]-labeled Met. (**A**) Elution profiles of ribosome-bound and free peptides after translation carried out without RF1 (red line), with RF1 (green line), or at conditions where translation was carried out without RF1 followed by cooling to 4^{o}C for 15 min and incubation with RF1 at 4^{o}C for 20 min (blue line). The first peak on the histogram corresponds to the peptide migrating together with ribosome (RNC), the second peak corresponds to the free peptide. (**B**) Quantification of the amount of stop-peptide remaining in RNC. Translation was carried out with the WT mRNA (gray) or –GC mRNA lacking a basepair in the A-site hairpin.

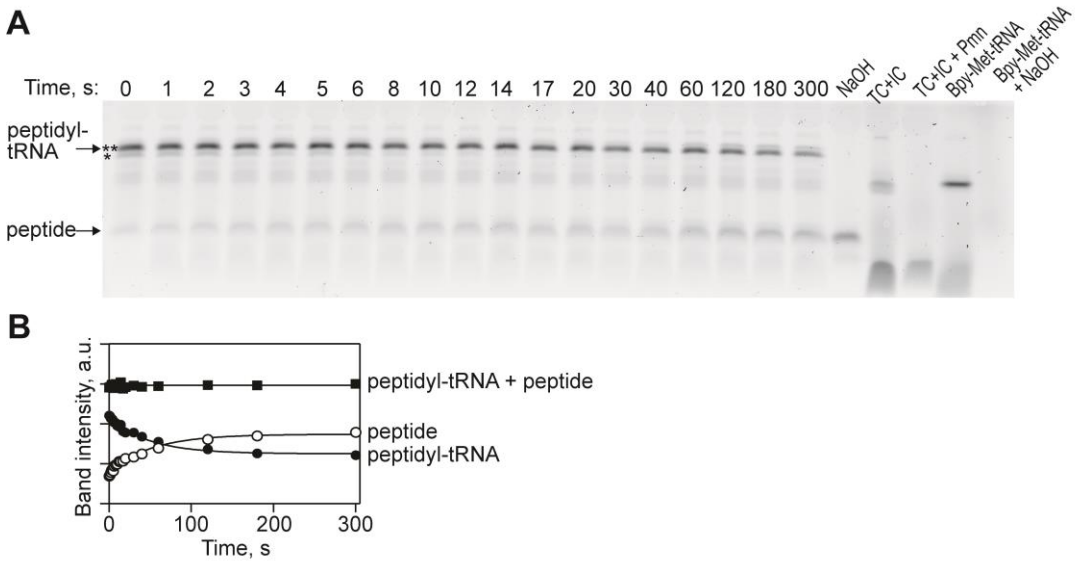


fig. S5. The reactivity of the PTC with Pmn at 10°C. During translation, Pmn, which resembles the 3´ end of tyrosyl-tRNA, enters the PTC A-site, and the peptidyl transferase reaction transfers the nascent peptide from tRNA to Pmn. Thus, the rate of nascent peptide chain transfer to Pmn is the indicator of PTC function. The advantage of using Pmn is that it binds regardless of the A-site (that is, it does not depend of the A-site stem-loop) and does not induce the active conformation of the PTC. (**A**) Time course of the Pmn reaction monitored as change in band intensities of the peptidyl-tRNA and peptide. The two bands of the peptidyl-tRNA correspond, respectively, to the stalled stop-peptide (major band indicated by **) and a shorter peptide that is a transient intermediate of translation at low temperature (minor band indicated by *). NaOH shows the full digestion control of peptidyl-tRNA. Other controls show the positions of Bpy-Met and Bpy-Met-tRNA. IC, initiation complexes with Bpy-Met-tRNA, TC, ternary complexes (not visible). (**B**) Quantification of the Pmn reaction by the reduction in band intensity of peptidyl-tRNA (closed circles) and the appearance of the band of the released peptide (open circles). As a control for consistency, the sum of band intensities corresponding to the free peptide and peptidyl-tRNA remains unchanged (closed squares).

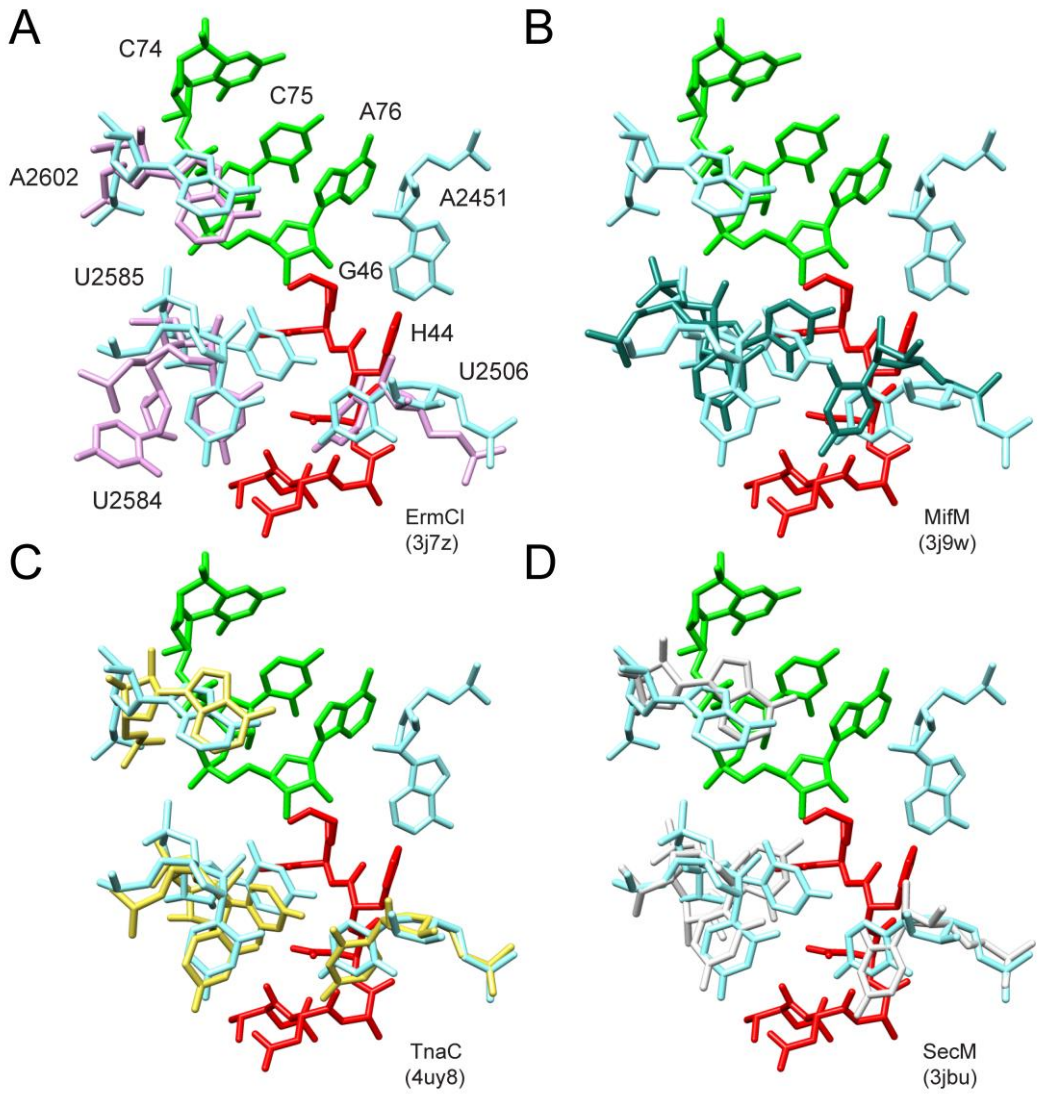


fig. S6. Conformation of the PTC. The take-off complex PTC configuration (blue) is compared to several complexes stalled by arrest peptides (lilac, green-blue, yellow and gray as indicated).