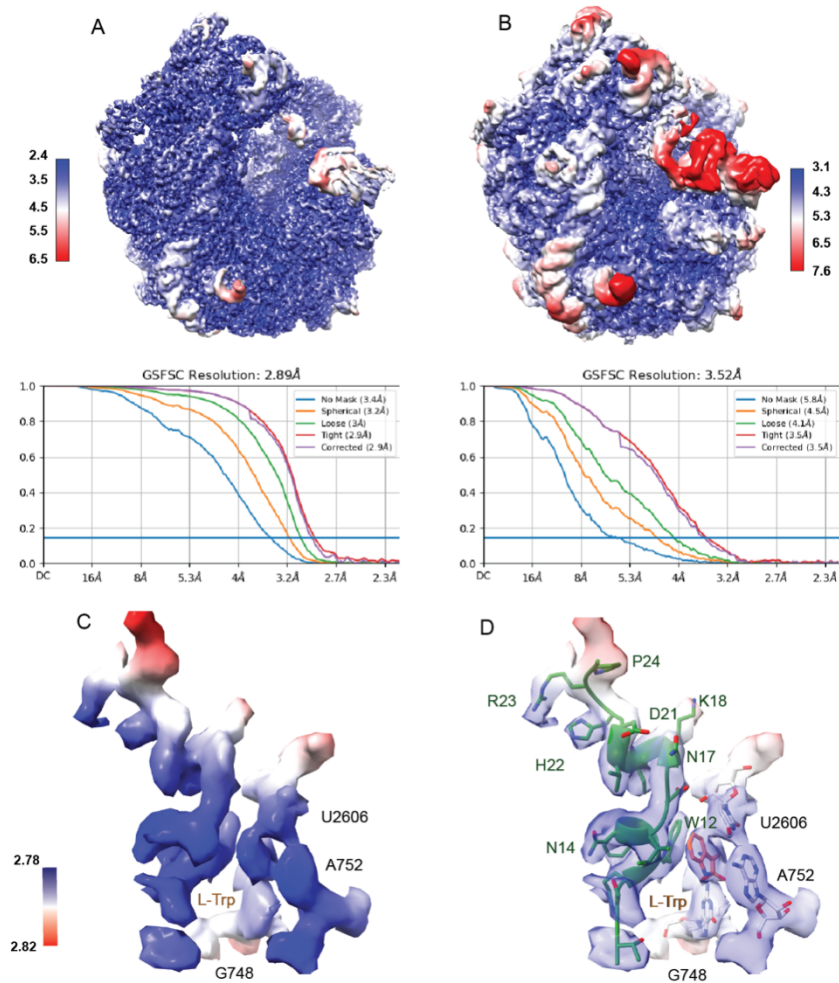
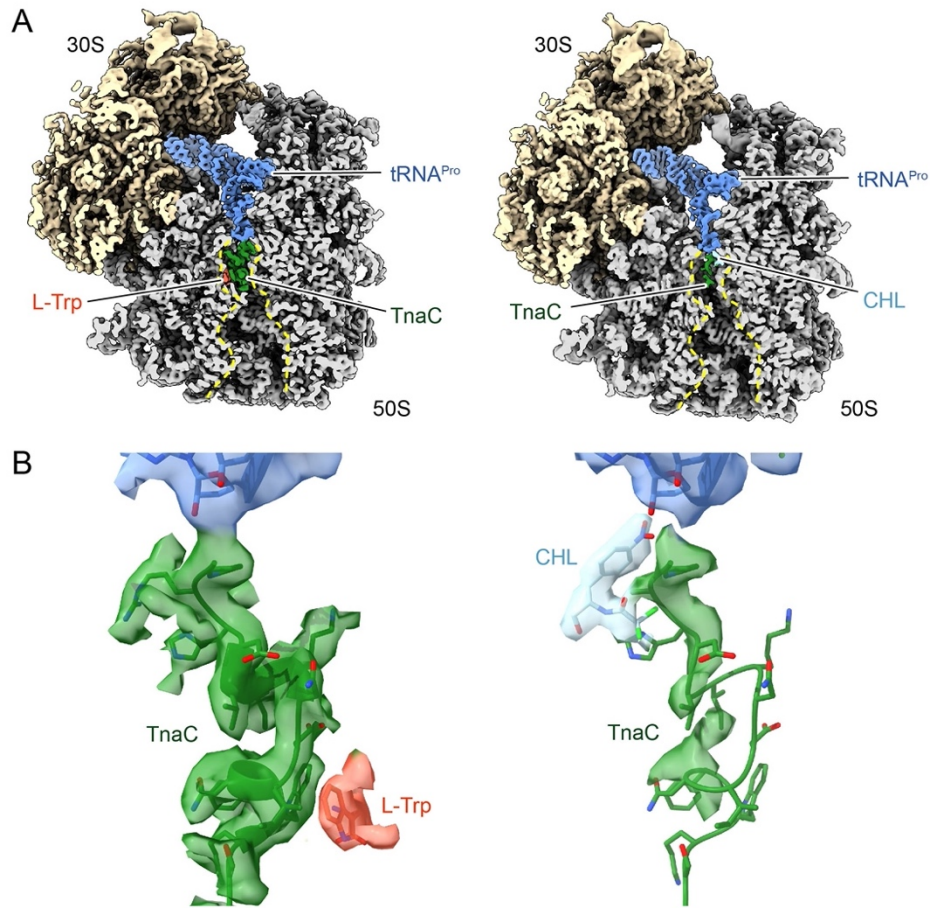


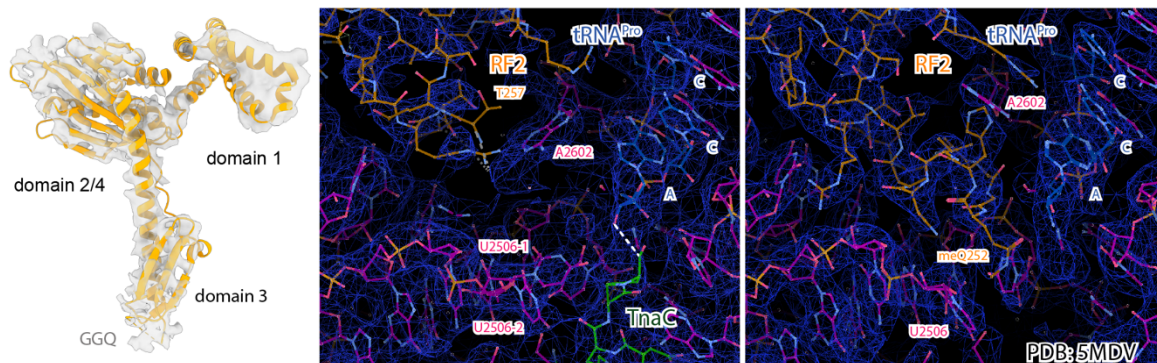
Supplementary Fig. S1: Single particle workflow for processing the RF2-TnaC-RNC dataset. In brief, particles picked with CrYOLO (version 1.7.5) were 2D classified in RELION-3.1. A major class representing TnaC-RNCs with a tRNA in the P-site was refined to a final resolution of 2.89 Å, including CTF refinement and correction of higher order aberrations with RELION-3.1 and CryoSPARC-2. A class with density present in the A-site was sub-sorted using a soft spherical mask around the translation factor binding and P sites. Several 3D classification steps were done in RELION-3.1 and RF2-containing particles were progressively enriched by removing unoccupied class particles. A small class (2.5 % of all particles) contained rigidly-bound RF2 as well as ArfA in the A-site of the 30S subunit. This class was refined similarly to the TnaC-RNC in RELION-3.1 and CryoSPARC-2. To analyse the structure, maps were post-processed and filtered according to local resolution.



Supplementary Fig. S2: Local resolution and FSC curves for the TnaC-RNC (A) and the RF2-TnaC-RNC (B) cryo-EM maps. Gold standard FSC curves (GSFSC) were exported from CryoSPARC-2. (C) and (D): local resolution of the isolated TnaC nascent chain and surrounding residues (C) and the model fit into the map (transparent and colored according to local resolution).



Supplementary Fig. S3: (A) Cryo-EM structure of the TnaC-RNA with L-Trp present (left) compared to the control TnaC-RNC lacking L-Trp (right). (B) Zoomed views on isolated densities for the TnaC nascent chain for L-Trp containing (left) and control (right) TnaC-RNC. Compared to the L-Trp containing structure, the TnaC arrest peptide is less structured, which indicates a higher degree of flexibility due to the lack of stabilizing L-Trp. CHL = chloramphenicol.



Supplementary Fig. S4: Isolated density and fitted model for RF2 (left panel) and zoomed views into the PTC focusing on the position of the GGQ-loop (middle panel). As a comparison, the same view on the PTC is shown for the ArfA-RF2-70S structure, where the GGQ-loop is accommodated (PDB: 5MDV).