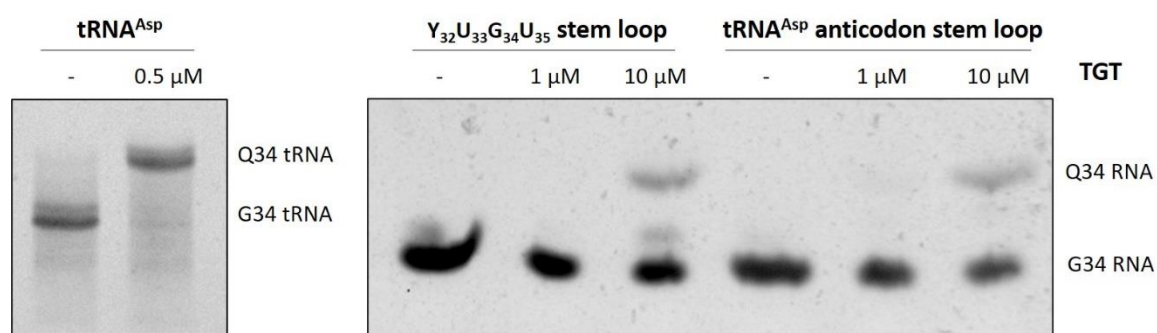
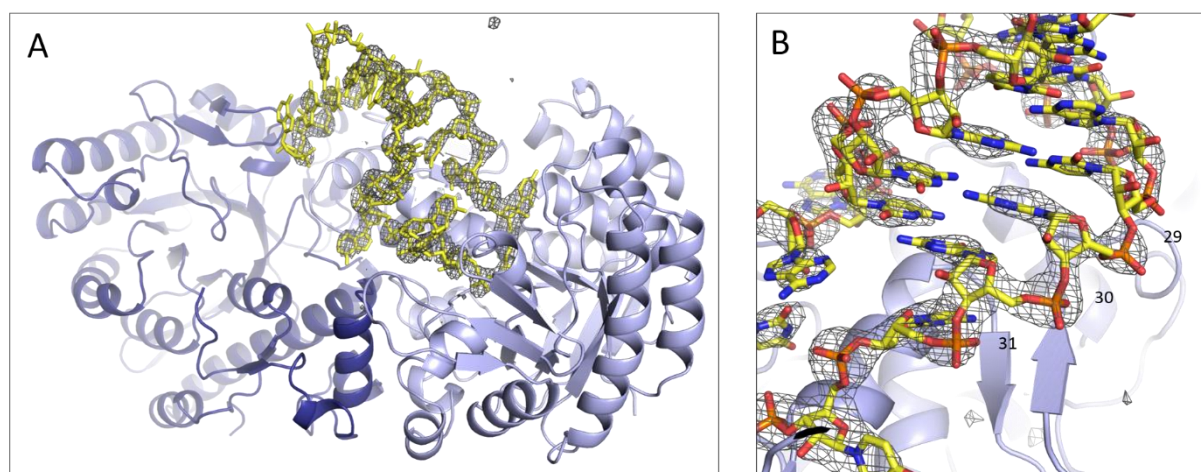


Supplementary Material



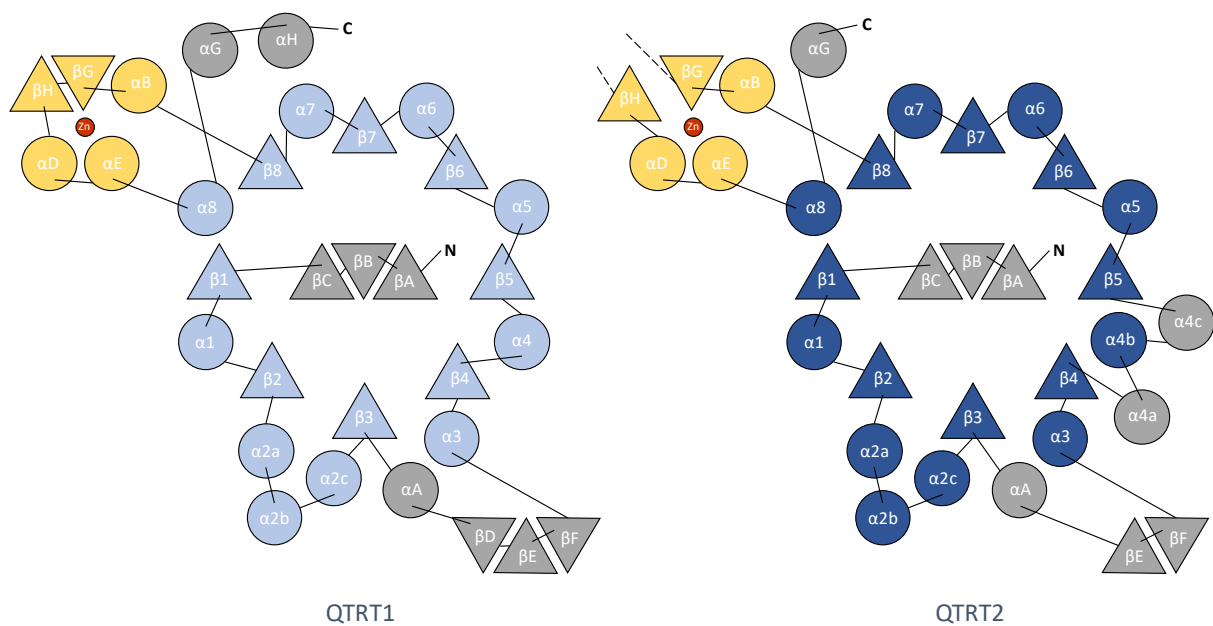
S 1 Q-incorporation into stem loop RNA

Q-incorporation by human TGT into tRNA^{Asp}, a tRNA^{Asp} anticodon stem loop or the Y₃₂U₃₃G₃₄U₃₅ stem loop construct used for crystallization. The incorporation reactions contained either 4 μ M tRNA or 5 μ M stem loop RNA, 1 mM queuine and 0.5 to 10 μ M TGT and were incubated for 1 h (tRNA^{Asp}) to 2.5 h (stem loops) at 37 °C. Reaction samples were separated on a boronate affinity electrophoresis gel, which causes retardation of queuine-containing RNA through interaction via its cis-diol.



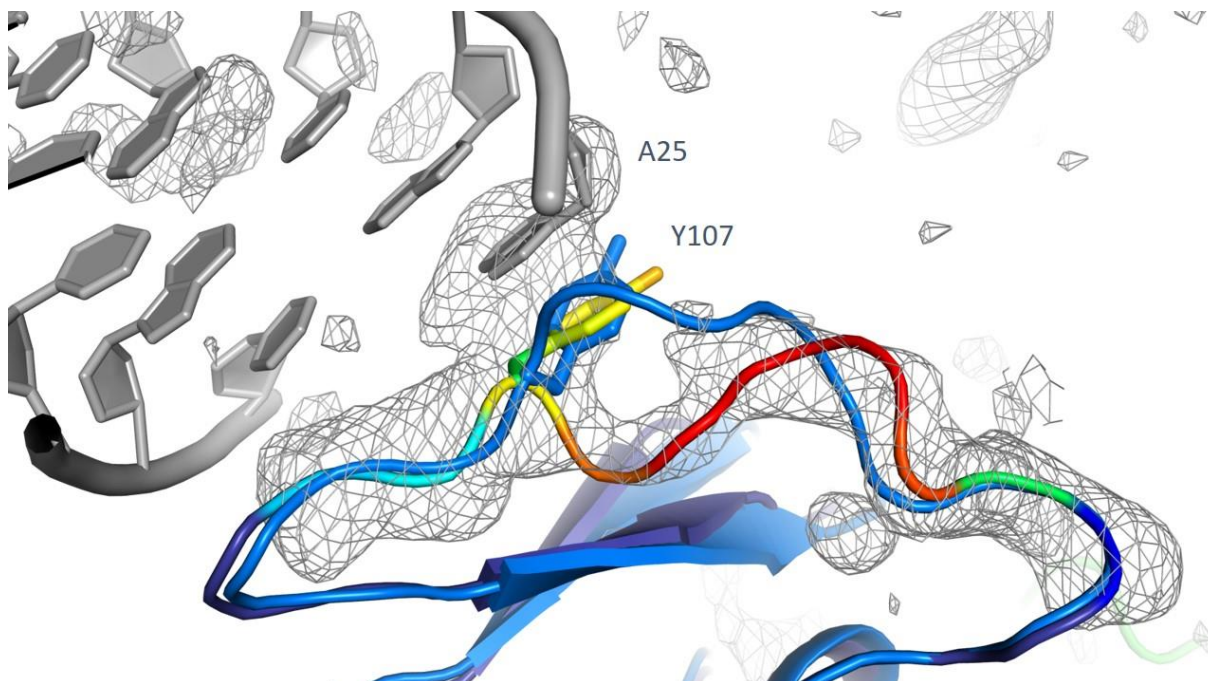
S 2 Electron density of the RNA stem loop

An mFo-DFc omit map of the RNA stem loop (chain C) contoured at $\sigma = 3.0$ is shown as grey mesh. (A): Overview of the hTGT-RNA structural model. (B): Close-up of the helical stem focusing on nucleotides 29-31.



S 3 Topology of human TGT

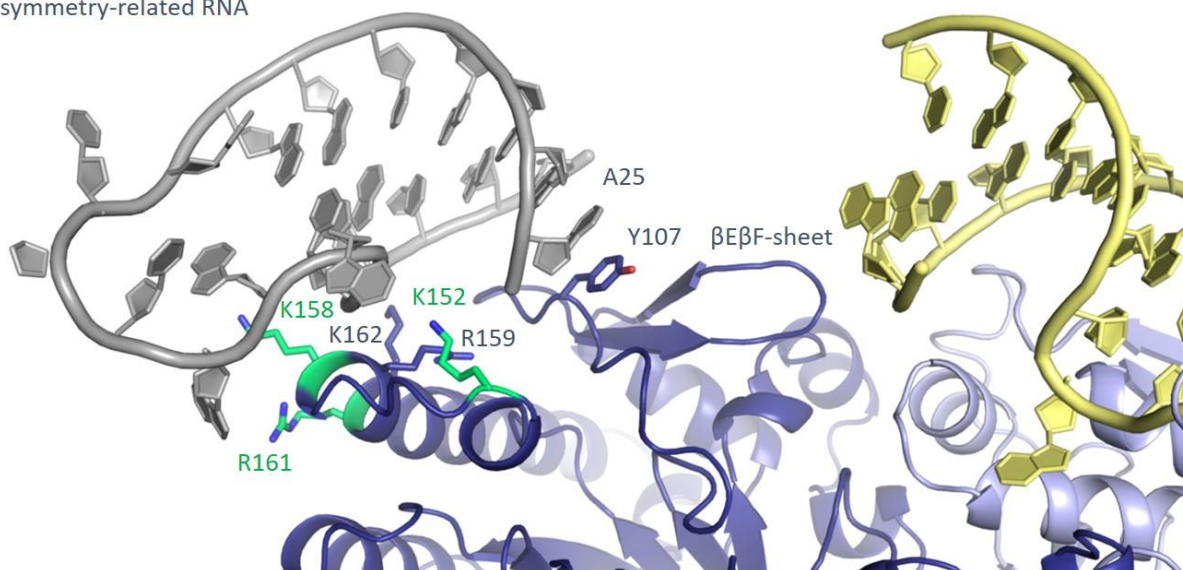
The topologies of QTRT1 (A) and QTRT2 (B) with adapted numbering of secondary structure elements. The (β/α)₈ core is shown in blue, the zinc binding domain coordinating a single zinc ion (red) is shown in yellow and additional insertions are shown in grey. Triangles represent β-sheets, circles represent α-helices.



S 4 Unpaired adenine 25 of a symmetry-related RNA forces QTRT2 main chain to adopt an alternative conformation

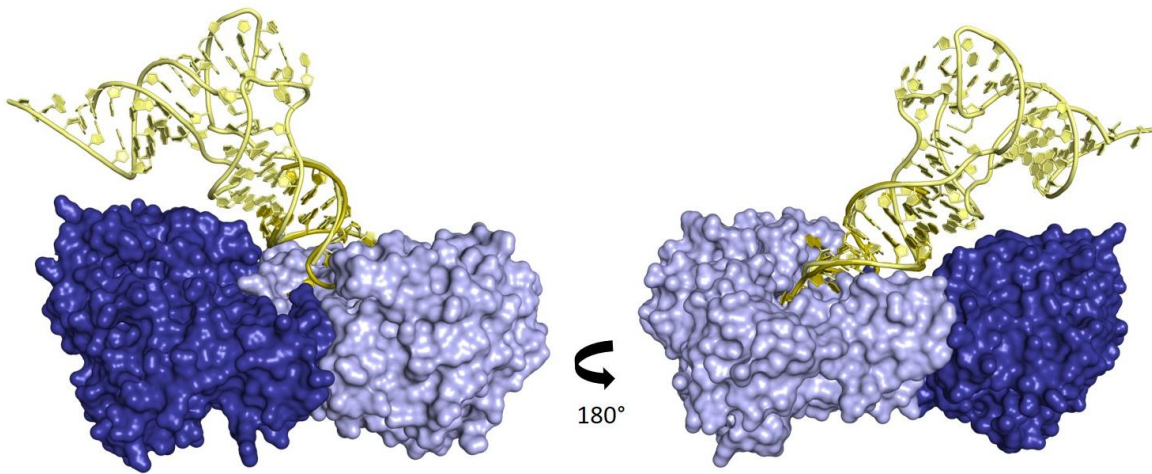
The course of the main chain of QTRT2 residues 100 - 109 deviates from that in the mouse structure (PDB-ID 6FV5, shown in marine blue). Y107 stacking to A25 of a symmetry-related RNA (grey) in the hTGT structure is shown in stick representation. QTRT2 as seen in the hTGT structure is shown in dark blue with residues 100 - 109 being colored according to B-factor (low: blue, high red). An mFo-DFc electron density omit map for QTRT2 residues 100 - 109 and adenine 25 contoured at $\sigma=2.5$ is shown as a grey mesh.

symmetry-related RNA



S 5 Interaction between positively charged QTRT2 patch and a symmetry-related RNA

QTRT2 (dark blue) interacts with a symmetry related RNA (grey). Positively charged QTRT2 residues in the area are shown in stick representation, residues that were a target for mutagenesis are colored in green. Additionally, QTRT2 residue Y107 stacking to A25 is also shown in stick representation.



S 6 Model of tRNA-bound complex

A model of human tRNA^{Asp} based on the crystal structure of *Saccharomyces cerevisiae* tRNA^{Asp} (light yellow) was superimposed to the RNA stem loop (dark yellow) in the hTGT structure. Human TGT is shown in surface representation (QTRT1: light blue, QTRT2: dark blue)