SUPPLEMENTARY INFORMATION
FOR

Recognition of the amber stop codon by release factor RF1

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Table S1: X-ray data collection and refinement statistics

Data collection	
Space group	$P2_1\,2_12_1$
Cell dimensions	
a, *b*, *c* (Å)	210.4, 452.7, 617.1
α, β, γ (°)	90, 90, 90
Resolution (Å)	50-3.62 (3.75-3.62)*
$R_{p.i.m}$ *(I)* **	0.11 (0.77)
$I/\sigma I$	11.0 (1.5)†
Completeness (%)	100.0 (100.0)
Redundancy	75.2 (37.7)
Refinement	
Resolution (Å)	50-3.62
No. reflections	663,328
R_{work}/ R_{free}***	0.26 / 0.29
No. atoms	294,206
Protein	96,414
RNA	197,382
Water/ion	410
R.m.s deviations	
Bond lengths (Å)	0.002
Bond angles (°)	0.7

* Parameters for the highest resolution shell are shown in parentheses.

** *Rp.i.m (I)* is the redundancy-independent merging R-factor (Weiss, 2001) calculated in SCALA (1994)

*** R_{work} is the R-factor calculated using working-set reflections, i.e. those that were used for structure refinement. R_{free} is the R-factor calculated using test-set reflections, i.e. those that were not used during structure refinement (Brunger, 1992).

† $I/\sigma I$=2.0 in the (3.7-3.85) resolution shell

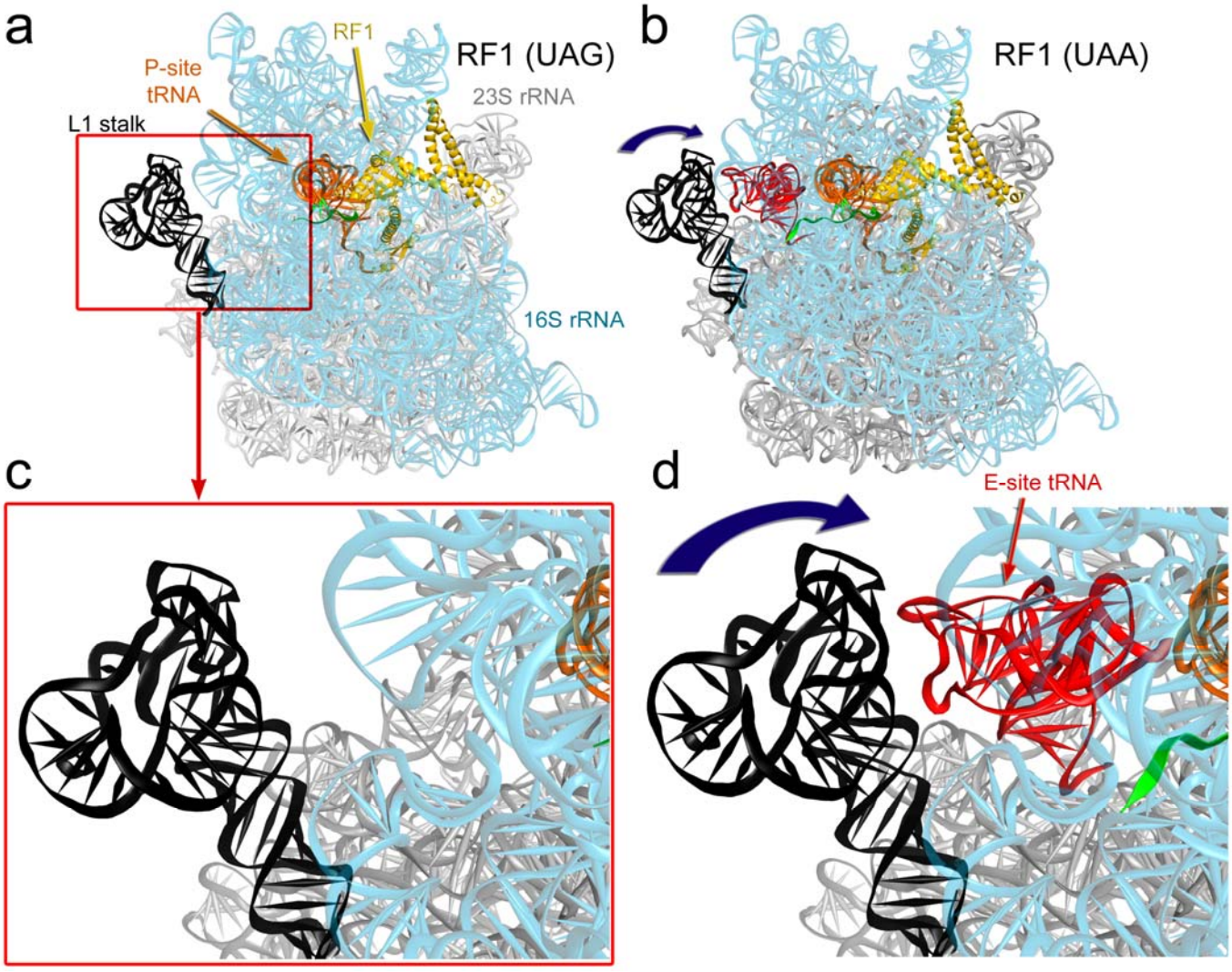

Figure S1.

Differences in the L1 stalk positions between the RF1-bound 70S termination complexes in the presence (Laurberg et al., 2008) and absence (current work) of the E-site tRNA. ***(a, c)***. In the RF1-UAG termination complex, described in this work, no density was found for the E-site tRNA. The absence of the E-site tRNA results in an opening of the L1 stalk. Relative to that in the RF1-UAA termination complex (Laurberg et al., 2008), formed in the presence of the E-site tRNA ***(b, d),*** the L1 stalk opens by 5-10 Å, which is less than distances observed in other ribosome complexes (Harms et al., 2001; Korostelev et al., 2006; Schuwirth et al., 2005). In the current crystal form, the L1 stalk is unable to open further likely due to a crystal contact with the neighboring ribosome.

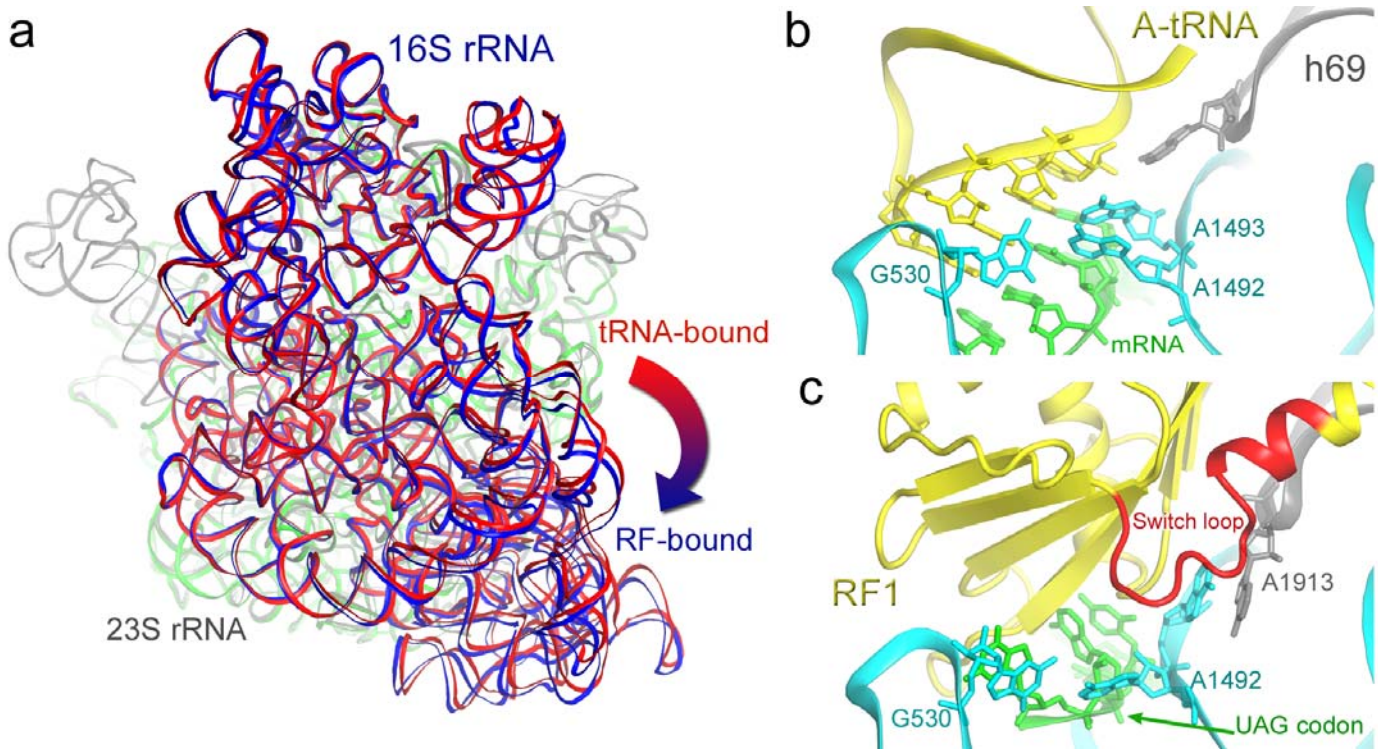

Figure S2. Conformational states of tRNA-bound and RF-bound ribosomes. ***(a)*** The small subunit of the ribosome with the A site occupied by a release factor (blue, this work) is rotated clockwise with respect to that occupied by a tRNA (blue) (Selmer et al., 2006). 23S rRNAs of the 70S termination complex (grey) and tRNA-bound complex (green) were superimposed using Pymol. The L1 and L11 portions of 23S rRNA were omitted from superposition. ***(b)*** and ***(c)*** Bridge B2a formed between helix 69 of 23S rRNA (grey) and helix 44 of 16S rRNA (cyan) undergoes significant rearrangement depending on the occupancy of the 30S A site. In a tRNA-bound complex ***(b)*** (Selmer et al., 2006), the tip of helix 69 (A1913) interacts with the tRNA (yellow), while in a termination complex ***(c)*** (this work and (Korostelev et al., 2008; Laurberg et al., 2008; Weixlbaumer et al., 2008)), A1913 stacks on A1493 of the 30S subunit.

In our first structures of 70S termination complexes (Korostelev et al., 2008; Laurberg et al., 2008), the planes of the stacked bases A1493 and A1913 were interpreted to be orthogonal to those by (Weixlbaumer et al., 2008) due to the ambiguity of the pattern of the electron density for two stacked purine bases. After careful examination of the electron density in our previous complexes and of the optimal stereochemistry of these nucleotides we have reinterpreted the structure in this region to be similar to that reported by Weixlbaumer et al. Since the overall packing of the stacked A1493 and A1913 is not affected by rotation of the bases by 90^{o}, this reinterpretation does not affect our initial conclusions regarding the roles of A1493 and A1913 in rearrangements of the switch loop (Korostelev et al., 2008; Laurberg et al., 2008).

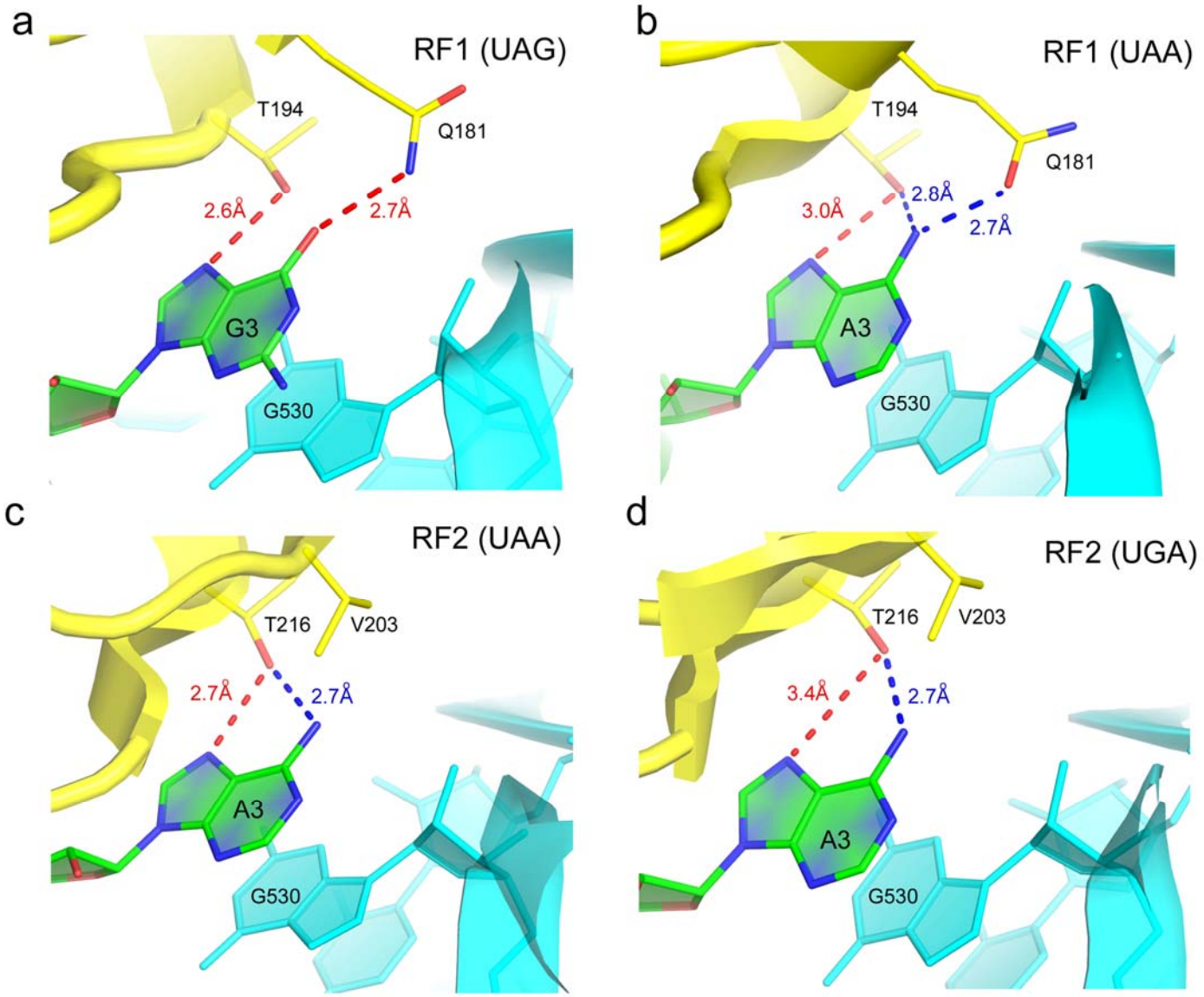

Figure S3. Recognition of the third nucleotide of a stop codon by release factors RF1 ***(a,b)*** (this work and (Laurberg et al., 2008)) and RF2 ***(c,d)*** (Korostelev et al., 2008; Weixlbaumer et al., 2008). The conformation of the decoding center around the third nucleotide of a stop codon suggests that the bonds from the hydroxyl group of T194 to N6 and N7 atoms of A3 (average lengths are 2.9 and 2.7 Å, respectively) have equivalent importance in recognition of adenosine at the third position of a stop codon.

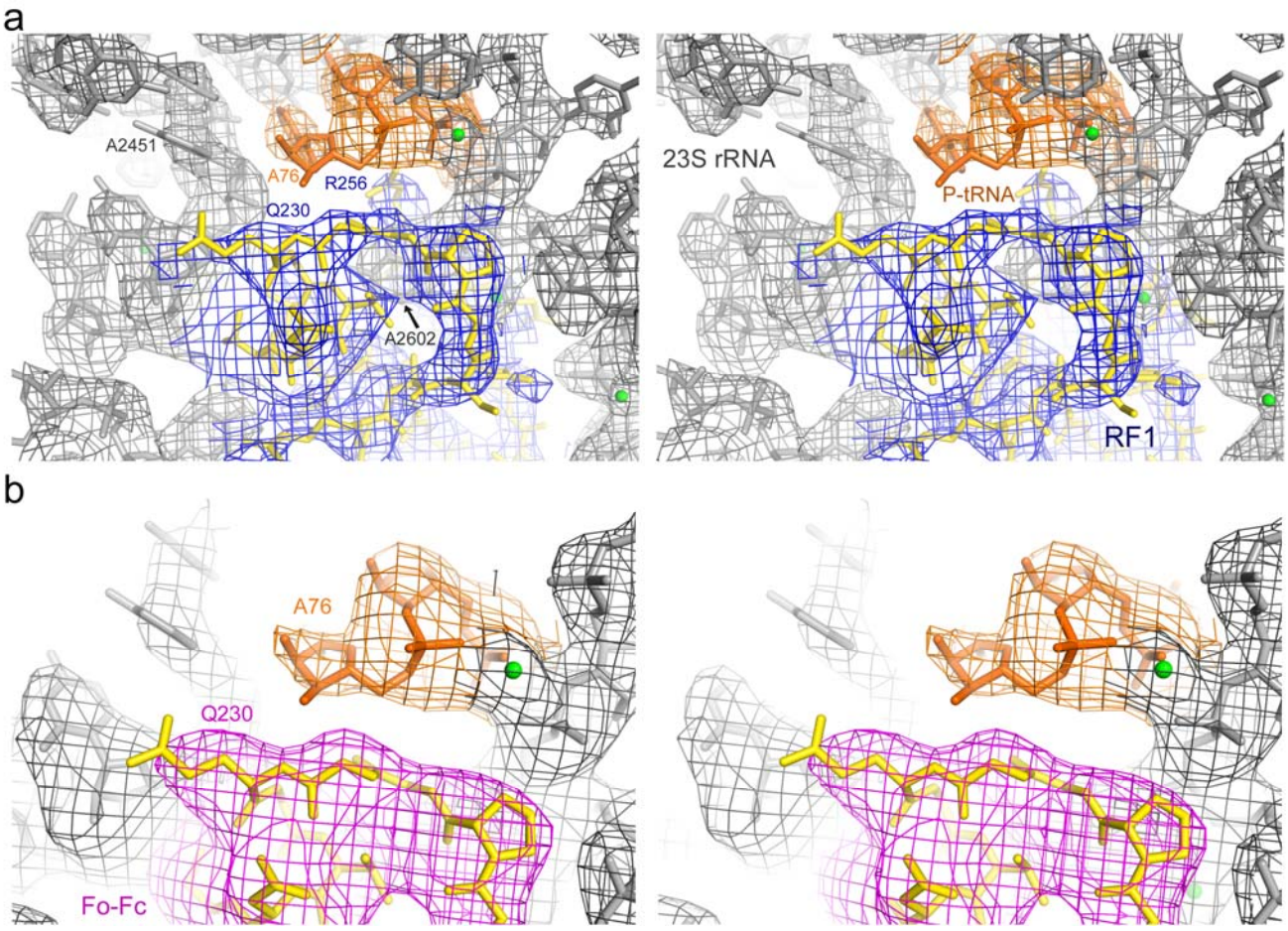

Figure S4.

a) Stereo view of σ_A-weighted $3F_{obs}–2F_{calc}$ electron density map of the GGQ motif of RF1 and the surrounding elements of the ribosomal peptidyl-transferase center and RF1. The density for RF1 was contoured at 1.4σ, and for 23S rRNA and P-site tRNA at 1.7σ. 23S rRNA is shown in grey, RF1 in yellow (density in blue), tRNA in orange.

b) Stereo view of σ_A-weighted $F_{obs}–F_{calc}$ simulated-annealing omit map, calculated in the absence of RF1 (magenta; contoured at 2.5σ) superimposed on the σ_A-weighted $3F_{obs}–2F_{calc}$ electron density map for the rest of the peptidyl-transferase center. The $F_{obs}–F_{calc}$ map was calculated from the 70S termination complex (this work), which underwent simulated-annealing refinement in the absence of RF1 (starting temperature=3000K). The $3F_{obs}–2F_{calc}$ density for 23S rRNA was contoured at 1.6σ (grey), P-site tRNA at 1.3σ (orange). Magnesium ions are rendered as green spheres.

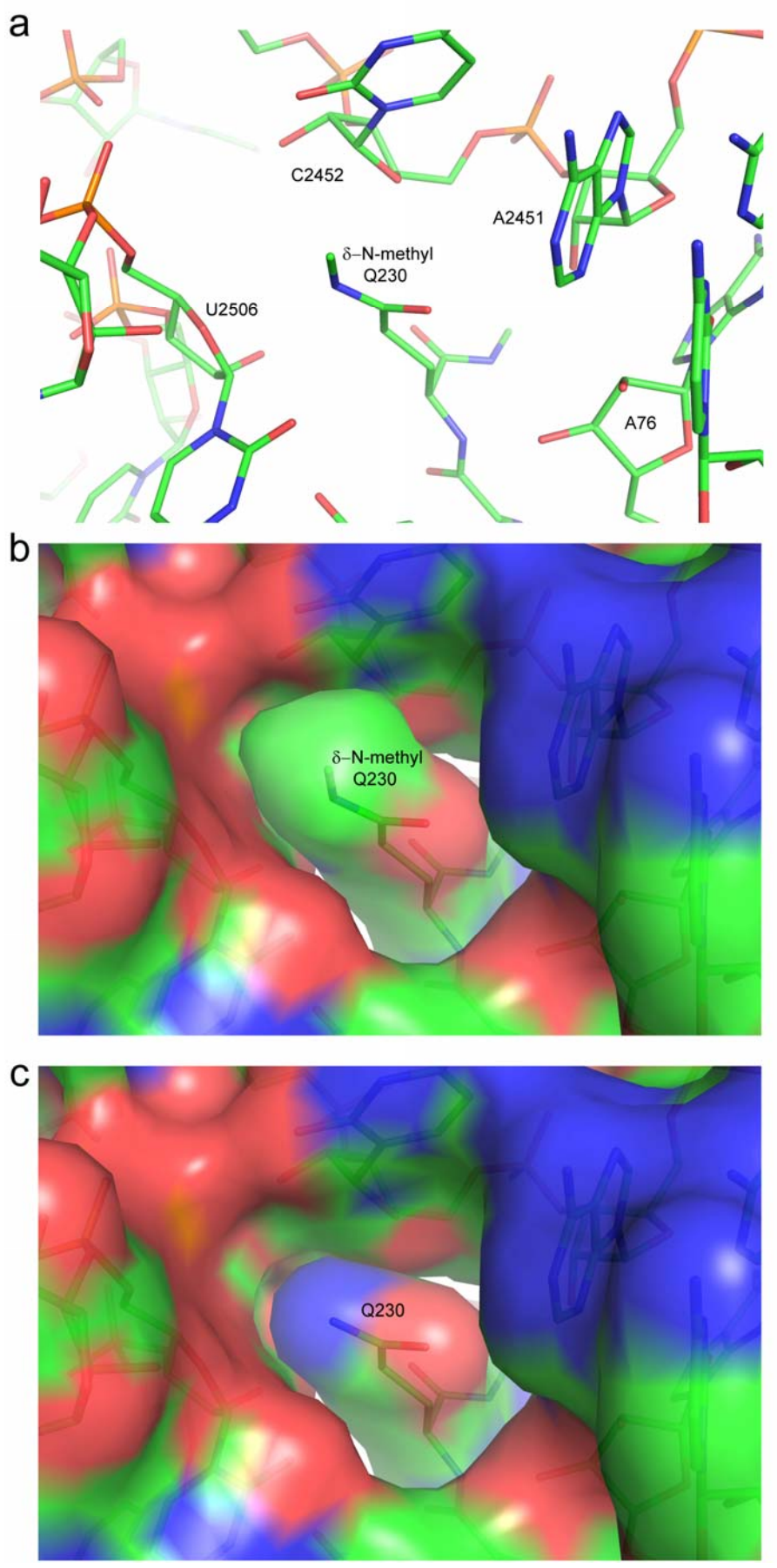
a
C2452
A2451
δ–N-methyl
Q230
U2506
A76
b
δ–N-methyl
Q230
c
Q230

Figure S5.

A model for the proposed interactions between the methyl group and ribose backbone of C2452 and U2506. These hydrophobic interactions ***(a, b)*** likely contribute to an increased affinity of methylated release factors to the ribosome (Pavlov et al., 1998) relative to that of unmethylated release factors ***(c)***. The model was created by adding a methyl group to the structure of the RF1 termination complex ***(c)*** using Pymol. The solvent radius of 1.4 Å was used to render the surfaces of the ribosomal peptidyl-transferase center and release factor. Surfaces were colored according to atom types: carbon atoms are in green, nitrogen in blue, oxygen in red.

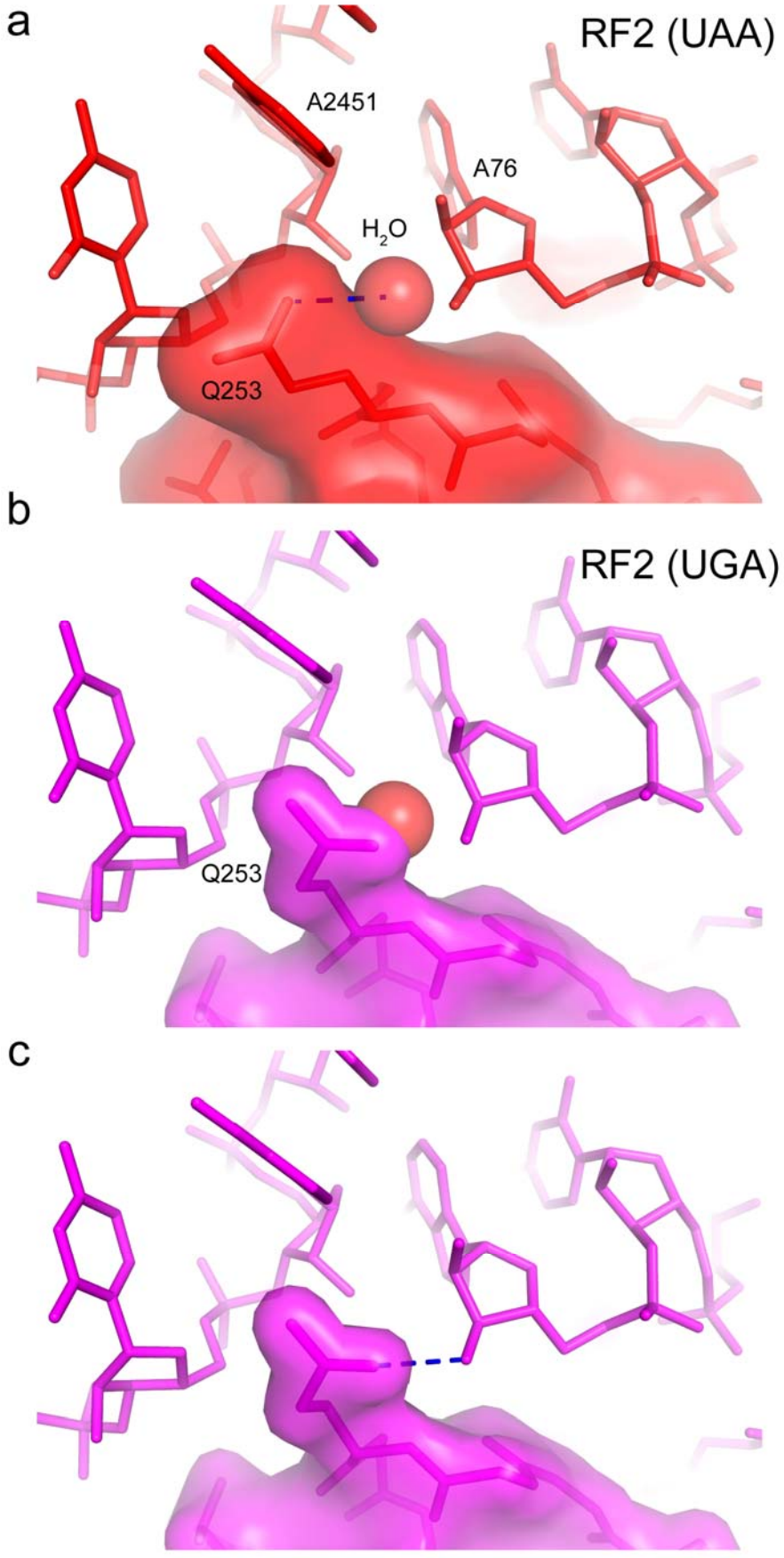
a
RF2 (UAA)
A2451
A76
H2O
Q253
b
RF2 (UGA)
Q253
c

Figure S6.

Two interpretations of the Q253 side chain conformation from the structures of RF2 bound to the ribosome in response to a UAA (Korostelev et al., 2008) ***(a)*** and UGA (Weixlbaumer et al., 2008) ***(b, c)*** stop codons. The side chain of the glutamine of the conserved GGQ motif was observed to be oriented away from the scissile bond position in three out of four termination complexes (this work and (Korostelev et al., 2008; Laurberg et al., 2008)). This position of the side chain is consistent with the proposed role of the side chain in orienting the nucleophilic water molecule for the attack on the ester bond of the peptidyl-tRNA ***(a)***.

In the RF2-UGA 70S structure (Weixlbaumer et al., 2008), X-ray data were found to be consistent with two alternative conformations for Q253: in addition to the "pointed away" conformation ***(a)***, the side chain was observed to be oriented toward the position of the scissile bond ***(b, c)***. This position of the glutamine side chain appears inconsistent with the proposed role in positioning the nucleophilic water molecule for the attack due to steric clash between the side chain carbonyl and the water molecule (putatively positioned in the active center as described in captions for Fig. 5) ***(b)***; this conformation, however, can contribute to stabilization of the leaving group (3'-OH of A76 of the P-site tRNA) via hydrogen bonding ***(c)***.

It should be pointed out that participation of the glutamine side chain in catalysis through water positioning and/or leaving group stabilization is at least modest as the side chain itself has been shown not to be essential for catalysis (Dincbas-Renqvist et al., 2000; Korostelev et al., 2008; Seit-Nebi et al., 2001; Seit Nebi et al., 2000; Shaw and Green, 2007).

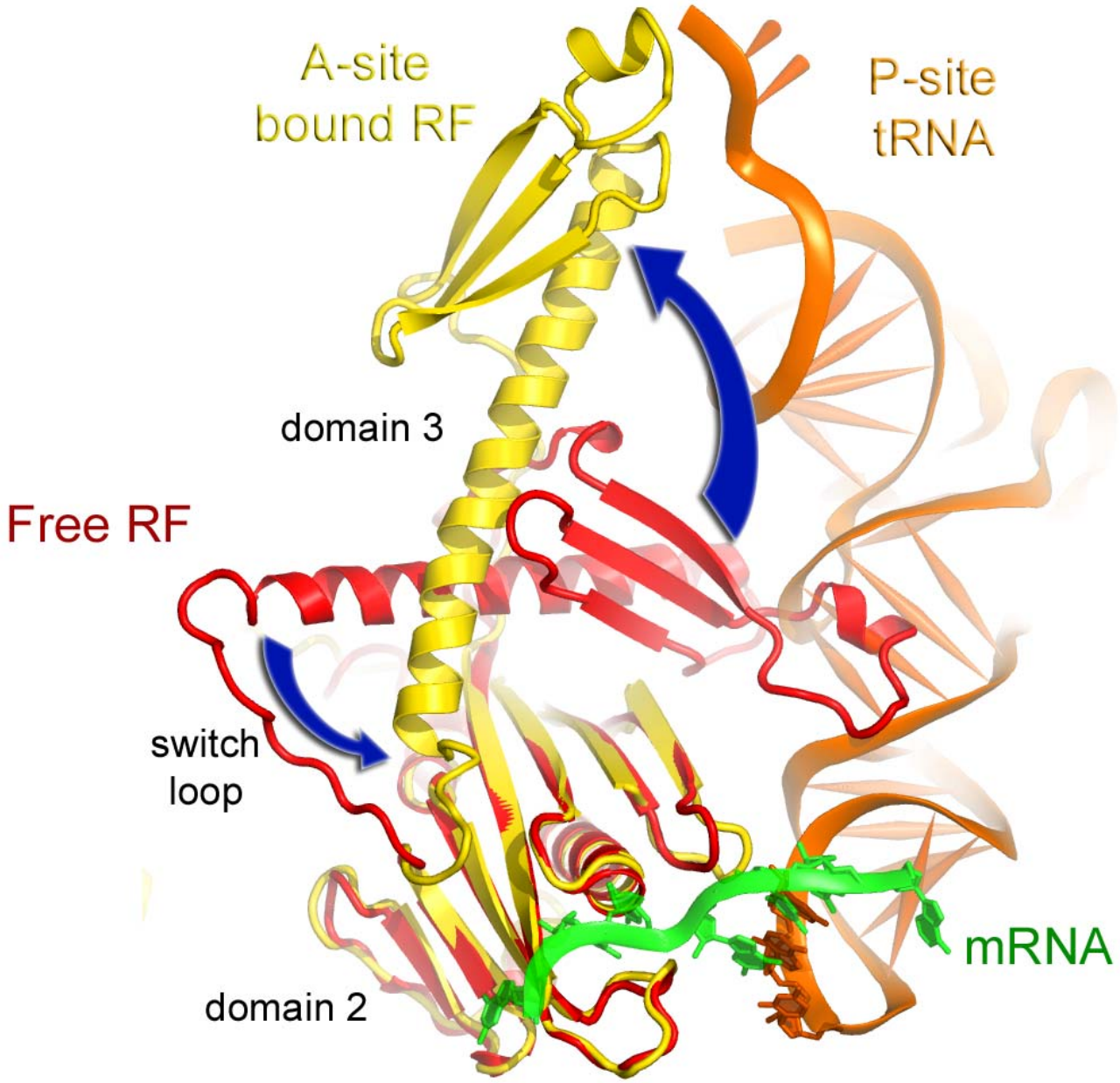

Figure S7.

Conformational rearrangements between the free and ribosome-bound forms of the release factor. The conformation of the free release factor (red, RF2) (Zoldak et al., 2007) is incompatible with ribosome binding due to multiple steric clashes including that with the P-site tRNA (orange). In the free state, domain 3 of the release factor is packed on the top of domain 2. In the ribosome-bound form (yellow, RF1, this work), domain 3 of the release factor is rearranged to interact with the peptidyl-transferase center of the ribosome. Domain 2 of *Thermus thermophilus* RF2 (PDB ID 2IHR) was superimposed on domain 2 of RF1 (this work) using Pymol (DeLano, 2002).

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