Supplemental Material

Structural characterization of an alternative-binding mode for tigecycline to the bacterial ribosome

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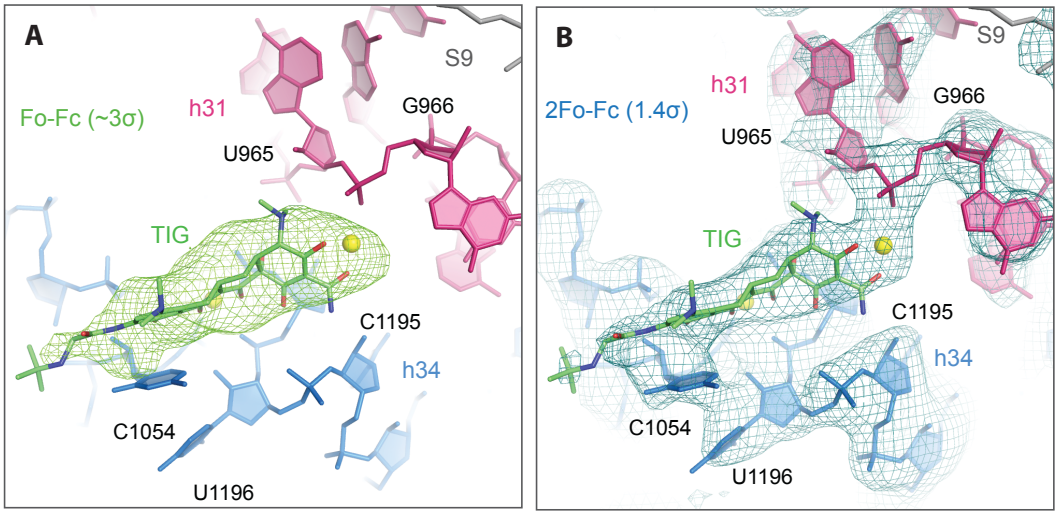

Figure S1: The initial unbiased Fo-Fc difference map calculated before tigecycline or the Mg ions were added to the model. Panel **A** shows the initial unbiased difference map indicating that TIG binds in a pocket formed by h31 and h34. Panel **B** shows the final 2Fo-Fc map in the same view as **Figure 2B** but at a slightly higher contour level (1.4 sigma).

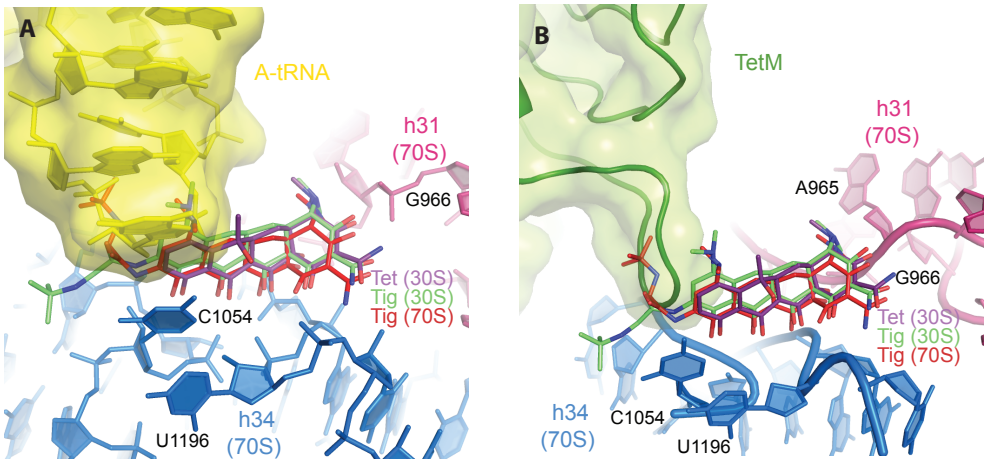

Figure S2 – The potential steric clash between TIG, A-tRNAs and the RPP, TetM.
To illustrate the inhibitory action of the various tetracyclines (TET, PDB 1HNW; TIG-bent, PDB 4G5T; TIG-extended, this study) we have aligned the indicated structures to a model of a 70S ribosome with a bound A-tRNA (panel **A**, PDB 2J00) and a 70S ribosome bound by TetM (panel **B**, PDB 3J25 and EMD-2183). Tetracyclines block protein synthesis by sterically hindering A-tRNA binding and this inhibition is prevented by the RPPs, which confer TET resistance (but not TIG resistance) by dislodging TET from its binding site via an interaction with C1054 (3). In panel **A** it is evident that the D rings of TET, and both TIG molecules clash with the base of the 3rd nucleotide of the A-tRNA anticodon while the tail of TIG observed in the 30S-TIG or 70S-TIG structures overlaps more extensively with the ribose of this same base. However, in the bent conformation (6) (70S-TIG, red) the tail of TIG follows the backbone of the A-tRNA (between positions 34 and 35) such that it shows more overlap than observed when the tail is in the extended conformation (30S-TIG, green). Similarly in Panel **B** it is observed that because residues forming loop 3 of TetM approach C1054 from above, more extensive overlap is seen when the side chain of TIG is in the bent conformation (red) rather than the extended conformation (green). Importantly, however, both conformations restrict access to C1054 and the polar interactions formed between the ribose/base of C1054 and the side chain of TIG when in the extended conformation (green; **Figure 2 C-D**) would restrict the flexibility of this base, which is presumed to be important for RPP activity (24).

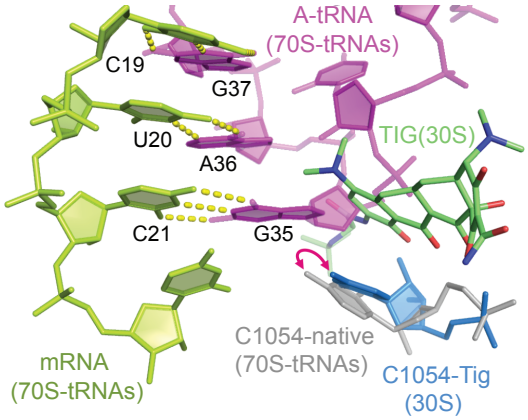

Figure S3 – The conformation of C1054 in the presence of TIG is different than that observed when an A-tRNA is bound to the A-site. By aligning the structure of a 70S ribosome with an A-tRNA bound (PDB ID: 3TVF) to the structure of the 30S-TIG complex it becomes evident that C1054 is in a distinctly different conformation in the two situations.