Supporting Information for

The Role of Pseudouridine in Structural Rearrangements of Helix 69 During Bacterial Ribosome Assembly

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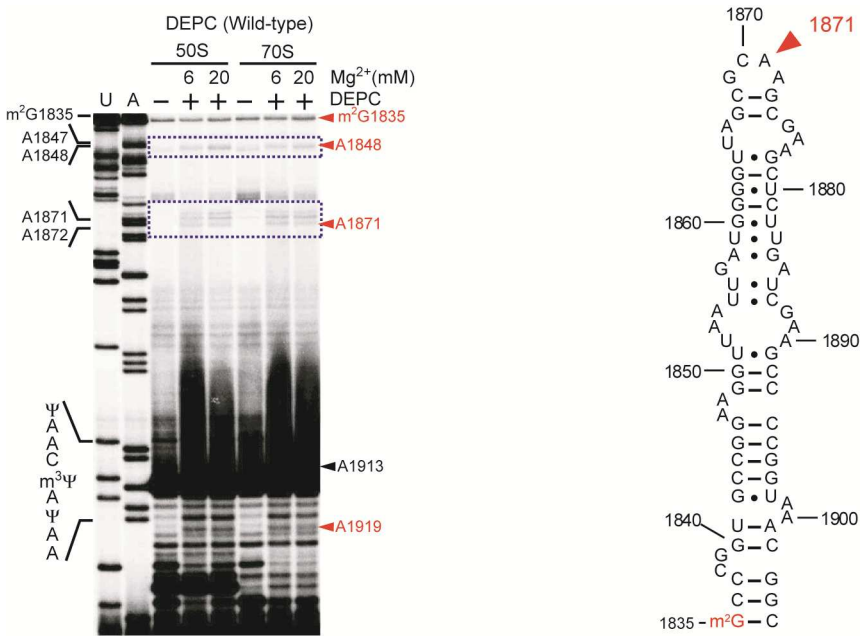

Figure S1. The autoradiogram for DEPC probing under low and high Mg^{2+} conditions and the secondary structure of H68 of 23S rRNA are shown. Analysis of the H68 region shows that changing the Mg^{2+} concentration does not alter DEPC reactivity at A N7 (positions 1871 and 1848), and the increased A1913 reactivity is predominantly attributed to H69 conformational changes. Because of the strong reverse transcription stop at $m^3\Psi$1915, it is challenging to show the H69 and H68 regions on the same image. Position 1835 is naturally modified at the N2 position of G in *E. coli* 23S rRNA, such that modified base-dependent reverse transcription stops can be used for band normalizations as described in the main text.

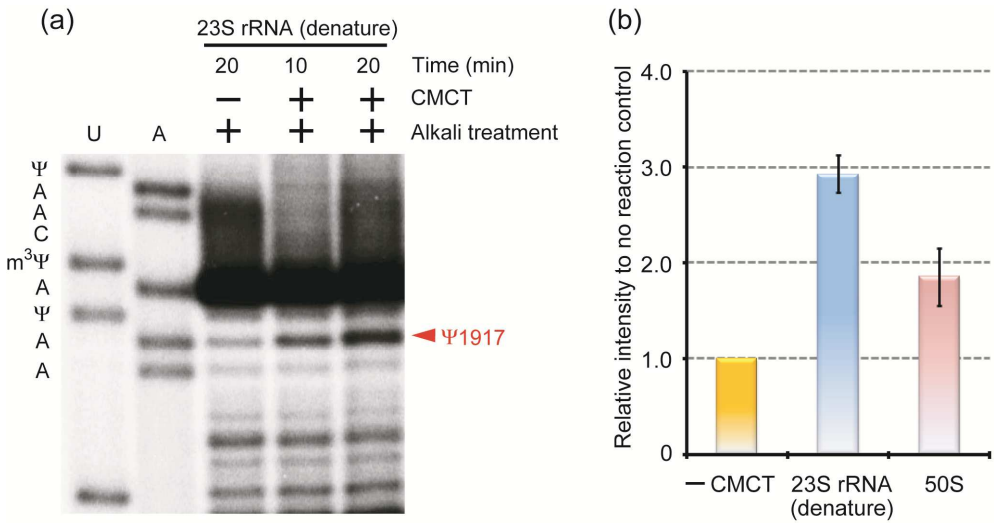

Figure S2. (a) Autoradiogram for CMCT reactivity under denaturing conditions and (b) relative band intensities compared to the no reaction (– CMCT) band are shown. CMCT probing conditions (10 min) used to probe 50S subunits as described in the main text are sufficient to label the N3 of Ψ1917, and comparison between the denaturing conditions and 50S subunit probing exhibits 40 to 50% protection of the N3 of Ψ1917 from CMCT in the structured H69 of 50S subunit.

Experimental procedure: Extracted 23S rRNA was dissolved in 15 µl of denaturing buffer (10 mM HEPES pH 7.5, 7 M urea, 4 mM EDTA). The rRNA solution was boiled for 2 min and quick chilled on ice for 2 min. The CMCT probing reaction was initiated by adding 15 µl of freshly prepared CMCT buffer (100 mM HEPES, pH 8.1 at 37 °C, 7 M urea, 4 mM EDTA, and 42 mg ml^{-1} CMCT) at 37 °C. After each time point, the probing reaction was stopped by adding cold ethanol, and the 23S rRNA was recovered. RNA pellets were dissolved in 50 mM Na_2CO_3 (pH 10.3 at 37 °C) and the RNA solution was incubated at 37 °C for 3.5 hours. The 23S rRNA was recovered and reverse transcription was performed.

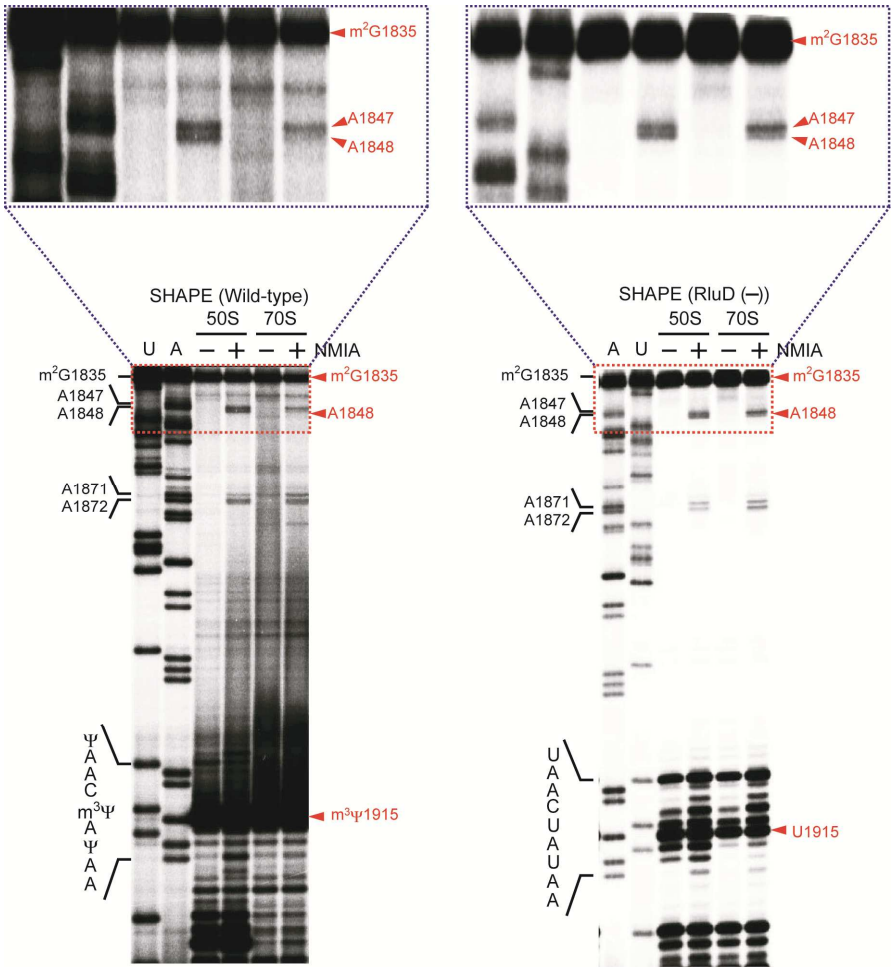

Figure S3. Autoradiograms for SHAPE analysis on wild-type (left) and RluD(–) ribosomes are shown. Position A1848 shows protection from the SHAPE reaction in 70S ribosomes.