Supporting Information

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Fig. S1. Binding of mature tRNA to the *Bacillus subtilis* ribonuclease P enzyme. (*A*) SHAPE reactivities in the absence of tRNA. (*B*) Comparison of absolute SHAPE reactivities obtained in the presence and absence of tRNA. Specificity and Catalytic domains are indicated explicitly. (*C*) Superposition of SHAPE reactivities (in color) and tRNA protection sites (gray shaded boxes) on the secondary structure for RNase P. Nucleotides that increase in reactivity in the presence of tRNA are indicated with an asterisk. (*D*) Superposition of SHAPE-detected tRNA protection sites and tRNA-induced conformational changes on the three dimensional structure of the B-type RNase P (PDB ID 2A64). All sites of protection upon tRNA binding (red) are solvent accessible and occur on the same face of the RNA. Sites of tRNA-induced increases in conformational flexibility (cyan) occur at the interface between the two domains.



Fig. 52. A130 and A374 experience slow conformational dynamics in the intact *Bacillus subtilis* RNase P RNA. (A) Comparison of absolute SHAPE reactivities obtained with slow (IA) and fast (1M7) SHAPE reagents. (B) Difference plot for SHAPE reactivities obtained with IA and 1M7. Red bars emphasize nucleotides showing the largest difference in SHAPE reactivity as a function of reagent. (C) RNase P secondary structure diagram showing the locations of A130 and A374.



Fig. S3. SHAPE analysis, performed with BzCN, showing that the starting and final states for the native sequence and Δ A130 mutant RNAs are identical.

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Fig. S4. Thermally induced unfolding of RNase P specificity domain RNAs, monitored by absorbance at 260 nm. Tertiary interactions, characterized by the intermediate transition at \approx 60 °C, are slightly more stable in the Δ 130 mutant.



Fig. S5. Comparison of emission spectra for free Oregon green versus dye-labeled native and mutant RNAs in the presence (open circles) and absence (closed circles) of Mg²⁺.