Supporting information for:

smFRET study of rRNA dimerization at the peptidyl transfer center

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This supporting information contains 7 supplemental figures.

Figure S1

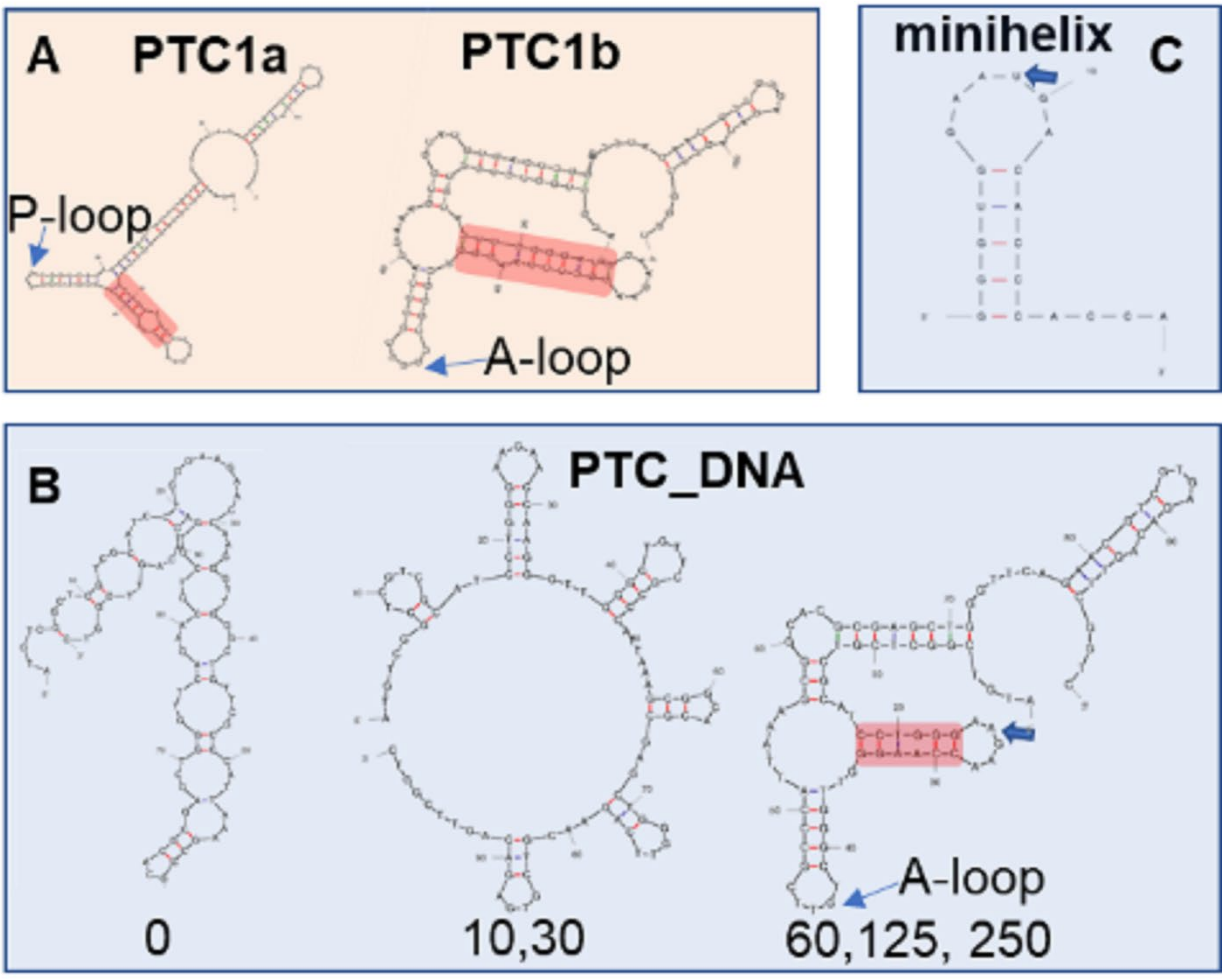


Figure S1. (A) Mfold prediction of 2D structures for PTC1a/b RNA molecules. The red shapes highlighted helix 2 of the 3-way junction. The canonical A- and P- loop residues were labelled. The two thick arrows pointed to the alkyl amino groups for labelling. (B) Mfold prediction of 2D structures for PTC_DNA molecule. The numbers beneath the structures are the mM of [Mg2+] in Mfold calculation. (C) The 2D structure of minihelix substrate.

Figure S2

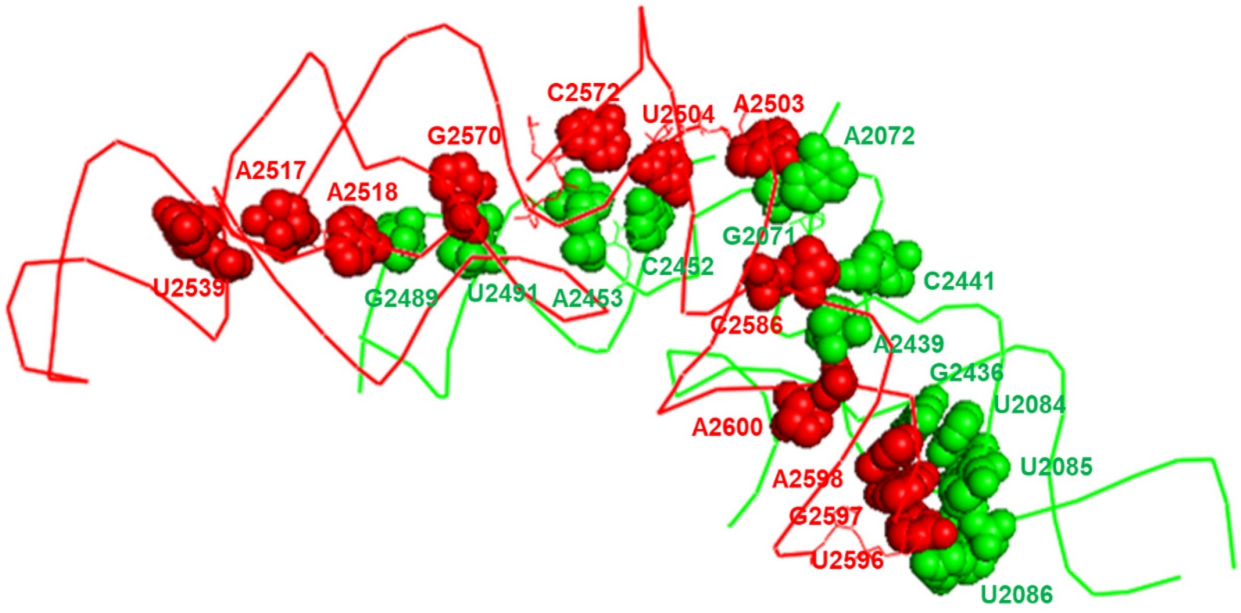


Figure S2. The e coli numbers of the bases are labeled, which are involved in dimerization interactions showed in Figure 1 of main text.

Figure S3

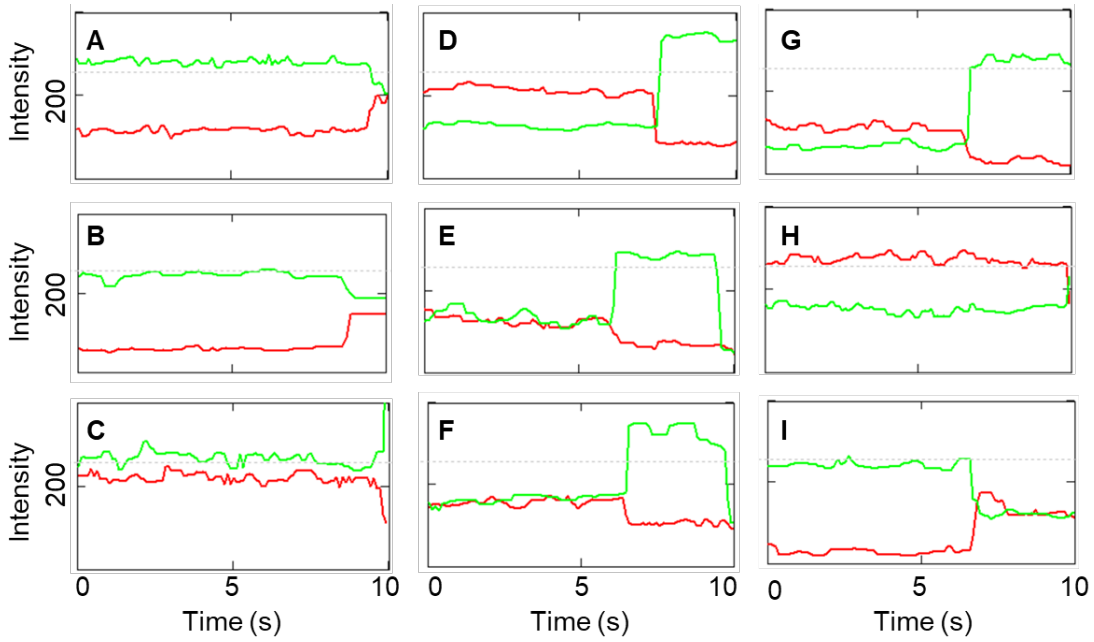


Figure S3. Representative smFRET traces. The green and red traces represent fluorescence intensities from the Cy3 and Cy5 Dyes, respectively. The X-axis is 100ms x 100 points. The Y-axis is intensity.

A, B, C are traces from Figure 3 row 1, 2, 3 at 15 mM Mg^{2+}, respectively.

D is trace from Figure 4 RNA heterodimer at 15 mM Mg^{2+}.

E is trace from Figure 5 RNA heterodimer at 15 mM Mg^{2+}.

F is trace from Figure 5 RNA homodimer at 15 mM Mg^{2+}.

G, H, I are traces from Figure 6 rows 1, 2, 3, respectively.

Figure S4.

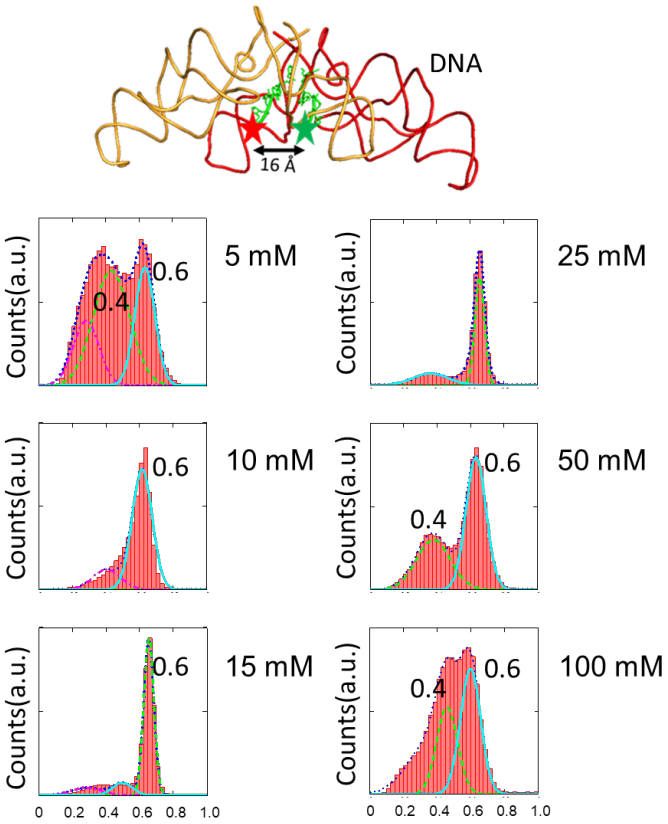
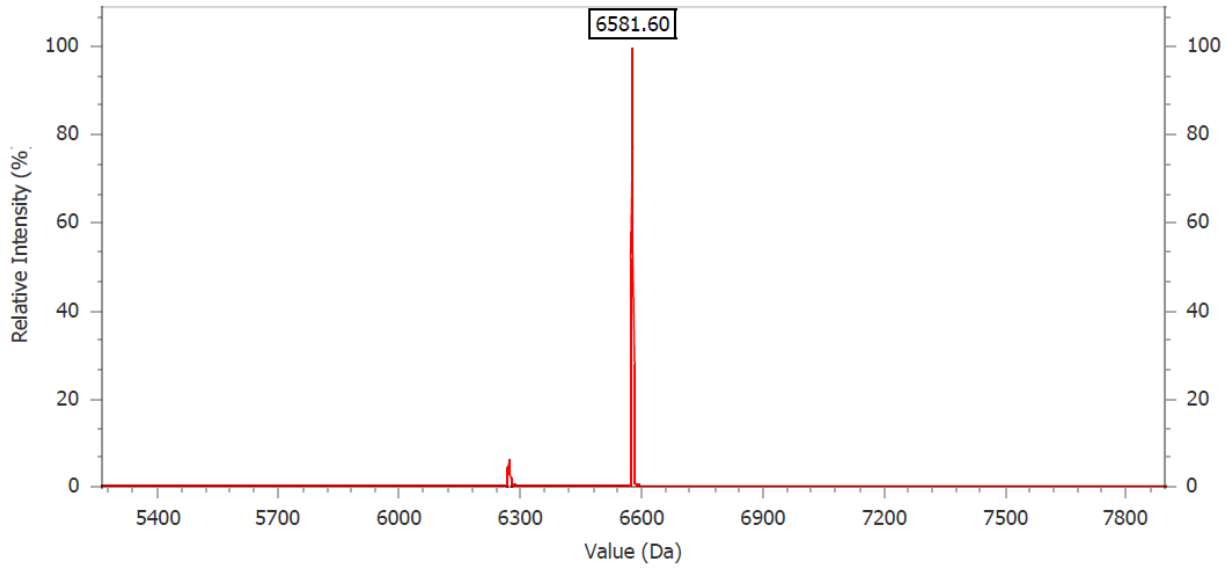


Figure S4. *FRET efficiency histograms between CA_DNA at varied concentration of* Mg^{2+}.

Figure S5.

Instrument:	MS-IALTQ-05	Operator ID:	4159
Acquired:	7/22/2020 9:55 AM	Reviewed:	7/22/2020 10:28 AM



Sequence Name:	**CCARNA**
Sequence:	**5'- rGrGrG rUrGrG rArA/iAmMC6T/ rGrArC rArCrC rCrArC rCrA -3'**
Calculated Molecular Weight:	**6580.2**
Measured Molecular Weight:	**6581.60**

Figure S5. Mass spec to show thr quality of the minihelix RNA oligo.

Figure S6

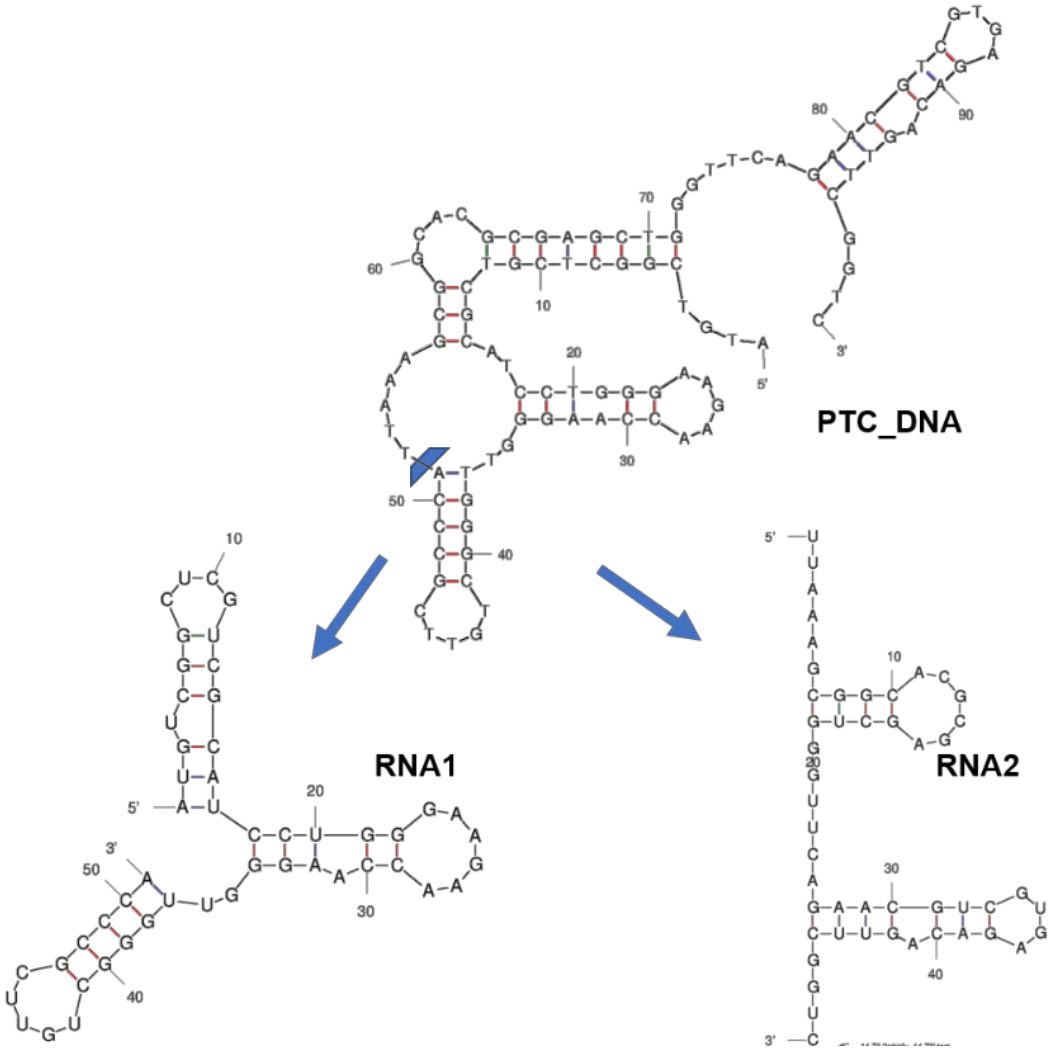


Figure S6. The PTC_DNA was incised into two halves, and RNAs were synthesized with the same sequence to test the minimal motif for ribozyme activity. Mixture of RNA1 and RNA2 did not generate FRET signals under the same conditions as in Figure 4 of the main text.

Figure S7

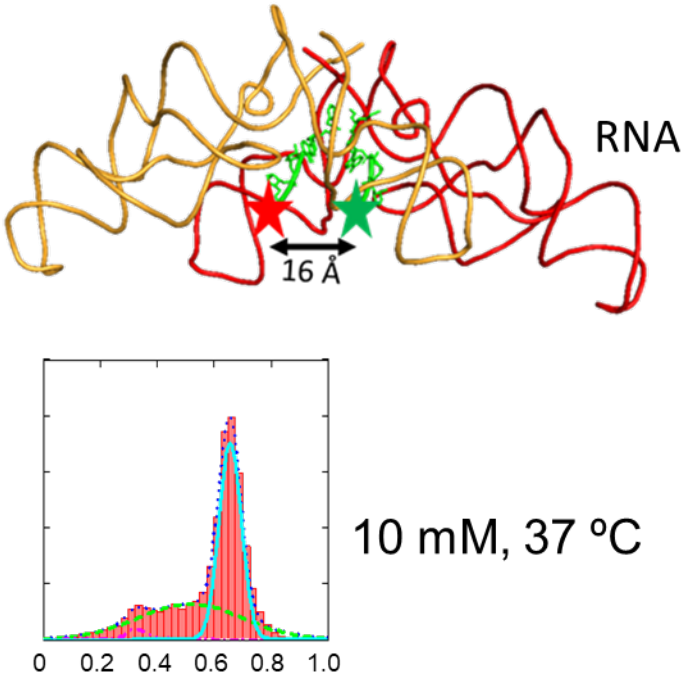


Figure S7. FRET efficiency histograms between un-charged minihelix and charged Lys-tRNA^{Lys}_T1.