Supporting information for:

Protein-Free ribosomal RNA scaffolds can assemble poly-lysine oligos from charged tRNA fragments

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Figure S1

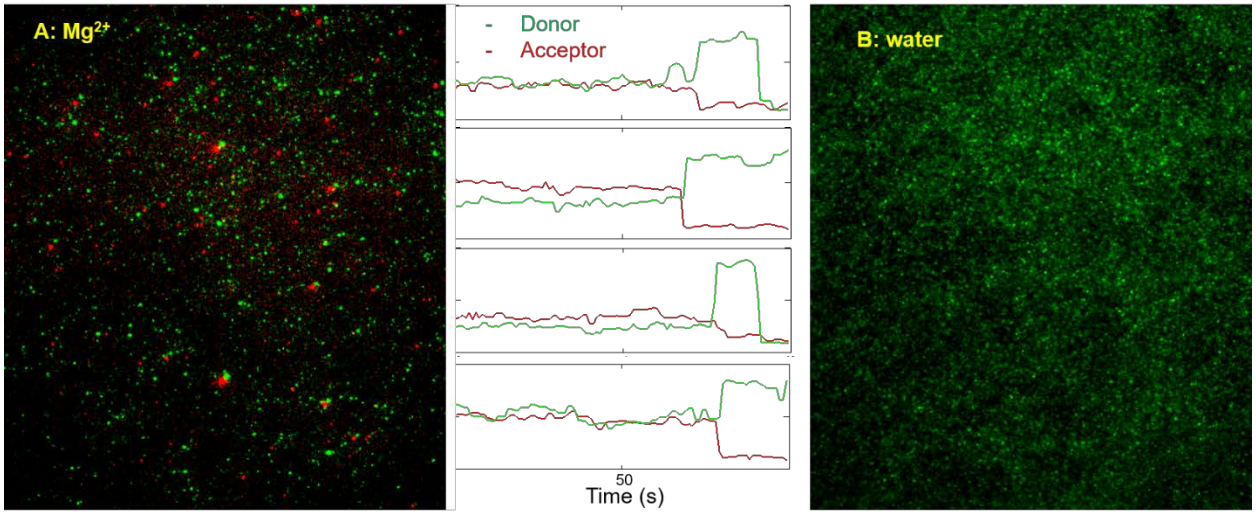

Figure S1. Examples of smFRET images and fitted fluorescence intensity time-lapse traces: (A) in the presence (high FRET formed) and (B) absence of Mg^{2+} (no FRET). The Cy3/Cy5 labels are shown in Figure 2. There are two type of Cy3/Cy5 experiments, both showed the similar images as above.

Figure S2.

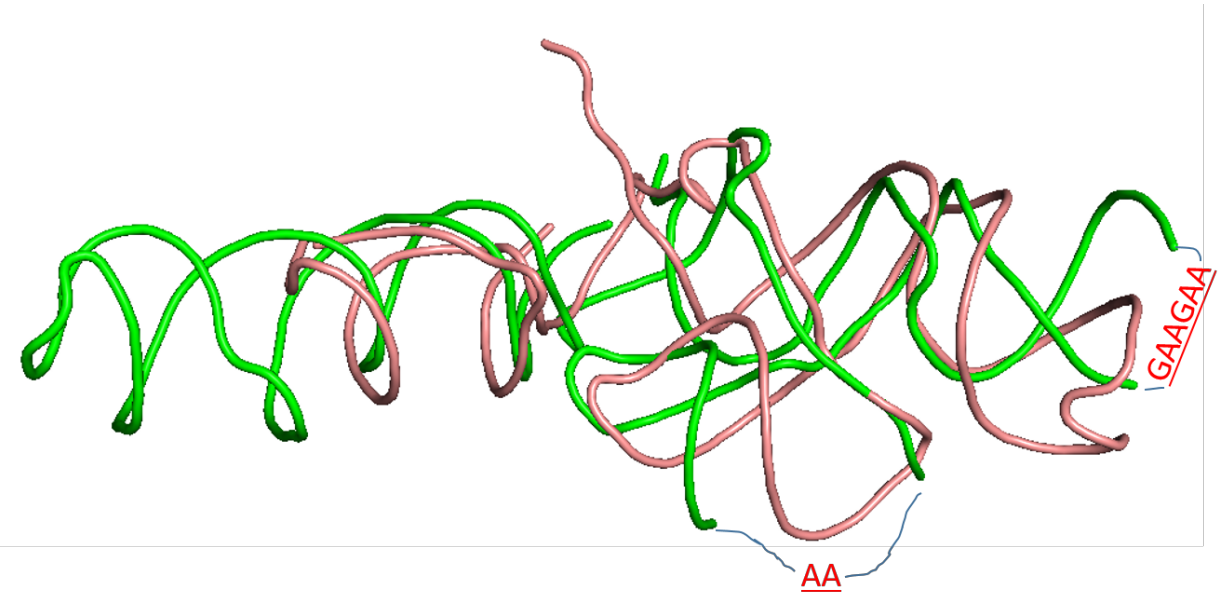

Figure S2. Alignment of ptc1b (red) onto ptc1a (green) fragments. Two long stems in ptc1a are truncated and replaced with the sequence of ptc1b.

Figure S3

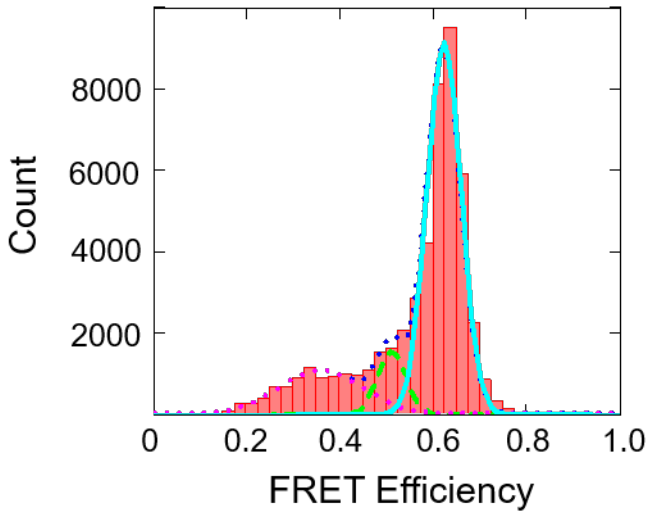

Figure S3. FRET efficiency histogram of heterodimer between ptc1a-Cy3 and ptc1b-Cy5. Both fragments are labeled at 3'-end. But one is labeled with Cy3 and the other one is labeled with Cy5.

Figure S4

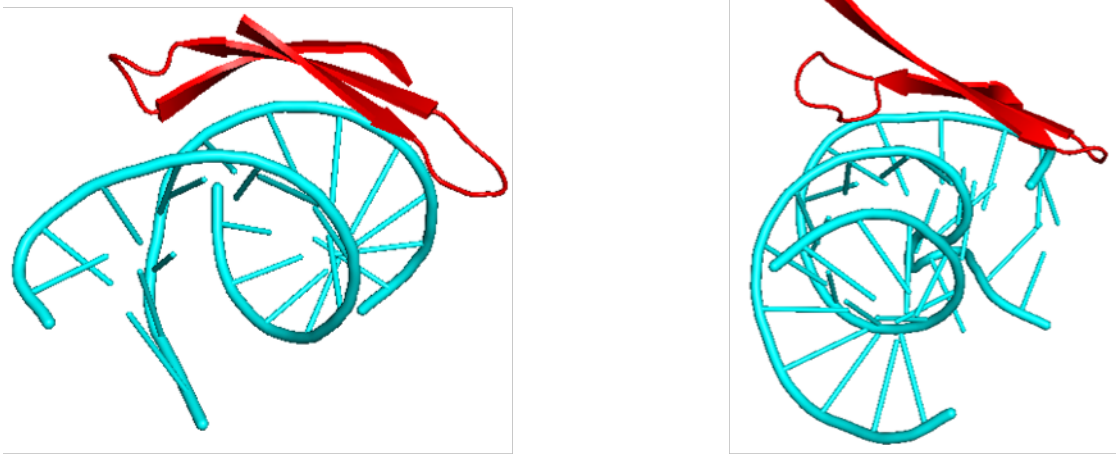

Figure S4. RNA duplex and protein b-sheet interactions between L15 and 5S; and between S11 and 16S. These 3D interactions are similar regardless of little sequence resemblance.

Table S1. Theoretical and measured Mass and analysis. Except for the substrates, only the N^{14}, N^{14}-lysine products are shown. The N^{15},N^{15}-lysine products shift +18 in all the mass spectrum.

species	Theoretical Mass (M+1)	Measured Mass	Difference	Assignment of difference (theoretical Mass)
ACCCACCA-$K^{N14,N14}$	2581.6	2579.6	-2	
ACCCACCA-$K^{N15,N15}$	2583.6	2581.5	-2	
CCA-K_9	2033.7	1897.3	-136.4	A_{base} (135.1)[1]
ACCA- K_9	2362.7	2273.4	-89.3	- A_{base} (135.1)[1] –OH(17)[2] +PO_2 (63)[3, 4]
CCACCA- K_9	2972.7	2931.5	-41.2	CO_2 (44)[5];
CCCACCA- K_9	3276.8	3189.5	-87.3	- A_{base} (135.1)[1] –OH(17)[2] +PO_2 (63)[3, 4]

Table S2. Theoretical and measured tandem MS/MS on 2273 (ACCA-K_9) and analysis. Only the N^{14}, N^{14}-lysine product 2273 MS/MS peaks are shown. The N^{15},N^{15}-lysine products 2291 MS/MS peaks shift +18 relative those of un-labeled spectra.

species	Theoretical Mass (M+1)	Measured Mass	Difference
2273-C_{base}[1]	2161.9	2162.2	+0.3
2273-A_{base}[1]	2137.9	2138.2	+0.3
2273-A	1943.8	1944.1	+0.3
2273-AC	1638.6	1638.2	-0.4

Reference:

[1] Andersen, T. E., Kirpekar, F., and Haselmann, K. F. (2006) RNA fragmentation in MALDI mass spectrometry studied by H/D-exchange: mechanisms of general applicability to nucleic acids, *J Am Soc Mass Spectrom 17*, 1353-1368.

[2] Banerjee, S., and Mazumdar, S. (2012) Selective deletion of the internal lysine residue from the peptide sequence by collisional activation, *J Am Soc Mass Spectrom 23*, 1967-1980.

[3] Joyner, J. C., Keuper, K. D., and Cowan, J. A. (2013) Analysis of RNA cleavage by MALDI-TOF mass spectrometry, *Nucleic Acids Res 41*, e2.

[4] Ni, J., Pomerantz, C., Rozenski, J., Zhang, Y., and McCloskey, J. A. (1996) Interpretation of oligonucleotide mass spectra for determination of sequence using electrospray ionization and tandem mass spectrometry, *Anal Chem 68*, 1989-1999.

[5] Shek, P. Y., Zhao, J., Ke, Y., Siu, K. W., and Hopkinson, A. C. (2006) Fragmentations of protonated arginine, lysine and their methylated derivatives: concomitant losses of carbon monoxide or carbon dioxide and an amine, *J Phys Chem A 110*, 8282-8296.