Supplementary Data:

A real-time fluorescence polarization activity assay to screen for inhibitors of bacterial ribonuclease P

Xin Liu¹, Yu Chen¹ and Carol A. Fierke^{1,2,*}

^{*}To whom correspondence should be addressed. Tel: 01-734-936-2678; Fax: 01-734-647-4865; Email: fierke@umich.edu

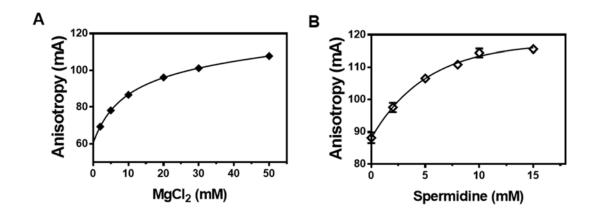


Figure S1. Fluorescence anisotropy (FA) of FI-pre-tRNA^{Asp} in response to cation concentrations. **(A)** FA of FI-pre-tRNA^{Asp} increased with increasing Mg²⁺ concentration measured in 50 mM Tris-HCl pH 7.2, 100 mM KCl, 20 mM DTT with 20 nM FI-pre-tRNA^{Asp}. Samples in duplicates were incubated in the plate for at least 15 min before reading at room temperature, G factor=0.99. (B) FA of FI-pre-tRNA^{Asp} increased with increasing concentration of spermidine. Measured in identical buffer conditions as A with 10 mM MgCl₂.

¹ Department of Chemistry, University of Michigan, Ann Arbor, Michigan, 48109, USA

² Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan, 48109, USA

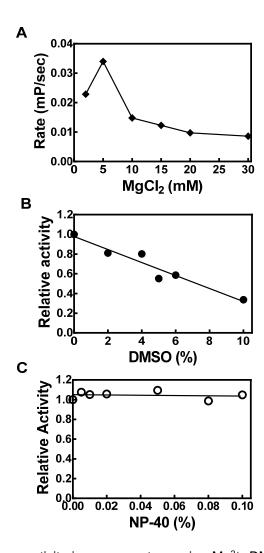


Figure S2. Test of RNase P cleavage activity in response to varying Mg²⁺, DMSO and NP-40 concentrations. **(A)** The RNase P activity was measured in duplicate in 50 mM Tris-HCl pH 7.2, varying MgCl₂, 100 mM KCl, 20 mM DTT, 10 mM spermidine with 20 nM Fl-pre-tRNA^{Asp}, 0.4 nM *B. subtilis* RNase P and 4 nM P protein at room temperature. **(B)** Activity measured as described in A with 10 mM MgCl₂, 50 nM Fl-pre-tRNA^{Asp}, 4 nM RNase P, 6 nM P protein and varying DMSO (v/v). **(C)** Activity measured as described in B except with 1 nM RNase P.

Table S1. List of kinetic parameters and goodness of fit from the global fit of RNase P activity to mixed mode of inhibitiona.

Best-fit values	Mixed			Mixed with Cooperativity		
K cat	0.159	±	0.003	0.159	±	0.003
K_{M}	15	±	1	16	±	1
n_{i}	1			1.4	±	0.1
<i>K</i> _i	130	±	10	220	±	20
nis	1			0.9	±	0.1
K is	480	±	20	470	±	30

Goodness of Fit

R square (weighted) 0.9775 0.9832

^a Assays were carried out as described in the legend of Figure 8 and the global fitting by Equation 7 was performed using GraphPad Prism 5.03 software.