Supplementary Data:

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

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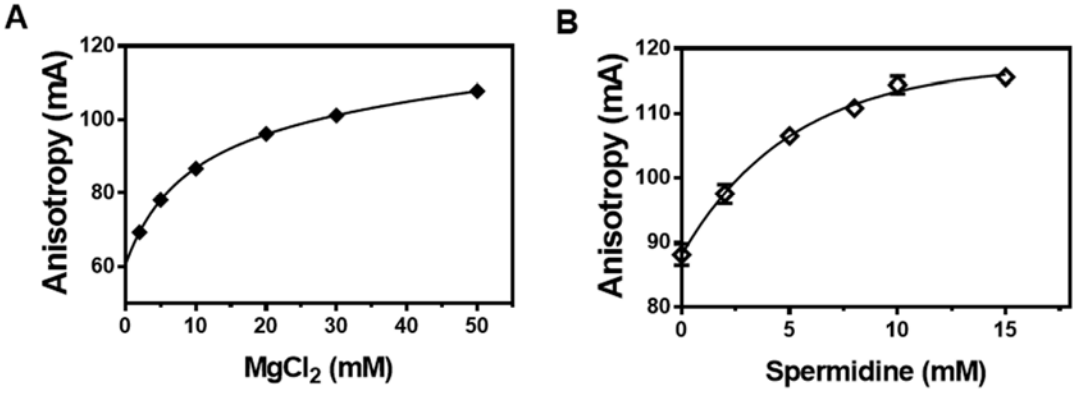

Figure S1. Fluorescence anisotropy (FA) of Fl-pre-tRNA^{Asp} in response to cation concentrations. **(A)** FA of Fl-pre-tRNA^{Asp} increased with increasing Mg^{2+} concentration measured in 50 mM Tris-HCl pH 7.2, 100 mM KCl, 20 mM DTT with 20 nM Fl-pre-tRNA^{Asp}. Samples in duplicates were incubated in the plate for at least 15 min before reading at room temperature, G factor=0.99. (B) FA of Fl-pre-tRNA^{Asp} increased with increasing concentration of spermidine. Measured in identical buffer conditions as A with 10 mM $MgCl_2$.

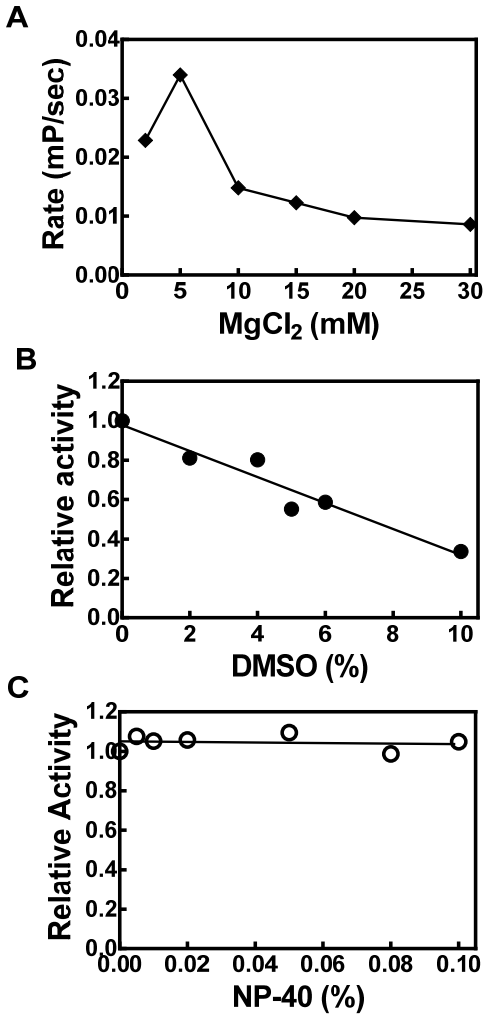

Figure S2. Test of RNase P cleavage activity in response to varying Mg^{2+}, DMSO and NP-40 concentrations. **(A)** The RNase P activity was measured in duplicate in 50 mM Tris-HCl pH 7.2, varying $MgCl_2$, 100 mM KCl, 20 mM DTT, 10 mM spermidine with 20 nM Fl-pre-$tRNA^{Asp}$, 0.4 nM *B. subtilis* RNase P and 4 nM P protein at room temperature. **(B)** Activity measured as described in A with 10 mM $MgCl_2$, 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 inhibition[a].

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
K_i	130	±	10	220	±	20
n_{is}	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.