

Supplemental Data | Sequences for riboswitch aptamers and their respective primers

All sequences are written 5' $\Rightarrow$ 3'. The extended T7 promoter sequence is shown in lower caps. Additional G nucleotides to enhance transcription initiation are bolded. Unnatural flanking nucleotides are underlined.

FMN *F. nucleatum impX* riboswitch aptamer (Serganov et al. 2009):

ccaagtaatacgactcactata**GGCT**AATCTTCGGGGCAGGGTGAAATTC**CCG**ACCGGTGGTATAGTCCAC
GAAAGTATTTGCTTTGATTTGGTGAAATTC**AAA**ACCGACAGTAGAGTCTGGATGAGAGAAGAAA
AGC

SAM-I *B. subtilis yitJ* riboswitch aptamer (Winkler et al. 2002):

ccaagtaatacgactcactata**GGG**TTCTTATCAAGAGAAGCAGAGGGACTGGCCCGACGAAGCTTCAGCA
ACCGGTGTAATGGCGATCAGCCATGACCAAGGTGCTAAATCCAGCAAGCTCGAACAGCTTGGA
GATAAGAAGAG

SAM-I *P. irgensii* riboswitch aptamer (Karns et al. 2013):

Genbank accession AAOG01000001.1, nucleotides 142961..142846

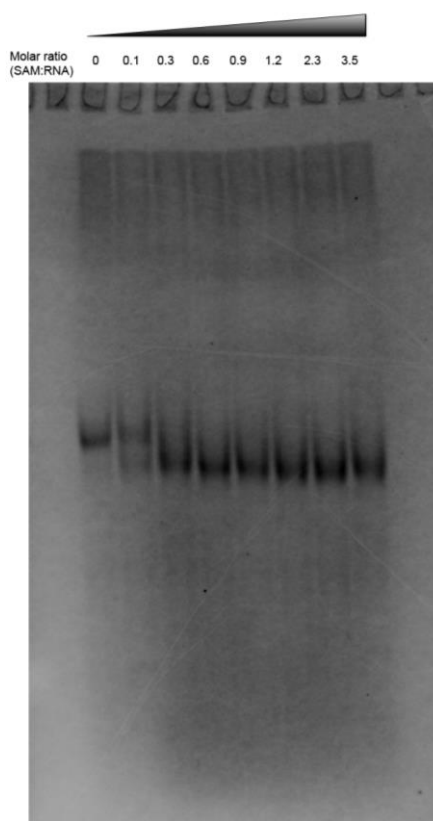
ccaagtaatacgactcactata**GGG**AAATGTTATCAAGAAAGGCGGAGGGATTAGACCCATTGAAGCCTTA
GCAACCCTTTAGTAATAAAGAAGGTGCTAAATTCTACTCAATTATTCGTAATTGGATAGATAACAA
AAA

c-di-GMP *V. cholerae tfoX* riboswitch aptamer (Sudarsan et al. 2008):

ccaagtaatacgactcactata**GG**AAAAATGTCACGCACAGGGCAAACCATTCGAAAGAGTGGGACGCAAA
GCCTCCGGCCTAAACCAGAAGACATGGTAGGTAGCGGGGTTACCGATGGCAAATGCATAC

Table S1. Literature extinction coefficients of nucleoside monophosphate regioisomers

Base	Phosphate Position	Wavelength (nm)	ϵ ($M^{-1} cm^{-1}$) $\times 10^{-3}$	Reference
C	5'	260	7.07	Cavaluzzi and Borer 2004
C	3'	260	7.60	Puglisi and Tinoco 1989
A	5'	260	15.04	Cavaluzzi and Borer 2004
A	3'	260	15.34	Puglisi and Tinoco 1989
A	2'-3'	257	14.9	Katz and Comb 1963
A	2'	260	15	Abrash et al. 1967
G	5'	260	12.08	Cavaluzzi and Borer 2004
G	3'	260	12.16	Puglisi and Tinoco 1989
G	2'-3'	257	11.8	Katz and Comb 1963
U	5'	260	9.66	Cavaluzzi and Borer 2004
U	3'	260	10.21	Puglisi and Tinoco 1989
U	2'-3'	260	9.6	Katz and Comb 1963



Supplementary Figure 1. Sub-stoichiometric amounts of SAM ligand results in apparent full binding of the *Pi* SAM-I riboswitch aptamer. Native gel shift with fluorescently labelled RNA was performed as described in (Karns et al. 2013). Concentration of the RNA sample was determined by neutral thermal hydrolysis. Concentration of the SAM ligand was determined by UV absorbance at 260 nm. Full complexation of the RNA to ligand appears to occur before 1:1 stoichiometry is achieved, which likely indicates that the total RNA includes sub-populations not represented by the two bands (unbound and bound) labelled in the figure. Diffuse fluorescence signal is observed in other regions of the gel, which may indicate oligomeric states and/or degradation products.

Supplemental References

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