Supporting information for:

Subsite ligand recognition and cooperativity in the TPP riboswitch: Implications for fragment-linking in RNA ligand discovery

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Two tables and five figures.

Table S1. X-ray crystallography data collection and refinement statistics for the thiamine pyrophosphate (TPP) riboswitch co-crystallized with thiamine, TPP and TPPc in the presence of Mn^{2+} or Ca^{2+}.

Data collection				
Data set	Thiamine, Mn^{2+}	TPP, Mn^{2+}	TPPc, Mn^{2+}	TPPc, Ca^{2+}
Space group	*P*3212	*P*3212	*P*3212	*P*3212
Cell dimensions				
a, b, c (Å)	61.11, 61.11, 103.41	65.41, 65.41, 101.50	64.96, 64.96, 101.52	65.01, 65.01, 101.27
α, β, γ (°)	90.00 90.00 120.00	90.00, 90.00, 120.00	90.00, 90.00, 120.00	90.00, 90.00, 120.00
Resolution (Å)	30.00–2.95 (3.06–2.95)[a]	30.00–2.25 (2.29–2.25)[a]	56.26–2.56 (2.67–2.56)[a]	56.30–2.46 (2.56–2.46)[a]
R_{merge}[b]	0.111 (0.555)	0.064 (1.297)	0.120 (3.817)	0.098 (3.730)
R_{pim}[c]	0.043 (0.237)	0.019 (0.437)	0.048 (1.470)	0.039 (1.551)
$CC_{1/2}$	0.985 (0.933)	0.994 (0.700)	0.995 (0.407)	0.997 (0.322)
$I/\sigma(I)$	47.6 (1.6)	82.7 (1.0)	14.0 (0.8)	13.9 (0.7)
Completeness (%)	98.8 (93.3)	100 (100)	100 (100)	99.9 (99.9)
Redundancy	8.4 (5.3)	12.4 (9.6)	14.1 (14.9)	13.8 (12.9)
No. unique reflections	4,766 (445)	12,071 (589)	8,128 (966)	9,133 (1,026)
Refinement				
Resolution (Å)	29.03 – 2.95	29.05 – 2.25	56.26 – 2.56	56.30 – 2.46
R_{work} / R_{free} (%)	22.9/28.5	20.9/23.1	21.7/24.5	21.1/24.7
No of atoms				
RNA	1594	1639	1639	1639
Lead	18	26	26	26
Other ligands[d]	3	64	58	44
Water	-	9	2	-
Average B-factors ($Å^2$)				
RNA	110.35	63.76	85.71	93.39
Lead	81.23	57.02	72.82	78.93

Ligand	97.45	60.11	85.38	90.01
Water	-	60.80	100.64	-
RMS deviations				
Bond lengths (Å)	0.005	0.004	0.003	0.005
Bond angles (°)	1.076	0.951	0.814	1.116
Clash score	13.55	5.84	10.15	20.80
PDB code	7TD7	7TDA	7TDB	7DTC

[a]The highest-resolution shell values are shown in parentheses.

[b]$R_{merge} = \Sigma_h \Sigma_i | I(h)_i - < I(h) > | / \Sigma_h \Sigma_i I(h)_i$, where $I(h)$ is the intensity for reflection h, Σ_h is the sum for all reflections, and Σ_i is the sum for i measurements of reflection h.

[c]$R_{pim} = \Sigma_{hkl} \sqrt{(1/(n-1))} \Sigma_i | I(hkl)_i - <I(hkl)> | / \Sigma_{hkl} \Sigma_i I(hkl)_i$

[d]Ligand indicates components of the crystallization solution (buffer, cations, etc.) except TPP, TPPc and thiamine molecules.

Table S2. RNA and ligand concentrations and c-values for ITC experiments used to determine K_d terms for ligand binding to the TPP riboswitch.

Titrating ligand (mM)	Pre-bound ligand (mM)	RNA (μM)	c-value	K_d (μM)
MDP (10)	–	400	0.3	1200
pyrithiamine (0.5)	–	50	3.8	13
HT (1)	–	50	8.3	6
THG (10)	–	450	0.6	780
TPPc (0.15)	–	9	470	0.019
TPP (0.15)	–	9	82	0.11
thiamine (0.5)	–	33	3.0	11
thiamine (0.1)	MDP (10)	500	0.8	620
pyrithiamine (0.1)	MDP (10)	500	0.7	720
HT (0.1)	MDP (10)	500	0.6	790
THG (2.5)	MDP (10)	398	0.8	470

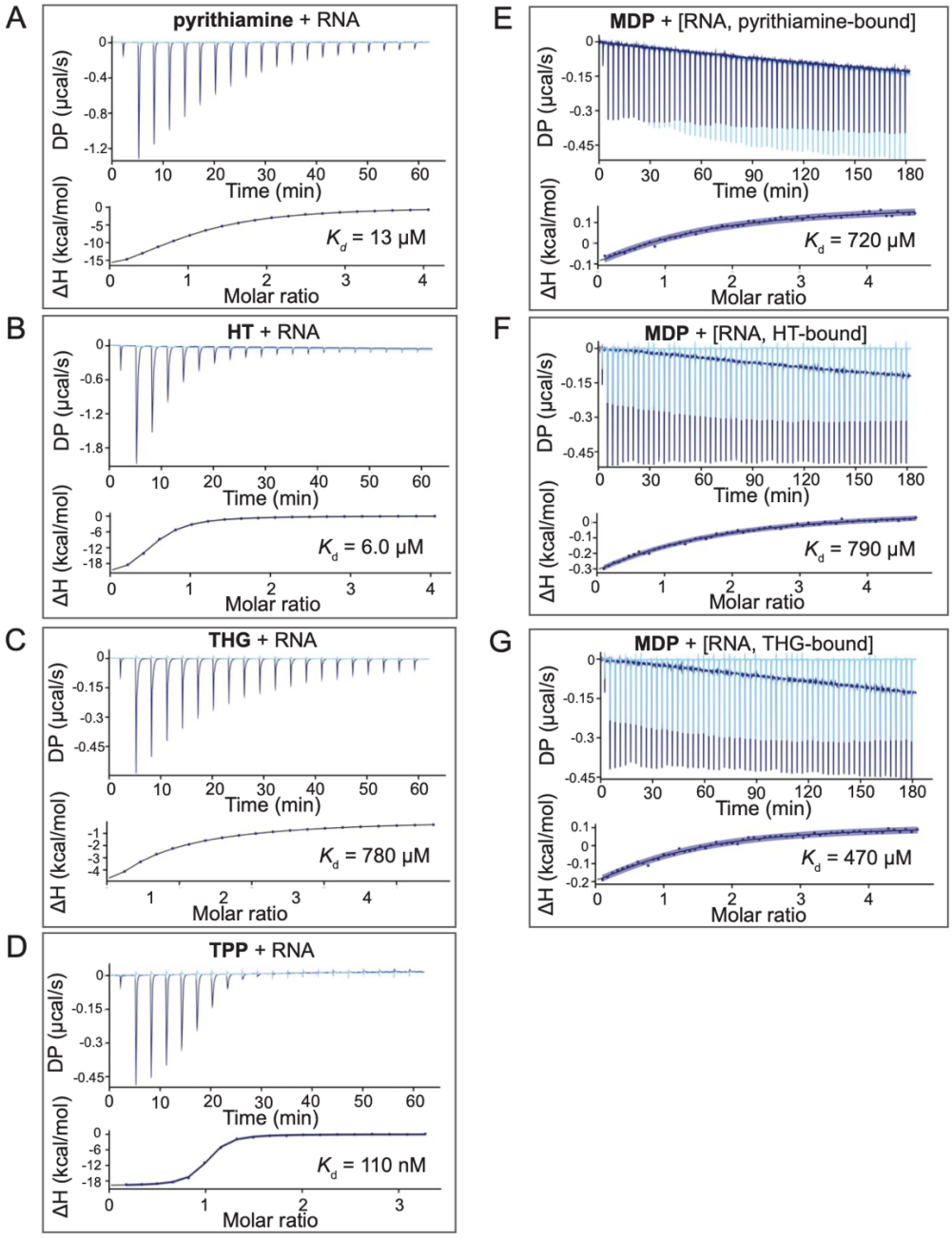

Figure S1. ITC data, integrated curve fits, and calculated dissociation constants for all experiments reported in Table 1 that are not shown in Fig. 2. Titrations of (A) pyrithiamine into the TPP riboswitch, (B) half-thiamine (HT) into the riboswitch, (C) THG into the riboswitch, (D) TPP (at 1 mM $MgCl_2$) into the riboswitch, (E) MDP into pyrithiamine-bound riboswitch, (F) MDP to HT-bound riboswitch, and (G) MDP into THG-bound riboswitch. Background traces (ligand titrated into buffer) are shown as light blue, experimental traces of ligand-RNA titrations in dark blue. Curve fits are shown with 95% confidence intervals in blue shading.

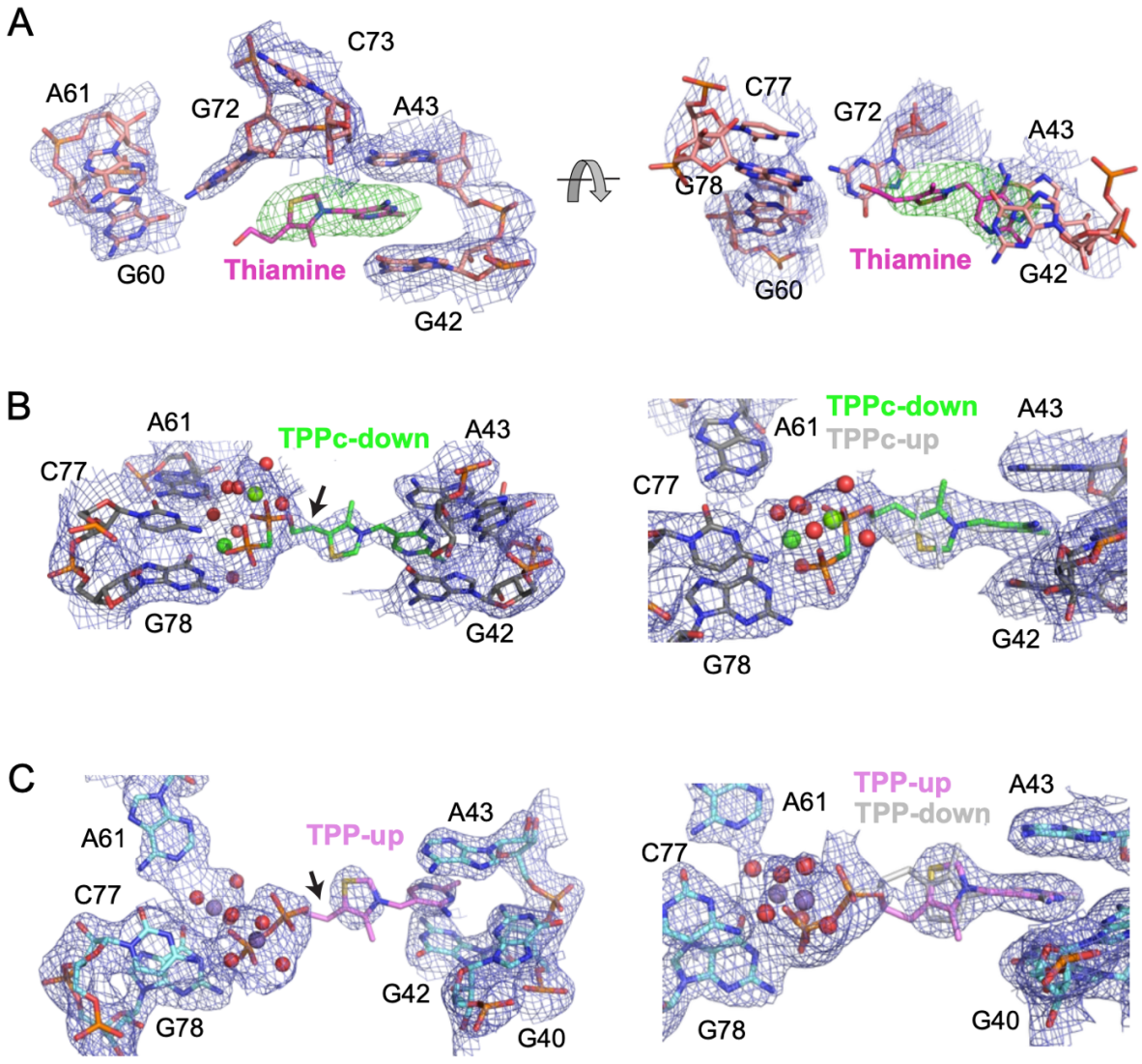

Figure S2. Electron density maps for riboswitch-ligand complexes. (A) Thiamine-bound structure. The refined structure of the thiamine-riboswitch complex shown with the refined $2F_o$-F_c map contoured at 1.0 σ level (light blue mesh). The map for thiamine is not shown. The green map around thiamine is an omit F_o-F_c map at 2.0 σ level obtained after initial rounds of refinement of the RNA. Panels (*left*, *right*) show side and top views. Note well-separated density map for the nucleotides of the thiamine senor and largely merged (less well defined) map in the pyrophosphate sensor region. (B) TPPc-bound structure. (*left*) $2F_o$-F_c simulated annealing omit map contoured at 0.9 σ level (light blue mesh) shown with the refined structure of the RNA-bound TPPc with thiazole in downward conformation (sulfur atom oriented down). (*right*) $2F_o$-F_c refined map contoured at 0.8 σ level (light blue mesh) shown with the refined structure of the

RNA-bound TPPc in downward conformation (green) and upward conformation (gray). The linker shows a better fit to the density map in the downward conformation. (C) TPP-bound structure. (*left*) $2F_o$-F_c simulated annealing omit map contoured at 0.8 σ level (light blue mesh) shown with the refined structure of the RNA-bound TPP with thiazole in upward conformation. Arrow points at lack of density for the linker. (*right*) $2F_o$-F_c refined map contoured at 0.8 σ level (light blue mesh) shown with the refined structure of the RNA-bound TPP in upward conformation (violet) and downward conformation (gray). In the TPP structure, the lack of clear density for the ethylene linker connecting thiazole with pyrophosphate is consistent with alternative conformation(s) of the linker and the thiazole ring, although the upward conformation is predominant.

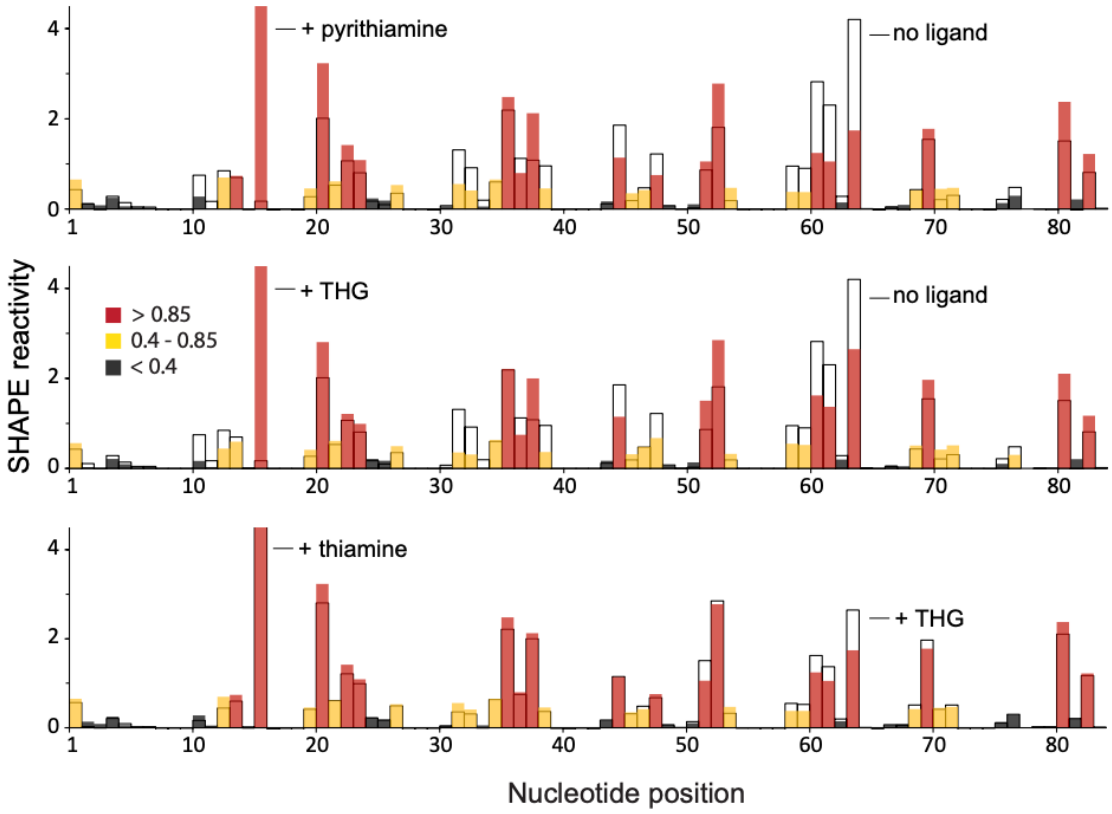

Figure S3. SHAPE reactivity profiles for thiamine-like ligands. *Top* to *bottom*: Pyrithiamine (solid bars) overlaid on a no-ligand trace (open bars), THG overlaid with no-ligand, and thiamine overlaid with THG trace. Comparison of thiamine to THG (strongest and weakest binding analogues, respectively) shows that ligand-bound RNA structures are highly similar, independent of RNA-binding affinity.

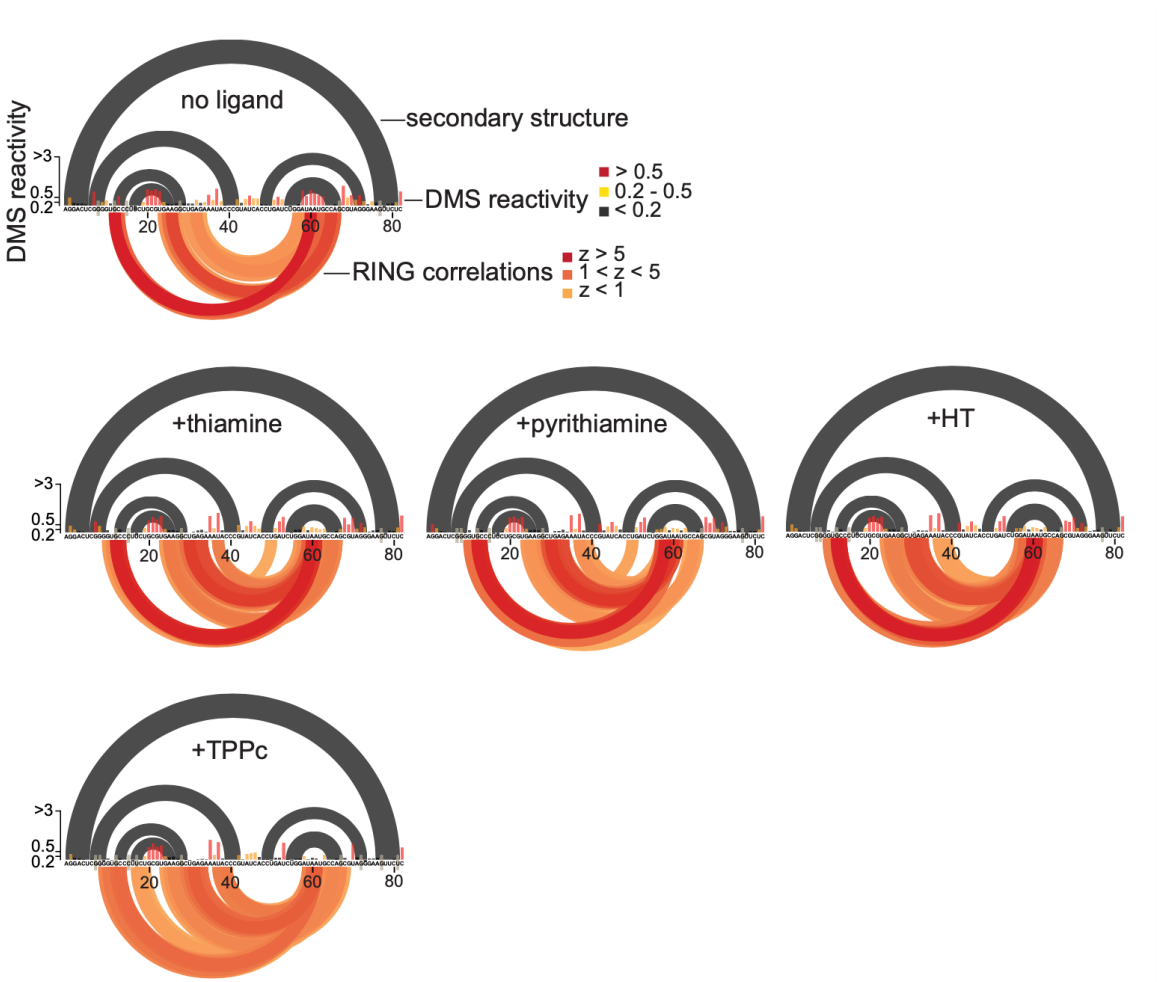

Figure S4. RING correlations and DMS reactivity for TPP riboswitch RNA bound to thiamine analogues. Black arcs show the accepted secondary structure; histograms show normalized DMS reactivities. Correlations are plotted as colored arcs and are not clustered, and all correlations are shown. From *top* to *bottom*, progression shows correlation changes from no-ligand, to thiamine- or thiamine-analogue-bound (from *left* to *right*; thiamine, pyrithiamine, and HT), to TPPc-bound.

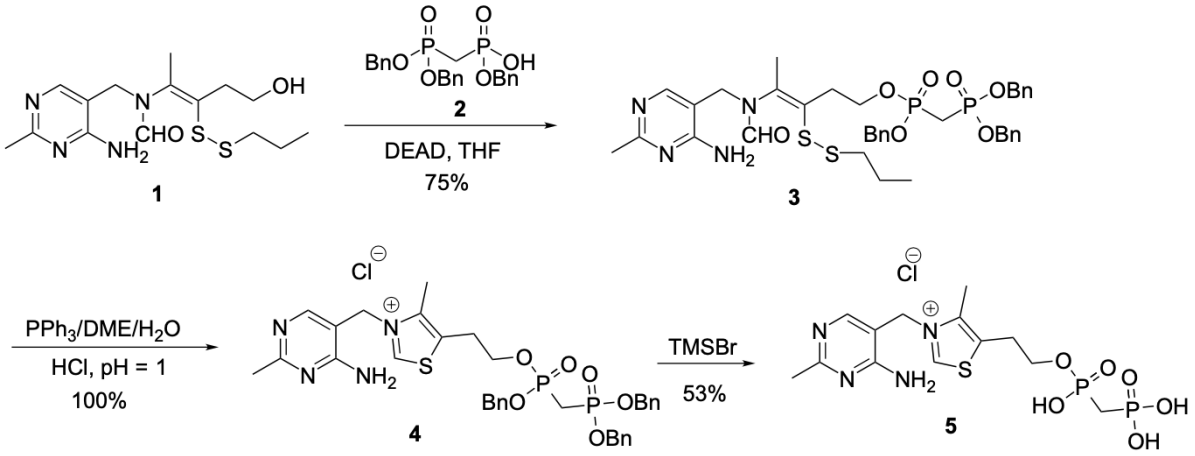

Figure S5. Scheme for synthesis of TPPc.