

Structural basis for gene regulation by a thiamine pyrophosphate-sensing riboswitch

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SUPPLEMENTARY INFORMATION

Supplementary Methods

Footprinting studies. For footprinting experiments, 166-nt long full-length riboswitch RNA was used either unlabelled or mixed with RNA (100,000 cpm) labeled by ^{32}P on 5'- or 3'-termini. Prior to experimentation, 30 pmoles of RNA (final concentration 1.5 μM) were preheated at 37 °C for 10 min in a 20 μl of buffer containing 50 mM Na^+ -HEPES (pH 7.9), 50 mM KCl and 2 mM MgCl_2 . TPP was added at 1:1, 1:3 and 1:10 ratio and the mixtures were incubated at 37 °C for 15 min. Cleavage reactions were initiated by addition of RNase V1 (0.0025 U) (Pierce) or T2 (0.1 U) (Sigma) and quenched by cooling and addition of 5 μg tRNA followed by phenol-chloroform extraction and ethanol precipitation. Radiolabeled RNA products were analyzed on 10% 8M urea/PAGE. Unlabelled RNAs were reverse transcribed with avian myeloblastosis virus polymerase using ^{32}P -labeled oligonucleotides complimentary to positions 82-102 or 156-168. Cleavage efficiency and TPP effects were estimated visually and with Image Gauge software (Fujifilm). RNA markers were prepared by partial digestion with alkaline solution or by partial digestion with ribonucleases T1, Bc or PhyM.

Primer extension experiments. Primer extension was conducted in 15 μl volume with 20 U of moloney murine leukemia virus reverse transcriptase from the ^{32}P -labeled oligonucleotides at 37 °C for 30 min. Sample preparation and analysis of the reaction products were performed as described for footprinting experiments. Sequencing reactions were run in parallel to provide product size markers.

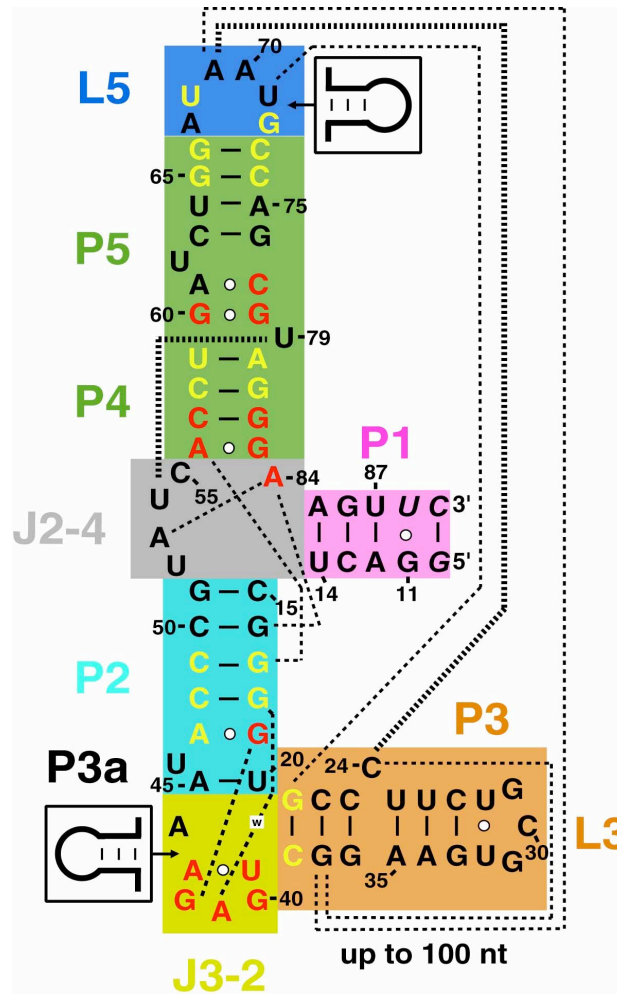
Supplementary Table S1. Data collection and refinement statistics

Data collection	TPP-riboswitch complexed with TPP		
Space group	C2		
Cell dimensions			
<i>a,b,c</i> (Å)	148.48, 29.34, 95.48		
α,β,γ (°)	90.0, 94.75, 90.0		
	Peak	Inflection	Remote
Wavelength (Å)	1.1407	1.1411	1.1201
Resolution ^a	20-2.7(2.8-2.7)	20-2.8(2.9-2.8)	20-3.0(3.0-2.9)
R _{sym} (%) ^a	8.1 (47.9)	8.4 (49.9)	8.3 (42.1)
<i>I</i> / $\sigma(I)$ ^a	15.1 (2.8)	15.6 (2.8)	15.8 (3.48)
Completeness (%) ^a	99.7 (99.9)	99.8 (100)	99.8 (100)
Measured reflections	78,204	75,520	67,893
Unique reflections ^a	22,249 (2,158)	20,054 (2,042)	17,995 (1,792)
Phasing			
Number of Os sites	11		
Initial figure of merit	0.53		
After density modification	0.69		
Refinement			
Resolution (Å) ^a	20-2.05 (2.103-2.050)		

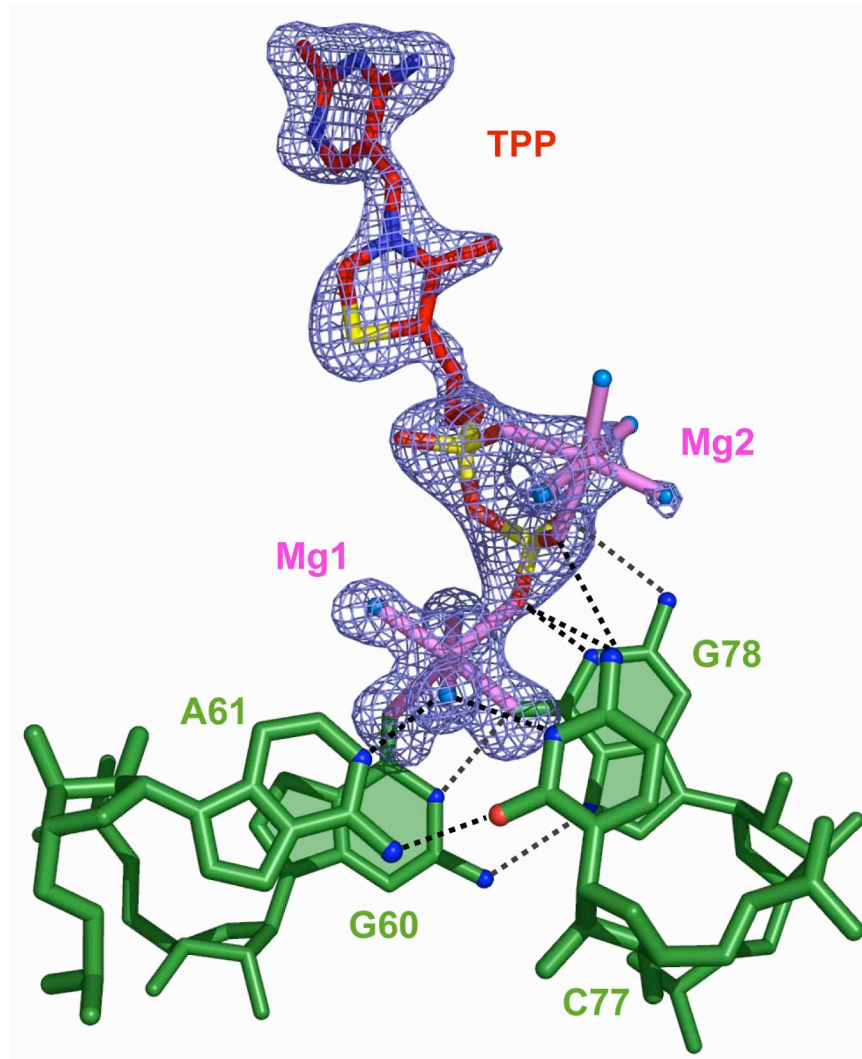
Number of reflections	25,258
Working set	23,921
Test set	1,337
Completeness (%) ^a	99.8 (100)
R _{factor} / R _{free} (%)	20.8 / 24.1
Number of atoms	3,817
RNA	3,442
TPP	52
Cations (with coordinated water)	35
Water	288
Average B-factors	34.95
RNA	34.95
TPP	31.32
Cations (with coordinated water)	35.95
Water	37.19
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	1.043
Estimated coordinate error ^b	0.135

^a Values for the highest-resolution shell are in parentheses.

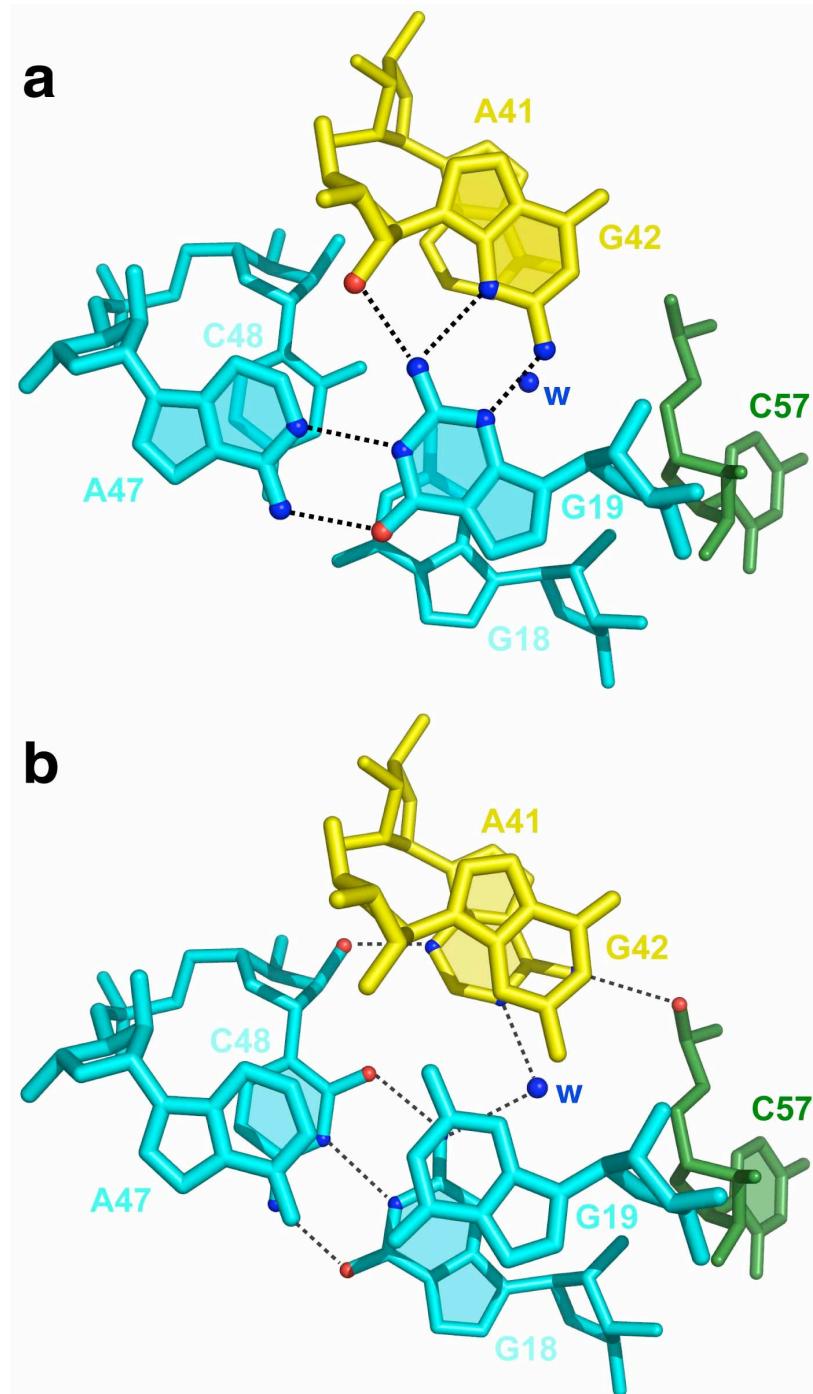
^b Estimated coordinate error based on maximum likelihood was calculated with REFMAC.



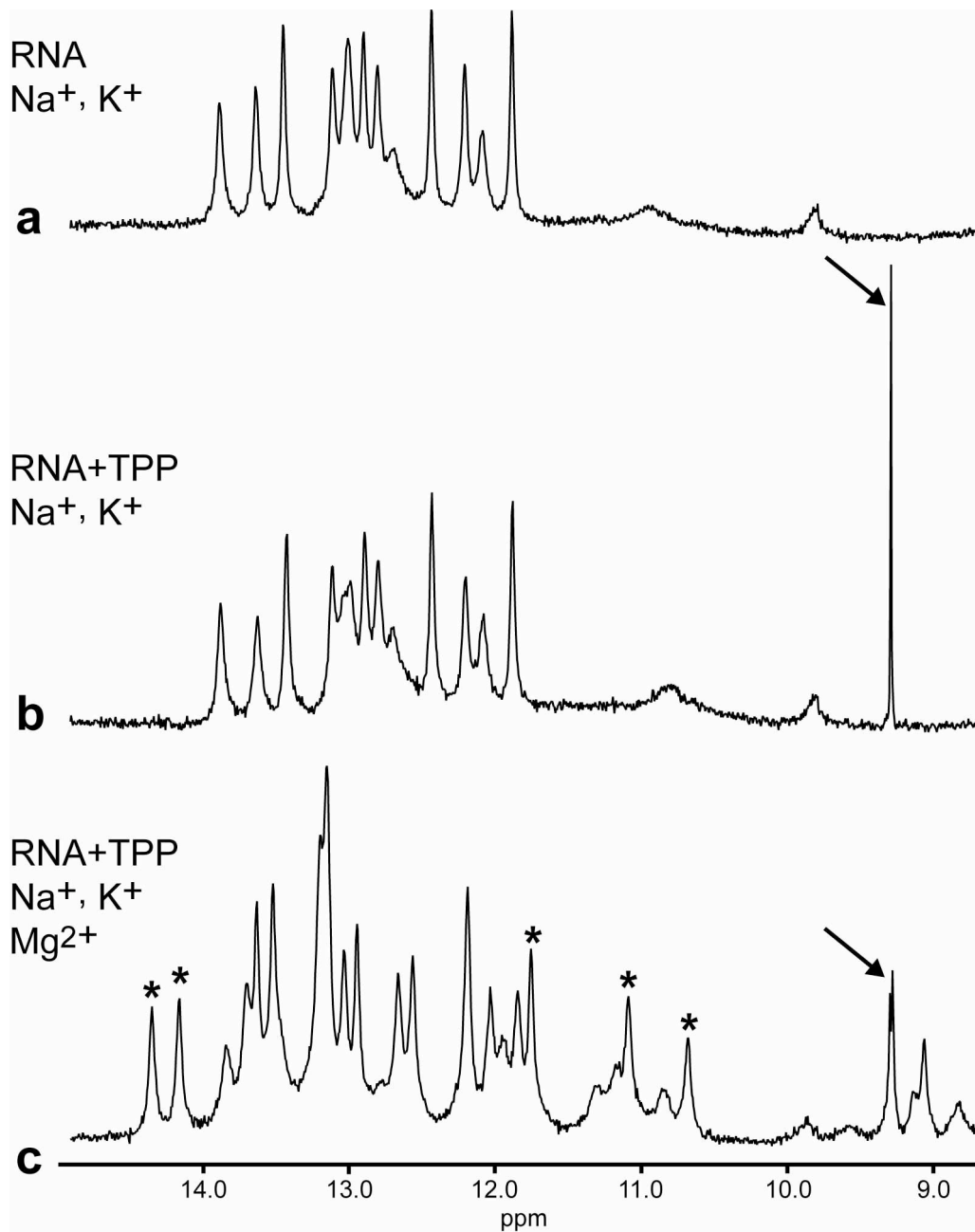
Supplementary Figure S1. Secondary structure and tertiary contacts in the complex between the TPP riboswitch and TPP. Schematic of the crystallized RNA fragment from *E. coli thiM* mRNA (nt 11-87, protein accession number P76423) is based on the traditional representation of TPP riboswitches. Stems, loops and junctions are color-coded as in Fig. 1. Red and yellow nucleotides, respectively, are conserved in more than 97% and 90% of known TPP riboswitches (J.E. Barrick and R.R.B., personal communication). Tertiary contacts formed by hydrogen bonds (*w* for water-mediated bond) between bases and stacking interactions are represented by thin and thick dashed lines, respectively. Base-pairing in non-canonical nucleotide pairs is shown by small white circles. Stem-loop elements, present in some TPP riboswitches, are shown in black. Nucleotides added for crystallization are in italics.



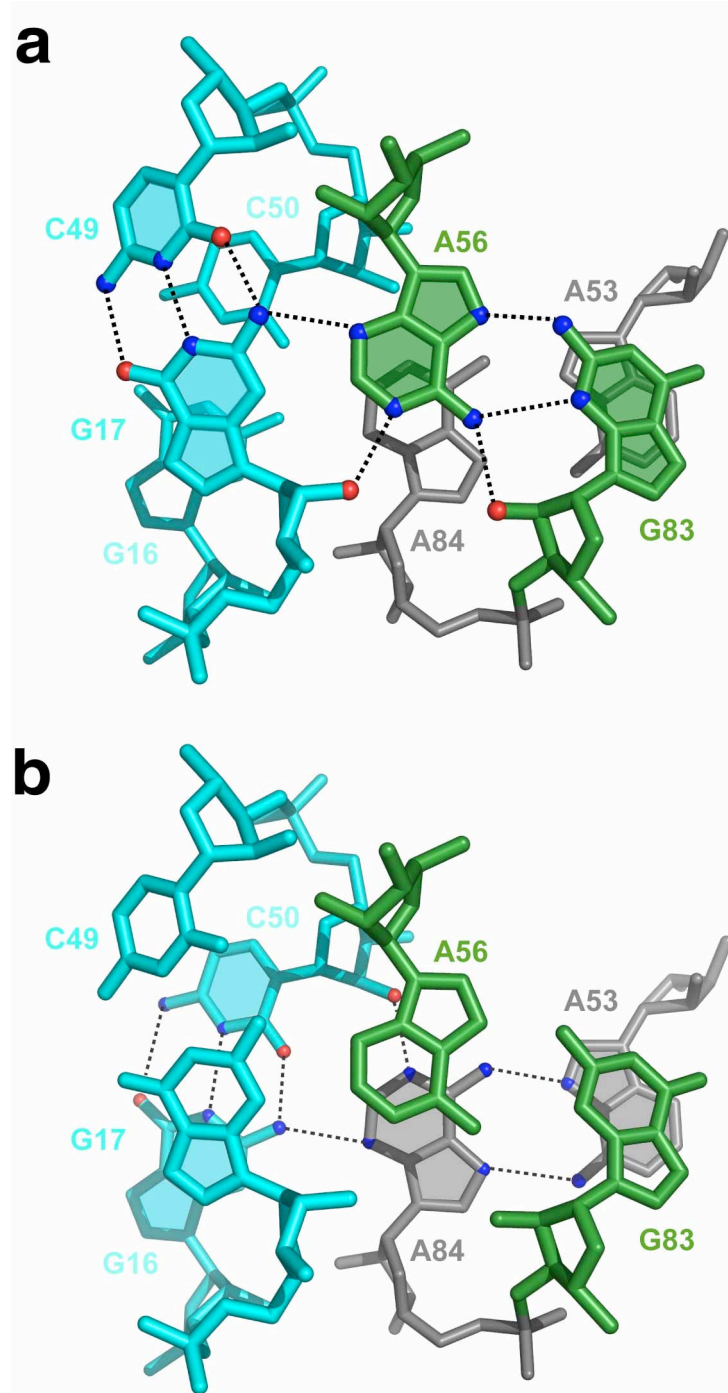
Supplementary Figure S2. Electron density map for TPP bound within the TPP riboswitch sensing domain. F_o-F_c omit electron density map (contoured at 2.5 σ level, blue mesh) calculated without TPP and Mg1 and Mg2 ions, superimposed with the final structure model for bound TPP in complex with the sensing domain. TPP is shown in red, Mg1 and Mg2 cations are shown in violet, metal-coordinated waters are shown as light blue spheres, and the non-canonical A61•C77 and Hoogsteen G60•G78 pairs are shown in green. The oxygen and nitrogen atoms of RNA are shown as red and blue spheres. The figure was prepared with PyMOL (<http://www.pymol.org>).



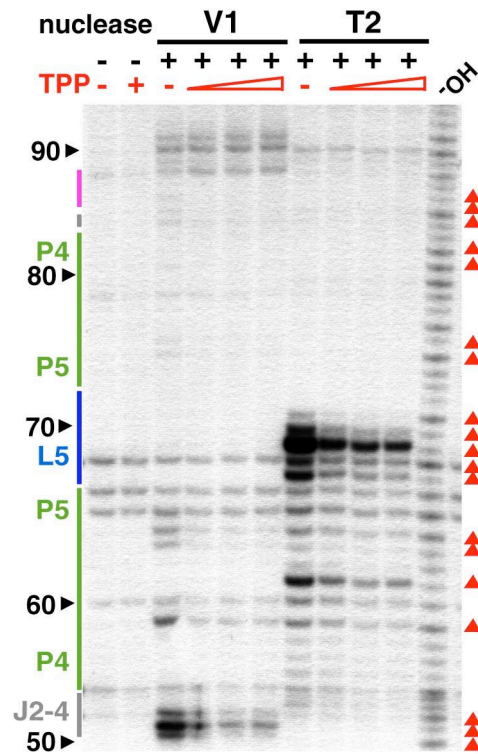
Supplementary Figure S3. Stacked base triples formed by purines of J3/2 and by base pairs of P2. **a**, Base triple between G42 and the minor groove of the G19•A47 non-canonical pair. **b**, Water-mediated (w) base triple between A41 and the minor groove of the G18-C48 Watson-Crick pair.



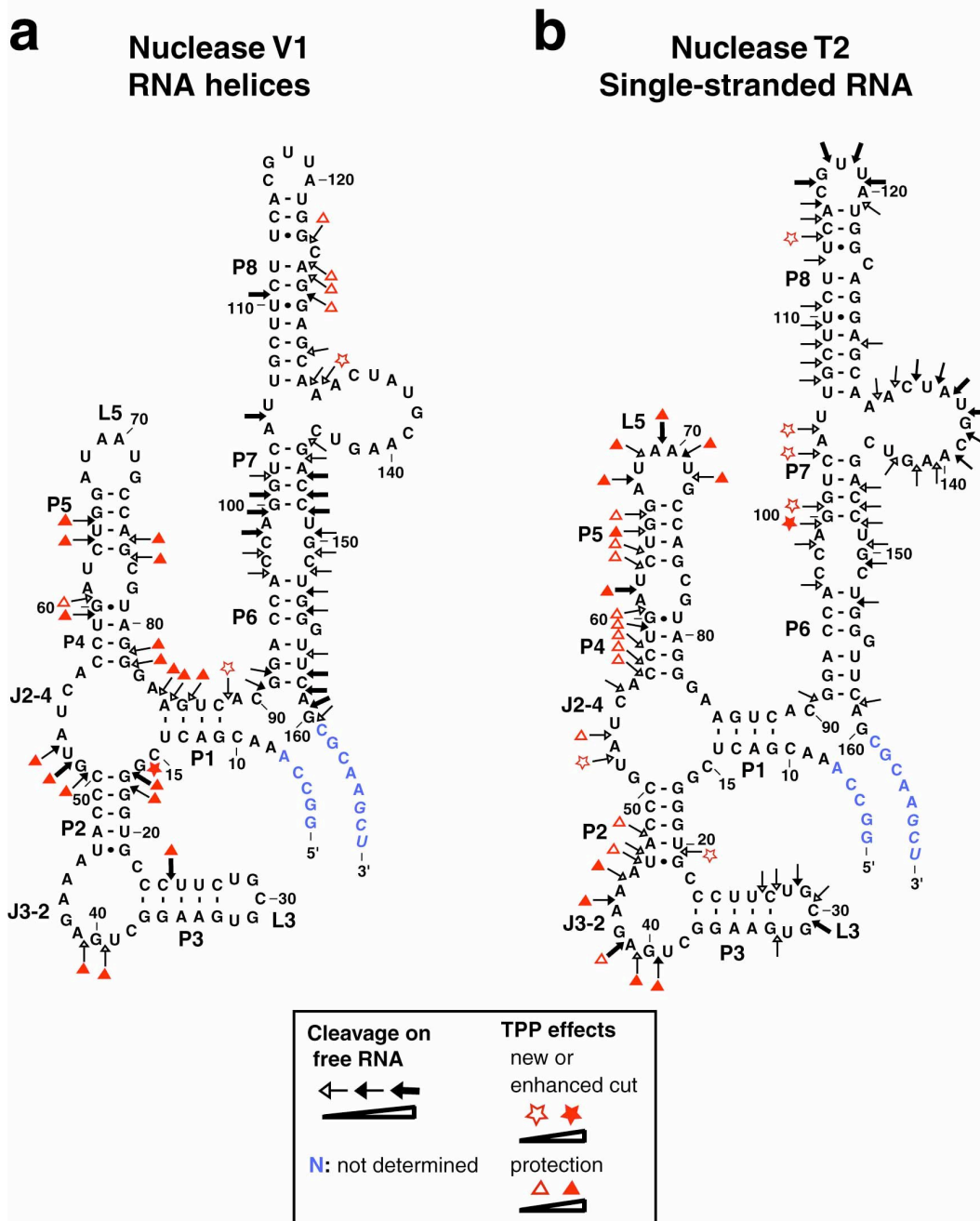
Supplementary Figure S4. Mg²⁺-mediated binding of TPP to its riboswitch monitored by NMR at 25 °C. **a**, Imino proton spectra (8.7 to 15 ppm) of the 80-nt long sensing domain of the TPP riboswitch in the free state (0.2 mM) in 25 mM K-acetate, pH 6.9, supplemented with 50 mM NaCl. **b**, Corresponding spectrum recorded after addition of ~2 equivalents of TPP. Arrow indicates free TPP. **c**, Corresponding spectrum recorded after addition of 6 mM MgCl₂. RNA is fully-bound to TPP as demonstrated by dramatic changes in the spectrum. Major new peaks are indicated by asterisks.



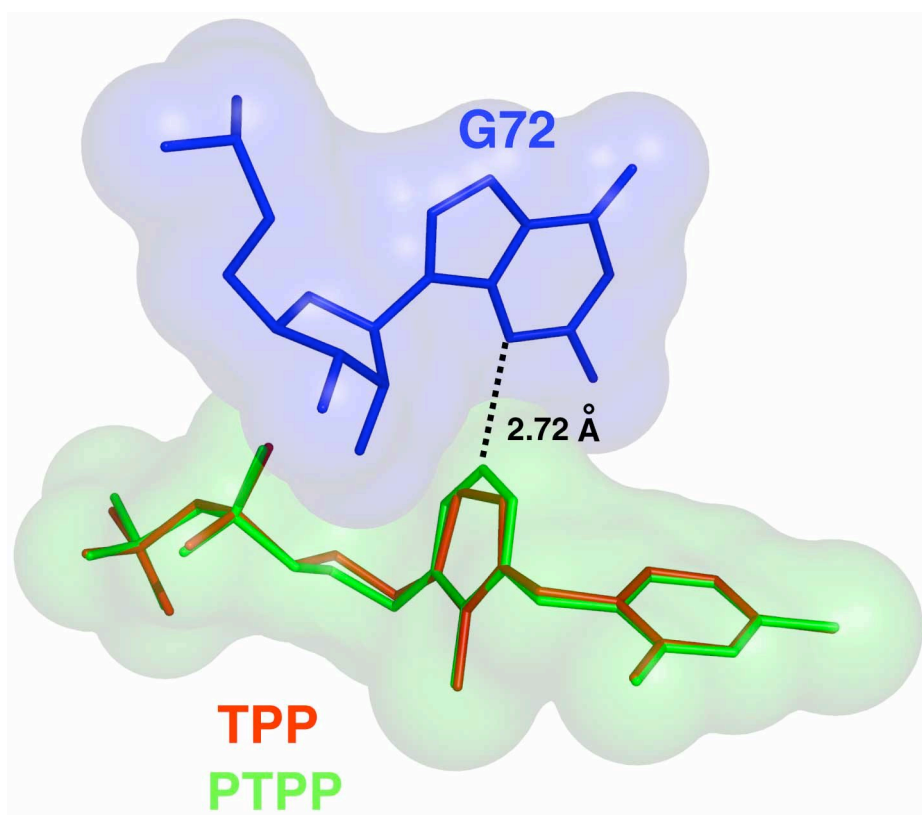
Supplementary Figure S5. Stacked base tetrads between nucleotides of J2/4, P2 and P4. **a**, Base tetrad formed between the non-canonical sheared A56•G83 pair and the minor groove of the Watson-Crick G17-C49 pair. **b**, Base tetrad formed between the non-canonical reverse A53•A84 pair and the minor groove of the Watson-Crick G16-C50 pair.



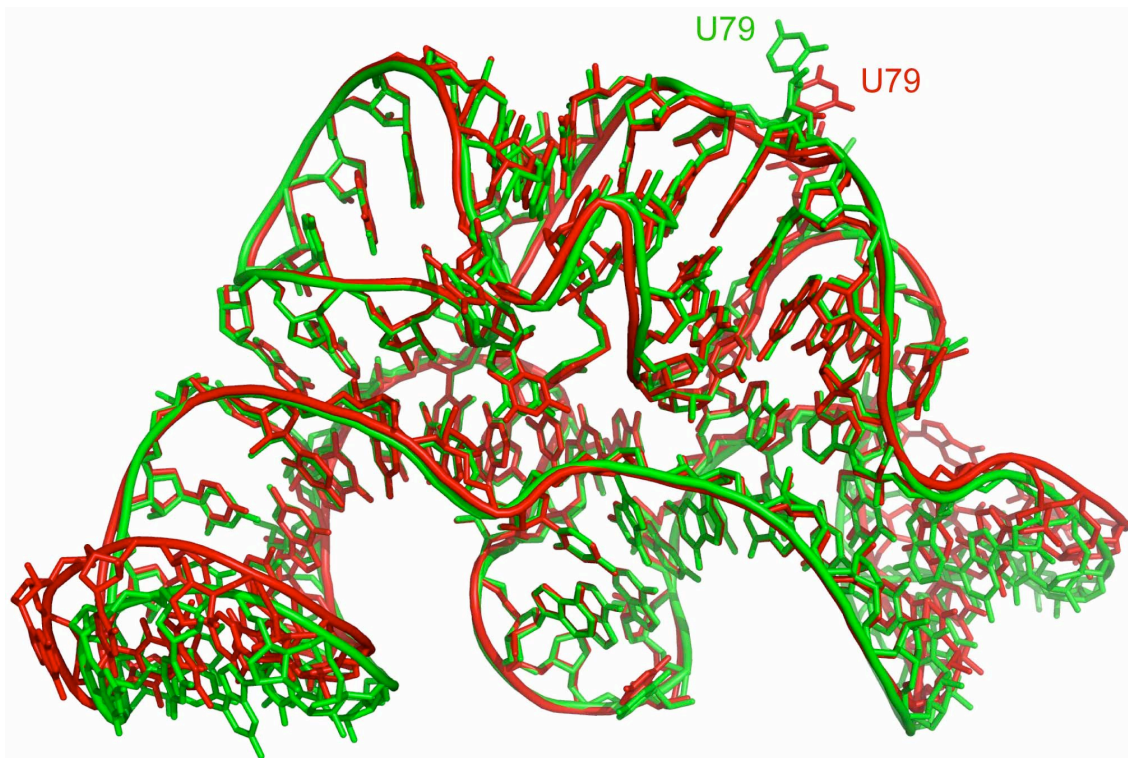
Supplementary Figure S6. Footprinting of the *thiM* TPP riboswitch. Representative (nt 49-94) ribonuclease V1 and T2 partial cleavage patterns for the *E. coli thiM* riboswitch (nt 1-166) visualized by PAGE. 5' ³²P-labeled RNAs were treated by nucleases in the absence (-) or presence (+) of 1, 3 and 10 equivalents of TPP. Experimental details are presented in Supplementary methods section. ⁻OH and Bc stand for ladders prepared by partial digestion with alkali or *B. cereus* ribonuclease. The major cleavage protections in the presence of TPP are labeled by triangles.



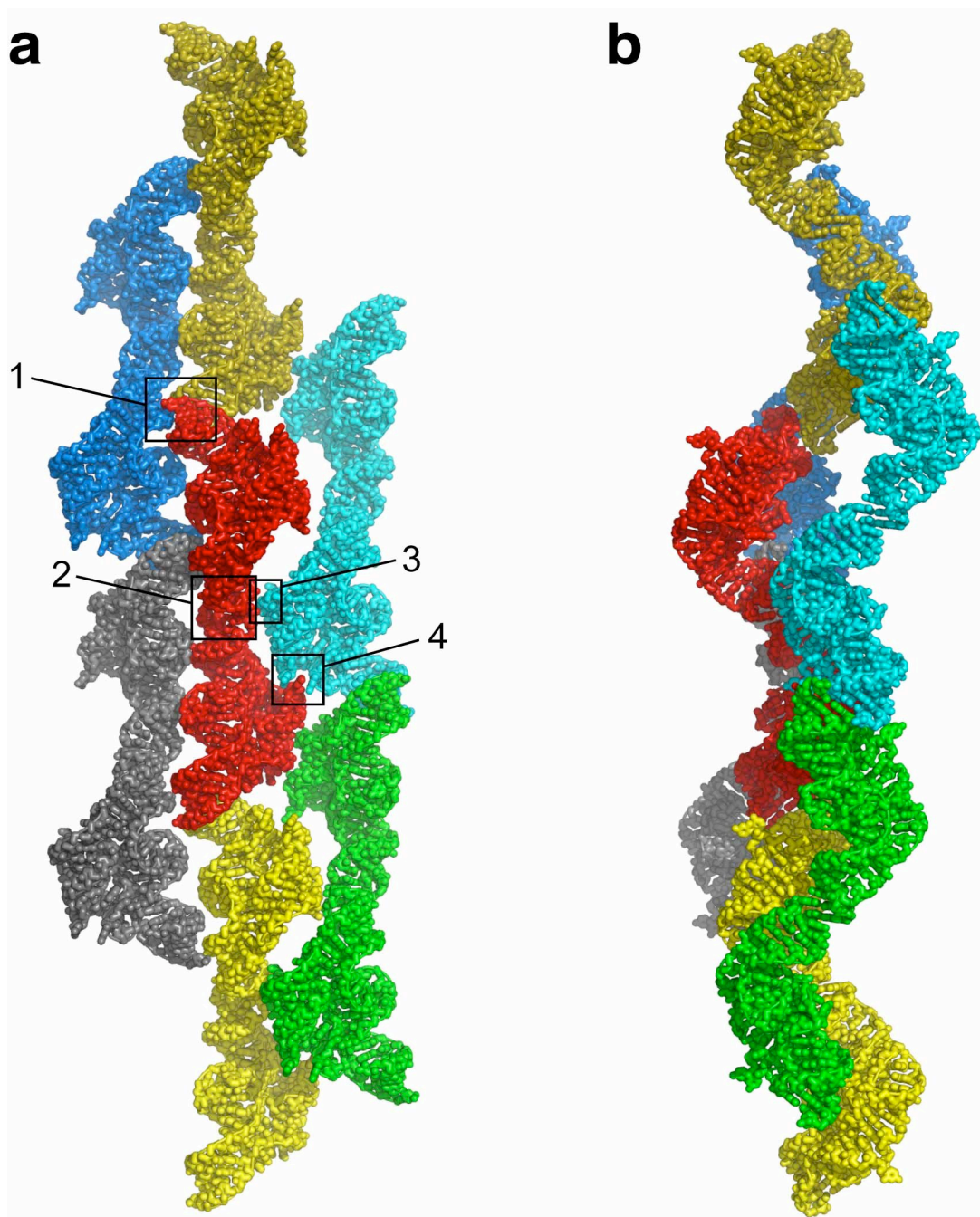
Supplementary Figure S7. Summary of V1 and T2 ribonuclease cleavage and footprinting experiments on free and TPP-bound riboswitch RNAs with the results depicted on the original secondary structure model for the *thiM* riboswitch. **a**, V1 nuclease and **b**, T2 nuclease. Cleavage intensities and TPP effects are indicated in the inserts. Footprinting experiments were performed on 5'- and 3'-³²P-labeled RNA molecules, as well as on non-labeled molecules, followed by reverse transcription.



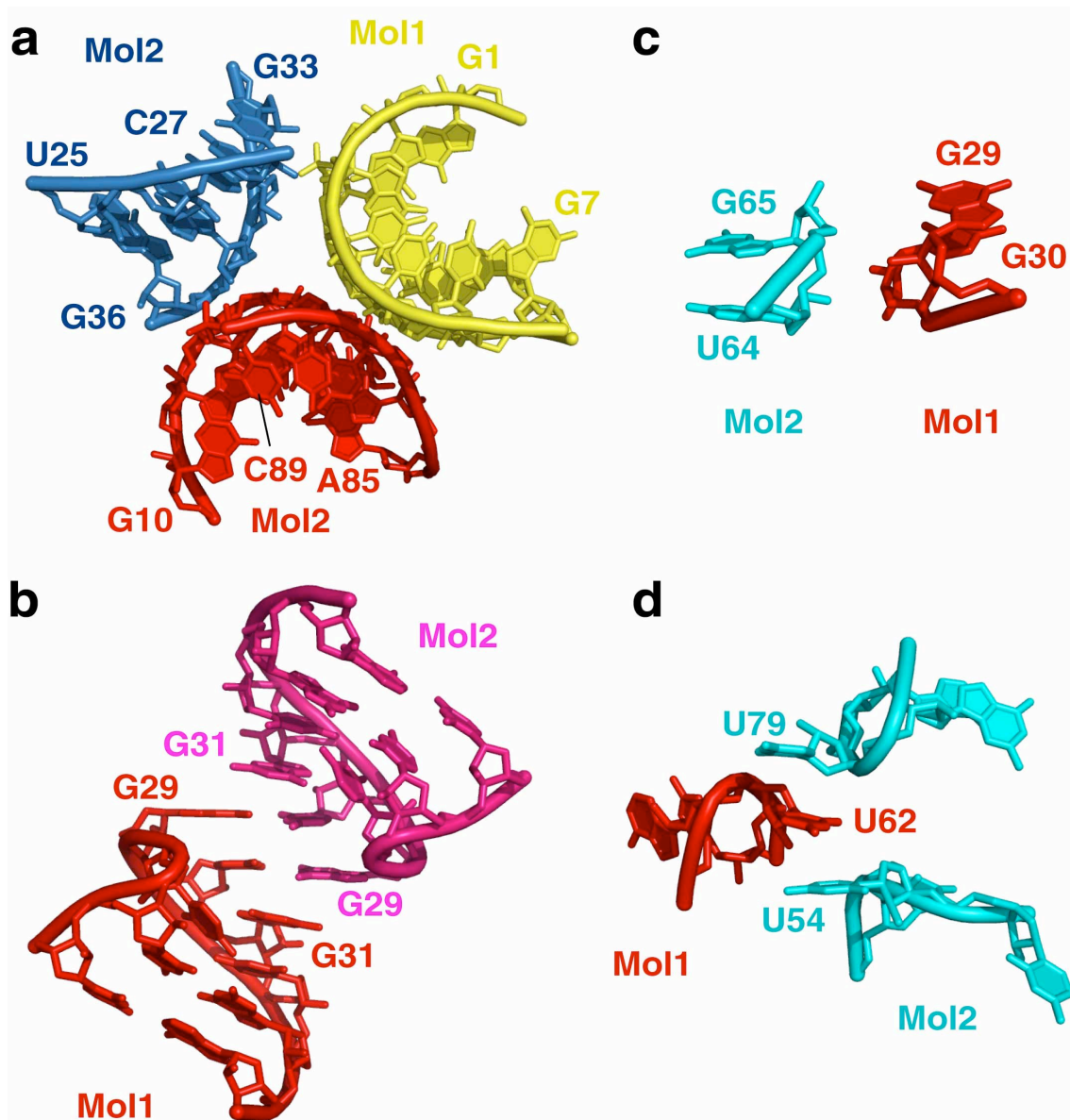
Supplementary Figure S8. Overlay of the structure of the bound TPP and a model of bound PTPP within the riboswitch binding pocket. The bound TPP and modeled PTPP are shown in red and green stick representations, respectively. A potential C-H...N hydrogen bond can be formed between the pyridine ring of PTPP and the N³ of G72, the closest residue to the bound ligand.



Supplementary Figure S9. Superposition of two riboswitch-TPP complexes from the asymmetric unit. Superposition was done using nt 16-25 and 36-85. Molecule 1, shown on other figures of the article, is in red, and molecule 2 is in green. The two complexes are similar and contain virtually all described interactions, including those formed between TPP, Mg^{2+} cations and RNA. The most visible exception is the absence of stacking between U79 and U54 in molecule 2 due to intercalation of residue U62 from another molecule. The central regions of the complexes, which lack P1 (nt 10-15 and 86-89) and P3-L3 (nt 26-35), have r.m.s.d. of 0.77 Å, and r.m.s.d. of the entire riboswitch-TPP complexes is 1.76 Å.



Supplementary Figure S10. Crystal packing of the riboswitch-TPP complexes. **a**, front view; **b**, side view. Contact regions, shown in detail in Supplementary Figure S11, are squared. RNA molecules 1 and 2, which form dimers, are shown in the same color. The dimers are formed by base-pairing between loops 3 of each molecule (region 2).



Supplementary Figure S11. Detailed view of the representative crystal contact regions from Supplementary Figure S10. **a**, region 1; **b**, region 2; **c**, region 3; **d**, region 4; RNA molecules from the asymmetric are indicated as Mol1 and Mol2.