Riboswitch Structure: An Internal Residue Mimicking the Purine Ligand

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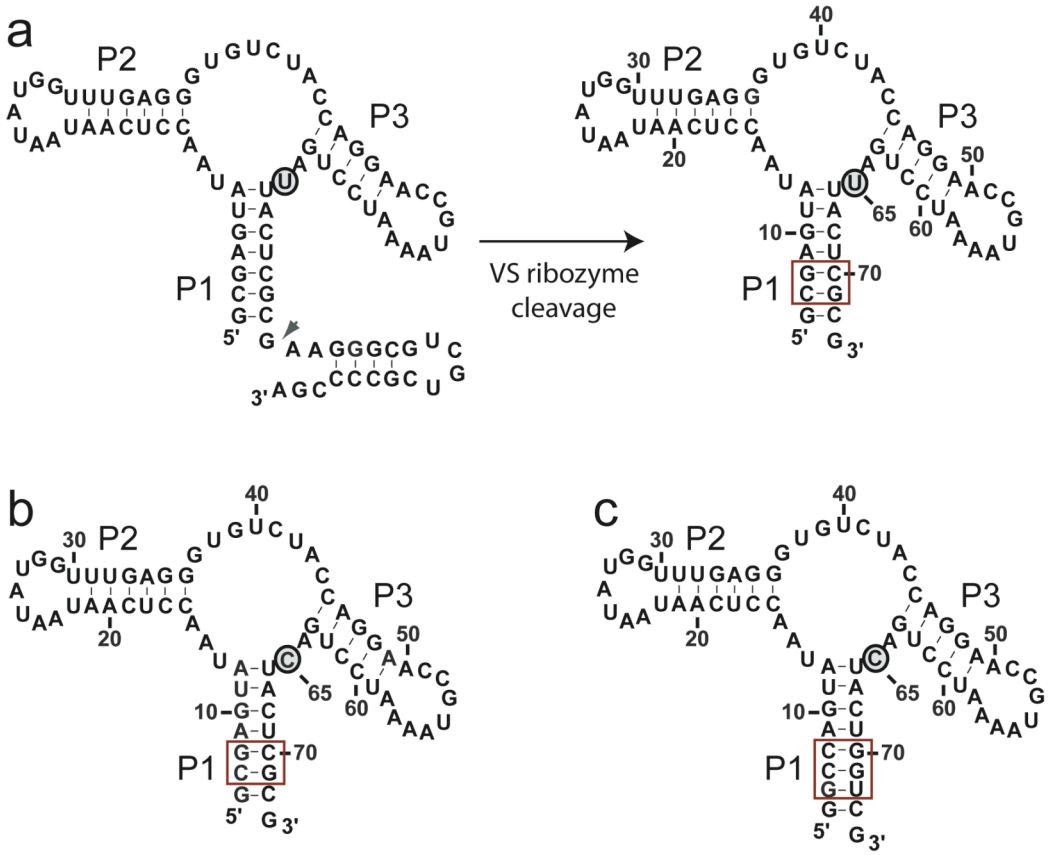
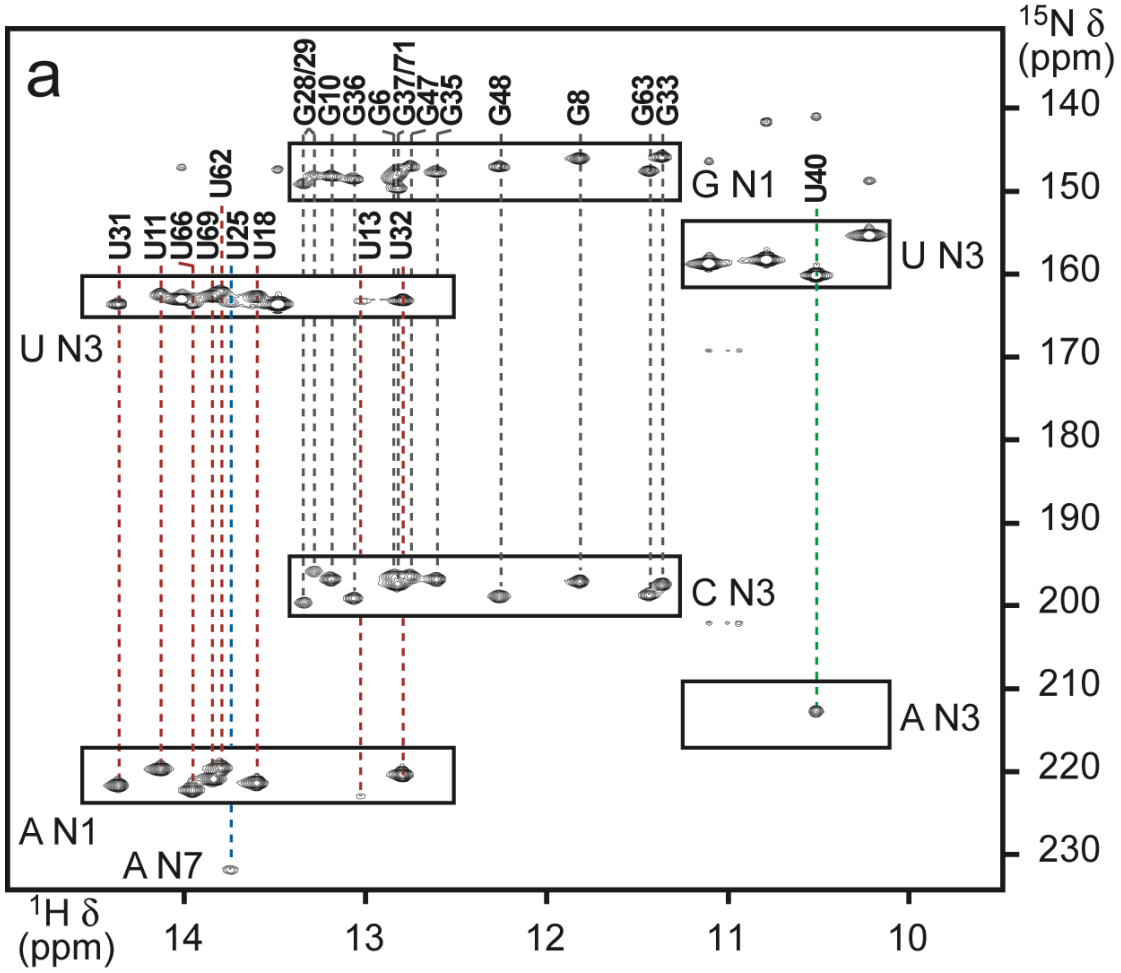
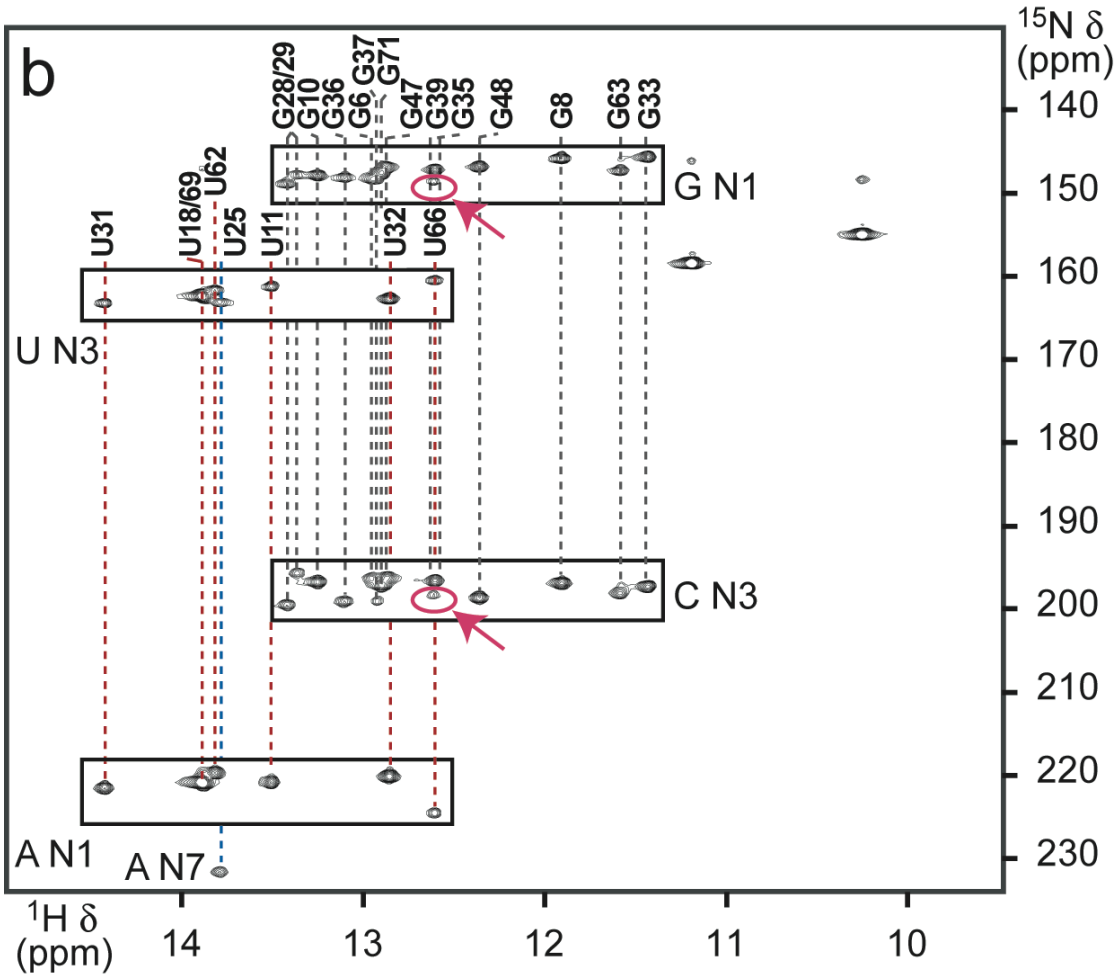
Supplementary Figures

Supplementary Figure 1. Summary of RNA sequences used for (**a**, **b**) NMR and (**c**) X-ray studies. (**a**) The RSA-VS is the precursor of the wild-type A-riboswitch aptamer (RSA) that contains a *Varkud* Satellite (VS) ribozyme substrate at its 3'-end. RSA was thus generated from cleavage of RSA-VS with a *trans*-cleaving VS ribozyme. The cleavage site is shown by a grey arrow. (**b**) The RSA(U65C) is the U65C mutant of RSA. (**c**) The RSA(U65C)$_{GU2}$ is a derivative of RSA(U65C) containing a G-U base pair in the P1 stem. The red box highlights the region that was mutated in RSA(U65C)$_{GU2}$.

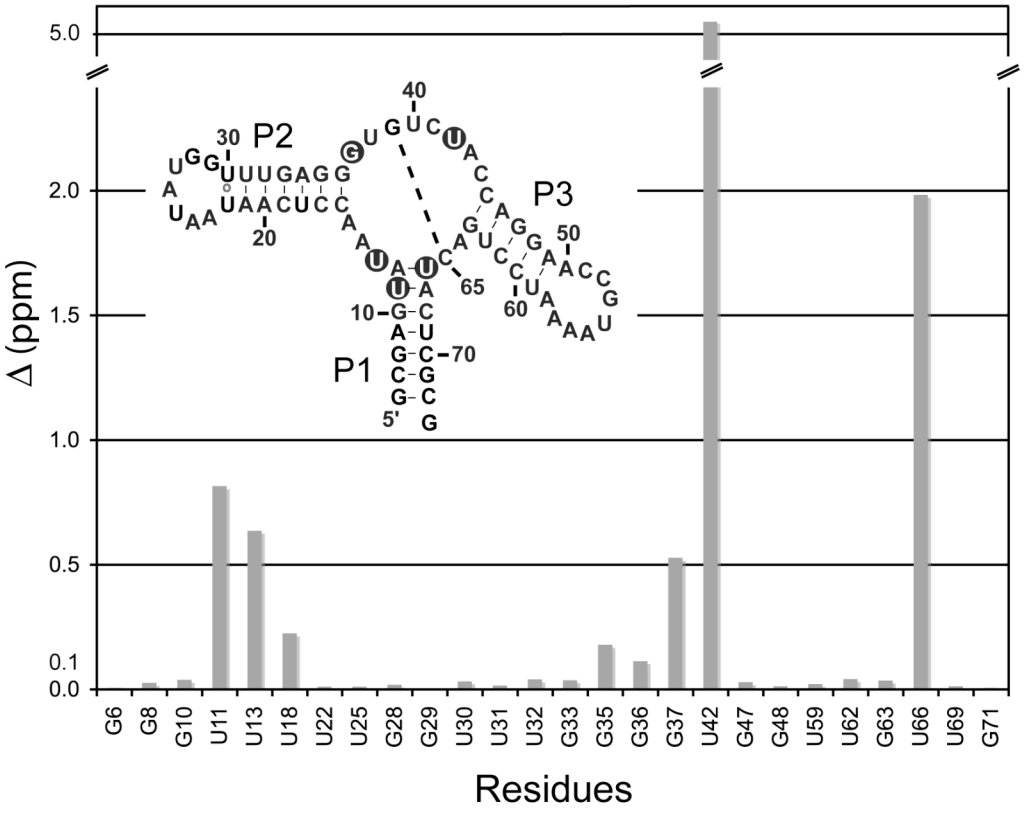


Supplementary Figure 2. Hydrogen bonding in the A-riboswitch aptamer derived from $^{2}J_{NN}$ coupling in the 2D HNN-COSY spectra of (**a**) the ^{15}N-labeled wild-type aptamer with unlabeled adenine and (**b**) the ^{15}N-labeled U65C mutant aptamer. The U(N3)-A(N1) and G(N1)-C(N3) correlations are indicated by red and grey dashed lines, respectively. The U25(N3)-A56(N7) and U40(N3)-A67(N3) correlations are indicated by blue and green dashed lines, respectively. The two peaks that define the G39(N1)-C65(N3) correlation are circled and marked with an arrow. Spectra were recorded on a 600 MHz NMR spectrometer at 298 K.

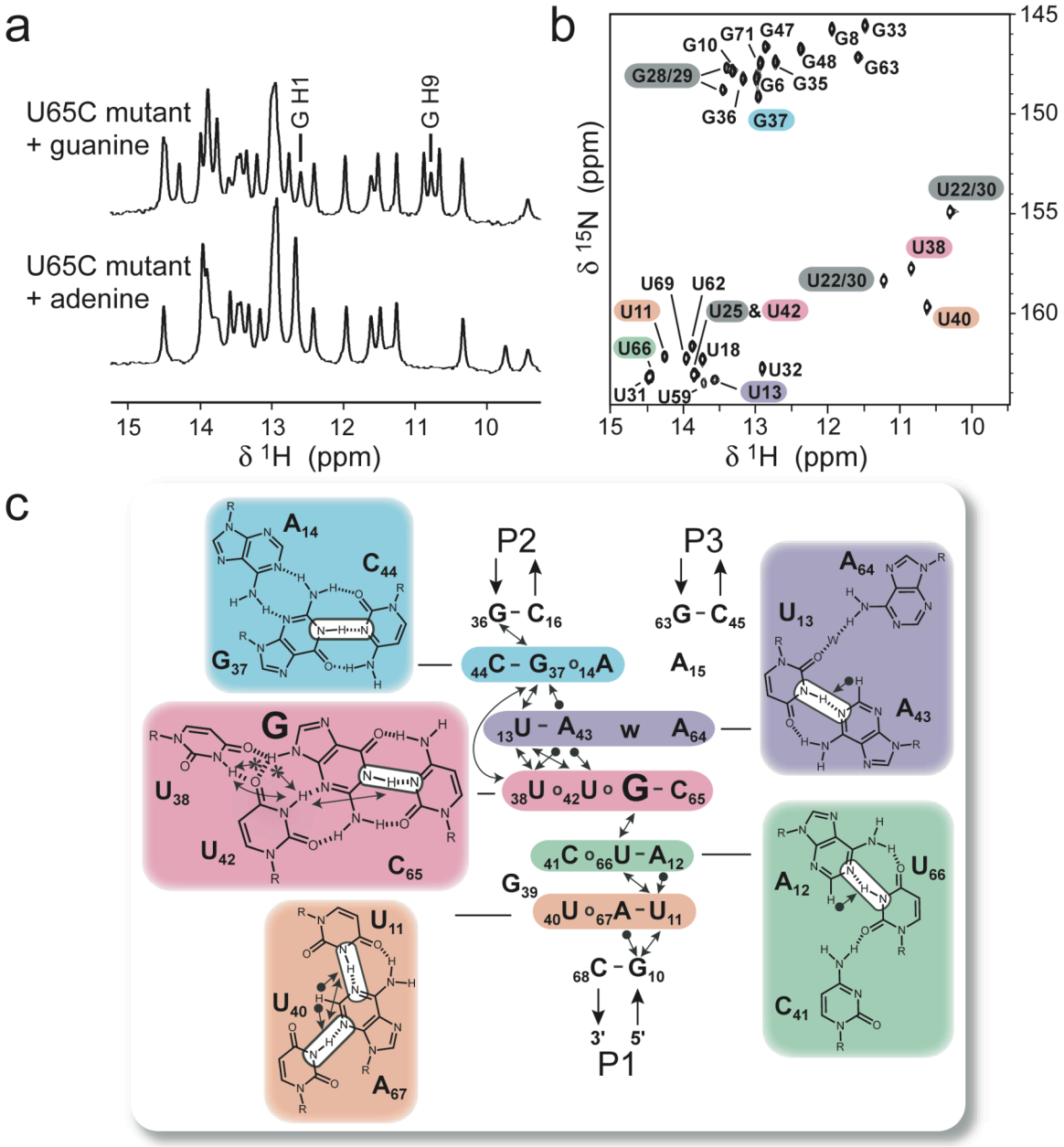



b
G28/29
G10
G36
G6
G37
G71
G47
G39
G35
G48
G8
G63
G33
G N1
U31
U18/69
U62
U25
U11
U32
U66
U N3
C N3
A N1
A N7
^{15}N δ (ppm)
140
150
160
170
180
190
200
210
220
230
^{1}H δ (ppm)
14
13
12
11
10

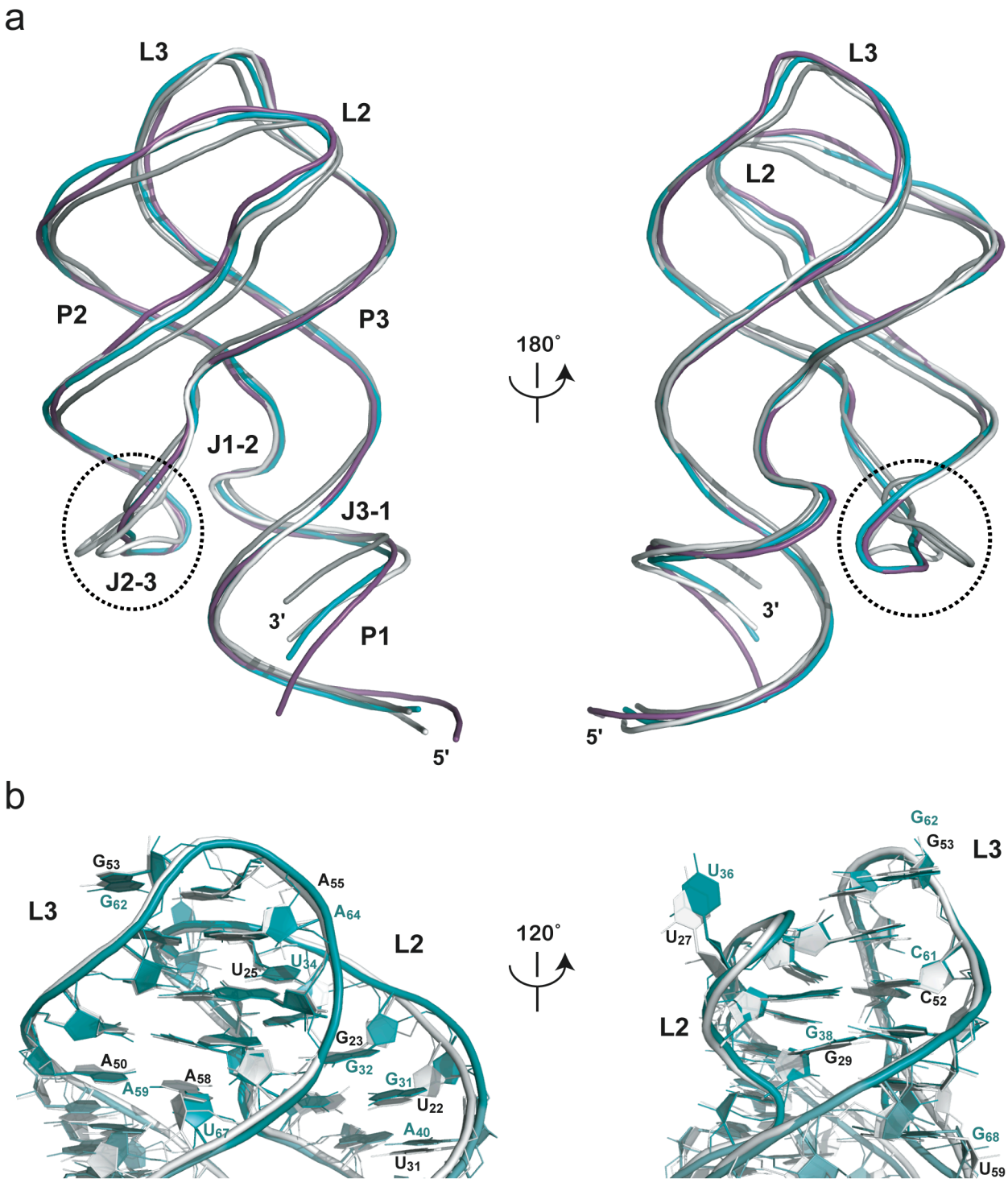
Supplementary Figure 3. Chemical-shift differences (Δ in ppm ± 0.03 ppm ; $\Delta = [(\Delta_H)^2 + (0.5 \times \Delta_N)^2]^{1/2}$) of imino protons and nitrogens detected in the 2D ^{1}H-^{15}N HSQC spectrum for both the wild-type and U65C mutant of the A-riboswitch aptamer. Significant differences in chemical shifts (Δ > 0.5 ppm) are mapped on the secondary structure of the U65C mutant (dark grey circles for U11, U13, G37, U42 and U66).



Supplementary Figure 4. Specific interaction of the U65C mutant of the *B. subtilis pbuE* A-riboswitch aptamer with guanine. (**a**) 1D ^{1}H NMR spectra were recorded on a 500 MHz NMR spectrometer at 288 K for samples of 0.35 mM U65C mutant in the presence of 2 mM adenine (+ adenine) or ~0.4 mM guanine (+ guanine). Two signals of the bound guanine are observed at 12.59 ppm (H1) and 10.76 ppm (H9). (**b**) Imino-optimized 2D ^{1}H-^{15}N HSQC spectra of the ^{15}N-labeled U65C mutant with unlabeled guanine. Crosspeaks are annotated by residue types and numbers. (**c**) Model of the core of the guanine-bound U65C mutant derived from the imino NMR data and in agreement with the X-ray structure of purine-bound aptamers. All through-bond $^{2}J_{NN}$ coupling detected in the 2D HNN-COSY spectra are in agreement with the G/U NH-N hydrogen bonds (depicted by white boxes) shown in these models. All NOEs involving imino protons in the 3D ^{15}N-edited NOESY-HSQC (**Supplementary Table S2**) are in complete agreement with the base pairing and stacking arrangement depicted in this model. Only the NOEs between two imino protons (lines with two arrowheads), between an adenine H2 and an imino proton (lines with a sphere and an arrowhead) and between the guanine ligand H9 and an imino proton (lines with a star and an arrowhead) were analyzed.


a
U65C mutant
+ guanine
U65C mutant
+ adenine
G H1
G H9
15
14
13
12
11
10
δ 1H (ppm)
b
δ 15N (ppm)
145
150
155
160
G10
G71
G47
G8
G33
G48
G63
G28/29
G6
G35
G36
G37
U22/30
U38
U22/30
U69
U62
U11
U25 & U42
U40
U66
U18
U32
U31
U59
U13
15
14
13
12
11
10
δ 1H (ppm)
c
A14
C44
G37
P2
P3
36G - C16
63G - C45
44C - G37 o14A
A15
A64
U13
A43
13U - A43 w A64
G
U38
38U o42U o G - C65
U42
C65
41C o66U - A12
G39
40U o67A - U11
68C - G10
3'
5'
P1
U11
U40
A67
A12
U66
C41

Supplementary Figure 5. Structural comparison between the U65C mutant and the A/G-riboswitch aptamers. (**a**) Superpositions of the U65C mutant structure (in white) with the *V. vulnificus add* A-riboswitch aptamer bound to adenine (in purple; PDB code 1Y26 and rmsd = 1.14 Å for residues 6-37 and 43-72), the *B. subtilis xpt-pbuX* G-riboswitch aptamer bound to guanine (in cyan ; PDB code 1Y27 and rmsd = 1.15 Å for residues 6-37 and 43-72); and a mutant of the *B. subtilis xpt-pbuX* G-riboswitch aptamer bound to 2'-deoxyguanosine (in grey ; PDB code 3DS7 and rmsd = 2.00 Å for residues 6-37 and 43-72). For each structure, a ribbon representation of the phosphate backbone is shown in two orientations that differ by 180˚. The most variable region is circled in dashed lines. (**b**) Close-up view of the loop-loop interaction in the superposition of the U65C mutant structure (in white) with the *B. subtilis xpt-pbuX* G-riboswitch aptamer bound to guanine (in cyan ; PDB code 1Y27). Here, a ribbon and stick representation is shown in two orientations that differ by 120˚. The residue numbering in (**b**) is given as in the PDB files.


a
L3
L2
P2
P3
J1-2
J3-1
J2-3
3'
P1
5'
180°
L3
L2
3'
5'
b
G53
G62
L3
A55
A64
L2
U25
U34
G23
G32
G31
U22
A40
U31
A50
A59
A58
U67
120°
G62
G53
L3
U36
U27
C61
C52
L2
G38
G29
G68
U59