Mutational analysis of the purine riboswitch domain

Supporting Information

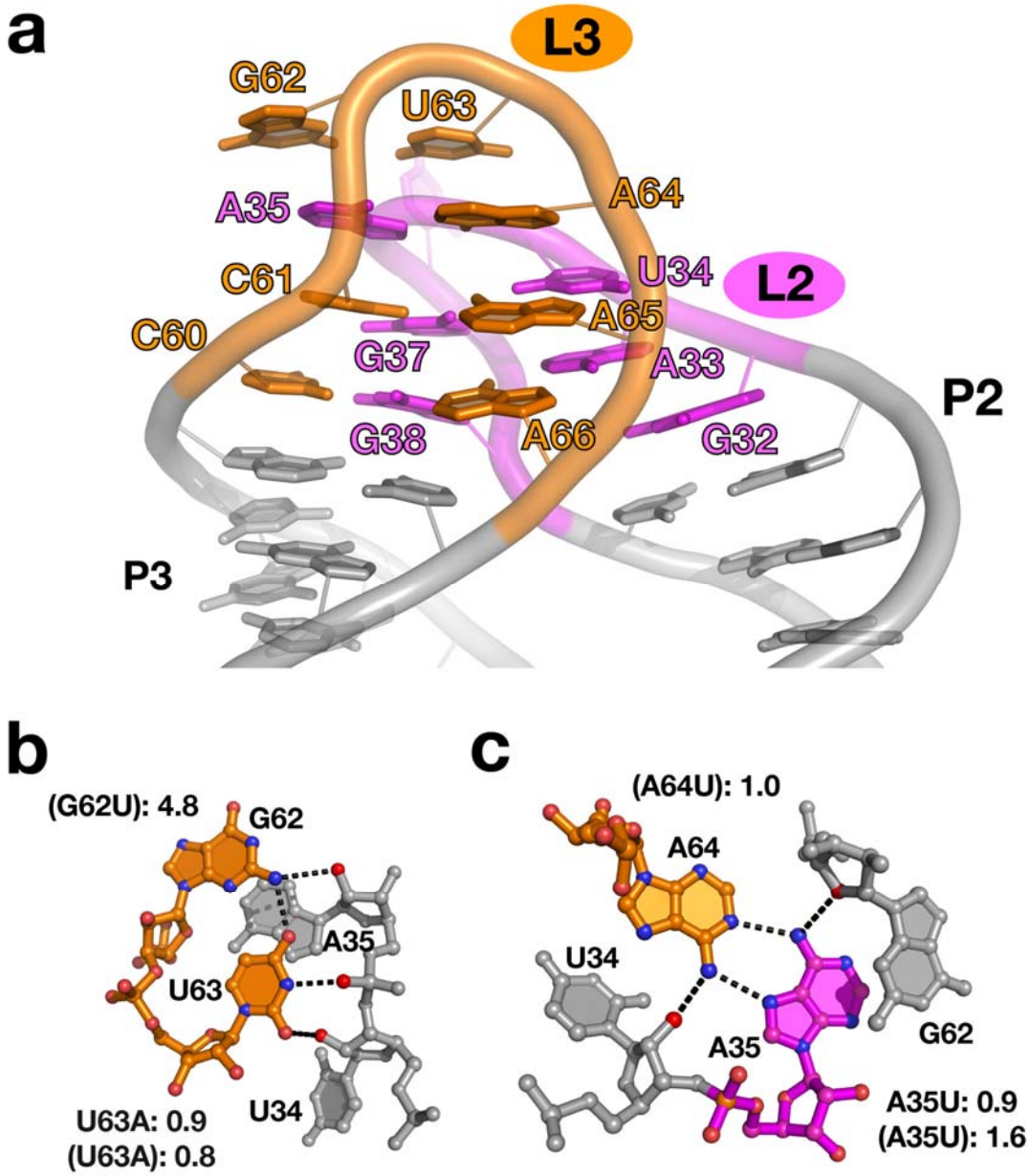
Crystal E. Love, Sunny D. Gilbert*, Andrea L. Edwards and Robert T. Batey‡*

Department of Chemistry and Biochemistry, University of Colorado at Boulder, Campus Box 215, Boulder, Colorado 80309-0215

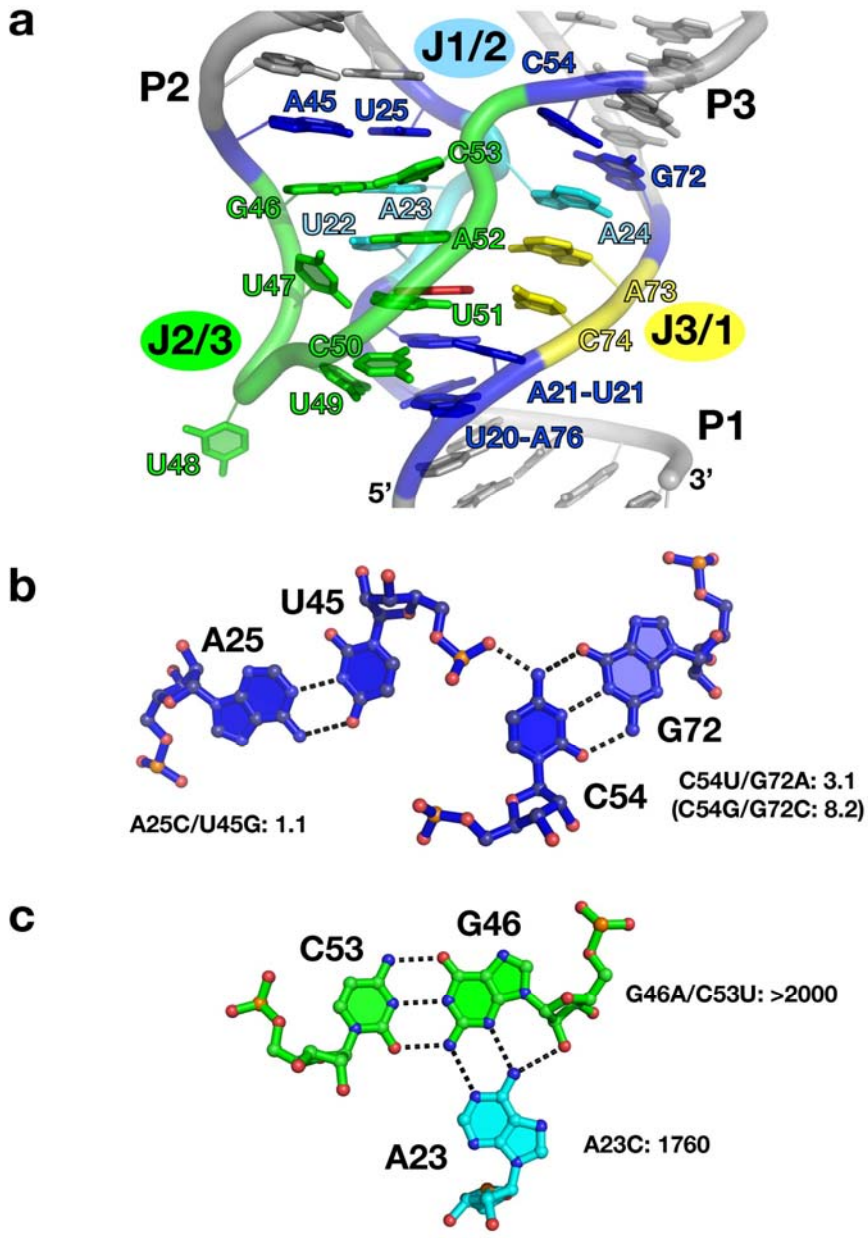
Supporting Table 1: Phylogenetic conservation of nucleotides in the purine riboswitch[a].

Sequence[b]	A	C	G	U	Δ
5'P1-J1/2-5'P2					
U20	0	0	0	100	0
A21	90	0	0	10[c]	0
U22	0	0	0	100	0
A23	100	0	0	0	0
A24	67	0	0	32	1
U25	9	15	7	32	13
Loop 2 (L2)					
G32	50	0	48	2	0
A33	100	0	0	0	0
U34	0	0	0	100	0
A35	88	0	0	0	12
U36	12	2	4	82	0
G37	0	0	100	0	0
G38	0	0	100	0	0
3'P2-J2/3-5'P3					
A45	73	16	6	5	0
G46	0	0	99	1[d]	0
U47	0	0	0	100	0
U48	2	30	2	66	0
U49	0	2	0	98	0
C50	0	100	0	0	0
U51	0	0	0	100	0
A52	100	0	0	0	0
C53	0	100	0	0	0
C54	10	87	0	1	2
Loop 3 (L3)					
C60	0	100	0	0	0
C61	0	100	0	0	0
G62	12	0	67	21	0
U63	1	1	2	96	0
A64	71	0	13	7	9
A65	100	0	0	0	0
A66	92	1	0	7	0
3'P3-J3/1-3'P1					
G72	12	0	87	1	0
A73	93	0	5	2	0
C74[e]	0	93	0	7	0
U75	10	0	0	90	0
A76	100	0	0	0	0

a. the 100 sequences used to compile this table are from version 7.0 of the Rfam database (*reference*).
b. sequence corresponds to the *xpt-pbuX* guanine riboswitch from *B. subtilis*
c. all 10 sequences containing U at this position contained a deletion at position 25.
d. the single sequence with a U at this position contained an insertion of a single uracil at between positions 52 and 53.
e. this position is always C in guanine-responsive riboswitches and U in adenine responsive riboswitches.



Supporting Figure 1. (a) Structure of the L2-L3 interaction from the *xpt-pbuX* guanine riboswitch from *B. subtilis*. Magenta nucleotides are in L2 and orange nucleotides are in L3. (b) Base interactions of the G62•U63 cis-Hoogsteen/sheared "side-by-side" pair. Dashed lines connect atoms that are in hydrogen bonding distance from one another (2.7-3.2 Å). K_{rel} values for mutants from Table 1 are shown by their respective bases. (c) Base interactions of the A35•A64 trans Watson-Crick/Hoogsteen pair.



Supporting Figure 2. (a) Structure of the three-way junction of the purine riboswitch aptamer domain from wild type *B. subtilis xpt-pbuX* guanine riboswitch bound to hypoxanthine. The structure of GRA RNA is identical except for the C74U mutation and the ligand is DAP; both changes do not appreciably change the structure. (b) Closing base pairs of the P2 and P3 helices adjacent to the three-way junction, emphasizing the hydrogen bonding interaction between C54(N4) and U45(O2P). $K_{D,rel}$ values for mutations of these pairs are shown adjacent, with values from Lemay et al shown in parenthesis (note that the numbering has been changed from Lemay et al to be consistent with our numbering). (c) Structure of the conserved (G46-C53)•A23 triple with $K_{D,rel}$ values for mutants shown.