Riboswitch Structure: An Internal Residue Mimicking the Purine Ligand

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Supplementary Tables

Supplementary Table 1. Resonance assignment of adenine H2 protons and G/U imino protons and nitrogens (δ in ppm) for the wild-type and U65C mutant of the *B. subtilis pbuE* adenine aptamer. [a]

Residue	Wild-type - Adenine			U65C mutant – No ligand			U65C mutant – Guanine		
	δ H2 Proton[b]	δ Imino Proton[c]	δ Imino Nitrogen[c]	δ H2 Proton[b]	δ Imino Proton[c]	δ Imino Nitrogen[c]	δ H2 Proton[b]	δ Imino Proton[c]	δ Imino Nitrogen[c]
G6		12.99	148.2		13.00	148.2		12.98	148.2
G8		11.96	145.7		11.94	145.7		11.94	145.7
A9	7.34			7.35			7.37		
G10		13.33	147.9		13.31	147.8		13.32	147.9
U11		14.26	162.0		13.57	161.1		14.26	162.2
A12	7.24			7.71			7.41		
U13		13.17	162.9		13.79	163.2		13.56	163.4
U18		13.75	162.4		13.95	162.6		13.73	162.3
A20	7.56			7.57			7.54		
A21	7.84			7.87			7.85		
U22/U30[d]		11.24	158.4		11.25	158.4		11.23	158.4
U25		13.88	163.1		13.88	163.1		13.84	163.1
G28/G29[e]		13.48	148.8		13.46	148.8		13.45	148.8
G29/G28[e]		13.43	147.7		13.43	147.7		13.40	147.7
U30/U22[d]		10.35	154.9		10.32	154.9		10.31	154.9
U31		14.50	163.3		14.50	163.2		14.48	163.2
U32		12.93	162.8		12.91	162.7		12.91	162.8
G33		11.50	145.6		11.47	145.6		11.49	145.6
A34	7.33			7.29			7.34		
G35		12.74	147.4		12.64	147.1		12.73	147.4
G36		13.20	148.3		13.15	148.1		13.17	148.3

G37		12.96	149.3		12.96	148.3		12.96	149.1
U38		10.92	157.9		Not detected			10.85	157.8
G39		Not detected			12.65	148.5		Not detected	
U40		10.65	159.8		Not detected			10.63	159.7
U42		13.62	163.3		9.72	156.7		13.85	163.1
A43	7.19			7.90			7.66		
A46	7.02			7.07			7.05		
G47		12.89	146.6		12.92	146.7		12.86	146.6
G48		12.40	146.7		12.40	146.8		12.38	146.7
A49	7.48			7.49			7.48		
A56									
U59		13.76	163.6		13.75	163.6		13.72	163.5
U62		13.93	161.7		13.90	161.7		13.88	161.7
G63		11.57	147.3		11.59	147.2		11.58	147.2
U65		14.15	162.6		U65 mutated to a C			U65 mutated to a C	
U66		14.09	163.2		12.67	160.5		14.45	163.2
A67	7.95			7.11			7.98		
U69		13.98	162.3		13.96	162.3		13.96	162.3
G71		12.96	147.5		12.95	147.5		12.94	147.5

a. The wild-type adenine aptamer was prepared in the presence of adenine and the U65C mutant was prepared in the absence (no ligand) or the presence of guanine as described in Materials and Methods.

b. Assignments of H2 protons were obtained from 3D ^{15}N-edited NOESY-HSQC spectra collected at 15 ˚C. The error on these values is ± 0.08 ppm.

c. Assignments of imino protons and nitrogens were obtained from the 2D ^{1}H-^{15}N HSQC spectra collected at 15 ˚C. For the adenine-bound wild-type aptamer and the ligand-free U65C mutant, the errors on the imino proton and nitrogen chemical shifts are ± 0.01 ppm and ± 0.1 ppm, respectively. For the guanine-bound U65C mutant, the errors on the imino proton and nitrogen chemical shifts are ± 0.02 ppm and ± 0.1 ppm, respectively.

d. These signals are assigned to either U22 or U30.

e. These signals are assigned to either G28 or G29.

Supplementary Table 2. List of NOEs involving imino protons in the wild-type and U65C mutant of the *B. subtilis pbuE* adenine aptamer.[a]

Wild-type - Adenine		**U65C Mutant – No ligand**		**U65C Mutant – Guanine**	
Imino proton	**NOE**	**Imino proton**		**Imino proton**	**NOE**
G6 H1	G6 H1 – G71 H1	G6 H1	G6 H1 – G71 H1	G6 H1	G6 H1 – G71 H1
G8 H1	G8 H1 – G71 H1	G8 H1	G8 H1 – G71 H1	G8 H1	G8 H1 – G71 H1
	G8 H1 – U69 H3		G8 H1 – U69 H3		G8 H1 – U69 H3
	G8 H1 – A9 H2		G8 H1 – A9 H2		G8 H1 – A9 H2
G10 H1	G10 H1 – U69 H3	G10 H1	G10 H1 – U69 H3	G10 H1	G10 H1 – U69 H3
	G10 H1 – U11 H3		G10 H1 – U11 H3		G10 H1 – U11 H3
	G10 H1 – A9 H2		G10 H1 – A9 H2		G10 H1 – A9 H2
	G10 H1 – A67 H2		G10 H1 – A67 H2		G10 H1 – A67 H2
U11 H3	U11 H3 – U66 H3	U11 H3	U11 H3 – U66 H3	U11 H3	U11 H3 – U66 H3
	U11 H3 – G10 H1		U11 H3 – G10 H1		U11 H3 – G10 H1
	U11 H3 – U40 H3				U11 H3 – U40 H3
	U11 H3 – A67 H2		U11 H3 – A67 H2		U11 H3 – A67 H2
	U11 H3 – A12 H2		U11 H3 – A12 H2		U11 H3 – A12 H2
U13 H3	U13 H3 – G37 H1	U13 H3	U13 H3 – G37 H1	U13 H3	U13 H3 – G37 H1
	U13 H3 – U38 H3				U13 H3 – U38 H3
	U13 H3 – U42 H3				U13 H3 – U42 H3
	U13 H3 – A43 H2		U13 H3 – A43 H2		U13 H3 – A43 H2
U18 H3	U18 H3 – G33 H1	U18 H3	U18 H3 – G33 H1	U18 H3	U18 H3 – G33 H1
	U18 H3 – G35 H1		U18 H3 – G35 H1		U18 H3 – G35 H1
	U18 H3 – A34 H2		U18 H3 – A34 H2		U18 H3 – A34 H2
U22 H3	U22 H3 – U30 H3	U22 H3	U22 H3 – U30 H3	U22 H3	U22 H3 – U30 H3

	U22 H3 – U31 H3		U22 H3 – U31 H3		U22 H3 – U31 H3
	U22 H3 – A21 H2		U22 H3 – A21 H2		U22 H3 – A21 H2
U30 H3	U30 H3 – U22 H3	U30 H3	U30 H3 – U22 H3	U30 H3	U30 H3 – U22 H3
	U30 H3 – U31 H3		U30 H3 – U31 H3		U30 H3 – U31 H3
	U30 H3 – A21 H2		U30 H3 – A21 H2		U30 H3 – A21 H2
U31 H3	U31 H3 – U30 H3	U31 H3	U31 H3 – U30 H3	U31 H3	U31 H3 – U30 H3
	U31 H3 – U22 H3		U31 H3 – U22 H3		U31 H3 – U22 H3
	U31 H3 – U32 H3		U31 H3 – U32 H3		U31 H3 – U32 H3
	U31 H3 – A20 H2		U31 H3 – A20 H2		U31 H3 – A20 H2
	U31 H3 – A21 H2		U31 H3 – A21 H2		U31 H3 – A21 H2
U32 H3	U32 H3 – G33 H1	U32 H3	U32 H3 – G33 H1	U32 H3	U32 H3 – G33 H1
	U32 H3 – U31 H3		U32 H3 – U31 H3		U32 H3 – U31 H3
	U32 H3 – A20 H2		U32 H3 – A20 H2		U32 H3 – A20 H2
	U32 H3 – A21 H2		U32 H3 – A21 H2		U32 H3 – A21 H2
G33 H1	G33 H1 – U18 H3	G33 H1	G33 H1 – U18 H3	G33 H1	G33 H1 – U18 H3
	G33 H1 – U32 H3		G33 H1 – U32 H3		G33 H1 – U32 H3
	G33 H1 – A20 H2		G33 H1 – A20 H2		G33 H1 – A20 H2
	G33 H1 – A34 H2		G33 H1 – A34 H2		G33 H1 – A34 H2
G35 H1	G35 H1 – G36 H1	G35 H1	G35 H1 – G36 H1	G35 H1	G35 H1 – G36 H1
	G35 H1 – U18 H3		G35 H1 – U18 H3		G35 H1 – U18 H3
	G35 H1 – A34 H2		G35 H1 – A34 H2		G35 H1 – A34 H2
G36 H1	G36 H1 – G35 H1	G36 H1	G36 H1 – G35 H1	G36 H1	G36 H1 – G35 H1
	G36 H1 – G37 H1		G36 H1 – G37 H1		G36 H1 – G37 H1
G37 H1	G37 H1 – U13 H3	G37 H1	G37 H1 – U13 H3	G37 H1	G37 H1 – U13 H3
	G37 H1 – G36 H1		G37 H1 – G36 H1		G37 H1 – G36 H1
	G37 H1 – U38 H3		G37 H1 – A43 H2		G37 H1 – U38 H3
	G37 H1 – A43 H2				G37 H1 – A43 H2

U38 H3	U38 H3 – Ade H9	U38 H3	Imino proton not detected	U38 H3	U38 H3 – Gua H9
	U38 H3 – G37 H1				U38 H3 – G37 H1
	U38 H3 – U13 H3				U38 H3 – U13 H3
	U38 H3 – U42 H3				U38 H3 – U42 H3
	U38 H3 – A43 H2				U38 H3 – A43 H2
G39 H1	Imino proton not detected	G39 H1	G39 H1 – U42 H3	G39 H1	Imino proton not detected
			G39 H1 – A12 H2		
U40 H3	U40 H3 – G10 H1	U40 H3	Imino proton not detected	U40 H3	
	U40 H3 – U11 H3				U40 H3 – U11 H3
	U40 H3 – A67 H2				U40 H3 – A67 H2
U42 H3	U42 H3 – Ade H9	U42 H3	U42 H3 – G39 H1	U42 H3	U42 H3 – Gua H1
	U42 H3 – Ade H2				U42 H3 – Gua H9
	U42 H3 – U38 H3				U42 H3 – U38 H3
	U42 H3 – U65 H3		U42 H3 – A43 H2		
	U42 H3 – U13 H3				U42 H3 – U13 H3
	U42 H3 – A43 H2				U42 H3 – A43 H2
G47 H1	G47 H1 – G48 H1	G47 H1	G47 H1 – G48 H1	G47 H1	G47 H1 – G48 H1
	G47 H1 – U62 H3		G47 H1 – U62 H3		G47 H1 – U62 H3
	G47 H1 – A46 H2		G47 H1 – A46 H2		G47 H1 – A46 H2
G48 H1	G48 H1 – G47 H1	G48 H1	G48 H1 – G47 H1	G48 H1	G48 H1 – G47 H1
	G48 H1 – U59 H3		G48 H1 – U59 H3		
	G48 H1 – A49 H2		G48 H1 – A49 H2		G48 H1 – A49 H2
U59 H3	U59 H3 – A49 H2	U59 H3	U59 H3 – A49 H2	U59 H3	
U62 H3	U62 H3 – G63 H1	U62 H3	U62 H3 – G63 H1	U62 H3	U62 H3 – G63 H1
	U62 H3 – G47 H1		U62 H3 – G47 H1		U62 H3 – G47 H1
	U62 H3 – A46 H2		U62 H3 – A46 H2		U62 H3 – A46 H2
G63 H1	G63 H1 – U62 H3	G63 H1	G63 H1 – U62 H3	G63 H1	

	G63 H1 – A46 H2		G63 H1 – A46 H2		G63 H1 – A46 H2
U65 H3	U65 H3 – Ade H2	C65	No imino proton	C65	No imino proton
	U65 H3 – U42 H3				
U66 H3	U66 H3 – Ade H2			U66 H3	U66 H3 – Gua H1
	U66 H3 – U11 H3		U66 H3 – U11 H3		U66 H3 – U11 H3
	U66 H3 – U65 H3				
	U66 H3 – A12 H2		U66 H3 – A12 H2		U66 H3 – A12 H2
	U66 H3 – A67 H2		U66 H3 – A67 H2		U66 H3 – A67 H2
U69 H3	U69 H3 – G10 H1		U69 H3 – G10 H1	U69 H3	U69 H3 – G10 H1
	U69 H3 – G8 H1		U69 H3 – G8 H1		U69 H3 – G8 H1
	U69 H3 – A9 H2		U69 H3 – A9 H2		U69 H3 – A9 H2
G71 H1	G71 H1 – G8 H1		G71 H1 – G8 H1	G71 H1	G71 H1 – G8 H1

a. NOEs were obtained from 3D ^{15}N-edited NOESY-HSQC spectra collected at 15 ˚C. Only NOEs involving two imino protons, an adenine H2 and an imino proton, and the purine ligand H9 and an imino proton were analyzed. The wild-type adenine aptamer was prepared in the presence of adenine and the U65C mutant was prepared in the absence (no ligand) or the presence of guanine as described in Materials and Methods.