Mechanistic insights into an engineered riboswitch: A switching element which confers riboswitch activity

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Supporting Information

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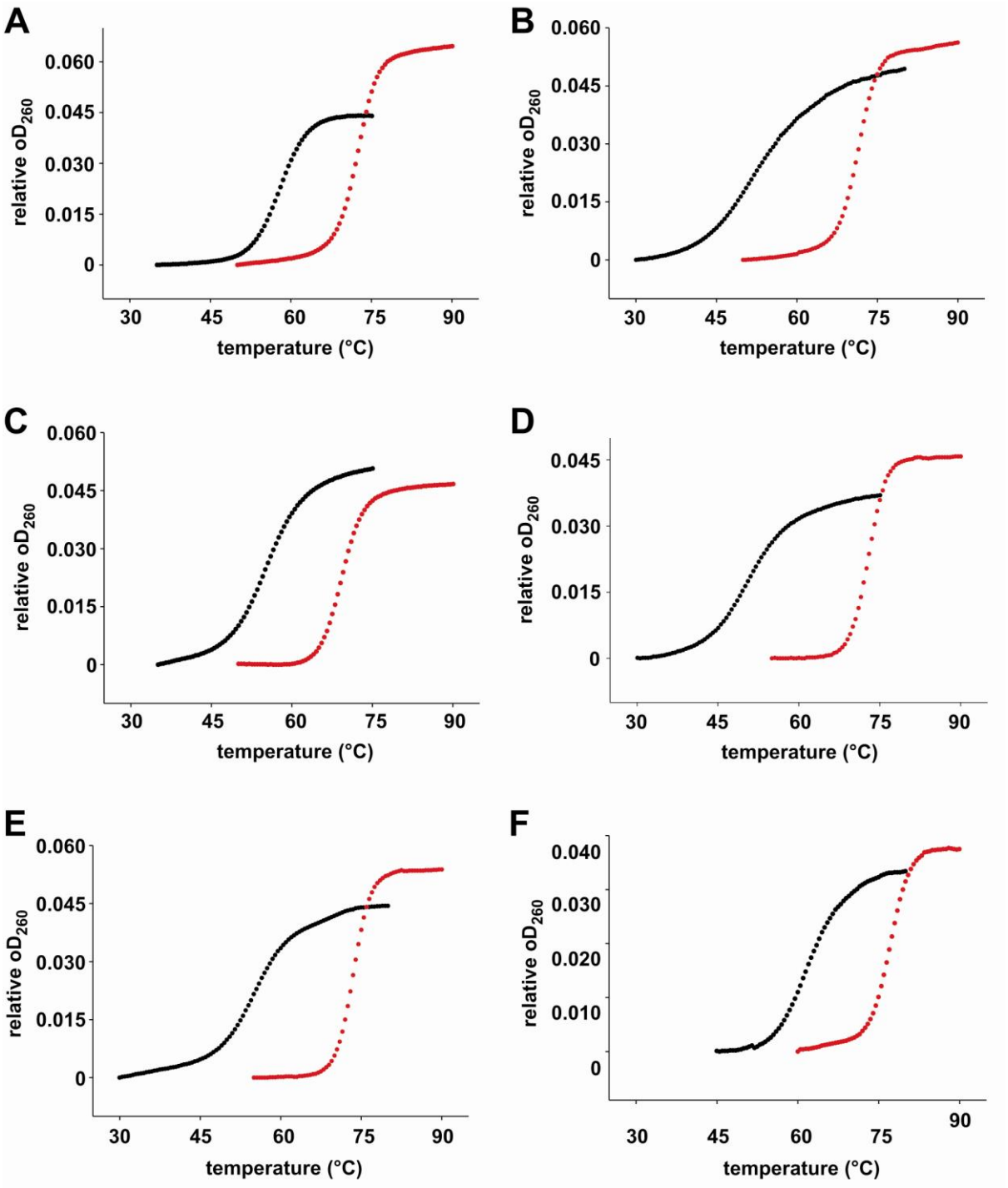
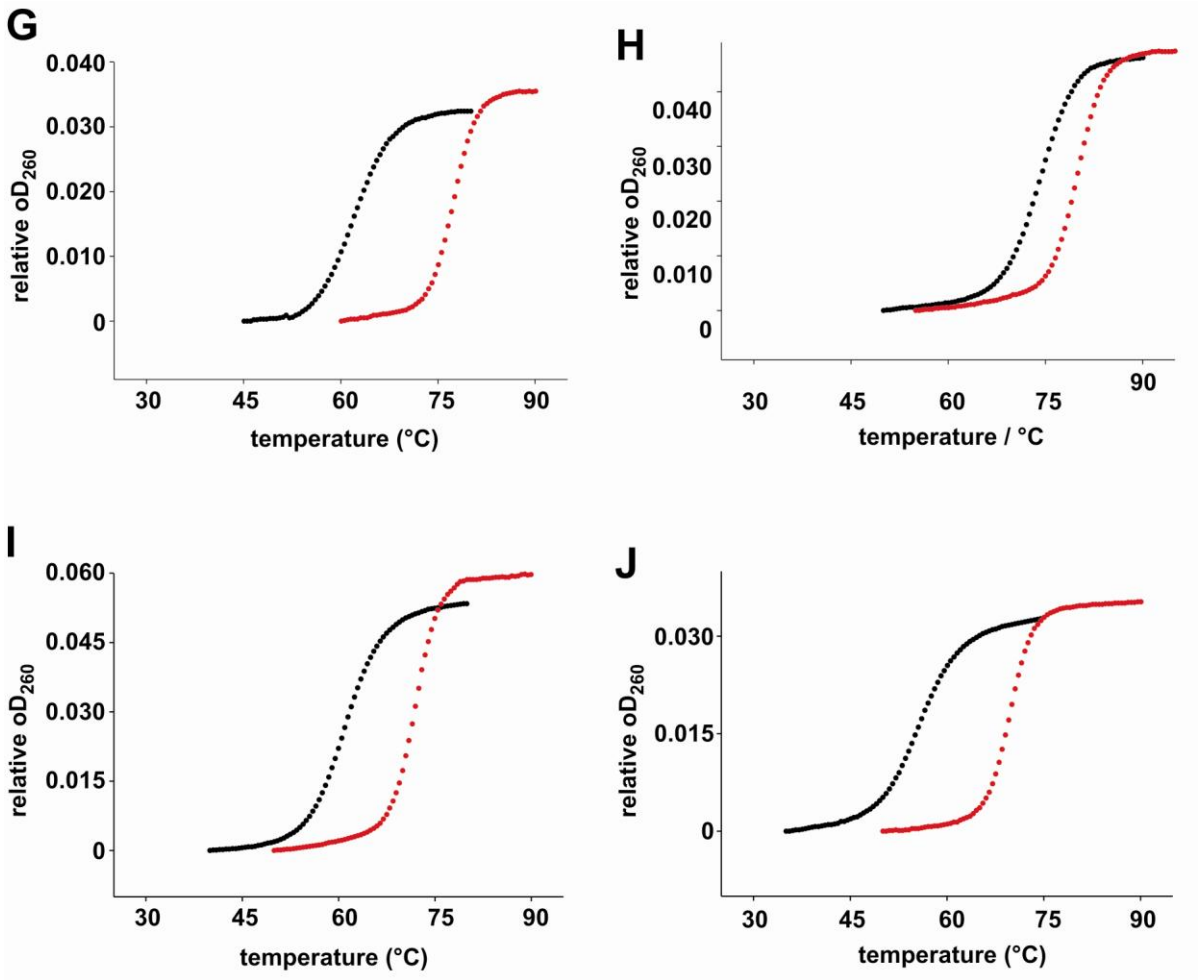
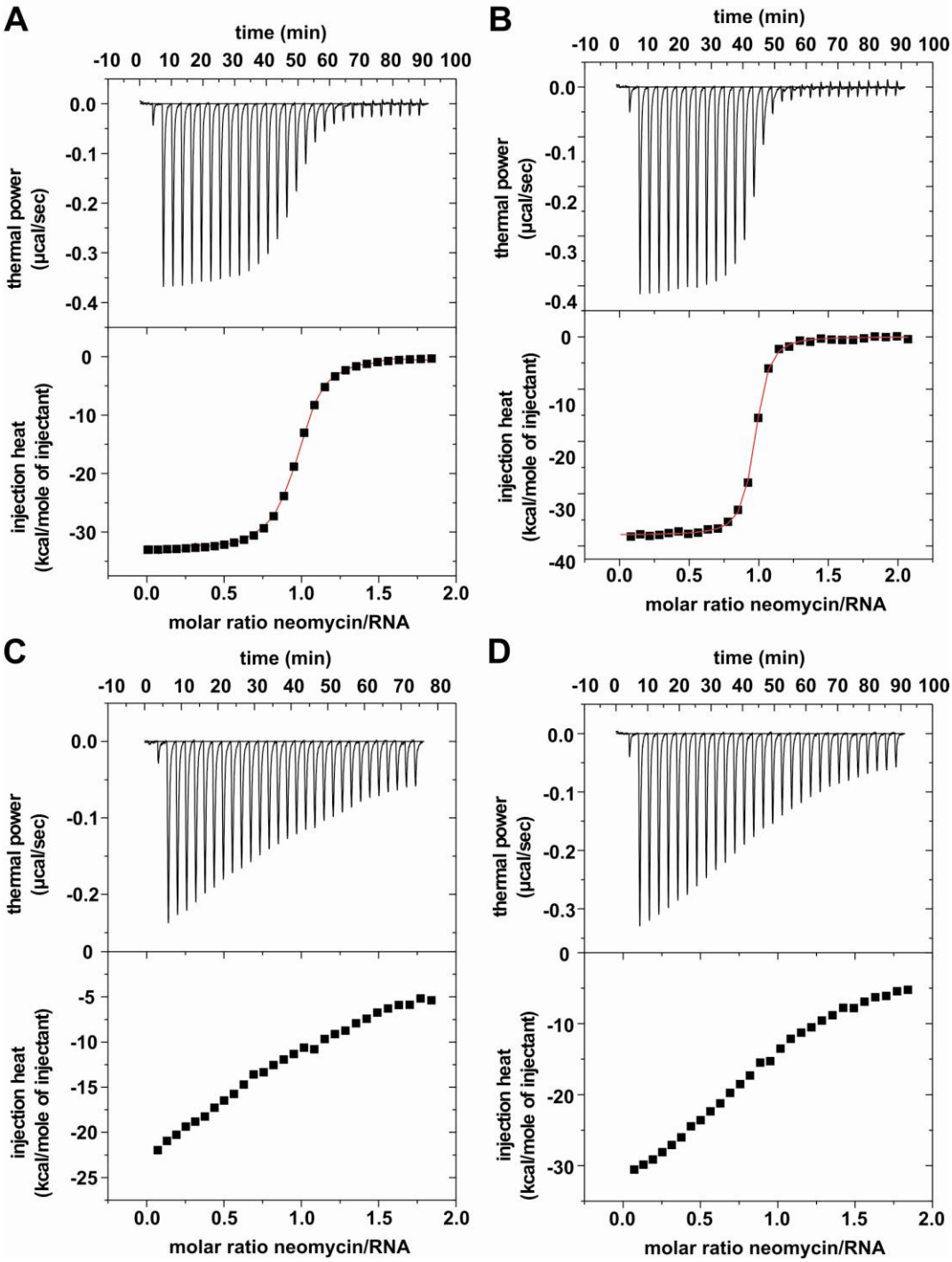
Supplementary Figure 1





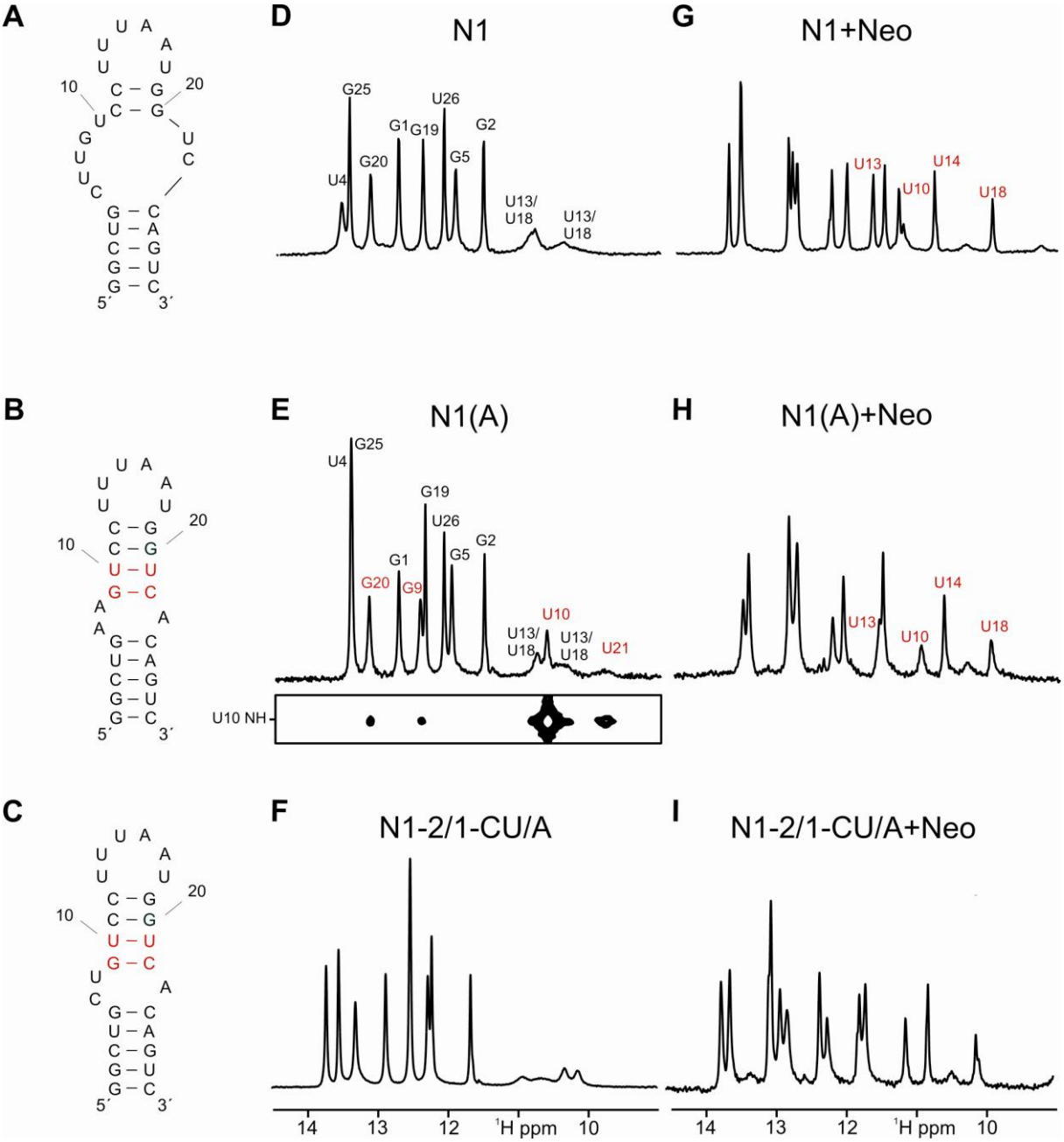
Supplementary Figure 1. Melting analysis of different neomycin binding aptamers. Melting analysis in the presence (red curves) and absence (black curves) of 10 µM neomycin for 1 µM R23 (**A**), N1(R23) (**B**), N1(A) (**C**), N1-3/0-CUC (**D**), N1-2/0-CU (**E**), N1-1/0-C (**F**), N1-1/0-U (**G**), N1-0/0 (**H**), N1-2/1-CU/A (**I**) and N1-3/1-CUU/A (**J**).

Supplementary Figure 2



Supplementary Figure 2. ITC measurements for different neomycin binding aptamers. Upper panel: power required to maintain the temperature of the RNA solution (37°C) recorded over the time of multiple injections (10 µl) of ligand (38 µM neomycin) until saturation was reached (baseline-corrected). Lower panels: integrated heats of interaction per mole of injectant plotted against the molar ratio of ligand over RNA. For R23 and N1(R23) fitted to a single binding site model. (**A**) 4.5 µM R23. **(B**) 4 µM N1(R23). (**C**) 4.5 µM N1(A). (**D**) 4.5 µM N1-2/1-CU/A.

Supplementary Figure 3



Supplementary Figure 3. Comparison of the conformation and neomycin binding of N1, N1(A) and N1-2/1-CU/A in the free and neomycin bound form. (**A-C**) Proposed secondary structure of the free form of N1, N1(A) and N1-2/1-CU/A based on the number of observable imino proton signals and NOE-patterns. The two additional base pairs in the upper helix of N1(A) and N1-2/1-CU/A are highlighted in red. (**D-I**) Comparison of the imino proton region of 1D-^{1}H-spectra of N1, N1(A) and N1-2/1-CU/A in the absence (**D-F**) and in the presence (**G-I**) of one equivalent of neomycin. Signal

assignments are indicated. Novel signals and chemical shift changes are observed as expected due to the formation of a stable 1:1 neomycin RNA-complex in slow exchange on the NMR-time scale. For N1(A) tentative assignments for the signals of U10, U13, U14 and U18 whose chemical shifts are indicative of a ligand binding mode similar to N1 are given in red. (**E** bottom) Section from a 2D-^{1}H,^{1}H-NOESY spectrum recorded for N1(A) including the diagonal signal of the U10 imino proton. This imino proton signal displays a strong cross peak to a second uridine imino proton at 9.8 ppm in agreement with the presence of the U10:U21 base pair and two weaker sequential NOEs to imino proton signals with chemical shifts typical for imino protons in Watson-Crick base pairs. These can be assigned to the imino protons of G9 and G20 (marked in red).

Supplementary Table 1. Summary of the *in vivo* screening of the N1 pools.

N1 Pool	Theoretical number of sequences	Primary transformants in *E. coli*	Pool coverage	Number screened
N1-3/0	64	247	3x	192
N1-2/0	16	418	12x	192
N1-2/1	64	228	3x	192
N1-3/1	256	114	0.4x	192

Pool coverage is given as the ratio of the number of colonies screened divided by the theoretical number of sequences.

Supplementary Table 2. Activity of different neomycin binding aptamers.

Aptamer	Relative fluorescence (%) without neomycin[1]	Relative fluorescence (%) 100 µM neomycin[1]	Regulatory factor[2]
N1-4/0-CUCC	46.9	28.2	1.7
N1-3/0-CCU	45.4	15.3	3.0
N1-3/0-CAU	46.7	18.0	2.6
N1-3/0-UUU	44.9	17.8	2.5
N1-3/0-CCC	60.9	25.0	2.4
N1-3/0-UCU	46.3	20.8	2.2
N1-3/0-CGG	18.9	9.4	2.0
N1-3/0-CCA	47.5	24.4	1.9
N1-3/0-CGC	38.8	20.2	1.9
N1-3/0-UUA	47.4	28.4	1.7
N1-3/0-UCG	20.6	12.4	1.7
N1-3/0-UAG	26.5	16.2	1.6
N1-3/0-AUC	47.6	29.7	1.6
N1-3/0-AUA	52.6	36.3	1.4
N1-2/0-UU	45.0	16.4	2.7
N1-2/0-CA	34.6	14.4	2.4
N1-2/0-AC	44.1	19.4	2.3
N1-2/0-UA	43.8	22.2	2.0
N1-2/0-AA	47.8	26.7	1.8

N1-2/1-GC/U	45.6	38.7	1.2
N1-2/1-AC/C	65.5	64.8	1.0
N1-2/1-UA/C	66.7	65.5	1.0
N1-2/1-UU/C	67.7	57.0	1.2
N1-2/1-CC/C	91.4	92.9	1.0
N1-2/1-CA/A	62.1	59.8	1.0
N1-3/1-GCC/C	20.5	3.8	5.4 [3]
N1-3/1-GUC/U	61.1	38.3	1.6
N1-3/1-GGA/U	19.1	16.9	1.1
N1-3/1-CAU/C	79.5	59.7	1.3
N1-3/1-GAG/A	50.0	47.0	1.1
N1-3/1-UUC/G	25.3	25.5	1.0
N1-3/1-UUA/A	59.2	54.2	1.1
N1-3/1-CCA/A	55.9	56.1	1.0

[1] *gfp* expression in the absence and presence of 100 µM neomycin. The fluorescence emission of the vector pWHE601 expressing *gfp* without an aptamer in its 5'UTR was set as 100%. Background level of a vector with no *gfp* expression was subtracted from all data. Values are the mean of three independently grown cultures with standard deviation below 10%. Measurements were repeated at least twice.

[2] Efficiency of regulation is given as the ratio of relative fluorescence with and without neomycin.

[3] The GCC/C sequence of the aptamer N1-3/1-GCC/C leads to the formation of an additional GC base pair and hence the formation of a six base pair long closing stem. This leads to an increase in regulation (1).

Supplementary reference

1. Weigand,J.E., Sanchez,M., Gunnesch,E.B., Zeiher,S., Schroeder,R. and Suess,B. (2008) Screening for engineered neomycin riboswitches that control translation initiation. *RNA*, **14**, 89-97.