

Table 1 Binding rate constants and affinities for wild-type and mutant c-di-GMP riboswitches

RNA	k_{off} (min^{-1})	$t_{1/2}^{\text{a}}$ (min)	k_{on} ($\text{M}^{-1} \text{min}^{-1}$)	K_{d} values (nM)		
				c-di-GMP, calculated ^c	c-di-GMP, measured	c-di-AMP, measured
110 Vc2	$1.1 \pm 0.082 \times 10^{-5}$	6.3×10^4	$1.0^{\text{b}} \pm 0.016 \times 10^6$	0.011	– ^d	n.d. ^e
C92U	$1.0 \pm 0.16 \times 10^{-2}$	69	$8.0 \pm 0.99 \times 10^5$	13	15 ± 1.1	n.d.
G20A, C92U	–	–	–	–	$4,900 \pm 960$	$1,200 \pm 130$
G20C, C92U	–	–	–	–	n.d. ^f	n.d.
G20U, C92U	–	–	–	–	n.d.	n.d.

^a $t_{1/2}$ is the half-life of the complex and was calculated from the dissociation rate. ^bThe on-rate data for 110 Vc2 was biphasic. This rate was calculated from the rate corresponding to 80% of the amplitude. ^cThe calculated K_{d} results from taking the ratio of the off-rate and the on-rate of ligand binding. ^dA dash means that the measurement was not attempted. ^en.d. means no binding was detected up to 100 μM ligand. ^fA small smear was observed at 100 μM RNA indicating some binding, but the K_{d} is not measurable with this assay. Reported values are the average of at least three trials \pm s.d.