Supplementary Information

Magnesium controls aptamer-expression platform switching
in the SAM-I riboswitch

Minimal model construction for full length SAM-I ribswitch:

Crystal structures of the aptamer domain of *Thermoanaerobacter tengcongensis*, both in its SAM-bound (pdb: 2GIS) and SAM-unbound (3IQP) forms are available (1,2). In addition, early small angle X-ray scattering (SAXS) experiment found a local difference in the binding site between these SAM-bound and SAM-unbound forms (2). In the absence of SAM, A46 base forms a base-triple with A45 and U57. In the SAM-bound form, the adenosyl moiety of SAM forms the base-triple with A45 and U57 replacing U46. The flipped away A46 in SAM-bound situation is shown in Supplementary Figure S1 that is included in our dual-basin model to distinguish the aptamer binding site in the presence and in the absence of ligand. As crystal structure of only aptamer domain is only known, for our theoretical model construction, we have essentially taken 2GIS pdb sequence and its three dimensional structural information, as our stable part of the aptamer domain, although 2GIS crystal structure has some missing part at helix P3. To correlate with our experiment, we have followed our earlier sequence information to construct the flexible part of aptamer domain which is more detailed(3). However, we have calculated folding free energy landscape for both, 2GIS and our experimental sequence construct of the aptamer domain. Both show similar folding temperature and folding landscape. This stability check has indeed given us much confidence about the stability of our designed construct. In order to improve primer extension reads, in our experiments, the aptamer sequence used includes sequence additions to the 5’ and 3’ (before and after P1 helix). Therefore, our experimental sequence numbering differs a little in comparison to our designed full-length SAM-I construct. The expression platform for both transcription OFF and transcription ON states are built using MacroMolecule Builder (MMB) software package, earlier known as RNA-builder (4), consistently following our early experimental base-pairing information.
Figure S1: SAM-binding site conformation in SAM-free and SAM-bound state.

(a) In SAM-free state, A45, A46 and U57 form a base triple. (b) In SAM-bound state adenosyl moiety of SAM forms base triple with A45 and U57. A46 flips away. **Methods:**

Potential for single and dual-basin structure-based generalized electrostatic model (GEM) simulation:

In this work, we have developed a dual basin structure based potential \( H_{DB-SBM} \) of RNA to investigate the transition between the transcription ON and the transcription OFF state. In order to investigate the folding energy landscapes of separated aptamer domain and anti-terminator helix at different buffer condition, we have used our previously developed single basin potential, \( H_{SBM} \). Here, we have implemented our previously developed ion interaction potential (5) within our dual basin structured based model(5)(5)(5). The functional form of the Hamiltonian used for our structure-based generalized electrostatic model (GEM) simulations consists of three terms as shown in Eq. S1:

\[
H = H_{SBM or DB-SBM} + H_{Excl} + H_{Elec}
\]

(Eq.S1)
Dual and Single Basin Structure Based Potential, $H_{SBM\ or\ DB-SBM}$:

$H_{SBM\ or\ DB-SBM}$ includes harmonic potentials that restraints bonds ($r$), angles ($\theta$), and improper/planar dihedrals ($\chi$). Proper/flexible dihedral angles ($\phi$) are treated with a cosine term, as shown in Eq. S4. The initial geometric parameters ($r_0^i$, $\theta_0^i$, $\chi_0^i$, $\phi_0^i$, $\chi_0^i$) are obtained from the crystal structure. Following our previous setup of SBM potential for proteins, the ratio of total contact energy to total dihedral energy is maintained, as $\sum_{i}^{contacts} k_c / \sum_{j}^{proper\ dihedral} k_\phi = 2$ (6,7). All the interaction coefficients used in this potential are given in Table 1. The difference between single basin and dual basin Hamiltonian is enclosed in the pairwise contact potential term, $C_{SBM\ DB-SBM}(r_{ij}, r_{ij}^0)$.

$$H_{SBM\ DB-SBM} = \sum_{i}^{bonds} \frac{k_c}{2} (r_i - r_i^0)^2 + \sum_{i}^{angles} \frac{k_\theta}{2} (\theta_i - \theta_i^0)^2 + \sum_{i}^{improper\ or\ planar\ dihedrals} \frac{k_\chi}{2} (\chi_i - \chi_i^0)^2 + \sum_{i}^{proper\ dihedrals} k_\phi F_D(\phi_i - \phi_i^0) + \sum_{ij}^{contacts} C_{SBM\ DB-SBM}(r_{ij}, r_{ij}^0) + \sum_{ij}^{non-contacts} k_{NC} \left( \frac{\sigma_{ij}^{NC}}{r_{ij}} \right)^{12} \quad (Eq\ S2)$$

Where, $F_D(\phi) = [1 - \cos(\phi)] + \frac{1}{2} [1 - \cos(3\phi)] \quad (Eq. S3)$

Contact Potential:

In single basin SBM, following our previous treatment, we have used Lennard Jones' 6-12 potential (8,9) to treat the pair-interactions present in the native crystal structure while all other non-native pair-interactions present are repulsive.

$$C_{SBM}(r_{ij}, r_{ij}^0) = k_c \left( \frac{r_{ij}^0}{r_{ij}} \right)^{12} - 2 \left( \frac{r_{ij}^0}{r_{ij}} \right)^6 \quad (Eq. S4)$$

Here, in dual basin RNA model, we have essentially two kinds of contacts: contacts those are unique in the transcription ON state, unique in the transcription OFF state, and those are shared by these two structures. To treat these unique contacts, we used an attractive Gaussian well coupled with a fixed hard wall-excluded volume which is rather user-friendly than the previous LJ contact potential as it separates
the effect of excluded volume from the potential minimum. The shared contacts are treated with a dual 
basin Gaussian potential.

\[
\begin{align*}
C_{DB-SBM}(r_{ij}, r_{ONij}, r_{OFFij}) &= \left\{ \begin{array}{l}
\varepsilon_{ON} \sum_{ij}^{\text{unique transON contacts}} \left[ 1 + \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} \right] \left[ 1 + G(r_{ij}, r_{ONij}) \right] - 1 \\
+ \varepsilon_{OFF} \sum_{ij}^{\text{unique transOFF contacts}} \left[ 1 + \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} \right] \left[ 1 + G(r_{ij}, r_{OFFij}) \right] - 1 \\
+ \varepsilon_{S} \sum_{ij}^{\text{shared contacts}} \left[ 1 + \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} \right] \left[ 1 + G(r_{ij}, r_{ONij}) \right] \left[ 1 + G(r_{ij}, r_{OFFij}) \right] - 1
\end{array} \right.
\end{align*}
\]

(Eq. S5)

where, 

\[
G(r_{ij}, r_{ij}^{0}) = -\exp \left[ \frac{-(r_{ij} - r_{ij}^{0})^2}{2\sigma_{G}^2} \right]
\]

(Eq. S6)

The Gaussian treatment for both unique and shared contacts fixes their minima at -1. The width of the
Gaussian, \( \sigma_{G} \), is set as the width of the 6-12 LJ potential such that 

\[
G(r_{ij} = 1.2 \cdot r_{ij}^{0}) = 0.5
\]

Excluded volume effect of explicit hexa-hydrated \( \text{Mg}^{2+} \), \( H_{Excl} \):

We have included the excluded volume effect for the explicit hexa-hydrated \( \text{Mg}^{2+} \) ions(10), \( H_{Excl} \) as a form of potential, as shown in Eq. S7:

\[
H_{Excl} = \sum_{ij}^{\text{Mg-RNA}} \varepsilon_{ij} \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12}
\]

(Eq. S7)
Electrostatic effects of explicit Mg$^{2+}$ ions and implicit KCl, $H_{Elec}$:

The electrostatic ion potential, $H_{Elec}$ in Eq. S1 consists of four free energy terms: 

$$ (G_E + G_{Mx} + G_{Hhe} + G_{Rec}) $$

As we stated earlier, in our GEM simulations, Mg$^{2+}$ ions are treated explicitly and buffer KCl is treated implicitly. Here electrostatic interactions of any explicit ions (RNA and Mg$^{2+}$ ions) are dealt with Debye-Hückel (DH) theory (11,12). Implicit KCl is dealt with generalized Manning counter-ion condensation theory(5). Although details of generalized Manning counter-ion condensation theory can be found elsewhere(5), here we will briefly summarize few primary equations which essentially describe our electrostatic potential.

Similar as classical Manning counter-ion condensation theory (13), our previously developed generalized Manning condensation model is a competition between the electrostatic energy and the mixing free energy which determine the counter-ion condensation (13,14) effect. In generalized Manning condensation model we have taken into account the effect of Debye-Hückel screening charges, in addition to the implicit condensed charges. The removal of redundant screening charges from the excluded volume of RNA has extended its applicability beyond regular polyelectrolytes to any irregular shaped polyionic systems. To model the heterogeneous charge distribution, three types of charge distribution have been included: (i) a Gaussian mixing charge ($P(r, \sigma_\mu)$) to address the mixing free energy, (ii) a Gaussian hole charge ($P(r, \sigma_\eta$)) to estimate the implicit charges that needs to be subtracted from the excluded volume of RNA, and (iii) a point charge ($\sigma_0$) for each particle (5).

Screening charges are accounted only in an effective Gaussian shell volume, 

$$ (V_{\mu,i} - V_{\eta,i}) = V_{eff,i}. $$

Therefore, the form of electrostatic free energy we used in our potential is given in Eq. S8:

$$ G_E = \frac{1}{2} \sum_{ij} \sum_{xy} q_{x,i} q_{y,j} \phi(r_{ij}, (\sigma_x^2 + \sigma_y^2)^{1/2}) + \frac{1}{2} \sum_{i} \sum_{s} z_{i,s} c_{s} \left( 1 - \frac{z_{i,s} \Phi_{\mu}(r_{i})}{k_B T} \right) V_{eff,i} \Phi_{\mu}(r_{i}), \quad \text{(Eq. S8)} $$

where, $\Phi_{\mu}(r) = \sum_{j} \sum_{n} q_{j,n} \phi((r - r_{j}), (\sigma_x^2 + \sigma_y^2)^{1/2})$
The indices, $x$ and $y$, are the pair-wise Debye-Hückel interactions ($\phi$) between any of these charge types. $r_{ij}$ is the distance between particles $i$ and $j$. $z_s$ is the charge number and $c_s$ is the concentration of a specific ion, $s$. For particle $i$, the condensed charges are, $\mu_{\text{mix},i} = \sum_s z_s \mu_{is}$ and $\eta_{\text{mix},i} = \sum_s z_s \eta_{is}$.

Mixing free energy within this effective Gaussian shell volume is given in Eq. S9,

$$G_{\text{Mix}} = \sum_i \sum_s k_B T n_{\text{Mix,}is} V_{\text{eff,}ij} \ln \left( \frac{n_{\text{Mix,}is}}{ec_s} \right)$$

(Eq. S9)

where $e$ is Euler's number. The mixing charge density, $n_{\text{Mix,}is}$, used in Eq. S9 includes both Gaussian mixing charges and the screening charges as in (Eq. S10),

$$n_{\text{Mix,}is} = c_s \left( 1 - \frac{z_s \Phi_{\mu}(\tilde{r}_i)}{k_B T} \right) + n_{\mu,i}(\tilde{r}_i)$$

(Eq. S10)

Two other Harmonic potentials $G_{\text{Hole}}$, $G_{\text{Rest}}$ are added to restrain the charges within their restricted volume region and are given in Eq. S11 and Eq. S12, respectively.

$$G_{\text{Hole,}is} = \frac{1}{2} k_{\text{Hole}} \sum_i \sum_s n_{\text{Hole,}is}^2$$

(Eq. S11)

where, $n_{\text{Hole,}is} = c_s \left( 1 - \frac{z_s \Phi_{\mu}(\tilde{r}_i)}{k_B T} \right) + n_{\mu,i}(\tilde{r}_i) + n_{\eta,i}(\tilde{r}_i)$

(Eq. S12)

and,

$$G_{\text{Rest}} = \frac{1}{2} k_w \sum_i \sum_s (\mu_{is} - n_{\mu,i}(\tilde{r}_i) V_{\mu,i})^2 + \frac{1}{2} k_w \sum_i \sum_s (\eta_{is} - n_{\eta,i}(\tilde{r}_i) V_{\eta,i})^2$$

(Eq. S13)

The parameter set we used for current simulation are given in Table S1. The related parameter set and their calibration are also available in our early studies (5,15).
Table S1: Values of parameter set used for current structure-based GEM simulation of full length SAM-1 riboswitch.

| Reduced parameter set |       |
|-----------------------|-------|
| $\tau_R$              | 2 ps  |
| $\mu_R$               | 15 amu|
| $\epsilon_R$          | 3.75 kJ/mol |
| $T_R$                 | 3.75 K |

| SBM potential        |       |
|----------------------|-------|
| $k_r$                | $2 \times 10^4 \epsilon_R/nm^2$ |
| $k_B$                | $40 \epsilon_R/rad^2$ |
| $k_{\chi}$           | $10 \epsilon_R/rad^2$ |
| $\sigma_{ij}^{NC}$   | 0.17 nm |
| $k_{NC}$             | 1.33 k_B T |

| Excluded volume potential |       |
|---------------------------|-------|
| $\sigma_{RNA-Mg^{2+}}$   | 0.34 nm |
| $\sigma_{Mg^{2+}-Mg^{2+}}$| 0.56 nm |

| Electrostatic potential  |       |
|--------------------------|-------|
| $\sigma_{\mu}$           | 0.7 nm |
| $\sigma_{\eta}$          | 0.34 nm |
| $k_{\text{Hole}}$        | $10^4 k_B T/P(0,\sigma_{\eta})$ |
| $k_W$                    | 1 k_B T |

**Equilibrium simulation detail**

Atomic coordinates and condensation variables, $\mu_{is}$ and $\eta_{is}$, are evolved with Langevin dynamics with a time step of 0.001 $\tau_R$. We used underdamped condition for rapid sampling. For explicit particles, reduced mass of $1 \mu_R$ and drag coefficient of $1 \tau_R^{-1}$ are used. Condensation coordinates, $\mu_{is}$ and $\eta_{is}$, are given a mass of $15 \mu_R nm^2$ and a drag coefficient of $0.05 \tau_R^{-1}nm^2$. Temperature was chosen such that both folded and partially unfolded states were accessible at physiological Mg$^{2+}$ concentration ([Mg$^{2+}$]≈2.0 mM) ($T=93T_R$ for separated aptamer and anti-terminator domain and 95 $T_R$ for full length SAM-I)
riboswitch). To prepare different Mg\(^{2+}\) composition we set up a large cubic box of length 75 nm. The number of Mg\(^{2+}\)/SAM molecule included in that box determines the overall concentration of the corresponding solutes. Periodic boundary conditions were applied. Each simulation was propagated for a total of 20 million time-steps and thus 5 set of trajectories were generated to calculate average physical quantities, such as radius of gyration, theoretical SHAPE, etc.

**Charges**

Effective charges were taken for each phosphate group as -1, each magnesium ion as +2 and each SAM molecule has effectively +1 charge. In SAM molecule, a positively charged sulfonium group, a positively charged amino group, and a negatively charged carboxylate group are important for SAM recognition (16).

**Umbrella sampling for free energy calculations and overall transition reaction coordinate:**

For free energy calculations, we used the Umbrella Sampling method(17). It is an efficient technique which helps to surmount the barrier by effectively sampling the near barrier region along the reaction coordinate with the help of an artificial biasing umbrella potential, V. The form of V over the reaction coordinate, N is expressed as, \( V = 0.5k(N - N_0)^2 \), where \( k \) is the harmonic force constant and \( N_0 \) represents reaction coordinate in the initial state. In the present case we find some unique native contacts which distinguish the transcription ON state (unique to transcription ON, \( N_{\text{transON}} \)) from the transcription OFF state (unique to transcription OFF, \( N_{\text{transOFF}} \)) and characterize barrier separated two well-funneled basins for full length SAM-I riboswitch (Figure 3 in main text). To monitor the overall transition progress, we chose a difference of contacts between \( N_{\text{transOFF}} \) and \( N_{\text{transON}} \), while we made sure to sample various configurations corresponding to every possible contact difference number chasing for every possible minimum energy path and let the Mg\(^{2+}\) concentration to decide which path would be energetically dominant. Contact calculation for two native fold is performed using Shadow criterion(6). The dual-basin SBM contact pair list was prepared combining all unique native contacts and shared contacts giving each contact of equal strength(18). Also, for folding free energy calculations, these unique contacts serve as good reaction coordinate. \( N_{\text{transOFF}} \) reaction coordinate characterizes folding free energy for the aptamer domain and \( N_{\text{transON}} \) reaction coordinate characterizes folding free energy for the expression platform (Figure 4 in the main text). While all these contact numbers, (\( N_{\text{transOFF}} \) and \( N_{\text{transON}} \)) are found to be the good reaction coordinates to estimate free energies, the contact counting potential (V) is a step function. However, an umbrella potential must be differentiable. Therefore, we use a continuous function of contact potential which is given as,

\[
V_{\text{contacts}} = \sum_{ij} \frac{1}{2} (1 - \tanh(a_{ij}))
\]

(Eq. S14)
where, \( \alpha_{ij} = \gamma \left( r_{ij} - 1.5 \mu_{ij} \right) \). \( r_{ij} \) is the separation between atoms \( i \) and \( j \), and \( \mu_{ij} \) is the same in the native state. The value of \( V_{\text{contacts}} \) can be used as argument for the harmonic umbrella constraint during sampling.

To begin with the umbrella sampling calculation we generated a series of initial structures at our desired transition coordinate values starting from each native structures (transcription ON and transcription OFF) at 2.0 mM Mg\(^{2+}\) condition. At each umbrella window, we ensure equilibration by reinitializing Mg\(^{2+}\) distributions. Each window ran for 10 million steps. Umbrella sampling has been performed slightly below the folder temperature (0.95\(T_m\)). We find folding temperature of the transcription ON and OFF state is close to each other (~100\(T_R\)). Every repeat used a total of 20 windows along the reaction coordinate, ensuring their substantial overlap in the conformational space. Finally, Weighted Histogram Analysis Method (WHAM) is used to calculate unbiased free energy profile(19). For each umbrella window at each Mg\(^{2+}\) and SAM concentration, four repeats were performed to check the consistency of the results with errors estimated. The error bars for the present study show the standard deviation from the mean, which is given by:

\[
\sum_{j=1}^{4} \frac{(x_j - \langle x \rangle)^2}{(N-1)^2}.
\]

Using this free energy calculation approach we have generated Figure 3(a) and 3(b) where have plotted \( G \) (transOFF - transON). The stability difference between the transcription ON and the transcription OFF states (\( \Delta G_{\text{transOFF-transON}} \)) quantified as a function of [Mg\(^{2+}\)] which shows a non-monotonic [Mg\(^{2+}\)] dependence. Now from thermodynamic equation of states we can write:

\[
\Delta G_{\text{transOFF-transON}} = \Delta H_{\text{transOFF-transON}} - T \Delta S_{\text{transOFF-transON}}.
\]

Where \( \Delta H_{\text{transOFF-transON}} \) is the difference in enthalpy between the OFF and ON states and \( \Delta S_{\text{transOFF-transON}} \) is the difference in entropy of the same. While we have calculated enthalpy from all possible interaction energies present in a given structure, the above equation gives us entropy of corresponding structure at a constant temperature, \( T \).

For the simulations, in the presence of SAM, the binding strength between the metabolite and the riboswitch was slightly tuned to ensure that we can obtain multiple binding-unbinding events. This may slightly lower SAM-induced stabilization of the closed state, although we have obtained substantial SAM-bound stabilization compared to SAM-free closed state. Three representative trajectories along the transcription ON-OFF transition are shown in Figure S5. These trajectories show higher number of
contact formation events in the transcription OFF state signifying their possible stable ligand binding events.

Figure S2: SAM-binding/unbinding contact profile (in the active site) during Umbrella sampling simulation at three different transition-coordinates along transcription ON-OFF conformational transition: (a) at N=200, towards transcription OFF state, (b) at N=5, intermediate level close to transition barrier, (c) at N=-200, towards transcription ON state. During enhanced sampling by umbrella sampling method, at
each restrained transition SAM ligand can bind and unbind which restore the equilibrium condition and
allow us measure right thermodynamics. Few steps of contact progression are only shown. Note that
larger number of SAM-RNA contact formation events gives rise to SAM-induced stabilization of the
transcription OFF state in the presence of SAM (as shown in Figure 3(a)) compared to that of
transcription ON state.
Figure S3: Contact profile of aptamer domain and anti-terminator platform along transcription ON-OFF conformational transition, in the absence and presence of SAM

The progress of aptamer domain growth and anti-terminator platform cleavage or vice versa as a function of the overall transition coordinate ($N_{\text{transOFF}} - N_{\text{transON}}$), at three different Mg$^{2+}$ concentration (a) 0.1 mM, (b) 2.0 mM, (iii) 10.0 mM, in the absence of SAM. Similar calculation but in the presence of 0.4 mM SAM at three different Mg$^{2+}$ concentration (a) 0.1 mM, (b) 2.0 mM, (iii) 10.0 mM. At 0.1mM Mg$^{2+}$ concentration, a sequential route is followed by the system, both in the presence and absence of SAM. As we increase [Mg$^{2+}$] up to 2.0 mM, a route bifurcation is observed close to transition midpoint. At 10.0 mM Mg$^{2+}$, we observe the system following a preferential cooperative route.

Figure S4: Mg$^{2+}$ dependence of transcription ON-OFF conformational transition of SAM-I riboswitch, in the absence and presence of SAM
(a) Two dimensional free energy landscape of transcription ON-OFF transition as a function transcription ON coordinate ($N_{\text{transON}}$) and transcription OFF state ($N_{\text{transOFF}}$) at four different buffer conditions: (a) 2.0 mM Mg$^{2+}$, (-) SAM, (b) 10.0 mM Mg$^{2+}$, (-) SAM, (c) 2.0 mM Mg$^{2+}$, (+) 0.4 mM SAM, (d) 10.0 Mg$^{2+}$, (+) 0.4 mM SAM. At 2.0 mM Mg$^{2+}$, the landscape explores two distinct possible pathways: a sequential path and a cooperative path, both in the presence and absence of SAM. At higher concentration ([Mg$^{2+}$]=10.0 mM), it predominantly follows a cooperative pathway where Mg$^{2+}$ induced aptamer-anti-terminator domains coexist. Here free energies scales are normalized for comparison. For colorbar comparison, Z-axis (free energy scale) is normalized by maximum free energy value as appeared at different buffer condition. Solid line represents dominant pathway and dotted line represents the alternate path.
**Figure S5:** Assessing the prevalence of magnesium ions in the ion-atmosphere of full-length SAM-I riboswitch for its different folds and at different magnesium concentrations.

(a) Radial distribution function magnesium around the phosphate groups of RNA. The boundary of 1st ion solvation layer in g(r) is highlighted by blue dotted line. (b) Calculation of average number of magnesium ions in the 1st ion solvation layer of RNA. Magnesium ions in the ion-solvation layer are categorized into two groups depending on their coordination with the
backbone phosphate groups of RNA: Single phosphate coordinated magnesium and multiple phosphate coordinated magnesium. (c) Calculation of average number of single phosphate coordinated magnesium as a function $[\text{Mg}^{2+}]$. (d) Calculation of average number of multiple phosphate coordinated magnesium as a function $[\text{Mg}^{2+}]$. It is evident that the transcription OFF state accommodates more number of multiple phosphate coordinated magnesium ion as compared to the same for the transcription ON state.

**Role of Site Specific Chelated Magnesium Ions:** There are two chelated magnesium ions spotted in the crystal structure of the aptamer domain (2gis.pdb) (20). The central chelated magnesium ion is coordinated with the phosphate group of residue A10 and U64. The 2nd chelated magnesium ion is coordinated with the phosphate group of residue A84 and A65. These residues are connected via magnesium ions those offer additional stability to the RNA fold. As the switching transition itself is a complex process, in order to avoid further complexity of the process, in the present model we have considered the implicit presence of these two inner sphere chelated magnesium ion. The implicit effect is embedded in terms of a number of direct all-atom nucleic acid contacts those are otherwise magnesium mediated. In our previous explicit solvent simulation we have observed that these site-specific chelated ions stays there throughout our ten 2μs simulations indicating its substantially high residence time, possibly much higher than the timescale of switching (21). In addition, as these chelated ions are not involved in any tertiary interactions associated with this switching transition, we believe that this assumption wouldn't affect our results. However, as very little is known about such strong magnesium chelation interactions and about its timescale, our work on this direction is under progress.
Figure S6: The presence of two site-specific chelated magnesium ions as spotted in the aptamer crystal structure (2gis.pdb).

(a) The central chelated magnesium ion mediates two phosphate groups of residue A10 and U64. The 2nd chelated magnesium ion mediates another two phosphate groups of residue A84 and A65. (b) In structure-based model if we delete those two site-specific magnesium ions, a number of additional contacts (highlighted by blue circle) those were otherwise screened by magnesium, mimic the explicit presence of chelated magnesium ion incurring the addition folding stability to the specific fold of an RNA.
Figure S7: Mg$^{2+}$ induced collapse transition of separated anti-terminator platform.

Average radius of gyration (Rg) obtained from equilibrium simulations of separated anti-terminator platform. Fitting into Hill equation allows us calculate transition midpoint, [Mg$^{2+}$]$^{1/2}$, which appears around 3.15 mM. This signifies that the anti-terminator helix requires more magnesium to stabilize its structural fold than that of flexible aptamer.

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