Supplementary Material

Atomistic basis for the on-off signaling mechanism in SAM-II riboswitch

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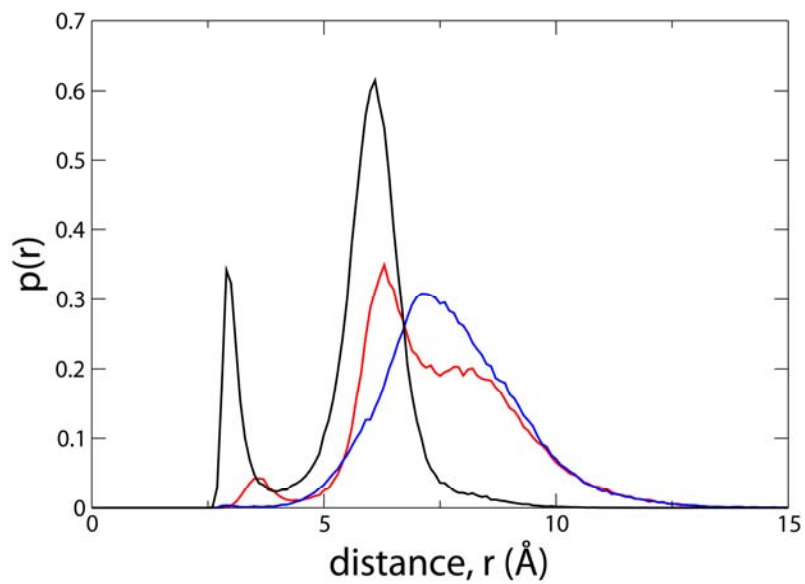Georgia State University, Atlanta, Georgia 30302-4098

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Figure S1. Probability distributions , p(r), of the distances between N3 of U10 and O4 of U44 (black), O4 of U11 and O4 of U21 (red), and O4 of U12 and N6 of A46 (blue) of the unbound form of the SAM-II riboswitch. The colors are consistent with the corresponding interactions shown in Figures 1 and 2.
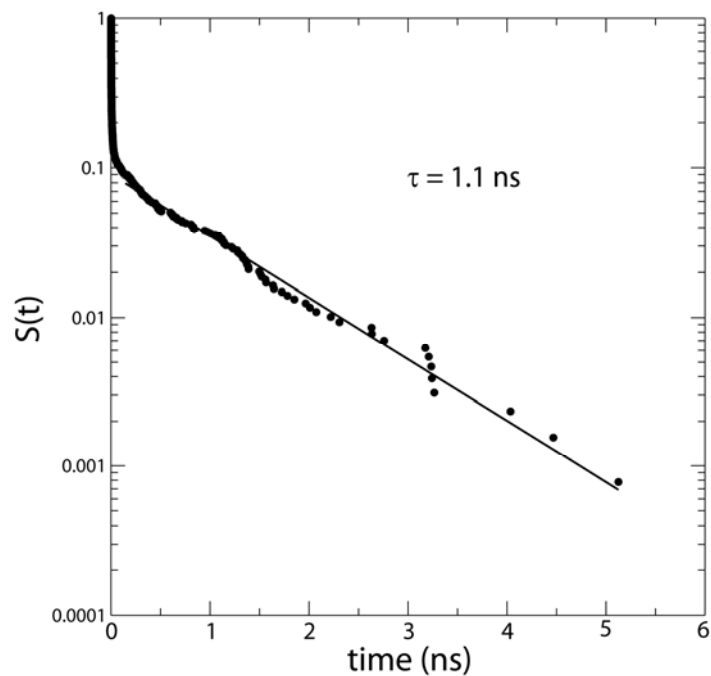
Figure S2. Survival probability, S(t), of forming interaction contacts between L1 and the major groove of the P2b helix necessary to switch the ensemble of conformations of the SAM-II riboswitch from the on state to the off state. The black solid line is a single exponential fit of the data points.

Timescale of switching between the different conformational states of the riboswitch

We have estimated the time it takes to establish any one of the first two contacts that are necessary to drive the ensemble of conformations of the riboswitch from the on state to the off state, as exemplified by the interactions between L1 and the major groove of the P2b helix (Figures 2 and S1). We have assumed a two state model and that the unbound form of the riboswitch samples the ensemble of conformations close to the ligand-bound (off) state when either one of the first two contacts between L1 and P2b is formed. We have calculated the survival probabilities, $S(t)$, of L1 forming these contacts with the major groove of P2, as shown in Figure S2. $S(t)$ is the probability that the contacts will be formed in time t or longer. Figure S2 shows the plot of the survival probabilities versus time that fits nicely to a single exponential. The transition time is estimated to be around 1.1 ns; therefore, switching between the different conformational states of the riboswitch in the unbound form seems to be very fast and in the nanosecond timescale.

Survival probabilities and rate of conformational transitions

The survival probabilities and the rate of going from the loosely formed pseudoknot structure to the ensemble of conformations close to the bound form was measured from the simulations of the unbound form as previously described (1). The time the riboswitch stays in one state was measured based on the interaction of L1 with the major groove of the P2 helix until it switches to the other state. As a result, a time series t_i is generated, where $i= 1, 2 \ldots$ N, and N is the total number of transitions. The survival probability $S(t) = \int_t^{+\infty} p(\tau)dt$ is calculated from the distribution of the escape times $p(\tau)$. $S(t)$ is the

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probability that the conformation will switch at time t or longer. $S(T_i) \approx (i-1)/N$, where

T_i is the sorted time series of t_i.

1.   Hamelberg, D., Shen, T. and Andrew McCammon, J. (2005) Relating kinetic rates and local energetic roughness by accelerated molecular-dynamics simulations. *The Journal of chemical physics*, **122**, 241103.