

# Supplementary Material

## Effect of Ligand binding on Riboswitch Folding: Theory and Simulations

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## A. The solution of GLE under an asymmetric potential

The steps for the conversion of the GLE with an asymmetric double well potential to an effective Fokker-Planck equation for calculating the probability distribution function,  $P(x, t)$ , may be outlined as follows. The asymmetric double well potential in Eqn (2) may be simplified as

$$U(x) = -\frac{k_S}{2}x^2 + 2k_B T \ln \left[ {}_1F_1 \left( \frac{A}{2} + \frac{1}{4}, \frac{1}{2}; \frac{k_S}{2k_B T} x^2 \right) + \mathcal{B} x \sqrt{\frac{k_S}{k_B T}} {}_1F_1 \left( \frac{A}{2} + \frac{3}{4}, \frac{3}{2}; \frac{k_S}{2k_B T} x^2 \right) \right] \quad (\text{S1})$$

Differentiation of Equation (S1) with respect to  $x$ , may be written as

$$\frac{d}{dx}U(x) = -k_S x(t) + \mathcal{W}(x(t)) \quad (\text{S2})$$

where,  $\mathcal{W}(x) = \frac{d}{dx} 2k_B T \ln \left[ {}_1F_1 \left( \frac{A}{2} + \frac{1}{4}, \frac{1}{2}; \frac{k_S}{2k_B T} x^2 \right) + \mathcal{B} x \sqrt{\frac{k_S}{k_B T}} {}_1F_1 \left( \frac{A}{2} + \frac{3}{4}, \frac{3}{2}; \frac{k_S}{2k_B T} x^2 \right) \right]$ .

Substituting the value of  $U'(x)$  from Eqn (S2) in Eqn (1), the form of the overdamped GLE may be written as

$$-k_S x(t) + \mathcal{W}(x(t)) = -m \int_0^t \gamma(t-t') \dot{x}(t') dt' + \xi(t) \quad (\text{S3})$$

Eqn (S3) may be solved via Laplace transform, which after some necessary simplifications may be expressed as

$$\tilde{x}(s) = \frac{m x_0 \tilde{\gamma}(s)}{ms\tilde{\gamma}(s) - k_S} + \frac{\tilde{\xi}(s)}{ms\tilde{\gamma}(s) - k_S} - \frac{\tilde{\mathcal{W}}(x(s))}{ms\tilde{\gamma}(s) - k_S} \quad (\text{S4})$$

where,  $\tilde{\cdot}$  represents the Laplace transform of any function, which is defined as  $\tilde{f}(s) \equiv \mathcal{L}[f(t)]$ .

The function  $\tilde{\mathcal{Z}}(s)$  obtained from Eqn. (S4) may be written as

$$\tilde{\mathcal{Z}}(s) = \frac{m \tilde{\gamma}(s)}{ms\tilde{\gamma}(s) - k_S} \quad (\text{S5})$$

here,  $\tilde{\gamma}(s)$  represents the Laplace transform of the memory kernel. For the power-law noise correlation in Eqn (4), the memory kernel may be calculated from the fluctuation-dissipation theorem as

$$\tilde{\gamma}(s) = \frac{\eta_\beta}{m} s^{\beta-1} \quad (\text{S6})$$

Substituting the value of  $\tilde{\gamma}(s)$  in Eqn (S5) and taking the inverse Laplace transform,  $\mathcal{Z}(t)$  may be represented as

$$\mathcal{Z}(t) = E_{\beta,1} \left( \frac{k_S}{\eta_\beta} t^\beta \right) \quad (\text{S7})$$

where,  $E_{a,b}$  represents the two-parameter Mittag-Leffler function.<sup>1</sup> The inverse Laplace transform of Eqn (S4) with Eqn (S5) may be expressed as

$$\begin{aligned} x(t) = & x_0 \mathcal{Z}(t) - \frac{1}{k_S} \int_0^t \phi(t-t') \xi(t') dt' \\ & + \frac{1}{k_S} \int_0^t \phi(t-t') \mathcal{W}x(t') dt' \end{aligned} \quad (\text{S8})$$

where, the function  $\phi(t)$  is obtained via inverse Laplace transform of the equation, given by

$$\tilde{\phi}(s) = -\frac{k_S}{ms\tilde{\gamma}(s) - k_S} \quad (\text{S9})$$

Expression for  $\dot{x}(t)$  may be derived from Eqn (S8) by differentiating with respect to  $t$ , by eliminating its dependence on the initial reaction coordinate,  $x_0$ , as

$$\begin{aligned} \frac{dx(t)}{dt} = & -\Upsilon(t)x(t) - \frac{1}{k_S} \mathcal{Z}(t) \frac{d}{dt} \frac{1}{\mathcal{Z}(t)} \int_0^t \phi(t-t') \xi(t') dt' \\ & + \frac{1}{k_S} \mathcal{Z}(t) \frac{d}{dt} \frac{1}{\mathcal{Z}(t)} \int_0^t \phi(t-t') \mathcal{W}(x(t')) dt' \end{aligned} \quad (\text{S10})$$

where,  $\Upsilon(t) = -\frac{\dot{\mathcal{Z}}(t)}{\mathcal{Z}(t)}$  and  $\mathcal{Z}(t)$  is obtained from Eqn (S7).

## B. Conversion of the GLE to an effective Fokker-Planck equation

The time evolution of the probability distribution function,  $P(x, t)$ , of the reaction coordinate may be obtained from the solution of the Fokker-Planck equation. Thus, the GLE may be transformed to a partial differential equation to account for the diffusion of the reaction coordinate in an asymmetric potential well. The probability distribution function of the reaction coordinate  $x$  at time  $t$  is given by

$$P(x, t) = \langle \delta(x(t) - x) \rangle \quad (\text{S11})$$

where, the angular brackets represent an ensemble average over the distribution of the noise. Applying the chain rule, the equation may be differentiated as

$$\frac{dP(x, t)}{dt} = -\frac{d}{dx} \langle \delta(x(t) - x) \frac{dx(t)}{dt} \rangle \quad (\text{S12})$$

Substituting the value of  $\frac{dx(t)}{dt}$  in this equation from Eqn (S10), we get

$$\frac{dP(x, t)}{dt} = -\frac{d}{dx} \langle \delta(x(t) - x) \left[ -\Upsilon(t)x(t) - \frac{1}{k_S} \bar{\xi}(t) + \frac{1}{k_S} \bar{\mathcal{W}}(x(t)) \right] \rangle \quad (\text{S13})$$

where, the functions  $\bar{\xi}(t)$  and  $\bar{\mathcal{W}}(x(t))$  correspond to

$$\bar{\xi}(t) = \mathcal{Z}(t) \frac{d}{dt} \int_0^t \frac{\phi(t-t') \xi(t')}{\mathcal{Z}(t)} dt' \quad (\text{S14})$$

and,

$$\bar{\mathcal{W}}(x(t)) = \mathcal{Z}(t) \frac{d}{dt} \int_0^t \frac{\phi(t-t') \mathcal{W}x(t')}{\mathcal{Z}(t)} dt' \quad (\text{S15})$$

A closed form analytical solution of Eqn (S14), is obtained in the small time limit as

$$\bar{\mathcal{W}}(x(t)) = \mathcal{Z}(t) \frac{d}{dt} \int_0^t \frac{\phi(t') \mathcal{W}(x(t-t'))}{\mathcal{Z}(t)} dt' \quad (\text{S16})$$

Expanding  $\mathcal{W}(x(t-t'))$  through the Taylor series, we have

$$\begin{aligned} \mathcal{W}(x(t-t')) &\approx \mathcal{W}(x(t)) - t' \mathcal{W}'(x(t)) \dot{x}(t) \\ &\quad + \frac{t'' \mathcal{W}''(x(t)) \ddot{x}(t)}{2!} + \dots \end{aligned} \quad (\text{S17})$$

Here the first term of the expansion is only taken into account as the higher order terms include derivatives of  $W$ . Calculations of these derivatives involve the fraction of two hypergeometric functions which assumes the form of continued fractions.<sup>2</sup> The exact calculations of such functions are mathematically complicated and are not accurate. Thus, for a closed form analytical solution, the higher order terms are neglected. Hence, Eqn (S16) be written as

$$\bar{\mathcal{W}}(x(t)) = -\frac{\mathcal{W}(x(t))}{\mathcal{Z}(t)} \frac{d\mathcal{Z}(t)}{dt} \quad (\text{S18})$$

Substituting the value of  $\bar{\mathcal{W}}(x(t))$  in Eqn (S13), it simplifies to

$$\frac{dP(x, t)}{dt} = -\frac{\Upsilon(t)}{k_S} \frac{d}{dx} U'(x) P(x, t) + \frac{1}{k_S} \frac{d}{dx} \langle \delta(x(t) - x) \bar{\xi}(t) \rangle \quad (\text{S19})$$

The second term of Eqn (S19) is solved by Novikov's theorem<sup>3</sup> as

$$\langle \delta(x(t) - x) \bar{\xi}(t) \rangle = -\frac{d}{dx} \int_0^t \langle \xi(t) \xi(t') \rangle \langle \delta(x(t) - x) \frac{dx(t)}{d\bar{\xi}(t')} \rangle dt' \quad (\text{S20})$$

The value of  $\frac{dx(t)}{d\bar{\xi}(t')}$  may be estimated from Eqn (S10) that may be functionally differentiated with respect to  $\bar{\xi}(t')$ , to obtain

$$\frac{d}{dt} \frac{dx(t)}{d\bar{\xi}(t')} = -\Upsilon(t) \frac{dx(t)}{d\bar{\xi}(t')} - \frac{1}{k_S} \delta(t-t') + \frac{1}{k_S} \Upsilon(t) \mathcal{W}'(x(t)) \frac{dx(t)}{d\bar{\xi}(t')} \quad (\text{S21})$$

This equation is approximated using Eqn (S17) and simplified as

$$\frac{d}{dt} \frac{dx(t)}{d\xi(t)} = -\frac{1}{k_S} H(t-t') \exp\left(-\int_{t'}^t \Upsilon(q) dq\right) \quad (\text{S22})$$

where,  $H(t-t')$  represents the Heaviside step function.<sup>2</sup> Further simplification of this equation yields

$$\langle \delta(x(t) - x) \bar{\xi}(t) \rangle = -k_B T \Upsilon(t) \frac{d}{dx} P(x, t) \quad (\text{S23})$$

Substituting Eqn (S19) and Eqn (S23) in Eqn (S13), the effective Fokker-Planck equation may be obtained as

$$\frac{dP(x, t)}{dt} = D(t) \left[ \frac{d^2}{dx^2} P(x, t) + \frac{1}{k_B T} \frac{d}{dx} U'(x) P(x, t) \right] \quad (\text{S24})$$

where,  $D(t)$  is the time dependent diffusion coefficient, which may be expressed as

$$D(t) = -\frac{k_B T}{k_S} \Upsilon(t) = \frac{k_B T}{k_S} \frac{\dot{\mathcal{Z}}(t)}{\mathcal{Z}(t)}.$$

## C. The solution of effective Fokker-Planck equation

$P(x, t)$  may be obtained from the solution of Eqn (S24). In this regard, we define another function<sup>4</sup>  $Q(x, t)$ , such that both functions satisfy Eqn (S24) and are related to each other as

$$P(x, t) = P_s(x)Q(x, t) \quad (\text{S25})$$

where,  $P_s(x)$  depicts a stationary solution of the FPE equation. Since,  $P_s(x)$  satisfies Eqn (S24)

$$\frac{d^2}{dx^2}P_s(x) + \frac{1}{k_B T} \frac{d}{dx}U'(x)P_s(x) = 0 \quad (\text{S26})$$

with,

$$P_s(x) = N \frac{\sqrt{\left(\frac{k_S}{k_B T}\right)}}{(\Phi(A, x))^2} \quad (\text{S27})$$

here,  $N$  denotes the normalization constant with  $N = \left(\frac{\mathcal{B}_c^2 - \mathcal{B}^2}{2 \mathcal{B}_c}\right)$ . Substituting  $Q(x, t)$  in Eqn (S24), satisfies the backward EFPE as

$$\frac{dQ(x, t)}{dt} = D(t) \left[ \frac{d^2}{dx^2}Q(x, t) - \frac{1}{k_B T} \frac{d}{dx}U'(x)Q(x, t) \right] \quad (\text{S28})$$

Both functions  $P(x, t)$  and  $Q(x, t)$  may be represented in the spatial and temporal domain via the method of separation of variables as

$$P(x, t) = P_\lambda(x) \exp \left( - \int_0^t \lambda D(t') dt' \right) \quad (\text{S29})$$

and

$$Q(x, t) = Q_\lambda(x) \exp \left( - \int_0^t \lambda D(t') dt' \right) \quad (\text{S30})$$

Eigenfunctions of these two equations may be represented as

$$\left[ \frac{d^2}{dx^2} + \frac{1}{k_B T} \frac{d}{dx}U'(x) + \lambda \right] P_\lambda(x) = 0 \quad (\text{S31})$$

$$\left[ \frac{d^2}{dx^2} - \frac{1}{k_B T} \frac{d}{dx} U'(x) + \lambda \right] Q_\lambda(x) = 0 \quad (\text{S32})$$

Solving these two equations simultaneously by partial integration and imposing the reflecting boundary condition, the coefficient  $Q(x, t)$  vanishes. Therefore,  $P_\lambda$  and  $Q_\lambda$  form a bi-orthogonal system, that may be represented as

$$\int_{-\infty}^{\infty} P_u(x) Q_v(x) dx = \delta_{u,v} \quad (\text{S33})$$

Thus the conditional probability distribution,  $P(x, t|x_0)$  may be obtained from the solution of Eqn (S24), with the initial condition  $P(x, 0) = \delta(x - x_0)$ , as

$$P(x, t|x_0) = \sum_{\lambda} \frac{1}{P_s(x_0)} P_\lambda(x) P_\lambda(x_0) \exp \left( - \int_0^t \lambda D(t') dt' \right) \quad (\text{S34})$$

$P_\lambda(x)$  is the solution of Eqn (S24) in the spatial domain, given by

$$P_\lambda(x) = P_s^{1/2}(x) \psi_\lambda(x) \quad (\text{S35})$$

Using this equation, Eqn (S31) may be represented in terms of eigenfunction as

$$\frac{d^2}{dx^2} \psi_\lambda(x) + \left\{ \lambda - \left[ \left( \frac{U'(x)}{2k_B T} \right)^2 - \frac{U''(x)}{2k_B T} \right] \right\} \psi_\lambda(x) = 0 \quad (\text{S36})$$

Comparing this equation with the solution of the Schrodinger equation,<sup>4</sup> for an asymmetric potential given in Eqn (2),  $V_{eff}(x)$  may be written as

$$V_{eff}(x) = 2 \left( \frac{\Phi'(A, x)}{\Phi(A, x)} \right)^2 - \frac{\Phi''(A, x)}{\Phi(A, x)} \quad (\text{S37})$$

Equation (S36), assumes the form

$$\frac{d^2}{dx^2} \psi_\lambda(x) + \left\{ \lambda - \left[ 2 \left( \frac{\Phi'(A, x)}{\Phi(A, x)} \right)^2 - \frac{\Phi''(A, x)}{\Phi(A, x)} \right] \right\} \psi_\lambda(x) = 0 \quad (\text{S38})$$



Considering the equation in  $z$  domain such that  $z = \sqrt{\frac{k_S}{k_B T}} x$ , the equation may be solved in terms of the solution of Weber equation via Darboux transform.<sup>5,6</sup> Thus, the equation in  $z$  domain may be represented as

$$\frac{d^2}{dz^2} \psi_\lambda(z) + \left\{ \frac{k_B T}{k_S} \lambda - \left[ 2 \frac{k_B T}{k_S} \left( \frac{\Phi'(A, z)}{\Phi(A, z)} \right)^2 - \left( \frac{z^2}{4} + A \right) \right] \right\} \psi_\lambda(z) = 0 \quad (\text{S39})$$

where,  $\Phi(A, z) = \exp\left(-\frac{z^2}{4}\right) \left[ {}_1F_1\left(\frac{A}{2} + \frac{1}{4}, \frac{1}{2}; \frac{z^2}{2}\right) + \mathcal{B} z {}_1F_1\left(\frac{A}{2} + \frac{3}{4}, \frac{3}{2}; \frac{z^2}{2}\right) \right]$ . Thus, the eigenfunctions of this equation may be derived from the Darboux transform method, and hence the eigensolutions of the equation may be written in terms of  $x$ . The solution of Eqn (S39) in terms of the eigenfunctions  $\psi_\lambda(x)$  as estimated from the Darboux theorem<sup>5-7</sup> may be represented as the ground state solution obtained for  $\lambda_0 = 0$

$$\psi_0(x) = C_0 \left( \frac{k_S}{k_B T} \right)^{1/4} \frac{1}{\Phi(A, x)} \quad (\text{S40})$$

and, the excited state solutions obtained for  $\lambda_{n+1} = \sqrt{\frac{k_B T}{k_S}} \left( n + A + \frac{1}{2} \right)$

$$\psi_{n+1}(x) = C_{n+1} \left( \frac{k_B T}{k_S} \right)^{1/4} \Phi(A, x) \frac{d}{dx} \frac{D_n(x)}{\Phi(A, x)} \quad (\text{S41})$$

which may also be expressed as

$$\psi_{n+1}(x) = -C_{n+1} \lambda_{n+1} \left( \frac{k_B T}{k_S} \right)^{1/4} \frac{1}{\Phi(A, x)} \int_0^x D_n(x') \Phi(A, x') dx' \quad (\text{S42})$$

where,  $D_n(x)$  denotes the parabolic cylinder function.<sup>2,8</sup>

## D. Calculation of the normalization constant $C_0$

The normalization constant of the eigenfunction for the ground state

$$\psi_0(x) = C_0 \left( \frac{k_S}{k_B T} \right)^{1/4} \frac{1}{\Phi(A, x)} \quad (\text{S43})$$

may be calculated by simplifying the function  $\frac{1}{\Phi(A, x)}$ . From Darboux theorem,  $\frac{1}{\Phi(A, x)}$  may be represented by an identity

$$\frac{1}{\Phi(A, x)} = \Phi(A, x) \frac{d}{dx} \left( \frac{\epsilon_1 y_1(A, x) + \epsilon_2 y_2(A, x)}{\Phi(A, x)} \right) \quad (\text{S44})$$

where,  $\epsilon_1$  and  $\epsilon_2$  are constants that may be calculated by considering  $x \rightarrow \infty$  and  $x = 0$ .

The function then becomes

$$\frac{1}{\Phi(A, x)} = \Phi(A, x) \frac{d}{dx} \left( \frac{y_2(A, x)}{\Phi(A, x)} \right) \quad (\text{S45})$$

following the normalization condition

$$\int_{-\infty}^{\infty} \psi_0(x) \psi_0^*(x) dx' = 1 \quad (\text{S46})$$

$$C_0^2 \sqrt{\left( \frac{k_S}{k_B T} \right)} \int_{-\infty}^{\infty} \frac{1}{(\Phi(A, x'))^2} dx' = 1 \quad (\text{S47})$$

Writing this equation in terms of the variable  $z$  such that  $z = \sqrt{\frac{k_S}{k_B T}} x$ ,

$$\frac{1}{C_0^2} = 2 \int_0^{\infty} \frac{1}{(\Phi(A, z'))^2} dz' \quad (\text{S48})$$

Using the identity given in Eqn (S45), the equation may be recast as

$$\frac{1}{C_0^2} = 2 \left. \frac{y_2(A, z)}{y_1(A, z) + \mathcal{B} z y_2(A, z)} \right|_{z=\infty} \quad (\text{S49})$$

Using the identities of the hypergeometric functions<sup>2,9</sup> associated with the respective functions  $y_1(A, x)$  and  $y_2(A, x)$  followed by some simplifications, Eqn (S49) may be represented as

$$\frac{1}{C_0^2} = \frac{2 \mathcal{B}_c}{\mathcal{B}_c^2 - \mathcal{B}^2} \quad (\text{S50})$$

where,  $\mathcal{B}_c = \sqrt{2} \frac{\Gamma(A/2+3/4)}{\Gamma(A/2+1/4)}$ . Therefore, the normalization constant  $C_0$  may be obtained as

$$C_0 = \left( \frac{\mathcal{B}_c^2 - \mathcal{B}^2}{2 \mathcal{B}_c} \right)^{1/2} \quad (\text{S51})$$

## E. Calculation of the normalization constant $C_{n+1}$

The calculation of the normalization factor for the eigenfunction  $\psi_{n+1}$ , may be calculated from the integral and the differential transforms given by Eqns (S42) and (S41), respectively.

$$\psi_{n+1}(x) = C_{n+1} \left( \frac{k_B T}{k_S} \right)^{1/4} \Phi(A, x) \frac{d}{dx} \frac{D_n(x)}{\Phi(A, x)} \quad (\text{S52})$$

Following the normalization condition

$$\int_{-\infty}^{\infty} \psi_{n+1}(x) \psi_{n+1}^*(x) dx = 1 \quad (\text{S53})$$

Substituting Eqns (S42) and (S41) in Eqn (S53), and recasting the resultant equation in terms of the variable  $z$ , where  $z = \sqrt{\frac{k_S}{k_B T}} x$ , the final expression may be obtained as

$$\frac{1}{C_{n+1}^2} = \int_{-\infty}^{\infty} \Phi(A, z) \frac{d}{dz} \frac{D_n(z)}{\Phi(A, z)} \left[ -\lambda_{n+1} \frac{1}{\Phi(A, z)} \int_0^{z/\sqrt{k_S/k_B T}} \Phi(A, z') D_n(z') dz' \right] dz \quad (\text{S54})$$

which may be further simplified to

$$\frac{1}{C_{n+1}^2} = -\lambda_{n+1} \left[ -D_n(z) \frac{\Phi(A, z)}{\lambda_{n+1}} \frac{d}{dz} \frac{D_n(z)}{\Phi(A, z)} \Big|_{-\infty}^{\infty} - \int_{-\infty}^{\infty} (D_n(z'))^2 dz' \right] \quad (\text{S55})$$

where, the first integral is equal to zero. Hence, the solution may be expressed as

$$\frac{1}{C_{n+1}^2} = \sqrt{2\pi} n! \lambda_{n+1} \quad (\text{S56})$$

Therefore the calculated normalization constant is

$$C_{n+1}^{-2} = \sqrt{2\pi} n! \left( n + A + \frac{1}{2} \right); \quad n \geq 0. \quad (\text{S57})$$

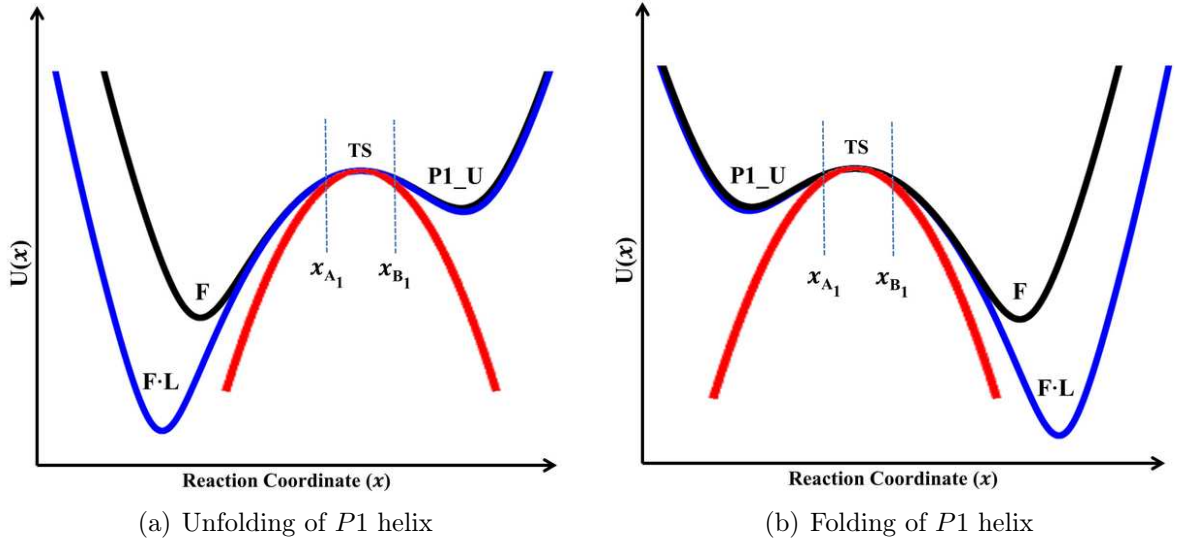


Figure S1: Schematic representation of the conformational transitions of the *add* A-riboswitch aptamer with and without ligand, conventionally modeled as a diffusive dynamics of the riboswitch along a reaction (or extension) coordinate,  $x$  in an asymmetric bistable potential. The riboswitch aptamer fluctuates between the fully folded (with and without ligand) ( $x_A$ ) and *P1* unfolded state ( $x_B$ ), located at the two minima of the potential well separated by a transition state located at the barrier top ( $x_{TS}$ ). Figures (a) and (b) represent the unfold and fold transition of the *P1* helix of the riboswitch aptamer both with (blue) and without (black) ligand, respectively. Transition path is defined as a part of the entire transition trajectory (i.e.,  $x_A < x < x_B$ ) that is confined in the transition region  $x_{A_1} < x_{TS} < x_{B_1}$ . Transitions paths for each of the unfold and fold transitions are denoted in red.

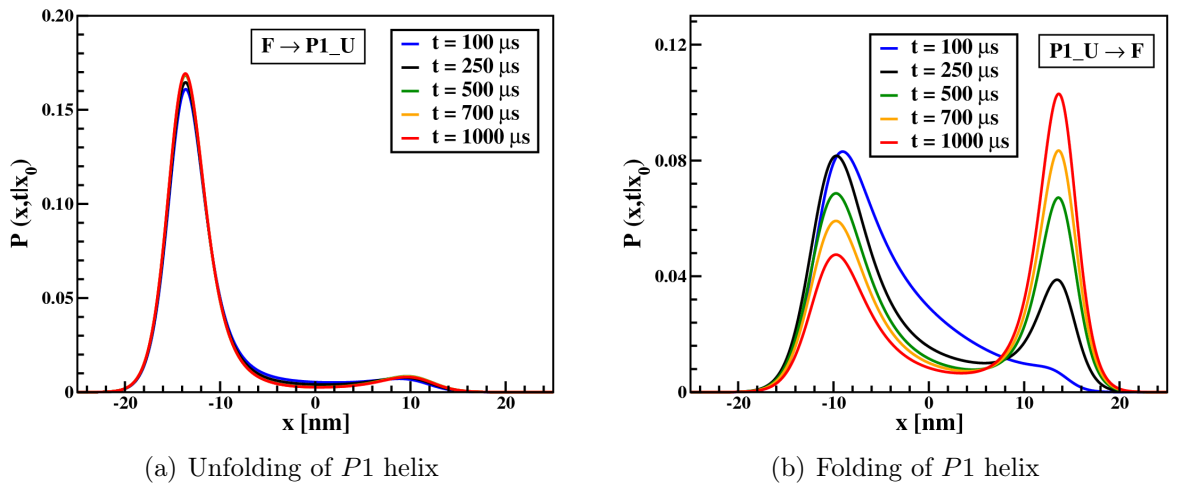


Figure S2: Probability distribution function,  $P(x, t|x_0)$  versus the reaction coordinate,  $x$  of the *add* A-riboswitch aptamer obtained from Eqn (10) at different time intervals. Plots (a) and (b) indicate the unfold and fold transition of the riboswitch aptamer in the absence of the ligand, respectively.

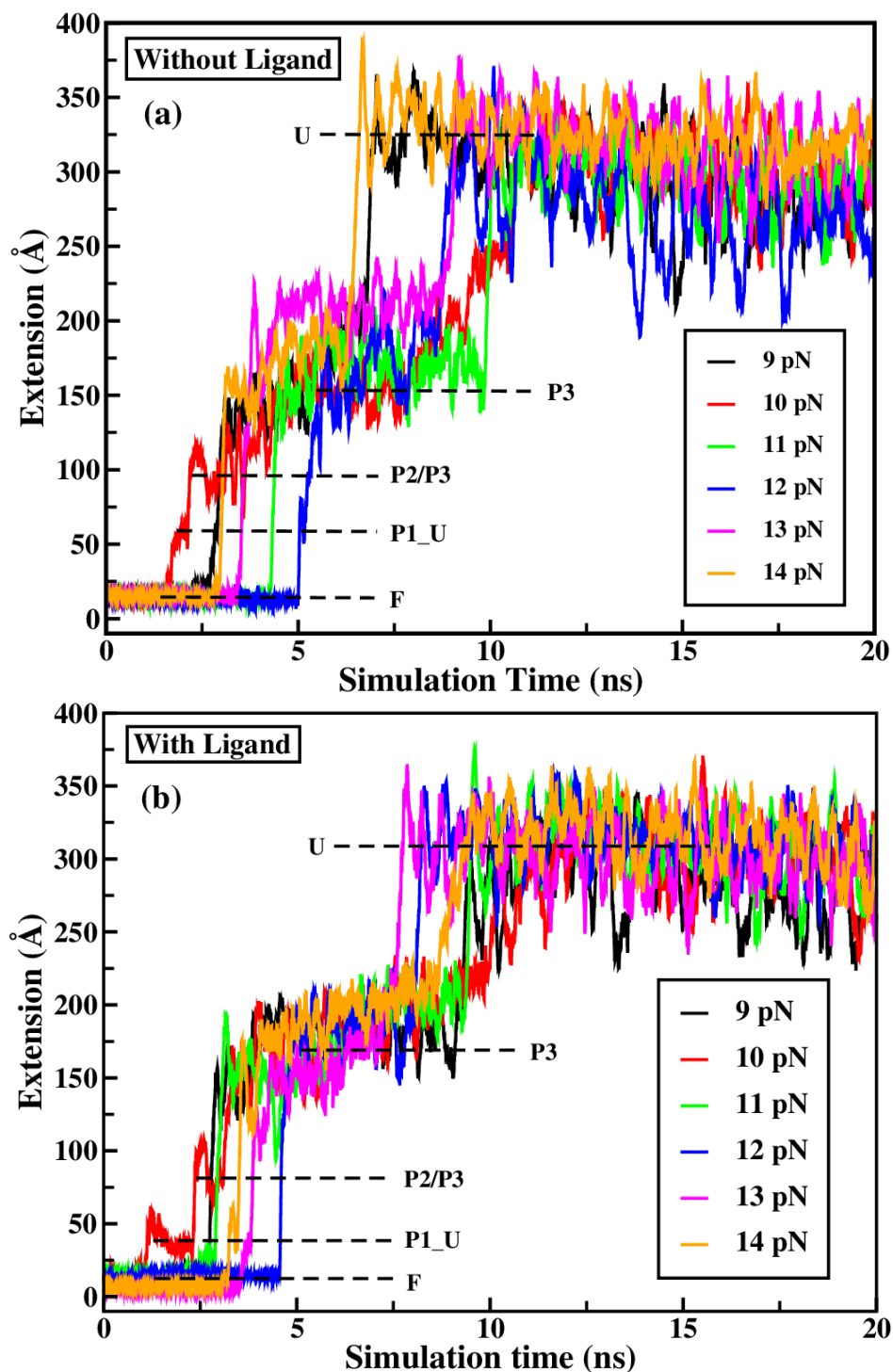


Figure S3: The end-to-end distance (between  $C3'$  and  $C5'$  atoms) of the riboswitch aptamer as a function of the simulation time for both (a) without and (b) with ligand binding SMD simulations at different forces.

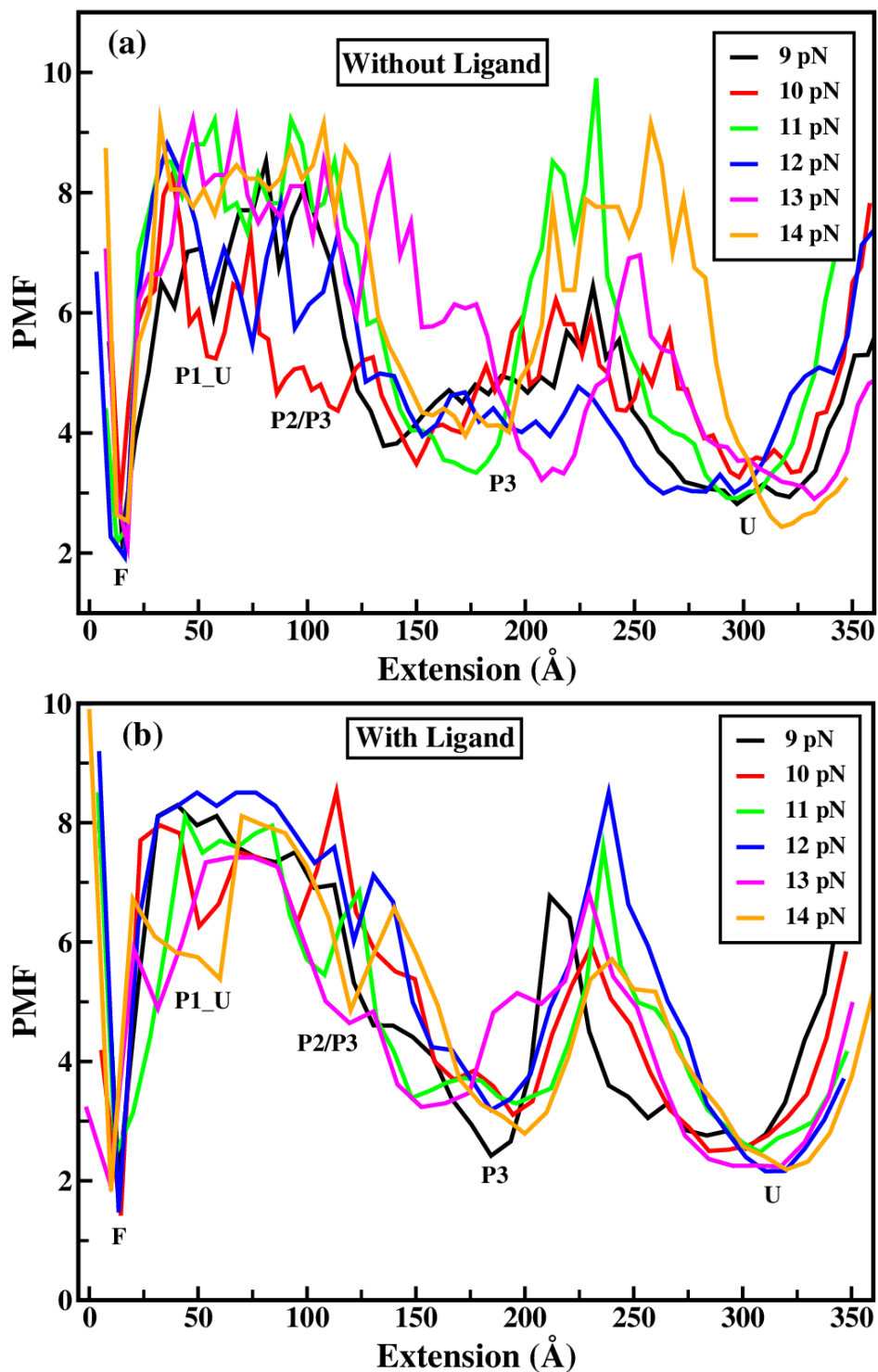


Figure S4: Potential of mean forces (PMFs) versus extension (end-to-end distance between  $C3'$  and  $C5'$  atoms) of the riboswitch aptamer for both (a) without and (b) with ligand binding SMD simulations at different forces.

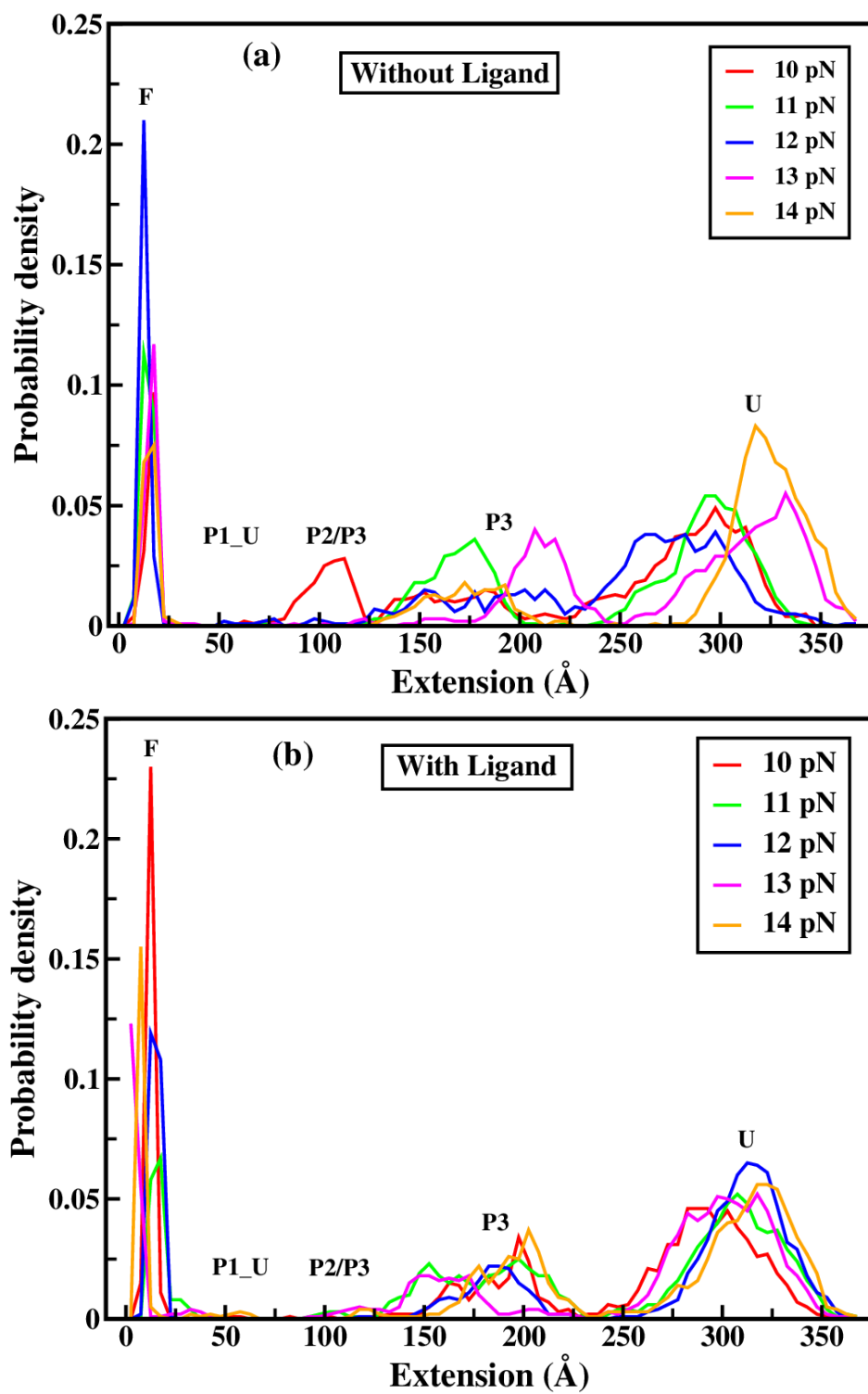


Figure S5: Probability density versus extension of the riboswitch aptamer for both (a) without and (b) with ligand binding SMD simulations at different forces.



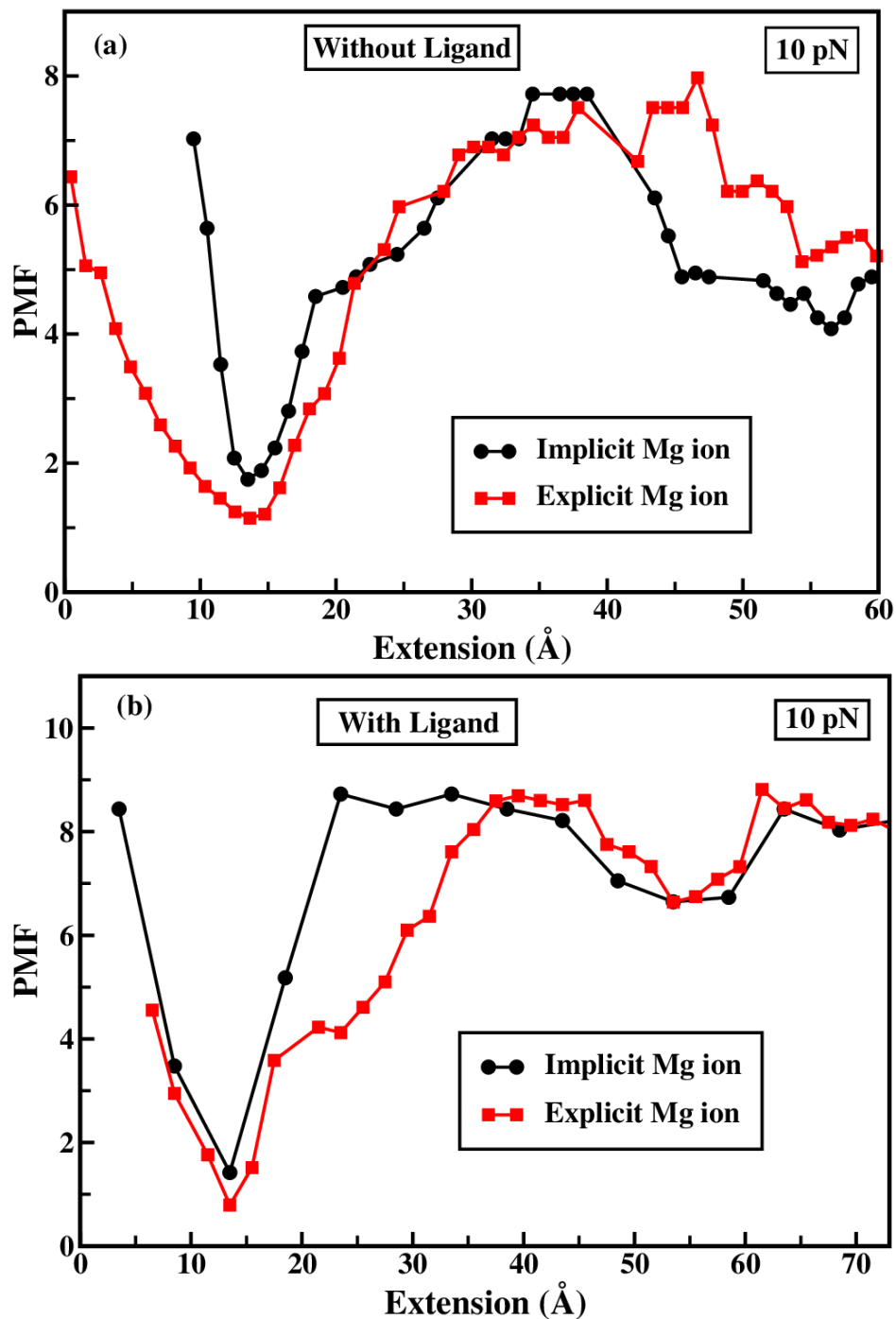


Figure S6: Comparison of Potential of mean forces (PMFs) versus extension (end-to-end distance between  $C3'$  and  $C5'$  atoms) of the riboswitch aptamer with implicit (black) and explicit (red)  $Mg^{2+}$  ion for both (a) without and (b) with ligand binding SMD simulations at 10 pN.

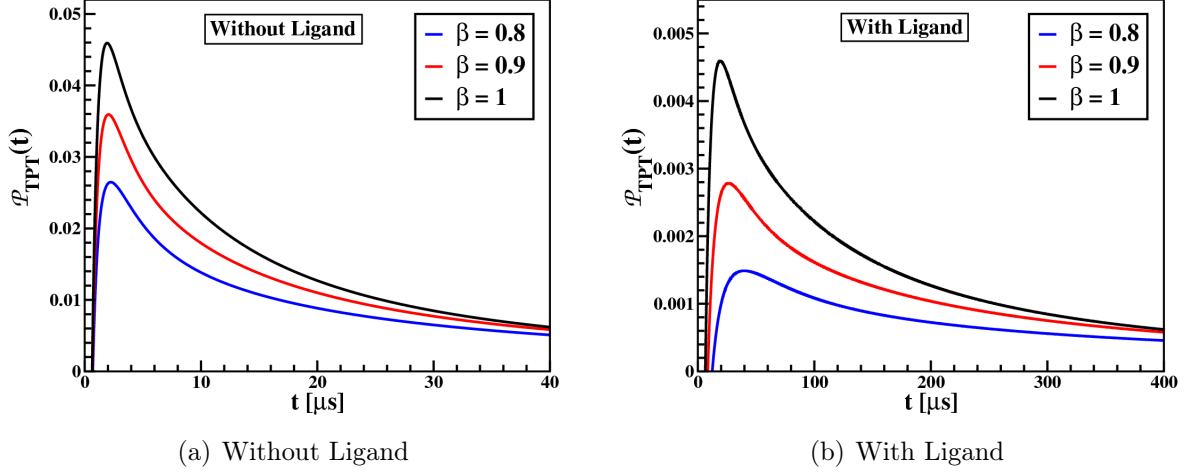


Figure S7:  $\mathcal{P}_{TPT}(t)$  versus time  $t$ , for the conformational transitions of the *add* A-riboswitch aptamer between the folded and unfolded states for different  $\beta$  values. Plots (a) and (b) depict the riboswitch transition without and with ligand binding, respectively. Different curves correspond to three different  $\beta$  values:  $\beta = 0.8$  (blue),  $\beta = 0.9$  (red) and  $\beta = 1$  (black) for without ligand  $D_\beta$  as  $D_{0.8} = 0.16 \text{ nm}^2/\mu\text{s}^{0.8}$ ,  $D_{0.9} = 0.18 \text{ nm}^2/\mu\text{s}^{0.9}$ , and  $D_1 = 0.2 \text{ nm}^2/\mu\text{s}$  and with ligand  $D_\beta$  as  $D_{0.8} = 0.016 \text{ nm}^2/\mu\text{s}^{0.8}$ ,  $D_{0.9} = 0.018 \text{ nm}^2/\mu\text{s}^{0.9}$ , and  $D_1 = 0.02 \text{ nm}^2/\mu\text{s}$ , respectively with  $k_S = 1 \text{ pN/nm}$  and  $x_0 = 1 \text{ nm}$ .

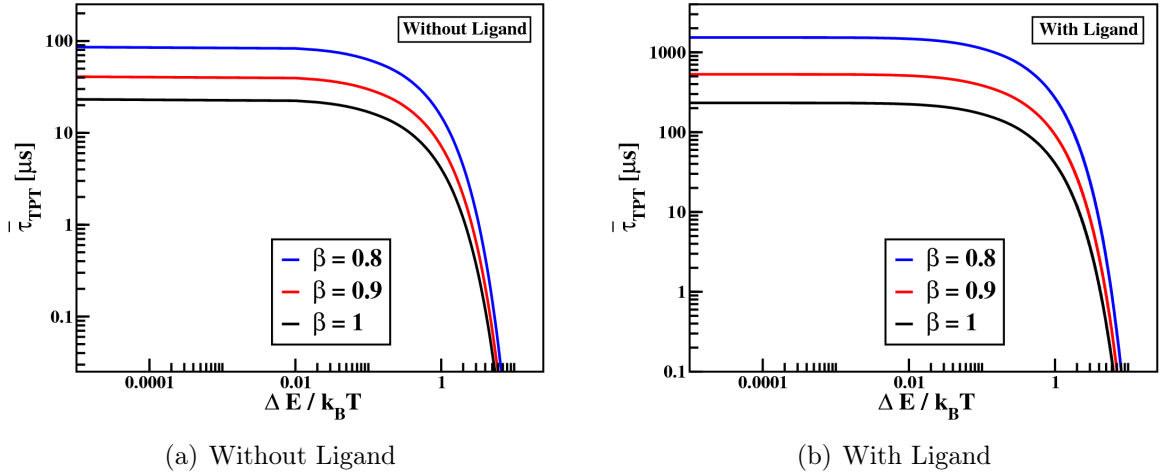


Figure S8: The mean transition path time,  $\bar{\tau}_{TPT}$  versus the barrier height,  $\Delta E/k_B T$  of an asymmetric potential obtained from Eqn (16). Plots (a) and (b) depict the MTPT of the unfold/fold transition of the riboswitch both without and with the ligand, respectively, for three different values of  $\beta$  as  $\beta = 0.8$ ,  $\beta = 0.9$  and  $\beta = 1$  with  $k_S = 1 \text{ pN/nm}$ .

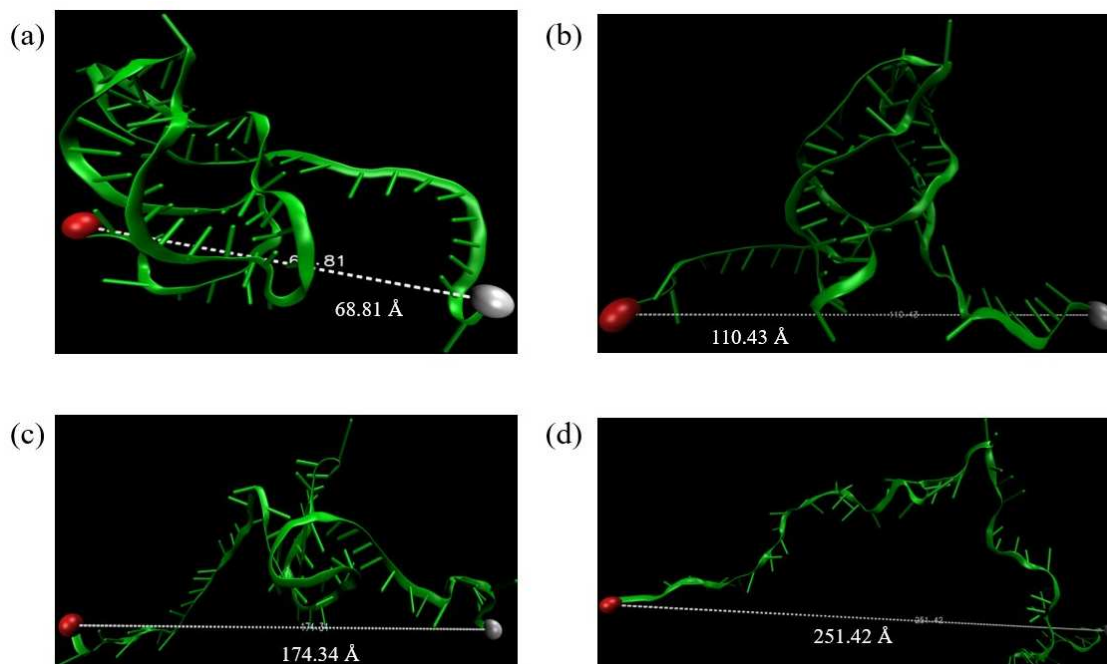


Figure S9: Snapshots of the unfolding events of the riboswitch aptamer in absence of the ligand from SMD simulation with a pulling force of 10 pN. The end-to-end distances between  $C3'$  (grey) and  $C5'$  (red) atoms are (a) 68.81 Å, (b) 110.43 Å, (c) 174.34 Å, and (d) 251.42 Å, respectively. (Multimedia view)

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