Supporting Information

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SI Text

Fluctuations of the handle polymer under tension. Using the force clamp simulations of the RNA hairpin in the presence of handles, the dynamics of the fluctuations of the handles can be independently extracted by probing the time-dependent changes in the 5' and 3' ends of the RNA molecule. The distribution of the longitudinal fluctuations $(z_H^{5'} \equiv z_{5'} - z_o \text{ and } z_H^{3'} \equiv z_p - z_{3'})$ and the dispersion in the transverse fluctuations $(x_5' \text{ or } y_{5'} \text{ and } x_{3'} \text{ or } y_{3'})$ are shown in Fig. S1. Assuming that the force $\mathbf{f} = f \| \hat{\mathbf{e}}_{\parallel} + f_{\perp} \hat{\mathbf{e}}_{\perp}$ is decomposed into $f = |f| \approx f_{\parallel} + f_{\perp}^2/2f_{\parallel} + \mathbb{O}(f_{\perp}^4)$ (with $f_{\parallel} \ll f_{\perp})$, and using the partition function of the worm-like chain polymer under tension, $Z = \int D[\mathbf{u}(5)]e^{-\beta}(H_{WLC} - \mathbf{f}\cdot\mathbf{R})$, one can express the longitudinal fluctuation as

$$\langle \delta R_{\parallel}^2 \rangle = k_B T \frac{d\langle R_{\parallel} \rangle}{df} \sim \begin{cases} L l_p & \text{for } f \leq \frac{k_B T}{l_p} \\ L l_p^{-1/2} (f/k_B T)^{-3/2} & \text{for } f \gg \frac{k_B T}{l_p} \end{cases},$$
[1]

where the force extension relations of a worm-like chain $R \parallel L \approx f l_p / k_B T$ for $f < k_B T / l_p$ and $R \parallel L \approx 1 - \sqrt{k_B T / 4 l_p} f$ for $f > k_B T / l_p$ are used for small and large forces, respectively (2).

These results are consistent with the fluctuations observed in the simulations. When $f < k_B T/l_p$, the transverse fluctuations are independent of the force, and are determined solely by the nature of the linker. For $f = f_m \approx 15.4$ pN, the tension is in the regime that satisfies $f > k_B T/l_p$ for both values of l_p used in the simulations, and the longitudinal fluctuations $\langle \delta R^{\parallel 2} \rangle$ decrease as the stiffness of the polymer increases for all L (Fig. S1a). The distribution of the extensions coincide for both the 3' and 5' ends of the handles for all L and l_p (Fig. S1a). This suggests that the constant force applied at the point z_p propagates uniformly throughout the whole system.

The transverse fluctuations are given by

$$\langle \delta R_{\perp}^2 \rangle = (k_B T)^2 \frac{\partial^2 \log Z}{\partial f_{\perp}^2} |_{f_{\parallel} = f_{f_{\perp}} = 0}$$
^[2]

$$= \frac{k_B T}{f} \langle R_{\parallel} \rangle \approx \frac{L k_B T}{f} \left(1 - \frac{1}{2} \sqrt{\frac{k_B T}{l_p f}} \right) \text{ for } f \gg \frac{k_B T}{l_p}.$$

The transverse fluctuations also decrease as $f(>k_BT/l_p)$ increases, with a different power. It is worth noting that the transverse fluctuations, which also increase as *L* increases, are nearly independent of the handle stiffness if $f > k_BT/l_p$. The standard deviations of distributions are plotted with respect to

the contour length at each bending rigidity (Fig. S1b). The fit shows that $\sigma \approx 0.28 \times L^{1/2}$ nm and $\sigma \approx 0.30 \times L^{1/2}$ nm for $l_p = 0.6$ nm and $l_p = 70$ nm, respectively, which is consistent with the analysis in Eq. 1.

Determination of the Persistence Length of the Handles. To determine the persistence length of the handles, we numerically generated the end-to-end distribution function P(R) of the free handles in the absence of tension, and fit the simulated distribution to the analytical result (3, 4)

$$P_{WLC}(R) = \frac{4\pi C(\alpha)\rho^2}{L(1-\rho^2)^{9/2}} \exp\left(-\frac{\alpha}{1-\rho^2}\right),$$
 [3]

with $\rho = R/L$ and $\alpha = 3L/4l_p$. The normalization constant $C(\alpha) = [\pi^{3/2}e^{-\alpha}\alpha^{3/2}(1 + 3\alpha^{-1} + 15\alpha^{-2}/4)]^{-1}$ ensures $\int_0^L dR P_{WLC}(R) = 1$.

Quantifying the Synchronization of $z_{sys}(t)$ and $z_m(t)$. The origin of the small discrepancy between $F_{eq}(z_{sys})$ and $F_{eq}(z_m)$ when the handles are flexible can be found by comparing $z_{sys}(t)$ with $z_m(t)$ for the two extreme cases in Fig. 2. To quantitatively express the synchronization between $z_{sys}(t)$ and $z_m(t)$, we defined a correlation function at each time t using

$$C(t) = \frac{z_{sys}(t) - z_{sys}^{TS}}{z_{sys}^{TS}} \times \frac{z_m(t) - z_m^{TS}}{z_m^{TS}},$$
 [4]

where z_{sys}^{TS} and z_m^{TS} are the positions of the transition states determined from $F_{eq}(z_{sys})$ and $F_{eq}(z_m)$ respectively. If C(t) > 0 at time t, both $z_{sys}(t)$ and $z_m(t)$ are in the same basins of attraction, i.e., the status of $z_m(t)$ is correctly detected by the measurement through the handles. If C(t) < 0, then the information of $z_m(t)$ is lost due to fluctuations or the slow response of the handles. The near-perfect synchronization between $z_{sys}(t)$ and $z_m(t)$ for $l_p = 60$ nm and L = 5 nm are reflected in C(t) > 0 for almost all t. Thus, when the handles are stiff, $z_m(t) \approx z_{sys}(t) - 2L$, which implies that $z_{sys}(t)$ faithfully reflects the dynamics $[z_m(t)]$ of the hairpin. In contrast, with $l_p = 0.6$ nm and L = 25 nm, $z_m(t)$ cannot be determined from $z_{sys}(t)$ using $z_{sys}(t) - 2 \times L \neq z_m(t)$. The amplitudes of $z_{sys}(t)$ are typically larger than that of $z_m(t)$, leading to C(t) < 0 occasionally (shown by an arrow on the right plot in Fig. S2).

The histograms of P(C) for the two extreme cases show that the dynamics between $z_{sys}(t)$ and $z_m(t)$ are more synchronous for the rigid and short handles (0.0 < P(C) < 0.5) than for the flexible and longer handles (-0.05 < P(C) < 0.2) (see the graph at the bottom of Fig. S2). The finding that short and stiff $[L/l_p ~ O (1)]$ handles minimize the differences between $F_{eq}(z_m)$ and $F_{eq}(z_{sys})$ is related to the tension-dependent fluctuations in the linkers.

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^{2.} Marko JF, Siggia ED (1995) Stretching DNA. *Macromolecules* 28:8759-8770.

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Fig. S1. Fluctuations of the handles with varying lengths and flexibilities at f = 15.4 pN. (a) Longitudinal fluctuations of the handle attached at the 5' and 3' sides of the RNA hairpin. (b) Transverse fluctuations are fit to a Gaussian distribution, and the standard deviation (σ) is plotted as a function of the contour length and flexibility.

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Fig. 52. The time traces, $z_{sys}(t)$ and $z_m(t)$, for two extreme cases that produce the free-energy profiles in the magenta and orange boxes in Fig. 2 of the main text are overlapped to show the differences. For $I_p = 70$ nm and L = 5 nm, both the phase and amplitude between $z_{sys}(t)$ and $z_m(t)$ coincide throughout the time series, whereas for $I_p = 0.6$ nm and L = 25 nm, the amplitude of $z_{sys}(t)$ is larger than $z_m(t)$, and the phase between $z_{sys}(t)$ and $z_m(t)$ is occasionally offset from one another. The correlation measure C(t) quantifies the synchrony between $z_{sys}(t)$ and $z_m(t)$ at time t. The histograms of C(t) show that the time trace for $I_p = 70$ nm, and L = 5 nm is more synchronized than one for $I_p = 0.6$ nm and L = 25 nm.



Fig. S3. The survival probabilities $P_F(t)$ and $P_U(t)$ are fit to a single exponential function to calculate τ_U and τ_F . For L = 0 nm, $\tau_U = 2.9$ ms, and $\tau_F = 1.9$ ms. For L = 20 nm, $\tau_U = 5.0$ ms and $\tau_F = 12.1$ ms. The quality of the fits for L = 20 nm (dashed lines) is not as good as for L = 0 nm. The survival probabilities show lag phases for both unfolding and refolding.

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Fig. S4. Time traces of molecular extension under tension f = 14.0, 15.4, and 16.8 pN, and corresponding distribution $P(z_m|f)$ at each force. The distributions are converted to the free energy profile in Fig. 6a by using $F_{eq}^o(z_m)/k_BT = -\log P(z_m)$. Note that the hairpin is pinned in the UBA at f = 16.8 pN ($>f_m$) with infrequent transitions to the NBA. Just as in experiments (5), accurate measurement of $F_{eq}(z_m)$ is possible only at $f \approx f_m$, where multiple hopping events between the NBA and UBA can be observed.