Supplementary Information for "Transition path times for nucleic acid folding determined from energy landscape analysis of single-molecule trajectories"

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Experimental Materials and Methods

Sample preparation: DNA hairpins were prepared as described previously [1]. Riboswitch RNA was transcribed in vitro from a DNA template containing the *add* riboswitch aptamer sequence flanked on each side by kilobase-long handle sequences, then annealed to complementary single-stranded DNA handles as described [2]. Pseudoknot samples were prepared similarly to the riboswitch samples. The handles were labelled at one end with biotin, at the other with digoxigenin. Samples were incubated with polystyrene beads 600-nm and 820-nm in diameter (labelled respectively with avidin- and anti-digoxinenin) to create dumbbells as in Fig 1b. The dumbbells were placed in measuring buffer as described for hairpins [1] and the riboswitch [2]; in the case of the pseudoknots, the measuring buffer was 50 mM Mops pH 7.0, 130 mM KCl, 4 mM $MgCl_2$, 50 U/mL Superase•In RNase inhibitor (Ambion), and oxygen scavenging system (40 U/ml glucose oxidase, 185 U/mL catalase, and 8.3 mg/mL glucose).

Force spectroscopy measurements: Measurements were made with custom-built, dual-beam optical tweezers as described previously [1, 2]. Non-equilibrium force-extension curves (FECs) were measured at pulling rates of 10–220 nm/s and trap stiffness of 0.3–0.9 pN/nm, sampling data at 20 kHz while filtering online at the Nyquist frequency. Equilibrium measurements were sampled at 50 kHz with a trap stiffness of 0.3 pN/nm, using a passive force clamp to maintain constant force [3].

Landscape reconstructions: Energy landscape profiles for the DNA hairpins were calculated by deconvolution of the extension histograms as described previously [4]. Curvature of the wells and barriers was found from a quadratic fit to the landscape profiles, and barrier heights were measured from the profiles for both folding and unfolding. D and τ_{tp} were calculated for both folding and unfolding. For all molecules, the values for the two directions were equal within error, and therefore the average values were reported.

The key energy landscape parameters were obtained from FEC measurements by fitting the unfolding force distributions, $p(F)$, to [5]:

$$p(F) \propto \frac{k(F)}{r} \exp\left\{ \frac{k_{\mathrm{off}}}{\Delta x^{\ddagger} r} - \frac{k(F)}{\Delta x^{\ddagger} r}\left(1 - \frac{2\Delta x^{\ddagger} F}{3\Delta G^{\ddagger}}\right)^{-1/2} \right\}, \tag{S1}$$

$$\text{where } k(F) = k_{\mathrm{off}} \left(1 - \frac{2\Delta x^{\ddagger} F}{3\Delta G^{\ddagger}}\right)^{1/2} \exp\left\{ \frac{\Delta G^{\ddagger}}{k_B T}\left[1 - \left(1 - \frac{2\Delta x^{\ddagger} F}{3\Delta G^{\ddagger}}\right)^{3/2}\right] \right\}, \tag{S2}$$

r is the loading rate, and $\nu = 2/3$ for a linear-cubic landscape profile. The same parameters were also obtained from fitting the force-dependent unfolding rate found from the survival probability [6] to Equation S2. For all RNA molecules, the results from the two methods were averaged.

References

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Table S1. Energy landscape parameters for pseudoknots and riboswitches found from fits to $p(F)$ and $k(F)$. Results from the two fits were averaged. Errors represent the standard error on the mean over all molecules.

Molecule	**ln k_{off} (s^{-1})**	**$\Delta x^{\ddagger}$ (nm)**	**$\Delta G^{\ddagger}$ ($k_B T$)**
Hairpins			
30R50/T4	−48 ± 1	19.2 ± 0.3	59 ± 2
20TS06/T4	−21 ± 2	9 ± 1	35 ± 2
Pseudoknots			
MMTV	−7 ± 1	2.0 ± 0.4	14 ± 2
PEMV1	−8 ± 1	1.9 ± 0.2	16 ± 3
ScYLV	−11.5 ± 0.7	1.7 ± 0.1	24 ± 2
ScYLV C27A	−9 ± 1	1.9 ± 0.2	20 ± 2
PT2G32	−9.4 ± 0.5	1.6 ± 0.1	26 ± 4
*add r*iboswitch			
without adenine	−11.0 ± 0.2	6.1 ± 0.5	20.5 ± 0.7
with adenine	−15 ± 1	6.2 ± 0.6	27 ± 3