Biophysical Journal, Volume *111*

Supplemental Information

Quantifying Instrumental Artifacts in Folding Kinetics Measured by Single-Molecule Force Spectroscopy

Krishna Neupane and Michael T. Woodside

Materials and Methods

Sample preparation: DNA hairpins attached to double-stranded (ds) DNA handles were prepared as described previously (1). Briefly, the hairpin was included as a 5′ overhang separated from a PCR priming sequence by abasic sites; the hairpin and 3′ handle (~800 bp long) were then made as a unit by PCR. A second handle (~1200 bp) made by PCR was then ligated to the 5′ end of the hairpin via complementary 5′ ligation sites. The hairpin-handle construct was attached to 600- and 820-nm polystyrene beads via biotin-avidin and dioxigenin and anti-dig pairs to generate bead-sample dumbbells for trapping. Hairpin samples were incubated for ~ 1 hr at ~ 100 pM with 250 pM polystyrene beads to form dumbbells. Dumbbells were diluted to ~ 500 fM in 50 mM MOPS, pH 7.5, with 200 mM KCl and oxygen scavenging system (8 mU/μL glucose oxidase, 20 mU/μL catalase, 0.01% w/v D-glucose), before insertion into a sample cell for optical trapping.

Measurements: All samples were measured on a dual-trap optical tweezers apparatus described previously (2). Briefly, two independently controlled traps were generated from a 4-W, 1064-nm, diode-pumped solid-state laser, splitting the traps by polarization. The stiffness and position of each trap were controlled respectively by acousto-optic and electro-optic deflectors. For constant force measurements, the stiffness of one trap was set to 0.3 pN/nm, whereas the other was used in the anharmonic region of the trap where the stiffness was zero, to achieve a passive force clamp (3). In constant trap position measurements, the stiffness set to 0.56–0.63 pN/nm in one trap and 0.75–1.1 pN/nm in the other; the effective system stiffness was thus several-fold higher in constant-position measurements, leading to a faster instrument response time (reflected in τ_A). Bead positions were detected by scattering a 7-mW, 633-nm HeNe laser off the beads and collecting the scattered light on position-sensitive detectors. Data were sampled at 20–256 kHz for constant-force measurements and 124–400 kHz for constant-position measurements, in each case filtered online at the Nyquist frequency.

Analysis: Rates were determined by thresholding analysis of the extension trajectories as described previously (1). The stiffness of the wells and barriers in $G_A(q)$ and $G_0(x)$ were found from quadratic fits to the energy profiles, as described (4).

All quantities were calculated using both unfolded-state and folded-state data. The results for measurements near $F_{½}$ are listed in Table S1 (all errors represent the standard error on the mean). The results from U and F were found to be the same within error for each quantity calculated ($\langle \delta q^2 \rangle$, τ_A, D_q, k_A, and k_M) and hence were averaged in each case to yield the values listed in Table 1.

The intrinsic molecular diffusion coefficient, D_x, was calculated from the average transition path time (τ_{tp}) obtained from constant trap position measurements, under the assumption of one-dimensional diffusive motion over a harmonic barrier in the high-barrier limit (5):

$$\tau_{tp} \approx \frac{\ln\left(2e^{\gamma}\beta\Delta G^{\ddagger}\right)}{\beta D \kappa_b}, \qquad \text{(S1)}$$

where $\Delta G^{\ddagger}$ is the barrier height in $G_0(x)$, κ_b is the barrier stiffness, γ is Euler's constant, and $\beta = 1/k_B T$ is the inverse thermal energy. Transition path times were measured directly from extension trajectories as described previously (6). Briefly, transition paths were identified as those parts of the trajectory traversing from the folded state F to the unfolded state U or vice versa. Defining the barrier region between F and U as the middle half of the distance from F to U, the transit time was measured as the time required to cross from one edge of the barrier region to the other. Transit times for all barrier crossings were averaged to obtain τ_{tp}. The diffusion coefficient D_x calculated using Eq. S1 agreed well with the value obtained from constant-force rate measurements using Kramer's equation (Table 1).

Table S1: Landscape and kinetic parameters near $F_{1/2}$

Constant force	30R50/T4		20TS06/T4	
	folded	unfolded	folded	unfolded
κ_{Aw} (k_BT/nm^2)	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.03	0.07 ± 0.02
κ_{Ab} (k_BT/nm^2)	0.08 ± 0.01	0.08 ± 0.01	0.11 ± 0.02	0.11 ± 0.02
κ_b (k_BT/nm^2)	0.29 ± 0.03	0.29 ± 0.03	0.4 ± 0.1	0.4 ± 0.1
$\Delta G^{\ddagger}_A$ (k_BT)	4.0 ± 0.1	3.7 ± 0.2	1.9 ± 0.3	2.0 ± 0.2
$\langle \delta q^2 \rangle$ (nm^2)	11 ± 2	8 ± 2	13 ± 2	14 ± 1
τ_A (μs)	40 ± 3	36 ± 3	32 ± 2	35 ± 1
k_A (s^{-1})	110 ± 10	100 ± 10	7 ± 2 × 10^2	7 ± 1 × 10^2
k_{MA} (s^{-1})	3.3 ± 0.3	3.3 ± 0.3	11 ± 5	11 ± 4
k_M (s^{-1})	4 ± 1	4 ± 1	16 ± 4	15 ± 4
D_q (×10^5 nm^2/s)	2.6 ± 0.2	2.2 ± 0.4	4.1 ± 0.5	4.0 ± 0.4
Constant position				
κ_{AW} (k_BT/nm^2)	0.10 ± 0.02	0.12 ± 0.02	0.08 ± 0.02	0.10 ± 0.01
κ_{Ab} (k_BT/nm^2)	0.11 ± 0.02	0.11± 0.02	0.18 ± 0.03	0.18 ± 0.03
$\Delta G^{\ddagger}_A$ (k_BT)	0.80 ± 0.05	0.73 ± 0.03	0.73 ± 0.06	0.68 ± 0.06
$\langle \delta q^2 \rangle$ (nm^2)	5.2 ± 0.1	5.1 ± 0.1	5.3 ± 0.2	5.5 ± 0.3
k_A (×10^3 s^{-1})	4.1 ± 0.5	4.7 ± 0.5	6 ± 1	6 ±1
τ_A (μs)	8.5 ± 0.3	8.7 ± 0.2	9.5 ± 0.04	9.5 ± 0.3
D_q (×10^5 nm^2/s)	6.1 ± 0.5	5.9 ± 0.4	5.6 ± 0.4	5.8 ± 0.4
τ_{tp} (μs)	27 ± 2	28 ± 2	22 ± 2	23 ± 2

Table S2: Parameters for hairpin 20TS06/T4 when 99.7% unfolded.

Constant force	folded	unfolded
κ_{Aw} (k_BT/nm^2)	0.037 ± 0.006	0.033 ± 0.003
κ_{Ab} (k_BT/nm^2)	0.04 ± 0.01	0.04 ± 0.01
κ_b (k_BT/nm^2)	0.4 ± 0.1	0.4 ± 0.1
$\Delta G^{\ddagger}_A$ (k_BT)	4.3 ± 0.2	0.18 ± 0.06
$\langle \delta q^2 \rangle$ (nm^2)	14 ± 1	14 ± 1
τ_A (μs)	54 ± 2	55 ± 2
D_q (nm^2/s)	2.6 ± 0.3 × 10^5	2.7 ± 0.3 × 10^5
k_A (s^{-1})	85 ± 5	5 ± 1 × 10^3
k_{MA} (s^{-1})	1.4 ± 0.2	290 ± 20
k_M (s^{-1})	1.5 ± 0.2	330 ± 20

References

1. Woodside, M.T., W.M. Behnke-Parks, K. Larizadeh, K. Travers, D. Herschlag, and S.M. Block. 2006. Nanomechanical measurements of the sequence-dependent folding landscapes of single nucleic acid hairpins. *Proc. Natl. Acad. Sci. USA*. 103:6190–6195.

2. Neupane, K., H. Yu, D.A.N. Foster, F. Wang, and M.T. Woodside. 2011. Single-molecule force spectroscopy of the add adenine riboswitch relates folding to regulatory mechanism. *Nucleic Acids Res*. 39:7677–7687.

3. Greenleaf, W.J., M.T. Woodside, E.A. Abbondanzieri, and S.M. Block. 2005. Passive all-optical force clamp for high-resolution laser trapping. *Phys. Rev. Lett*. 95:208102.

4. Neupane, K., D.B. Ritchie, H. Yu, D.A.N. Foster, F. Wang, and M.T. Woodside. 2012. Transition path times for nucleic acid folding determined from energy-landscape analysis of single-molecule trajectories. *Phys. Rev. Lett*. 109:68102.

5. Chung, H.S., J.M. Louis, and W.A. Eaton. 2009. Experimental determination of upper bound for transition path times in protein folding from single-molecule photon-by-photon trajectories. *Proc. Natl. Acad. Sci. USA*. 106: 11837–11844.

6. Neupane, K., D.A.N. Foster, D.R. Dee, H. Yu, F. Wang, and M.T. Woodside. 2016. Direct observation of transition paths during the folding of proteins and nucleic acids. *Science*. 352: 239–242.