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Supporting Material

Laser-Assisted Single-Molecule Refolding (LASR)

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Supplementary Methods

Determination of Activation Barrier from LASR experiments. To determine transition state parameters, we begin with the assumption that, during the temperature jump, the kissing complex (KC) is in equilibrium with an activated complex ($TS^{‡}$) to form the extended duplex, similarly to Eyring's Transition State theory:

$$KC \rightleftarrows [TS^{‡}] \rightarrow ED \quad \text{(Eq. 2)}$$

The equilibrium constant between the KC and $TS^{‡}$ can be defined as

$$K^{\pm} = \frac{\left[TS^{\pm}\right]}{\left[KC\right]} \quad \text{(Eq. 3)}$$

Once the reactant reaches across the transition state, it proceeds to form the extended duplex. Therefore, we can estimate $[TS^{‡}]$ as the fraction of molecules that form the extended duplex at a given jump temperature (f_{ED}), whereas [KC] can be estimated as the fraction of molecules that did not form the extended duplex (1 - f_{ED}). The equilibrium constant can be rewritten as:

$$K^{\pm} = \frac{f_{ED}}{1 - f_{ED}} \quad \text{(Eq. 4)}$$

Thus, (Eq. 4) can be rearranged as:

$$f_{ED} = \frac{K^{\pm}}{1 + K^{\pm}} \quad \text{(Eq. 5)}$$

From the LASR melting curves (Fig. 4), f_{ED} is determined experimentally as:

$$f_{ED} = \frac{f(T) - f_0}{f_{\max} - f_0} \quad \text{(Eq. 6)}$$

where $f(T)$ is the fraction reacted at a temperature T, f_0 is the fraction reacted at low temperature and f_{max} is the maximum fraction reacted. The fraction reacted, $f(T)$, can be expressed as:

$$f(T) = f_0 + (f_{\max} - f_0) f_{ED} = f_0 + (f_{\max} - f_0) \frac{K^{\pm}}{1 + K^{\pm}} \quad \text{(Eq. 7)}$$

The constant, $K^{‡}$, can be expressed in terms of transition state Gibbs free energy as

$$K^{\pm} = \exp\left(-\frac{\Delta G^{\pm}}{RT}\right) \quad \text{(Eq. 8)}$$

The temperature dependence of $\Delta G^{\ddagger}$ can be obtained using the Gibbs-Helmotz equation,(1)

$$\Delta G^{\pm} = \Delta H_r^{\pm}\left(1 - T/T_r\right) \quad \text{(Eq. 9)}$$

where $\Delta H_r^{\ddagger}$ is the activation energy barrier for the refolding reaction and T_r is the refolding temperature obtained using (Eq. 1). Finally, the fraction reacted during a LASR temperature jump, *f(T)*, can be expressed as

$$f(T) = f_0 + \left(f_{\max} - f_0\right)\frac{e^{-\frac{\Delta H_r^{\pm}}{RT}\left(1-T/T_r\right)}}{1 + e^{-\frac{\Delta H_r^{\pm}}{RT}\left(1-T/T_r\right)}} \quad \text{(Eq. 10)}$$

This expression was used to fit the LASR curves in Fig. 4 to determine the activation energy barrier of extended duplex formation. A similar expression can be derived for the dissociation reaction.

Determination of Activation Barrier Parameters by Eyring Analysis. The dissociation activation barrier can be obtained by measuring the temperature dependence of the kissing complex dissociation rate constants (k_{off}) and using Eyring analysis (2). The kinetic rate constant was obtained by fitting the distribution of dwell times in the kissing complex to a single exponential decay, as previously described (3). Using a microscope stage temperature controller, the rate constants k_{off} was determined at temperatures ranging from 15 to 23 ˚C. At higher temperatures, the number of molecules forming the kissing complex decreased dramatically. The resulting rate constants were linearized in an Eyring's plot (Supplementary Fig. 4) and fit to Eyring's equation):

$$\ln\left(\frac{k_{off}h}{k_B T}\right) = -\frac{\Delta H_d^{\pm}}{R}\frac{1}{T} + \frac{\Delta S_d^{\pm}}{R} \quad \text{(Eq 11)}$$

to obtain the activation energy barrier $\Delta H_d^{\ddagger}$.

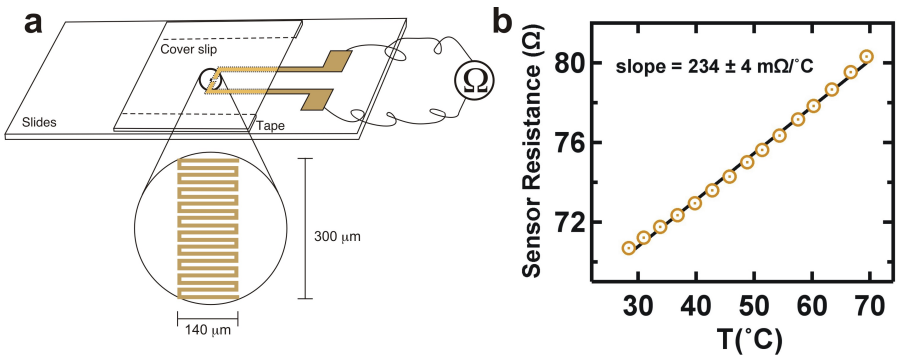
Supplementary Table 1. DNA and RNA sequences used in this study.

Name	Description	Sequence[1,2]
dsDNA1	Short duplex	5'-ATC TAT AAA AAT ATA GAT-3' 3'-TAG ATA TTT TTA TAT CTA-5'
dsDNA1'	Fluorophore labeled short duplex	5' Cy3-ATC TAT AAA AAT ATA GAT dT(Cy5)TTT-3' B 3'-TAG ATA TTT TTA TAT CTA-5'
dsDNA2	Intermediate duplex	5'-ATC ATC TCT CTC TAA GAT GAT-3' 3'-TAG TAG AGA GAG ATT CTA CTA-5'
dsDNA2'	Fluorophore labeled Intermediate duplex	5' Cy3-ATC ATC TCT CTC TAA GAT GAT dT(Cy5)TTT-3' B 3'-TAG TAG AGA GAG ATT CTA CTA-5'
dsDNA3	Long duplex	5'-ATT GCG ATA GAG AGA GAT CGC AAT-3' 3'-TAA CGC TAT CTC TCT CTA GCG TTA-5'
dsDNA3'	Fluorophore labeled Long duplex	5' Cy3-ATT GCG ATA GAG AGA GAT CGC AAT dT(Cy5)TTT-3' B 3'-TAA CGC TAT CTC TCT CTA GCG TTA-5'
HP1	RNA hairpin 1	5' B-AUA ACA AGG GGA AAU GCC UUG U-3' Cy3
HP2	RNA hairpin 2	5' Cy5-ACG AGG CAU UUC CCC UUG U-3'
HP3	RNA hairpin 3	5' B-AUA ACA AGG GG**C CC**U GCC UUG U-3' Cy3
HP4	RNA hairpin 4	5' Cy5-**UGU UC**G CAU UUC CC**G AGC A**-3'

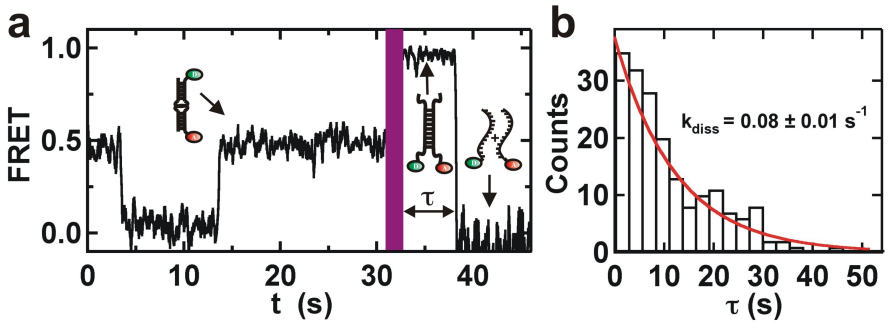
[1] B = biotin, Cy3 and Cy5 are linked to the nucleic acid by a 6-carbon amino linker.

[2] The underlined bases in HP1 and HP2 were also mutated to G and C, respectively.

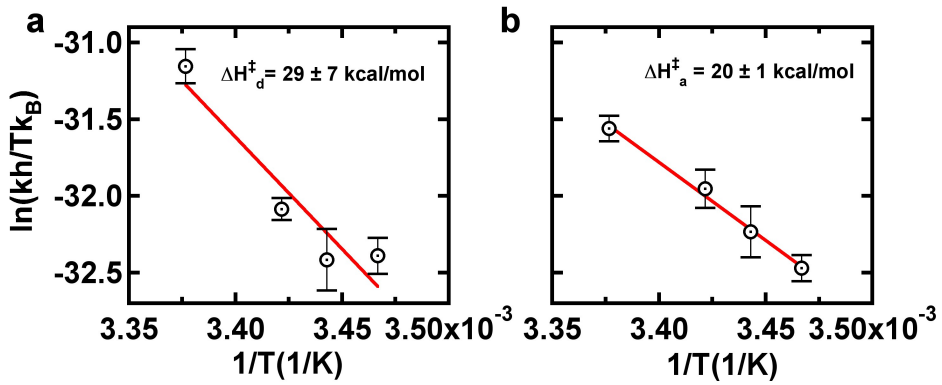
[2] Bold bases in HP3 and HP4 are modifications from HP1 and HP2, respectively.



Supplementary Figure 1. a. Micrometer-size gold sensor for temperature calibration. The gold micro-sensor is fabricated by depositing gold onto the masked pre-cleaned glass surface. A thin layer (<1mm) of polydimethylsiloxane (PDMS) film is deposit on the sensor to insulate. Circle shows the sensor with a size of 140 x 300 μm. **b. Calibration of the micro-sensor in a temperature-controlled oven.** Gold wire sensor was placed in a temperature-controlled oven, where the temperature was increased with a step of 3˚C with 5 minutes of equilibrating time. The resistance of the gold wire sensor is monitored using a multi-meter (Agilent).



Supplementary Figure 2. a. FRET trajectory of transient stable duplex formation and dissociation. τ is the dwell-time in the transient stable duplex state. **b**. Histogram distribution of τ. k_{off} is obtained by fitting the distribution to an exponential decay.



Supplementary Figure 3. a. Eyring analysis of the HP1 and HP2 kissing complex dissociation reaction. **b**. Eyring analysis of the HP1 and HP2 kissing complex association reaction.

Supplementary References

1. Stancik, A. L., and E. B. Brauns. 2008. Rearrangement of partially ordered stacked conformations contributes to the rugged energy landscape of a small RNA hairpin. Biochemistry 47:10834-10840.
2. Fiore, J., and D. Nesbitt. 2010. Personal communication.
3. Zhao, R., and D. Rueda. 2009. RNA folding dynamics by single-molecule fluorescence resonance energy transfer. Methods 49:112-117.