Single-molecule measurement of the effective temperature in non-equilibrium steady states

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Figure S1. Harmonic Oscillator System - (a) Verification of the equilibrium FDT. The main frame shows the response function \( \chi(t) \equiv (x(t) - x(0))/\delta f \) (dark red) and the correlation function \( C(t) \equiv (x(0) - x(t))x(0) \) (light red), while the inset depicts the corresponding parametric plot (red) together with the equilibrium FDT (grey). The size of the perturbation is \( \delta f \approx 7 \text{pN} \) and \( \delta \lambda \approx 1700 \text{nm} \). In both (a) and (e), the error bars show the standard errors of single bead response and correlation functions ((c), darker colours: their average). - (b) Contour plot of the logarithmic histogram of a stochastically driven trajectory with \( \Delta f \approx 0.7 \text{pN} \) and \( \tau_e = 100 \text{ms} \). The bead (grey dot) in average tends to relax towards the minimum of the harmonic potential. As the position of this minimum is permanently changed in a dichotomous fashion (vertical arrows), the bead in average shows clockwise motion in the \((x, f)\) plane leading to dissipation of heat. - (c) Results for single beads. In addition to the averages over several beads which we have shown in Fig. 2 of the main text, here, we give the results for some single beads (in the respective lighter colour). The experimental parameters are \( \Delta f \approx 0.7 \text{pN} \), \( \delta f \approx 1.0 \text{pN} \) and \( \tau_e = 67 \text{ms} \) (red), \( \tau_e = 100 \text{ms} \) (blue) and \( \tau_e = 333 \text{ms} \) (green). - (d) Corresponding parametric plots where the equilibrium FDT prediction is shown in grey.
S1. HARMONIC OSCILLATOR SYSTEM

A. Verification of the Equilibrium FDT

We have measured the response and correlation function in equilibrium to verify that the FDT is satisfied. Fig. S1a shows that the FDT is fulfilled well except for a small deviation in the short time regime which, however, covers the first ms of \( \chi \) and \( C \) only. Note that the overall good agreement with the equilibrium FDT also confirms the linearity of the response of the driven harmonic oscillator even for large perturbation sizes such as the value \( \delta f \approx 1.7 \text{ pN} \) used here.

B. Results for Single Beads

Figs. S1c and S1d demonstrate that the variability among different beads is small. Moreover, the parametric plots of single beads all show a linear regime if \( \tau_e < \tau_s \) and none if \( \tau_e > \tau_s \). In the former case, the variability among the beads therefore just leads to different estimates of the effective temperature. Its very existence is, however, demonstrated not only for the average but also at the level of single beads. Finally, we note that even though averaging data over several beads smooths the curves by decreasing the statistical error, single bead curves still are quite smooth.

C. Theoretical Model for Driven Harmonic Oscillator

For the Langevin dynamics of the driven harmonic oscillator described by equation (5) of the main text, the response

\[
\chi(t) \equiv \langle x(t) - x(0) \rangle / \delta f \tag{S1}
\]

of the bead position \( x \) to a step-like perturbation \( \delta f \) setting in at \( t = 0 \) is

\[
\chi(t) = \frac{1}{k} \left( 1 - e^{-t/\tau_s} \right), \tag{S2}
\]

with \( \tau_s = 1/\mu k \). The correlation function

\[
C(t) \equiv \langle (x(0) - x(t))x(0) \rangle \tag{S3}
\]
is evaluated over the unperturbed NESS and follows as

\[ C(t) = \frac{k_B T}{k} \left( 1 - e^{-t/\tau_s} \right) + \frac{2(\Delta f/k)^2}{\tau_e (1 - (2\tau_s/\tau_e)^2)} \left( \frac{\tau_e}{2} \left( 1 - e^{-2t/\tau_e} \right) - \tau_s \left( 1 - e^{-t/\tau_s} \right) \right). \]  (S4)

With these expressions, we find the parametric plot

\[ C(\chi) = k_B T \chi + \frac{2(\Delta f/k)^2}{\tau_e (1 - (2\tau_s/\tau_e)^2)} \left( \frac{\tau_e}{2} \left( 1 - (1 - k\chi)2\tau_s/\tau_e \right) - \tau_s k\chi \right) \]  (S5)

and its curvature

\[ \frac{\partial^2 C(\chi(t))}{\partial (\chi(t))^2} \sim \exp \left( -\frac{2(\tau_s/\tau_e - 1)t}{\tau_s} \right) \equiv \exp (-t/\tau_c) \]  (S6)

with

\[ \tau_c/\tau_s = \frac{1}{2(\tau_s/\tau_e - 1)}. \]  (S7)

For \( \tau_e \ll \tau_s, \tau_c \approx \tau_e/2. \) The effective temperature of this system is the slope of the parametric plot in the limit \( t \gg \tau_c \) where its curvature has become negligible, leading to

\[ \frac{T_{\text{eff}}}{T} = \frac{1}{k_B T} \left. \frac{\partial C(\chi(t))}{\partial \chi(t)} \right|_{t \gg \tau_c} \approx 1 + \frac{2\alpha\tau_s}{\tau_e(2\tau_s/\tau_e)^2 - 1}. \]  (S8)

with the scaled force amplitude \( \alpha = ((\Delta f^2)/k)/k_B T. \)

In the limit, where the external forces behave like thermal forces, i.e., for \( \alpha \to \infty \) and \( \tau_e \to 0 \) such that \( \alpha\tau_e \) remains constant, the effective temperature becomes

\[ \frac{T_{\text{eff}}}{T} \approx 1 + \frac{\alpha\tau_e}{2\tau_s}. \]  (S9)

If \( \tau_e \gg \tau_s \), the expression for the correlation function (S4) turns into

\[ C(t) \approx \frac{k_B T}{k} \left( 1 - e^{-t/\tau_s} \right) + (\Delta f/k)^2 \left( 1 - e^{-2t/\tau_e} \right). \]  (S10)

while the response (S2) remains the same. In this limit, the equilibrium FDT is restored for times \( t \ll \tau_e \) since

\[ C(t \ll \tau_e) \approx \frac{k_B T}{k} \left( 1 - e^{-t/\tau_s} \right) = k_B T \chi \quad (\tau_e \gg \tau_s). \]  (S11)
Figure S2. Harmonic Oscillator System - (a) Experimental results for the response $\chi$ (main frame) and the correlation function $C$ (inset) in the limit $\tau_e \gg \tau_s$. We show results for six single beads (various colours) and their average (violet) for the experimental parameters $\Delta f \simeq 0.9\,\text{pN}$, $\delta f \simeq 1.1\,\text{pN}$ and $\tau_e = 10\,\text{s}$. The error bars show the standard errors of single bead response and correlation functions (violet: their average). The theoretical prediction from the Langevin model is given in black. - (b) Corresponding parametric plots where the grey line represents the equilibrium FDT. As comparison, we include from Fig. 2c of the main text the experimental (dark green) and theoretical (black) parametric plot for $\Delta f \simeq 0.7\,\text{pN}$, $\delta f \simeq 1.0\,\text{pN}$ and $\tau_e = 333\,\text{ms}$.

To further illustrate this limit, we have experimentally measured the response $\chi$ and the correlation function $C$ for a large force switching time $\tau_e \gg \tau_s$ (see Fig. S2a). The corresponding parametric plot Fig. S2b demonstrates that in comparison to a more moderate $\tau_e \simeq \tau_s$, the system is noticeably closer to equilibrium in the short-time regime (i.e., for small $\chi$ and $C$). We point out that the larger variability between results for single beads is due to the large force switching time used which causes the system to relax more slowly into the stationary state. Since the trajectory cannot exceed a certain length to avoid a change of the experimental conditions over time, the statistics of $\chi$ and $C$ thus gets worse in the limit $\tau_e \gg \tau_s$. However, the statistics is still good enough to clearly resolve that the system is closer to equilibrium in the short-time regime.

Comparing Theory and Experiment We compare the predictions from the Langevin model with the experimental response and correlation functions shown in the main text (Fig. 2b and 2c therein) and the SI (Fig. S2a and S2b). For all four values of $\tau_e$ considered, the experimental results can be reproduced well by plugging the parameters listed in Table S1 in the respective theoretical expression for $\chi$ and $C$.

The difference between these parameters and their experimental counterparts is less than five percent in most cases, with the comparatively large deviation between the experimental $\tau_e = 67\,\text{ms}$
<table>
<thead>
<tr>
<th>( \tau_e ) [ms]</th>
<th>( \Delta \lambda ) [nm]</th>
<th>( k ) [pN/nm]</th>
<th>( \tau_s ) [ms]</th>
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<tr>
<td>experiment 67</td>
<td>163.5</td>
<td>( C : 0.00424 ) ( \chi : 0.00428 )</td>
<td>( \approx 200 - 250 )</td>
</tr>
<tr>
<td>theory: ( C )</td>
<td>55</td>
<td>0.00424</td>
<td>222</td>
</tr>
<tr>
<td>theory: ( \chi )</td>
<td>-</td>
<td>-</td>
<td>0.00440</td>
</tr>
<tr>
<td>experiment 100</td>
<td>162</td>
<td>( C : 0.00425 ) ( \chi : 0.00429 )</td>
<td>( \approx 200 - 250 )</td>
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<tr>
<td>theory: ( C )</td>
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<td>0.00425</td>
<td>226</td>
</tr>
<tr>
<td>theory: ( \chi )</td>
<td>-</td>
<td>-</td>
<td>0.00441</td>
</tr>
<tr>
<td>experiment 333</td>
<td>162.5</td>
<td>( C : 0.00423 ) ( \chi : 0.00414 )</td>
<td>( \approx 200 - 250 )</td>
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<tr>
<td>theory: ( C )</td>
<td>346</td>
<td>0.00423</td>
<td>219</td>
</tr>
<tr>
<td>theory: ( \chi )</td>
<td>-</td>
<td>-</td>
<td>0.00401</td>
</tr>
<tr>
<td>experiment 10 s</td>
<td>191.5</td>
<td>( C : 0.00481 ) ( \chi : 0.00473 )</td>
<td>( \approx 300 - 400 )</td>
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<tr>
<td>theory: ( C )</td>
<td>10.56 s</td>
<td>0.00481</td>
<td>298</td>
</tr>
<tr>
<td>theory: ( \chi )</td>
<td>-</td>
<td>-</td>
<td>0.00448</td>
</tr>
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</table>

Table S1. Comparing experimental and theoretical parameters for the driven harmonic oscillator. We show the values of the position amplitude \( \Delta \lambda \), the stiffness \( k \) and the system relaxation time \( \tau_s \) for experiments at four different position switching times \( \tau_e \). Among these parameters, we use \( \tau_s \) and \( \tau_e \) as fit parameters in equation (S4) for \( C \), and \( k \) and \( \tau_s \) as fit parameters in equation (S2) for \( \chi \). The experimental estimate of the system relaxation time \( \tau_s \) has been obtained from repeated measurements of the equilibrium force autocorrelation function.

and the theoretical \( \tau_e = 55 \text{ ms} \) as the sole exception. This deviation can be attributed to the fact that for fast switching, i.e., for small \( \tau_e \), the optical trap struggles to accurately follow the external protocol for its position. In these conditions, the experimental \( \tau_e \) can therefore be somewhat different from the externally chosen parameter.

S2. SHORT DNA HAIRPINS

A. Information on the Experimental Setup

In the hopping experiments with short hairpins, a single DNA hairpin is tethered between two polystyrene beads. One of them is confined in the optical trap and the other is subjected by air suction on the tip of a micropipette (Fig. S3). As explained in the main text, at forces similar to the critical force \( f_c \) of the hairpin, the folded and unfolded conformation coexist and the molecule
hops between the two states. In the constant-force ensemble, the optical trap is displaced using feedback in order to maintain the force constant during these transitions. The folding (unfolding) of the DNA hairpin is therefore observed as a decrease (increase) of the distance $\lambda$ of the optical trap with respect to the tip of the pipette. As shown in Fig. S3, this distance can be expressed as

$$\lambda = x_b + 2x_h + x,$$

where $x_b$ is the displacement of the bead from the centre of the optical trap, $x_h$ the extension of the 29 bp dsDNA handles and $x$ the extension of the unfolded hairpin (ssDNA). In the range of forces in which these experiments are performed ($12 - 17 \, \text{pN}$), $2x_h \approx x_0$ can be assumed to be constant and $x_b$ can be determined from $x_b = f/k$ (where $k$ is the stiffness of the optical trap and $f$ the measured force). Thus, the molecular extension $x$ follows from the experimental $\lambda$ and $f$ as

$$x = \lambda - f/k - x_0.$$
Figure S4. Short Hairpin Systems. - (a) Trace of the force (grey) and the molecular extension (orange) for the fast (top) and the slow (bottom) hopping hairpin in the stochastically driven NESS. The NESS is generated with $\tau_e = 50\text{ ms}$ and with $\Delta f = 1\text{ pN}$, $f_c = 15\text{ pN}$ for the fast hairpin and $\Delta f = 1.5\text{ pN}$, $f_c = 14\text{ pN}$ for the slow one. - (b) Verification of the equilibrium FDT for the slow hopping hairpin. The main frame shows the response function $\chi(t) \equiv \langle x(t) - x(0) \rangle / \delta f$ (dark red) and the correlation function $C(t) \equiv \langle (x(0) - x(t))x(0) \rangle$ (light red). The corresponding parametric plot (red) is depicted in the inset together with the equilibrium FDT (grey). Experiments use the protocol parameters $f_c = 14.7\text{ pN}$ and $\delta f = 0.5\text{ pN}$. The error bars show the standard error of the single molecule response and correlation function.

B. Verification of the Equilibrium FDT

In analogy to the harmonic oscillator system, we verify the FDT by measuring the response and correlation function in equilibrium which, for the DNA hairpin systems, corresponds to the application of a constant force (Fig. 3b and 4b of main text). The results for $\chi$ and $C$ of such an equilibrium experiment shown in Fig. S4b demonstrate that the FDT is fulfilled well across the whole time axis for our experimental setup.

C. Feedback

The force cannot be controlled directly in this experimental setup but is accessible to manipulation using feedback. Since this feedback operates on a finite time scale $\tau_f$, the change of force associated with transitions of the hairpin cannot be immediately compensated. In the following, we consider different quantities characterizing the force feedback to show that the feedback is nevertheless good enough not to compromise our observation of an effective temperature. We first note that the force autocorrelation function along the NESS is compatible with the theoretical prediction from equation (4) of the main text, although deviations are observed, which can be at-
Figure S5. Force feedback for the short (left) and the long hairpin (right). (a) Experimental (colours) and theoretical (black) force autocorrelation function along the NESS for the slow (red, \( \Delta f = 1.5 \text{ pN} \), \( \tau_e = 50 \text{ ms} \)) and the fast (green, \( \Delta f = 1.0 \text{ pN} \), \( \tau_e = 100 \text{ ms} \)) hopper and for the third hairpin type considered in Sec. S2 F (blue, \( \Delta f = 1.5 \text{ pN} \), \( \tau_e = 200 \text{ ms} \)). In both (a) and (c), the error bars show standard errors. - (c) Corresponding histograms of force switching times. - (e) Experimental mean force trajectory after a change of the target force (grey) from \( f_c - \Delta f \) to \( f_c + \Delta f \) and vice versa for the short hairpins (same colours as in (a)). The average is performed along the NESS. In the short-time regime, the mean force trajectories can be fitted to an exponential function (black) that converges on a time scale of around 5 ms. - (g) Force histograms for the short hairpins (same colours as in (a)). - (b, d, f, h) Corresponding plots for the long hairpin of 6.8kbp (red) where \( \Delta f = 1.6 \text{ pN} \) and \( \tau_e = 1.33 \text{ s} \).
tributed to mechanical resonances of the optical fibre and to the finite bandwidth of the feedback algorithm (Fig. S5a).

Second, we observe that the experimental histograms of the force switching times decay on the time scale $\tau_e$ for all but very small times, corresponding to a quick succession of two force transitions which the feedback cannot realize because of its finite operation time. This short-time regime is, however, small compared to both the time scales $\tau_e, \tau_s$ and, in particular, the large times for which we observe the effective temperature (Fig. S5b). We reproduce this observation from the perspective provided by the mean force trajectory after a change of the target force. This quantity illustrates that the feedback only needs several milliseconds to adjust the force to the new value determined by the external protocol (Fig. S5c). On the time scales $\tau_e$ of the external switching and $\tau_s$ of the system relaxation, the change of the force is therefore fast.

Finally, we consider the force histogram in the NESS as a complementary quantity to the above measures displaying the time scales of the feedback. This histogram shows that the force fluctuations are small compared to the force amplitude $\Delta f$ for all hairpins, thus demonstrating that the force feedback can resolve the external forcing generating the effective temperature (Fig. S5d).

Summing up, we have discussed different quantities characterizing the feedback which have shown that the feedback in general controls the force well except for times much shorter than $\tau_e, \tau_s$ and the times for which we observe the effective temperature. Further evidence that the feedback control of the force is sufficient is given by the good match we observe between experiment and theory for the response $\chi$ and the correlation function $C$ for all short hairpins (see Supplementary Sec. S2 E): Since the driven two-state model assumes perfect feedback, this good match suggests that the imperfection of the force feedback takes no significant effect on the measurement of $\chi$ and $C$.

### D. Results for Single Molecules

Fig. S6a and Fig. S6b show that the variability among different experiments is noticeably larger for the short hairpin experiments than it is for the harmonic oscillator (Sec. S1 B). In particular, the response functions for the slow short hairpin show large variability. Still, the finding that there is a linear regime in the parametric plot, i.e., an effective temperature, for $\tau_e < \tau_s$ but none for $\tau_e > \tau_s$ is recovered at the level of single molecules.
Figure S6. Results for single short and long hairpins. - For each, the fast short hairpin (a), the slow short hairpin (b) and the long hairpin (c), we show the response $\chi$ and the correlation function $C$ on the left hand side and the corresponding parametric plot $\chi(C)$ on the right hand side. In addition to the average over different molecules already presented in Figs. 3 and 4 of the main text (red), results for single molecules are given (various other colours). The error bars show the standard errors of single molecule response and correlation functions (red: their average). Theoretical predictions are displayed in black. In the parametric plots, we draw the equilibrium FDT prediction as comparison (grey).

E. Toy Model for a Driven Two-State System

For any applied force, the short hairpin can either be in the unfolded state $UF$ or in the folded state $F$. If one applies the stochastic driving where the force is changed in a dichotomous fashion
between two values \( \pm = f_c \pm \Delta f \), the system therefore has four states (Fig. 3a and 3c in main text). Following the notation of the main text, we refer to these states as \( \{ F^+, UF^+, F^-, UF^- \} \). The transitions between the states occur at rate \( 1/\tau_e \) if the force is changed and at the rate \( \omega_{F/UF}^{\pm} \) if the hairpin folds/unfolds at force \( \pm \).

The probability to be in state \( m \in \{1, 2, 3, 4 \} \equiv \{ F^+, UF^+, F^-, UF^- \} \) at time \( t \) is governed by the master equation\(^1\)

\[
\frac{\partial p_m(t)}{\partial t} = \sum_n L^0_{mn} p_n(t), \quad (S14)
\]

with

\[
L^0_{mn} = \begin{pmatrix}
-(1/\tau_e + \omega_{UF}^+) & \omega_F^+ & 1/\tau_e & 0 \\
\omega_{UF}^- & -(1/\tau_e + \omega_F^+) & 0 & 1/\tau_e \\
1/\tau_e & 0 & -(1/\tau_e + \omega_{UF}^-) & \omega_F^- \\
0 & 1/\tau_e & \omega_{UF}^- & -(1/\tau_e + \omega_F^-)
\end{pmatrix} \quad (S15)
\]

\[
\equiv k_{mn} - \delta_{mn} \sum_l k_{ml},
\]

where the rates \( k_{mn} \) comprise both the (un)folding rates \( \omega_{(U)F}^{\pm} \) and the force switching rate \( 1/\tau_e \).

With each state \( m \), we can associate a molecular extension according to

\[
x_1 = 0 \quad x_2 = x_{UF} \quad x_3 = 0 \quad x_4 = x_{UF}, \quad (S16)
\]

where \( x_{UF} \) equals the distance between the states \( F \) and \( UF \). The linear response of this molecular extension \( x \) to a stepwise force perturbation \( \delta f \) setting in at \( t = 0 \) is defined as in equation (S1) with

\[
\chi(t) \equiv \langle x(t) - x(0) \rangle / \delta f. \quad (S17)
\]

Exploiting the general theory of the response in a NESS developed in Ref. 1, \( \chi \) can be expressed by

\[
\chi(t) = \int_0^t \langle x(\Delta t)B(0) \rangle \, d\Delta t \quad (S18)
\]

as the time integral over a correlation function involving the variable \( B \) which is conjugate to the perturbation. The integral over time is necessary to change from the response to a delta-like perturbation used in Ref. 1 to the response to a stepwise perturbation considered here. We point out that \( B \) is the nonequilibrium steady state analogue to the equilibrium conjugate variable \( B^{eq} \).
which appears in the equilibrium FDT

\[ k_B T \chi^\text{eq}(t) = \int_0^t \partial_{t_1} \langle x(t_2) B^{\text{eq}}(t_1) \rangle \, dt_2 - t_1. \]  

(S19)

This quantity \( B^{\text{eq}} \) is conjugate to the perturbation \( f \) and takes the form

\[ B^{\text{eq}} = -\partial_f G \]  

(S20)

for our systems, where \( G \) is the free energy of the hairpin (see equation (S32)). In the NESS, the conjugate variable \( B \) has been shown to have different, yet equivalent representations all reproducing with the FDT (S18) the NESS response \( \chi \) of the system to a perturbation \( f \) (see Ref. 1). For the driven two state system, equation (S18) turns into

\[ \chi(t) = \int_0^t \sum_{mn} x_m \text{prob}(m, \Delta t | n, 0) B_{n} p_n^0 \, d\Delta t, \]  

(S21)

where \( p_n^0 \) is the stationary distribution and where \( \text{prob}(m, \Delta t | n, 0) \) is the conditional probability for the system to be in state \( m \) at time \( \Delta t \) provided that it was in state \( n \) at time \( t = 0 \). This probability can be calculated by solving the master equation (S14), leading to

\[ \chi(t) = \int_0^t \sum_{mn} x_m \left[ \exp(L^0 t) \right]_{mn} B_n p_n^0 \, d\Delta t. \]  

(S22)

The conjugate variable \( B \) can be written in the "Agarwal form" (among other equivalent representations, as pointed out above)

\[ B_n = \sum_l \left( p_l^0 / p_n^0 \right) k_l n_l - \sum_l k_{nl} n_{nl}, \]  

(S23)

with \( n_{nl} \equiv \partial_f \ln k_{nl} \) (with \( f \) being the perturbation). The response (S22) is compared to the correlation function defined in equation (S3) which takes the expression

\[ C(t) \equiv \langle (x(0) - x(t))x(0) \rangle = \sum_{mn} x_m \left( \delta_{mn} - \left[ \exp(L^0 t) \right]_{mn} \right) x_n p_n^0. \]  

(S24)
for the driven two-state system.

Both $\chi$ and $C$ can be simplified by expanding with

$$
B_n p_n^0 \equiv \sum_i c^{(i)}_\chi v_n^{(i)} \quad x_n p_n^0 \equiv \sum_i c^{(i)}_C v_n^{(i)} \quad n = 1, 2, 3, 4
$$

(S25)

in eigenstates $v^{(i)}$ of $L^0$ with eigenvalues $\lambda^{(i)}$. Then,

$$
\chi(t) = -\sum_{im} x_m v_m^{(i)} c^{(i)}_\chi (1 - \exp(\lambda^{(i)} t))/\lambda^{(i)},
$$

(S26)

$$
C(t) = \sum_{im} x_m v_m^{(i)} c^{(i)}_C (1 - \exp(\lambda^{(i)} t)).
$$

(S27)

For this system, apart from the trivial eigenvalue

$$
\lambda^{(0)} = 0
$$

(S28)

corresponding to the NESS, the three eigenvalues characterizing the relaxation in this NESS are

$$
\lambda^{(1)} = -2/\tau_e
$$

(S29)

and

$$
\lambda^{(2,3)} = -\left(1/\tau_e + (\omega_{UF}^+ + \omega_F^+ + \omega_{UF}^- + \omega_F^-)/2 \right)
\pm \left[1/\tau_e^2 + (\omega_{UF}^- - \omega_{UF}^+ + \omega_F^- - \omega_F^+)^2/4\right]^{1/2}.
$$

(S30)

The fact that $\tau_e$ is independent of the perturbation leads to $c^{(1)}_\chi = 0$. In contrast to the correlation function, the response therefore has no contribution from the time scale $\tau^{(1)} \equiv -1/\lambda^{(1)} = \tau_e/2$.

The time scales $\tau^{(2,3)} \equiv -1/\lambda^{(2,3)}$ differ from $\tau^{(1)}$ in that they reflect properties of the hairpin via the (un)folding rates $\omega_{(U|F)}$. Since $\tau^{(2)} < \tau^{(1)}$, the contribution of $\tau^{(2)}$ converges faster than the violation (curvature) term associated with $\tau^{(1)}$. Moreover, in the experimental conditions, this fast time scale $\tau^{(2)}$ can be neglected even in the short-time regime (Sec. S2 E below). In analogy to the harmonic oscillator system, the two relevant relaxation times of the driven two state system are $\tau^{(1)} = \tau_e/2$ and $\tau^{(3)} \equiv \tau_s$. We thus recover both, $\tau_e < \tau_s$ as the condition for an effective temperature, and $\tau_e$ from equation (S7) as the time scale beyond which the parametric plot $\chi(C)$ becomes linear.
The effective temperature of the driven two-state system then follows as

\[
T_{\text{eff}} / T = \frac{1}{k_B T} \frac{\partial C(\chi(t))}{\partial \chi(t)} \bigg|_{\tau \gg \tau_e} \approx \frac{c_C^{(3)}}{k_B T c_C^{(3)} \tau^{(3)}}. \tag{S31}
\]

This expression illustrates that the effective temperature only depends on the constants \( c_C^{(3)} \) and \( c_C^{(3)} \) and the time scale \( \tau^{(3)} \). Through equation (S25) and (S30), respectively, these quantities are connected with the molecular extension and the (un)folding rates of the hairpin, the change of these rates to a force perturbation and the driving parameters \( \Delta f \) and \( \tau_e \) of the external forcing. We stress that by replacing the observable ”molecular extension \( x_m \)” by an arbitrary other observable \( A_m \), the expression (S31) for the effective temperature remains the same. The effective temperature (S31) of the driven two-state system is thus independent of the choice of the observable.

**Comparing Theory and Experiment** On the level of single base pairs, the free energy of the short hairpin can be modelled as

\[
G(f, n) = G_{\text{DNA}}(n) - \int_0^f x(f', n) \, df'
\]

(S32)
given an applied force \( f \) and for the first \( n \) base pairs open (see equation (S47) below Sec. S3 D). The four-state model, however, describes the hairpin on a more coarse-grained level since it only distinguishes between the folded state \( F \) and the unfolded state \( UF \). We model the free energy of these two states as

\[
G_F = G_F^0 \quad \text{and} \quad G_{UF} = G_{UF}^0 - af x_{UF}
\]

(S33)

where \( G_{F/UF}^0 \) is the respective free energy at zero force. Here, we have exploited that in the folded state the hairpin has no molecular extension. We need to keep \( a \) as a fit parameter which, however, will turn out to take the naively expected value \( a = 1 \) for both equilibrium and the NESS of the fast hairpin.

We demand that detailed balance is fulfilled for the unfolding and folding rate at each applied force. In particular, detailed balance has to hold for the two forces \( \pm = f_c \pm \Delta f \) used in the stochastic driving, so that

\[
\frac{\omega_{UF}^{\pm}}{\omega_F^{\pm}} = \exp\left(-(G_{UF}^0 - G_F^0 - a(f_c \pm \Delta f)x_{UF})/k_B T\right)
\]

(S34)
Since experimentally the unfolded and folded state are almost equally populated when subject to the stochastic driving, we can assume that $f_c$ is the coexistence force of the hairpin so that $G_{UF}^0 - G_F^0 = af_c x_{UF}$ and

$$\frac{\omega_{UF}^+}{\omega_F^+} = \exp(\pm a \Delta f x_{UF}/k_B T). \tag{S35}$$

Moreover, these two states are equally populated for any value of the force switching time $\tau_e$ and the force amplitude $\Delta f$ only if

$$\omega_{UF}^+ = \omega_F^- \quad \text{and} \quad \omega_{UF}^- = \omega_F^+, \tag{S36}$$

which suggests that a symmetric splitting of the ratio in equation (S35) can be used. Hence, we model the experimental unfolding and folding rate at force ± by

$$\omega_{UF}^+ = k_0 \exp(\pm a \Delta f x_{UF}/2k_B T) \tag{S37}$$
$$\omega_F^\pm = k_0 \exp \left( \mp a \Delta f x_{UF}/2k_B T \right), \tag{S38}$$

with the hopping frequency at coexistence $k_0$.

Experimentally, the force amplitude $\Delta f$ is large so that

$$\omega_{UF}^+ \gg \omega_F^+ \quad \text{and} \quad \omega_{UF}^- \ll \omega_F^- \tag{S39}.$$

Referring to the fastest rates by

$$\omega^* \equiv \omega_{UF}^+ = \omega_F^- \tag{S40}.$$

the expressions (S26) for the response and (S27) for the correlation function become

$$\chi(t) = \frac{a x_{UF}^2}{2k_B T(2 + \tau_e \omega^*)} \left( 1 - \exp(-\omega^* t) \right) \tag{S41}$$

and

$$C(t) = \frac{x_{UF}^2}{4(4 - (\tau_e \omega^*)^2)} \left( 4(1 - \exp(-\omega^* t)) - (\tau_e \omega^*)^2 (1 - \exp(-2t/\tau_e)) \right) \tag{S42}$$

in the experimentally relevant regime of $\tau_e$ in the order of $1/\omega^*$ and smaller. Fitting these expressions to the experimental response and correlation functions gives $\omega^* \approx 8.2 \text{ s}^{-1}$, $a \approx 0.68$ and $x_{UF} \approx 18.1 \text{ nm}$ for the slow hopper (red curve in Fig. 3d in the main text) and $\omega^* \approx 20.7 \text{ s}^{-1}$, $a \approx 1.03$ and $x_{UF} \approx 17.8 \text{ nm}$ for the fast one (blue curve in the same figure). The relaxation time
of the response is $\tau_s \equiv 1/\omega^* \simeq 120 \text{ ms}$ for the slow and $\tau_s \simeq 50 \text{ ms}$ for the fast hairpin. Finally, we note that the effective temperature of the driven two-state system is

$$T_{\text{eff}}/T = \frac{1}{a(1 - \tau_e \omega^*/2)}$$

(S43)

under the experimental conditions, as follows by evaluating the general expression (S31) with the response (S41) and the correlation function (S42).

**F. Further Single-Molecule Measurements of the Effective Temperature for Short Hairpins**

In addition to the short hairpin experiments presented in Fig. 3 of the main text, we have performed further measurements with another molecule type which has the same hairpin sequence as the slow hopper but a larger loop, leading to a slower hopping kinetics. As a result, the system relaxation time of this molecule has increased to $\tau_s \simeq 400 - 500 \text{ ms}$ which has allowed us to vary the force switching time $\tau_e$ more widely while still matching the effective temperature condition $\tau_e < \tau_s$. In particular, we have increased the force switching time to $\tau_e = 200 \text{ ms}$ to restore the ratio $\tau_e/\tau_s \simeq 0.4$ that we have observed for the original slow hopper with a smaller loop. The experiment then confirms well the theoretical prediction (see equation (S41) and (S42)) that using the same ratio $\tau_e/\tau_s$ leaves the parametric plot unchanged even though we have considered a different molecule with a different system relaxation time $\tau_s$ which has been driven with a different force switching time $\tau_e$ (see Fig. S7a and S7b). For a smaller $\tau_e/\tau_s$, the experiment clearly shows that the effective temperature approaches room temperature, as is predicted by the four-state model (see equation (S43)). This result can be intuitively understood to reflect how for smaller $\tau_e/\tau_s$ the hairpin is driven more weakly and is thus closer to equilibrium.

Finally, we point out that some of the parametric plots of single molecules shown in Fig. S7d and S7f tend to be a bit noisy since molecules break after a while so that on some occasions we could not collect long traces for both the correlation function and the response for the same molecule. However, for all molecules, at least a long NESS or a long perturbed NESS trace could be measured, so that reliable single-molecule estimates of the response and the correlation function are available, as is demonstrated by Fig. S7c and S7e.
Figure S7. Further single-molecule measurements of $T_{\text{eff}}$. - (a) For the slow hopper with a larger loop, the response $\chi$ and the correlation function $C$ (inset) is shown for $f_c \simeq 12.8$ pN, $\Delta f = 1.5$ pN and $\tau_e = 50$ ms (green) and $\tau_e = 200$ ms (blue). The relaxation time is $\tau_s \simeq 400 - 500$ ms for both $\tau_e$. - Panel (b) shows the corresponding parametric plots. In both (a) and (b), an average over five (four) molecules for $\chi$ and six (five) molecules for $C$ for the condition $\tau_e = 50$ ms ($\tau_e = 200$ ms) is displayed. Theoretical results with $\omega^* \simeq 2.5$ s$^{-1}$, $a \simeq 0.80$, $x_{UF} \simeq 18.0$ nm ($\omega^* \simeq 1.9$ s$^{-1}$, $a \simeq 0.62$, $x_{UF} \simeq 19.1$ nm) for $\tau_e = 50$ ms ($\tau_e = 200$ ms) are shown in black. In red, we include from Fig. 3e of the main text the parametric plot for the slow hopper with a smaller loop with $\tau_e = 50$ ms and $\tau_s \simeq 120$ ms. - For $\tau_e = 50$ ms ($\tau_e = 200$ ms), panels (c) and (d) (e) and (f) give the results for single molecules (various colours) together with their average (same colour as in (a)). The error bars show the standard errors of single molecule response and correlation functions ((a): their average). In all parametric plots, the grey line represents the equilibrium FDT.
S3. LONG DNA HAIRPIN

A. Details about the Experimental Setup

In the long hairpin experiments, a DNA hairpin of 6848 base pairs is used (\sim 300-fold longer than the short constructs). As illustrated in the main text, intermediate states with a given number of open base pairs \( n \) \( (0 < n < 6848) \) are observed for this hairpin. The experimental unfolding setup is the same for the short and long hairpins and the distance \( \lambda \) of the optical trap to the pipette is

\[
\lambda = x_b + 2x_h + x, \tag{S44}
\]

with the displacement \( x_b \) of the bead from the centre of the optical trap, the extension \( x_h \) of the 29 bp dsDNA handles and the extension \( x \) of ssDNA released during the unzipping process (Fig. S8). In the experimental force range \((14 - 19 \text{ pN})\), \( 2x_h \simeq x_b \) is approximately constant and the trap is harmonic so that \( x_b = f/k \) with the trap stiffness \( k \) and the measured force \( f \). Thus, for every \( \lambda \) and \( f \), the molecular extension \( x \) can be obtained with

\[
x = \lambda - f/k - x_b. \tag{S45}
\]
Finally, for every data point \((x, f)\) determined in this way, one can associate the number of open base pairs \(n\) by modelling the released single stranded DNA with the freely jointed chain model using the generally accepted parameters for ssDNA\(^2\).

**B. Feedback**

For the long hairpin, the control of the force via feedback is not as accurate as for the short hairpin systems. While the full opening and closing of the short hairpins implies \(\Delta \lambda < 20\text{ nm}\), transitions of the long hairpin mean changes of the trap position in the order of \(\mu\text{m}\). Thus, the feedback needs more time to compensate the force jumps connected to the unfolding and folding of the long hairpin.

However, in analogy to the short hairpin systems, the feedback is fast enough with respect to the relevant system time scales \(\tau_e\) and \(\tau_s\). Again, we support this claim by considering, as experimental quantities characterizing the feedback, the force autocorrelation function in the NESS (Fig. S5e), the histogram of force switching times (Fig. S5f) and the mean force trajectory after a change of the target force (Fig. S5g). Similarly to what has been observed for the short hairpins, we note that the experimental force correlation deviates somewhat from the theoretical prediction due to the same reasons discussed in Supplementary Sec. S2 C. The histogram of force switching times on the other hand decays on the time scale \(\tau_e\) as expected, except in the regime of very short times. These times are, however, much smaller than \(\tau_e, \tau_s\) and the times on which we observe the effective temperature. Finally, as for the short hairpins, we note that at short times, the mean force trajectory approximately relaxes on a single time scale (\(\simeq 20\text{ ms}\)) much smaller than \(\tau_e\) and \(\tau_s\).

Overall, both the mean force trajectory and the histogram of force switching times thus confirm the earlier statement that the feedback works well on the relevant time scales of the experiment.

As a further quantity characterizing the feedback, the experimental force histogram along the NESS shows that while the force fluctuations are a bit larger than in the case of the short hairpins, they are still small compared to the force amplitude \(\Delta f\), reflecting that the feedback can resolve the external driving for the long hairpin as well (Fig. S5h).

Finally, we note that in analogy to the short hairpins, the good agreement between experiment and theory observed for the response \(\chi\) and the correlation function \(C\) of the long hairpin (see Supplementary Sec. S3 D) suggests that the imperfection of the force feedback does not significantly affect the experimental \(\chi\) and \(C\).
C. Results for Single Molecules

For the long hairpin, the variability among different molecules is small, especially considering the complexity of the hairpin (Fig. S6c). In particular, all single molecules clearly show a linear regime in their parametric plot, thus demonstrating the existence of an effective temperature at the level of single experiments as well. Moreover, even the value of this effective temperature is similar for different molecules.

D. Theoretical Model for Base-pairwise Dynamics

The base pair interactions of the hairpin are described with a nearest neighbour model with energies previously measured in DNA unfolding experiments\(^3\). For a given sequence of base pairs, these energies add up to the free energy \(G_{\text{DNA}}(n)\) needed to break the first \(n\) base pairs open. However, this free energy does not include the stretching of the single stranded DNA which is released in this process. If the force \(f\) is applied, this second contribution to the total free energy can be obtained from the molecular extension \(x\) as

\[
G_s(f, n) = -\int_0^f x(f', n) \, df'.
\]  
(S46)

The total free energy \(G(f, n)\) of the DNA hairpin at the applied force \(f\) and with the first \(n\) base pairs open follows as\(^3\)

\[
G(f, n) = G_{\text{DNA}}(n) + G_s(f, n).
\]  
(S47)

The stochastically driven dynamics of the long hairpin can be modelled as a Markov process within this free energy profile with rates \(\omega_{nn\pm 1}\) to break or close one base pair if \(n\) base pairs are open. For these rates, we use an asymmetric splitting of Kramers type rates as suggested in Ref. 4

\[
\omega_{nn+1} = k_0 \exp\left( -\frac{G_{\text{DNA}}(n+1) - G_{\text{DNA}}(n)}{k_B T} \right)
\]  
(S48)

\[
\omega_{nn-1} = k_0 \exp\left( -\frac{G_s(f, n-1) - G_s(f, n)}{k_B T} \right)
\]  
(S49)

with the attempt frequency \(k_0\).

We have taken into account that experimentally the force baseline can change along the \(x\) axis. Specifically, we observe that the actual applied force can vary by a few 0.1 pN across a distance...
of several μm. In the simulations, these conditions are included by adding an offset to the force decreasing linearly from zero at the most zipped state of the dynamics \((n \simeq 2000)\) to some minimal value \(f_{\text{off}}\) at the fully unfolded state \((n \simeq 6800)\). Finally, we point out that we have neglected the contribution from the loop which brings in an entropic barrier that has to be overcome to close the first base pair when the hairpin is fully unfolded. In the experiments, single base pair transitions cannot be resolved, though. Instead, we observe transitions in which many base pairs break or open at once\(^5\). On this more coarse-grained level, the entropic barrier caused by the loop is smoothed out so that it can be expected to have little effect on the dynamics.

**Comparing Theory and Experiment** For the simulations shown in Fig. 4c and 4d of the main text, we have used \(f_{\text{off}} = -0.35\) pN and \(k_0 = 6.1 \cdot 10^5\) Hz. All theoretical parameters but \(f_c\), i.e., \(\tau_c\), \(\Delta f\) and \(\delta f\), take the respective experimental value. The theoretical mean force \(f_c\) had to be adjusted somewhat since we observe that the experimental mean unzipping force of the FDC can change by up to 1 pN between molecules of the same type. In order to similarly populate the states of the hairpin for different molecules, the experimental mean force was always set \(0.1 - 0.2\) pN larger than the respective mean unzipping force. Theoretically, the mean unzipping force is around 16.9 pN, and therefore, the experimental conditions have been reproduced using \(f_c = 17.0\) pN in the simulations.

**S4. COUPLING A HARMONIC OSCILLATOR TO THE SYSTEM**

**A. The Oscillator Temperature**

Following Cagliano et al.\(^6\), we measure the temperature of the stochastically driven system by coupling a harmonic oscillator to it. Assuming the coupling is weak and linear and exploiting the equipartition theorem, Cagliano et al.\(^6\) found that an oscillator with resonance frequency \(\omega\) measures the temperature

\[
\bar{T}_{\text{eff}}(\omega) \equiv \frac{\omega \dot{\tilde{C}}''(\omega)}{k_B \tilde{R}(\omega)}. \tag{S50}
\]

Here, we have introduced the Fourier transforms

\[
\tilde{R}(\omega) \equiv \text{Im} \left( \int_0^\infty R(t) \exp(i\omega t) \, dt \right) \tag{S51}
\]
and
\[ \tilde{C}'(\omega) \equiv \text{Re} \left( \int_{0}^{\infty} C'(t) \exp(i\omega t) \, dt \right) \]  
(S52)

of the response
\[ R(t) \equiv \frac{\delta \langle x \rangle(t)}{\delta f(0)} \]  
(S53)

of the system to a delta-like perturbation \( f \) at time \( t = 0 \) and of the correlation function
\[ C'(t) \equiv \langle (x(t) - \langle x \rangle)(x(0) - \langle x \rangle) \rangle. \]  
(S54)

In the following, we will compare this temperature \( \tilde{T}_{\text{eff}}(\omega) \) with the effective temperature \( T_{\text{eff}} \) which the quasi-FDT predicts for large enough times. In the frequency domain, large times roughly correspond to small frequencies. Therefore, we expect that \( \tilde{T}_{\text{eff}}(\omega) \) and \( T_{\text{eff}} \) coincide for small \( \omega \). Since according to the FDT, the system equilibrates at room temperature \( T \) in the short time regime, we moreover expect to obtain \( T \) for large enough \( \omega \).

We present plots of both \( \tilde{R}(\omega) \) and \( \omega \tilde{C}'(\omega) \) at the experimental conditions for all of our systems (Fig. S9). These plots show that at small frequencies, \( \tilde{T}_{\text{eff}}(\omega) \), i.e., the ratio of \( \omega \tilde{C}'(\omega) \) and \( k_B \tilde{R}(\omega) \), is almost constant. Equivalently, we observe that the parametric plots \( \tilde{R}(\omega \tilde{C}'(\omega)) \) show a linear regime at small frequencies in analogy to the linear regime which the corresponding parametric plot \( \chi(C) \) shows at large times. This analogy breaks down, however, for force switching times \( \tau_e \) larger than \( \tau_s \): In the frequency domain, a linear regime then still exists (data not shown) whereas in the time domain it does not.

As a consequence, there is strictly speaking no crossover frequency (indicating the breakdown of the concept of an effective temperature) which is analogous to the crossover time scale \( \tau_c \) since \( \tau_e \) diverges as \( \tau_e \) approaches \( \tau_s \). Finding the frequency ranges corresponding to the time ranges \( t \ll \tau_c \) and \( t \gg \tau_c \) for which the quasi-FDT predicts room temperature and the effective temperature, respectively, is thus not straightforward. Roughly, however, we can use the limits \( \omega \gg 1/\tau_e, 1/\tau_s \) and \( \omega \ll 1/\tau_e, 1/\tau_s \). In the following, these limits will be discussed for the harmonic oscillator and the different hairpin systems using both experimental results and theoretical predictions for the respective model system.
Figure S9. Coupling a harmonic oscillator to the three stochastically driven systems. For each, the harmonic oscillator (a), the slow short hairpin (b) and the long hairpin (c), we present the experimental $R(\omega)$ (red) and $\omega \tilde{C}'(\omega)/k_B T$ (blue) on the left hand side and the experimental parametric plot $\tilde{R}(\omega \tilde{C}'/k_B T)$ (green) on the right hand side. The respective theoretical prediction is displayed in black. As a comparison, the equilibrium FDT prediction is shown in the parametric plots (grey). For each system, we give the theoretical (black) and experimental (green) value of the effective temperature $T_{\text{eff}}$ and the temperature $\tilde{T}_{\text{eff}} \equiv \tilde{T}_{\text{eff}}(\omega \ll 1/\tau_c, 1/\tau_s)$ which an oscillator with small resonance frequency would measure. The experimental parameters are $\Delta f \approx 0.7$ pN, $\delta f \approx 1.0$ pN and $\tau_c = 100$ ms for the harmonic oscillator, $\Delta f = 1.5$ pN, $\delta f = 0.5$ pN and $\tau_c = 50$ ms for the slow short hairpin and $\Delta f = 1.6$ pN, $\delta f = 0.5$ pN and $\tau_c = 1.33$ s for the long hairpin. Note that for the short hairpin, the large frequency regime of the parametric plot has been cut off since it shows strong fluctuations. We also point out that we have added zeros to the ends of the experimental and simulated time signals $R(t)$ and $C'(t)$ before calculating their Fourier transforms in order to make the low frequency regime better visible. This zero-padding is possible here since both $R(t)$ and $C'(t)$ decay almost entirely within the observed time span.
B. Harmonic Oscillator

For the driven harmonic oscillator, the Langevin model introduced in equation (5) of the main text gives

$$\tilde{R}(\omega) = \frac{\mu \omega \tau_s^2}{1 + (\omega \tau_s)^2}$$  \hspace{1cm} (S55)

and

$$\omega \tilde{C}^\prime(\omega)/k_B T = \tilde{R}(\omega) + \frac{2\alpha \tau_s/\tau_e}{(2\tau_s/\tau_e)^2 - 1} \left( \tilde{R}(\omega) - \frac{\mu \omega (\tau_e/2)^2}{1 + (\omega \tau_e/2)^2} \right).$$  \hspace{1cm} (S56)

The temperature measured by an oscillator with resonance frequency $\omega$ follows as

$$\tilde{T}_{\text{eff}}(\omega)/T = \frac{\omega \tilde{C}^\prime(\omega)/k_B T}{\tilde{R}(\omega)} = 1 + \frac{2\alpha \tau_s/\tau_e}{(2\tau_s/\tau_e)^2 - 1} \left( 1 - \left( \frac{\tau_e}{2\tau_s} \right)^2 \frac{1 + (\omega \tau_e)^2}{1 + (\omega \tau_s/2)^2} \right).$$  \hspace{1cm} (S57)

In the limits $\omega \gg 1/\tau_e, 1/\tau_s$ and $\omega \ll 1/\tau_e, 1/\tau_s$, we find

$$\tilde{T}_{\text{eff}}(\omega \gg 1/\tau_e, 1/\tau_s)/T \approx 1$$  \hspace{1cm} (S58)

and

$$\tilde{T}_{\text{eff}}(\omega \ll 1/\tau_e, 1/\tau_s)/T \approx 1 + \frac{2\alpha \tau_s/\tau_e}{(2\tau_s/\tau_e)^2 - 1} \left( 1 - \left( \frac{\tau_e}{2\tau_s} \right)^2 \right) = 1 + \frac{\alpha \tau_e}{2\tau_s},$$  \hspace{1cm} (S59)

respectively. Hence, while at large frequencies the oscillator measures room temperature as expected, the temperature at small frequencies is different from the expression derived in equation (S8)

$$T_{\text{eff}}/T \approx 1 + \frac{2\alpha \tau_s/\tau_e}{(2\tau_s/\tau_e)^2 - 1}.$$  \hspace{1cm} (S60)

In fact, these two temperatures are strictly the same only if $\tau_e \ll \tau_s$. However, their deviation is still small for larger force switching times $\tau_e < \tau_s$ outside this limit. This statement is illustrated by Fig. S9a which demonstrates that for both, theory and experiment, the difference between $\tilde{T}_{\text{eff}}(\omega \ll 1/\tau_e, 1/\tau_s)$ and $T_{\text{eff}}$ is just around six percent even for a force switching time $\tau_e \simeq \tau_s/2$.

On a side note we point out that the discrepancy between the experimental and the theoretical estimate of $\omega \tilde{C}''(\omega)$ visible in Fig. S9a is caused by the finite experimental time resolution which at large frequencies leads to errors in $\tilde{C}''(\omega)$. 

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C. Short Hairpins

As a toy model for the short hairpins, the driven two state system discussed in Sec. S2 E predicts that
\[ \tilde{R}(\omega) = \frac{a x_{UF}^2}{2 k_B T (2 + \tau_e/\tau_s) 1 + (\omega \tau_s)^2} \]  
(S61)

and
\[ \omega \tilde{C}'(\omega)/k_B T = \frac{x_{UF}^2}{4 k_B T (4 - (\tau_e/\tau_s)^2)} \left( \frac{4 \omega \tau_s}{1 + (\omega \tau_s)^2} \left( \frac{\tau_e}{\tau_s} \right)^2 - \frac{\omega \tau_e/2}{1 + (\omega \tau_e/2)^2} \right) \]  
(S62)

which leads to the oscillator temperature
\[ \tilde{T}_{\text{eff}}(\omega)/T = \frac{\omega \tilde{C}'(\omega)/k_B T}{\tilde{R}(\omega)} = \frac{1}{a(1 - \tau_e/2\tau_s)} \left( 1 - \left( \frac{\tau_e}{2\tau_s} \right)^3 \frac{1 + (\omega \tau_s)^2}{1 + (\omega \tau_e/2)^2} \right). \]  
(S63)

In analogy to the driven harmonic oscillator, we consider the limits
\[ \tilde{T}_{\text{eff}}(\omega \gg 1/\tau_e, 1/\tau_s)/T \approx 1/a \]  
(S64)

and
\[ \tilde{T}_{\text{eff}}(\omega \ll 1/\tau_e, 1/\tau_s)/T = T_{\text{eff}} \left( 1 - \left( \frac{\tau_e}{2\tau_s} \right)^3 \right). \]  
(S65)

We note that an oscillator with a large resonance frequency reproduces the short-time limit of the FDT. For small resonance frequencies, the oscillator measures a temperature which is slightly different from the effective temperature. This deviation is, however, even smaller than in the case of the harmonic oscillator. This statement is exemplified by Fig. S9b which on the theory side shows good agreement between the effective temperature and the oscillator temperature at small resonance frequencies. The agreement is less good for the experimental data which is, however, quite noisy in the frequency domain leading to a rather inaccurate estimate of the oscillator temperature.

D. Long Hairpin

For the long hairpin, the theoretical predictions are given by simulations rather than by analytical expressions. Since these simulations operate with a finite time resolution like the experiments...
do, the theoretical estimate for $\omega \tilde{C}'(\omega)$ is affected by errors for large frequencies as is the experimental one. These estimates are good enough, however, to suggest that $\tilde{T}_{\text{eff}}(\omega)$ becomes the room temperature $T$ at large $\omega$ (corresponding to the upper branch for small $\omega \tilde{C}'(\omega)$ in Fig. S9c). For small $\omega$ (lower branch for small $\omega \tilde{C}'(\omega)$ in Fig. S9c), we find little difference between the effective temperature and the oscillator temperature in both theory and experiment.