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Supplemental Information

Overstretching Double-Stranded RNA, Double-Stranded DNA, and RNA-DNA Duplexes

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I. FITTING THE MEASURED FORCE-EXTENSION RELATIONS

In figure S4, we present average values and mean-square deviations of measured force-displacement curves for the four different constructs. The part below the overstretching plateau is fitted to the twistable worm-like chain model. The fit function is shown as a blue solid line. It relates the imposed displacement to the measured force F. The displacement equals the sum of the length x of the molecule and the shifts F/k_{trap} of the beads compared to their equilibrium positions in the optical traps. The length of the molecule x is the product of the number of base pairs N_b ($N_b = 4050$ for our constructs) and the length per base pair l_{ds}. The latter is given by the analytical expression [1, 2]

$$l_{ds}(F) = L_c^{ds}\left(1 - 1/2\sqrt{\frac{k_B T}{F L_p}} + \frac{F}{K - g(F)^2/C}\right), \tag{1}$$

where L_c^{ds}, L_p and K are respectively the crystallographic length per base pair, the persistence length and the stretch modulus per base pair of the nucleic acid (NA) duplex. The twist-stretch coupling is parametrized by the twist rigidity C and the function $g(F)$. The latter is described by

$$g(F) = \begin{cases} g_0 + g_1 F_c & \text{for} \quad F \leq F_c \\ g_0 + g_1 F & \text{for} \quad F > F_c \end{cases}, \tag{2}$$

with three parameters, g_0, g_1 and F_c. The parameters used to describe the average force-extension relations are presented in Table S1.

Although we measured a significant number of force-displacement relations for each of the four NA duplexes, the experimental data do not allow determining the seven parameters in a unique way. The parameter set shown in Table S1 is consistent with the information available from the literature. Our lengths L_c^{ds} and L_p agree with published values for dsDNA and dsRNA [3–7]. The parameters K, C, g_0, g_1 and F_c affect the shape of the force-extension curve at high force. For the twist rigidity C of all duplexes, we take a value reported in the literature for dsDNA [1, 8]. Moreover we assume a common critical force F_c that is close to the value published for dsDNA [1]. Our parameter set displays the reported opposite sign of the twist-stretch-coupling value $g_0 - g_1 F_c$ for dsDNA as compared to dsRNA [9–13]. We did not find corresponding literature information for hetero-duplexes.

II. THEORETICAL DESCRIPTION OF OVERSTRETCHING

A. Peeling

The force-induced peeling phenomenon can be described by a conversion of a double stranded nucleic acid into two single strands, only one of which stays under tension. This transition implies rupture of hydrogen bonds, modified stacking interactions as well as a change in elastic energy of the molecular construct. In free energy terms this process will have the following representation:

$$E(F) = E_b + E_{ss} - E_{ds} - F\,(l_{ss} - l_{ds}) \tag{3}$$

Let us consider each energy term separately.

1. E_b term

The base pair binding energies E_b are phenomenological free energies for opening a base pair of the duplex. As such, they contain both enthalpic and entropic contributions and are sequence-dependent. Neglecting sequence heterogeneity and assuming a GC-content of 50 % (the average GC-content of our constructs is 52 %), we simply use an arithmetic average of the ΔG^0_{37} values reported for the different base pairs in the nearest-neighbor models of the literature. For 1M monovalent salt and no divalent salt, we thus obtain $E_b = 2.30$ for dsDNA from SantaLucia [14], $E_b = 3.33$ for dsRNA from Xia et al [15] and $E_b = 2.38$ for the heteroduplex from Sugimoto et al [16], with energies expressed in units of k_BT at the sample temperature of 306 °K.

The variation of E_b with monovalent salt concentration was estimated using the DNA formula available from the literature [14]. We find that all E_b values decrease by about 0.6 k_BT when the salt concentration decreases from 1M to 150 mM. A rough estimate of the variation with divalent salt was also performed, using the approach proposed by Qi et al [17]. We thus find an increase by about 0.8 k_BT when the salt conditions change from 150 mM monovalent salt to 150 mM monovalent salt plus 50 mM divalent salt. For the sake of simplicity, we use the E_b values corresponding to 1M monovalent salt and no divalent salt for all calculations presented in this paper.

E_b also depends on temperature. The values presented in main text Table 1 correspond to

the sample temperature of T=33°C. At 25°C, we have 2.42, 3.48 and 2.52 k_BT for dsDNA, dsRNA and the hybrids, respectively. At 37°C, the series reads 2.05, 3.08 and 2.10 k_BT. To roughly estimate the corresponding change in the transition force F_t, we extract a factor of about 17 pN/k_BT from the slopes of the $E(F)$ relations for peeling (main text Fig.7). F_t is thus predicted to decrease by almost 7 pN for a temperature increase from 25 to 37°C. Moreover, we estimated the change in F_t caused by temperature-dependence of NA elasticity. This temperature effect is of opposite sign, but much smaller in magnitude. Temperature-dependence of NA elasticity causes increases in F_t of less than 1 pN when temperature increases from 25 to 37°C. The temperature dependence of E_t is thus dominated by the influence of E_b.

2. E_{ss} term

E_{ss} denotes the energy per nucleotide required to stretch a single-stranded nucleic acid (ssNA) from zero-force to a force F. We theoretically describe the elasticity of the ssNA by the worm-like chain model [2],

$$l_{ss}(F) = L_c^{ss}\left(1 - 1/2\sqrt{\frac{k_BT}{FL_p}}\right). \tag{4}$$

We use $L_c^{ss} = 0.70$ nm and $L_c^{ss} = 0.65$ nm for the crystallographic length per nucleotide of ssDNA and ssRNA, respectively. The persistence lengths are $L_p = 1.20$ nm for ssDNA and $L_p = 1.37$ nm for ssRNA. These values were obtained by fitting force-displacement measurements of DNA and RNA hairpin structures [18]. Integration of Eq.4 leads to the energy E_{ss},

$$E_{ss}(F) = F\, l_{ss}(F) - \int_0^F l_{ss}(f)\, df = \frac{L_c^{ss}}{2}\sqrt{\frac{k_BT}{L_p}F}.$$

3. E_{ds} term

E_{ds} is the energy per base pair required to stretch a double-stranded nucleic acid (dsNA) from zero-force to a force F. It is obtained by analytical integration of the force-displacement relation $l_{ds}(F)$ of Eq.1, using

$$E_{ds}(F) = F\, l_{ds}(F) - \int_0^F l_{ds}(f)\, df.$$

The simple theoretical description of twist-stretch coupling according to Eq.1 and 2 exhibits an unphysical divergence. Approaching a critical force $F = \left(\sqrt{C\,K} - g_0\right)/g_1$ from below, the predicted dsNA length goes to infinity. This softening also causes a divergence of E_{ds}. To avoid using the model outside its validity range, we restrict our calculation to forces below a threshold value. The force for which the dsNA length becomes equal to the length of two parallel non-interacting ssNA strands defines our threshold; we denote it divergence force F_d. The curves presented in main text figures 7 and 8 are restricted to forces below F_d. For illustration, the theoretical length of dsRNA is compared to the length of two parallel non-interacting ssRNA strands in Fig.S5.

4. $F\ (l_{ss} - l_{ds})$ term

The length of the molecular construct under tension changes when a base pair opens, since a dsNA fragment of one base pair under force F is then converted to an ssNA nucleotide under force F. The corresponding mechanical work $W = F\ (l_{ss} - l_{ds})$ is performed by the force measuring device; a double optical trap with two beads in our case. The force-dependent lengths l_{ss} and l_{ds} are given by Eqs.1 and 4. Typically, a single-stranded NA is longer than a double-stranded NA at given force, with the notable exception of the low-force entropic regime. In Fig.S5, we present theoretical force-extension relations of ssRNA, dsRNA and two parallel strands of non-interacting ssRNA molecules.

B. Melting bubble formation

Overstretching by melting bubble formation involves rupture of dsNA base pairs; the mechanism is similar to peeling in this respect. As a difference, however, melting bubble formation results in single strands that both remain under tension, while one strand relaxes in the peeling case. The applied force F is distributed among the two strands, either equally if the two strands are of the same nature ($F_1 = F_2 = F/2$; for dsDNA and dsRNA), or unequally if the two strands are of different nature ($F_{DNA} \neq F_{RNA}$; $F_{DNA} + F_{RNA} = F$; for RNA-DNA and DNA-RNA). The process is described in main text section III.C.2. Here we complement the description by one detail. It regards how the couple $\{F_{DNA}, F_{RNA}\}$, occuring in the heteroduplex case of main text Eq.2, can be calculated. For given F, we

numerically determine F_{DNA} as the zero of the function

$$y(F_{DNA}) = l_{ss}^{DNA}(F_{DNA}) - l_{ss}^{RNA}(F - F_{DNA}),$$

where $l_{ss}^{DNA}(F)$ and $l_{ss}^{RNA}(F)$ are given by Eq.4. F_{RNA} is obtained afterwards by calculating $F_{RNA} = F - F_{DNA}$.

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TABLE S1: Parameters used to describe the measured average force-extension relations.

	L_c^{ds} (nm)	L_p (nm)	K (pN)	C (pN nm^2)	g_0 (pN nm)	g_1 (nm)	F_c (pN)
dsDNA	0.316	50	1054	440	-530	19.55	25
dsRNA	0.290	60	1484	440	-513	21.0	25
RNA/DNA	0.313	55	1254	440	-525	18.0	25
DNA/RNA	0.313	55	1254	440	-525	18.0	25

There are uncertainties in the parameter values presented in this table. As described in section I of this supplementary information, the effects of most of the individual parameters on the force versus displacement curves are coupled. A quantitative determination of the errors is impossible in these cases. The crystallographic length L_c^{ds} is an exception, because the horizontal position of the major force increase in the force versus displacement curve is dominated by this parameter, while the other parameters are of minor influence. Analysing our experimental uncertainties of these positions, we can estimate the errors in the crystallographic lengths L_c^{ds}. In terms of standard deviation they are 0.03 nm for dsDNA, 0.04 nm for dsRNA, 0.02 nm for RNA-DNA and 0.02 nm for DNA-RNA. We attribute most of these errors to variation in the bead sizes from one measurement to another (according to the commercial suppliers the variation in the bead diameters can amount to 10%).

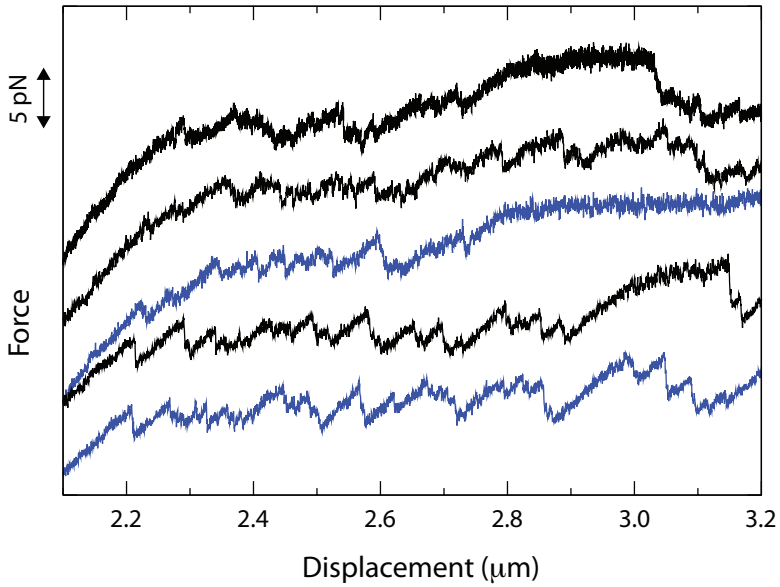

FIG. S1: Representative force-displacement curves of the overstretching plateau of DNA-RNA hybrid duplexes. Curves have been shifted vertically for clarity. Buffer conditions are the same as in main text Fig. 2. Displacement velocities are 30 nm/s (top curve) and 100 nm/s (other curves). The second and the third (from the top) curves correspond to successive stretch/release cycles of the same molecule. The two bottom curves also correspond to successive stretch/release cycles on another single molecule. Please note the similarity of the pattern of sawteeth even originating from different molecules or from slightly different experimental conditions (in this case, displacement velocity). One also observes on some of these curves the simultaneous occurrence on the same overstretching plateau of (mainly) sawtooth-shaped portions and smooth portions, indicating coexistence of peeling and of (at least) one other mechanism.

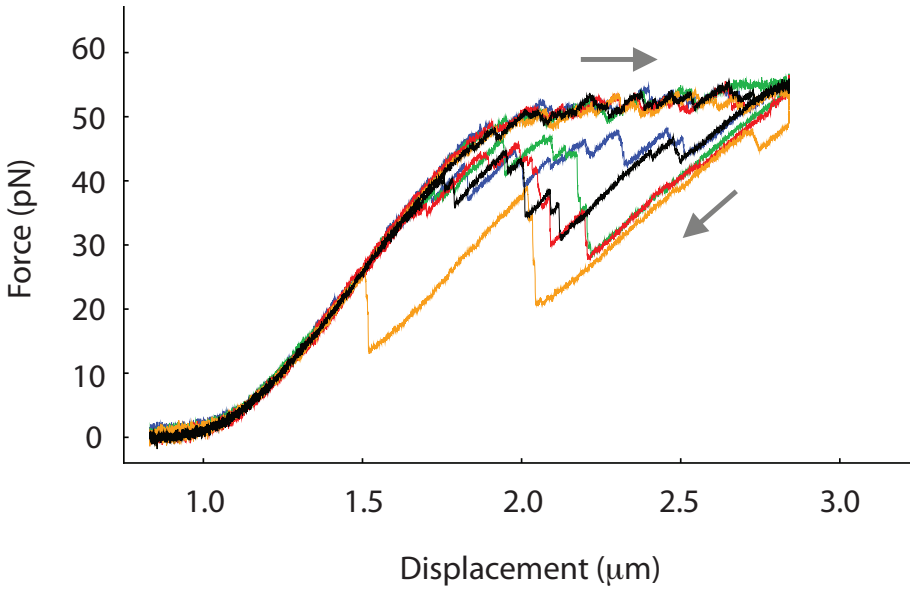

FIG. S2: One single dsDNA molecule was subjected to five successive stretch/release cycles, corresponding to the following color code: first cycle in blue, second in green, third in red, fourth in orange and fifth cycle in black. Buffer conditions and displacement velocities are as in main text Fig.2.

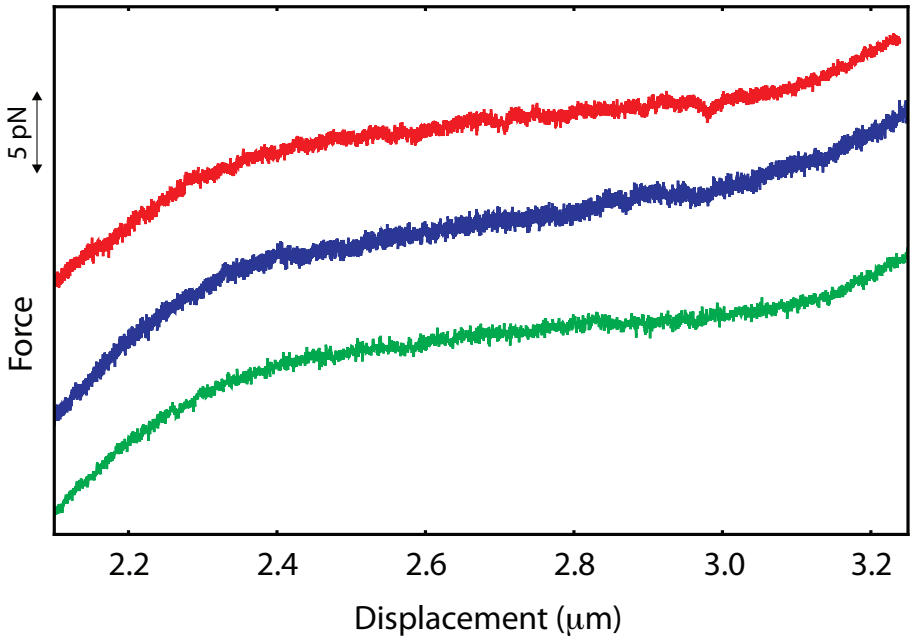

FIG. S3: Representative force versus displacement curves of the overstretching plateau of dsRNA molecules, measured upon increasing the distance between the optical traps for different salt conditions and displacement velocities. Green curve corresponds to 100 mM KCl, 20 mM Hepes pH 7.6, 5 mM $MgCl_2$ and displacement velocity of 100 nm/s (this curve is the same as the one displayed in main text Fig.2). Blue curve corresponds to 100 mM KCl, 20 mM Hepes pH 7.6 and displacement velocity of 30 nm/s. Red curve corresponds to 10 mM KCl, 10mM Hepes pH 7.6 and displacement velocity of 10 nm/s. The curves are shifted vertically for clarity.

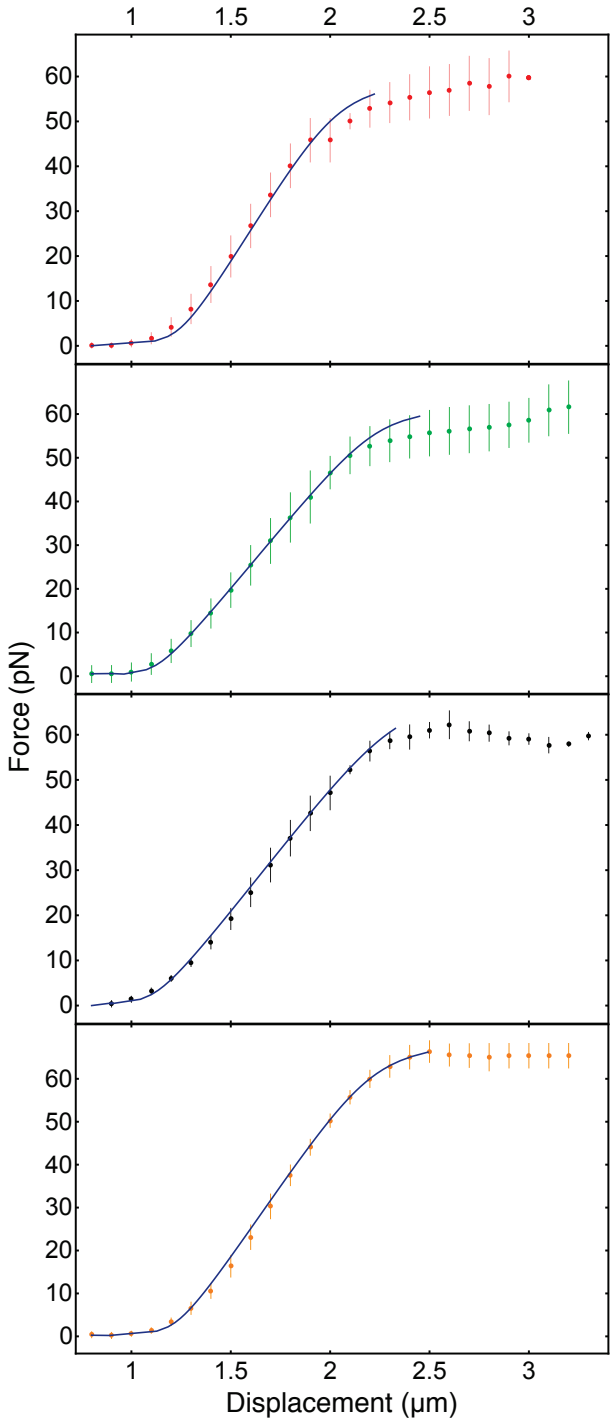

FIG. S4: Average force-displacement curves of the four different molecular duplexes. Data for dsDNA, dsRNA, RNA-DNA and DNA-RNA are presented from top to bottom and are based on 76, 92, 57 and 24 individual measurements, respectively. Dots and vertical bars show average force and mean-square deviation. Solid blue lines represent fits to a twistable worm-like chain model. In all measurements used for this figure we used the buffer condition and displacement velocity given in the caption of main text Fig.2.

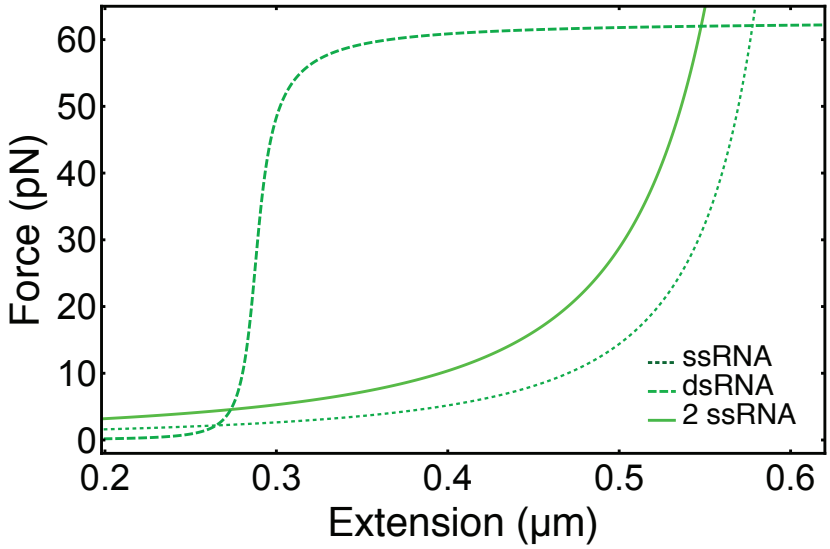

FIG. S5: Comparison of theoretical force-extension relations of dsRNA (dashed), ssRNA (dotted) and two parallel non-interacting ssRNA strands (solid).