Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Mechanochemical Properties of DNA Origami Nanosprings Revealed by Force Jumps in Optical Tweezers

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Materials

Chemicals like KCl, $MgCl_2$, EDTA and MES with >99% purity were obtained from VWR. All the DNA used for synthesis of DNA origami, such as staple strands and bridge strands were acquired from Eurofins Genomics Tokyo (Tokyo, Japan) while scaffold DNA was obtained from Tilibit Nanosystems (Garching, Germany). Polystyrene beads coated with either streptavidin or anti-digoxigenin were purchased from Spherotech (Lake Forest, IL, USA). The pET-26b (+) plasmid used as PCR template was purchased from Novagen (Darmstadt, Germany) while PCR primers were made available from Japan Bio Service (Saitama, Japan). Restriction enzymes were purchased from New England Biolabs (Ipswich, MA, USA).

Preparation of the "DNA-only" construct.

The "DNA-only" construct was prepared by incorporation of a ssDNA fragment, 5′-CTA GAC GGT GTG AAA TAC CGC ACA GAT GCG TTG AAC TAT ACA ACC TAC TAC CTC ATT TTT GAG GTA GTA GGT TAT CGC CAG CAA GAC GTA GCC CAG CGC GTC, between a 2028-bp dsDNA handle[1] prepared from the PCR of pBR322 plasmid on the one end and a 2690-bp dsDNA handle[2] obtained from the cleavage of the pEGFP plasmid by endonucleases (EagI and XbaI) on the other end. The process was carried out first by annealing the phosphorylated DNA fragment with two DNA oligos: 5′-CGC ATC TGT GCG GTA TTT CAC ACC GT and phosphorylated 5′-GGC CGA CGC GCT GGG CTA CGT CTT GCT GGC. The slow annealing process was started at 95 °C for 15 min, and then reduced to 20 °C at a rate of -1.0 °C/min. This annealed product was then ligated with biotin labeled 2028 bp dsDNA handle and agarose gel purified. Finally, the purified product was ligated with poly digoxigenin labeled 2690-bp dsDNA handle.

DNA origami nanosprings.

Using a caDNAno software[3] for strand routing and CanDo[4-5] for their final structure prediction, DNA origami structures were designed. The whole structure assembly of the origami structures was mediated by mixing 10 nM circular single-stranded scaffold DNA (p8064) with ~40 nM staple strands, including bridge strands in 40 μL of the folding buffer containing 5 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 15 mM $MgCl_2$. The mixture solution was then incubated at 65 °C for 15

min, and then annealed by reducing the temperature from 60 °C to 45 °C at a rate of -1.0 °C/hr which facilitated the Watson-Crick base pairing of complementary sequences. Using PEG-precipitation, the obtained assembled mixture was purified.[6] In brief, the annealed mixture was mixed with a precipitation buffer (15% PEG 8000 (w/v), 5 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 505 mM NaCl) in the volume ratio 1:1. Next, centrifugation was carried out at 16,000 ×g for 25 min, then, the supernatant was removed and the pellet was dissolved in the buffer of targeted pH for experimental use.

Preparation of the Biotin and poly-Digoxigenin labeled dsDNA handles.

Two 2520 bp dsDNA handles were prepared via PCR amplification of pET-26b (+) plasmid. Each handle sequence had a forward primer comprised of "5'-*Staple sequence*-O-$(CH_2)_2$-O-$CH_2)_2$-O-*Primer sequence*" (see below). The staple sequence provided a unique single-stranded overhang in one end of each handle that could hybridize with either end of the nanospring origami. The other end of the handle was labeled with either biotin or digoxigenin. For biotin labeled handle, the reverse primer was directly modified with 5' biotin while for digoxigenin labeled handle, the PCR amplified product was cleaved with RPSacI and further labeled with poly-digoxigenin-dUTPs using terminal transferase.

Primers for the digoxigenin labeled handle
Forward primer:
5'- TTT AAA GGG CAG TGT TGT TCC AGT TTG CCC GAG ATA GGG TTG GAA AAA CCG TCT ATC A -X- CGC CGA TCA ACT GGG TGC CAG CGT

Reverse primer:
5'- AAA AAA AAG AGC TCG GGT TCG TGC ACA CAG CCC AGC TT

Primers for the biotin labeled handle
Forward primer:
5'- TTT CAT AGT TAC TGA GTT TCG TCA CCA CCC ATG TAC CGT AAC AGC GTA ACG ATC TAA AGT TTT GTC-X- CGC CGA TCA ACT GGG TGC CAG CGT

Reverse primer:
5'-[Biotin]-GGGTTCGTGCACACAGCCCAGCTT

X= O-$(CH_2)_2$-O-$CH_2)_2$-O

Preparation of handle-conjugated DNA origami nanosprings.

The dsDNA handle-conjugated origami nanosprings were assembled by mixing 10 nM scaffold DNA (p8064) with ~40 nM staple strands, including bridge strands, and two DNA handles (biotin-handle and poly-DIG handle), 10 nM each) in 60 μL of the folding buffer containing 5 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 15 mM $MgCl_2$. The mixture was incubated at 65 °C for 15 min, then annealed by reducing the temperature from 60 °C to 45 °C at a rate of -1.0 °C/hr. The assembled structure was purified by PEG-precipitation as described in the above section.

Single-molecule force ramping assays.

The nanospring constructs for single-molecule force ramping assays were prepared by its ligation with 2520-bp dsDNA handles having labeled with digoxigenin and biotin on either end. These constructs were then diluted to ~2 ng and incubated with 0.1% solution of digoxigenin (Dig)-antibody coated polystyrene beads for 10 min at room temperature, resulting in the formation of Dig-anti-dig complex. Next, the sample was further diluted in 1 mL of 5 mM MES buffer (pH 5.0) along with 15 mM $MgCl_2$ and 1 mM EDTA. This sample solution was then injected from the top channel of the 4-channel microfluidic chamber. Likewise, the adjacent channel (upper middle) of the chamber was injected with the same composition of the buffer (pH 5.0), while that on the lower middle channel a buffer with pH at 7.4 was used. Finally, the lowermost channel was injected with polystyrene beads coated with streptavidin. Once the respective buffers were injected in each channel, streptavidin coated bead was trapped by laser in the lower middle channel and moved to the upper middle channel, where anti-Dig coated bead was trapped by another laser focus. Eventually, the nanospring construct was anchored between these two beads having the Dig-anti-Dig linkage on one end and the streptavidin-biotin linkage on the other. Next, force ramping assays were performed with a loading rate of ~5.5 pN/s in the upper middle channel (pH 5.0) reaching a maximum force of 40 pN. The F-X curve at this pH was recorded and then, the beads containing the same molecule were moved to the lower middle channel (pH 7.4) where other sets of F-X curves were recorded.

Force-jump experiments.

A similar microfluidic setup as used for force ramping experiment was prepared for the force-jump experiments. The tethered nanospring construct in the upper middle channel (pH 5.0) was fully

stretched and maintained at 25 pN and then suddenly released to a force of 0.5 pN within 10 ms. The data showing the changes in force, recoiling distance, and time were recorded. Similarly, other force jump transitions were performed ranging from initial high force of 25 pN to the low forces of 1, 2, 3…10 pN respectively. Next, the same molecule was then moved to the lower middle channel (pH 7.4) and then other data sets of the changes in force, recoiling distance, and time were recorded for similar transitions from the 25 pN to 0.5, 1, 2…10 pN.

Fitting model of the force-extension curves of the DNA origami nanodevices.

In the low force region (F = 0~10 pN), F-X curve of a nanospring (extended in pH = 7.4 or compacted in pH = 5.0) can be described by the Hooke's law,

$$F = kx + x_0 \tag{1}$$

where k and x_0 are the spring constant and initial spring length (F=0 pN), respectively. F-X curve of the dsDNA handles can be described by the Worm-like Chain (WLC) model,[7]

$$F = \left(\frac{k_B T}{L_p}\right)\left[\frac{1}{4\left(1-\frac{x_0}{L}+\frac{F}{K_0}\right)^2} - \frac{1}{4} + \frac{x}{L_0} - \frac{F}{K_0}\right] \tag{2}$$

where $k_B T$, L_p, L_0, and K_0 are Boltzmann's constant times absolute temperature, persistence length, contour length, and elastic modulus, respectively. Consequently, F-X curves of the nanospring and dsDNA handles can be fitted by the combination of the Hooke's law and worm-like chain (WLC) model. Since WLC model is an implicit function for force F, MATLAB was used to solve the equation to get the explicit function,

$$x = f_{WLC}(F) \tag{3}$$

where $f_{WLC}(F)$ is the numerical formula about parameter F obtained from MATLAB. Then the equation $x = f_{WLC}(F) + F/k + x_0$ was used to fit F-X curve in the low force region. For the multivariable fitting, the first step is to hold the dsDNA handle parameters (L_p, L_0, and K_0 or persistent length, contour length, and stretch modulus, respectively) to get the force calibration value, which can eliminate experimental deviation near the zero force. After the force calibration, we fitted the F-X curves by the equation calibrated by F_0,

$$x = f_{WLC}(F - F_0) + (F - F_0)/k + x_0 \tag{4}$$

with all parameters changing freely to obtain the final fitting results, which were shown below,

Table S1: Fitting parameters of the nanospring

Parameter	pH = 7.4	pH = 5.0	Theoretical values
L_p for handles/nm	48 ± 3	46 ± 2	~50
L_0 for handles/nm	1729 ± 11	1738 ± 7	~1713
K_0 for handles/pN	1189 ± 127	1061 ± 154	~1008
k for nanospring/(pN.nm^{-1})	0.146 ± 0.034	0.070 ± 0.011	N/A
x_0 for nanospring/nm	374 ± 39	271 ± 56	N/A

Figure S1. Representative atomic force microscopy (AFM) images of dsDNA-handle-labelled DNA origami nanosprings possessing the i-motif-forming sequences in the bridge regions.

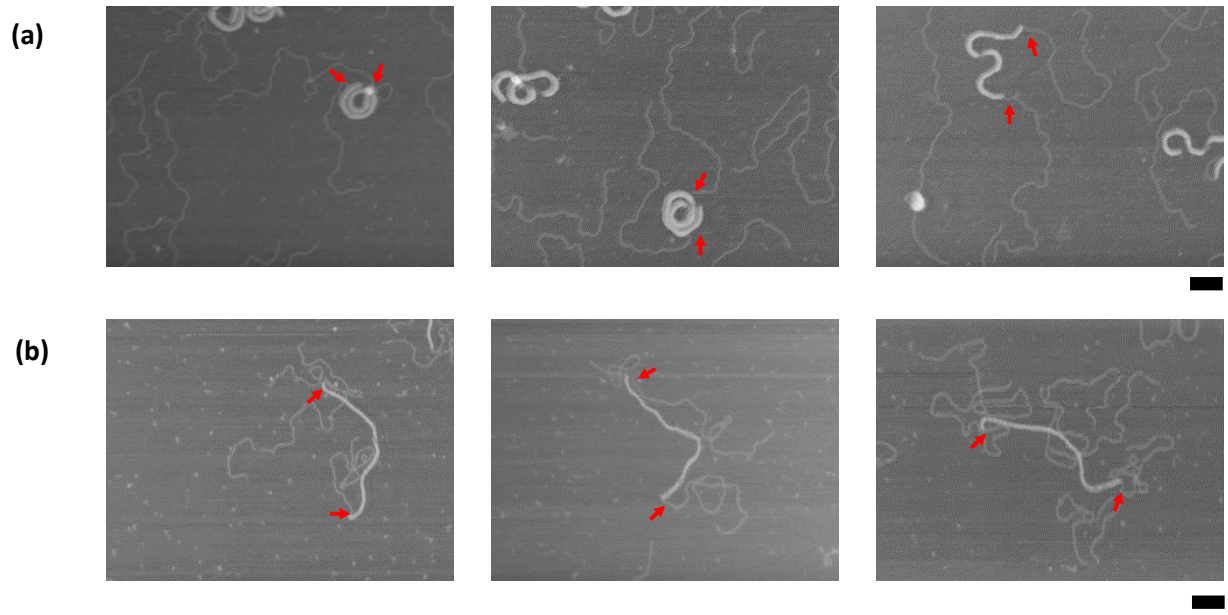


Figure S1. Atomic Force Microscopy. (a) AFM images obtained at pH 5.0. Scale bars: 200 nm. (b) AFM images obtained at pH 7.4. Scale bars: 200 nm. AFM images show proper ligation of the DNA origamis with the 2520 bp dsDNA handles (arrows). Also, the coiling at respective pH is not affected by the dsDNA handles.

Figure S2. Representative atomic force microscopy (AFM) images of DNA origami nanospring possessing the 21T bridge linkers.

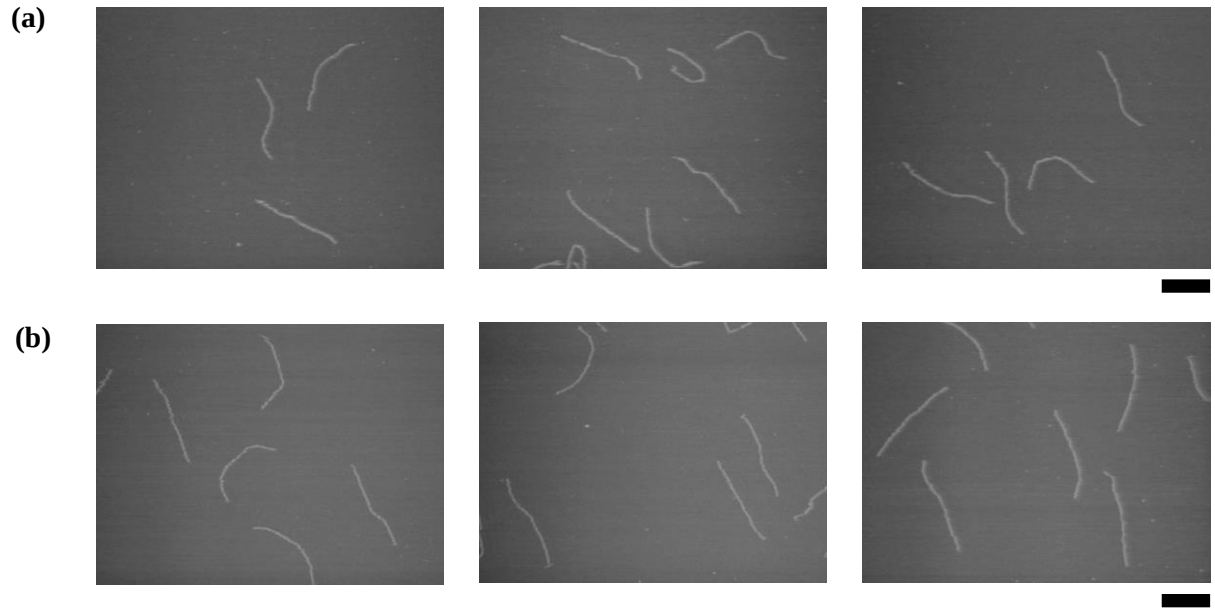


Figure S2. Atomic Force Microscopy.[8] (a) AFM images obtained at pH 5.0. Scale bars: 400 nm. (b) AFM images obtained at pH 7.4. Scale bars: 400 nm. The 21T bridge linkers incorporated into the DNA origami nanospring do not show pH responsive coiling of the origami structure.

Figure S3. Representative atomic force microscopy (AFM) images of DNA origami nanosprings (2T-NS) with 2T bridge linkers at pH 5.0.

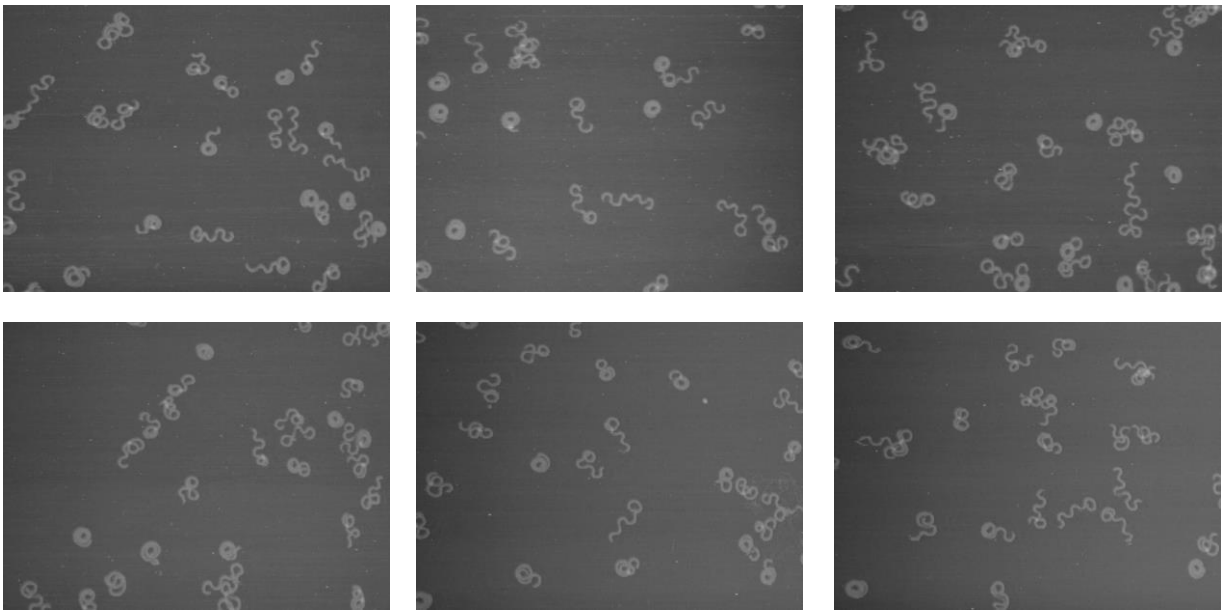


Figure S3. Atomic Force Microscopy.[8] AFM images (2000×1500 nm) obtained at pH 5.0. The 2T linkers in the bridge of the DNA origami nanospring lead to its coiling.

Figure S4. Representative atomic force microscopy (AFM) images of the DNA origami nanosprings (2T-NS) with 2T bridge linkers at pH 7.4.

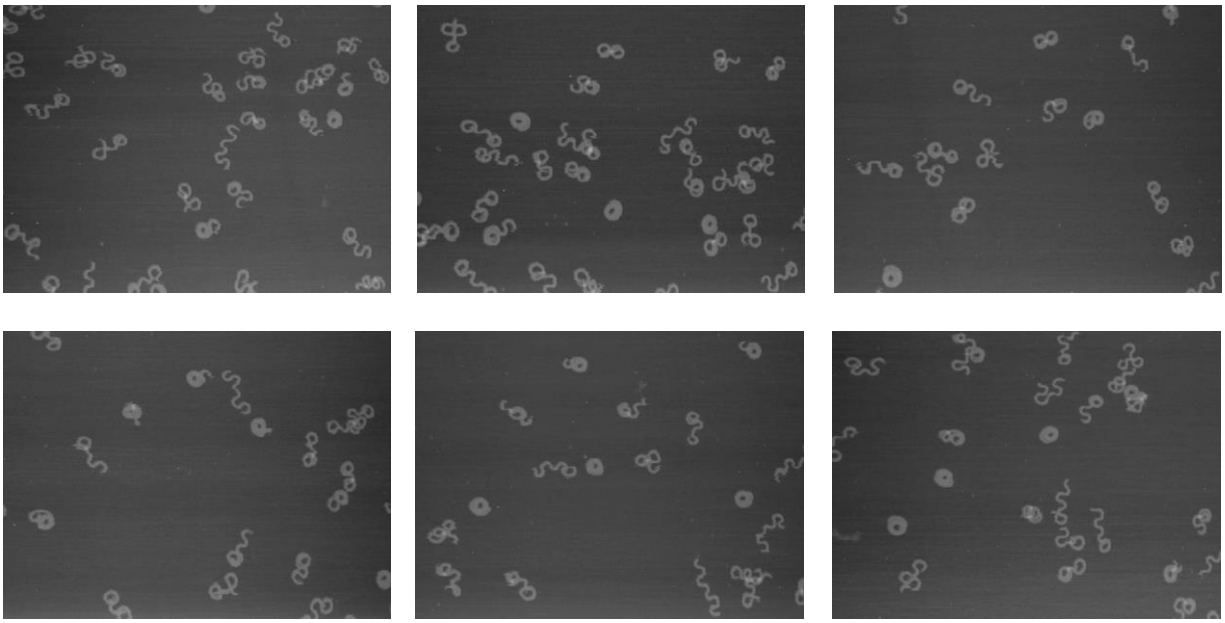


Figure S4. Atomic Force Microscopy.[8] AFM images (2000×1500 nm) obtained at pH 7.4. Coiling mediated by the 2T linkers in the bridges of the DNA origami nanospring does not show pH responsive behavior.

Figure S5. Unfolding force histograms and work histograms for the telomere i-motif at different pHs.

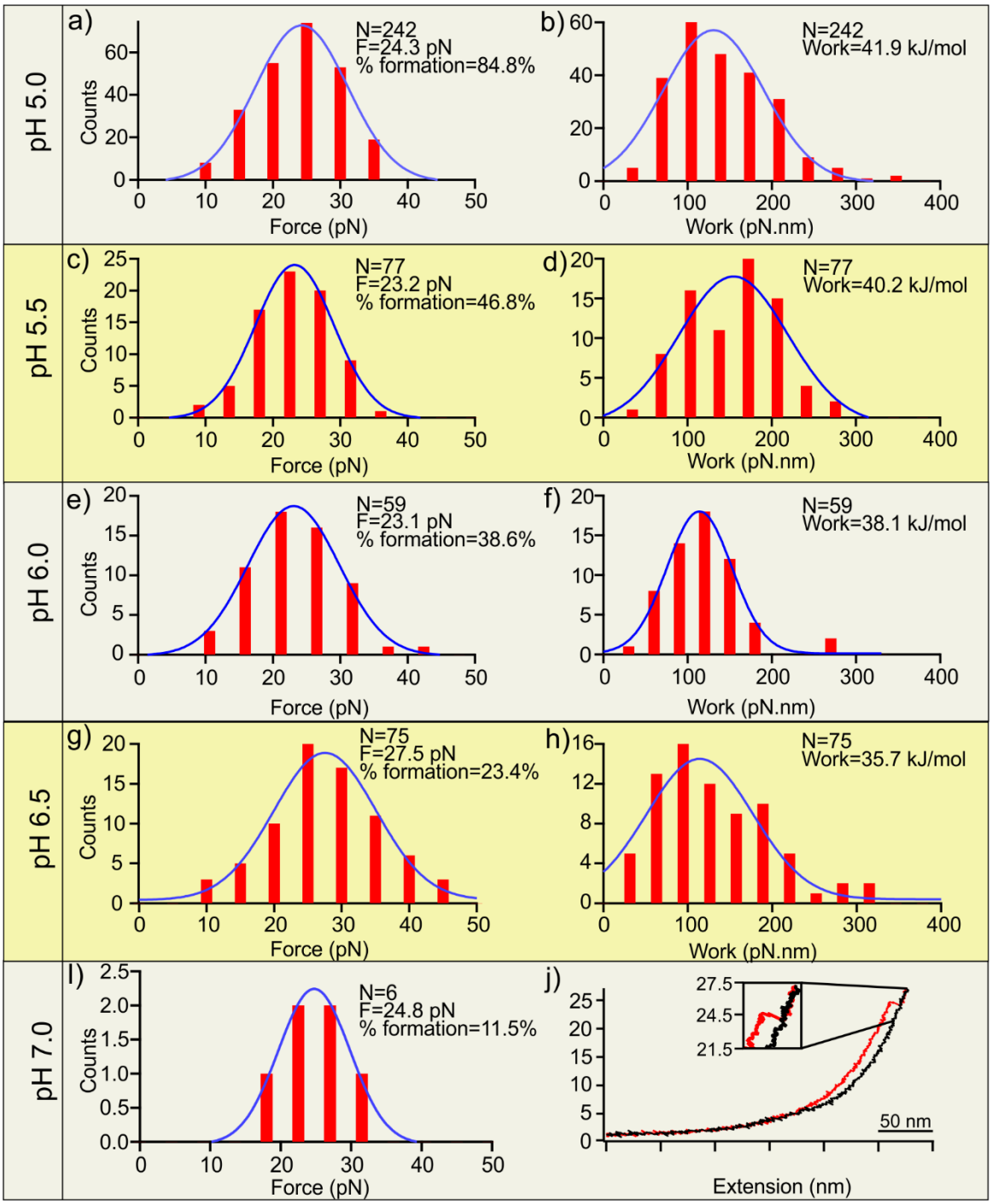


Figure S5. Force and work histograms for unfolding of a telomere i-motif at different pH. An unfolding force of approximately 25 pN was required for the unfolding of telomere i-motif formed in DNA fragment 5'-CCCTAACCCTAACCCTAACCC-3'. The unfolding force was

measured via force-ramp assay (see below for experimental description). I-motif starts to unfold at a force about 10 pN indicated by unfolding force histograms. N represents the numbers of total unfolding features. Figure j) represents a typical F-X curve for unfolding an i-motif at pH 5.0.

For the single-molecule force-ramping assay, the DNA construct was prepared by integrating the telomere i-motif DNA fragment between a 2028-bp dsDNA handle on one end and a 2690-bp dsDNA handle on the other end using the protocol in literature.[2] The free end of 2028 bp dsDNA handle was labeled with biotin which could form a complex with the streptavidin coated polystyrene beads on that end, while the 2690 bp handle was labeled with Digoxigenin (Dig) on its free end that could form a linkage with the Digoxigenin-antibody coated beads. Those two types of beads were then trapped using two laser foci separately and then moved apart at a loading rate of ~5.5 pN/s reaching to a maximum of 40 pN. The buffer solution was maintained at respective pHs using 50 mM MES which served as a medium to perform telomere i-motif unfolding assay. Between successive force ramping curves, the delay time was adjusted to 30 s.

Figure S6. Representative F-X curves for i-motif NS.

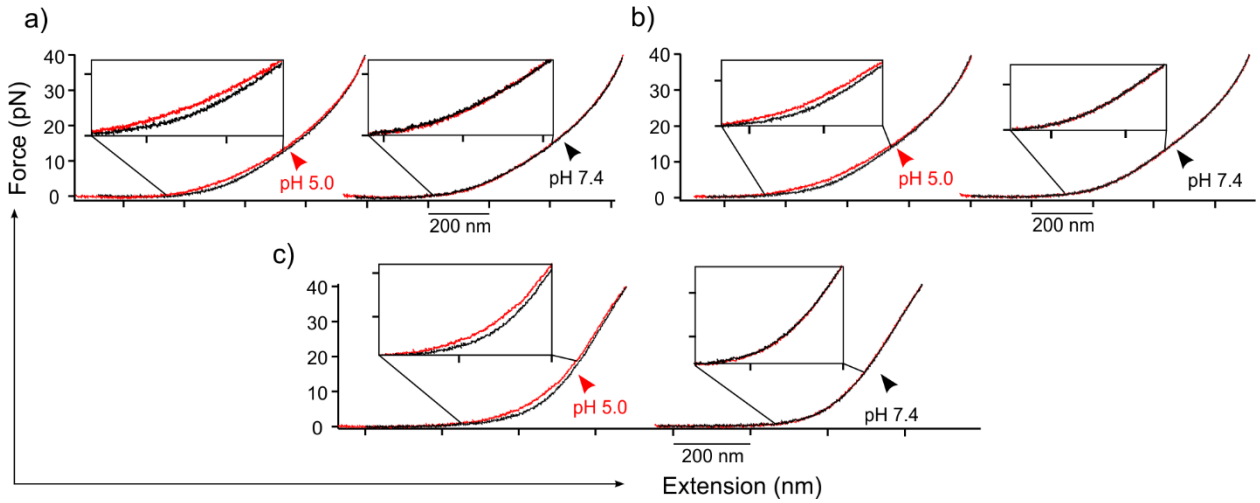


Figure S6. F-X curves of the i-motif NS at different pH. Red and black curves depict stretching and relaxing traces, respectively. Each F-X curve obtained at pH 5.0 showed hysteresis between stretching and relaxing traces due to the mechanical unfolding of i-motif contained in the bridges of the nanosprings. At pH 7.4, no hysteresis was observed between stretching and relaxing traces due to the fact that the i-motifs are unfolded at pH 7.4.

Figure S7. Force jump assays.

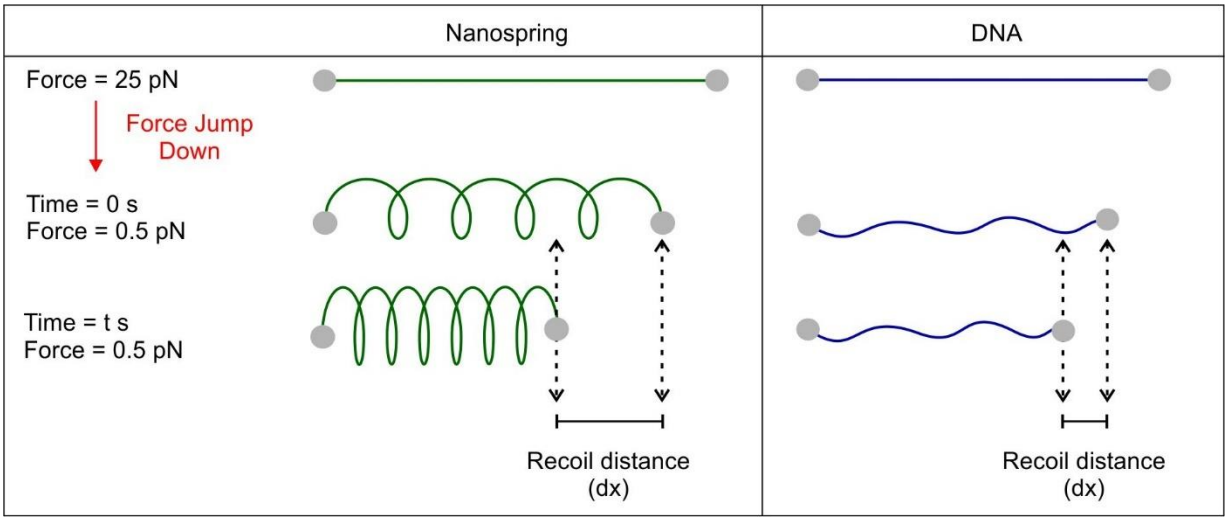


Figure S7. Schematics of the events during recoiling of a nanospring or DNA during the force jump processes. At a high force (here 25 pN), both nanospring and DNA are fully stretched. With the sudden release to low force (e.g. 0.5 pN), nanospring starts to form coil (time = 0 s). After certain time (t s), the nanospring is fully coiled demonstrating a certain recoiling distance (dx). A very small recoiling distance observed in DNA is due to the elasticity of DNA.

Figure S8. Representative atomic force microscopy (AFM) images of DNA origami nanosprings with i-motif-forming sequences in the bridge regions.

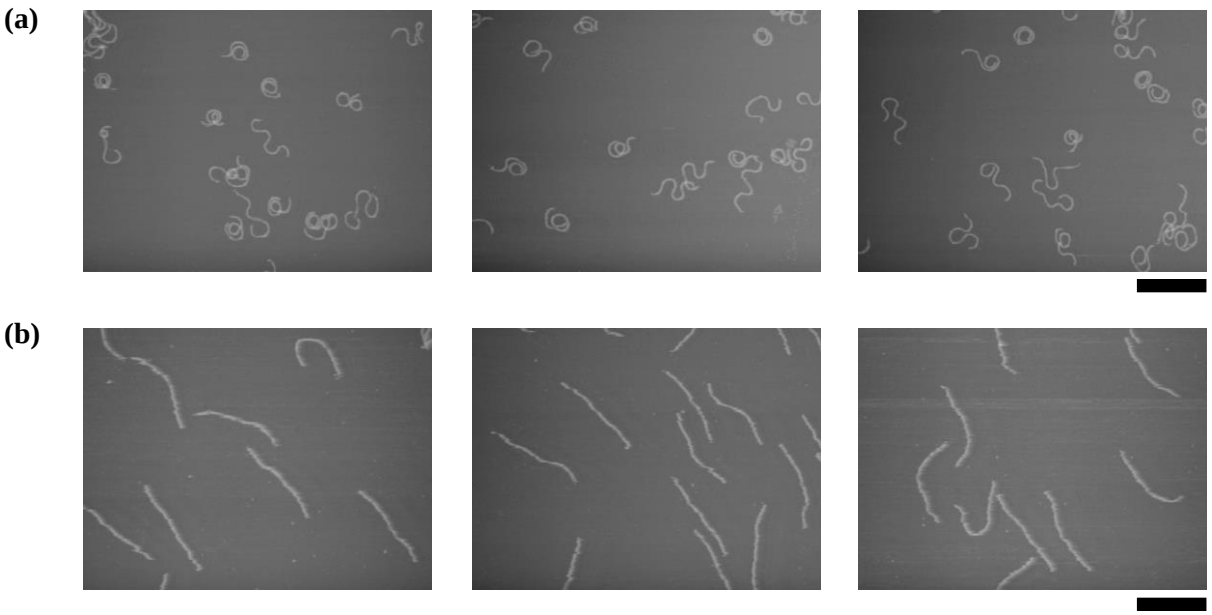
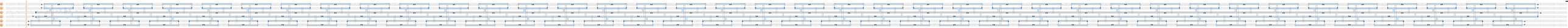


Figure S8. Atomic Force Microscopy.[8] (a) AFM images obtained at pH 5.0. Scale bars: 400 nm. Formation of telomere i-motif in the nanospring leads to the coiling of the nanosprings at pH 5.0. (b) AFM images obtained at pH 7.4. Scale bars: 400 nm. No i-motif formation at pH 7.4 makes nanosprings linear.

Figure S9. Origami Nanospring Design.

Figure S9. General structure with detailed sequence information for the DNA origami nanospring.[8] See next page for this figure. Please blow up to see the details.



Supporting References

1. Mandal, S.; Zhang, X.; Pandey, S.; Mao, H., Single-Molecule Topochemical Analyses for Large-Scale Multiplexing Tasks. *Anal. Chem.* 2019, *91*, 13485-13493.
2. Dhakal, S.; Schonhoft, J. D.; Koirala, D.; Yu, Z.; Basu, S.; Mao, H., Coexistence of an ILPR i-Motif and a Partially Folded Structure with Comparable Mechanical Stability Revealed at the Single-Molecule Level. *J. Am. Chem. Soc.* 2010, *132* (26), 8991–8997.
3. Douglas, S. M.; Marblestone, A. H.; Teerapittayanon, S.; Vazquez, A.; Church, G. M.; Shih, W. M., Rapid prototyping of 3D DNA-origami shapes with caDNAno. *Nucleic Acids Res* 2009, *37* (15), 5001-5006.
4. Castro, C. E.; Kilchherr, F.; Kim, D. N.; Shiao, E. L.; Wauer, T.; Wortmann, P.; Bathe, M.; Dietz, H., A primer to scaffolded DNA origami. *Nat Methods* 2011, *8* (3), 221-9.
5. Kim, D. N.; Kilchherr, F.; Dietz, H.; Bathe, M., Quantitative prediction of 3D solution shape and flexibility of nucleic acid nanostructures. *Nucleic Acids Res* 2012, *40* (7), 2862-8.
6. Stahl, E.; Martin, T. G.; Praetorius, F.; Dietz, H., Facile and scalable preparation of pure and dense DNA origami solutions. *Angew Chem Int Ed Engl* 2014, *53* (47), 12735-40.
7. Baumann, C. G.; Bloomfield, V. A.; Smith, S. B.; Bustamante, C.; Wang, M. D.; Block, S. M., Stretching of Single Collapsed DNA Molecules. *Biophysical Journal* 2000, *78* (4), 1965-1978.
8. Karna, D.; Stilgenbauer, M.; Jonchhe, S.; Ankai, K.; Kawamata, I.; Cui, Y.; Zheng, Y.-R.; Suzuki, Y.; Mao, H., Chemo-Mechanical Modulation of Cell Motions Using DNA Nanosprings. *Bioconjugate Chemistry* 2021, *32* (2), 311-317.