Electronic Supplementary Material (ESI) for ChemComm.

Studies of G-quadruplexes formed within self-assembled DNA mini-circles

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Supplementary Information

Experimental details

All DNA oligos were purchased from Eurogentec (Belgium) HPLC-purified and in a solubilised form. The sequences of the oligos used are given in the **Table S1**. To make stock solutions DNA oligos were dissolved in MilliQ water to final 100 μM concentration using manufacturer recommended volumes and stored at -20^{o}C until use.

Table S1. DNA oligonucleotides used for the preparation of DNA mini-circles. 3C-FAM and 4C-TAMRA are labelled oligonucleotides for the assembly of the mini-circles while $3C_2$-FAM and $4C_2$-TAMRA are labelled oligonucleotides for the assembly of the linearised mini-circles (L-mini-circles).

Name	Sequence (5'-3')
1T	CAGTCTTATTGCCAGTTTTTGCGACTGAGGGTGGGGAGGGTGGG GAATTATTGTACGTTTTTGCTAGTTAATT
2T	CCGCATTATTGAGCCTTTTTTCTAGCTTAATTCGGCCTTATTGGCC GTTTTTTGGCTCTTAATT
3C	AATTAAGCTAGAAAAAAGGCTCAATAATGCGGAATTAACTAGCA AAAACGTACAATAA
3C-FAM	AATTAAGCTAGAAAAAAGGCTCAATAATGCGGAATTAACTAGCA AAAACGTACAAT^{FAM}
4C	AGTCGCAAAAACTGGCAATAAGACTGAATTAAGAGCCAAAAAA CGGCCAATAAGGCCG
4C-TAMRA	^{TAMRA}GTCGCAAAAACTGGCAATAAGACTGAATTAAGAGCCAAAA AACGGCCAATAAGGCCG
5C	TTCCCCACCCTCCCCACCCTC
$3C_2$-FAM	AATTAACTAGCAAAAACGTACAAT^{FAM}
$4C_2$-TAMRA	^{TAMRA}GTCGCAAAAACTGGCAATAAGACTG
24-mer-G4	^{FAM}TTAAGGGGTGGGAGGGGTGGGAGT^{TAMRA}

Pyridostatin (Fig. S1) was purchased from Aldrich and stock solution at 10 mM concentration was prepared in MilliQ water and then stored at -20^{o}C.

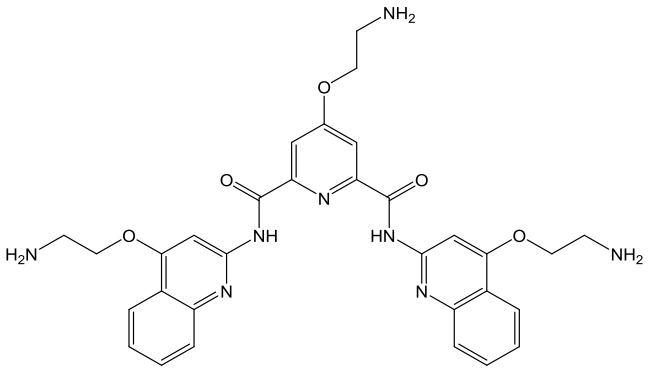


Figure S1. Chemical structure of the G-quadruplex ligand pyridostatin (PDS).

Preparation of DNA mini-circles. DNA oligos **1T**, **2T**, **3C** and **4C** (Figure S1) were diluted to the final 0.2 μM concentration in 100 mM LiCl/10 mM Tris-HCl buffer (pH 7.4) at equimolar amounts. The oligo solution was heated to 95 °C for 5 min then left to cool down to room temperature over 3 h and then left at room temperature for another 12 h. The sample was stored at 4 °C until purification. For the FAM/TAMRA labelled minicircles the same protocol as described above was followed except that **3C-FAM** and **4T-TAMRA** (instead of **3C** and **4C**) and 100 mM NaCl (instead of LiCl) were used. The solution was also buffered to 7.4 with 10 mM Tris-HCl buffer.

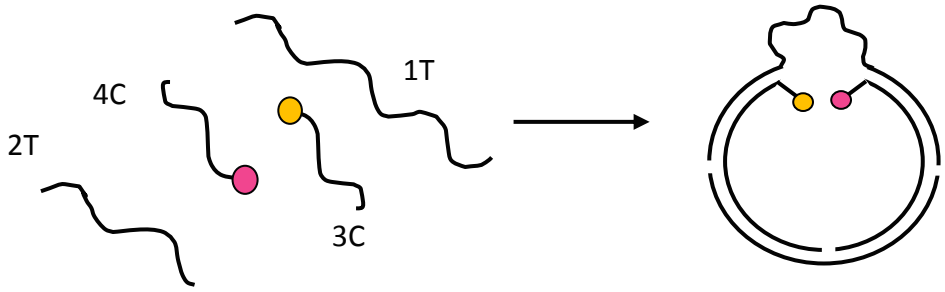


Figure S2. Schematic representation of the assembly of mini-circles.

Preparation of linearised DNA half mini-circle (L-mini-circle). As in the above procedure, DNA oligos **1T**, **3C$_2$** and **4C$_2$** were diluted to the final 1 μM concentration in 100 mM NaCl/10 mM Tris-HCl buffer (pH 7.4) at equimolar amounts. The oligo solution was heated to 95 °C for 5 min then left to cool down to room temperature over 3 h and then left at room temperature for another 12 h. Then sample was stored at 4 °C until purification.

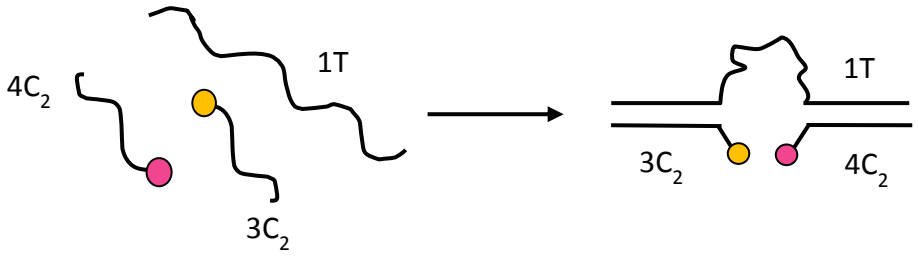


Figure S3. Schematic representation of the assembly of linearised half-mini-circles (L-mini-circles).

***Native gel electrophoresis*.** 6% native polyacrylamide gels and 1xTBE buffer were used for gel electrophoresis. Into each well 10 μL of annealed DNA mini-circles or DNA L-mini-circles supplemented with 2 μL of 30% glycerol solution in water were loaded. 50bp DNA ladder was used as a marker. Gels were run at room temperature using mini-Protean (Bio-Rad) tank, at 90V constant voltage for 60 min. Then they were stained with SYBR® Gold (Invitrogen) for 10 min and imaged with an Ettan DIGE scanner (GE Healthcare, UK) on the Cy2 channel (Figure S2, A). Mini-circles labelled with FAM and TAMRA were imaged without staining on the Cy2 and Cy3 channels (Figure S4, B).

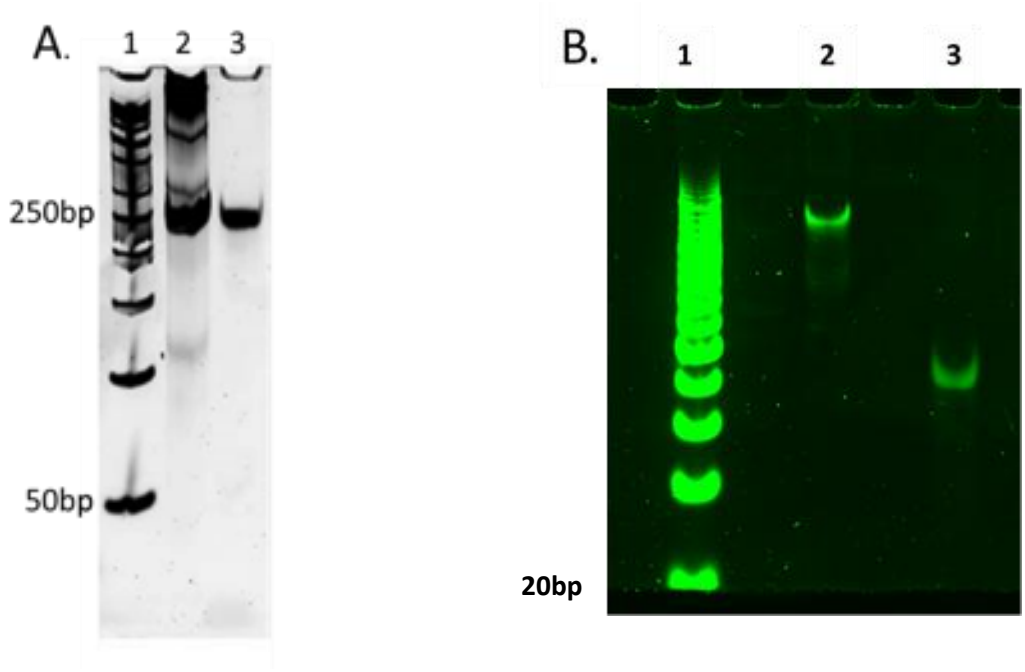


Figure S4. Characterization of DNA mini-circles native polyacrylamide by 6% polyacrylamide gel electrophoresis. A) unlabelled DNA mini-circles. Lanes: 1) 50bp size marker; 2) mini-circles before purification; 3) mini-circles after purification. B) Labelled mini-circles and L-mini-circles. Lanes: 1) 20bp size marker; 2) Mini-circles after purification; 3) L-mini-circles after purification. Gels were run in the presence of 1xTBE buffer for 1h at constant 90 V.

Purification of unlabelled and FAM/TAMRA labelled mini-circles. DNA mini-circles were purified by loading 50 μL of the annealed 0.2 μM sample into wide-wells of 1.5 mm mini-gels. The gel was run for 2 h at 90 V then stained with SYBR® Gold (labelled mini-circles were imaged without staining) for 10 min. The required band was detected with the Ettan Imager scanner, cut out and crushed through a 1mL syringe into an Eppendorf tube. 200 μL of 100 mM LiCl buffer (for the unlabelled mini-circles) or 100 mM NaCl buffer (for the FAM/TAMRA labelled mini-circles) was added into the Eppendorf and left to soak overnight at 4 ^{o}C. After that the crushed gel mixture was loaded onto Pierce® Centrifuge Columns (Thermo Scientific) and centrifuged at 12 000 rpm for 5 min to collect the supernatant. Purified DNA mini-circles were then stored at 4 ^{o}C until use or at -20 ^{o}C for longer storage.

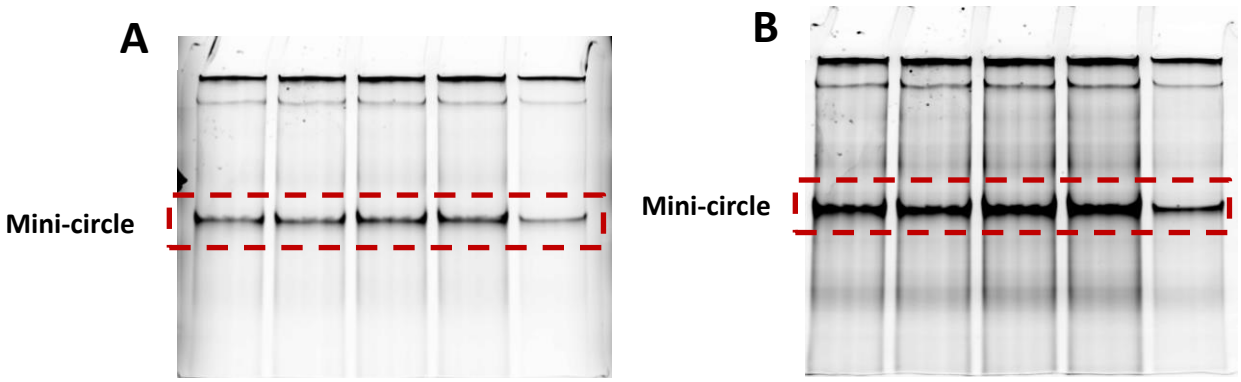


Figure S5. Purification of DNA mini-circles by 6% native polyacrylamide gel electrophoresis. A) FAM-TAMRA labelled DNA mini-circles (FAM filter). B) FAM-TAMRA labelled DNA mini-circles (TAMRA filter). All wells contain impure annealed FAM-TAMRA mini-circle solution. Gels were run in the presence of 1xTBE buffer for 2 h at constant 90 V. The required band indicated by the dashed red box was cut out, and the mini-circles extracted.

Purification of FAM/TAMRA-labelled L-mini-circles. DNA L-mini-circles were purified by loading 50 μL of the annealed 1 μM sample into wide-wells of 1.5 mm mini-gels. After pre-running for 30 mins at 90 V, the gel was run for 1 h at 90 V. The required band was detected with the Ettan Imager scanner, cut out and crushed through a 1 mL syringe into an eppendorf tube. 200 μL of 100 mM NaCl buffer was added into the eppendorf and left to soak over 3 days at 4 ^{o}C. After that the crushed gel mixture was loaded onto Pierce® Centrifuge Columns (Thermo Scientific) and centrifuged at 12 000 rpm for 5 min to collect the supernatant. Purified DNA mini-circles were then stored at 4 ^{o}C until use or at -20 ^{o}C for longer storage.

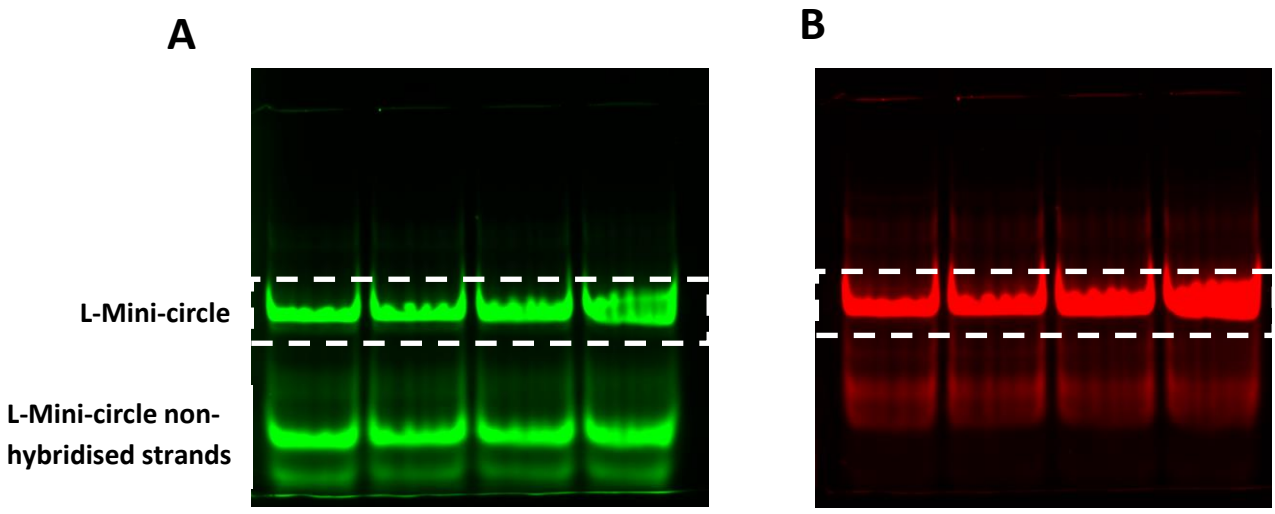


Figure S6. Purification of L-mini-circles by 6% native polyacrylamide gel electrophoresis. A) FAM-TAMRA labelled DNA L-mini-circles (FAM filter). B) FAM-TAMRA labelled DNA L-minicircles (TAMRA filter). All wells contain the impure annealed L-mini-circle solution. Gels were run in the presence of 1xTBE buffer for 1 h at constant 90 V. The required band indicated by the dashed white box was cut out, and the L-mini-circles extracted.

Atomic Force Microscopy - Sample Preparation. For AFM experiments, DNA mini-circles were adsorbed onto freshly cleaved mica specimen disks (diameter 6 mm, Agar Scientific, UK) at room temperature, using either Ni^{2+} divalent cations or cationic polymer monolayers formed by poly-L-lysine (PLL) (Sigma) and polyquaternium 6 (PQ) (Sigma) to bridge between the negative charges on the DNA and on the cleaved mica substrate.[1] For adsorption using $NiCl_2$, the freshly cleaved mica was incubated for 10 minutes with 2 µL (50-100 nM) purified DNA mini-circles, diluted in 25 µL 10 mM Tris-HCl, 10 mM $NiCl_2$ (pH 7.4) buffer to yield a 2-5 nM final DNA concentration. Excess solution (~20 µL) containing unbound mini-circles was removed, and the supernatant further diluted in 10 mM Tris-HCl pH 7.4 thereby reducing the concentration of Ni^{2+} cations on the surface to approximately 1 mM. For adsorption using cationic polymers (PLL and PQ), the mica surface was treated with 10 µL PLL or PQ for 1 minute, then washed 3x with MilliQ water, removing all remaining liquid. The cationic surface was incubated for 10 minutes with 25 µL buffer (10 mM Tris-HCl pH 7.4 or 10 mM Tris-HCl 100 mM KCl pH 7.4) and 3 µL (50-100 nM) purified DNA mini-circles to yield a 2-5 nM final

DNA concentration. Excess solution (~20 µL) containing unbound mini-circles was removed and the volume replaced with fresh buffer solution for imaging. The resulting samples were left to equilibrate over 10 minutes for the DNA mini-circles to adsorb on the mica surface. Unless indicated otherwise, mini-circles were immobilised using $NiCl_2$.

Atomic Force Microscopy – Imaging: All AFM measurements were performed in liquid. Images were obtained at a frame rate of 0.1-1 /min, using a Multimode 8 AFM with E-scanner (Bruker, Santa Barbara, CA) and a FastScan Bio AFM operated in PeakForce Tapping mode. Cantilevers used were: FastScan D (Bruker) and Biolever mini (Olympus, Tokyo, Japan). Force-distance curves were recorded over 10 or 20 nm (PeakForce Tapping amplitudes of 5 and 10 nm) at 4 kHz (Multimode) and 8 kHz (FastScan Bio). Figures 2B and S7 were recorded on the Multimode using biolever mini cantilevers. All other images were recorded using FastScan D cantilevers on the FastScan Bio. Imaging was carried out at PeakForce setpoints in the range of 5-20 mV, corresponding to peak forces of <70 pN.[2] *Image Processing:* Images were processed using zeroth-order line-by-line flattening to centre data and first order plane fitting to remove sample tilt in the Nanoscope Analysis software (Bruker AXS, CA, USA). A 3 pixel (~1 nm) Gaussian filtering was applied to remove high frequency noise.

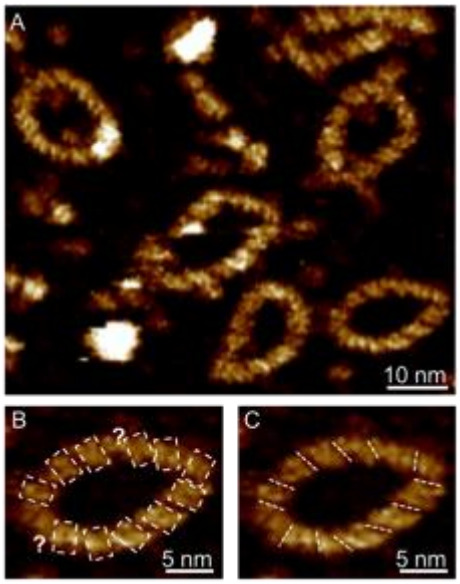


Figure S7. High resolution AFM images of purified mini-circles showing secondary structure. (B,C) Tentative identification of 12 turns of the double helix, as indicated by dashed lines. AFM data taken from the mini-circle bottom right in (A). Height scale (see inset in Fig. 1A: 2.75 nm).

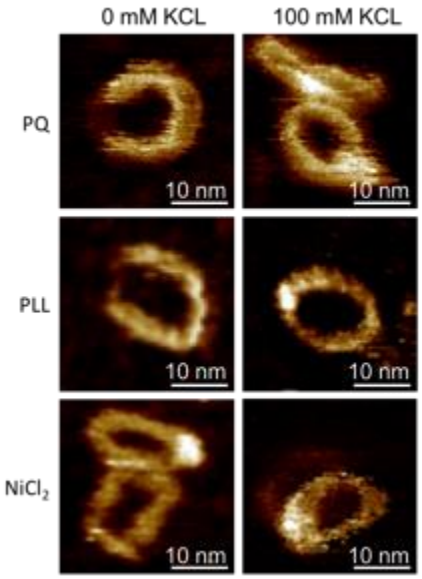


Figure S8. AFM images of purified mini-circles adsorbed using $NiCl_2$, PLL and PQ. Both PQ and PLL show an 'open' configuration without the addition of 100 mM KCl, and a 'closed' configuration for adsorption with 100 mM KCl, The 'open' configuration corresponds to a non-quadruplex containing state, with the 'closed' configuration showing the formation of a quadruplex. In the case of adsorption using $NiCl_2$ both states are seen for adsorption without KCl. For adsorption with KCl, the mini-circles then adopt the closed state. Height scale (see inset in Fig. 1A: 2.5 nm)

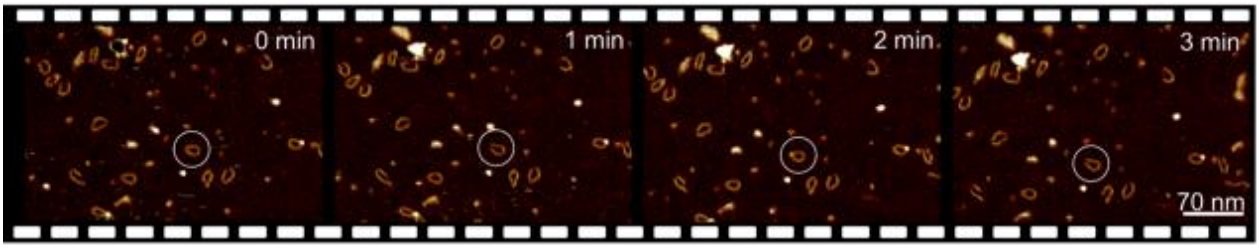


Figure S9. Timelapse imaging of quadruplex formation on the mica surface for DNA mini-circles immobilised using $NiCl_2$. Four successive images taken at 1 minute per frame show a mini-circle (highlighted by a white circle) switching from the open state to closed state at minute 2 and back again at minute 3. Height scale (see inset in Fig. 1A: 3 nm).

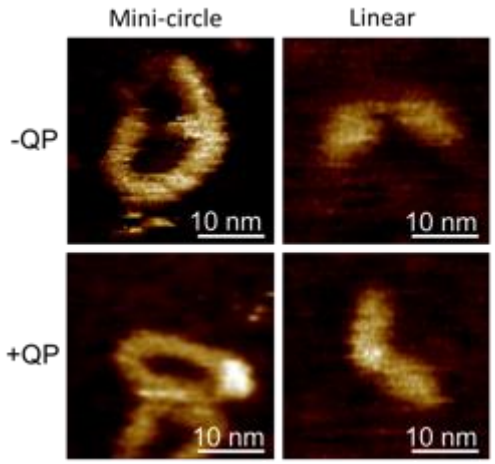


Figure S10. Comparison of the 'open' (-QP) and 'closed' (QP) states for both the mini-circle and the L-mini-circle. All images are 30 nm wide. Height scale (see inset in Fig. 1A: 2.5 nm).

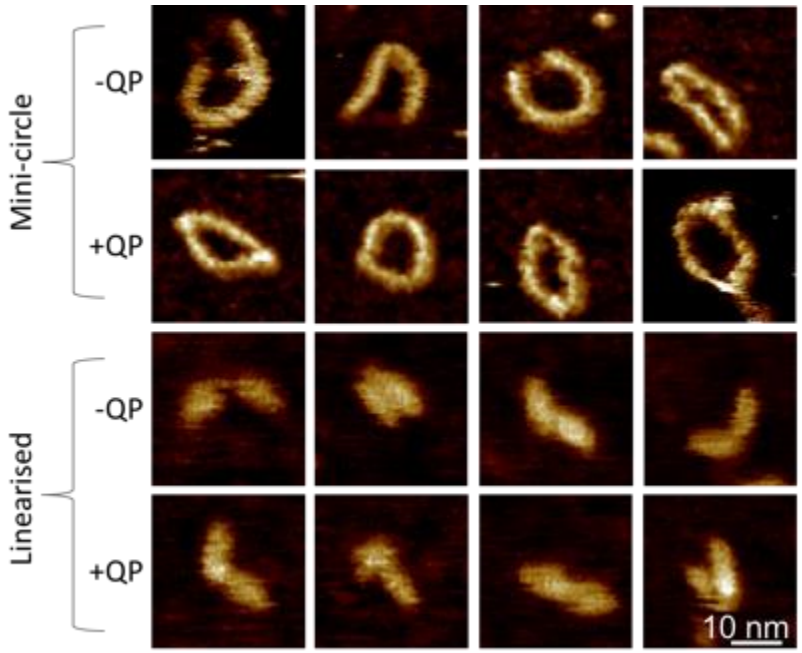
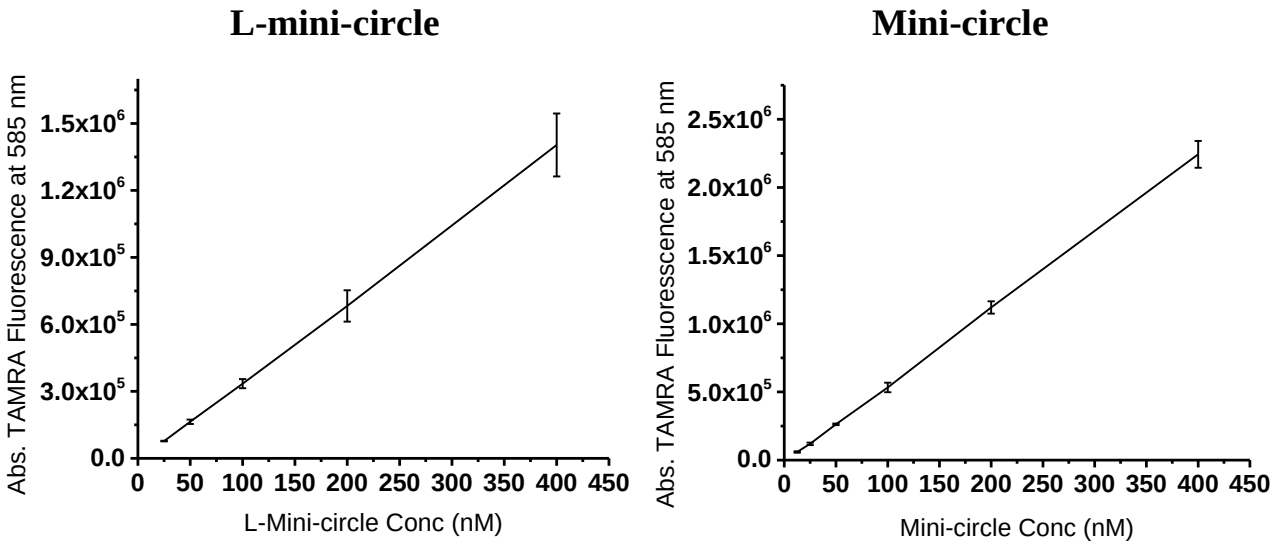


Figure S11. A gallery of images showing the 'open' (-QP) and 'closed' (QP) states for both the mini-circle and the L-mini-circle (linearised). All images are 30 nm wide. Height scale (see inset in Fig. 1A: 2.5 nm).

Determining the concentration of mini-circles and L-mini-circles. The concentrations of the purified nucleic acid structures were approximated as follows: calibration curves for both the L-mini-circles and mini-circles were constructed by using a TAMRA containing dimer (**1T** + **4C** or **4C**$_2$) annealed in sodium buffer as a mimic. Each solution was transferred into a Hellma Ultra-Micro cuvette (105.251-QS), after which a fluorescence spectrum was taken (Excitation 545 nm, 2 mm slit width, Emission 560-620 nm, 10 mm slit width, Horiba FluoroMax 4). The fluorescence maxima at 585 nm was used to determine the concentration in both cases.



$$F_{585} = 3464c - 9222 \quad [1] \qquad F_{585} = 5545c - 12619 \quad [2]$$

Figure S12. Fluorescence calibration curves to determine the concentration of the mini-circles and L-mini-circles. The curves were obtained by using a TAMRA containing dimer (**1T** + **4C** or **4C**$_2$) of known concentration annealed in sodium buffer as a mimic. The concentration (c) of a solution of L-mini-circle [1] or mini-circle [2] can be determined by reading off the TAMRA fluorescence at 585 nm (F_{585}) and applying this value to the appropriate calibration curve.

FRET studies for buffer determination. The samples for the kinetic FRET experiments were prepared as follows: the mini-circle solution (40 µL, 10 nM) in sodium/lithium buffer (100 mM, 10 mM Tris) was added to an Eppendorf tube, followed by 32 µL lithium/sodium/potassium buffer (100 mM, 10 mM Tris). This solution was gently agitated, before being stored at 4 °C for a period of approximately 36 hours.

The solution was transferred into a Hellma Ultra-Micro cuvette (105.251-QS), after which an initial fluorescence spectrum was recorded (Excitation 480 nm, 2 mm slit width, Emission 495-600 nm, 10 mm slit width, Horiba FluoroMax 4). The previously annealed complementary strand (8 µL, 5 µM) in sodium/lithium buffer was subsequently added, after which the emission of FAM (Excitation: 480 nm, 2 mm slit width, Emission: 520 nm, 10 mm slit width) was recorded every 4 seconds over 1 hour.

Kinetic FRET studies. The samples for the kinetic FRET experiments were prepared as follows: The nucleic acid structure (40 µL, 10 nM) in sodium buffer (100 mM, 10 mM Tris) was added to an Eppendorf tube, followed by either the PDS (20 µL, 100 nM) in sodium buffer (100 mM, 10 mM Tris) or sodium buffer (20 µL, 100 mM, 10 mM Tris). To this solution, potassium buffer (0.8 µL, 100 mM, 10 mM Tris) was added, after which the solution was made up to 72 µL with the sodium buffer. This solution was gently agitated, before being stored at 4 °C for a period of approximately 36 hours.

The solution was transferred into a Hellma Ultra-Micro cuvette (105.251-QS), after which an initial fluorescence spectrum was taken (Excitation 480 nm, 2 mm slit width, Emission 495-600 nm, 10 mm slit width, Horiba FluoroMax 4). The previously annealed complementary strand (8 µL, 5 µM) in sodium buffer was subsequently added, after which the emission of FAM (Excitation: 480 nm, 2 mm slit width, Emission: 520 nm, 10 mm slit width) was recorded every 4 seconds over 1 hour.

Data fitting. To obtain the decay constant from this data, the fluorescence readings were divided by the initial value of fluorescence at 520 nm (to generate the 'Relative Fluorescence' parameter). Relative fluorescence was then plotted against time for each system using Origin 2015, to generate the exponential decay curves shown in the main body of text. Each curve was fitted with either the mono-exponential *ExpDec1* (for mini-circles and L-mini-circles) or bi-exponential *ExpDec2* (for G4-mer-24) function using the Levenburg Marquardt iteration algorithm. The fitting function was applied over the entire time range (3600 seconds) for all curves, with the exception of the G4-24-mer with 0 eq. PDS, which reached a plateau after 500 seconds. The bi-exponential fitting function was therefore only applied over the first 800 seconds for this decay curve, to improve the quality of the data-fit, and thus the reliability of the obtained

decay constant. For the mono-exponential decay curves, the τ_D values could be found from the output of the fitting function [3]. For the bi-exponential decay curves, an amplitude weighted τ_D value was generated using the following equations [4] and [5] (3):

$$F_t = \mathrm{A}e^{-\frac{t}{\tau_D}} \quad \textbf{[3]}$$

$$F_t = A_1 e^{-\frac{t}{\tau_{D,1}}} + A_2 e^{-\frac{t}{\tau_{D,2}}} \quad \textbf{[4]}$$

$$\tau_D = \frac{(\mathrm{A}_1\tau_{D,1} + \mathrm{A}_2\tau_{D,2})}{(\mathrm{A}_1 + \mathrm{A}_2)} \quad \textbf{[5]}$$

Table S2. Fitting parameters generated from the application of the mono-exponential [3] and bi-exponential [4] fitting functions on the fluorescence decay curves.

System	Run	Reduced Chi-Sqr	Residual Sum of Squares	Adj. R-Square
Mini-circle 0 eq. PDS	Run 1	2.60E-05	2.34E-02	0.997
	Run 2	1.25E-05	1.12E-02	0.996
	Run 3	1.16E-05	1.05E-02	0.999
Mini-circle 5 eq. PDS	Run 1	1.11E-05	9.93E-03	0.998
	Run 2	7.95E-06	7.14E-03	0.998
	Run 3	8.74E-06	7.85E-03	0.998
L-Mini-circle 0 eq. PDS	Run 1	1.11E-04	9.97E-02	0.997
	Run 2	7.37E-05	6.61E-02	0.998
	Run 3	1.96E-04	1.76E-01	0.994
L-Mini-circle 5 eq. PDS	Run 1	3.84E-05	3.45E-02	0.998
	Run 2	3.22E-05	2.89E-02	0.998
	Run 3	3.92E-05	3.52E-02	0.998
G4-24-mer 0 eq. PDS	Run 1	7.95E-05	1.56E-02	0.999
	Run 2	8.61E-05	1.69E-02	0.999
	Run 3	1.45E-04	2.85E-02	0.998
G4-24-mer 5 eq. PDS	Run 1	4.48E-05	4.01E-02	0.998
	Run 2	3.38E-05	3.03E-02	0.999
	Run 3	3.94E-05	3.53E-02	0.999

References

(1) Pyne ALB, Hoogenboom BW. *Imaging DNA Structure by Atomic Force Microscopy*. In: Chromatin Protocols. New York, NY: Springer New York; **2016**. pp. 47–60. (Methods in Molecular Biology; vol. *1431*)

(2) Pyne ALB, Thompson R, Leung C, Roy D, Hoogenboom BW, *Small*, **2014,** *10* (16), 3257–3261.

(3) J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd ed. (Kluwer Academic/Plenum, New York, 2006), Chap. 4