Supporting Information

Monitoring G-quadruplex formation with DNA carriers and solid-state nanopores

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Section 1. Nanopore chip preparation and measurement conditions

1.1. Nanopore fabrication

Glass quartz capillaries with filaments with an outer diameter of 0.5 mm and inner diameter 0.2 mm, 7 cm in length were purchased from Sutter Instruments, USA. Capillaries were pulled to the desired pore diameter using P-2000 model of laser puller (Sutter Instruments, USA). The pulling parameters for 5 ± 1 nm nanopores were:[1]

HEAT=575, FIL=0, VEL=25, DEL=170, PUL=225

For the streptavidin-biotin assay, the inner nanopore diameter was 12 ± 3 nm (mean ± s. d.). This estimation is based on a previously described nanopore fabrication.[1,2]

The parameters have to be adjusted to glass quartz capillary batch and are variable between instruments.

1.2. Nanopore chip assembly

Capillaries were cut to the desired length using ceramic blade to connect the chip *cis* and *trans* chamber. Chip is produced by mixing PDMS and curing agent in the ratio 10:1. Mould was filled with PDMS mixture and left overnight to remove the bubbles. The mould with PDMS was solidified by heating on 60°C for 1 hour. The holes in the *trans* chambers were made by biopsy puncher with 1.5 mm diameter and holes between chambers were 1 mm in diameter. Pulled capillaries were placed to connect *cis* and *trans* chamber and attached to a glass slide using plasma etching. The 1 mm holes between cis and trans chambers were filled with 10:1 PDMS mixture and heated for 15 minutes on 100°C to separate possible connections between chambers.

1.3. Nanopore diameter estimation

A glass nanopore diameter is estimated using a scanning electron microscopy (SEM). The inner diameter of nanopores is estimated based on the previous paper.[1] An example of an SEM micrograph is shown below. We used 5 nm and 15 nm nanopores in our experiments. For detection of Gq and kinetics of Gq folding, we used 5 nm nanopores, and for streptavidin-biotin duplex-quadruplex transition, 15 nm nanopores are used. The estimation of nanopore diameter can be made based on the base current. For the 5 nm nanopore at 600mV applied voltage, in 4 M LiCl, 100 mM, 1×TE the base current has to be between 2.94 nA and 4.2 nA. Under the same conditions for 15 nm nanopore, the base current should be between 9 nA and 12 nA.

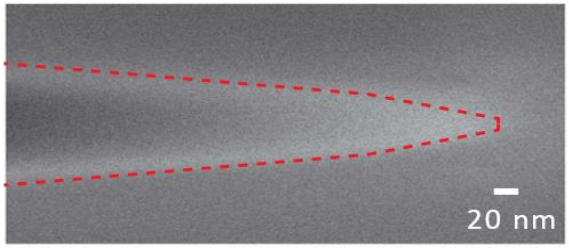

Figure S-1. An example SEM micrograph of the glass nanopore used in this study. Scale bar is 20 nm. The tip diameter represents the outer diameter of the nanopore.

1.4. Nanopore measurement

Glass nanopores were plasma cleaned for 5 minutes on maximum generator power to make glass hydrophilic. Immediately after filling chamber with working buffer (for instance 4 M LiCl, 1×TE). DNA in the concentration of 0.3 nM to 0.5 nM were mixed with the same volume of 8 M LiCl, with/without 200 mM KCl, 2×TE, pH=9.44 and then we added 4 M LiCl, with/without 100 mM KCl, 1×TE, pH = 9.44 (adjusted with 2 M LiOH) up to 15 µL. The nanopore measurements were recorded using an Axopatch 200B (Molecular Devices, Sunnyvale, CA, USA) and filtered with 50 kHz Bessel filter (Frequency devices, Ottawa, IL, USA). We digitized recordings at a 250 kHz sampling rate with a data card (PCI 6251; National Instruments, Austin, TX, USA).

1.5. Materials

Lithium chloride, lithium hydroxide, potassium chloride, sodium chloride, and 100×TE buffer were purchased from Sigma-Aldrich (1 M Tris-HCl, 0.1 M EDTA, pH = 8.0). All buffers were filtered before any experiment using 0.22 μm pore size membrane filter from (Millipore).

Section 2. DNA carrier preparation

2.1. DNA carrier annealing

The protocol is modified based on the previous paper.[2]

Single-stranded DNA carrier – M13 is bought from New English Biolabs - NEB (M13mp18, catalogue number N4040S). As previously described, a DNA carrier with the desired design is prepared as follows:
A 39nt long ssDNA was hybridised to the M13 carrier by mixing
40 μl M13 (stock concentration 250ng/μl),
8 μl 10× NEB cutsmart buffer (Catalog number B7204S),
2 μl of oligonucleotide (100μM, purchased from Integrated DNA technologies - IDT),
28 μl of milli-Q water
Followed by heating to 65°C and cooling to 25°C in a thermocycler over 40 minutes. Then 1 μl of BamHI-high fidelity (R3136T, NEB) and 1 μl of EcoRI-high fidelity (R3101T, NEB) restriction enzymes each at 100000 units/ml, were added to the reaction and incubated for 1 hour at 37°C. The linearized M13 was purified from reaction products using a NucleoSpin gel and PCR clean-up kit (Machery-Nagel), the PCR clean-up protocol was used and the purified M13 is eluted in 2 times 30 μl elution buffer to have high recovery of M13.

The next step was annealing of the designed oligonucleotides to the carrier. The reaction mixture contained
20.6 μl of milli-Q water,
6.35 μl of cut M13 (126 nM),
4.55 μl of oligonucleotide mixture (527.5 nM of each oligonucleotide),
5.6 μl of 100 mM $MgCl_2$,
2.9 μl of 10×TE (100 mM Tris-HCl pH = 8.0, 10 mM EDTA)

which was heated to 85°C for the 30 s, and then on 84.5°C followed by cooling over 1 hour to 25°C. The oligonucleotides are at 3 times excess to the carrier. Nonannealed oligonucleotides were removed using Amicon Ultra 0.5 ml 100kDa filters. One tube of reaction (40 μl) was added to 460 μl of washing buffer (10 mM Tris-HCl pH = 8.0, 0.5 mM $MgCl_2$) and centrifuged at 6000×g for 10 minutes at 4°C. This process is repeated three times. After leftover oligonucleotide removal, around 30 μl of purified DNA nanostructure was collected and 3 μl of stabilizing solution was added (100 mM NaCl, 2 mM $MgCl_2$, 10 mM Tris-HCl, pH=8.0). The concentration of DNA was measured on the Nanodrop UV/Vis spectrophotometer.

*For preparation of the DNA carriers used in direct detection of Gqs (Figure 1-3), purification is done in only filtered 10 mM Tris-HCl solution and stabilizing solution was not added.

To cover M13 carrier with a length of 7228 nt after restriction digestion, 190 oligonucleotides were hybridized (Section 2.2), and desired modified oligonucleotides were in 6 times excess to the carrier. Each oligonucleotide was 38 nt long except the last and the first with 46 nt in length.

2.2. Oligonucleotides

Below are shown oligonucleotides used to anneal to carrier M13.

Table S-1. Oligonucleotide sequences added to anneal a DNA carrier.

Oligonucleotide number	Sequence (5'-3')	Length (nt)
1	TTTTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATC	46
2	CGCTCACAATTCCACACAACATACGAGCCGGAAGCATA	38
3	AAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACT	38
4	CACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGT	38
5	CGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGC	38
6	CAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCCA	38
7	GGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGC	38
8	TGATTGCCCTTCACCGCCTGGCCCTGAGAGAGTTGCAG	38
9	CAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAAT	38
10	CCTGTTTGATGGTGGTTCCGAAATCGGCAAAATCCCTT	38
11	ATAAATCAAAAGAATAGCCCGAGATAGGGTTGAGTGTT	38
12	GTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGT	38
13	GGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGG	38
14	GCGATGGCCCACTACGTGAACCATCACCCAAATCAAGT	38
15	TTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAA	38
16	CCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAA	38
17	AGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCG	38
18	AAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGT	38
19	CACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATG	38
20	CGCCGCTACAGGGCGCGTACTATGGTTGCTTTGACGAG	38
21	CACGTATAACGTGCTTTCCTCGTTAGAATCAGAGCGGG	38
22	AGCTAAACAGGAGGCCGATTAAAGGGATTTTAGACAGG	38
23	AACGGTACGCCAGAATCCTGAGAAGTGTTTTTATAATC	38
24	AGTGAGGCCACCGAGTAAAAGAGTCTGTCCATCACGCA	38
25	AATTAACCGTTGTAGCAATACTTCTTTGATTAGTAATA	38
26	ACATCACTTGCCTGAGTAGAAGAACTCAAACTATCGGC	38
27	CTTGCTGGTAATATCCAGAACAATATTACCGCCAGCCA	38
28	TTGCAACAGGAAAAACGCTCATGGAAATACCTACATTT	38
29	TGACGCTCAATCGTCTGAAATGGATTATTTACATTGGC	38
30	AGATTCACCAGTCACACGACCAGTAATAAAAGGGACAT	38
31	TCTGGCCAACAGAGATAGAACCCTTCTGACCTGAAAGC	38
32	GTAAGAATACGTGGCACAGACAATATTTTTGAATGGCT	38
33	ATTAGTCTTTAATGCGCGAACTGATAGCCCTAAAACAT	38
34	CGCCATTAAAAATACCGAACGAACCACCAGCAGAAGAT	38
35	AAAACAGAGGTGAGGCGGTCAGTATTAACACCGCCTGC	38
36	AACAGTGCCACGCTGAGAGCCAGCAGCAAATGAAAAAT	38
37	CTAAAGCATCACCTTGCTGAACCTCAAATATCAAACCC	38
38	TCAATCAATATCTGGTCAGTTGGCAAATCAACAGTTGA	38
39	AAGGAATTGAGGAAGGTTATCTAAAATATCTTTAGGAG	38
40	CACTAACAACTAATAGATTAGAGCCGTCAATAGATAAT	38
41	ACATTTGAGGATTTAGAAGTATTAGACTTTACAAACAA	38
42	TTCGACAACTCGTATTAAATCCTTTGCCCGAACGTTAT	38
43	TAATTTTAAAAGTTTGAGTAACATTATCATTTTGCGGA	38
44	ACAAAGAAACCACCAGAAGGAGCGGAATTATCATCATA	38
45	TTCCTGATTATCAGATGATGGCAATTCATCAATATAAT	38
46	CCTGATTGTTTGGATTATACTTCTGAATAATGGAAGGG	38
47	TTAGAACCTACCATATCAAAATTATTTGCACGTAAAAC	38
48	AGAAATAAAGAAATTGCGTAGATTTTCAGGTTTAACGT	38
49	CAGATGAATATACAGTAACAGTACCTTTTACATCGGGA	38
50	GAAACAATAACGGATTCGCCTGATTGCTTTGAATACCA	38
51	AGTTACAAAATCGCGCAGAGGCGAATTATTCATTTCAA	38
52	TTACCTGAGCAAAAGAAGATGATGAAACAAACATCAAG	38
53	AAAACAAAATTAATTACATTTAACAATTTCATTTGAAT	38
54	TACCTTTTTTAATGGAAACAGTACATAAATCAATATAT	38
55	GTGAGTGAATAACCTTGCTTCTGTAAATCGTCGCTATT	38
56	AATTAATTTTCCCTTAGAATCCTTGAAAACATAGCGAT	38
57	AGCTTAGATTAAGACGCTGAGAAGAGTCAATAGTGAAT	38
58	TTATCAAAATCATAGGTCTGAGAGACTACCTTTTTAAC	38
59	CTCCGGCTTAGGTTGGGTTATATAACTATATGTAAATG	38
60	CTGATGCAAATCCAATCGCAAGACAAAGAACGCGAGAA	38
61	AACTTTTTCAAATATATTTTAGTTAATTTCATCTTCTG	38
62	ACCTAAATTTAATGGTTTGAAATACCGACCGTGTGATA	38
63	AATAAGGCGTTAAATAAGAATAAACACCGGAATCATAA	38
64	TTACTAGAAAAAGCCTGTTTAGTATCATATGCGTTATA	38
65	CAAATTCTTACCAGTATAAAGCCAACGCTCAACAGTAG	38
66	GGCTTAATTGAGAATCGCCATATTTAACAACGCCAACA	38
67	TGTAATTTAGGCAGAGGCATTTTCGAGCCAGTAATAAG	38
68	AGAATATAAAGTACCGACAAAAGGTAAAGTAATTCTGT	38
69	CCAGACGACGACAATAAACAACATGTTCAGCTAATGCA	38
70	GAACGCGCCTGTTTATCAACAATAGATAAGTCCTGAAC	38
71	AAGAAAAATAATATCCCATCCTAATTTACGAGCATGTA	38
72	GAAACCAATCAATAATCGGCTGTCTTTCCTTATCATTC	38
73	CAAGAACGGGTATTAAACCAAGTACCGCACTCATCGAG	38
74	AACAAGCAAGCCGTTTTTATTTTCATCGTAGGAATCAT	38
75	TACCGCGCCCAATAGCAAGCAAATCAGATATAGAAGGC	38
76	TTATCCGGTATTCTAAGAACGCGAGGCGTTTTAGCGAA	38
77	CCTCCCGACTTGCGGGAGGTTTTGAAGCCTTAAATCAA	38
78	GATTAGTTGCTATTTTGCACCCAGCTACAATTTTATCC	38
79	TGAATCTTACCAACGCTAACGAGCGTCTTTCCAGAGCC	38
80	TAATTTGCCAGTTACAAAATAAACAGCCATATTATTTA	38
81	TCCCAATCCAAATAAGAAACGATTTTTTGTTTAACGTC	38
82	AAAAATGAAAATAGCAGCCTTTACAGAGAGAATAACAT	38
83	AAAAACAGGGAAGCGCATTAGACGGGAGAATTAACTGA	38
84	ACACCCTGAACAAAGTCAGAGGGTAATTGAGCGCTAAT	38
85	ATCAGAGAGATAACCCACAAGAATTGAGTTAAGCCCAA	38
86	TAATAAGAGCAAGAAACAATGAAATAGCAATAGCTATC	38
87	TTACCGAAGCCCTTTTTAAGAAAAGTAAGCAGATAGCC	38
88	GAACAAAGTTACCAGAAGGAAACCGAGGAAACGCAATA	38
89	ATAACGGAATACCCAAAAGAACTGGCATGATTAAGACT	38
90	CCTTATTACGCAGTATGTTAGCAAACGTAGAAAATACA	38
91	TACATAAAGGTGGCAACATATAAAAGAAACGCAAAGAC	38
92	ACCACGGAATAAGTTTATTTTGTCACAATCAATAGAAA	38
93	ATTCATATGGTTTACCAGCGCCAAAGACAAAAGGGCGA	38
94	CATTCAACCGATTGAGGGAGGGAAGGTAAATATTGACG	38
95	GAAATTATTCATTAAAGGTGAATTATCACCGTCACCGA	38
96	CTTGAGCCATTTGGGAATTAGAGCCAGCAAAATCACCA	38
97	GTAGCACCATTACCATTAGCAAGGCCGGAAACGTCACC	38
98	AATGAAACCATCGATAGCAGCACCGTAATCAGTAGCGA	38
99	CAGAATCAAGTTTGCCTTTAGCGTCAGACTGTAGCGCG	38
100	TTTTCATCGGCATTTTCGGTCATAGCCCCCTTATTAGC	38
101	GTTTGCCATCTTTTCATAATCAAAATCACCGGAACCAG	38
102	AGCCACCACCGGAACCGCCTCCCTCAGAGCCGCCACCC	38
103	TCAGAACCGCCACCCTCAGAGCCACCACCCTCAGAGCC	38
104	GCCACCAGAACCACCACCAGAGCCGCCGCCAGCATTGA	38
105	CAGGAGGTTGAGGCAGGTCAGACGATTGGCCTTGATAT	38
106	TCACAAACAAATAAATCCTCATTAAAGCCAGAATGGAA	38
107	AGCGCAGTCTCTGAATTTACCGTTCCAGTAAGCGTCAT	38
108	ACATGGCTTTTGATGATACAGGAGTGTACTGGTAATAA	38
109	GTTTTAACGGGGTCAGTGCCTTGAGTAACAGTGCCCGT	38
110	ATAAACAGTTAATGCCCCCTGCCTATTTCGGAACCTAT	38
111	TATTCTGAAACATGAAAGTATTAAGAGGCTGAGACTCC	38
112	TCAAGAGAAGGATTAGGATTAGCGGGGTTTTGCTCAGT	38
113	ACCAGGCGGATAAGTGCCGTCGAGAGGGTTGATATAAG	38
114	TATAGCCCGGAATAGGTGTATCACCGTACTCAGGAGGT	38
115	TTAGTACCGCCACCCTCAGAACCGCCACCCTCAGAACC	38
116	GCCACCCTCAGAGCCACCACCCTCATTTTCAGGGATAG	38
117	CAAGCCCAATAGGAACCCATGTACCGTAACACTGAGTT	38
118	TCGTCACCAGTACAAACTACAACGCCTGTAGCATTCCA	38
119	CAGACAGCCCTCATAGTTAGCGTAACGATCTAAAGTTT	38
120	TGTCGTCTTTCCAGACGTTAGTAAATGAATTTTCTGTA	38
121	TGGGATTTTGCTAAACAACTTTCAACAGTTTCAGCGGA	38
122	GTGAGAATAGAAAGGAACAACTAAAGGAATTGCGAATA	38
123	ATAATTTTTTCACGTTGAAAATCTCCAAAAAAAAGGCT	38
124	CCAAAAGGAGCCTTTAATTGTATCGGTTTATCAGCTTG	38
125	CTTTCGAGGTGAATTTCTTAAACAGCTTGATACCGATA	38
126	GTTGCGCCGACAATGACAACAACCATCGCCCACGCATA	38
127	ACCGATATATTCGGTCGCTGAGGCTTGCAGGGAGTTAA	38
128	AGGCCGCTTTTGCGGGATCGTCACCCTCAGCAGCGAAA	38
129	GACAGCATCGGAACGAGGGTAGCAACGGCTACAGAGGC	38
130	TTTGAGGACTAAAGACTTTTTCATGAGGAAGTTTCCAT	38
131	TAAACGGGTAAAATACGTAATGCCACTACGAAGGCACC	38
132	AACCTAAAACGAAAGAGGCAAAAGAATACACTAAAACA	38
133	CTCATCTTTGACCCCCAGCGATTATACCAAGCGCGAAA	38
134	CAAAGTACAACGGAGATTTGTATCATCGCCTGATAAAT	38
135	TGTGTCGAAATCCGCGACCTGCTCCATGTTACTTAGCC	38
136	GGAACGAGGCGCAGACGGTCAATCATAAGGGAACCGAA	38
137	CTGACCAACTTTGAAAGAGGACAGATGAACGGTGTACA	38
138	GACCAGGCGCATAGGCTGGCTGACCTTCATCAAGAGTA	38
139	ATCTTGACAAGAACCGGATATTCATTACCCAAATCAAC	38
140	GTAACAAAGCTGCTCATTCAGTGAATAAGGCTTGCCCT	38
141	GACGAGAAACACCAGAACGAGTAGTAAATTGGGCTTGA	38
142	GATGGTTTAATTTCAACTTTAATCATTGTGAATTACCT	38
143	TATGCGATTTTAAGAACTGGCTCATTATACCAGTCAGG	38
144	ACGTTGGGAAGAAAAATCTACGTTAATAAAACGAACTA	38
145	ACGGAACAACATTATTACAGGTAGAAAGATTCATCAGT	38
146	TGAGATTTAGGAATACCACATTCAACTAATGCAGATAC	38
147	ATAACGCCAAAAGGAATTACGAGGCATAGTAAGAGCAA	38
148	CACTATCATAACCCTCGTTTACCAGACGACGATAAAAA	38

149	CCAAAATAGCGAGAGGCTTTTGCAAAAGAAGTTTTGCC	38
150	AGAGGGGGTAATAGTAAAATGTTTAGACTGGATAGCGT	38
151	CCAATACTGCGGAATCGTCATAAATATTCATTGAATCC	38
152	CCCTCAAATGCTTTAAACAGTTCAGAAAACGAGAATGA	38
153	CCATAAATCAAAAATCAGGTCTTTACCCTGACTATTAT	38
154	AGTCAGAAGCAAAGCGGATTGCATCAAAAAGATTAAGA	38
155	GGAAGCCCGAAAGACTTCAAATATCGCGTTTTAATTCG	38
156	AGCTTCAAAGCGAACCAGACCGGAAGCAAACTCCAACA	38
157	GGTCAGGATTAGAGAGTACCTTTAATTGCTCCTTTTGA	38
158	TAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTG	38
159	CTGAATATAATGCTGTAGCTCAACATGTTTTAAATATG	38
160	CAACTAAAGTACGGTGTCTGGAAGTTTCATTCCATATA	38
161	ACAGTTGATTCCCAATTCTGCGAACGAGTAGATTTAGT	38
162	TTGACCATTAGATACATTTCGCAAATGGTCAATAACCT	38
163	GTTTAGCTATATTTTCATTTGGGGCGCGAGCTGAAAAG	38
164	GTGGCATCAATTCTACTAATAGTAGTAGCATTAACATC	38
165	CAATAAATCATACAGGCAAGGCAAAGAATTAGCAAAAT	38
166	TAAGCAATAAAGCCTCAGAGCATAAAGCTAAATCGGTT	38
167	GTACCAAAAACATTATGACCCTGTAATACTTTTGCGGG	38
168	AGAAGCCTTTATTTCAACGCAAGGATAAAAATTTTTAG	38
169	AACCCTCATATATTTTAAATGCAATGCCTGAGTAATGT	38
170	GTAGGTAAAGATTCAAAAGGGTGAGAAAGGCCGGAGAC	38
171	AGTCAAATCACCATCAATATGATATTCAACCGTTCTAG	38
172	CTGATAAATTAATGCCGGAGAGGGTAGCTATTTTTGAG	38
173	AGATCTACAAAGGCTATCAGGTCATTGCCTGAGAGTCT	38
174	GGAGCAAACAAGAGAATCGATGAACGGTAATCGTAAAA	38
175	CTAGCATGTCAATCATATGTACCCCGGTTGATAATCAG	38
176	AAAAGCCCCAAAAACAGGAAGATTGTATAAGCAAATAT	38
177	TTAAATTGTAAACGTTAATATTTTGTTAAAATTCGCAT	38
178	TAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAG	38
179	GAACGCCATCAAAAATAATTCGCGTCTGGCCTTCCTGT	38
180	AGCCAGCTTTCATCAACATTAAATGTGAGCGAGTAACA	38
181	ACCCGTCGGATTCTCCGTGGGAACAAACGGCGGATTGA	38
182	CCGTAATGGGATAGGTCACGTTGGTGTAGATGGGCGCA	38
183	TCGTAACCGTGCATCTGCCAGTTTGAGGGGACGACGAC	38
184	AGTATCGGCCTCAGGAAGATCGCACTCCAGCCAGCTTT	38
185	CCGGCACCGCTTCTGGTGCCGGAAACCAGGCAAAGCGC	38
186	CATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCG	38
187	ATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGA	38
188	AAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACG	38
189	CCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGC	38
190	CAGTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCTTTT	46

2.3. G-quadruplex (Gq) sequences

The full modified oligonucleotide sequences with attached Gq sequences and sequences of complementary strands with biotin modifications. The Gq sequence itself is bolded at the table. Number/position of oligonucleotide from the table S-1. which is modified is indicated at the table S-2.

Table S-2. Gq sequences and complementary sequences used in this study.

G-quadruplex full name	Code	Sequence (5'-3')	Modified oligonucleotide	Length (nt)
HIV integrase G-quadruplex	T30695	AATTAACCGTTGTAGCAATACTTCTTTGATTAGTAATA TTT **GGGTGGGTGGGTGGGT**	25	57
	T30695	GAAACAATAACGGATTCGCCTGATTGCTTTGAATACCA TTT **GGGTGGGTGGGTGGGT**	50	57
	T30695	TACCGCGCCCAATAGCAAGCAAATCAGATATAGAAGGC TTT **GGGTGGGTGGGTGGGT**	75	57
	T30695	TTTTCATCGGCATTTTCGGTCATAGCCCCCTTATTAGC TTT **GGGTGGGTGGGTGGGT**	100	57
	T30695	CTTTCGAGGTGAATTTCTTAAACAGCTTGATACCGATA TTT **GGGTGGGTGGGTGGGT**	125	57
	T30695	AGAGGGGGTAATAGTAAAATGTTTAGACTGGATAGCGT TTT **GGGTGGGTGGGTGGGT**	150	57
Single stranded poly-deoxythymidine DNA	polydT	AATTAACCGTTGTAGCAATACTTCTTTGATTAGTAATA TTT **TTTTTTTTTTTTTTTT**	25	57
	polydT	GAAACAATAACGGATTCGCCTGATTGCTTTGAATACCA TTT **TTTTTTTTTTTTTTTT**	50	57
	polydT	TACCGCGCCCAATAGCAAGCAAATCAGATATAGAAGGC TTT **TTTTTTTTTTTTTTTT**	75	57
	polydT	TTTTCATCGGCATTTTCGGTCATAGCCCCCTTATTAGC TTT **TTTTTTTTTTTTTTTT**	100	57
	polydT	CTTTCGAGGTGAATTTCTTAAACAGCTTGATACCGATA TTT **TTTTTTTTTTTTTTTT**	125	57
	polydT	AGAGGGGGTAATAGTAAAATGTTTAGACTGGATAGCGT TTT **TTTTTTTTTTTTTTTT**	150	57

Human telomere G-quadruplex	hTel	GTAGCACCATTACCATTAGCAAGGCCGGAAACGTCACCT TTT **TAGGGTTAGGGTTAGGGTTAGGG**	97	65
	hTel	TGGGATTTTGCTAAACAACTTTCAACAGTTTCAGCGGAT TTT **TAGGGTTAGGGTTAGGGTTAGGG**	121	65
	hTel	GATGGTTTAATTTCAACTTTAATCATTGTGAATTACCTT TTT **TAGGGTTAGGGTTAGGGTTAGGG**	142	65
Human minisatellite 25CEB motif	26CEB	GTAGCACCATTACCATTAGCAAGGCCGGAAACGTCACCT TTT **AAGGGTGGGTGTAAGTGTGGGTGGGT**	97	68
	26CEB	TGGGATTTTGCTAAACAACTTTCAACAGTTTCAGCGGAT TTT **AAGGGTGGGTGTAAGTGTGGGTGGGT**	121	68
	26CEB	GATGGTTTAATTTCAACTTTAATCATTGTGAATTACCTT TTT **AAGGGTGGGTGTAAGTGTGGGTGGGT**	142	68
T30695 5’-biotin complementary strand	TC	/5Biosg/TTTACCCACCCACCCACCC	/	19
hTel 5’-biotin complementary strand	HC	/5Biosg/TTTCCCTAACCCTAACCCTAACCCTAA	/	27
26CEB 5’-biotin complementary strand	CC	/5Biosg/TTTACCCACCCACACTTACACCCACCCTT	/	29

Section 3. Circular dichroism recordings

G-quadruplexes were purchased from Integrated DNA Technologies and dissolved in water to 100 µM (IDT). For the CD measurement oligos were diluted in the respective buffer. Control samples were diluted in either control buffer (4 M LiCl, 1×TE) Gq buffer (4 M LiCl, 1×TE, 100 mM KCl). With CD measurements we wanted to see if Gqs are folded in control buffer and afterwards if in Gq buffer they are folded. To do that, we diluted Gq oligonucleotides to 10 µM in the respective buffer and leave samples overnight at 4°C. CD measurements were recorded on JASCO J – 810 with a temperature-controlled cuvette holder and incubated/measured for 1 h at 20°C. A quartz cuvette with a path length of 1 mm was used. CD spectra were obtained as the average of 5 individual measurements in a range between 210 nm and 400 nm, with data interval 0.5 nm, bandwidth 1 nm, scanning speed 50 nm/min and response time of 1 s. CD spectra of Gqs were normalized to the molar ellipticity using the formula $\Delta\varepsilon$ ($M^{-1}\cdot cm^{-1}$) = $\theta/(32980*c*l)^3$ where θ represents the CD ellipticity in millidegrees (mdeg), c is the Gq concentration in mol/L, and l is the path length in cm. CD spectra of all Gqs used in this study obtained in control buffer are shown in Figure S-2.

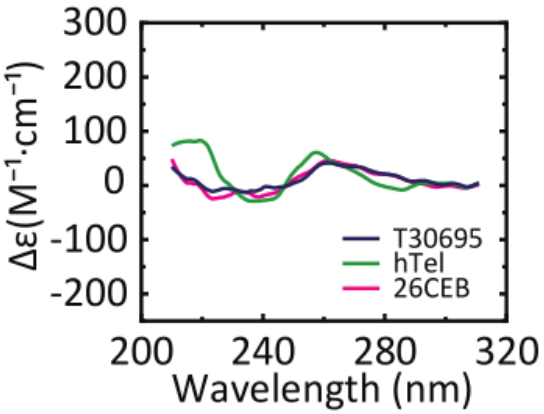

Figure S-2. Control CD spectra of Gqs used in this study in 4M LiCl, 1×TE measurement buffer without KCl.

Section 4. Data analysis

We used the home-built Python code to find translocations. Since we are collecting single molecule data, short DNA fragments and large aggregates are excluded from further analysis and code distinguish them based on event charge deficit (event surface). Translocation finder isolates events with 100 points before and after an event. Secondly, we set the threshold for peak detection and used Bayesian fitting.[4] Data consist of folded DNA and unfolded DNA. In further analysis, we used only linear-unfolded DNA events.

Positional analysis is done by relative measuring of peak position from the closest end of an event. In this way, we normalize the peak position regardless of the direction in which DNA translocates through a nanopore.

Detail description of nanopore data analysis are presented previously[1–3]. In the case of direct detection of G-quadruplexes, we normalized peak drop to DNA event as shown in Figure 3. This difference is used as $\mathbf{\Delta I_{peak}}$. All data are plotted using OriginPro 2018 version.

Section 5. Statistics of nanopores

Here we presented the characteristics of each nanopore measurement we used in this paper including the base current at 600 mV and respective RMS noise.

Table S-3. Data for individual nanopores including base current and a root mean square (RMS) noise for the 600 mV voltage used to obtain data.

Experiment	Nanopore number	Base current at 600 mV (nA)	RMS noise at 600 mV (nA)
Detection of T30695	1	4.22	0.00571
	2	3.71	0.00524
	3	3.27	0.00559
	4	2.94	0.0058
Detection of hTel	1	3.43	0.00531
	2	3.42	0.00542
	3	3.43	0.00555
	4	2.93	0.00516
Detection of 26CEB	5	3.2	0.00573
	6	3.05	0.00564
	7	3.84	0.00509

	8	3.6	0.00511
	9	3.06	0.00508
Kinetics of T30695 Gq folding	1	3.72	0.00539
	2	3.12	0.00519
	3	3.57	0.00545
	4	3.21	0.00547
	5	3.82	0.00530
	6	3.41	0.00593
	7	3.77	0.00537
	8	3.31	0.00559
	9	3.27	0.00556
	10	3.31	0.00557
T30965+TC	1	12.59	0.00677
	2	10.17	0.00676
	3	9.86	0.00683
hTel+HC	1	9.28	0.00674
	2	9.83	0.00660
	3	9.86	0.00654
26CEB+CC	1	9.9	0.00659
	2	9.29	0.00656
	3	9.13	0.00661

The IV curves for the nanopores used for data obtaining are shown below. On Figure S-3a. IV curves of nanopores used in direct detection of Gqs and kinetics of Gq folding are plotted. On Figure S-3b. the IV curves used for the quadruplex-duplex competition are plotted.

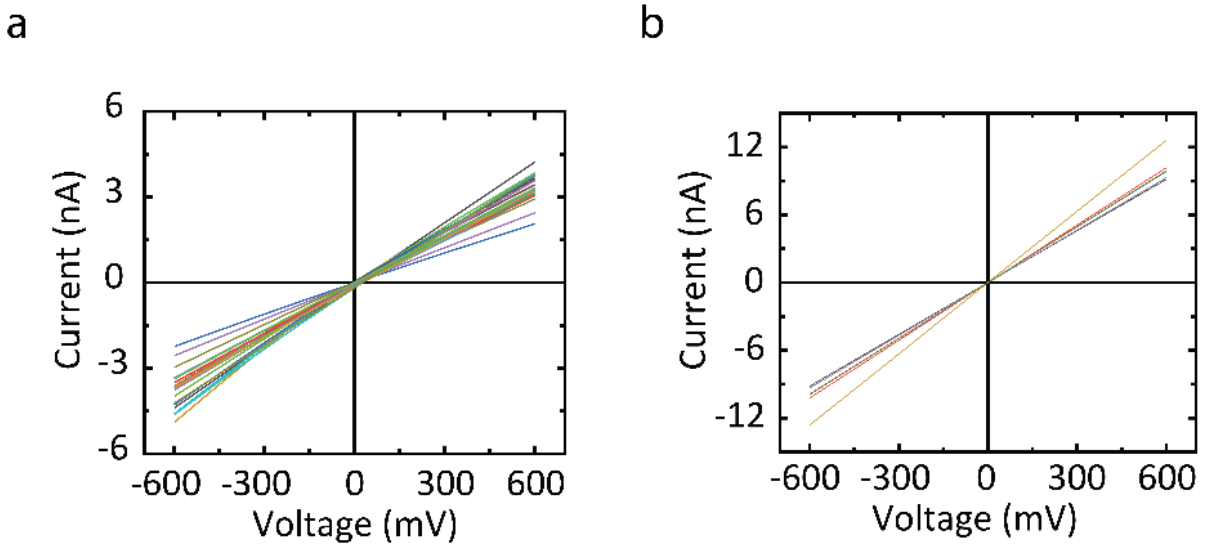

Figure S-3. IV curves of nanopores for a) direct detection of Gq and for b) streptavidin-biotin assay.

Example of 2s current trace obtained in this study is shown below. Two events can be observed. The first one unfolded DNA and the second one represents folded DNA.

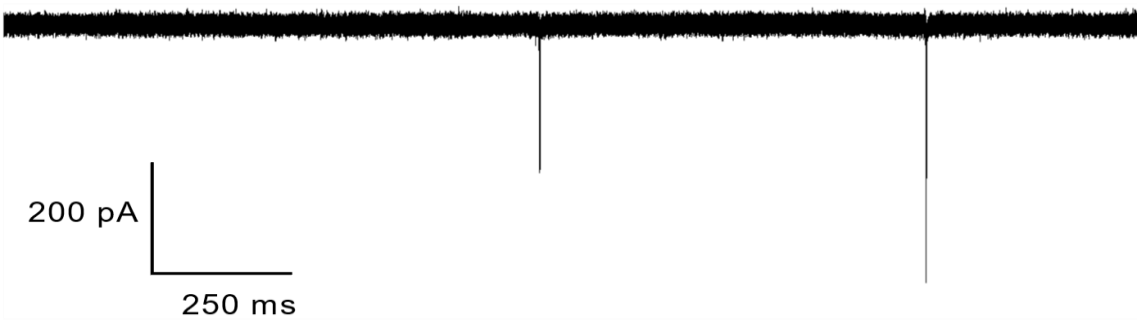

Figure S-4. An example of a current trace from the data.

Section 6. Sample events

For data presented in Figure 2. and Figure 4. we designed following DNA carriers. Firstly, for T30695 Gq modified oligonucleotides with Gq-sequence were added to 3' end of oligonucleotide positions 25, 50, and 75. Thus, the peaks in positions 25, 50, and 75 corresponds to 13%, 27%, and 40% of event duration, respectively. In Figure 2 peaks positions are shown as 87%, 73%, and 60% (100% minus 13%, 27%, and 40%) for easier graphical understanding of data. Secondly, for hTel and 26CEB Gq modified oligonucleotides with respective Gq-sequence were added to 3' end of oligonucleotide positions 97, 121, and 142. The peaks in positions 97, 121, and 142 corresponds to 51%, 64%, and 75% of event duration. In our data analysis we measure peak position from the closest end of event. In this way we normalize peak position regardless of the DNA carrier direction of entering in nanopore.

The DNA carrier for Gq folding kinetics experiments (Figure 3) had T30695 Gq modified oligonucleotides with Gq-sequence added to 3' end of oligonucleotide positions 25, 50, 75, 100, 125, and 150. In Figure S-4., we show additional sample events for folded T30695 Gqs in 100 mM KCl.

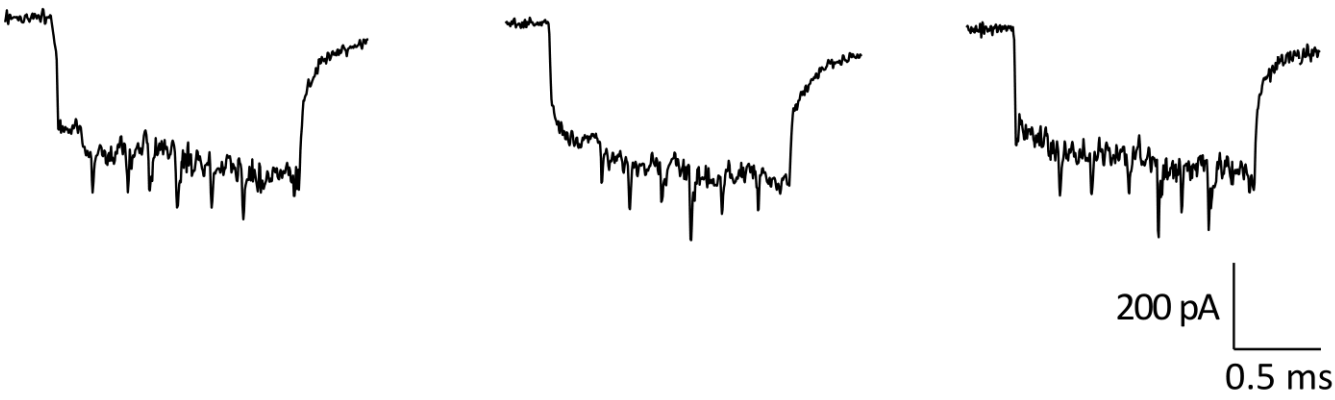

Figure S-5. Sample events for folded T30695 Gq in 100 mM KCl are presented. The six peaks corresponding to folded Gq are shown.

For the DNA carrier designs used for quadruplex-duplex competition we exchange one of the 190 oligonucleotides with oligonucleotide with desired Gq-sequence at the 3' end. This was done in step of assembly of DNA carrier as aforementioned. The designed DNA carriers with sample events for both conditions are shown in Figure S-5. For the quadruplex-duplex structural transition data, we have the possibility of a maximum three folded/unfolded Gq so we have either three peaks (all unfolded Gq), two peaks (two unfolded Gq), 1 peak (one unfolded Gq), or no peaks (all folded Gq). Below we show sample events regarding all possible combinations for the quadruplex-duplex competition for T30695 (Figure S-6.), hTel (Figure S-7.) and 26CEB Gq (Figure S-8.). The position is indicated as place where Gq is added to the 3'-sequence of oligonucleotides shown in Table S-1.

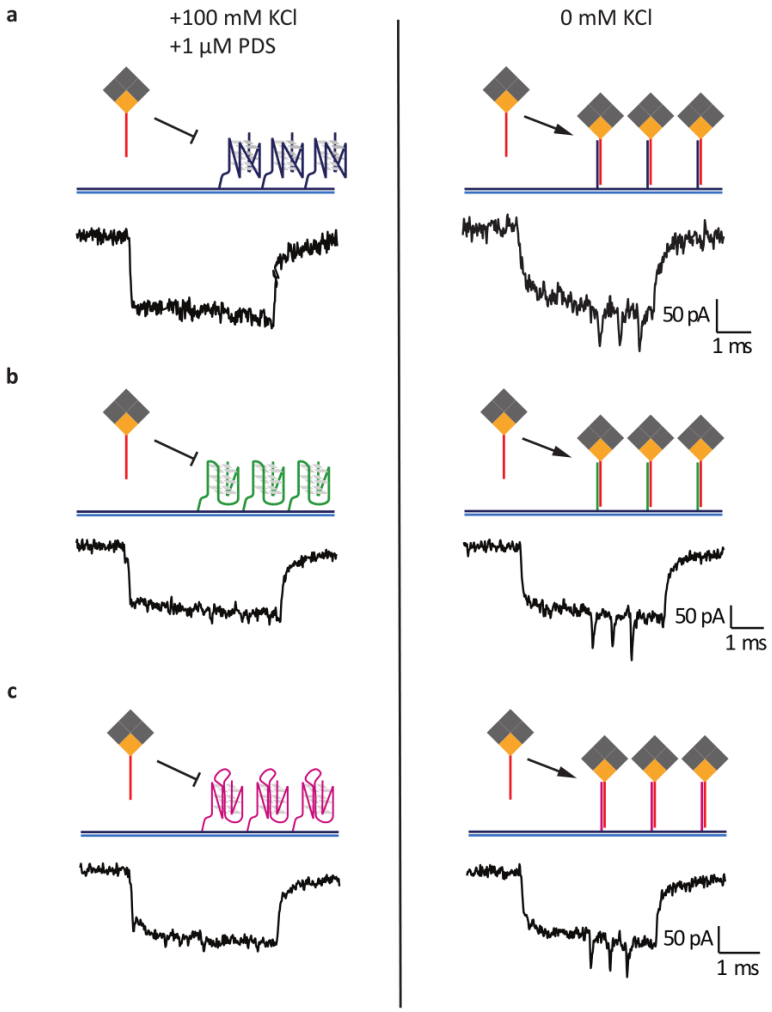

Figure S-6. smGNA quantifies quadruplex-duplex competition. In the presence of Gq stabilizing conditions including 1 µM high-specific ligand pyridostatin (PDS) and 100 mM KCl majority of Gq are folded (left panel) even in the presence of complementary strand in the same concentration. In the absence of stabilizing conditions duplex forms in different ratios compare to Gq (right panel). The positions of Gq-forming sequences are identical as in Figure 2. Three Gqs are placed at the specific positions (percentage of the whole DNA carrier). Observed peaks correspond to duplex-biotin-streptavidin (duplex-sb) complex i.e. formed duplex. Here we present the DNA carriers and sample events for a) T30695, b) hTel, and c) 26CEB quadruplex-duplex competition.

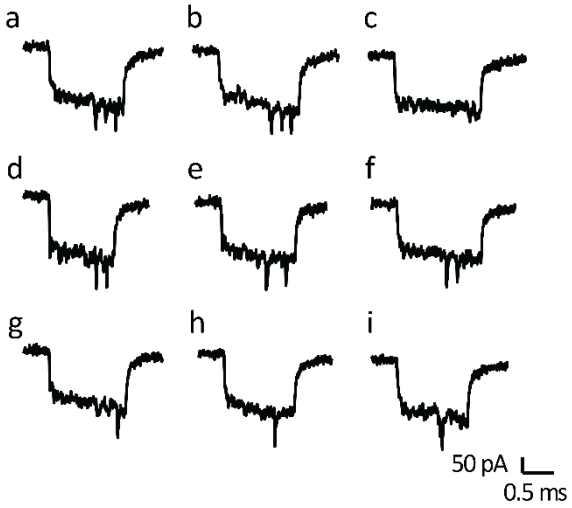

Figure S-7. Events as results of the quadruplex-duplex competition for T30695 Gq. Each peak corresponds to duplex with the streptavidin-biotin complementary strand. Three Gqs were attached to the positions 25, 50 and 75. a), b) all three unfolded, c) all three folded quadruplexes, d) quadruplexes at the positions 25 and 50 unfolded, e) quadruplexes at the positions 25 and 75 unfolded, f) quadruplexes at the positions 50 and 75 unfolded, g) quadruplex at the position 25 unfolded, h) quadruplex at the position 50 unfolded, i) quadruplex at the position 75 unfolded.

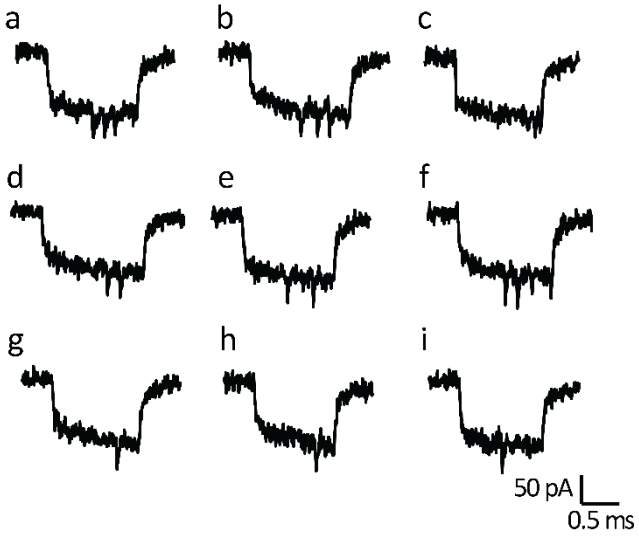

Figure S-8. Events as results of the quadruplex-duplex competition for hTel. Each peak corresponds to duplex with the streptavidin-biotin complementary strand. Three Gqs were attached to the positions 97, 121 and 142. a), b) all three unfolded, c) all three folded quadruplexes, d) quadruplexes at the positions 97 and 121 unfolded, e) quadruplexes at the positions 97 and 142 unfolded, f) quadruplexes at the positions 121 and 142 unfolded, g) quadruplex at the position 142 unfolded, h) quadruplex at the position 121 unfolded, i) quadruplex at the position 97 unfolded.

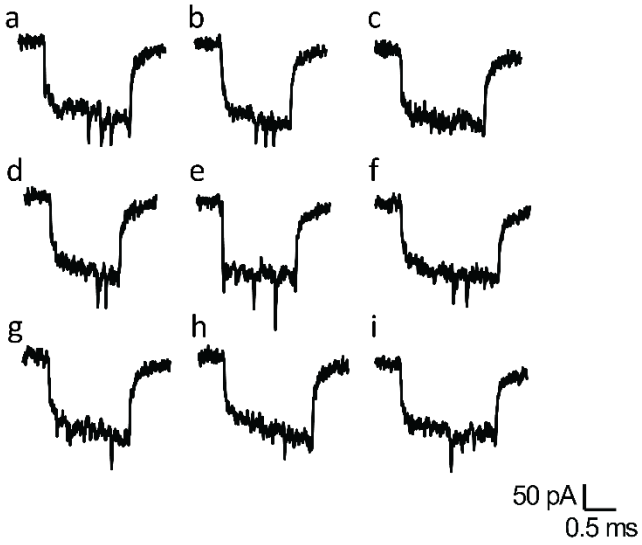

Figure S-9. Events as results of the quadruplex-duplex competition for 26CEB. Each peak corresponds to duplex with the streptavidin-biotin complementary strand. Three Gqs were attached to the positions 97, 121 and 142. a), b) all three unfolded, c) all three quadruplexes folded, d) quadruplexes at the positions 97 and 121 unfolded, e) quadruplexes at the positions 97 and 142 unfolded, f) quadruplexes at the positions 121 and 142 unfolded, g) quadruplex at the position 121 unfolded, h) quadruplex at the position 142 unfolded, i) quadruplex at the position 97 unfolded.

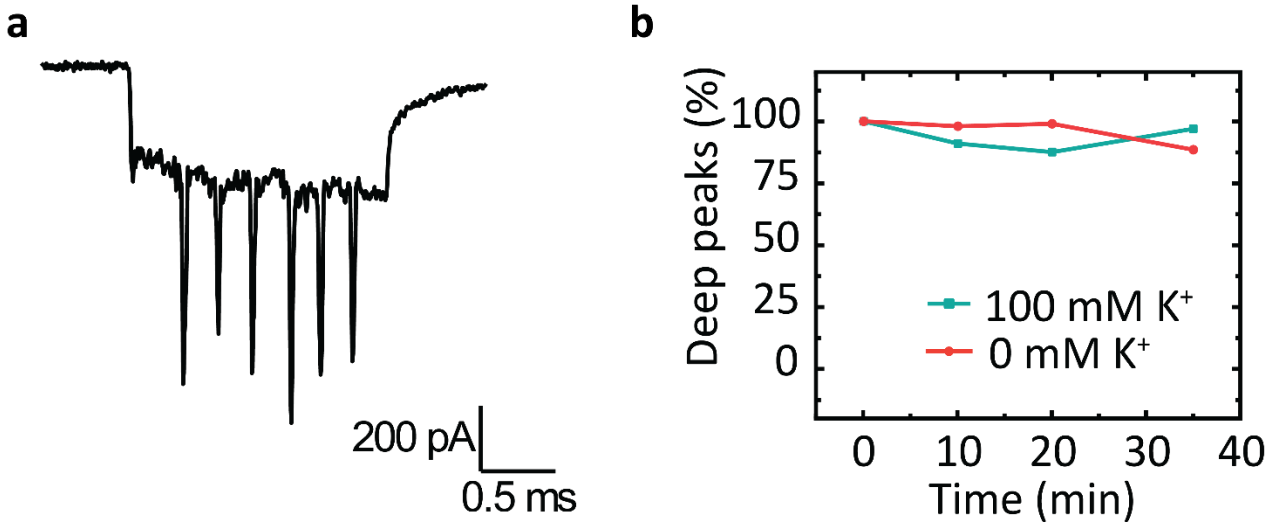

Figure S-10. DNA carrier with six single-stranded poly-deoxythymidine DNA (polydT) at the same positions as for the DNA carrier used for kinetics of Gq folding (Figure 3.). a) Six deep peaks can be observed, b) for 35 minutes six deep peaks still can be observed in both measurement buffer supplemented with 100 mM KCl and 0 mM KCl. The number of peaks are reduced if less than six peaks per event is observed.

References

1. Chen K, Kong J, Zhu J, Ermann N, Predki P, Keyser UF. Digital Data Storage Using DNA Nanostructures and Solid-State Nanopores. *Nano Lett*. 2019;19(2):1210-1215. doi:10.1021/acs.nanolett.8b04715

2. Bell NAW, Keyser UF. Digitally encoded DNA nanostructures for multiplexed, single-molecule protein

sensing with nanopores. *Nat Nanotechnol*. 2016;11(7):645-651. doi:10.1038/nnano.2016.50

3. Villar-Guerra R del, Gray RD, Chaires JB. Quadruplex DNA Structure Characterization by Circular Dichroism. *Curr Protoc Nucleic Acid Chem*. 2017;68:17.8.1. doi:10.1002/CPNC.23

4. Ermann N, Chen K, Keyser UF. Bayesian inference for nanopore data analysis. April 2019. http://arxiv.org/abs/1904.01040. Accessed July 11, 2019.