## Supporting Information

**Nanopore Detection of 8-Oxoguanine in the Human Telomere Repeat Sequence**

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**Figure S33.** Time histograms for events observed on the oxidized and labeled Q5 sample.
**Figure S1.** Sequences studied for the hTelo G4 with site-specific OG.

hTelo Wild Type  \(5`-\text{TAGGGTTAGGGTTAGGGTTAGGGTT}-3`\)

hTelo OG position 3  \(5`-\text{TAGO\text{-}GGTTAGGGTTAGGGTTAGGGTT}-3`\)

hTelo OG position 4  \(5`-\text{TAGO\text{-}GTTAGGGTTAGGGTTAGGGTT}-3`\)

hTelo OG position 9  \(5`-\text{TAGGGTATAGGGTTAGGGTTAGGGTT}-3`\)

hTelo OG position 10  \(5`-\text{TAGGGTTAGGGTTAGGGTTAGGGTT}-3`\)

hTelo OG position 15  \(5`-\text{TAGGGTTAGGGTTAGGGTTAGGGTT}-3`\)

hTelo OG position 17  \(5`-\text{TAGGGTTAGGGTTAGGGTTAGGGTT}-3`\)

hTelo OG position 21  \(5`-\text{TAGGGTTAGGGTTAGGGTTAGGGTT}-3`\)

hTelo OG position 23  \(5`-\text{TAGGGTTAGGGTTAGGGTTAGGGTT}-3`\)

\(O = \text{8-oxo-dG}\)
Figure S2. Nanopore analysis of wild type hTelo.

Data for hTelo in NaCl

|        | 120 mM NaCl | 1 M NaCl |
|--------|-------------|----------|
| $T_m$ (°C) | 56.0 ± 0.7 | 72.3 ± 0.5 |

G-Quad 5'-TAGGGTTAGGGTTAGGGTTAGGGTT

Analysis Conditions: 25 mM Tris (pH 7.9), 1 M NaCl, 25 °C.
Nanopore: 120 mV (trans vs. cis). The data were collected with a 100 kHertz filter, and the presented data were filtered at 20 kHertz.

Data taken from An, N.; et al., Proc. Nat. Acad. Sci. U.S.A. 2014, 111, 14325-14331.
Figure S3. Nanopore analysis of hTelo with OG at position 3.

Data for hTelo with OG at Position 3 in NaCl

| Concentration | Tm (°C) |
|---------------|---------|
| 120 mM NaCl   | 33.1 ± 1.0 |
| 1 M NaCl      | 65.0 ± 0.5 |

CD Data

Wavelength (nm)

Representative i-t Trace

Analysis Conditions: 25 mM Tris (pH 7.9), 1 M NaCl, 25 °C.
Nanopore: 170 mV (trans vs. cis). The data were collected with a 100 kHz filter, and the presented data were filtered at 20 kHz.
Figure S4. Nanopore analysis of hTelo with OG at position 4.

**Data for hTelo with OG at Position 4 in NaCl**

| [\(T_m\) (°C)] | 120 mM NaCl | 1 M NaCl |
|-----------------|-------------|---------|
|                 | 33.0 ± 0.2  | 54.4 ± 0.7 |

**G-Quad 5'-TAGGTTAGGTTAGGTTAGGTT**

**Representative \(i\)-\(t\) Trace**

Analysis Conditions: 25 mM Tris (pH 7.9), 1 M NaCl, 25 °C.
Nanopore: 120 mV (trans vs. cis). The data were collected with a 100 kHz filter, and the presented data were filtered at 20 kHz.

**Time vs. Count Histogram**

\[\tau = 2.13 \pm 0.17 \text{ ms}\]
Figure S5. Nanopore analysis of hTelo with OG at position 9.

**Solution Data for Quad with OG at Position 9 in NaCl**

| T_m (°C)          | 120 mM NaCl | 1 M NaCl |
|-------------------|-------------|----------|
| 39.0 ± 1.1        | 58.0 ± 0.4  |

G-Quad 5′-TAGGGTTAOGGTTAGGGTTAGGGTT

**Representative i-t Trace**

Analysis Conditions: 25 mM Tris (pH 7.9), 1 M NaCl, 25 °C.
Nanopore: 120 mV (trans vs. cis). The data were collected with a 100 kHz filter, and the presented data were filtered at 20 kHz.

**Time vs. Count Histogram**

τ = 15.56 ± 1.30 ms
Figure S6. Nanopore analysis of hTelo with OG at position 10.

**Solution Data for Quad with OG at Position 10 in NaCl**

|        | $T_m$ (°C) |
|--------|------------|
| 120 mM NaCl |              |
| 1 M NaCl    | 52.0 ± 0.5  |

G-Quad 5'-TAGGGTTAGGGTTAGGGTTAGGGTT

**Representative $i$-$t$ Trace**

**$I$ vs. Count Histogram**

Analysis Conditions: 25 mM Tris (pH 7.9), 1 M NaCl, 25 ºC. Nanopore: 120 mV (trans vs. cis). The data were collected with a 100 kHz filter, and the presented data were filtered at 20 kHz.
**Figure S7.** Nanopore analysis of hTelo with OG at position 17.

### Solution Data for Quad with OG at Position 17 in NaCl

|                | 120 mM NaCl | 1 M NaCl |
|----------------|-------------|----------|
| Tm (°C)        | 36.8 ± 0.3  | 53.9 ± 0.5 |

G-Quad 5′-TAGGTTAGGGTTAGGGTTAGGGTT

**Representative i-t Trace**

**I vs. Count Histogram**

*Analysis Conditions: 25 mM Tris (pH 7.9), 1 M NaCl, 25 °C. Nanopore: 120 mV (trans vs. cis). The data were collected with a 100 kHz filter, and the presented data were filtered at 20 kHz.*
Figure S8. Nanopore analysis of hTelo with OG at position 21.

**Solution Data for Quad with OG at Position 21 in NaCl**

| T<sub>m</sub> (°C) | 120 mM NaCl | 1 M NaCl |
|-------------------|--------------|----------|
|                   | 41.7 ± 0.2   | 57.5 ± 0.2 |

G-Quad 5′-TAGGGTTAGGTTAGGTTAOGGT

Representative i-t Trace

Analysis Conditions: 25 mM Tris (pH 7.9), 1 M NaCl, 25°C. Nanopore: 120 mV (trans vs. cis). The data were collected with a 100 kHz filter, and the presented data were filtered at 20 kHz.
Figure S9. Nanopore analysis of hTelo with OG at position 23.

### Solution Data for Quad with OG at Position 23 in NaCl

| [Tm (°C)] | 120 mM NaCl | 1 M NaCl |
|-----------|-------------|----------|
|           | 47.2 ± 0.2  | 60.0 ± 0.8 |

G-Quad 5'-TAGGGTTAGGGTTAGGGTTAGGOTT

### Representative i-t Trace

![Image of i-t Trace]

**Analysis Conditions:** 25 mM Tris (pH 7.9), 1 M NaCl, 25 °C.

Nanopore: 120 mV (trans vs. cis). The data were collected with a 100 kHz filter, and the presented data were filtered at 20 kHz.

### I vs. Count Histogram

![Image of I vs. Count Histogram]

**τ = 11.12 ± 1.29 ms**
**Figure S10.** Thermal melting studies on OG-containing G4s and van’t Hoff analysis.

### van’t Hoff Thermodynamic Analysis for a G-Quadruple

**Abs vs. Temp**

\[ \Theta_T = \frac{(\text{Abs}_T - \text{Abs}_U)}{\text{Abs}_F - \text{Abs}_U} \]

**Fraction Folded (\( \Theta \)) vs. Temp**

\[ K_a = \frac{\Theta_T}{1 - \Theta_T} \]

### Calculation of Thermodynamic Values

\[ y = mx + b \]
\[ m = -\frac{\Delta H}{R} \quad b = \frac{\Delta S}{R} \]
\[ R = 1.987 \text{cal/mol K} \]

*Mergny, J.-L.; Lacroix, L. Curr. Protocols in Nucleic Acids Chem. 2009, 17.1.1 - 17.1.15.*
Figure S11. Comparison of translocation time vs. ΔG or melting temperature.

**Correlation between duration of nanopore translocation and Tm/ΔG**
Figure S12. Analysis of Q1-G and Q1-OG in 1 M NaCl electrolyte. (A) Q1-G, (B) Q1-OG, and (C) Q1-Sp-18c6. The $i$-$t$ traces were collected under 120 mV ($trans$ vs. $cis$). The Q1-G data was taken from our previous publication (An, N.; et al., Proc. Nat. Acad. Sci. U.S.A. 2014, 111, 14325-14331).

| Voltage (mV) | $\tau$ (ms) |
|--------------|-------------|
| 100          | 241 ± 25    |
| 120          | 65 ± 10     |
| 160          | 23 ± 3      |

| Voltage (mV) | $\tau$ (ms) |
|--------------|-------------|
| 100          | 29 ± 2.0    |
| 120          | 5.6 ± 0.8   |
| 160          | 1.8 ± 0.5   |

| Voltage (mV) | $\tau$ (ms) |
|--------------|-------------|
| 100          | 34 ± 3.0    |
| 120          | 7.5 ± 0.8   |
| 160          | 2.5 ± 0.5   |
Figure S13. Continuous $i$-$t$ trace for Q1-G in 1 M NaCl at 120 mV.

The trace represents 10 seconds of recording on one $\alpha$-HL nanopore to show the event frequency. Expanded examples are provided in Figure S13. The data were collected at 120 mV ($trans$ vs. $cis$) at 25 °C. The data is shown sideways to provide the maximum resolution (top = right).
Figure S14. Expanded examples of \(i-t\) traces (Figure S12) for the Q1-G sample in 1M NaCl.

The omission of data between each example is shown to depict that these are not one continuous \(i-t\) trace. The data were collected at 120 mV (trans vs. cis) at 25 °C. These data were obtained from two individual experiments (\textit{i.e.}, different nanopores and DNA samples).
The trace represents 10 seconds of recording on one α- HL nanopore to show the event frequency. Expanded examples are provided in Figure S15. The data were collected at 120 mV (trans vs. cis) at 25 °C. The data is shown sideways to provide the maximum resolution (top = right).
Figure S16. Expanded examples of $i$-$t$ traces (Figure S14) for the Q1-OG sample in 1M NaCl.

The omission of data between each example is shown to depict that these are not one continuous $i$-$t$ trace. The data were collected at 120 mV ($trans$ vs. $cis$) at 25 °C. These data were obtained from two individual experiments ($i.e.$, different nanopores and DNA samples).
The labeling reaction for OG was conducted in a buffer and salt system that minimized folding of the hTelo sequences to any stable G4 structures (20 mM cacodylate pH 8.0, 100 mM LiCl). The DNA strand (10 μM) to be labeled was mixed with methylamino-[18-crown-6] (2 mM) and in a 200-μL reaction vessel followed by incubation at 45 °C for 20 min prior to starting the reaction. The reaction was initiated by a bolus addition of K₂IrBr₆ (200 μM), and the reaction progressed for 30 min. The reaction salts and oxidant were removed by a NAP-5 column (GE Healthcare) following the manufacturer’s protocol. The two product peaks observed represent the two diastereomers of the labeled product. The DNA strand studied in this reaction had the following sequence 5’-TGTTCATCATCGTCXTCGGTATATCCCAT-3’ X = site of labeling.
Figure S18. Reaction monitoring for labeling an hTelo containing OG with 18c6 and CD analysis of the product.

The reaction was conducted as described in Figure S17 on the sequence 5’-TAGGGTTAGGGTTAOOGGTAGGGTT-3’ (where O = 8-oxoG). During oxidation of OG to an electrophilic intermediate two possible nucleophiles can trap this species, water or the aminomethyl-[18c6]. The reaction labeled hTelo Sp was conducted without the amine present to yield only the water adduct Sp. When the amine was added to the reaction only two new peaks that elute before the starting material were observed, this is consistent with the 18c6 label outcompeting water as the nucleophile. See McGibben, P.; et al. JACS, 2013, 135, 13851 for example elution profiles and characterization of water (Sp) and amine adducts to OG in an oligomer.

CD Spectrum for hTelo Sp-18c6 Adduct

CD analysis of the hTelo 18c6 yields a spectrum that is similar to a triplex-like species (An, N.; JACS, 2013, 135, 8562). This result supports the claim that these adducts are highly disrupting to the G-quadruplex structure.
Figure S19. Continues \(i-t\) trace for Q1-Sp-18c6 in 1 M NaCl.

The trace represents 10 seconds of recording on one \(\alpha\)-HL nanopore to show the event frequency. Expanded examples are provided in Figure S18. The data were collected at 120 mV (\textit{trans} vs. \textit{cis}) at 25 \(^\circ\)C. The data is shown sideways to provide the maximum resolution (top = right).
Figure S20. Expanded examples of $i$-$t$ traces (Figure S17) for the Q1-Sp-18c6 sample in 1 M NaCl.
Continued

The omission of data between each example is shown to depict that these are not one continuous $i$-$t$ trace. The data were collected at 120 mV ($trans$ vs. $cis$) at 25 °C. These data were obtained from two individual experiments ($i.e.$, different nanopores and DNA samples).