Supporting Information

Oxidative modification of the potential G-quadruplex sequence in the

PCNA gene promoter can turn on transcription

Samuel C.J. Redstone, Aaron M. Fleming, and Cynthia J. Burrows*

Department of Chemistry, University of Utah, Salt Lake City, UT 84112-0850, USA

*To whom correspondence should be addressed

E-mails: <u>burrows@chem.utah.edu</u>

Table of Contents

ltem Page **S2** Figure S1. Example of a Sanger sequencing chromatogram **Figure S2.** Native PAGE analysis of lesion-containing *PCNA* 4- and 5-track G4s **S**3 **Figure S3.** T_m data supporting the *PCNA* G4 adopts an intramolecular fold **S4** Figure S4. Thioflavin-T data of PCNA 4-track and CD data for PCNA i-motif **S5** Figure S5. Overexposed oxidation gel to visualize the Maxam-Gilbert G lane S6 Figure S6. Thioflavin-T experimentation of lesion-containing PCNA 4and 5-track G4s **S7 Figure S7.** CD spectra of the lesion-containing *PCNA* 4- and 5-track G4s **S8**





Sanger sequencing chromatogram showing successful incorporation of the *PCNA* 5-track and flanking Nt.BspQ1 recognition sequences in the target plasmid. The sequence inserted was 5`-pTT CAG GGA GGC AGG GCG ACG GGG GCG GGG CGG GGC GG CTC TTC T and the red Gs are the positions studied with OG and p = phosphate.

Figure S2. Native PAGE analysis of lesion-containing PCNA 4- and 5-track G4s

<u>Lane</u> 1 2 3	<u>ODN</u> Ladder G5 WT G4 WT	1 :	23	4	5	6	78	89	10 11	12 13	14 15	
4 5 6	G5 F Loop 2 G5 F Loop 1 G5 F Core											
7 8 9	G5 OG Loop 2 G5 OG Loop 1 G5 OG Core	-	-				ſ					
10 11 12	G4 F Loop 2 G4 F Loop 1 G4 F Core	14		-								
13 14 15	G4 OG Loop 2 G4 OG Loop 1 G4 OG Core				/	/						

Non-denaturing PAGE analysis of lesion-containing *PCNA* 4- and 5-track sequences. *We would like to reiterate that the five-track *PCNA* sequence in all of its modified forms was extremely challenging to work with, and this is why a number of the lanes failed to give satisfactory bands. Previous studies have determined that folded G4s migrate faster than the single strands (e.g., Shrestha, P.; et al., *Nucleic Acids Res.* **2014**, *42*, 7236-7246).

Strand	Sequence
PCNA G5	5`-CAGGGAGGCA <mark>G</mark> GGC <mark>G</mark> ACGGGGGC <mark>G</mark> GGGCGGGCG-3`
PCNA G4	5'-CAGGGCGACGGGGGGGGGGGGGGGGGGGGGGGGGGGGG

The Gs labeled red represent the sites that OG or F was studied individually.

Figure S3. T_m data supporting the PCNA G4 adopts an intramolecular fold



The T_m values measured when the ODN concentration was increased to support intramolecular G4 folding. The lack of a T_m value change across the concentrations studied support this claim. The *PCNA* G4 sequence studied was 5'-CAG GGC GAC GGG GGC GGG GCG GGG CG- 3'



Figure S4. Thioflavin-T data of PCNA 4-track and CD data for PCNA i-motif

(A) CD spectra on the C-rich complementary strand showing the *PCNA* i-motif to be single stranded at the pH conditions of the study on the basis of the lambda max for this sequence to be at 278 nm at pH 5.0, 7.4, and 8.0. (B) Data showing the thioflavin-T fluorescence enhancement values for the *PCNA* G4 and C-rich complementary strand compared to the *c-MYC* positive control. The low fluorescence enhancements found for the C-rich strand supports the single-strand state for this sequence and not an i-motif fold. The *PCNA* G4 sequence studied was 5'-CAG GGC GAC GGG GGC GGG GCG GGG CG-3', the *PCNA* C4 sequence was 5'-CAG GGC T GGGG A GGG T GGGG AA-3'.

Figure S5. Overexposed oxidation gel to visualize the Maxam-Gilbert G lane



This is the overexposed PAGE shown in Figure 5 to illustrate the Maxam-Gilbert G lane (left lane) utilized to identify the G nucleotides oxidized.



Figure S6. Thioflavin-T experimentation of lesion-containing PCNA 4- and 5-track G4s



Figure S7. CD spectra of the lesion-containing PCNA 4- and 5-track G4s