

1 **DNA G-quadruplexes in the human genome: detection, functions and therapeutic**  
2 **potential**

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12  
13 **Abstract**

14 Single-stranded guanine rich DNA sequences can fold into four-stranded DNA  
15 structures called G-quadruplexes (G4s) that arise from self-stacking of two or more  
16 guanine-quartets. There has been considerable recent progress on the detection and  
17 mapping of G4 structures in the human genome and in biologically relevant contexts.  
18 These advancements have provided important new insights into their functions, for  
19 example in regulating transcription and genome stability, and their potential for  
20 therapeutic applications, much of which is aligned to predictions previously made in  
21 computational studies.

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23  
24 **Introduction**

25 Single-stranded guanine rich DNA sequences can fold into stable intramolecular and  
26 intermolecular four-stranded non-B DNA structures called G-quadruplexes (G4s,  
27 Figure 1)<sup>1</sup>. G4s arise from Hoogsteen hydrogen bonding of four guanines arranged  
28 within a planar quartet (G-quartet)<sup>1</sup>. Self-stacking of two or more G-quartets generates  
29 a G4 structure that is further stabilised by monovalent cations in the order  
30 ( $K^+ > Na^+ > NH_4^+ > Li^+$ ) (Figure 1)<sup>1</sup>. G4 formation has been observed in oligonucleotide  
31 sequences derived from the human genome, particularly gene promoters and  
32 telomeres. Such studies, along with chemical biological approaches using G4 targeted  
33 small molecules or antibodies, and also computational predictions, have suggested  
34 that G4s may be important in gene regulation and telomere biology.

35 This article will focus on recent, significant progress in detecting and mapping G4s in  
36 the human genome and the new insights into their functions and their potential for  
37 therapeutic applications. More comprehensive reviews on functional roles of DNA G4  
38 can be found elsewhere<sup>2,3</sup>.

39

## 40 **Imaging and Mapping G4s**

41

42 Computational G4 predictions using simple algorithms have suggested that over  
43 300,000 sequence motifs (of the type  $G_{\geq 3}N_{1-7}G_{\geq 3}N_{1-7}G_{\geq 3}N_{1-7}G_{\geq 3}$ ) in the human  
44 genome have the potential to form a G4 structure<sup>4,5</sup>. A more recent algorithm has  
45 predicted the number of potential G4 sequences to be substantially higher<sup>6</sup>. These  
46 computational studies showed that G4 motifs are enriched in telomeres, promoters  
47 and the first intron of genes, but have highlighted the need to generate explicit  
48 experimental data about the existence and function(s) of G4s in biologically relevant  
49 contexts. G4-selective probes have been developed and employed to capture G4s in  
50 cells by fluorescence microscopy and also by DNA chromatin immuno-precipitation  
51 followed by sequencing.

52

53 **Cellular visualisation.** One approach to visualise particular DNA structures in cells is  
54 to employ structure-selective molecular probes. Antibody proteins can have exquisite  
55 specificity in their recognition of molecular structures and are widely used to bind to  
56 and visualise proteins within cells or map their binding sites in DNA or RNA.  
57 Antibodies can be generated by immunisation or *via in vitro* affinity selection to  
58 recognise a particular DNA structure or chemical feature. The first reported  
59 visualisation of G4 formation in a biologically relevant context used a G4-selective  
60 single-chain antibody (scFV) probe (Sty3)<sup>7</sup> to show G4 formation at telomeres in the  
61 macronuclei of the ciliate *Stylonychia lemnae*<sup>7</sup>. The same antibody was used to  
62 elucidate the dynamic, formation and loss of telomeric G4 under the cooperative  
63 control of telomere-end-binding proteins, and a cell-cycle dependent phosphorylation  
64 of one of them<sup>8</sup>. Recently G4s have been visualised in human cells by  
65 immunofluorescence microscopy using two G4-specific antibodies BG4<sup>9</sup> and 1H6<sup>10</sup>,  
66 each generated from separate labs, with the use of secondary and tertiary antibodies  
67 for signal sensitivity. These independent and complementary studies were each  
68 performed on *in situ* fixed nuclei and showed punctate staining of G4 in genomic  
69 DNA in the nuclei of a range of human cell lines. Cell synchronisation experiments

70 revealed cell cycle dependent G4 dynamics with the quantity of G4s reaching a  
71 maximum during the S-phase<sup>9</sup>. Immunofluorescence staining of metaphase  
72 chromosome spreads revealed G4s at telomeres with the majority of foci occurring  
73 away from the telomeres<sup>9</sup>. The number of detected G4-antibody *foci* increased after  
74 exposure of live human cells to G4 ligands, that include PDS, PhenDC3 and TMPyP4,  
75 demonstrating that such ligands do indeed trap out G4 structures once they form in  
76 cells (Figure 2b)<sup>9,10</sup>. The number of G4 foci in the presence of G4-stabilising ligand  
77 telomestatin in DT40 chicken cells was higher when FANCI, a G4-specific helicase,  
78 was deficient, consistent with FANCI controlling the susceptibility of G4s as  
79 molecular targets for ligands (Figure 2f)<sup>10</sup>. BG4 Immunofluorescence has shown  
80 colocalisation of human telomerase with a subset of endogenous telomeric G4  
81 structures in cells, suggesting telomerase might be recruited to G4 to extend telomeres  
82 during meiosis<sup>11</sup> and presenting an alternative perspective to the earlier views that  
83 telomeric G4 structure may preclude telomerase recognition and action<sup>12</sup>. Synthetic  
84 small molecules that recognise G4s have also been employed to detect these DNA  
85 structures. A derivative of the G4-ligand Pyridostatin (PDS) called PDS- $\alpha$  enabled  
86 nuclear detection of G4s by bio-orthogonal ligation of a fluorophore to the ligand after  
87 cellular incubation and formaldehyde fixation<sup>13</sup>. PDS- $\alpha$  staining was significantly  
88 colocalised with the G4-helicase Pif1 in osteosarcoma (U2OS) cells, by super high-  
89 resolution spectroscopy, consistent with Pif1 processing of G4 structures in human  
90 cells<sup>13</sup>. The intrinsically fluorescent G4 ligands BMVC and DAOTA-M2, have also  
91 been used to visualise G4s suggesting higher G4 prevalence in some cancer cell lines  
92 compared to normal cells<sup>14,15</sup>. These studies have complemented earlier work that  
93 visualised accumulation of a radiolabeled small molecule G4 ligand at telomeres in  
94 human cells<sup>16</sup>. Be they antibodies or small molecules, it is a fundamental consequence  
95 that probes that bind to particular DNA structures can alter the intrinsic stability of  
96 those structures by the very act of binding. Thus, molecular probes can alter the  
97 apparent lifetimes of these dynamic structures, from their natural states. Probe-based  
98 observations of natural biological dynamics (e.g. during the cell cycle)<sup>8,9</sup> or  
99 perturbation experiments (by ligands or manipulation of key enzymes)<sup>9,10,17</sup>, are  
100 helpful to visualise changes that are unlikely to be attributed to the binding effect of  
101 the probes.

102

103 **Genome mapping.** A method for combining G4-dependent DNA polymerase stalling  
104 and next-generation sequencing, called G4-seq, has been developed to map G4  
105 structures in purified, single-stranded DNA on a human genome scale<sup>18</sup>. Typically,  
106 genomic DNA isolated from cells, is sequenced first under conditions that do not  
107 favour G4 structure formation, then the same DNA fragments are re-sequenced under  
108 conditions that stabilize G4 structure formation, either by addition of K<sup>+</sup> or the G4-  
109 ligand PDS. G4-specific polymerase stalling is detected at specific sites during the  
110 second sequencing run by a precipitous loss of sequencing data quality, as compared  
111 to the first sequencing run. G4-seq identified over 700,000 G4s in the human genome,  
112 the majority (70%) of which comprised extra-long loops and/or bulges in their G-  
113 tracts, which precluded their prediction by earlier algorithms, e.g. (G<sub>≥3</sub>N<sub>1-7</sub>G<sub>≥3</sub>N<sub>1-7</sub>  
114 G<sub>≥3</sub>N<sub>1-7</sub>G<sub>≥3</sub>)<sup>4</sup>. Together with other studies<sup>6</sup>, this suggests the breadth and number of  
115 potential G4s is greater than originally envisaged. The G4s were enriched in  
116 regulatory regions that included promoter, 5'UTR, splicing sites and were also  
117 overrepresented in cancer-related genes and in somatic copy number alterations  
118 (SCNAs) amplified in cancer genomes<sup>18</sup>. There are now a number of G4 predictor  
119 algorithms available that vary considerably in the details and the types of G4s that are  
120 captured. While, computational predictors and G4-seq provide a framework for  
121 understanding the potential for G4 structures to form in genomes, it is important to  
122 experimentally consider the profile and genome dynamics of G4 DNA in a biological  
123 context. A step towards this is to employ probes that bind to and enrich G4s from  
124 chromatin, followed by sequencing (e.g. Chromatin Immuno Precipitation  
125 Sequencing; ChIP-seq). An early attempt was to map the sites of DNA double-strand  
126 breaks (DSBs) induced by the G4-targeting ligand PDS in human immortalized  
127 fibroblast (MRC5-SV40) cells by ChIP-seq using an antibody for the DSB marker  
128 γH2AX in fixed chromatin<sup>13</sup>. A significant enrichment of DSBs was observed in  
129 particular genomic regions rich in computationally predicted G4 motifs consistent  
130 with the G4-ligand binding to G4 target structures and causing DSBs at those sites<sup>13</sup>.  
131 The binding sites of endogenous cellular proteins that can bind or resolve G4s *in vitro*,  
132 such as human ATRX<sup>19</sup> and XPB/ XPD<sup>20</sup> and yeast PIF-1 (an inactive mutant form)<sup>21</sup>  
133 and RIF-1<sup>22</sup>, have been mapped by ChIP-seq to regions that comprise predicted G4  
134 motifs that occur for example at telomeres and gene promoters (Figure 2g). Whilst the  
135 proteins that feature in such studies may also be capable of binding to other sequences

136 or structures, the data are consistent with hypotheses linking their biological functions  
137 to G4 structures or genomic regions that are enriched in G4 motifs. The mapping of  
138 G4 structures in chromatin was recently achieved using the G4 antibody BG4 as a G4  
139 structure-specific ChIP-seq probe (G4 ChIP-seq) to map endogenous G4 structures in  
140 fixed chromatin in normal (NHEK) and spontaneously immortalized pre-cancerous  
141 (HaCaT) human epidermal keratinocyte cells (Figure 2e)<sup>17</sup>. In this study, about 10,000  
142 and 1,000 G4s were detected in HaCaT and NHEKs, respectively, which is only ~1%  
143 of those identified by G4-seq, and by G4 predictors. This suggests G4 structure  
144 formation is largely suppressed in the context of chromatin, possibly due to  
145 chromatin-associated proteins and other proteins that control the duplex vs. non-  
146 duplex folded states of DNA. Most G4s observed were in regulatory, nucleosome-  
147 depleted chromatin regions that that were on average highly transcribed<sup>17</sup> and also  
148 significantly overlapped with G4 predicted sequences enriched in earlier ChIP-Seq  
149 mapping of the transcriptional helicases XPB/XPD<sup>20</sup>. Furthermore, endogenous G4s  
150 are enriched in promoter and 5'UTR regions of cancer-related genes and genes  
151 strongly associated with somatic copy number aberrations in cancer, such as *MYC*<sup>23</sup>.  
152 A perturbation experiment using the histone deacetylase inhibitor Entinostat, caused  
153 dynamic reprogramming of the G4 landscape by G4 ChIP-seq with the loss and  
154 emergence of G4s showing, on average, a coupling to transcriptionally active  
155 chromatin<sup>17</sup>. The observation of G4 dynamics goes some way towards addressing the  
156 possibility that the G4s may be an artifact of antibody stabilization, which was  
157 discussed earlier in relation to antibody imaging of G4s. It will nonetheless be  
158 important to consider orthogonal approaches to detect G4 structures in chromatin and  
159 in cells to further consolidate these findings.

160

### 161 **Biological Significance and Therapeutic Opportunities**

162

163 Much of the early work on G4s focused on biophysical studies and functional studies  
164 on telomeres and telomerase. This section will focus primarily on some of the insights  
165 that recent imaging and G4 mapping has provided on the biology of G4s in non-  
166 telomeric regions. Such observations suggest a broader number of biological  
167 processes and the associated possibilities for therapeutic intervention.

168

169 ***Transcription, Replication and Intrinsic function(s)***. There have been a number of  
170 cellular studies describing G4 targeting ligands that alter levels of mRNA transcripts

171 for genes that have G4 motifs in their upstream (promoter) elements, for example the  
172 proto-oncogenes *MYC*<sup>23</sup> and *KRAS*<sup>24</sup>. Recent studies on zebrafish embryos  
173 demonstrated the use of G4-targeting small molecules or synthetic oligonucleotides to  
174 target conserved G4 motifs in promoters of developmental genes to lower  
175 transcription of the targeted genes and cause the expected phenotypic change<sup>25</sup>.  
176 Further work is needed to confirm and fully elucidate the mechanistic details of cause  
177 and effect in such studies. That genes physically targeted by the small molecule PDS,  
178 as judged by localised DNA damage, cause a concomitant reduction in transcript  
179 levels<sup>13</sup>, suggests the relationship between G4 ligands and transcriptional changes at  
180 proximal genes may, at least for some cases, be more complex than a simple  
181 reversible binding mechanism. Recent data that includes G4 ChIP-seq<sup>17</sup>, the genomic  
182 binding sites of proteins XPB, XPD<sup>20</sup> and SP1<sup>17</sup>, the colocalization of G4-antibodies  
183 BG4 or 1H6 with transcriptionally active regions (marked by RNA polymerase II and  
184 H3K4me3)<sup>17</sup>, support that G4 structures form in transcriptionally active chromatin in  
185 human cells. Dysfunctional mutations in WRN and BLM helicases cause altered  
186 regulation of genes that are enriched in predicted G4 motifs, consistent with a link  
187 between G4s and transcription<sup>26-28</sup>. It is noteworthy that in *D. mel.* G4 structure  
188 formation has actually been observed in the heterochromatin of polytene  
189 chromosomes during embryonic development, by immuno-gold labeling using 1H6  
190 and microscopy<sup>29</sup>, revealing differences in this very different genome, from  
191 mammalian systems. Overall, the weight of recent data from mammalian cells is  
192 consistent with a functional and dynamic link between G4 structures and  
193 transcription. Further studies are needed to elucidate the mechanistic details of this  
194 link, including the specific roles of proteins associated with transcription, such as SP1  
195 or XPB/XPD, and their interaction(s) with loci where G4 structures have been  
196 observed to exhibit dynamic formation or unfolding.

197

198 DNA replication is a carefully regulated process and is initiated at many thousands of  
199 sites called DNA replication origins. Conserved DNA structures have been found at  
200 origin of replications sites in prokaryotes<sup>30</sup>. Recently, locations of human replication  
201 origins have been mapped via deep sequencing of short nascent strands and predicted  
202 to contain G4-motifs<sup>31</sup>. In addition, the human origin recognition complex (ORC) has  
203 been shown to bind G4-forming DNA and RNA sequences *in vitro*<sup>32</sup>. Such studies  
204 have led to the hypothesis that G4 DNA structures may somehow be involved in the

205 mechanism of initiating replication origins. Further studies that experimentally  
206 support G4 structure formation at origins more directly are necessary to advance our  
207 understanding of these findings.

208

209 **Genomic Instability.** In the absence of helicases that resolve G4 structures in DNA,  
210 stable G4 structures can pose an impediment for DNA polymerase progression,  
211 leading to replication stalling, DNA-damage and genomic instability (Figure 3a).  
212 Quantitative assays have been used to monitor G4 induced genome instability in *S.*  
213 *cerevisiae*, to show that Pif1 and Rrm3 helicases are essential to suppress and prevent  
214 G4-induced DNA strand breakage<sup>33,34</sup>. The RTEL1 helicase has been shown to  
215 resolve telomeric G4 to maintain telomere integrity in mouse cells<sup>35</sup>. Similarly,  
216 FANCI, BLM and WRN helicases have been shown to recognise and unwind G4  
217 structures *in vitro*<sup>36</sup>. Mutations causing dysfunction in these helicases have been  
218 associated with premature ageing and predisposition to cancer development, though  
219 since the helicases also operate on duplex DNA the extent to which this is a G4-  
220 related effect must be further elucidated. Regulators of DNA synthesis, such as REV1  
221 or PrimPol, affect gene expression by a mechanism proposed to ordinarily maintain  
222 epigenetic stability at replicated G4 DNA, which when compromised, e.g. by REV1  
223 deficiency, leads to gene activation<sup>37</sup> or repression<sup>38</sup> depending on the location of the  
224 predicted G4-forming sequence.

225 Ligands that stabilise G4 structures can induce DNA breakage in humans<sup>13</sup> as well as  
226 insertion and deletions at predicted G4 motifs in yeast<sup>33,34</sup>. In *S. cerevisiae* the G4  
227 ligand PhenDC3 triggers G4-induced<sup>39</sup> and G4-stability<sup>40</sup> dependent genomic  
228 instability, as measured by the increased genetic insertion and deletion at a level  
229 comparable to when Pif1 function is impaired. Interestingly, the yeast system  
230 provided a useful platform in which G4 stability could be systematically varied by  
231 mutagenesis to demonstrate a clear correlation between G4 stability and DNA  
232 instability at CEB-1 microsatellite, with short G4 loops ( $\leq 4$  nt) causing higher levels  
233 of genome instability<sup>40</sup>. Given that G4-seq<sup>18</sup> and G4 ChIP-seq<sup>17</sup> both show G4s are  
234 enriched in SCNA-amplifications associated with cancer and that a similar association  
235 has been reported by computational analysis of SCNA associated breakpoints<sup>18</sup>, it  
236 appears that G4 structures also represent vulnerable regions in the genome (Figure  
237 3a). These associations are consistent with the immunohistochemistry observations  
238 from matched normal and cancerous gut/stomach tissues using BG4 revealed higher

239 apparent levels of G4 in the cancerous state<sup>41</sup>. This empirical data suggests, at least  
240 for some cases, that there are aspects of a cancer genome that exhibit more G4  
241 structures, which immediately suggests G4s may have potential as both a cancer-  
242 biomarker and as a therapeutic target.

243

244 ***Therapeutic Opportunities.*** The data supporting concepts that link G4s with telomere  
245 biology, transcription regulation (of cancer genes) and trigger points for instability  
246 and DNA strand breakage, have stimulated rationales for targeting G4s with small  
247 molecules for therapeutics. Several small molecule ligands with high selectivity, as  
248 determined biophysically, for G4 relative to double-stranded DNA have been  
249 designed and evaluated for their therapeutic potential. Recent examples include the  
250 tetra-substituted naphthalene diimide MM41 caused an 80% decrease in tumour  
251 growth in MIA PaCa-2 pancreatic cancer xenograft<sup>42</sup>. While this may be explained in  
252 part by the accompanied strong reduction of *KRAS* and *BCL-2* gene expression, there  
253 may be other G4-related modalities that also contribute to its efficacy. The G4-ligands  
254 PDS and RHPS4 trigger an ATM-dependent DNA damage response (DDR), DNA  
255 double strand breaks (DSB) and activation of DNA repair pathways, such as  
256 Homologous Recombination (HR) and Non Homologous End Joining (NHEJ), and  
257 signalling of single-strand DNA breaks by the synthesis of poly ADP-ribose chains  
258 (PARs) by PAR protein (PARP)<sup>13,43</sup>. This has inspired the application of G4 ligands  
259 in combination with DNA-PK (NHEJ) or PARP inhibitors, as well as with G4  
260 helicases (WRN) inhibitors for a greater effect (i.e. synergy) than the observed sum of  
261 the individual effects. Exposure of HeLa and U2OS cancer cells to the WRN inhibitor  
262 NSC-19630 sensitize the cells to the G4 ligand telomestatin, showing exacerbated S  
263 phase prolongation and DNA damage response<sup>44</sup>. Similarly, treatment of HT29  
264 human colon cancer xenografts with the G4 ligand RHPS4 in combination with the  
265 PARP1 inhibitor GPI resulted in a 50% reduction of tumor weight and an increase of  
266 45% of mice survival, significantly higher of what could be obtained by treating the  
267 mice with either RHPS4 or GPI individually<sup>43</sup>. Equally, by inhibiting NHEJ repair  
268 with the DNA-PK inhibitor NU7441, a significant sensitization to the G-quadruplex  
269 ligand PDS can be observed in human HT1080 fibrosarcoma cells (~ 45% synergy, as  
270 calculated by a Bliss independent score model)<sup>13,45</sup>. Furthermore, HCT116 colon  
271 cancer cells deficient in HR (*BRCA2*<sup>-/-</sup>) displayed a ~10 fold increase in sensitivity  
272 against PDS compared to their isogenic counterpart that is HR-proficient



273 (BRCA2<sup>+/+</sup>)<sup>45</sup>. DNA repair deficiencies have been further demonstrated to stimulate  
274 sensitization to the G4 ligand PDS in DLD1 human cells BRCA2<sup>-/-</sup>, as well as human  
275 HEK-293T subjected to knock-down of the DNA repair proteins BRCA1 and  
276 RAD51<sup>45,46</sup>. PDS sensitization is further retained in HR deficient cells after they have  
277 acquired resistance to the drug Olaparib, highlighting the potential of G4 ligands as  
278 therapeutic agents against HR compromised tumors with acquired drug resistance  
279 (Figure 3b)<sup>46</sup>. These recent findings indicate clear potential for G4-ligands to be  
280 considered as cancer therapeutics especially for tumours genetically deficient in  
281 DNA-repair machinery such as HR<sup>45,46</sup>.

282

283

## 284 **Conclusions and Perspectives**

285 Recent advances have provided a substantial body of new data to support the  
286 existence of G4 structures in the genomes of human cells. There is now more explicit  
287 experimental data to show G4s form throughout the human genome and in regulatory  
288 regions, largely aligned to previous computational predictions. These findings have  
289 provided new insights into the fundamental biology of G4 structures, suggesting roles  
290 in marking regulatory chromatin, whilst also being hotspots for genome instability  
291 particularly in cases where there are specific genetic/functional deficiencies.  
292 Fundamental insights into endogenous G4 function(s) enabled by advances in  
293 experiments with probe molecules that bind G4s suggest rationales for therapeutic  
294 strategies against cancer that may provide the window of selectivity that would be  
295 required for future clinical development. Whilst there is much more to be understood  
296 about the mechanistic details relating to DNA G4s in biology, the developments of the  
297 past five or so years would appear to have moved the field substantially closer to the  
298 realm of functional biology.

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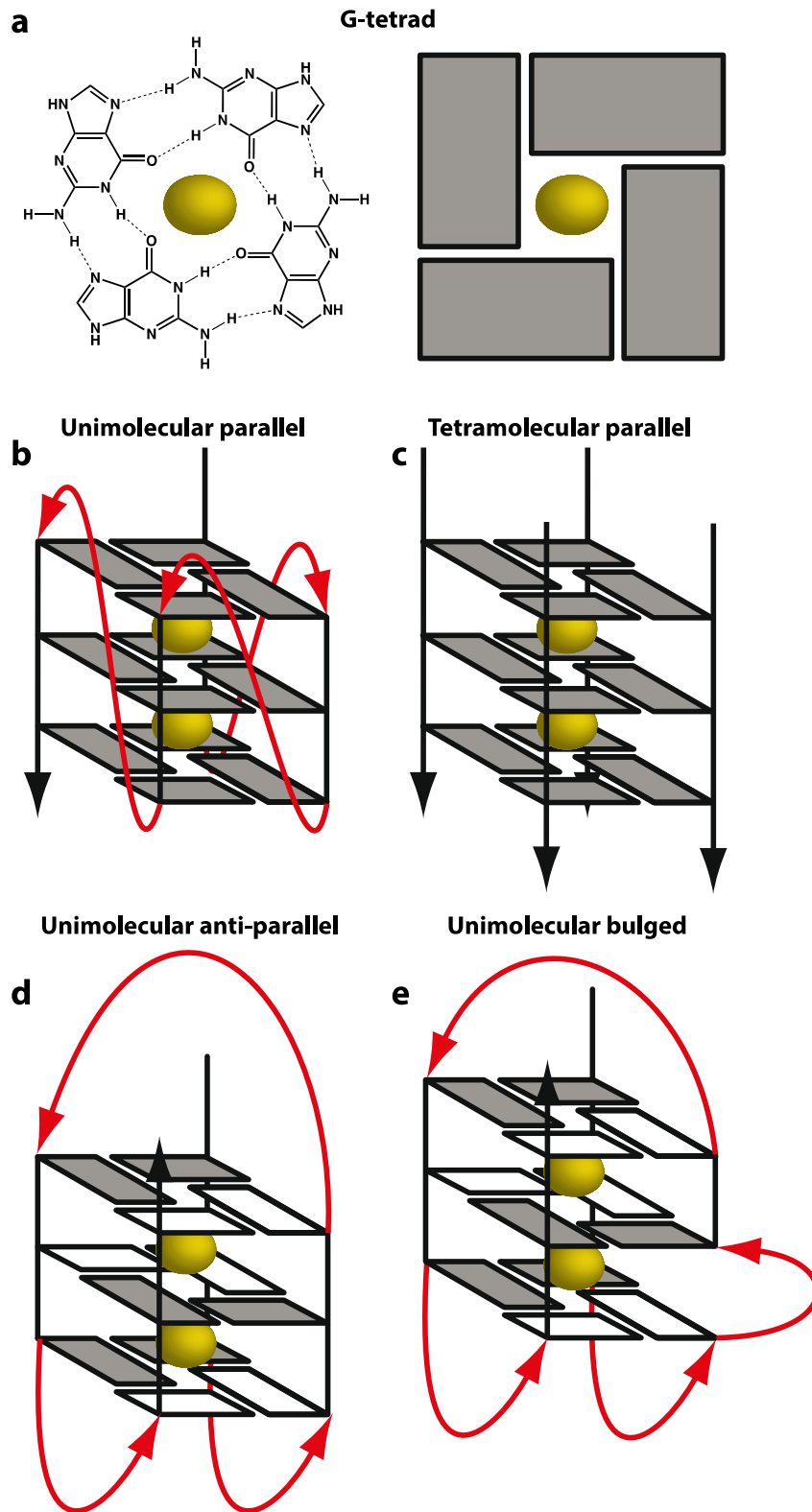
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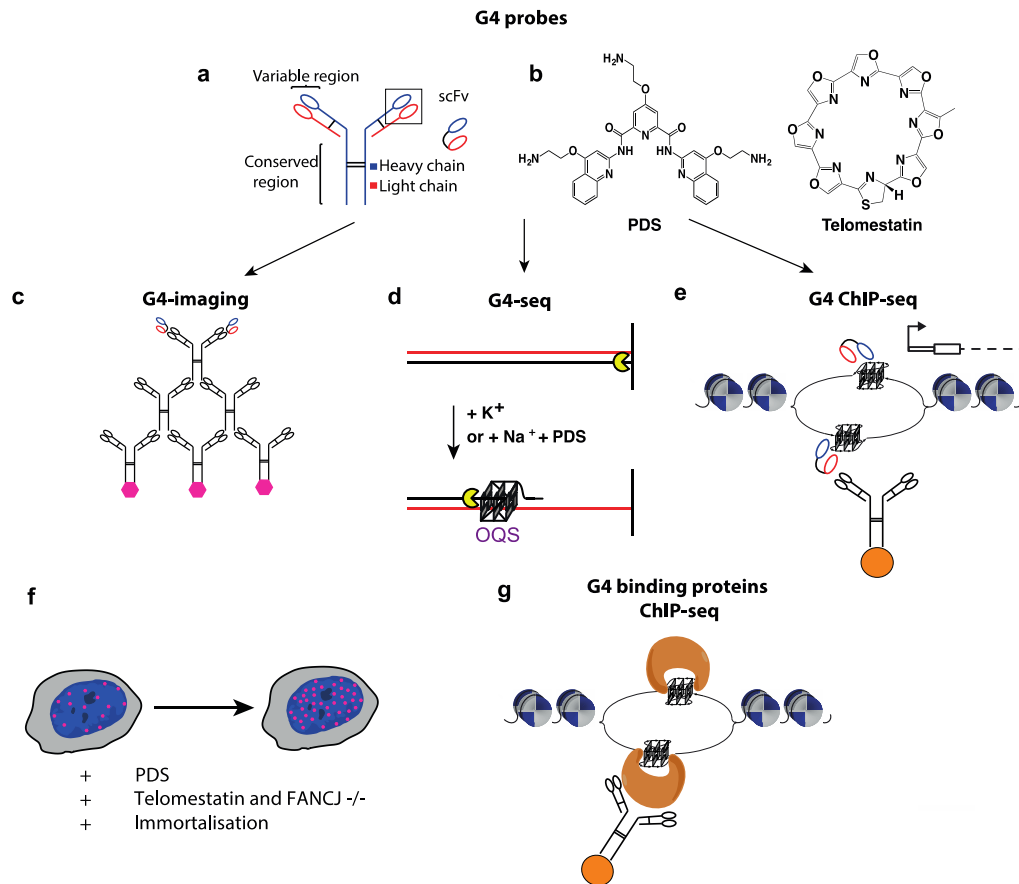


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416 **Figure 1: G-quadruplex structures.** G-quadruplex structures can be generated from  
 417 one DNA strand (unimolecular) or multiple DNA strands coming together (e.g. bi- or  
 418 tetra molecular). G4 structures can be classified by the relative strand orientations:

419 parallel G4s have the same strand orientation within the structure whereas antiparallel  
 420 G4s have alternating strand orientations. **a**, Structural (left) and schematic (right)  
 421 representations of a G-tetrad that makes up the core of G-quadruplex structures. **b**,  
 422 Schematic representation of unimolecular parallel G4s **c**, Schematic representation of  
 423 a tetramolecular G4s . **d**, Schematic representation of an antiparallel intramolecular G-  
 424 quadruplex structure **e**, Schematic representation of an antiparallel intramolecular G-  
 425 quadruplex structure containing a bulge.



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428 **Figure 2: Visualisation and mapping of G-quadruplex structures.** **a**, Schematic  
 429 representation of a single chain antibody (scFv) as used to probe G4s, such as the  
 430 BG4 or 1H6. **b**, Chemical structures of the selective G4 ligands pyridostatin (PDS)  
 431 and telomestatin. **c**, Visualization of G4 structure can be achieved using G4 antibodies  
 432 (e.g. BG4 or 1H6) together with secondary or tertiary antibodies that carry a  
 433 fluorescent label. **d**, Schematic representation of the G4-seq method. DNA templates  
 434 are sequenced a first time under non G4-stabilising conditions and a second time after

435 the addition of G4 stabilising agents (e.g.  $K^+$  or PDS). Only DNA templates  
436 containing a G4 forming sequence will cause stalling of sequencing polymerase under  
437 G4-stabilising conditions, enabling selective detection of G4-forming genomic  
438 sequences. **e.** Schematic representation of G4 ChIP-seq: isolated chromatin is  
439 immuno-precipitated with BG4 and G4 structures detectable in chromatin are  
440 enriched and detected by sequencing. **f.** BG4 and 1H6 *foci* (red) detected in the  
441 nucleus of human cells (blue) are markedly increase in upon treatment with  
442 pyridostatin (PDS), telomestatin and FANCI knock-down, and after immortalisation  
443 of normal human epidermal keratinocytes. **g.** Schematic representation of a typical  
444 ChIP-Seq of endogenous G4 binding proteins: isolated chromatin is immuno-  
445 precipitated using a selective antibody against the protein of interest (e.g. ATRX,  
446 PIF1, XPB/XPD). DNA sequences associated with those proteins are detected by  
447 sequencing.

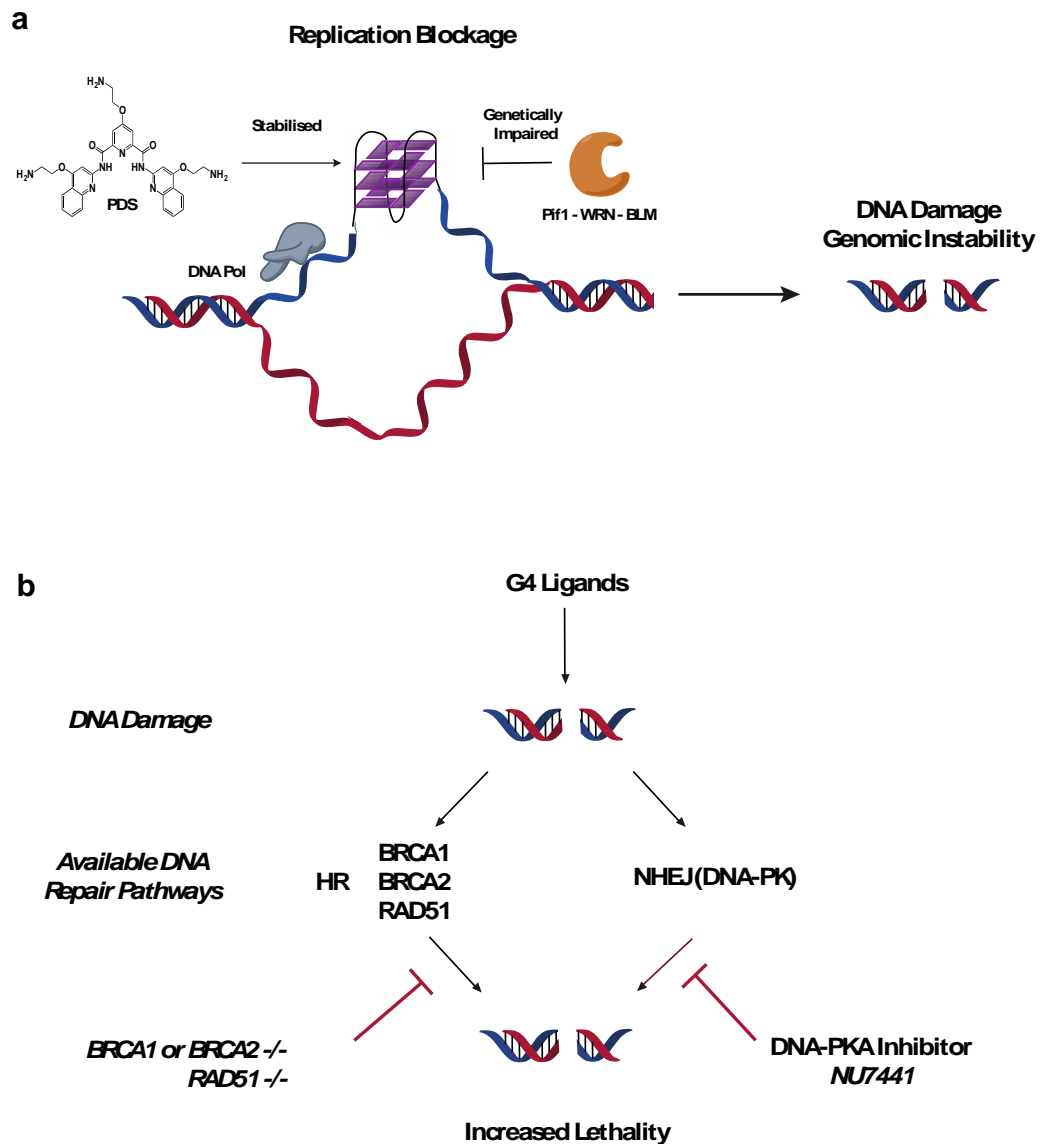
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454 **Figure 3: Therapeutic opportunities.** **a**, Schematic representation of DNA damage  
 455 response and genomic instability events that can be triggered by DNA G-  
 456 quadruplexes either by stabilization with small molecules or by impairment of  
 457 helicases that resolve G-quadruplexes. **b**, G-quadruplex ligands have been explored  
 458 for their potential as cancer therapeutic agents. Representative scheme illustrating one  
 459 the possible rationale behind the use of G4 ligands in combined therapies. DNA  
 460 damage is triggered by exposure to a G4 ligand: sensitivity to ligand exposure can be  
 461 obtained in cells genetically impaired in BRCA1/2 and RAD51, which regulate one of  
 462 the two DNA repair pathway (HR). Selective killing of BRCA1/2 and RAD51  
 463 impaired cells can be achieved by combined treatment with G4-ligands and a

464 chemical inhibitor of the kinase DNA-PK that regulates the alternative DNA repair  
465 pathway (NHEJ).  
466