Summary

G-quadruplexes (G4) play crucial roles in biology, analytical chemistry and nanotechnology. The stability of G4 structures is impacted by the number of G-quartets, the length and positions of loops, flanking motifs, as well as additional structural elements such as bulges, capping base pairs, or triads. Algorithms such as G4Hunter or Quadparser may predict if a given sequence is G4-prone by calculating a quadruplex propensity score; however, experimental validation is still required. We previously demonstrated that this validation is not always straightforward, and that a combination of techniques is often required to unambiguously establish whether a sequence forms a G-quadruplex or not. In this article, we adapted the well-known FRET-melting assay to characterize G4 in batch, where the sequence to be tested is added, as an unlabeled competitor, to a system composed of a dual-labeled probe (F21T) and a specific quadruplex ligand. PhenDC3 was preferred over TMPyP4 because of its better selectivity for G-quadruplexes. In this so-called FRET-MC (melting competition) assay, G4-forming competitors lead to a marked decrease of the ligand-induced stabilization effect (ΔT), while non-specific competitors (e.g., single- or double-stranded sequences) have little effect. Sixty-five known sequences with different typical secondary structures were used to validate the assay, which was subsequently employed to assess eight novel sequences that were not previously characterized.

Summary

Dynamic combinatorial libraries of acylhydrazones were prepared from diacylhydrazides and several cationic or neutral aldehydes in the presence of 5-methoxyanthranilic acid catalyst. Pull-down experiments with magnetic beads functionalized with a G-quadruplex (G4)-forming oligonucleotide led to the identification of putative ligands, which were resynthesized or emulated by close structural analogues. G4-binding properties of novel derivatives were assessed by fluorimetric titrations, mass spectrometry and thermal denaturation experiments, giving evidence of strong binding (Kd < 10 nM) for two compounds.
Anton Granzhan, Rodrigo Prado Martins, Robin Fåhraeus, Marc Blondel and Marie-Paule Teulade-Fichou (2020 Jun 30)

**Quadruplex-interacting compounds for regulating the translation of the Epstein–Barr virus nuclear antigen 1 (EBNA1) mRNA: A new strategy to prevent and treat EBV-related cancers**

*Quadruplex Nucleic Acids As Targets For Medicinal Chemistry, Annual Reports in Medicinal Chemistry*: Chap 8, 54 : 243-286 : [DOI: 10.1016/bs.armc.2020.05.001](https://doi.org/10.1016/bs.armc.2020.05.001)

**Summary**

The Epstein–Barr (EBV) virus is linked to at least 1% of human cancers that include Burkitt’s and Hodgkin’s lymphomas, nasopharyngeal carcinoma, and 10% of gastric cancers. EBV is a latent virus that possesses a genome maintenance protein, EBNA1, which is both essential for the virus and highly antigenic. Hence, EBV has evolved a mechanism by which EBNA1 self-limits the translation of its own mRNA, thereby minimizing the production of EBNA1-derived antigenic peptides. Although not fully elucidated, this mechanism involves the Gly-Ala-rich (GAr) motif of EBNA1, encoded by a G-repeat-containing mRNA sequence able to form clusters of G-quadruplexes (G4s). This chapter summarizes recent significant advances in understanding this phenomenon. Mechanistic investigations based on yeast chemical genetics, cellular assays and *in vitro* experiments have shown that the host cell factor nucleolin (NCL) is involved in this limitation of EBNA1 translation through binding to the G4s of EBNA1 mRNA. This interaction can be disrupted by the benchmark G4-ligand PhenDC3 acting as a NCL competitor for binding to G4-RNA. Finally, exploration of the chemical space around PhenDC3 using combinatorial chemistry approach led to the generation of 20 compounds based on a bis(acylhydrazone) scaffold. Among these, two hits (PyDH2, PhenDH2) exhibit optimized properties with regard to the disruption of NCL/G4 interaction in cells, along with lower cytotoxicity. Consequently, treatment
by PyDH2 or PhenDH2 increases EBNA1 production and stimulates the GAr-restricted antigenic response. Altogether, this innovative concept of antigenic stimulation sets the basis for further identification of lead candidates that may become promising candidate drugs for treating EBV-related cancers.

Michela Zoffa, Aurélie Gandolfini, Brahim Heddi, Anton Granzhan (2020 Apr 20)
Harnessing intrinsic fluorescence for typing of secondary structures of DNA

Summary

High-throughput investigation of structural diversity of nucleic acids is hampered by the lack of suitable label-free methods, combining fast and cheap experimental workflow with high information content. Here, we explore the use of intrinsic fluorescence emitted by nucleic acids for this scope. After a preliminary assessment of suitability of this phenomenon for tracking conformational changes of DNA, we examined steady-state emission spectra of an 89-membered set of oligonucleotides with reported conformation (G-quadruplexes (G4s), i-motifs, single- and double-strands) by means of multivariate analysis. Principal component analysis of emission spectra resulted in successful clustering of oligonucleotides into three corresponding conformational groups, without discrimination between single- and double-stranded structures. Linear discriminant analysis was exploited for the assessment of novel sequences, allowing the evaluation of their G4-forming propensity. Our method does not require any labeling agent or dye, avoiding the related bias, and can be utilized to screen novel sequences of interest in a high-throughput and cost-effective manner. In addition, we observed that left-handed (Z-) G4 structures were systematically more fluorescent than most other G4 structures, almost reaching the quantum yield of 5′-d[(G3T3)3G3]-3′ (G3T, the most fluorescent G4 structure reported to date).

Year of publication 2019

Katerina Duskova, Pauline Lejault, Élie Benchimol, Régis Guillot, Sébastien Britton, Anton Granzhan, David Monchaud (2019 Dec 13)
DNA junction ligands trigger DNA damage and are synthetic lethal with DNA repair inhibitors in cancer cells
*Journal of the American Chemical Society*: 142 : 424-435 : [DOI: 10.1021/jacs.9b11150]

**Summary**

Translocation of DNA and RNA polymerases along their duplex substrates results in DNA supercoiling. This torsional stress promotes the formation of plectonemic structures, including three-way DNA junction (TWJ), which can block DNA transactions and lead to DNA damage. While cells have evolved multiple mechanisms to prevent the accumulation of such structures, stabilizing TWJ through *ad hoc* ligands offer an opportunity to trigger DNA damage in cells with high level of transcription and replication, such as cancer cells. Here, we develop a series of azacryptand-based TWJ ligands, we thoroughly characterize their TWJ-interacting properties in vitro and demonstrate their capacity to trigger DNA damage in rapidly dividing human cancer cells. We also demonstrate that TWJ ligands are amenable to chemically induced synthetic lethality strategies upon association with inhibitors of DNA repair, thus paving the way towards innovative drug combinations to fight cancers.


**Monitoring DNA–Ligand Interactions in Living Human Cells Using NMR Spectroscopy**
*Journal of the American Chemical Society*: 141 : 13281-13285 : [DOI: 10.1021/jacs.9b03031]

**Summary**

Studies on DNA–ligand interactions in the cellular environment are problematic due to the lack of suitable biophysical tools. To address this need, we developed an *in-cell* NMR-based approach for monitoring DNA–ligand interactions inside the nuclei of living human cells. Our
method relies on the acquisition of NMR data from cells electroporated with preformed DNA–ligand complexes. The impact of the intracellular environment on the integrity of the complexes is assessed based on in-cell NMR signals from unbound and ligand-bound forms of a given DNA target. This technique was tested on complexes of two model DNA fragments and four ligands, namely, a representative DNA minor-groove binder (netropsin) and ligands binding DNA base-pairing defects (naphthalenophanes). In the latter case, we demonstrate that two of the three in vitro-validated ligands retain their ability to form stable interactions with their model target DNA in cellulo, whereas the third one loses this ability due to off-target interactions with genomic DNA and cellular metabolites. Collectively, our data suggest that direct evaluation of the behavior of drug-like molecules in the intracellular environment provides important insights into the development of DNA-binding ligands with desirable biological activity and minimal side effects resulting from off-target binding.

Xiao Xie, Michela Zuffo, Marie-Paule Teulade-Fichou, Anton Granzhan (2019 Aug 6)
Identification of optimal fluorescent probes for G-quadruplex nucleic acids through systematic exploration of mono- and distyryl dye libraries

Summary

A library of 52 distyryl and 9 mono-styryl cationic dyes was synthesized and investigated with respect to their optical properties, propensity to aggregation in aqueous medium, and capacity to serve as fluorescence “light-up” probes for G-quadruplex (G4) DNA and RNA structures. Among the 61 compounds, 57 dyes showed preferential enhancement of fluorescence intensity in the presence of one or another G4-DNA or RNA structure, while no dye displayed preferential response to double-stranded DNA or single-stranded RNA analytes employed at equivalent nucleotide concentration. Thus, preferential fluorimetric response towards G4 structures appears to be a common feature of mono- and distyryl dyes, including long-known mono-styryl dyes used as mitochondrial probes or protein stains. However, the magnitude of the G4-induced “light-up” effect varies drastically, as a function of both the
molecular structure of the dyes and the nature or topology of G4 analytes. Although our results do not allow to formulate comprehensive structure–properties relationships, we identified several structural motifs, such as indole- or pyrrole-substituted distyryl dyes, as well as simple mono-stryryl dyes such as DASPMI [2-(4-(dimethylamino)styryl)-1-methylpyridinium iodide] or its 4-isomer, as optimal fluorescent light-up probes characterized by high fluorimetric response ($I/II$ of up to 550-fold), excellent selectivity with respect to double-stranded DNA or single-stranded RNA controls, high quantum yield in the presence of G4 analytes (up to 0.32), large Stokes shift (up to 150 nm) and, in certain cases, structural selectivity with respect to one or another G4 folding topology. These dyes can be considered as promising G4-responsive sensors for in vitro or imaging applications. As a possible application, we implemented a simple two-dye fluorimetric assay allowing rapid topological classification of G4-DNA structures.

![Diagram of 61 di- and mono-stryryl dyes and G4 and non-G4 DNA and RNA](image)

![Optimal fluorescent probes](image)

Oksana Reznichenko, Alicia Quillévéré, Rodrigo Prado Martins, Nadège Loaëc, Hang Kang, María José Lista, Claire Beauvineau, Jorge González-García, Régis Guillot, Cécile Voisset, Chrysoula Daskalogianni, Robin Fåhraeus, Marie-Paule Teulade-Fichou, Marc Blondel, Anton Granzhan (2019 May 23)

**Novel cationic bis(acylhydrazones) as modulators of Epstein-Barr virus immune evasion acting through disruption of interaction between nucleolin and G-quadruplexes of EBNA1 mRNA**


**Summary**
The oncogenic Epstein-Barr virus (EBV) evades the immune system through limiting the expression of its highly antigenic and essential genome maintenance protein, EBNA1, to the minimal level to ensure viral genome replication, thereby also minimizing the production of EBNA1-derived antigenic peptides. This regulation is based on inhibition of translation of the virally-encoded EBNA1 mRNA, and involves the interaction of host protein nucleolin (NCL) with G-quadruplex (G4) structures that form in the glycine–alanine repeat (GAr)-encoding sequence of the EBNA1 mRNA. Ligands that bind to these G4-RNA can prevent their interaction with NCL, leading to disinhibition of EBNA1 expression and antigen presentation, thereby interfering with the immune evasion of EBNA1 and therefore of EBV (M.J. Lista et al., Nature Commun., 2017, 8, 16043). In this work, we synthesized and studied a series of 20 cationic bis(acylhydrazone) derivatives designed as G4 ligands. The in vitro evaluation showed that most derivatives based on central pyridine (Py), naphthyridine (Naph) or phenanthroline (Phen) units were efficient G4 binders, in contrast to their pyrimidine (Pym) counterparts, which were poor G4 binders due to a significantly different molecular geometry. The influence of lateral heterocyclic units (N-substituted pyridinium or quinolinium residues) on G4-binding properties was also investigated. Two novel compounds, namely PyDH2 and PhenDH2, used at a 5 μM concentration, were able to significantly enhance EBNA1 expression in H1299 cells in a GAr-dependent manner, while being significantly less toxic than the prototype drug PhenDC3 (GI_{50} > 50 μM). Antigen presentation, RNA pull-down and proximity ligation assays confirmed that the effect of both drugs was related to the disruption of NCL–EBNA1 mRNA interaction and the subsequent promotion of GAr-restricted antigen presentation. Our work provides a novel modular scaffold for the development of G-quadruplex-targeting drugs acting through interference with G4–protein interaction.
The human genome is replete with repetitive DNA sequences that can fold into thermodynamically stable secondary structures such as hairpins and quadruplexes. Cellular enzymes exist to cope with these structures whose stable accumulation would result in DNA damage through interference with DNA transactions such as transcription and replication. Therefore, chemical stabilization of secondary DNA structures offers an attractive way to foster DNA transaction-associated damages to trigger cell death in proliferating cancer cells. While much emphasis has been recently given to DNA quadruplexes, we focused here on three-way DNA junctions (TWJ) and report on a strategy to identify TWJ-targeting agents through a combination of in vitro techniques (TWJ-Screen, PAGE, FRET-melting, ESI-MS, dialysis equilibrium and SRB assays). We designed a complete workflow and screened 1200 compounds to identify promising three-way DNA junction ligands selected on stringent criteria in terms of TWJ folding ability, affinity and selectivity.


Summary
Ligands interacting with abasic (AP) sites in DNA may generate roadblocks in base-excision DNA repair (BER) due to indirect inhibition of DNA repair enzymes (e.g., APE1) and/or formation of toxic byproducts, resulting from ligand-induced strand cleavage or covalent cross-links. Herein, we prepared and systematically studied a series of 12 putative AP-site ligands, sharing the common naphthalenophane scaffold but endowed with a variety of substituents. Our results demonstrate that most naphthalenophanes bind to AP-sites in DNA and inhibit the APE1-induced hydrolysis of the latter in vitro. Remarkably, their APE1 inhibitory activity, as characterized by IC50 and Ki values, can be directly related to their affinity and selectivity to AP-sites, assessed from the fluorescence-melting experiments. On the other hand, the molecular design of naphthalenophanes has crucial influence on their intrinsic AP-site cleavage activity (i.e., ligand-catalyzed β- and β,δ-elimination reactions at the AP site), as illustrated by the compounds either having an exceptionally high AP-site cleavage activity (e.g., 2,7 BisNP-S, 125-fold more efficacious than spermine) or totally devoid of this activity (four compounds). Finally, we reveal the unprecedented formation of a stable covalent DNA adduct upon reaction of one ligand (2,7-BisNP-NH) with its own product of AP-site cleavage.

Michela Zuffo, Xiao Xie, Anton Granzhan (2018 Dec 6)
Strength in Numbers: Development of a Fluorescence Sensor Array for Secondary Structures of DNA.

Summary
High-throughput assessment of secondary structures adopted by DNA oligonucleotides is currently hampered by the lack of suitable biophysical methods. Fluorescent sensors hold great potential in this respect; however, the moderate selectivity that they display for one DNA conformation over the others constitutes a major drawback to the development of accurate assays. Moreover, the use of single sensors impedes a comprehensive classification of the tested sequences. Herein, we propose to overcome these limitations through the development of a fluorescence sensor array constituted by easily accessible, commercial dyes. Multivariate analysis of the emission data matrix produced by the array allows to explore the conformational preferences of DNA sequences of interest, either by calculating the probability of group membership in the six predefined structural categories (three G-quadruplex groups, double-stranded, and two groups of single-stranded forms), or by revealing their particular structural features. The assay enables rapid screening of synthetic DNA oligonucleotides in a 96-wells plate format.

**Year of publication 2018**

Abhijit Saha, Sophie Bombard, Anton Granzhan, Marie-Paule Teulade-Fichou (2018 Oct 27)

Probing of G-Quadruplex Structures via Ligand-Sensitized Photochemical Reactions in BrU-Substituted DNA.

*Scientific Reports*: 8 : 15814 : [DOI: 10.1038/s41598-018-34141-z](https://doi.org/10.1038/s41598-018-34141-z)

**Summary**

We studied photochemical reactions of BrU-substituted G-quadruplex (G4) DNA substrates with two pyrene-substituted polyazamacrocyclic ligands, M-1PY and M-2PY. Both ligands bind to and stabilize G4-DNA structures without altering their folding topology, as demonstrated by FRET-melting experiments, fluorimetric titrations and CD spectroscopy. Notably, the bis-pyrene derivative (M-2PY) behaves as a significantly more affine and selective G4 ligand, compared with its mono-pyrene counterpart (M-1PY) and control compounds. Upon short UVA irradiation (365 nm) both ligands, in particular M-2PY, efficiently sensitize photoreactions at BrU residues incorporated in G4 structures and give rise to two kinds of photoproducts, namely DNA strand cleavage and covalent ligand-DNA photoadducts. Remarkably, the photoinduced strand cleavage is observed exclusively with G4 structures presenting BrU residues in lateral or diagonal loops, but not with parallel G4-DNA structures presenting only propeller loops. In contrast, the formation of fluorescent photoadducts is observed with all BrU-substituted G4-DNA substrates, with M-2PY giving significantly higher yields (up to 27%) than M-1PY. Both ligand-sensitized photoreactions are specific to BrU-modified G4-DNA structures with respect to double-stranded or stem-loop substrates. Thus,
ligand-sensitized photoreactions with BrU-substituted G4-DNA may be exploited (i) as a photochemical probe, allowing “photofootprinting” of G4 folding topologies in vitro and (ii) for covalent trapping of G4 structures as photoadducts with pyrene-substituted ligands.

Xiao Xie, Oksana Reznichenko, Ludovic Chaput, Pascal Martin, Marie-Paule Teulade-Fichou, Anton Granzhan (2018 Aug 27)

**Topology-Selective Fluorescent “Light-Up” Probes for G-Quadruplex DNA Based on Photoinduced Electron Transfer.**


**Summary**

Six novel probes were prepared by covalent attachment of a G4-DNA ligand (PDC) to various coumarin or pyrene fluorophores. In the absence of DNA, the fluorescence of all probes is quenched due to intramolecular photoinduced electron transfer (PET) evidenced by photophysical and electrochemical studies, molecular modeling and DFT calculations. All probes demonstrate similarly high thermal stabilization of various G4-DNA substrates belonging to different folding topologies, as assessed by fluorescence melting experiments; however, their fluorimetric response is strongly heterogeneous with respect to structures of the probes and G4-DNA targets. Thus, the probes containing the 7-diethylaminocoumarin fluorophore demonstrate significant fluorescence enhancement in the presence of G4-DNA, with the strongest “light-up” response (20- to 180-fold) observed for antiparallel G4 structures as well as for hybrid G4 structures, formed by the variants of human telomeric sequence and capable of a conformation change to the antiparallel isoform. These results shed light on the influence of the linker and electronic properties of fluorophores on the efficiency of G4-DNA “light-up” probes operating via PET.

Ludivine Guyon, Marc Pirrotta, Katerina Duskova, Anton Granzhan, Marie-Paule Teulade-Fichou, David Monchaud (2018 Feb 16)

**TWJ-Screen: an isothermal screening assay to assess ligand/DNA junction interactions in vitro**

Summary

The quest for chemicals able to operate at selected genomic loci in a spatiotemporally controlled manner is desirable to create manageable DNA damages. Mounting evidence now shows that alternative DNA structures, including G-quadruplexes and branched DNA (or DNA junctions), might hamper proper progression of replication fork, thus triggering DNA damages and genomic instability. Therefore, small molecules that stabilize these DNA structures are currently scrutinized as a promising way to create genomic defects that cannot be dealt with properly by cancer cells. While much emphasis has been recently given to G-quadruplexes and related ligands, we report herein on three-way DNA junctions (TWJ) and related ligands. We first highlight the biological implications of TWJ and their strategic relevance as triggers for replicative stress. Then, we describe a new in vitro high-throughput screening assay, TWJ-Screen, which allows for identifying TWJ ligands with both high affinity and selectivity for TWJ over other DNA structures (duplexes and quadruplexes), in a convenient and unbiased manner as demonstrated by the screening of a library of 25 compounds from different chemical families. TWJ-Screen thus represents a reliable mean to uncover molecular tools able to foster replicative stress through an innovative approach, thus providing new strategic opportunities to combat cancers.

Year of publication 2017

Jiyeon Choi, Mai Xu, Matthew M Makowski, Tongwu Zhang, Matthew H Law, Michael A Kovacs, Anton Granzhan, Wendy J Kim, Hemang Parikh, Michael Gartside, Jeffrey M Trent, Marie-Paule Teulade-Fichou, Mark M Iles, Julia A Newton-Bishop, D Timothy Bishop, Stuart MacGregor, Nicholas K Hayward, Michiel Vermeulen, Kevin M Brown (2017 Aug 1)

A common intronic variant of PARP1 confers melanoma risk and mediates melanocyte growth via regulation of MITF.

Nature genetics : 49 : 1326-1335 : DOI : 10.1038/ng.3927

Summary

Previous genome-wide association studies have identified a melanoma-associated locus at 1q42.1 that encompasses a ∼100-kb region spanning the PARP1 gene. Expression quantitative trait locus (eQTL) analysis in multiple cell types of the melanocytic lineage consistently demonstrated that the 1q42.1 melanoma risk allele (rs3219090[G]) is correlated with higher PARP1 levels. In silico fine-mapping and functional validation identified a common intronic indel, rs144361550 (-/GGGCC; r(2) = 0.947 with rs3219090), as displaying allele-specific transcriptional activity. A proteomic screen identified RECQL as binding to rs144361550 in an allele-preferential manner. In human primary melanocytes, PARP1 promoted cell proliferation and rescued BRAF(V600E)-induced senescence phenotypes in a PARylation-independent manner. PARP1 also transformed TERT-immortalized melanocytes expressing BRAF(V600E). PARP1-mediated senescence rescue was accompanied by transcriptional activation of the melanocyte-lineage survival oncogene MITF, highlighting a new role for PARP1 in melanomagenesis.
Team publications
Chemistry for Nucleic Acid Recognition