ANNA 2025

Advances in Noncanonical Nucleic Acids:

Book of Abstracts

Bohinj, Slovenia, October 23rd – 25th, 2025

Organized by
Slovenian NMR Centre @ National Institute of Chemistry

ANNA 2025 Advances in Noncanonical Nucleic Acids: Book of Abstracts

Published by: Slovenian NMR Centre
National Institute of Chemistry
Hajdrihova 19
SI-1000 Ljubljana, Slovenia

Ljubljana, Slovenia, 23rd October 2025

Editors: Peter Podbevšek
Janez Plavec

Design: Klemen Pečnik
Peter Podbevšek

https://slonmr.si/anna_2025/ANNA2025BookOfAbstracts.pdf

Kataložni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani
COBISS.SI-ID 253506307
ISBN 978-961-7238-15-0 (PDF)

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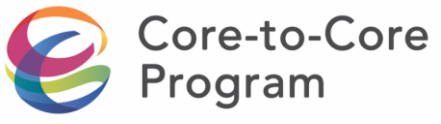

PROGRAMME

Thursday, October 23rd, 2025

10:00	**Bus transfer to Bohinj**
12:00 – 13:00	**Lunch,** *Hotel Bohinj*
13:00 -	**Registration**

Afternoon session *Chair: Naoki Sugimoto*

13:20 – 13:30	**Opening remarks,** *Janez Plavec, Head of NMR centre*
13:30 – 14:00	Katrin Paeschke, *University Clinic Bonn, Germany*
14:00 – 14:30	Ilaria Frasson, *University of Padua, Italy*
14:30 – 15:00	Chuanzheng Zhou, *Nankai University, Tianjin, China*
15:00 – 15:30	Massimo Carraro, *University of Sassari, Italy*
15:30 – 16:00	**Coffee break**
16:00 – 16:30	James Bardwell, *University of Michigan, Ann Arbor, USA*
16:30 – 17:00	Daniela Montesarchio, *University of Naples Federico II, Italy*
	Flash talks
17:00 – 17:15	Filippo Doria, *University of Pavia, Italy*
17:15 – 17:30	Lionel Guittat, *Ecole Polytechnique, Palaiseau, France*
17:30 – 17:45	Lucia Comez, *Istituto Officina dei Materiali, Perugia, Italy*
17:45 – 18:00	Nataša Medved, *National Institute of Chemistry, Ljubljana, Slovenia*
18:00 – 18:15	Chiara Platella, *University of Naples Federico II, Italy*
18:15 – 18:30	Gurudas Chakraborty, *Leibniz Institute for Interactive Materials, Aachen, Germany*
19:00	**Dinner,** *Restaurant "Pri lovcu", Ribčev Laz 4a, Bohinj*

Friday, October 24th, 2025

Morning session *Chair:* Zhen Xi

9:00 – 9:30	Naoki Sugimoto, *FIBER, Kobe, Japan*
9:30 – 10:00	Roberto Improta, *National Research Council, Naples, Italy*
10:00 – 10:30	Viktor Viglasky, *P. J. Šafárik University, Košice, Slovakia*
10:30 – 11:00	**Coffee break**
11:00 – 11:30	Katherine Seley-Radtke, *University of Maryland, Baltimore, USA*
11:30 – 12:00	Bruno Pagano, *University of Naples Federico II, Italy*
12:00 – 12:30	Valentina Pirota, *University of Pavia, Italy*
12:30 – 14:00	**Lunch**, *Hotel Bohinj*

Afternoon session *Chair:* Katrin Paeschke

14:00 – 14:30	Chaoyong Yang, *Xiamen University, China*
14:30 – 15:00	Akimitsu Okamoto, *University of Tokyo, Japan*
	Flash talks
15:00 – 15:15	Emanuela Ruggiero, *University of Padua, Italy*
15:15 – 15:30	Miha Modic, *National Institute of Chemistry, Ljubljana, Slovenia*
15:30 – 15:45	Václav Brázda, *Czech Academy of Sciences, Brno, Czech Republic*
15:45 – 16:00	Anna Artese, *University of Catanzaro, Italy*
16:00 – 16:15	Lea Spindler, *University of Maribor, Slovenia*
16:15 – 16:30	Lutan Liu, *FIBER, Kobe, Japan*
17:00 – 19:00	**Poster session**
19:00	**Dinner,** *Hotel Bohinj*

Saturday, October 25th, 2025

Morning session *Chair:* Katherine Seley-Radtke

9:00 – 9:30	Zhen Xi, *Nankai University, Tianjin, China*
9:30 – 10:00	Claudia Sissi, *University of Padua, Italy*
10:00 – 10:30	Hisae Tateishi-Karimata, *FIBER, Kobe, Japan*
10:30 – 11:00	**Coffee break**
11:00 – 11:30	Antonio Randazzo, *University of Naples Federico II, Italy*
11:30 – 12:00	Hanyang Yu, *Nanjing University, China*
12:00 – 12:30	San Hadži, *University of Ljubljana, Slovenia*
13:00 – 14:00	**Lunch**, *Hotel Bohinj*
14:00	**Bus transfer to Ljubljana**

INVITED LECTURES

G4 stabilization impacts macrophage function

Philipp Schult, Melanie Kastl, Katrin Paeschke

Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, Venusberg Campus, Bonn, Germany

G-quadruplexes (G4s) are stable secondary structures forming in guanine-rich regions of DNA and RNA. G4s are involved oncogenesis, immunity and inflammation and thus G4 stabilization by small molecule ligands has emerged as a potential anti-cancer immunotherapy. Tumour-associated-macrophages (TAMs) are some of the most abundant immune cells in solid tumours and provide a link between cancer and inflammation. Although G4s were shown to impact several biological processes in different organisms, little is known about their steady state and role in immune cells like macrophages. Hence, we monitored G4s in mouse macrophages and show that while G4 stabilization does not directly influence phagocytosis and bacterial lysis, significant transcriptome-wide changes occur after dual treatment of macrophages with PDS and bacterial lipopolysaccharide (LPS). Particularly genes modulating the immune response to bacterial infection become downregulated after treatment with PDS and LPS and several chemokines are no longer secreted. We show that G4 stabilization modulates the immune response via decreasing signalling downstream of NF-κB activation. Mechanistically, we suggest that G4 formation directly competes with NF-κB DNA occupancy at important target genes like IL1B. Based on our data we propose a novel model in which altered G4 levels affect the immune response after bacterial infection in macrophages.

Identification of i-Motifs in Alphaherpesvirus Immediate Early promoters and their dynamic folding with G4s during infection

Emanuela Ruggiero[1], Filippo Mattellone[1], Daniel Christ[2], Sara N. Richter[1], Ilaria Frasson[1]

[1]Department of Molecular Medicine, University of Padova, Padova 35121, Italy
[2]Garvan Institute of Medical Research, Immunology Department, Darlinghurst, Sydney, NSW 2010, Australia

G-quadruplexes (G4s) and i-motifs (iMs) are non-canonical nucleic acid structures that play an increasingly recognised role in key biological processes.[1,2] Computational and experimental studies have revealed the widespread presence of putative G4- sequences across the genomes of different families and types of human viruses.[3–5] In contrast, the presence and function of iMs in viruses remain largely unexplored.

Alphaherpesviruses (αHHVs), which include herpes simplex virus 1 (HSV-1) and 2 (HSV-2) as well as varicella-zoster virus (VZV), are widespread human pathogens that cause lifelong infections. They cause a range of diseases, from common cold sores to severe conditions such as keratitis and encephalitis, posing significant public health challenges.[6] Current treatment options are limited to symptom management, with no cure available to eradicate the infection. Therefore, investigating the presence and role of G4s and iMs in αHHV genomes could provide important insights into their pathobiology and reveal novel antiviral targets.

The immediate-early (IE) promoters of αHHVs are critical for initiating and maintaining an efficient viral cycle. We previously described the presence of multiple characteristic G4s embedded in the IE promoters of the three αHHVs.[7] As these regions have the potential to form iMs on the opposite strand to G4s, we investigated the presence of iMs in the same regions in vitro and in infected human cells. We identified highly conserved iM-forming sequences in all IE promoters. Biophysical characterization using circular dichroism, thermal difference spectroscopy and bromine footprinting revealed which sequences were capable of forming iMs. Our results revealed differences in conformations, pH stabilities and distributions of iMs among the three viruses. This suggested the presence of virus-specific regulatory mechanisms, modulated by dynamic G4/iM folding. To investigate the folding of these structures *in vivo*, we set up a viral-CUT&Tag-qPCR assay in human cells and detected both iMs and G4s in the HSV-1 genome during viral infection. We observed the dynamic formation of iMs and G4s in HSV-1 IE promoters, with enrichment levels changing during the initial stages of infection. Notably, these structural changes correlated with variations in IE gene expression, suggesting a functional role in HSV-1 biology.

Our study has broadened our understanding of the organization of the αHHV genome and the regulation of viral genes through iM folding alongside G4s in this viral family.

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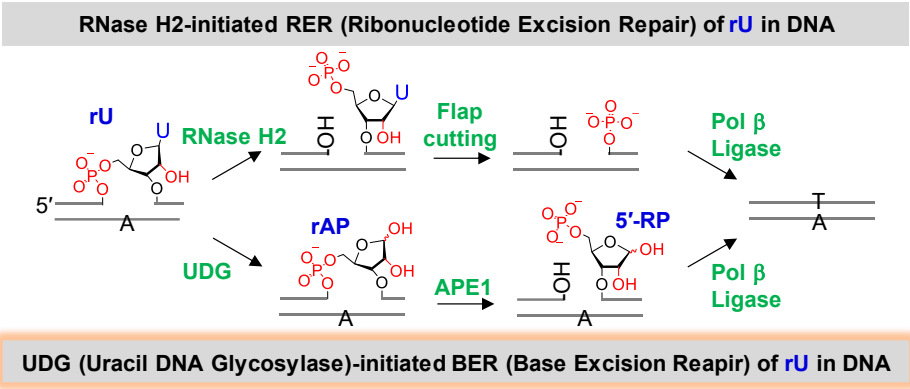
Uridine Embedded within DNA is Repaired by Uracil DNA Glycosylase via a Mechanism Distinct from That of Ribonuclease H2

Chuanzheng Zhou

State Key Laboratory of Elemento-Organic Chemistry, Frontiers Science Center for New Organic Matter, Department of Chemical Biology, College of Chemistry, Nankai University, Tianjin 300071, China

Uridine (rU) and 2′-deoxyuridine (dU) are common DNA lesions. dU is repaired through a base excision repair (BER) pathway initiated by uracil DNA glycosylase (UDG), while rU is typically removed from DNA via ribonucleotide excision repair, mediated by RNase H2. In this study, we report that rU is also repaired through the UDG-mediated BER pathway. We found that UDG catalyzes the removal of uracil from rU embedded in DNA but exhibits no activity toward rU in RNA. Biochemical and crystallographic analyses revealed that the 2′–OH group of rU is effectively accommodated by UDG and directly participates in catalyzing the hydrolysis of the N-glycosidic bond. The abasic site product generated upon removal of uracil from rU by UDG is further processed by downstream BER enzymes to restore undamaged DNA. Our findings suggest that UDG-initiated BER constitutes a previously unrecognized pathway for the repair of rU-specific ribonucleotides. Additionally, we developed a method for selectively quantifying rU content in DNA. Using this method, we determined that rU repair by UDG is not a major pathway in human cells. This discovery expands our understanding of the diverse biological functions of UDG and inspires further investigation to determine the role of its rU-removal in cells.



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Fan, C.; Zhan, X.; Guo, F.; Li, Q.; Lu, K.; Shan, X.; Zhou, Y.; Ren, M.; Greenberg, M. M.; Liu, Y.; Zhou, C., Uridine embedded within DNA is repaired by uracil DNA glycosylase via a mechanism distinct from that of ribonuclease H2. J. Am. Chem. Soc. 147, 11574-11583 (2025)

Acknowledgements: This work was supported by National Key Research and Development Program of China (no. 2023YFA0913800), the National Natural Science Foundation of China (no. 22377059), Haihe Laboratory of Sustainable Chemical Transformations and Frontiers Science Center for New Organic Matter, Nankai University.

Targeting G-quadruplexes with Ligands from Renewable Sources

Jussara Amato[1], Valentina Arciuolo[1], Massimo Carraro[2], Lidia De Luca[2], Silvia Gaspa[2], Francesca Mocci[3], Giuliana Novarni[2], Bruno Pagano[1], Luisa Pisano[2], Janez Plavec[4], Andrea Salis[3], Giuseppe Satta[4,5]

[1]Department of Pharmacy, University of Naples Federico II, Naples, Italy
[2]Department of Chemical, Physical, Mathematical and Natural Sciences, University of Sassari, Sassari, Italy
[3]Department of Chemistry and Geological Science, University of Cagliari, Monserrato, Italy
[4]Slovenian NMR Centre, National Institute of Chemistry, Ljubljana, Slovenia
[5]CERIC-ERIC, AREA Science Park, Basovizza, Trieste, Italy

The use of small molecules as ligands for non-canonical nucleic acid structures, like G-quadruplexes, is actively investigated as a tool to expound their function and a mean to tune gene expression and telomere maintenance for therapeutic purposes. The use of renewable, rather than depleting, raw materials or feedstocks is one of the Green Chemistry principles driving the development of Green Economy but medicinal and bio- chemistry are probably the fields where penetration of this idea is slower. We tried to contribute to the field by choosing a variety of compounds designed to incorporate building blocks from renewable sources. The design was applied to known G4s ligands by decorating them with “renewable” moieties as well as by functionalizing a “renewable” core with moieties that could enhance their affinity for nucleic acids. Furthermore, this approach offers the opportunity to design potential drugs with improved biodegradability.

The study involves a combination of molecular docking and dynamics, organic synthesis and multiple spectroscopic and spectrometric determinations on cationic compounds and their interaction with different DNA sequences[1], subsequently the investigation was expanded to G4 forming RNA sequences.

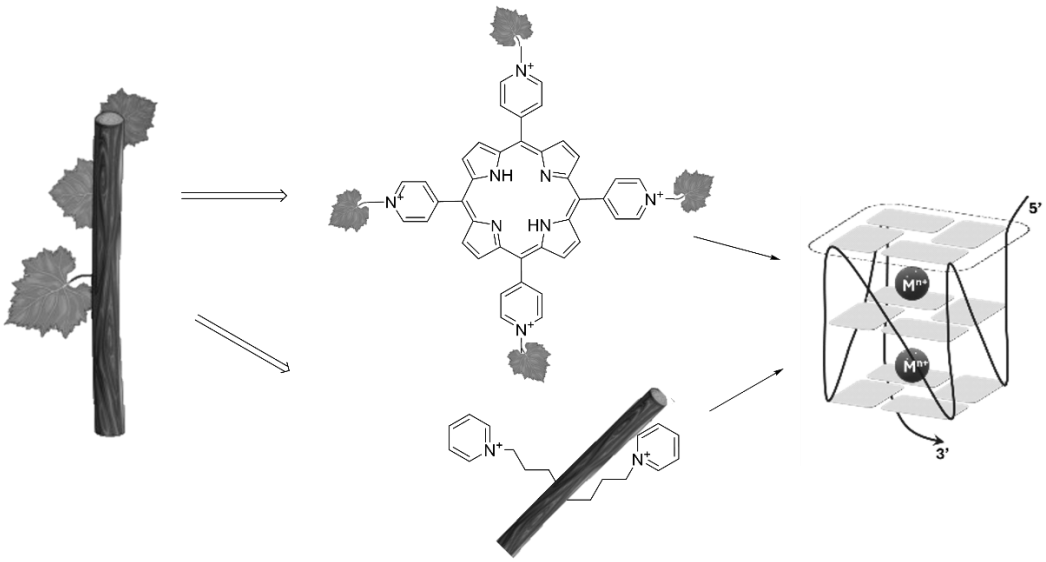

Figure 1 Use of renewable building blocks as decoration or core for G4s binders.

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Small proteins that modulate intracellular granule formation and RNA aggregation

Bikash Sahoo, Xiexiong Deng, Alexey Kovalenko, Nathan W Clark, Ammar Ali, James C. A. Bardwell

Dept. of Molecular Cellular and Developmental Biology, Howard Hughes Medical Institute, University of Michigan 1105 N. University Ave., 48104, Ann Arbor, Michigan, USA

We find that the small partially disordered proteins of the SERF family are important components of stress granules and serve as modulators of RNA condensation. Stress granules and other biomolecular condensates have been implicated in neurodegeneration, but their large and heterogeneous nature has hindered structural understanding of how they form and function. The small size and high biophysical tractability of SERF family proteins and their interacting G-quadruplex partners had enabled us to address these questions and to obtain mechanistic and structural insights into liquid-liquid phase transitions at atomic resolution. Using NMR spectroscopy, single-molecule microscopy and large-scale all-atom simulations, we have uncovered how SERF2 specifically recognizes RNA G-quadruplexes, including ALS-linked *C9orf72* hexanucleotide repeat expansions. SERF2 uses conserved lysine- and arginine-rich sequence motifs to stabilize dynamic ribonucleoprotein condensates into ring-like forms. Disruption of these motifs abolishes G-quadruplex binding and liquid-liquid phase transitions. SERF related proteins remodel gel-like RNA aggregates into liquid-like droplets, constraining RNA dynamics while preventing irreversible gelation. *In vivo*, SERF2 localizes to stress granules, is important for their assembly, and SERF2 depletion perturbs G4-mediated gene expression. These findings establish the small SERF-related proteins are important for RNA-protein condensates and provide detailed structural information about ribonucleoprotein condensation.

G-quadruplex-forming aptamers to inhibit HMGB1 pathological activity: exploring multivalency effects

Daniela Montesarchio

Department of Chemical Sciences, University of Napoli Federico II, via Cintia 21, I-80126 Napoli, Italy

Among the known aptamers, many are based on G-rich oligonucleotides.[1] This is not surprising if we consider that these oligonucleotides share a distinctive ability to fold into stable but also extremely different G-quadruplex (G4) structures, which constitute very useful scaffolds to build efficient aptamers. Indeed, even very similar G-rich DNA sequences can exhibit extraordinarily wide structural variability, thus providing aptamers with very different recognition abilities.[2]

In this context, we recently investigated several G4-forming aptamers specifically targeting inflammation-,[3] coagulation-[4], neurodegeneration-[5,6] and cancer-related[7-10] proteins. Here, special focus will be devoted to G4-forming aptamers selectively recognizing HMGB1, a chromatin-associated, non-histonic protein released under inflammatory conditions in the extracellular environment, where it acts as a cytokine contributing to the pathogenesis of cancer. The newly discovered anti-HMGB1 L12[9,10] is a polymorphic aptamer whose conformational behavior dramatically depends on sample manipulation and the chosen buffer. In physiological buffers mimicking extracellular media and without annealing, this aptamer folds into very stable dimeric parallel G-quadruplex structures, which recognize the target protein with higher affinity than the monomeric counterpart.[9,10] To obtain L12 analogues more effective for *in vivo* studies and early detection of this cancer-relevant protein in biological samples, a mini library of covalent dimers of L12 has been designed and investigated.[11] Moreover, a new approach in which the L12 aptamer was immobilized on biocompatible silica nanoparticles has been undertaken to exploit multivalency effects.[12]

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Acknowledgements:

The research leading to these results has received funding from: i) Fondazione AIRC under IG 2020—ID.25046 — P.I. Montesarchio Daniela; 2) CN00000041 National Center for Gene Therapy and Drugs based on RNA Technology and the European Union-NextGeneration EU – P.I. Montesarchio Daniela.

cRGD-mediated delivery of G4 ligands: dual targeting strategies for precision cancer therapy

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G-quadruplexes (G4s) are increasingly recognized as promising anticancer targets due to their role in regulating essential biological processes.[1] To enhance the therapeutic potential of G4-targeted agents, we designed novel delivery systems exploiting the overexpression of integrins αvβ3/αvβ5 on tumour cells, using the cyclic peptide Arg-Gly-Asp (cRGD) as a targeting vector.[2] Two complementary strategies were explored. First, cRGD was conjugated by click chemistry to either silk fibroin nanoparticles encapsulating a tetrasubstituted naphthalene diimide (cRGD-SFNs-NDI)[3] or to a structurally analogous NDI (cRGD-NDI).[4] Both systems selectively enhanced ligand uptake and activity in glioblastoma cell lines U373 and U251, while sparing integrin-negative cells, thus reducing the off-target toxicity typically associated with free NDIs. Second, the cRGD platform was extended to address triple-negative breast cancer (TNBC), an aggressive malignancy with limited therapeutic options. We engineered cRGD-decorated liposomes for targeted delivery of doxorubicin. Functionalization with cRGD promoted integrin-mediated endocytosis, leading to increased cytotoxicity against TNBC cells overexpressing αvβ3 integrins.[5] The incorporation of a fluorescent dye and a gadolinium complex further provided dual imaging capabilities, establishing these liposomes as potential theranostic agents.

Together, these studies demonstrate the versatility of cRGD-based strategies for precision oncology. By guiding either G4 ligands or conventional chemotherapeutics to integrin-overexpressing tumours, cRGD functionalization enhances selectivity, reduces systemic toxicity, and opens the way to new targeted therapies with diagnostic potential.

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Ovejero-Paredes K., Bisbano G., Flier I., Gonzalez-Sim A., Marciello M., Oliveira S., Zupi M., Rubes D., Ayash F., Doria F., Pirota V., Terreni M., Serra M. and Filice M., European Journal of Pharmaceutical Sciences, 213, 107216-107234 (2025).

G-Quadruplexes in Archaea

Zackie Aktary[1], Kate Sorg[1], Anne Cucchiarini[1], Guglielmo Vesco[1,2], Dorian Noury[1], Rongxin Zhang[1], Thomas Jourdain[1], Daniela Verga[3], Pierre Mahou[1], Nicolas Olivier[1], Natalia Bohalová[4], Otilia Porubiaková[4], Václav Brázda[4], Marie Bouvier[5], Marta Kwapisz[5], Béatrice Clouet-d'Orval[5], Thorsten Allers[6], Roxane Lestini[1], Jean-Louis Mergny[1], Lionel Guittat[1,7]

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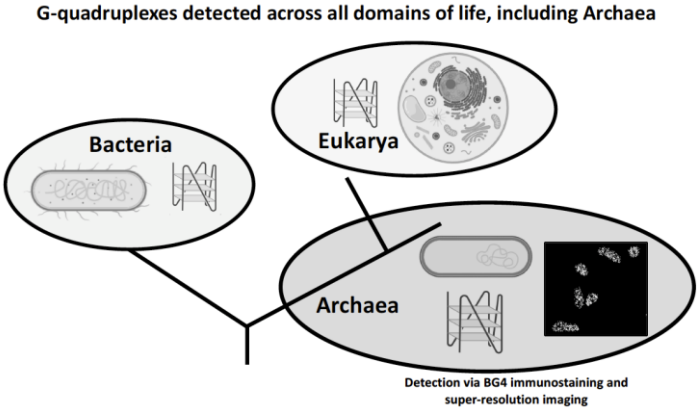
The archaeal domain comprises a diverse group of microorganisms that thrive in a wide range of environments and are considered the closest known relatives of eukaryotes. This evolutionary relationship suggests that eukaryotes may have originated from an archaeal ancestor. G-quadruplexes (G4s) are secondary DNA and RNA structures involved in key biological processes. However, their formation and function in archaea remain largely unexplored. A bioinformatics analysis of the genome of *Haloferax volcanii*, a halophilic archaeon, identified potential G4-forming sequences (PQS). Biophysical studies confirmed that these PQSs can form stable G4 structures in vitro under physiological conditions. Using the BG4 antibody and super-resolution microscopy, we detected G4 structures in both DNA and RNA at the single-cell level across different growth phases. Similarly, in the thermophilic archaeon *Thermococcus barophilus*, we observed G4 structures along with helicases that may assist in their resolution. Moreover, combining fluorescence in situ hybridization (FISH) with G4 detection allows for the simultaneous visualization of G4 structures and chromosomal organization. This approach enables the study of G4 dynamics in relation to cellular ploidy at the single-cell level. By establishing archaea as new model systems for G4 research, this study bridges bacterial and eukaryotic systems, offering new insights into the evolutionary conservation of G4s across the tree of life.[1]



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Spectroscopic Investigation of Hydration Interactions in G-Quadruplex Solutions

Valeria Libera[1], Sara Catalini[1], Luca Bertini[1], Martina Alunni Cardinali[2], Francesca Ripanti,[3] Francesco D'Amico[4], Caterina Petrillo[1], Marco Paolantoni[2], Alessandro Paciaroni[1], Lucia Comez[5]

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The intrinsic polymorphism of G-quadruplexes (GQs) renders their structure highly sensitive to environmental conditions, with solvation playing a pivotal role in their folding and stability. We employ a dual-spectroscopy strategy to investigate dilute GQ solutions during thermal unfolding. UltraViolet Resonance Raman (UVRR) scattering enhances the vibrational features of GQs.[1,2] While this method has been previously applied to other biosystems,[3] its use in probing solute-solvent interactions in GQs is novel. Analysis of the OH stretching vibrational band—an indicator of the hydrogen-bonded water network—provides insight into how nearby water molecules interact with two distinct G-quadruplex conformers: hybrid and parallel. We demonstrate that coupling UVRR with circular dichroism spectroscopy allows for the correlation of vibrational properties with secondary structural features, even in spectral regions dominated by solvent contributions.[4] Extending this approach to other G-quadruplexes may provide deeper insights into the crucial role of solvation in their stability and function. Notably, the subtraction method used to calculate residual water in UVRR spectra was recently applied to cells,[5] paving the way for characterizing structural water in G-quadruplexes, even in crowded environments.

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We acknowledge Elettra Sincrotrone Trieste for providing access to its synchrotron radiation facilities and for financial support (Proposal No. 20225297)

Cytosine methylation controls folding kinetics and stability of the bcl2Mid G-quadruplex

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DNA methylation is an important epigenetic modification with a well-established role in the regulation of gene expression.[1] In addition to its classical function of modulating transcription through promoter accessibility, methylation can also influence the formation and properties of non-canonical DNA structures.[2] Among these, G-quadruplexes (G4) are of particular interest as they form in guanine-rich regions of the genome and play an important role in transcriptional regulation.[3] However, the effects of cytosine methylation on G4s are still poorly understood, particularly in terms of how a single methyl group can alter their folding behaviour, thermodynamic stability and recognition by proteins.

We investigated the effects of a single 5-methylcytosine residue (C^m) on the bcl2Mid G4, a structure that forms in the GC-rich region upstream of the P1 promoter of *BCL2* gene.[4] Using solution-state NMR spectroscopy and complementary biophysical techniques, we observed a distinct sequence-specific effect of C^m on G4 folding kinetics. Substitution of cytosine residue at position C6 by $C6^m$ slowed down the folding process and shifted the equilibrium between major and minor G4 structures in the presence of K^+ ions. The increased population of the minor G4 structure allowed us to characterize its previously unidentified topology. In contrast to the major G4 structure, which adopts a (3+1) hybrid topology, the minor structure exhibits parallel topology and contains a snapback element that fills a vacancy in the G-quartet at the 5'-end. In the major G4 structure, C^m induced subtle local structural rearrangements and decreased thermodynamic stability. Furthermore, we demonstrated that the zinc finger 3 motif of transcription factor Sp1 preferentially binds to the minor G4 structure, indicating a possible mechanism by which cytosine methylation can modulate protein-DNA interactions.

Our results show that even a single 5-methylcytosine residue can significantly influence G4 polymorphism, folding kinetics, thermodynamic stability and recognition by transcription factors. This provides new insights into the intricate interplay between epigenetic modifications and non-canonical DNA structures in the regulation of gene expression.

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Acknowledgements: Slovenian Research and Innovation Agency [ARIS, grants P1-0242, P1-0201 and J1-60019] and CERIC – ERIC consortium for access to experimental facilities and financial support.

Structural investigation of G-quadruplex aptamers as potent inhibitors of HMGB1 pathological activity

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Targeting High Mobility Group Box 1 (HMGB1) protein emerged as a valuable therapeutic strategy for the treatment of various inflammatory and autoimmune diseases as well as cancer, considering its main role in these pathological processes.[1,2] Recently, we identified a set of anti-HMGB1 aptamers as potent inhibitors of the protein pathological activity, the most active of which were L12 and L41, able to form G-quadruplex structures of different topology and molecularity in relation to the different solution conditions and preparation methods used.[3,4]

Intrigued by the potent anti-HMGB1 activity of L12 and L41, NMR spectroscopy, complemented by circular dichroism (CD) analyses, was exploited to define the structural features of the main G-quadruplex structures formed by the two aptamers in pseudo-physiological conditions[5]. In-depth structural details were obtained for the monomeric forms of both aptamers, which were further investigated in their interaction with HMGB1 by CD and biolayer interferometry (BLI) analyses, and finally tested for their anti-HMGB1 activity in biological assays.[5]

In K^+-rich solutions L12 and L41 proved to fold in hybrid-2 G-quadruplex topologies endowed with similar features, overall suggesting that both aptamers exploit similar recognition patterns at the level of DNA/protein interfaces in HMGB1 targeting. However, L12 showed ability to fold in a hybrid-2 G-quadruplex structure in a higher amount than L41. Remarkably, BLI analysis demonstrated a preference of HMGB1 for L12 over L41, which well matched the in vitro assays results showing higher ability to inhibit the HMGB1-induced cell migration for L12 than L41. Altogether these results suggest that HMGB1 well recognizes the hybrid-2 G-quadruplex structure adopted by these aptamers, present to a higher extent in L12 than in L41.[5]

The precious structural insights obtained on the conformational preferences of L12 and L41 aptamers in pseudo-physiological solutions will be crucial for the future rational design of modified analogues as even more effective and selective anti-HMGB1 aptamers.

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Acknowledgements: The authors acknowledge the CERIC-ERIC consortium for access to NMR facilities. The research received funds from: i) Slovenian Research and Innovation Agency [ARIS, grants P1-0242 and J1-60019] – P.I. Janez Plavec; ii) the European Union - NextGeneration EU, National Recovery and Resilience Plan (PNRR), Project CN00000041 "National Center for Gene Therapy and Drugs based on RNA Technology": iii) Fondazione AIRC under IG 2020—ID. 25046 — P.I. Daniela Montesarchio; and iv) Federico II University, FRA2022 project (CUP: E65F22000050001) – P.I. Domenica Musumeci.

Supramolecular Noncanonical DNA in Organic Solvents: From Structure to Catalysis

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The discovery of noncanonical DNA structures, particularly G-quadruplexes and i-motifs, has expanded DNA's role from biology to remarkable applications in supramolecular chemistry and nanotechnology.[1-3] However, their highly polar nature and the need for stabilizing factors, such as metal cations for G-quadruplexes and hemi-protonated cytosines for i-motifs, have largely confined their use to aqueous media. In contrast, we recently achieved the formation of both G-quadruplexes and i-motifs in organic solvents by employing a noncovalent PEGylation strategy based on electrostatic interactions.[4] This approach enabled the first metal-free G-quadruplexes in organic solvents. Moreover, incorporation of an iron-containing porphyrin rendered these self-assembled, metal-free G-quadruplexes catalytically active, giving rise to "supraG4zymes".[5] In parallel, we have established solvent-selective folding of C-rich strands into i-motifs, whose thermostability can be finely tuned by PEG molecular weight and crowding effects in organic media.[6] These advances demonstrate how noncanonical DNA structures can be formed and controlled in non-aqueous solvents, opening new avenues for DNA-based supramolecular assemblies and catalysis beyond water.

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Acknowledgements:
G.C. is indebted to the Erasmus Mundus Programme for economic assistance through the NAMASTE scholarship. These studies were supported by the European Research Council Advanced Grant SUPRABIOTICS (No. 694610) and the NARRATE Grant (No. 101138690). Additional support was provided by the National Institutes of Health GM077422.

“To B or not to B” in Nucleic Acids Chemistry

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In this lecture, I will provide an overview of the basic concepts, methods, and recent applications of predicting the stabilities of nucleic acid structures. I will explain the theory of the most successful prediction method based on a nearest-neighbour (NN) model. To improve the versatility of prediction, corrections for various solution conditions considered hydration have been investigated. I also describe advances in the prediction of non-canonical structures of G-quadruplexes and i-motifs. A new physicochemical approach will be introduced to apply the analysis of nucleic acids behaviours in cell. Finally, studies of intracellular analysis and stability prediction are discussed for the application of NN parameters for human health and diseases.

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Acknowledgements. The author is grateful to the colleagues named in the cited papers from my laboratory, institute (FIBER), and others. This work was supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and the Japan Society for the Promotion of Science (JSPS), especially, Grant No. JP25K21727, Grant-in-Aid for Scientific Research (S) (JP22H04975), JSPS Core-to-Core Program (JPJSCCA20220005), and The Chubei Itoh Foundation.

A DNA fluorescent probe provides new insights on the flipping mechanism of 5-methylcytosine in hemi-methylated CpG sequences by the epigenetic UHRF1 protein

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We shall report some of the results obtained in the development of perfectly isofunctional and isosteric fluorescent nucleoside analogues (FNAs)[1-3] and in their use to provide new information on the interaction between biological macromolecules.[4-8] In particular, we shall discuss the interaction of UHRF1 protein with DNA fragments containing a hemi-methylated (HM) CpG site. UHRF1, via its SET- and RING-associated (SRA) domain, recognizes indeed hemi-methylated (HM) CpG sites and flips 5-methylcytosine (5mC) nucleobases (see Figure 1).[4] This is a key biological process, necessary to preserve epigenetic patterns during cell replication. In our study, thienoguanosine (thG), a fluorescent guanosine analogue, was introduced at four positions in HM and non-methylated duplexes, and the interaction with SRA were monitored combining fluorescence measurements, molecular dynamics simulations, and quantum mechanical calculations. 5mC and C residues are flipped with similar rate constants, but only SRA complexes with flipped 5mC undergo a slow conformational rearrangement, leading to the final conformation crucial for the recruitment of the DNA methyltransferase 1, which can then methylate C in the complementary strand. In this contribution we shall discuss the role of Quantum Mechanical calculations to achieve such a picture, provide a rational for the sensitivity of the thG fluorescence on 5mC flipping and showing that the photophysical properties of thG are also sensitive to the conformation adopted by the residues of the complementary strand.

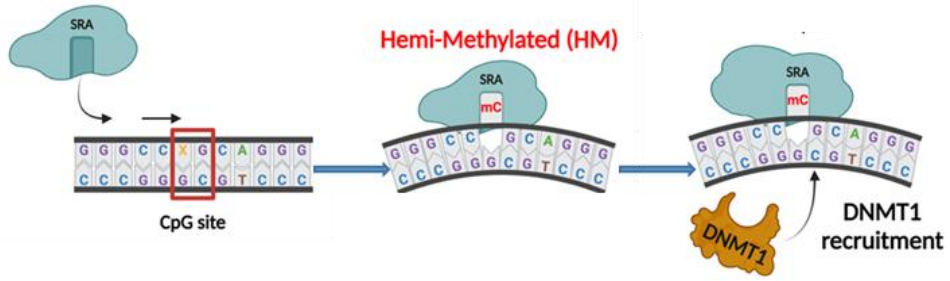

Figure 1 Schematic description of the processes involving the SRA domain of UHRF1 protein and a hemi-methylated CpG site.

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Acknowledgements: RI, thanks the CNR program “Progetti di Ricerca @cnr'', project UCATG4, NUTRAGE funded by FOE-2021 DBA.AD005.225, and CN3, National Center for GeneTherapy and Drugs based on RNA technology, funded by the European Union-NextGenerationEU-PNRR for financial support.

Identification of Non-Canonical Motifs in the Genome and their Potential Relationship with Genetic Incompatibility.

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There is no longer any doubt that non-canonical structural motifs (NCSs) formed by nucleic acids play a crucial role in regulating essential cellular processes. These include G-quadruplexes, i-motifs, cruciforms, triplexes, and other structures. Recently, we introduced an alternative approach to representing nucleic acid sequences in 3D space.[1] This system enables the discovery of novel, unusual structures within long sequences, and we developed a software tool for identifying sequences prone to forming NCSs[1]. Comparative analysis has so far revealed unexpected results: i) the relationship between gene expression and NCSs, ii) the colocalization of predicted G-quadruplexes with cruciforms, iii) high heterogeneity in biologically incompatible organisms. For these purposes we applied the G-finder searching tool.[2]

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Acknowledgements: This work was supported by research grants VEGA 1/0347/23

Development of Pan-viral Nucleoside Analogues

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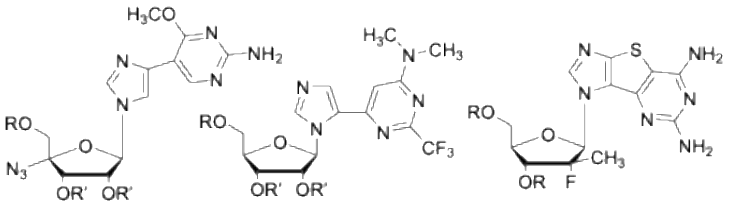

R= McGuigan ProTide prodrug; R'=isobutyrate

The severity and rapid spread of the coronavirus pandemic served to drive home that we were completely unprepared to fight such an outbreak. As a result, it became clear that there was a critical need for small molecule, orally bioavailable, broad-spectrum drugs that could be stockpiled and readily distributed when the next outbreak occurs. In that regard, for many years nucleos(t)ides have maintained a prominent role as one of the cornerstones of antiviral and anticancer therapeutics, and numerous scaffolds in nucleos(t)ide and nucleic acid drug design have been pursued.[1,2] One such approach involves adding flexibility to the sugar moieties of nucleos(t)ides, for example, in the highly successful anti-HIV/HBV drug Tenofovir developed by Antonín Holý. [1,2] In contrast, introduction of flexibility to the nucleobase scaffold has only more recently gained significance with the invention of our fleximers. This modification has led to a significant improvements in antiviral activity, and in some cases endowing the nucleoside with potent broad-spectrum activity across several viral families, when the parent rigid nucleoside was inactive.[3-6] Another advantage observed is the ability to avoid resistance mechanisms related to point mutations by engaging secondary amino acid residues not previously involved in the mechanism of action. A second series of nucleosides being pursued in our group, involves insertion of a heterocyclic spacer ring in between the two moieties of the bicyclic purine ring system, forming an expanded tricyclic nucleoside with increased aromaticity.[7] This modification has also led to potent antiviral activity, again targeting several different pathogens of pandemic concern.[8] A brief history of their design, synthesis, and recent antiviral findings for these innovative nucleos(t)ide scaffolds will be discussed.

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Acknowledgements: Thanks to our many collaborators over the years, particularly our NIH AntiVIral Drug Development (AViDD) Center for Pathogens of Pandemic Concern collaborators, and NIH/NIAID and the North Carolina State Appropriation fund for funding our research for the past several years.

Beyond small molecules: Bioinspired molecular tools for selective G-quadruplex targeting

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Noncanonical nucleic acid structures such as G-quadruplexes (G4s) are increasingly recognized as key regulators of gene expression and genome stability, as well as attractive targets for therapeutic intervention.[1,2] However, the search for selective recognition of individual G4 sites is hindered by the intrinsic similarity among G4 structures, with available ligands providing only limited discrimination.

To address these limitations, novel strategies are being explored that move beyond conventional small-molecule design. These include hybrid systems combining the sequence specificity of nucleic acid analogues with G4-binding ligands, as well as peptide-based approaches inspired by natural G4-recognizing proteins.[3-6] Such bioinspired and modular systems promise high levels of precision, enabling selective interaction with G4s at specific genomic loci and potentially unlocking new modes of therapeutic control. This conceptual shift may open new perspectives for G4-targeted therapeutic strategies and for dissecting the biological functions of individual G4 structures.

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Acknowledgements: Italian Association for Cancer Research (AIRC) IG 2020 - ID. 24590; PRIN 2022 - ID. 2022SP38PS.

Targeting G-Quadruplexes in Neurodegenerative Disorders: A Dual Approach to Modulate Pathogenic Pathways

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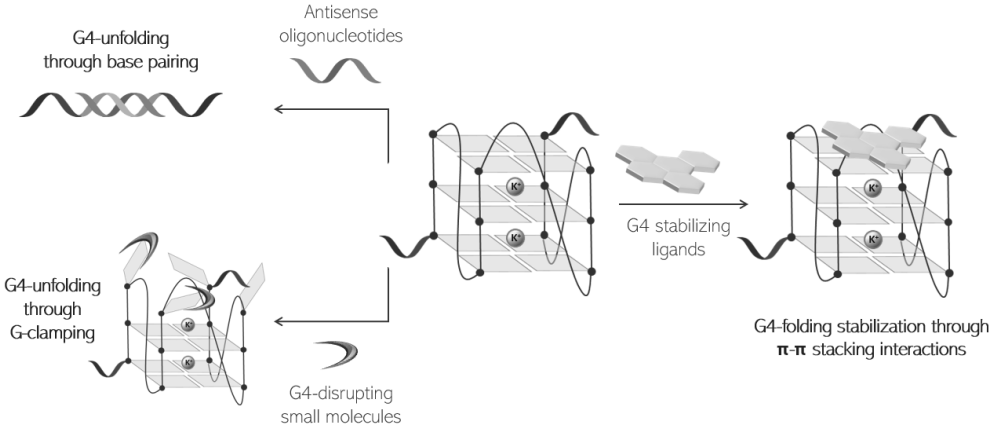
As key regulators of gene expression and genomic stability, G-quadruplexes (G4s) represent compelling targets in neurodegenerative diseases.[1] Here, our research underscores the transformative potential of dynamic G4 modulation, via stabilization or destabilization,[2] as a novel therapeutic strategy for neurodegeneration. In Parkinson's disease, we demonstrated that G4-stabilizing ligands effectively reduced α-synuclein expression by targeting mSNCA-G4 within SNCA mRNA. In parallel, peptide nucleic acids (PNAs) selectively disrupted pSNCA-G4 at the transcription start site (TSS), leading to a remarkable SNCA transcription and protein level reduction by ~70%.[3] This dual-targeting approach highlights the therapeutic versatility of SNCA-G4s modulation against synucleinopathies.

For Alzheimer's disease, we identified the highly stable pApoE-G4 near the transcription start site of the APOE gene, within a critical transcription factor binding region. Similarly, we reach an effective modulation of pApoE-G4 folding via G4-ligands and PNA conjugates, providing a robust framework for fine-tuning ApoE expression.[4]

In CANVAS syndrome, we highlighted that pathogenic RFC1 repeats formed stable G4s, unlike normal sequences, allowing us to suggest the diagnostic and therapeutic potential of targeting G4s in repeat expansion disorders.[5]

Likewise, in amyotrophic lateral sclerosis, we compared antisense strategy with disrupting small molecules to unfold toxic G4s formed by C9orf72 repeat expansions, offering new insights into G4 disruption as a therapeutic strategy. By harnessing the ability to manipulate G4-folding, our work bridges biophysical insights with clinical applications, paving the way for innovative therapeutic strategies in neurodegenerative diseases.



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Spatially resolved whole transcriptomics and translatomics reveals tissue translational regulation mechanism

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Spatially resolved transcriptome and translatome co-mapping unlocks gene expression and translational regulation underlying tissue development, homeostasis, or disease. However, there is a need for genome-wide, high-sensitive, single-section transcriptome and translatome co-profiling methods. Here, we developed a dendrimeric DNA coordinate barcoding design for spatial ribosome-bound RNA sequencing (Decoder-Ribo-seq) to address these challenges, which integrates genome-wide probe design, tri-probe-based proximity ligation chemistry to simultaneously capture transcriptome and translatome, and high-density dendrimeric spatial barcoded arrays. Decoder-Ribo-seq achieves high-throughput detection of 19,954 translated genes of mouse brain tissues and reveals translation-active regions and a set of translationally regulated genes, verifying its potential for advancing understanding of tissue translational regulation mechanism during physiological and pathological processes.

Unusual base pairing induced by imidazo[1,2-c]pyrimidine

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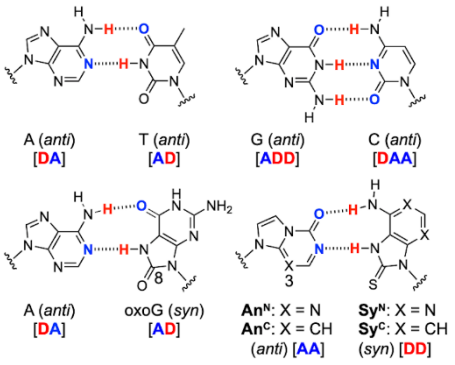
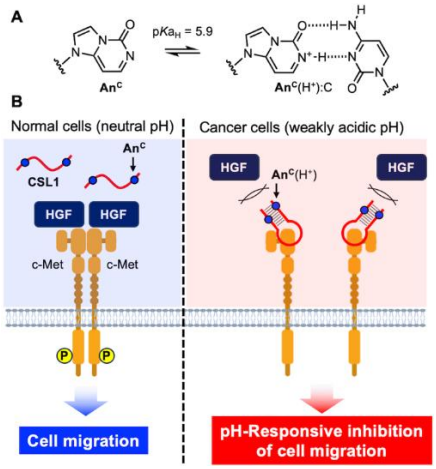
I. New base pairs with 8-thiopurines[1]



The unique self-assembly properties of DNA and its capacity to form diverse higher-order structures have enabled the development of programmable molecular tools. However, the practical application of these DNA-based systems is often limited by their lack of orthogonality to natural nucleic acids, particularly in complex molecular environments. To overcome this limitation, we present a strategy for orthogonal control of DNA self-assembly using an unnatural base pair (UBP). We have developed a novel UBP, An^N:Sy^N, which forms through a combination of anti and atypical syn glycosidic conformations, exhibiting high thermal stability and strong selectivity. In addition, the artificial base An^C functions as a pH-responsive nucleobase, forming a stable base pair with cytosine under mildly acidic conditions (pH 6.0). Notably, the orthogonal An^N:Sy^N pair successfully initiated a hybridization chain reaction (HCR), resulting in the formation of long, nicked double-stranded DNA structures approximately 1000 base pairs in length. This study marks the first demonstration of orthogonal DNA self-assembly that remains unreactive to triggers composed of the natural four-letter DNA alphabet. Our findings broaden the scope of programmable molecular tools operable in complex biological and synthetic environments.

II. Design of pH-sensitive aptamer[2]



Due to the characteristically acidic extracellular micro-environment of cancer cells, pH-responsive molecules have become essential components in both bioanalytical applications and the development of targeted therapies. Among them, pH-responsive DNA aptamers, which can selectively bind to cancer-associated proteins, have emerged as a promising focus in therapeutic research. However, conventional pH-responsive aptamers suffer from significant limitations. Their reliance on specialized nucleic acid structures, such as i-motifs and DNA triplexes, results in complex architectures, strict sequence constraints, and challenges in large-scale synthesis. To overcome these drawbacks, we employed An^C, which is an unnatural nucleobase with a pKa_H of 5.9, to design a structurally simple yet effective pH-responsive DNA aptamer, designated CSL1. This aptamer selectively targets the *c*-Met protein, which is overexpressed in various cancer cells. Under mildly acidic conditions, CSL1 demonstrated enhanced inhibitory activity against the HGF/*c*-Met signaling pathway and significantly suppressed cancer cell migration and spreading. Our approach offers a streamlined and adaptable platform for the development of pH-responsive DNA aptamers, and represents the first report of a *c*-Met-selective antagonist that exerts cancer-specific inhibition of cellular migration.

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G-Quadruplexes Promote HTLV-1 Antisense Transcription through SP1 Binding

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The human T-cell lymphotropic virus type 1 (HTLV-1) is a highly oncogenic delta-retrovirus responsible for uncurable malignancies. It presents two long terminal repeat (LTR) regions that are enriched in putative G-quadruplex (G4)-forming sequences. G4s, non-canonical nucleic acid structures, play crucial roles in regulating key biological processes in both human and viral genomes.[1]

We investigated the presence and functional role of G4s within the HTLV-1 LTRs, focusing on the 3'-LTR, which governs antisense transcription of the viral bZIP factor (HBZ).[2] We identified seven highly conserved putative G4-forming sequences and confirmed their ability to fold into two-layer G4s in both single- and double-stranded DNA contexts in vitro. Using chromatin immunoprecipitation, we demonstrated G4 folding at the 3'-LTR in infected cells. Remarkably, stabilization of these G4s with pyridostatin enhanced antisense transcription from the 3'-LTR by facilitating recruitment of the SP1 transcription factor.

Our findings unveil a novel regulatory mechanism underpinning HTLV-1 antisense transcription and highlight G4 structures as critical regulatory elements within the provirus. This work provides new insights into the complex interplay between viral genomic structures and host cellular factors in HTLV-1 pathogenesis, contributing to our understanding of retroviral replication strategies.

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Integrative analysis of RNA condensation principles

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Complex RNA–protein networks play a pivotal role in the formation of many types of biomolecular condensates. How intrinsic RNA features contribute to condensate formation however remains unclear. Here, we integrate tailored transcriptomics assays to identify a distinct class of developmental condensation-prone RNAs termed 'smOOPs' (semi-extractable, orthogonal organic phase separation-enriched RNAs). These transcripts are localised to larger intracellular foci, form denser RNA-RNA interaction subnetworks than expected and are heavily bound by RNA binding proteins (RBPs). Using an explainable deep learning framework, we reveal that smOOPs harbour characteristic sequence composition with lower sequence complexity, increased intramolecular folding and specific RBP binding patterns. Intriguingly, these RNAs encode proteins bearing extensive intrinsically disordered regions and are markedly predicted to be involved in biomolecular condensates, indicating an interplay between RNA- and protein-based features in phase separation. This work advances our understanding of condensation-prone RNAs and provides a versatile resource to further investigate RNA-driven condensation principles.

G-quadruplex structures in mitochondrial DNA: Evolutionary conservation, regulatory roles, and a link to Neanderthal extinction

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G-quadruplexes (G4s) are non-canonical nucleic acid structures increasingly recognized for their regulatory roles in both nuclear and mitochondrial genomes. In our comprehensive analysis of 11,883 mitochondrial DNA (mtDNA) sequences across 18 taxonomic sub-groups, we observed significant variability in the frequency and distribution of putative G4-forming sequences (PQS). PQS abundance was inversely correlated with evolutionary age, suggesting a gradual refinement or loss of these motifs over time. Notably, PQS were consistently over-represented in key regulatory regions—such as 3'UTRs, D-loops, replication origins, and stem loops—highlighting their potential involvement in mitochondrial gene expression and replication.

Extending this investigation to extinct hominin lineages, we analysed G4 motifs in the mtDNA of *Homo neanderthalensis*, Denisovans, and *Homo sapiens*. While overall G4 patterns were similar, a striking difference was found in the D-loop region, which governs mtDNA replication. *H. neanderthalensis* harbours a long uninterrupted guanine tract capable of forming a stable G4 structure, potentially impeding replication efficiency. In contrast, *H. sapiens* typically exhibits a shorter, interrupted motif, which may facilitate more efficient replication and energy metabolism. This structural difference could have conferred a selective advantage to modern humans and may represent a molecular factor contributing to Neanderthal extinction.

Together, these findings underscore the evolutionary conservation and functional importance of G4s in mitochondrial genomes. The presence, variability, and lineage-specific adaptations of G4 motifs across diverse taxa—including extinct hominins—highlight their potential role in shaping mitochondrial function, species fitness, and evolutionary trajectories.

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Acknowledgements: This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic under the INTER-EXCELLENCE II program, project number LUAKR25078, and by the European Regional Development Fund through the INTERREG Austria–Czech Republic programme, project number ATCZ0052.

Targeting TERRA G-Quadruplexes: from computational prediction to experimental validation

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Telomeric repeat-containing RNA (TERRA) transcripts are long non-coding RNAs transcribed from telomeric regions, characterized by repetitive UUAGGG sequences. These transcripts can fold into stable G-quadruplex (G4) secondary structures, which are implicated in the regulation of telomere maintenance, chromatin organization and genome stability. Increasing evidence indicates that TERRA G4s play a pivotal role in cancer cell survival and proliferation, making them promising targets for the development of innovative therapeutic strategies.[1]

In this context, our research group has extensively investigated these challenging targets. In a first study, we combined structure-based virtual screening with biophysical profiling, leading to the identification of three novel stabilizers of both bimolecular telomeric DNA (Tel_2) and TERRA ($TERRA_2$) G4s.[2] These compounds were subsequently characterized through a combination of computational and experimental approaches against monomeric Tel/TERRA G4s.[3] More recently, the pharmacological stabilization of TERRA G4s has been validated as a novel therapeutic strategy in multiple myeloma models.[4] In parallel, we designed chromene derivatives as TERRA-selective ligands, showing remarkable specificity for TERRA G4s over Tel G4s.

Figure 1 Graphical overview of the computational–experimental workflow for the identification of novel chromene derivatives as selective TERRA G4 stabilizers.

Collectively, these advances provide a strong rationale for the further development of TERRA G4 binders as innovative anticancer agents.

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The polyelectrolyte character of G-quadruplex dynamics in solution

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Guanine-rich DNA segments have an interesting ability to self-assemble into quadruple helices named G-quadruplexes. The folding patterns of G-quadruplexes are highly diverse and intensively studied. Much less attention, however, is on the physical aspects of quadruplexes, especially their polyelectrolyte nature. The DNA molecule in solution is found in the form of a negatively charged polyion surrounded by co-ions and counterions. The electrostatic interactions between them alter the solution dynamics and become evident if we study DNA solutions with dynamic light scattering (DLS). They affect the apparent diffusion coefficient that is directly related to the length of the DNA molecule. Even more interestingly, DNA molecules, and polyelectrolytes in general, exhibit an additional slow diffusive mode that is still not clearly resolved, but usually attributed to large globular clusters of molecules. G-quadruplexes are due to their quadruple helix structure considered as even stronger polyelectrolytes than the standard dsDNA.

In this work we focused on polyelectrolyte properties of G-quadruplex solutions, especially how they affect solution dynamics. We prepared a set of G-rich oligonucleotides with GC-overhangs, some of them previously studied.[1,2] They were selected to cover a wide range of aggregate lengths, from very short quadruplexes and dimers[1], over intermediate lengths[2] to extremely long stacks of quadruplexes. With DLS we determined their solution dynamics, both the fast and slow diffusive mode. A thorough analysis of the fast mode was applied to test the analysis standardly used to determine the length of rod-like quadruplexes and their polydispersity.

The slow DLS mode was also carefully detected and studied. It appeared in all investigated solutions, but its presence was more prominent in solutions with shorter G-quadruplex assemblies. To obtain more insight on the slow mode and to extend the range of accessible scattering vectors a new method, differential dynamic microscopy (DDM) was used.[3,4] This enabled us to visualize large globular aggregates under the microscope that are responsible for this slow diffusion and measure their apparent diffusion coefficients. The combination of DLS and DDM methods enabled us to gain additional knowledge on the polyelectrolyte properties of the slow DLS mode. It is, however, still unclear how the slow DLS mode is related to the structure of the G-quadruplexes.

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Acknowledgements This work was supported by the Slovenian Research Agency in the framework of the research program P1-0192.

Backbone Conformational Entropy is the Spine of Nucleic Acid Duplex Stability and Functions

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The living systems utilize DNA and RNA separately, but it is still a mystery how the roles of DNA and RNA have differentiated. The functional variance among DNA/DNA, RNA/RNA, and RNA/DNA duplexes stem from their thermodynamic properties, which are modulated by bulk factors including hydrogen bonding, base stacking, and conformational entropy (Figure 1A).[1] Of these factors, conformational entropy control hydrogen bonding and base stacking interactions by posing geometric constraints. However, although the energetic contributions of hydrogen bonding and base stacking have been studied extensively in the past, the contribution by conformational entropy remains elusive. The importance of backbone is highlighted in duplex stability prediction in various cellular conditions[2,3] and their conformational preferences[4]. Thus, the effect of backbone conformational entropy on duplex formation may be implicated in the distinct functions of RNA and DNA during various cellular processes. Therefore, in this study, we will elucidate the role of backbone conformational entropy in the cellular functions of RNA/RNA, DNA/DNA and RNA/DNA duplexes. The conformational entropy contributions were evaluated based on the complementary relationship between the free energy of hydrogen bonding (ΔG^{o}_{HB}) and base stacking (ΔG^{o}_{ST}) on the terminal base pair (Figure 1B). In addition, the conformational entropy contribution of propagation of a base pair was determined by subtracting the free energy contributions of dangling ends and hydrogen bonding from the total free energy of paired ends (Figure 1B). Then, polymerase assays were conducted to determine the dependence of polymerase efficiency on the entropic penalty in comparison to stacking efficiency. Our results showed that there is a clear sequence dependence on polymerase efficiencies, with flexible duplex (RNA/DNA) showing opposite pattern from the rigid duplex (RNA/RNA). This indicates that the polymerase efficiencies do not solely depend on the stacking and hydrogen bonding but also the conformational entropy of the propagating base pair. This is the first direct report showing the connection between the conformational entropy of the duplexes with the biological process involving nucleic acids. Our findings will provide valuable insights into the role of duplex backbone properties in functional specialization of RNA and DNA during various cellular processes.

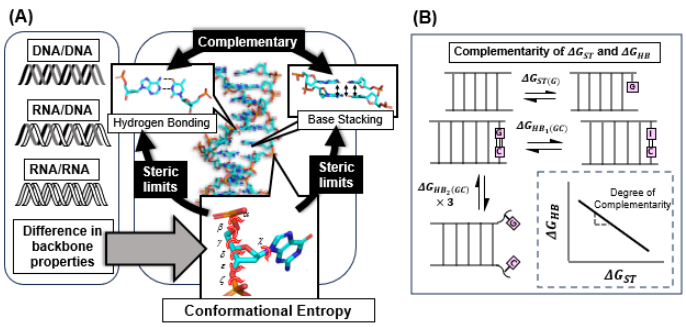

Figure 1 (A) Modulation of hydrogen bonding and base stacking by conformational entropy, and (B) Schematics of measurement of duplex conformational entropy.

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Genome Therapy Meeting Promises for Tumour Growth Inhibition

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According to the central dogma of molecular biology, chromatin DNA is subject to spatial and temporal regulation at multiple hierarchical levels, including chromatin unfolding, DNA transcription, post-transcriptional processing, mRNA translation, and post-translational modification. Correspondingly, regulatory tools acting at these distinct stages have been identified and artificially harnessed for fundamental biological investigations as well as therapeutic applications. With the increasing understanding of the evolution and progression of complex diseases such as cancer, single-target gene therapy has encountered considerable challenges, particularly with respect to minimizing adverse effects and overcoming drug resistance.

The rapid advancement of gene delivery platforms and regulatory technologies has driven a paradigm shift in gene therapy, expanding its focus from monogenic disorders to multifactorial, polygenic diseases. This transition has enabled therapeutic interventions to become more personalized, precise, safe, and efficient. To achieve optimal outcomes, an emerging strategy is to emulate chromatin-mediated regulation by orchestrating gene expression through the rational combination of diverse regulatory modalities across multiple molecular levels. Such an integrative framework provides a promising avenue to address complex diseases in a manner that more closely resembles natural biological regulation.

Within this conceptual framework, various gene regulatory tools can be strategically integrated into a unified toolbox and incorporated into chromatin-like delivery vehicles, thereby recapitulating the chromosome-mediated decoding of genetic information for therapeutic purposes. We propose the term genome therapy to describe this artificial, chromosome-inspired regulation of gene networks through the concurrent deployment of multiple regulatory mechanisms. In this work, we present our efforts toward developing multi-gene regulatory strategies for antitumor applications, with particular emphasis on branch-PCR–assembled gene nanovectors designed to mimic chromatin-like activity.

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Beyond the sequence–structure correlation: The complexity of DNA folding

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Since the discovery of the DNA double helix, our understanding of the diverse structures and functions of this fundamental biomolecule has greatly expanded, continuously incrementing the interest in exploiting DNA as a therapeutic target for a wide range of diseases. Although many approved chemotherapeutic agents act through DNA targeting, the original limitation mostly related to their poor selectivity remains mostly an open question.

To solve it, different strategies have been developed. Among them, significant examples refer to the design of agents working at the protein-nucleic acids interface, to take advantage of the protein selectivity in terms of localization and/or functions. An alternative, highly promising approach rests on the search for ligands able to recognize selected underrepresented DNA architectures.[1] Indeed, according to its primary sequence, a DNA chain can accommodate domains folded into the so called "non-canonical structures". For these elements, a strong correlation between their structural organization and the environmental conditions is well validated. Indeed, the effects of the environment in terms of co-solutes type and concentrations on the stability and shape of these nucleic acid arrangements have been extensively described and characterized.

Conversely, a still poorly addressed issue is related to the plastic structural features of this target when inserted in a more variegate genomic context. With the aim to shed light on this point, here, we will discuss the diverse structural behaviour of short DNA domains when analysed as isolated single stranded elements or embedded in a genomic context of increasing complexity, i.e. with flanking ends and, more intriguingly, a complementary strand.[2] The description of the equilibria behind these higher order systems is expected to represent an innovative support for the rational development of novel targeted drug projects.

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Acknowledgements: AIRC (grant IG 2021 - ID26474, PI CS), CERIC-ERIC (grants No. 20207052 and 20202174) and European Union-Next GenerationEU (PNRR M4 C2-Investimento 1.4- CN00000041).

Development of a pseudo-organelle system to explore how the molecular environment shapes G-quadruplex behaviour

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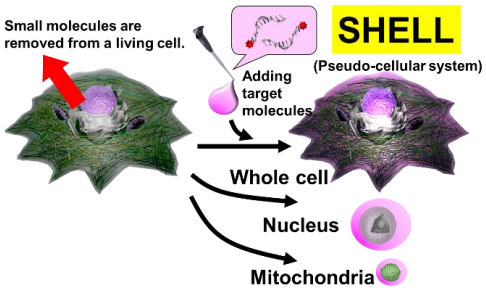
The structural diversity of nucleic acids within living cells is attracting increasing attention across medical, pharmaceutical, and materials sciences. While duplex DNA represents the canonical form, non-canonical structures such as triplexes, G-quadruplexes, and i-motifs are now recognized as critical regulators of transcription, translation, and replication.[1,2] Intracellular environments undergo significant changes, particularly during disease progression, leading to alterations in ion concentrations due to the inactivation or activation of ion channels, as well as fluctuations in cosolute levels resulting from the overexpression of disease-related proteins and metabolic abnormalities. Previously, we have found that the expression level of template DNA containing the G-quadruplexes of c-*Myc* is reduced during cancer progressions.[3] However, analysing the behaviour of crucial targets, such as nucleic acid-ion interactions, in cellular experiments proves exceedingly challenging due to the intricate nature of intracellular processes.

Living cells contain various organelles, cytoskeletons, and soluble and insoluble biomolecules, both of low molecular weight. Biomolecules occupy a significant portion of the cellular volume, accounting for up to 40%, resulting in crowded and intricate intracellular environments referred to as the molecular crowding effect. Previously, we developed novel pseudo-cellular systems (SHELL; system for highlighting the environment inside of the cell) using various types of cancer cells[4]. Specifically, we used cells as pseudo-cellular systems by creating cracks in the cell membrane to allow the contents to leak out. In this study, to further quantify the effects of the local cellular environment, we extracted nuclei and mitochondria from cells and attempted to create SHELLs. Furthermore, we analysed the behaviour of G-quadruplexes in SHELLs composed of whole cells, nuclei, and mitochondria.

First, fluorescently labelled G-quadruplexes were added to each SHELL, and G-quadruplex incorporation into the SHELLs was confirmed by confocal microscopy. Furthermore, we varied the temperature of these SHELLs to analyse the thermal melting behaviour of G-quadruplexes. As a result, in a pseudo-cellular system composed of whole cells, G-quadruplexes were stabilized by favourable enthalpy contributions. In the presentation, we will discuss the effects of the local cellular environment on nucleic acid stability and transcription efficiency.



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The G-quadruplex ligand PhenDC3 as a Novel Suppressor of NF-κB–Driven Inflammatory Responses

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Immune and inflammatory responses are governed by intricate regulatory networks involving chromatin architecture, gene expression, and RNA processing. G-quadruplexes (G4s), four-stranded secondary structures formed by guanine-rich DNA or RNA, have emerged as key structural regulators within these pathways. While G4s are known to influence genome stability, transcription, and translation, their role in regulating immune signalling remains controversial, with evidence suggesting both pro- and anti-inflammatory functions depending on the cellular context, stimulus, and the specific G4-targeting ligand. To unravel this paradox, we conducted a comprehensive immunological and mechanistic investigation of PhenDC3 — one of the most widely studied G4 ligands — across *in vivo*, *ex vivo*, and *in vitro* models. To capture the global molecular landscape under defined biological conditions, we integrated untargeted transcriptomic profiling with targeted proteomic analysis.

We demonstrate that the well-known G4 ligand PhenDC3 exerts profound anti-inflammatory effects in a murine model of zymosan-induced peritonitis, significantly reducing leukocyte recruitment and the levels of key pro-inflammatory mediators in the peritoneal cavity. Crucially, mechanistic interrogation *in vitro* using J774A.1 macrophage cell line revealed that PhenDC3 operates at the transcriptional level, directly suppressing the expression of a network of pro-inflammatory genes, particularly those associated with the NF-κB pathway. This compelling concordance between the phenotypic rescue observed *in vivo* and the transcriptional repression measured *in vitro* suggest a possible role of G4 structures as critical regulatory nodes in the immune response. This study provides the first integrated multi-system assessment of PhenDC3's immune activity and offers critical mechanistic insights into the therapeutic potential of G4 ligands in immune-mediated diseases.

Acknowledgements: The authors acknowledge the Italian Association for Cancer research (AIRC)

in vitro selection of RNA ligase TNAzymes

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Threose nucleic acid (TNA) is a synthetic genetic polymer of both prebiotic significance and practical utility. Identification of TNA molecules of enzymatic activities (TNAzymes) not only lends experimental support for TNA as a potential primitive catalyst but also offers intrinsically stable biotechnological and biomedical molecular tools. TNAzymes capable of catalysing RNA transformations are particularly appealing, not only for their potential role in the emergence of the RNA world, but also for their utility in fundamental research and as RNA-targeted therapeutics. For instance, we previously isolated an RNA-cleaving TNAzyme capable of discriminating single point mutation on EGFR transcript, which could be used to mediate allele-specific gene silencing.[1]

TNAzymes with RNA ligase activities are exceptionally intriguing because they could enable the synthesis of longer RNA strands and even functional RNA molecules, potentially facilitating a transition from a hypothetical TNA world to the RNA world. Here we report *in vitro* selection of RNA ligase TNAzymes. Use of magnesium ion as cofactor resulted exclusively in TNAzymes that facilitated formation of a noncanonical 2′-5′ phosphodiester bond between two RNA fragments.[2] In contrast, selection experiment in the presence of zinc ion allowed the identification of TNAzymes catalysing native 3′-5′ ligation of RNA instead.[3] Interestingly, further directed evolution of this 3′-5′ RNA ligase TNAzyme shifted the site selectivity toward the 2′ hydroxyl, leading to 2'-5' RNA ligase TNAzymes. These findings showcase the potential role of TNA as a primordial catalyst during the emergence of the RNA world, as well as its prospective application in RNA synthesis.

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Structural basis of G-quadruplex recognition by a camelid antibody fragment

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Apart from the iconic Watson-Crick duplex, DNA can fold into different non-canonical structures, of which the most studied are G-quadruplexes (G4). Despite mounting structural and biophysical evidence, their existence in cells was controversial until their detection using G4-specific antibodies. However, it remains unknown how antibodies recognize G4 at the molecular level and why G4-specific antibodies have low selectivity and are unable to distinguish different G4 sequences. Nanobodies derived from camelid heavy-chain-only antibodies have a broad capacity to recognize structurally diverse antigens such as non-canonical DNA structures. We show here that camelid immune libraries can be used to obtain binders against G-quadruplexes. Among them we isolated Nb55, which shows strong affinity toward the thrombin-binding aptamer (TBA), and we present the crystal structure of a nanobody bound to TBA. The nanobody exhibits strong selectivity against different G4 sequences and utilizes an unusual scaffold-based paratope, with very limited involvement of the complementarity-determining region (CDR). The nanobody effectively mimics the binding interface of thrombin, a natural binding partner of TBA, by using isosteric interactions at key positions. The presented structure sheds light on the molecular basis of how antibodies, essential G4-detection tools, recognize non-canonical G4 structures.

POSTER PRESENTATIONS

Insights into the effects of TERRA G-quadruplex stabilization by ligands

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Telomeric DNA is transcribed into a G-rich long non-coding RNA known as TERRA (telomeric repeat-containing RNA), which is crucial for telomere protection and maintenance[1,2]. In cancer cells, telomere elongation occurs through telomerase reactivation or the alternative lengthening of telomeres (ALT), where TERRA is highly overexpressed and functionally implicated[3,4]. Yet, the lack of effective tools to investigate TERRA biology has limited our understanding of its role in cancer progression and its therapeutic potential. Both telomeric DNA and TERRA form noncanonical G-quadruplex (GQ) structures, which can be targeted by small-molecule ligands[5-7].

In this study, by performing a ligand-based virtual screening of FDA-approved drugs, we identified novel TERRA GQ ligands that stabilize TERRA association with chromatin. This stabilization promoted telomeric DNA:RNA hybrid formation, triggered telomeric defects, and increased ALT-associated PML body formation in both telomerase- and ALT-positive cancer cells in an RNaseH1-dependent manner. These ligands also partially increased C-circle levels. Furthermore, in vitro analyses revealed that they recognize and stabilize DNA:RNA GQ hybrids, unveiling a previously uncharacterized mechanism of TERRA interaction with telomeric DNA. Together, our findings provide new insights into TERRA's intricate roles in telomere dynamics and their implications in cancer biology.

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Acknowledgements: PRIN 2022 PNRR (EU-Next Generation EU, No. P20223RKJ7 to J.A.).

Solution NMR Study of an RNA G-Quadruplex in a Long Non-Coding RNA Associated with Multiple Myeloma

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Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides that do not encode proteins. They play critical roles in the regulation of gene expression and are implicated in different biological processes, including cancer initiation and progression.[1] Many lncRNAs adopt complex secondary and tertiary structures that enable specific interactions with proteins, nucleic acids, and small molecules.[2] Among these motifs, G-quadruplexes (G4s) are noncanonical structures formed by guanine-rich sequences, characterized by stacked G-tetrads, planar arrangements of four guanines, connected by loops.[3] G4s have gained increasing attention for their regulatory functions in transcription, translation, and DNA replication, representing potential targets for therapeutic intervention.

In this study, we investigated a G4-forming sequence located within the lncRNA RP11-350G8.5, a transcript associated with Multiple Myeloma (MM).[4] To achieve a high-resolution structural characterization, we employed a comprehensive NMR approach, complemented by circular dichroism (CD) analyses. Solution conditions were optimized by acquiring 1D ^{1}H NMR spectra under different buffers, cation concentrations, and temperatures. DOSY experiments confirmed that the RNA sequence folds into a single G4 species. CD spectra supported the presence of a parallel G4 containing a G-A hexad, while CD melting and annealing experiments revealed significant hysteresis, consistent with the formation of a non-monomolecular assembly. To facilitate the unambiguous assignment of guanine H1 and H8 resonances, we acquired ^{15}N- and ^{13}C-edited HSQC NMR spectra of the RNA sequence. Partially residue-specifically ^{15}N- and ^{13}C-labeled oligonucleotides were synthesized to aid this assignment. Information from these site-specifically labeled samples was then used to guide the assignment. Structural details were further elucidated through 2D NMR experiments, including TOCSY, NOESY, and HSQC, which provided the restraints necessary to calculate the three-dimensional solution structure. The analysis revealed a parallel G4 composed of one G-tetrad and one G-A hexad, with two molecules associating through a hexad-hexad interface. These findings provide key insights into the structural properties of this G4-forming RNA sequence and establish a basis for the rational development of ligands targeting its G4 structure with potential therapeutic applications in MM.

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From Resistance to Response: Re-sensitizing Colorectal Cancer to 5-FU with G-quadruplex Ligands

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Chemoresistance represents a critical hurdle in the management of colorectal cancer (CRC). Building on the evidence that the ribosomal protein uL3 is positively correlated with chemoresistance, our study explores a novel approach to overcome uL3-mediated chemoresistance by combining 5-fluorouracil (5-FU), the first-line treatment for CRC, with G-quadruplex (G4) ligands. These compounds are promising antitumor agents that bind and stabilize G4 structures, non-canonical DNA conformations involved in regulating oncogenes and cancer-related signaling pathways.[1]

Our immunofluorescence experiments with the specific G4-recognizing BG4 antibody revealed that chemoresistant CRC cells, lacking functional p53 and with silenced uL3 expression, exhibit significantly elevated levels of G4 structures, both in the nucleus and in the mitochondria. This accumulation made them particularly vulnerable to the cytotoxic effects of the G4 ligands PDS and RHPS4. Surprisingly, the combination of these ligands with 5-FU produced synergistic effects in reducing the viability of CRC cells, allowing for a tenfold reduction in the required 5-FU dose. The effectiveness of this combined therapy was also confirmed using an *in vivo* chicken chorio-allantoic membrane (CAM) model derived from CRC cells with silenced uL3.[2]

Overall, our results suggest that targeting G-quadruplexes may be a promising approach to overcome chemoresistance in CRC. Such strategy could potentially restore the sensitivity of tumor cells to 5-FU and allow for reduced drug dosages, offering the dual advantage of increased treatment effectiveness and reduced toxicity.

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Discovering G-Quadruplex Motifs in Aptamers

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Aptamers are short single-stranded DNA or RNA molecules that can fold into defined structural conformations and bind specific targets, including proteins, small molecules, and nucleic acids. They are often described as chemical antibodies due to their high specificity and affinity. The ability of aptamers to form G-quadruplexes (G4), which are four-stranded structures formed by guanine-rich sequences, has often been overlooked. To investigate how frequently G4-prone motifs occur, over 1400 aptamer sequences from the UTexas Aptamer Database, covering publications up to 2023, were analyzed for their potential to adopt G4 or i-motif structures. While i-motif formation was extremely rare, approximately 25 percent of DNA aptamers and 16 percent of RNA aptamers were predicted to form stable G4 folds. Most of these motifs had not been previously identified as quadruplexes, with only 17 % explicitly described as "quadruplex" in the original reports. Experimental testing was performed on 30 aptamers, and G4 formation was confirmed in all sequences with a G4Hunter score of 1.31 or higher. These findings show that G4 motifs are more common than generally recognized and should be routinely considered during aptamer design and characterization.

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Cytosine FRET Analogs as Dynamic Reporters of Holliday Junction Topology and Stability

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The Holliday Junction (HJ) is a four-stranded DNA structure formed by the pairing of two complementary duplexes during processes such as homologous recombination, DNA repair, and the alternative lengthening of telomeres (ALT) pathway.[1-3] HJs adopt two interconvertible conformations: an open, square-planar form with a central cavity of 20–30 Å, and a stacked form, in which crossover strands interact via π-stacking, closing the cavity. Divalent cations such as Mg^{2+} promote the stacked conformation, whereas enzymatic processing often requires junction opening.[4,5] The central role of HJs in genome stability highlights their potential as therapeutic targets and underscores the need for reliable methods to probe their structure and dynamics. Förster resonance energy transfer (FRET) is well-suited to this task due to its sensitivity to conformational changes in cavity size.[6] While previous studies have employed bulky base modifications for FRET applications, here we demonstrate that cytosine base analogues can serve as effective FRET probes to monitor HJ conformational transitions, small-molecule binding, and enzymatic digestion.[7] Importantly, We show that these analogues minimally perturb HJ stability and structure, ensuring that the modified junctions remain representative of their native counterparts.

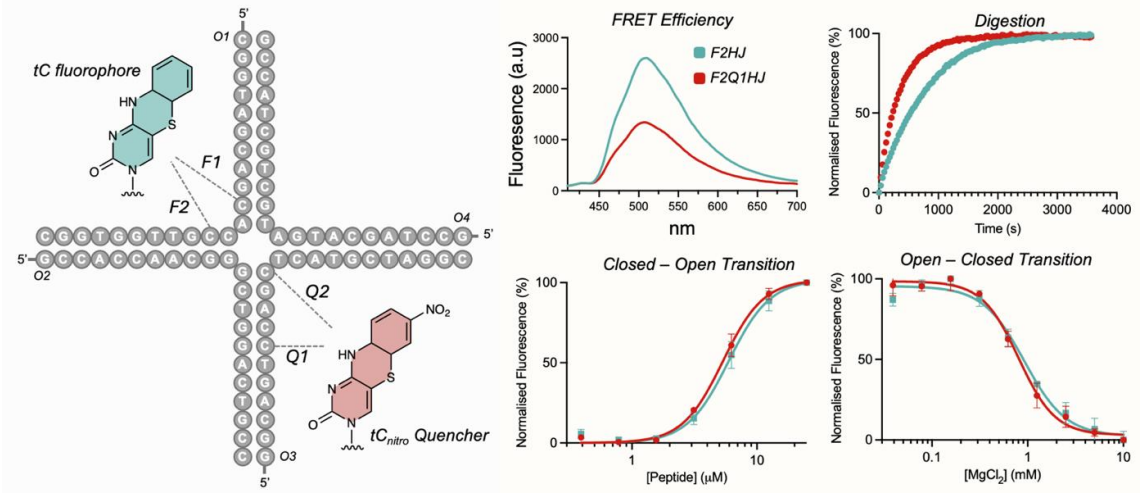

Figure 1 This work incorporates tC and tC nitro as cytosine FRET analogs at varying positions in a HJ sequence. The resulting FRET efficiencies in four FRET-HJ constructs were measured and used to visualize topology transition of the HJ in addition to its enzymatic digestion.

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Fluorescent Pt(II) metallacyles as potential G4 DNA sensors

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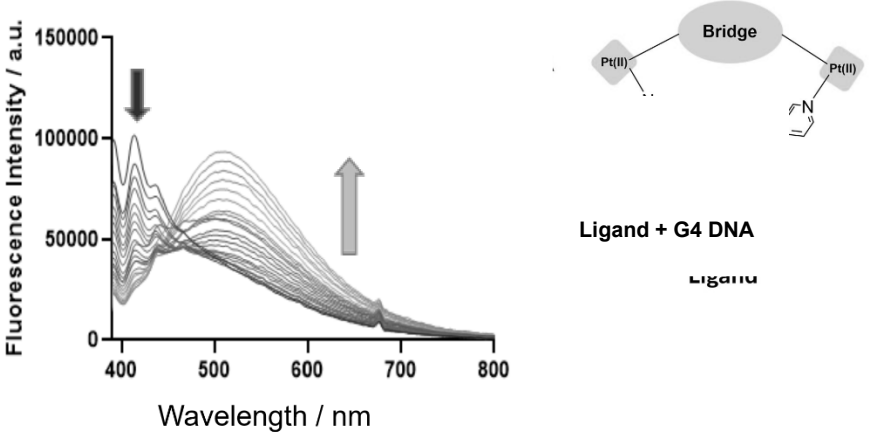
G4s are nucleic acid secondary structures formed from guanine rich sequences that have been identified as potential targets for anti-cancer, anti-parasitic and antimicrobial activity. In order to aid in the study and exploitation of the role and function of G4s in biology, it is critical to develop G4-selective sensors. Despite many efforts in the field, the design of topology-selective G4 sensors remains a challenge.

In this project, we have developed a supramolecular platinum metallacycle derived from a tunable fluorophore scaffold[1] and a naphthalene diimide (NDI), a known G4 ligand[2]. Biophysical assays show the sensor's selective binding and thermal stabilisation of model G4 sequences over duplex DNA. Moreover, the probes display turn-on fluorescence on introduction of G4-forming DNA, with an 8x greater fluorescence output for a parallel G4 sequence over a duplex sequence. Overall, the structural features and modular synthetic approach combined with its tunable fluorescence emission profile, may allow us to generate a fluorescence signature for distinct G4 topologies.



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A Tetraaza Macrocyclic Co(III) Complex Recognizes the M2 Parallel G-Quadruplex DNA Structure: Spectroscopic and Molecular Modeling Studies

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Current antimicrobial research integrates novel approaches with traditional ones, positioning bacterial G-quadruplex (G4) DNA structures as key targets for drug development1. In line with our recent reports2,3, the present study has employed a combination of spectroscopic and computational methods to explore the binding of a novel Co(III) coordination compound [Co(HL)Cl2]•CH3OH (1) endowed with selective antimicrobial properties to the M2 G4 structure, featuring parallel topology4. Circular dichroism experiments pointed at the ability of ligand 1 to produce stabilizing effects on the investigated parallel G4 DNA structure. The 1H NMR titration data are indicative about the 3'-end stacking as the binding mode between 1 and the model G4. Particularly, this conclusion is supported by the observation of both the signals for the NH protons of G5, G10, G19 and G14, identifying the G-quartet at the 3'-end that is mainly affected upon titration, and the signals for the aromatic, anomeric and methyl protons characterizing T20 residue, as well. To gain deeper insight into the atomistic binding mode of ligand 1 with the M2 G4 structure, molecular modeling studies have been conducted by firstly assessing its electron density distribution using DFT calculations, followed then by molecular docking and molecular dynamics simulations. These analyses consistently confirmed the binding of ligand 1 at the 3'-end of the G4 engaging hydrogen bond interactions with T20 (Figure 1b).

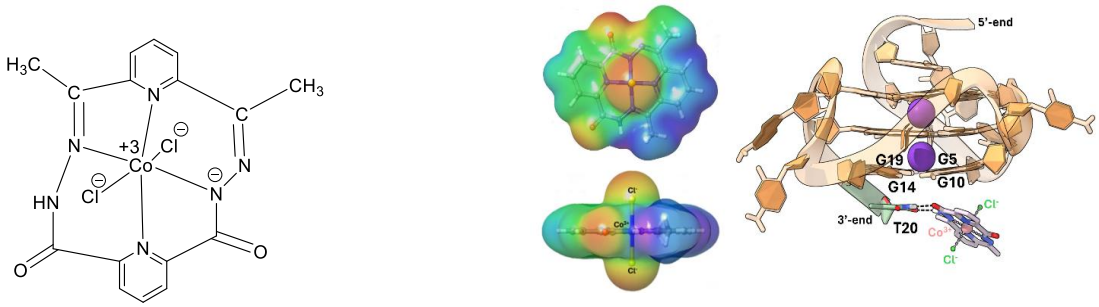

Figure 1 Bioactive macrocyclic Co(III) complex 1: a) chemical structure; b) ED isosurfaces and best-clustered 1/M2-G4 complex extracted from MDs, highlighting hydrogen bond interaction with T20.

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Acknowledgements: E.G. thanks the CERIC-ERIC for the access to facilities of Slovenian NMR Centre for NMR studies on DNA.

Structural characterization of interactions between pre-let-7 RNA and the Lin28A protein

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Lethal-7 miRNA (*let-7*) family was one of the first miRNAs to be discovered[1] and it acts as essential regulator of differentiation and is a fundamental tumor suppressor.[2] The biogenesis of *let-7* members is tightly regulated at the post-transcriptional level, with small RNA binding proteins LIN28A and LIN28B.[3] Upon binding of LIN28, *pre-let-7* RNAs are efficiently terminally uridylated and subsequently degraded.[4-6] The molecular determinants for LIN28 binding and regulation have been widely studied.[3,6-9] Despite advances in understanding the molecular basis of Lin28A interactions with pre-let-7 miRNAs,[6] information about RNA conformational changes remains limited. X-ray crystallography and cryo-EM structures often fail to capture the dynamic regions of RNAs. Here, we aim to investigate the role of conformational dynamics in the pre-miRNA loop region and their influence on recognition and interaction with Lin28A, both in buffer conditions and in cell-like environments (nucleus and cytoplasm).

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Acknowledgements: The authors acknowledge the financial support from the Slovenian Research and Innovation Agency – ARIS (grant no. P1-0242). A.K. acknowledges the financial support from ARIS (grant: Z1-3198).

Conformational insights into let-7 miRNA biogenesis

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MicroRNAs (miRNAs) are central regulators of gene expression, controlling diverse cellular processes through post-transcriptional silencing of messenger RNA.[1] The production of individual miRNAs requires tight regulation, since even slight imbalances in their levels can contribute to disease.[2,3] Among miRNAs, the let-7 family is of particular interest, as it serves as a key tumour suppressor in adults.[4,5] In this poster, we investigate the conformational space adopted by precursor let-7 miRNA and examine how these conformations may influence, and possibly regulate, RNA processing. We designed mutants that stabilize only one of the conformations present in the wild type RNA and assessed their impact on recognition and cleavage by the Dicer–TRBP complex. These findings provide structural and mechanistic insights into miRNA biogenesis with important implications for the development of RNA-targeted anticancer therapeutics.

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NMR study of the binding effect of RG-rich peptides to DNA G-quadruplexes

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Interactions between proteins and nucleic acids are crucial for the regulation of many cellular pathways. However, exact mechanisms at the atomic level are often still poorly understood due to difficulties *in vitro* mimicking of intracellular conditions that are needed for breakthrough structural studies.

One example of such important biological interactions are the ones between non-canonical nucleic acid secondary structures called G-quadruplexes and the arginine/glycine-rich (RGG/RG) domains of DNA/RNA binding proteins.[1] G-quadruplexes are structurally diverse and capable of performing a broad range of cellular functions, most notably regulation of gene expression, which may be facilitated by the binding of various DNA or RNA processing proteins. Nucleolin, a multifunctional nucleolar protein, contains an intrinsically disordered C-terminal RG/RGG-rich domain. It plays a role in various cellular functions and is also capable of G-quadruplex binding.[2]

We investigated the interaction between the nucleolin-derived RG/RGG-rich peptides and two different G-quadruplexes, one being a well-studied anti-parallel TBA G-quadruplex and the other parallel M2 G-quadruplex. We show that the investigated binding has moderate strength and that the binding is influenced even by the smallest differences in the amino acid sequence of RG/RGG-peptides, while a specific amino acid sequence may be responsible for the major contribution towards the binding affinity. Binding strength and stabilization of G4 structures is heavily impacted by arginine sidechain methylation, a well-known post translational modification. Interestingly, we show that TBA oligonucleotide adopts a G4 topology in the presence of RG-rich peptide even in the absence of the G4 promoting cations. Our results may become of greater interest considering the importance of the interaction investigated for the development of G-quadruplex-based anti-cancer aptamers that are incorporated into the target cells via interaction with the cell surface nucleolin proteins.

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G-quadruplexes and their ligands influence both basal and inducible transcriptional activity in yeast isogenic system

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G-quadruplexes (G4s) are non-canonical four-stranded DNA and RNA structures formed in guanine-rich regions of the genome. They are involved in the regulation of transcription, replication, and translation, and their occurrence in oncogene promoters requires precise cellular control, as improper G4 formation may contribute to the development of cancer. Due to their ability to modulate gene expression and interact with small molecules, G4s are being intensively studied in the context of novel anticancer drug development. In this study, we investigated the effect of G-quadruplexes and their ligands on basal and induced expression of proteins from the p53 and NF-κB families in an isogenic yeast system carrying native as well as artificially designed human promoter sequences. Our results show that the presence of G4 structures increased basal transcription and more strongly promoted the activity of weaker members of the p53 family (p63, p73) and selected mutants (A161T, N235S, R283C) compared to p53. The influence of G4s was also evident on the opposite strand relative to the TSS. In contrast, the effect on NF-κB proteins was less consistent and depended on genomic context. Furthermore, we observed that the impact of G4 ligands (curcumin, TmPyP4, Quarfloxin) on basal transcriptional activity was dependent on both ligand concentration and exposure time. Our data thus contribute to a better understanding of the interplay between G-quadruplexes, transcriptional regulation, and their modulation by natural and synthetic ligands, which may facilitate the development of targeted anticancer therapies.

Acknowledgements: This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic under the INTER-EXCELLENCE II program, project number LUAKR25078, and by the European Regional Development Fund through the INTERREG Austria–Czech Republic programme, project number ATCZ0052.

Targeting *PIM1* quadruplex-duplex hybrids: Structural insights and in-cell NMR characterization of bis-quinolinium ligand interactions

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Quadruplex-duplex hybrids (QDHs) represent emerging noncanonical nucleic acid motifs that combine double-stranded helices with tetra-stranded elements within a single fold. Their unique architectures are increasingly recognized for their biological importance and potential as therapeutic targets, yet their conformational preferences in living cells and their interactions with small molecules remain poorly understood[1-6]. The *PIM1* oncogene, frequently overexpressed in triple-negative breast cancer, provides a compelling model system as it adopts both hybrid and antiparallel QDH conformations *in vitro*[7].

Using *Xenopus laevis* oocytes as a cellular model, we demonstrate that the intracellular environment strongly favors the antiparallel topology[8]. In contrast, *in vitro* and in-cell NMR experiments reveal that bis-quinolinium ligands Phen-DC3 and 360A selectively recognize and stabilize the hybrid QDH form, counteracting the cellular bias toward the antiparallel fold. High-resolution NMR structures highlight distinct ligand binding modes at the quadruplex–duplex (Q–D) junction: Phen-DC3 engages in rigid stacking interactions, whereas 360A displays dynamic reorientation within the binding site.

To further explore these interactions in a cellular context, we developed an in-cell ^{19}F NMR approach based on 2'-FANA-modified DNA constructs, enabling direct detection of ligand–QDH complexes in live cells. This methodology not only validates ligand binding under physiological conditions but also establishes a versatile, high-throughput platform for screening QDH-targeting compounds. Together, these results provide mechanistic insights into ligand recognition of *PIM1* QDHs and establish in-cell NMR as a powerful tool for advancing drug discovery efforts against noncanonical DNA targets.

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Acknowledgements: The presenting author acknowledges funding from the Slovenian Research and Innovation Agency (P1-0242) and access to research infrastructure through the CERIC-ERIC consortium (project no. 20217158).

New Anticancer Molecular Hybrids Based on Homodrimane, Thiosemicarbazone and Oxadiazole Core Target M2 G-Quadruplex DNA Featuring Parallel Topology

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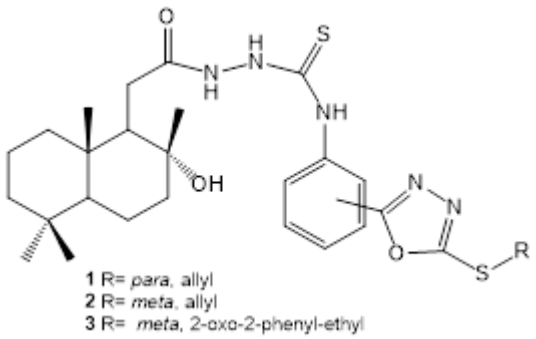
Our recent data prove terpeno–heterocyclic molecular hybrids incorporating benzothiazole, benzimidazole, thiadiazole and other heterocyclic fragments as promising drug candidates in virtue of the demonstrated notable antimicrobial effects.[1]

The current study was focused on synthesis and evaluation of the anticancer activity for some novel homodrimane derivatives bearing thiosemicarbazone and oxadiazole cores (**1**-**3**), prepared in two steps from commercially available (+)-sclareolide. The structures of the synthesized compounds were established by modern spectral methods of analysis: ATR-FTIR, ^{1}H, ^{13}C and ^{15}N NMR. Cytotoxicity evaluated by MTS assay indicated good antitumor properties for **1-3** against human osteoblasts (HOS) and human breast (MCF-7) cancer cell lines, with cytotoxic effects observed starting from 10 µM in both cell types. To investigate the plausible molecular mechanisms driving uncontrolled cell growth, we have studied the interactions of compounds **1-3** (Figure 1) with G-quadruplex (G4) DNA, as targeting G4 structures represents a promising broad-spectrum anticancer strategy.[2] The DNA models of choice comprised two G4s with different conformations: M2[3] and m-Tel24 (2GKU),[4] featuring parallel and hybrid topologies, respectively. Circular dichroism (CD) experiments demonstrated that ligands **1-3** stabilize M2 but not 2GKU. CD thermal denaturation experiments showed that the addition of ligand **2** increased the melting temperature of M2 by approximately 5-6 °C. These findings may guide the further development of optimized analogs of the molecular hybrids investigated as potential agents for anticancer targeted therapies.



1 R= *para*, allyl
2 R= *meta*, allyl
3 R= *meta*, 2-oxo-2-phenyl-ethyl

Figure 1 Chemical structures of molecular hybrids 1-3.

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Acknowledgements: This research was carried out within the framework of the project 25.80012.5007.28TC “Targeted synthesis and study of the biological activity of terpene compounds with heterocyclic structural units”.

Structural characterization of mitochondrial isoleucine tRNA fragments

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The mitochondrial tRNA derived fragments (mt-tRFs) are a group of highly conserved non-coding RNAs that are broadly gaining attention in the scientific community due to their role in regulating a wide spectrum of cellular processes including homeostasis, cancer cell viability, tumorigenesis and others[1]. Increasing evidence links aberrant expression of mt-tRFs to diverse cancers, where they contribute to proliferation, apoptosis, and metastasis, underscoring their potential as biomarkers and therapeutic targets[2,3]. However, despite their growing biological and clinical relevance, there exists no structural information on mt-tRFs, leaving behind a critical gap in understanding their mechanism of actions.

With the recent advances in the state-of-the-art experimental techniques over the past decade, clinically relevant mt-tRFs have been recognized as potential biomarkers for cancer diagnosis and prognosis. To bridge the gap between their function and mechanism of action we have aimed to achieve critical insights into their structural dynamics and conformational states at atomic level of resolution utilizing NMR spectroscopy. In this work, we have combined state-of-the-art NMR with complementary biophysical approaches, to establish the first structural framework for mt-tRFs derived from mt-tRNA^{ILE}. This will provide mechanistic insights into their biogenesis and regulation, thereby advancing the understanding of how mt-tRFs contribute to disease processes and paving the way for their future exploitation in RNA-based diagnostics and therapeutics.

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Acknowledgements: The authors acknowledge the financial support from the Slovenian Research and Innovation Agency-ARIS and the Scientists4Future (S4F) grant funded by The Ministry of Higher Education, Science and Innovation of Republic of Slovenia (grants: P1-0242 and Z1-3192).

Structural study of a G-Quadruplex from the 5’-UTR of TMPRSS2 and its interaction with the bisquinolinium ligand PhenDC3

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The TMPRSS2 receptor is involved in diverse inflammatory processes and has been shown to facilitate viral cell entry during influenza and SARS-CoV-2 infections. Downregulation of TMPRSS2 expression through stabilization of a G-quadruplex structure in its promoter region has been demonstrated to reduce viral infection.[1,2] While transcriptional regulation could be achieved via regulatory elements in promoter region, gene expression can also be modulated at the level of mRNA translation. In particular, G-quadruplexes located close to 5’-end of 5’-UTR have been reported to have inhibitory effect on translation.[3]

In this study, we examined a G-rich region within the mRNA of TMPRSS2 isoform 1, which is highly expressed in epithelial cells of the lung. The G-tracts within the 5’-UTR of this transcript vary in length, suggesting the presence of polymorphic G-quadruplex structures. NMR spectroscopy confirmed that a family of G-quadruplexes is formed both by a short G-rich oligonucleotide and within a longer UTR fragment. CD data indicated that the ensemble adopts a parallel topology, with minor structural variations such as differences in the stacking of flanking nucleotides.

G-to-U mutations enabled identification of G-register isomers within the ensemble, and a representative mutant was selected for high-resolution analysis. NMR spectra exhibited spectral pattern characteristic of a parallel RNA G-quadruplex. Notably, stacking of a flanking adenine residue upon the 5’-terminal G-quartet was observed. This G-quadruplex was strongly stabilized by the bisquinolinium ligand PhenDC3, which binds to both the 3’- and 5’-terminal G-quartets without changing of G-quadruplex topology. Detailed data on the structure of this complex provide the basis for future development of selective ligands for stabilization of G-quadruplex from 5'-UTR of TMPRSS2 and the control of the protein expression.

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Immune library derived nanobody against noncanonical DNA

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The existence of G-quadruplexes (G4) in human cells was proven more than 10 years ago using specific anti-G4 antibodies[1]. These antibodies have since become an essential tool for genome-wide mapping of G4 structures and are widely used as molecular probes to study G4-related biological processes. The development of G4 antibodies was based on synthetic libraries derived from conventional antibody framework or synthetic nanobody library[2].

Here, we present nanobody Nb55, which was raised against a new type of noncanonical DNA, AGCGA-quadruplex. Unlike broadly specific G4 antibodies, Nb55 was derived from a llama immune library. It exhibits nanomolar affinity toward the AGCGA-quadruplex, as determined by isothermal titration calorimetry. Interestingly, Nb55 also binds with high affinity to the thrombin binding aptamer (TBA) quadruplex. We determined the structure of the Nb55-TBA complex using X-ray crystallography[3].

The examples of the presented Nb55–AGCGA and Nb55-TBA complexes highlight the potential to develop highly specific antibodies targeting individual G4 sequences. Furthermore, the high-resolution structure of Nb55-TBA complex provides a molecular basis for understanding the interactions between antibodies and G4s, thereby enabling the rational design of highly specific G4-targeting antibodies.

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Acknowledgements:
We acknowledge the support of EUTOPIA, The Centre for Research Infrastructure at the University of Ljubljana, Faculty of Chemistry and Chemical Technology and Slovenian Research and Innovation Agency.

Exploring the LTR-III G-Quadruplex for Therapeutic Strategies Against HIV

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G-quadruplex (G4) structures within the HIV-1 long terminal repeat (LTR), and in particular the LTR-III motif, play a critical role in regulating viral transcription and have emerged as promising targets for antiviral drug development.[1] In this study, we employed structure-based virtual screening to identify small molecules with selective affinity for the LTR-III G4.[2] Our strategy focused on two structural domains: the junction region, investigated through the repurposing of naphthalene diimide derivatives and the screening of chemically diverse libraries, and the stem-loop region, a flexible element essential for the structural plasticity of LTR-III. The latter was further characterized through molecular dynamics simulations, which provided insights into its conformational landscape and guided the identification of ligands capable of acting synergistically to promote G4 stabilization. The results of these integrated computational approaches underscore the potential of G4-directed ligands as selective anti-HIV agents and provide a framework for the rational design of next-generation compounds that merge the most effective scaffolds for both the junction and stem-loop regions, thereby maximizing specificity and promoting cooperative stabilization of the G4 structure.

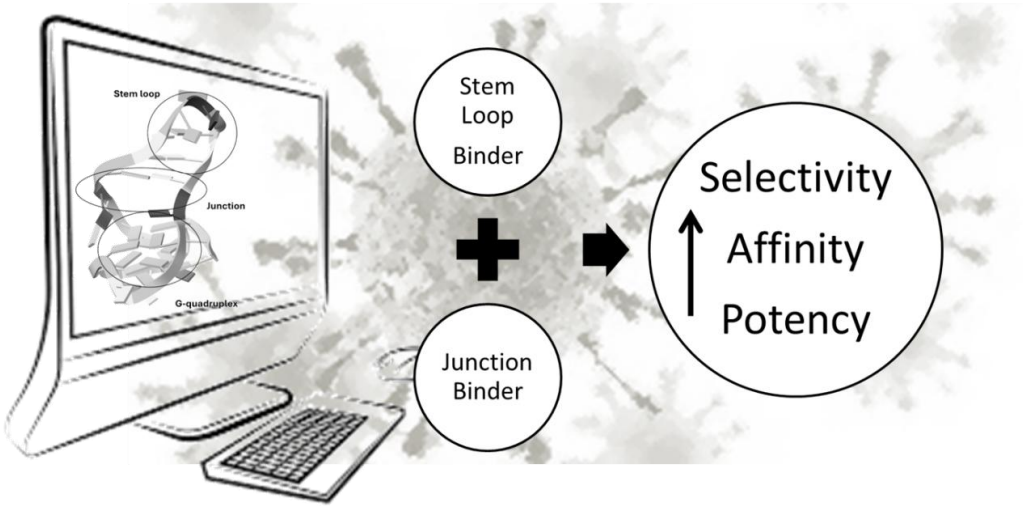

Figure 1 Computational design of selective, high-potency ligands targeting the LTR-III G-quadruplex in HIV-1.

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Acknowledgements: This work was supported by "Tackle HIV-1. Silencing viral LTR promoter by Target-tailored G-quAdruplex ligaNDs. (STRAND)" PRIN 2022BL3L7A.

Dissecting DNA G-Quadruplex Ligand Mechanisms via Multi-Omics integration

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DNA G-quadruplexes (G4s) are non-canonical secondary structures abundant in key genomic loci such as telomeres, promoters, and first introns of genes[1], where they influence genome stability, transcription, and mitochondrial function. Notably, G4 motifs are particularly enriched in oncogenes and occur with higher prevalence in cancer cells than in their normal counterparts[2], making them attractive and innovative anticancer targets. Although many small molecules have been identified as G4 ligands[3], their precise cellular mechanisms remain poorly defined and only few have advanced into clinical testing.

In this work, we applied an integrative multi-omics strategy, combining transcriptomics, proteomics, and metabolomics, to systematically characterize the effects of three representative G4 binders (berberine, pyridostatin, and RHPS4) in human cervical adenocarcinoma cells (HeLa). The ligands elicited strikingly divergent biological effects. Berberine induced minimal changes across all omics layers, while pyridostatin provoked extensive reprogramming at the transcriptional, proteomic, and metabolic levels. Finally, RHPS4 predominantly impaired mitochondrial bioenergetics, most likely via stabilization of mitochondrial G4s, suggesting a plausible mechanistic basis (Fig. 1).

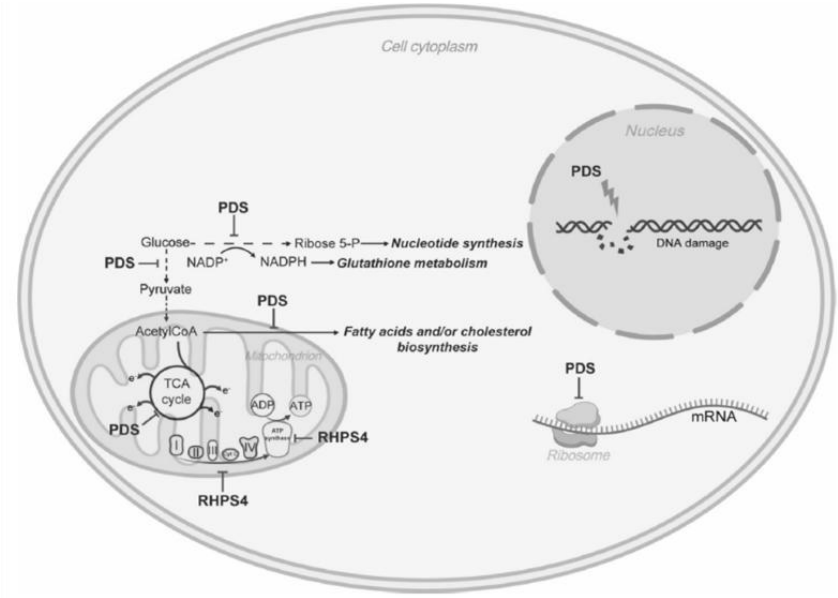

Figure 1 Illustration of the major cellular pathways modulated by PDS and RHPS4 in HeLa cells

Overall, our findings offer an unprecedented multi-omics perspective on how G4 ligands disrupt key metabolic pathways in cancer cells, thereby advancing the mechanistic understanding of their anti-cancer activity and informing the rational development of more effective G4-targeted therapeutics.

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Targeting G-rich lncRNA and Its Structural Polymorphism with Selective G-Quadruplex Ligands: An NMR Study

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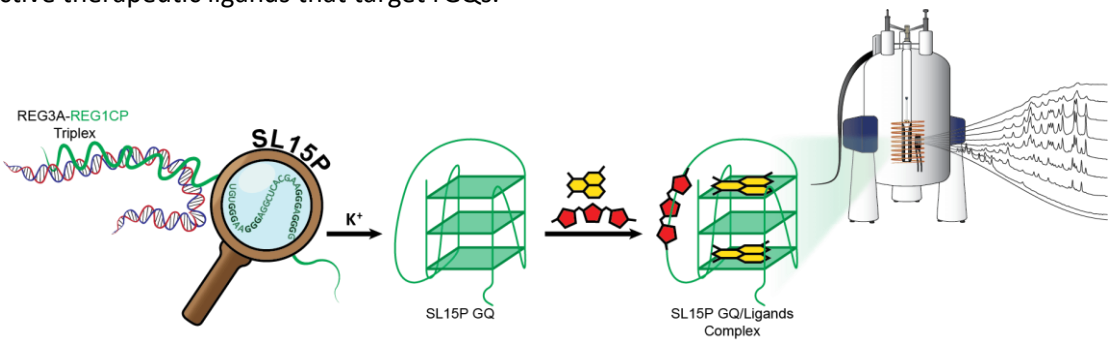
Long non-coding RNAs (lncRNAs) have emerged as key regulators of gene expression, chromatin architecture, and RNA metabolism.[1,2] Initially considered as transcription noise due to their low conservation and expression, they are now recognised as key factors in disease development.[3] Compared to DNA, RNA can assume a broader array of structural motifs. Among theme, RNA G-quadruplexes (rGQs) have attracted particular attention. GQs are four-stranded non-canonical structures formed by guanine-rich sequences that stack into planar G-quartets, stabilised by Hoogsteen hydrogen bonding and monovalent cations. As RNA function is largely determined by its three-dimensional conformation rather than sequence alone, detailed structural characterisation is essential to understand their regulatory roles.[4] For example, REG1CP is a lncRNA upregulated in colorectal cancer that regulates REG3A transcription through a dual mechanism: at one end it forms an RNA–DNA triplex with a homopurine region in the distal promoter of REG3A, at the other it includes a G-quadruplex putative region required for recruiting the helicase FANCJ.[5] In this study, we focused on how small molecules interact with the rGQ formed by the lncRNA REG1CP, since understanding such interactions may reveal how ligand binding modulates RNA–protein recruitment and influences associated functions. To that end, we characterised a 30-nt guanine-rich RNA oligonucleotide derived from REG1CP (referred to as SL15P). SL15P exists in a dynamic equilibrium between a GQ conformation and a hairpin, allowing us to assess how ligands shift this balance and stabilise the rGQ state. We evaluated a panel of twelve known DNA GQ ligands for their ability to bind to and stabilise the SL15P rGQ. A series of ^{1}H NMR titration experiments were performed to detect complex formation and to evaluate the ligand's ability to influence rGQ folding, even under the hairpin's favoured conditions. Circular dichroism melting analyses were conducted to confirm ligand-induced stabilisation. Out of all the compounds tested, 360A, PhenDC3 and PDS were found to be the most effective, forming well-defined rGQ–ligand complexes and significantly enhancing thermal stability. Notably, PhenDC3 exhibited evidence of dual binding modes. These results highlight the heterogeneous nature of rGQ–ligand interactions and support the rational design of selective therapeutic ligands that target rGQs.



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Ultra-low multiplexed detection of nucleic acids with nitrogen-vacancy centres in diamond

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Over the past decade, significant efforts have been dedicated to creating innovative biosensor platforms for detecting a wide variety of analytes. Among these, magneto-DNA assay systems have gained substantial attention due to their ability to deliver highly sensitive and specific detection while also enabling target manipulation. In this work, we introduce a hydrogel-based, multiplexed magneto-DNA assay that leverages nitrogen-vacancy (NV) centres in diamond as transducers for magnetic nanotags (MNTs). This approach combines near-background-free sensing through diamond imaging with the non-invasive control of chemically stable nanotags, making it a promising tool for medical diagnostics, life sciences, and pharmaceutical research. To showcase its practical potential, we applied this sensor platform to a sandwich DNA hybridization assay and achieved attomolar detection sensitivity with single-base mismatch discrimination.

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Mutations alter the conformational dynamics of mitochondrial tRNA fragments and can thereby influence their functionality and contribute to pathology

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tRNA fragments (tsRNAs and mt-tsRNAs) are generated by cleavage of mitochondrial (mt-) and cytosolic tRNAs mediated by enzymes such as DICER and Angiogenin.[1] These fragments are involved in various biological processes, including modulation of protein translation,[2] gene expression regulation,[3] and cellular stress response,[4] among others. Notably, studies on structure – function relationship of tsRNAs, and especially of mt-tsRNAs, are practically absent. Consequently, it remains unclear how mutations in (mt-)tRNAs influence the functionality of the corresponding (mt-)tsRNAs.

Here, we investigate the structural implications of a known pathological A-to-G mutation[5] on a 30-nucleotide-long mt-tsRNA originating from mt-tRNA^{Ala}.[6] We structurally analysed the wild-type RNA (WT) and its A-to-G mutant (A4G) by NMR and UV-VIS spectroscopy. Our data showed that WT forms a stable hairpin with an A•G mismatch in the stem and a 7-nt loop. On the other hand, the A-to-G mutation introduces a G•G mismatch, leading to significant changes in the structural and dynamic properties of A4G. Although the hairpin loop of A4G is identical to that of WT, the A4G adopts two distinct conformations in the stem region, which are in chemical exchange. Interestingly, the A4G mutant is more thermally stable compared to WT. To explore how this structural change affects the function of our model mt-tsRNAs, we transfected WT, A4G, and scrambled control into HeLa cells. Our RNA-seq and splicing analyses revealed significant differences between WT and A4G in the number and types of affected genes, which may arise from mutation-induced structural changes. These findings demonstrate that mutations in (mt-)tsRNAs can induce important conformational changes that alter the functionality of these regulatory small RNAs. Our work highlights the importance of considering the structural aspects of (mt-)tsRNAs, which are often overlooked, when investigating their biological and pathological roles.

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Acknowledgements:
The authors acknowledge the financial support from the Slovenian Research Agency (grant: no. P1-0242).

Selective and topology dependent unfolding of G-Quadruplexes via guanine clamps

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G-quadruplexes (G4s) are non-canonical nucleic acid structures whose implication in a wide range of biological contexts is now well established[1]. Their folding modulation is implicated in gene expression, translation initiation, and telomerase activity. Furthermore, G4s can regulate epigenetic environment, genomic stability, and DNA replication. G4s' research has historically been dominated by studies focused on their stabilization, but evidence has emerged suggesting that G4 stabilization can, in certain contexts, promote oncogenic activity, cause genomic instability by interfering with replication machinery, and act as a pathogenic driver in neurodegenerative disorders[2-3]. According to these studies, in recent years, the unfolding of G4s has been more deeply investigated[4-5]. In this work, the synthesis and unfolding ability of three G4-ligands, an already known guanine binder (GClamp, Figure 1) and two novel dipeptides of non-natural amino acids (DIP1 and DIP2, Figure 1), have been described. These compounds are small molecules that can be classified as guanine clamps. They can interact with single guanines by hydrogen bonds destroying the secondary structure of G4s. Circular dichroism (CD) titrations of ten oligonucleotides (nine G4s with different topology and one double strand as comparison) have confirmed ligands unfolding ability. GClamp has shown the best results, highlighting high values of G4 destruction and a topological preference for parallel G4s and sequences of telomeric origin. Following these results, a dimeric form of GClamp (GClamp dimer, Figure 1) has been synthesised and studied. CD titrations of the ten oligonucleotides with GClamp dimer have shown a greater unfolding ability than the monomeric one, but with a loss in selectivity.

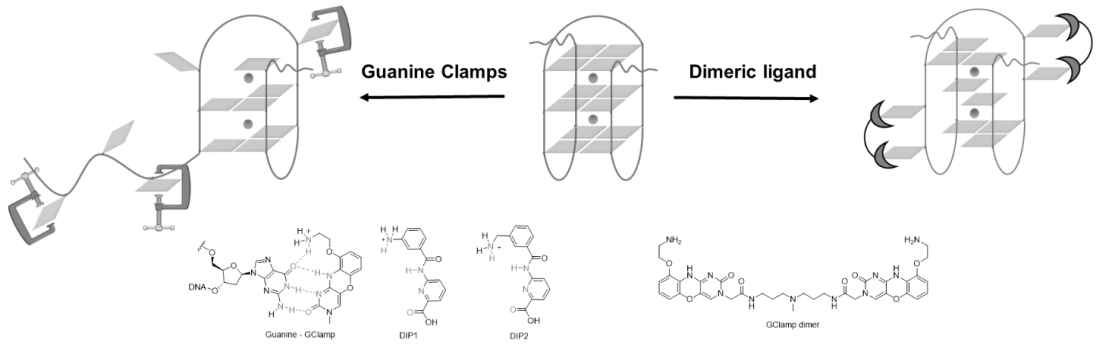

Figure 1 Schematic representation of G4 unfolding by monomeric ligands (GClamp, DIP1, and DIP2) and dimeric one (GClamp dimer).

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The application of SG4 nanoparticles for immunostaining cells and tissue samples and their usage for verifying the ability of natural compounds to interact with G-quadruplexes in cells

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G-quadruplexes (G4) have been the subject of many research studies since their discovery. These structures play an important role in the genome and its stability and influence some of the basic cellular processes such as transcription, translation or replication.[1,2] Their specific structure allows proteins and chemicals to bind specifically to a particular site in the genome, affecting not only the stability of these secondary structures but also the transactivation of nearby genes.[3,4] G-quadruplexes are formed non-randomly at sites with a high frequency of guanines and are made up of at least two G-tetrads consisting of four guanines. Determining their occurrence in cells or directly in tissue samples is therefore very interesting for further experiments and a deeper understanding of the mechanism of cellular processes that are influenced by the presence of G4. In our work, protocols for the use of SG4 nanobody for fluorescent staining of cell cultures and histological samples were optimized.

Many studies have now been carried out on this topic and it is clear that substances that bind specifically to G-quadruplexes, known as G-quadruplex ligands, can be found in natural products, but can also be synthesized, for example as more specific analogues of natural products.[5–8] For this reason, the next step was to test the ability of natural substances to interact with G-quadruplexes by measuring their ability to replace or occupy the binding site for SG4. If SG4 was replaced by a natural substance, the resulting SG4 fluorescence signal was reduced. A total of five natural substances were selected for these measurements: gallic acid, epigallocatechin gallate (EGCG), epicatechin, ellagic acid and brucine. The results of tests on HDF and HCT cell lines show that some of the selected substances have the ability to bind to G4 and thus reduce the resulting SG4 signal.

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