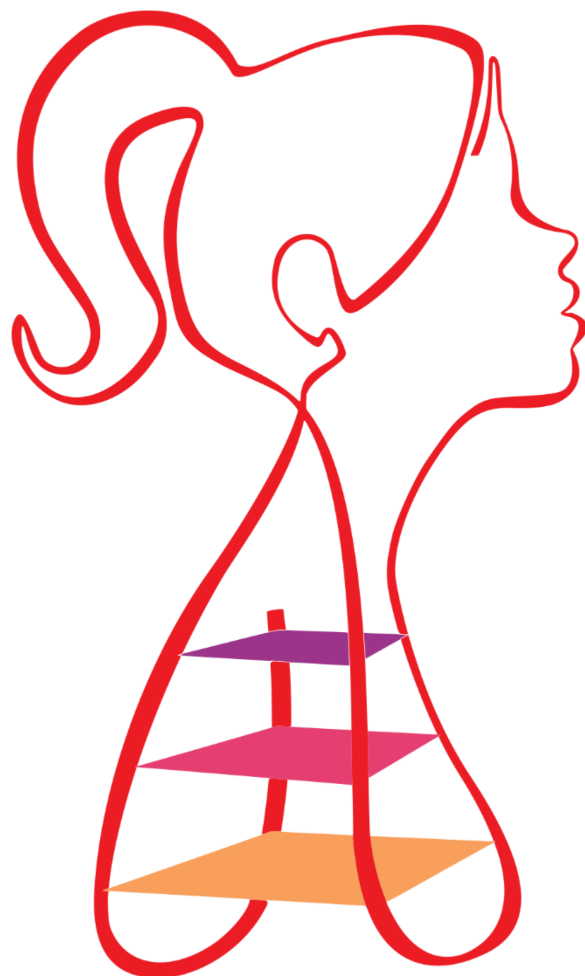

ANNA 2023

Advances in Noncanonical Nucleic Acids:

Book of Abstracts

Maribor, Slovenia, October 18th – 21st, 2023

Organized by
Slovenian NMR Centre @ National Institute of Chemistry

ANNA 2023 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, 18th October 2023

Editors: Peter Podbevšek
Janez Plavec

Design: Klemen Pečnik
Peter Podbevšek

https://slonmr.si/anna_2023/ANNA2023BookOfAbstracts.pdf

Kataložni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani
COBISS.SI-ID 167710211
ISBN 978-961-6104-88-3 (PDF)

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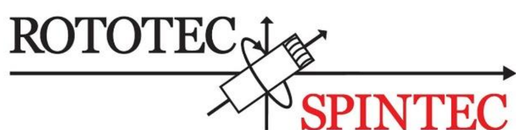


PROGRAMME

Wednesday, October 18th, 2023

18:00	**Welcome reception,** *Ljubljana*

Thursday, October 19th, 2023

10:00	**Bus transfer to Maribor**
12:00 – 13:00	**Lunch,** *City Hotel, Maribor*

Afternoon session *Chair: Katherine Seley-Radtke*

13:00 – 13:30	**Opening remarks,** *Janez Plavec, Head of NMR centre*
13:30 – 14:00	Sara Richter, *University of Padua*
14:00 – 14:30	Lukáš Trantírek, *CEITEC, Brno*
14:30 – 15:00	Chuanzheng Zhou, *Nankai University, Tianjin*
15:00 – 15:30	Anna Di Porzio, *University of Naples Federico II*
15:30 – 16:00	**Coffee break**
16:00 – 16:30	Naoki Sugimoto, *FIBER, Kobe*
16:30 – 17:00	Eriks Rozners, Binghamton University
17:00 – 17:30	Masayuki Fujii, *Kindai University, Fukuoka*
17:30 – 18:00	Domenica Musumeci, *University of Naples Federico II*
19:00	**Dinner,** *Rožmarin, Maribor*

Friday, October 20th, 2023

Morning session	*Chair: Naoki Sugimoto*
9:00 – 9:30	Katrin Paeschke, *University Clinic, Bonn*
9:30 – 10:00	Ambadas Rode, *Regional Centre for Biotechnology, Haryana*
10:00 – 10:30	Martina Lenarčič Živković, *National Institute of Chemistry, Ljubljana*
10:30 – 11:00	**Coffee break**
11:00 – 11:30	Jean-Louis Mergny, *Ecole Polytechnique, Palaiseau*
11:30 – 12:00	Luigi Petraconne, *University of Naples Federico II*
12:00 – 12:30	Viktor Víglaský, *P. J. Šafarik University, Košice*
12:30 – 13:30	**Lunch**, *City Hotel, Maribor*

Afternoon session	*Chair: Katrin Paeschke*
13:30 – 14:00	Steven Rokita, *Johns Hopkins University, Baltimore*
14:00 – 14:30	Shigeori Takenaka, *Kyushu Institute of Technology, Fukuoka*
14:30 – 15:00	Jurij Lah, *University of Ljubljana*
15:00 – 15:30	**Coffee break**
15:30 – 16:00	Zhen Xi, *Nankai University, Tianjin*
16:00 – 16:30	Roberto Improta, *National Research Council, Naples*
16:30 – 17:00	Claudia Sissi, *University of Padua*
19:00	**Dinner,** *Gostilna Anderlič, Maribor*

Saturday, October 21st, 2023

Morning session	*Chair: Steven Rokita*
9:00 – 9:30	Antonio Randazzo, *University of Naples Federico II*
9:30 – 10:00	Tamaki Endoh, *FIBER, Kobe*
10:00 – 10:30	Emanuela Ruggiero, *University of Padua*
10:30 – 11:00	**Coffee break**
11:00 – 11:30	Katherine Seley-Radtke, *University of Maryland, Baltimore*
11:30 – 12:00	Masayasu Kuwahara, *Nihon University, Tokyo*
12:00 – 12:30	Hisae Tateishi-Karimata, *FIBER, Kobe*
12:30 – 13:00	Anita Kotar, *National Institute of Chemistry, Ljubljana*
13:00 – 14:00	**Lunch**, *City Hotel, Maribor*
14:00 – 17:00	**Guided city tour and wine tasting,** *Hiša Stare trte, Maribor*
17:00	**Bus transfer to Ljubljana**

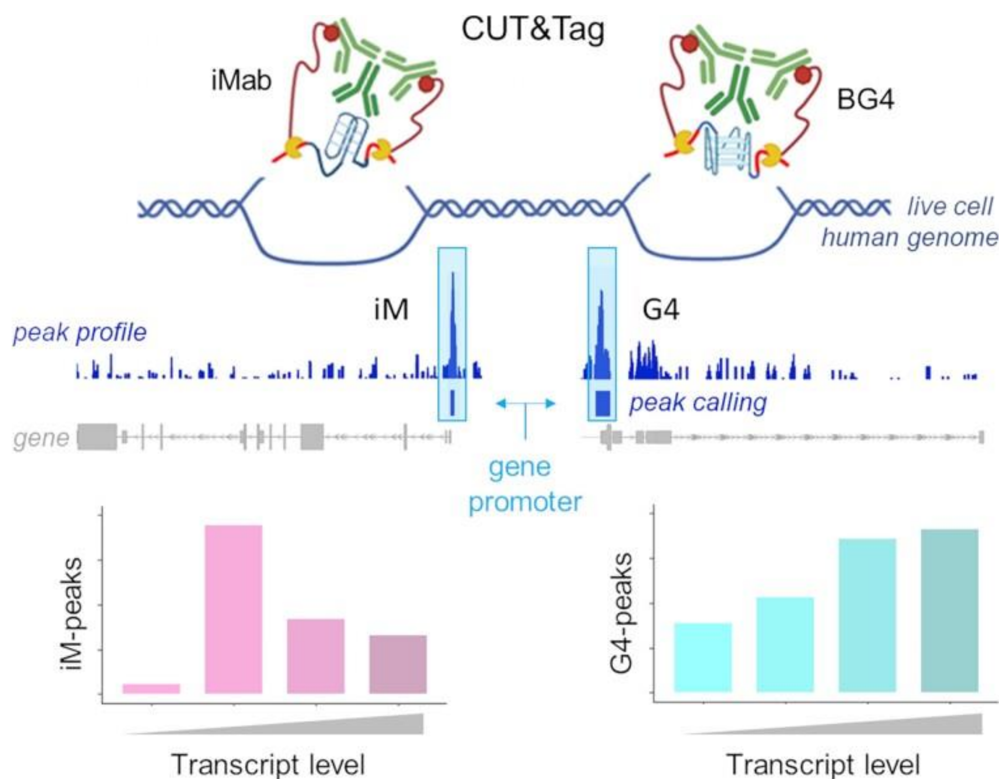
INVITED LECTURES

Genome-wide mapping of i-motifs reveals their association with transcription regulation in live human cells

Irene Zanin[1], Emanuela Ruggiero[1], Giulia Nicoletto[1], Sara Lago[2], Ilaria Maurizio[1], Irene Gallina[1], Sara N Richter[1,3]

[1] *Department of Molecular Medicine, University of Padua, Padua, Italy*
[2] *Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Trento, Italy*
[3] *Microbiology and Virology Unit, Padua University Hospital, Padua, Italy*

i-Motifs (iMs) are four-stranded DNA structures that form at cytosine (C)-rich sequences in acidic conditions in vitro. Their formation in cells is still under debate. We performed CUT&Tag sequencing using the anti-iM antibody iMab and showed that iMs form within the human genome in live cells. We mapped iMs in two human cell lines and recovered C-rich sequences that were confirmed to fold into iMs in vitro. We found that iMs in cells are mainly present at actively transcribing gene promoters, in open chromatin regions, they overlap with R-loops, and their abundance and distribution are specific to each cell type. iMs with both long and short C-tracts were recovered, further extending the relevance of iMs. By simultaneously mapping G-quadruplexes (G4s), which form at guanine-rich regions, and comparing the results with iMs, we proved that the two structures can form in independent regions; however, when both iMs and G4s are present in the same genomic tract, their formation is enhanced. iMs and G4s were mainly found at genes with low and high transcription rates, respectively. Our findings support the in vivo formation of iM structures and provide new insights into their interplay with G4s as new regulatory elements in the human genome.[1]



References:
1. Zanin I. et al, Nucleic Acids Research, 2023, 51, 8309-8321

Acknowledgements: Italian Foundation for Cancer Research, grant #21850

…about DNA i-motifs in living human cells at physiological temperature: an in-cell NMR story

Lukáš Trantírek

Central European Institute of Technology, Masaryk University, Brno, Czech Republic

In addition to the double helix, there are other secondary structures in human genomic DNA, including i-Motifs (iMs). Until recently, it was believed that iMs only formed under laboratory conditions in vitro due to the iMs' stability under acidic conditions or sub-physiological temperatures. However, recent research using an i-motif-specific antibody, iMab, revealed that the human genomic DNA is widely interspersed with regions that form i-motif structures.[1,2] A more recent study, using the iMab, demonstrated that the iM-forming regions are mainly present at actively transcribing gene promoters in open chromatin regions, suggesting their active roles in gene regulation.[3]

In our research, we utilized in-cell NMR spectroscopy to examine iM formation using oligonucleotides as models, directly within living human cells at physiological temperatures. Our results suggest that many of the iMab-detected genomic sites may have biological roles linked to their unfolded states. This study represents the first instance in which the in-cell NMR approach, traditionally limited to monitoring structural equilibria in asynchronous cell suspensions, has been directly applied to cells in specific physiological states.

References:

1. Zeraati M., Langley D.B., Schofield P., Moye A.L., Rouet R., Hughes W.E., Bryan T.M., Dinger M.E., Christ D. Nat Chem. 2018 10, 631-637.
2. Peña Martinez C.D., Zeraati M., Rouet R., Mazigi O., Gloss B., Chan C.-L., Bryan T.M., Smith N.M., Dinger M.E., Kummerfeld S., Christe D. Preprint *DOI:10.1101/2022.04.14.488274*
3. Zanin I., Ruggiero E., Nicoletto G., Lago S., Maurizio I., Gallina I., Richter S.N. *Nucleic Acids Res*. 2023 51, 8309-8321.

Acknowledgments: This project was supported by grants from the Czech Science Foundation (GX19–26041X)

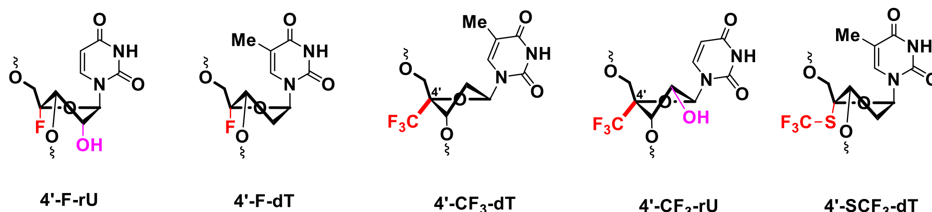
4′-Fluorinated nucleic acids: Synthesis, Structure, and Applications

Qiang Li, Chaochao Fan, Chuanzheng Zhou

State Key Laboratory of Elemento-Organic Chemistry and Department of Chemical Biology, College of Chemistry, Nankai University, Tianjin, China

Fluorinated nucleic acids have attracted considerable interest in recent decades. Owing to the unique physical properties of the fluorine atom (e.g., its small atomic radius and high electronegativity), introducing a fluorine atom into nucleic acid molecules markedly affects their structure, lipophilicity, nuclease resistance, and interactions with other molecules.[1] Fluorine atoms are generally introduced onto either the nucleobase or the ribose. Introducing electron-withdrawing group such as a fluorine atom to the C4′ position generally makes the glycosidic bond prone to hydrolysis. Thus, synthesis of 4′-fluorinated nucleic acids remains a challenge.

In the past few years, we developed a strategy that allowed us to circumvent the instability of 4′-fluorinated nucleosides and achieved the successful synthesis of oligonucleotides containing a 4′-F-rU, 4′-F-dT, 4′-CF_3-dT, 4′-CF_3-rU and 4′-SCF_3-dT. We found the electron-withdrawing moieties, such as 4′-F and 4′-CF_3 constrain both ribose and deoxyribose in the North conformation, whereas electron-donating moieties, such as CH_3 and CF_3, constrain the pentose sugar in the South conformation.[2-6]



4′-Fluorinated nucleic acids demonstrate striking properties for elucidating the structures and functions of nucleic acids by means of ^{19}F NMR spectroscopy. The ^{19}F NMR signal of 4′-F-rU is sensitive to secondary structure but not to sequence context, making 4′-F-rU an ideal probe for monitoring RNA structural dynamics and enzyme-mediated processing. 4′-SCF_3 group exhibited a flexible orientation in the minor groove of DNA duplexes and was well accommodated by various higher order DNA structures. The three magnetically equivalent fluorine atoms in 4′-SCF_3-DNA constitute an isolated spin system, offering high ^{19}F NMR sensitivity and excellent resolution of the positioning of $T^{4'-SCF3}$ within various secondary and tertiary DNA structures. In addition, 4′-CF_3-dT modification increases the lipophilicity of antisense oligonucleotides (AONs), which enable direct cellular uptake of the modified AONs without any delivery reagents.

Taken together, 4′-fluorinated nucleic acids show striking biophysical and biochemical properties and have found broad applications in the structural and functional studies of nucleic acids and in nucleic acid-based therapeutics.

References:
Guo, F.; Li, Q.; Zhou, C., Org. Biomol. Chem. 2017, 15, 9552-9565.
Guo, F. et al., J. Am. Chem. Soc. 2018, 140, 11893-11897.
Zhou, Y. et al., Org. Biomol. Chem. 2019, 17, 5550-5560.
Li, Q. et al., J. Am. Chem. Soc. 2020, 142, 4739-4748.
Zhou, Y.; Lu, K.; Li, Q.; Fan, C.; Zhou, C., Chem. Eur. J. 2021, 27, 14738-14746.
Li, Q. et al., Angew. Chem. Int. Ed. 2022, 61, e202201848.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (Nos. 22377059, 21877064 and 91953115) and the Slovenian Research Agency (ARRS, grants P1-0242 and J1-1704). The authors acknowledge the CERIC-ERIC consortium for access to experimental facilities and for financial support. We thank Prof. Janez Plavec and Dr. Marko Trajkovski for the fruitful collaboration.

The G-quadruplex ligand RHPS4 sensitizes melanoma cells to traditional chemotherapy

Anna Di Porzio[1], Carolina Persico[1], Nunzia Iaccarino[1], Gelsomina Riccardi[1], Francesca Romano[1], Laura Schembri[1], Stefano De Tito[2], Antonio Randazzo[1]

[1] Department of Pharmacy, University of Naples Federico II, Via D. Montesano 49, Naples, Italy
[2] Molecular Cell Biology of Autophagy Laboratory, The Francis Crick Institute, London, UK

Cancer is a global health problem responsible for one in six deaths worldwide, with chemotherapy being the standard strategy to treat it. Although conventional chemotherapeutics, including alkylating agents, antimetabolites, and plant alkaloids, have been and are being successfully used in cancer therapy, their side effects on the patient's physical and psychological health are often severe. Additionally, tumour cells can spontaneously or adaptively become resistant to nearly all kinds of chemotherapeutic drugs, eventually leading to treatment failure. In this frame, chemo-sensitization of cancer cells to conventional drugs using small molecules is gaining momentum as an innovative strategy to overcome the mechanisms underlying chemoresistance, reduce the chemotherapy-induced adverse effects and improve the clinical outcome.[1,2] In the present investigation, taking into consideration the emerging role of G-quadruplex (G4) structures as anti-cancer targets,[3] we report encouraging preliminary data about the use of the pentacyclic acridinium salt RHPS4, one of the most effective and selective G-quadruplex ligands, to potentiate the antitumor activity of traditional chemotherapeutics against A375MM melanoma cancer cells. These results might pave the way towards the use of G4-interacting molecules to synergize the activity of standard antineoplastic drugs without increasing their toxicity on healthy cells.

References:
1. Sawyer C.L., Nature, 2007, 449, 993–6.
2. Daub H., Specht K. and Ullrich A., Nature Reviews Drug Discovery, 2004, 3, 1001–10.
3. Kosiol N., Juranek S., Brossart P., Heine A. and Paeschke K., Molecular Cancer, 2021, 20, 40.

Acknowledgements: The authors acknowledge the Italian Association for Cancer Research for the financial support (IG 26313 to Antonio Randazzo). Moreover, Anna Di Porzio is a recipient of a FIRC-AIRC postdoctoral fellowship (26644).

Physical Chemistry of Nucleic Acids: “To *B* or not to *B*”

Shuntaro Takahashi[1], Saptarshi Ghosh[1], Hisae Tateishi-Karimata[1], Dipanwita Banerjee[1], Tamaki Endoh[1], Tatsuya Ohyama[1], Naoki Sugimoto[1,2]

[1] *Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, Kobe, Japan*
[2] *Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, Kobe, Japan*

The stability of nucleic acids structures cannot be determined from only the sequence composition, as this property critically depends on the surrounding environment of the solution. The intracellular condition is greatly different from that of the diluted buffer typically used for standard experiments and is not constant in each local area of the cell. Thus, stability predictions should reflect the situation under intracellular conditions and thus are required importantly, especially after the COVID-19.

In this presentation, we will provide an overview of the basic concepts, methods, and applications of predicting the stabilities of nucleic acid structures. We will explain the theory of the most successful prediction method based on a nearest-neighbour (NN) model. To improve the versality of prediction, corrections for various solution conditions considered hydration have been investigated. We will also describe advances in the prediction of non-canonical structures. Finally, studies of intracellular analysis and prediction are discussed for the application of NN parameters in the post-COVID-19 era.

References:
Sugimoto N. et al., *Nucleic Acids Res.* **2023**, *51*, 4101-4111; *Sci. Adv*. **2022**, *8*, eadc9785; *Chem. Commun.* **2022**, *58*, 12459-12462; *J. Am. Chem. Soc.* **2022**, *144*, 5956-5964; *Anal. Chem.* **2022**, *94*, 7400-7407; *Chem. Commun*. **2022**, *58*, 5952-5955, *Sci. Rep.*, **2022**, *12*,1149; *J. Am. Chem. Soc.* **2021**, *143*, 16458–16469; *Bull. Chem. Soc. Jpn.* **2021**, *94*, 1970-1998; *ACS Chem. Biol.* **2021**, *16*, 1147–1151; *RSC Adv*. **2021**, *11*, 37205-37217; *Nucleic Acids Res.* **2021**, *49*, 7839–7855; *Topics Curr. Chem.* **2021**, *379*, 17; *Nucleic Acids Res.* **2021**, *49*, 8449–8461; *Acc. Chem.Res.* **2021**, *54*, 2110-2120; *Chem. Soc. Rev.* **2020**, *49*, 8439–8468; *Chem. Commun.* **2020**, *56*, 2379–2390; *RSC Adv.* **2020**, *10*, 33052–33058; *Biochemistry.* **2020**, *59*, 2640–2649; *Proc. Natl. Acad. Sci. U.S.A.* **2020**, *117*, 14194–14201; *Anal. Chem.* **2020**, *92*, 7955–7963; *Biochemistry.* **2020**, *59*, 1972–1980; *Ncleic Acids Res.* **2020**, *48*, 3975–3986; *Biochem. Biophys. Res. Commun.* **2020**, *525*, 177–183; *Chem. Commun.* **2020**, *56*, 2379–2390; *Sci. Rep.* **2020**, *10*, 2504 and Sugimoto, N. “**Chemistry and Biology of Non-Canonical Nucleic Acids**” *WILEY.* **2021**, 1–288.

Acknowledgment: The authors are grateful to the colleagues named in the cited references from our 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 for Grant-in-Aid for Scientific Research (S) (22H04975), JSPS Core-to-Core Program (JPJSCCA20220005), The Hirao Taro Foundation of Konan Gakuen for Academic Research, and The Chubei Itoh Foundation.

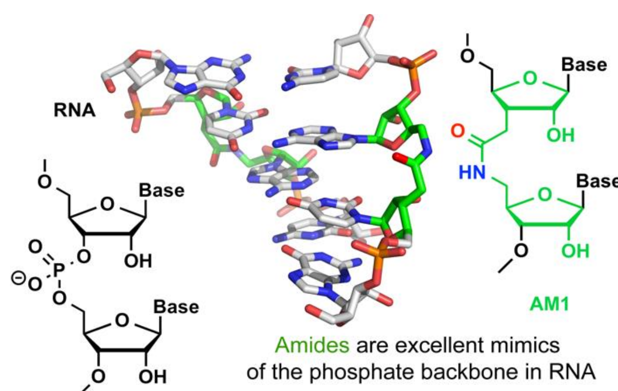
Amide-Modified Oligonucleotides for Chemical Control of Functional RNAs

Michael Richter, Venubabu Kotikam, Chandan Pal, Praveen Kumar Gajula,
Michael Richter, Julien A. Viel, Lamorna Coyle, Eriks Rozners

Department of Chemistry, Binghamton University, The State University of New York, Binghamton, NY, USA

RNA-based technologies to control gene expression, such as, RNA interference (RNAi) and CRISPR have become powerful tools in molecular biology and genomics. The exciting advances of RNAi and CRISPR as new therapeutic approaches has reinvigorated interest in chemically modifying RNA to improve its properties for in vivo applications. Chemical modifications can improve enzymatic stability, in vivo delivery, cellular uptake, and sequence specificity; as well as minimize off-target activity of short interfering RNAs (siRNAs) and CRISPR associated RNAs (crRNAs). The long-term goal of our research is to develop chemical modifications for optimization of in vivo potential of siRNAs and crRNAs. Our current work is focused on the development of non-ionic analogues of RNA that have the phosphodiesters replaced by amide linkages (AM1 in Figure).[1]

Structural studies show that amides are excellent mimics of the phosphodiester inter-nucleoside linkages in RNA. The local conformational changes caused by the amide linkages where accommodated easily by small adjustments in RNA structure. On the other hand, amides are more rigid than phosphodiesters and strongly prefer the trans conformation, which fits well the A-type helix, but disfavours alternative RNA structures. We hypothesize that the reduced negative charge and conformational preferences of amide linkages can be used to optimize potency, cellular uptake, and reduce off-target effects of siRNAs and crRNAs.



Chemical structure of amide-modified RNA.

This presentation will discuss synthesis, structure, and RNAi and CRISPR activity and specificity of amide-modified RNA. RNAi activity assays show that amides are well tolerated at internal positions in both strands of siRNAs. Surprisingly, amide modifications in the middle of the guide strand and at the 5´-end of the passenger strand increased the RNAi activity compared to unmodified siRNA. Most remarkably, replacement of certain phosphate linkages with amides significantly reduced the off-target activity of guide and passenger strands.[2] Recent studies by our group showed that amide modifications did not interfere with CRISPR-Cas9 activity when placed in the protospacer adjacent motif distal region of crRNAs.[3] Taken together, our results suggest that amides are excellent mimics of phosphate backbone in RNA and may have potential to optimize biological and pharmacological properties of siRNAs and crRNAs for in vivo applications. These findings are unexpected and raise the possibility that functional RNAs may tolerate and benefit from even more substantial modifications than the ones tried so far.

References:
1. Kotikam, V.; Rozners, E. *Acc. Chem. Res.* **2020**, *53*, 1782-1790.
2. Richter, M.; Viel, J. A.; Kotikam, V.; Gajula, P. K.; Coyle, L.; Pal, C.; Rozners, E. *ACS Chem. Biol.* **2023**, *18*, 7-11.
3. Kotikam, V.; Gajula, P. K.; Coyle, L.; Rozners, E. *ACS Chem. Biol.* **2022**, *17*, 509-512.

Acknowledgements: This work was supported by US National Institutes of Health (R35 GM130207 to E.R.).

Crosstalk Between Chemical Biology and Structural Biology of RNA Interference

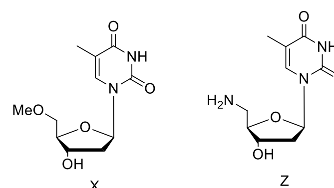
Masayuki Fujii

School of Biological & Environmental Chemistry, Kindai University, Fukuoka, Japan

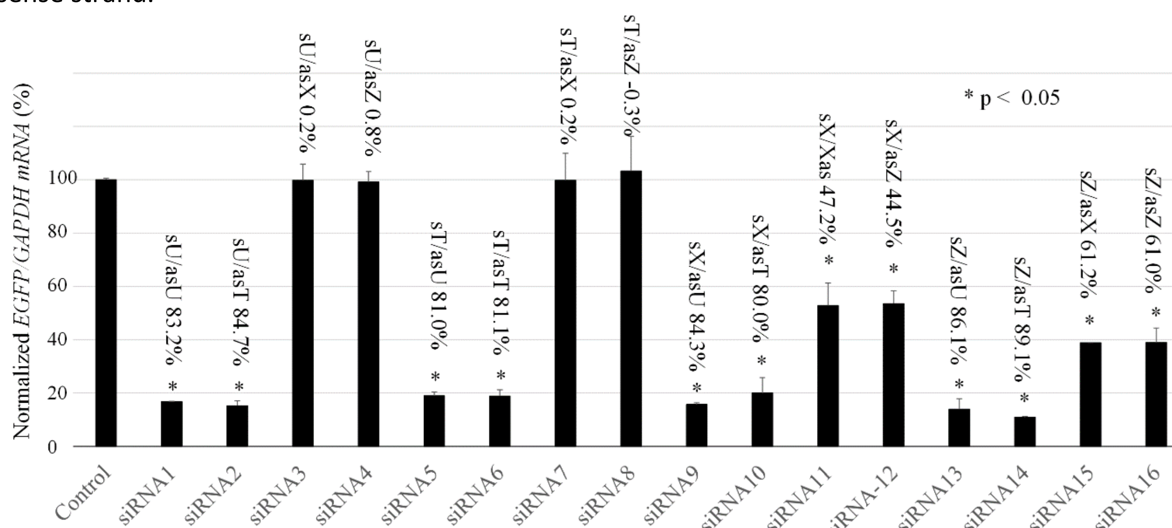
Small interfering RNA (siRNA) represents the most common and the most effective method to inhibit target gene expression in human cells. In order to optimize the chemical structure of siRNA for biological and medical applications, DDS and minimization of off-target effect are critical issues.

In the present study, we investigated RNA interference (RNAi) efficiencies of chemically modified siRNAs and the relationship with the structure of human Argonaute 2 protein.[1] Modifications include 5'-ends, major groove side of bases, and 3'-overhangs. Especially, we would like to focus on siRNAs bearing 5'-O-methylthymidine (X) and 5'-aminothymidine (Z) at 5'-end of the strands.[2]

Ant-EGFP siRNAs (214-234)
sense; 5'-RACGGCAAGCUGACCCUGAag-3'
antisensne; 5'-RCAGGGUCAGCUUGCCGUAgg-3'
R = U, T, X, Z



The results showed that modification of the 5'-end of siRNA with X or Z significantly affected on the recognition of asymmetry of double stranded siRNA, namely, strand selection during RLC and RISC formation and also on the stability of RISC bearing X/Z-modified guide strand. Modification of the 5'-end of the sense strand with X or Z significantly increased the chance for the antisense strand to be selected as the guide strand. Modification of the 5'-end of the guide strand with X or Z destabilized RISC and decreased silencing efficiency of siRNA. These results strongly suggested that modification of 5'-end of the sense strand with X and Z will eliminate the off-target effect of the sense strand.



Silencing of EGFP mRNA by siRNA1-siRNA16. HeLa (5 x 10^4 cells /well, 10% FBS/MEM) maintained in 5% CO_2, at 37 °C, for 24 h were transfected with siRNA at the final concentration of 100 nM using Lipofectamine 2000. The values represent the mean ± SD of 3 independent experiments. The results were evaluated by Kruskal-Wallis ($p < 0.0001$) and multiple comparisons uncorrected Dunn's test. *$p < 0.05$ versus values of the negative control (scramble siRNA).

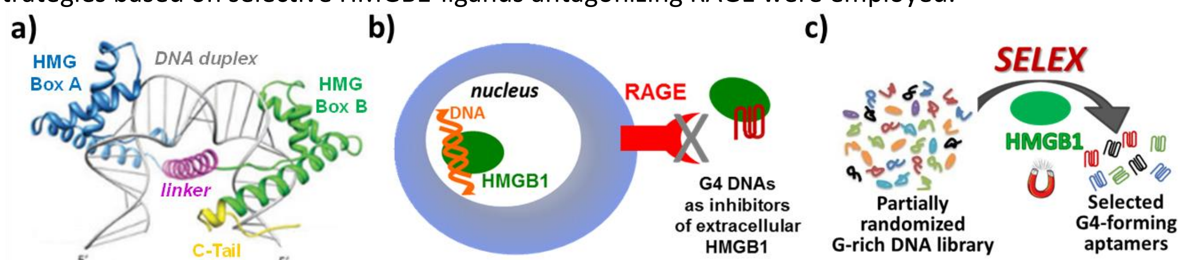
References:
1. Schirle, NT. and MacRae, IJ. (2012) The crystal structure of human argonaute2. Science, 336 (6084) 1037–1040.
2. Masayuki Fujii, et al, (2022) Elimination of Off-target Effect by Chemical Modification of 5'-End of siRNA Nucleic Acid Therapeutics, 2022, 32(5):438-447.

Directing in vitro selection towards G-quadruplex-forming aptamers for the development of efficient inhibitors of HMGB1 pathological activity

Domenica Musumeci[1,2], Ettore Napolitano[1], Andrea Criscuolo[1], Carla Esposito[3], Claudia Riccardi[1], Giovanni N. Roviello[2], Daniela Montesarchio[1]

[1] Department of Chemical Sciences, University of Naples Federico II, Naples, Italy
[2] Institute of Biostructure and Bioimaging (IBB)-CNR, Naples, Italy
[3] Institute Experimental Endocrinology and Oncology "Gaetano Salvatore" (IEOS) - CNR, Naples, Italy

HMGB1, a protein which acts as an architectural factor for chromatin when in the nucleus, and as a chemokine/alarmin when released in serum, was established as a good therapeutic target for a wide number of diseases including inflammatory states, sepsis, rheumatoid arthritis, atherosclerosis, as well as cancer.[1] Interaction of released HMGB1 with the cell-surface receptor for advanced glycation end products (RAGE) is one of the main signaling pathways triggering these diseases.[2] Efficient inhibition of the HMGB1-RAGE interaction represents a promising approach for the modulation of the inflammatory and tumor-facilitating activity of HMGB1.[2,3] In this context, several strategies based on selective HMGB1-ligands antagonizing RAGE were employed.[3]



a) HMGB1 bound to a DNA duplex. b) Schematic representation of our approach to inhibit HMGB1 pathological activity by using G4 DNA structures. c) Adapted SELEX procedure applied on a doped library of G-rich oligonucleotides to select G4-forming aptamers

Considering the role of HMGB1 in cell nuclei, where it interacts with DNA leading to distortion and bending of the double helix (Figure a),[1] and taking into account the high affinity of the protein for non-canonical DNA structures (cruciform, hemicatenane, etc.),[4] including the DNA G-quadruplex (G4) structure of the human telomeric sequence (the 26-mer named tel_{26}),[5] we decided to investigate the use of G4-forming oligonucleotides, variants of tel_{26}, as potential inhibitors of extracellular HMGB1 in pathological conditions (Figure b). In particular, starting from tel_{26}, we designed a focused library of G-rich-oligonucleotides potentially forming 3-planes G4 structures and, employing SELEX, we identified from this DNA pool the sequences able to specifically bind HMGB1 (Figure c). The results of the doped SELEX, the biophysical characterization of the selected aptamers in pseudo-physiological buffer mimicking the extracellular medium – where HMGB1 exerts its pathological activity – and preliminary data on their interaction with the protein as well as on their biological activity in cellular assays will be here presented.

References:
1. Müller S., Scaffidi P., Degryse B., et al. The double life of HMGB1 chromatin protein: architectural factor and extracellular signal. EMBO J, 2001, 20, 4337-40.
2. Sims G.P., Rowe D.C., et al. HMGB1 and RAGE in inflammation and cancer. Annu Rev Immunol, 2010, 28, 367-88.0
3. Musumeci D., Roviello G.N., Montesarchio D. An overview on HMGB1 inhibitors as potential therapeutic agents in HMGB1-related pathologies. Pharmacol Ther, 2014, 141, 347-57.
4. Bianchi M.E., Beltrame M., Paonessa G. Specific recognition of cruciform DNA by nuclear protein HMG1. Science, 1989, 243, 1056-59.
5. Pagano B., Margarucci L., Zizza P., Amato J., Iaccarino N., Cassiano C., Salvati E., Novellino E., Biroccio A., Casapullo A., Randazzo A. Identification of novel interactors of human telomeric G-quadruplex DNA. Chem Commun, 2015, 51, 2964-67.

Acknowledgements: AIRC grant (IG 2020 – ID. 25046 – P.I. Montesarchio Daniela)

UV-induced G4 DNA structures recruit ZRF1 which prevents UV-induced senescence

Katrin Paeschke

University Clinic Bonn, Biomedical Center, Bonn, Germany

Senescence has two roles in oncology: it is known as a potent tumour-suppressive mechanism, which also supports tissue regeneration and repair, but it is also known to contribute to reduced patient resilience, which might lead to cancer recurrence and resistance after therapy. Senescence can be activated in a DNA damage-dependent and -independent manner. It is not clear which type of genomic lesions induces senescence, but it is known that UV irradiation can activate cellular senescence in photoaged skin. Proteins that support the repair of DNA damage are linked to senescence but how they contribute to senescence after UV irradiation is still unknown. Here, we unravelled a mechanism showing that upon UV irradiation multiple G-quadruplex (G4) DNA structures accumulate in cell nuclei, which leads to the recruitment of ZRF1 to these G4 sites. ZRF1 binding to G4s ensures genome stability. The absence of ZRF1 triggers an accumulation of G4 structures, improper UV lesions repair and the entry into senescence. On the molecular level loss of ZRF1 as well as high G4 levels lead to the upregulation of DDB2, a protein associated with the UV-damage repair pathway, which drives cells into senescence.

Role of alternate RNA conformations in human health and disease

Ambadas B. Rode

Regional Centre for Biotechnology, NCR Biotech Science Cluster, Faridabad, India

Beyond the transfer of genetic information RNA drives a large number of cellular processes e.g., transcription, splicing, translation, and its own stability etc. through its three-dimensional structures.[1] The RNA molecules adopt simple secondary to complex tertiary structures using Watson-Crick base-pairing and tertiary interactions such as loops, bulges, helical junctions, and long-range interactions. The RNA secondary and tertiary structures are in equilibrium with competitive alternative conformations to form a different population of substructures. The different RNA substructures give rise to a distinct biological outcome and plays crucial role in human health and disease. The alternative RNA conformations equilibrium can be shifted in response to external cues such as small molecule ligands for the therapeutic and biotechnological applications. Our group research focuses on harnessing the nucleic acids structure-mediated gene regulation in human,[2] bacteria[3] and viruses[4] for biomedical applications. In the talk, I will provide an overview of some of the alternate RNA conformations e.g., G-quadruplex, riboswitches etc. present in human, bacteria and viruses, and use of these structures for therapy.[2-4]

References:

1. Pandey M, Ojha D, Bansal S, Rode AB*, Chawla G* *Molecular Aspects of Medicine*, 2021, 81, 101003.
2. Gupta P, Ojha D, Nadimetla DN, Bhosale SV, Rode AB* *ChemBioChem*, 2022, 23, e20220013.
3. Harale B, Kidwai S, Ojha D, Singh M, Chouhan D, Singh R, Khedkar V, Rode AB* *Bioorg Med Chem Lett.* 2012, 48, 128236.
4. Gupta P, Khadake RM, Mirgane H, Bhosale SV, Gupta D, Vrati S, Rode AB* *ChemRxiv. Cambridge*, 2023, DOI:10.26434/chemrxiv-2023-jsrz0.

Acknowledgements: This research was supported in part by a Ramalingaswami Re-Entry Fellowship (BT/RLF/Re-entry/19/2016) from Department of Biotechnology (DBT) of the Government of India.

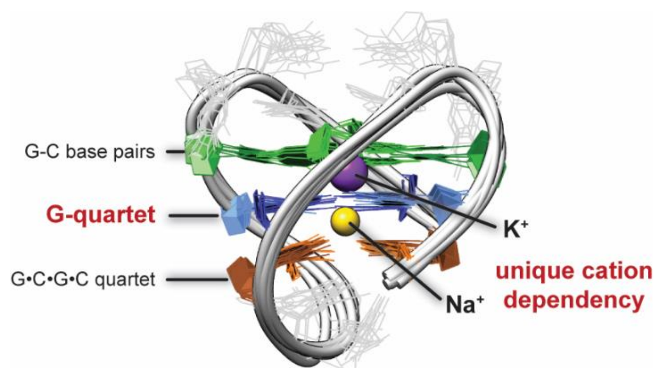
It takes two to tango: Unique cation dependency of telomeric DNA quadruplex

Martin Gajarský[1], Petr Stadlbauer[2], Jiři Šponer[2], Anne Cucchiarini[1,3], Michaela Dobrovolna[2,4], Vaclav Brazda[2,4], Jean-Louis Mergny[2,3], Lukaš Trantírek[1], Martina Lenarčič Živković[1,5]

[1] *Central European Institute of Technology, Masaryk University, Kamenice 753/5, Brno, Czech Republic*
[2] *Institute of Biophysics, Czech Academy of Sciences, Kralovopolska 135, Brno, Czech Republic*
[3] *Laboratoire d'Optique et Biosciences, Ecole Polytechnique, CNRS, INSERM, Palaiseau, France*
[4] *Faculty of Chemistry, Brno University of Technology, Purkynova 464, Brno, Czech Republic*
[5] *Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, Ljubljana, Slovenia*

Non-canonical DNA structures play important roles as regulators of key biological processes, such as transcription, translation, and telomere homeostasis.[1] Numerous biophysical studies have substantiated the presence of G-quadruplexes (GQs) within telomeric DNA fragments across different species, establishing the formation of GQ as an evolutionarily conserved structural hallmark.[2-6] However, recent reports have presented exceptions to this notion.[5,7]

Here, we investigated one of the proposed exceptions to the telomeric *GQ rule*. We demonstrate that a native G-rich DNA sequence originating from the telomeric region of *Caenorhabditis elegans* forms a distinctive tetrastranded structure, which we termed the KNa-quadruplex (KNaQ). The structure is defined by a single G-quartet sandwiched between different GC-based structural elements and concurrently coordinates K^+ and Na^+ ions at two distinct binding sites (see Figure). In addition to the absence of stacked G-quartets and unique cation dependency, the KNaQ structure differs from closely related GQs by a different groove width and its susceptibility toward GQ binding ligands. Furthermore, we show that two well-established GQ binders can be used as turn-on fluorescent probes, allowing us to distinguish between KNaQ and GQ structures with different topologies. Additionally, the absence/presence of KNaQ motifs in the host/parasite introduces an intriguing possibility of exploiting the KNaQ fold as a plausible antiparasitic drug target.



High-resolution NMR structure of a novel tetrastranded DNA motif, called KNa-quadruplex (KNaQ)

References:
1. Bansal A., Kaushik S. and Kukreti S., Front. Genet., 2022, 13, 959258
2. Henderson E., Hardin C. C., Walk S. K., Tinoco I., Blackburn E. H., Cell, 1987, 51, 899 – 908
3. Phan A. T., FEBS J., 2010, 277, 1107 – 1117
4. Tran P. L. T., Mergny J.-L., Alberti P., Nucleic Acids Res., 2011, 39, 3282 – 3294
5. Školáková P., et al., Nucleic Acids Res., 2015, 43, 4733 – 4745
6. Wu W.-Q., Zhang M.-L., Song C.-P., J. Biol. Chem., 2020, 295, 5461–5469
7. Gajarský M., et al., J. Am. Chem. Soc., 2017, 139, 3591–3594

Acknowledgements: This work was supported by the Czech Science Foundation [19-26041X to L.T. and M.G., 21-23718S to J.S. and P.S.] and Czech Ministry of Education, Youth and Sports [MSCAfellow2@MUNI, grant no. CZ.02.2.69/0.0/0.0/18.070/0009846 to M.L.Z., SYMBIT, grant no. CZ.02.1.01/0.0/0.0/15_003/0000477 to J.L.M., J.S. and V.B.]. The authors acknowledge projects enabling access to research infrastructure, namely projects financed by the Ministry of Education, Youth and Sports of the Czech Republic [EATRIS-ERIC-CZ: LM2018133, Czech-Bioimaging: LM2018129, and CIISB LM2018127] and the CERIC-ERIC Consortium [project number 20192131].

Quadruplexes are everywhere!*

Jean-Louis Mergny[1,2]

[1] *Laboratoire d'Optique et Biosciences, Ecole Polytechnique, Institut Polytechnique de Paris, Palaiseau, France*
[2] *Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic*

We are developing tools to understand G-quadruplex folding and polymorphism, both *in vitro* and in cells [1]. In parallel, we are applying the G4Hunter algorithm [2] (for the prediction of G4 propensity) to a variety of genomes, including cancer [3], parasite [4] or virus genomes [5], as well as extinct genomes. We have analysed genomes of hepatitis B viruses (HBV) for the presence of G-quadruplex-forming sequences [6]. Our work used genomes from ancient and modern HBV stains and represents the first paleogenomic analysis of the propensity for G4 formation in any genome. We then performed a detailed analysis of G-quadruplex sequences in Neandertal mitochondrial DNA [7]. Relatively similar patterns were found compared to modern humans, in mitochondrial DNA with one notable exception, corresponding to a motif found in the D-loop region of mtDNA, which is responsible for mitochondrial DNA replication. This area is directly responsible for the number of mitochondria and consequently for the efficient energy metabolism of cell. Neandertals harbour a long uninterrupted run of guanines in this region, which may cause problems for replication, in contrast with anatomically modern humans, for which this run is generally shorter and interrupted.

*I know this title s*cks, and is actually the same as last year. But, I will show now that quadruplexes are not only everywhere, but also elsewhere.

References:
1. Luo et al, **Nucleic Acids Res.** (2022), 50, e93. Luo et al, **Biochimie** (2023), doi: 10.1016/j.biochi.2022.12.019. Esnault et al, **Nat Genet** (2023) 55, 1359-1369.
2. Bedrat et al, **Nucleic Acids Res.** (2016), 44: 1746. Brázda et al, **Bioinformatics** (2019), 35, 3493.
3. Zhang et al, **Cancer Research** (2023), 83, 1234.
4. Cantara et al, **Nucleic Acids Research** (2022), 50, 2719-2735.
5. Abiri et al, **Pharmacol Rev**. (2021) 73, 897. Bohálová et al, **Biochimie** (2021) 186, 13-27. Lavigne et al. **Nucleic Acids Res**. (2021) 49, 7695. Lista et al, **Retrovirology** (2023) 20, 10.
6. Bohálová et al, **Nucleic Acids Res**. (2023) 51, 9548.
7. Brázda et al, in preparation

Acknowledgements: This work would not have been possible without the constant support of my colleagues in Palaiseau and Brno. Special mention to V. Brázda (IBP). Supported by INCa PL-BIO [2020-117] and ANR [ANR-20-CE12-0023] G4Access grants.

The anticancer peptide LL-III: a model for a new class of peptide-based G4 ligands

Marco Campanile[1], Rosario Oliva[1], Pompea Del Vecchio[1], Roland Winter[2], Luigi Petraccone[1]

[1] *Department of Chemical Sciences, University of Naples Federico II, Via Cintia 4, Naples, Italy*
[2] *Department of Chemistry and Chemical Biology, TU Dortmund, Otto-Hahn Str. 4a, Dortmund, Germany*

Anticancer peptides (ACPs) are a promising class of compounds for the development of novel drugs due to their low toxicity and high selectivity. Although most ACPs act by targeting the lipid bilayer of cancer cells, some peptides have been shown to translocate into the cell cytoplasm interacting with intracellular targets.[1] The ACP LL-III, a natural cationic peptide from the family of Lasioglossins, was found to selectively recognize and cross the negatively charged tumour membrane, localizing in the nucleolus and suggesting that nuclear DNA could be an intracellular target.[2-4] In this study, we explored the ability of LL-III to interact with cancer-relevant DNA sequences known to adopt G-quadruplex (G4) structure.[5] Using biophysical techniques such as fluorescence, circular dichroism (CD), and isothermal titration calorimetry (ITC), we investigated the molecular basis underpinning the binding of LL-III to different G4 structures, including the human telomeric sequence (Tel-23), cMyc, and cKIT1. Our results showed that LL-III discriminates among different DNA structures, in terms of sequence and topology, with a marked preference towards G4s when compared to duplex and disordered DNA. Interestingly, we measured the highest affinity ($K_D \sim$ nM) for the parallel cMyc and the mixed-type Tel-23, two well-known relevant anticancer targets. The binding process was found to be endothermic and entropically driven for all G4s, with the higher $\Delta_b H°$ values observed in the complexes where LL-III adopts a partially helical conformation, suggesting that the peptide/DNA recognition occurs through hydrophobic interactions between the most solvent-exposed G-tetrads and the peptide aromatic and apolar residues. Further, the characterization of the G4 interaction with mutated LL-III sequences allowed us to identify some of the key residues involved in the binding process. Our findings support the idea that G4s recognition could be involved in the mechanism of action of LL-III, and that this peptide could represent a lead sequence for the development of new and highly selective peptide-based G4 ligands.

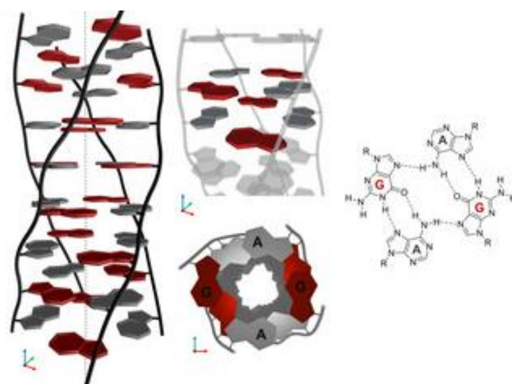
References:
1. Harris F., Dennison S.R., Singh J. and Phoenix D.A., Medicinal Research Reviews, 2013, 33, 190-234
2. Čeřovský V., Buděšínský M., Hovorka O., Cvačka J., Voburka Z., Slaninová J., Borovičková L., Fučík V., Bednárová L., Votruba I. and Straka J., ChemBioChem, 2009, 10, 2089-2099
3. Slaninová J., Mlsová V., Kroupová H., Alán L., Tumová T., Monincová L., Borovičková L., Fučík V. and Čeřovský V., Peptides, 2012, 33, 18-26
4. Campanile M., Oliva R., D'Errico G., Del Vecchio P. and Petraccone L, Physical Chemistry Chemical Physics, 2023, 25, 3639-3650
5. Neidle S., Nature Reviews Chemistry, 2017, 1, 0041

Unusual features of $d(GT)_n$ and $d(GA)_n$ repeats

Lukáš Trizna, Viktor Víglaský

Department of Biochemistry, Institute of Chemistry, Faculty of Sciences, P. J. Šafarik University, Moyzesova 11, Košice, Slovakia

The recently introduced semi-orthogonal system of nucleic acid imaging offers a greatly improved method of identifying DNA sequences that can adopt noncanonical structures[1]. Our newly developed G-QINDER tool highlighted the unusual properties of repetitive sequences that could adopt unique structural motifs in DNA: $d(TG)_n$ and $d(AG)_n$[2]. Structures consisting of these repetitive sequences were found to be very likely to adopt a left-handed G-quadruplex form and/or a unique tetrahelical motif, but only under certain conditions. The tetrahelical structure likely consists of stacked AGAG-tetrads but, unlike G-quadruplexes, their stability does not appear to be dependent on the type of monovalent cation present. There is a strong analogy with the VK motif described earlier.[3]



Proposed noncanonical motif based on analogies with the noncanonical tetrahedral VK structure, in which the guanines in one AGAG-quartet are oriented in *syn*- conformation in the adjacent quartet.[2]

Interestingly, the occurrence of TG and AG repeats in the genomes of living organisms is not rare, and they are also frequently found in nucleic acid regulatory regions, suggesting that putative structural motifs, like other non-canonical forms, could play an important regulatory role in cells. This hypothesis is supported by the structural stability of the AGAG motif; its unfolding can occur even at physiological temperatures since the melting temperature is primarily dependent on the number of AG repeats in the sequence.

References:

1. Viglasky V., Int. J. Mol. Sci. 23(3), 1804 (2022)
2. Trizna L., Osif B. and Viglasky V., Int. J. Mol. Sci. 24(8), 7565 (2023)
3. Kocman, V., Plavec, J. Nat. Commun., 5, 5831 (2014)

Acknowledgements: This work was supported by the Slovak Grant Agency (1/0347/23).

The Reversibility of Cyclobutane Pyrimidine Dimer Accumulation in DNA

Ravina Moirangthem, Manusha N. Gamage, Steven E. Rokita

Department of Chemistry, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD, USA

Cyclobutane pyrimidine dimers (CPD) are the product of a photochemical [2 + 2] reaction of DNA and responsible for many of the mutagenic hotspots induced by sun and ultraviolet light. Distribution of these lesions is highly variable in cells[1] and in vitro models have implicated DNA conformation as a major basis for this observation.[2,3] Past efforts to predict hotspots of CPD have primarily focused on CPD formation and have rarely considered contributions of CPD reversion. However, reversion is competitive under the standard conditions of 254 nm irradiation as illustrated in this presentation by the dynamic response of CPD to changes in DNA conformation.[4] A periodic profile of CPD[2] was recreated in DNA held in a bent conformation by λ repressor. After linearizing this DNA, the CPD profile relaxed to its characteristic uniform distribution over an equivalent time of irradiation that is required to generate the initial profile. Similarly, when a T tract was released from a bent conformation, its CPD profile converted under further irradiation to that consistent with a linear T tract. This interconversion of CPD suggests that both its formation and reversion exert control on CPD populations long before photo steady state conditions are achieved and predicts that CPD hotspots will evolve as DNA conformation changes in response to natural cellular processes.

References:
1.Premi, S., Han, L., Mehta, s., Knight, J., Zhao, D., Palmatier, M.A., Kornacker, K. and Brash, D.E., Proc. Natl. Acad. Sci. (USA) Genomic sites hypersensitive to ultraviolet radiation, 2019, 116, 24196-24205
2. Pehrson, J.R. and Cohen, L.H., Nucleic Acids Res. Effects of DNA looping on pyrimidine dimer formation, 1992, 20, 1321-1324
3. Wang, K. and Taylor, J.-S., Nucleic Acids Res. Modulation of cyclobutane thymine photodimer formation in T_{11}-tracts in rotationally phased nucleosome core particles and DNA minicircles, 2017, 45, 7031-7041
4. Moirangthem, R., Gamage, M.N. and Rokita, S.E., Nucleic Acids Res. Dynamic accumulation of cyclobutane pyrimidine dimers and its response to changes in DNA conformation, 2023, 51, 5341-5350.

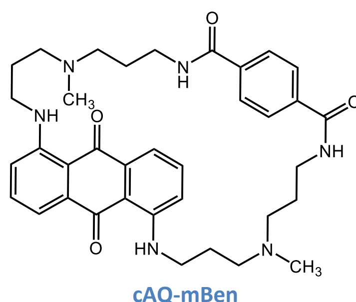
Acknowledgements: This project was supported in part by the National Science Foundation [MCB-1914560].

Novel G-quadruplex binders as cyclic anthraquinone derivatives

Shigeori Takenaka

Department of Applied Chemistry, Kyushu Institute of Technology. Kitakyushu 804-8550 Japan

We have previously shown that cyclic intercalators such as cyclic naphthalene diimide derivatives act as specific ligands for G-quartet (G4) DNA.[1] Here, we present cyclic anthraquinone derivatives (cAQs), which are linked to two side chains of 1,5-disubstituted anthraquinones.[2] Anthraquinone derivatives are known as intercalation molecules to double stranded DNA and have long been used as antibiotics for anticancer drugs.[3] We synthesized cAQs to improve the specificity of anthraquinones for G4 DNA. Among the cAQs, cAQ-mBen linked through the 1,3-position of benzene had the strongest affinity for G4 recognition and stabilization in vitro and was confirmed to bind to the G4 structure in vivo, selectively inhibiting cancer cell proliferation in correlation with telomerase expression levels and triggering cell apoptosis. RNA-sequencing analysis further indicated that differentially expressed genes regulated by cAQ-mBen were profiled with more putative G4 sequences (PGSs). In the treatment of tumour-bearing mouse model, cAQ-mBen could effectively reduce tumour tissue and had less adverse effects on healthy tissue. These results suggest that cAQ-mBen can be a novel potential cancer therapeutic agent as a G4 binder.



cAQ-mBen

References:
1. S. Takenaka, *Polymer J*., **53**, 415-427 (2021).
2. H. Fukuda, T. Zou, S. Fujii, S. Sato, D. Wakahara, S. Higashi, T.-Y. Tseng, T.-C. Chang, N. Yada, K. Matsuo, M. Habu, K. Tominaga, H. Takeuchi, S. Takenaka, *PNAS Nexus*, **2**, pgad211 (2023).
3. M. Shaheer Malik, R. I. Alsantali, R. S. Jassas, A. A. Alsimaree, R. Syed, M. A. Alsharif, K. Kalpana, M. Morad, I. I. Althagafi, S. A. Ahmed, *RSC Adv.*, **11**, 35806-35827 (2021).

Acknowledgements: ST appreciates the significant contribution made by the researchers who appear in the reference papers of Takenaka's group. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

The thermal stability of DNA shows a linear pressure dependence - Why?

Jurij Lah, San Hadži

Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia.

DNA adopts unique structures that are required to perform its biological function. The stability of these structures is determined mainly by base stacking, base pairing, electrostatic interactions, and the composition of the surrounding medium, with hydration being one of the most important factors. Increasing the pressure affects the non-covalent interactions and can disrupt the structure and function of DNA. According to Le Chatelier's principle, the population of DNA structures shifts toward those occupying a smaller volume. Thus, a change in pressure affects the thermal stability of DNA, T_m. The pressure dependence of T_m may be described by the Clapeyron equation

$$dT_m/dp = T_m \Delta V/\Delta H$$

where ΔV is the volume and ΔH the enthalpy of unfolding. The linear dependence of T_m on p is systematically observed up to $p \approx 200$ MPa. Why dT_m/dp is independent of p, even though T_m depends on p and ΔV and ΔH are functions of T and p, is not well understood. Moreover, it is not clear why dT_m/dp depends on the composition of the solution (e.g. counterion and water activity) in a specific way as observed experimentally.[1,2]. The thermodynamic reasons for such behaviour of DNA duplexes and quadruplexes will be discussed.

References:

1. Takahashi S. and Sugimoto N. *Angew. Chem. Int. Ed*. 52, 13774 –13778 (2013)
2. Rayan G. and Macgregor Jr R.B. *Biophysical Chemistry* 144, 62–66 (2009)

Acknowledgements: The financial support of the Slovenian Research Agency projects P1-0201 and J1-1706 is gratefully acknowledged.

Genome Therapy: A New Approach for Tumour Growth Inhibition

Zhen Xi

State Key Laboratory of Elemento-organic Chemistry, Department of Chemical Biology,
National Pesticide Engineering Research Centre, College of Chemistry, Nankai University, Tianjin, China

By central dogma, chromatin DNA was spatial-temporally regulated at multiple levels, including chromatin unfolding, DNA transcription, post-transcription, mRNA translation, post-translation. Accordingly, gene regulation tools at multiple levels were also discovered and artificially exploited for different biological studies and gene therapy applications. With the deep knowledge of the evolution and progression of complex diseases such as cancers, single target-based gene therapy has met with great challenges in reducing side-effect and drug resistance. The fast development of novel gene delivery methods and gene regulation technologies moved gene therapy from single gene causing illnesses to multiple gene-associated disorders in a more personalized, precise, safe and efficient manner. To find an efficient therapy solution, the strategies of mimicking chromatin DNA to precisely regulate gene expression through combining various gene regulation tools at different levels as an integrative toolbox are promising to combat complex diseases in the near natural way. In this way, a number of gene regulation tools could be rationally integrated as a smart toolbox and loaded into chromatin-like payloads to mimic the chromosome-mediated gene decoding process for disease therapy. Therefore, we here termed this artificial chromosome-like gene network regulation at multiple levels with different tools simultaneously as genome therapy. In this talk, we will discuss our efforts towards multiple gene regulations for antitumor efficacy with branch-PCR assembled gene nano-vector mimicking chromatin-like activity.

References:

1. Liu, J.; Wang, R.; Ma, D.; Ouyang, D.; Xi, Z. Efficient construction of stable gene nanoparticles through polymerase chain reaction with flexible branched primers for gene delivery. *Chem Commun* **2015**, *51*, 9208-9211.

2. Liu, J.; Wang, R.; Ma, D.; Li, Y.; Wei, C.; Xi, Z. Branch-PCR constructed stable shRNA transcription nanoparticles have long-lasting rnai effect. *ChemBioChem* **2016**, *17*, 1038-1042.

3. Cheng, L.; Deng, H.; Ma, D.; Zhai, B.; Zhang, Q.; Li, L.; Xi, Z. Branch-PCR constructed tp53 gene nanovector for potential cancer therapy. *Chem Commun* **2018**, *54*, 9687-9690.

4. Cheng, L.H.; Ma, D.J.; Lu, L.Q.; Ouyang, D.; Xi, Z. Building customizable multisite-targeting c-myc shRNA array into branch-PCR-constructed DNA nanovectors for enhanced tumor cell suppression. *Chemistryselect* **2020**, *5*, 10250-10255.

5. Lu, L.; Fang, T.; Pang, T.; Chen, Z.; Cheng, L.; Ma, D.; Xi, Z. The potential application of branch-PCR assembled PTEN gene nanovector in lung cancer gene therapy. *ChemBioChem* **2022**, *23*, e202200387.

6. Lu, L.; Rao, D.; Niu, C.; Cheng, L.; Ma, D.; Xi, Z. Dibenzocyclooctyne-branched primer assembled gene nanovector and its potential applications in genome editing. *ChemBioChem* **2022**, *23*, e202100544.

7. Cheng, L.H.; Lu, L.Q.; Chen, Z.; Ma, D.; Xi, Z. Multiple gene regulation for enhanced antitumor efficacy with branch-PCR assembled TP53 and MYC gene nanovector. *Molecules*. 2022, *27*(20), 6943.

8. Ma, D., Xi, Z. (2022). Gene Nanovector for Genome Therapy. In: Sugimoto, N. (eds) Handbook of Chemical Biology of Nucleic Acids. Springer, Singapore. https://doi.org/10.1007/978-981-16-1313-5_60-1.

Photophysics and photochemistry of I-motifs: some insights from quantum mechanical calculations.

Roberto Improta

Consiglio Nazionale delle Ricerche, Istituto di Biostrutture e Bioimmagini (IBB-CNR), Naples, Italy

By using a computational approach already profitably used in the study of canonical and non-canonical DNA structures,[1-4] we try to provide a comprehensive picture of the photoactivated behaviour of the core of I-motifs, from the absorption to the emission, also considering the possible photochemical reactions.[5,6] Our model includes up to four intercalated hemi-protonated $(CH{\bullet}C)^+$ pairs, formed by a cytosine (C) and a protonated cytosine (CH^+).[5,6] We reproduce and assign their spectral signatures, i.e. infrared, absorption, fluorescence and circular dichroism spectra, disentangling the underlying chemical-physical effects. We suggest the most populated decay pathways involve two 'stacked' C and CH^+ bases, participating in excited states with a certain degrees of CT character, whereas monomer-like decay paths, where the excitation is localized on a single base, should play some role only in the ultrafast dynamics, and, on this ground, provide an interpretation for the available time-resolved spectra. We propose that a photodimerization reaction can occur on an excited state with strong $C \rightarrow CH^+$ charge transfer character and examine some of the possible photoproducts.

References:
1. Improta, R.; Douki, T. DNA Photodamage: From Light Absorption to Cellular Responses and Skin Cancer; Royal Society of Chemistry: London, UK, 2021.
2. Martínez Fernández, L., Santoro, F, Improta, R. Acc. Chem. Res. 2022, 55, 2077–2087
3. Martínez-Fernández L., Esposito L., Improta R. Photochem. Photobiol. Sci. 2020, 19, 436–444
4. Improta R., Santoro F., Blancafort L. Chem. Rev. 2016, 116, 3540–3593
5. Improta R., Int. J. Mol. Sci. 2023, 24, 12614
6. Martinez-Fernandez L., Improta, R. Photochem Photobiol. 2023; 00: 1-9. doi:10.1111/php.13832

Acknowledgements: RI thanks financial support from CNR (progetti@cnr/UCATG4 and Nutrage FOE2022) and from CN3, National Center for GeneTherapy and Drugs based on RNA technology, funded by the European Union-NextGenerationEU-PNRR.

Functional non-canonical secondary structures of nucleic acids: What do we need more of?

Claudia Sissi

Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy

After the discovery of the DNA double helix, the interest for the structural polymorphism of nucleic acids (in particular of DNA) has been confined to the lab. However, the discovery of telomerase and the dissection of its mechanism of action at molecular level remarkably prompted the interest for the so-called “non-canonical structures” as potential drug targets. Indeed, their three-dimensional features largely diverge from the most common double helix and, additionally, they occur at a small subset of genomic sites where they can exert unique functional roles. These evidences might envisage the design of selective binders that are expected to result into targeted therapeutic approaches.

In the past decades, an increasing number of high-resolution structures of these domains has been delivered. At the same time, to support their relevance in the intracellular environments, along with functional studies (i.e. luciferase assay), several sequencing approaches have been developed to map the distribution of these non-canonical arrangements along the genome under variable conditions.

Nevertheless, more data are required to efficiently identify the best targets in order to properly set up a rational drug-design approaches. Indeed, it emerged that the folding of these non-canonical structures is frequently a multi-step process with multiple “non-canonical” stable intermediates endowed with half-life comparable with the time-scale of several physiological processes. Moreover, the dynamic behaviour of the short single stranded domains (that are the most convenient to study models) might be remarkably altered when they are inserted in a longer double stranded genomic frame. Last but not least, the energetic contributions that determine the resulting population distribution are not always fully dissected.

Here, we will discuss on how different experimental evidences must be more extensively complemented to fruitfully derive a comprehensive picture of the functions of these structural elements at molecular level.

Novel Anticancer Multi-Target-Directed Ligands (MTDLs) Targeting G-Quadruplexes and Human Carbonic Anhydrases

Alessio Nocentini[1], Anna Di Porzio[2], Alessandro Bonardi[1], Carla Bazzicalupi[3], Andrea Petreni[1], Tarita Biver[4], Silvia Bua[1], Simona Marzano[2], Jussara Amato[2], Bruno Pagano[2], Nunzia Iaccarino[2], Stefano De Tito[5], Stefano Amente[6], Claudiu T. Supuran[1], Paola Gratteri[1], Antonio Randazzo[2]

[1] NEUROFARBA Department, Pharmaceutical and Nutraceutical Section and Laboratory of Molecular Modeling Cheminformatics & QSAR, University of Florence, Florence, Italy
[2] Department of Pharmacy, University of Naples Federico II, Naples, Italy
[3] Department of Chemistry "Ugo Schiff", University of Florence, Florence, Italy
[4] Department of Chemistry and Industrial Chemistry, University of Pisa, Pisa, Italy
[5] Molecular Cell Biology of Autophagy Laboratory, The Francis Crick Institute, London, UK
[6] Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Naples, Italy

Compared to the association of medicines, multi-target-directed ligands (MTDLs) can potentially offer a more predictable pharmacokinetic profile, reduced risk of drug-drug interactions, and a higher adherence to therapy. In the present investigation, we propose MTDLs hitting two highly promising anticancer targets: G-quadruplex (G4) structures and human carbonic anhydrases (hCAs) IX and XII. G4s are noncanonical four-stranded nucleic acid secondary structures which can arise from guanine-rich regions, including telomeres and oncogene promoters. Induction and/or stabilization of G4s by means of small molecules represent a potential anticancer tool, leading to telomere maintenance problems and reduced oncogene expression.[1,2] Carbonic anhydrases IX and XII are two proteins that have been found to be upregulated in many hypoxic tumors, contributing to an aggressive metastatic phenotype.[3] Inhibition of hCAs IX and XII has been consolidated over the last two decades as an innovative chemotherapeutic strategy against solid (and hypoxic) tumors.[4] We synthesized a library of molecular conjugates containing both a well-known G4 stabilizer (berberine) and an inhibitor of hCAs IX/XII as new potential multi-target anticancer agents. The in vitro ability of the newly synthesized compounds to stabilize G4 structures and inhibit the tumour-associated hCAs IX and XII was assessed. The most promising derivatives were subjected to a further biological characterization, leading to good cytotoxic effect on CA IX-positive human cervix cancer cells, even greater under hypoxic conditions, as well as to the ability to stabilize G4 structures also in the cellular environment.

References:
1. Zahler A.M., Williamson J.R., Cech T.R. and Prescott D.M., Nature, 1991, 350 (6320), 718–20.
2. Siddiqui-Jain A., Grand C.L., Bearss D.J. and Hurley L.H., Proc Natl Acad Sci USA, 2002, 99 (18), 11593–8.
3. Stock C. and Schwab A., Pflügers Archiv - Eur. J. Physiol., 2009, 458, 981–992.
4. Neri D. and Supuran C.T., Nat Rev Drug Discov., 2011, 10, 767–77.

Acknowledgements: The authors acknowledge the Italian Association for Cancer Research for the financial support (IG 26313 to Antonio Randazzo). Moreover, Anna Di Porzio is a recipient of a FIRC-AIRC postdoc fellowship (26644).

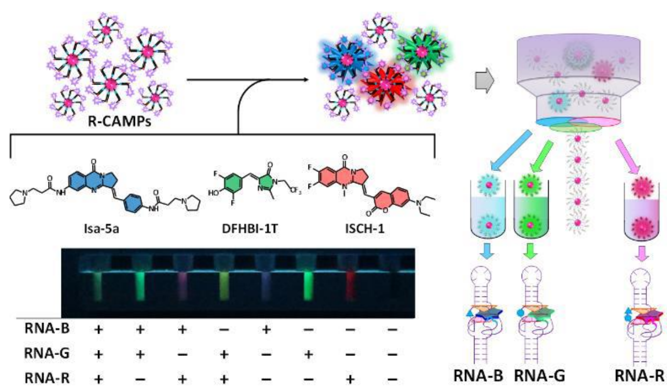
Versatile binding core for small fluorogens consisting of noncanonical base pairs

Tamaki Endoh[1], Jia-Heng Tan[2], Shuo-Bin Chen[2], Sinjan Das[1], Shuntaro Takahashi[1], Naoki Sugimoto[1,3]

[1] *Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, Kobe, Japan*
[2] *School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China*
[3] *Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, Kobe, Japan*

Aptamers, which dramatically enhance fluorescence of small ligands called fluorogens, are known as light-up aptamers. A ligand biding core of Spinach, which is one of the pioneering light-up aptamers, consists of U-A-U triad and G-quartet. These non-canonical base pairs sandwich the small ligand. Based on the structure information, we envisioned that the core structure accommodates other small molecules with relatively planer structure. Based on the assumption, we have performed selection of orthogonal light-up aptamers. Randomized nucleotides were introduced at and around the ligand binding core of Spinach. Then, we have selected RNAs, which distinguish different fluorogens, by using RNA-capturing microsphere particles (R-CAMPs).[1] We have succeeded to obtain the orthogonal light-up aptamers that emit blue, green, or red fluorescence with their ligand.[2]

Stimulated by the results, we have tried to construct light-up DNA aptamers by using selection based on DNA-immobilizing particles. Since the strategy to sandwich the small chemicals with unique core structure seemed efficient, we have focused on an i-motif structure. The first and the third loop regions, which parallelly arching out from the core structure of i-motif, are likely able to sandwich small molecules. For example, we recently found that crystal violet interacts with i-motif structure derived from *BCL2* gene and emits its fluorescence.[3] Millions of particles, each of which individually immobilizes unique i-motif structure derived from library having randomized loop sequence, were constructed and mixed with fluorogens at pH 7 or 5. We have confirmed that particles showed strong fluorescence at pH 5 comparing to those at pH 7, suggesting some i-motif structures on the particles interacted with the fluorogens.



Orthogonal light up aptamers, which distinguish target fluorogen and emit blue, green, or red fluorescence

Based on these results, the stable core unit containing non-canonical base pairs can be a versatile structure to accommodate fluorogens to variate light-up aptamers.

References:

1. a) Endoh, T., Ohyama, T. and Sugimoto, N., ***Small***, 2019, 15, 1805062; b) Satpathi, S., Endoh, T., Podbevšek, P., Plavec, J. and Sugimoto, N., ***Nucleic Acids Res***., 2021, 49, 8449–8461; c) Endoh, T., Takahashi, S. and Sugimoto, N., ***Chem. Commun.***, 2023, 59, 872-875
2. Endoh, T., Tan, J.-H., Chen, S.-B. and Sugimoto, N., ***Anal. Chem.***, 2023, 95, 976-985
3. Das, S., Takahashi, S., Ohyama, T., Bhowmik, S. and Sugimoto, N., ***Sci. Rep.***, 2023, 13, 14338.

Acknowledgements: 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) (Grant No. 18KK0164, and 21H05108), especially for Grant-in-Aid for Scientific Research (S) (22H04975), JSPS Core-to-Core Program (JPJSCCA20220005), JSPS A3 Foresight Program, The Hirao Taro Foundation of KONAN GAKUEN for Academic Research, and the Chubei Itoh Foundation.

G-quadruplexes in the antisense promoter of HTLV-1 virus

Emanuela Ruggiero, Irene Zanin, Beatrice Tosoni, Sara N. Richter

Department of Molecular Medicine, University of Padua, Via Gabelli, 63, Padua, Italy

The HTLV-1 virus is a delta-retrovirus (RV) responsible for the onset of two distinct pathologies, the adult T-cell leukemia (ATL) and the HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). To date, the virus is estimated to affect nearly 15 million people worldwide and the lack of successful treatments provides an extremely unfortunate prognosis. Given that HTLV-1 is the most carcinogenic of all oncoviruses, with 5-10% of infected individuals developing cancer, new insights into viral pathogenesis and the disclosure of feasible antiviral targets, are imperative.[1] Upon entry into the cell, the HTLV-1 genome is integrated into the host cell DNA: at this stage, the viral genome, called the provirus, is flanked by two identical long terminal repeats (LTRs) at the 5'- and 3'- ends. The 5'-LTR serves as the promoter for all structural and most accessory and regulatory genes, while the 3'-LTR initiates transcription from the negative strand of the provirus and acts as the promoter for the sole antisense transcript of the virus, the HTLV-1 basic leucine zipper factor (HBZ).[2]

We have recently demonstrated that the LTRs of most RVs are highly enriched in guanines, making them prone to form non-canonical DNA structures such as G-quadruplexes (G4s). G4s have already been shown to regulate virus transcription and progression, resulting in promising antiviral targets. Among RVs, delta-RVs were the most enriched in G4-forming sequences, with low interspecies variability.[3] These findings prompted a deeper investigation into the HTLV-1 virus, leading to the identification of seven, highly conserved putative G4-forming sequences, all characterized by GG-tracts and located in the reverse strand of the provirus LTR. Such a peculiar location suggests a possible involvement of HTLV-1 LTR G4s in the regulation of the viral antisense transcription.

Here, we show that HTLV-1 LTR putative G4 sequences do indeed fold into G4s, which are further stabilized upon binding to the G4 ligand BRACO-19. G4 stabilization was also observed in stop assays, where the HTLV-1 G4s were able to halt polymerase progression, both in a single- and in a double-stranded context. Finally, we directly captured HTLV-1 3'-LTR G4s by chromatin immunoprecipitation using the G4-specific antibody BG4, followed by qPCR.

Overall, our data demonstrate that the 3'-LTR antisense promoter is enriched in two-layered G4s, which interfere with polymerase progression, thus inferring a possible modulation of antisense transcription. Our data provide new insights into viral pathogenesis and lay the basis for a novel antiviral approach in the fight against HTLV-1.

References:

1. Bangham C.R.M. HTLV-1 persistence and the oncogenesis of adult T-cell leukemia/lymphoma. Blood. 141, 2299-2306 (2023).
2. Ma, G., Yasunaga, Ji. & Matsuoka, M. Multifaceted functions and roles of HBZ in HTLV-1 pathogenesis. Retrovirology 13, 16 (2016).
3. Ruggiero,E., Tassinari,M., Perrone,R., Nadai,M. and Richter,S.N. Stable and Conserved G-Quadruplexes in the Long Terminal Repeat Promoter of Retroviruses. ACS Infect. Dis., 5, 1150–1159 (2019).

Flex-Nucleosides – A Strategic Approach to Broad-Spectrum Antiviral Therapeutics

Katherine Seley-Radtke

Department of Chemistry & Biochemistry, University of Maryland, Baltimore County, Baltimore, MD, USA

Over the past several decades nucleos(t)ides have maintained a prominent role as one of the cornerstones of antiviral and anticancer therapeutics.[1] As a result, numerous approaches to nucleos(t)ide and nucleic acid drug design have been pursued. 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]. In contrast, introduction of flexibility to the nucleobase scaffold has only more recently gained significance with the invention of our fleximers.[2] This modification has led to a significant improvement in antiviral activity, and in some cases endowing the nucleoside with potent broad-spectrum activity when the parent rigid nucleoside was inactive.[2-5] 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.[2] A brief history of their development, and recent antiviral findings for this innovative class of nucleos(t)ides will be discussed.

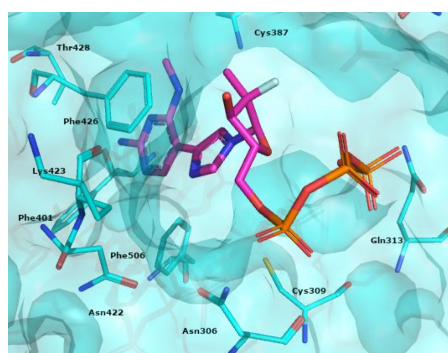


Illustration of Flex-AT-527-TP in the CoV-2 Nsp-14/Nsp10 complex

References:

1. *Seley-Radtke, K. L. and Yates, M. *Antiviral Res*. **2018**, *154*, 66-86; and **2019**, *162*, 5-21.
2. *Seley-Radtke, K. L. *Antiviral Chem Chemoth*, **2018**, 26, 1-12.
3. Thames, J. E.; Waters III, C. D.; Valle, C.; Bassetto, M.; Aouadi, W.; Martin, B.; Falat, A.; Coutard, B.; Brancale, A.; Canard, B.; Decroly, E.; *Seley-Radtke, K. L. *Bioorg Med Chem* **2020**, *28*, 115713.
4. Yates, M.; Raje, M.; Chatterjee, Soloveva, V.; Bavari, S.; *Seley-Radtke, K. L. *Bioorg Med Chem Lett* **2017**, *27*, 2800-2802.
5. Peters, H. L.; Jochman, D.; Posthuma, C. C.; de Wilde, A.; Snijder, E.; Neyts, J.; *Seley-Radtke, K. L.; *Bioorg Med Chem Lett* **2015**, *25*, 2923-2926.

Acknowledgements: The author thanks these funding sources: National Institutes of Health/NIH AViDD U19 AI171292, and North Carolina State Appropriation grant #22-4446, and the UNC READDI AViDD center and her many other collaborators for screening, modeling, biochemical and mechanistic studies.

Aptamers involving base-appended bases

Masayasu Kuwahara

Graduate School of Integrated Basic Sciences, Nihon University, Tokyo, Japan

Generally, single-stranded nucleic acids have a unique three-dimensional structure depending on their base sequence. Thus, the nucleic acid strand acts as a ligand molecule (key) or receptor molecule (keyhole) for a specific target molecule, like a “lock-and-key” relationship. Nucleic acid strands (DNA, RNA) that exhibit this specific affinity are called nucleic acid aptamers. Nucleic acid aptamers, unlike antibodies, do not rely on immunization of small animals, and can be used to obtain nucleic acids that specifically bind to a target by the SELEX method using a mixture (library) of nucleic acid strands with different base sequences. Therefore, although the SELEX method is limited to nucleic acid strands, it has the advantage that in principle the desired ligand and receptor molecules for any target can be generated *in vitro*. Nucleic acid aptamers created in this way are expected to be applied to biosensors and therapeutic drugs. Our previous studies[1–10] have shown that base modification by introducing nitrogen-containing fused bicyclic compounds (*e.g.*, base-appended bases) *via* linkers is effective in terms of improving target binding affinity and specificity. Although there have been reports using X-ray crystallography to verify the interaction between a target and an artificial nucleic acid aptamer complex, it has not yet reached the level where it is possible to design and predict effective base modifications. Much research remains regarding the selection of chemically modified aptamers.

In recent years, the problem of substrate specificity of polymerases in artificial nucleic acid strand synthesis,[11,12] which had been a bottleneck until then, has been greatly improved by a group of *KOD* DNA polymerase variants that we have constructed.[13] A powerful tool, the group of variants, allows us to further investigate the effects of optimizing the linker structure of modified bases and introducing modifying groups into nitrogen-containing fused bicyclic compounds. In this presentation, we will show the examples of aptamers involving base-appended bases, and tolerance of the variants for the modifications of nucleotides.

References:

1. Kasahara Y, Irisawa Y, Fujita H, Yahara A, Ozaki H, Obika S, Kuwahara M. *Anal Chem.* 2013, 85, 4961–4967.
2. Imaizumi Y, et al. *J Am Chem Soc.* 2013, 135, 9412–9419.
3. Kuwahara M, Hagiwara K, Ozaki H. Modified nucleic acids in biology and medicine (RNA technologies) 2016, 429–453.
4. Minagawa H, et al. *Sci Rep.* 2017, 7, 42716.
5. Minagawa H, Kataoka Y, Fujita H, Kuwahara M, Horii K, Shiratori I, Waga I. *Int J Mol Sci.* 2020, 21, 2683.
6. Minagawa H, Kataoka Y, Kuwahara M, Horii K, Shiratori I, Waga I. *Anal Biochem.* 2020, 594, 113627.
7. Minagawa H, Shimizu A, Kataoka Y, Kuwahara M, Kato S, Horii K, Shiratori I, Waga I. *Anal Chem.* 2020, 92, 1780–1787.
8. Idili A, Arroyo-Currás N, Ploense KL, Csordas AT, Kuwahara M, Kippin TE, Plaxco KW. *Chem Sci.* 2019, 10, 8164–8170.
9. Kataoka Y, Kuwahara M. *CSJ Curr Rev.* 2021, 41, 76–85.
10. Existing Registered Patents (as of May 31, 2023), Kuwahara M *et al.*, JP6744028, JP6763552, JP6763551, JP6795211, JP6763553, JP6963221, JP7168920, US10760084, US10781230, US10611791, US11236342, ZL201780056368.1, ZL201780056298.X, ZL201780056678.3, ZL201780072997.3, EP3514165, EP3514164, EP3514237, EP3783105, EP3546471.
11. Sawai H, Ozaki AN, Satoh F, Ohbayashi T, Masud MM, Ozaki H. *Chem Commun.* 2001, 24, 2604–2605.
12. Kuwahara M, et al. *Nucleic Acids Res.* 2006, 34, 5383–5394.
13. Hoshino H, Kasahara Y, Kuwahara M, Obika S. *J Am Chem Soc.* 2020, 142, 21530–21537.

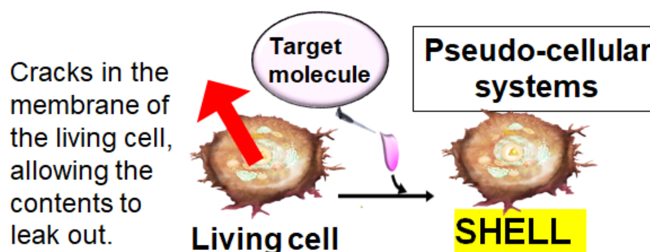
Acknowledgements: The author is grateful to all the collaborators and graduate students cited in the publications. The author is especially grateful to Professor Naoki Sugimoto of Konan University, Dr. Ikuo Shiratori and co-workers of NEC Solution Innovators, Ltd., and Dr. Hiroto Fujita and Dr. Yuka Kataoka of Nihon University. This study was partly supported by Research Grant of Institute of Natural Science at Nihon University for 2023, the Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (JSPS), and a grant from the Adaptable and Seamless Technology Transfer Program through Target-Driven R&D, no. AS2525029M, from the Japan Science and Technology Agency (JST), and by the Basic Science and Platform Technology Program for Innovative Biological Medicine, no. JP18am0301026, from the Japan Agency for Medical Research and Development (AMED).

Development of pseudo-cellular systems to understand effects of molecular environments on G-quadruplex behaviours depending on the type of cells

Hisae Tateishi-Karimata[1], Keiko Kawauchi[2], Shuntaro Takahashi[1], Naoki Sugimoto[1,2]

[1] *Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, Kobe, Japan*
[2] *Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, Kobe, Japan*

The structure and stability of nucleic acids in living cells hold significant interest across various research fields, encompassing medical, pharmaceutical, and materials sciences1. While the canonical structure of nucleic acids is a duplex, recent reports have highlighted the formation of non-canonical structures such as triplexes, G-quadruplexes, and i-motifs within cells. These non-canonical structures have emerged as key regulators of essential biological processes, including 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 progressions3. 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. In this study, we developed a novel pseudo-cellular system using different types of cancer cells. We used the cells as the pseudo-cellular system by cracking the membrane of the cells and allowing the contents to flow out. Then, we introduced fluorescently labelled G-quadruplexes into the pseudo-cellular system and analysed the behaviour of G-quadruplexes.

As results, the G-quadruplexes were stabilized in the pseudo-cellular system due to a favourable enthalpy contribution. During cancer progression, overexpression of K^+ channels results in fluctuating intracellular K^+ concentrations.[3] Therefore, we analysed the stability of G-quadruplexes at different K^+ concentrations. Interestingly, the G-quadruplexes in the pseudo-cellular system were stable even at low potassium concentrations. We will discuss quantitatively how the intracellular environment alters the interaction between ions and G-quadruplexes and affect biological reactions in the presentation.

References:
1. Tateishi-Karimata, H. and Sugimoto, N., ***Nucleic Acids Res.***, 2021, 49, 7839-7855.
2. a) Tateishi-Karimata, H., Isono, N. and Sugimoto, N., ***PLoS ONE***, 2014, 9, e90580; b) Matsumoto, S., Tateishi-Karimata, H. and Sugimoto, N., ***Chem. Commun.***, 2022, 58, 12459-12462.
3. Tateishi-Karimata, H., Kawauchi, K. and Sugimoto, N., ***J. Am. Chem. Soc***. 2018, 140, 642–651.

Acknowledgements: 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 for Grant-in-Aid for Scientific Research (S) (22H04975), Grant-in-Aid for Transformative Research Areas (B) (21H05109), and JSPS Core-to-Core Program (grant number: JPJSCCA20220005), The Hirao Taro Foundation of KONAN GAKUEN for Academic Research, the Naito Foundation, and the Chubei Itoh Foundation.

The role of pre-miRNA structures in their biogenesis

Vito Šuklje[1,2], Sicong Ma[3], Janez Plavec[1,2], Sarah C. Keane[3,4], Anita Kotar[1]

[1] *National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia*
[2] *Faculty of Chemistry and Chemical Technology, Večna pot 113, Ljubljana, Slovenia*
[3] *Biophysics Program, University of Michigan, Ann Arbor, MI 48109, USA*
[4] *Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA*

microRNAs (miRNAs) are short non-coding RNAs which play important roles in the regulation of gene expression through targeting messenger RNAs for post-transcriptional gene silencing.[1] Mature miRNAs are produced via a series of enzymatic processing events from longer primary and precursor transcripts (primary miRNA > [processed by DROSHA] > precursor miRNA > [processed by DICER]> mature miRNA). The production of individual miRNAs must be strictly controlled, as dysregulation of miRNA levels often leads to disease states.[2] While proteins are common regulators of various steps in the miRNA biogenesis pathway, miRNA precursors (for example pre-miR-31 and members of let-7 family) for which no proteins partners in processing events have been identified are known. Indicating, that these pre-miRNAs are not only passive actors in their biogenesis, but they actively explore their conformational space in order to regulate their own processing. Recently, we revealed a mechanism by which pre-miR-31 processing by the Dicer–TRBP complex is regulated.[3] We are continuing our study on the structure of pre-let-7f-2, a member of miRNA family,[4] which functions in adults as a fundamental tumor suppressor. Insights into the structure and molecular determinants of miRNA biogenesis would have great implications for RNA-targeted drug development.

References:
1. Gebert, L.F.R., MacRae, I.J., *Nat. Rev. Mol. Cell Biol.*, 2019, 20, 21 - 37
2. Lin S., Gregory R.I., *Nat. Rev. Cancer.*, 2015, 15, 321 - 333
3. Ma S., Kotar A., Hall I., Grote S., Rouskin S., Keane S.C. *Proc. Natl. Acad. Sci. USA*, 2023, 120(39): e2300527120
4. Ma Y., Shen N., Wicha M.S, Luo M. *Cells*, 2021, 10(9): 2415

Acknowledgements: V.Š., J.P., A.K. acknowledge the financial support from the Slovenian Research and Innovation Agency – ARiS (grants: P1-0242 and Z1-3198). S.C.K thank National Institute of General Medical Sciences of the National Institutes of Health (grant R35 GM138279), Research Corporation for Science Advancement Cottrell Scholar Award 28248, and the Pew Charitable Trusts Scholars Program.

POSTER PRESENTATIONS

Effect of oxidative stress on nucleic acid structural equilibria

Simon Aleksič[1,2], Peter Podbevšek[1], Janez Plavec[1,2,3]

[1] Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia
[2] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia
[3] EN-FIST Centre of Excellence, Trg OF 13, 1000 Ljubljana, Slovenia

Disproportionate production of reactive oxygen species in cells leads to oxidation of deoxyribonucleic acids, with guanines being most susceptible to oxidation among the nucleobases. Guanines exhibit an even lower redox potential when several concurrent guanine nucleobases are stacked in a nucleotide sequence, forming a G-tract. Guanine-enriched regions are predominantly found in promoter and telomere regions of the genome and can form G-quadruplexes. The main building block of a G-quadruplex is the G-quartet, a planar arrangement of four guanine nucleobases, which are hydrogen bonded in the Hoogsteen geometry. Since oxidized nucleotides exhibit altered hydrogen bonding capabilities and conformational changes due to the presence of bulky oxo groups, oxidative damage of guanine-rich DNA may lead to structural rearrangements and therefore affect cellular mechanisms, such as replication and transcription.[1,2]

Structural changes caused by an oxidative product of guanine were probed by incorporating 8-oxoguanine nucleotides into a model sequence. Using NMR spectroscopy, we determined that 8-oxoguanine nucleotides do not hinder G-quadruplex formation and that 8-oxoguanine moieties can form a fully substituted 8-oxoguanine quartet with a distinct hydrogen-bonding scheme. DFT optimization revealed that the oxidized quartets exhibit a larger central cavity compared to G-quartets, allowing binding of larger cations.[3] In some cases, two species differing only in cation coordination were identified and were found to be in the intermediate exchange regime on the NMR timescale. Our further studies are focused on the effect of guanine oxidation on structural equilibria of dsDNA sequences.

References:
1. Marsico G., Chambers V.S., Sahakyan A.B., McCauley P., Boutell J.M., Di Antonio M., Balasubramanian S., Nucleic Acids Res., 2019, 47, 3862 – 3874
2. Redstone S.C.J., Fleming A.M., Burrows C.J., Chem. Res. Toxicol., 2019, 32, 437 – 446
3. Aleksič S., Podbevšek P., Plavec J., Biochemistry, 2022, 61, 2390 – 2397

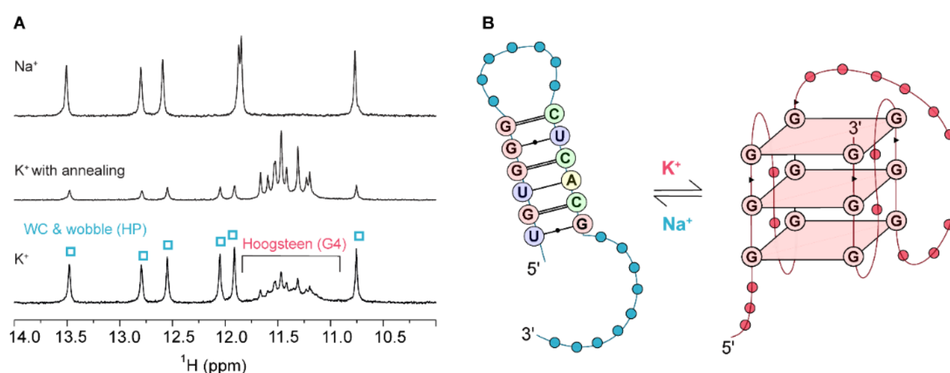
Acknowledgements: This work is part of the PhD “The effect of oxidative stress on guanine-rich genomic regions” supported by CERIC-ERIC and ARRS research programme P1-0242.

A cation-dependent structural switch in the G-rich region of lncRNA REG1CP

Jasna Brčić[1], Janez Plavec[1,2,3]

[1] Slovenian NMR Center, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia
[2] EN-FIST Center of Excellence, Trg Osvobodilne fronte 13, 1000 Ljubljana, Slovenia
[3] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia

Long-noncoding RNA (lncRNA) REG1CP was shown to promote cancer cell proliferation and tumorigenicity by activating the REG3A gene in colorectal cancer. The mechanism involves the lncRNA acting as a two-site address code which necessitates an RNA: DNA triplex formation (anchoring REG1CP) at one end and a structure-dependent binding and recruitment of helicase FANCJ to the REG3A locus on the other end[1]. FANCJ facilitates transcription by unwinding the duplex, thus enhancing DNA accessibility within the REG3A promoter and activating transcription which is normally repressed. A G-rich sequence within REG1CP was proposed to fold into a non-canonical structure called G-quadruplex (G4)[1] which is recognized by FANCJ, a helicase known to bind G4 structures[2]. We initiated an NMR study on a 30-nt oligoribonucleotide (ORN) derived from the G-rich region of REG1CP to gain insight into its structural properties and the basis of its interaction with FANCJ. ^{1}H NMR study clearly revealed the presence of Hoogsteen hydrogen-bonded imino protons and imino- protons involved in canonical Watson-Crick base pairs. This suggests formation of two stable, mutually exclusive structures; a canonical hairpin (HP) and a noncanonical G4. Two structures coexist in slow exchange in K^+ solution, while only HP is formed in Na^+ ion containing solution (Fig. 1A). Variable temperature and time-dependent observation of folding in the presence of K^+ ions also showed that HP is kinetically favored, while G4 is thermodynamically more stable. The folding topologies of the two structures are elucidated by 2D NMR (Fig. 1B). ^{1}H NMR titration with a peptide derived from FANCJ was used to establish its binding preference for the target G-rich ORN and the alternate structures it forms. Our structural study suggests that interaction between REG1CP and FANCJ might be modulated by conformational switching between the G4 and HP structures within REG1CP that could affect REG1CP-mediated expression of REG3A in colon cancer.



Folding characteristics of the G-rich ORN derived from REG1CP. A) Imino regions of the ^{1}H NMR spectra of ORN in the presence of K^+ and Na^+ ions. B) Folding topologies of the G4 and HP.

References:
1. Yari, H.; Jin, L.; Teng, L.; Wang, Y.; Wu, Y.; Liu, G. Z.; Gao, W.; Liang, J.; Xi, Y.; Feng, Y. C.; et al., *Nat Commun*, **2019**, *10* (1), 5334.
2. Wu, C. G.; Spies, M., *Nucleic Acids Res*, **2016**, *44* (18), 8742–8753.

Acknowledgements: This research was supported by the Slovenian Research Agency (ARRS P1-0242).

The genomic landscape of vimentin-G4 repeats interactions within human metastatic cancer cells

Silvia Ceschi[1], Marco Grazioli[2], Riccardo Rigo[1], Alessia Paro[1], Marta Cozzaglio[1], Greta Mucignat,[3] Mery Giantin[3], Stefano Toppo[2], Claudia Sissi[1]

[1] *Dept. of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy*
[2] *Dept. of Molecular Medicine, University of Padua, Padua, Italy*
[3] *Dept. of Comparative Biomedicine and Food Science, University of Padua, Legnaro, Italy*

Research in the field has come a long way since when G4s were considered only as unwanted structures that could pose a threat to DNA replication and transcription. Indeed, recent advances suggest that both transcription factors and chromatin remodelling proteins can bind to G4s to ultimately tune the epigenetic landscape, pointing to G4s as important genomic regulatory elements. The recent release of the complete gapless sequence of the human genome allowed the characterization of highly repetitive genomic regions and highlighted the presence of tandem repeats of G4 forming motifs. Here, the folding of G4 repeats (higher order arrangements that arise from the close proximity of multiple G4s) is feasible. Vimentin, an intermediate filament protein, is the first identified protein that displays selective binding to G4 repeats vs individual G4s.[1] Vimentin is highly expressed within migratory mesenchymal cells. These are physiologically present at the early stage of embryonic development and can be pathologically reactivated through epithelial to mesenchymal transition when epithelial cancers become metastatic.[2] In the present study, we mapped the genomic distribution of vimentin within metastatic cancer cells, highlighting its colocalization with G4 repeats. Our data suggest that G4 repeats may exist as primary structural elements, able to drive the recruitment of architectural proteins such as vimentin to ultimately reshape the higher-order genome folding during important physiological processes such as cell development and migration.

References:
1. Ceschi, S.; Berselli, M.; Cozzaglio, M.; Giantin, M.; Toppo, S.; Spolaore, B.; Sissi, C. Nucleic Acids Res. 2022 Feb 22;50(3):1370-1381.
2. Strouhalova, K.; Přechová, M.; Gandalovičová, A.; Brábek, J.; Gregor, M.; Rosel, D. Cancers (Basel) 2020 Jan 11;12(1):184.

Acknowledgment: This work was supported by University of Padua and AIRC.

Exploring the properties of dimeric analogues of anti-HMGB1 G-quadruplex-forming aptamers

Andrea Criscuolo, Ettore Napolitano, Claudia Riccardi,
Domenica Musumeci, Daniela Montesarchio

Department of Chemical Sciences, University of Naples Federico II, Naples, Italy

High-Mobility Group Box 1 (HMGB1) is an abundant, highly conserved, non-histonic nuclear protein present in almost all eukaryotic cells.[1,2] It is also a DNA binding protein, involved in critical biological processes, such as DNA transcription, replication, repair, and recombination.[3] In an inflammatory state, HMGB1 is actively secreted from immune cells in the extracellular matrix, where it behaves as a proinflammatory cytokine,[4] contributing to the pathogenesis of various chronic inflammatory and autoimmune diseases as well as cancer.[5] Given the multiple roles of the protein in these pathologies, identification of HMGB1-inhibitors is of considerable interest.[6,7]

Considering the ability of this protein to induce bending in double-stranded DNA,[8,9] as well as the identification of HMGB1 as a telomeric and non-telomeric G-quadruplex (G4)-interacting protein,[10,11] in a recent work we identified a set of G4-forming aptamers from a well-designed library of G-rich oligonucleotides able to interact with high affinity with the protein and also inhibit the HMGB1-induced cell migration.[12] A more in-depth biophysical and biological characterization of one of the best anti-HMGB1 aptamers revealed that its efficacy was mostly due to its ability to spontaneously form dimeric species. In this context, obtaining dimeric analogues in which the monomeric aptamers are covalently linked could produce a significant improvement in terms of protein binding affinity and inhibition of the HMGB1-pathological activity. Here we present the design, synthesis and evaluation of the biophysical properties of a set of covalent dimers of one of the best G4-forming anti-HMGB1 aptamer. These novel analogues have been designed so to incorporate linkers with different features – in order to develop optimized constructs that can better interact with HMGB1 and inhibit the protein pathological activities - and have been studied using different techniques, such as Circular Dichroism, UV-vis spectroscopy, gel electrophoresis.

References:
1. G. H. Goodwin, C. Sanders, E. W. Johns, P. Electrophoresis, Eur. J. Biochem. 1973, 38, 14–19.
2. G. H. Goodwin, E. W. Johns, Method Cell Biol. 1977, 16, 257–267.
3. T. Ueda, M. Yoshida, BBA-Gene Regul Mech. 2010, 1799, 114–118.
4. U. G. Andersson, K. J. Tracey, J. Intern. Med. 2004, 255, 318–319.
5. R. Kokkola, Å. Andersson, G. Mullins, T. Östberg, C. J. Treutiger, B. Arnold, P. Nawroth, U. Andersson, R. A. Harris, H. E. Harris, Scand. J. Immunol. 2005, 61, 1–9.
6. D. Musumeci, G. N. Roviello, D. Montesarchio, Pharmacol Ther. 2014, 141, 347–357.
7. E. Venereau, F. De Leo, R. Mezzapelle, G. Careccia, G. Musco, M. E. Bianchi, Pharmacol. Res. 2016, 111, 534–544.
8. C. M. Read, P. D. Cary, C. Crane-robinson, P. C. Driscoll, D. G. Norman, Nucleic Acids Res. 1993, 21, 3427–3436.
9. C. H. Hardman, R. William Broadhurst, A. R. C. Raine, K. D. Grasser, J. O. Thomas, E. D. Laue, Biochemistry-US. 1995, 34, 16596–16607.
10. J. Amato, T. W. Madanayake, N. Iaccarino, E. Novellino, A. Randazzo, L. H. Hurley, B. Pagano, Chem. Commun. 2018, 54, 9442–9445.
11. J. Amato, L. Cerofolini, D. Brancaccio, S. Giuntini, N. Iaccarino, P. Zizza, S. Iachettini, A. Biroccio, E. Novellino, A. Rosato, M. Fragai, C. Luchinat, A. Randazzo, B. Pagano, Nucleic Acids Res. 2019, 47, 9950–9966.
12. E. Napolitano, A. Criscuolo, C. Riccardi, C. L. Esposito, S. Catuogno, G. N. Roviello, D. Montesarchio, D. Musumeci, *submitted*.

Acknowledgements: The research leading to these results has received funding from AIRC (Associazione Italiana per la Ricerca sul Cancro) under IG 2020-ID. 25046-P.I. Montesarchio Daniela.

G-quadruplex binding and unwinding activity of the bacterial FeS helicase DinG

Federica D'Aria[1], Elisa De Piante[2], Luisa M. R. Napolitano [2], Jussara Amato [1], Silvia Onesti [2], Concetta Giancola[1]

[1] Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy
[2] Structural Biology Laboratory, Elettra-Sincrotrone Trieste S.C.p.A, 34149 Trieste, Italy

The *Escherichia coli* DinG protein is a DNA damage-inducible helicase implicated in DNA repair. It belongs to the iron-sulphur (FeS) cluster family and unwinds DNA duplex with a 5′ to 3′ polarity.[1] In addition, DinG shows specificity for several unusual DNA structures, being active on D-loops, R-loops and G-quadruplexes (G4s) structures.[2] Although both *E. coli* and *Mycobacterium tuberculosis* DinG were shown to be able to resolve G4 DNA structures, some of the reported results are inconsistent.

With the aim of better understanding the role of DinG in modulating G4s metabolism, the *E. coli* DinG protein was expressed, purified, and systematically investigated for its interaction with different G4 topologies, focusing on the more physiologically relevant unimolecular G4s. We performed the physicochemical and biochemical analysis of the interaction between a variety of G4s and DinG, to better understand the energetics of molecular interactions and dynamical behaviour of G4/DinG complexes. Surface Plasmon Resonance (SPR) provided the energetics and equilibrium binding constants and elucidated the thermodynamic and kinetic aspects of G4-helicase interactions. A two-step fluorescence-based helicase assay allowed testing the unwinding of DinG towards many G4 structures. The G4/DinG interactions have also been investigated in the presence of well-known G4 ligands, which can significantly interfere with several biological processes involving G4s. Our results demonstrate that DinG binds to most of the investigated G4s with little discrimination, while it exhibits a clear degree of unwinding specificity towards different G4 topologies. In addition, when the G4 structures were stabilized by ligands (Pyridostatin, PhenDC3, BRACO-19 or Netropsin), the DinG unwinding activity decreased and in most cases was abolished, with a pattern that is not simply explained by a change in binding affinity. Overall, these results have important implications for the biochemistry of helicases, strongly suggesting that when analysing the G4 unwinding property of an enzyme, it is necessary to investigate a variety of G4 substrates.[3]

References:
1. Voloshin, O.N., Vanevski, F., Khil, P.P., Camerini-Otero, R.D, J. Biol. Chem., 2003, 278, 28284–28293.
2. Thakur, R.S., Desingu, A., Basavaraju, S., Subramanya, S., Rao, D.N., Nagaraju, G., J. Biol. Chem., 2014, 289, 25112–25136.
3. De Piante E., D'Aria F., Napolitano L.M.R., Amato J., Pirrello S., Onesti S., Giancola C. Sci. Rep., 2023, 13, 12610.

Acknowledgements: This work was supported by Italian Association of Cancer Research, AIRC (grant IG 23198 to CG and IG 20778 to SO)

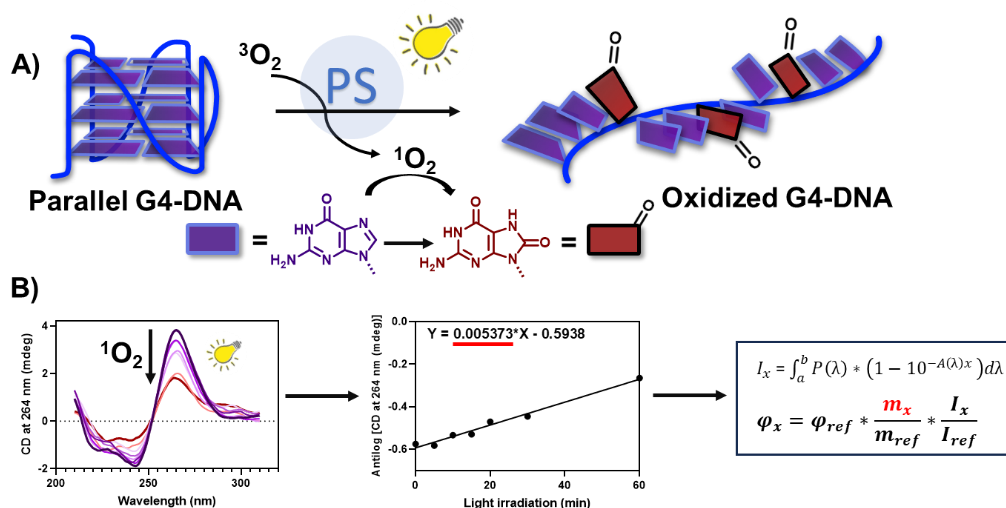
Reversing the causality: Exploiting DNA Damage as a Tool to Quantify Singlet Oxygen Production

Lessandro De Paepe[1], Annemieke Madder[1], Enrico Cadoni[1]

[1] *Department of Organic and Macromolecular Chemistry, Organic and Biomimetic Chemistry Research Group, Ghent University, Ghent, Belgium*

Singlet oxygen ($^1O_2$), the lowest excited electronic state of molecular oxygen, plays a pivotal role in a multitude of areas, ranging from material science to medicinal and pharmaceutical applications, with photodynamic therapy as a prominent example thereof. For these purposes, $^1O_2$ is intendedly produced by light irradiation of a photosensitizer (PS).[1] An important aspect in selecting a suitable PS includes its $^1O_2$-generation capacity or $^1O_2$-quantum yield ($^1O_2$-QY), and therefore, reliable methods to quantify this parameter are needed.

Various methods have been reported, either based on the direct measurement of the phosphorescence/luminescence, or the indirect reaction with chemical or fluorescent probes. However, most of these methods cannot be performed in water, which limits the extrapolation to a cellular context since the $^1O_2$-QY is highly dependent on the used solvent.[2] Here, we present an alternative methodology for the existing indirect probes, using a water-soluble parallel G-Quadruplex (G4)-sequence. Guanine-rich sequences are highly susceptible to oxidation and structural changes in G4-folding can be easily detected via Circular Dichroism (CD). Therefore, we reasoned that G4s can be used as tools to quantify the $^1O_2$-QY production of a specific visible light or UV-light excited PS, by following the transition in the CD signature in function of irradiation time. In contrast to the two most-used chemical probes, Singlet Oxygen Sensor Green and anthracene derivatives, our method does not suffer from inherent $^1O_2$ production or probe degradation, allowing us to achieve unequivocal quantification in a cell-relevant medium.[3]



A) Illustration of Singlet Oxygen mediated G-Quadruplex unfolding. B) Stepwise procedure to calculate the 1O2-QY.

References:

1. Aerssens D., Cadoni E., Tack L., Madder A. A Photosensitized Singlet Oxygen (1O2) Toolbox for Bio-Organic Applications: Tailoring 1O2 Generation for DNA and Protein Labelling, Targeting and Biosensing. *Molecules*. 2022.
2. Entradas T, Waldron S, Volk M. The detection sensitivity of commonly used singlet oxygen probes in aqueous environments. *J Photochem Photobiol B Biol.* 2020; 204:111787.
3. De Paepe L., Madder A., Cadoni E., Exploiting DNA damage as a tool to quantify singlet oxygen production, *Manuscript in submission*

Modulation of the tetrameric I-motif folding of C-rich Tetrahymena telomeric sequences by hexitol nucleic acid (HNA) modifications

Michele Ghezzo[1], Luca Grigoletto[1,2], Riccardo Rigo[1], Piet Herdewijn[2], Elisabetta Groaz[1,2], Claudia Sissi[1]

[1] *Dept. of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy.*
[2] *KU Leuven, Rega Institute for Medical Research, Medicinal Chemistry, Leuven, Belgium.*

I-motifs are non-canonical DNA structures formed by hemiprotonated cytosine-cytosine⁺ base pairs, resulting in two parallel duplexes that intercalate to create an ordered quadruplex structure. These intriguing arrangements are attractive in various fields, including gene regulation and biotechnology. Meanwhile, there is a growing interest in modified sugars to enhance nucleic acid resistance to nuclease degradation, crucial for therapeutic applications.

A unique structural feature of I-motifs is their extremely narrow minor grooves, allowing for sugar-sugar interactions. Indeed, oligonucleotides with pentose derivatives such as ribose, 2'-deoxyribose, arabinose, and 2'-deoxy-2'-fluoroarabinose exhibit very distinct folding behavior.1 Conversely, hexitol-based nucleic acids (HNA) are a still an unexplored in the I-motif field. Hexitol is a stable six-membered ring analogue compatible with A-like double helices.2. In this study, we examined the folding of HNA and RNA oligonucleotides, derived from two DNA C-rich Tetrahymena telomeric sequences which are known to form tetrameric I-motif structures. Our investigation, employing circular dichroism, differential scanning calorimetry, and NMR, compared their folding behaviors to DNA counterparts. Notably, ribose and hexitol prevented I-motif formation. However, strategic placement of hexitol residues at the 3'-end allowed the folding into I-motifs and modulated the equilibrium of different topological species in solution.

References:
1. Abou Assi,H., Garavís,M., González,C. and Damha,M.J. (2018) i-Motif DNA: structural features and significance to cell biology. *Nucleic Acids Research*, **46**, 8038–8056.
2. Ruben Declercq, Arthur Van Aerschot, Randy J. Read, Piet Herdewijn, Luc Van Meervelt, (2002) Crystal Structure of Double Helical Hexitol Nucleic Acids. *ACS Publications*.

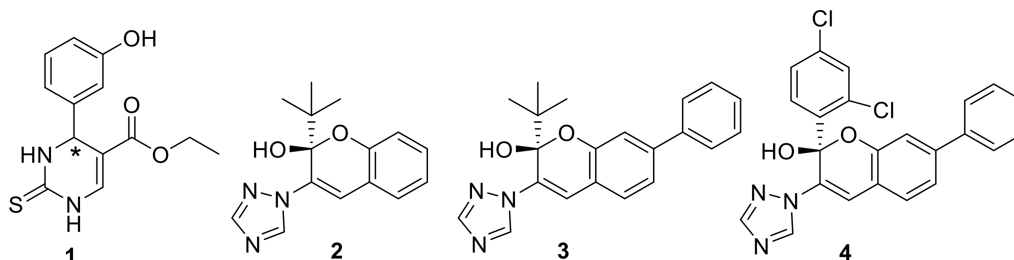
New non-natural bioactive heterocycles as promising binders to G-quadruplex DNA

Elena Gorincioi[1,2], Anastasia Verdes[2], Marina Zveaghintseva[2], Gheorghe Duca[2], Fliur Macaev[2]

[1] Institute of Chemistry, Moldova State University, Chisinau, Republic of Moldova
[2] State Pedagogical University „Ion Creanga", Chisinau, Republic of Moldova

Recent reports regarding the alternative DNA conformations, particularly human telomeric G-quadruplex DNA structures (G4s) eloquently demonstrate the regulatory roles of small molecule non-natural ligands in various key biological cellular processes, *e.g.* ranging from transcription and translation to genome instability and cancer.[1] Also, G4-binding molecules have been gained much attention lately as propitious antiviral targets, in response to the impetuous necessity of finding some intelligent solutions against the newly emerging viruses and mutations of the existing ones.[2]

We herein propose some bioactive synthetic compounds as novel candidates for solution NMR studies of the G4s DNA-ligand interactions. As DNA binders (±)-monastrol **1** and three chromenol–triazole hybrids **2-4** are advanced. Compound **1** is an important representative of 3,4-dihydropyrimidin-2-(1*H*)-ones and an attractive target molecule for organic chemists due to its remarkable biological effects, *e.g.* antitumor activity and inhibition of the motility of the mitotic motor protein kinesin Eg5, thus serving as a useful tool for studying the mechanisms of mitosis.[3] We have recently presented its synthesis *via* an eco-friendly procedure of Biginelli multicomponent reaction and its full ^{1}H, ^{13}C and ^{15}N NMR characterisation,[4] now work being in progress on its stereospecific preparation.



The heterocyclic chromenol–triazole hybrids **2-4** for which synthesis, *in silico* and *in vitro* evaluation has been recently reported are novel antifungal agents that have demonstrated more efficacious properties than the reference drugs ketoconazole and bifonazole and a low cytotoxicity, as well.[5] It may present interest to investigate if there is a relationship between elaboration of the carbon skeleton of these compounds and their potential for targeting the G4s. For the G4 DNA model M2 G-quadruplex has been chosen, with the d(TAGGGACGGGCGGGCAGGGT) oligonucleotide sequence, exhibiting all strands in parallel orientation, which is a common feature of most G4-forming DNA segments in oncogene promoter regions.[6]

As a result of the proposed study, the new high-potential 'actors' in DNA recognition may be portrayed, to be directly involved in rational drug design.

References:
1. Varshney, D., Spiegel, J., Zyner, K., Tannahill, D., Balasubramanian, S. Nat. Rev. Mol. Cell Biol., 2020, 21, 459–474.
2. Ruggiero, E., Richter, S.N. Bioorg. Med. Chem. Lett., 2023, 79, 129085-129092.
3.Matković, J., Ghosh, S., Ćosić, M., Eibes, S., Barišić, M., Pavin, N., Tolić, I. Nature Communications, 2022, 13(1), 7307.
4. Verdeș A., Gorincioi E., Macaev F. The Central European NMR Symposium & Bruker Users Meeting 2023/solid-state NMR workshop, 13th-15th September, 2023, Prague, Czech Republic. Book of Abstracts, pag. 33.
5. Zveaghintseva, M., Stingaci, E., Pogrebnoi, S., et al. Molecules, 2021, 26(14), 4304.
6. Trajkovski, M., et al. Nucleic Acids Res., 2018, 46, 4301–4315.

Acknowledgements: This study is supported by NAIR of the Republic of Moldova under projects 20.80009.5007.17 and 20.80009.5007.27, as well as by CERIC-ERIC.

Identification of G-quadruplex structures in a long non-coding RNA involved in multiple myeloma

Raffaele Graziano, Bruno Pagano, Antonio Randazzo, Jussara Amato

Department of Pharmacy, University of Naples Federico II, Naples, Italy

Multiple myeloma (MM) is a hematologic malignancy characterized by the presence of abnormal clonal plasma cells in the bone marrow, with potential for uncontrolled growth causing destructive bone lesions, kidney injury, anemia, and hypercalcemia.[1] Despite significant advances in the treatment of MM, which have led to unprecedented response and survival rates, patients still relapse, and cure remains elusive: about 100000 deaths occur worldwide each year.[2]

Approximately 75% of the human genome is transcribed into RNA, while only 3% is transcribed into protein-coding mRNAs. Therefore, most of the human genome is transcribed into RNAs that do not encode proteins. Among them, long non-coding RNAs (lncRNAs, more than 200 nucleotides long) are involved in various biological processes and play crucial roles in regulating gene expression, cancer initiation and progression.[3] Aberrant expressions of carcinogenic or tumor-suppressive lncRNAs have been identified in a broad spectrum of cancer types. All this suggests important applications of lncRNAs in the diagnostic, prognostic, and therapeutic evaluation of cancer.[4] LncRNAs can fold into various secondary structures, including G-quadruplexes (G4s), which can facilitate their interactions with DNA, RNA, and proteins, and also represent potential molecular targets in the MM treatment strategy.[5]

Here, we investigated the structural features of a lncRNA identified in patients' MM cells and their Bortezomib-resistant subclone. We used *in silico* approaches to assess the presence of potential G4-forming motifs within the lncRNA and identified ten putative G4-forming sequences, each containing four repeats of two consecutive guanines. Among these, we selected the top five highest scoring sequences for a further experimental characterization in vitro in K^+ solution, employing three biophysical methodologies. First, the Thioflavin T assay was performed to distinguish G4 and non-G4 RNA structures (such as hairpin and single-stranded RNA).[6] Then, NMR spectra were recorded to further confirm or exclude the formation of G4 structures,[7] and CD experiments were performed to evaluate the conformational properties of the oligonucleotides in solution.[8] Finally, melting and annealing CD experiments were used to investigate the thermal stability of the secondary structures formed by the RNA molecules, and the reversibility of their unfolding process. Our results clearly show that one out of the five RNA sequences adopts a G4 structure, while the others probably form hairpin-like structures.

References:
1. Cowan A.J., Green D.J., Kwok M., Lee S., Coffey D.G., Holmberg L.A., Tuazon S., Gopal A.K., Libby E.N., JAMA, 2022, 327, 464-77.
2. Zhou L., Yu Q., Wei G., Wang L., Huang Y., Hu K., Hu Y., Huang H., BMC Cancer, 2021, 21, 606.
3. Batista P.J., Chang H.Y., Cell, 2013, 152, 1298-307.
4. Yan H., Bu P., Essays Biochem., 2021, 65, 625-39.
5. Li F., Zhou J., J. Mol. Med., 2023, 101, 621-35.
6. Renaud de la Faverie A., Guédin A., Bedrat A., Yatsunyk L.A., Mergny J.L., Nucleic Acids Res., 2014, 42, e65.
7. Adrian M., Heddi B., Phan A.T., Methods, 2012, 57, 11-24.
8. Kypr J., Kejnovská I., Renciuk D., Vorlícková M., Nucleic Acids Res., 2009, 37, 1713-25.

The effect of CGAG repeats on the structure of non-canonical hairpins formed by oligonucleotides from the promoter region of the neurodevelopmental regulator AUTS2 gene

Aleš Novotný[1], Janez Plavec[1,2,3], Vojč Kocman[1,2]

[1] Slovenian NMR centre, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia
[2] EN-FIST Centre of Excellence, 1000 Ljubljana, Slovenia
[3] Faculty of Chemistry and Chemical Technology, University of Ljubljana, 1000 Ljubljana, Slovenia

It is known that the AUTS2 protein can be expressed as two major isoforms, a long and a short isoform, with their expression highly dependent on different stages of brain development[1]. The two AUTS2 isoform levels are highly regulated and misregulations in their expression have been correlated to developmental delay and intellectual disability[2]. It is still unknown how the expression switch, between long and short isoforms, is regulated on the molecular level. We offer some insights into a possible regulation mechanism by focusing on a CGAG-rich region comprising a putative protein binding site (PPBS), d(AGCGAAAGCACGAA), found in the promoter region of AUTS2 gene located approximately 150 base pairs upstream of the transcription start site of the long isoform[3]. It was expected, based on the data from the literature, that CGAG-rich oligonucleotides will form structurally polymorphic noncanonical folds stabilized by different non-Watson-Crick base pairs[4,5]. To overcome the polymorphic nature of the CGAG-rich region we chose to focus on three truncated variants, with lengths between 32 and 38 residues, to explain the general sequence-structure relationship. By utilizing NMR spectroscopy we were able to ascertain that all three studied variants form thermally stable non-canonical hairpins. The different number of CGAG repeats contained in each variant had major effects on the arrangement of the loop region whereas the composition of the stem was similar in all three variants and dominated by G:C and sheared G:A base pairs. Since the loop regions contain the predicted protein binding site the described different structural arrangements are potentially biologically relevant. We were able to provide important information on how the structural landscape of the CGAG-rich region of AUTS2 promoter responds to the different number of CGAG repeats surrounding the PPBS site. The presented NMR approaches, used to obtain structural data about the noncanonical hairpins, are extremely valuable to future structural studies of highly repetitive sequences and characterization of their complicated conformational landscapes.

References:
1. Monderer-Rothkoff G. *et. al.*, Mol. Psychiatry 2021, 26 (2), 666–681
2. Biel A. *et. al.*, Front. Mol. Neurosci., 2022, 15:858582
3. Novotný A., Plavec J. and Kocman V., Nucleic Acids Res., 2023, 51(6), 2602-2613
4. Kocman V. and Plavec J., Nat. Commun., 2017, 8, 15355
5. Novotný A. *et. al.*, Nucleic Acids Res., 2021, 49 (20), 11425–11437

Acknowledgements: This work was supported by Slovenian Research Agency (ARRS, grants P1-0242 and Z1-3192 as well as Young researcher grant for A.N.).

Unraveling the specificity of the G-quadruplex/RG-rich peptide interactions by NMR

Matic Kovačič[1,2], Janez Plavec[1,2,3]

[1] *Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova ulica 19, 1000 Ljubljana, Slovenia*
[2] *EN-FIST Centre of Excellence, Trg OF 13, 1000 Ljubljana, Slovenia*
[3] *Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia*

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 the parallel DNA G-quadruplex adopted by the oligonucleotide with four d(G_4C_2) hexanucleotide repeats, that are characteristic for the gene C9orf72 and the onset of ALS neurodegenerative disease.[3] We showed that the investigated interaction is weak and 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. Folding of the oligonucleotide into the G-quadruplex during temperature annealing is also potentially affected by the presence of the peptides, resulting in altered G-quadruplex topology. Our results may become of greater interest considering the importance of the investigated interaction for the development of ALS and FTD diseases.

References:
1. Thandapani P., O'Connor T. R., Bailey T. L., Richard S. *Mol. Cell,* 50, 613–623 (2013).
2. Saha A., Duchambon P. *Biochemistry*, 59, 1261-1272 (2020).
3. Brčić J., Plavec J. *Nucleic Acids Res.,* 46, 11605–11617 (2018).

Targeting interface of SARS-CoV-2 Nsp3c and human mRNAs

Maja Marušič[1], Janez Plavec[1,2,3]

[1] *Slovenian NMR center, National Institute of Chemistry, Hajdrihova 19, Ljubljana, Slovenia*
[2] *EN-FIST Center of Excellence, Trg OF 13, Ljubljana, Slovenia*
[3] *Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, Ljubljana, Slovenia*

Papain-like proteinase Nsp3 is the largest non-structural protein of SARS-CoV-2, functioning as an essential component of the replication and transcription complex, a proteinase that cleaves non-structural proteins 1–3 and a blocker of host's innate immune response. Among Nsp3c domains is macrodomain Nsp3c that is conserved for SARS coronaviruses and is missing in all other coronaviruses. Consequently, it is linked to increased pathogenicity of SARS-CoV and SARS-CoV-2 compared to other less pathogenic coronaviruses.[2,3] Nsp3c is moreover suggested to bind the host's guanine-rich 3'-UTR RNA sequences to influence processes of apoptosis and immune response and with it the outcome of viral infection.

We have characterized the interaction of short Nsp3c peptides with several guanine-rich sequences found in 3'-UTR of human mRNAs with NMR spectroscopy and complementary biophysical methods. Several lysine, tyrosine, and arginine amino-acid residues were identified to be crucial for interaction, while electromobility shift assay ascertained that Nsp3c peptides preferentially bind high-order guanine-rich RNA structures. Finally, exchange processes on an intermediate time scale suggest that the interface site is expanded well further from the one originally identified.

References:
1. Yoshimoto, F. K. Protein J 2020, 1–19.
2. Tan, J.; Kusov, Y.; Mutschall, D.; Tech, S.; Nagarajan, K.; Hilgenfeld, R.; Schmidt, C. L. Biochemical and Biophysical Research Communications 2007, 364 (4), 877–882.
3. Tan, J.; Vonrhein, C.; Smart, O. S.; Bricogne, G.; Bollati, M.; Kusov, Y.; Hansen, G.; Mesters, J. R.; Schmidt, C. L.; Hilgenfeld, R. PLoS Pathog 2009, 5 (5).

Acknowledgments: This work was supported by Slovenian Research Agency (grant no. P1-0242)

Insights into G-quadruplexes recognition by a new hit compound: An NMR approach

Simona Marzano[1,2], Jasna Brčić[2], Jussara Amato[1], Janez Plavec[2], Bruno Pagano[1]

[1] Department of Pharmacy, University of Naples Federico II, Via D. Montesano 49, Naples, Italy
[2] Slovenian NMR Center, National Institute of Chemistry, Hajdrihova 19, Ljubljana, Slovenia

G-quadruplexes (G4s) are highly polymorphic noncanonical DNA/RNA secondary structures formed by stacking of at least two G-tetrads, a planar association of four guanines, connected by intervening loops.[1] Low-molecular-weight compounds affecting nucleic acid conformational equilibria by preferentially binding to a given form could represent a promising strategy for therapeutic applications.[2,3] Recently, a new hit compound, namely BPBA, was found to be able to bind both telomeric repeat-containing RNA (TERRA) G4 and several DNA G4s derived from oncogene promoters. Biological assays showed that BPBA is endowed with a preferential cytotoxic effect on osteosarcoma cancer cells, where it induces a DNA damage response at the telomere level.[4]

In this study, 1D ^{1}H NMR titration was carried out to obtain information regarding how BPBA binds to various G4-forming DNA and RNA sequences by ^{1}H chemical shift perturbation analysis of the target. BPBA induced the highest perturbations in both imino and aromatic protons of *c-kit2* G4, thus we considered the BPBA/*c-kit2* G4 complex as the promising system for a detailed structural characterization. Fluorescence titration of fluorescently labeled *c-kit2 G4* with increasing amounts of BPBA helped us to determine both the dissociation constant of the complex (in the range of high nanomolar) and its stoichiometry. Structural details of the interaction between BPBA and *c-kit2 G4* were elucidated through 2D NMR analysis of the BPBA/*c-kit2* G4 complex in a 2:1 ratio. Observed NOE contacts between ckit2 and BPBA clearly indicate a strong interaction of BPBA with both external tetrads of *c-kit2 G4*. Obtaining the 3D structure of this complex will hopefully allow us to upgrade the molecular scaffold of BPBA and synthesize derivatives based on structure-guided, rational design, thus paving the way for a new class of ligands with improved pharmacological properties.

References:
1. Burge S., Parkinson G.N., Hazel P., Todd A.K., and Neidle S., Nucleic Acids Res., 2006, 34, 5402 – 15
2. Balasubramanian S., Hurley L.H. and Neidle S., Nat. Rev. Drug Discov., 2011, 10, 261 – 75
3. Kosiol N., Juranek S., Brossart P., Heine A. and Paeschke K., Mol. Cancer, 2021, 20, 40
4. Marzano S., Pagano B., Iaccarino N., Di Porzio A., De Tito S., Vertecchi E., Salvati E., Randazzo A. and Amato J., Int. J. Mol. Sci., 2021, 22, 10315 – 35

Acknowledgements: Italian Association for Cancer Research (AIRC) (IG 24590 to B.P.), and Central European Research Infrastructure Consortium (CERIC) (Proposal no. 20232146 to S.M.).

The Effect of Cytosine Methylation on the Structural and Thermodynamic Features of the bcl2Mid G-Quadruplex

Nataša Medved[1], Mirko Cevec[1], San Hadži[3], Jurij Lah[3] and Janez Plavec[1,2,3]

[1] *National Institute of Chemistry, Slovenian NMR Centre, Ljubljana, Slovenia*
[2] *EN-FIST Centre of Excellence, Ljubljana, Slovenia*
[3] *University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia*

The most studied DNA epigenetic modification in mammals is cytosine methylation, occurring within CpG dinucleotides. CpG islands (CGIs), clusters of CpGs, are found in approximately 40% of mammalian genes, primarily in G/C-rich promoter and exonic regions.[1] In healthy human cells, CGIs are typically unmethylated, enabling gene transcription when appropriate transcriptional factors are present. Importantly, these genes can become extensively methylated under particular conditions, leading to the suppression of the associated gene.[2] Cytosine methylation, catalysed by the enzyme DNA methyltransferase (DNMT), leads to the formation of 5-methylcytidine (mC).[3] Remarkably, mC forms a Watson-Crick base pair with guanine, yet this pairing can perturb stability and groove dimensions.[4]

Recent research indicates that the formation of G-quadruplex structures in G-rich regulatory regions, such as promoters and amplification sequences, can disrupt DNA methylation patterns[5] and can positively or negatively affect gene expression and thus cause transcriptome changes.[6] Bcl-2 (B-cell lymphoma 2), an anti-apoptotic protein, controls carcinoma growth in many tumors.[7] The aim of our study was to gain a deeper insight into how epigenetic modifications impact the structure and thermodynamic stability of the bcl2mid G-quadruplex, which is formed within the G/C-rich promoter region. Importantly, the effects of epigenetic modifications on noncanonical structures are poorly studied.

Our findings, based mainly on 1D ^{1}H, ^{31}P and 2D ^{1}H-^{1}H NOESY, complemented with CD spectroscopy and differential dynamic calorimetry (DSC) data, indicate that the presence of mC residues does not hinder the formation of G-quadruplex structures within investigated oligonucleotides. Furthermore, the introduction of mC is well-tolerated, preserving the original G-quadruplex topology. Nevertheless, we did observe some local structural rearrangements associated with cytosine methylation at positions C4 and C6, which are also reflected by alterations in thermodynamic stability.

References:
1. Larsen, F.; Gundersen, G.; Lopez, R.; Prydz, H., Genomics, 1992, 13 (4), 1095–1107
2. Deaton, A. M.; Bird, A., Genes Dev., 2011, 25 (10), 1010– 1022
3. Okano, M.; Bell, D. W.; Haber, D. A.; Li, E., Cell, 1999, 99 (3), 247 – 257
4. Jeltsch, A, Chembiochem, 2002, 3 (4), 274 – 293
5. Mao, S.-Q. et al., Nat Struct Mol Biol, 2018, 10, 951 – 957
6. Spiegel, J., Trends Chem., 2020, 2, 123 – 136
7. Dai, J.et al., Nucleic Acids Res, 2006, 34 (18), 5133 – 5144

Acknowledgements: The Slovenian Research Agency [ARRS, grant P1-0242] supported this project.

Self-assembly of $d(G_4C_2)_n$ repeats in concentrated solutions

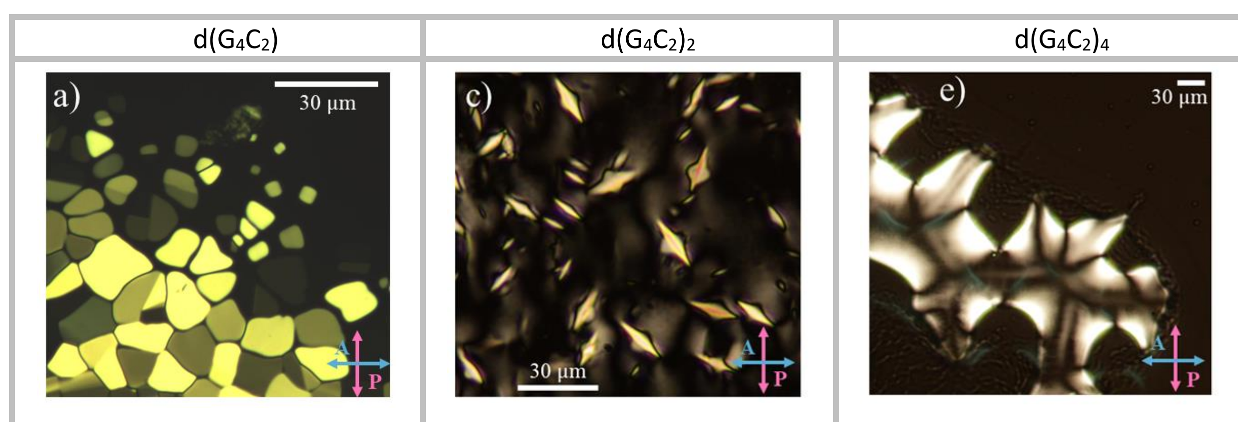
Melani Potrč[1,2], Irena Drevenšek-Olenik[2,3], Lea Spindler[2,4]

[1] *University of Maribor, Faculty of Natural Sciences and Mathematics, Maribor, Slovenia*
[2] *Jožef Stefan Institute, Department for Complex Matter, Ljubljana, Slovenia*
[3] *University of Ljubljana, Faculty of Mathematics and Physics, Ljubljana, Slovenia*
[4] *University of Maribor, Faculty of Mechanical Engineering, Maribor, Slovenia*

Guanine-rich DNA sequences self-assemble into highly stable fourfold helical structures known as G-quadruplexes. We studied quadruplex formation of sequences $d(G_4C_2)_n$ with n = 1, 2, and 4 in concentrated aqueous solutions. Increased numbers of these $d(G_4C_2)$ repeats within the *C9orf72* gene were identified as the most common mutation associated with neurological disorders amyotrophic lateral sclerosis and frontotemporal dementia. While the normal repeat includes up to 25 copies, it can expand to several thousand in patients with the mutation.[1]

DNA oligonucleotides $d(G_4C_2)$, $d(G_4C_2)_2$ and $d(G_4C_2)_4$ were previously confirmed to form G-quadruplexes and their higher order structures. By dynamic light scattering we were able to determine that $d(G_4C_2)$ formed extremely long stacks of quadruplexes with lengths beyond 80 nm. The $d(G_4C_2)_2$ formed a relatively short stacked dimeric quadruplex, while $d(G_4C_2)_4$ formed multimers corresponding to seven stacked intramolecular quadruplexes.[2]

In this work we investigated how the stacked $d(G_4C_2)_n$ quadruplexes behave under crowding conditions. Highly concentrated (c > 50 mM) aqueous solutions were incorporated into thin glass cells and imaged by polarization optical microscopy. All three sequences showed extensive orientational ordering of quadruplex aggregates and the formation of liquid crystalline (LC) phases. For long rod like aggregates formed by $d(G_4C_2)$ this was not surprising and the columnar LC phases were similar to those formed by long DNA molecules at high concentration. The shorter stacks of $d(G_4C_2)_2$ and $d(G_4C_2)_4$ forming columnar LC phases, however, were surprising. We explain their LC formation by enhanced stacking under crowding conditions, something that was reported previously for short duplex DNA.[3] The strong tendency of short $d(G_4C_2)_n$ quadruplexes to stack and orientationally organize could have implications for their behaviour in cell-like environments.



G-quadruplexes from $d(G_4C_2)_n$ repeats stack and orientationally order to form columnar liquid crystalline phases in concentrated solutions. Polarization optical microscopy images show the onset of the nematic LC phase in surrounding isotropic solution.

References:
1. DeJesus-Hernandez, M.; et al.; *Neuron* **2011,** *72***,** 245-256.
2. Potrč, M. et al; *Int. J. Mol. Sci.* **2021**, *22*, 4532.
3. Nakata, M.; et al; *Science* **2007**, *318*, 1276-1279.

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

G-rich oligonucleotides for cancer therapy: the effects of AS1411 on the metabolome of MCF-7 cells

Francesca Romano, Nunzia Iaccarino, Gelsomina Riccardi, Marialuisa Piccolo, Maria Grazia Ferraro, Carlo Irace, Bruno Pagano, Antonio Randazzo, Jussara Amato

Department of Pharmacy, University of Naples Federico II, Via Domenico Montesano 49, Naples, Italy

G4-forming guanine-rich oligonucleotides (GROs) are small synthetic oligomers that can form G-quadruplexes giving their ability to adopt a peculiar three-dimensional structural motif composed of planar arrangements of four guanine (G) bases stabilized by eight Hoogsteen hydrogen bonds known as G-tetrads. Interestingly, these characteristic secondary structures adopted by GROs seems to be responsible for the oligomers' binding to target proteins involved in carcinogenesis and tumor progression.[1] Indeed, certain GROs, including AS1411, demonstrated a great potential to be used in cancer therapy, being more specific against cancer cells and much less toxic for the organism than conventional methods of genotoxic chemotherapy.[2] The biological activity of GROs is primarily ascribed to their ability to bind to nucleolin, a multifunctional protein overexpressed in the cytoplasm and on the cell surface of many tumor types, resulting in the inhibition of nucleolin-mediated phenomena.[3] However, multiple nucleolin-independent biological effects of GROs have also been reported,[4,5] like AS1411 ability to both recognize STAT3 and inhibit Topoisomerase I[6] or the cytotoxicity of GROs guanine-based degradation products.[7] These studies support the hypothesis that GROs could be promising candidates for multi-targeted cancer therapy, therefore it would be of benefit to elucidate the molecular mechanisms involved in their activity.

Herein, we present the preliminary results obtained from a metabolomic-based investigation performed by employing one dimensional nuclear magnetic resonance (1D ^{1}H-NMR) on breast adenocarcinoma MCF-7 cells treated with AS1411. The aim of the study was to investigate the potential metabolic alterations induced by the treatment with AS1411, thus providing insight into the mechanisms that underlie the oligonucleotide antiproliferative activity. Intriguingly, the Principal Component Analysis, employed to extrapolate information from the metabolomic dataset, showed a clear separation between the two studied groups (AS1411-treated cells and non-treated cells), shedding light on the metabolic alterations that occur as a result of the treatment.

References:
1. Collie G.W. and Parkinson G.N., Chem. Soc. Rev., 2011, 40, 5867–5892
2. Ogloblina A.M., Iaccarino N., Capasso D. et al., Int J Mol Sci., 2020, 21, 7781
3. Rosenberg J.E., Bambury R.M., Van Allen E.M. at al., Invest New Drugs, 2014, 32, 178-187
4. Bates P.J., Reyes-Reyes E.M., Malik M.T. et al., Biochim. Biophys. Acta Gen. Subj., 2017, 1861, 1414–1428
5. Kabirian-Dehkordi S., Chalabi-Dchar M., Mertani H.C. et al., Nanomed. Nanotechnol. Biol. Med., 2019, 21, 1020-60
6. Ogloblina A.M., Khristich A.N., Karpechenko N.Y. et al., Biochimie 2018, 145, 163–173
7. Zhang N., Bing T., Liu X. et al., Chem. Sci. 2015, 6, 3831–3838

Unravelling the secrets of an aptamer-small molecule complex using NMR spectroscopy, the case of the testosterone binding TESS.1 aptamer

Sofie Schellinck[1], Nataša Medved[2], Dieter Buyst[1,4], Annemieke Madder[1], Karolien De Wael[3], Janez Plavec[2], José C. Martins[1,4]

[1] Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium.
[2] Slovenian NMR Centre, National Institute of Chemistry, Ljubljana, Slovenia.
[3] Department of Bioscience Engineering, University of Antwerp, Antwerp, Belgium.
[4] NMR Expertise Centre, Ghent University, Ghent, Belgium

Since their discovery in 1990,[1,2] DNA aptamers have shown to be promising bioreceptors for small molecule detection. Numerous articles have appeared describing newly raised aptamers capable of binding with the desired small molecule target with high affinity and selectivity. These aptamers can be converted into a biosensor by using a fairly simple and logical design that exploits the conformational change of the aptamer induced by the target. Although a number of these biosensors appear successful, there is still a general lack of knowledge about the underlying molecular events taking place during an aptamer-target interaction. Such knowledge could aid in further optimisation towards real-world applications. We describe our efforts to apply NMR based strategies to this end, using the structure-switching testosterone binding TESS.1 DNA aptamer, developed and extensively characterized by the Stojanovic group, as model system.[3]

While NMR spectroscopy is a uniquely suited technique to acquire molecular level information about conformational changes and intermolecular interactions, the TESS.1 aptamer, being 51 nucleotides long, did present some challenges when attempting to go towards an assignment. Therefore, the sequence has been truncated and further optimized, generating a more 'NMR optimal' 30-nucleotide long construct, labelled TESS.1_s_mod, which interacts with the testosterone in a similar fashion as the originally sized TESS.1 aptamer. Continuing with this new construct, partial assignments provided a preliminary insight into the conformation of the aptamer and interaction with the testosterone. Finally, analysis of single-nucleotide ^{13}C and ^{15}N labelled sequences allowed a more complete assignment leading to an improved analysis and interpretation of the aptamer-target interaction.

We will present and discuss recent results that provide the first detailed molecular view on this aptamer and its interaction with the testosterone target.

References:
1. Tuerk, C. and L. Gold, Science, 1990. 249(4968): p. 505-510.
2. Ellington, A.D. and J.W. Szostak, Nature, 1990. 346(6287): p. 818-822.
3. Yang, K.A., et al., Acs Chemical Biology, 2017. 12(12): p. 3103-3112.

Investigation of structural characteristics of mitochondrial tRNA fragments

Vito Šuklje[1,2], Vojč Kocman[1], Janez Plavec[1], Anita Kotar[1]

[1] National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia,
[2] Faculty of Chemistry and Chemical Technology, Večna pot 113, Ljubljana, Slovenia

There are 2 sets of tRNAs in human cells – cytosolic tRNAs needed for translation of nuclear-encoded proteins and mitochondrial tRNAs (mt-tRNAs) for mitochondrial-encoded proteins. Human genome contains over 500 tRNA genes, while the mitochondrial genome encodes only 22 different mt-tRNA genes.[1,2] Thus, it is evident how mutations present in these genes can have a big influence on mitochondrial protein synthesis and mitochondrial functionality, whilst mutations in cytosolic tRNA genes are rarely clinically relevant.[1] Both sets of tRNAs are also precursors for tRNA fragments (tRFs), short non-coding RNAs involved in regulation of protein translation, gene silencing, cell stress response, cell growth and differentiation.[3] Most of what we know about tRNA fragments, comes from studies on cytosolic tRNAs, however biogenesis and functions of mitochondrial tRNA fragments (mt-tRFs) remain mostly unexplored for now. We know that production of some mt-tRFs is specific to certain diseases such as cancers and mitochondrial disorders, although it is not clear if mutations in mt-tRNA genes lead to disorders solely due to structural defects in mt-tRNAs or whether changes in mt-tRFs are also responsible for onset of such diseases.[4]

Herein, by utilizing NMR spectroscopy, we investigate the structure of mt-tRF originating from mt-$tRNA^{Ala}$.[5] We want to elucidate the effects of a pathological A-to-G mutation, connected to chronic progressive external ophthalmoplegia – a mitochondrial myopathy[6], in the mt-tRF structure. By extensive analysis of NMR spectra of wild-type (WT) mt-tRF and its A-to-G mutant we were able to confirm that the WT mt-tRF forms a hairpin secondary structure containing an internal bulge formed by A•G mismatch. In the A-to-G mutant, the G•G mismatch forms a base pair and therefore the internal bulge is not present in the stem region. Additionally, NMR results suggest that A-to-G mutant adopts 2 distinct conformations as reveled by doubling of several imino and H8/H6 signals in close proximity to the G•G mismatch. Interestingly, all doubled imino signals remain in the chemical shift ranges typical of their original base pairs suggesting there is no slippage present in the stem of A-to-G mutant hairpin, but that the G residues in the G•G mismatch adopt two different orientations.

References:

1. Biela A., Hammermeister A., Kaczmarczyk I., Walczak M., Koziej L., Lin T., and Glatt S., *Journal of Biological Chemistry*, 2023, 299(8), 104996
2. Suzuki T., Nagao A., and Suzuki T., *Annual Review of Genetics*, 2011, 45, 299 – 329
3. Yu M., Lu B., Zhang J., Ding J., Liu P., and Lu Y, *Journal of Hematology & Oncology*, 2020, 13(1), 121
4. Shaukat A., Kaliatsi E., Stamatopoulou V., and Stathopoulos C., *Frontiers in Physiology*, 2021, 12, 729452
5. Pliatsika V., Loher P., Magee R., Telonis A.G., Londin E., Shigematsu M., Kirino Y., Rigoutsos I., *Nucleic Acids Res.*, 2018, 46(D1), D152 – D159
6. Spagnolo M., Tomelleri G., Vattemi G., Filosto M., Rizzuto N., and Tonin P., *Neuromuscular Disorders*, 2001, 11(5): 481 – 484

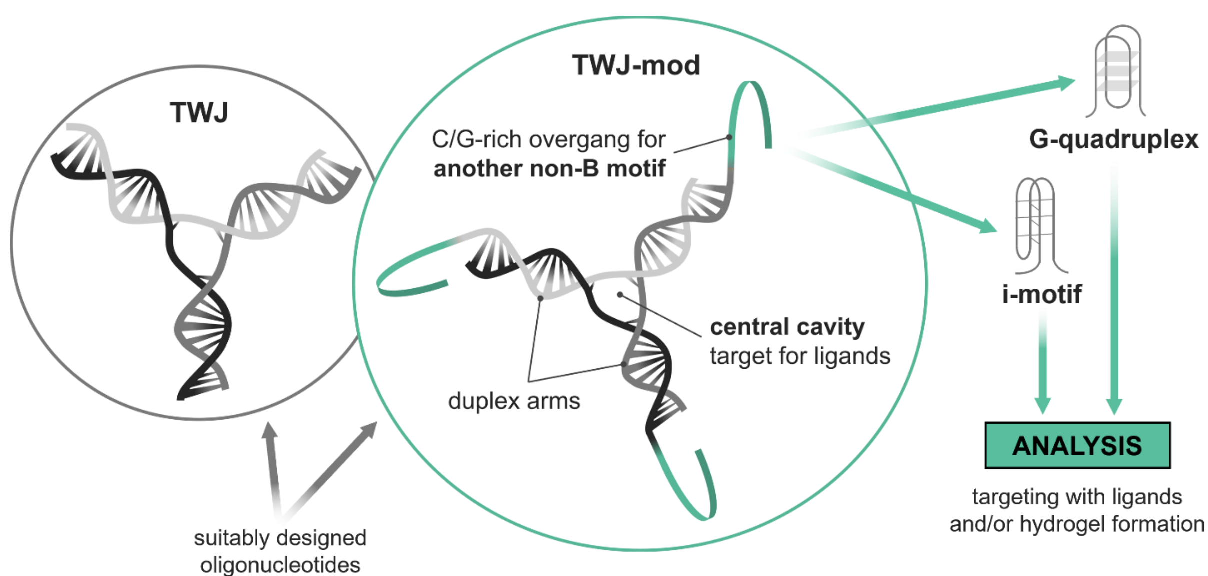
Acknowledgements: The authors acknowledge the financial support from the Slovenian Research and Innovation Agency – ARiS (grants: P1-0242, Z1-3192 and Z1-3198).

TWJ DNA motif: structural modification, stability and targeting with ligands

Lukáš Trizna, Diana Pitková, Viktor Víglaský

Department of Biochemistry, Institute of Chemistry, Faculty of Science, Pavol Jozef Šafárik University, Košice, Slovak Republic

Suitably designed single-stranded nucleic acids are capable to form a structural motif called TWJ (three-way junction) DNA[1]. TWJs have considerable potential in nanotechnology. It´s well known, that metallosupramolecular helicate-like ligands with trigonal geometry could bind into the central cavity of TWJ[2]. Moreover, partial terminal modification of TWJ single-stranded overhangs can ensure the formation of a non-B structure such as G-quadruplex or i-motif (TWJ-mod). Thanks to this, it is possible to create a hydrogel dependent on pH and/or different salt concentration[3]. In our work, we used known as well as newly synthesized ligands. We analysed different types of TWJ-based nanostructures. Interesting physicochemical properties of these motifs were shown by targeting of different parts of this nanosystem by various types of ligands, as well as by elongation of oligonucleotide scaffold. Such a macromolecular system represents a biopolymer that can be used as salt/pH-dependent fluorescent and/or coloured sensors in nanotechnology. The stability of this macromolecular arrangement can be controlled either by the sequence itself or also by the use of metallohelicates.



References:

1. Wu B., Girard F., Van Buuren B., Schleucher J. Tessari M. and Wijmenga S., Nucleic Acids Res., 2004, 32, 3228 – 3239
2. Oleksi A., Blanco A.G., Boer R., Usón I., Aymamí J., Rodger A., Hannon M.J. and Coll M., Angew. Chem. Int. Ed., 2006, 45, 1227 – 1231
3. Cheng E., Xing Y., Chen P., Yang Y., Sun Y., Zhou D., Xu L., Fan Q. and Liu D., Angew Chem Int Ed., 2009, 48. 7660 – 7663

Acknowledgements: This work was supported by the Grant Agency of the Slovak Ministry of Education, Science, Research and Sport No. 1/0347/23

PARTICIPANT LIST

A

Aleksič, Simon	Ljubljana
Amato, Jussara	Naples
Ampenberger, Willi	Munich

B

Brčić, Jasna	Ljubljana

C

Ceschi, Silvia	Padua
Criscuolo, Andrea	Naples

D

D'Aria, Federica	Naples
De Paepe, Lessandro	Ghent
Di Porzio, Anna	Naples

E

Endoh, Tamaki	Kobe

F

Fujii, Masayuki	Fukuoka

G

Giancola, Concetta	Naples
Ghezzo, Michele	Padua
Gorincioi, Elena	Chisinau
Graziano, Raffaele	Naples
Gundy, Marcel	Munich

I

Iaccarino, Nunzia	Naples
Improta, Roberto	Naples

J

Javornik, Uroš	Ljubljana

K

Kocman, Vojč	Ljubljana
Kotar, Anita	Ljubljana
Kovačič, Matic	Ljubljana
Kuwahara, Masayasu	Tokyo

L

Lah, Jurij	Ljubljana
Lenarčič Živković, Martina	Ljubljana

M

Marušič, Maja	Ljubljana
Marzano, Simona	Naples
Medved, Nataša	Ljubljana
Mergny, Jean-Louis	Palaiseau
Montesarchio, Daniela	Naples
Musumeci, Domenica	Naples

N

Novak Kramberger, Anamarija	Ljubljana

P

Paeschke, Katrin	Bonn
Pagano, Bruno	Naples

Petraccone, Luigi	Naples
Pišek, Lucija	Ljubljana
Platella, Chiara	Naples
Plavec, Janez	Ljubljana
Podbevšek, Peter	Ljubljana
Potrč, Melani	Maribor

R

Randazzo, Antonio	Naples
Riccardi, Gelsomina	Naples
Richter, Sara	Padua
Rode, Ambadas	Haryana
Rokita, Steven	Baltimore
Romano, Francesca	Naples
Rozners, Eriks	Binghamton
Ruggiero, Emanuela	Padua

S

Schellinck, Sofie	Ghent
Seley-Radtke, Katherine	Baltimore
Sissi, Claudia	Padua
Spindler, Lea	Maribor
Sugimoto, Naoki	Kobe

Š

Šket, Primož	Ljubljana
Šuklje, Vito	Ljubljana

T

Takenaka, Shigeori	Fukuoka
Tateishi-Karimata, Hisae	Kobe
Trajkovski, Marko	Ljubljana
Trantírek, Lukáš	Brno
Trizna, Lukáš	Košice

V

Víglaský, Viktor	Košice

X

Xi, Zhen	Tianjin

Z

Zhou, Chuanzheng	Tianjin