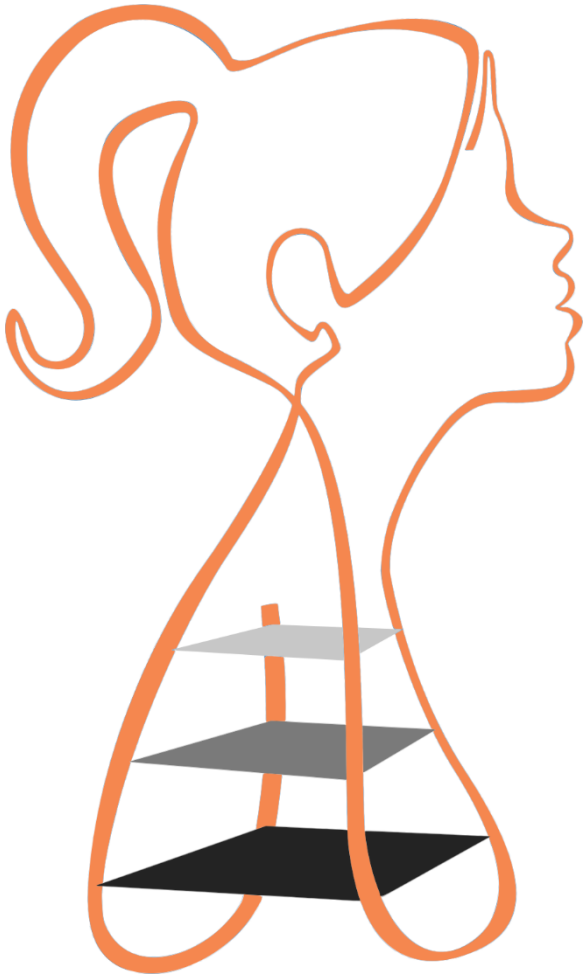

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PROGRAMME

Monday, October 17th, 2022

19:00	**Welcome reception,** *Šestica, Slovenska cesta, Ljubljana*

Tuesday, October 18th, 2022

Morning session *Chair: Jean-Louis Mergny*

9:20 – 9:30	**Opening remarks,** *Janez Plavec, Head of NMR Centre*
9:30 – 10:00	Naoki Sugimoto, *FIBER, Kobe*
10:00 – 10:30	Claudia Sissi, *University of Padova*
10:30 – 11:00	Bruno Pagano, *University of Naples Federico II*
11:00 – 11:30	**Coffee break**
11:30 – 12:00	Sara N. Richter, *University of Padova*
12:00 – 12:30	Saki Matsumoto, *FIBER, Kobe*
12:30 – 14:00	**Lunch**, *Ljubljana Castle*

Afternoon session *Chair: Antonio Randazzo*

14:00 – 14:30	Daniela Montesarchio, *University of Naples Federico II*
14:30 – 15:00	Marko Trajkovski, *National Institute of Chemistry, Ljubljana*
15:00 – 15:30	Emanuela Ruggiero, *University of Padova*
15:30 – 16:00	**Coffee break**
16:00 – 16:30	Jurij Lah, University of Ljubljana
16:30 – 17:00	Shuntaro Takahashi, *FIBER, Kobe*

19:00	**Dinner,** *Vodnikov hram, Vodnikov trg, Ljubljana*

Wednesday, October 19th, 2022

Morning session *Chair: Naoki Sugimoto*

9:30 – 10:00	Dimitra Markovitsi, *Universite Paris-Saclay*
10:00 – 10:30	Roberto Improta, *National Research Council, Naples*
10:30 – 11:00	Jussara Amato, *University of Naples Federico II*
11:00 – 11:30	**Coffee break**
11:30 – 12:00	Jean-Louis Mergny, *Ecole Polytechnique, Palaiseau*
12:00 – 12:30	Chiara Platella, *University of Naples Federico II*
12:30 – 14:00	**Lunch**, Ljubljana Castle

Afternoon session *Chair: Daniela Montesarchio*

14:00 – 14:30	Viktor Víglaský, *P. J. Šafarik University, Košice*
14:30 – 15:00	Tatsuya Ohyama, *FIBER, Kobe*
15:00 – 15:30	Daša Pavc, *National Institute of Chemistry, Ljubljana*
15:30 – 16:00	**Coffee break**

Young investigator presentations

16:00 – 16:15	Marta Cozzaglio, *University of Padova*
16:15 – 16:30	Kateřina Peterková, *National Institute of Chemistry, Ljubljana*
16:30 – 16:45	Lukáš Trizna, *P. J. Šafarik University, Košice*
16:45 – 17:00	Aleš Novotný, *National Institute of Chemistry, Ljubljana*

19:00 **Dinner,** *Pod vrbo, Ziherlova ulica, Ljubljana*

INVITED LECTURES

Beyond "To *B* or not to *B*" in Nucleic Acids Chemistry

Naoki Sugimoto[1,2]

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Phase separations are important not only for cancers, but also for the progression of neurodegenerative diseases, which involve a gradual damage to specific nerve cells that are subsequently eliminated from the brain and spinal cord. Neurodegenerative disease occurred in the central nervous system is characterised by a decrease in the number of specific groups of neurons and the accumulation of fibrous substances inside and outside the neurons. Recently, RNA transcripts of these neurodegenerative disease-related genes were demonstrated to form non-canonical structures and undergo liquid–liquid phase separation and RNA accumulation. Thus, evidence for the association of aberrant phase separation has been accumulating. The speed and efficiency of phase separation depends on the higher-order structure of RNA such as hairpin and G-quadruplexes, suggesting that the structures of nucleic acids may play an important role in cancer and neurodegeneration. In this study, the effect of chemical environments on the accumulation of RNA with different structures was quantitatively investigated.

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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; 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; N. 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.

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C-rich sequences and iM: the same old story?

Claudia Sissi

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Non-canonical tetrahelical DNA structures are peculiar structural elements that attract the attention for their potential role as target in medicinal chemistry as well as for technological applications.

Among them, the most studied are G-quadruplexes (G4). The sequences that can fold according this motif (PQS) can be predicted quite efficiently by common bioinformatic tools based on the required pattern of repetitive G-runs. Even focusing on intramolecular structures, G-quadruplexes are highly polymorphic and their preferred final topology is difficult to be anticipated.

Sequences complementary to those folding into G-quadruplex can give rise to I-motif (iM) in slightly acidic conditions. The folding of iM corresponds to two parallel duplexes intercalated with an antiparallel orientation. This requirement reduces their polymorphism which is essentially limited to a shift in the intercalation frame. A possible conclusion should be that they should be easier to be predicted.

This hold true if we focus mainly on the repetitive G- or C- pattern. However, less clear is the definition of the role of the loops. In particular it is not clearly addressed if their length/composition play comparable role in G4 and iM.

Here, we will present some examples to illustrate the divergent behavior of complementary G- and C- rich sequences. On these bases, we set up a screening to address the role of the loop length in iM to identify the minimal sequence requirements compatible with the tetrahelix formation. Due to the limited number of solved iM, these data aim to provide new insights in the rationalization (and eventually prediction) these attractive DNA motifs.

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Ligand-based drug repurposing strategy identified SARS-CoV-2 RNA G-quadruplex binders

Federica Moraca[1], Simona Marzano[1], Francesco D'Amico[2], Antonio Lupia[1], Silvia Di Fonzo[2], Eleonora Vertecchi[3], Erica Salvati[3], Anna Di Porzio[1], Bruno Catalanotti[1], Antonio Randazzo[1], Bruno Pagano[1] and Jussara Amato[1]

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So far, almost all new therapeutic strategies against SARS-CoV-2 have focused on targeting viral proteins.[1,2] However, the threat posed by SARS-CoV-2 infection requires exploring also plausible alternative approaches, such as targeting viral RNA and, in particular, its secondary structures.[3,4] Indeed, the folding of specific regions of the viral genomic RNA into certain secondary structures may hinder the viral genome expression and replication by acting as roadblocks for RNA transcription and/or as hallmarks for the attachment of RNA processing machinery.

The single-stranded RNA genome of SARS-CoV-2 contains some G-quadruplex-forming G-rich elements which are putative drug targets.[4] Here, we performed a ligand-based pharmacophore virtual screening of FDA approved drugs to find candidates targeting such RNA structures. Further *in silico* and *in vitro* assays identified three drugs as emerging SARS-CoV-2 RNA G-quadruplex binders.[5]

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DNA G-quadruplexes in X-linked Dystonia Parkinsonism disease

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Biomedical Center, Faculty of Medicine, LMU Munich, Munich, Germany

X-linked Dystonia Parkinsonism (XDP) is a genetic neurodegenerative movement disorder. All patients share the same haplotype in the X chromosome. Retrotransposon insertion within intron-32 of the TAF1 gene has been proposed to be the most crucial mutation. In fact, TAF1 transcript levels are lower in XDP patients compared to healthy controls. It is still not clear how this insertion impairs TAF1 transcription. Because the retrotransposon is very GC-rich, we hypothesised that G-quadruplex (G4) structures form and affect the transcription process.

We first retrieved by bioinformatic analysis all possible G4 putative sequences and characterized the four most stable *in vitro* by circular dichroism, DMS footprinting and Taq Pol stop assays, which all indicated formation of parallel and stable G4 structures in all selected sequences. Increasing KCl concentration and G4 ligands inhibited PCR amplification of the retrotransposon from genomic DNA, further supporting G4 formation. To evaluate G4 folding and assess its role in cells we performed G4-ChIP-seq and retrotransposon G4-ChIP-qPCR in primary fibroblasts from skin biopsies of XDP affected patients and healthy relatives. We found different G4-landscapes between XDP patients and healthy controls, suggesting the existence of G4-mediated pathways that could be relevant for the XDP disease. Upon incubation with G4 ligands, TAF1 mRNA levels decreased in a concentration-dependent manner only in XDP-affected patient-derived cells. Our data indicate that G4s are present in the XDP retrotransposon in cells and that potentially their folding has a key role in the pathogenesis of the disease.

DNA methylation depending on stability and topology of G-quadruplex

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DNA methylation on the CpG sequences in the human genome regulates gene expression. The pattern of methylation is precisely regulated in biologically important processes such as development, differentiation, cancer, and aging. However, the regulatory mechanisms of these methylations remain unclear. CpG islands (CGIs) have GC-rich sequences and can form non-canonical DNA structures such as G-quadruplex (G4) and i-motif. Interestingly, since the stability and topology of G4, resulting in the changes in transition between duplex and G4 are regulated by the surrounding environments,[1-4] the formation of G4 possibly regulates methylation by changing their stability and topology by responding to surrounding environments. G4 formation has been shown to regulate transcription,[5] translation,[6] and replication.[7] DNA methylation may also be regulated by G4 formation on DNA. Indeed, methylome analysis suggested that G4 may suppress methylation of CGI. However, quantitative and systematic knowledge of the effect of G4 formation on methylation is still lacking.

Here, we systematically investigated the effect of G4 formation on methylation. Methylation reactions were performed using G4-forming sequences with various thermal stability and topology as substrates.[8] As a result, methylation efficiency decreased with increasing G4 stability. Moreover, DNA methylation was regulated by not only the stability of G4 but also the topology of G4. Because investigation of equilibrium between duplex and quadruplexes before methylation showed that the equilibrium could be determined only by the stability of G4, regulation of methylation efficiency by G4 topology was suggested to be caused by differences in unfolding processes during methylation reaction. Our findings may explain how CGIs are hypermethylated in specific tissues during aging or in cancer cells.

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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, 21H05109, and 21K14742), especially for Grant-in-Aid for Scientific Research (S) (22H04975), JSPS Core-to-Core Program (JPJSCCA20220005), JSPS Bilateral project, The Hirao Taro Foundation of Konan Gakuen for Academic Research, The Asahi Glass Foundation, and the Chubei Itoh Foundation.

G-quadruplex forming aptamers for therapeutic applications

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G-quadruplex (G4) structures exhibit an extraordinarily wide structural variability compared to canonical duplex structures.[1] Thus, their ability to recognize very different targets is not surprising and in part explains the high abundance of guanine-rich oligonucleotides, able to fold into stable but also extremely different G4 conformations, identified as aptamers by SELEX.[2] In this context, several G-quadruplex forming aptamers have been studied for their therapeutic applications, also in consideration of the good cell uptake - also in the absence of transfecting agents - and high nuclease resistance generally associated with these oligonucleotides.[3]

As representative case studies, recent data concerning the design, biophysical characterization and biological activity of novel G-quadruplex-forming aptamers targeting: 1) High-Mobility Group Box 1 (HMGB1)[4,5] and 2) mutated huntingtin,[6] respectively of interest in anticancer and anti-Huntington disease treatments, will be presented.

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PhenDC$_3$ intercalates into human telomeric G-quadruplex

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Non-canonical nucleic acid structures are important from several essential biological aspects, including maintenances of genome integrity and regulation of gene expressions.[1] Amongst, G-quadruplexes that are formed by guanine-rich DNA are particularly inciting, as they represent potential targets for treatment of various cancers, neurological and other disorders. Moreover, the progress of the ongoing quest of designing or finding small molecules that bind G-quadruplex in a specific and high-affinity manner relies on detailed insights into ligand-DNA interfaces.[2] Considering the reported structural studies, drug-like properties of small molecules mostly relate to (extent of) their stacking to outer G-quartets and interactions with loops that connect the guanine moieties in the core of a G-quadruplex. Additionally, intercalation of a small molecule between outer G-quartets of separate entities remains particularly promising strategy of modulating longer guanine-rich segments, such as telomeres, where several closely-spaced G-quadruplex may form. Moreover, this approach is fundamentally based on unlikeliness that a drug-like agent could bind between consecutive G-quartets of a sole G-quadruplex.

The essence of guanine-rich DNA polymorphism has been most extensively explored on different oligonucleotide variants originating from human telomeric region, as this genomic segment represents one of the most appealing targets for novel cancer chemotherapies. On the other hand, there is a lack of structural data on interactions between G-quadruplexes that may form in telomere and PhenDC$_3$ that is renowned as the 'golden standard' amongst the most curious G-quadruplex stabilizer.[3] Our work which addresses this gap will be presented, with the focus on NMR-based structural characterization of the interactions between PhenDC$_3$ and guanine-rich oligonucleotide originating from human telomeric DNA.[4]

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Fused in liposarcoma protein, a new player in the regulation of HIV-1 transcription, binds to known and newly identified LTR G4s

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The human immunodeficiency virus type-1 (HIV-1) integrated long terminal repeat (LTR) region is the viral unique promoter and is highly enriched in guanines (Gs). The LTR G-rich segment has been previously demonstrated to fold into non-canonical nucleic acids structures, such as G quadruplexes (G4s), and its activity is finely modulated by G4s interaction with cellular proteins.[1] In detail, the nucleolin protein has been shown to bind and stabilize LTR G4s, downregulating viral transcription,[2] whereas the ribonucleoprotein A2/B1 promoted the transcription machinery through G4 unfolding.[3] The mechanism also involves the folding of an i-motif structure in the complementary (cytosine-rich) strand, which is promoted by ribonucleoprotein K.[4] Therefore, the virus exploits alternative DNA secondary structures as regulatory elements in HIV-1 progression and in establishing host/pathogen interactions.[5]

To further characterize HIV-1 at the G4 level, we sought to investigate additional players in the LTR activity, and consequent viral transcription, modulation. Through a combined pull down/mass spectrometry/western blot approach, we identified the fused in liposarcoma (FUS) protein and found it to preferentially bind and stabilize the least stable LTR G4, especially in the cell environment. The outcome of this interaction is the down-regulation of viral transcription, as assessed in a reporter assay with LTR G4 mutants in FUS-silencing conditions. In addition, we observed that FUS binding to the full-length LTR sequence induced the folding of a new LTR G4, which was never reported before. Interestingly, the higher stabilized LTR G4s contain a bulged G-tract, making them unconventional G4s with unique characteristics, thus amenable for selective recognition. These data indicate that the complexity and dynamics of HIV-1 LTR G4s are much greater than previously envisaged. The G-rich LTR region, with its diverse G4 landscape and multiple cell protein interactions, stands out as prime sensing center for the fine regulation of viral transcription. Indeed, LTR G4 recognition by different cellular proteins could regulate the progression of the virus towards an active or a silent transcriptional state. Therefore, targeting this region with compounds could interfere with G4/protein interaction, representing a promising antiviral target for inhibiting both the actively transcribing and latent viruses.

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Ligand binding-induced diversity of a G-quadruplex stability phase space

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The structural diversity of G-quadruplexes is important for their recognition by proteins and small-molecule ligands. However, why the binding of several ligands alters the topology of G-quadruplexes is not clearly understood. We addressed this question by following the (un)folding and binding of the human telomeric fragment 5'-(GGGTTA)3GGGT-3' (22GT) by calorimetry (DSC, ITC) and spectroscopy (CD). Analysis of the measured data led to the thermodynamic parameters of folding and binding of 22GT, which were decomposed into specific driving forces and interpreted by molecular modeling.[1-3] This allows a detailed description of the topological phase space of stability (phase diagram) of 22GT and shows how it changes in the presence of a specific bisquinolinium ligand (360A). Various 1:1 and 2:1 ligand-quadruplex complexes were observed. As the temperature increases, the 1:1 complexes change to 2:1 complexes, which can be attributed to the preferential binding of the ligand to the folding intermediates. Overall, our thermodynamic analysis suggests why ligand binding alters the phase space of conformational stability of human telomere quadruplexes.

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Volumetric study for the functions of non-canonical nucleic acids structures

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Nucleic acids typically form a double helix structure through Watson–Crick base-pairing. This canonical structure is for the storage and transfer of genetic information. On the other hand, nucleic acids can also form base pairs within the strand and different types of base pairs, such as Hoogsteen types, resulting in formations of non-canonical structures such as triplexes and quadruplexes. Thus, non-canonical structures are for functions of genetic materials. It has been recently clarified that these structural changes of nucleic acid in cells dynamically occur. Such dynamical changes of nucleic acid structures accompany the volumetric changes of the structures with (de)hydration. In living cells, the solution condition is far from the test tube conditions, because various biomacromolecules exist in extremely high concentrations (~400 g/L). In these cellular conditions, the nucleic acid structures are affected by these cosolutes due to changes in water activity, volume exclusion, and other factors. High pressure is a physical tool to directly study volumetric changes of macromolecules. Therefore, it is helpful to elucidate the biological functions of non-canonical nucleic acid structures in cells using high pressure approaches.[1-7] These studies are beneficial to develop new materials to regulate these structures of nucleic acids.[8-13] One of the targets of our study is a G-quadruplex ligand. The volumetric parameters obtained by thermodynamic analyses under high pressure can provide the quantitative information about the fit of the ligand on G-quadruplex.[8,11] Furthermore, the volumetric parameters can be also used as an index to predict the binding manner of the ligand on G-quadruplex, which is useful to predict and design the novel G-quadruplex ligands.[13] In our talk, we will present our latest research works and perspectives about high pressure studies on nucleic acids in the talk.

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Guanine radicals generated in G-Quadruplexes by low-energy photoionization

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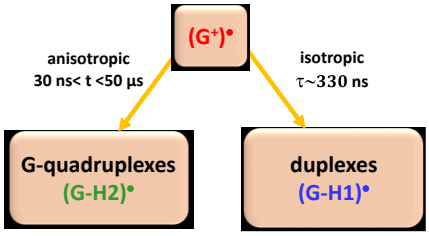
We discovered recently that G-quadruplexes undergo one-photon ionization at energies significantly lower that the ionization potential of their constituents (≥ 7e V ≈ 177 nm). The generated electrons and electron holes [guanine radical cations: $(G^+)^\bullet$] are important both in respect to the DNA damage and for the development of photoconductivity based nanodevices. Quantum yields ϕ related to this process were determined by nanosecond transient absorption exciting at 266 nm.

The ϕ values found for a series of G-quadruplexes range from 3.5×10^{-3} to 15×10^{-3}, being much larger than that found for double stranded genomic DNA (2×10^{-3}). Moreover, they strongly depend on structural characteristics, such as the type of metal cations in the central cavity and the nature and position of the peripheral bases. The effect of structural parameters of G-Quadruplexes on ϕ helped us to propose a mechanism explaining low-energy photoionization of DNA in general. The latter involves formation of excited charge transfer states during the excited state relaxation and subsequent charge separation.



Time-resolved spectroscopy using low-energy photoionization also offers a unique possibility to characterize the time-evolution of the $(G^+)^\bullet$ population. This is due to the fact that, under such well-controlled conditions, $(G^+)^\bullet$ are formed on zero-time, without intermediation of electron donors, whose presence may modify the reaction dynamics.

$(G^+)^\bullet$ in neutral aqueous solution tends to lose a proton. While deprotonation in duplex DNA takes place with a time constant of ~330 ns, in G-Quadruplexes it is highly anisotropic; it consists of a fast step (< 1 µs), which is followed by a slower one, completed within tens of ns. Another specificity of the $(G^+)^\bullet$ deprotonation in G-Quadruplexes is that the released proton stems from a different site of the G residue, giving rise to $(G\text{-}H2)^\bullet$ radicals, instead of $(G\text{-}H1)^\bullet$ in duplexes. The final lesions originating from $(G\text{-}H2)^\bullet$ radicals remain to be identified.

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Computing the electronic spectra of guanine quadruplexes by an excitonic Hamiltonian

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The measurement of an absorption or electronic circular dichroism (ECD) spectrum is one of the first and most basic steps to identify and characterize the static and dynamical behavior of a G-Quadruplex (GQ). We here describe a computational procedure to simulate the absorption and ECD spectra of GQs, also including the effect of thermal fluctuations and the loop, attaining a good compromise between accuracy and computational cost. Our approach is based on a new excitonic model (FrDEx)[1-3] able to include the contribution to the spectra of charge transfer transitions and to take into account the effect of the surrounding bases on the excited states of each base. We report the spectra computed for FrDex Quantum Mechanical calculations. In this work we compute the ECD spectra of GQs of different topology, obtaining spectra close to the reference full quantum mechanical (QM) ones (obtained with time-dependent density functional theory), with significant improvements with respect to "standard" excitonic Hamiltonians. Furthermore, we get interesting insights into the chemical–physical effects modulating the spectral signals. FrDEx appears particularly suitable for the treatment of closely stacked multichromophore assemblies and, thus, to investigate many other biological and nanotechnological materials, from DNA to (opto)electronic polymers.

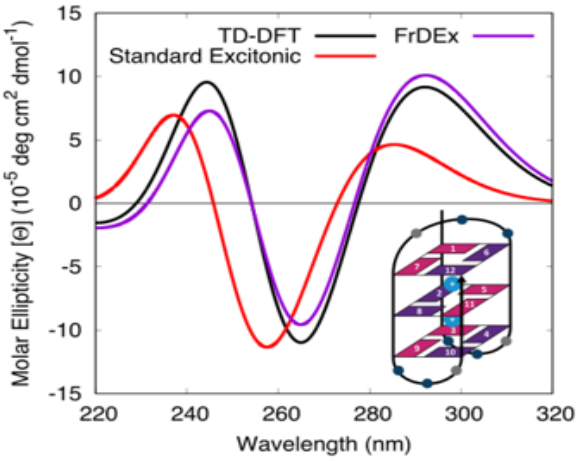

Figure: Calculated ECD spectra of the G-Quadruplex via TD DFT calculations and FrDEx model, compared to Standard Excitonic Model

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Studying noncanonical DNA structures and their drug targeting: new insights from ultraviolet resonance Raman spectroscopy

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The conformational plasticity of nucleic acids is essential for several biological functions, including the specific regulation of DNA transcription, replication, or repair.[1] The so-called "noncanonical" DNA secondary structures represent sequence-dependent conformational topologies, frequently clustered in regulatory regions of oncogenes and in telomeres.[1] For example, G-rich strands can form G-quadruplex (G4) structures which, depending on the DNA sequence, may switch into several interconvertible polymorphs in solution upon changes in DNA or cation concentration.[2] Similarly, depending on the environmental conditions (particularly pH variations), some C-rich sequences can experience polymorphism between i-motif (iM) and hairpin structures.[3,4] It has now been unambiguously demonstrated that G4s and iMs are present in living cells and can be involved in important cancer-related biological processes. The identification of small organic molecules able to selectively bind and stabilize G4s is considered a promising strategy for the development of new anticancer drugs.[5] Noteworthy, the high conformational polymorphism of G4 structures increases the potential modes of ligand binding and represents a major challenge of the present research efforts devoted to the search of effective G4-targeting compounds.[6]

Several experimental techniques including nuclear magnetic resonance, X-ray diffraction, mass spectrometry, as well as Raman, UV-VIS, fluorescence, and circular dichroism spectroscopies are currently employed to investigate noncanonical DNA and their interactions with putative drugs.[7] In this frame, in addition to conventional Raman spectroscopy, ultraviolet resonance Raman (UVRR) spectroscopy can provide valuable information about noncanonical DNA structures and their interactions with drugs in solution.[8] Indeed, an interesting feature of UVRR is represented by the possibility of gaining information about ligand and DNA chemical groups involved in the interaction from the same spectrum through the enhanced response of the resonant groups.

In this communication, I will discuss how UVRR provided a useful method for our investigations on the pH-dependent equilibrium between iM and hairpin structures,[9] on the conformational polymorphism of G4s in crowding and dilute conditions,[10] and to shed light on the binding modes of a ligand to different G4 structures.[11]

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Quadruplexes are everywhere!

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G-quadruplexes are unusual nucleic acid structures which can find applications in biology, medicine, as well as biotech- and nano-technologies [1]. We are developing tools to understand their folding and polymorphism [2]. In parallel, we proposed a new algorithm for the prediction of G4 propensity [3]. We are now applying this G4-Hunter prediction tool to a number of genomes.

We became interested in quadruplexes quadruplex-prone regions conserved in the genome of a number of viruses [4]. We recently demonstrated that viruses regularly causing persistent infections are enriched in G4 motifs, while viruses causing acute infections are significantly depleted in these structures [5], including SARS-CoV2 [6]. Interestingly, one of SARS-CoV2 proteins, Nsp3, can bind to G4s. These interactions can be disrupted by molecules called ligands specific for these G4s. Our results pave the way for further studies on the role of SUD/G4 interactions during SARS-CoV-2 replication and the use of inhibitors of these interactions as potent antiviral compounds.

We are also interested in the role of quadruplexes in parasites such as *Plasmodium falciparum* or *Trypanosoma brucei* [7] and, more recently on parasitic helminths [8], which are highly prevalent and infect approximately two *billion* people worldwide. A nematode, *Ascaris lumbricoides*, was found to be highly enriched in stable quadruplexes. We demonstrated that small compounds able to recognize these structures called G-quadruplex ligands were able to selectively recognize G4 found in the *Schistosoma mansoni* genome. Two of these compounds demonstrated potent activity both against larval and adult stages of this helminth, opening new perspectives for the use of G4 ligands to fight diseases caused by these parasites.

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An insight into targeting cancer-related G-quadruplex structures by small-molecule ligands

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G-quadruplexes play key roles in the regulation of cancer-specific genes as well as in molecular pathways involved in uncontrolled proliferation mechanisms common to all tumour types. Thus, selectively targeting G-quadruplex structures in vivo represents a very general and promising anticancer strategy.[1] The appealing possibility to treat common features of different cancers without impairing normal cells stimulated the synthesis of large libraries of putative G-quadruplex ligands.

To rapidly and effectively select 'true hits', we have recently developed an affinity chromatography-based method, i.e. the G4-CPG (G-quadruplex on Controlled Pore Glass) assay to identify ligands able to specifically recognize biologically relevant G-quadruplex structures.[2] More specifically, we recently focused on libraries of small-molecule ligands including both synthetic and natural compounds.[3-7]

Within the series investigated by the G4-CPG assay, the most attractive compounds proved to be a naphthalene diimide and an alkaloid derivative. Most notably, in vitro cell viability tests indicated these compounds as very promising candidate drugs for their strong bioactivity against human cancer cells, which well correlated with their ability to target genomic G-quadruplexes.[3,5,7]

Encouraged by these results, we deemed it essential to undertake in-depth biophysical studies on their interaction with G-quadruplex models to better elucidate the details of the strong and specific binding.[3-7]

Altogether the obtained insights are now directing the design of optimized analogues of the best synthetic and natural ligands of G-quadruplexes identified thus far as effective anticancer candidate drugs to be advanced to in vivo targeted therapies.

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Non-Canonical Structure: from Digital Information to Real Structures

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Currently, there are several bioinformatic approaches enabling the prediction of the occurrence of non-canonical structural motifs in certain sequences of nucleic acids. The G4Hunter algorithm is currently a popular method of identifying G-quadruplex forming sequences in nucleic acids and offers promising scores despite its lack of the substantial rational basis.1 A new quasi-orthogonal 3D presentation of sequence has been designed in our laboratory. The linear sequence of nucleic acids is mathematically transformed into an orthogonal representation; G–C and A–T pairs are shown in different planes, originally designed as orthogonal, but the slight declination from perpendicularity allows some non-canonical structures to be more precisely identified, especially G-quadruplexes and VK structures2.

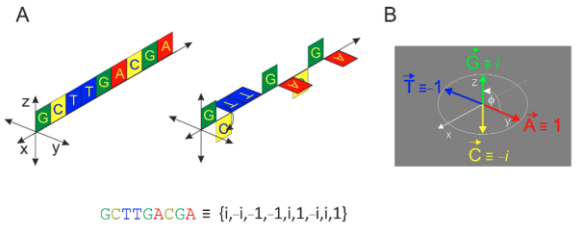

Figure: Basic properties of an (quasi-)orthogonal system. Sequence visualization is performed on two planes, where nucleotides A + T are on the xy-planes and C + G are on the xz-planes. The nucleotide order is expressed by an integer value on the x-axis (A). There is a close analogy with the representation of complex integers (B). In the complex space, any oligonucleotide in the DNA sequence can be expressed instead of A, T, C, and G by four values: −1, 1, −i, and i, respectively.

The base allocation enables the evaluation of any nucleic acid and predicts the likelihood of a particular region to form non-canonical motifs[3]. In addition, our sequence representation facilitates the search for other sequences that can adopt non-canonical motifs, such as direct and palindromic repeats. The technique can also be used for various RNA molecules, including any aptamers. This powerful tool based on an orthogonal system offers a considerable potential for a wide range of applications. We are currently finalizing a public software tool that will offer a highly accurate prediction of non-canonical motifs based on nucleic acid sequences.

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Analysis of dynamic behaviors of G-quadruplexes using molecular simulations

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Non-canonical structures of nucleic acids such as triplex and G-quadruplex are involved in biological reactions such as replication, transcription, and translation in the cell.[1–3] As the formation of these structures is highly affected by surrounding environment, it is crucial to understand the physicochemical properties of non-canonical structure nucleic acids with changing the surrounding environment for not only elucidation of biological functions of nucleic acids but also development of drugs and nanomaterials. The thermodynamics of the formation of these structures provides quantitative information. Pressure was used to analyze the partial molar volume of the biopolymer, which can be calculated by the sum of the molecular volume of the biopolymer and the solvation volume.[4,5] Therefore, structural analysis at high pressure is informative to know both the conformational change of nucleic acids and (de)hydration on them. Previously, we found that the stability of thrombin binding aptamer (TBA) G-quadruplex DNA destabilized with increasing pressure, but the magnitude of the destabilization was reduced in the presence of polyethylene glycol 200 (PEG200).[4] In this study, we investigated the destabilization mechanism of TBA by pressure change using molecular dynamics (MD) simulations under 0.1 to 1000 MPa. As a result, hydration water molecules disrupted hydrogen bonds in G-quartet and destabilized TBA by increasing pressure. In addition, the destabilization mechanism which can be described only in terms of pressure and volume in the experiment was demonstrated in the form of hydrogen bonds between the TBA and hydration water molecules, which is more easily understood. In the presentation, we will discuss more detailed structural and dynamics information of the G-quadruplex.

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Structural insights into self-assembly of DNA G-wire

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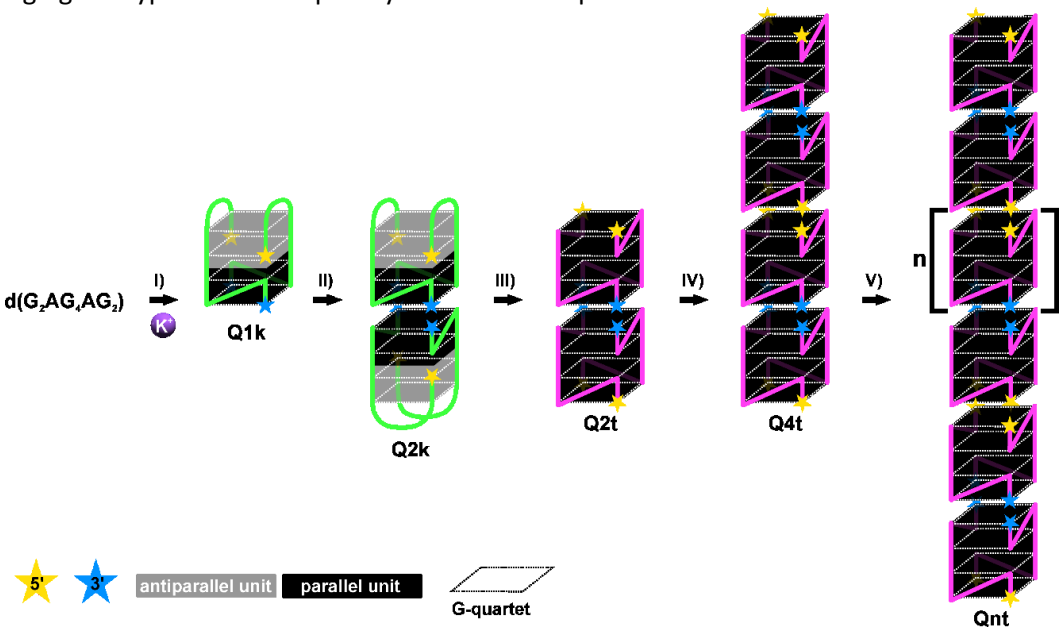
Materials with various bio-nanoapplications can be engineered by utilizing self-assembly of DNA nucleotides, e.g., short, guanine-rich oligonucleotides can self-assemble into elongated nanostructures, termed G-wires. Their structural details and self-assembly mechanism are crucial for optimization of G-wire's properties, however they remain poorly understood.

Herein, we have utilized nuclear magnetic resonance to understand how chosen short, guanine-rich DNA oligonucleotide self-assemble into G-wires and thus obtained insights on behavior of these nanostructures at molecular level. Complementary methods, e.g., CD, DLS, AFM, SEM, TEM were used for further characterization of G-wires. The crucial step of self-assembly mechanism includes structural rearrangement of kinetically favored G-quadruplex building block into a thermodynamically preferred one. Unravelling mechanistic details enable us to guide G-wire self-assembly in a controlled manner. We have showed that length of resulting G-wires can be tailored by changing the type and consequently features of loop residues.[1]



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YOUNG INVESTIGATOR PRESENTATIONS

G-quadruplexes formation within the promoter of TEAD4 oncogene and their interaction with Vimentin

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G-quadruplexes (G4s) are nucleic acid secondary structures detected within human chromosomes, that cluster at gene promoters and enhancers. This suggests that G4s may play specific roles in the regulation of gene expression. Within a distinct subgroup of G-rich domains, the formation of two or more adjacent G4 units (G4-repeats) is feasible. Recently it was shown that Vimentin, a protein highly expressed within mesenchymal cells, selectively recognizes these arrangements. Putative G4 repeats have been searched within the human gene proximal promoters by the bioinformatics tool QPARSE and they resulted to be enriched at genes related to epithelial-to-mesenchymal transition (EMT). This suggested that Vimentin binding at these sites might be relevant for the maintenance of the mesenchymal phenotype. Among all the identified sequences, in the present study we selected the one located within the promoter of the *TEAD4* oncogene. *TEAD4* codifies for a transcriptional enhancer factor, TEAD4, that actively promotes EMT, supports cell proliferation and migration. Moreover, in colorectal cancer cells TEAD4 directly enhances the expression of Vimentin. Thus, the possible interaction of Vimentin with *TEAD4* promoter could highlight a positive feedback loop between these two factors, associated to important tumor metastasis related events. Here, we exploited spectroscopic and electrophoretic measurements under different conditions to address the folding behavior of the selected sequence. This allowed us to validate the folding of *TEAD4* promoter into a G4-repeat able to interact with Vimentin.

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Design of G-quadruplex decoys derived from KIT proto-oncogene

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The *KIT* proto-oncogene encodes a transmembrane tyrosine kinase receptor, which participates in a broad range of physiological processes.[1] KIT abnormalities, typically mutations, play important roles in human cancer development, which makes KIT an attractive target for anti-cancer therapy.[2] Proximal promoter region of KIT contains three G-rich regions (c-kit1, kit* and c-kit2) that are able to fold into G-quadruplexes. They are closely clustered and separated from each other by only a few nucleotides. Importantly, the promoter segment comprising kit* and c-kit2 contains a putative binding site for the Sp1 transcription factor, which can bind to the G-quadruplex motif.[3] Considering that Sp1 binding is critical for activity of the human KIT promoter,[4] highly stable G-rich oligonucleotides mimicking G-quadruplexes from KIT could be used as decoys to sequester these proteins and modulate KIT expression.

In an attempt to design stable G-quadruplexes that could be used as decoy molecules against KIT, we investigated the impact of covalently attached pyrene on the folding, stability and structure of c-kit2 G-quadruplex. We found that individual incorporation of U^{py} (5-(1-pyrenylethynyl)-2'-deoxyuridine) in the pentaloop of c-kit2 caused structural polymorphism and in some cases destabilization. On the other hand, incorporation of U^{py} at individual or both termini of the c-kit2 sequence resulted in highly stable G-quadruplex structures with preserved parallel topology. Interestingly, detailed structural analysis revealed major difference in structural dynamics of Upy between the two terminal analogues. While $U^{py}1$ appeared structurally rigid with one well-defined stacking mode of the pyrene moiety, $U^{py}21$ exhibited multiple conformational states. We believe that the contrast between structural dynamics of $U^{py}1$ and $U^{py}21$ stems from an intrinsic asymmetry of c-kit2 G-quartets. This way U^{py} acts as a probe for local G-quadruplex dynamics. This is a vice-versa effect to the binding of ligands comprised of unfused aromatic rings to G-quadruplexes, where ligand planarity is key for efficient stacking.[5]

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Non-canonical Motifs in Artificial Circular DNA Nanosystem

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It is generally known that the DNA molecule can create non-canonical structural motifs. Various biological processes directly depend on the creation of non-canonical structures in DNA.[1] In addition to some *in vivo* studies, the research is also focused on the development of nucleic acid-based nanotechnologies. For example, the development of DNA switchers, which is an assembly of supramolecular nucleic acid that undergoes cyclic, switchable transitions between two distinct states in the presence of appropriate triggers such as pH value, metal ions/ligands, photonic and electrical stimuli. Applying of switchable DNA systems for tailoring switchable DNA hydrogels and controlled drug release or switchable enzyme activation have been described recently. Other possible perspectives for applications of such systems are still in the process of development.[2]

In our work, we have prepared and characterized a circular artificial DNA nanosystem. Suitably designed single-stranded sequences are used as a building block to prepare circular DNA molecules. The sequences that make up the overall structure of the circle can create non-canonical structures under certain conditions. To favorize the creation of non-canonical motifs in the overall nanostructure of circle is achieved by the inserts that are not complementary. In certain cases, the formation of G-quadruplexes and i-motifs can be moderated by changing the pH or increasing the salt concentration, respectively. In principle, this system imitates biological circular objects such as plasmid, but it is also possible to use it in nanotechnologies as pH-switchers and/or salt-dependent biosensors.

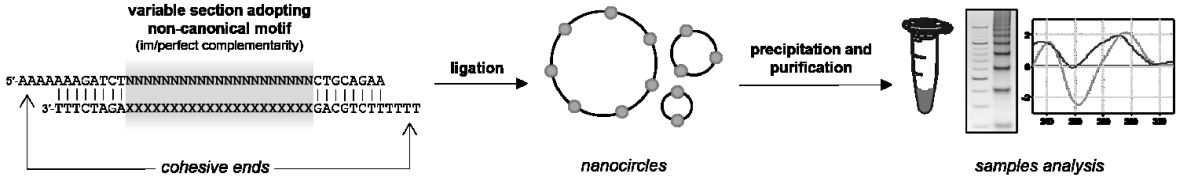

Figure: Principle of artificial DNA nanosystem preparation

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Non-canonical structures formed by a purine-rich sequence found in AUTS2 promoter

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The AUTS2 protein is expressed as a long or short isoform based on the stage of brain development.[1] Misregulation of their expression has been correlated with developmental delay and intellectual disability.[2] The molecular mechanism responsible for switching between the two isoforms is unknown. We identified a CGAG-rich region located approximately 150 base pairs upstream of the transcription start site of the long isoform. The region has a high potential to form a variety of stable non-canonical secondary structures, which may be involved in the expression switch. As long repetitive sequences are prone to polymorphism,[3,4] we focused on three truncated variants to explore the sequence-structure relationship of the CGAG-rich region. We show that the variants form thermally stable hairpins stabilized predominantly by G:C and G:A base pairs. The number of CGAG repeats critically affects the arrangement of the loop. The structural differences are rationalized using obtained high-resolution structures. The acquired knowledge will enable us to study the entire CGAG-rich region of the promoter. Our approach will aid structural studies of repetitive sequence motifs and characterization of their complicated conformational landscapes.

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