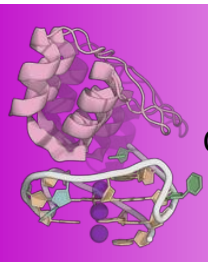

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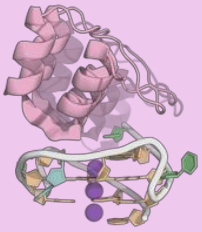

Book of Abstracts

Poster session

Characterization of a variant of Replication Protein A (RPA) found in a patient with telomeropathy

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Abstract:

Telomeres are nucleoprotein structures that protect the ends of chromosomes. The human Replication Protein A (hRPA) complex, a key single-stranded DNA-binding protein, plays a critical role in telomere maintenance by resolving G-quadruplex (G4) structures that can hinder replication. Mutations in RPA subunits have been linked to telomere biology disorders (TBDs), a group of rare and heterogeneous diseases characterized by premature telomere shortening and genomic instability. In this study, we characterized a novel hRPA variant with a mutation located in an intrinsically disordered region of the protein. *In vitro* studies with purified recombinant proteins showed that this mutation does not affect ssDNA binding or G4 unfolding properties of RPA. However, phase separation assays revealed that this mutation led to the formation of smaller molecular condensates, indicating a potential role of the intrinsically disordered region in regulating condensate dynamics. These findings enhance our understanding of hRPA phase separation properties and provide new perspectives on how specific mutations may contribute to telomere-associated dysfunctions in TBDs.

SubG4s: A Novel Class of Dehydration-Induced G-Quadruplexes

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Abstract:

Guanine-rich DNA sequences can form G-quadruplexes (G4s), yet most studies have focused on canonical right-handed forms. Here we report a novel class of inducible G4s (called SubG4s) and revealed through systematic analysis of left-handed G4s (LHG4s). We identify strict sequence constraints, a parity-dependent folding rule, and induction by dehydrating cosolvents, peptides, and a rationally designed ligand. Unexpectedly, certain variants adopt achiral quadruplexes lacking circular dichroism signatures, expanding G4 conformational space beyond handedness. A predictive model establishes millions of potential subG4 loci across the human genome, enriched in repetitive and regulatory regions. These results uncover a previously overlooked, dehydration-sensitive layer of quadruplex regulation with broad implications for genome organization and gene control.

Selective ethidium-based G-quadruplex ligands with antiparasitic and antitumoral activity

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Abstract:

Vector-borne parasitic diseases such as leishmaniasis, Chagas disease and sleeping sickness are caused by the kinetoplastid parasites *Leishmania*, *Trypanosoma cruzi* and *Trypanosoma brucei*, respectively. Current therapies are inappropriate for different reasons including low efficacy, high toxicity, and the emergence of drug-resistant parasites. Thus, new drug candidates are needed to improve the therapeutic arsenal against these Neglected Tropical Diseases (NTDs).

Recent studies have shown that the genome of protozoan parasites, especially kinetoplastid parasites,[1-4] has a high prevalence of putative quadruplex-forming sequences (PQSs).[5] In *Leishmania* and *T. brucei* they are distributed in 5'UTR regions (12% and 44%, respectively) or gene promoters (14% and 36%, respectively). This high prevalence of G-quadruplexes (G4s) and the crucial roles they play in the regulation of vital processes of these organisms, including immune evasion and virulence,[7] underscores G4s as a promising drug target in kinetoplastid parasites.[5]

In this work, we designed and synthesised G4 ligands based on the phenanthridin-5-ium structure. This scaffold is interesting because it displays intrinsic fluorescence, G4 binding properties, and it has antitrypanosomal and antitumoral activity. Molecular docking studies were performed to observe the binding modes and interaction energies with G4s. We found that several phenanthridinium derivatives present optimal interactions with these targets. The biophysical studies (FRET and CD) confirmed the interaction of these compounds with human and parasite G4s as well as selectivity versus duplexes. The new compounds were also tested in vitro against *Leishmania* parasites and tumoral cell lines. Several hit compounds displayed submicromolar activities and adequate selectivity against these targets.

Acknowledgements

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Human TPP1 contacts telomeric DNA into the ternary POT1:TPP1:DNA complex

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Abstract:

Telomeres are DNA repeated sequences that associate with Shelterin proteins and protect the ends of eukaryotic chromosomes. Human telomeres are composed of 5' TTAGGG repeats and ends with a 3' single-stranded tail, called G-overhang. This overhang can fold into stable G-quadruplex structures that are specifically bound and unfolded by the Shelterin protein POT1 (human Protection of Telomeres 1). hPOT1 forms a heterodimer with the human telomeric protein TPP1 (hTPP1), which recruits the telomerase and acts as a telomerase processivity factor. *In vitro* studies have shown that hTPP1 enhances hPOT1's affinity for its substrate, however the underlying mechanism remains unclear. In the present study we performed Electrophoretic Mobility Shift Assay, Surface Plasmon Resonance assays and Photo Affinity Labelling to explore the binding mode and contacts within the ternary hPOT1-hTPP1-DNA complex. Our data show that hTPP1 increases the DNA binding dynamics of hPOT1 and contacts the DNA within the ternary POT1-TPP1-DNA complex.

Interplay Between G-Quadruplex Structures and Helicases DDX5/17 in HBV mRNA Regulation

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Abstract

Chronic infection with the Hepatitis B virus (HBV) is still a major global health burden and the leading cause of liver cancer. No curative treatments are available, primarily due to the persistence of the viral reservoir, the covalently closed circular DNA (cccDNA). This viral minichromosome serves as the template for the transcription of viral mRNAs, crucial for viral replication and liver pathogenesis. These transcripts undergo a series of co-transcriptional processing, including polyadenylation and splicing, whose regulatory mechanisms, yet remain incompletely understood.

Several studies, including ours, pointed out the critical role of G-quadruplexes (G4s) secondary structures in cccDNA transcription. Those structures also regulate the processing of host transcripts. Interestingly, a peculiar G4, the conserved PQS8, is located upstream of the HBV polyadenylation signal (PAS) and of a particular HBV splicing donor site at position 2070 (DS2070). Strikingly, the recognition of these 2 sites is hampered by two RNA helicases, DDX5 and DDX17 acting as host restriction factors. Silencing of both proteins resulted in the induction of a HBV transcriptional readthrough and the significant increase of two splice variants (SVs), SP03 and SP17. These helicases are well-known G4 resolvases, prompting us to hypothesise that part of their contribution is dependent on this PQS8.

First, trans-complementation assays or expression of wild-type (wt) or helicase dead proteins showed that the role of both DDX5 and DDX17 in regulating HBV RNA metabolism depended on their helicase activity. Then, we tested the role of PQS8 in HBV RNA metabolism by introducing point mutations in the PQS8 sequence of a cccDNA-mimicking model that circular dichroism experiments showed to disrupt its ability to form a G4. HepG2-NTCP cells were then transfected with either wt or the PQS8 mutated cccDNA molecules. Interestingly, ddPCR experiments revealed that SP03 and SP17 SVs proportion were decreased. Furthermore, HBV RNA levels were also decreased, which is concordant with their readthrough-mediated destabilisation. 3' RACE-PCR are currently being performed to test this possibility combined with RNA stability assays. Finally, chromatin immunoprecipitation experiments showed a decreased association of both DDX5 and DDX17 to cccDNA when the PQS8 is mutated.

Overall, these findings support a mechanism wherein PQS8 serve as docking site for DDX5/17 helicases, coordinating recognition of cPAS and DS2070 to regulate HBV mRNA metabolism. This G4-helicase axis unveils a novel vulnerability of HBV lifecycle and represents a promising antiviral strategy for chronic hepatitis B.

Alternative splicing of the apoptosis regulator gene MCL-1 is controlled by G-quadruplex of its pre-mRNA

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Abstract

Apoptosis is a genetically-programmed cell death mechanism which plays a crucial role notably in cancer. *MCL-1* is a major regulator of apoptosis. Its pre-mRNA undergoes an alternative splicing (AS) which leads to two major antagonistic isoforms: a long canonical anti-apoptotic isoform (Mcl-1_L), and a short alternative pro-apoptotic isoform (Mcl-1_S). This AS pattern is similar to the one of *BCL-x,* another master regulator of apoptosis of the Bcl-2 family. Deregulation of AS of these genes in favor of the long antiapoptotic isoforms is almost systematically found associated to cancers. We have recently shown that *BCL-x* AS is regulated by the interaction between the splicing factor RBM25 and an RNA G-quadruplex (rG4) that forms in its pre-mRNA. Here we found that overexpression of RBM25 leads to an increase of Mcl-1_S. *In silico* predictions unraveled two potential rG4 sequences, PQS3 and PQS4, near the alternative splice sites of *MCL-1*. Destabilization of at least one of these PQS, strongly favors the pro-apoptotic isoform, Mcl-1_S. Furthermore, the G4 ligand PhenDH8 also leads to an increase of Mcl-1_S in various cell lines with, as observed for *BCL-x*. Finally, downregulation of RBM25 also favors Mcl-1_S. Taken together, these results suggest that a common mechanism involving RBM25 binding to rG4 of their pre-mRNA may control AS of both *BCL-x* and *MCL-1*, two major apoptosis regulatory genes, and points out to relevant therapeutic targets to promote both Mcl-1_S and Bcl-xS isoforms and therefore interfere with chemotherapy resistance in cancer.

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FRET-unfold: a novel in vitro assay to characterize G-quadruplex (G4)-destabilizing molecules

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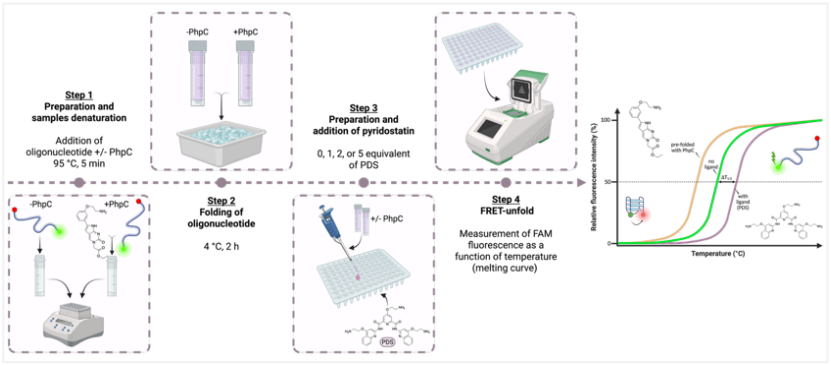
Abstract

Neidle and Hurley reported in 1997[1] on the very first prototype of a G-quadruplex (G4)-interacting molecule (or G4 ligand) aimed at being used for therapeutic approaches[2]. Since then, massive efforts have been invested to uncover ever more efficient G4 ligands[3], which now culminates with >4,000 different molecules in the G4 ligand database (G4LDB)[4]. To make this possible, a series of *in vitro* protocols have been developed to characterize their G4-interacting properties -that is, their G4-stabilizing properties- in a fast and reliable manner. Undoubtedly, the most commonly used assay is the Fluorescence Resonance Energy Transfer (FRET)-melting[5,6] assay, which has been subsequently declined into several other assays (*e.g.*, FRET-MC[7] and iso-FRET[8]) to tackle the known limitations of the initial protocol. Herein, we would like to report on a novel addition to the portfolio of FRET-based *in vitro* techniques, with the FRET-unfold: inspired by the FRET-MC assay, which relies on the displacement of the well-established G4 ligand PhenDC3[9] from a labeled to an unlabeled G4-forming sequence, the FRET-unfold relies on the modulation of the interaction between another well-established G4 ligand, the pyridostatin (PDS)[10], and a labeled G4-forming sequence (F-S100P-T)[11] by putative G4 destabilizing molecules. This assay could thus lead to the identification of promising G4 unfolders, in a fast and reliable manner. In this poster, we will 1/ explain why it is important to uncover and characterize G4 unfolders[12], 2/ describe how this new class of molecules were identified so far (the so-called G4-unfold workflow)[13], and 3/ present the results obtained with FRET-unfold, which led to the identification of highly promising candidates.



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Detecting G-quadruplexes in *Haloferax volcanii* using Super Resolution Microscopy

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Abstract:

Interest in G-quadruplex research has grown over the past decades to understand more about what cellular functions these G4 may have. G4 have been extensively studied in eukaryotes and well-described in bacteria, but very little is known about the presence of G4 in the third domain of life, archaea.

While archaea are morphologically similar to bacteria (i.e. small, single-celled prokaryotes), archaea more closely resemble eukaryotes on an enzymatic level, particularly with respect to nucleic acid metabolism. Recent bioinformatic searches have indicated a strong likely presence of G4 in different archaeal species, but this work presents the first *in cellulo* evidence that G4 exist in archaea, specifically *Haloferax volcanii*. *H. volcanii* was originally isolated from the Dead Sea and exists as a mesophilic halophile, growing optimally at 45°C and 2.5 M NaCl. This high sodium condition combined with the fact that the genome of *H. volcanii* is also significantly GC rich (~65% GC content) provides an optimal model for the first investigation of G4 in an archaeal species.

Fixed *H. volcanii* samples were incubated with the BG4 antibody and imaged with Structured Illumination Microscopy (SIM) where they demonstrated distinct, punctuate G4 foci dispersed throughout the cell. After several rounds of protocol adaptation, imaging with Stochastic Optical Reconstruction Microscopy (STORM) demonstrates a similar G4 foci pattern as seen with SIM. These two different techniques can provide different levels of quantification of BG4 signal: SIM allows the quantification of signal intensity (where higher intensity indicates a higher amount of G4). STORM allows for a more direct quantification of the number of G4 foci. Using both techniques, we can compare the amount of G4 in *H. volcanii* with and without treatment of RNase A; both techniques show a statistically significant decrease in BG4 signal after RNase A treatment, indicating that *H. volcanii* expresses both DNA-based and RNA-based G4.

Due to the very heterogeneous nature of *H. volcanii*, any quantification generally requires a large sample size to be statistically powerful (>800 cells per condition). To achieve direct quantification with the higher throughput required in a more practical manner, this work is extending to incorporate Expansion Microscopy (ExM) with SIM. This continuing work will not only shed more light on the general role of G-quadruplexes, but will also highlight evolutionary similarities and key differences between archaea and eukaryotes by leveraging super-resolution techniques.

Unraveling Viral peptide-G4 Interactions: NS3 Protease Domain of Yellow Fever Virus Binds G-Quadruplexes with High Specificity and Affinity.

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Abstract :

G-quadruplexes (G4s) are critical nucleic acid secondary structures that play pivotal roles in regulating gene expression. In this study, we conducted a proteome-wide *in silico* analysis across multiple viruses causing hemorrhagic fevers to identify candidate proteins containing a conserved G4-binding motif. Four peptides belonging to Marburg, Ebola, Hantaan and Yellow fever viruses were shown to bind to G4 *in vitro*. We selected the NS3 protease domain of Yellow Fever virus for further validation. Biochemical assays demonstrated that the NS3 protease domain binds G4 structures with high specificity and affinity, particularly favoring the parallel conformation. Molecular docking and simulations further revealed that the NS3 protease domain interacts with the terminal G-tetrads and loop regions of G4 via key residues, including Arg37, adopting an insertion and stacking composite binding mode. These findings expand our understanding of virus–G4 interactions and offer novel potential targets for G4-based antiviral strategies.